Point of care (POC) tests for diagnosis of sexually transmissible infections (STIs) in Australia

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Sexually transmissible infections (STIs) are responsible for a huge health, economic and social burden globally with an estimated almost 360 million new infections in 15–49 year olds annually due to curable microbial STIs. Access to accurate diagnostic testing is a key control strategy, yet in many settings laboratory infrastructure is inadequate or as is the case in remote Australia, significant physical distances and highly mobile patient populations are barriers. Point-of-care (POC) tests have the potential to provide timely testing and treatment for STIs, yet their poor accuracy or in the case of syphilis, their inability to differentiate current from past/treated infection, has limited their use. New POC tests, which overcome these limitations, need rigorous laboratory and field-based evaluations before being introduced programmatically. The research presented in this thesis provides new information from evaluations of novel POC tests for STIs.

The first evaluation was a multi-site laboratory-based assessment of one new and three traditional POC test for syphilis; more than 1000 sera were tested. The new test identified >93% of active syphilis infections, whereas just over half of past infections were diagnosed as past or not syphilis, thereby avoiding unnecessary treatment compared with traditional POC tests. Its sensitivity (89.8%) was lower than traditional tests, possibly limiting its usefulness. The second evaluation was field-based in two remote Aboriginal communities and included two traditional tests and one new molecular-based POC test for chlamydia (CT) and gonorrhoea (NG); this followed a laboratory-based investigation which already confirmed test accuracy. The new test was easy-to-use and exceptionally more sensitive (CT:100%; NG:100%) than traditional tests (CT:27.3%; NG:66.7%), and was subsequently included in a large cluster-randomised controlled trial involving 12 remote Aboriginal health services to evaluate clinical benefit and accuracy in the hands of health care staff. Over 2500 POC tests were performed, demonstrating very high concordance (CT:99.4%; NG:99.9%) with laboratory results.

The findings presented here contribute new knowledge to the field of STI POC testing and highlight the importance of the evaluation pathway. The research has already informed the direction of further STI POC program implementation in Australia. These findings are also likely to be applicable more broadly in similar settings globally.

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3. **Causer LM**, Hengel B, Natoli L, Tangey A, Badman SG, Tabrizi SN, Whiley D, Ward J, Kaldor JM, Guy RJ. *A field evaluation of a new molecular-based point-of-care test for chlamydia and gonorrhoea in remote Aboriginal health services in Australia.* **Sex Health**. 2014 Nov 27. doi: 10.1071/SH14158. [Epub ahead of print]

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Abstract

Sexually transmissible infections (STIs) are responsible for a huge health, economic and social burden globally with an estimated almost 360 million new infections in 15–49 year olds annually due to curable microbial STIs. Access to accurate diagnostic testing is a key control strategy, yet in many settings laboratory infrastructure is inadequate or as is the case in remote Australia, significant physical distances and highly mobile patient populations are barriers. Point-of-care (POC) tests have the potential to provide timely testing and treatment for STIs, yet their poor accuracy or in the case of syphilis, their inability to differentiate current from past/treated infection, has limited their use. New POC tests, which overcome these limitations, need rigorous laboratory and field-based evaluations before being introduced programmatically. The research presented in this thesis provides new information from evaluations of novel POC tests for STIs.

The first evaluation was a multi-site laboratory-based assessment of one new and three traditional POC test for syphilis; more than 1000 sera were tested. The new test identified >93% of active syphilis infections, whereas just over half of past infections were diagnosed as past or not syphilis, thereby avoiding unnecessary treatment compared with traditional POC tests. Its sensitivity (89.8%) was lower than traditional tests, possibly limiting its usefulness. The second evaluation was field-based in two remote Aboriginal communities and included two traditional tests and one new molecular-based POC test for chlamydia (CT) and gonorrhoea (NG); this followed a laboratory-based investigation which already confirmed test accuracy. The new test was easy-to-use and exceptionally more sensitive (CT: 100%; NG: 100%) than traditional tests (CT: 27.3%; NG: 66.7%), and was subsequently included in a large cluster-randomised controlled trial involving 12 remote Aboriginal health services to evaluate clinical benefit and accuracy in the hands of health care staff. Over 2500 POC tests were performed, demonstrating very high concordance (CT: 99.4%; NG: 99.9%) with laboratory results.

The findings presented here contribute new knowledge to the field of STI POC testing and highlight the importance of the evaluation pathway. The research has already informed the direction of further STI POC program implementation in Australia. These findings are also likely to be applicable more broadly in similar settings globally.

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List of abbreviations

AGSP	Australian Gonococcal Surveillance Program
AHW	Aboriginal health worker
ANC	Antenatal care
AMR	Antimicrobial resistance
ASSURED	Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, Deliverable
CARPA	Central Australian Rural Practitioners Association
CI	Confidence interval
CT	Chlamydia trachomatis (organism)
DNA	Deoxyribonucleic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
FTA-ABS	Fluorescent treponemal antibody absorption
GASP	Gonococcal Antimicrobial Surveillance Program
GUD	Genital ulcer disease (syndrome)
HIV	Human immunodeficiency virus
IA	Immunoassay
IC	Immunochromatographic
LAP	Lower abdominal pain (syndrome)
MTCT	Maternal-to-child transmission
NAAT	Nucleic acid amplification test
NATA	National Association of Testing Authorities, Australia
NG	Neisseria gonorrhoeae (organism)
NHANES	National Health and Nutrition Examination Survey
NNDSS	National Notifiable Disease Surveillance System
NPV	Negative predictive value
NRL	National Reference Laboratory, Australia
NSP	Needle and syringe program
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
PNG	Papua New Guinea

POC	Point-of-care
PPNG	Penicillinase-producing Neisseria gonorrhoeae
PPV	Positive predictive value
QA	Quality assurance
QAAMS	Quality Assurance for Aboriginal and Torres Strait Islander Medical Services
QC	Quality control
RACGP	Royal Australian College of General Practitioners
RCPA	Royal College of Pathologists of Australasia
RPR	Rapid plasma reagin test
SEAR	South East Asian Region
SDI	(WHO) Sexually Transmitted Diseases Diagnostics Initiative
SMS	Short message service
STARD	Standards for Reporting of Diagnostic Accuracy
STI/STD	Sexually transmissible infection/sexually transmitted disease
STRIVE	STIs in Remote communities: ImproVed & Enhanced primary health care
TPHA	*T. pallidum* haemagglutination assay
TPPA	*T. pallidum* particle agglutination assay
TTANGO	Test, Treat And GO (trial)
TV	Trichomonas vaginalis (organism)
UD	Urethral discharge (syndrome)
UNAIDS	Joint United Nations Programme on HIV/AIDS
VD	Vaginal discharge (syndrome)
VDRL	Venereal Disease Research Laboratory test
WA	Western Australia
WHO	World Health Organisation
WPR	Western Pacific Region

Thesis overview

Rapid point-of-care (POC) tests offer a new approach to improve timeliness of diagnosis, an important pillar of infectious disease control. Bacterial sexually transmissible infections (STIs) such as syphilis, chlamydia and gonorrhoea are good candidates for POC test diagnosis as most infections are asymptomatic, highly transmissible, and easily treatable. Globally the greatest burden of illness from STIs is in resource-limited settings where traditional laboratory diagnosis is often unavailable or impractical. In Australia, despite the availability of curative treatment and reliable laboratory diagnosis, rates of STIs are highest in gay men residing in urban settings where testing rates are suboptimal and in remote Aboriginal communities where there are significant physical distances to laboratories and populations are highly mobile.

Constraints to widespread uptake of POC testing include the inability to distinguish current from past/treated infection for syphilis and the very poor performance of traditional lateral flow tests for chlamydia and gonorrhoea. New and novel POC tests for STIs are have recently become available including a syphilis test able to distinguish current from past/treated infection and a molecular-based test for simultaneously detecting *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoea* (NG). When being considered for programmatic implementation, these new tests require rigorous laboratory and field-based investigations to ensure accuracy, acceptability and suitability in a variety of settings, and most importantly relevant patient and community-important outcomes.

This thesis aims to contribute new knowledge to the field of POC testing for STIs, particularly with respect to innovative advances in POC test technology. The findings will facilitate and support decision making for programmatic STI POC test implementation in Australia. Furthermore, the conclusions and recommendations of this thesis are also likely to be applicable more broadly to similar settings globally.

The portfolio of research with which I have been involved during my candidature, has complemented and enhanced my previous experience in POC test evaluations in both disease area and context (which focused primarily on malaria diagnosis and case management in sub-Saharan Africa). This thesis was completed while employed in my current position as a lecturer at the Kirby Institute, UNSW Australia. In this role I have contributed significantly to a series of research projects evaluating POC tests for STIs in Australia (as presented in this thesis) and in PNG (including the first pilot study of a full suite

of POC testing for STIs in an antenatal setting). I have been integrally involved in every aspect of the research described in this these including study design, ethics submissions, planning, logistics, training and implementation, data collection, analysis and dissemination of findings (including peer-reviewed publications), and generation of recommendations. Importantly, all these projects have been highly collaborative, bringing together researchers, government, community and pathology organisations and their staff across several regional and jurisdictional boundaries. In particular, the ability to conduct the important field work has hinged upon the establishment and development of highly respectful relationships and negotiations conducted by the investigators with the communities, stakeholders and their representatives. Without the support of the communities, none of this research would have been possible. Future collaborations and ongoing research depends upon upholding these respectful and reciprocal relationships.

In summary, the work presented in this thesis arises from three specific evaluations of POC test performance in the Australian setting. The first, a multi-centre, laboratory-based evaluation of three traditional and one novel POC test for syphilis; the second, a pilot field evaluation of two traditional and one new molecular-based POC tests for chlamydia and gonorrhoea which was conducted to inform a cluster –randomised controlled trial; and the third, a large scale operational performance evaluation of the new molecular-based POC test for chlamydia and gonorrhoea in the context of this cluster-randomised controlled trial.

The thesis commences with a review of the relevant literature (Chapter 1) and is followed by two chapters containing peer-reviewed publications reporting on the laboratory-based syphilis evaluation (Chapter 2 and 3). The next chapter (Chapter 4) contains a peer-reviewed publication (not counted as contributing towards this thesis) summarising the large cluster-randomised controlled trial of POC testing for chlamydia and gonorrhoea in remote communities in Australia. This manuscript offers some background and context to the work presented in the subsequent chapters (Chapter 5 and 6) which containing one published manuscript (reporting on the pilot field evaluation of three POC tests for chlamydia and gonorrhoea) and one publication-ready manuscript to be submitted to an international journal for consideration (reporting on the large operational performance evaluation of the novel molecular-based POC test for CT and NG). The thesis ends with conclusions and recommendations chapters (Chapter 7 and 8).

A summary of each chapter is provided below:

Chapter 1 is a review of the relevant literature including burden of bacterial STIs globally and a more detailed account of the epidemiology of STIs in Australia. A discussion of data sources informing the epidemiology of STIs in Australia is also presented, including their benefits and limitations. A brief description of the geography of Australia and the circumstances of the Aboriginal population are presented to provide important context to the rationale for the research in the thesis. From this point forward, the thesis focuses on the three curable infections (syphilis, chlamydia and gonorrhoea) which are at this time most likely to benefit from new POC test technologies. Clinical aspects of these infections are presented including current reference diagnostics and recommended management approaches. STI control approaches are summarised including particular diagnostic and treatment approaches such as laboratory-based diagnosis, syndromic, presumptive and mass treatment, and POC testing. The next section is dedicated to reviewing the ideal evaluation research pathway from development to laboratory and field evaluations and formal trials. The chapter concludes with a summary of currently available POC tests for syphilis, chlamydia and gonorrhoea, their benefits and limitations, and the POC testing landscape in Australia. Research needs are identified in the context of the evaluation pathway to inform program implementation in Australia and the research aims of the thesis are summarised.

Chapter 2 contains a peer-reviewed, published manuscript describing a laboratory-based head-to-head evaluation of the performance of four currently available POC tests for syphilis including a new test designed to distinguish current infection from past/treated infection. The evaluation was conducted using stored sera (including reference treponemal negative and positive specimens across a range of non-treponemal titres) at two large reference laboratories in Australia, performed by trained (double-blinded) laboratory staff. The evaluation is unique in its ability to determine the performance of these POC tests by clinical stage of syphilis and HIV status.

> Manuscript (published) #1: **Causer LM**, Kaldor JM, Fairley CK, Donovan B, Karapanagiotidis T, Leslie DE, Robertson PW, McNulty AM, Anderson D, Wand H, Conway DP, Denham I, Ryan C, Guy RJ. *A Laboratory-Based Evaluation of Four Rapid Point-of-Care Tests for Syphilis. PLoS One. 2014 Mar 11;9(3):e91504.*

Chapter 3 contains a peer-reviewed, published manuscript based on the syphilis POC test evaluation described in Chapter 2. This manuscript uniquely evaluates the clinical implications and performance of the DPP Screen and Confirm Assay, the only currently

available POC test for detection of both treponemal and non-treponemal antibodies, in regard to its ability to distinguish active from past syphilis.

Manuscript (published) #2: **Causer LM**, Kaldor JM, Conway DP, Leslie DE, Denham I, Karapanagiotidis T, Ryan C, Wand H, Anderson DA, Robertson PW, McNulty AM, Donovan B, Fairley CK, Guy RJ. *An evaluation of a novel dual treponemal/nontreponemal point-of-care test for syphilis as a tool to distinguish active from past treated infection.* Clin Infect Dis.2015 Jul 15;61(2):184-91

Chapter 4 contains a peer-reviewed publication describing the TTANGO (Test, Treat and Go) trial to provide context and background to the research presented in Chapters 5 and 6. *This publication does not count as one of the publications contributing to this thesis.* TTANGO is a cluster-randomised controlled trial designed to comprehensively evaluate the clinical effectiveness, cost-effectiveness, feasibility and acceptability of POC testing for chlamydia and gonorrhoea (using the GeneXpert CT/NG assay) in remote primary health services (where STI rates among the Aboriginal population are very high and where there are great distances between health services and their jurisdictional reference laboratories) in the hands of clinical staff. The TTANGO trial itself represents the first use globally of the GeneXpert CT/NG assay by clinical staff in primary health care settings for routine STI management. The TTANGO trial results will provide evidence to support the programmatic implementation of this technology in Australia in settings where STI rates are highest.

Chapter 5 contains a peer-reviewed published manuscript describing a field-based pilot evaluation of three chlamydia and gonorhoea POC tests using fresh specimens collected from patients as they presented for STI testing in two remote primary health services in Australia. The evaluation included two available “best-performing”, traditional format (lateral flow) POC tests and a new molecular-based format for detection of CT and NG. This prospective evaluation represents the first experience using the molecular assay for CT/NG (GeneXpert CT/NG assay) at the point-of-care in remote locations and assessed not only performance in a field setting but also describes the relative feasibility and acceptability of this new technology in this setting. This work directly facilitated the choice of POC test evaluated as part of the large TTANGO trial.

Manuscript (published) #3: **Causer LM**, Hengel B, Natoli L, Tangey A, Badman SG, Tabrizi SN, Whiley D, Ward J, Kaldor JM, Guy RJ. *A field evaluation of a new molecular-based*

point-of-care test for chlamydia and gonorrhoea in remote Aboriginal health services in Australia. Sex Health. 2014 Nov 27. doi: 10.1071/SH14158. [Epub ahead of print]

Chapter 6 contains a manuscript ready for submission for publication to a peer-reviewed journal which describes the findings of an evaluation of the operational performance of the GeneXpert CT/NG assay conducted in the context of the TTANGO trial. The GeneXpert POC test was provided at 12 remote health services where testing was performed by the clinical staff at the service (health workers, nurses and doctors) as part of routine screening and diagnosis. POC test results guided clinical management. Specimens continued to be sent to reference laboratories as usual for routine testing (nucleic acid amplification tests [NAATs]) throughout the study and estimates of POC test concordance with these reference results were calculated. A further analysis of discordant results was conducted at another reference laboratory to explore possible explanations for these few discordant results.

Manuscript (submission-ready) #4: **Causer LM**, Tabrizi SN, Whiley D, Speers D, Tangey A, Badman SG, Hengel B, Natoli L, Ward J, Anderson DA, Wand H, Wilson D, Regan DG, Shephard M, Donovan B, Fairley CK, Kaldor JM, Guy RJ. *Prospective evaluation of the diagnostic performance of a new, molecular point-of-care test for chlamydia and gonorrhoea in routine primary health care use.* Submitted for publication. Proposed for submission - April 2016

Chapters 7 provides a summary of conclusions and **Chapter 8** presents recommendations and suggests possible areas for future research.

Chapter 1: Review of relevant literature

1.1 The burden of curable sexually transmissible infections (STIs)

1.1.1 Global

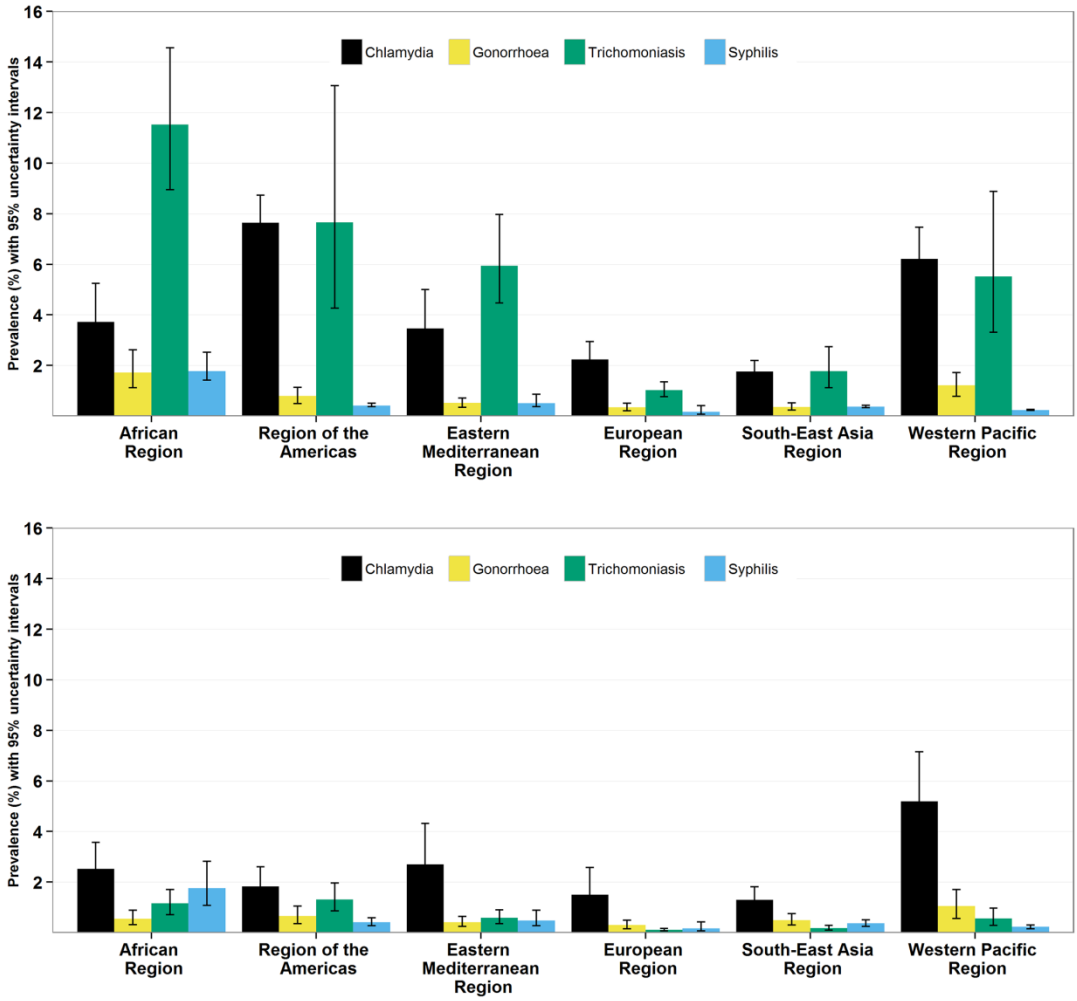
Sexually transmissible infections (STIs) are among the most widespread infectious diseases, exacting substantial social and economic burden on families and communities worldwide. Among the most prevalent and curable STIs (syphilis, chlamydia, gonorrhoea and trichomoniasis), global estimates from the World Health Organization (WHO) suggest that in 2012, there were about 273 million case among adults aged 15–49 years: 128 million prevalent cases of chlamydia, 27 million prevalent cases of gonorrhoea, 101 million prevalent cases of trichomoniasis, and 18 million prevalent cases of syphilis.(1) These correspond to a global incidence of 357 million infections in 15–49 year olds, or an average of nearly one million new infections each day; 131 million incident chlamydia cases, 78 million incident gonorrhoea cases, 143 million incident trichomoniasis cases, and 5.6 million incident cases of syphilis. (1) These most recent estimates were based on models using data among low risk or general populations for genitourinary infection with chlamydia, gonorrhoea and trichomoniasis, and nationally reported data on syphilis seroprevalence among antenatal care attendees.(1) STI co-infection is common, and therefore, it is unclear exactly how many individuals this represents. Global estimates suggest that 9.5% of HIV positive adults are co-infected with syphilis.(2)

Undoubtedly STIs are a global epidemic however there are distinct regional variations with the highest prevalence and incidence rates for syphilis found in the WHO African region which may partly reflect differences in access to antenatal syphilis screening (see Figure 1). (1) All four infections were lowest in the European and South East Asian region regions, possibly explained by the lower risk of infection due to lower numbers of partners and increase condom use, or better social and economic conditions including clinical STI services.(1) An alternative explanation might rest in the variable quality of the data sourced and possible lack of representativeness of the data from these regions.(1)

Syphilis in pregnancy is an important cause of mortality and morbidity in pregnancy, with significant adverse pregnancy outcomes including stillbirth, early foetal death, low birth weight, preterm delivery, neonatal death, or infection or disease in the newborn.(3) In

2008, globally, nearly 1.4 million pregnant women had probable active syphilis infection and were at risk of transmitting the disease perinatally to their unborn children, with an estimated over 520,000 adverse pregnancy outcomes.(3)

Figure 1: Estimated prevalence (and 95% UI) of chlamydia, gonorrhoea, trichomoniasis, and syphilis in women and men aged 15–49 years by WHO region, 2005–2012 data



*From Newman et al (2015)

While developing countries data are scarce, in developed nations, the highest rates of STIs occur in adolescents and young adults under the age of 30 years.(4, 5)

In the recent Global Burden of Disease study, chlamydia, gonorrhoea and trichomoniasis accounted for 1.2 million disability-adjusted life years lost in 2010.(6) These STIs also impose a substantial economic burden on health systems as demonstrated in the United

States, where it was estimated that more than USD$705 million was spent in 2008 to diagnose and treat the complications of chlamydia, gonorrhoea and trichomoniasis.(7)

1.1.2 Australia

To provide some context to the epidemiology of STIs in Australia, sources and methods of data collection, including strengths and limitations, are presented in the following paragraphs.

Sources and systems for STI data collection

In Australia, STI data are collected through national notification systems including passive, enhanced, sentinel, antimicrobial resistance surveillance systems, routine reports and periodic prevalence and incidence surveys. Together these data provide a relatively comprehensive indication of the burden and epidemiology of STIs in Australia, though there are some limitations that should be acknowledged.(8)

Passive surveillance

A select number of STIs (HIV, chlamydia, gonorrhoea, syphilis, donovanosis and hepatitis B) are mandated for notification by legislation at the state and territory level and contribute to The National Notifiable Disease Surveillance System (NNDSS) which is maintained by the Australian Government Department of Health and Ageing.(9) All cases of laboratory-confirmed chlamydia, gonorrhoea and syphilis are captured through reporting via the network of public and private laboratories across Australia (which provide pathology services to hospitals, specialist clinics and primary health services). Clinicians are required to provide additional demographic and clinical data to enhance these notifications, particularly for cases of gonorrhoea. HIV diagnoses are sent directly to the Kirby Institute for Immunity and Infection (UNSW Australia). Trichomoniasis is only mandated at the state level for reporting in one jurisdiction (Northern Territory). These STI notification data are analysed and reported annually, and are essential to inform, monitor and evaluate STI control programs and policy in Australia.(4, 10)

Notification data provide a simple, population level picture of STI diagnoses, and are useful for monitoring trends over time. As data relating to the denominator (total number of people tested) are not collected, any trends identified need to be interpreted in the context

of changes in testing patterns and may not equate directly with actual changes in disease transmission or prevalence.(11) Furthermore, incompleteness of socio-demographic identifiers, such as Aboriginality status in many jurisdictions, can result in over or under estimation of STI rates in population subgroups.(12)

Enhanced surveillance

Enhanced surveillance, where follow-up is conducted with clinicians to gather missing or additional information including Aboriginal status and mode of exposure (e.g. male-to-male sex), can overcome some of these shortcomings but this is only feasible nationally for infections like HIV and syphilis where numbers of diagnoses are manageable (~1000 cases annually). Some enhanced surveillance is conducted for gonorrhoea in some jurisdictions, however none is conducted for other STIs given the large number of diagnoses reported (>80,000 for chlamydia). As a result, information related to exposure risk and Aboriginal status varies by infection and jurisdiction. Notification rates of STIs generally are likely to underestimate the true picture in the broader population, as testing is generally undertaken for clinical reasons that prompt health service attendance (symptoms, risk behaviour or as a consequence of contact tracing).(13) As a consequence, the vast majority of infections which are asymptomatic go undetected and are not notified at all.

Sentinel surveillance

Systematic collection of routinely recorded data at suitable clinical sites can supplement information from passive surveillance systems. This 'sentinel surveillance' allows for more detailed collection of important data around specific diagnoses such as risk behaviours and also allows for collection of testing denominator data in order to calculate positivity (the proportion of tests taken which were positive over a specified time frame and population).(11) If test uptake is high, positivity can represent prevalence, however in situations where uptake is low, these data may over-estimate prevalence as testing may only be done on those at risk or with symptoms.

Since 2007, the Australian Collaboration for Chlamydia Enhanced Sentinel Surveillance (ACCESS), which is a collaboration of 76 primary care sites (sexual health clinics, family planning, general practice and Aboriginal community controlled health services), has been providing consultation, demographics, risk factor, chlamydia testing and results for every

individual attending the clinic.(14) In 2011, ACCESS expanded to include gonorrhoea and a range of other STIs and blood borne viruses.

Surveys and studies

Special surveys and studies (repeated or one off) are sometimes undertaken in certain populations groups to understand in more depth risk factors for STI disease transmission or to more accurately estimate disease positivity or prevalence. Some examples of repeated cross-sectional surveys for STIs using a convenience sample conducted in Australia include the Gay Community Periodic Survey undertaken annually among gay men in most cities in Australia to monitor self-reported testing and risk behaviour among gay men (15) and the National Survey of Australian Secondary Students and Sexual Health undertaken regularly since 1992 with year 10 and 12 students.(16) Although these surveys provide important data regarding behaviours and potential risk factors for acquisition of STI infections, biological samples are not collected, thus STI positivity was not able to be estimated.

True population-based prevalence estimation requires a high level of uptake in a representative population sample and is often resource intensive so can be difficult to obtain. An example of a population-based prevalence survey is the National Health and Nutrition Examination Survey (NHANES) in the United States which is conducted every year on a representative sample of individuals (around 5000 people) and combines both a behavioural survey and physical examination.(17) The survey findings are used to determine the prevalence of major diseases and risk factors for diseases.

One-off studies describing the prevalence of STIs in Australia have been undertaken and measure test positivity in convenience samples (usually routine health centre attendees) as an indicator of the extent of infection within a population, though these primarily have reported on chlamydia and few have been conducted in Aboriginal populations.(8, 18-20) Estimates of incidence of infection, in addition to the prevalence, provides insight into the duration of infection, a key parameter in determining the frequency of screening needed to reduce rates of infection and thereby the burden of disease. In the context of the STRIVE trial,(19) conducted across 65 remote communities in central and northern Australia, the reported findings among almost 18,000 individuals of incidence of chlamydia, gonorrhoea and trichomoniasis in men and women represents the largest study conducted of the incidence in any population.(20)

Gonococcal antimicrobial resistance (AMR) monitoring

The Australian Gonococcal Surveillance Programme (AGSP) has continuously monitored antimicrobial resistance in clinical isolates of *Neisseria gonorrhoea* (NG) from all states and territories since 1981. (21) Given the inherent difficulties in specimen collection, transport and culture methodologies (described in more detail in Section 1.2.2 Diagnosis of chlamydia and gonorrhoea), and often vast distances between clinical services and gonococcal reference laboratories in Australia, less than 20% of specimens testing positive for NG are successfully cultured.(22) As a result, some laboratories in Western Australia routinely use non-culture molecular-based techniques to detect markers of resistance in NG (PPNG) to selected antibiotics.(22)

The WHO recommends that only drugs with an expected efficacy of 95% and above should be used to treat gonorrhoea(23) and results of AMR monitoring are used to inform recommendations on antibiotic choice for gonorrhoea in national and state based guidelines in Australia.

Additional information related to AMR can be found in Section 1.3.2 and Section 1.4.1)

Geographic locations in Australia

Australia's population distribution across geographical areas has been defined by the Australian Bureau of Statistics (ABS) under the *Australian Statistical Geographical Standard (ASGS*) in order to provide a classification for the release of statistics that inform policy development by classifying Australia into large regions that share common characteristics of remoteness. Areas within Australia are classified as Major Cities, Inner Regional, Outer Regional, Remote and Very Remote.(24)

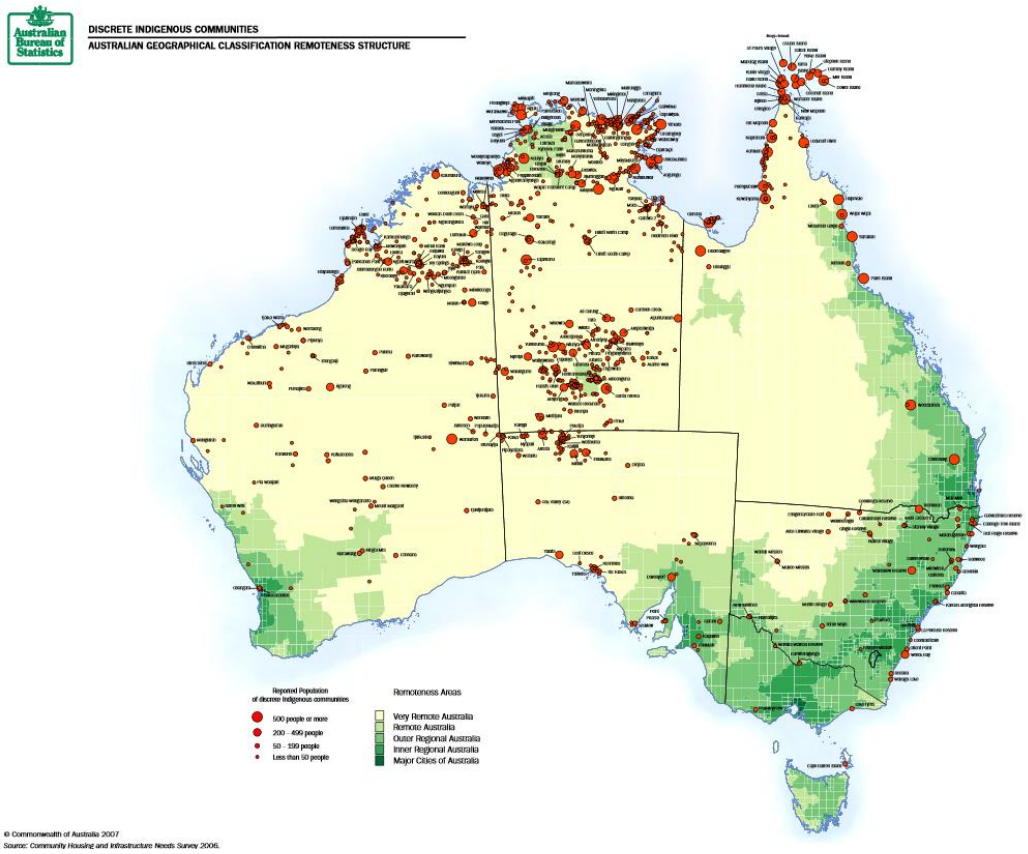
Aboriginal and Torres Strait Islander people

Aboriginal and Torres Strait Islander people are the Indigenous people of Australia. They are not one group, but comprise hundreds of groups that have their own distinct set of languages, histories and cultural traditions. The Australian Government defines Indigenous Australians as people who are of Aboriginal or Torres Strait Islander descent, who identify as being of Aboriginal or Torres Strait Islander origin and who are accepted as such in the communities in which they live, or have lived. In most data collections, a person's Indigenous status is based on the first 2 parts of the definition.(25)

The Indigenous (hereafter called Aboriginal) population make up approximately 3% (669,900 people in 2011) of the Australian population and is much younger than the non-Indigenous population. In 2011, half of the Aboriginal population was aged 22 or under compared with 38 or under for the non-Indigenous population.(25)

In 2006, almost one-fifth of Australia's Aboriginal population lived in a discrete Indigenous community, with the majority of those being classified as Remote or Very Remote (see Figure 2). (26) Virtually all of Australia's remote Aboriginal communities are in the northern half of the country, either in arid, central regions, or tropical zones of Queensland, Northern Territory and Western Australia. People living in remote Indigenous communities may experience more difficulty than other Australians in accessing basic housing, infrastructure and community services due to their isolation from large population centres.(27)

Figure 2: Discrete Indigenous Communities and the Australian Standard Geographical Classification Remoteness Structure



In 2006, just one in ten people were in a community which had a hospital. However, three-quarters (75%) of people in remote Indigenous communities had an Aboriginal Health Care Centre or other community health centre located in their community (see Figure 3). Access to health facilities was influenced by the size of the community.(27) While most people in Indigenous communities have access to Aboriginal Health Care Centres or community health centres, not all centres have full-time trained medical staff able to provide treatment services. Instead, health professionals such as nurses and doctors are required to visit communities on a regular basis.(27)

Figure 3: Health service and community airstrip in remote central Australia

Aboriginal Australians have poorer health than other Australians and are more likely to die at younger ages, experience disability and report their health as fair or poor.(25)

The mortality gap for Aboriginal people compared with non-Indigenous Australians is 12 years for males and 10 years for females. (28) Aboriginal people living in remote and very remote areas experience higher age-standardised rate of years of life lost compared with Aboriginal people in other geographical areas and have higher hospitalisation rates for preventable diseases (ten times) than the national rate and have a higher death rate (three times).(29)

About two-thirds of the Aboriginal–non-Indigenous health gap has been attributed to chronic diseases such as cardiovascular disease, diabetes, mental disorders and chronic lung disease.(25) Other factors contributing to poorer health relate to behavioural and social disadvantages including education, unemployment, low income, discrimination and poor quality housing.(25)

In 2008, the Council of Australian Governments (COAG; peak intergovernmental forum in Australia) agreed to strive to address the disadvantages faced by Aboriginal Australians across a range of areas including life expectancy, child mortality, education and employment.(30) To monitor change, the following targets have been agreed to:

- close the gap in life expectancy within a generation (by 2031);
- halve the gap in mortality rates for Indigenous children under five by 2018;
- ensure access to early childhood education for all Indigenous four year olds in remote communities by 2013;
- halve the gap in reading, writing and numeracy achievements for children by 2018;
- halve the gap for Indigenous students in Year 12 (or equivalent) attainment rates by 2020; and
- halve the gap in employment outcomes between Indigenous and other Australians by 2018.

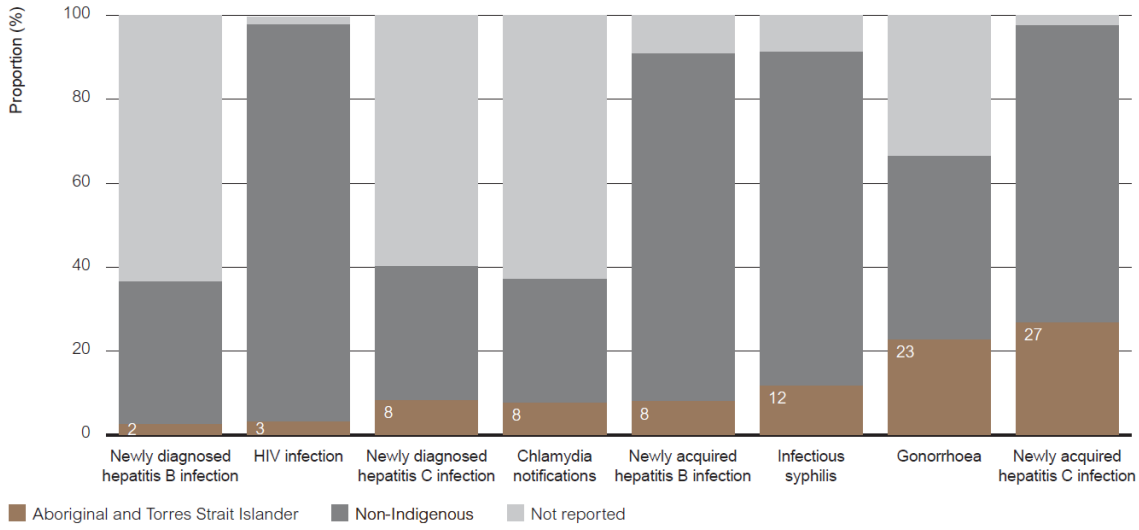
The most recent report from the Prime Minister indicates mixed levels of success in meeting the agreed targets, however there is acknowledgement that achievement of these will take a national commitment and resolve across Indigenous-specific and mainstream programs.(31)

Epidemiology of STIs in Australia

The epidemiology of the curable STIs causing the greatest burden in Australia - syphilis, chlamydia, gonorrhoea, trichomoniasis - and HIV are presented in more detail below to give context and support the rationale for research conducted in this thesis.

Overall, Aboriginal people accounted for a disproportionate level (3 – 27%) of all STIs (and blood-borne viruses) diagnoses in 2014, and for many infections, this proportion may be a lower limit, due to the incompleteness of reporting of Aboriginal status as noted earlier (Figure 4).(10) Young people aged 15–19 years and those living in outer regional, remote and very remote communities experience substantially higher rates of diagnoses per capita of chlamydia, gonorrhoea and infectious syphilis compared to non-Indigenous people.(4, 10)

Figure 4: Proportion of all STI s and blood-borne virus diagnoses by Aboriginal and Torres Strait Islander status, 2014



Source: Australian National Notifiable Disease Surveillance System

<u>Syphilis</u>

Syphilis in Australia is concentrated among men, having male-to-male sex, primarily residing in urban settings, and young heterosexual Aboriginal people, living in remote and outer regional areas. (4)

Among the almost 2000 notifications of cases of infectious syphilis (infections of less than 2 years duration) in 2014, the majority of cases were in males (92%) and 75% were in people residing in major cities. (4) The rate of diagnosis of among men has increased (from 5.1 per 100,000 in 2005 to 15.9 per 100,000 in 2014) whereas the rate among women has remained stable between 1.0-1.9 per 100,000 population.(4)

Very high rates of syphilis have been reported among gay men,(32) particularly those with HIV, where the incidence of syphilis in gay men with HIV infection is up to five-times higher than in those without HIV (2.5 vs. 0.5 per 100 person years). (33)

Data from the ACCESS sentinel surveillance system of sexual health services show that of infectious syphilis diagnoses in males in 2014, 89% (722) were in men who have sex with men. The infectious syphilis positivity was 4 times higher in men who have sex with men with HIV infection than HIV-negative men (8% vs 2%).(4)

Based upon the 91% of diagnoses with Indigenous status reported, the rate in the Aboriginal population was 4 times higher than that in the non-Indigenous population, and over 300 times higher for those living in remote Australia compared to the non-Indigenous urban population.(4) The male-to-female ratio of infectious syphilis notifications among the Aboriginal population in 2014 was 1.1:1, 46% resided in remote/very remote areas, 38% in outer regional areas and 31% were aged 15 – 19 years. This is in contrast to the non-Indigenous population, where the male-to-female ratio was 33:1, 91% resided in urban settings and 99% were aged >20 years.(4)

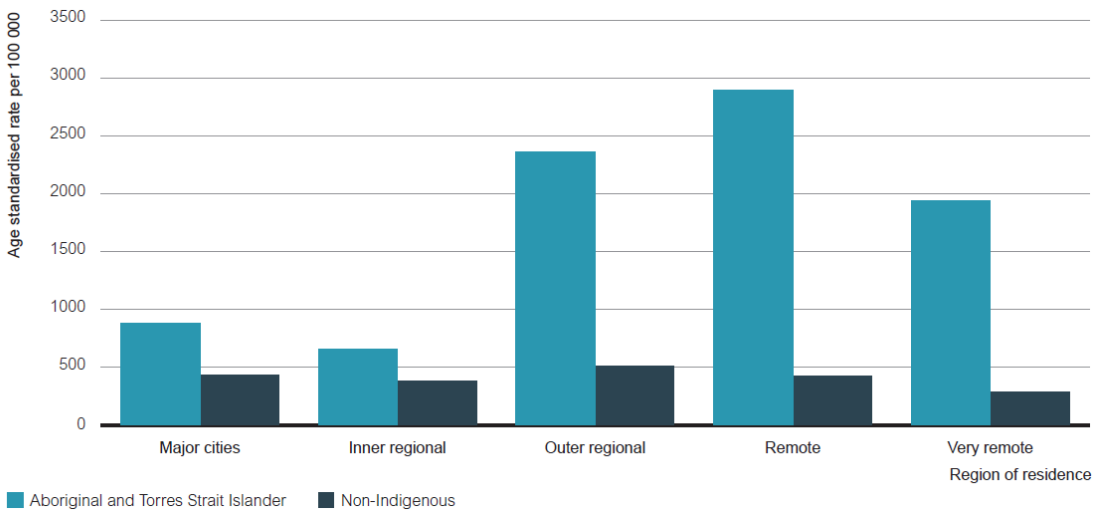
Congenital cases of syphilis are reported in the Aboriginal population with a peak in 2014 (5 cases) which coincided with a peak in infectious syphilis notifications during the same period.(4)

Chlamydia

Chlamydia is by far the most frequently reported notifiable infection in Australia.(4) There were 86,136 new chlamydia infections reported through the NNDSS for 2014, with 8% among the Aboriginal population, 29% among the non-Indigenous population and Indigenous status was not reported for 63%. Females accounted for 57% of diagnoses, 78% occurred in those aged 15-29 years old, and 63% occurred in people residing in major cities.(4)

The rate of chlamydia diagnosis is higher in females than males (436 per 100,000 versus 310 per 100,000 in 2014), and after increasing for a number of years, the rate has remained stable since 2011. Other groups with particularly high rates include the Aboriginal population and people residing in regional and remote locations.(4) The rate of chlamydia notifications overall in the Aboriginal population was over 3 times that in the non-Indigenous population (1341 per 100,000 versus 389 per 100,000), with rates in remote locations 7 times that for the non-Indigenous population (Figure 5).(4, 10)

Figure 5: Chlamydia notification rate (per 100,000) in 2014 by Aboriginal status and area of residence



Source: Australian National Notifiable Diseases Surveillance System; ABS Catalogues: 3101051 – 3101058; 3100DO003_201212; 1270055006_CG_POSTCODE_2012_RA_2011; ABS SuperTable 2011 Census; 2380do001_2011; Includes jurisdictions (NT, QLD, SA, WA) in which Aboriginal and Torres Strait Islander status was reported for more than 50% of diagnoses for each year

Using a modelling approach incorporating a number of data sources (including NNDSS notifications, Medicare testing, and ABS population estimates),(34) there were estimated to be just over 256,000 new cases of chlamydia in 15 – 29 year olds in 2014, only 26% of whom were diagnosed.(4) These data highlight that the vast majority of infections remain undiagnosed and hence untreated, emphasising the need for testing to be routinely offered to sexually active adolescents, young adults and other populations at risk in Australia.(4)

A recent systematic review and meta-analysis of chlamydia prevalence studies in Australia suggested the pooled prevalence in community or general practice settings was in the range of 3.9-5%.(18) Prevalence was higher in younger people (<25 years) attending sexual

health, family planning or youth clinics, with an estimated prevalence of 6.2% for women and 10.2% for men.(18) Among the studies in remote areas where there was a predominantly Aboriginal population, a pooled prevalence of 22.1% and 14.6% for Aboriginal women and men <25 years, respectively, was reported.(18) Among those studies that included age-stratified data, there was a clear trend of increasing prevalence with decreasing age.(18)

Data from the STRIVE trial (19) suggest an overall prevalence of chlamydia (based on detection of CT by NAAT in urine or vaginal/endocervical swab specimen at a laboratory) of 11.1% (women 12.1% vs men 9.7%), with rates of new infection extremely high, particularly among 16-19 year olds.(20) In other analyses from this study, co-infections among young people in this population were also very common, with nearly half of patients aged 16-19 years having one or more of chlamydia, gonorrhoea or trichomonas infection during the study period.(35)

Chlamydia incidence among women attending primary care clinics in Australia has been reported at a rate of 4.4 cases per 100 person-years and is associated with being aged 16 to 20 years, being employed and having two or more new sex partners. Reinfection was common (22.3 cases per 100 person-years), with the median time to reinfection of 4.6 months.(36) In contrast, in an analysis of clinical service data as part of the STRIVE trial, incident chlamydia among young Aboriginal women (16-19 year olds) was reported to be 29.2 per 100 person-years; and among men of the same age, the rate of new chlamydia infections was also very high at 23.4 per 100 person-years.(20)

Gonorrhoea

Similar to the picture for syphilis, gonorrhoea in Australia occurs primarily in men having male-to-male sex in urban settings, and in young heterosexual Aboriginal people living in remote communities.

There were 15,786 cases of gonorrhoea notified in 2014; 23% of these notifications were among the Aboriginal population, 44% among the non-Indigenous population and Indigenous status was not reported for 33% of diagnoses.(10) The majority of notifications were among males (73%) and more than half (67%) reported residing in major cities. (4) These notifications represent a more than doubling of notifications in the last 5 years and with diagnoses increasing in both males and females over this timeframe. One explanation

for the observed increase in gonorrhoea diagnoses may be related to laboratories having recently switched to using dual chlamydia and gonorrhoea tests recently where if a chlamydia test was ordered, a gonorrhoea test would be conducted automatically. Strategies to increase chlamydia testing in young people may therefore have led to a substantial rise in the number of tests conducted for gonorrhoea.(4)

In 2014, the rate of diagnosis of gonorrhoea in the Aboriginal population was 18 times that in the non-Indigenous population (859 vs 49 per 100,000) increasing to 69 times among those residing in remote communities.(4, 10) Given the roughly equal numbers of males and females contributing to diagnoses in the Aboriginal population, transmission is likely to be predominantly heterosexual in this population.(4, 10)

In contrast, gonorrhoea diagnoses in non-Indigenous people in 2014 were predominantly in men, suggesting that transmission is primarily related to sex between men, and most (87%) resided in urban settings.(4)

Data from the STRIVE trial suggest a baseline prevalence of gonorrhoea (based on detection of NG by NAAT in urine or vaginal/endocervical swab specimen at a laboratory) of 9.5% across the remote study communities, with the prevalence in men higher than women (10.4% versus 8.9%) and greatest in the youngest age group for both men (21.6%) and women (20.1%).(20) There were an estimated 26.1 and 23.4 cases of gonorrhoea per 100 person-years in men and women respectively, with those aged 16-19 years having the highest incidence. (20)

Trichomoniasis

Trichomoniasis is a notifiable infection in the Northern Territory. In this jurisdiction, most infections reported are in women (84.7%) and Aboriginal people (96.7%), with the rate 82 times higher than that in the non-Indigenous population.(37) The youngest age groups (15-19 and 20-24 year olds) had the highest recorded rates, however sustained high rates are seen in those up to the age of 49 years.(37) The majority (83%) of trichomoniasis notifications are in females and Aboriginal people (96.9%).(37) The upward trend in the last few years may indicate increased testing rather than increased prevalence, with clinical guidelines recommending annual testing since 2010. (37)

Prevalence studies indicate that trichomonas infections are relatively more common in remote areas and among Aboriginal women than in urban settings. A study undertaken in sexual health clinics in rural and remote New South Wales (using NAATs for diagnosis) found prevalence to be higher (8.4%) than that reported in urban centres, especially among Aboriginal women and those living in more remote areas.(38)

From the STIVE trial,(19) the estimated prevalence of trichomonas infection (based on detection of TV by NAAT in urine or vaginal/endocervical swab specimen at a laboratory) was 17.6%.(20) Women were 3.6 times more likely than men to be tested. Prevalence was highest among women aged 16-19 years (31.5%), whilst among men the highest prevalence was in those aged 35 years and older (7.8%). Interestingly, prevalence remained steady across all age groups for men (range 5.6-7.8%) and declined slightly with age in women (31.5%-20.2%).(20) One possible explanation suggested by Silver et al is that routine screening for TV, introduced during the late 1990s in many Aboriginal communities, has lowered the prevalence of trichomonas infection in the older women but among younger women, who have a higher incidence, prevalence has been sustained through reinfection.(20)

The incidence rate of trichomonas infection overall was 7.3 per 100 person-years, with the rate being significantly higher in women (10.6 per 100 person-years) than men (2.4 per 100 person-years). (20) Trichomonas infection was associated with both chlamydia and gonorrhoea infection, but the association was much stronger with gonorrhoea and in women.(35)

HIV

In 2014 in Australia the estimated HIV prevalence among adults aged 15 years and older was 0.14%, which is low compared to other high income countries and other countries in the region.(4) HIV infection in Australia is concentrated among men who have sex with men, with a prevalence of 14-18% among gay men in the past ten years. (4) This estimate of prevalence is based on self-reported HIV status and as such is prone to bias and likely not representative of the community of men who have sex with men.

In 2014, based on newly diagnosed cases, sexual contact between men accounted for 70% of cases.(4) Due to the availability of needle and syringe programs (NSPs) in Australia (initiated in 1987), HIV infection is low among people who inject drugs with a prevalence of

1-2% among people tested when attending NSPs each year.(4) Prevalence is <1% if gay and bisexual men are excluded from the sample (0.5% in 2014).(4) The prevalence of HIV among women involved in sex work is extremely low (incidence of 0.07% in 2014), and is attributed to the promotion of safer sex practices.(4)

The annual number of new HIV diagnoses has gradually increased by 13% over the past 10 years, from 953 diagnoses in 2005 to 1,064 in 2012 and stabilised since then with 1081 cases of HIV infection newly diagnosed in Australia in 2014.(4) Based on 33 cases, the rates of HIV diagnosis in 2014 among Aboriginal population was higher than in the Australian-born non-Indigenous population (5.9 vs 3.7 per 100,000). (4) In the most recent five year reporting period (2010 – 14), a greater proportion of HIV diagnoses in the Aboriginal population were attributed to injecting drug use (16%) or heterosexual sex (20%) compared with the Australian-born non-Indigenous population (3% and 13%, respectively).(10)

Among cases attributed to heterosexual sex, 23% were in people born in countries recognised by the UNAIDS as having a national HIV prevalence above 1% (sub-Saharan African and South East Asia), and 16% in people with sexual partners of people born in these countries.(4)

Between 2012 and 2014, the notification rate of newly diagnosed HIV infections was higher for the Aboriginal population compared to the non-Indigenous population (5.9 per 100,000 vs 3.7 per 100,000 in 2014).(10) In contrast to the situation for chlamydia and gonorrhoea, where the burden among Aboriginal people is greatest in regional and remote locations, the rate of diagnosis of HIV among Aboriginal people was highest in major cities.(10)

The above summary of the epidemiology of the four curable STIs in Australia highlights the importance of these infections at the national level and the disproportionate burden experienced by Aboriginal people living in remote communities. The remaining sections of this introduction and thesis focus on three of these STIs - syphilis, chlamydia and gonorrhoea – all of which are curable and for which new diagnostic technologies have become available and have the potential to make a significant impact in terms of control of these infections in Australia.

1.2 Clinical features, diagnosis and treatment

STIs can lead to a range of short and longer term consequences relating to sexual, reproductive and maternal-child health. A diagnosis not only includes these physical and clinical sequelae, but can also have a profound psychological impact, contributing to feelings of stigma, shame and anxiety, and can negatively impact on sexual relationships and result in intimate partner violence.(5)

1.2.1 Syphilis

Syphilis results from infection with the spirochaete *Treponema pallidum* subsp. *pallidum*, and can manifest in three stages: (1) primary syphilis, which occurs within a few weeks to months after infection; (2) secondary syphilis, which presents after a few months up to a year; and (3) tertiary syphilis, which presents years to decades after primary infection. The lesions of both primary and secondary syphilis may wax and wane, but they eventually resolve without treatment. Latent syphilis is characterised by the absences of signs or symptoms of active syphilis, along with evidence of syphilis infection (serological tests are positive for *T. pallidum)*. These stages can overlap, and they are frequently asymptomatic.(39)

Clinical stages of syphilis

Primary syphilis is characterized by a firm, painless chancre at the site of inoculation, most frequently in the genital, perineal or anal area.(40) The chancre lasts 3 to 6 weeks and heals regardless of treatment. Most patients subsequently diagnosed with secondary syphilis do not recall the lesions of primary syphilis. If the infected person does not receive adequate treatment, the infection progresses to the secondary stage.(40) Secondary syphilis typically starts with a non-pruritic rash, usually macular or papular, on the trunk and extremities, classically including the palms and soles. Other symptoms may include alopecia, lymphadenopathy, condylomata, and systemic symptoms. Without treatment, the infection can progress to latent syphilis, which is further categorized as early (acquired during the preceding year), late latent, or of unknown duration.(40) Tertiary syphilis is characterised by cardiovascular syphilis (aortic lesions), gummatous syphilis (bone, skin and mucosal lesions), and neurosyphilis (meningitis, dementia, general paresis and tabes dorsalis).(39) In

the era of antibiotics, all but neurosyphilis are now infrequently seen in the developed world.(40)

Table 1: Clinical stages of syphilis and common associated manifestations

Clinical Stage	Clinical manifestations
Primary	Painless ulcer(s)/chancre; commonly genital or perineal or perianal area; lesion generally resolves without treatment
Secondary	Systemic illness (fever, malaise, headache, muscle aches, itch, lymph node enlargement); generalised macular/papular rash; self-limiting
Latent	Evidence of untreated syphilis (serological) with no clinical symptoms of active infection Early: up to 1 year since inoculation Late: greater than 1 year since inoculation Unknown: duration unknown
Tertiary	End organ involvement (usually 1-20 years from time of acute infection); includes cardiovascular syphilis (aortic lesions), gummatous syphilis (bone, skin and mucosal lesions), and neurosyphilis (meningitis, dementia, general paresis and tabes dorsalis)

Syphilis and HIV co-infection

Syphilis is associated with an increased risk of acquisition and transmission of HIV through a variety of mechanisms including disruption to the physical barrier of the epithelium and mucosa by syphilitic ulcers, thus aiding the passage of HIV; and increased immune activation of host cells, altered secretion of cytokines, and upregulation of transcription factors altering cell cycles, and thus enhancing HIV replication.(41, 42) Co-infection with syphilis in pregnant women has been reported to increase transmission of HIV to their infants in utero compared to those who are infected with HIV alone.(43)

Co-infection with HIV may alter the host response to syphilis(44) although clinical manifestations of primary and secondary syphilis are generally reported to be unchanged.(45) However, concurrent HIV infection may have an impact on neurological involvement of syphilis with the risk of neurosyphilis increased in HIV-infected patients with RPR titres ≥1:32 or in whom CD4 counts are <350.(46)

Mother to child transmission and congenital syphilis

Mother-to-child transmission (MTCT) of syphilis occurs via the placenta at any stage of gestation. Because the transmission is haematogenous there is no primary lesion (chancre) and infection is disseminated from the outset. Without treatment, pregnant women with early syphilis will almost certainly transmit the condition to the fetus via the placenta, though infectivity progressively declines to below 10% in late latent infection.(39) MTCT of syphilis can result in fetal loss, stillbirth, or neonatal or childhood disease (congenital syphilis).(39) Manifestations of congenital syphilis include a wide spectrum of severity, from unapparent infection to severe cases that are clinically apparent at birth. An infant or child (aged less than 2 years) may have signs such as hepatosplenomegaly, rash, condyloma lata, snuffles, jaundice (nonviral hepatitis), pseudoparalysis, anemia, or edema (nephrotic syndrome and/or malnutrition). An older child may have stigmata of syphilis (e.g., interstitial keratitis, nerve deafness, anterior bowing of shins, frontal bossing, mulberry molars, Hutchinson teeth, saddle nose, rhagades, or Clutton joints).(47)

Diagnosis of Syphilis

Dark-field examinations and tests to detect *T. pallidum* directly from lesion exudate or tissue are the preferred definitive methods for diagnosing early syphilis.(48) Although no direct *T. pallidum* detection tests are commercially available, some laboratories provide locally developed PCR tests for the detection of *T. pallidum*.(49, 50) As diagnosis by direct observation is limited to patients presenting with an ulcer or appropriate lesion and access to laboratories capable of the required detection techniques, this is not feasible in the majority of circumstances outside specialised STI services.

Therefore in most cases, a presumptive diagnosis of syphilis relies on a combination of serological tests (non-treponemal and treponemal tests). In resource-limited environments syndromic management(39) and/or POC tests may be the only option available.(51)

a) Non-treponemal (non-specific) tests

The RPR (rapid plasma reagin) and VDRL (Veneral Diseases Reference Laboratory) tests are flocculation tests targeting detection of the antibody to the phospholipid, known as cardiolipin. Antibodies to cardiolipin are not specific for syphilis and can be elevated in patients with other inflammatory conditions including other infectious diseases (e.g. malaria), older age and some autoimmune disorders.

Broadly equivalent, RPR tests can be read macroscopically while VDRL tests require a microscope. The toluidine red unheated serum test (TRUST) is a variant of the RPR test. The VDRL test is the only syphilis test recommended for CSF evaluation.(39, 52) These non-treponemal (non-specific) tests are relatively sensitive in early infection (77 to 88% for primary syphilis and 100% for secondary syphilis),(52) but tend to decline over the next several years without treatment.(40)

Non-treponemal tests tend to correlate with disease activity and are used in follow-up to monitor response to treatment. Active infection is suspected in patients who produce a reactive RPR/VDRL, especially with a titre of >1:16, although lower titres should take into account clinical presentation to detect early cases.(53) In general, a fourfold change in titre is taken as evidence of cure, relapse, or reinfection. Test titres usually decline after treatment and might become non-reactive with time; however, in some persons, non-treponemal antibodies can persist for a long period of time - a response referred to as the "serofast reaction."(48)

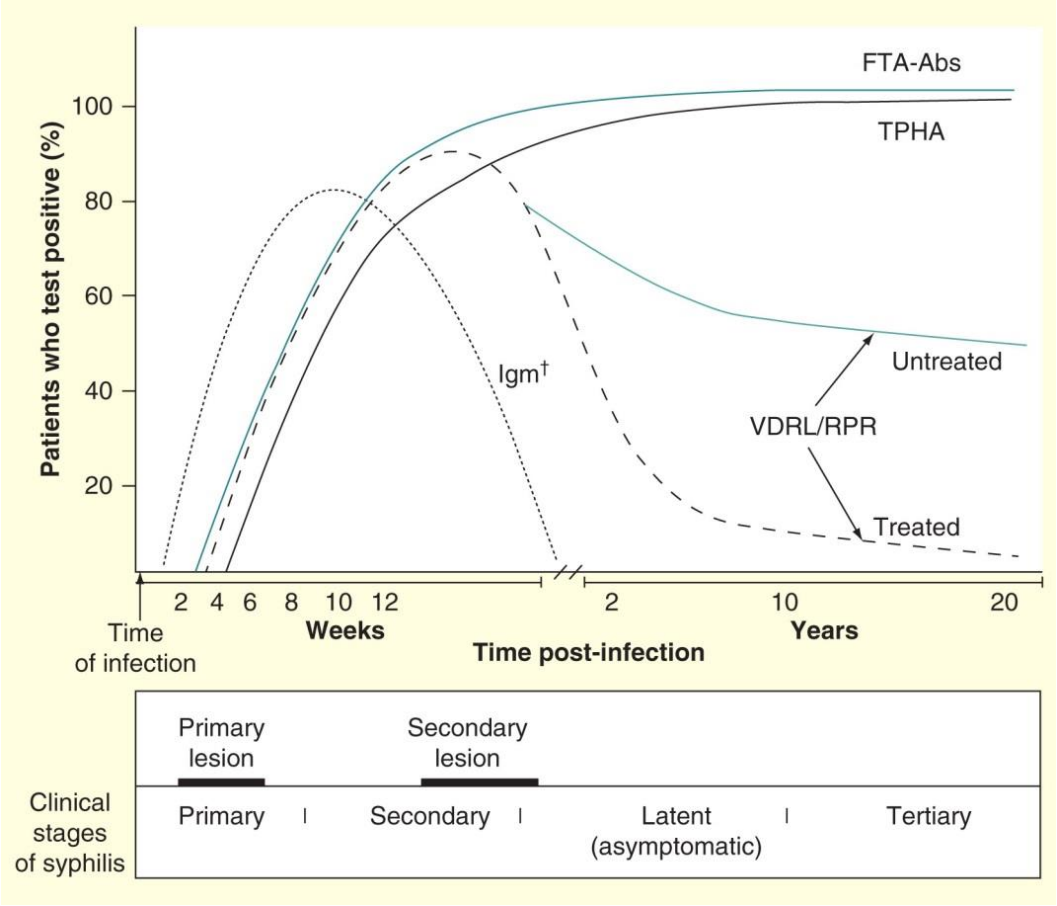
Although most frequently performed in a laboratory setting as part of a screening algorithm, RPR can be performed at the point-of-care though performance is highly user-dependent and the test is inherently difficult to perform and interpret for inexperienced personnel.(44)

b) Treponemal (specific) tests

These tests detect the presence of antibodies to *T. pallidum* and may become positive shortly before the non-specific tests.(39) They are considered specific for infection with *Treponemal sp.* However, treponemal antibodies are also elevated in individuals with infections with other *Treponemal sp* (non-venereal treponematoses) including yaws, pinta and endemic syphilis; the epidemiology and transmission of these non-venereal

treponematoses is very different - yaws primarily affects young children and transmission is via close contact in humid tropical regions including Africa, Asia and the Western Pacific).(54) Unlike syphilis, mother-to-child transmission does not occur.(54)

Figure 6: Common patterns of serological reactivity in syphilis



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There are a number of different test platforms available including indirect immunofluorence (fluorescent treponemal antibody absorbed [FTA-ABS] tests), agglutination testing using sensitized erythrocytes or gelatin particles (*T. pallidum* passive particle agglutination [TP-PA] assay and T. pallidum haemagglutination [TP-HA], ELISAs (EIAs), including variants that use chemiluminescence technology (CIAs).(52) All these assays are highly specific (Table 2) and were initially designed for use as confirmatory tests. However, those tests that are amenable to automation, namely some EIAs and CIAs, have been licensed in some countries for use as initial screening tests. (52)

The sensitivity of these tests can vary depending on clinical stage of syphilis infection, with most tests being at their most sensitive during secondary syphilis (Table 3). (52)

Table 2: Summary characteristics of serological tests for syphilis

	Non-treponemal tests		**Treponemal tests**		
Characteristic	RPR	VDRL	EIA	TPHA-TPPA	FTA-ABS
Sensitivity	86 -100%	78-100%	84-98%	82-100%	70-100%
Specificity	93-98%	89%	97-100%	98-100%	94-100%
Average cost (USD)	$0.5	$0.5	$3.00	$3.00	$3.00

(adapted from Peeling et al. 2004)

EIA: Enzyme immunoassay; FTA-ABS fluorescent treponemal antibody absorption; RPR: rapid plasma reagin; TPHA: T. pallidum haemagglutination assay; TPPA: T. pallidum passive particle agglutination assay; VDRL: Veneral Disease Research Laboratory

Table 3: Performance characteristics for selected syphilis serological tests by clinical stage

Tests	Sensitivity (%)				Specificity
	Clinical stage of syphilis				
	Primary	Secondary	Latent	Tertiary	
FTA-ABS	98 (93-100)	100	100	96	99
TPHA/PA	82 (69 – 90)	100	100	94	99
RPR	86 (81 -100)	100	80 (53 -100)	73 (36 – 96)	98
VDRL	80 (74-87)	100	80 (71-100)	71 (37 – 94)	98

(adapted from Ballard et al. 2013)

FTA-ABS: fluorescent treponemal antibody absorption; RPR: rapid plasma reagin; TPHA: T. pallidum haemagglutination assay; TPPA: T. pallidum passive particle agglutination assay; VDRL: Veneral Disease Research Laboratory

Most patients who have reactive treponemal tests will have reactive tests for the remainder of their lives, regardless of treatment or disease activity. However, 15%-25% of

patients treated during the primary stage revert to being serologically nonreactive after 2--3 years.(55) Treponemal test antibody titers are not recommended for use to assess treatment response or to assess stage or reinfection.(39, 48)

Treponemal IgM tests are available but these are more technically difficult to perform and may be limited to specialist centres. Detection of IgM is most useful in congenital cases as IgM does not pass through the placenta, so its presence indicates infection in the neonate rather than transfer of the maternal antibody.(53)

The use of only one type of serologic test is generally insufficient for diagnosis. As most patients with a history of syphilis remain treponemal antibody seropositive for life, treponemal-specific tests are unable to distinguish current, new infection from past infection. To overcome this problem, a non-treponemal test is employed as part of the diagnostic algorithm. As described above, non-treponemal tests are quantitative and can be used to distinguish status of infection in the treponemal-antibody positive patient as the non-specific antibodies being detected by these tests decline with duration of infection (accelerated with successful treatment).

Another limitation associated with these tests is the possibility of false-positive test results (in persons without syphilis). False-positive non-treponemal test results can be associated with various medical conditions unrelated to syphilis, including other infectious conditions, autoimmune conditions, older age, and injection-drug use; depending on the sequence of recommended testing, persons with a reactive test should receive the alternative (treponemal-specific or non-treponemal) test to confirm the diagnosis of syphilis.(48) As a rule, the vast majority of false-positive sera show antibody titres of ≤1:4.(52) Although pregnancy has long been considered a condition possibly associated with false-positive non-treponemal tests, the rate of erroneous positive tests in pregnant women appears to be no greater than in their non-pregnant counterparts.(52, 56)

Australian and many other laboratories prefer to screen using treponemal tests due to their greater sensitivity in early primary syphilis and lower rate of biological false positive reactions which may outnumber true positives in a low prevalence population.(48, 57) If a treponemal test is used as the screening assay all reactive sera should be retested using another type of treponemal assay and a non-treponemal test. A quantitative RPR or VDRL is also essential for assessing the stage of infection and to establish a base line for response to

treatment. Referral to a reference laboratory for further analysis of the humoral response by immunoblot may also be useful.(48, 57)

Syphilis testing is recommended in clinical guidelines as part of antenatal screening, for people at high risk, for those with symptoms and following sexual contact with confirmed cases.(48, 58) In Australia, the STIs in Gay Men Action Group (STIGMA) recommend annual screening for syphilis among all men who have had any type of sex with another man in the previous year, and more frequent screening (up to four times a year) for those at high risk (having any unprotected anal sex; more than 10 sexual partners in six months; participate in group sex; use recreational drugs during sex; or are HIV positive).(58) Syphilis screening should be offered to all pregnant women. In regions where STIs are considered endemic, screening is recommended in the first trimester (or at the first antenatal visit) and between 28 and 36 weeks of pregnancy (or at delivery).(59)

Treatment of Syphilis

Parenteral penicillin G is the original, and remains the preferred, treatment for syphilis globally. In most parts of the world this takes the form of long-acting benzathine penicillin G injections, although some prefer daily injections with procaine penicillin, sometimes boosted with probenecid, because treponemicidal CSF levels may be achieved.(39)

The recommended regimen for adults with primary, secondary and early latent syphilis is benzathine penicillin G (2.4 million units) IM in a single dose, and for children 50,000 units/kg IM, up to the adult dose of 2.4 million units in a single dose.(48) For those with late latent/unknown duration or tertiary syphilis, benzathine penicillin G 7.2 million units total, administered as 3 doses of 2.4 million units IM each at 1-week intervals is recommended.(48) An extended regimen is recommended if neurosyphilis is diagnosed, usually involving intravenous aqueous penicillin or intramuscular procaine penicillin plus oral probenecid for 10-14 days. Alternative non-penicillin based regimens (in the case of allergy to penicillin-based regimens) include doxycycline 100 mg orally twice daily for 14 days or tetracycline (500 mg four times daily for 14 days).(48)

Treatment failure can occur with any regimen. However, assessing response to treatment frequently is difficult, and definitive criteria for cure or failure have not been established. Clinical and serologic evaluation should be performed 6 months and 12 months after treatment; more frequent evaluation might be prudent if follow-up is uncertain.(48)

1.2.2 Chlamydia and gonorrhoea

Chlamydia and gonorrhoea are presented together in the following section as these infections are both frequently asymptomatic and when clinical symptoms do develop, their presentation is often similar. Furthermore, individuals are frequently co-infected, screening and diagnosis often involves detection of both causative organisms from a single specimen as a matter of routine practice, and management guidelines direct clinicians to syndromic or presumptive treatment that covers the possibility of both infections. Finally, without treatment these infections can result in similar sequelae.

Clinical presentation

Chlamydia infection is caused by the bacterium *Chlamydia trachomatis (CT).* In women, symptoms depend on the site of infection; infection of the urethra and lower genital tract may cause dysuria and abnormal vaginal discharge or post coital bleeding, whereas infection of the upper genital tract can causer irregular uterine bleeding and abdominal or pelvic discomfort.(60) In males, urethral chlamydia infection may cause dysuria and a clear discharge.(61) Other sites of infection include the rectum (causing proctatitis) and pharynx (causing pharyngitis) both of which correlate strongly with age and sexual preference in men.(62) However, the majority of chlamydia infections in both men and women are asymptomatic. Detels et al estimated chlamydia was asymptomatic in 31.2- 98.4% of women and 87.5-100% of men (63, 64) and if untreated, around half (54%) will persist beyond 12 months, with a small proportion (6%) persisting for up to 4 years.(65)

Gonorrhoea, caused by the bacterium *Neisseria gonorrhoeae (NG)*, can cause abnormal vaginal discharge, bleeding and dysuria in women, and may result in urethral discharge and dysuria in men.(63, 66) As with chlamydia, the majority of gonorrhoea infections in women are asymptomatic; and in men approximately half of gonorrhoea infections are asymptomatic.(63, 64) On average, untreated gonorrhoea infections will clear within about 4 months.(67)

As noted earlier, chlamydia and gonorrhoea infections are frequently asymptomatic, remaining undetected, untreated and thus transmissible for long periods of time.(63, 68, 69) If left untreated, chlamydia and gonorrhoea can result in serious sequelae. This is especially true for women, where infections can lead to pelvic inflammatory disease (PID) and fallopian tube scarring, and can also result in ectopic pregnancy and tubal factor

infertility.(40, 70) A number of studies have found that with just a few weeks delay between testing and treatment, 2-5% of women with chlamydia infection have already developed PID, and that up to 18% of these women may develop infertility.(71) Furthermore, there is evidence from a study in the UK that almost 10% of women developed PID within 12 months of chlamydia infection.(72) In men, the more severe sequelae include prostatitis and balanoposthitis, (69) and orchitis and epididymitis, which can in turn impact on sperm quality and male fertility.(69, 70, 73)

A range of adverse pregnancy outcomes are associated with these infections. There is overwhelming evidence that gonorrhoea and chlamydia are strongly associated with both preterm birth and low birth weight, (74-84) as well as miscarriage ,(78, 83) stillbirth,(83, 85) premature rupture of membranes,(74, 83, 84, 86) postpartum endometritis,(83) and ophthalmia neonatorum.(83)

As with syphilis, chlamydia and gonorrhoea can increase susceptibility to HIV infection. This is believed to occur via a range of biological mechanisms, including as a result of disruption to the protective integrity of the genital mucosa, and also through the associated stimulation of the immune system which increases the production of CD4 lymphocytes, both a target and source of HIV infection.(87) Among men who have sex with men, a number of studies have shown that syphilis and rectal chlamydia and gonorrhoea place these men at increased risk of HIV acquisition.(88, 89) STIs also appear to increase the infectiousness of HIV from men to women (evidence is more equivocal for the reverse), in that individuals with HIV and inter-current STIs have higher rates of genital shedding of HIV, which diminishes when STIs resolve.(87, 90) It is also well established that inter-current STIs enhance the risk of MTCT transmission of HIV.(91)

Diagnosis of chlamydia and gonorrhoea

Urogenital infection with CT and NG can be generally be diagnosed by testing urine or swab (endocervical/vaginal in women or urethral in men) specimens. Extra-genital infections require anatomically suitable specimens, such as rectal swabs and oropharyngeal swabs.(92)

For many years the accepted standard for the diagnosis of chlamydia has been cell culture. However, its stringent requirements including particular aspects related to specimen collection (columnar epithelial cells from the cervix or urethra), transport (cold-chain) and

technical expertise to culture (established cell culture system) make this technique impractical in many settings.(62) Other non-culture techniques developed to overcome the difficulties of cell-culture include enzyme immunoassay (EIA) and amplification of *C. trachomatis* DNA or RNA. Amplification of target nucleotides on the CT plasmid by polymerase chain reaction (PCR) methods is now considered the gold standard diagnostic method. This diagnostic method using a nucleic acid amplification test (NAAT) can be fully automated and are highly sensitive and specific. NAATs allow detection of very low level organism making them suitable for use with self-collected urine and swab specimens as well as standard clinician collected swabs.(53, 62, 93, 94)

Traditionally, as with CT, culture of NG was the gold standard for diagnosis of infection. This methodology requires a well-collected specimen (clinical collected urethral [in men] or cervical [in women] swab), inoculated on a highly nutritious medium, rapid transport from the patient to the laboratory and incubation in appropriate conditions. The yield can be variable and the methodology is intolerant of delays or inadequacies in the process as often may occur when used for patients attending health services distant from pathology providers.(53) The development of highly sensitive and specific NAATs, which are more forgiving in terms of specimen collection and transport and are able to be highly automated, has led to these assays becoming the diagnostic of choice across laboratories in the developed world.

NAATs for CT and NG are often coupled as dual infection is not uncommon in high risk patients and dual testing of common specimens for two STIs adds little or no extra cost compared with the single test.(53) Although convenient and potentially cost saving, the routine dual testing of CT and NG in Australia has led to an increase in false positive NG results among low prevalence populations.(95)

At present, regulatory approvals are still pending for use of NAATs to detect NG and CT from extra-genital sites.(48, 53) For NG in particular, these samples are likely to be contaminated with large numbers of commensal species of *Neisseria,* potentially leading to false positive results. Some laboratories have established performance specifications for using NAATs with rectal and pharyngeal swab specimens, making sure that specificity is not compromised by cross-reaction with non-gonococcal *Neisseria* species. The sensitivity of NAATs for the detection of NG in genital and non-genital anatomic sites is superior to culture but varies by NAAT type. (48, 53)

Despite the advantages of NAATs for detection of NG, isolation and culture of viable NG organism continues to be essential for the monitoring of antimicrobial susceptibilities, particularly in cases of suspected or document treatment failure, and surveillance for emerging resistance.(96, 97) With clinical microbiology laboratories having moved away from using culture-based methods to diagnose gonorrhoea, their ability to detect antimicrobial resistance (AMR) has been undermined. Recent advances in molecular techniques however have demonstrated their utility for the direct detection of genetic markers of resistance or by association with genotype. Though in their infancy, molecular AMR surveillance could be used to enhance current culture-based surveillance to detect the threat of AMR.(96) AMR surveillance is described in more detail in Section 1.3.2.

Retesting at 3 months post treatment is recommended to detect potential reinfections while proof of cure testing is not generally recommended, especially as NAATs at <3 weeks after completion of therapy may detect the continued presence of non-viable organisms and lead to false positive results.(48)

Treatment of chlamydia and gonorrhoea

Chlamydia infections are susceptible to antibiotics that interfere with DNA and protein synthesis, including tetracyclines, macrolides, quinolones and rifamycins. Azithromycin continues to be the treatment of choice for uncomplicated urogenital chlamydial infection.(98) Recommended regimens for CT include oral azithromycin (1g) as single dose or doxycycline 100mg twice a day of 7 days.(48)

Drug resistant NG is an increasing public health concern. In recent years there has been widespread resistance to the first-line and second-line antibiotics used to treat gonorrhoea including penicillin-based regimens and fluoroquinolones (i.e., ciprofloxacin, ofloxacin, or levofloxacin).(21, 97) There are also reports of increasing resistance to third generation cephalosporins, the last class of effective treatment available.(99) As a consequence, some countries have increased the recommended dose of cephalosporin, and most now advise dual therapy using an extended spectrum cephalosporin plus azithromycin which also provided adequate coverage in the case of co-infection with chlamydia. (48, 100) In some settings where antimicrobial resistance surveillance indicates continued low levels of penicillin resistance,(21) penicillin-based regimens remain the treatment of choice for uncomplicated urogenital NG infection.

In Australia, ceftriaxone 500mg IMI, PLUS azithromycin 1g PO is recommended however, penicillin-based regimens (oral amoxycillin 3gm and probenecid 1gm) are still recommended in some remote locations in Australia where penicillin resistance remains low.(59, 101, 102)

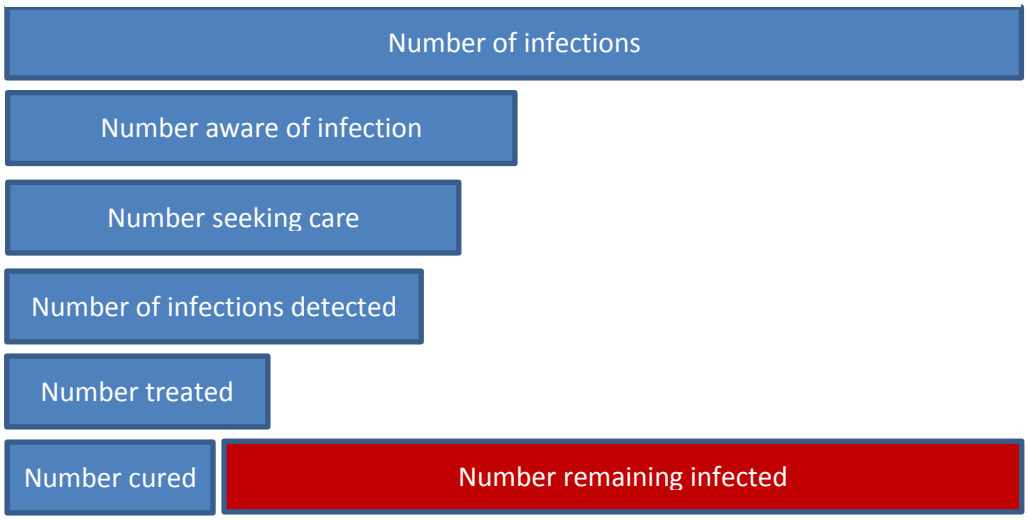
1.3 Approaches to STI control

1.3.1 Models and control strategies

As with any infectious disease, control strategies for STIs may be understood in the context of the "transmission dynamics" model.(103) In this model, transmission of an STI is dependent on the reproductive number (average number of new or secondary cases generated by an index case) in a population over a period of time. The reproductive number is a function of rate of partner change, probability of transmission of the STI during sexual intercourse and the duration of infection. Reduction in the reproductive rate by one or a combination of strategies theoretically will lead to a decrease in transmission and consequently the number of cases and associated sequelae. STI control programs designed with prevention as the primary focus, aim to modify risk behaviours (e.g. decrease numbers of sexual partners or increase condom use) and/or reduce the duration of infections (e.g early detection and treatment of infected individuals).

Another way of conceptualising control efforts is the "operational model" which identifies the many different steps a patient with a curable STI will pass through before they can be considered cured.(104, 105) This model represented in Figure 7 (also similar to a care cascade model) is useful to identify opportunities for improvement at different stages of care where patients are missed or "lost". This model highlights the potentially large cohort of untreated infections remaining in a population and represents a significant source for ongoing transmission.

Figure 7: Overview of STI care cascade



* (adapted from WHO Sexually transmitted diseases: policies and principles for prevention and care 1999)

Relative proportions at each stage will vary depending on the infection epidemiology, symptoms, health care setting, access to diagnostics and treatment, and individual health seeking behavior. The proportion of the population not aware of their infection (largely as a result of being asymptomatic) represents an enormous opportunity to improve STI control through screening strategies that encourage and enable early diagnosis and treatment among this group. This might include opportunistic screening for those who are asymptomatic but present to a health service for another unrelated episode of care or annual community screening where testing services might be extended beyond the clinical facility to include outreach or testing at large sporting or cultural events.(106)

Primary objectives of STI control programs are to reduce infection-associated morbidity and mortality, prevent HIV infection, and reduce reproductive health complications including adverse pregnancy outcomes.(107) Key strategies that have benefits across a broad range of STIs include promoting healthy sexual behavior, providing condoms and other barrier methods, delivering acceptable, comprehensive and effective case management including ensuring access to medicines and appropriate technologies (including rapid diagnostic or POC tests and vaccines), partner notification and treatment.

The most recent WHO global strategy makes a strong case for integrating STI care more widely into primary health care, sexual and reproductive health services and services that provide HIV management.(107) It emphasizes opportunities to increase coverage by working collaboratively with other government sectors, and with community-based organisations and private providers.

Global strategies for STIs are relatively rare, however in 2007 the WHO launched the global initiative for the elimination of congenital syphilis as a public health problem.(108) The initiative is based around four guiding principles: *country-driven*, adapted to the local level; *integrated,* with other HIV,STI, reproductive and antenatal services; *rights-based*, individuals are informed to be capable of participating in decision-making about their health; *collaborative*, requiring a combined commitment of governments and other partners in order to optimise scarce resources. (109) Though this is clearly a targeted initiative, this strategy can greatly support current global STI control efforts, including those outlined in the Millennium Development Goals of reducing child mortality, improving maternal health, and combating HIV, malaria and other infectious diseases.(109) The elimination strategy focuses on: ensuring advocacy and political commitment; increasing

access to and quality of services; increasing screening of pregnant women and their partners; and establishing surveillance and monitoring programs. Increasing screening of pregnant women and partners, through the provision of diagnosis and treatment at the point-of-care, is considered paramount.(109) To raise funds for and awareness of the initiative, WHO and partners developed an investment case document that highlights the cost-effectiveness, technical feasibility and political acceptability of the approach.(110) The success of this global initiative depends on a comprehensive approach as no one strategy is able to achieve the end goal.

1.3.2 STI control in Australia

The following sections provide more detail on STI control in Australia through description of key components including the national strategy, management guidelines, screening/testing, partner notification, national STI surveillance and antimicrobial resistance monitoring (AMR). National STI and AMR surveillance is also mentioned earlier in Section 1.1.2.

National STI Strategy

Australia's *3rd National STI Strategy* is informed by guiding principles encompassing human rights, ensuring access and equity of health and community care, health promotion, prevention, harm reduction, shared responsibility, commitment to evidence-based policy and programs, and partnership and meaningful involvement of affected communities.(111) As reflected by the epidemiology of STIs in Australia presented in earlier (Section 1.1.2), the strategy identifies priority populations in order to maximize the impact and sustainability of responses. These include young people (<30 years of age), Aboriginal people, gay men and other men who have sex with men, sex workers, culturally and linguistically diverse groups, travelers and mobile workers, and people in custodial settings.(111)

Primary prevention strategies (i.e. safer sexual behaviour and condoms), combined with secondary prevention strategies (testing, treatment and partner notification), are considered to constitute the most effective response to the control of STIs.(111) Safer sexual behaviour, including the use of condoms and water-based lubricants, remains the primary tool for preventing transmission of STIs. The early detection of STIs (through timely and appropriate testing and treatment and partner notification) is recognised as essential to reduce further transmission, limiting the facilitation of HIV transmission and preventing the development of complications. The *National Strategy* identifies the exploration of the

feasibility, accessibility and cost-effectiveness of a range of existing and emerging testing methods such as rapid and POC testing as a priority action. For those STIs which are largely asymptomatic (in particular chlamydia and gonorrhoea), testing is largely through screening strategies rather than diagnosis of conditions presenting with clinical symptoms. Improved testing coverage and timely treatment of STIs are both priority areas of action detailed in the *Fourth National Aboriginal and Torres Strait Islander Blood Borne Viruses and Sexually Transmissible Infections Strategy*.(112)

STI Management Guidelines

National (101) and state-based guidelines (59, 102, 113, 114) are available to guide clinical staff in the diagnosis (including specimen types to collect and test to request) and management of STIs (including antibiotic treatment recommendations and necessary follow-up procedures). More targeted and specific guidelines are also available for general practice (115) and for those providing care and services to clients of men who have sex men.(58) These guidelines are regularly updated to ensure recommendations are in line with the most recent evidence base, particularly with respect to antimicrobial resistance profiles (primarily for gonorrhoea). Guidelines take into account epidemiology of the infections which, as described earlier, varies considerably between urban and non-urban settings and among different population groups.

Despite the widespread availability of highly accurate laboratory-based diagnosis and highly effective antibiotic therapies in Australia, a 'syndromic management' approach,(116) whereby immediate treatment is provided for suspected STIs on the basis of specified signs and symptoms, is practiced in most remote communities.(102, 117, 118) Specimen collection and laboratory testing continues to occur in parallel to support the diagnosis made and ensure that appropriate treatment was provided. In these remote settings, syndromic management is considered an appropriate strategy as the prevalence of STIs is high, time to diagnosis can be long given the vast distances between clinical and pathology services, and the population is highly mobile. Delayed treatment is associated with the risk of ongoing STI transmission, and frequently patients do not return or cannot be found to deliver the test results and treatment (if needed). For asymptomatic patients who test positive for an STI, being recalled for treatment and initiation of partner notification is done upon receipt of the result by the health service. Partner notification is discussed in more detail in a following section.

‘Presumptive treatment’ (regardless of symptoms or a confirmed diagnosis) is also recommended in some circumstances,(58, 101, 102, 114) and refers to providing treatment on the basis that a patient has a high probability of infection. For example, for an individual who is the sexual contact of another individual with a known STI, or in settings where background STI prevalence is high and an individual’s behaviour has placed them at risk. Presumptive treatment given at regular intervals is known as periodic presumptive treatment. (116) More discussion of presumptive treatment is provided in Section 1.4.3.

Some of the issues related to access and timeliness of diagnosis are likely to be less significant in urban settings (119) however loss to follow-up may still be of concern among certain individuals. Consequently, some patients presenting with symptoms may be treated on the basis of clinical judgement while awaiting results of the laboratory diagnosis.(101) As in remote settings, management of asymptomatic patients in urban areas is largely guided by laboratory-based diagnostic testing.

STI testing

Testing for STIs in a population may encompass the following approaches: offering testing to symptomatic individuals presenting to a health centre; opportunistic screening whereby clinicians test asymptomatic individuals at risk of STIs who present for any reason; or through targeted screening of individuals at risk of STIs who may not access health care regularly, often called “outreach”.

Testing recommendations take into consideration a number of factors including risk by population (men who have sex with men, Aboriginal, pregnant women, sex workers, co-infection with other STI/HIV), residential location (remoteness), change and number of sexual partners and other risk behaviours such as condom use and unprotected anal intercourse. Given the asymptomatic nature of chlamydia and gonorrhoea, Australian guidelines generally advise annual (at least) screening for these infections in those who are sexually active and aged 15 – 29 years (residing in urban areas) or less than 35 years (residing in remote areas) or <39 years (residing in remote Queensland).(101) In Western Australia (WA), the most recent guidelines now recommend screening those at risk twice per year.(120) More frequent screening is recommended for high-risk individuals including those with a previously positive test (recommended to be retested at 3 months) and men who have sex with men (3-6 monthly).(101)

Partner notification

Partner notification (also sometimes called "contact tracing") is the process sexual partners of a person found to have an STI are informed of their potential exposure and consequent need for testing and treatment. For the person (index patient) with a curable STI such as chlamydia, gonorrhoea or syphilis, partner notification aims to cure infection and prevent re-infection. For sexual partners, partner notification aims to identify and treat undiagnosed STIs. The public health aim of partner notification is to interrupt chains of STI transmission.(121) Testing of sexual partners of individuals diagnosed with STIs generally yields a higher rate of STIs compared with individuals unselectively screened for STIs, warranting efforts supporting partner notification.(122)

A number of approaches are available, but their implementation and success depend highly on the cultural, legal and economic constraints of the setting. A recent Cochrane review of partner notification interventions categorised these approaches as: (i) **patient referral** whereby the index patient notifies their sexual partners directly; (ii) **expedited partner therapy** whereby the index patient delivers medication or a prescription for medication to their partners(s) without the need for medical examination of the partner; (iii) **provider referral** whereby the health service notifies partners as named by the index patient); and (iv) **contract referral** whereby the index patient is encouraged to notify partner(s) with the understanding that partners will be contacted if they do not visit the health service by a certain date.(121) This review of 26 trials across developing and developed countries was not able to identify a single optimal strategy for partner notification for any particular STI but suggested that expedited partner therapy was more successful than simple patient referral in reducing repeat infection in index patients.

In most jurisdictions in Australia, current partner notification strategies rely on patient or provider-initiated referral to notify sexual partners of their STI risk.(101) However, in practice, given the sensitive nature of STI diagnoses and potentially labour intensive nature of the process, partner notification is often carried out poorly, if at all. New attempts to expedite the treatment of partners, who are either the source of the infection or at risk themselves, have evolved as a pragmatic response to the failure of partners to attend clinical services for testing and treatment.(122) One such approach is patient-delivered partner therapy (PDPT). There is interest in implementing PDPT where suitable in Australia, however in some jurisdictions the ambiguous legal state for clinicians to prescribe PDPT is a

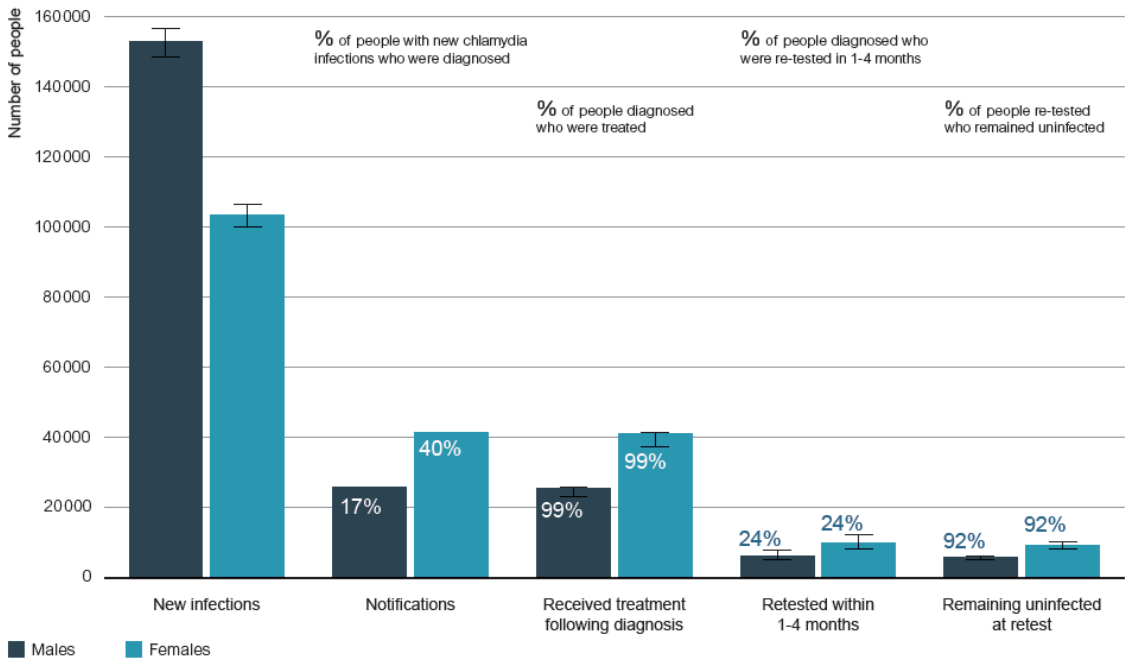
major barrier to implementing this potentially beneficial strategy.(123) Despite this, in one Australian study almost half the general practitioners reported 'sometimes' or 'always' using PDPT for chlamydia.(124)

STI surveillance

In Australia, STI data are collected through national notification systems including passive, sentinel, enhanced, antimicrobial resistance surveillance systems and periodic prevalence and incidence surveys, and provides an indication of the burden and epidemiology of STIs. A more detailed discussion is provided earlier in Section 1.1.2.

Using a modeling approach incorporating a number of data sources (including NNDSS notifications, Medicare testing, and ABS population estimates) to estimated chlamydia infections,(34) clearly demonstrates the diagnosis step as the greatest gap in the STI care cascade in Australia (Figure 8).(4) This highlights the need to increase coverage of testing in Australia, particularly among young people in this 15 -29 year old age group, which represents by far the highest risk group for chlamydia.

Figure 8: 2014 chlamydia diagnosis and care cascade in 15-29 year olds



Source: The Kirby Institute. Annual Surveillance Report (2015)

Gonococcal antimicrobial resistance (AMR) monitoring

The WHO recommends that only drugs with an expected efficacy of 95% and above should be used to treat gonorrhoea.(23) The continued emergence and spread of antimicrobial resistance (AMR) in NG is widely recognised as a global public health threat.(125, 126) The WHO Gonococcal Antimicrobial Surveillance Programme (GASP) conducts surveillance of antimicrobial resistance in NG in the WHO Western Pacific Region (WPR) and South East Asian Region (SEAR) to optimise antibiotic treatment and control of gonococcal disease.(97) In these regions, the progressive development of AMR in NG within and across antibiotic classes over many years has compromised the treatment and public health management of gonococcal disease.(97)

As described earlier (Section 1.1.2), the Australian Gonococcal Surveillance Programme (AGSP) has continuously monitored antimicrobial resistance in clinical isolates of NG from all states and territories since 1981. (21) Given the inherent difficulties in specimen collection, transport and culture methodologies described earlier, and often vast distances between clinical services and gonococcal reference laboratories in Australia, less than 20% of specimens testing positive for NG are successfully cultured.(22) As a result, some laboratories in WA have developed and routinely use non-culture molecular-based techniques to detect markers of resistance in NG (PPNG) to selected antibiotics.(22) More recently, a real-time PCR method to predict NG ciprofloxacin susceptibility directly in clinical samples has been developed.(127)

As over 90% of gonorrhoea notifications in the remote regions of WA are based on molecular diagnosis, it is no longer possible to rely on the NG phenotypic susceptibility testing of isolates to ensure that empirical therapy maintains adequate efficacy. Consequently, the molecular detection of PPNG using molecular-based techniques to determine whether combination oral azithromycin, amoxicillin and probenecid continues to meet the WHO criteria for effective treatment of gonorrhoea has proven highly valuable.(22) Most recently, the development in detection of molecular markers aimed at identifying susceptibility to ciprofloxacin,(127) has demonstrated the potential in the recycling of previously effective antibiotics via the use of such markers inform individualized treatment as strategy for the future.(99)

On the background of current guidelines that recommend a dual therapy strategy of ceftriaxone with oral azithromycin for uncomplicated gonococcal infection,(101) the most recent AGSP report indicates AMR rates are increasing, with high level resistance to azithromycin reported for the second consecutive year.(126) The report recommends as a matter of priority the expansion of sentinel site surveillance programs involving patient follow up and "test of cure" cultures after treatment of infections with NG, in particular those with involvement of oropharyngeal sites.(126) As the next direction for treatment is uncertain, continued monitoring of AMR to inform treatment and monitor interventions is paramount.

1.3.3 Barriers to STI control in Australia

As highlighted in Figure 8, failure to diagnose curable STIs results in the greatest lost opportunity to control the transmission these infections and subsequent associated morbidity. Therefore, encouraging regular STI testing among young sexually active persons, whether symptomatic or not, is critical.

In remote Australia, where care and management of STIs is provided mostly through primary care services, many populations fail to access health services regularly for STI testing as a result of lack of services themselves, transport, stigma, perceived confidentiality issues, and lack of awareness about STIs.(128)

A recent literature review of outreach screening programs as a strategy to improve STI testing coverage found the most successful were those that offered testing at existing venues outside the usual health service (community centre or sports venue) but were also targeted to specific under-tested, most at-risk sub-populations rather than simply aiming to increase numbers tested overall.(128) Patients who do present with recognized symptoms attributable to an STI are eligible for treatment according to principles of syndromic management. (59, 102, 129) This approach has advantages in providing rapid treatment, however it has poor sensitivity and specificity for detecting infection, leading to a missed and/or over-treated infections when not combined with another laboratory-based diagnostics.(130)

The majority of patients tested for STIs in these remote setting are asymptomatic.(131) These asymptomatic patients who have a positive test result will need to be recalled for treatment at a later date once the positive test result is available. A recent review of STI

programs in Aboriginal communities found up to 1 in 4 people remain untreated for diagnosed STIs and the average time to treatment is 21 days,(132) compared to 2 days in urban settings.(119) Barriers preventing or delaying the provision of treatment to a patient with a positive test result in these settings are well described in a recent paper by Hengel et al (106) and include physical barriers such as the vast distances between health services and reference laboratories and infrequent transportation of specimens; staff related barriers such as delays in actioning results as a consequence of competing clinical demands and more complex clinical consultations, and high staff turnover; infrastructure issues such as under-utilised or failed IT systems; or patient related such as adverse health seeking behaviours reducing the likelihood of returning for results or adherence to prescribed treatment.(106) Approaches to overcome these barriers include strengthening systems for orientation and support for new staff, checking of results and auctioning recalls, home-delivery of treatments, and new technologies to allow for diagnosis at the time of consultation.(106)

1.4 Diagnostic and treatment approaches to control STIs

As highlighted in the previous section, early diagnosis and treatment, including the treatment of sexual partners, are critical components of STI control strategies in all settings. Improved diagnostic and treatment approaches, including the use of point-of-care (POC) tests, may be an avenue to improve STI control by providing the opportunity to offer rapid and accurate diagnostic-directed treatment. In resource-limited settings where access to any diagnostic tests is often impossible or unfeasible, the major benefit of POC testing is through the identification of infections in asymptomatic patients who would otherwise be missed (when syndromic management is primarily directing treatment); while in remote Australia and similar settings where high-quality laboratory diagnostics are available yet distances to laboratories are vast and population mobility is high, the benefits of POC testing are most likely to result from improved treatment uptake (minimising loss-to-follow up) and reduction in time to treatment (minimizing individual sequelae and onward transmission risk in the community).

The following sections summarise the diagnostic and treatment approaches to control STIs currently in use, including laboratory-based testing, syndromic management, presumptive and mass treatment. Newer approaches using POC tests are discussed including their associated benefits and limitations, and their potential impact.

1.4.1 Laboratory-based testing

The vast majority of tests for STI diagnosis are performed in laboratory settings using sophisticated, expensive, highly-sensitive equipment and conducted by trained technical staff. Detail as to the specific diagnostic tests available and those currently recommended for each infection (syphilis, chlamydia and gonorrhoea) have already been described (Diagnosis of syphilis - 1.2.1; Diagnosis of chlamydia and gonorrhoea – Section 1.2.2).

The benefits of laboratory-based diagnosis include the use of very accurate testing platforms which are capable of processing large numbers of specimens at any one time, and thus being relatively cost-efficient. The processes themselves are often highly-automated reducing opportunities for human error or contamination. Testing processes are implemented and supported by trained laboratory technicians and the technologies utilised have continuous support from manufacturers in the case of ongoing platform maintenance.

In addition, laboratories usually participate in rigorous quality assurance processes and adhere to stringent regulations regarding testing performance. In Australia, accreditation is awarded by the National Association of Testing Authorities (NATA), the authority in the assurance of technical standards.(133) NATA ensures that member facilities comply with relevant international and Australian standards and so are competent to provide consistently reliable testing, calibration, measurement and inspection data to government, industry and the wider community. NATA accreditation requires a determination, formal recognition and promotion of the competence of facilities to perform specific types of testing, measurement, calibration and inspection against the relevant national and international standards.(133)

Laboratories generally have robust laboratory information systems (LIS) that maintain a database of processed specimens and results, which can be delivered to health services and clinicians in an electronic format in many cases. As is the case in Australia, laboratories can play a crucial role in alerting jurisdictional public health departments of positive tests which meet case definitions for notifiable diseases, contributing to the NNDSS (described earlier in Section 1.1.2). The reporting of these positive tests for selected STIs is mandated through public health legislation in most states in Australia and in some states, this may be the only reliable source of notifications. Enhanced surveillance (laboratory and clinician notifications) does occur but tends to be limited to particular diseases (such as syphilis, and in some jurisdictions, gonorrhoea).

Though there are some regional laboratories in Australia, many of these do not routinely perform diagnostic tests for STIs. Thus the larger, centralised laboratories usually process all specimens for STI testing from a wide geographic area and as a result, are in a unique position to monitor for the development of gonococcal AMR and may be able to detect early signs of increased resistance in these areas.

In Australia, these large laboratories are located in capital cities and may be thousands of kilometres from remote communities and their health services. Specimen transport is often limited to once or twice a week by plane from these services and during the time between collection from the patient and testing at the laboratory, specimens may deteriorate, reducing the viability of the target for detection and diagnosis by traditional culture-based methods. With diagnosis of chlamydia and gonorrhoea having shifted to be predominantly molecular-based (using NAATs), this degradation issue is most pertinent for specimens

being used to culture NG isolates for monitoring of AMR.(22) For the patient and health service, the long delay in obtaining diagnostic results because of the significant distances involved to transport specimens, means infectious patients are at prolonged risk of individual sequelae and ongoing transmission to their partners.(40, 70)

See Section 1.3.2 for more detail on AMR monitoring.

1.4.2 Syndromic management

For the vast majority of the world where STIs are endemic, laboratory access is limited or simply not available.(134) In these resource-limited and other remote settings and where there is high likelihood of patient loss to follow-up, syndromic case management is a key strategy for STI control.(23) The syndromic management algorithms developed by the WHO (and adapted locally to suit the disease epidemiology and antimicrobial resistance patterns) identify well-described clusters of symptoms and easily recognised signs associated with common STIs and recommend effective treatment regimens.(23) The main syndromes identified include urethral discharge (UD), genital ulcer disease (GUD), vaginal discharge (VD), and lower abdominal pain (LAP). The guidelines are designed to treat the usual pathogens responsible for these syndromes without requiring confirmation by traditional laboratory diagnostics.(23)

Syndromic management is simple, assures rapid, same-day treatment, and avoids expensive or unavailable diagnostic tests. The approach works well for men with UD and GUD but algorithms for VD lack both sensitivity and specificity for the identification of chlamydia and gonorrhoea.(130, 135-137) Most women with the VD syndrome do not have these cervical infections, but rather many have other vaginal infections (bacterial vaginosis or trichomoniasis).(138) Because some STIs lack obvious signs and symptoms, particularly those associated with cervical and vaginal infection, patient risk-score assessments were developed to improve the accuracy of these syndromic algorithms with some improvement reported.(130)

Despite the success in rationalising and improving management of STI and its impact on the STI epidemic, the success of syndromic management depends on the aetiological patterns of the syndrome, and algorithms and guidelines need regular review to ensure they are adapted to the epidemiological pattern of STIs in a given setting.(139) In 2008, WHO reviewed its guidelines focusing the syndromes of GUD and VD – the former because of the

observed increase of herpes simplex virus type 2 (HSV-2) as the main cause of GUD in developing countries, and the latter for its continued complexity and controversy as an entry point for managing cervical gonococcal and chlamydial infections.(140)

It should be remembered that syndromic management was designed as a diagnostic tool in symptomatic patients, and was not intended for use as a screening tool. As such, the use of a syndromic approach to STI control when a large proportion of STIs are known to be asymptomatic, inevitably means that many infections remain completely undiagnosed and untreated,(141) contributing to ongoing transmission in the community, and subsequent morbidity and mortality.

The advantages and disadvantages of syndromic management (as summarized by Maynaud et al.) are shown in table 4.(142)

Table 4: Advantages and disadvantages of syndromic management of STIs

Advantages
• Problem oriented (response to patient's symptoms) • Highly sensitivity for the detection of infections among symptomatic patients and does not miss mixed infections • Treatment given at first visit • Provides opportunity and time for education and counselling • Avoids expensive laboratory tests • Avoids unnecessary return visit for laboratory results • Curtails referral to specialist centres • Can be implemented at primary care level
Disadvantages

• Over-diagnosis and over-treatment with the following consequences:
  ○ Increased drug costs
  ○ Possible side effects of multiple drugs
  ○ Changes in vaginal flora
  ○ Potential for increased drug resistance
  ○ Domestic violence
• Requires (re)training of staff
• Possible resistance to its introduction from medical establishment
• Cannot be used to detect infection among asymptomatic individuals

As outlined in an earlier section (1.3.2 – STI management guidelines), in remote Australia, despite the availability of laboratory diagnosis, syndromic management is recommended.(59, 102, 117)

1.4.3 Presumptive treatment

'Presumptive treatment' (possibly in the absence of symptoms or a confirmed diagnosis) refers to providing treatment on the basis that a patient has a high probability of infection. For example, where an individual is the sexual contact of another individual with a known STI, or where background STI prevalence is high and an individual's behaviour has placed them at risk. Presumptive treatment is recommended in some circumstances in Australia,(58, 101, 102, 114) with the patients being offered treatment in addition to specimens being sent to the laboratory for STI testing.

Presumptive treatment given at regular intervals is known as **periodic presumptive treatment (PPT).** (116) PPT has been shown to reduce prevalence of gonorrhoea, chlamydia and ulcerative STIs among sex workers in whom prevalence is high.(143) Important operational considerations include use of single-dose combination antibiotics for high cure rates, reinforced condom promotion to reduce rates of reinfection, strong outreach and peer interventions to increase coverage and utilization of services, and use of PPT together with other intervention components to reinforce STI control and HIV prevention.(144)

1.4.4 Mass treatment

Mass treatment or mass drug administration (MDA) refers to the treatment of whole populations irrespective of disease status and tends to include not only those at highest risk (as are the target with PPT) but also those at lower risk as a result of a population level approach. Mass treatment, as with PPT, is most useful in settings of high prevalence where infection is frequently asymptomatic.

Mass treatment approaches have been used successfully to gain rapid control of epidemics and as an integrated strategy for control and elimination of a number of "neglected tropical diseases" including soil-transmitted helminthiasis, schistosomiasis, lymphatic filariasis, onchocerciasis and trachoma. As part of the WHO roadmap for neglected tropical diseases, there is a goal to eradicate yaws worldwide by 2020 through mass treatment with azithromycin.(54, 145)

MDA programs rely on the availability of inexpensive, effective and safe drugs that can be administered to affected communities during annual or biannual programs. (146) Fundamental to the expected success of such programs is the assumption that once the prevalence of infection is reduced to below a critical threshold level, transmission will remain low and re-emergence of the disease as a public-health problem is unlikely and may be controlled by other factors, such as natural immunity. Although MDA is the cornerstone of the control programs, they all require additional measures, such as vector control, improved hygiene and environmental sanitation, and health education and information.(146)

Mass treatment for STIs is less widespread, though it has been shown to be an effective approach in several studies. In Rakai, Uganda, as part of an HIV prevention trial in the mid-1990s, mass treatment of STIs resulted in a reduction in prevalence of syphilis and trichomoniasis in the general population.(147) In Vancouver, Canada in 2000, a targeted mass treatment approach was implemented in response to an outbreak of syphilis in urban areas. The approach was considered because of the geographical concentration of people at risk, good access to and support from the community, the failure of standard control measures, and the availability of a suitable treatment drug (azithromycin). The approach appeared to help control the syphilis outbreak in the short term with a reduction in numbers of cases, however this reduction was not sustained.(148)

A mass treatment campaign targeting chlamydia was conducted in the Cook Islands and Nauru in late-2000s with significant reductions in prevalence (more than 50%) reported.(149) In the Solomon Islands, a recent study reported a 40% reduction in prevalence of genital CT infection as a consequential benefit to an MDA initiative aimed at eliminating ocular CT infection (trachoma).(150) The MDA approach may be an effective strategy for use in some Pacific Island setting with small populations and discrete island geography, however it is recommended that this approach only be considered if resources and capacity to implement and sustain control measures are available.(149)

1.4.5 Point-of-care (POC) testing

Point-of-care (POC) tests are generally understood to be tests that are performed by trained staff at the consultation interface between the patient and the health care provider with the result guiding management decisions at the time of the same consultation. The

setting itself may vary – from a large district health facility or an urban clinic but equally may be a remote, low level dispensary or aid station, or even a temporary consultation point as might be experienced by those providing mobile health services to highly mobile or displaced populations. Shephard et al (151) provide a comprehensive definition of POC testing: "pathology testing which performed by or on behalf of the treating medical practitioner by a trained operator in an onsite clinical setting while the patient is in attendance, allowing the rest result to be generated and used to make and immediate informed decision that contributes to an improved health outcome for the patient".

POC tests (also known as rapid tests in some contexts) are being heralded as a solution to overcome many of the barriers experienced in settings with limited access to laboratory-based diagnostics.(51, 152-156) The WHO Sexually Transmitted Diseases Diagnostics Initiative (SDI) has developed the ASSURED criteria as a benchmark to decide if tests address disease control needs.(51) These criteria are to be **A**ffordable to those at risk of infection; **S**ensitive (few false negatives); **S**pecific (few false positives); **U**ser-friendly (simple to perform in 3-4 manual steps with minimal training); **R**apid (to enable treatment at first visit with results in under 30 minutes) and **R**obust (reagents do not require refrigerated storage); **E**quipment-free; and **D**eliverable to those who need them. The ASSURED criteria have provided a useful structure to frame advocacy for research and development around POC tests and guide evaluation of these tests once developed. More recently, with the availability of new platforms based on more advanced technology some of these criteria seem less important in terms of the ideal characteristics for POC testing.(157) Understanding the relative importance of particular test characteristics to stakeholders, and particularly the end-users, is important and likely will vary depending on a variety of conditions including the infection type and sequelae, availability of treatment and alternative approaches, and cultural, social and economic contexts in which the test will be used.

Other important criteria needing consideration included acceptability to operators, other health staff and patients; feasibility (in terms of implementation at the clinical site and also in terms of program logistics, supply and support); cost effectiveness (affordability in the context of a sustainable program including supply and delivery infrastructure, implementation of quality assurance (QA) programs, training and maintenance of

competency, waste control); availability of alternative management of presenting condition when POC test is negative.

Benefits and limitations of POC testing

The potential benefits and limitations of POC testing are many and may be realised at a number of levels (health service, clinicians and health care providers, patient and community). These are summarized in Table 5.

The major benefit of POC testing relates to the ability to test and offer specific diagnosis-directed treatment to the patient at the time of the initial consultation. Whether testing is offered as part of an opportunistic screening approach or based on assessment of risk or presence of symptoms, the outcome of reduced time to diagnosis and treatment has significant downstream benefits. These include reduced time with infection (and therefore reduced risk of individual sequelae and ongoing transmission) and reduced time to initiate partner notification (limiting potential infectivity, ongoing transmission and reinfection). In populations where there are barriers to returning for treatment such as in remote Australia, the potential reduction in numbers of patients who do not receive treatment is critically important. Also important, POC testing has the potential to increase coverage of STI testing as ideally these tests are portable and require minimal equipment, making them suitable for use in outreach or mobile service settings to provide screening to traditionally hard-to-reach populations.

Other benefits of POC testing include the potential immediate cost savings to the health system associated with no longer needing to store and transport specimens to centralised laboratories, reduced overuse of antibiotics and reduced efforts for health staff to recall patients for required treatment.

POC tests should be easy to use and require minimal training to become competent in performing the test, meaning current staff are usually able to be the test operators. Having the new responsibility and skills to perform a POC test which directly informs clinical management can directly benefit staff and health systems through capacity building, improved job satisfaction and staff commitment.

Table 5: Potential benefits and limitations with POC testing

Benefits	Limitations
• Cost savings on specimen transport and reporting as testing is performed on-site • Minimal or no ancillary equipment required (e.g. centrifuges) • Can be performed by current staff (i.e. non-laboratory trained staff) • Ensure targeted use of available resources (i.e. reduced unnecessary treatments if results used to replace syndromic management) • Reduce time to result and thus timely initiation of treatment and partner notification (leading to reduced sequelae and onward transmission and reinfection) • Reduce number of patients who do not receive treatment because of loss-to-follow up • Reduce number of visits by patient (no need to return for result and treatment) • Reduce resources employed by clinic staff recalling patients needing treatment • Health systems strengthening through staff capacity building, improved job satisfaction and health worker commitment; • Expanded reach of STI testing (to outreach settings and hard-to-reach populations)	• STI management guidelines need revision and updating • Training and refresher training of staff required to perform POC test • Increased workload for staff • Time away from other clinical duties while performing the test • Need adequate physical space and infrastructure for POC testing (including IT connectivity for some newer molecular POC tests) • Responsibility of stock management POC test and equipment supply • Implementation of QA program • Ongoing maintenance of POC test equipment • Generation and disposal of addition (biohazard) waste • Maintenance of confidentiality of results • Recording of POC test results (as part of patient medical record)

Use of POC tests is accompanied by a number of limitations. At the local level this includes identifying suitable physical space to store POC tests and any associated supplies. Such spaces may need a stable power supply, lighting, refrigeration, and relatively stable temperature conditions as some POC tests do not store or perform well at extremes of temperature and humidity as are often experienced in remote Australian and other settings. Identifying processes for managing waste (biohazard) generation and disposal must also be considered and may require engagement of a new disposal service, at additional cost.

Providing adequate initial training supplemented with refresher training to ensure those performing the test (clinicians and health workers) are always competent to do so and have confidence in the results is vital.(157) This is particularly difficult in environments where staff turnover is high.(106) The need for already busy staff to set aside time to learn new skills and then perform the POC test as part of routine clinical work can be problematic. In addition to performing the test, staff may need to be responsible for POC test stock management and test maintenance, again in addition to their already substantial workload.

Unlike test results generated through a centralised laboratory, POC test results may need to be recorded manually in a patient record (if available) adding extra work and potential opportunities for data entry error. Newer POC technologies may overcome this difficulty with the generation of an electronic results record, however most POC tests still require manual recording of test results. Maintaining patient and result confidentiality may be problematic in small health services or outreach settings as POC tests may need to be performed in a space shared by other staff or patients. Thought as to how to maintain patient confidentiality in these circumstances is a high priority.

Implementation of a robust QA program (described in more detail later in this section) along with well-monitored logistics and supply infrastructure can ensure the test quality and end-user confidence in the POC test is maintained.

A major disadvantage of relying solely on POC testing is that antibiotic susceptibility for individual patient management may not available through the same testing process, and thus for monitoring and anticipating the development of resistance more broadly. As described earlier (Section 1.3.2), current surveillance relies on isolates for culture to monitor antimicrobial resistance patterns. Until molecular methods to determine resistance

patterns become routine and more widely available in laboratories, (22, 96, 127) and possibly incorporated as standard into POC test platforms (as is available for rifampicin resistance testing with tuberculosis diagnosis using the GeneXpert test platform),(156) the supply of viable specimens for susceptibility testing at laboratories will need to continue.(21, 48)

Evidence of impact of POC testing for STIs

As discussed above, the benefits of POC tests for STIs have the potential to change clinical practice. Whether these benefits translate to real patient and population outcomes can be explored with modelling and operational research and trial methodologies.

Modelling studies

Mathematical modelling has demonstrated POC testing for STIs can have a significant impact on prevalence of STIs. These models also demonstrate the importance of multiple factors that influence the successes of POC tests. Gift et al (158) reported that screening women with a POC test for chlamydia even with relatively poor sensitivity (63%) compared to highly sensitive laboratory-based NAAT and culture tests could result in more treatments despite fewer cases detected because POC testing allowed immediate treatment of patients and avoided loss of patients who fail to return for treatment. This and a subsequent modelling study by Vickerman et al (159) highlights the trade-off between test performance and opportunity to treat at the time of consultation in settings where loss to follow-up is frequent and delay in treatment may result in significant transmission.

A modelling study by Mitchell et al (160) explored the impact of implementing rapid POC testing among female sex workers in China. The authors reported substantial reductions in syphilis prevalence among this traditionally hard-to-reach population but highlighted the importance of including strategies to reduce reinfections from regular non-commercial partners in order to maximize the impact.

Kuznik at al (161) reported antenatal syphilis screening with POC tests was highly cost-effective in 43 countries in Sub Saharan Africa, and that substantial reductions in DALYs can be achieved with a relatively modest cost. Universal screening of pregnant women in clinics could lead to a substantial reduction in adverse pregnancy outcomes (stillbirths, neonatal deaths and congenital syphilis cases).(161)

In the Australian context, Hui et al (162) investigated the impact of annual screening with high performing chlamydia and gonorrhoea POC tests in settings of high endemicity. The model predicts that over a 10 year period with high screening coverage (80%) using a highly sensitive POC test (95%), chlamydia and gonorrhoea prevalence could be reduced from rates of 11% and 7% respectively to less than 0.1%. The authors note the importance of the combined effects of reduced time to treatment and increased treatment rate resulting from use of POC test in screening, and that this also offers opportunity to improve other control strategies such as partner notification and (where possible) partner-delivered treatment.(162)

Operational research

Antenatal screening and treatment of pregnant women for syphilis is well established as a highly cost-effective intervention(163) and investment in the prevention of MTCT of syphilis is likely to contribute to improved maternal and child health globally.(110) Implementation of syphilis POC tests is a fundamental intervention to increase access and coverage of ANC screening of pregnant women in the strategy for the global elimination of MTCT of syphilis.(109)

A recently published systematic review by Swartzendruber et al (164) evaluated the impact of the introduction of POC (also called rapid) syphilis testing in ANC settings in low and middle income countries. The review reported on six studies from eight countries in Africa, Asia and Latin America where rapid syphilis testing in antenatal settings was recently introduced and found that in all studies and all settings (urban and rural, low-level clinics and tertiary referral hospitals) substantial increases in antenatal syphilis screening followed introduction of rapid syphilis testing.(164) The authors note that introduction of rapid testing did not appear to negatively impact antenatal HIV screening levels at sites already conducting this intervention and, in fact, may have increased HIV screening in some settings.(164) Other benefits noted included those for health care providers included increased efficiency and job satisfaction. Barriers to effective implementation were reported included ensuring adequate training, consistency of staff and quality of test devices. The evidence reported in this review supports the strategy promoting rapid syphilis testing in ANC settings with the goal of elimination of MTCT of syphilis (and HIV).(164)

Unlike POC tests for syphilis (which are based on detection of specific and non-specific antibodies in blood, plasma and sera), traditional POC tests for chlamydia and gonorrhoea (which detect specific antigens in genital specimens), have not been widely implemented to date as a result of a generally very poor performance.(165-167) However, recent technological advances in POC tests for these infections have led to the development of a highly accurate molecular-based platform, the GeneXpert.(168, 169) GeneXpert assays are available for the diagnosis of a number of infectious diseases, including tuberculosis for which it is most well-known and widely used at the POC following endorsement from WHO in 2010.(156) [See following sections for more detailed discussion: Section 1.6.1 – POC test formats, Section 1.6.2 – Syphilis POC tests, Section 1.6.3 – Chlamydia and gonorrhoea POC tests]

Though not yet widely implemented or evaluated for routine use for STI diagnosis, in a service evaluation published in 2014, a specialized genitourinary clinic in London (UK) piloting the use of this new molecular POC test for CT and NG reported a reduced time from testing to treatment compared with standard of care for asymptomatic clinic attendees.(170) These early data demonstrate some of the anticipated short-term benefits of POC testing using this technology and encourage further evaluations in a routine use setting.

Quality assurance (QA) programs

Accuracy and reliability of any diagnostic or laboratory testing is critical to the success of a diagnostic test program. In order to ensure testing reliability and reduce errors to a minimum, to improve consistency between testing sites and monitor costs, a quality system that addresses all aspects of testing process, from test procurement to use at the POC, is essential.(51, 171-173)

While the principles of quality assessment should be the same in the POC testing environment, the methods by which they are applied will vary, depending on factors such as frequency of testing, complexity of the device, inbuilt checks manufactured into the device, cost and practicality of providing the quality management system, and the fact that non-laboratory-trained operators will most likely be performing the tests.(173) Such schemes can be developed in conjunction with QA schemes for other diagnostic testing and

could be supported technically by a network of expert laboratories, inside and/or outside of the country to take advantage of expertise and existing infrastructure.(51)

Quality assurance (QA) is an integrated management function that deals with setting policy and running an administrative control system to ensure the usability of a test. It ensures that a test is of the quality needed and expected by the operator. The objectives of a QA program include: to assess the quality of specimen/sample collection and processing; to document the validity of test methods, to monitor reagents, equipment, and the performance of test procedures and personnel; to review test results; to provide feedback for corrective action.(171)

Quality control (QC) is a system of routine and standard technical activities undertaken to measure and control the quality of testing against a defined set of criteria or standards. It ensures that testing procedures are performed correctly, that the environment is suitable for reliable testing, and the test works as expected to produce accurate and reliable results.(171) QC is an immediate check on the integrity of the POC test device (173) and usually includes testing specimens of a known value using the same reagents and equipment that are used for the specimen being measured. For POC tests this is not possible as these devices are single use. While it is not possible to test each device, QC specimens can be used to detect damage at a batch or lot level at different points in the distribution of the test, including the POC.(171)

Internal quality control evaluates the accuracy of the test and verifies the operator's ability to perform the test and interpret the test result correctly. Through *external quality* assessment, the performance of a testing site can be independently evaluated from outside the laboratory or testing site. Methods for external quality assessment include traditional proficiency testing, re-testing of specimens, and careful on-site monitoring using a checklist and knowledgeable assessors.(171)

Considerations for introduction of STI POC testing programs

Through a regional consultation process, the WHO SDI identified and defined a number of key elements and enabling activities that need to be addressed for the successful introduction of POC tests for syphilis (through this could be applied more broadly to POC tests for STIs). These are summarized in Table 6 (adapted from Peeling et al), and highlights

the complexity and variety of stakeholders and evidence required to bring a new test into program use.(51)

Table 6: Key elements and enabling activities for the introduction of rapid STI tests

Key element	*Enabling activities*
Advocacy	Define disease burden, problems and areas for improvement, benefits of new POC test (morbidity and mortality averted, cost-effectiveness)
Policy	Accrue evidence required for change; determine fiscal and human resource implications of change; feasibility of implementation
Test algorithms and treatment guidelines	Discuss with stakeholders and experts; review current local/relevant guidelines
Test selection and regulatory approval	Review test performance and operational performance data; conduct (if required) local assessments/evaluations; select test; review regulatory processes and ensure approvals in place; negotiate test costs
Procurement and logistics	Identify procurement processes including importation and distribution in country; consider also medicines for treatment; processes to avoid stockouts
Training and supervision	Develop and adapt training modules and processes for training across health system (including pre- and in-service, and refresher)
Quality assurance	Develop a quality assurance scheme for ensuring validity of tests in remote settings and proficiency of health workers (test operators)
Monitoring and evaluation	Evaluate effectiveness and impact of POC test introduction
Surveillance	Monitor disease trends to assess benefit attributable to POC test introduction
Communications campaign	For stakeholders and target populations
Partnerships	Develop partnerships to coordinate efforts and ensure synergies, minimise duplication
Academic sensitization	Workshops for professional societies and introduction of new POC test strategy to continuing medical education and clinical curriculum

The following section (Section 1.5) describes the pathway from test development to program implementation, providing detail as to the processes and methodologies for acquiring the ideal evidence base upon which to base decisions regarding implementation a new POC test and strategy, with a focus on use in the Australian context.

1.5 The evaluation research pathway: from POC test development to program implementation

Public need and country policies can drive diagnostic innovation.(174) Innovative POC tests have the potential to transform management and control of STIs, not only in high-burden and resource-limited settings but also in remote locations where distances to traditional laboratories and loss-to-follow up are significant, with rapidly available results enabling treatment to be offered at this same consultation. Without the complementary evidence of operation research, good performance and ease of use may fail to translate into impact.(174)

Tests are rarely used in isolation, and therefore studies are needed that evaluate not only the performance of the test but also the testing strategy itself, taking into account when and how a new test is used within a diagnostic pathway, how results are incorporated into clinical management, and the effect of improved performance on decision making. Then, as improved decision making is only one route by which tests affect patient health, further empirical evaluations are needed to compare the effect of test strategies on patient health.(175)

At the patient level, whether a new test will improve health outcomes, depends upon a number of components including how the test is delivered, how the test result is produced, how the diagnosis is made, how management is decided and how treatment is implemented.(175) These components and their underlying mechanisms can be considered as part of a “test-treatment” pathway and are summarised in Table 7.(175)

Table 7: Attributes of the test-treatment pathway that affect patient health

Pathway component and mechanism	Definition
Diagnostic test delivered	
Timing of test	Speed with which a test is performed within the management pathway
Feasibility	Completion of test process. Reasons for non-completion are: patient acceptability, tests contraindicated, technical failure
Test process	Patient interaction with test process
Test result produced	
Interpretability	Degree to which test data can be used to inform a diagnostic classification
Accuracy	Ability of test to distinguish between patients who have disease and those who do not
Timing of results	Speed with which test results are available
Diagnosis made	
Timing of diagnosis	Speed with which a diagnostic decision is made
Diagnostic yield	Degree to which the test contributes to a patient diagnosis in any form, including: provision of a definitive diagnosis, confirmation of a suspected diagnosis, ruling out a working diagnosis, and distinguishing between alternative diagnoses with different treatment implications. Diagnostic yield is difference from accuracy because it also incorporates any other information used by a clinician to make a diagnosis (such as previous test results/history/physical signs and symptoms)
Diagnostic confidence	Degree of confidence that clinicians and patients have in the validity or applicability of a test result
Management decided	
Therapeutic yield	Degree to which diagnostic decision affect treatment plans
Therapeutic confidence	Certainty with which doctors and patient pursue a course of treatment
Treatment implemented	
Timing of treatment	Speed with which patient receive treatment
Treatment efficacy	Ability of the treatment intervention to improve patient outcomes
Adherence	Extent to which patients participate in the management plan, as advised by their clinician, to attain therapeutic goal.

Beyond the confines of the patient focused test-treatment pathway, other factors influencing the impact of a new test and strategy include local disease epidemiology, treatment availability, health seeking behaviours of the population (including loss to follow up), current diagnostic practices (including specimen type collected and reference tests available), health service infrastructure (including staffing), and acceptability of the new test and its strategy for implementation to staff and clients. Potential difficulties associated with the introduction of POC testing need consideration including the impact of POC testing on current disease notification processes, antimicrobial resistance surveillance, clinical management practices, and pre- and post-service clinical training.

A recent qualitative study by Natoli et al (176) highlighted the numerous anticipated benefits and limitations encountered with the use of POC testing for STIs in remote communities in Australia. Among the public health benefits identified were a reduction in the duration of infection through prompt treatment and partner notification, reduction in prevalence and STI morbidity, more targeted prescribing possibly leading to a reduction in overtreatment and mitigation of the development of antibiotic resistance, and an increase in testing coverage through enhanced acceptability of STI testing.(176)

POC tests should be first considered in the context of the WHO endorsed 'ASSURED' criteria,(177) as described earlier (Section 1.4.5). Next, exploratory work should be undertaken to assess the suitability of the population and setting being considered for POC testing. The populations and settings in which POC tests are likely to have greatest benefits are those in which disease prevalence is high, most infections are asymptomatic, there are serious complications without treatment of asymptomatic infections, there is a lack of laboratory infrastructure, low treatment uptake, and high loss-to-follow up rate. If introduction of a POC test in such a setting could significantly improve the uptake and timeliness of treatment, the next steps are to: (i) evaluate the accuracy of the test in the laboratory (analytical performance) and the field (operational performance including ease-of-use); and (ii) assess staff and patient acceptability of using the POC test in the identified setting.

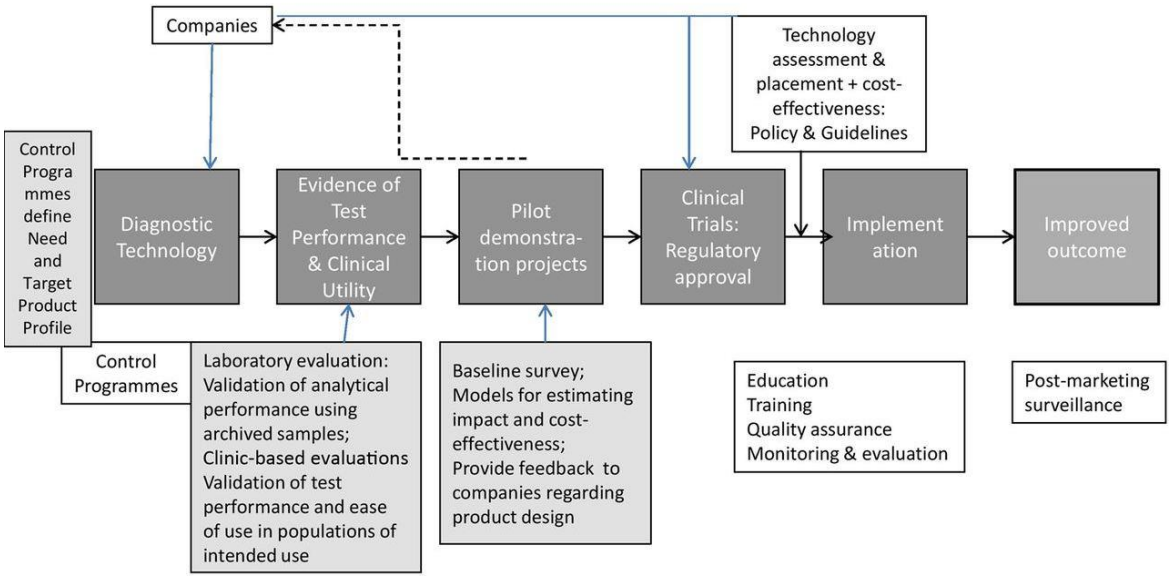
Laboratory assessments of new diagnostic tests are usually conducted in highly controlled environments with relatively skilled staff. Specimens are generally well-characterised and selected in order to determine technical accuracy, that is confirm the ability of the test to correctly identify the presence of absences of the target in the specimen. (178) These

assessments also allow for exploration of concentration thresholds and determination of "cut-offs", above or below which a test might be considered positive or negative. The technical performance resulting from laboratory-based assessments often represents the best possible performance that may be expected from the test.

A range of factors can influence test performance and impact outside of the laboratory setting (i.e. in the field) including technical and operational errors, low coverage in the target population, health systems constraints, and other political, cultural, socioeconomic and behavioural factors. Field research is needed to demonstrate the diagnostic performance of the test in a "real world setting", along with an assessment of ease of use, conditions of use, storage and shelf life(179) and may also offer the opportunity to evaluate the impact of the POC testing program on disease incidence and associated morbidity (if possible). This is particularly important for the introduction of STI diagnostic technologies, as STI testing in primary care settings can be a low priority and health care staff may find it difficult to discuss sensitive issues about sexual behaviours, may be unfamiliar with best clinical practice, and there may be hesitancy to perform genital examinations. From the patient perspective there is also significant stigma, shame and denial that accompany a diagnosis of an STI. Other factors that may influence the impact of an STI POC program included the asymptomatic nature of most STI infections; the significant harms (psychosocial and societal) of false positive and negative results; frequency of co-infections with HIV and other STI pathogens; and considerable heterogeneity in behaviours (social/sexual and health seeking) among infected people.(174)

Planning, financing and conducting a portfolio of research that is comprehensive and provides evidence in a timely manner is complex. Peeling et al (174) have described this program science approach as "the systematic application of theoretical and empirical scientific knowledge to improve the design, implementation and evaluation of public health programs; its end point is a reduction in the incidence of disease at the population level". Figure 9 summarises the importance of program science in supporting the translation of evidence of acceptable performance to policy and implementation.

Figure 9: Evidence to Implementation: The importance of Programme Science



(Source: Peeling et al. 2013)

Steps in the evaluation pathway may overlap and be implemented in parallel, however often the results of the prior step critically inform the subsequent step and ensure its most appropriate scope, design, and implementation.

The subsequent paragraphs in this section provide more detail on the variety of methodologies within the program science pathway

- Section 1.5.1 describes evaluations to determine test performance, operational characteristics and clinical utility (choice of reference test, test accuracy and performance characteristics, study settings, study designs, limitations)
- Section 1.5.2 describes evaluations to assess impact of test implementation patient and population outcomes, health service delivery, and costs (models, pilot projects and randomized controlled trials).

1.5.1 Test performance and clinical utility assessments

When a new POC test becomes available, evaluations of performance and clinical utility are important first steps, and are likely to be conducted in both laboratory and field-based studies.

Characteristics to be assessed include:

1. Performance characteristics (including sensitivity, specificity, PPV and NPV; accuracy; concordance/agreement; reliability and reproducibility)
2. Operational characteristics (time taken to perform the test, technical simplicity or ease-of-use, acceptability and stability of the test under field conditions)

Choice of reference test

Calculation of test performance indices, such as estimates of sensitivity and specificity, requires a carefully selected clinically-relevant reference standard. Generally, the reference standard most often utilised is one that is considered to be the most accurate available. This is sometimes referred to as the "gold standard". In the context of collecting evidence for program implementation, the most suitable comparator may be the current method of diagnosis being utilised. Evaluations of new tests that may be more sensitive than existing standards usually require a composite reference standard as the comparator. Similarly, where no reference standard exists or there is no agreement on the reference standard, a composite can be constructed.(180)

Depending on the choice of comparator, there are different recommended ways to report test performance. These are described in more detail below.

Test accuracy and performance characteristics

Test accuracy refers to the ability of a test to discriminate between those who have and those who do not have the condition of interest. Accuracy is assessed by comparing the results of the test under evaluation with the results of the reference standard, which aims to classify patients as having or not having the condition.(181) Test accuracy is most often expressed as the test's sensitivity and specificity. **Sensitivity** refers to how often the diagnostic test is positive when the condition of interest is present and **specificity** refers to how often the same test is negative when the condition of interest is absent. In order to estimate sensitivity and specificity, the new test is compared to the reference standard test (also called the "gold standard") among individuals who are representative of the population in which the test will be used. This population should contain individuals both with and without the condition of interest. Together, sensitivity and specificity describe how well a test can determine whether a specific condition is present or absent. They should be reported together as each provides distinct and equally important information.

These results are typically reported in a 2x2 table (Table 8).(180)

Table 8: Standard 2x2 table to calculate sensitivity and specificity

		Reference Standard		
		Condition present	Condition absent	
		+	-	
New Test	+	TP	FP	**(PPV=TP/TP+FP)**
	-	FN	TN	**(NPV=TN/FN+TN)**
		(sensitivity=TP/TP+FN)	**(specificity=TN/FP+TN)**	

(adapted from USDOH, 2007)

Where TP = number of true positives; FP = number of false positives; TN = number of true negatives; FN = number of false negatives.

The estimated sensitivity is the proportion of individuals with the condition of interest (that is who are "reference standard positive") that are "New Test positive" (TP/TP+FN); the estimated specificity is the proportion of individuals without the condition of interest ("reference standard negative") that are "New Test negative" (TN/FP+TN). Sensitivity and specificity are inversely proportional, meaning as one increases, the other decreases.

Two other important measures of test performance are ***positive predictive value*** (PPV) and ***negative predictive value*** (NPV). Positive predictive value reflects the proportion of positive new test results that are truly positive (TP/TP+FP), and NPV indicates the proportion of negative new test results that are truly negative (TN/FN+TN). (151)

It is important to remember that PPV and NPV are both influenced by the prevalence of the condition of interest in the population as well as sensitivity and specificity. For this reason, even if a new test has excellent sensitivity, it will have a poor PPV in a population where the prevalence of the STI is low, meaning most of the "new test" positives will be false positives. In this type of low prevalence setting, the performance of the test in the population is driven by its specificity.

If a new test is compared to a non-reference standard rather than to a reference standard, the usual sensitivity and specificity type calculations from the 2x2 table will produce biased estimates of sensitivity and specificity because the non-reference standard is not always correct. In addition, quantities such as PPV and NPV cannot be computed since the subjects' condition status (as determined by a reference standard) is unknown. However, being able to describe how often a new test agrees with a non-reference standard may be useful.(180)

Table 9: Standard 2x2 table to calculate concordance estimates

		Non-reference Standard	
		+	-
New Test	+	a	b
	-	c	D
Total		a+c	b+d

(adapted from USDOH, 2007)

Data from Table 9 provides information on how often the new test agrees with a non-reference standard rather than how often the new test is correct (as in Table 8).

The simplest measure to present is overall percent agreement: the percentage of total subjects where the new test and the non-reference standard agree, calculated as follows:

Overall percent agreement = 100% x (a+d)/(a+b+c+d)

Overall percent agreement does not by itself adequately characterize the agreement of a test with the non-reference standard. Therefore, it is more useful to report a pair of agreement measures, **positive percent agreement** (PPA) and **negative percent agreement** (NPA): PPA = 100% x a/(a+c) ; NPA = 100% x d/(b+d)

All agreement measures have two major disadvantages: "agreement" does not mean "correct" and agreement changes depending on the prevalence (the relative frequency of the condition of interest in a specified group; also called pre-test probability). It is important to understand these differences in the way new POC tests performances are reported when considering and comparing test evaluations in the literature.

Precision refers to the ability of a test to give the same result upon repeated use with the same sample. A test can be precise (reliable reproducibility) without being accurate or vice versa. Although a test that is 100% accurate and 100% precise is ideal, in practice, test methodology, instrumentation, and operations all contribute to small but measurable variations in results. Precision is often reported as a kappa statistic and is intended to give the reader a quantitative measure of the magnitude of agreement between tests or observers (in the case of determining "interobserver" agreement) and corrects for agreement by chance alone.(182) A kappa statistic of 1 indicates perfect agreement, where as a kappa of 0 indicates agreement equivalent to chance.(182) The level of precision and accuracy that can be obtained with a test is specific to each test method and is ideally monitored for reliability through comprehensive quality control and quality assurance procedures.

Reproducibility of results is the ability to obtain the same test result on repeated testing. Reproducibility is influenced by analytical variability and observer interpretation. Analytical variability is due to inaccuracy (systematic error) and imprecision (random error). (178)

Study settings

i. Laboratory-based studies

Analytical performance (or technical accuracy) of a test is assessed using well-characterised samples and usually performed under laboratory conditions. Analytical sensitivity is the ability to detect a specified quantity of the measure component/target. In these types of studies, reference material that contains known concentrations of the component of interest are used. Likewise, the analytic specificity is measured on samples that do not contain the component of interest, but contain another component that may cause false positive results. Such an assessment should be done for every new test under evaluation. (178) A test's analytical performance contributes to its diagnostic accuracy, however, determining how the test performs in the clinical setting in which it will eventually be used is crucial as the next step.

ii. Field studies

As noted earlier, there are many factors that can influence test performance (disease prevalence, genetic variation of the pathogen, type of target antigen or antibody in test, physical format of the test, local experience conducting diagnostic tests etc.). Field trials

offer an opportunity to validate test performance in the setting in which testing will be used, performed by the clinical staff providing care, among the target population.

Depending on resources and timeframes, field studies might be conducted in a number of settings independently, however in some situations (to ensure adequate sample size, logistical feasibility, and generalizability across a range of potentially important clinical settings) a more coordinated, multi-centre approach may be warranted.

Study designs

Cross-sectional and cohort studies can provide high quality evidence of test performance. They may be conducted retrospectively or prospectively. Studies in which data collection occurs after the index test and reference test are done are considered retrospective, while in prospective studies data collection is planned before the test and reference test are done. The advantages and disadvantages for each summarized below and in table 10.(183)

Table 10: Study designs for evaluation of POC test performance

Study type	Site	Samples
Retrospective	Laboratory	Well-characterised samples; archived specimens
	Advantages	Speed; economy; convenience; very amenable to head-to-head (simultaneous) comparisons
	Disadvantages	Poorer specimen quality; limited patient/clinical information; consent waiver usually required
Prospective	Laboratory or field	Fresh/recently collected specimens
	Advantages	Superior specimen quality; more generalizable (depending on population); able to collect more clinical/demographic information; potential to mimic use in clinical setting; amenable to other study objectives (assessment of cost effectiveness,

		acceptability, clinical impact)
	Disadvantages	Costly; logistically challenging; if prevalence is low, may take time to reach desired sample size

For studies aiming to evaluate more than one POC test simultaneously (also known as "head-to-head" comparisons) using either a retrospective or prospective study design, there are additional disadvantages of being more complicated to implement and analyse but can provide individual and comparative accuracy assessment in a relatively economic and timely manner.

Limitations of POC test evaluations

Studies of diagnostic accuracy are a vital step in the evaluation of new and existing diagnostic technologies however several factors threaten the internal and external validity of these studies. Common factors include inappropriate study group (such as mostly symptomatic), inappropriate setting (low-prevalence leading to greater number of false positives), inappropriate purpose (different performance characteristics depending on whether used for screening asymptomatic patients, surveillance, verification of elimination etc), inappropriate reference standard test, inadequate sample size (leading to wide confidence intervals around performance indices), lack of blinding (possible inflating the agreement between test modalities) and also inappropriate execution of the test.(179)

To improve the quality of reporting of studies of diagnostic accuracy and to allow readers the opportunity to detect potential biases and judge the generalizability and applicability of the results, the STARD (Standards for Reporting of Diagnostic Accuracy) initiative commenced in 2003.(184) A checklist has been developed to guide the reporting of the study abstract, introduction, methods, results and discussion and is summarised in Table 11 below.(185)

Table 11: STARD checklist for reporting diagnostic accuracy studies

Section/Topic	Subsection	Comments
Title, Abstract, Keywords		Identify as study of diagnostic accuracy e.g. MESH heading "sensitivity and specificity"
Introduction		State research question, study aims
Methods	Participants	Study population (inclusion, exclusion criteria, setting locations); recruitment and sampling process; data collection (retrospective or prospective)
	Test methods	Reference test and rationale; technical specifications of materials and methods; definition and rational for categories and cutoffs for tests; training and expertise for test operators; blinding
	Statistical methods	Methods for calculating or comparing accuracy; statistical methods for quantifying uncertainty and reproducibility (if done)
Results	Participants	Study timeframe; clinical and demographic characteristics of study population; number of participants satisfying inclusion who did/did not have tests; flow diagram recommended
	Test results	Time interval between index test and reference test; distribution of severity of disease (define criteria) in those with target condition; other diagnoses in those without the condition; cross tabulation of index test and reference test results; adverse events
	Estimates	Diagnostic accuracy and measure of uncertainty; estimates of variability of accuracy between subgroups, readers, centres; reproducibility (if done)
Discussion		Discuss the clinical applicability of the study findings

*adapted from Bossuyt et al 2003 (185)

Comprehensive guidelines have been developed and published outlining the recommended approach to evaluating POC tests for syphilis and CT/NG. As with the STARD initiative, these guidelines aim to standardize the approach to POC testing and to minimize bias and inaccurate performance estimates.(179, 183)

Certainly, test accuracy is important and can act as a surrogate for patient-important outcomes, however it is not enough to simply know the accuracy of a new diagnostic test,

as no matter their accuracy, diagnostic tests are of value only if they result in improved outcomes for patients.(186) Inferring from data on accuracy that a diagnostic test or strategy improves patient outcomes will require the availability of effective treatment, reduction of test related adverse effects or anxiety, or improvement of patients' wellbeing from prognostic information.(187) Thus, if performance results are acceptable and address a public health need, then consultation with policy makers and stakeholders should guide where the new test could be deployed to maximize health impact, and indicate the target population for the next step in the evaluation pathway.

1.5.2 Patient and population level impact evaluations

The impact a test (and testing strategy) has on patient health and community outcomes can be evaluated by decision analysis models that integrate multiple sources of evidence and/or (where feasible) by a randomised controlled trial (RCT).(175, 188) Decision models use existing clinical data to extrapolate, through a number of assumptions, the link between intermediate outcomes (such as accuracy) and long term outcomes. RCTs of testing and treatment interventions randomly allocate patients to tests, follow up subsequent management and measure outcomes only after treatment has been received. A key issue for these methods of evaluation is the selection of appropriate outcomes to be measured or modelled in order to evaluate how the testing strategy affects patients and their communities.

Modelling studies

Modelling studies can facilitate the interpretation of test performance measures by connecting the link between testing and patient outcomes, accounting for uncertainties and explaining assumptions, and allowing the systematic study of trade-offs and uncertainty.(189) Models can explore clinical and population-level impact with sensitivity analyses taking into account varying disease epidemiology and different testing conditions (testing coverage, test accuracy, treatment success/failure). Modelling also may allow for comparisons of different testing strategies.

Some of the difficulties and limitations encountered with modelling new testing strategies includes scarcity of data on important parameters, transferring estimates of test performance across studies, choosing modelling outcomes, and obtaining summary estimates for test performance data.(189)

Models exploring and providing an estimate of test impact at the population level can provide evidence that complements accuracy studies and strengthen the case for further evaluation of a testing strategy in research trials. Some key advantages of modelling studies include generally requiring fewer resources and providing more rapid results than a larger trial. A variety of testing scenarios can be explored to identify the optimal combination of attributes (POC test accuracy, prevalence of infection, coverage of testing) in order to achieve the primary health outcome.

When effectiveness data become available (following analysis of clinical trial data) models can be used to evaluate cost-effectiveness of the testing strategy. Cost and cost-effectiveness data are critical for policy makers when considering whether such a program should be a priority for funding using scare health resources.

Pilot projects

Pilot projects, in addition to allowing small scale evaluations of diagnostic accuracy of the index test (and possible alternative options through a head-to-head comparison) also offer opportunities to gather information on feasibility, logistics of implementation and appropriateness and effectiveness of training materials. Pilot projects also allow the opportunity to confirm assumptions made about disease epidemiology and other factors that might influence test performance. Pilot projects can strengthen collaborative relationships and allow further engagement with communities and their health care providers in which larger scale trials may be implemented in the future. The trust and reciprocity garnered during these smaller scale projects is invaluable.

Information from pilot projects can be relayed back to the test manufacturer for consideration prior to full scale research trial implementation.(174) This pilot environment may also allow the opportunity for preliminary information to be collected on acceptability of the POC test and strategy to stakeholders, operators and patients.

Other obvious advantages of the pilot project include the smaller scale and consequently more feasible logistics and economics than a full trial, however it may be more difficult to achieve an adequate sample size to allow for a statistically robust analysis of diagnostic accuracy.

Lessons learned from the pilot and modelling studies can inform the design of the larger scale RCT.

Randomised Controlled Trials (RCTs)

In order to evaluate clinical impact and address key program design, implementation and policy questions, larger scale trials are usually required. Such trials may also be well-placed to evaluate training options, QA programs, large-scale logistics, jurisdictional roles and responsibilities, sustainability, potential adverse effects on community, health service staff, health system and cost.

Properly conducted RCTs are the gold standard for assessing the effectiveness and safety of interventions.(190, 191) Key generic features of the RCT include: random allocation of patients to intervention groups; patients and investigators should remain unaware of which intervention is being implemented until the study is completed (although such double blind studies are not always feasible or appropriate); patients are analysed within the group to which they were allocated, irrespective of whether they experienced the intended intervention (intention to treat analysis); the analysis is focused on estimating the size of the difference in predefined outcomes between intervention groups.(192)

A *diagnostic* RCT may be defined as a randomised comparisons of two diagnostic interventions (one standard and one novel) with identical therapeutic interventions based on the results of the competing diagnostic interventions (for example, disease: yes or no) and with the study outcomes being clinically important consequences of diagnostic accuracy.(190) Such RCTs of tests can measure patient and population outcomes and the important management processes directly to understand why and how changes to patient health have occurred (if indeed they have).(175) Diagnostic performance (as described earlier in Section 1.5.1) can also be determined when the gold standard and the novel test are both conducted but the results of one test are randomly kept blinded.(190) The process of randomisation ensures that differential contextual biases are eliminated as both competing strategies are evaluated in groups with similar characteristics including disease prevalence.

Interventions that operate at a group level, manipulate the physical or social environment, or cannot be delivered to individuals are best evaluated using a *group* or *cluster-randomised* design.(193) Differences between the cluster-randomised and individual-

randomised trial include that the unit of assignment is an identifiable cluster or group (such groups are formed not at random but rather through some physical, social, geographic, or other connection among their members); different clusters are assigned to different interventions; the units of observation are members of the cluster; and usually only a limited number of groups are assigned to each intervention.(194)

Diagnostic RCT of tests may be more difficult to design than randomised studies of treatments as the benefits from testing may be limited to a subset of those tested, so sample size requirements can be substantial. Another limitation for diagnostic RCTs is that patients should not ethically be placed in a position of being randomised to a known inferior diagnostic procedure for the sake of research. Thus investigators must substantiate clearly that the interventions are (highly likely to be) in diagnostic equipoise.(190)

Diagnostic RCTs need a well-defined protocol that links testing, results, and downstream decisions. As a result, such trials evaluate the effectiveness of testing as well as that of downstream management. These protocols may not always mimic the way the test will ultimately be used in practice, and in addition clinical staff compliance with such protocols may be difficult, limiting the external validity of the trail results. (188)

Another variation on the standard RCT is the *cross-over* design. Cross-over trials are another way of overcoming differences in groups by keeping the patients as matched as possible. Instead of having different patients in each intervention group, patients receive first one intervention and then the other, in a random order. Within-patient differences are then compared. Thus each patient effectively becomes their own 'test' and 'control'. (195) An advantage of the cross over design is the increased power to detect an effect: that is, a crossover trial requires a smaller sample size than parallel-group trials to meet the same criteria in terms of type I and type II error risks.(196) The importance of the "washout period", time between switching between one intervention and the other, will depend on the intervention itself and will need to be considered in the design and analysis of study results.(196)

Clinical impact and patient relevant outcomes

In designing an RCT, it is important to determine the most appropriate clinical outcome and how it will be measured. In some cases, when the time between the intervention and the ultimate clinical outcome is long and thus impossible to measure in the timeframe of the

RCT, the outcome selected for measurement may be a proxy or an intermediate outcome. For example, although the hypothesis behind implementing POC testing for STIs is that programmatic use will lead to a reduction in incidence and population prevalence of STIs, the RCT designed may not be able to detect such change in the available time frame. Thus a short-to-intermediate outcome might be chosen in place, such as reduction in re-infections at 3 months among patients with a positive.

Acceptability

The mere availability of a high-performing POC test does not ensure uptake by service providers or end users.(157) The needs and wishes of the end-user are very important and are an often neglected focus of POC test research. (197) In her review of the literature, Natoli (198) notes that test acceptability to health care workers and patients is critical to support the introduction and sustainability of POC testing and includes a range of factors including the perceived reliability/accuracy of the test and confidence in the test result; complexity of test (manual, timed steps particularly); ease of interpretation of test result (subjectivity of reading result); nature of specimen collection (complexity and patient discomfort); time taken to perform the test (hands-on and total time to result); impact of POC testing on clinic workflow; and cost and maintenance of POC test.

The context of an RCT can provide the opportunity to formally assess the acceptability of a new test and testing strategy among healthcare workers and clinicians who will ultimately be the test operators, and among patients and the communities who will be the immediate beneficiaries of the new strategy. Other stakeholders, including those in management and policy positions, are also important subjects for this research. Such research might involve a mix of qualitative and quantitative methodologies (198) and aims to contribute to the understanding of the potential benefits and barriers to POC test implementation, thus improving design and opportunities for program success, and ideally the realisation of anticipated benefits at the population level.

Quality assurance (QA)

As described earlier in Section 1.4.5, a QA program is an essential element to any POC testing program.(173) As part of the RCT, the QA program might be designed to reflect what might be suitable should POC testing be expanded or implemented programmatically following the trial.

The trial setting allows for investigation of alternative or novel methods for QC testing in collaboration or consultation with existing QA programs and implementing bodies such as NRL (199) or Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP).(55) Evidence can be collected as to the feasibility and predicted cost of the QA program for programmatic expansion.

Cost and cost-effectiveness

As with any program, whether government or non-government, limited health resources need to be prioritised and having relevant data to estimate of the costs associated with implementation of a new STI POC testing strategy (and compared to alternative strategies) is critical to make informed decisions about resource allocation. In the context of introducing STI POC tests, cost and cost-effectiveness analyses can help determine how these tests can contribute to reducing the burden of STIs across a range of settings and help prioritise optimal strategies. Collection of site specific costs generates data necessary to model the costs of scaling projects up regionally or nationally. It will also contribute to exploring the best use of optimal testing strategies and more generally aid decisions about the best use of present and new resources for STI screening.(200)

In general, the cost per person screened and treated depends largely on the cost of the test and the prevalence of the STI, with a higher prevalence usually resulting in a lower cost per person screened. In some circumstances, integrating a POC testing strategy into an already established program (as with syphilis POC testing added to PMTC HIV screening in ANC settings) may be particularly cost-effective as personnel time and building space can be considered shared costs, especially in countries that have made a strong commitment for the joint elimination of MTCT HIV and syphilis.(174)

As part of the formal trial setting, an economic evaluation may be conducted from a defined perspective (societal, provide or household) to determine costs of all relevant health care resources (e.g. training, equipment, consumables, staff time, treatment, follow-up etc.) used in STI testing and in managing patients after testing. Analyses may involve a comparison of costs between POC tests and conventional laboratory-based tests, health care resources used in the syndromic management of patients, and management of patients after testing (standard care).

Should the POC testing intervention prove to be effective in terms of the clinical outcome being measured (e.g. reduction in reinfections or prevalence in the community), cost-effectiveness can be reported in terms of incremental cost per additional STI prevented. However, if feasible, this incremental cost-effectiveness ratio (ICER) may also be extrapolated to the cost per additional quality-adjusted life year (QALY) gained.

Costs and benefits can be examined over the time horizon of the formal trial and then extrapolated over the life-time of a cohort of a target population. Some of these outputs from such mathematical models will be critical inputs to economic analyses. Sensitivity analyses of the modelled outcomes to variations in assumptions underpinning the model and in input parameters can also be undertaken.

1.5.3 Program implementation and evaluation

The robust and comprehensive evidence generated through the research studies described along the evaluation pathway will critically inform the design and implementation of any new POC strategy. This can be supplemented with an understanding of cultural contexts, training and site preparation needs, funding and reporting arrangements. Engagement of policy makers, stakeholders and other potential partners as peers throughout all the study periods, complemented by well communicated results, is critical to the next phase of program implementation.(174)

As described earlier in Section 1.4.5 , there are a number of key elements and activities to facilitate implementation of a new program of POC testing. The monitoring and evaluation element fits well within the program science pathway. A well-designed monitoring and evaluation plan will ensure that the program is achieving the desired and expected outcomes, especially those longer term outcomes that may have been beyond the scope of direct measurement with a RCT. Program monitoring and evaluation is also very important to identify unintended negative or detrimental impacts resulting from implementation. For example, making sure the introduction of a novel POC test which is only able to detect a subset of potential STIs (chlamydia and gonorrhoea) does not lead to neglect of traditional testing for other STIs (such syphilis, HIV, trichomoniasis) or loss of viable specimens for monitoring antimicrobial resistance.

1.6 Current status of POC tests: syphilis, chlamydia and gonorrohea

POC tests are considered to be those that are performed by trained staff at the consultation interface between the patient and the health care provider. Though there is some variation in what constitutes a "simple" or "easy to use" test or a "rapid" result, the critical element that supports the major benefit of the POC test is that the result generated is able to inform clinical decision making and patient management during the same consultation.

This section provides an overview of technical aspects of POC tests, with a comprehensive summary of the currently available POCs for syphilis, chlamydia and gonorrhoea including their advantages and limitations. The section concludes with a synopsis of POC testing in Australia.

Note: Currently utilised laboratory-based reference tests for these infections are described in earlier sections (Section 1.2.1 and Section 1.2.2)

1.6.1 POC test formats

A variety of test formats and detection methodologies are utilised for STI POC tests. POC tests for a specific infection may detect either/or a combination of

1. Serological markers (such as specific antibodies or non-specific antibodies associated with the presence of the target pathogen)
2. Antigens (unique to the pathogen)

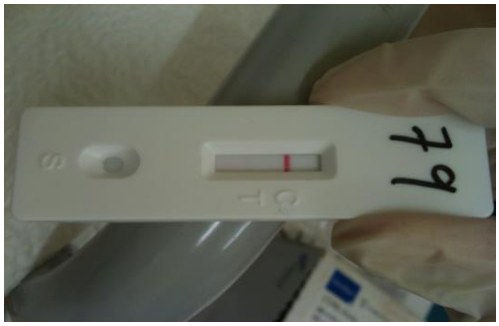
The most widely available format utilises a lateral flow technology, which is based on similar general principles as enzyme-linked immunoassays (ELISA) as performed in laboratories. In general, a specified volume (usually 1-2 drops) of suitable specimen (e.g. swab fluid, urine, blood or serum) is added and washed with a buffer solution along a nitrocellulose (immunochromatographic [IC]) strip. As the sample progresses via capillary flow, it encounters regions on the test strip with immobilised anti-immunoglobulin (bound with a dye or colloidal gold) capable of capturing either the desired infection-specific antibody or antigen. If capture occurs, a visible colour change is generated at that location on the strip. The test operator observes this colour change and records this as a positive test result. Such platforms always include a control region on the test strip. If this control line is not activated upon completion of the test, then the test result is invalid. Lateral flow

tests may contain one or more regions for detection and can also support a mix of antigen and serological elements for detection. In some circumstances, test manufacturers have developed electronic readers capable of detecting the colour change on the test strip, relieving the operator of this subjective interpretation.

An alternative format used in some POC test devices uses particle agglutination technology. These test formats use gelatin particles coated with disease specific antigens that clump together on a test tray when combined with the specimen containing the target antibodies.(201)

Advantages of this lateral flow format (see Figure 10) include: rapid time to results (15 -20 minutes), small, compact, robust and highly portable, stable at room temperature, no electricity required, simple and easy to use, and minimal waste generation. Disadvantages of this format include: non-quantifiable result (therefore not useful to monitor response to treatment), critical timed steps and time to read result (requires timing device and availability at precise time to read result), results not valid if read beyond recommended time (and thus no permanent record of result), specimen preparation required in some circumstances (e.g. need to centrifuge specimen), subjective interpretation of result (inter-observer variability), potential exposure to (bio)hazards through self- or workspace contamination (which may depend on levels of operator skill and experience).

Figure 10: Example of a typical lateral flow test in cassette format



Sample well (S), test result (T) and control (C) regions as indicated

Technological advances in recent years have led to the development of POC tests based upon molecular detection methodologies, which have until now been restricted to use in well-controlled laboratories with trained technicians. These molecular methods utilise the

technique of polymerase chain reaction (PRC) to amplify unique pathogen-specific nucleic acid sequences. When the amplified target reaches a critical predetermined cycle threshold, the target is considered to be "detected". In the case of using this technique as a diagnostic test, reaching the threshold indicates a positive test result. This technique for use at the POC has only recently become available for a selection of STIs (chlamydia, gonorrhoea and trichomoniasis), however it has been available for some time as a POC diagnostic for tuberculosis and has demonstrated excellent sensitivity and specificity for detection of this infection (in those with and without HIV).(156, 202)

1.6.2 Syphilis POC tests

Currently available POC tests for syphilis are primarily IC assays that use lateral flow technology and are capable of detecting target antibodies in a specimen (whole blood or serum or plasma). Traditionally, these tests use treponemal-specific antibody as the target; these "treponemal-only" tests have been demonstrated to have good performance in laboratory and field trials, acceptability and cost effectiveness, (51, 155, 203-207) costing generally less than $1-2USD per test. In a comprehensive systematic review of 15 published evaluations encompassing nine different syphilis POC tests, Tucker et al (155) reported a median test sensitivity of 86% (interquartile range [IQR]: (75 – 94) and median test specificity of 99% (IQR: 98 – 99) compared to laboratory-based treponemal reference tests (TPPA, TPHA, etc). These indices were similar in STI and ANC settings. The authors highlighted, however, the lack of data available from many of the evaluations on test performance by clinical stage, HIV co-infection status and non-treponemal titre.(155)

A more recent meta-analysis of published studies by Jafari et al (205) reviewed performance of 18 POC syphilis test from 131 studies, the vast majority evaluating lateral flow based assays. In their meta-analysis, the authors corrected for imperfect reference standards across the studies (that allow for variability between index tests, studies, populations, and chance that impact evaluation of the accuracy of an index test) which generally improved the accuracy estimates of the tests. The Determine rapid test has the best sensitivity in serum (92.03%, 95% CI: 87.22 – 95.77) while the Syphicheck had the best specificity in blood (99.44%; 95% CI: 98.96 – 99.81) compared to laboratory-based treponemal tests. Performance estimates in serum were consistently higher for all tests than estimates in blood, possibly because of higher concentrations of biomarkers in serum and the absence of interfering substances in whole blood. (205) The authors note that

results in whole blood are most relevant to decision makers as they best capture circumstances encountered in resource-limited settings.(205)

Supported by this available evidence indicating the relatively good performance of these treponemal-only syphilis POC tests, there has been substantial enthusiasm and support for the implementation of POC tests for syphilis in resource-limited settings,(51, 208, 209) with several countries having recently adopted POC tests into their national antenatal screening policies. (152) Although successes with syphilis POC test implementation have been reported,(164) there remain a number of barriers to implementation in some countries including limited financing and external support, poorly functioning supply chains, and busy clinical practice and adequate training.(164, 210) The use of syphilis POC tests in ANC settings as a strategy in the prevention of MTCT of syphilis is also discussed in Section 1.4.5.

As described in Section 1.2.1, treponemal antibodies are specific for syphilis (outside areas with endemic yaws or other non-venereal treponematoses) and remain positive for life following infection in most cases, even after successful treatment. Thus the use of POC tests detecting treponemal antibody alone provide no information about current status of syphilis. Tests for non-treponemal antibodies, such as RPR (rapid plasma reagin) and TRUST (toluidine red unheated serum test), used in combination with treponemal antibodies tests are useful indicators of active infection and can provide a quantitative result (particularly useful to monitor response to therapy) but, if used alone, can yield false-positive results due to cross-reactions with other antigens. They are also subject to false negativity in active syphilis because of biological fluctuations. Non-treponemal tests require a serum specimen and although these tests (e.g. RPR) are sometimes used at the health service level and in a near patient settings,(44) they are not considered to be well-suited to these environments.

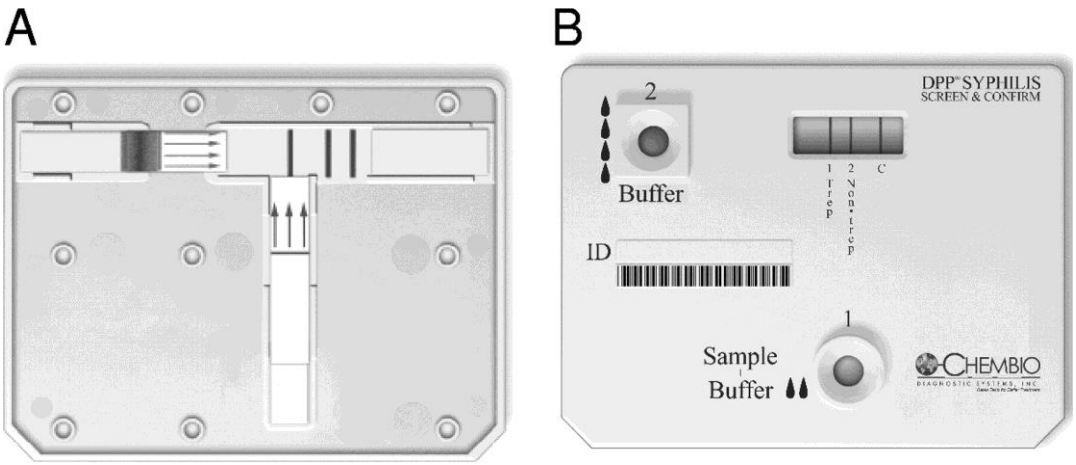
Recent advances in syphilis POC test technology include the combination of tests that detect both treponemal and non-treponemal antibodies using a single device with one sample and offer the potential advantage to distinguish between active and past/treated infection. One such commercially available test is the DPP Syphilis Screen and Confirm Assay (Chembio Diagnostic Systems Inc, Bedford, NY, USA) (Figure 10).(211)

Figure 11: DPP Syphilis Screen and Confirm Assay

The DPP Screen and Confirm assay simultaneously detects treponemal and non-treponemal antibodies from the same specimen. It is designed for use with serum, plasma or whole blood and is stable at room temperature for 1 year. The test cost is around $2.50USD (which is two to five times more than single treponemal tests).(212) The test is based on lateral flow technology comprising of a cassette containing two nitrocellulose membrane strips perpendicular to each other in a T-formation and is shown below in Figure 12. (213)

Figure 12: Structure of the Chembio dual POC test for syphilis

Location of antigen lines and orientation of test strips (A), and front cover plate (B).



(Source: Castro et al. 2010)

At the time of initiating this thesis, the one published evaluation of the dual POC test by Castro et al (213) suggested that among 1601 stored samples, the reactive concordance of the non-treponemal line was 98.4% when the RPR titers of sera were ≥1:2 and the non-reactive concordance was 98.6% (compared to the reference RPR test). The reactive and non-reactive concordances of the treponemal line were 96.5% and 95.5%, respectively

(compared to the TPPA reference test).(213) Sensitivities appeared to be higher among specimens with non-treponemal test titres ≥1:4. In a subsequent multisite evaluation by Yin et al in China, this test also appears to perform consistently across different specimen types (whole blood, finger prick, and plasma) in clinic and non-clinic settings.(214) At the time of commencing this thesis, no evaluation had directly compared this novel test to currently available treponemal-only tests, investigated its performance by clinical stage of syphilis or attempted to quantify the test's ability to reduce the extent of false-positive diagnoses in past treated syphilis.

Other advances in syphilis POC tests include the combination of tests for different infections, such as syphilis and HIV costing around $2USD.(215) These combination tests rely on detection of treponemal antibody for syphilis alone and thus experience similar limitations with regards to determining current syphilis status.(216) The development of these combination tests addresses and aligns with the significant dual opportunity to interrupt the transmission of syphilis as well as HIV, particularly in setting where co-infection is common. In ANC settings, this development also takes advantage of the major investment in prevention of MTCT of HIV which is now a global health priority,(217) with Cuba recently being the first country in the world to have WHO validation of this achievement.(218) There are currently 6 dual tests in development, at least 2 of which are ready for evaluation.(219)

Another new technology in the realm of POC testing is the advent of electronic readers whereby digital imaging technology is used to produce an image of the entire test device or test window. Readers are designed to be either universal (one device can read multiple test types from different manufacturers) or dedicated (requires customised software, specific to one test type/manufacturer). A number of these readers for POC tests are available or under development.(220) Though this technology is still in its infancy, given the widespread adoption of smartphone technology across the globe, these devices which can combine high resolution test images with the computing capability required to run image analysis software and transmit data could change the nature of POC testing practice entirely.(220) These devices could be used for real-time surveillance monitoring, assessment of interventions, supply chain management and quality assurance.

Outstanding questions around syphilis POC tests

POC tests are not routinely used in Australia and there is no data available on performance of syphilis POC tests using specimens derived from the Australian population. Further, there is little evidence available regarding the performance of syphilis POC tests during different clinical stages of infection and among those with HIV co-infection which may influence the host response to syphilis. The findings of the head-to-head laboratory evaluation of four selected syphilis POC tests presented in Chapter 2 and Chapter 3 of this thesis address these gaps in knowledge. Chapter 3 focuses specifically on the performance characteristics of the new dual Chembio test and discusses the potential tradeoffs with the ability to distinguish current from past, treated infection.

1.6.3 Chlamydia and gonorrhoea POC tests

Traditional lateral flow POC tests

The first generation chlamydia and gonorrhoea specific POC tests are based on lateral flow platforms. Evaluations of these tests to date have generally demonstrated very poor performance (165-167, 221, 222) and consequently, despite being relatively inexpensive, their use has been very limited.

Natoli (198) in her recent review of the literature, reported that sensitivities for chlamydia and gonorrhoea later flow tests varied depending on specimen type, symptom status, background prevalence of infection and reference method, however the vast majority were below 80%. Some explanations for the observed poor sensitivities include the higher threshold for detection of antigen (10^4 – 10^5 bacteria) by lateral flow tests compared to laboratory reference NAATs (10 bacteria); the antibody-antigen reaction takes place over a much shorter time frame compared to laboratory-based tests, meaning there is an inevitable trade-off between sensitivity and speed of result generation.(174)

New molecular-based POC tests

Recent technological advances have led to the development of new molecular-based assays for POC testing for CT and NG. The first platform commercially available utilising this technology is the GeneXpert CT/NG assay (Cepheid, Sunnyvale CA). As noted earlier, GeneXpert assays are available for the diagnosis of a number of infectious diseases

including tuberculosis, for which it is most well-known and widely used at the POC following endorsement from WHO in 2010.(156)

In summary, the GeneXpert CT/NG (Xpert) assay is a rapid NAAT that detects nucleic acid of CT and NG from endocervical, vaginal, and urine specimens of females, as well as from urine specimens of males, from both symptomatic and asymptomatic individuals.(223) Although not formally validated, swab specimens from extragenital sites are also able to be processed. The GeneXpert is a self-contained, automated test system performed using a modular cartridge-based platform for testing each specimen by nucleic acid amplification, and it can process from 1 to 96 specimens in 90 minutes (depending on the platform configuration) with easy-to-use cartridges that minimize processing steps and contamination.(223) The GeneXpert CT/NG assay includes two targets for NG, with both targets requiring detection for the assay to give a result indicating detection of NG. The design and ability of the GeneXpert assay to detect both NG targets reduces the possibility of a false positive result that could occur with commensal NG species. Commercial NAAT assays detect one or two targets on the same gene, and laboratories are recommended to conduct a second confirmatory test for NG using a different assay with a different target for specimens from genital sites before reporting the results as positive, at additional time and cost.(129) (See also Section 1.2.2)

Figure 13: GeneXpert four-module platform and laptop setup in a remote health service

Since this assay became available in 2012, evaluations have demonstrated high analytical (169) and clinical (223) performance compared to established commercial nucleic acid

amplification tests (NAATs), in highly-controlled laboratory environments with dedicated staff.(134)

The table below summarises selected characteristics of the Cepheid CT/NG assay.

Table 12: Selected characteristics of the Cepheid CT/NG assay

Characteristic		Detail
Test details	Name	GeneXpert (Xpert)
	Company	Cepheid
	Assay type	PCR
	Organism detected	CT/NG
Operational characteristics	Recommended specimens	Vaginal swab (VS), Endocervical swab (ECS), Urine (female and male-UR)
	Volume of sample + volume diluents	1mL
	Preparation steps	Add specimen to directly to cartridge from collection tube
	Results available for reading	90 minutes
Independent laboratory evaluation (169)	Sensitivity	NG: 100% (10/10) CT: 100% (15/15)
	Specificity	NG: 100% (75/75) -non-gonococcal *Neisseria* isolates were assessed CT: not done
	Reference test	Well-characterised cultured isolates
Manufacturer reported performance	Sensitivity	Females (n=648) NG VS: 100%, ECS: 100%, UR: 91% CT VS: 96%, ECS: 94%,UR:96% Males n=272 NG UR: 100% CT UR:96%
	Specificity	Females (n=648) NG VS: 99.7%, ECS: 99.5%, UR: 99.8% CT VS: 99.7%, ECS 99.7%, UR: 99.7% Males (n=272) NG UR: 100% CT UR:100%
	Reference test	Patient Infected Status (PIS) Standard: Concordant results for two molecular

		assays on at least one specimen type Including industry-leading commercial assays, the BD Probe Tec and Gen-Probe Aptima

Though manufacturer and laboratory-based performance evaluations using clinical specimens indicate the GeneXpert is highly accurate (169, 223) and has the potential for use in a POC setting,(134) evidence on test performance in the hands of clinical staff, who would be required to use it in routine practice, was not yet available.(174) In addition to determining if test performance is maintained under routine conditions, patient-important and population outcomes must also be evaluated in the target populations. Operational research as part of the program science pathway (discussed in Section 1.5) can provide evidence to inform policy decisions at a programmatic level and is a critical next step. It should include assessments of cost, effectiveness, feasibility, acceptability, training materials and delivery, and QA program implementation.

Although it has been shown that use of POC test alone can reduce prevalence of infection if the use of the POC test increases treatment uptake, to have a maximal impact at the population level, testing coverage needs to be high.(162) Consequently, complementary strategies to increased coverage of STI testing will be important to assess in order for the impact of new strategies such as POC testing to be fully realised.

Given the paucity of data on field operational performance, and clinical and populations-level outcomes associated with this new POC test platform for CT and NG, there is a very clear need for research to provide evidence on the use of this new diagnostic technology on a larger scale and in a variety of settings in the hands of the end user. The technology is unlike most other POC platforms available currently (i.e. lateral flow based) and there are likely to be a number of unique implementation challenges experienced as a result. Some of these issues are addressed in the research presented in Chapters 5 and Chapter 6 pertaining to the operational performance of the GeneXpert CT/NG in remote Australia.

1.6.4 POC testing in Australia

Programmatic implementation of POC testing of any type in Australia is very limited. Currently there are no mandatory standards or guidelines specifically for POC testing and

such testing falls outside the regulations governing the established laboratory-based pathology services.(224)

POC testing for chronic diseases has been acknowledged to be a potentially useful frontline clinical management tool, relieving stress on primary care services and expanding the reach of pathology.(224) Examples of local operational networks of POC testing include the *Quality Assurance for Aboriginal and Torres Strait Islander Medical Services (QAAMS)* program which provides culturally appropriate diabetes management services through on-site POC testing with over 170 Aboriginal medical services enrolled, currently representing every State and Territory; the *i-STAT network*, operated by Northern Territory and Queensland Health; and the integrated *Cardiovascular Clinical Network South Australia* using POC testing for cardiac markers to direct clinical pathways.(224)

As an example of a successful POC testing program designed to address a specific clinical need and contemporary health issue confronting Aboriginal populations in Australia, the QAAMS program is the largest and longest running national POC testing program in Australia and has been continuously funded by the Australian Government's Department of Health and Ageing since commencing in 1999.(225) The program targets training at Aboriginal Health Professionals who then act as key drivers of the program at the service level, further encouraging community control and ownership of the program. This role is highly valued by the community and the health service staff develop a sense of empowerment, responsibility and commitment.(225) The program has demonstrated it is analytically sound, culturally and clinically effective, cost effective and sustainable, adaptable and transferable to other clinical settings.(225) Being able to have a pathology test, obtain the result and see the doctor on-site during the one visit has not only improved the patient's self-motivation to improve their health but also improved their relationship with the treating medical practitioner.(225)

Currently there is no routine use of POC for STIs or any other infectious disease and all such approved diagnostics are conducted in a laboratory setting. Though the QAAMS program described above was established for the use of POC testing to support the management of a chronic disease condition (diabetes), the program experience, established platform and current infrastructure are likely to be highly relevant, adaptable and potentially receptive to a POC for STIs in these same communities where there is a high STI burden.

A recent qualitative study by Natoli et al (176) reports stakeholders across a number of fields including sexual health, primary care, remote Aboriginal health and laboratory services are highly supportive of wider access to POC testing for STIs citing potential benefits including earlier, more accurate treatment and expedited partner notifications and reductions in ongoing transmission. POC testing could also improve acceptability of STI testing, increase testing coverage and result in more targeted prescribing. (176) The study also highlighted the need for new systems to avoid adverse impact on disease surveillance (notifications and AMR), with concerns raised regarding the possible reduction in completeness of case reporting and AMR surveillance given the heavy reliance on laboratories currently in Australia for these responsibilities.(176)

1.7 Summary and Thesis Research Aims

In summary, this chapter has described the substantial burden of STIs in the Australian setting with Aboriginal people (especially those residing in remote communities) and other marginalised populations (such as gay men) disproportionately affected by these infections.

POC tests for STIs have the potential to overcome a number of unique barriers in Australia, a relatively wealthy, developed country with a good laboratory infrastructure and supply of effective treatments, to the control of STIs through their ability to reduce time to treatment and avoid loss to follow up of patients.

The application of the framework of an evaluation pathway to the planning, design and implementation of research ensures critical evidence is generated in a reliable and robust manner to support the movement of particular POC testing options towards programmatic implementation, ultimately resulting in patient and community-important outcomes.

It is in this context that this thesis aims to evaluate a selection of POC tests for syphilis, chlamydia and gonorrhoea in laboratory and/or field settings to establish their accuracy and operational performance characteristics in the Australian context.

This was achieved through the conducted of the following studies:

1. Laboratory-based evaluation of performance of four POC syphilis tests in Australia
2. Field-based pilot evaluation of performance of POC tests for CT and NG in remote Aboriginal communities in Australia
3. Operational performance of molecular-based POC test for CT and NG in context of cluster-randomised cross-over trial of POC test CT and NG in remote Aboriginal communities in Australia

List of publications

The table below details the chapters presented as publications contributing to fulfilment of this thesis, together with their respective aims, data sources and method of data analysis.

Table 13: Summary of chapters and first-author publications

Chapter number and title	Aim	Data source	Analytical method	Journal
Chapter 2: A Laboratory-Based Evaluation of Four Rapid Point-of-Care Tests for Syphilis	To evaluate the sensitivity, specificity and operational characteristics of selected syphilis POC tests in a laboratory setting; to explore associations between clinical parameters (syphilis stage, HIV status, CD4 count) and POC test performance	Retrospective, head-to-head laboratory-based evaluation of POC tests using stored Australian sera (n=1203)	Sensitivity and specificity compared to reference treponemal tests; by subcategory (HIV status, clinical stage etc)	PLoS One. 2014 Mar 11;9(3):e91504. doi: 10.1371/journal.pone.0091504. eCollection 2014
Chapter 3: An evaluation of a novel dual treponemal/nontreponemal point-of-care test for syphilis as a tool to distinguish active from past treated infection	To evaluate overall performance (both treponemal and non-treponemal components) and the potential clinical implications of the novel DPP syphilis POC test	Retrospective, head-to-head laboratory-based evaluation of POC tests using stored Australian sera (n=1005)	Sensitivity and specificity compared to reference treponemal and non-treponemal tests	Clinical Infectious Diseases. 2015 Jul 15; 61(2):184-91. doi:10.1093/cid/civ243. Epub 2015 Mar 25
Chapter 5: A field evaluation of a new molecular-based point-of-care test for chlamydia and gonorrhoea in remote Aboriginal health services in Australia	To determine the operational performance, and explore the feasibility, acceptability and ease-of-use of the GeneXpert CT/NG assay alongside two of the better performing lateral flow tests available	Prospective, pilot study in two remote Australian communities (n=198)	Sensitivity and specificity compared to routinely performed NAATs for CT and NG	Sexual Health. 2014 Nov 27. doi: 10.1071/SH14158. [Epub ahead of print]
Chapter 6: Prospective evaluation of the diagnostic performance of a new, molecular point-of-care test for chlamydia and gonorrhoea in routine primary health care use	To determine the operational performance of the GeneXpert CT/NG assay in the hands of clinical staff at remote primary health services	Prospective, cross-over cluster-RCT in 12 remote primary health services in Australia (n=1486)	Concordance compared to routinely performed NAATs for CT and NG	Submission-ready for peer-reviewed journal

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Chapter 2: A Laboratory-Based Evaluation of Four Rapid Point-of-Care Tests for Syphilis

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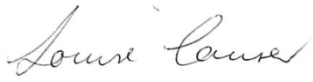

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A Laboratory-Based Evaluation of Four Rapid Point-of-Care Tests for Syphilis

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Abstract

Background: Syphilis point-of-care tests may reduce morbidity and ongoing transmission by increasing the proportion of people rapidly treated. Syphilis stage and co-infection with HIV may influence test performance. We evaluated four commercially available syphilis point-of-care devices in a head-to-head comparison using sera from laboratories in Australia.

Methods: Point-of-care tests were evaluated using sera stored at Sydney and Melbourne laboratories. Sensitivity and specificity were calculated by standard methods, comparing point-of-care results to treponemal immunoassay (IA) reference test results. Additional analyses by clinical syphilis stage, HIV status, and non-treponemal antibody titre were performed. Non-overlapping 95% confidence intervals (CI) were considered statistically significant differences in estimates.

Results: In total 1203 specimens were tested (736 IA-reactive, 467 IA-nonreactive). Point-of-care test sensitivities were: Determine 97.3%(95%CI:95.8–98.3), Onsite 92.5%(90.3–94.3), DPP 89.8%(87.3–91.9) and Bioline 87.8%(85.1–90.0). Specificities were: Determine 96.4%(94.1–97.8), Onsite 92.5%(90.3–94.3), DPP 98.3%(96.5–99.2), and Bioline 98.5%(96.8–99.3). Sensitivity of the Determine test was 100% for primary and 100% for secondary syphilis. The three other tests had reduced sensitivity among primary (80.4–90.2%) compared to secondary syphilis (94.3–98.6%). No significant differences in sensitivity were observed by HIV status. Test sensitivities were significantly higher among high-RPR titre (RPR≥8) (range: 94.6–99.5%) than RPR non-reactive infections (range: 76.3–92.9%).

Conclusions: The Determine test had the highest sensitivity overall. All tests were most sensitive among high-RPR titre infections. Point-of-care tests have a role in syphilis control programs however in developed countries with established laboratory infrastructures, the lower sensitivities of some tests observed in primary syphilis suggest these would need to be supplemented with additional tests among populations where syphilis incidence is high to avoid missing early syphilis cases.

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Introduction

Timely diagnosis and treatment of syphilis is crucial to reduce morbidity, and onward transmission of syphilis to sexual partners and newborns, in whom consequences can be devastating [1]. Congenital syphilis is almost entirely preventable with early identification of maternal infection followed by prompt treatment [2]. Syphilis can also increase susceptibility to acquiring HIV infection and increase transmissibility [3,4]. In resource limited settings with poor access to laboratories or syphilis screening, rapid point-of-care (POC) tests have the potential to increase numbers of people tested, and provide clinical services with the opportunity to offer treatment immediately during the same consultation.

Syphilis testing is recommended in clinical guidelines as part of antenatal screening, for people at high risk, for those with symptoms and following sexual contact with confirmed cases [5–7]. There has been substantial enthusiasm and support for the implementation of POC tests for syphilis in resource limited settings [8–10], with several countries having recently adopted POC tests into their national antenatal screening policies [11]. This approach is supported by evidence from laboratory and field evaluations of syphilis POC tests which demonstrate good performance, acceptability and cost effectiveness [12–18], however very little has been published on the performance of these tests during different clinical stages of infection and among those with HIV co-infection which may influence the host response to syphilis [19].

This paper presents results of a large dual-site, laboratory-based, head-to-head evaluation of performance of four commercially available rapid POC syphilis tests using archived sera that includes analysis by syphilis stage, HIV status and non-treponemal antibody titre.

Methods

Ethical approval

Ethical approval was granted by the South East Sydney and Illawarra Area Health Service Human Research Ethics Committee (HREC), Melbourne Health HREC and University of New South Wales HREC. Consent was not required from individuals as it was a retrospective study of de-identified stored samples that has previously been tested with the goal standard syphilis assays. Linked clinical data were anonymized and de-identified prior to analysis.

Setting

Rates of syphilis in Australia are highest among two distinct populations - gay men living in urban centres and Aboriginal and Torres Strait Islander people living in remote regions across Australia [20]. The incidence of syphilis in gay men with HIV infection is five-times higher than the incidence in MSM without HIV (2.5 vs. 0.5 per 100 person years) [21]. Study sera were selected from two major laboratories in Australia, both conducting a high volume of syphilis testing. These laboratories also are the primary pathology providers for two large, urban sexual health clinics in Sydney and Melbourne from which corresponding patient demographic and clinical information was extracted. These clinics have extensive clinical expertise in STI diagnosis and management and provide sexual health services to a significant proportion of the gay population in these cities.

Study design

We assessed the performance of four syphilis POC tests by comparing results to routinely performed reference treponemal tests (immunoassays [IA]) conducted at the laboratories. Linked clinical data for a subset of specimens were extracted from patient medical records. Inter-technician variability and operational characteristics of the tests were also assessed.

Syphilis POC tests

POC tests were selected to include commercially available tests with traditional as well as novel test platforms: (i) Determine Syphilis TP® (Inverness Medical Japan Co, Ltd, Chiba, Japan); (ii) Onsite Syphilis Ab Combo Rapid Test® (CTK Biotech, San Diego, CA, USA); (iii) SD Bioline Syphilis 3.0® (Standard Diagnostics Inc, Kyonggi-do, Korea); and (iv) DPP Syphilis Screen and Confirm Assay® (Chembio Diagnostic Systems Inc, Bedford, NY, USA). All four tests use an immunochromatographic strip design with the appearance of a visible coloured line if treponemal antibodies are detected in the specimen. The DPP Syphilis test, in addition, simultaneously detects non-treponemal antibodies by a separate coloured line on the same test strip.

Laboratories and serological reference tests

The respective reference treponemal immunoassay (IA) tests used by Victorian Infectious Diseases Reference Laboratory (VIDRL) in Melbourne and South Eastern Area Laboratory Service (SEALS) in Sydney are: (i) Trepanostika TP recombinant enzyme immunoassay® (bioMerieux, Boxtel, The Netherlands) [22] and (ii) Architect Syphilis TP chemiluminescence assay® (Abbott, Wiesbaden, Germany) [23,24]. Both laboratories perform quantitative rapid plasma reagin (RPR) tests using BD Macro – Vue RPR Card Tests® (Becton, Dickinson and Co, MD, USA). In Australia, a "reverse" screening algorithm is used, i.e. treponemal IA followed by a quantitative RPR for IA reactive specimens. Both laboratories participate in an ongoing external quality assurance program (Royal College of Pathologists of Australia Quality Assurance Program).

Reference test results were extracted from the laboratory databases or patient medical records for comparison with POC test results. A specimen from a patient with a previously documented reactive treponemal IA result was not retested and was considered to be IA reactive for the study analyses.

Specimen selection and categorisation

Specimens were selected to include approximately equal numbers of IA reactive and IA non-reactive samples, and a range of RPR titres.

a. Serological categorisation. Specimens were categorised by treponemal IA and by non-treponemal (RPR) reference test results as *high RPR titre syphilis* (IA reactive, RPR≥8); *low RPR titre syphilis* (IA reactive, RPR = 1, 2 or 4); *inactive* syphilis (IA reactive, RPR non-reactive); *no syphilis* (IA and RPR non-reactive); and *biological false positives* (IA non-reactive, RPR ≥2).

b. Clinical categorisation. Demographic (age, gender, gender of sexual partners in last 12 months) and clinical data (HIV status, CD4 count, syphilis stage) were extracted from medical records. Specimens were categorised by clinical stage according to the definitions in Table 1.

POC testing

Technicians performing the POC tests completed training to ensure consistency in the conduct and interpretation of results. Specimens were tested according to the manufacturer's instructions by one study technician blinded to the reference results. The POC test results were interpreted and recorded on a laboratory data record sheet. A second blinded study technician, independently interpreted and recorded the POC test result within 1 minute of the first read. Discrepancies were discussed and a consensus reached and recorded. Reasons for discrepancies were noted. Invalid tests were repeated using a new test and recorded as such.

Operational characteristics

We assessed selected operational characteristics of each test by self-administered questionnaire among the technicians performing the POC tests. Questions focused on ease of test use and interpretation of the results.

Data analysis

The sensitivity and specificity for each syphilis POC test compared to the treponemal IA reference test results was calculated by standard methods and a kappa (k) value was calculated for each test as a summation of the overall performance using Stata (StataCorp. Release 12. College Station, TX). Results were stratified by HIV status and RPR titre (among IA reactive specimens). POC test sensitivity was also compared to clinical stage of syphilis. For the analyses presented here, DPP POC test sensitivity refers the treponemal line result compared to reference IA to allow for consistent comparison across all four POC tests.

To compare performance between POC tests and between subcategories, 95% confidence intervals (CI) were calculated [25] for each estimate (sensitivity and specificity). Statistical significance of a difference in estimates was based on non-overlapping CI.

Table 1. Clinical Syphilis Case Definitions.

Primary syphilis – primary chancre with reactive syphilis serology and/or PCR and/or dark ground positive test(s)
Secondary syphilis – systemic symptoms typical of syphilis with reactive syphilis serology, plus mucocutaneous lesions (which may be dark field microscopy and/or PCR positive)
Early latent syphilis – asymptomatic with reactive syphilis serology (on two occasions) and non-reactive serology results within the last 2 years or, if not previously tested, likely time of infection in the last 2 years
Late or unknown duration latent syphilis – asymptomatic with reactive syphilis serology and no prior testing or prior testing >2 years since the last test
Past treated syphilis – documented adequate treatment for syphilis and has achieved adequate serological and clinical response on follow up

Positive syphilis serology – either reactive immunoassay ± reactive RPR (need two sets of positive results to confirm if asymptomatic if no prior history of syphilis or reactive RPR with a fourfold titre increase if past treated syphilis).
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P-values were calculated using a Chi-squared test for overall test sensitivities only.

Inter-observer variability to determine the discrepancy rate between the two technicians recording results was calculated as the number of test results which differ between the two technicians x 100/total number of tests performed using the same serum specimens.

Results

Sample characteristics

In total, 1203 specimens (678 from Melbourne and 525 from Sydney) were tested with each of the four syphilis POC tests. The median age of the cases was 35 years (range 18–85) and 83.4% were men. Additional patient demographic and clinical information were available for 878 (73%) specimens. About half (50.9%) of the male cases identified as having a least one same sex partner during the 12 months, prior to specimen collection. One hundred and fifty four (12.8%) cases were HIV positive and 67 (5.6%) had a most recent CD4 count of <500 cells/mm^3.

Clinical categorisation

As presented in Table 2, 53 cases (4.4%) had primary syphilis, 70 (5.8%) had secondary syphilis, 91 (5.8%) had early latent syphilis, and 25 (2.1%) had late latent infection or infection of unknown duration; 248 (20.6%) were documented as having past treated syphilis infection; four cases had a clinical diagnosis of syphilis with no stage specified; 387 (32.2%) did not have syphilis nor a history of syphilis infection. Among those with primary syphilis, 64.7% had an RPR titre >1:8 while among those with secondary syphilis, 92.9% had an RPR>1:8.

Serological categorisation

Among the 1203 specimens, 736 (61.2%) were reference IA reactive and 467 (38.5%) were non-reactive. RPR results were available for 1,005 (83.5%) specimens and used to further stratify the specimens as described in Table 2. Among the IA reactive specimens, 404 were high RPR titre (RPR≥1:8) syphilis, 121 were low RPR titre (RPR = 1:1, 2, or 4) syphilis, and 211 were RPR non-reactive (inactive syphilis). Among the IA non-reactive, 242 were RPR nonreactive or RPR≤1 (not syphilis) and 27 had an RPR ≥1:2 (biological false positives).

Sensitivity and specificity

POC sensitivities and specificities overall, stratified by HIV status, CD4 count are shown in Table 3. Determine TP showed the highest sensitivity (97.3%) and the difference was statistically significant to the other three tests ($p<0.001$). POC test specificities ranged from 96.4–98.5% with no significant differences between tests. The kappa statistic for Determine (0.94, 95% CI: 0.92–0.96) was statistically significantly higher than the other three tests which ranged from 0.84–0.88.

By HIV status, there were no statistically significant differences observed in POC test sensitivities. POC test specificities were lower among HIV positive compared to HIV negative specimens; however the difference was only statistically significant for the Determine test (81.5% vs. 97.8%). Though not statistically significant, POC test specificities were lower among those with CD4 count≥500 cells/mm^3 compared to <500 cells/mm^3 across all POC tests.

Table 4 shows sensitivity of syphilis POC tests compared to clinical syphilis stage. Sensitivity of the Determine test was 100% for both primary and secondary syphilis however was lower for each of the other three tests among primary compared to secondary syphilis (none were statistically significant). The Bioline test was however statistically significantly less sensitive compared to the Determine test among primary syphilis cases. No other differences were noted between any of the tests across any other these clinical categories.

Table 5 shows POC test sensitivities among IA reactive specimens stratified by RPR titre. POC test sensitivity was significantly higher among high titre (RPR≥8) syphilis infections (range: 94.6–99.5%) than for RPR non-reactive infections (range: 76.3 - 92.9%), with the Determine test demonstrating superior sensitivity to the other three tests across high and low RPR titres and non-reactive RPR specimens. The Determine test sensitivity was statistically significantly higher compared to the Bioline test in the high and low titre categories and compared to all other tests in the non-reactive RPR category.

Inter-reader variability analysis (discrepancy rate between results recorded by two technicians) revealed a range of discrepancies across the tests: Determine 0.2% (n = 3), Bioline 1.2% (n = 15), DPP 1.5% (n = 18) and Onsite 2.7% (n = 33). Technician's comments indicated that many of these discrepancies occurred when results lines on the tests kits were faint.

Technicians (n = 2) found all the four POC tests were "simple" or "very simple" to conduct. Overall interpretation of the POC result was considered to be "relatively easy" for all POC test devices, however technicians raised concerned regarding interpretation of faint or weak bars. Faint bars were reported to have occurred "sometimes" with all the devices except the Determine, leading to discrepancies in initial results between technician 1 and 2. Technicians noted some problems with the nitrocellulose strip in the Determine test and the cassette casing for the DPP and Onsite devices.

Table 2. Selected demographic and clinical characteristics of specimens tested (n = 1203).

Variable	N (%)
Male	1003 (83.4)
Female	200 (16.6)
Median age in years	35
Age range in years	18–85
Additional clinical Information	
Available	878 (73.0)
Unavailable	325 (27.0)
Among men, same sex partner in last 12 months	
Yes	612 (50.9)
No	125 (10.4)
Unavailable	466 (38.7)
HIV status	
Positive	154 (12.8)
Negative	724 (60.2)
Unavailable	325 (27.0)
CD4 count (cells/mm^3) among HIV positive	
<200	3 (0.2)
200–<500	64 (5.3)
≥500	63 (5.2)
Unknown	24 (2.0)
Unavailable	325 (27.0)
Clinical syphilis diagnosis	
Primary	53 (4.4)
Secondary	70 (5.8)
Early latent	91 (7.6)
Late Latent/unknown duration	25 (2.1)
Past treated	248 (20.6)
Syphilis, no stage specified	4 (0.3)
Not syphilis	387 (32.2)
Unavailable	325 (27.0)
Serological Syphilis Categorisation	
IA reactive/RPR reactive (R≥8)	404 (33.6)
IA reactive/RPR reactive (R = 1:1, 2 or 4)	121 (10.1)
IA reactive/RPR non-reactive	211 (17.5)
IA non-reactive/RPR reactive (R≥2)	27 (2.2)
IA non-reactive/RPR non-reactive (RPR≤1)	242 (20.1)
IA non-reactive/RPR not done*	198 (16.5)

* Syphilis testing in Australia involves screening with a treponemal antibody test, followed by RPR, if positive, to stage disease. 198 specimens with non-reactive IA did not have routinely performed RPR results available.
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Discussion

This paper reports results from a large, dual-site, head-to-head evaluation of performance of four commercially available syphilis POC test devices using stored sera. This is the first such study exploring the impact of syphilis stage and HIV co-infection on POC performance. Our results provide evidence supporting the good performance of treponemal tests currently available, with the Determine test demonstrating overall superior performance and with technicians experiencing the least number of challenges in interpretation of results. Uniquely demonstrated in our study, test sensitivities did not differ by HIV status but did differ by clinical stage and RPR titre.

Though much of the current published literature focuses on the use of these POC tests as part of antenatal screening programs in developing countries, there may be a role for these tests, particularly those able to differentiate potentially active from inactive/past infection, in other settings, including developed countries among populations with high rates of syphilis and HIV co-infection. Our results suggest that such a role be considered in the context of ongoing routine serology to identify false negative results, given the possible poorer sensitivity among primary syphilis of some of these tests.

Overall, the Determine test had the highest sensitivity (97.3%), while all four had similarly very high specificities (97.2–98.5%). The overall kappa statistic for each test, a summation of the overall performance of each test against the reference standard, was highest for Determine TP suggesting very substantial agreement between the POC test and laboratory reference results. These individual test performances are not dissimilar to others reported in the literature [12,15,16,19,26–34] and support their use as screening tools for syphilis, particularly in resource-limited settings where routine laboratory-based screening is limited.

We observed no difference in sensitivity by HIV status, however, we did note a trend towards reduced test specificity among HIV positive compared to negative specimens. Determine was the only test showing a statistically significant lower specificity among HIV positive specimens. This phenomenon has been described elsewhere [29,35] and may be the result of immune activation and subsequent deranged B-cell function in HIV [36] which presumably can lead to false positive tests. This is clearly important in settings such as Europe, USA and Australia where among gay men, up to 60% the syphilis infections are in HIV-positive individuals [37–39], such that approximately 1 in 6 reactive tests could be a false positive in this population. We were unable to detect any differences in performance by CD4 count though specificities appeared to be somewhat lower among those with HIV and CD4 counts ≥500 cells/mm^3. As numbers were small, these results should be interpreted with some caution.

The Determine test was the best performing POC test and was able to identify 100% of specimens in both primary and secondary stage infection. The other three tests appeared to have a lower sensitivity among primary infection, particularly the Bioline test with a sensitivity of only 77.3%. In all of these tests the sensitivity increased in secondary infections but did not reach 100%. These tests would therefore miss a number of primary and secondary infections. In many countries all patients with a genital ulcer are treated for syphilis as identification of early syphilis infections is important to prevent ongoing transmission and is a key STI control strategy.

Although the DPP Screen and Confirm syphilis test has a lower sensitivity compared with the Determine test, it does have the ability to detect both treponemal and non-treponemal antibodies, and therefore help distinguish past, treated infection from possible active infection with some encouraging lab and field results [34,40]. However in this paper, as we wished to compare performance across tests and investigate clinical and serological factors that might affect performance, we have focused on reporting only the sensitivities and specificities of the treponemal line of the DPP test compared to reference IA tests.

The strength of our study was the majority of specimens tested were collected from clients attending large urban sexual health clinics with substantial expertise in syphilis and HIV and providing

Table 3. Syphilis POC test sensitivity and specificity (compared to reference Treponemal immunoassay), overall and by HIV status and CD4 cell count.

			Determine		Onsite		DPP (Trep)		Bioline	
		N	Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec
		(IA+/IA −)*	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
Overall		1203	97.3	96.4	92.5	97.0	89.8	98.3	87.8	98.5
		(736/467)	(95.8–98.3)	(94.1–97.8)	(90.3–94.3)	(94.9–98.3)	(87.3–91.9)	(96.5–99.2)	(85.1–90.0)	(96.8–99.3)
HIV status	Neg	724	95.9	97.8	91.2	97.2	89.8	98.6	86.5	98.9
		(364/360)	(93.1–97.6)	(95.5–99.0)	(87.7–93.8)	(94.8–98.6)	(86.1–92.7)	(96.6–99.5)	(82.5–89.8)	(97.0–99.6)
	Pos	154	96.9	81.5	94.5	85.2	89.8	88.9	87.4	88.9
		(127/27)	(91.6–99.0)	(61.3–93.0)	(88.6–97.6)	(65.4–95.1)	(82.8–94.2)	(69.7–97.1)	(80.1–92.4)	(69.7–97.1)
CD 4 count	<500	67	94.6	91.7	90.9	100.0	89.1	100.0	81.8	100.0
(cells/mm3)		(55/12)	(84.0–98.6)	(59.8–99.6)	(79.3–96.6)	(69.9–100.0)	(77.1–95.5)	(70.0–100.0)	(68.6–90.5)	(69.9–100)
	≥500	63	98.0	76.9	96.0	76.9	88.0	84.6	92.0	84.6
		(50/13)	(88.0–99.9)	(46.0–93.8)	(85.1–99.3)	(46.0–93.8)	(75.0–95.0)	(53.7–97.3)	(79.9–97.4)	(53.7–97.3)

*IA+/IA- = Treponemal immunoassay reactive/Treponemal immunoassay nonreactive.
Sens = sensitivity; Spec = specificity; SYD = Sydney (Architect Syphilis Chemiluminescence IA); MEL = Melbourne (Trepanostika TP recombinant Enzyme IA)
Differences between estimates were considered to be statistically significant where 95% CI were not overlapping.
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for a population at highest risk of syphilis (urban gay men) making our study sample well-suited to evaluate potential differences in POC test sensitivity by clinical stage and HIV status. A smaller proportion of our study population were women and although information regarding pregnancy status was not collected, it is likely that fewer than 1% of these women would have been pregnant in this setting (B.Donovan, personal communication).

There are also a few limitations to consider. The two laboratories providing reference results each used a different treponemal IA. Both are highly sensitive and specific and as relative ranking of POC test performance did not differ by site, we believe the use of two different reference IAs did not bias our results. Our study was conducted in a laboratory environment and the tests were performed by skilled technicians with experience in POC test devices, therefore likely represent a best performance scenario. Though results may differ in a clinical setting, the relative performance of the tests would be expected to remain similar. As these tests are not quantitative, training regarding the interpretation of results required technicians to record a positive result if any band appeared on the test strip at the test line site. It is possible that some faint lines were interpreted by our technicians as positive that may have been called negative by others. Though some evaluations have demonstrated lower test sensitivities using whole blood in field settings [12,16] compared to sera in a laboratory setting, a recent evaluation has contradicted these findings showing similar performance across specimen type [34].

In conclusion, our results support the potential role for these tests to be implemented more broadly supplementing existing

Table 4. Sensitivity of syphilis POC tests compared to clinical syphilis stage.

		Determine	Onsite	DPP (Trep)	Bioline
Clinical stage	N	Sens	Sens	Sens	Sens
		(95% CI)	(95% CI)	(95% CI)	(95% CI)
Primary	53*	100.0	88.7	84.9	77.3
		(91.6–100.0)	(76.3–95.3)	(71.9–92.8)	(63.5–87.3)
Secondary	70	100.0	98.6	94.3	94.3
		(93.5–100.0)	(91.2–99.9)	(85.3–98.2)	(85.3–98.2)
Early latent	91	95.6	93.4	92.3	86.8
		(88.5–98.6)	(85.7–97.3)	(84.3–96.6)	(77.7–92.7)
Late latent/unknown duration	25	96.0	88.0	84.0	84.0
		(77.7–99.8)	(67.7–96.8)	(63.1–94.7)	(63.1–94.7)
Past/treated	248**	93.1	89.9	88.3	84.7
		(89.1–95.8)	(85.3–93.2)	(83.5–91.9)	(79.4–88.8)

Clinical stage = documented in medical records for patient specimens; Sens = sensitivity; *Among Primary syphilis, 2 specimens were IA non-reactive but PCR positive.
** Among past/treated syphilis, 5 specimens were IA non-reactive.
Differences between estimates were considered to be statistically significant where 95% CI were not overlapping.
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Table 5. POC test sensitivity among reference Treponemal immunoassay reactive specimens, by RPR reactivity and titre.

Reference results			Sensitivity (95% Confidence Intervals)			
IA	RPR	N	Determine	Onsite	DPP (Trep line)	Bioline
R	R:≥8	404	99.5	98.3	95.3	94.6
			(98.0–99.9)	(96.3–99.2)	(92.6–97.1)	(91.7–96.5)
R	R:1,2 or 4	121	97.5	93.4	93.4	85.1
			(92.4–99.4)	(87.0–96.9)	(87.0–96.9)	(77.2–90.7)
R	NR	211	92.9	81.0	77.3	76.3
			(88.3–95.8)	(75.0–86.0)	(70.9–82.6)	(69.9–81.8)

Sensitivity of POC test result compared to reference treponemal immunoassays (IA); RPR = reactive plasma reagin; R = reactive; NR = non-reactive
Differences between estimates were considered to be statistically significant where 95% CI were not overlapping.
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antenatal screening strategies in resource limited settings. Their use in developed countries might also be considered in populations with high rates of syphilis and HIV co-infection and where loss to follow-up is a significant risk. This might be either as part of a screening strategy using the Determine test or to distinguish potentially active from past/treated infection using a newer dual platform test. Field evaluations of selected POC tests are needed to determine the local performance in the hands of end users, relevance, acceptability, economic costs and potential impact associated with implementation in each setting.

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Author Contributions

Conceived and designed the experiments: LC JK CF BD DL PR DA AM HW CR RG. Performed the experiments: LC TK DC ID CR DL PR CF. Analyzed the data: LC JK RG HW TK ID DC CR DA. Contributed reagents/materials/analysis tools: PR DL TK CF AM ID DC. Wrote the paper: LC JK CF TK BD DL PR DA AM HW CR ID DC RG.

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Chapter 3: An Evaluation of a Novel Dual Treponemal/Nontreponemal Point-of-Care Test for Syphilis as a Tool to Distinguish Active From Past Treated Infection

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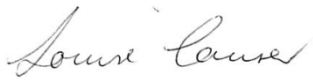

MAJOR ARTICLE

An Evaluation of a Novel Dual Treponemal/Nontreponemal Point-of-Care Test for Syphilis as a Tool to Distinguish Active From Past Treated Infection

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Background. Most syphilis point-of-care (POC) tests detect treponemal antibodies, which persist after successful treatment. Subsequent POC tests are positive, despite no active infection, and can lead to unnecessary treatment. We evaluated a new POC test, incorporating a nontreponemal component, to distinguish active from past infection.

Methods. Sera stored at 2 Australian laboratories were tested with DPP Screen and Confirm Assay. Treponemal and nontreponemal test lines were compared to corresponding conventional treponemal and nontreponemal reference test results: immunoassays and rapid plasma reagin (RPR), respectively, with RPR quantification by endpoint titration. POC test outcome concordance with conventional test results was assessed according to serological and clinical categories.

Results. Among 1005 serum samples tested, DPP treponemal line sensitivity was 89.8% (95% confidence interval [CI], 87.3%–91.9%) and specificity was 99.3% (95% CI, 97.0%–99.9%). DPP nontreponemal line sensitivity was 94.2% (95% CI, 91.8%–96.0%) and specificity was 62.2% (95% CI, 57.5%–66.6%). DPP test outcome (pair of test lines) was concordant with both reference test results for 94.3% of 404 high-titer infections, 90.1% of 121 low-titer infections, 27.5% of 211 past/treated infections, and 78.1% of 242 infections classified as not syphilis. Among 211 past/treated infections, 49.8% were incorrectly identified as active infection and a further 22.8% as not syphilis.

Conclusions. DPP test use would result in identification of >93% of active syphilis infections, whereas just over half of past infections would be diagnosed as past or not syphilis, avoiding unnecessary treatment compared with other POC tests. This may be at the expense of missing some active infections; thus, its potential benefits will depend on the prevalence of past vs active infection in a population.

Keywords. syphilis; point-of-care test; diagnosis.

Timely diagnosis and treatment of syphilis is crucial to minimize morbidity and onward transmission. Of particular concern is maternal syphilis, estimated at 1.36 million cases globally in 2008 [1], which can lead to fetal loss, perinatal death, prematurity, low birth weight, and a range of serious malformations in surviving children [2, 3]. These outcomes are preventable with accurate screening and prompt treatment for maternal infection [4–7], but many pregnant women with syphilis remain undiagnosed, particularly in developing countries. Screening for syphilis is also indicated for population groups that are at increased risk through sexual contact. These groups vary by setting but may include sex workers, men who have sex with men, and sexual contacts of those found to have syphilis.

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Despite the importance of testing [8–10], it is widely recognized that the reliable diagnosis of active syphilis is challenging. Tests detecting treponemal antibodies are specific for syphilis (outside areas with endemic yaws or other treponemal infections) but are positive for life following infection in most cases, even after successful treatment. Tests for "nontreponemal" antibodies, such as RPR (rapid plasma reagin) and TRUST (toluidine red unheated serum test), are better indicators of active infection and can provide a quantitative result but, if used alone, can yield false-positive results due to cross-reactions with other antigens. They are also subject to false negativity in active syphilis because of biological fluctuations. Moreover, their accuracy and safe use depend on levels of operator skill and experience that are not insubstantial.

The recent development of easy-to-use, accurate, rapid point-of-care (POC) treponemal tests can transform syphilis control strategies. In endemic situations, these tests are now recommended for antenatal screening [11], even though it is understood that a proportion of women detected as positive and offered treatment will have past adequately treated syphilis rather than active infection. In addition to unnecessary treatment, the diagnosis of an infection that is primarily sexually transmitted may lead to psychosocial trauma including stigma and partner violence. In many settings, it is already challenging for women to deal with the consequences of such a true diagnosis, and false-positive test results can compound this burden further. There is therefore an urgent need for a syphilis POC test that can make the distinction between active and past treated infection.

The DPP Syphilis Screen and Confirm Assay is a commercially available POC test for syphilis that simultaneously detects treponemal and nontreponemal antibodies from the same specimen. Published evaluations suggest that the treponemal and nontreponemal test components have good sensitivity and specificity compared with selected treponemal and nontreponemal laboratory reference tests [12–14]; however, no studies have quantified the test's ability to reduce the extent of false-positive diagnoses in past treated syphilis. We recently reported a laboratory-based evaluation of 4 syphilis POC tests [15], including the DPP assay, in a side-by-side comparison of the tests' treponemal components using a well-characterized panel of stored serum specimens. Here we report specifically on the unique dual detection capability of the DPP test.

METHODS

Study Design

We used a cross-sectional design to assess the performance of the DPP test using stored sera. DPP test results were compared to the results of routinely performed conventional laboratory reference treponemal and nontreponemal tests. Routinely recorded clinical data were extracted from patient medical records and used to characterize sera in regard to demographic and clinical factors.

Setting

Sera were selected from 2 major laboratories in Australia's 2 largest cities, the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Melbourne and South Eastern Area Laboratory Service (SEALS) in Sydney. These laboratories are the primary pathology providers for large, urban sexual health clinics that have extensive clinical expertise in sexually transmitted infection diagnosis and management and provide health services to substantial numbers of gay men. In both cities, very high rates of syphilis have recently been reported among gay men [16], particularly those with human immunodeficiency virus (HIV) [17].

DPP Screen and Confirm Assay

The DPP Syphilis Screen and Confirm Assay (Chembio Diagnostic Systems Inc, Bedford, New York) is designed as a POC test. It uses an immunochromatographic strip platform for simultaneous detection of treponemal and nontreponemal antibodies indicated by separate colored lines (T1 and T2), with a separate control line, C. It is can be used with serum, plasma or whole blood (including fingerprick specimens [13]) without requiring refrigeration. Visual interpretation is made at 10–15 minutes.

Laboratories and Serological Reference Tests

VIDRL and SEALS routinely conduct reference treponemal testing using the Trepanostika TP recombinant enzyme immunoassay (bioMérieux, Boxtel, the Netherlands) and Architect Syphilis TP chemiluminescence assay (Abbott, Wiesbaden, Germany), respectively. Both assays are highly sensitive and specific [18, 19]. In this paper, these treponemal reference tests are collectively referred to as immunoassays (IA). Both laboratories also use TPPA (*Treponema pallidum* particle agglutination) to confirm the reference treponemal IA. In both laboratories, the nontreponemal reference test was a quantitative RPR assay using BD Macro–Vue RPR Card Tests (Becton, Dickinson and Co). Following Australian guidelines, a "reverse" screening algorithm is used, with the treponemal test conducted first, followed by a quantitative nontreponemal test for reactive IA specimens [20]. Both laboratories participate in an ongoing external quality assurance program (Royal College of Pathologists of Australia Quality Assurance Program).

Clinical and Laboratory Data

For each specimen, information on demographic, behavioral, and biological characteristics were obtained from the laboratory and clinical records (for those attending Melbourne and Sydney Sexual Health Centres).

Specimen Categorization

Serological

Specimens were categorized by treponemal (IA) and by nontreponemal (RPR) reference test results as active high RPR titer syphilis (IA reactive, RPR ≥8); active low RPR titer syphilis (IA reactive, RPR of 1, 2, or 4); past syphilis (IA reactive, RPR nonreactive); no syphilis (IA and RPR nonreactive/RPR ≤1); and biological false positives (IA nonreactive, RPR ≥2). As specimens from a person with a previously documented reactive treponemal reference test go directly for RPR testing, these were considered IA reactive for the study analyses.

Clinical

Using methods previously described [15], specimens were categorized into clinical stages (primary syphilis, secondary syphilis, early latent syphilis, late latent or unknown duration syphilis, past treated syphilis) based on clinicians' diagnosis documented in the corresponding medical records and according to the definitions in Supplementary Text Box 1.

Sample Size and Specimen Selection

We selected approximately 1000 specimens, aiming for adequate numbers in each serological category to ensure that the estimate for sensitivity and specificity would have a 95% confidence interval (CI) of ±10% or less. Stored specimens were sequentially selected by date until the desired quota was reached.

Conduct of the DPP Test

Technicians performing the DPP test completed training to ensure consistency in test conduct and interpretation. Specimens were tested according to the manufacturer's instructions by 1 study technician blinded to the reference results. DPP test results were recorded on a data record sheet. A second study technician, blinded to both the reference test and first technician's results, interpreted and recorded the test result within 1 minute of the first read. Discrepancies were discussed and consensus recorded.

Data Analysis

The sensitivity and specificity of the individual DPP test lines (T1 and T2) compared to corresponding reference test results (IA and RPR), with quantitation by RPR endpoint titration, were calculated by standard methods (Stata software, version 12; StataCorp, College Station, Texas).

The DPP test outcome (pair of DPP test lines) was compared with dual reference test results categorized (*i*) by serological status and (*ii*) within clinical categories, to determine concordance. CIs were calculated [21] for estimates (sensitivity and specificity) with statistical significance of a difference between categories based on nonoverlapping CI.

Clinical consequence of using the DPP test—that is, the potential reduction in overdiagnosis of syphilis—was assessed among serum specimens determined to be "past syphilis" based on the reference test results. Among these, we calculated the proportion yielding a reactive T1 and nonreactive T2 result on the DPP test.

Ethical Approval

Ethical approvals were granted by relevant committees: South East Sydney and Illawarra Area Health Service, Melbourne Health,

Table 1. Selected Characteristics of Study Population (N = 1005)

Variable	No. (%)
Sex	
Male	852 (84.8)
Female	153 (15.2)
Age, y	
Median	37
Range	18–85
Additional clinical information	
Available	681 (67.8)
Unavailable	324 (32.2)
Among men, same sex partner in last 12 mo	
Yes	504 (50.1)
No	83 (8.3)
Unknown	94 (9.4)
Unavailable	324 (32.2)
HIV status	
Positive	139 (13.8)
Negative	542 (53.9)
Unavailable	324 (32.2)
CD4 count, cells/μL, among HIV positive	
<200	2 (0.2)
200–499	59 (5.9)
≥500	55 (5.5)
Unknown	23 (2.3)
Unavailable	866 (86.2)
Clinical syphilis diagnosis[a]	
Primary	53 (5.3)
Secondary	70 (7.0)
Early latent	91 (9.1)
Late latent/unknown duration	25 (2.5)
Past treated	248 (24.7)
Syphilis, no stage specified	4 (0.4)
Not syphilis	190 (18.9)
Unavailable	324 (32.2)
Serological syphilis categorization	
IA reactive/RPR reactive (R ≥8)	404 (40.2)
IA reactive/RPR reactive (R = 1:1, 2, or 4)	121 (12.0)
IA reactive/RPR nonreactive	211 (21.0)
IA nonreactive/RPR reactive (R ≥2)	27 (2.7)
IA nonreactive/RPR nonreactive (RPR ≤1)	242 (24.1)

Abbreviations: HIV, human immunodeficiency virus; IA, immunoassay; R, reactive; RPR, rapid plasma reagin.

[a] As documented in medical records for patient specimens.

Melbourne Sexual Health Centre Alfred Health and University of New South Wales prior to commencing of the evaluation.

RESULTS

Sample Characteristics

A total of 1005 specimens (678 from Melbourne and 327 from Sydney), characterized by treponemal and nontreponemal reference tests, were tested using the DPP assay. The median age of the cases was 37 years (range, 18–85 years), and 852 (84.8%) were men. Additional patient demographic and clinical data were available for 681 (67.8%) specimens. Table 1 describes the selected demographic, clinical, and laboratory characteristics of specimens tested.

Clinical Syphilis Categorization

Clinical and laboratory diagnoses of syphilis were documented for 491 cases, and 190 were documented as not having syphilis. On the basis of the records, 53 cases were classified as primary (5.3%), 70 (7.0%) secondary, 91 (9.1%) early latent, 25 (2.5%) late latent or unknown duration, 248 (24.7%) past/treated infections, and 4 (0.4%) as syphilis stage unspecified.

Serological Syphilis Classification

Of the 1005 sera tested, 525 (52.2%) were classified as having active syphilis infection, 211 (21.0%) were classified with past syphilis infection, and 27 (2.7%) were biological false positives. The remaining 242 (24.1%) were classified as not syphilis.

Stratifying active syphilis specimens by RPR titer, there were 404 specimens with high-titer and 212 with low-titer syphilis.

Sensitivity and Specificity of Individual T1 and T2 Lines

Table 2 describes the sensitivity and specificity of the 2 DPP test lines individually compared with the corresponding reference tests. Sensitivity of T1 compared to reference IA was 89.8% (95% CI, 87.3%–91.9%) and specificity was 99.3% (95% CI, 97.0%–99.9%). Sensitivity of T2 compared to reference RPR (reactive = RPR ≥1) was 94.2% (95% CI, 91.8%–96.0%) and specificity 62.2% (95% CI, 57.5%–66.6%).

Among specimens with a reactive RPR (n = 553) stratified by category of RPR titer, T2 sensitivity was 98.3% (95% CI, 96.3%–99.2%) and 83.2% (95% CI, 76.2%–88.8%) at high and low titers, respectively. These were significantly different as indicated by nonoverlapping CIs. Restricting the analysis to IA reactive specimens (n = 525) only, sensitivity among low-titer specimens was 95% (95% CI, 98.1%–98.0%) and no longer significantly different from that of high-titer specimens (98.3% [95% CI, 96.3%–99.2%]). Figure 1 shows distribution of RPR titers among treponemal (IA) reference test–reactive specimens (n = 525) and DPP test T2 reactivity by RPR titer.

There was no statistically significant difference in performance of T1 or T2 by HIV status (Supplementary Table 1).

Concordance of DPP Test Outcome by Serological Syphilis Category

The DPP test outcome was concordant for 93.3% of 525 (correctly identified) specimens classified as active infections. Table 3 describes the concordance of DPP test outcomes with the 2 reference test results defined by serological category.

The DPP outcome was concordant with both treponemal and nontreponemal reference test results (correctly identified) for 94.3% of 404 high-titer infections, 90.1% of 121 low-titer infections, 27.5% of 211 past infections, and 78.1% of 242 classified as no syphilis. Among those with no syphilis but reactive DPP test lines (n = 53), 51 had reactive T2 line only, 1 had reactive T1 line only, and 1 had reactive T1 and T2 lines.

As T2 is not a quantitative result, concordance within the high- and low-titer categories is based only on reactive or nonreactive T2 result within the category.

Among 211 past infections, 49.8% were incorrectly identified as active infection and an additional 22.8% incorrectly as not syphilis. Table 4 describes further the concordance of DPP outcome among those with past infection by reference tests.

Concordance of DPP Test Outcome by Clinical Syphilis Category

Table 5 presents concordance of the DPP test outcome with both reference test results by clinical syphilis category. DPP test outcome is concordant with both reference tests for 88.6% of 44 primary syphilis cases, 90.0% of 69 secondary

Table 2. DPP T1 (Treponemal) Line Performance Versus Immunoassay (Treponemal) Reference Result and DPP (Nontreponemal) T2 Line Performance Versus Rapid Plasma Reagin (Nontreponemal) Reference Test Result (N = 1005)

DPP Test Line	DPP Reactive	Reference Laboratory Test Reactive	Sensitivity, % (95% CI)	DPP Nonreactive	Reference Nonreactive	Specificity, % (95% CI)
DPP T1[a]	661	736	89.8 (87.3–91.9)	267	269	99.3 (97.0–99.9)
DPP T2[b]	521	553	94.2 (91.8–96.0)	281	452	62.2 (57.5–66.6)

Abbreviation: CI, confidence interval.

[a] Compared to reference immunoassay result.

[b] Compared to reference rapid plasma reagin (RPR) result; for T2 sensitivity calculation, a reactive RPR was R ≥1; for specificity calculation, RPR was nonreactive.

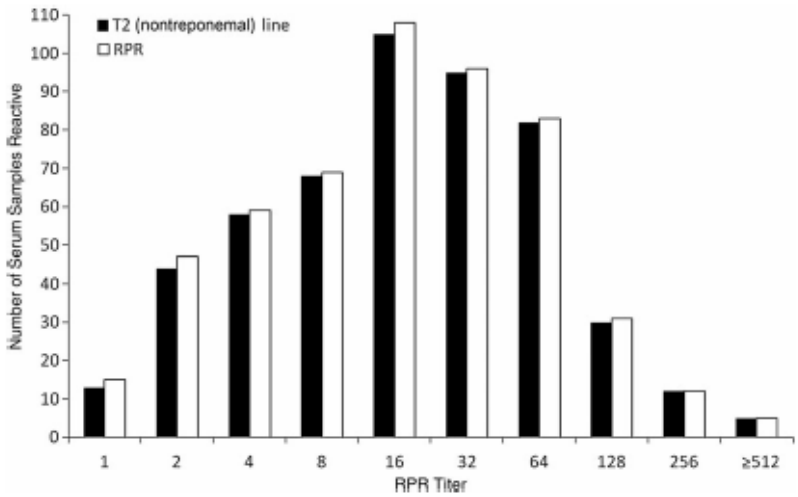

Figure 1. Distribution of rapid plasma reagin (RPR) titers among RPR (nontreponemal) reactive and immunoassay (treponemal) reactive reference test specimens (n = 525) and DPP T2 test line reactivity.

syphilis cases, 94.8% of 77 early latent syphilis cases, and 85.0% of 20 late latent syphilis cases with dual reactive reference tests.

Clinical Diagnostic and Treatment Consequences Based on DPP Test Outcome

Among those with past syphilis (n = 211), 50.2% (n = 106) would be correctly attributed to this category by the DPP test outcome whereas 49.8% (n = 105) would be incorrectly classified as having active syphilis. Assuming that a highly sensitive treponemal-only POC test would have detected all of these cases as positive, the use of the DPP test would have successfully categorized 50.2% of them as not active syphilis.

Among those with active syphilis (n = 525), 93.3% (n = 490) would be correctly diagnosed, with 35 being incorrectly classified. In this instance, assuming as above that a highly sensitive treponemal-only POC test would have detected all of these cases as reactive, the use of the DPP test would have incorrectly classified 35 cases (which subsequently would have received no treatment).

DISCUSSION

Our laboratory-based study is the first to evaluate clinical implications and performance of the DPP Screen and Confirm Assay, the only currently available POC test for detection of both treponemal and nontreponemal antibodies, in regard to its ability to distinguish active from past syphilis. We found that the DPP test was able to correctly identify 93% of active syphilis infections, 28% of past syphilis infections, and 78% nonsyphilis infections.

Table 3. Concordance of DPP Outcome With Treponemal and Nontreponemal Reference Test Results by Serological Syphilis Category

	Reference Test Result			DPP Result				
Serological Syphilis Category	IA	RPR	No.	T1	T2	No.	% Concordance	95% CI
Active (overall)	R	R	525	R	R	490	93.3	90.8–95.2
Active (high titer)	R	≥8	404	R	R	381	94.3	91.6–96.4
Active (low titer)	R	<8	121	R	R	109	90.1	83.3–94.7
Past	R	NR	211	R	NR	58	27.5	21.6–34.0
No syphilis	NR	NR/≤1	242	NR	NR	189	78.4	72.3–83.1
Biological false positive	NR	≥2	27	NR	R	8	29.6	13.7–50.2

Abbreviations: CI, confidence interval; IA, reference immunoassay (treponemal) result; NR, nonreactive; R, reactive; RPR, reference rapid plasma reagin (nontreponemal) result (reactive = R ≥1); T1, DPP treponemal line; T2, DPP nontreponemal line.

Table 4. Concordance of DPP Test Outcome Among Past Serological Syphilis Category

DPP			Past Syphilis (n = 211)[a]	
T1	T2	No.	Concordance, %	95% CI
R	NR	58	27.5	21.6–34.0
R	R	105	49.8	42.8–56.7
NR	R	15	7.1	4.0–11.4
NR	NR	33	15.6	11.0–21.3

Abbreviations: CI, confidence interval; NR, nonreactive; R, reactive; T1, DPP treponemal line; T2, DPP nontreponemal line.

[a] Past syphilis category defined by serological reference tests as immunoassay (treponemal) reactive and rapid plasma reagin (nontreponemal) nonreactive.

Among those past syphilis infections, compared to a highly sensitive treponemal-only POC as the next best alternative in POC testing, the use of the DPP test would have successfully detected 50.2% as not active syphilis.

Serological testing to detect treponemal and nontreponemal antibodies is critical to determine the infection status of an individual and need for treatment. Treatment is usually indicated when both these antibodies are detected, indicating active infection. Except in very early primary syphilis, presence of treponemal antibody in the absence of nontreponemal antibody suggests past treated infection. Among populations with high prevalence of syphilis infection, this may account for a large proportion of the population. In addition, infections with other *T. pallidum* subspecies causing illnesses such as yaws and bejel (endemic syphilis) can produce an indistinguishable antibody profile, complicating diagnosis of syphilis further in regions where these infections are also endemic.

Table 5. Concordance of DPP Outcome With Reference Test Results by Clinical Syphilis Category

Clinical Syphilis Category (Total)	Reference Test			DPP Test			DPP Test Outcome Concordance With Reference Tests, %
	IA	RPR	No.	T1	T2	No.	
Primary (n = 53)[a]	R	≥1	44	R	R	39	88.6
	R	NR	7	R	NR	1	14.3
	NR	NR	2	NR	NR	1	50.0
Secondary (n = 70)	R	≥1	69	R	R	62	90
	R	NR	1	R	NR	0	0.0
Early (n = 91)	R	≥1	77	R	R	73	94.8
	R	NR	14	R	NR	2	14.3
Late (n = 25)	R	≥1	20	R	R	17	85.0
	R	NR	5	R	NR	0	0.0

Clinical stage = documented in medical records for patient specimens.

Abbreviations: IA, reference immunoassay result; NR, nonreactive; R, reactive; RPR, reference rapid plasma reagin result (reactive = R ≥1); T1, DPP treponemal line; T2, DPP nontreponemal line.

[a] Among "primary" syphilis, 2 specimens were IA nonreactive but polymerase chain reaction positive.

POC tests capable of detecting treponemal antibodies alone are widely available and have been shown to perform well both in the field and laboratory settings compared to standard reference treponemal assays [15, 22–26]. POC syphilis tests have also been shown to be cost-effective in some settings [27–29]. In a number of countries, POC syphilis tests have been adopted as national policy, particularly as part of antenatal screening programs aiming to eliminate the mother-to-child transmission of syphilis [30]. As our study population included few women, with very few likely to be pregnant (B. Donovan, personal communication), the generalizability of our results to such screening programs may be limited.

The major limitation of these more widely used POC syphilis tests remains their inability to distinguish active, infectious syphilis from past treated infection. As a result, overdiagnosis and subsequent unnecessary treatment inevitably occur. This situation is likely to be of most concern among populations where syphilis (or other treponemal infection resulting in presence of treponemal antibodies such as yaws) is prevalent. Furthermore, the psychosocial ramifications of a "positive" syphilis test are often far-reaching and worthy of consideration.

As we have previously reported [15], the sensitivity of the individual DPP treponemal line compared to the conventional laboratory reference treponemal test was lower (89.8%) than that previously published [12–14], but specificity (99.3%) was similar.

In our study, the DPP nontreponemal line sensitivity compared to a reactive nontreponemal laboratory test (RPR ≥1:1) was 94.2% (95% CI, 91.8%–96.0%). The increased sensitivity among high-titer specimens (98.3% [95% CI, 96.3–99.2]) is consistent with findings reported elsewhere [12, 13, 31]. Among those with active syphilis infection, T2 sensitivity among low-titer RPR specimens improved to 95.0% (95% CI, 89.1%–98.0%). In our study, T2 specificity compared to a nonreactive reference nontreponemal test was poor (62.2%), and is in contrast to specificities noted by Castro et al (98.6%) [12], Yin et al (95%) [13], and Ayoye et al (92.5%) [31]. There was no statistically significant difference in T2 specificity by HIV status, although the number of HIV-infected participants was limited. Despite standardized training on performing these tests, this poor specificity might be explained by technicians being overly cautious and recording even very faint shadows at the nontreponemal line region as reactive. Further exploration of this finding by stratifying DPP nontreponemal line specificity by reference treponemal test reactivity suggested that specificity was lower among treponemal reactive specimens (43.1%) vs nonreactive specimens (78.8%).

To our knowledge, we are the first to report the clinical implications of the DPP test's ability to distinguish active from past

syphilis. Among those with serologically determined past syphilis, the DPP test outcome correctly identified more than one-quarter (27.5%) of cases. In a clinical setting, these cases would not receive treatment. The incorrectly identified cases here could be considered as biological false positives (7.1%), no syphilis (15.6%), and misdiagnosed active infection (49.8%). Of these, only those called active infection would be offered treatment, and represent the ongoing "overdiagnosis" and "overtreatment" occurring with the use of a POC test.

Our conclusion that the use of the DPP test would successfully detect 50.2% of past cases as not active syphilis is likely to be true only if patients who do have lesions of early primary syphilis are excluded. This is explained by the fact that a number of patients with polymerase chain reaction–positive early primary lesions and some treponemal antibody marker positivity have negative nontreponemal tests (up to 20% at VIDRL; D. Leslie, personal communication). In light of this, the DPP test may have a different potential under screening and clinical presentation with a genital ulcer.

Although it appears that most active infections would be detected (>93%), our previously published data show that the sensitivity of the DPP treponemal line is lower than that of some other treponemal-only syphilis POC tests, such as Determine TP [15]. In considering the use of this novel dual test, although there is evidence that it can improve identification of past cases and avoid unnecessary treatment, it is important to note that this may come at the expense of a somewhat reduced treponemal sensitivity, resulting in missing cases that may truly require treatment. The balance between possible reduction in overtreatment offered by the DPP test and loss of sensitivity is important to consider in the context of the population at risk. Unless there is a substantial prevalence of past/treated infection (or yaws), a treponemal-only POC test may be preferred or, alternatively, a treponemal-only test might be considered as first line, followed by the dual test to minimize overdiagnosis and subsequent treatment. Another setting in which the DPP test may prove useful is that of yaws eradication and monitoring programs, which usually occur in regions where standard laboratory methods are highly impractical.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Chapter 4: A randomised trial of point-of-care tests for chlamydia and gonorrhoea infections in remote Aboriginal communities: Test, Treat ANd GO- the "TTANGO" trial protocol

This chapter provides context relating to the 'Test, Treat ANd GO' (TTANGO) trial, and sets the scene for the subsequent publications presented in Chapters 5 and 6.

The peer-review publication contained in this chapter is not counted as one of the publications contributing to this thesis, as I was fourth author.

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BMC Infectious Diseases

STUDY PROTOCOL Open Access

A randomised trial of point-of-care tests for chlamydia and gonorrhoea infections in remote Aboriginal communities: Test, Treat ANd GO- the "TTANGO" trial protocol

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Abstract

Background: High prevalence rates of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) have been reported in Aboriginal people in remote and regional areas of Australia for well over two decades, and repeat positivity rates are high. To interrupt disease transmission and reduce the risk of complications, early diagnosis and treatment is important. However in many remote and regional areas there are long delays between testing for these curable sexually transmissible infections and providing treatment, due to both physical distance from laboratories and difficulties when recalling patients for subsequent management once results are available. Point-of-care (POC) tests have the potential to provide more timely diagnosis, to increase treatment and contact tracing, and in turn reduce CT and NG infection rates.

Methods/design: TTANGO (Test, Treat, ANd GO) is a cross-over cluster randomised controlled trial in 12 regional or remote Australian health services, which predominantly provide clinical services to Aboriginal people. The overall aim of TTANGO is to measure the clinical effectiveness, cost-effectiveness and cultural and operational acceptability of molecular POC testing for CT and NG infection. The primary outcome is repeat positivity at three months after treatment of an initial CT or NG infection.

Participating health services will undertake the clinical management of CT and NG under two different modalities for one year each. In the first year, six health services will be randomly assigned to manage these infections under current diagnostic guidelines. The other six will supplement current diagnostic guidelines with POC testing, whereby diagnosis is made and subsequent treatment for those with positive POC tests is offered at the initial consultation. In the second year, the health services will cross over to the opposite management modality.

TTANGO will be conducted over four years; 1.5 years of trial initiation and community consultation, 2 years of trial conditions and evaluation, and 6 months of data analysis and feedback.

Discussion: TTANGO is the first cluster randomised trial of POC testing for CT and NG internationally. The results of this trial will provide crucial information to guide sexual health clinical practice in remote Aboriginal communities and other high prevalence settings.

Trial registration: Australian and New Zealand Clinical Trials Registry ACTRN12613000808741

Keywords: Point-of-care testing, Sexually transmitted infections, Randomized controlled trial

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Background

Chlamydia trachomatis (CT) and *Neisseria gonorrhoea* (NG) are sexually transmissible infections (STIs) that are fully curable with single dose treatment but are often asymptomatic for long periods of time [1]. Both infections can lead to serious complications [1] including pelvic inflammatory disease (PID) [2], ectopic pregnancy and tubal factor infertility [3,4], and a range of adverse pregnancy and neonatal outcomes [5,6]. The risk of PID increases by 4–6 fold in women with repeated CT infections [7].

Along with several other STIs, CT and NG have been reported at highly elevated rates in Aboriginal and Torres Strait Islander peoples (hereafter referred to as 'Aboriginal') in Australia. According to the most recent national data, CT and NG were 3.6 and 30.6 times [8] more likely to be diagnosed in Aboriginal people, respectively, compared to the non-Indigenous population. As in many areas of health, the disadvantage experienced by Aboriginal people is amplified in regional and remote communities, where more Aboriginal Australian's reside. Aboriginal people comprise approximately 2.5% of the total Australian population, yet 25% live in remote or very remote areas compared with < 2% of non-Indigenous people [9]. In 2011, CT and NG were diagnosed 8.3 and 61 times more often among Aboriginal people in remote areas, respectively, compared to non-Indigenous people in remote areas, and diagnoses rates were highest in young people aged 16–19 years [8]. Marked differences in rates of diagnosis among Aboriginal people are also evident according to area of residence; in 2011 CT and NG were diagnoses were 2.5 and 11 times higher in remote areas, respectively, compared to Aboriginal people in major cities [8]. Although the complications of CT and NG are not systematically monitored in Australia, clinical reports from remote Aboriginal communities in Central and Northern Australia indicate that PID and infertility occur at rates far higher than in other parts of Australia [10-12].

In regional and remote areas, primary health services play an important role in the prevention, testing, treatment and management of CT and NG. These health services are either State or Territory government funded or Federal government funded Aboriginal Community Controlled Health Services (ACCHS) and are the primary health care provider for Aboriginal people. There are an estimated 150 ACCHS located across Australia. Current clinical practice in most remote Aboriginal communities [13-15] follows guidelines which provide for immediate treatment if NG or CT are suspected on the basis of specified symptoms or risk [16]. Although symptom-driven diagnosis has the advantage of providing a rapid pathway to treatment, it has poor sensitivity and specificity for detecting infection, particularly in women [17,18]. Used as a diagnostic strategy on its own it therefore leaves many infections untreated, and results in unnecessary treatment in others.

As CT infections are mainly asymptomatic, and NG infections are mainly asymptomatic in women and in about 50% of men [19-21], treatment and contact tracing is usually initiated once a specimen has been tested and found positive by the laboratory. Treatment for these infections is almost inevitably delayed due to a range of factors including the time taken to transport the specimen from remote health services to laboratories [22], and delays in recalling patients when a positive diagnosis occurs. Some health services are more than 1000 kilometers away from the laboratory and pathology is sent on a weekly basis by small plane. A recent review of STI programs in remote Aboriginal communities showed that in a 12 month period only 75-89% of people diagnosed with an STI were treated, and the average time from diagnosis to treatment in asymptomatic people was 21 days [23] , far longer than the 2–3 day interval experienced in most urban settings [24].

In order to interrupt disease transmission, it is essential to diagnose and provide treatment as early as possible to reduce the average duration of infection among those with CT and NG [25]. Without treatment, infection with CT and NG can persist for on average 12 months and 6 months, respectively [26-28]. Timely diagnosis also reduces the risk of sequelae. A number of studies have found that within just a few weeks between testing and treatment, 2-3% of patients with CT infection have already developed PID [29,30]. Timely diagnosis also enables potentially infected sex partners to be contacted and treated (contact tracing), and in turn reducing the risk of re-infection in the index case which often occurs due to subsequent sex with an untreated existing partner [31]. Repeat chlamydial infections following treatment are also common. In a prospective cohort of young women in urban Australia, the repeat CT positivity rate was found to be 22.3% by 12 months [32]. Data based on routine re-testing remote Australian Aboriginal communities found the CT and NG repeat positivity rates was 50% within 12 months [33].

The use of point-of-care (POC) testing in the Australian Indigenous health sector is well established for chronic diseases (diabetes and renal diseases) through the national 'QAAMS' (Quality Assurance for Aboriginal and Torres Strait Islander Medical Services) Program, which currently operates in over 170 Aboriginal medical services nationally [34,35]. POC tests for CT and NG would be the first use of infectious disease POC tests in this sector and could enable clinical services to offer treatment and begin the process of contact tracing at the initial consultation. However to date, use of CT and NG POC tests has been limited as only lateral flow devices have been commercially available with

poor sensitivity and specificity [36,37]. Recently, molecular CT and NG POC tests have been developed, which have very high sensitivity and specificity of >95%, providing an opportunity to utilize these tests to control infections rates in high prevalence settings [38]. Mathematical modeling by Hui *et al.* [39] has shown that CT/NG POC tests with 95% sensitivity could reduce the prevalence of CT in remote Aboriginal communities from 11.9% to 8.9% and NG from 7.1% to 5.7% and if the annual STI screening coverage is increased from 44% (estimated current rates) to 60% the prevalence of the infections could drop further to 3.1% and 1.8% for CT and NG, respectively.

The overall aim of the TTANGO trial is to measure the clinical effectiveness, cost-effectiveness and cultural and operational acceptability of POC testing for CT and NG infections in remote Aboriginal communities. The primary objective is:

1. To determine whether the addition of POC testing to standard diagnostic procedures significantly decreases rates of repeat positive infections at three months among people with CT or NG infection in remote communities.

The secondary objectives are to determine:

1. Whether the addition of POC testing to standard diagnostic procedures significantly increases the proportion of CT and NG infections treated within seven days of specimen collection;
2. The cultural acceptability of POC tests to patients.
3. The operational acceptability to health service staff in remote community settings.
4. The cost effectiveness of the addition of POC testing to standard diagnostic procedures in remote primary health care services.
5. The diagnostic performance characteristics (sensitivity, specificity and positive and negative predictive value) of the POC test in a field setting compared to conventional laboratory testing.
6. A best practice model for quality management of STI/POC testing in remote health services.

Methods

Study design

TTANGO is a crossover cluster randomised controlled trial set in 12 health services in regional or remote areas of Queensland, West Australia and South Australia (Figure 1: Study overview). Participating health services will undertake the clinical management of CT and NG under two different parallel modalities for one year each, in a randomly assigned order. In the first year, six of the health services will be randomly assigned to manage these infections under current diagnostic guidelines ("standard practice" phase or "control" phase). The other six will supplement current diagnostic guidelines with POC testing ("POC" phase), such that diagnosis is made and subsequent treatment for those with positive POC tests is offered at the time of initial consultation. In the second year, the health services will cross over to the opposite management modality. The study will require four years to complete, including 1.5 years preparation and community consultation, 2 years for the intervention and evaluation, and 6 months for analysis and reporting. The fundamental premise of the trial is that more timely treatment for STIs (both index case and partners), will result in a substantial reduction in the percentage of people with repeat positive CT and NG infections.

A randomised trial is required because it will provide the most compelling evidence to guide future use of a novel technology which is currently not routinely used for STI management. The cross-over design allows each health service to act as its own control, thereby eliminating the effect of between-health service variation in the study endpoints. Furthermore, health services are more inclined to participate in a trial of an innovative approach to service delivery if they are guaranteed to receive the intervention during the course of the trial. Individual patient randomisation would not be feasible as the POC tests would then be used for some patients but not others within the same health service, potentially causing disruption and confusion for health service staff and patients.

CT and NG are the focus of the trial, because they occur at very high rates in remote Aboriginal communities and are easily detected and curable with single dose treatment, making them good candidates for strategies which aim to improve detection and treatment of infections with antibiotics. Although syphilis is also curable and has been endemic in some remote communities, rates have decreased considerably over the past decade and is now much rarer than the other STIs [8]; also at the time of the trial design syphilis point-of-care tests were unable to differentiate recent from past syphilis infection [40]. Despite its focus on CT and NG, TTANGO encourages best practice for the detection and treatment of all STIs.

Ethics and informed consent

The TTANGO study protocol has been approved by five ethics committees from participating jurisdictions: the Western Australian Aboriginal Health Information Ethics Committee, the Western Australian Country Health Service Board Research Ethics Committee, the Cairns and Hinterland Health Service Human Research Ethics Committee, the Townsville Health Service District Human Research Ethics Committee and the Aboriginal Health Research Ethics Committee of South Australia.

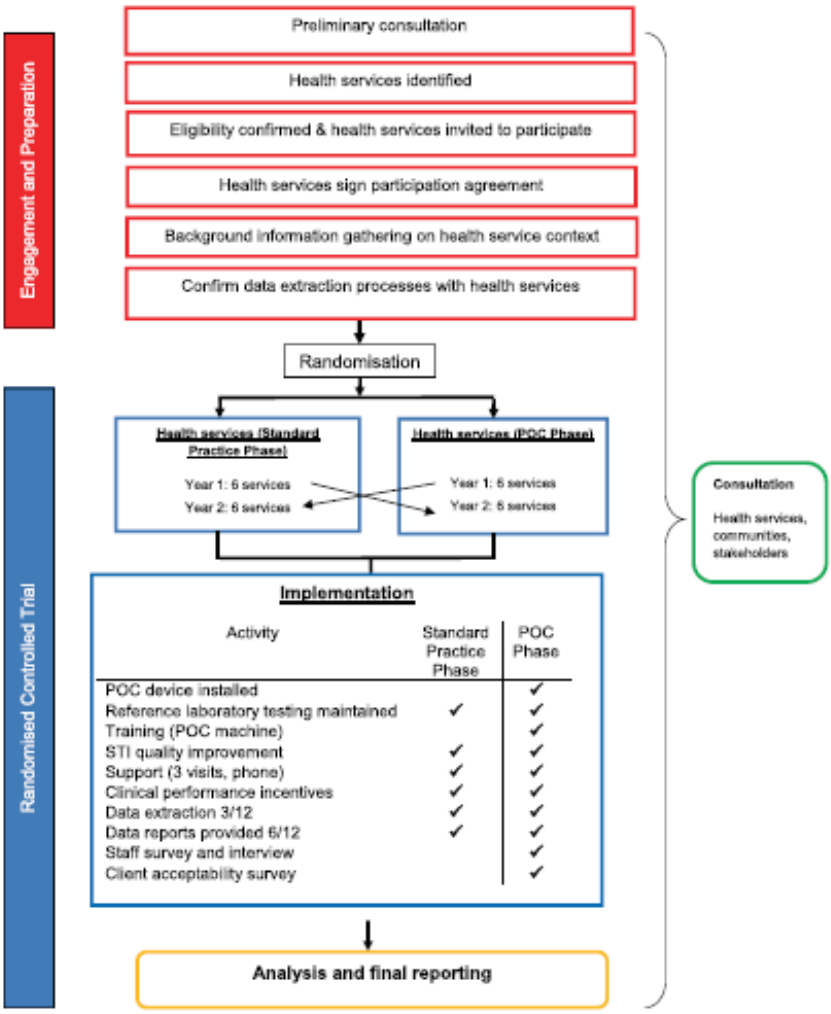

Figure 1 Trial overview.

Participating health services sign a Site Participation Agreement prior to commencement of their involvement in TTANGO. This agreement outlines trial details, responsibilities of the research team and participating services, principles of confidentiality and data ownership, and defines processes for reporting of findings and public release of study results.

Community engagement and collaboration

Engagement with stakeholders including Government, state-based Aboriginal Community Controlled Health Organisations (which represent ACCHSs in each state), and laboratories will be a key principle of the TTANGO trial. Effective community engagement will ensure that an appropriate framework is developed to guide project implementation, and that transparency is maintained at all levels.

Identification of communities and health services and eligibility criteria

In the first instance, potentially eligible health services will be identified in consultation with Government and Aboriginal Community Controlled Health Organisations. Identified health services will then be approached and invited to participate in TTANGO if they meet the following inclusion criteria: located in communities classified as regional, remote or very remote by the Australian Bureau of Statistics [41]; annually offer CT and NG testing to a minimum of 150 people aged 16–29 years (see sample size section), with a CT and NG positivity of at least 10% in 16–29 year olds; agree to be randomly assigned in one year out of two to the

POC phase; have an electronic patient management system; and have the staff and resource capacity to fulfil protocol requirements.

Randomisation

Health services within the four geographic regions will be randomised to intervention ("POC" phase) or control arm ("standard practice" phase) strategies for year one in a 1:1 ratio, and will cross over to the opposite arm in year two. Randomisation within geographic regions is mainly for pragmatic reasons so that the TTANGO coordinators are able to manage workload appropriately, and avoid the possibility of all services in one region being randomised to the same study arm. The randomisation scheme will be developed by the TTANGO statistician using SAS statistical software (Version 9.3) and will be communicated to participating health services once participation agreements have been signed.

During the ***POC Phase*** health services will continue routine STI testing and management practices (syndromic management, and sending off specimens to laboratories for routine NAAT tests) but will supplement their activity with CT and NG POC testing, so treatment and management of positive CT and NG POC tests occurs at the time of initial consultation. The POC test selected for the trial is the GeneXpert CT/NG* (Cepheid) which is a new test based on nucleic acid amplification technology, with dual detection of CT and NG in under 90 minutes. The GeneXpert CT/NG POC test had been demonstrated in laboratory and field evaluations in Australia [42,43] and the US [44] to have similar sensitivity and specificity to routine laboratory based NAAT tests, and was easy to use. The test has 5 targets, including a specimen processing control, specimen adequacy control, one CT target and two targets for NG. Two separate targets for NG allows for more specific detection and confirmation of NG [45]. The GeneXpert CT/NG test was approved by the Therapeutic Goods Administration for diagnostic purposes in Australia in March 2013, and obtained Conformité Européenne (CE) marking and US Food and Drugs Administration (FDA) clearance in 2012.

During the ***Standard Practice phase*** health services will maintain routine STI testing and management practices involving syndromic management, and sending off specimens to laboratories for routine NAAT tests, with treatment and management of asymptomatic infections based on the results that are received from the laboratory. The NAAT tests commonly used at the laboratories are the Roche Cobas and the Gen Probe Aptima.

Health services in both trial phases will receive *clinical performance incentives* and will participate in *STI quality improvement* (see further details below). Health services operating in the POC Phase will receive *POC training and ongoing support* and will participate in *laboratory quality management procedures* (see below).

STI quality improvement

In both trial phases, de-identified clinical and laboratory data will be used to generate regular site specific reports on STI screening and management. Reports will show the percentage of health centre attendees patients tested for STIs, the percentage testing positive, time to treatment, the percentage re-tested 3 months after treatment, and the percentage with a repeat positive result at re-test. The reports will be discussed at initial and subsequent visits, and are intended to assist services to reflect on current practice and develop strategies to achieve best practice in STI service delivery [16,46,47]. Accordingly, health services will be supported to increase and appropriately target opportunistic STI testing, provide treatment promptly where indicated, and follow recommended guidelines for re-testing and contact tracing. Where necessary, TTANGO coordinators will respond to identified training needs to strengthen sexual health service delivery.

Clinical performance incentives

In both trial phases, health services will receive incentive payments at six monthly intervals. Payments will be slightly higher in the POC phase, in recognition of the additional time spent fulfilling trial data collection requirements. Over the course of the trial (POC phase and Standard Practice phase), health services will be eligible to receive a maximum of $5000. In the POC phase, payments will be on the basis of $20 per POC test, up to a maximum of 150 tests, and $30 per re-test in clients who test positive. In the standard practice phase, payments will be on the basis of $10 per test, up to a maximum of 150 tests, and $30 per re-test in clients who test positive. Trial coordinators will provide support in both phases of the trial to strengthen testing and re-testing practices.

POC training and support

In the POC phase, health service personnel will be trained onsite in use of the GeneXpert machine, complete a competency assessment, and will be provided with all materials necessary to perform POC testing throughout the POC phase. 'Real-time' monitoring and support will be provided remotely by TTANGO coordinators, through the use of 'LogMeIn' software. Trial coordinators will also provide support at their 6-monthly visits and by phone/email.

Quality management for POC processes

The trial will incorporate Quality Management procedures to ensure that QM is conducted and that data are

generated, documented and reported in compliance with good clinical laboratory practice. Standard operating procedures (SOPs) for POCT have been developed and are reinforced through POCT training and competency assessment processes, and compliance with SOPs is monitored during site visits. Any specimens generating discordant results (between laboratory reference test and the GeneXpert) will be stored at the local laboratory, and at the end of the trial transferred to a research laboratory for re-testing with the COBAS 4800 test. Whilst the GeneXpert machine has a range of sophisticated built-in quality test processes, health services will be required to test a known positive "in house" quality control sample (consisting of non-infectious DNA of both CT and NG and prepared to simulate an infection of average bacterial load) each month and four blinded external quality assurance samples twice during the trial. The quality assurance procedures were developed by the National Serology Reference Laboratory for this trial and involve sending out swabs with freeze-dried organism (CT, CT and NG, NG and negative) [48].

Outcome measures

The primary outcome is repeat positivity at three months after treatment among people with CT or NG infection.

The secondary outcomes are:

1. Proportion of infections treated within seven days of specimen collection.
2. Cultural acceptability of POC tests to patients.
3. Operational acceptability of POC tests to health service staff in remote community settings.
4. The cost effectiveness of the addition of POC testing to standard diagnostic procedures.
5. The sensitivity, specificity, negative and positive predictive value of the POC test in a field setting compared to the reference laboratory test.
6. A best practice model for quality management of STI/POC testing in remote health services.

Data collection methods and variables

The trial will utilise a number of methods to collect information to calculate the trial outcomes above and these are described in further details in Table 1. Confidentiality will be maintained at all levels of data collection, transfer and analysis by de-identifying participating health services, staff, patients and other key stakeholders.

Sample size

Power calculations were performed using the methods of Julious et al. [49], for paired binary comparisons and Hayes et al. [50] for community randomised trials. We assumed that the prevalence of CT and/or NG will be 15% at recruitment to the trial in each year, that 30% of patients will have a positive re-test within three months (in the standard practice phase), and that at least 150 patients aged 16–29 years will be tested at each health service in both POC and standard practice years. Table 2 shows the total number of health services needed to detect a reduction in CT and NG repeat positivity rate from 30% in control health services to 15% in intervention health services with 80% and 90% power, differing community coefficients of variation (0.25, 0.30) and equal numbers of health services in each arm. At least ten services will provide data in both the intervention and control arms. This will ensure 88% power to detect a decrease in CT and NG repeat positivity rate from 30% to 15%, and 78% power to detect a reduction from 20% to 10%. Conservatively, 12 services will be included in the trial.

Statistical analysis

We will compare results between the two years (with and without POC testing) from within the 150 patients aged 16–29 tested each year in participating health services. Characteristics of participating health services will be summarized at baseline and across trial arms (standard practice or POC phase). The primary endpoint of the study will be the health service specific difference between the two years in repeat positivity rates at three months. The secondary endpoint will be the health service specific difference between the two years in the proportion of true CT and NG infections that were treated within seven days of presentation. For both the primary and secondary endpoints, the average difference between the two years (with and without POC tests) will be calculated across participating health services. Other secondary outcomes will be measured as follows:

Cultural acceptability of POC tests to patients: will be assessed by a descriptive analysis of questionnaire feedback received from patients.

Operational acceptability of POC tests to health service staff in remote community settings: will be assessed by calculating the proportion of staff who indicated that they agreed or strongly agreed on the Likert scale with the acceptability questions in the staff questionnaire.

The cost effectiveness of the addition of POC testing to standard diagnostic procedures: The treatment interval, repeat positivity rate and contact tracing data collected in this trial will enable us to parameterise the models. Our analyses will apply similar techniques to those used by other studies in the context of POC tests [51,52], but explicitly represent regional and remote Australian Aboriginal community settings. Our approach will extend previous work by developing a more realistic model based on simulated sexual networks rather than population averages. The mathematical approach will use an individual-based model to enable us to accurately

Table 1 Tools, methods and description of information collected in the trial

Tool	Method and frequency	Description of data collected
Site assessment tool	• Completed by TTANGO Coordinators at baseline, and updated 6 monthly in all health services.	*Broad description of:* (i) Clinical services and staffing (ii) STI testing practices (iii) Patient information system (iv) Clinic setting as it relates to location of GeneXpert machine and consumables
Clinical data (quantitative)	• Collated from one or more of the following sources: (i) Health service patient information management systems (ii) Laboratory data • Extracted 3 monthly for all health services	(i) Patient consultation data and associated demographics (ii) STI testing (point of care and laboratory), retesting and treatment outcomes
Quality management data	• Services record results of EQA and forward to coordinators, • twice during POC phase • Internal quality control testing, once per month • Temperature monitoring of test consumables during transport • Knowledge and competency assessment of staff following training in GeneXpert usage	• External quality assurance and internal quality control test results • Temperature range of consumables during transport • Multiple choice quiz and observed practice against competency standards
Surveys (quantitative)	• Staff acceptability survey at end of POC phase • Client acceptability survey, with attending clients in last month of POC phase	• Confidence using the test; satisfaction with training; trust in the results; ease of use; experience of discordant results; and impact on health service operations. • Preference for testing modality (POC test vs conventional); convenience; satisfaction with test process and communication of results
In-depth interviews (qualitative)	• Stakeholders (national) • Stakeholders (national and international) • Health service staff	• Challenges and implications of introducing POC testing • Quality management and training considerations • Acceptability

simulate and track repeat positivity rates, which is not possible with previous models. The epidemiological transmission models will project the estimated prevalence, incidence and repeat positivity rates expected from implementing POC tests in communities.

These model outputs will become inputs in a health economics analysis. The cost of using POC tests in regional and remote health services including the increased clinician and patient waiting time for POC tests and the patient costs incurred in a return visit to the health service for medication after a positive test result will be directly measured in the trial. These data will be used to estimate the additional cost of implementing POC tests in combination with NAAT testing, compared to standard diagnostic procedures. Costs related to research will be excluded.

Table 2 Sample size estimates based on reduction in repeat positivity rate from 30% to 15%

Coefficient of variation	Number of services	
	80% power	90% power
25%	8	10
30%	10	14

The total costs associated with the widespread implementation of POC tests, including estimated logistical, clinical and administrative costs, will be compared with the long-term health and financial savings associated with their use (including the prevention of new infections, co-morbidities, and saving in reduced quality of life as well as costs of treatment saved due to decreased infections). As is standard with health economic analyses, discounting will be applied at levels of 0%, 3% and 5%. The health economic analyses will be carried out from the perspectives of the provider and the client. The integrated epidemiological and economic modelling will examine issues of allocation, efficiency, and cost-effectiveness.

The sensitivity, specificity, negative and positive predictive value of the POC test in a field setting compared to the reference laboratory test: POC test performance will be assessed by comparing the results of the POC tests performed during the POC phase with the reference test (NAAT).

Discussion

Rates of CT and NG in remote Aboriginal communities in Australia are unacceptably high, and TTANGO will evaluate whether POC testing for these STIs can improve the timeliness of treatment and in turn reduce repeat infection rates. To our knowledge, TTANGO will be the first randomized trial of molecular POC tests for CT/NG infection internationally.

It is anticipated that one of the main challenges for TTANGO will be to sustain the involvement of health service staff amidst the numerous competing demands and health priorities, and also ensure POC operator competency is maintained in the context of high staff turnover. Regular communication and training with trial sites from a dedicated TTANGO coordinator should help to mitigate this risk. Another challenge will be if the services randomised to the POC arm in the first year prefer to keep the POC machines due to realized improvements in STI management. In the consultation phase we emphasized to health services the importance of the cross-over design to ensure long term policy change and funding. Finally, repeat positivity rates are calculated from those who re-tested and these need to be similar in both POC and non-POC arms to minimize biases – lower re-testing rates are often associated with higher repeat positivity due to re-testing in those with symptoms [53]. To mimimise any imbalance in re-testing rates, a quality improvement program and incentives associated with re-testing have been provided in both arms.

This research is significant as study outcomes will make an important contribution to the international understanding of STI control using POC tests. The research may also lead to significant decreases in STI prevalence and reinfection across multiple sites with long term benefit for the sexual health and wellbeing of young Aboriginal people. Finally, it has the potential to influence public health policy and result in [54] uptake of CT and NG POC tests into further remote clinical services in Australia, and other settings.

Competing interests

No financial support was received by Cepheid. Cepheid has provided GeneXpert machines on loan for the duration of TTANGO.

Authors' contributions

RG and LN prepared the manuscript. All other authors reviewed the manuscript and contributed to the research protocol. All authors read and approved the final manuscript.

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Chapter 5: A field evaluation of a new molecular-based point-of-care test for chlamydia and gonorrhoea in remote Aboriginal health services in Australia

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A field evaluation of a new molecular-based point-of-care test for chlamydia and gonorrhoea in remote Aboriginal health services in Australia

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Abstract. ***Background*:** Point-of-care (POC) tests could be important public health tools in settings with treatment delays and high rates of sexually transmissible infections (STIs). Use is limited due to suboptimal performance. The performance and ease-of-use of a new molecular-based POC test for simultaneous detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) was assessed, alongside two single-organism immunochromatographic tests (ICT). ***Methods*:** The evaluation occurred between May 2012 and March 2013 during community STI screens in two remote Aboriginal health services. Urine was tested with the GeneXpert® CT/NG and if sufficient volume, also with Diaquick CT and Gonorrhoea Card. The gold standard comparison was laboratory nucleic acid amplification testing (NAAT). Operational characteristics were also assessed. ***Results*:** Among 198 samples, GeneXpert CT sensitivity and specificity was 100% [95% confidence intervals (CI): 75.9–100] and 99.5% (95% CI: 96.5–100), and NG was 100% (95% CI: 96.5–100) and 100% (95% CI: 97.5–100), respectively. Among a sample subset, Diaquick CT ($n = 104$) sensitivity and specificity was 27.3% (95% CI: 7.3–60.7) and 66.7% (95% CI: 12.5–98.2), and Gonorrhoea Card ($n = 29$), was 66.7% (95% CI: 12.5–98.2) and 76.9% (95% CI: 56.0–90.2), respectively. GeneXpert required 1 mL of urine, four steps, 1 min specimen preparation and 90 min to result. ICTs required 15 mL of urine, eight steps, 18 min preparation and 10–15 min to result. ***Conclusion*:** The accuracy and operational benefits of GeneXpert CT/NG make it very suitable in these settings where delays to treatment are encountered.

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Background

Sexually transmissible infections (STIs; chlamydia, gonorrhoea and trichomonas) remain at high levels in remote Australian Aboriginal communities despite the wide availability of accurate diagnostic laboratory tests and effective treatment. A recent study in 68 remote Aboriginal communities[1] estimated the prevalence of chlamydia (*Chlamydia trachomatis;* CT) to be 9% in 16–34 year olds, 7% for gonorrhoea (*Neisseria gonorrhoeae*; NG) and 18% for trichomonas (TV) among females. For all three STIs, rates were highest in 16–19 year olds.[2] These STIs are often asymptomatic[3–5] for long time periods and, if untreated, can lead to serious complications[6] including pelvic inflammatory disease (PID)[7–9] and adverse neonatal outcomes,[10] and untreated NG can also lead to disseminated infection.[6]

In regional and remote areas of Australia, primary health services play an important role in the prevention, testing, treatment and management of CT and NG. These health services are either government funded or provided by Aboriginal Community-Controlled Health Services (ACCHS) and are the primary healthcare provider for Aboriginal people. Current clinical practice in most remote Aboriginal

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communities[11,12] follows guidelines that provide for immediate treatment if CT or NG are suspected on the basis of specified symptoms or risk.[13] For those patients who are asymptomatic, which constitutes the majority of cases, specimens are sent to laboratories and treatment is initiated once the result is received and actioned by the health service.

In these remote locations, the control of STIs is compromised by delays in diagnosis and treatment due to considerable distances between remote health services and urban laboratories, as well as difficulties locating and recalling patients for treatment when results are received. Reports from eight remote health services in central Northern Territory in Australia indicated that 15–20% of patients remain untreated and that the average time to treatment is 21 days,[14] compared with 3 days in urban centres.[15] Among pregnant Indigenous women attending primary healthcare centres in Townsville in 2000–03, 18% remained untreated, the average time to treatment was 21 days and contact tracing occurred in 17% of cases.[16] Also, symptom-driven diagnosis has poor sensitivity and specificity for detecting infection, particularly in women.[17,18]

Point-of-care (POC) testing in this setting, defined as 'pathology testing which is performed on behalf of the treating medical practitioner by a trained operator in an on-site clinical setting while the patient is in attendance, allowing the test result to be generated and used to make an immediate informed decision that contributes to an improved health outcome for the patient'[19] may have public health benefits by increasing the uptake and timeliness of treatment and, in turn, reducing STI transmission in these communities. Mathematical modelling has shown that a POC test, even with a moderate sensitivity of 60% in a setting of high prevalence, could reduce community prevalence if coupled with conventional laboratory testing.[20] Recent mathematical modelling focussed on remote Aboriginal communities in Australia, suggests that if a more accurate POC test was used (>95% sensitivity/specificity), the prevalence of NG and CT could fall from 7.1% and 11.9% to 5.7% and 8.9% respectively after 10 years.[20] This impact could be increased further by increasing screening coverage.

While a POC test has these theoretical benefits, it can only be used in practice if it combines a high level of diagnostic performance with ease of use for both the clinician and the patient. The first generation STI POC tests, based on lateral flow or immunochromatographic test (ICT) platforms for the diagnosis of CT and NG, have generally demonstrated poor performance[21–25] and as a result, have not been widely implemented. Newer POC technologies have recently provided the opportunity to reconsider the use of POC tests for STI control in high prevalence settings. In order to inform the choice of test for a large scale, cross-over, randomised controlled trial of STI POC testing in remote Aboriginal communities in Australia, we evaluated the operational performance, ease-of-use and feasibility of a new molecular-based POC test and two ICTs in remote field settings. The subsequent larger trial, called TTANGO (Test, Treat and Go), will investigate the feasibility, acceptability, cost effectiveness and impact of POC testing on the timeliness of STI treatment testing and reinfections in 12 remote communities over a 2-year period.[26]

Methods

Setting

Following consultation with communities and their associated health services, and supported by the study reference group consisting of representatives from the government, ACCS and pathology providers, the field evaluation was conducted at two remote Aboriginal health services. The services were purposefully selected for their high rates of STIs (as is the case in many remote areas in Australia), their willingness to participate in the evaluation and their STI community screening programs that provided the greatest number of specimens within the study timeframe. The annual community STI screening programs at these services aim to screen the population most at-risk of STIs and provide treatment for those with positive tests over a 3–4 week period.

POC tests selected

Based on preliminary laboratory evaluations of nine ICTs tests (six for CT and three for NG) conducted at the Queensland Paediatric Infectious Diseases Laboratory (D. Whiley, pers. comm.,) and a laboratory evaluation of a molecular-based POC assay at the Royal Women's Hospital,[27] the chosen tests for this field evaluation were the best performing devices for detection of CT and NG in urine available at the time to the researchers.[28] These selected tests included: the (i) GeneXpert®CT/NG [Cepheid, Sunnyvale, CA, USA] (simultaneous detection of CT and NG), (ii) Diaquick CT [DIALAB, Neudorf, Austria] (CT only), and (iii) Gonorrhea Card Test [Immuno-Diagnostics, Foster City, CA, USA] (NG only). Although the package insert of the Gonorrhea Card Test recommended genital swabs only be used, the preliminary laboratory evaluation showed that it has superior performance compared with a range of other ICTs when urine was used.

Briefly, the GeneXpert System is a closed, self-contained, fully-integrated and automated platform that is designed to purify, concentrate, detect and identify targeted nucleic acid sequences using real-time polymerase chain reaction (PCR) assays, thereby delivering results directly from unprocessed samples. The system has a modular design. Patient samples are added to diseases-specific, single use, disposable cartridges, containing all PCR reagents, buffers, primers and probes, and these are then loaded into one of the system's modules. The test cartridge also contains an internal control to verify adequate processing of the target sequence and the system monitors the presence of inhibitors in the PCR assay to avoid a false-negative result. Test results (detected or not detected), as well as assay details including sample adequacy and quantitative crossing points for each organism, are accessed from a laptop connected to the system. Results can be stored and viewed at the convenience of staff at any time after the test is completed. For this field evaluation, we used urine, CT/NG detection cartridges, and 2×4-module systems side-by-side to perform the tests (Fig. 1).

The Diaquick and Gonorrhea Card tests are membrane-based immunochromatographic assays in which a patient sample, pretreated with selected buffers to release the target antigen into solution, is added to a well in a cassette containing a solid substrate test strip. The sample mixes with dried, coloured

Fig. 1. Side-by-side setup of 2 × 4 module GeneXpert CT/NG.

conjugate (containing antibody to either CT or NG) and flows along the strip via capillary action. As the mixture migrates along the strip, it encounters the test zone, embedded with antibody to the conjugate, which turns red when bound. These ICTs also contain a control region which, when red, indicates a valid test and functional reagent. Results are subjective and the test strip is viewed to determine the presence or absence of a coloured test line, indicating a positive or negative test, at the designated time recommended by the manufacturer.

POC testing process

Urine specimens are the most commonly collected specimen for diagnosis of NG and CT in remote communities and were self-collected by patients attending the two sites during their annual community screening. These specimens were aliquotted and tested onsite at the services by the study team using the three POC devices. The study team had no formal laboratory technical qualifications, but participated in a 1-day training program before the field evaluation commenced. POC tests were performed according to the manufacturer's instructions; however, as the Gonorrhea Card Test is recommended for use with genital swabs, the use of urine samples here was a deviation from the kit protocol. Where delays to immediate testing were apparent, specimens were refrigerated until testing was conducted (as per manufacturer recommendations). All were tested within 24 h of specimen collection.

Reference test

Urine specimens were transported for reference laboratory CT and NG testing using either the Cobas® 4800 CT/NG Test (Roche Molecular Diagnostics, Pleasanton, CA, USA) or Aptima Combo 2 Assay (Hologic Gen-Probe Inc., San Diego, CA, USA) nucleic-acid amplification tests (NAAT), as per routine laboratory practice. As reported in the literature, both these assays have very high sensitivities and specificities for the detection of both CT and NG in urine.[29–31]

Patient management

POC test results were not provided back to patients, as the tests were not approved at the time by the Therapeutic Goods Administration (TGA) and therefore were considered experimental. To maintain patient anonymity, specimens were de-identified before study staff performed the POC tests. POC testing did not influence the clinical management of patients and clinical staff provided care as per usual practice.

Test performance analysis

POC test results were recorded manually, then entered into a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) database. Results were compared with respective laboratory reference NAAT results (CT or NG; Cobas or Aptima depending on the site), with sensitivity and specificity and 95% confidence intervals (CI) calculated by using standard methods.[32] McNemar's test for agreement was used to calculate *P*-values for each POC test (StataCorp.; Release 12, College Station, TX, USA).

Assessment of operational characteristics

The study staff who conducted the POC testing formally assessed selected operational characteristics and ease-of-use using a standardised checklist, which included urine volume and equipment required, number of steps, time for specimen preparation and to conduct the test and time-to-result. Both study staff who conducted the tests ($n = 4$) and clinical staff (Aboriginal health workers) who closely observed the testing ($n = 2$) were also asked about their perceptions and acceptability of POC testing. The information was recorded by hand and analysed descriptively.

Ethical approvals

The research received approval from relevant jurisdictional human research ethics committees (HREC): West Australian Aboriginal Health Ethics Committee, Townsville Health Service District HRECs (Queensland Health) and University of New South Wales HREC.

Results

Study population

In total, 198 urine specimens were collected for testing. Equal numbers were collected from each of the two sites, with more specimens from men than women (64% vs 36%) tested. All 198 specimens were tested with GeneXpert CT/NG, and due to limited urine volumes, only 104 were tested with Diaquick and 29 with Gonorrhea Card.

POC test sensitivity and specificity

POC test sensitivity and specificity [with 95% confidence intervals (CI) and *P*-values] compared with laboratory NAAT

tests are shown in Table 1. For GeneXpert, CT sensitivity and specificity was 100% (95% CI: 75.9–100) and 99.5% (95% CI: 96.5–100) respectively, and for NG, it was 100% (95% CI: 56.1–100) and 100% (95% CI: 97.5–100) respectively. The one GeneXpert result (CT) discordant with the commercial NAAT result had a crossing point of 36.9 for CT, indicating a low bacterial load.

The sensitivity of Diaquick CT was 27.3% (95% CI: 7.3–60.7) and the specificity was 98.5% (95% CI: 93.9–99.9), while for Gonorrhea Card, the sensitivity was 66.7% (95% CI: 12.5–98.2) and the specificity was 76.9% (95% CI: 56.0–90.2).

Operational characteristics

The operational characteristics of the three POC tests are summarised in Table 2. Equipment required for the GeneXpert CT/NG test included the GeneXpert machine itself, a configured laptop with GeneXpert software connected to the machine, a protected power supply for both the machine and laptop and the CT/NG test cartridge and transfer pipette. Only 1 mL of urine and a total of four steps were required to perform the GeneXpert CT/NG test; the total time to conduct the test was 91 min, including 1 min for specimen preparation and test initiation, then 90 min to result. Once initiated, the GeneXpert CT/NG test did not require any further intervention. Staff did not have to return immediately at the completion of the test as the CT and NG result is displayed on the laptop screen (and electronically saved) once the test is complete.

Equipment required for the ICTs included the test cassette itself, transfer pipettes and two buffer solutions specific to the ICT and a centrifuge device with an appropriate tube for spinning the specimen before testing. A minimum of 15 mL of urine and a total of eight steps were required to perform each ICT (a total of 30 mL to conduct both tests); the total time to conduct the ICT was 28–33 min: 18 min for specimen preparation (including centrifuging) and test initiation, then 10–15 min to result depending on the ICT being performed. The test start time needed to be noted and the test cassettes were left to process for the designated time, according to

Table 1. Poin-of-care (POC) test sensitivity and specificity compared with reference laboratory nucleic acid amplification testing (NAAT)
CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoea*; STI, sexually transmissible infection; CI, confidence interval. POC test type numbers vary as urine volume was not always sufficient to perform all three POC tests; priority was given to GeneXpert, followed by Diaquick then the Gonorrhoea Card Test.
"The Gonorrhea Card Test is recommended for use with genital swabs so the use of urine samples in this study was a deviation from the kit protocol

POC test	STI	*N*	NAAT* result (+/−)	POC result (+/−)	Sensitivity (95% CI)	Specificity (95% CI)	*P*-value#
GeneXpert	CT	198	16/182	17/181	100.0 (75.9–100)	99.5 (96.5–100)	0.32
GeneXpert	NG	198	7/191	7/191	100.0 (56.1–100)	100.0 (97.5–100)	1.00**
Diaquick	CT	104	11/93	4/100	27.3 (7.3–60.7)	98.5 (93.9–99.9)	0.02
Gonorrhea Card Test"	NG	29	3/26	8/21	66.7 (12.5–98.2)	76.9 (56.0–90.2)	0.06

*Reference laboratory NAAT: Cobas 4800 or Aptima Combo 2 (depending on health service).
#*P*-value calculated using McNemar's test.
**Exact McNemar's *P*-value.

Table 2. Operational characteristics of point-of-care (POC) tests (preparation time, number of steps, ease of use and equipment required)
Note: Preparation time was the median time measured between commencing transfer of urine from the collection container to the beginning of test processing. Qualitative comments related to ease of interpretation of results were noted during informal feedback from researchers and staff

POC test	Specimen preparation	Preparation time (min)	Time to result (min)	Number of steps	Ease of interpretation of results	Equipment required
GeneXpert® CT/NG	Transfer 1 mL urine to cartridge, scan cartridge, enter patient ID, load cartridge in machine, commence test.	1	90	4	Very easy (quantitative result on the computer).	Power supply and GeneXpert machine and laptop computer, CT/NG GeneXpert cartridges, transfer pipettes, gloves.
Diaquick CT®	Transfer 15 mL urine to centrifuge tube, centrifuge for 10 min, tip off supernatant, resuspend pellet with buffer, wait, add portion to second buffer, mix, add specimen to kit.	18	10	8	Moderate (qualitative results –weak bands were subjective).	Diaquick test kit, buffer, centrifuge plus power supply, tubes, transfer pipettes, gloves, timer/stopwatch.
Lateral Flow Gonorrhea Card®	Transfer 15 mL urine to centrifuge tube, centrifuge for 10 min, tip off supernatant, resuspend pellet with buffer, wait, add portion to second buffer, mix, add specimen to kit.	18	15	8	Moderate (qualitative results –weak bands were subjective).	Gonorrhea Card test kit, buffer, centrifuge plus power supply, tubes, transfer pipettes, gloves, timer/stopwatch.

manufacturer's instructions (Diaquick CT = 10 min; Gonorrhea Card = 15 min). Staff were required to visually inspect the cassette and record the result by hand at the designated end time (different of each ICT) to ensure a valid result. As per the manufacturer's instructions, results interpreted after the designated end times are liable to be invalid.

Study and clinical staff performing the POC tests identified several advantages of the GeneXpert over the ICT devices, including: the small volume of urine required, no additional specimen preparation needed, fewer steps performed, ability to test both CT and NG simultaneously, multiple specimens tested at the same time, results reported objectively (did not require visual interpretation) and the result stored electronically on the GeneXpert laptop computer. Disadvantages included: the need for electricity and the close attention required to avoid potential cross-contamination between specimens while transferring samples into the test cartridges.

Study and clinical staff noted the advantages of the ICTs over the GeneXpert, which included: ease of transportation and storage of test kits and familiarity with other POC tests with similar lateral flow platforms such as pregnancy tests. Disadvantages of the ICTs included: large volumes of urine required, the need for the centrifugation of the specimen, numerous steps to perform the test, one infection detected per test, visual interpretation of the result and no immediate permanent record of the result. Taking all this into consideration overall, clinical staff reported that the GeneXpert was easier to use and feasible for use in their health service while the ICT devices were more complicated.

Discussion

To the best of our knowledge, this is the first report of the field use, in a remote setting, of these POC tests for the diagnosis of sexually transmitted CT and NG. The results demonstrate that the GeneXpert CT/NG test had a very high sensitivity and specificity in this remote field setting, with all chlamydia or gonorrhoea infections detected and only one discordant chlamydia result (with a high crossing point suggesting the bacterial load was close to the detectable threshold), using the laboratory results as the reference test. The GeneXpert CT/NG test required a minimal volume of urine (1 mL) and preparation time (1 min), and despite taking 90 min to result, there was no additional intervention required once the test was initiated. Staff considered the GeneXpert easy to perform and identified several advantages over other POC tests, including the simultaneous detection of both CT and NG and an objective, electronically stored result at end of testing.

The performance of the GeneXpert CT/NG test in this field study is consistent with published literature and manufacturer information[27,33] and also consistent with early results from the TTANGO trial. In the TTANGO trial, more than 1500 STI POC tests have been performed across the six participating health services randomised to POC in the first year. Routine nucleic acid laboratory tests for CT and NG conducted in parallel to POC testing have shown that the POC test results (primarily performed by Aboriginal health practitioners and nurses) for CT and NG have excellent concordance with laboratory results.[34] The operational sensitivity of the GeneXpert POC test for CT as of May 2014 was 100% (95% CI: 96.4–100) and the specificity was 99.6% (95% CI: 99.0–99.9), and for NG, it was 100% (95% CI: 94.7–100) and 99.9% (95% CI: 99.4–100) respectively,[34] and as was seen in this field study, the rare discordant results occurred mainly as the organism load was close to the assay detection threshold.[35]

Both the Diaquick and Gonorrhea Card ICTs performed poorly, which is consistent with other published evaluations of these types of ICT tests.[21–25,36] The estimates of performance of the ICTs should be interpreted with caution, as numbers tested were small, particularly for Gonorrhea Card ($n = 29$). However, despite the small numbers tested, the upper limit of confidence for sensitivity of the Diaquick test was only 60%, which was significantly lower than that of the GeneXpert test for detecting CT. The upper limit for specificity of the Gonorrhea Card Test (90%) remains concerning, given the potential psycho-social consequences of reporting false-positive results to patients. The performance of these ICTs is in stark contrast to syphilis and HIV ICTs, with reported sensitivities and specificities of >95%.[37] However, as syphilis and HIV infections result in a sustained antibody response, ICTs for diagnosis of these infections are designed to detect this antibody response and are compared with similar laboratory-based enzyme immunoassays, while ICTs for CT and NG are designed to detect antigens and are compared with laboratory-based nucleic acid amplification methods that detect the bacteria DNA, and thus have lower-limits of sensitivity.[38]

The main strength of our evaluation is that it was conducted at sites with community STI screening programs, which enabled the POC tests to be evaluated using fresh specimens and occurred in a close-to-real-world setting. Some challenges and limitations were also experienced. We had anticipated 600 samples were required to provide confidence intervals ±13% around the sensitivity estimates; however, various factors meant this sample size was not achieved, including traditional events occurring in the communities leading to reduced numbers of patients attending the services for STI screening during the study time period. Additional sites were considered; however, after completing the evaluation at the two sites, the GeneXpert CT/NG test became TGA approved, meaning that he GeneXpert CT/NG test could be used as an approved diagnostic and treatment offered to those patients with positive results. Given these circumstances, the evaluation was closed despite the reduced sample size. In addition, urine volumes were not always sufficient to allow testing of all three POC tests, particularly as each ICT required 15 mL of urine for processing. POC testing was prioritised to favour the GeneXpert, followed by Diaquick, then the Gonorrhea Card Test; CT testing was prioritised over NG in order to collect specimens for a CT substudy, with the net result that fewer specimens were tested using ICTs. The reduced overall sample size and limited urine volumes resulted in wide confidence intervals, particularly around performance estimates for the ICTs and, therefore, these results should be interpreted with some caution.

Although tests were performed by study staff rather than health service staff, we believe the experiences of study staff, who had not had any formal laboratory training, provides a preliminary indication of feasibility and ease-of-use in this

setting. A formal assessment of acceptability, once integrated into routine clinical practice and used to initiate treatment, will be conducted as part of the larger TTANGO trial. Although our evaluation was implemented over a short time period to coincide with busy annual screening programs at each of the two health services, staff indicated that the GeneXpert test could be incorporated into the service and used for routine testing. Preliminary results from the TTANGO trial indicate that the GeneXpert CT/NG test is being incorporated as a screening tool in routine adult health checks[39] and that patients are willing to wait either in or close by the health service until their test results are available (at least 90 min).

In conclusion, our results demonstrate that the GeneXpert test has very high sensitivity and specificity for the detection of both CT and NG, and study and health service staff indicated that the GeneXpert test was easier to use than the ICTs evaluated. Although not evaluated here, cost is an important factor that will influence wider uptake and access to this POC testing modality. Medicare rebates, similar to that obtained for existing chronic disease POC testing in the QAAMS (Quality Assurance for Aboriginal and Torres Strait Islander Medical Service) program, will be important in the long term and POC costs will need to be weighed against costs saved in regards to health service staff recalling patients and potential public health benefits associated with more timely treatment.

The results of this field evaluation have informed the design and implementation strategies of the TTANGO trial, which aims to formally explore the acceptability, effectiveness and cost-effectiveness of the GeneXpert CT/NG test in a randomised controlled trial in remote communities across Australia.[26] Also, the findings have informed a study of GeneXpert CT/NG use in antenatal screening in Papua New Guinea later this year. These studies will provide empirical evidence to support the wider implementation of the technology.

Conflicts of interest

None declared.

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Chapter 6: Prospective evaluation of the diagnostic performance of a new, molecular point-of-care test for chlamydia and gonorrhoea in routine primary health care use

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Prospective evaluation of the diagnostic performance of a new, molecular point-of-care test for chlamydia and gonorrhoea in routine primary health care use

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ABSTRACT

Background: A new molecular point-of-care (POC) test for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) has been shown to accurately replicate the results of conventional nucleic acid tests when used by dedicated personnel. We examined the diagnostic performance of the POC test when used routinely by primary care clinicians, both medical and non-medical, working in a setting of high prevalence for these two infections.

Methods and Findings: After receiving training, clinical staff at 12 remote primary health services in central and northern Australia were instructed to use the GeneXpert®CT/NG assay to test specimens from all patients attending the health service for routine care for whom the test would normally be indicated according to clinical guidelines. Specimens from the same patients were also sent for laboratory-based CT/NG nucleic acid amplification tests (NAATs) as per standard practice. During the study period, 2486 specimens were tested with the GeneXpert with a valid result obtained. Overall CT concordance was 99.4% (95% confidence interval [CI]: 99.1–99.7) with positive concordance of 98.6% (95% CI: 95.9–99.7) and negative concordance of 99.5% (95%CI: 99.1–99.8). Overall NG concordance was 99.9% (95%CI: 99.7–100.0) with positive concordance of 100.0% (95%CI: 97.5–100.0) and negative concordance of 99.9% (95%CI: 99.7–100.0). Most discordant results (14/16) were for CT, with 11 positive on GeneXpert. The two discordant NG results were both positive on GeneXpert. Mean cycle threshold for discordant GeneXpert CT positive results (35.3) was higher than for concordant results (29.1) ($p<0.001$).

Conclusions: In the first study to report on the routine use of the GeneXpert CT/NG in the hands of primary health service staff, we found excellent concordance compared with laboratory-based NAATs. Discordant results have arisen mostly in specimens with low organism loads, possibly from degradation due to the long journey to the laboratory. The use of the GeneXpert CT/NG at the POC has the potential to transform management and control of sexually transmissible infections in high burden settings with rapid results ensuring timely treatment.

Introduction

Ensuring access to accurate diagnosis and timely treatment is a critical strategy in the control of sexually transmissible infections (STIs). There could, therefore, be potential benefit in shifting diagnostic capability from centralised laboratories to more locations where patients are encountered such as at primary health services or outreach locations.(1-6) Point-of-care (POC) testing for STIs would allow clinicians to offer appropriate treatment to patients at the time of consultation rather than waiting for a laboratory result to return and requiring patient attendance at a second visit, which may be delayed or missed, thus potentially limiting complications and ongoing transmission.

STIs such as chlamydia (caused by *Chlamydia trachomatis;* CT) and gonorrhoea (caused by *Neisseria gonorrhoeae*; NG) are ideal candidates for POC testing: they can be diagnosed with easily collected specimens such as urine and treated with single doses of antibiotics at the time of consultation. Furthermore, these infections continue to represent a significant global public health burden (7), with common complications such as pelvic inflammatory disease (PID),(8) infertility and ectopic pregnancy,(9) and a range of adverse pregnancy and neonatal outcomes.(10) These infections contribute to a range of psychosocial consequences, from feelings of discomfort and shame,(11) to relationship breakdown(12) and can also play a role in the spread of HIV.(13-15)

In remote Australian Aboriginal communities, rates of infection with CT and NG remain high despite the wide availability of accurate diagnostic laboratory tests and effective treatment.(16) Clinical reports from these communities indicate that PID and infertility occur at rates far higher than in other parts of Australia.(17-19) With diagnostic laboratories located far from health services, and a highly mobile population, there can be substantial delays between specimen collection and treatment, with some reports showing about 20% of those diagnosed with an STI do not receive treatment, and for those treated, the average time to initiation of treatment is around three weeks.(20, 21) In Australian urban settings the corresponding figures are 97% and three days.(22)

Until recently, commercially available POC tests for CT and NG were mainly single antigen detection lateral flow platforms which were inexpensive but required considerable specimen preparation(23) and had poor sensitivity.(24-29) Recent technological advances have resulted in the availability of new molecular-based assays and systems suitable for use at the POC.

The first GeneXpert assay for CT/NG (GeneXpert®CT/NG, Cepheid, Sunnyvale, CA) became available in 2013 and has high analytical(30) and clinical(31) performance compared to established commercial nucleic acid amplification tests (NAATs), in highly-controlled laboratory environments with dedicated staff.(32) However, the true test of a POC diagnostic test's value is in the hands of clinical staff, who would be required to use it in routine practice.(33)

TTANGO (Test, Treat ANd GO) is a cross-over randomized control trial which commenced in mid-2013 and was designed to comprehensively evaluate the clinical effectiveness, cost-effectiveness, feasibility and acceptability of the GeneXpert CT/NG assay (hereafter referred to as GeneXpert) for POC testing of STIs in primary health services.(34-36) We report here on the operational performance of the GeneXpert when used at the POC by primary health care staff compared to conventional laboratory-based NAATs.

Methods

Setting

Approximately one-fifth (21.1%) of the Australian Aboriginal population live in remote or very remote areas of Australia, compared to 1.8% of the non-Indigenous population.(16) Primary health care in remote Australia is generally provided through government or Aboriginal Community Controlled Health Services. Services are typically staffed by registered nurses and Aboriginal health practitioners/workers who provide the majority of clinical care and reside in the community, along with resident or fly in/fly out medical practitioners and visiting specialists.(37) There is frequent staff turnover in these settings.

In remote Australia where STIs are endemic, guidelines recommend annual CT/NG testing for people sexually active and younger than 30 years, and up to 34 years in some regions. Urine is the most commonly collected specimen for CT/NG testing in Australia, followed by vaginal swabs (self-collected) and cervical swabs (provider-collected). Diagnosis of CT and NG infections in Australia currently relies on testing of specimens (either urine or swabs) using commercial or in-house NAATs, which are conducted at centralised private or public laboratories. In remote Australia, these laboratories are often significant distances (up to 3200 kilometres) from the primary health service which not only impacts turn-around time for results but may also adversely affect specimen viability.

The TTANGO trial and study design

Within the context of the TTANGO trial,(34) we conducted a prospective study to assess the operational performance of the POC test used in the hands of primary health care staff compared to routine conventional laboratory-based tests.

Briefly, 12 health services were enrolled in the TTANGO trial, with eight classified(38) as being located in very remote areas, two in remote and two in outer regional areas. The health services covered five geographical regions across Australia. Participating health services were randomly assigned to either POC or routine laboratory testing for CT and NG for one year before crossing to the opposite modality for another year. The recommended target age group for STI testing was in accordance with clinical guidelines. During the 12 month period when the GeneXpert was available at each service, specimens were tested using the GeneXpert by health service staff according to manufacturer recommendations using either 1mL of urine or a swab specimen. All staff performing the POC test had completed training as described below. Specimens continued to be sent for conventional laboratory testing throughout the trial.

Clinical management of patients was informed by the POC test results according to local guidelines. (39, 40) Where POC test results were not available, or the POC result was negative but conventional laboratory NAAT was positive, the laboratory-based results informed further patient management.

Training to perform the POC test

Study coordinators delivered standardised training to nominated clinical staff (Aboriginal health workers/practitioners, nurses, doctors) at each participating health service. Training was conducted in-person over one or two days on-site and included instruction on the TTANGO protocol, trial and results documentation and data collection; performing the GeneXpert test, maintenance of the device and quality assurance processes; and concluded with an assessment of competency for those staff designated to be responsible to perform the test at the service ("operators"). Staff received a competency certificate valid for 2 years if successful. On-line refresher training was also available. Study coordinators monitored POC testing remotely (test numbers and results, including errors) and made regular visits to services (approximately every 6 months) to discuss STI testing reports generated from data collected during TTANGO and encourage best practice STI management at the service.

GeneXpert CT/NG test

The GeneXpert CT/NG test was approved by the Therapeutic Goods Administration (TGA) in March 2013 for use in Australia. GeneXpert devices, test cartridges and specimen collection kits were supplied to the participating health services free of charge to services for the duration of the TTANGO trial.

The GeneXpert test is a molecular-based test platform suited to use at the POC. Details of the operation of the GeneXpert platform and the CT/NG assay are described elsewhere. (30, 41) Results generated by the GeneXpert at the conclusion of testing (approximately 90 minutes) for each specimen are reported separately for CT and NG and also include information relating to test cycle thresholds, sample adequacy and test running time. Any specimen generating an error result was retested where sufficient specimen volume remained. Error results were investigated by study coordinators to identify possible causes.

Quality assurance

As part of TTANGO, all participating services were enrolled in an external quality assurance (EQA) program supported by National Reference Laboratory (NRL) Australia, a not-for-profit scientific organisation and WHO Collaborating Centre for Diagnostics and Laboratory Support in Australia and internationally. Participating services were provided with blinded samples for testing with the GeneXpert prior to commencement of routine patient testing and again within the first 6 months of implementing the test at the service. EQA results from all services were sent to NRL Australia for aggregation and reporting purposes. Individual service results were reported back and any errors at the service level investigated by the TTANGO coordinators. Quality control (QC) was monitored monthly with services performing the GeneXpert test using positive CT and NG specimens supplied by The University of Queensland. The results of the EQA and QC will be reported separately elsewhere.

Comparative laboratory-based NAAT tests

Specimens collected for STI testing during the TTANGO trial were sent as per usual practice by the health services with requests for conventional STI testing to their respective laboratories. Across the six laboratories, a range of NAATs for CT and NG detection were used including: Aptima Combo 2 (Gen-Probe, San Diago, CA, USA), Cobas® 4800 (Roche Diagnostics, Pleasanton, CA, USA); or in-house CT and NG assays.(42) Health service staff

cross-checked incoming laboratory results with POC test results in patient records and manual POC test logs at the service, and alerted TTANGO coordinators to any discordant results (where a difference between laboratory NAAT and the POC test result on the GeneXpert was observed) as soon as they were identified. The corresponding laboratory was then notified, and the original specimen stored (frozen) for additional reference testing.

Additional reference laboratory testing of discordant specimens

Stored samples with discordant POC and laboratory results were transported from the laboratories to the study reference laboratory in Melbourne (Royal Women's Hospital) for further investigation. Urine or samples received in Cobas transport media was tested using Cobas 4800 (for both CT and NG), in-house *OmpA* CT assay,(43) *PorA* and *Opa* NG assays.(44, 45) Any swab or Aptima sample received was extracted on MagNA pure 96 (Roche Diagnostic) and tested with the in-house *OmpA* CT assay, *PorA* and *Opa* NG assays. A final reference laboratory "positive" result was assigned to any sample in which CT or NG was detected by either the repeat Cobas 4800 assay or the in-house CT or NG assays. These final reference results were not used to revise original results but rather provide additional information as recommended in diagnostic evaluation guidelines.(46)

Data collection and statistical analyses

De-identified POC test results from the GeneXpert platform were captured via middleware (ONDAS, Medical Communication Associates, Adelaide, Australia) and entered in a secure SQL database (Microsoft SQL Server 2008, Microsoft Corporation, USA). GeneXpert results were compared to corresponding laboratory NAAT results as reported to the health service. Positive, negative and overall concordance(46) was determined separately for CT and NG between POC test and corresponding laboratory NAAT results, along with 95% confidence intervals (CI) by standard methods. Error results generated by the POC test were investigated informally by study coordinator and remedial actions implemented as needed.

POC test result cycle thresholds were analysed. Rank-sum and t-tests were used to assess if there was a significant difference in the median and mean cycle threshold values for concordant and discordant categories of POC CT positive samples, respectively. (47) As a proxy indicating low-organism load, proportions with cycle thresholds greater than 35 were also calculated.

Sample size calculation

Sample size calculations for the TTANGO trial are described elsewhere.(34) For this operational performance assessment, assuming a positivity of 10% in 16-29 year olds for CT/NG and POC test concordance of 95% or above, we aimed to include a minimum of 2000 POC tests (i.e. 200 POC test positives) and estimated confidence intervals around positive concordance would be +/- 4% and negative concordance +/-2%.

Ethics approvals

TTANGO was approved by the following Human Research Ethics Committees (HREC): West Australian Aboriginal Health Ethics Committee; Kimberley Aboriginal Health Planning Forum; West Australian Community Health Board Research Ethics Committee; Townsville Hospital and Health Service HREC; Aboriginal Health Council (South Australia).

Results

There were 2509 GeneXpert tests performed from 1 July 2013 to 30 April 2015. Among these, there were 2426 samples (96.7%) with a valid result at first test and 83 (3.3%) with an error. Of the 83 errors, 60 results gave a valid result on repeat test, 8 an error at repeat test and 15 had no repeat test conducted (Figure 1). The 2486 valid results (2426 results and 60 retest results) formed the basis of the concordance analysis.

Among the 2486 samples included in the concordance analysis, the laboratory NAATs gave a positive CT result in 212 (8.5%) samples and a positive NG result in 145 (5.8%) samples. Overall CT concordance was 99.4% with positive concordance of 98.6% and negative concordance of 99.5% (Table 1).

Table 1: Concordance between GeneXpert and conventional laboratory NAAT results for *Chlamydia trachomatis* (CT)

	NAAT Result		
GeneXpert	**Positive**	**Negative**	**Total**
Positive	209	11	220
Negative	3	2263	2266
Total	212	2274	2486
% concordant (95% CI)	**98.6 (95.9 – 99.7)**	**99.5 (99.1 – 99.8)**	**99.4 (99.1-99.8)**

Overall NG concordance was 99.9% with positive concordance of 100.0% and negative concordance of 99.9% (Table 2).

Table 2: Concordance between GeneXpert and conventional laboratory NAAT results for *Neisseria gonorrhea* (NG)

	NAAT Result		
GeneXpert	**Positive**	**Negative**	**Total**
Positive	145	2	147
Negative	0	2339	2339
Total	145	2341	2486
% concordant (95% CI)	**100.0 (97.5 – 100.0)**	**99.9 (99.7 – 100.0)**	**99.9 (99.7 – 100.0)**

A total of 16 (0.6%) discordant results were identified. Discordant results occurred in samples tested at most (5/6) laboratories and among both swab (n=6) and urine (n=10) specimens. The majority of the discordant results identified were for CT tests (n=14), with 11 positive on GeneXpert and negative on laboratory NAAT and 3 negative on GeneXpert

but positive on laboratory NAAT. Both discordant NG results were positive on GeneXpert but negative on the laboratory NAAT.

The difference in the mean cycle threshold between discordant (35.3) and concordant (29.1) GeneXpert CT positive results was statistically significant (p<0.001). The proportion of GeneXpert CT positive samples with cycle thresholds greater than 35.0 was 63.6% among discordant results compared to 6.2% among concordant results (p<0.001) (Table 3).

Table 3: Cycle threshold analysis among GeneXpert CT positive samples

GeneXpert CT positive	**N**	**Median* (IQR)**	**Mean[¥] (SD)**	**% Ct > 35.0∞ (95% CI)**
Discordant	11	35.7 (31.6 – 37.4)	35.3 (3.7)	63.6 (30.8 – 89.1)
Concordant	209	29.0 (26.3 – 32.6)	29.1 (4.2)	6.2 (3.4 – 10.4)

Tests of significance: *Ranksum : p<0.001. [¥]T-test: p<0.001. ∞Chi-squared (Fisher's exact): p<0.001. IQR = interquartile range. SD = standard deviation. CI = Confidence interval

Ten of 16 discordant samples had sufficient remaining volume for additional reference testing. Reference laboratory testing was in final agreement with GeneXpert in six samples and in disagreement for four samples (Table 4).

Table 4: Select characteristics of specimens with discordant GeneXpert and conventional laboratory NAAT results

Spec ID	Pat ID	Lab ID	Service ID	Spec type	Conventional laboratory NAAT* Results		GeneXpert Results		GeneXpert Ct[∞]	Reference laboratory NAAT[¥] result	
					CT	NG	CT	NG		CT	NG
1	1	1	2	Urine	CT neg	NG neg	CT pos	NG neg	34.2	Neg	-
2	2	1	2	Urine	CT neg	NG neg	CT pos	NG neg	37.2	Pos	-
3	3	2	6	Urine	CT neg	NG neg	CT pos	NG neg	29.6	N/A	-
4	4	3	1	Urine	CT neg	NG neg	CT pos	NG neg	30.3	Pos	-
5	5	4	4	LVS	CT neg	NG neg	CT pos	NG neg	37.7	N/A	-
6	6	4	4	Urine	CT neg	NG neg	CT pos	NG neg	37.4	N/A	-
7	7	2	8	Urine	CT neg	NG neg	CT pos	NG neg	37.2	Pos	-
8	8		3	Urine	CT neg	NG neg	CT pos	NG neg	31.6	Neg	-
9	9	2	6	Urine	CT neg	NG pos	CT pos	NG pos	42.4	Neg	-
10	10	3	12	Urine	CT neg	NG neg	CT pos	NG neg	35.5	Pos	-
11	11	3	12	Urine	CT neg	NG pos	CT pos	NG pos	35.7	Pos	-
12	6	4	4	LVS	CT pos	NG neg	CT neg	NG neg	0	N/A	-
13	12	2	3	LVS	CT pos	NG pos	CT neg	NG pos	N/A	Neg	-
14	13	4	4	LVS	CT pos	NG pos	CT neg	NG pos	N/A	N/A	-
15	14	4	4	LVS	CT neg	NG neg	CT neg	NG pos	37.5/35.5	-	N/A
16	15	5	7	LVS	CT neg	NG neg	CT neg	NG pos	35.3/33.9	-	Neg

LVS = Lower vaginal swab. * Aptima Combo 2 (Gen-Probe, San Diago, CA, USA), Cobas® 4800 (Roche Diagnostics, Pleasanton, CA, USA); In-house CT and NG (42).[∞] Ct=Cycle threshold for discordant result only (CT-one threshold; NG – two thresholds [2 targets]) [¥] Reference laboratory result for discordant infection only: performed at Royal Womens Hospital (Melbourne) - samples in Cobas transport media/urine retested by Cobas 4800 (for both CT and NG) and by In-house *Omp CT* assay, In-house *PorA* and *Opa NG* assays. Swab or Aptima samples were extracted and run on in-house *Omp CT* assay,(43) In house *PorA* and *Opa NG* assays(43-45) only. Positive result reported if detected on any one of these assays. N/A = sample not available. (-) =result not applicable (not discordant)

An error rate of 3.3% was observed across all 12 sites (83/2509), with a median of ten errors (IQR 5-12) per site. Almost one quarter of all errors (21/83) occurred at one site in a one month period; due to faulty cartridges. The timing of the remaining errors was mixed with some services experiencing errors in the initial months of POC test implementation while others on occasion throughout the study. These errors appeared to be primarily related to the test operator including inadequate volume of specimen (<1mL) being added to the GeneXpert cartridge.

Discussion

To our knowledge, the TTANGO trial represents the first use of the GeneXpert test platform for STI diagnosis at the POC, integrated into routine primary health care and performed by clinical staff. In our setting, clinical staff performing the test included doctors, but more often nurses and Aboriginal health practitioners, a professional category that has been established in Australia in recent decades to provide health care in services with primarily Aboriginal patients.(37) The analysis and results reported here demonstrate very high concordance of the GeneXpert for both CT (99.4%) and NG (99.9%) undertaken at the POC by clinical staff, compared with conventional NAATs routinely performed by scientists in laboratories. The POC results are consisted with those obtained under more controlled conditions and in the hands of dedicated, non-clinical operators. (23, 30, 41, 48)

POC test performance has been presented here as concordance rather than sensitivity and specificity given the comparison to a non-reference gold standard.(46) Concordance does not equate with accuracy as the non-reference standard used for comparison may also be incorrect. Concordance in this setting represents agreement with the best available test alternative currently being used, in this case laboratory NAATs. There is no evidence from the TTANGO study to confirm superiority of either test, and therefore commentary on false positives or false negatives with respect to the POC test has not been provided.

Very few (0.6%) results were identified as discordant and these appeared to be unrelated to operator, comparator NAAT or specimen type. The generally higher GeneXpert cycle thresholds observed with discordant results suggests the presence of lower organism loads, potentially at the limit of test detection. Further testing of these specimens by the

study reference laboratory resulted in mixed agreement, supporting the very high accuracy of both the GeneXpert and laboratory-based NAATs.

Around 3% of the GeneXpert tests led to an error result when first run, but the vast majority returned a valid result when repeated. Some errors occurred during the initial period of implementation when the POC test was new to the service, while others occurred during the more established period of implementation when staff turnover may have occurred. These errors appeared to be generally related to operator inexperience. Investigation by TTANGO coordinators identified inadequate sample volume resulting from poor pipetting technique as a likely cause. Experience with using plastic pipettes seemed limited at some study sites, thus staff experiencing such difficulties were retrained in pipetting techniques and offered alternative methods such as using a syringe to extract the required sample volume. TTANGO training programs were also modified to include a greater emphasis on achieving competency in extraction of adequate sample volumes. During the study, faulty cartridges appeared to be associated with a cluster of error results at one service. Replacement of stock rectified this problem. The outcomes of the investigation of errors highlights the need for good communication and logistics support to minimize disruptions to STI testing at services and maintaining staff confidence in performing the test and the result generated. We do not formally report here on incomplete or aborted tests where no result is generated and which usually are related to instrument failure or disruptions to power supply. Feedback from coordinators indicated that this outcome was infrequently observed, however during the study, some hardware including test modules and laptop computers, required maintenance or replacement.

Although testing was integrated as part of routine clinical practice at services, the study provided standardised training supported with regular telephone contact and in-person site visits by study coordinators. Staff turnover is generally high in remote primary care settings, however the training support offered as part of our study and the engagement of Aboriginal health workers/practitioners as POC test operators (who mostly reside locally in these communities) likely assisted in maintaining a relatively low error rate under these circumstances. Further evaluations are warranted to provide evidence of sustained high performance and clinical benefits of POC testing for CT and NG in these and other settings outside a well-supported research environment.

Remote monitoring of results in real-time allowed for the rapid identification of problems and implementation of remedial actions to avoid any further misuse or technical issues. The errors observed in this study appeared to have been associated with both operator and equipment and if they had not been rectified quickly may have reduced the uptake of POC testing in these services, particularly if alternative testing methods (laboratory-based) continue to be available. Though not described here in detail, the EQA and QC quality testing procedures implemented in TTANGO provided a complementary objective snapshot of the quality of GeneXpert use at each participating service. These quality procedures will be an essential component of any further expansion of STI POC testing both in pilot programs and beyond.

This study was done in a population with a very high prevalence of both CT and NG in very remote settings. It is possible that the POC test accuracy may be different when the prevalence is lower however the same would apply to laboratory NAATs. As noted recently by Chow and colleagues,(49) the use of highly sensitive testing in populations with a very low prevalence of NG can lead to greater numbers of false positive results. An advantage of the GeneXpert is the inclusion of two targets for NG, essentially having a screen and confirmation in one step. Commercial NAAT assays (Cobas 4800 and Aptima) detect one or two targets on the same gene, and laboratories are recommended to conduct a second confirmatory test for NG using a different assay with a different target for specimens from genital sites before reporting the results as positive, at additional time and cost.(50) For extra-genital NG testing (not performed in this study), three targets or additional genetic sequencing is required.

In conclusion, the results presented here suggest the GeneXpert integrated into remote primary health service and performed at the POC by trained clinical staff has excellent diagnostic performance, comparable with laboratory-based commercial and in-house NAATs. These findings, coupled with the logistic, clinical and public health advantages of being able to offer appropriate management to patients and their partners at the time of their initial consultation, strongly support the use of this POC test technology on a broader scale. However, to fully achieve the benefits and maximize its impact, POC testing must be combined with other strategies including increasing opportunistic testing, partner notification, retesting and health promotion in these remote Aboriginal communities with high rates of CT and NG.

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Competing interests

All authors declare that no competing interests exist.

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Chapter 7: Conclusions

The aim of the research presented in this thesis was to generate new evidence to support decision-making in relation to the use of POC tests for STIs. The research projects reported in this thesis specifically sought to provide evidence required to determine whether select POC tests meet requirements to advance along various steps of the pathway from initial technical development, through to laboratory and field evaluations, with the ultimate objective of program implementation.

7.1 Syphilis POC tests

Laboratory and operational research studies had previously demonstrated that traditional treponemal-only POC tests for syphilis had excellent performance characteristics in a variety of laboratory, field and programmatic settings.(1-5) However, there was comparatively little information available on the relative performance of competing POC syphilis tests. Also, at the time of commencing this thesis, it was unclear if the performances of these tests were affected by HIV co-infection. In many populations, syphilis occurs more frequently in people with HIV infection, so the question is of considerable practical importance. For example in Australia nearly half the new syphilis cases diagnosed among men who have sex with men are among those with HIV co-infection.(6) Another important question was the extent to which test performance was affected by syphilis stage.

As described in Chapter 2, a large, multisite, head-to-head laboratory-based evaluation of four POC tests for syphilis was conducted using archived Australian sera that could be classified according to syphilis stage and HIV status. The aim of the study was to evaluate the performance and ease of use of these tests, exploring potential differences by clinical stage of syphilis and HIV status. The evaluation included three treponemal-only tests selected based upon their commercial availability and suitability for future field use (Determine TP, SD Bioline, CTK Biotech) and one newly available treponemal/non-treponemal assay (Chembio DPP).

In the evaluation, all four tests were considered easy to use and interpret by the technicians performing the tests. When compared against the laboratory reference treponemal immunoassays (CIA and EIA), the Determine test was the best performing of

the POC tests, with a sensitivity of 100% in both primary and secondary stage syphilis. The other three tests had lower sensitivities among primary infection (77.3-88.7%) and while their sensitivities increased in secondary infections (94.3-98.6%), none reached 100%, so they would fail to detect a proportion of active infections. As described in Chapter 1, missed infections are of particular concern for antenatal populations, in whom untreated syphilis can lead to serious pregnancy outcomes in the short-term including stillbirth, early foetal death, low birth weight, preterm delivery, neonatal death, or infection or disease in the newborn.(7) Among men who have sex with men, untreated infection could increase the likelihood of onward transmission of syphilis and the risk of HIV transmission.(8) The performance of all four tests appeared to be unaffected by HIV status.

The treponemal performance and ease-of-use findings across the four POC tests for syphilis are similar to other evaluations and support the role for these tests, particularly the Determine test, for antenatal and other screening in countries that do not have access to standard laboratory tests for syphilis, as per WHO and other recommendations.(3, 9) Further programmatic efforts are needed to ensure coverage reaches 100% in a number of countries, particularly in settings of high prevalence. Combined HIV and syphilis POC tests may assist in improving uptake.(3)

The use of syphilis POC tests in high income countries such as Australia might be considered in populations with high rates of syphilis and HIV co-infection, and in which loss to follow-up is a significant risk. An important limitation of treponemal-only tests is their inability to distinguish past, treated infection from new infection, an issue that is of particular importance for high incidence populations such as men with HIV infection in Australia. In this context treponemal-only tests could be utilised in a screening strategy with any positive POC test leading to venepunture for routine laboratory-based syphilis serology and retesting with a non-treponemal assay.

One of the four POC tests included in the performance comparison was a novel test capable of detecting both treponemal and non-treponemal antibodies (Chembio DPP Screen and Confirm), which had been newly introduced in 2010. At the time of commencing this thesis in early 2011, there were limited performance data available for the test,(10) and none quantifying the extent to which it could be used to avoid over-diagnosis (and treatment) compared to traditional treponemal-only POC tests. No laboratory-based or field-based operational performance data were available from Australia.

Chapter 3 is based on a sub-analysis of data from the laboratory-based evaluation and focuses on the performance of the Chembio DPP test. The study also explored the potential clinical implications of the test. We found that although the DPP test would detect most active infections (>93%), only around half (50.2%) of the past, treated cases would be determined as not requiring treatment. These findings suggest that this POC test could lead to a reduction in unnecessary treatment, but it may come at the expense of a somewhat reduced treponemal sensitivity, resulting in missing cases that may truly require treatment. When comparing to the best available treponemal-only assay (Determine) it is important to consider the balance between the reduction in over-treatment offered by the DPP test and the relatively lower sensitivity, resulting in some false negative results. The population context will be important to consider in this comparison. If there is a substantial proportion of past/treated infection (or yaws, which is serologically indistinguishable from syphilis), a treponemal-only POC test may be preferred or, alternatively, a treponemal-only test might be considered as first line, followed by the dual test to minimise over-diagnosis and subsequent treatment. The DPP test may prove useful to support the monitoring of programs to eradicate yaws, a disease that is generally endemic in regions where standard laboratory methods are unavailable.

The potential benefit of using the dual test as a secondary test was recently explored in a model-based cost-effectiveness evaluation comparing a number of different syphilis screening strategies using available POC tests. The study focused on pregnant women attending antenatal clinics in Peru, Tanzania and Zambia.(11) The model considered 10 screening and treatment approaches including testing algorithms involving RPR only, treponemal-only POC tests and combined treponemal/non-treponemal (dual) POC tests, as well as no treatment and mass treatment. The authors concluded that treponemal-only tests were the most cost-effective option given the current costs of dual tests ($2.50), while the sequential testing regimens were more costly but could reduce over-treatment.(11) The authors also found that cost-effectiveness of these interventions could be improved with increased screening coverage.(11) Mass treatment, though controversial, was considered to be relatively inexpensive and effective in the absence of screening supplies, but would result in unnecessary treatment of many women.(11) With a reduced cost of dual tests and increased coverage, it is possible that a sequential approach may become more cost effective than treponemal-only strategies in these settings.

Since we conducted and reported our study, three additional papers have appeared reporting on the performance of the Chembio DPP test.(12-14) These studies report good performance of the DPP test (for each of the treponemal and non-treponemal components) with treponemal and non-treponemal sensitivities ranging from 90.0 - 99.5% and 87.2 - 98.8%, respectively.(12-14) The study from China found the performance of the test in outreach settings was as good as that in clinical settings and that there was no difference in performance between specimen types (whole blood, finger prick and serum).(13) These results demonstrate that the test can be performed to a high standard with different types of specimens, in clinical and non-clinical settings, and by clinical staff or outreach team members. The papers published since our evaluation did not consider the performance of these tests according to HIV status.

The DPP test has also been evaluated recently for screening and diagnosis of yaws utilising samples collected as part of a community prevalence survey in the Solomon Islands(15) and among samples (blood and plasma) collected among clinically suspected cases of yaws as part of a follow-up survey in the eradication effort in Papua New Guinea.(16) In the Solomon Islands, the sensitivity of the DPP test components (treponemal and non-treponemal) appeared to be much lower (59% and 42% respectively) than reported estimates for syphilis (>85%), possibly due to generally lower antibody titres often found in people with yaws compared to those with syphilis.(17) As in syphilis, the DPP test sensitivity in yaws increased (to 92%) with higher RPR titres (>1:16).(15) In contrast, sensitivities were higher in the PNG study (both treponemal and non-treponemal were 88%), with non-treponemal sensitivity increasing to 94% at higher RPR titres (>1:8).(16) Given these mixed results, further evaluations may be required to better define the role of the DPP test in assessing patients presenting with yaws-like lesions in the setting of an eradication environment.

Also subsequent to our reports, the dataset of more than 1000 records that we used for syphilis laboratory evaluation in Australia has contributed to a recently conducted meta-analysis of DPP test performance, including a total of nine datasets. The combined large sample size for the meta-analysis will allow for more accurate performance estimates to be calculated across a range of sub-groups defined by syphilis stage, RPR titres, infection type (yaws or syphilis), and comparator reference test.

7.2 Chlamydia and gonorrhea POC tests

As explained in Chapter 1, chlamydia and gonorrhea infections contribute substantially to the global burden of STIs.(18, 19) In Australia the highest incidence and prevalence of both infections are reported among young people in remote Aboriginal communities, so there is urgent need to identify new strategies to improve the uptake of testing and treatment.(6) POC tests offer one potential solution to the problem of delays in treatment resulting from the use of long distance laboratories.

Evaluations and reviews of the evidence of a variety of traditional lateral flow POC tests for detection of CT and NG have demonstrated dismal performance and inconvenient timed testing processes.(20-22) Recent technological advances have led to the development of molecular-based POC testing devices capable of using methodologies previously limited to well-resourced reference laboratories with trained technicians. The first of these devices to be commercially available was the Cepheid GeneXpert, which uses pathogen-specific cartridges and was initially promoted globally for the diagnosis of tuberculosis.(23) The development of the CT/NG cartridge opened up the possibility of using this device for point of care diagnosis of these infections. The first evaluations of the GeneXpert CT/NG assay in laboratory (24) and in clinical settings (with specimens collected routinely at clinical services and testing performed at a mix of investigational sites and a study laboratory)(25) showed that it had a very high sensitivity and specificity, but it had not yet been shown to be feasible for clinical POC use or that its high performance could be maintained in the hands of clinicians in a primary care setting.

We therefore conducted a pilot field study of CT and NG POC tests (Chapter 5), to provide evidence in the next step of the evaluation pathway. The pilot field study aimed to determine the operational performance of the test, and explore the feasibility, acceptability and ease of use of the GeneXpert CT/NG assay as compared to two of the better performing lateral flow tests in two clinics providing health services to Aboriginal people in remote communities. The evaluation was conducted in the context of annual STI screening that the clinics were conducting in their communities, thereby allowing for the desired sample size to be achieved in a relatively short time period.

In contrast with laboratory-based studies, the field-based evaluation allowed us to assess performance of the assays in a more “real world” setting characterised by: (i) non-laboratory technicians performing the tests and (ii) tests conducted on fresh specimens as

they were collected from patients attending the health service for routine care or screening. As with a laboratory-based study, staff performing the tests were blinded to all clinical information and the reference test results. During this pilot field evaluation laboratory results continued to guide patient management according to regional guidelines as the GeneXpert POC test was not yet approved for diagnostic use by Australia's regulatory body (TGA). As a result, this study was not able to evaluate the impact on time to treatment by using the POC tests.

The results demonstrated substantially superior performance of the GeneXpert, with CT sensitivity of 100% and specificity of 99.5%, compared to 27.3% and 66.7% respectively for the lateral flow test; and NG sensitivity and specificity of 100%, compared to 66.7% and specificity of 76.9% for the lateral flow test.

Unlike a laboratory-based study, the pilot field study provided the opportunity to ascertain how these POC tests might be incorporated into the clinic workflow and to explore the perspectives of the operators and clinic staff regarding the use of the tests, acceptability and likely suitability in similar remote settings. Despite the length of time to result with the GeneXpert test being longer than traditional lateral flow tests (90 minutes vs 10 -15 minutes), there were fewer specimen preparation steps and minimal preparation time for the GeneXpert (four steps; 1 minute preparation) compared to the lateral flow tests (eight steps; 18 minutes preparation).

The field study provided some insight into the feasibility and potential scale of logistics that might be required to include a molecular-based POC test such as GeneXpert for STI diagnosis into a large scale trial of STI POC testing performed by clinical staff as part of routine care. The GeneXpert CT/NG failed to meet some of the ASSURED criteria (26) (rapidity of result and scope of equipment required), but its high accuracy and substantial operational benefits over the traditional lateral flow are good indicators of its potential suitability for use in such remote settings where delays to treatment, and loss to follow-up following a positive test are frequently encountered.

Guided by the findings from this pilot field study, a large scale trial known as TTANGO (Test, Treat and Go) was implemented as the next step in the evaluation pathway to determine clinical impact and quantify operational performance and acceptability, feasibility and cost/cost-effectiveness of a POC testing strategy for STIs using the GeneXpert CT/NG assay. As described in Chapter 4, the design was a cross-over RCT trial in which 12 remote health

services (clusters) were randomised to commence with either standard STI care or standard care plus POC testing for CT and NG for 12 months. At the completion of the first period, each service then crossed over to the alternative care strategy for the next 12 months. Services were selected based upon a number of criteria including likely sufficient number of positive CT and NG results to achieve the required sample size (a limitation already noted for the pilot study). In both phases (standard care and POC testing), specimens continued to be sent to jurisdictional laboratories for routine NAATs. This not only provided a reference test result as the comparator for an estimation of POC test performance but also ensured no disruption to processes for mandated STI notifications and culture/isolation for gonococcal AMR monitoring conducted by these reference laboratories.

In contrast to the pilot study (and as TGA had by then approved the GeneXpert for diagnosis on CT/ NG in 2013), the POC test result guided STI management for asymptomatic patients (syndromic management continued as per guidelines for symptomatic and high-risk clients). In the standard care phase, results of routine laboratory NAATs continued to guide STI management as usual. Designated clinical staff from the health service were trained to perform the POC test (rather than study staff as was done in the pilot study) and incorporated the POC test as part of routine clinical practice. Treatment could therefore be offered immediately to those found to be positive by the POC test.

Chapter 6 reports on the operational performance of the GeneXpert CT/NG assay in the context of this trial. With more than 2500 POC tests performed, overall concordance with laboratory-based NAATs for CT was 99.4% (95% CI: 99.1-99.8) and for NG was 99.9% (95% CI: 99.7-100.0). The large sample size provided more precise performance estimates (narrower confidence intervals) compared to those in the earlier pilot study. Concordance in this setting represents agreement with the best available test alternative currently being used, in this case laboratory NAATs. There is no evidence from the TTANGO trial to confirm superiority of either test, and therefore commentary on false positives or false negatives with respect to the POC test is not provided. Analysis of the GenXpert cycle thresholds and the additional testing at the study reference laboratory of the discordant results (using a combined algorithm of both a commercial and in-house molecular CT and NG assays) suggests that these discordant results have arisen mostly in specimens with low organism loads, possibly from degradation due to the long journey to the laboratory. The results of this discordant testing were not used to modify our estimates of concordance, which is in keeping with recommendations for reporting.(27)

As far as we are aware, the TTANGO trial represents the first use of the GeneXpert CT/NG assay for point of care use in a general, primary care setting anywhere in the world, although it has been used in specialised STI primary care settings.(28) The findings strongly suggest that the GeneXpert CT/NG assay, integrated into these settings and performed at the POC by trained clinical staff is both highly practical and diagnostically accurate to a level comparable with laboratory-based commercial and in-house NAATs. As mentioned in Chapter 4, the TTANGO trial aimed to provide evidence not only on operational performance of the POC test but also on clinical impact (measured in the short term in this trial by a change in numbers of reinfections), acceptability of the POC test and testing to patients and staff (measured through qualitative and quantitative interviews and questionnaires), cost and cost-effectiveness. Some of the results of these objectives which complement the work in this thesis have been presented and published already by co-investigators as briefly noted below.

Acceptability of POC testing was assessed through a series of qualitative studies (in-depth interviews and self-administered questionnaires) through the perspective of key informants and stakeholders (29, 30), and staff (31) and patients (unpublished). Stakeholders believed POC testing could achieve anticipated outcomes of reduced time to treatment and expedited partner notification though they also highlighted the need for systems to be developed to maintain the completeness of notification reports and gonococcal antimicrobial resistance (AMR) surveillance.(29, 30) Most staff reported the GeneXpert CT/NG test was both easy to use and improved STI management, resulting in more timely and targeted treatment, earlier commencement of partner notification, and reduced time and effort associated with client recall.(31) For some, barriers included the manual documentation processes and managing positive test results in a shorter time frame.(31) Preliminary data on time-to-treatment suggests that even at a health service with a strong and comprehensive STI control program further improvement in STI management can be achieved with POC tests, with treatment occurring on average 8 days sooner for those treated on the basis of a test result.(32)

Beyond the topics considered in this thesis, key issues for POC test implementation that were incorporated in TTANGO include training, and quality assurance. The experience gained through designing and implementing these programs will require formal assessment to ensure that findings will be available to guide future implementation of POC testing in

Australia. Funding of tests and linkage to public health notification systems for STIs will also need to be taken into consideration.

Building upon the lessons learned and findings from the TTANGO trial, and enthusiasm from the participating health services to continue POC testing, funding (through NHMRC and Australian Department of Health) was secured in 2015 to design and implement larger scale roll out of POC testing for CT/NG in 33 remote health services in Australia. The expansion of POC testing will be undertaken in concert with other key strategies to increase testing coverage, retesting and partner notification. The program will also introduce POC testing for trichomoniasis using the same GeneXpert platform, as this new assay has recently been demonstrated to have good performance in the laboratory (33) and to be feasible for use alongside testing for chlamydia and gonorrhoea.(34) This expanded program, known as TTANGO2, includes a formal monitoring and evaluation component to comprehensively assess the clinical impact of POC testing on STIs, impact on testing for HIV and syphilis (non-point of care diagnosis), program cost, training methodologies, alternative gonococcal AMR surveillance and STI notification strategies.

Availability and use of the GeneXpert at the POC for STI diagnosis has the potential to transform management and control of STIs in remote Australia and other high burden settings with rapid results ensuring timely treatment. Ideally, to measure the impact of this strategy, significant longer term clinical outcomes such as PID should be assessed. In these settings with very high incidences and prevalences of curable STIs, control is likely to require more than just the availability of POC testing; a combination of approaches will be needed including increasing opportunistic testing, partner notification, retesting and health promotion in these remote Aboriginal communities with high rates of chlamydia and gonorrhoea.(6, 35) Determination of costs and cost-effectiveness of these approaches will be essential to advocate sustainable funding over the longer term.

Chapter 8: Recommendations

The following recommendations are based upon the findings generated through the research described in this thesis and are proposed in the context of other relevant work being implemented and recently published around POC testing for STIs. These recommendations have been presented and discussed with co-investigators and colleagues but not formally endorsed by them. As such they represent the views of the author and are intended to promote discussion of next steps and future work.

8.1 Syphilis POC tests

Our research demonstrated that a selection of treponemal-only POC tests for syphilis and one novel dual test perform well in an Australian laboratory setting and could be incorporated into a comprehensive POC STI testing strategy among populations at greatest risk. Whether the benefits of using the DPP test (being able to distinguish some cases of past, treated syphilis from current active cases), outweighs the limitations (a number of missed cases) needs further consideration through both empirical research and cost-effectiveness modelling.

For syphilis in urban, gay men in Australia, time to treatment and loss-to follow-up are of lesser concern than they might be in an endemic low resource setting in which maternal transmission is a threat. POC tests for syphilis may nevertheless have a role when there is a need to rapidly and substantially increase testing coverage. For example, they have been used to respond to the recent outbreaks of infection in remote communities of central and northern Australia (36) and they could also be of relevance in an at-risk population in an urban setting. Circumstances where opportunistic screening is suitable may also be amenable to POC testing such as cultural or sporting events where increased testing coverage may lead to identification of potential cases for further follow-up.

At this time, we recommend:

- Continue traditional laboratory-based serological diagnosis of syphilis in Australia
- Conduct field research to explore circumstances in Australia in which POC testing for syphilis might add benefit through increased testing coverage and treatment completion, e.g. outbreaks, outreach or opportunistic settings, possibly in combination with HIV POC testing

- Conduct laboratory-based and/or field research as appropriate on other new, promising dual tests (treponemal and non-treponemal), and combination/dual infection tests such as for HIV plus syphilis

8.2 Chlamydia and gonorrhoea

Our operational research has demonstrated that the GeneXpert CT/NG test used clinically in remote health services performs as well as currently available laboratory based NAATs and is feasible for use in this setting. Other complementary findings generated from the TTANGO trial suggest that the POC test and strategy are welcomed by high-level stakeholders,(29) and both clinical staff (31) and patients (unpublished). Preliminary results on clinical impact suggest a significant reduction in time to treatment,(32) but analysis of the primary RCT endpoint (reduction of reinfections) has not been undertaken yet.

As a next step in the evaluation pathway, the recently funded expansion of POC testing for CT and NG strategy (TTANGO2) will be implemented and formally assessed to evaluate its impact, sustainability, costs and acceptability. To reduce the STI prevalence in this high burden setting, POC testing will need to be accompanied by health promotion and other strategies to increase testing, partner notification, retesting and treatment timeliness. For example, in Western Australia, clinical guidelines have recently changed to recommend bi-annual STI screening in endemic regions.(37)

In addition to its broader goals, it is important that the evaluation of the TTANGO2 POC testing program assesses

- Impact of the multi-faceted STI program on STI clinical practices in health services including changes in the uptake and frequency of STI testing and timeliness of treatment
- Impact on re-infections, STI prevalence and if possible, sequelae of STIs such as PID. These outcomes are difficult to measure in implementation studies, so may need to be explored using mathematical modelling
- Cost and cost-effectiveness of testing, with a view to providing evidence to inform applications for sustainable funding mechanisms (through government schemes such as Medicare)

- Impact on other STI testing (HIV and syphilis in particular) - to ensure the introduction of POC testing for CT/NG and TV does not have any unintended detrimental effects to non-POC testing for other STIs
- Whether the program meets public health surveillance goals, particularly STI notification and gonococcal AMR surveillance
- Acceptability of the training model to clinical staff and quality management processes
- Compatibility of POC device with current electronic patient management systems to record POC test results (and mimic, where possible, electronic delivery processes for standard laboratory results)
- Training methodologies to ensure competency of clinical staff/test operators

This GeneXpert device has the potential to be used for screening or diagnosis of other infections that are related to sexual and other risk behaviours including HIV (for the detection of very early infection), HPV (human papilloma virus), and hepatitis C. In 2017, Australia will implement a new program for cervical cancer screening, based on 5-yearly HPV screening rather than traditional Pap tests. In remote Aboriginal communities, health services will likely face similar issues to those that arise with STI testing, requiring that specimens be sent to centralised urban laboratories, the need to follow up in a mobile population and difficulties recalling patients once results are received.

We recommend future research include:

- Investigation of how the GeneXpert POC can be used to expand access to HPV screening in remote Aboriginal communities and how best to integrate STI and HPV screening (recognising that target age groups for HPV screening are likely to be older than for other STIs)

As recently reported by Natoli et al, there is also potential for the GeneXpert POC for chlamydia and gonorrhoea to have benefits in other populations and settings.(38) The recent release (2015) of the small, portable, battery operated, single cartridge “Omni” platform from Cepheid is likely to be more feasible for use in mobile outreach programs, when available for STI assays, than the current more bulky versions used in our clinic-based studies.

We recommend future research include:

- Investigation of use of POC testing for STIs using new, more portable devices in outreach programs and prison or other settings where high-risk individuals may experience barriers to access regular clinical facilities and there may be significant loss to follow-up before treatment.

As described in Chapter 1, curable STIs are important causes of adverse maternal and neonatal health outcomes but in many resource-limited settings these infections remain undiagnosed and therefore untreated in pregnancy, because of a lack of suitable diagnostic technology. Evaluations of the effectiveness and costs of diagnosis and treatment of STIs in pregnant women using the GeneXpert assays for STIs (including CT, NG and TV) in antenatal clinics in resource-limited settings are a priority.

One such trial is about to commence at a number of antenatal clinics in Papua New Guinea. This trial will use a cluster-randomised design to compare birth outcomes between women attending antenatal clinics who were offered POC STI testing with those who were not, and will evaluate the implementation requirements, costs and acceptability of antenatal POC STI testing and treatment compared to routine care. The trial will have a potentially major impact on practice in all low-resource, high-burden settings.

In conclusion, the research presented in this thesis has contributed substantially to the knowledge surrounding the use of POC tests for STIs in Australia and already influenced approaches to programmatic implementation. Although much remains to be done to realise the potential benefits of POC testing for STIs at the patient and community level, this research along with future work conducted in the framework of the evaluation pathway will ensure that these tests and testing strategies are in implemented in the most appropriate and effective ways possible.

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