Optimizing *Chlamydia trachomatis* and *Treponema pallidum* diagnostics

Laura van Dommelen

OPTIMIZING *CHLAMYDIA TRACHOMATIS* AND TREPONEMA PALLIDUM DIAGNOSTICS

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PROMOTOREN

Prof. dr. C.J.P.A. Hoebe

Prof. dr. C.A. Bruggeman

COPROMOTOR

Dr. F.H. van Tiel

BEOORDELINGSCOMMISSIE

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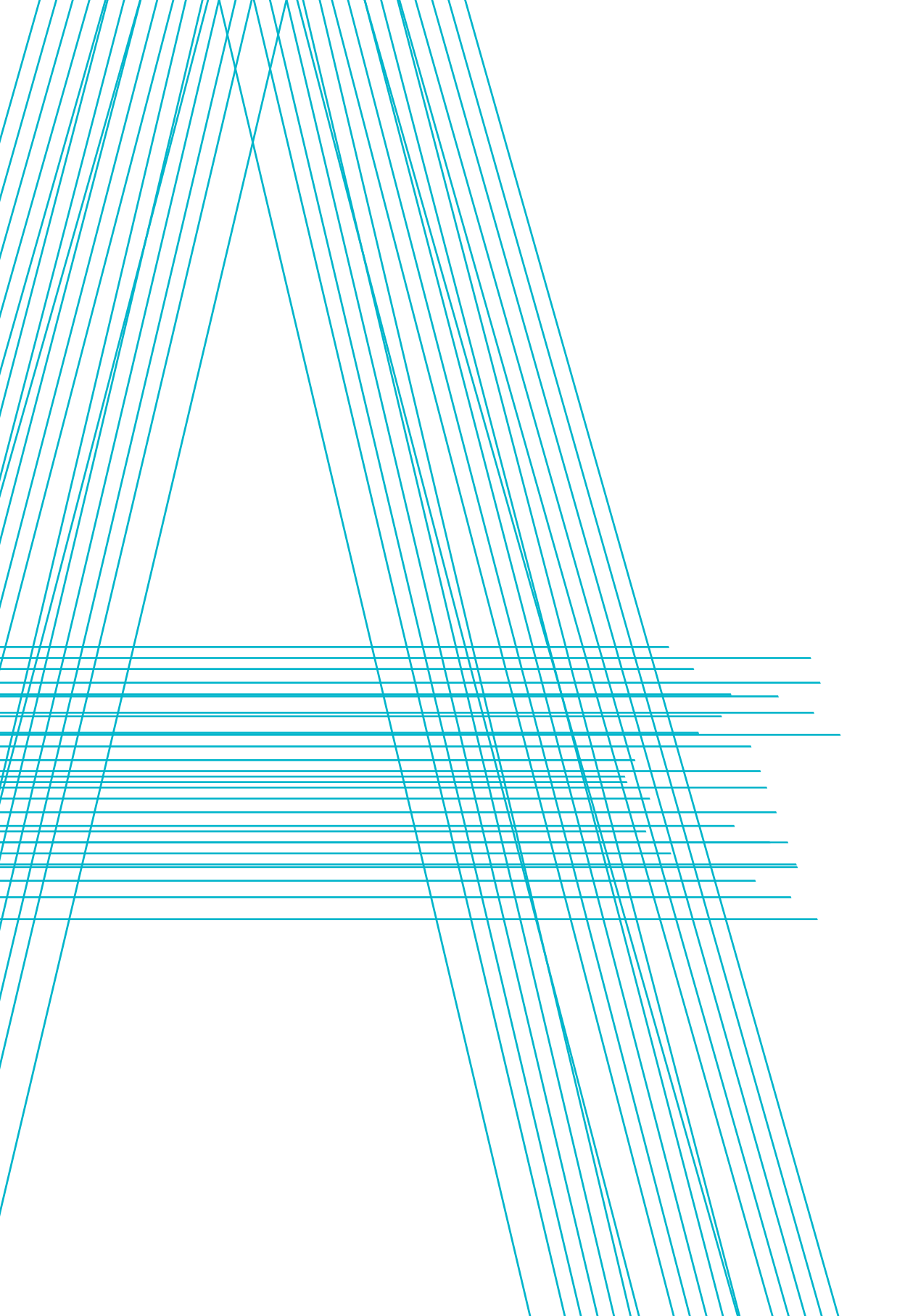
ABBREVATIONS

CDC	Centers for Disease Control and Prevention
CI	Confidence interval
Ct/CT	*Chlamydia trachomatis*
EB	Elementary bodies
ELISA	Enzyme-linked immunosorbent assay
FCU	First-catch urine
HBV	*Hepatitis B virus*
HIV	*Human immunodeficiency virus*
HSV	*Herpes simplex virus*
IFU	Inclusion forming units
LGV	Lymphogranuloma venereum
MSM	Men who have sex with men
NA	Not assessed
NAAT	Nucleic acid amplification test
ND	Not detected
Ng	*Neisseria gonorrhoeae*
NPV	Negative predictive value
nvCT	New variant *Chlamydia trachomatis*
Omp A	Outer membrane protein A
PCR	Polymerase strain reaction
PID	Pelvic inflammatory disease
POC(T)	Point-of-care (test)
PPV	Positive predictive value
qNAAT	Quantitative nucleic acid amplification test
RB	Reticulate bodies
SOA	Seksueel overdraagbare aandoeningen
SDA	Strand displacement amplification
STD	Sexually Transmitted Diseases
STI	Sexually Transmitted Infections
SVS	Self-taken vaginal swab
swCT	Swedisch variant *Chlamydia trachomatis*
Tp	*Treponema pallidum*
Tv	*Trichomonas vaginalis*
VDRL	Venereal Disease Research Laboratory test



Introduction

1. Introduction on sexually transmitted infections with a focus on *Chlamydia trachomatis* and *Treponema pallidum*.

Laura van Dommelen

GLOBAL BURDEN OF SEXUALLY TRANSMITTED INFECTIONS WITH A FOCUS ON *CHLAMYDIA TRACHOMATIS* AND *TREPONEMA PALLIDUM*

Sexually transmitted infections (STI) are a major medical and public health challenge, due to their high incidence and emerging threat of drug resistance. In 2008, WHO estimated 498.9 million curable STI, caused by *Treponema pallidum* (Tp), *Neisseria gonorrhoeae* (Ng), *Chlamydia trachomatis* (Ct) and *Trichomonas vaginalis* (Tv). Moreover, millions of viral STI occur every year, mainly caused by *human immunodeficiency virus* (HIV), *herpes simplex viruses* (HSV), *human papilloma viruses* en *hepatitis B virus* (HBV). The number of newly acquired infections was 105.7 million for Ct and 10.6 million for syphilis according to WHO. Most curable STI occur in sub-Saharan Africa (incidence 92.6 million) and the Americas (incidence 125.7 million). All figures however are hampered by the lack of good prevalence studies, limited healthcare seeking and inadequate access to healthcare, among others.[1]

Syphilis infection, caused by Tp, during pregnancy contributes to 650,000 fetal and neonatal deaths in developing countries, while prevention of congenital syphilis only costs $1.50 per person. Large scale screening in pregnant females in high burden countries is however not practised yet.[2] With Ct infection, under-reporting is huge because most people are not aware of their infections and do not seek help. People with genital *chlamydia* may experience symptoms of genital tract inflammation including urethritis and cervicitis, but the majority remains asymptomatic. Ct is a significant public health problem because untreated Ct may lead to pelvic inflammatory disease, subfertility and poor reproductive outcomes in some women.[3] Concurrent STI facilitates the transmission of HIV, which is the case in 40% of all newly acquired HIV infections, which is a concern in countries with high HIV prevalence. Furthermore, when STI coexist with HIV, higher loads of HIV are shed into genital fluids, compared to individuals who do not have a coexisting STI. All STI together cause 17% of the economic loss due to health related causes in developing countries. In developed countries, the costs for STI screening, HIV treatment, managing infertility due to STI and cervical cancer treatment are substantial.[4]

Sexually Transmitted Infections in The Netherlands

STI's continue to be a serious health problem in the Netherlands. Certain ethnic minorities (especially (former) inhabitants of Surinam and the former Netherlands Antilles), young persons (<25 years of age) and men having sex with men (MSM) are important risk groups for STI.[5] Ct is the most prevalent STI in the Netherlands and the incidence is highest in the heterosexual population under 25 years of age. At the different STI centres, the test positivity rate was 11.5% in the year 2011. Lymphogranuloma venereum (LGV) was only detected in MSM and 79% is known to be also HIV positive. The test positivity rate for Ng was much lower, 3.2%, and was mostly detected in MSM. Syphilis was rare: only 476 cases were detected in 2011. Almost 90% of the cases concerned MSM and the positivity rate has decreased since 2007 (from 4.3% to 2.0% in 2011). The prevalence of syphilis among pregnant women was estimated at 0.2% in 2009, which is a slight increase compared to previous years.[5]

Most first-line STI related consultations are handled by the general practitioner (63%) [6]; the remaining consultations are mainly managed in STI clinics (e.g. Public Health Service). Standard STI testing includes Ct, Ng and syphilis. HIV opting out is now widely practiced, which means HIV testing is performed unless the client refuses.[7] HBV, *hepatitis C virus*, genital herpes and Tv are only tested when deemed necessary.[5] Pregnant women are all screened for syphilis, HBV and HIV before the 13th week of pregnancy.

The prevention and control of STI is based on five major strategies described by the Centers for Disease Control and Prevention (CDC): education and counselling of persons at risk in order to achieve changes in sexual behaviour; identification of asymptomatically infected persons and of symptomatic persons unlikely to seek diagnostic and treatment services; effective diagnosis and treatment of infected persons; evaluation, treatment, and counselling of sexual partners of persons who are infected with an STI and pre-exposure vaccination of persons at risk for vaccine-preventable STI.[8] In general, the Dutch population is well informed concerning sexual health, although this is not the case in adolescents, lower educated individuals and men with a Turkish or Moroccan background. National campaigns on sexual health are effective in creating awareness and condom use is considered important to prevent STI, but its use is not always practiced. The number of STI consultations are increasing every year and the percentage of positive STI tests has increased from 12% to 14% between 2004 and 20105 (Figure 1). MSM have a higher percentage of positive STI tests compared with heterosexuals.

CHLAMYDIA TRACHOMATIS

Ct infection is the most prevalent bacterial STI worldwide and causes most cases of infection-related female infertility worldwide.[4]

Organism

Ct is an obligatory intracellular, non-motile, Gram negative organism. The developmental cycle consists of an extracellular and an intracellular part (Figure 2). The extracellular form of Ct consists of elementary bodies (EB). The cell wall of EB does not contain peptidoglycan, but has a rigid wall as a result of a highly cross-linked outer-membrane complex.[9] EB are able to survive outside the cell and stay dormant until entering the host cell. Cell entry consists of two stages; in the first stage, the EB attaches through reversible electrostatic interactions

between the outer membrane protein A (OmpA), expressed on the Ct cell surface and the heparan sulphate containing glycosaminoglycans on the cell. During the second, irreversible binding stage the EB probably interact with protein disulfide isomerase, although this has not yet been fully elucidated.[9] After this step, translocated actin-recruiting phosphoprotein is released into the host cell via type three secretions system, leading to actin remodelling, which in turn allows EB to enter the host cell and inhibits apoptosis.[10] Intracellularly, EB transform into metabolically active, non infectious, reticulate bodies (RB). RB replicate and in the end of the intracellular cycle transform back into EB. The EB are again released into the extracellular space by exocytosis or cell lysis, able to infect other epithelial cells. This replication cycle takes about 48-72 hours.

Figure 1 **Number of consultations and percentage of positive STI tests (*chlamydia*, gonorrhoea, infectious syphilis, HIV , infectious hepatitis B) in the national STI surveillance in the Netherlands, 1995-2011** [5]

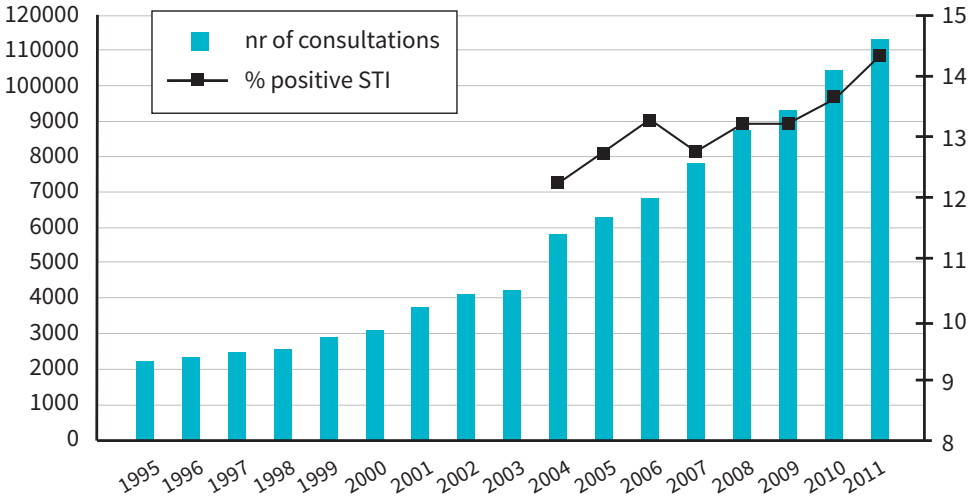
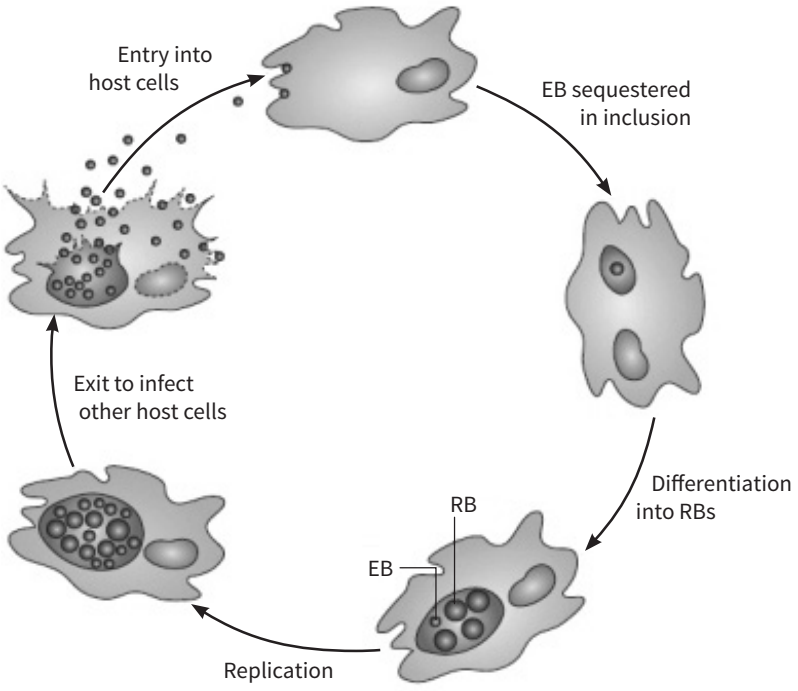


Figure 2 *Chlamydia trachomatis* developmental cycle [11]



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The family *Chlamydiaeceae* consists of three genera: *Chlamydia*, *Chlamydophila* and *Clavochlamydia*. The genus *Chlamydia* can be subdivided in three species: *C. trachomatis* (human), *C. suis* (swine) and *C. muridarum* (mice). The human variant comprises of biovar trachoma and lymphogranuloma venereum (LVG). The biovar trachoma consists of 14 serovars (A, B, Ba, C, D, Da, E, F, G, H, I, Ia, J, K) of which A-C have most affinity to conjunctivae and D-K to urogenital epithelium; LGV consists of serovar L1, L2, L2a and L3. Serovars E and F are most prevalent in The Netherlands.[12] This thesis will focus on *C. trachomatis* biovar trachoma serovars D-K.

Pathogenesis

Histological studies on Ct infection show both signs of acute and chronic inflammation in the affected tissue. Epithelial cells are the primary target of Ct and initiate the immune response.[13] Secondly, neutrophils infiltrate the vagina and subsequently the uterus during the first week after infection.[14] Subsequently, epithelial cells and innate immune cells produce proinflammatory cytokines and chemokines which further activate the innate and adaptive immune cells. Ultimately, resolution of infection occurs, but sometimes leaving a destroyed oviduct. Tissue damage can be the result of chronic infection (continues low grade tissue damage) and/or reinfections which leads to the predominance of CD8 T-cell, associated with scarring and fibrosis.[15] The mechanisms behind chronic infection due to Ct infections are not clear. The degree of infection is host dependent and may be dependent on genetic polymorphisms.[16-18] Cervical lymphocytes of females with or females without fertility problems, for instance, produce different cytokine profiles when stimulated with Ct elementary bodies.[16] Also, a study on monozygotic and dizygotic twins on the influence of genetic trait on the immune response to Ct (serovar A), suggested that genetic factors contribute for 39% to the variation in lymphoproliferative responses.[19]

Transmission and natural course

Ct can be transmitted via sexual contact or perinatally. The incubation period for acute infection is 7-14 days. Infection concordance for Ct between sexual partners is approximately 70%.[20-22] The transmission and natural course of Ct infection depends on Ct virulence factors, host immune response and the presence of co-infections. Approximately 50% of all uncomplicated Ct infections in females resolves without therapy during the first year after acquisition.[23] Almost 20% of all infections is already cleared within the interval between testing and treatment, which is on average 13 days.[24]

Clinical manifestations and complications

Infection with Ct is asymptomatic in the majority of the women and in more than 50% of the men. When symptomatic, women can experience abnormal vaginal discharge, abdominal pain or post-coital bleeding for instance. Other clinical manifestations in women include cervicitis, urethritis, conjunctivitis, endometritis and pelvic inflammatory disease (PID). The incidence of PID is heavily debated, since methodology and results vary between different studies and is suggested to be between 5 and 20%.[24-29] PID due to Ct can progress subclinically and may eventually might lead to tubal infertility.[30, 31] It is also associated with chronic abdominal pain.[32, 33] Another important complication is adverse pregnancy outcome. In neonates, perinatally

acquired infection may result in for instance pneumonia and/or conjunctivitis.[34] In men, Ct can cause urethritis, prostatitis, epididymitis, conjunctivitis and Reiter's syndrome (arthritis, conjunctivitis and urethritis).

Diagnosis

The mainstay for the diagnosis of Ct are nucleic acid amplification tests (NAAT). Table 1 gives an overview of the different, commercially available, NAAT used to detect Ct. NAAT are usually performed on self-taken vaginal swabs (SVS) in females and on urine in men. When indicated, NAAT can also be performed on many other materials, for instance pharyngeal, rectal of corneal swabs and peritoneal fluid – although these samples are not always officially validated and approved by the manufacturer.

Table 1 Currently most used commercially available NAAT to detect *Chlamydia trachomatis*

System	Assay	Company	Technique	Target	Validated material
ProbeTec ET or VIPER-XTR	ProbeTec CT/GC	Beckton Dickinson (BD)	SDA	Cryptic plasmid	Endocervical swabs, vaginal swabs, male urethra swabs, urine
COBAS Taqman	COBAS CT/NG	Roche	Real-time PCR	Cryptic plasmid and CT genome (MOMP gene)	Endocervical swabs, urine
COBAS 4800	COBAS CT/NG	Roche	Real-time PCR	Cryptic plasmid and CT genome (MOMP gene)	Endocervical swabs, urine
M2000 RealTime	RealTime CT/NG	Abbott	Real-time PCR	Cryptic plasmid	Endocervial, male urethra swabs, vaginal swab, urine
Versant kPCR	Versant CT/GC DNA 1.0	Siemens	Real-time PCR	Cryptic plasmid	Endocervical swab, urethral swabs, urine
TIGRIS or Panther	Aptima Combo AC2	Hologic/ Gen-Probe	TMA	CT rRNA NG rRNA	Endocervical swabs, male urethra swabs, urine
GeneXpert	Xpert® CT/NG	Cepheid	Real-time PCR	CT genomic DNA NG genomic DNA	Urine (male and female), endocervical swab, and patient-collected vaginal swab

SDA = strand displacement amplification, TMA = transcription mediated amplification

The overall sensitivity and specificity of NAAT for Ct detection in SVS ranges between 97-99% and 95-100% [36,37] and between 96-100% and 99-100% for urine, respectively.[38] Ct detection in SVS is equally sensitive as detection in endocervical specimens.[39] Furthermore, SVS is an acceptable and feasible specimen in women.[40]

Before NAAT became available, Ct culture was considered as the gold standard. The sensitivity of culture however is low and is no longer practised in clinical laboratories.[41] Another technique to diagnoses an infection due to Ct is direct fluorescent antibody testing, but as with Ct culture, this technique is laborious, requires experienced technicians, is less sensitive than NAAT and not necessarily detects acute infection. Also, enzyme immunoassays to detect *chlamydia*l lipopolysaccharide are widely available, but these again are less sensitive than NAAT. Point of care tests (POCT) are upcoming and are currently being evaluated. However, results obtained thus far do not warrant their use, instead of NAAT, in clinical practice yet.[35,42,43]

For research purposes, Ct typing methods are available. Results can be used to reveal transmission patterns, can be useful to detect associations with clinical manifestations, to study pathogenicity and can help to differentiate between persistent or new infections.[44] Variants in the major outer membrane protein (MOMP), encoded by the omp1 (ompA) gene, are used for decades to serotype, or genotype, Ct. Several typing methods have been developed, with different capabilities and limitations and variation in laboriousness.[44] Also, typing can be useful in identifying new Ct variants. In 2006, an unexpected drop in Ct prevalence was noticed in Halland County, Sweden.[45] Thorough analysis revealed the existence of a new variant of Ct, the so-called Swedish variant of Ct (swCt), which was not detected by the most commonly used NAAT. This was due to a deletion on the Ct plasmid which is the target of the used NAAT.

Treatment, follow-up, antibiotic resistance and prevention

According to the guidelines of the Dutch Society of Dermatology and Venereology, urogenital infections due to Ct are treated with azithromycin 1g orally, once only (www.soaaids.nl). Anal infections (non-LGV) are treated with doxycycline 100mg twice daily for 7 days. Azithromycin is also first choice in pregnant women. Patients are advised not to have sexual contact during and one week after treatment.[46] Re-testing after treatment is not advised, except when amoxicillin has been used in pregnant women ([46], www.soaaids.nl). It is important to trace and test all sexual contacts, since at least 1 out of 2 of these contacts on average are Ct positive and can again lead to additional cases and repeat cases.[47] Additionally, re-testing within one year after the first positive Ct test is advisable, since the percentage of positive patients is much higher in case of a previously positive Ct test compared to patients with a negative Ct test (10.4% vs 2.9% respectively).[48] The challenges of repeat and persistent infections after

single dose treatment with azithromycin have emerged over the past 10 years. Treatment failures might occur due to reduced effectiveness of azithromycin and doxycycline in a hypoxic environment.[49,50] Increasing number of studies suggest that more than 5% treatment failure can occur during use of azithromycin.[51]

Although inexpensive and effective treatment of symptomatic patients is available, control of Ct is challenging since most infected persons are asymptomatic. Despite the fact that over the past two decades huge resources were put into attempts to reach these asymptomatic patients, the impact of widespread screening for Ct has been disappointing. The reason for this is that screening has not been shown to reach sufficient numbers of participants and thereby reduce either transmission of infection [52] or the incidence of reproductive complications. Besides screening and early treatment, effective prevention obviously relies on having safe sex, e.g. using a condom. Another potentially effective measure may be offered by vaccination. A good vaccine against Ct could eliminate Ct within 20 years after its introduction and high coverage.[10] Vaccination could potentially prevent Ct to pass the cervix and cause damage to the oviduct. Many candidate proteins have been explored for their vaccine capacity, but no vaccine is yet available nor expected in the near future.[10,53]

TREPONEMA PALLIDUM

Early syphilis infections during pregnancy cause 25% of stillbirths and 14% of neonatal deaths.[4]

Organism

Treponema pallidum (Tp) belongs to the family *Spirochaetaceae* which is characterized by its typical spiral configuration. Other species belonging to the *Spirochaetaceae* are for instance *Leptospira spp.* and *Borrelia spp.* Tp can be subdivided in the subspecies *pallidum*, *endemicum*, *pertenue* and *carateum* which cause the disease syphilis, bejel, yaws and pinta, respectively. In this thesis, the primarily focus is on *Treponema pallidum* subsp. *pallidum*.[54] Tp has an cytoplasmic membrane which is surrounded by an outer membrane. Within these membranes, there is a thin layer of peptidoglycan. At both ends of the spirochete, flagellar motors are present. Tp has only few intergral proteins in its outer membrane.[55] The genome of Tp is very small compared to other bacteria, e.g. *Escherichia coli*: 1.14 Mb vs. 4.6 Mb. The metabolic activity of Tp is very limited.[54]

Pathogenesis

Tp is capable of attachment to a variety of cell types, including epithelial and endothelial cells.[56] It is very motile, even in less fluid material, due to its corkscrew-like movements using its endoflagella.[55] Compared with other bacteria, the flagellar construction of Tp is well developed and comprises several proteins instead of just one. These proteins have strong antigenic properties.

Tp is extremely invasive. It induces the production of matrix metalloproteinase-1 which is involved in collagen breakdown, which facilitates penetration and Tp is found in deeper tissues, just hours after (mucosal) inoculation.[54] Endothelial cells are promoted to express adhesion molecules (intercellular adhesion molecule 1, vascular cell adhesion protein 1 and E-selectin) which cause migration and adhesion of leukocytes.[56] Although polymorphnuclear lymphocytes are able to reduce the infectivity of Tp [57], these do not halt disease progression. TLR-2 (toll like receptor 2; present on human cell surface) is essential in recognising Tp, for instance by immature dendritic cells (DC's).[58] Immature DC's subsequently activate T-cells by taking up Tp and migrating to the lymphnodes. After maturation, the DC's produce inflammatory cytokines (interleukin 12 and tumor necrosis factor alfa) and express maturation markers. A delay in this process, facilitates Tp dissemination. Macrophages opsonise and kill Tp, although it has been shown that a subpopulation of organisms is resistant to ingestion, a phenomenon which is unexplained thus far.[59] The most important lipoproteins for the immune response, are TpN47, TpN17 and TpN15. At approximately day 10 after infection, the number of T-cells and macrofages reaches its peak, after which the Tp load decreases.[54]

After dissemination, Tp is also capable of causing chronic infection, as will be discussed in the next sections. The latent phase is possibly due to the small numbers of organisms present at different anatomical sites and (very) slow replication during this stage and its relatively non-antigenic surface. There are, however, outer membrane proteins on its cell surface which are encoded by the tpr gene family. Gene conversion occurs and gene expression can vary depending on the human immune response. This variable gene expression is called phase variation and helps Tp to constantly evade the immune system.[54]

Transmission and natural course

Tp can only infect humans and can be transmitted via contact between lesions in the primary and secondary stage and mucosal membranes. The incubation period is between 10-90 days. At least 60 organisms are needed to cause an infection [54] and approximately 60% of the sexual partners will become infected.[60]

Clinical manifestations

In primary syphilis, a lesion, so called chancre, develops at the inoculation site and is usually accompanied by regional lymphadenopathy. The chancre usually occurs 3 weeks after exposure, is painless and heals spontaneously within 4-6 weeks. Due to is painless presentation and rapid resolution, diagnosis of primary syphilis is hampered.

Secondary syphilis presents approximately 3 months after initial infection. The most classical finding is maculopapular rash which can also be present on palms of hand and soles of feet. Other accompanying symptoms are sore throat, muscle aches and lymphadenopathy. Sometimes the dermal lesions can become necrotic, which is called lues maligna. Condylomata lata can be present in the anal region in 10% of the patients and are highly infectious. The kidneys and liver can be involved. Neurosyphilis occurs in 40% of patients with early syphilis and in 25% of individuals with late syphilis. Most patients can resolve the infection by themselves and never become (noticeably) symptomatic.

After the secondary stage, the latent phase starts which is variable in duration. In the first year after secondary syphilis, it is considered the early latent phase and afterwards the late latent phase (also if duration is unknown). Sexual transmission is not likely during the latent phase, although foetal infection can occur. Before the use of antibiotics, approximately 30% of the patients with latent syphilis, subsequently developed tertiary syphilis. Symptoms can occur as long as 40 years after initial infection and can include gummata, cardiovascular infection and neurological complications.

The probability of congenital infection is highest when the mother is in her first year of infection. Treatment in the first or second trimester usually prevents the newborn from being symptomatic. In the third trimester, primary syphilis can result in abortion or stillbirth. Symptoms of congenital syphilis resemble those in adults only worse, and become apparent 2-10 weeks after birth. The baby can suffer from chronic rhinitis (snuffles), skin lesions, condylomata lata, osteochondritis, among other symptoms. Late manifestations, occurring after 2 years, are for instance keratitis, damage of the vestibulocochlear nerve, neurosyphilis, arthropathy and gummata.[54]

Diagnosis

Treponema pallidum cannot be cultured in vitro. Darkfield microscopy is still practised, but can only visualize spirochetes in fresh lesions. NAAT is not currently available in most laboratories, thus, diagnosis relies on serology. IgM antibodies can be detectable as early as 2 weeks post-infection and are produced in response to Tp surface lipids, flagellar proteins and lipoproteins, among others. IgG is formed 2 weeks hereafter and is capable of blocking the binding capacity of Tp and causes organism to be unable to produce dermal lesions, but do not kill Tp nor prevent infection. The IgM response wanes when infection persists for longer periods of time or when a patient has been adequately treated.[61] The attributes of the serological assays are displayed in table 2.[11]

The first serological assay for diagnosing syphilis was developed in the beginning of the 20th century and was called the Wasserman test. It detects antigens in the Tp membrane which is originally derived from the host. These antigens comprise of lecithin, cholesterol and cardiolopin, are not is not unique for Tp, therefore the Wasserman test is called a non-treponemal assay. Other non-treponemal tests are the rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) which utilize the same antigens and are still used to determine disease activity.[61] Unfortunately, these antigens are also released in different other (infectious) diseases, causing false positive results.[62-64]

Specific Tp immunoglobulins (Ig), IgM and IgG, are detected in the fluorescent treponemal antibody absorption test (FTA-ABS) and *Treponema pallidum* hemagglutination assay (TPHA) and *Treponema pallidum* particle-agglutination assay (TPPA). The FTA-ABS utilize anti-human Ig to detect antibodies attachted to Tp fixed on slides. The TPHA is based on red blood cells sensitized with Tp antigen which agglutinate in the presence of specific antibodies. TPPA uses gel particles instead of red blood cells. During recent years, enzyme-linked immunosorbent assays (ELISA) are upcoming. ELISA are designed to detect IgG or IgM antibodies, or both. These assays have the great advantage that these can be performed on an automated system.[61]

Table 2 Sensitivity and specificity of serological tests for syphilis [11]

Test characteristic	Non-treponema l tests		Treponemal tests		
	RPR	VDRL	EIA	TPHA / TPPA	FTA-ABS
Specimen	Serum or plasma	Serum or plasma	Serum or plasma	Serum or plasma	Serum or plasma
Sensitivity	86-100%	78-100%	82-100%	85-100%	70-100%
Specificiry	93-98%	98-100%	97-100%	98-100%	94-100%
Ease of use	Easy	Easy	Moderate	Complex	Complex
Level ofuse	Examination room, on-site laboratory	Examination room,on-site laboratory	lntermediate laboratory, reference laboratory	Reference laboratory	Reference laboratory
Equipment	Rotator, refrigerator	light microscope, refrigerator	Incubator, microwell plate washer and reader	Incubator	Fluorescence microscope
Training	Minimal	Minimal	Moderate	Extensive	Extensive
Average cost	US$ 0,5	US$ 0,5	US$ 3	US$3	US$ 3
Comments	Most RPR reagents require refrigeration	Reagents require refrigeration	Allows high-throughput screening; does not distinguish between prior treated and active infection	Confirmatory test, so does not distinguish between prior treated and active infection	Confirmatory test, so does not distinguish between prior treated and active infection

EIA, enzyme immunoassay; FTA-ABS, fluorescent treponemal antibody-absorption test; RPR, rapid plasma reagin test; TPHA/TPPA, *Treponema pallidum* haemagglutination assay/*T. pallidum* particle agglutination assay; VDRL, venereal disease research laboratory test.)

Treatment, follow-up, antibiotic resistance and prevention

Penicillin is the antibiotic of choice for treatment of all known syphilis stages. It can be given intramuscularly for the early and latent stage and should be given intravenously for neurosyphilis. In case of penicillin intolerance or allergy, doxycycline can be used. Partner notification is essential to prevent secondary cases. After treatment of primary, secondary or early latent syphilis, the VDRL must be checked every quarter for the first year and every half year for the second year. In all other cases, follow-up should be guided by an experienced physician (www.saoaids.nl)

Individuals with syphilis are mainly infectious -when having dermal lesions- during the first year of infection. Contact with lesions and blood should be avoided. Mother to child transmission, however, can also occur beyond this period, albeit rarely. To prevent symptomatic congenital syphilis, all females in the Netherlands are serologically tested before their 13th week of pregnancy.[65] No vaccine against syphilis is presently available, nor expected to become available in the near future.

AIMS AND OUTLINE OF THIS THESIS

The subject of this thesis is STI diagnostics in a broad sense, with a focus on syphilis and *chlamydia*. Several hypotheses have been formulated. For instance what kind of samples can be used in clinical practice and for research purposes? What are the assays of choice and how should we interpret results? All chapters are designed to give answers to these questions.

Currently, Ct detection by NAAT is performed on SVS or urine in females, since dual testing is not considered cost effective. Since SVS is considered the specimen of choice in women, urinary tract only Ct infection could be missed. Chapter 2 explores the possible sensitivity gain when using NAAT on combined swab and urine samples when testing for Ct in females.

As mentioned in this introduction, a new Ct variant (swCt) was discovered in 2006 which could not be detected by the most commonly used NAAT.[45] Rapid exploration of the extent of this problem was needed. Chapter 3 describes a newly developed assay to specifically detect the swCt, which can be used to monitor the spread of swCt.

Different genotyping assays are developed to distinguish between different CT serovars to understand more about Ct. Genotyping can provide information that can be helpful in understanding epidemiology, transmission and pathogenicity. Chapter 4 evaluates a rapid and easy Ct genotyping assay.

Another 'hot' issue in Ct research are the POCT. POCT can usually be performed within 30 minutes and treatment can be given instantly when positive. This could potentially mean a great step forward in limiting Ct spread in the population compared to the current standard (e.g. NAAT). POCT also could be very useful in remote areas in which STI diagnostics are currently not available due to absence of facilities and financial means. The World Health Organisation advices practising syndromic management if STI diagnostics are unavailable, but its sensitivity and specificity is poor.[66,67] Chapter 5 discusses the evaluation of three Ct rapid antigen tests in comparison to NAAT.

POCT are also upcoming in the field of syphilis diagnostics. As with Ct, early syphilis can be treated with single dose of penicillin and instant treatment would mean an improvement compared to the current situation. Also, ELISA are being introduced in favour of the TPPA/TPHA or a non-treponemal assay due to the ability to perform the assay on an automated system. Chapter 6 discusses the evaluation of a rapid test and two immunoenzymatic assays to detect antibodies against Tp.

As a follow-up to chapter 6, chapter 7 discusses the validity of using a selected sample set to evaluate diagnostic assays. In case of syphilis, the prevalence in The Netherlands is very low as mentioned before, but for the project described in chapter 6, a high number of syphilis seropositive samples were used. Does the evaluation of a diagnostic assay using this sample selection result in data which can be used in the general population?

In the afore mentioned chapters, many of the samples tested to answer a research question were stored, frozen samples. Literature regarding the use of stored samples in the evaluation of Ct NAAT is lacking. Do stored samples generate the same result as 'fresh' samples, in other words can storage of samples lead to false negative results of NAAT testing? In chapter 8 the stability of Ct DNA is explored by using NAAT under different circumstances.

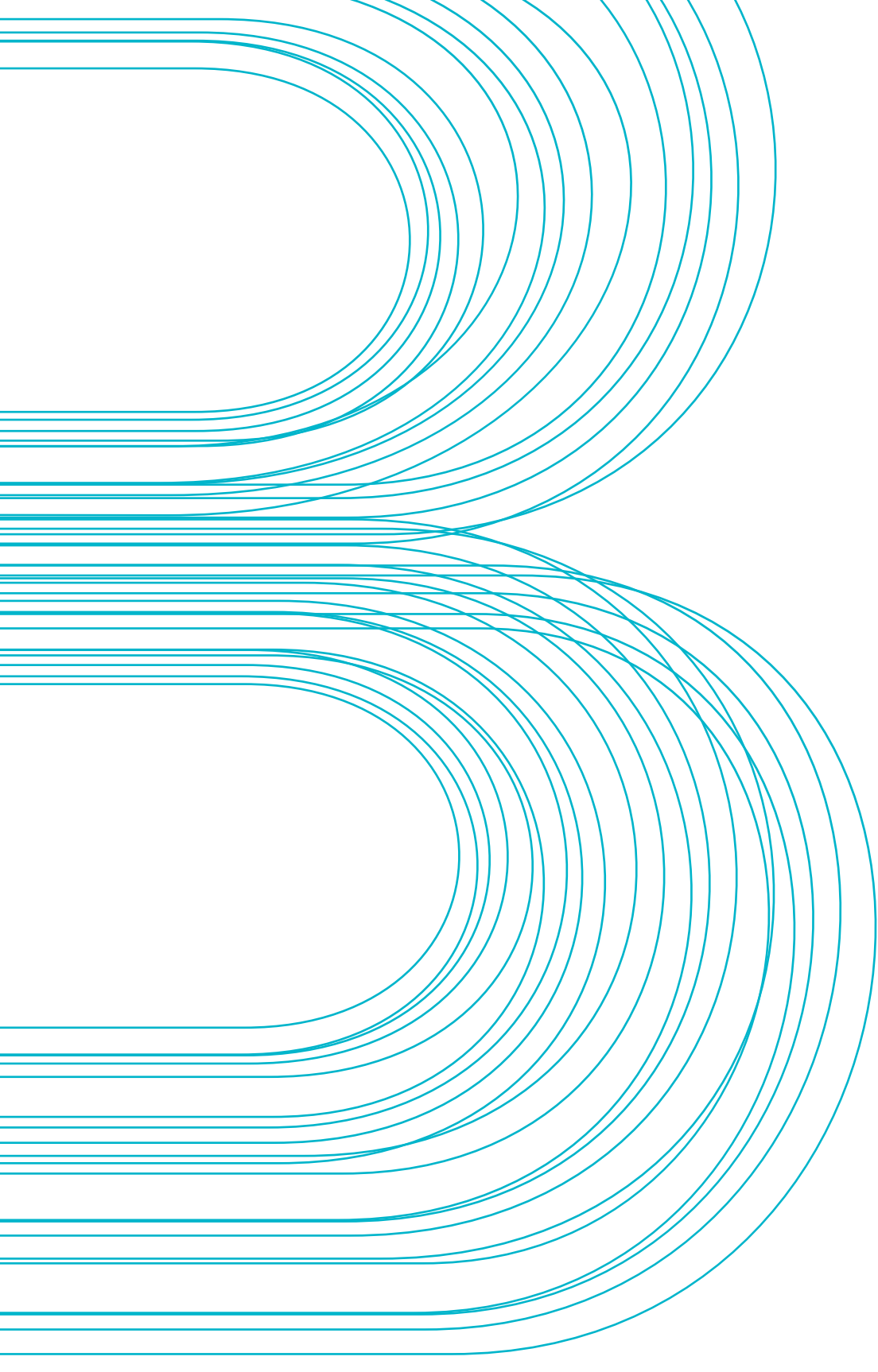
Finally, chapter 9 gives an overview of the results of this thesis and how they relate to recent literature.

REFERENCES

1. Global incidence and prevalence of selected curable sexually transmitted infections - 2008 World Health Organization, 2012.
2. Advancing MDG 4, 5 and 6: impact of congenital syphilis elimination: World Health Organization, 2010.
3. Carey AJ, Beagley KW. *Chlamydia trachomatis*, a hidden epidemic: effects on female reproduction and options for treatment. Am J Reprod Immunol 2010;63(6):576-86.
4. Global strategy for the prevention and control of sexually transmitted infections: 2006-2015: WHO, 2007.
5. S.C.M. Trienekens FDHK, I.V.F. van den Broek, H.J. Vriend, E.L.M. Op de Coul, M.G. van Veen, A.I. van Sighem, I. Stirbu-Wagner, M.A.B. van der Sande. Sexually transmitted infections, including HIV, in the Netherlands in 2011: National Institute for Public Health and the Environment, 2012.
6. van Bergen JE, Kerssens JJ, Schellevis FG, Sandfort TG, Coenen TJ, Bindels PJ. Prevalence of STI related consultations in general practice: results from the second Dutch National Survey of General Practice. Br J Gen Pract 2006;56(523):104-9.
7. Dukers-Muijrers NH, Niekamp AM, Vergoossen MM, Hoebe CJ. Effectiveness of an opting-out strategy for HIV testing: evaluation of 4 years of standard HIV testing in a STI clinic. Sex Transm Infect 2009;85(3):226-30.
8. Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep 2010;59(RR-12):1-110.
9. Abdelrahman YM, Belland RJ. The *chlamydia*l developmental cycle. FEMS Microbiol Rev 2005;29(5):949-59.
10. Howie SE, Horner PJ, Horne AW, Entrican G. Immunity and vaccines against sexually transmitted *Chlamydia trachomatis* infection. Curr Opin Infect Dis 2011;24(1):56-61.
11. Peeling RW, Mabey D, Herring A, Hook EW, 3rd. Why do we need quality-assured diagnostic tests for sexually transmitted infections? Nat Rev Microbiol 2006;4(12):909-21.
12. Spaargaren J, Verhaest I, Mooij S, Smit C, Fennema HS, Coutinho RA, et al. Analysis of *Chlamydia trachomatis* serovar distribution changes in the Netherlands (1986-2002). Sex Transm Infect 2004;80(2):151-2.
13. Rasmussen SJ, Eckmann L, Quayle AJ, Shen L, Zhang YX, Anderson DJ, et al. Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in *chlamydia*l pathogenesis. J Clin Invest 1997;99(1):77-87.
14. Morrison SG, Su H, Caldwell HD, Morrison RP. Immunity to murine *Chlamydia trachomatis* genital tract reinfection involves B cells and CD4(+) T cells but not CD8(+) T cells. Infect Immun 2000;68(12):6979-87.
15. Van Voorhis WC, Barrett LK, Sweeney YT, Kuo CC, Patton DL. Repeated *Chlamydia trachomatis* infection of Macaca nemestrina fallopian tubes produces a Th1-like cytokine response associated with fibrosis and scarring. Infect Immun 1997;65(6):2175-82.
16. Agrawal T, Gupta R, Dutta R, Srivastava P, Bhengraj AR, Salhan S, et al. Protective or pathogenic immune response to genital *chlamydia*l infection in women-a possible role of cytokine secretion profile of cervical mucosal cells. Clin Immunol 2009;130(3):347-54.
17. den Hartog JE, Ouburg S, Land JA, Lyons JM, Ito JI, Pena AS, et al. Do host genetic traits in the bacterial sensing system play a role in the development of *Chlamydia trachomatis*-associated tubal pathology in subfertile women? BMC Infect Dis 2006;6:122.
18. Lyons JM, Morre SA, Airo-Brown LP, Pena AS, Ito JI. Comparison of multiple genital tract infections with *Chlamydia trachomatis* in different strains of female mice. J Microbiol Immunol Infect 2005;38(6):383-93.
19. Bailey RL, Natividad-Sancho A, Fowler A, Peeling RW, Mabey DC, Whittle HC, et al. Host genetic contribution to the cellular immune response to *Chlamydia trachomatis*: Heritability estimate from a Gambian twin study. Drugs Today (Barc) 2009;45 Suppl B:45-50.
20. Markos AR. The concordance of *Chlamydia trachomatis* genital infection between sexual partners, in the era of nucleic acid testing. Sex Health 2005;2(1):23-4.
21. Rogers SM, Miller WC, Turner CF, Ellen J, Zenilman J, Rothman R, et al. Concordance of *chlamydia trachomatis* infections within sexual partnerships. Sex Transm Infect 2008;84(1):23-8.
22. Quinn TC, Gaydos C, Shepherd M, Bobo L, Hook EW, 3rd, Viscidi R, et al. Epidemiologic and microbiologic correlates of *Chlamydia trachomatis* infection in sexual partnerships. Jama 1996;276(21):1737-42.

23. Geisler WM. Duration of untreated, uncomplicated *Chlamydia trachomatis* genital infection and factors associated with *chlamydia* resolution: a review of human studies. J Infect Dis 2010;201 Suppl 2:S104-13.
24. Geisler WM, Wang C, Morrison SG, Black CM, Bandea CI, Hook EW, 3rd. The natural history of untreated *Chlamydia trachomatis* infection in the interval between screening and returning for treatment. Sex Transm Dis 2008;35(2):119-23.
25. Risser WL, Risser JM. The incidence of pelvic inflammatory disease in untreated women infected with *Chlamydia trachomatis*: a structured review. Int J STD AIDS 2007;18(11):727-31.
26. Aghaizu A, Atherton H, Mallinson H, Simms I, Kerry S, Oakeshott P, et al. Incidence of pelvic inflammatory disease in untreated women infected with *Chlamydia trachomatis*. Int J STD AIDS 2008;19(4):283.
27. Simms I, Horner P. Has the incidence of pelvic inflammatory disease following *chlamydia*l infection been overestimated? Int J STD AIDS 2008;19(4):285-6.
28. Boeke AJ, van Bergen JE, Morre SA, van Everdingen JJ. [The risk of pelvic inflammatory disease associated with urogenital infection with *Chlamydia trachomatis*; literature review]. Ned Tijdschr Geneeskd 2005;149(16):878-84.
29. Herzog SA, Althaus CL, Heijne JC, Oakeshott P, Kerry S, Hay P, et al. Timing of progression from *Chlamydia trachomatis* infection to pelvic inflammatory disease: a mathematical modelling study. BMC Infect Dis 2012;12:187.
30. Haggerty CL, Gottlieb SL, Taylor BD, Low N, Xu F, Ness RB. Risk of sequelae after *Chlamydia trachomatis* genital infection in women. J Infect Dis 2010;201 Suppl 2:S134-55.
31. den Hartog JE, Land JA, Stassen FR, Kessels AG, Bruggeman CA. Serological markers of persistent *C. trachomatis* infections in women with tubal factor subfertility. Hum Reprod 2005;20(4):986-90.
32. Kortekangas-Savolainen O, Makinen J, Koivusalo K, Mattila K. Hospital-diagnosed late sequelae after female *Chlamydia trachomatis* infections in 1990-2006 in Turku, Finland. Gynecol Obstet Invest 2012;73(4):299-303.
33. Oakeshott P, Kerry S, Aghaizu A, Atherton H, Hay S, Taylor-Robinson D, et al. Randomised controlled trial of screening for *Chlamydia trachomatis* to prevent pelvic inflammatory disease: the POPI (prevention of pelvic infection) trial. Bmj 2010;340:c1642.
34. Rours IG, Hammerschlag MR, Ott A, De Faber TJ, Verbrugh HA, de Groot R, et al. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. Pediatrics 2008;121(2):e321-6.
35. Bebear C, de Barbeyrac B. Genital *Chlamydia trachomatis* infections. Clin Microbiol Infect 2009;15(1):4-10.
36. Schachter J, Chernesky MA, Willis DE, Fine PM, Martin DH, Fuller D, et al. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results from a multicenter evaluation of the APTIMA assays for both infections. Sex Transm Dis 2005;32(12):725-8.
37. Van der Pol B. COBAS Amplicor: an automated PCR system for detection of *C. trachomatis* and N. *gonorrhoeae*. Expert Rev Mol Diagn 2002;2(4):379-89.
38. Gaydos CA, Theodore M, Dalesio N, Wood BJ, Quinn TC. Comparison of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* in urine specimens. J Clin Microbiol 2004;42(7):3041-5.
39. Keane FE, Bendall R, Saulsbury N, Haddon L. A comparison of self-taken vulvovaginal and cervical samples for the diagnosis of *Chlamydia trachomatis* infection by polymerase chain reaction. Int J STD AIDS 2007;18(2):98-100.
40. Hoebe CJ, Rademaker CW, Brouwers EE, ter Waarbeek HL, van Bergen JE. Acceptability of self-taken vaginal swabs and first-catch urine samples for the diagnosis of urogenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with an amplified DNA assay in young women attending a public health sexually transmitted disease clinic. Sex Transm Dis 2006;33(8):491-5.
41. Livengood CH, 3rd, Wrenn JW. Evaluation of COBAS AMPLICOR (Roche): accuracy in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by coamplification of endocervical specimens. J Clin Microbiol 2001;39(8):2928-32.
42. Steingrimsson O, Pawlak C, Van Der Pol B, Turner BP, Hjaltalin Olafsson J, Dolphin L, et al. Multicenter comparative evaluation of two rapid immunoassay methods for the detection of *Chlamydia trachomatis* antigen in endocervical specimens. Clin Microbiol Infect 1997;3(6):663-667.
43. Rani R, Corbitt G, Killough R, Curless E. Is there any role for rapid tests for *Chlamydia trachomatis*? Int J STD AIDS 2002;13(1):22-4.
44. Pedersen LN, Herrmann B, Moller JK. Typing *Chlamydia trachomatis*: from egg yolk to nanotechnology. FEMS Immunol Med Microbiol 2009;55(2):120-30.
45. Ripa T, Nilsson P. A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill 2006;11(11):E061109 2.

46. Kalwij S, Macintosh M, Baraitser P. Screening and treatment of *Chlamydia trachomatis* infections. Bmj 2010;340:c1915.
47. Forbes G, Clutterbuck DJ. How many cases of *chlamydia*l infection would we miss by not testing partners for infection? Int J STD AIDS 2009;20(4):267-8.
48. Veldhuijzen IK, Van Bergen JE, Gotz HM, Hoebe CJ, Morre SA, Richardus JH. Reinfections, persistent infections, and new infections after general population screening for *Chlamydia trachomatis* infection in the Netherlands. Sex Transm Dis 2005;32(10):599-604.
49. Hong KC, Schachter J, Moncada J, Zhou Z, House J, Lietman TM. Lack of macrolide resistance in *Chlamydia trachomatis* after mass azithromycin distributions for trachoma. Emerg Infect Dis 2009;15(7):1088-90.
50. Shima K, Szaszak M, Solbach W, Gieffers J, Rupp J. Impact of a low-oxygen environment on the efficacy of antimicrobials against intracellular *Chlamydia trachomatis*. Antimicrob Agents Chemother 2011;55(5):2319-24.
51. Horner P. The case for further treatment studies of uncomplicated genital *Chlamydia trachomatis* infection. Sex Transm Infect 2006;82(4):340-3.
52. van den Broek IV, van Bergen JE, Brouwers EE, Fennema JS, Gotz HM, Hoebe CJ, et al. Effectiveness of yearly, register based screening for *chlamydia* in the Netherlands: controlled trial with randomised stepped wedge implementation. Bmj 2012;345:e4316.
53. Finco O, Frigimelica E, Buricchi F, Petracca R, Galli G, Faenzi E, et al. Approach to discover T- and B-cell antigens of intracellular pathogens applied to the design of *Chlamydia trachomatis* vaccines. Proc Natl Acad Sci U S A 2011;108(24):9969-74.
54. Lafond RE, Lukehart SA. Biological basis for syphilis. Clin Microbiol Rev 2006;19(1):29-49.
55. Liu J, Howell JK, Bradley SD, Zheng Y, Zhou ZH, Norris SJ. Cellular architecture of *Treponema pallidum*: novel flagellum, periplasmic cone, and cell envelope as revealed by cryo electron tomography. J Mol Biol 2010;403(4):546-61.
56. Riley BS, Oppenheimer-Marks N, Radolf JD, Norgard MV. Virulent *Treponema pallidum* promotes adhesion of leukocytes to human vascular endothelial cells. Infect Immun 1994;62(10):4622-5.
57. Cox DL, Sun Y, Liu H, Lehrer RI, Shafer WM. Susceptibility of *Treponema pallidum* to host-derived antimicrobial peptides. Peptides 2003;24(11):1741-6.
58. Salazar JC, Pope CD, Moore MW, Pope J, Kiely TG, Radolf JD. Lipoprotein-dependent and -independent immune responses to spirochetal infection. Clin Diagn Lab Immunol 2005;12(8):949-58.
59. Cruz AR, Ramirez LG, Zuluaga AV, Pillay A, Abreu C, Valencia CA, et al. Immune evasion and recognition of the syphilis spirochete in blood and skin of secondary syphilis patients: two immunologically distinct compartments. PLoS Negl Trop Dis 2012;6(7):e1717.
60. Singh AE, Romanowski B. Syphilis: review with emphasis on clinical, epidemiologic, and some biologic features. Clin Microbiol Rev 1999;12(2):187-209.
61. Sena AC, White BL, Sparling PF. Novel *Treponema pallidum* serologic tests: a paradigm shift in syphilis screening for the 21st century. Clin Infect Dis 2010;51(6):700-8.
62. I. Hernandez-Aguado FB, R. Moreno, F.J. Pardo, N. Torres, J. Belda, A. Espacio, and the Valencian Study Group on HIV Epidemiology. False-positive tests for syphilis associated with *human immunodeficiency virus* and *hepatitis B virus* infection among intravernous drug abusers. Eur J Clin Microbiol Infect Dis 1998;17(11):784-787.
63. Geusau A, Kittler H, Hein U, Dangl-Erlach E, Stingl G, Tschachler E. Biological false-positive tests comprise a high proportion of Venereal Disease Research Laboratory reactions in an analysis of 300,000 sera. Int J STD AIDS 2005;16(11):722-6.
64. Rompalo AM, Cannon RO, Quinn TC, Hook EW, 3rd. Association of biologic false-positive reactions for syphilis with *human immunodeficiency virus* infection. J Infect Dis 1992;165(6):1124-6.
65. Op de Coul EL, Hahne S, van Weert YW, Oomen P, Smit C, van der Ploeg KP, et al. Antenatal screening for HIV, hepatitis B and syphilis in the Netherlands is effective. BMC Infect Dis 2011;11:185.
66. Yin YP, Wu Z, Lin C, Guan J, Wen Y, Li L, et al. Syndromic and laboratory diagnosis of sexually transmitted infection: a comparative study in China. Int J STD AIDS 2008;19(6):381-4.
67. Vuylsteke B. Current status of syndromic management of sexually transmitted infections in developing countries. Sex Transm Infect 2004;80(5):333-4.



Different Sample Types and *Chlamydia trachomatis* Detection

2. Evaluation of one-sample testing of self-obtained vaginal swabs and first catch urine samples separately and in combination for the detection of *Chlamydia trachomatis* by two amplified DNA assays in women visiting an sexually transmitted disease clinic.

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Laura van Dommelen, Nicole Dukers-Muijrers,
Frank H. van Tiel, Elfi E. H. G. Brouwers, Christian J. P. A. Hoebe

This study evaluates the performance of self-obtained vaginal swabs (SVS)/first-catch urine (FCU) combination samples in comparison to testing FCU or SVS alone. The *Chlamydia trachomatis* detection rate for the SVS, FCU, and SVS/FCU combination were 94%, 90%, and 94%, respectively. Self-obtained vaginal swabs are therefore the specimen of choice for *Chlamydia trachomatis* Nucleic Acid Amplification Tests in females.

Chlamydia trachomatis (CT) represents the most common bacterial sexually transmitted disease (STD) in women with major public health consequences due to its frequent asymptomatic nature and its part in reproductive morbidity.[1–3] However, willingness to undergo traditional gynecologic STD testing is limited, and therefore efforts to enhance compliance with testing among at-risk women are needed.[4] Prior studies have shown that testing self-obtained vaginal swabs (SVS) is equivalent in sensitivity and reliability to testing traditional endocervical swabs for the diagnosis of CT by Nucleic Acid step forward, because internal pelvic examination was an im-portant reason for females to be hesitant to visit an STD clinic.[4]

Genital swabs and FCU have been compared numerous times when it comes to CT detection by NAAT.[10–14] The results with SVS are usually slightly better than with FCU, and testing both SVS and FCU results in highest sensitivity. However, in most laboratories, 1-sample testing is performed for reasons of cost efficiency. To further improve 1-sample testing, we hypothesized that combining SVS with FCU in a single test will result in better performance than using either SVS or FCU alone as sample. The objective of this study was to assess the laboratory performance of 3 different testing approaches to find the most sensitive 1-sample test procedure: SVS versus FCU versus a combined specimen of FCU/SVS.

All women visiting our STD clinic were asked to participate in the study. Before visiting the clinic, each participant was instructed by telephone not to urinate within 2 hours before the FCU collection. During the consultation, clients received instructions from educated STD-nurses on how to take SVS and FCU. The FCU was taken before SVS. In order to have 2 SVS, a dual swab was used, which was separated at the laboratory. The specimens were stored and transported at 2°C to 8°C until processing.

The samples were tested for the presence of CT at 2 medical microbiology laboratories using the following 2 different NAAT: (1) Strand Displacement Amplification (SDA) assay of Becton Dickinson (ProbeTec ET system, MD) and (2) polymerase chain reaction (PCR) by Roche Diagnostics Inc. (Cobas Amplicor system, CA); both assays are commercially available and widely used. The SDA and PCR were performed according to the manufacturer's instructions.

Clients with at least 1 of 3 sample types (SVS, FCU, SVS/FCU combination) tested as positive for CT by NAAT were regarded as CT positive (comparison standard). CT detection rate (including 95% confidence interval [CI]) per sample type was calculated using the number of positive samples, divided by the total number of clients in which this sample type was tested by NAAT. The detection rates were compared between testing SVS alone, FCU alone, and SVS/FCU combination in 1 assay and in a 2-test algorithm. Concordance was evaluated using the McNemar test. Analyses were performed with the SPSS package version 17.0 (SPSS, Inc., Chicago, IL).

Table 1 Overview of *Chlamydia trachomatis* NAAT Results for the Different Sample Types

SVS	FCU	SVS / FCU	SDA	PCR	Total
+	+	+	38	36	74
+	+	-	0	1	1
+	-	-	0	3	3
-	+	+	2	2	4
-	-	+	0	0	0
-	+	-	1	1	2
+	-	+	4	2	6
+	NA	+	6	0	6
-	NA	-	44	0	44
-	-	-	302	349	651
			397	394	791

NAAT indicates nucleic acid amplification tests; SVS, self-obtained vaginal swabs; FCU, first-catch urine; SDA, strand displacement amplification; PCR, polymerase chain reaction.

Between 2006 and 2007, 791 females were included. In all, 96 of 791 (12%) females tested positive for CT by NAAT in SVS, FCU, and/or SVS/FCU combination. Results are presented in Table 1. The CT detection rate (CI) for SVS, FCU, and SVS/FCU combination were 94% (89%–99%), 90% (84%–96%), and 94% (89%–99%), respectively, if results of NAAT by SDA and by PCR were analyzed together. If SVS and FCU would be tested in separate assays (2-test algorithm), all CT-positive clients would have been detected (100%), which was not the case when using SVS alone (P = 0.031, data not shown). The detection rate was not significantly different between any of the sample types, when tested solely. The concordance rates between results for SVS and for FCU, SVS, and SVS/FCU combination and FCU and SVS/FCU combination were 98.0% (P = 0.61), 99.0% (P = 1.00), and 98.8% (P = 0.51), respectively. Discordance in NAAT results within the different sample types was found in 16 of 96 CT-positive results. If we choose "CT positive in at least 2 out of 3 samples" as the comparison standard (instead of 1 of 3), CT prevalence was 11.5% (91/791). In this case, none of the aforementioned results changed significantly (data not shown).

Our results show that the detection rate of SVS/FCU combination is equal to that of SVS alone. In single testing, the possibility of false-positive results exists, but when changing the comparison standard in "CT positive in at least 2 out of 3 samples," the performance results remained the same. A few limitations to our study should be mentioned. First, not all FCU

samples were tested for CT (93%). Theoretically, "urinary tract only" CT-positive clients could therefore have been missed. Second, CT DNA degradation could occur during the transport of dry swabs, but in our experience this will not result in false-negative results.[15] Lastly, 2 different NAAT have been used in this study (PCR and SDA), but every individual sample has only been tested by a single NAAT. No head-to-head comparison of performance between the 2 assays can therefore be made. The overall reported performance of NAAT by PCR and by SDA is however comparable, as was illustrated by our study, and results can therefore be analyzed as 1 group.[5,16,17] In light of these facts, using 2 different NAAT also represents a strength of our study, because our data can be extrapolated even better to other STD populations. Other strengths of our study are the large, representative STD population with high CT prevalence (12%) and prospective inclusion of clients without knowledge of STD status, which allows adequate comparison of different diagnostic methods. Moreover, our study is based on well-standardized and documented laboratory procedures. Also, extensive instructions and guidance regarding sampling have been given to the clients, with results in highly reliable data.

In a recent study, Falk et al evaluated the sensitivity of CT NAAT on SVS, FCU, and SVS/FCU combination.[18] Many women were already known to be CT positive before inclusion, which resulted in a CT positivity rate of 54%. This selection makes it difficult to extrapolate their results to another population, because previous knowledge of CT status and the CT "prevalence" of 54% are not representative for an STD clinic population. Nevertheless, their results are surprisingly similar to the results of our study. No significant difference in sensitivity was found between SVS, endocervical specimen, and SVS/FCU combination. These authors however found a significantly poorer sensitivity for FCU (95% CI of 81.8%–92.2%) compared with the endocervical specimen and SVS (95% CI of 93.3%–99.0% and 92.5%–98.7%, respectively). If SVS and FCU results would have been combined, sensitivity in this study would have been 98.2% (95% CI of 96.3%–100%). Our results confirm that SVS and SVS/FCU combination perform equally well. Although FCU did not perform significantly poorer in our sample set, our results were similar to those found by Falk et al. The percentage of discrepant results was also comparable: 15.6% (27/171) in their study and 16.6% (16/96) in our study. Also, no significant difference in sensitivity for the tested sample types between symptomatic and asymptomatic females in both studies was found.

Our results are the "missing link" in the ongoing discussion on whether a single SVS is the appropriate specimen to test for CT by NAAT in females. SVS is an acceptable and feasible[10] specimen for females. When using CT NAAT, the detection rate is not significantly different between using SVS/ FCU combination or testing SVS plus FCU separately in 2 assays, in symptomatic nor in asymptomatic females. Moreover, SVS is the most cost-effective sample type for an STD clinic population.[19] We can therefore conclude that SVS is the specimen of choice to detect CT in females.

REFERENCES

1. Boeke AJ, van Bergen JE, Morre SA, et al. The risk of pelvic inflammatory disease associated with urogenital infection with *Chlamydia trachomatis*; literature review [in Dutch]. Ned Tijdschr Geneeskd 2005; 149:878 –884.
2. Mardh PA. Tubal factor infertility, with special regard to *chlamydia*l salpingitis. Curr Opin Infect Dis 2004; 17:49–52.
3. van de Laar MJ, Morre SA. *Chlamydia*: A major challenge for public health. Euro Surveill 2007; 12:E1–E2.
4. Arkell J, Osborn DP, Ivens D, et al. Factors associated with anxiety in patients attending a sexually transmitted infection clinic: Qualitative survey. Int J STD AIDS 2006; 17:299–303.
5. Cosentino LA, Landers DV, Hillier SL. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by strand displacement amplification and relevance of the amplification control for use with vaginal swab specimens. J Clin Microbiol 2003; 41:3592– 3596.
6. Hook EW III, Smith K, Mullen C, et al. Diagnosis of genitourinary *Chlamydia trachomatis* infections by using the ligase chain reaction on patient-obtained vaginal swabs. J Clin Microbiol 1997; 35:2133–2135.
7. Knox J, Tabrizi SN, Miller P, et al. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. Sex Transm Dis 2002; 29:647–654.
8. Schachter J, McCormack WM, Chernesky MA, et al. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with *Chlamydia trachomatis*. J Clin Microbiol 2003; 41:3784–3789.
9. Stary A, Najim B, Lee HH. Vulval swabs as alternative speci-mens for ligase chain reaction detection of genital *chlamydia*l infection in women. J Clin Microbiol 1997; 35:836 –838.
10. Hoebe CJ, Rademaker CW, Brouwers EE, et al. Acceptability of self-taken vaginal swabs and first-catch urine samples for the diagnosis of urogenital *Chlamydia trachomatis* and Neisseria gonorrhoeae with an amplified DNA assay in young women attending a public health sexually transmitted disease clinic. Sex Transm Dis 2006; 33:491–495.
11. Schachter J, Chernesky MA, Willis DE, et al. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and Neisseria gonorrhoeae: Results from a multicenter evaluation of the APTIMA assays for both infections. Sex Transm Dis 2005; 32:725–728.
12. Michel CE, Sonnex C, Carne CA, et al. *Chlamydia trachomatis* load at matched anatomic sites: Implications for screening strat-egies. J Clin Microbiol 2007; 45:1395–1402.
13. Skidmore S, Horner P, Herring A, et al. Vulvovaginal-swab or first-catch urine specimen to detect *Chlamydia trachomatis* in women in a community setting? J Clin Microbiol 2006; 44:4389 – 4394.
14. Shafer MA, Moncada J, Boyer CB, et al. Comparing first-void urine specimens, self-collected vaginal swabs, and endocervical specimens to detect *Chlamydia trachomatis* and Neisseria gon-orrhoeae by a nucleic acid amplification test. J Clin Microbiol 2003; 41:4395–4399.
15. Catsburg A, van Dommelen L, Smelov V, et al. TaqMan assay for Swedish *Chlamydia trachomatis* variant. Emerg Infect Dis 2007; 13:1432–1434.
16. Van Dyck E, Ieven M, Pattyn S, et al. Detection of *Chlamydia trachomatis* and Neisseria gonorrhoeae by enzyme immunoassay, culture, and three nucleic acid amplification tests. J Clin Microbiol 2001; 39:1751–1756.
17. Templeton K, Roberts J, Jeffries D, et al. The detection of *Chlamydia trachomatis* by DNA amplification methods in urine samples from men with urethritis. Int J STD AIDS 2001; 12:793– 796.
18. Falk L, Coble BI, Mjornberg PA, et al. Sampling for *Chlamydia trachomatis* infection—a comparison of vaginal, first-catch urine, combined vaginal and first-catch urine and endocervical sampling. Int J STD AIDS 2010; 21:283–287.
19. Blake DR, Maldeis N, Barnes MR, et al. Cost-effectiveness of screening strategies for *Chlamydia trachomatis* using cervical swabs, urine, and self-obtained vaginal swabs in a sexually trans-mitted disease clinic setting. Sex Transm Dis 2008; 35:649 –655.

Newly Developed Nucleic Acid Amplification Tests to Detect *Chlamydia trachomatis*

3. TaqMan assay for Swedish *Chlamydia trachomatis* variant.

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Arnold Catsburg, Laura van Dommelen, Vitaly Smelov, Henry J.C. de Vries, Alevtina Savitcheva, Marius Domeika, Björn Herrmann, Sander Ouburg, Christian J.P.A. Hoebe, Anders Nilsson, Paul H.M. Savelkoul, Servaas A. Morré

Chlamydia trachomatis (CT) is the most prevalent bacterial sexually transmitted infection worldwide. Recently, a new variant of CT (swCT) has been reported in Halland County, Sweden. A total of 12 swCT specimens were sequenced and found to have the same deletion, a 377-bp deletion in the cryptic plasmid.[1] Because the deletion was found in the target area of 2 commercial CT nucleic acid amplification tests (Roche, Basel, Switzerland, and Abbott Laboratories, Abbott Park, IL, USA), screening tests have produced false-negative results for patients infected with this new Swedish variant.[1] In specific regions of Sweden, the proportion of all detected CT cases attributable to swCT ranges from 13% to 39%; a considerable number of *chlamydia* infections have escaped detection by commonly used test systems[1].

Although the first 2 studies to monitor potential spread of the swCT variant outside Sweden (Ireland and the Netherlands) did not detect swCT, a third study (Norway) did identify this variant .[2-4] Subsequently, the European Surveillance of Sexually Transmitted Infections network and the European Center for Disease Prevention and Control launched an initiative, consisting of a short questionnaire, to learn more about this swCT variant problem outside Sweden.[5]

However, quick monitoring of the spread of the swCT variant has been hampered by lack of a direct test to detect this swCT variant and by lack of a readily available positive control. We therefore constructed a positive control by using a clinical specimen of the swCT variant in which the deletion was present (forward swCT 5′-TCC GGA TAG TGA ATT ATA GAG ACT ATT TAA TC-3′ reverse swCT 5′GGT GTT TGT ACT AGA GGA CTT ACC TCT TC-3′).[2] The specimen was obtained in Sweden (by B.H.) and confirmed as swCT by the method described by Ripa and Nilsson.[6] The obtained 98-bp amplicon was subsequently cloned in a pGEM-T Easy Vector (Promega Benelux b.v., Leiden, the Netherlands) and transformed in *Escherichia coli* DH5 α. After extraction the plasmid was verified for the correct insert by sequencing and quantified as described.[7] This positive control is available for researchers and clinicians free of charge.

Subsequently, we developed a real-time PCR (TaqMan assay) that specifically detects the swCt variant by using a probe that spans the 377-bp left and right gap border sequences: probe-swCT 5′-^{FAM} GGA TCC GTT TGT TCT GG ^{MGB} -3′. One copy of cloned positive swCT control could be detected in our swCT assay. We selected 10 copies per PCR as positive swCT control for each run. A total of 239 recent samples known to be CT positive and identified with techniques detecting the swCT variant were retrospectively analyzed with our new swCT real-time PCR for 3 cohorts: 1) 30 real-time PCR CT-positive clinical samples (CT prevalence in the population, 1.8%) from the Department of Medical Microbiology and Infection Prevention, VU University Medical Center, Amsterdam, the Netherlands; 2) 57 Becton Dickinson (Franklin Lakes, NJ, USA) CT-positive samples (CT prevalence in the sexually transmitted disease population, 7.3%) from the Department of Infectious Diseases, South Limburg Public Health Service, Heerlen, the Netherlands; and 3) 152 CT-positive culture samples (CT prevalence in the population, average 15% [8]) from the Faculty of Medicine, St. Petersburg State University, St. Peters-burg, Russia, and from the Laboratory of Microbiology, D.O. Ott Research Institute of Obstetrics and Gynaecology, St. Petersburg, Russia.

Cohort 1 consisted of cervical swabs in 2-sucrose-phosphate (2SP) transport medium, stored at –80°C. Cohort 2 consisted of frozen dry swabs that had been shaken for 10 s in 1 mL 2SP transport medium before sample preparation. Cohort 3 consisted of positive cultured samples. DNA extraction used 200 µL 2SP and was performed with the NucliSens easyMAG (bioMérieux, Boxtel, the Netherlands); the DNA was eluted in 110 µL 2SP.[7] Presence of CT DNA was reconfirmed for all samples with our in-house PCR. Sensitivity of this assay was determined by using a previously described serial dilution of lymphogranuloma venereum (LGV) strain L2 and was assessed at 0.01 inclusion-forming units.[9] Amplification and detection were performed with an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA) by standard PCR conditions of the manufacturer, with 45 cycles. The Swedish variant was found in none of the 3 cohorts tested. Sensitivity and specificity were confirmed by using 12 swCT variant samples from Sweden, which were all positive according to our swCT TaqMan assay.

Our new swCT TaqMan assay, combined with the positive control (which can be obtained by contacting S.M.), will be a helpful tool for determining whether this Swedish CT variant is present outside Sweden, other than in the 2 case-patients identified in Norway. We did not find any evidence of the swCT variant in the Netherlands or St. Petersburg, Russia, each of which is near Scandinavia (Table). Recently, the *C. trachomatis* LGV strain was discovered in the Netherlands in a population of men who have sex with men. In this instance, the real-time TaqMan assay also proved helpful in determining spread.[10]

Table. Published studies and the current study on screening for the swCT variant*

Location	Ct+, no. detected	swCT variant, no. detected	Reference
Amsterdam, the Netherlands	75	ND	3
Dublin, Ireland	750	ND	4
Oslo, Norway†	47	2	5
St. Petersburg, Russia	152	ND	This study
Heerlen, the Netherlands	57	ND	This study
Amsterdam, the Netherlands	30	ND	This study

*swCT, Swedish *Chlamydia trachomatis* variant identified in Halland County, Sweden; Ct+, C. trachomatis DNA; ND, not detected.

†2 female patients: 1 originally from Sweden, 1 from Norway.

REFERENCES

1. Soderblom T, Blaxhult A, Fredlund H, Herrmann B. Impact of a genetic variant of *Chlamydia trachomatis* on national detection rates in Sweden. Euro Surveill. 2006;11:E061207.1.
2. de Vries HJC, Catsburg A, van der Helm JJ, Beukelaar EC, Morré SA, Fennema JSA, et al. No indication of Swedish *Chlamydia trachomatis* variant among STI clinic visitors in Amsterdam. Euro Surveill. 2007;12:E070208.3.
3. Lynagh Y, Crowley B, Walsh A. Investigation to determine if newly-discovered variant of *Chlamydia trachomatis* is present in Ireland. Euro Surveill. 2007;12: E070201.2.
4. Moghaddam A, Reinton N. Identification of the Swedish *Chlamydia trachomatis* variant among patients attending a STI clinic in Oslo, Norway. Euro Surveill. 2007;12:E070301.3.
5. de Laar V, Ison C. Europe-wide investigation to assess the presence of new variant of *Chlamydia trachomatis* in Europe. Euro Surveill. 2007;12:E070208.4.
6. Ripa T, Nilsson PA. A *Chlamydia trachomatis* strain with a 377-bp deletion in the cryptic plasmid causing false-negative nucleic acid amplification tests. Sex Transm Dis. 2007;34:255–6.
7. Catsburg A, van der Zwet WC, Morré SA, Ouburg S, Vandenbroucke-Grauls CM, Savelkoul PH. Analysis of multiple single nucleotide polymorphisms (SNP) on DNA traces from plasma and dried blood samples. J Immunol Methods. 2007;321:135–41.
8. Savitcheva A, Smirnova T, Pavlova N, Bashmakova M, Shishkina O, Novikov B, et al. Diagnosis and treatment of genital *Chlamydia trachomatis* infection in St. Petersburg and Leningradskaya Oblastj. In: Domeika M., Hallen A., editors. *Chlamydia trachomatis* infection in Eastern Europe. Uppsala (Sweden): Uppsala University; 2000.
9. Morré SA, Sillekens P, Jacobs MV, van Aarle P, de Blok S, van Gemen B, et al. RNA amplification by nucleic acid sequence-based amplification with an internal standard enables reliable detection of *Chlamydia trachomatis* in cervical scrapings and urine samples. J Clin Microbiol. 1996;34:3108–14.
10. Morré SA, Spaargaren J, Fennema JSA, de Vries HJC, Peña AS. Real-time PCR for the rapid one-step diagnosis of *Chlamydia trachomatis* LGV infection to help manage and contain the current outbreak in Europe and the USA. Emerg Infect Dis. 2005;11:1311–2

4. High concordance of test results of the rapid and easy *Chlamydia trachomatis* Detection and genoTyping Kit compared to the COBAS Amplicor CT/NG test in females visiting an STD clinic.

» Submitted

Laura van Dommelen, Antoinette A.T.P. Brink, Frank H. van Tiel, Wim G.V. Quint, Servaas A. Morré, Petra F. Wolffs, Christian J.P.A. Hoebe

We have compared *Chlamydia trachomatis* (Ct) detection in 672 self obtained vaginal swab samples by the DNA enzyme immunoassay, part of Ct Detection and genotyping Kit (Ct-DT), with the COBAS Amplicor CT/NG. Detection results were highly concordant between the two tests. Furthermore, information on Ct load was available to further analyze results. In addition, the Ct-DT proved to be a reliable and user-friendly genotyping method. These results are essential for researchers interested in large scale epidemiological Ct projects.

INTRODUCTION

The prevalence of genital *Chlamydia trachomatis* (Ct) infections has increased in the last decade. Improving diagnostic methods and increased knowledge of the epidemiology, transmission and pathogenicity, can contribute to a reduction in Ct infections. Typing Ct plays an important role in this process and several typing methods have been published in recent years, as reviewed by Pedersen et al. [5] In the present study, we have compared the performance of the polymerase chain reaction (PCR) based Ct Detection and genoTyping Kit [9](Ct-DT; Labo Bio-medical Products B.V., Rijswijk, The Netherlands) with the COBAS Amplicor CT/NG (Roche Diagnostics Systems, Basel, Switzerland) for the detection of Ct in a well described female population consulting a sexually transmitted diseases (STD) clinic.[13] Contrary to the COBAS Amplicor CT/NG, the Ct-DT is directed at two targets (on the cryptic plasmid and Omp1 gene) to detect Ct, which potentially reduces the number of false negative results.[1]

Although several studies have been published concerning the Ct-DT [2, 6-9], none addressed the sensitivity in a representative population using a commercially available screening method. With our study, we aim at providing data to validate the use of the Ct-DT as a Ct screening method for large scale epidemiologic Ct research.

MATERIAL AND METHODS

Between September 2007 and April 2008, self obtained vaginal swabs (SVS) were collected from females visiting an STD clinic, as described previously.[13] In short, every client was asked to take several SVS (pre-numbered 1-6). The presence of Ct DNA in the original study was primarily determined on SVS 2 by the COBAS Amplicor CT/NG, which was performed according to the manufacturer's instructions (reference standard). SVS 1 and 6 were subsequently used to determine the Ct load using a quantitative PCR (qPCR).[13] The COBAS Amplicor CT/NG is not licensed for SVS, but previous studies have not demonstrated a significant difference in performance between SVS and practioner-collected endocervical swabs. [4,11] For the current study, 200 µL of COBAS Amplicor CT/NG medium from SVS 2 was used for DNA extraction , using the Qiagen DNA mini kit (Qiagen GmbH, Hilden, Germany). COBAS Amplicor medium was used in agreement with Labo Bio-medical Products. All samples were previously stored at -80°C and only thawed once for the current study. DNA was eluted in 100 µL, of which 10 µL was used for the Ct-DT PCR-DNA enzyme immunoassay (DEIA). Ct genotyping was performed using the Ct-DT reverse hybridization assay (RHA) on all samples showing a DEIA optical density (OD) of at least 0.75 times the OD of the borderline DEIA control. An OD between 0.75-1.00 of the borderline DEIA control is considered a borderline result and anything above 1.00 a positive result. The Ct-DT was performed according to the manufacturer's instruction as described previously.[9] Discrepant samples were retested by the DEIA and COBAS Amplicor CT/NG and, if the discrepancy was still present, by the COBAS TaqMan CT Test v2.0 (Roche Diagnostics Systems, Basel, Switzerland). A sample was considered true Ct positive (comparison standard)

if the primary COBAS Amplicor CT/NG was positive and if Ct plasmids were detected with the Ct-DT RHA. Statistical analyses were performed with the SPSS package version 14.0 (SPSS, IBM corp., New York, USA) and www.statpages.org.[3,10]

RESULTS

In all, 772 patients were included in the original study.[13] COBAS Amplicor medium was available from 672 cases, resulting in a Ct prevalence of 10.9% (original study Ct prevalence 10.9%) when using the COBAS Amplicor CT/NG as a single assay. With the Ct-DT, 70 out of 73 COBAS Amplicor CT/NG Ct positive samples (96%) tested positive or borderline (2 samples), leaving three discrepant results (Table 1 and 2). In both samples with borderline results, the plasmid could be detected using RHA and therefore these samples were considered true positives. In the original study, one borderline sample was also tested using the qPCR, indicating a low Ct load (269 infection forming units (IFU)/SVS).

Table 1 Performance characteristics of Ct-DT compared with Cobas Amplicor CT/NG.[3,10]

			Cobas Amplicor CT/NG (Ct)						
			Positive		*Negative*	*Total*			*95% CI*
Ct-DT	*Positive*		*70*		*0*	*70*	*PPV*	*100%*	*94.9-100%*
	Negative		*3*		*599*	*602*	*NPV*	*99.5%*	*98.6-99.9%*
	Total		*73*		*599*	*672*			
		Se	*95.9%*	*Sp*	*100%*				
		95% CI	*88.5-99.1%*	*95% CI*	*99.4-100%*				

Ct-DT = *Chlamydia trachomatis* Detection and genoTyping Kit, CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value, Se = sensitivity, Sp = specificity

Retesting of the discrepant samples using the COBAS Amplicor CT/NG resulted in one positive and two negative results. Repeated DEIA assays of discrepant samples were all negative. The COBAS TaqMan also gave a positive result in the repeated COBAS Amplicor CT/NG positive sample. In the original study the average qPCR result (average Ct load of SVS 1 and 6) in this sample was 630 IFU/SVS. The qPCR was also performed on one of the (repeated) COBAS Amplicor CT/NG negative samples, which resulted in an average Ct load of 38 IFU/SVS. The average IFU/SVS measured in Ct positive samples (performed on 58/73 Ct positive samples)

in the original study was 38110 IFU/SVS. All COBAS Amplicor Ct negative samples were also negative with the Ct-DT, suggesting that plasmidless Ct strains were not present in this population. The agreement between the Ct-DT and COBAS Amplicor CT results was 99.6% (κ = 0.98) and the sensitivity of the Ct-DT relative to COBAS Amplicor CT was 96% (95% confidence interval 88.5-99.1%).

Genotyping results are presented in Table 2. In 97% (68/70) of the DEIA positive/borderline samples, the serogroup could be determined; the serovar could be determined in 93% of these samples (65/70). The average Ct loads in 3 out of 5 non-typable samples were 269, 6007 and 2197 IFU/SVS. Serovars E and F were most prevalent: 57% (37/65) and 28% (18/65), respectively. Two patients were infected with two different serogroups/serovars (I/F plus C/? and B/E plus C/K).

Table 2 Nucleic acid amplification test results, including serovar distribution

COBAS Amplicator CT/NG	DEIA	RHA		N
		Serogroup	Serovar	
Positive	Positive	B complex	D/Da	4
			E	37*
			Not detected	1
		Intermediate complex	F	18**
			G/Ga	3
		C complex	I/Ia	2
			J	1
			Not detected	1
		Only plasmid detected		1
	Borderline	B complex	Not detected	1
		Only plasmid detected		1
	Negative	NA	NA	3
Negative	Negative	NA	NA	599
				672

* One double infection with serogroup C, serovar K.
** One double infection with serogroup C (no serovar detected)
NA Not assessed

DISCUSSION

We have shown that the Ct-DT is a sensitive and highly specific assay to detect Ct compared to the COBAS Amplicor CT/NG and serovars could be determined in almost all Ct positive samples. As described above, in two out of three discrepant samples the Ct load was shown to be low in the original study (average of 38 and 630 IFU/SVS). Moreover, the repeated COBAS Amplicor CT/NG was negative in one of these two samples, as well as in the third discrepant sample. Therefore the limits of sensitivity of the Ct-DT become apparent in these samples with a low Ct load. Regarding the non-typable samples, again the average Ct load, in the 3 out of 5 samples for which the data were available, was much lower than the group average (2824 vs 38110 IFU/SVS, respectively) which could explain the results. Also, sequence variation in the primer target region of the omp1 gene could result in non-typable samples.[7]

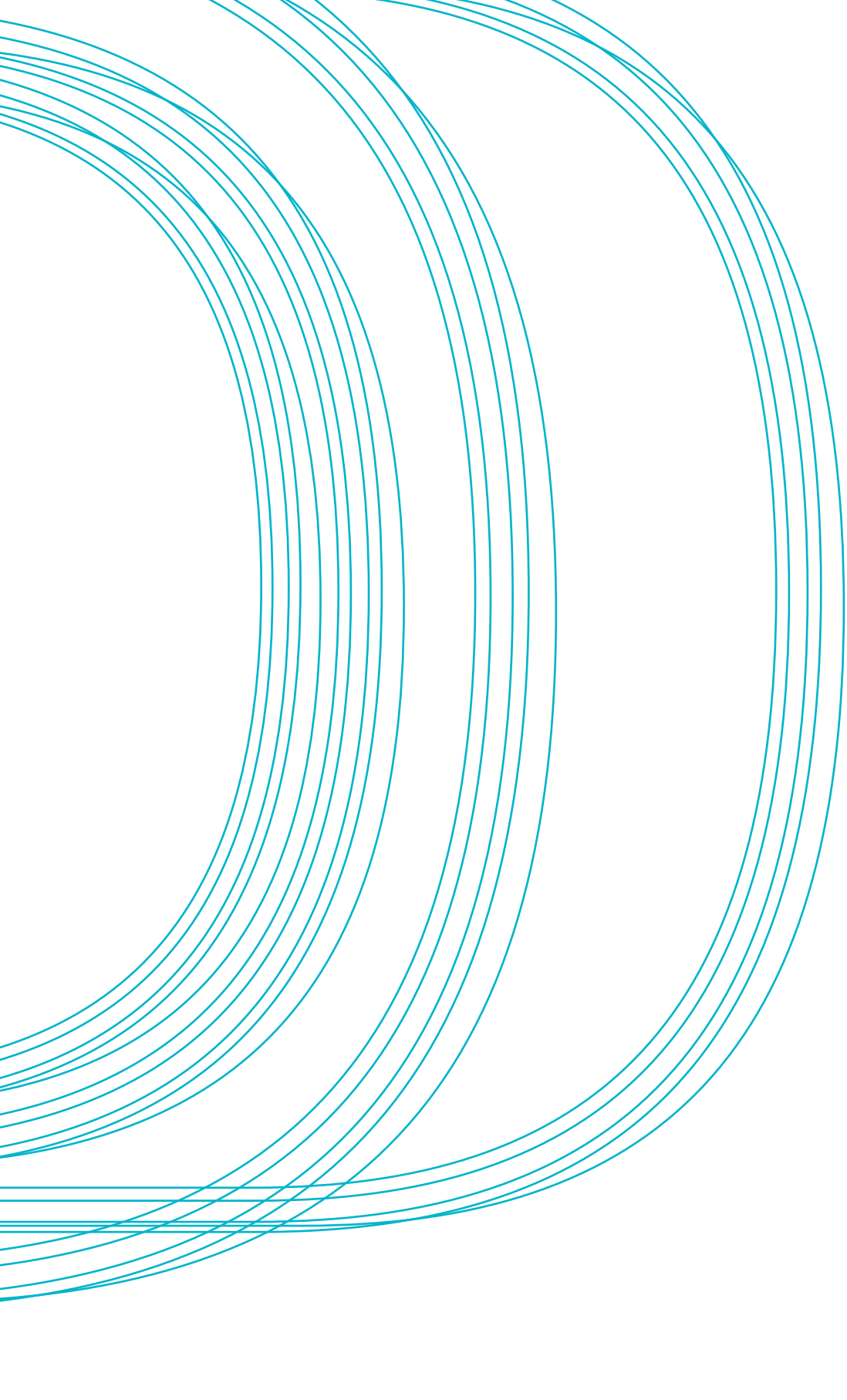
A limitation of the study lies in the fact that retesting was only performed on discrepant samples. This could lead to overestimation of test performance. However, considering the previously reported agreement and specificity of the Ct-DT with other assays, this seems unlikely.[7,9] Strengths of this study are the well described population and the thorough evaluation according to standardized clinical and laboratory procedures. Moreover, in the large group of Ct positive females, quantitative Ct PCR data were available to make excellent performance analysis.

Although assessing genotyping performance of the Ct-DT kit was not the primary goal of this study, we have shown that the assay was able to determine serogroup/serovar in most Ct positive samples. Quint et al. have already compared the Ct-DT genotyping with Omp1 sequencing and found a very good agreement ($\kappa = 0.875$)[8] and therefore we expect our results to be valid. The Ct-DT is a more rapid and easier to perform method to detect the most commonly detected serovars than PCR-RFLP typing. Since the sensitivity and specificity for Ct detection is comparable with the COBAS Amplicor CT/NG, the Ct-DT is an excellent assay for large, epidemiological studies or diagnostic purposes. Moreover, it can detect multiple serovars in one sample, which is not the case in Omp1 sequencing (using standard protocol sequencing methods), unless the load difference between the two mixed serovars is small. The serovar distribution we found in our study, is comparable to previously published Dutch data in an STD clinic population.[12] We did not find L1, L2 or L3 (LGV serovars) in our population, since these serovars are most prevalent in men who have sex with men.

In conclusion, we have found a high agreement between the Ct-DT and COBAS Amplicor CT/NG and were able to determine the serovar in 93% of the Ct positive samples. Due to its excellent performance, we believe this rapid and easy to perform assay can play a major role in future epidemiological studies.

REFERENCES

1. An Q, Radcliffe G, Vassallo R, Buxton D, O'Brien WJ, et al. (1992) Infection with a plasmid-free variant *Chlamydia* related to *Chlamydia trachomatis* identified by using multiple assays for nucleic acid detection. J Clin Microbiol 30: 2814-2821.
2. Bax CJ, Quint KD, Peters RP, Ouburg S, Oostvogel PM, et al. (2011) Analyses of multiple-site and concurrent *Chlamydia trachomatis* serovar infections, and serovar tissue tropism for urogenital versus rectal specimens in male and female patients. Sex Transm Infect 87: 503-507.
3. Clopper CJ, Pearsen ES. (1934) The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 26: 404-413 (http://statpages.org). Accessed October 7th 2012
4. Knox J, Tabrizi SN, Miller P, Petoumenos K, Law M, et al. (2002) Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. Sex Transm Dis 29: 647-654.
5. Pedersen LN, Herrmann B, Moller JK. (2009) Typing *Chlamydia trachomatis*: from egg yolk to nanotechnology. FEMS Immunol Med Microbiol 55: 120-130.
6. Porras C, Safaeian M, Gonzalez P, Hildesheim A, Silva S, et al. (2008) Epidemiology of genital *Chlamydia trachomatis* infection among young women in Costa Rica. Sex Transm Dis 35: 461-468.
7. Quint K, Porras C, Safaeian M, Gonzalez P, Hildesheim A, et al. (2007) Evaluation of a novel PCR-based assay for detection and identification of *Chlamydia trachomatis* serovars in cervical specimens. J Clin Microbiol 45: 3986-3991.
8. Quint KD, Bom RJ, Bruisten SM, van Doorn LJ, Nassir Hajipour N, et al.(2010) Comparison of three genotyping methods to identify *Chlamydia trachomatis* genotypes in positive men and women. Mol Cell Probes 24: 266-270.
9. Quint KD, van Doorn LJ, Kleter B, de Koning MN, van den Munckhof HA, et al. (2007) A highly sensitive, multiplex broad-spectrum PCR-DNA-enzyme immunoassay and reverse hybridization assay for rapid detection and identification of *Chlamydia trachomatis* serovars. J Mol Diagn 9: 631-638.
10. Rosner, B. (2006) Fundamentals of Biostatistics (http://statpages.org). Accessed October 7th 2012
11. Skidmore S, Kaye M, Bayliss D, Devendra S. (2008) Validation of COBAS Taqman CT for the detection of *Chlamydia trachomatis* in vulvo-vaginal swabs. Sex Transm Infect 84: 277-278
12. Spaargaren J, Verhaest I, Mooij S, Smit C, Fennema HS, et al. (2004) Analysis of *Chlamydia trachomatis* serovar distribution changes in the Netherlands (1986-2002). Sex Transm Infect 80: 151-152.
13. van Dommelen L, van Tiel FH, Ouburg S, Brouwers EE, Terporten PH, et al. (2010) Alarmingly poor performance in *Chlamydia trachomatis* point-of-care testing. Sex Transm Infect 86: 355-359.



Point-of-Care Tests to Detect Sexually Transmitted Infections

5. Alarmingly poor performance in *Chlamydia trachomatis* point-of-care testing.

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Laura van Dommelen, Frank H van Tiel, Sander Ouburg, Elfi E H G Brouwers, Peter HW Terporten, Paul H M Savelkoul, Servaas A Morré, Cathrien A Bruggeman, Christian J P A Hoebe

Background Infection by *Chlamydia trachomatis* (CT) is the most prevalent sexually transmitted infection (STI) world wide. The most frequently used diagnostic test for CT is a nucleic acid amplification test (NAAT), which is highly sensitive and specific. To further shorten time delay until diagnosis has been made, in order to prevent CT spread, the use of point-of-care (POC) tests may be the way forward. **Objectives** The diagnostic performance of three POC tests, Handilab-C, Biorapid *CHLAMYDIA* Ag test and QuickVue *Chlamydia* test, was evaluated and compared with NAAT. **Methods** All women, above the age of 16 years, attending for a consultation at an STI clinic between September 2007 and April 2008, were asked to participate. Women were asked to complete a short questionnaire and to collect six self-taken vaginal swabs (SVS). SVS 2 was used for NAAT and SVS 3 to 5 were randomised for the different POC tests. SVS 1 and 6 were used for determining quantitative CT load to validate the use of successive SVS. All POC tests were performed without knowledge of NAAT results. NAAT was used as the 'gold standard'. **Results** 772 women were included. CT prevalence was 11% in our population. Sensitivities of the Biorapid *CHLAMYDIA* Ag test, QuickVue *Chlamydia* and Handilab-C test were 17%, 27% and 12%, respectively. **Conclusions** The evaluated POC tests, owing to their very low sensitivities, are not ready for widespread use. These results underline the need for good-quality assurance of POC tests, especially in view of the increased availability of these tests on the internet.

INTRODUCTION

World wide, *Chlamydia trachomatis* (CT) remains the most prevalent bacterial sexually transmitted infection (STI), with increased incidence in Europe over the past decade.[1] CT infection is a major cause of reproductive morbidity,[2,3] bacterial conjunctivitis in neonates,[4] and may facilitate HIV transmission.[5] The use of nucleic acid amplification tests (NAAT) with self-taken vaginal swabs (SVS) or urine have made CT testing more sensitive, specific and acceptable.[6] Nevertheless, case finding and case recognition is hampered first by the limited willingness of patients at risk to undergo STI testing because of fear of pelvic examination and stigmatisation, and second owing to the frequently asymptomatic nature of these infections.[7] Moreover, with the use of NAAT, there is still a time delay between first consultation and treatment, usually around 1-2 weeks.[8] Although some infections may resolve during this period, secondary transmission can take place and infection can progress. Therefore, a point-of-care (POC) test with proven diagnostic accuracy may well help limit the spread of and morbidity associated with CT.

Over the past few years, an increase in the availability of POC tests in drug stores and on the internet has been noticeable. In general, there appears to be a trend towards producing diagnostics, which are faster and easier to use. WHO has formulated criteria for a POC test which is adequate[9]: a new STI diagnostic test should be affordable by those at risk, sensitive (sensitivity between 43% and 65%), specific (specificity of 98%), user-friendly, rapid and robust, equipment-free and deliverable to those in need (ASSURED criteria; http://www.who.int/std_diagnostics, accessed 14 June 2010). We have selected three widely available POC CT diagnostic tests, which might meet these criteria but which have not yet been evaluated thoroughly. We assessed laboratory performance and the potential acceptability, when used in optimal conditions compared with NAAT, to maximise POC test results before evaluation in non-laboratory and/or less developed settings. Moreover, the use of successive SVS was validated using a quantitative CT NAAT.

METHODS

Study setting, specimen collection and population

Women above the age of 16 years applying for STI consultation between September 2007 and April 2008 were included in the study. The medical ethics committee of Maastricht University Medical Centre approved this study (MEC LLL06srs) and all participants signed a written consent form. At the STI clinic, each patient was asked to take six number-marked SVS in the order of number (SVS 1 to 6). Patients were shown how to insert the vaginal swab by approximately 4-5 cm and with 10 s vaginal rotation and rubbing time and how to position the swab into each capped tube. During the consultation, demographic and behavioural data were collected and, if indicated, samples were collected for other STI diagnostics. All data and

SVS were anonymised and transported to the hospital while refrigerated. Patients who tested positive for CT were treated with a single dose of 1000 mg azithromycin. CT prevalence was expected to be 11% in this population with no loss to follow-up.[6]

Point-of-care tests

SVS 3 to 5 were used for the POC tests. The three POC tests that were validated were the Handilab-C (Zonda, Dallas, USA), Biorapid *CHLAMYDIA* Ag test (Biokit, S.A., Barcelona, Spain) and QuickVue *Chlamydia* test (Quidel Corporation, San Diego, USA). All POC tests had a CE mark and were commercially available. In order to control for possible differences in CT load in successively taken SVS, the POC tests were randomised before distribution, into SVS groups (named A, B and C) with Handilab-C, Biorapid *CHLAMYDIA* Ag test and QuickVue *Chlamydia* tests being performed on SVS 3-4-5 in group A, SVS 4-5-3 in group B and SVS 5-3-4 in group C, respectively. The Handilab-C is an enzymatic test with a detection limit of 16 inclusion bodies/test (package insert). The Biorapid *CHLAMYDIA* Ag test and QuickVue *Chlamydia* test are antigen tests; the detection limit of the Biorapid *CHLAMYDIA* Ag test is 57-570 elementary bodies/test and the QuickVue *Chlamydia* should have a sensitivity of 81% when <100 inclusion forming units (IFU)/ml are present (package inserts).

All POC tests were stored and performed under optimal conditions in the medical microbiology laboratory, after training provided by the suppliers, and according to the manufacturers' instructions. One exception was the use of an SVS instead of an endocervical specimen with the Biorapid *CHLAMYDIA* Ag test and QuickVue *Chlamydia* test. The POC tests were performed in the medical microbiology laboratory, but the Handilab-C test was started at the STI clinic: 'fluid A' was allowed to mix with the specimen and left standing for 10 min. After transportation to the laboratory, the swab was pushed through the foil in order to make a short contact with 'fluid B'. This procedure was discussed and supported by the manufacturer. The Handilab-C cannot be used during menstruation and the second step of the test performance must be completed within 24 h (definition of an 'on time' result). Both the Biorapid *CHLAMYDIA* Ag test and QuickVue *Chlamydia* test had to be performed within 72 h after collecting the SVS (definition of an 'on time' result). POC tests were performed and read by LvD and three fully qualified microbiological technicians. NAAT results and clinical data were linked to the POC test results no sooner than at the end of the study. Stratification by menstruation and time to test performance was therefore done retrospectively.

NAAT tests

The COBAS Amplicor CT/NG (Roche Diagnostics Systems, Basel, Switzerland) on SVS 2 was used as 'gold standard' for determining CT presence. Although the COBAS Amplicor CT/NG is not licensed for SVS, previous studies have demonstrated no significant difference in performance between the use of SVS and that of endocervical swabs.[10 11] SVS 2 was placed in 1 ml lysis buffer and after rotation for 10 s the swab was squeezed by pressing against the plastic tube and then removed. Next, 1 ml diluent was added, mixed, centrifuged and 50 ml of the supernatant was added to 50 ml PCR Mix. The sample was processed further according to the standard operating procedure for CT PCR. A result of more than 10 000 DNA copies was considered positive. All low positive samples between 2000 and 9999 copies of CT DNA were retested to confirm the presence of CT. Samples with repeatedly borderline (n=1) or inhibited (n=8) NAAT results were excluded from analysis.

For quantitative CT load determination, a real-time PCR (TaqMan assay) targeting the cryptic plasmid of CT (sensitivity of 0.01 IFU as compared with 1 IFU for the COBAS Amplicor and able to detect the recently reported Swedish variant of CT) or the human HLA was developed with Primer Express v2.0 (Applied Biosystems, Foster City, California, USA), described previously by Catsburg et al.[12] Real-time PCR reactions were performed in a volume of 30 ml PCR volume, consisting of TaqMan Mastermix (Applied Biosystems), 300 nM of each primer, 150 nM of each probe and 5 ml prepared sample. Amplification and detection was performed with an ABI Prism 7000 sequence detection system (Applied Biosystems) using standard PCR conditions of the manufacturer, with 45 cycles. By using a *chlamydia*l and a human target, the average *chlamydial*/ human cell load ratio, and IFU/swab were calculated. All samples were spiked with an optimal amount of internal control to validate the sample preparation as well as the RT-PCR procedure.

Statistical analysis

Sensitivity, specificity, negative (NPV) and positive (PPV) predictive values of the different POC tests compared with 'gold standard' PCR were calculated. Categorical variables were analysed with the Pearson χ^2 test for independence and with Fisher's exact test where appropriate. Binary logistic regression was used to determine the influence of different variables (including randomisation) on the outcome of NAAT and POC tests. A p value <0.1 was used for selecting variables and a p value <0.05 was used to determine significant adjusted OR. Quantitative CT results were compared using the t test for paired samples. A p value <0.05 was considered statistically significant. Analyses were performed with the SPSS package version 14.0.

Role of POC test providers

None of the POC test providers had any role in the study design, collection or interpretation of the data or writing of the manuscript.

RESULTS

Population and questionnaire

Between September 2007 and April 2008, 772 women were included with a median age of 23 years (range 16-64). Over 95% of all clients filled in the questionnaire. The median age of first sexual contact was 16 years (range 6-36). The median lifetime number of sexual partners was nine (range 1->99) and almost half of these contacts were considered as unsafe sexual contact. During the past 6 months, the median number of newly acquired sexual partners was three (mean 4; range 0->99). Only two out of 772 women were coinfected with Neisseria gonorrhoeae. No cases of syphilis or HIV were detected. In the month before visiting the outpatient clinic, 13% (99/772) of the clients had used antibiotics, five of whom were CT positive with NAAT. The CT-positive clients could not recall which antibiotic they had used.

POC tests compared with NAAT

C trachomatis testing by COBAS Amplicor resulted in a CT prevalence of 11% in our population (84/772 clients). Sensitivities, specificities, NPV and PPV of the different POC tests compared with NAAT are presented in table 1. Results are presented according to time between collecting the SVS and performance of the POC test and subdivided for women with self-reported symptoms. Owing to logistical limitations, 49% of the Handilab-C results were performed in time. On time Handilab-C results are depicted for non-menstruating clients, since this test is not validated in the case of menstruation. Sensitivities of the Biorapid *CHLAMYDIA* Ag test, QuickVue *Chlamydia* test and Handilab-C were 17%, 27% and 12%, respectively. The failure rate (meaning an invalid or missing test result) of 5% when including all Handilab-C results is mainly caused by presence of blood on the SVS, which hinders interpretation of the test result; self-reported menstruation was the probable cause of 85% (23/27) of the bloody samples. If all POC tests were included, sensitivity only decreased significantly in the QuickVue *Chlamydia* test. Binary logistic regression was performed using all POC test results, taking into account factors that might influence diagnostic test results [13 14] (details on the binary logistic regression are available in the supplementary online table). This assessment suggested no relevant influences.

Table 1 Performance of the different point-of-care tests

	N	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV (%)	NPV (%)	Failure (%)
Biorapid *CHLAMYDIA* Ag test						
Performed within 72 h	737	17,3 (8,8 to 25,9)	93,5 (91,6 to 95,4)	23,2	90,9	1,2
Clients with symptoms	359	17,0 (6,3 to 27,8)	92,6 (89,7 to 95,5)	25,8	88,1	0,8
All results	763	17,1 (8,9 to 25,2)	93,7 (91,9 to 95,5)	24,6	90,4	1,2
QuickVue *Chlamydia* test						
Performed within 72 h	737	27,3 (17,3 to 37,2)	99,7 (99,3 to 100)	91,3	92,2	1,2
Clients with symptoms	357	28,6 (15,9 to 41,2)	99,7 (99,0 to 100)	93,9	89,8	1,4
All results	763	25,0 (15,7 to 34,3)	99,7 (99,3 to 100)	91,3	91,5	1,2
Handilab-C						
Performed within 24h in non-menstruating women	378	11,6 (2,0 to 21,2)	91,9 (89,0 to 94,9)	15,6	89	1
Clients with symptoms	180	11,1 (0,0 to 23,0)	91,5 (87,1 to 95,9)	18,8	85,4	0,6
All results	735	22,5 (133 to 31,7)	88,9 (86,4 to 91,3)	19,8	90,4	4,8

Quantitative CT NAAT results

Quantitative CT NAAT (qNAAT) was used on 70/84 positive CT samples. The qNAATwas inhibited in six paired samples and in a single SVS 6; all other samples tested CT positive. Almost 30% of the bacterial loads were identical between the first and sixth swab taken. Higher bacterial loads were seen in SVS 1 (mean: 445678 IFU/swab, median: 19410 IFU/swab: this is excluding extreme values with the Grubb test for outlier detection[15]) compared with SVS6 (mean: 29963 IFU/swab, median: 12180 IFU/swab: excluding extreme values). The CT load was <100 (but >20 CFU/ml) in one paired sample and in three single SVS 1 and two single SVS 6. Statistical analysis demonstrated no significant difference in POC test performance in relation to CT load for the different tests (data not shown). On average 14.6×10^6 HLA targets per swab were seen in SVS 1 (median: 5.0×10^6 HLA targets/swab), compared with an average of 706.7×10^6 HLA targets/swab in SVS 6 (median: 167.9×10^6 HLA targets/swab). The Grubb test was used to detect and remove outliers. The average bacterial load per cell was higher in SVS 1 than in SVS 6, probably owing to mucus removal by the immediately preceding five SVS.

DISCUSSION

The development and marketing of POC tests for CT has taken place in response to the demand for more rapid diagnosis, with the obvious goal of earlier treatment and prevention of secondary cases. In this study, three POC tests were evaluated under optimal laboratory conditions, in a population with a high CT prevalence (11%). Overall, our data show that all POC tests perform alarmingly poorly.

A few limitations in our study should be mentioned. First, choosing patients solely from a western laboratory setting, limits direct translation of our results to other settings. However, the poor performance of POC tests in our setting is unlikely to improve under conditions with lower resources. Second, reproducibility of POC tests could not be assessed, since each swab could only be used for one POC test. Third, the COBAS Amplicor does not detect the Swedish variant of CT (swCT or new variant nvCT) and POC test results might therefore be worse since some CT-positive samples might have been missed. The swCT, however, has been detected in The Netherlands in only one case so far and directly linked to a swCT-positive Swedish women (Morré SA, personal correspondence).[16 17] Finally, for the Biorapid *CHLAMYDIA* Ag test and QuickVue *Chlamydia* test a SVS was used instead of an endocervical swab as stated in the package insert. As we have shown, the CT loads in the SVS were almost all above the detection limit of the different POC tests and statistical analysis demonstrated no significant influence of CT load on test performance. Moreover, the bacterial loads found in our study using SVS, are comparable to results found for endocervical swabs in a previous study.[18]

The strengths of our study are the large study population, the comparison of three POC tests in one and the same study, the experiments performed to control for CT load differences in successively taken SVS and, finally, the use of the ASSURED criteria as a reference enabling objective reviewing of results.

In our experience, all POC tests were easy to perform with respect to laboratory handling, but the Handilab-C was difficult to interpret, even after multiple tests had been carried out. In a previously published evaluation, a small-scale Norwegian study [19] has already raised questions about the performance of the Handilab-C. In this study, 50% of all participating women, who were asked to perform the test themselves, were not certain how to interpret their Handilab-C result. Sixteen out of 157 participating women were CT positive with NAAT (used as 'gold standard'). The Handilab-C result was interpreted as positive by only four, and as uncertain by nine clients, which resulted in sensitivity between 25% and 57%. Michel et al recently evaluated the Handilab-C in a group of 231 women (38/ 231 CT NAAT positive), again demonstrating a low sensitivity, and discussed this in view of the value of a CE mark.[20]

The QuickVue *Chlamydia* has been evaluated twice thus far. In a 1997 publication, the QuickVue *Chlamydia* was evaluated in a population of 724 women divided into two high-risk and one low-risk population.[21] Sensitivity and specificity were on average 90.1% and 99.5%, respectively, in the high-risk populations (n=366, CT prevalence 14.1%). Performance of the QuickVue *Chlamydia* in this study was compared with culture. Samples with false-positive QuickVue *Chlamydia* results, however, were retested with NAAT and added to the true positive results if found positive with NAAT. In contrast, culture-negative samples, with a negative QuickVue *Chlamydia* result, were not retested with NAAT despite a sensitivity of culture of only 65%.[22] Therefore, false-negative QuickVue *Chlamydia* test results would not have been detected, and performance of the QuickVue *Chlamydia* in this study has been overestimated. In 2002, a second evaluation was published comparing QuickVue *Chlamydia* with NAAT in two groups of 100 women.[23] In the high-risk population, sensitivity and specificity were 65% and 100%, respectively, with 16 women being positive with NAAT. In the low-risk population however, the sensitivity was only 25% (1/4). If both groups in the study by Rani et al are taken into account, the CT prevalence in their study is 10% (20/200), which is comparable to the CT prevalence of 11% in our population. Recalculating sensitivity and specificity when using both populations of Rani et al, rendered a sensitivity of 55.0% (95% CI 33.3% to 76.8%) and a specificity of 100%, which is not significantly different from our results.

As can be extrapolated from our results, a POC test with excellent performance may make a difference; assuming a primary CT transmission of 65% (without further transmission) when having sexual contact,[24] a treatment delay of 2 weeks [8, 25] and a POC test sensitivity of 100%, eight additional new CT cases would have been avoided compared with NAAT. In contrast, when applying the same calculation to our data, the result is negative compared with NAAT and owing to false-positive results, participants would have been treated unnecessary, especially in case of the Biorapid *CHLAMYDIA* Ag and Handilab-C test. In a recent evaluation, the *Chlamydia* Rapid Test showed promising results[26]; this POC test primarily would have detected fewer CT cases than NAAT, but owing to instant treatment prevent more CT cases, resulting in equal outcome in our model. A sensitivity of 83.5% is not sufficient to replace NAAT in a setting with minimal loss to follow-up; costebenefit analysis therefore may determine if combining NAAT and a POC test is beneficial to avert additional CT cases.

In summary, results of this study, performed in a large population, show poorer laboratory performance of the different POC tests than has previously been described. The ASSURED criteria for POC testing including a sensitivity 43-65% and a specificity 98%,[9] are not met by any of the POC tests. The poor performance of all POC tests evaluated in our study has implications

for public health, since the Handilab-C test remained commercially available via the internet (€29.95) during the entire inclusion period. The distributor has claimed a reliability of 98.15% (not further specified) on his website while, for instance, sensitivity in our study population was only 12%. Our results underline the need for good quality assurance of POC tests, especially in view of their availability on the internet.[27] Although excellent guidelines on CT POC test evaluation exist,[28] these guidelines are regularly ignored, and thus tighter regulations are urgently needed to prevent unrestrained marketing.[9] In our opinion, the CT POC tests we have evaluated, are not ready for widespread use.

REFERENCES

1. van de Laar MJ, Morre SA. *Chlamydia*: a major challenge for public health. Euro Surveill 2007;12:E1e2.
2. Boeke AJ, van Bergen JE, Morre SA, et al. The risk of pelvic inflammatory disease associated with urogenital infection with *Chlamydia trachomatis*; literature review. Ned Tijdschr Geneeskd 2005;149:878e84.
3. Mardh PA. Tubal factor infertility, with special regard to *chlamydia*l salpingitis. Curr Opin Infect Dis 2004;17:49e52.
4. Rours IG, Hammerschlag MR, Ott A, et al. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. Pediatrics 2008;121:e321e6.
5. Laga M, Manoka A, Kivuvu M, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. Aids 1993;7:95e102.
6. Hoebe CJ, Rademaker CW, Brouwers EE, et al. Acceptability of self-taken vaginal swabs and first-catch urine samples for the diagnosis of urogenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with an amplified DNA assay in young women attending a public health sexually transmitted disease clinic. Sex Transm Dis 2006;33:491e5.
7. Arkell J, Osborn DP, Ivens D, et al. Factors associated with anxiety in patients attending a sexually transmitted infection clinic: qualitative survey. Int J STD AIDS 2006;17:299e303.
8. Geisler WM, Wang C, Morrison SG, et al. The natural history of untreated *Chlamydia trachomatis* infection in the interval between screening and returning for treatment. Sex Transm Dis 2008;35:119e23.
9. Peeling RW, Holmes KK, Mabey D, et al. Rapid tests for sexually transmitted infections (STIs): the way forward. Sex Transm Infect 2006;82(Suppl 5):v1e6.
10. Knox J, Tabrizi SN, Miller P, et al. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. Sex Transm Dis 2002;29:647e54.
11. Skidmore S, Kaye M, Bayliss D, et al. Validation of COBAS Taqman CT for the detection of *Chlamydia trachomatis* in vulvo-vaginal swabs. Sex Transm Infect 2008;84:277e8; discussion 8e9.
12. Catsburg ASPHM, Vliet A, Algra J, et al. Development and evaluation of an internally controlled Real-Time quantitative PCR assay for the detection of *Chlamydia trachomatis*. In: Chernesky M, Caldwell H, Christiansen G, et al, Eds. Eleventh International Symposium on Human *Chlamydia*l Infections. Niagara-on-the-Lake, Ontario, Canada, 2006:521e4.
13. Marrazzo JM, Johnson RE, Green TA, et al. Impact of patient characteristics on performance of nucleic acid amplification tests and DNA probe for detection of *Chlamydia trachomatis* in women with genital infections. J Clin Microbiol 2005;43:577e84.
14. Ghanem KG, Johnson RE, Koumans EH, et al. Cervical specimen order and performance measures of *Chlamydia trachomatis* diagnostic testing. J Clin Microbiol 2005;43:5295e7.
15. Grubbs F. Procedures for detecting outlying observations in samples. Technometrics 1969;11:1e21.

16. Morre SA, Catsburg A, de Boer M, et al. Monitoring the potential introduction of the Swedish *Chlamydia trachomatis* variant (swCT) in the Netherlands. Euro Surveill 2007;12:E9e10.
17. de Vries HJ, Catsburg A, van der Helm JJ, et al. No indication of Swedish *Chlamydia trachomatis* variant among STI clinic visitors in Amsterdam. Euro Surveill 2007;12:E070208 3.
18. Michel CE, Sonnex C, Carne CA, et al. *Chlamydia trachomatis* load at matched anatomic sites: implications for screening strategies. J Clin Microbiol 2007;45:1395e402.
19. Moi H. Handilab C *Chlamydia* for home testing is not what it claims. Tidsskr Nor Laegeforen 2007;127:2083e5.
20. Michel CE, Saison FG, Joshi H, et al. Pitfalls of internet-accessible diagnostic tests: inadequate performance of a CE-marked *Chlamydia* test for home use. Sex Transm Infect 2009;85:187e9.
21. Steingrimsson O, Pawlak C, Van Der Pol B, et al. Multicenter comparative evaluation of two rapid immunoassay methods for the detection of *Chlamydia trachomatis* antigen in endocervical specimens. Clin Microbiol Infect 1997; 3:663e7.
22. Livengood CH 3rd, Wrenn JW. Evaluation of COBAS AMPLICOR (Roche): accuracy in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by coamplification of endocervical specimens. J Clin Microbiol 2001;39:2928e32.
23. Rani R, Corbitt G, Killough R, et al. Is there any role for rapid tests for *Chlamydia trachomatis*? Int J STD AIDS 2002;13:22e4.
24. Lin JS, Donegan SP, Heeren TC, et al. Transmission of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among men with urethritis and their female sex partners. J Infect Dis 1998;178:1707e12.
25. Fernando I, Oroz C, Steedman N, et al. Factors affecting time to treatment following diagnosis of genital *Chlamydia trachomatis* infection in Scottish genitourinary medicine clinics. Int J STD AIDS 2007;18:819e22.
26. Mahilum-Tapay L, Laitila V, Wawrzyniak JJ, et al. New point of care *Chlamydia* Rapid Testebridging the gap between diagnosis and treatment: performance evaluation study. BMJ 2007;335:1190e4.
27. Owens SL, Arora N, Quinn N, et al. Utilising the internet to test for sexually transmitted infections: results of a survey and accuracy testing. Sex Transm Infect 2010;86:112e16.
28. Herring A, Ballard R, Mabey D, et al. Evaluation of rapid diagnostic tests: *chlamydia* and gonorrhoea. Nat Rev Microbiol 2006;4(Suppl 12):S41e8.

6. Evaluation of a rapid one-step immunochromatographic test and two immunoenzymatic assays for the detection of anti-*Treponema pallidum* antibodies.

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L van Dommelen, A Smismans, V J Goossens,
J Damoiseaux, C A Bruggeman, F H van Tiel, C J P A Hoebe

Background The control of syphilis depends on screening of the population at risk and is usually performed using the *Treponema pallidum* particle agglutination test (TPPA). Outside Europe the rapid plasma reagin test (RPR) or Venereal Disease Research Laboratory Test is most often used for screening purposes. Because of the drawbacks in current diagnostic procedures, ie, long turnaround time, the need is felt for a rapid and simple test that can potentially be performed on whole blood. **Objective and study design** In this study a one-step immunochromatographic test (Biorapid Syphilis) and two ELISA, the Bioelisa Syphilis 3.0 and ETI-Treponema Plus, were evaluated. **Methods** Serum samples were collected between February 2000 and May 2006 at the University Hospital in Maastricht, The Netherlands. 145 TPPA-positive sera, confirmed by fluorescent treponemal antibody absorption (FTA-Abs, treponemal test) and/or RPR (non-treponemal) were included. Furthermore, 41 sera from healthy controls and 144 TPPA-negative sera from controls with underlying conditions that might interfere with *T pallidum* serology were collected. **Results** The sensitivity and specificity of the Biorapid Syphilis, Bioelisa Syphilis 3.0 and ETI-Treponema Plus were 92% and 79%, 100% and 100% and 100% and 100%, respectively, with our selected sera. **Conclusions** The performance of both ELISA was excellent in our study and is favoured over the TPPA because of its ability to be run on an automated system. The sensitivity and specificity of the Biorapid Syphilis were considered too low to implement the test in a hospital laboratory in a developed country but it might be useful in primary healthcare settings in developing countries.

INTRODUCTION

Syphilis, a sexually transmitted disease caused by the spirochete *Treponema pallidum*, constitutes a major public health problem. According to the World Health Organisation estimate of 1999, approximately 12 million new cases of syphilis occur worldwide every year, with a wide variation in prevalence between countries.[1,2] In Europe the incidence of syphilis infections declined in the early 1990s as a result of the global health campaigns related to the HIV pandemic. By the end of the 1990s, however, the incidence had started to rise again because of an increase in unsafe sex.[3] In developing countries, in sub-Saharan Africa for example, syphilis in pregnant women is of particular concern because congenital syphilis causes 26% of all stillbirths and 11% of neonatal deaths.[4] In The Netherlands, most of the new syphilis cases were attributed to men having unsafe sex with men, with an increase of 340% between 2000 and 2004.[5]

Serological testing for treponemal antibodies is the cornerstone for the diagnosis and control of syphilis. In general, the diagnosis of syphilis can be made by different types of diagnostics: (1) direct microscopic examination, which has many logistical disadvantages; (2) non-treponemal serological tests, which lack sensitivity in some stages of syphilis and specificity; (3) treponemal serological tests, which are highly sensitive and specific and can remain positive for life and (4) PCR, under evaluation for *T pallidum*.[6] In The Netherlands, the *T pallidum* particle agglutination test (TPPA; also known as TPHA: *T pallidum* haemagglutinin agglutination test), a treponemal serological test, is most frequently used for screening purposes. Positive screening results are confirmed by fluorescent treponemal antibody absorption (FTA-Abs; a treponemal serological test) and the rapid plasma reagin test (RPR; a non-treponemal serological test). The TPHA screening test is a manually performed test with a turnaround time of two hours and requires laboratory facilities. Its performance, including interpretation, depends on the skills and experience of the laboratory technician. The sensitivity and specificity of the TPHA are 76–100% and 98–100%, respectively, depending on disease stage.[6] Considering the drawbacks of a long turnaround time, the need for human serum instead of whole blood and the availability of a laboratory with experienced personnel, new tests without one or more of these drawbacks in current diagnostic procedures are needed.

Several rapid syphilis tests have been developed that may enhance active case finding, provided the test is easy to perform, robust and affordable. Used alone, they would be unable to distinguish active from non-active disease because only antibodies against *T pallidum* are detected, similar to the TPPA. Nevertheless, these tests may prove to be an effective tool in the control of syphilis in difficultto-reach risk groups in field settings where they can facilitate a crucial intervention. Several rapid tests have been evaluated, resulting in sensitivities ranging between 40% and 100% in different settings.[7–12] An overview of the evaluations of various ELISA-based detection methods for *T pallidum* antibodies is given in table 1. In a recent article by Cole et al,[13] different ELISA were tested on a panel of 114 serum and plasma samples from

syphilis patients and 249 samples from blood donors. No significant differences in sensitivity or specificity were detected between these ELISA. To process large numbers of samples, ELISA-based methods are still preferred to rapid tests.

Table 1 Overview of different ELISA evaluated for detecting *T pallidum* antibodies in serum

Author	Year	ELISA	*T pallidum* antibodies	Sample total / positive	Sensitivity (%)	Specificity (%)	Remarks
Ebel et al21	1998	Bioelisa Syphilis	IgG	824/434	99,5	99,4	Positives are TPHA and FTA-Abs positive
Gutiérrez et al22	2000	Enzygnost Syphilis	IgG / IgM	821/401	100	99,5	13 Positive samples were only RPR positive, remainder are MHA-TP positive
Sambri et al23	2001	RecomWell Treponema	IgG	122/122	98,2		Positives are MHA-TP positive
Castro et al24	2003	ETI-Syphilis-G	IgG	441/313	100	93	Positives are MHA-TP positive
Aktas et al25	2005	Syphilis ICE	IgG / IgM	124/100	100		Positives are TPHA positive
		Enzywell T *pallidum*	IgG / IgM	124/100	100		
Viriyataveekul et al26	2006	Enzygnost Syphilis	IgG / IgM	3055/102	100	97,9	Positives are positive in two out of three ELISA and TPHA and / or FTA-Abs
		Syphilis EIA 480		3055/102	100	99,6	
		ICE* Syphilis		3055/102	99,1	99,8	

FTA-Abs, fluorescent treponemal antibody absorption; MHA-TP, microhaemagglutination assay for *T pallidum*; RPR, rapid plasma reagin test; TPHA, *T pallidum* haemagglutinin agglutination test.
*ICE is a trademark of Abbott Murex.

The aim of this study was to assess the validity and reproducibility of a one-step immunochromatographic rapid test, the Biorapid Syphilis (Biokit SA, Barcelona, Spain) and two commercial ELISA, the Bioelisa Syphilis 3.0 (Biokit) and the ETI-Treponema Plus (DiaSorin SpA, Saluggia, Italy), which can be run on an automated system. All three tests detect specific *T pallidum* antibodies. To the best of our knowledge, an evaluation of the Biorapid Syphilis and

current versions of both ELISA has not been published so far. Furthermore, we have included a broad range of sera from patients with underlying pathological and physiological conditions, which are known to interfere with syphilis serology.[14–20]

MATERIALS AND METHODS

Patient samples

All TPPA-positive samples collected between February 2000 and May 2006 in the laboratory of Medical Microbiology at the University Hospital in Maastricht, The Netherlands, were selected for this study. The gold standard for a ''true TPPA-positive sample'' was defined as a sample with a TPPA titre of 1 : 80 or above, confirmed by a positive FTA-Abs and/or a positive RPR and/or a positive immunoblot at a reference centre (National Institute of Public Health and the Environment, Bilthoven, The Netherlands). Ultimately 145 true TPPA-positive serum samples were included, with no more than one sample per patient. RPR was positive in 80 of these samples (55%), ranging from 1 : 1 to 1 : 256; all other samples were negative. A positive RPR result, especially values above 1 : 8, suggests active disease. The FTA-Abs was positive in 141 of the 145 TPPA-positive samples; two samples were negative and two had a borderline result. The immunoblot was performed and found positive in all four of these samples. The exact clinical status and possible drug usage of the patients was not known because most samples were collected at an anonymous venereal disease clinic. We know from clinical experience that in the TPPA-positive population approximately 70% is in the latent phase; primary and tertiary syphilis are rare and occasionally a rash is seen (secondary syphilis; data not published). In our study the control group consisted of 41 healthy TPPA-negative controls and 144 TPPA-negative controls with known underlying conditions that may interfere with *T pallidum* serology: pregnancy (n = 21); high antistreptodornase titre (n = 10); high anti-cardiolipin antibodies (n = 10); systemic lupus erythematosus (n = 10); diabetes mellitus (n = 7); rheumatoid factor IgM positive (n = 10); leptospirosis (n = 10); borreliosis (n = 10); recent Epstein–Barr virus infection (n = 10); recent cytomegalovirus infection (n = 10); current *hepatitis B virus* infection (n = 8); current hepatitis C virus infection (n = 9) or current HIV infection (n = 19). Samples were unlinked from any possible patient identifiers and kept at -20°C until evaluation.

T pallidum particle agglutination test, RPR and FTA-Abs

The TPPA (MHA-TP; Fujirebio, Tokyo, Japan) utilises gelatin particle carriers sensitised with purified pathogenic *T pallidum* (Nichols strain), which agglutinate with antibodies against *T pallidum*, if present, in serum. The RPR (Syfacard-R*; Abbott Murex, Dartford, UK) utilises tissue lipid cardiolipin (antigen) to detect ''reagin'', ie, antibodies, directed against tissue

components, which appear in serum as a reaction to tissue damage caused by *T pallidum*. Finally, in the FTA-Abs (Trepo Spt IF; BioMerieux SA, Marcy l'Etoile, France), human anti-*T pallidum* immunoglobulins bind to the *T pallidum* on the slide, which in turn binds fluorescein-labelled goat anti-human immunoglobulins, which can be seen with a fluorescence microscope. All tests were performed according to the manufacturers' instructions.

One-step immunochromatographic test

The one-step immunochromatographic test Biorapid Syphilis (Biokit) combines anti-human immunoglobulin dyed conjugate and p15 and p17 *T pallidum* recombinant antigens to detect anti-*T pallidum* IgG, IgA and IgM antibodies in plasma, serum or whole blood. Briefly, after inserting 25 ml human serum in zone A, the *T pallidum*-specific antibodies, if present, will bind to the anti-human immunoglobulin dyed conjugate to form an antigen–antibody complex. This complex will fix the recombinant protein on the band in zone B (test zone). When no human *T pallidum* antibodies are present, no antigen–antibody complexes will form and no fixation will take place in zone B. The superfluous conjugate will flow to zone C and bind to the reactions in zone B should be considered positive, according to the manufacturer. The test is invalid without a reaction in zone C.

Enzyme-linked immunosorbent assays

Two ELISA were evaluated in this study: the Bioelisa Syphilis 3.0 (Biokit) and the ETI-Treponema Plus (DiaSorin). Both ELISA are able to detect IgG and IgM antibodies, separately or together, against *T pallidum* in serum or plasma. In both assays, human antibodies against *T pallidum*, if present, will bind to p15, p17 and p47 *T pallidum* recombinant antigens in the microtitre plate coating. Next, the conjugate containing the enzyme peroxidase is added, which will bind to the *T pallidum* recombinant antigen–human antibody complex. Subsequently, the enzyme substrate, chromogen and after incubation sulphuric (stop) reagents are added. While performing the ETI-Treponema Plus the chromogen can be inserted into the automated system at the beginning of the assay, whereas for the Bioelisa Syphilis 3.0 it should be prepared and inserted 5– 10 minutes before usage. The absorbance value for the sample tested was divided by the mean absorbance value of the low positive control (cut-off), if this ratio equalled or was above 1.0, the result was considered positive in both ELISA. A result below 0.9 was considered negative. Both ELISA were performed according to the manufacturers' instructions, using an automated system (DSX automated system; Dynex Technologies, Inc, USA).

The laboratory characteristics, reported by the manufacturers, of the TPPA and the evaluated tests, are compared in table 2.

Table 2 An overview of the diagnostic characteristics of all tests used

	TTPA	Biorapid Syphilis	Bioelisa Syphilis 3.0	ETI-Treponema Plus
Turnaround time minutes/sample	130	20	150	150
Material required	Serum/plasma	Whole blood/serum/plasma	Serum/plasma	Serum/plasma
Storage temperature	2–10°C	2–25°C	2–8°C	2–8°C
Automated	No	No	Yes	Yes
Additional laboratory equipment needed	Yes	No	Yes	Yes
Interpretation	Visual	Visual	Automated reader	Automated reader

TPPA, *T pallidum* particle agglutination test.

Statistics

A true positive result in either the immunochromatographic test or one of both ELISA was defined as a positive result in a true TPPA-positive sample. A false-positive result was defined as a positive result in a TPPA-negative sample and a false-negative result as a negative result in a true TPPA-positive sample. A true negative result was defined as a negative result in a TPPA-negative sample. The sensitivity of a test was defined as the number of true positives divided by the number of true positives plus false negatives. Specificity was defined as the number of true negatives divided by the number of true negatives plus false positives.

RESULTS

One-step immunochromatographic test

Out of the 145 true TPPA-positive samples included, 12 samples were false negative in the Biorapid Syphilis, resulting in an overall sensitivity of 92% in our sample collection (table 3). The TPPA titre was below or equal to 1 : 320 in eight out of 12 false-negative samples (67%), two out of 12 had an RPR of 1 : 1 and one an RPR of 1 : 16. In selected samples with a TPPA titre above or equal to 1 : 2560 (n = 47), sensitivity increased to 100% but decreased to 83%

in selected samples with a TPPA titre below or equal to 1 : 320 (n = 46). The specificity of the Biorapid Syphilis was 79%; all false-positive results are categorised in table 4. Although the Biorapid Syphilis uses a one-step procedure, interpretation was difficult in some cases (fig 1).

Table 3 A comparison of Biorapid Syphilis, Bioelisa Syphilis and ETI-Treponema Plus test results

		TPPA			
		Positive	Negative	Total	Validity (95% CI)
Biorapid Syphilis	Positive	133	39	172	Sensitivity 92% (87 to 96)
	Negative	12	146	158	Specificity 79% (75 to 83)
Bioelisa Syphilis	Positive	145	0	145	Sensitivity 100% (96 to 104)
	Negative	0	185	185	Specificity 100% (96 to 104)
ETI-Treponema Plus	Positive	143*	0	143	Sensitivity 100% (96 to 104)
	Negative	0	171	171	Specificity 100% (96 to 104)

TPPA, *T pallidum* particle agglutination test.
*Including two borderline results.

Figure 1 Biorapid Syphilis. (A) Sample insertion. (B) Test result. (C) Internal control; 1. Positive result (strong); 2. Positive result (weak); 3. Negative result.

Table 4 Biorapid Syphilis false-positive results per category

	False positives (%)
Healthy controls	8/41 (17)
Pregnancy	2/21 (10)
Cytomegalovirus	5/10 (50)
Epstein–Barr virus	2/10 (20)
Hepatitis B virus	2/8 (25)
Hepatitis C virus	1/9 (11)
HIV	6/19 (32)
Leptospirosis	4/10 (40)
Borreliosis	0/10 (0)
Diabetes mellitus	3/7 (43)
Rheumatoid factor IgM	5/10 (50)
Anti-cardiolipin antibodies IgG	1/5 (20)
Anti-cardiolipin antibodies IgM	0/5 (0)
Systemic lupus erythematosus	0/10 (0)
Elevated antistreptodornase	0/10 (0)
Total	39/185 (21)

Enzyme-linked immunosorbent assays

The sensitivity of the Bioelisa Syphilis 3.0 was 100%. As a result of too low a volume of some samples, the ETI-Treponema Plus was performed on samples from 143 out of 145 patients and 171 out of 185 controls. The missing control samples include 10 samples from leptospirosis patients. The sensitivity of the ETI-Treponema Plus was 100%, if two borderline results (absorbance/cut-off ratio 0.988 and 0.936, respectively) are considered positive. Specificity was 100% for both ELISA.

DISCUSSION

In this study, we report on the sensitivity and specificity of a one-step immunochromatographic rapid test, the Biorapid Syphilis and two ELISA, the Bioelisa Syphilis 3.0 and ETI-Treponema Plus, when compared with the TPPA. Regarding the Biorapid Syphilis, the 12 false-negative reactions resulted in a sensitivity of 92%, which we considered insufficient in our setting. In particular, having a negative Biorapid Syphilis when the RPR is 16 (0.7%), which suggests active disease, is worrisome. Although very easy to perform, the Biorapid Syphilis was often difficult to interpret, which renders the test less suitable for use by non-laboratory personnel at, for example, clinics specialising in sexually transmitted diseases. According to the manufacturer, every visible reaction should be considered positive. As can be seen in fig 1, differentiating between a positive, a weak positive and a negative result is subjective.

In this study, the specificity of the Biorapid Syphilis was determined to be 79%, based on the selection of samples used for the negative control test panel in this study. This specificity may, however, actually be higher when tested on a population at risk. Six out of 19 HIV-positive sera were false positive in the Biorapid Syphilis, which is unfortunate because most syphilis cases occur in areas with the highest HIV prevalence.[1,2,7] Such false-positive reactions in syphilis serology in HIV-infected patients have been described before.[15, 16,18,28] Five out of 10 cytomegalovirus-positive control samples showed false-positive results with the Biorapid Syphilis, which has not been described before in syphilis serological studies. Furthermore, four out of 10 leptospirosis samples also gave false-positive reactions. Surprisingly, no samples from patients with borreliosis gave false-positive reactions, although these have been described in the literature.[29,30] False-positive reactions in syphilis serology have also been described in several autoimmune disorders and indeed were found in our study, especially in the rheumatoid factor IgM-positive group.[6,31,32]

The largest multicentre evaluation of point-of-care tests with archived sera so far has recently been published by Herring et al.[12] Nine rapid point-of-care syphilis tests were evaluated at eight laboratories on different continents. Sensitivities and specificities in that trial ranged between 84.5–97.7% and 92.8– 98%, respectively. The sensitivity confidence interval of the Biorapid Syphilis was comparable with the rapid tests used by Herring et al but the specificity confidence interval was somewhat lower. This could be explained by the difference in sample collection. Although the Biorapid Syphilis is not sufficiently sensitive to be used in our hospital laboratory, it might be useful in primary healthcare settings in developing countries, as suggested by Herring et al, although further testing (using whole blood) is needed.

The ELISA tested in our study have excellent sensitivities and the results were equal or better when compared with the results of other ELISA (table 1).[13] The specificity of both tests (100%) is especially good, considering the fact that serum samples from patients with most known possible cross-reacting pathological and physiological conditions were included in our population.[6,15–18,20,28,29,33] The turnaround time of the ELISA was comparable with that of the TPPA. Both ELISA can be performed on an automated system, which may reduce the handling time and because of the electronic transfer of information, fewer administrative errors will be made. One small disadvantage of the Bioelisa Syphilis 3.0 is that the substrate-chromogen solution must be prepared and inserted in the automated system 5–10 minutes before usage, requiring additional handling by the laboratory technician. This is not the case with the ETI-Treponema Plus substrate-chromogen, which is ready to use and can be inserted at the beginning of the assay.

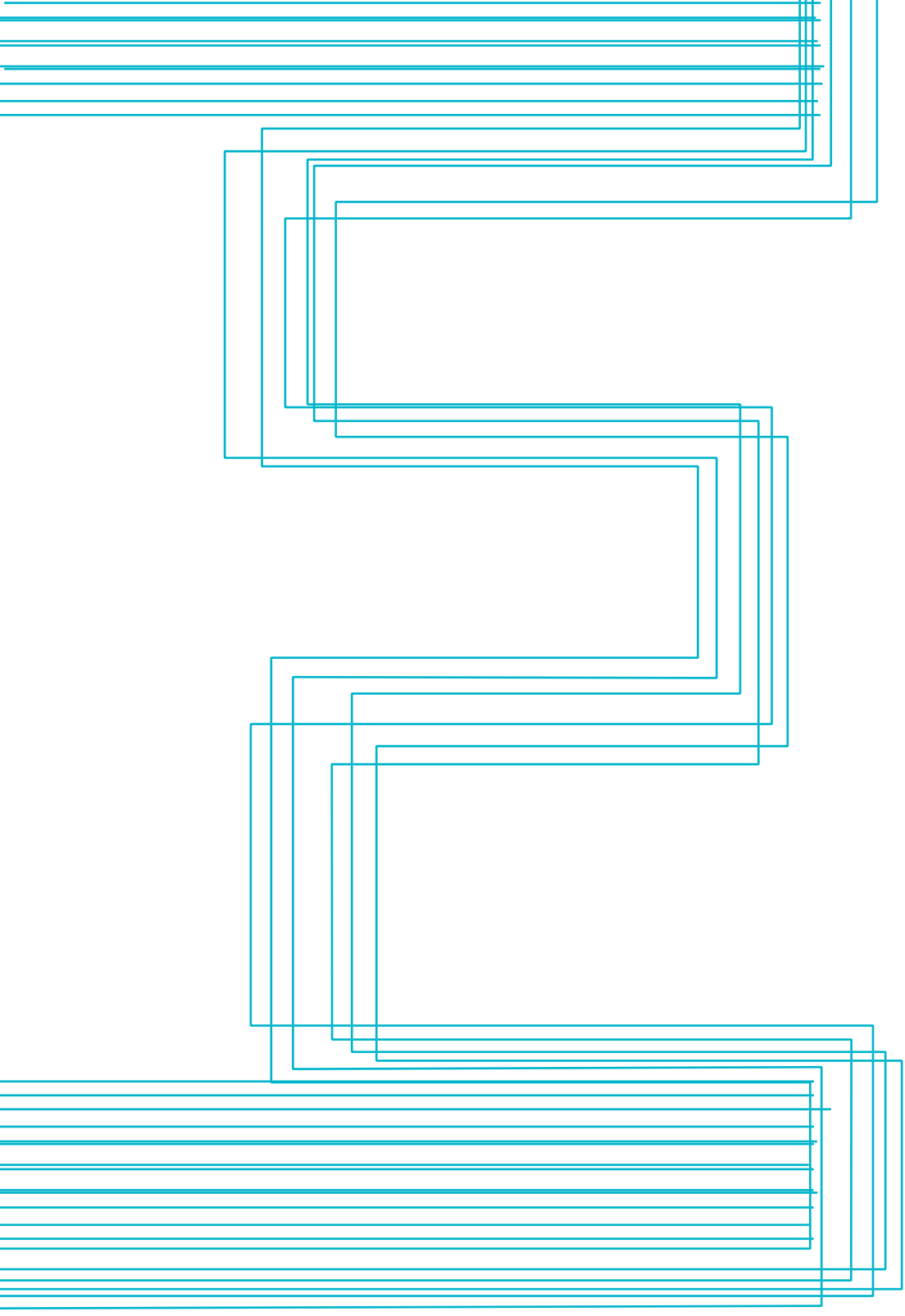
In conclusion, the sensitivity and specificity of the Biorapid Syphilis are considered too low to implement the test for screening purposes in a hospital laboratory but it might be useful in primary healthcare settings in developing countries. In contrast, both the Bioelisa Syphilis 3.0 and the ETI-Treponema Plus were found to have excellent sensitivities in this study and no false-positive reactions were detected in the control groups. As the ELISA can be run on an automated system, these have a clear advantage over the TPPA as a screening test.

REFERENCES

1. Peeling RW, Mabey DC. Syphilis. Nat Rev Microbiol 2004;2:448–9.
2. Singh AE, Romanowski B. Syphilis: review with emphasis on clinical, epidemiologic, and some biologic features. Clin Microbiol Rev 1999;12:187–209.
3. Fenton KA, Lowndes CM. Recent trends in the epidemiology of sexually transmitted infections in the European Union. Sex Transm Infect 2004;80:255–63.
4. Walker DG, Walker GJ. Forgotten but not gone: the continuing scourge of congenital syphilis. Lancet Infect Dis 2002;2:432–6.
5. Laar MJW van de, Boer IM de, Koedijk FDH, Op de Coul ELM. HIV and Sexually transmitted infections in the Netherlands in 2004. An update: November 2005. Bilthoven, The Netherlands: National Institute for Public Health and the Environment (RIVM). RIVM rapport 441100022 2005.
6. Larsen SA, Steiner BM, Rudolph AH. Laboratory diagnosis and interpretation of tests for syphilis. Clin Microbiol Rev 1995;8:1–21.
7. West B, Walraven G, Morison L, et al. Performance of the rapid plasma reagin and the rapid syphilis screening tests in the diagnosis of syphilis in field conditions in rural Africa. Sex Transm Infect 2002;78:282–5.
8. Campos PE, Buffardi AL, Chiappe M, et al. Utility of the Determine Syphilis TP rapid test in commercial sex venues in Peru. Sex Transm Infect 2006;82(Suppl 5):v22–5.

9. Montoya PJ, Lukehart SA, Brentlinger PE, et al. Comparison of the diagnostic accuracy of a rapid immunochromatographic test and the rapid plasma reagin test for antenatal syphilis screening in Mozambique. Bull WHO 2006;84:97–104.
10. Siedner M, Zapitz V, Ishida M, et al. Performance of rapid syphilis tests in venous and fingerstick whole blood specimens. Sex Transm Dis 2004;31:557–60.
11. Diaz T, Almeida MG, Georg I, et al. Evaluation of the Determine Rapid Syphilis TP assay using sera. Clin Diagn Lab Immunol 2004;11:98–101.
12. Herring AJ, Ballard RC, Pope V, et al. A multi-centre evaluation of nine rapid, point-of-care syphilis tests using archived sera. Sex Transm Infect 2006;82(Suppl 5):v7–12.
13. Cole MJ, Perry KR, Parry JV. Comparative evaluation of 15 serological assays for the detection of syphilis infection. Eur J Clin Microbiol Infect Dis 2007;26:705–13.
14. Marangoni A, Sambri V, Accardo S, et al. Evaluation of LIAISON Treponema Screen, a novel recombinant antigen-based chemiluminescence immunoassay for laboratory diagnosis of syphilis. Clin Diagn Lab Immunol 2005;12:1231–4.
15. Rompalo AM, Cannon RO, Quinn TC, et al. Association of biologic false-positive reactions for syphilis with *human immunodeficiency virus* infection. J Infect Dis 1992;165:1124–6.
16. Hernandez-Aguado FBI, Moreno R, Pardo FJ, et al. False-positive tests for syphilis associated with *human immunodeficiency virus* and *hepatitis B virus* infection among intravernous drug abusers. Eur J Clin Microbiol Infect Dis 1998;17:784–7.
17. Brauner A, Carlsson B, Sundkvist G, et al. False-positive treponemal serology in patients with diabetes mellitus. J Diabetes Complicat 1994;8:57–62.
18. Geusau A, Kittler H, Hein U, et al. Biological false-positive tests comprise a high proportion of Venereal Disease Research Laboratory reactions in an analysis of 300,000 sera. Int J STD AIDS 2005;16:722–6.
19. Rodriguez I, Alvarez EL, Fernandez C, et al. Comparison of a recombinant-antigen enzyme immunoassay with *Treponema pallidum* hemagglutination test for serological confirmation of syphilis. Mem Inst Oswaldo Cruz 2002;97:347–9.
20. Tramont ED. *Treponema pallidum* (syphilis). In: Mandell GL, Bennett JE, Dolin R. Principles and practice of infectious diseases. London, UK: Churchill Livingstone. 2005;2:2780.
21. Ebel A, Bachelart L, Alonso JM. Evaluation of a new competitive immunoassay (BioElisa Syphilis) for screening for *Treponema pallidum* antibodies at various stages of syphilis. J Clin Microbiol 1998;36:358–61.
22. Gutierrez J, Vergara MJ, Soto MJ, et al. Clinical utility of a competitive ELISA to detect antibodies against *Treponema pallidum.* J Clin Lab Anal 2000;14:83–6.
23. Sambri V, Marangoni A, Simone MA, et al. Evaluation of recomWell Treponema, a novel recombinant antigen-based enzyme-linked immunosorbent assay for the diagnosis of syphilis. Clin Microbiol Infect 2001;7:200–5.
24. Castro R, Prieto ES, Santo I, et al. Evaluation of an enzyme immunoassay technique for detection of antibodies against *Treponema pallidum.* J Clin Microbiol 2003;41:250–3.
25. Aktas G, Young H, Moyes A, et al. Evaluation of the serodia *Treponema pallidum* particle agglutination, the Murex Syphilis ICE and the Enzywell TP tests for serodiagnosis of syphilis. Int J STD AIDS 2005;16:294–8.
26. Viriyataveekul R, Laodee N, Potprasat S, et al. Comparative evaluation of three different treponemal enzyme immunoassays for syphilis. J Med Assoc Thai 2006;89:773–9.
27. CDC. The Global HIV/AIDS pandemic, 2006. MMWR Morb Mortal Wkly Rep 2006;55:841–4.
28. Augenbraun MH, DeHovitz JA, Feldman J, et al. Biological false-positive syphilis test results for women infected with *human immunodeficiency virus.* Clin Infect Dis 1994;19:1040–4.
29. Raoult D, Hechemy KE, Baranton G. Cross-reaction with Borrelia burgdorferi antigen of sera from patients with *human immunodeficiency virus* infection, syphilis, and leptospirosis. J Clin Microbiol 1989;27:2152–5.
30. Magnarelli LA, Anderson JF, Johnson RC. Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J Infect Dis 1987;156:183–8.

31. Contreras MA, Andreu JL, Isasi C, et al. False positive treponemal test result in a patient with active systemic lupus erythematosus. J Rheumatol 2000;27:2059.
32. Henquet CJ, de Vries RR. [Problems in the interpretation of serological results of syphilis]. Ned Tijdschr Geneeskd 1994;138:1705–8.
33. Thomas DL, Rompalo AM, Zenilman J, et al. Association of hepatitis C virus infection with false-positive tests for syphilis. J Infect Dis 1994;170:1579–81.



Validation of Methodology Used in Sexually Transmitted Infections Research

7. Confirmation of high specificity of an automated ELISA test for serological diagnosis of syphilis - results from confirmatory testing after syphilis screening and sensitivity analysis in the absence of a gold standard.

» Submitted

Laura van Dommelen, Christian J.P.A. Hoebe, Frank H. van Tiel, Carel Thijs, Valère J. Goossens, Cathrien A. Bruggeman, Inge H.M. van Loo

In clinical microbiology laboratories, serological diagnostic assays are usually implemented after evaluation using a selected sample collection. In the current study we have compared the specificity of Bioelisa Syphilis 3.0 after clinical implementation as a syphilis screening test with the specificity found in a previous evaluation using a selected sample collection. We included 14,622 sera (positivity rate 1.4%) sent to the laboratory for syphilis serology in the period between October 2007 and February 2010. We confirm the initially reported specificity and further narrow down its confidence interval (specificity 99.5%, 95%CI 99.4-99.6%), and show that this high specificity is valid across diverse patient categories. Here we demonstrate that differences in positive predictive values between patient categories reflect the prevalence of syphilis in these categories, and are not due to differences in specificity. In addition, in a sensitivity analysis we show that these conclusions are robust for several assumptions. Our re-evaluation shows that the use of a selected serum sample collection is validated in the evaluation of syphilis serological diagnostic assays. Confirmatory syphilis testing is mandatory in low prevalence populations, even when the screening test has a very high specificity.

In clinical microbiology laboratories, serological diagnostic assays are usually implemented after evaluation using a selected sample collection. We have previously evaluated the performance of the Bioelisa Syphilis 3.0 compared with the *Treponema pallidum* Particle Agglutination (TPPA) in a selected collection of serum samples.[1] This enzyme-linked immunosorbent assay (ELISA) detects *T. pallidum* antibodies. The sample collection included 145 sera from syphilis patients with active or latent disease, 41 sera from healthy controls and 144 sera from patients with underlying conditions which might influence *T. pallidum* antibody testing. The sensitivity and specificity were both 100% (95% confidence interval (95%CI) sensitivity 97.5-100%, specificity 98.0-100%, table 1, A). Since the Bioelisa Syphilis 3.0 can be used on an automated system, this ELISA was implemented in our laboratory to replace the TPPA as a screening method.

Since the initial evaluation was performed on a selected sample collection with an artificially high syphilis prevalence, it was not surprising that we noticed a high number of false positive results in the first year after implementation: a positive Bioelisa with confirmative testing with TPPA, immunofluorescense, rapid plasma reagin and/or additional testing with an immunoblot at a reference laboratory, being negative. In the current study we have therefore retrospectively evaluated the Bioelisa Syphilis 3.0, to assess whether the high specificity would stand up in clinical practice.

The Bioelisa Syphilis 3.0 (Biokit SA, Barcelona, Spain) was introduced in our laboratory on October 1st 2007. If the Bioelisa was positive, the TPPA (MHA-TP, Fujirebio, Tokyo, Japan), Rapid Plasma Reagin Test (RPR; Syfacard-R*, Abbott Murex, Dartford, UK) and/or fluorescent treponemal antibody-absorption test (FTA-Abs; Trepo Spot IF, BioMerieux SA, Marcy l'Etoile, France) were used for confirmation. If the previously mentioned serological tests gave an inconclusive serological profile, according to the microbiologist on duty, the serum was sent to the national reference laboratory (National Institute of Public Health and the Environment, Bilthoven, The Netherlands) for additional testing with an immunoblot. The microbiologist in duty decided if a sample was a true positive or negative sample. The diagnostic assays were performed according to the manufacturers' instructions, as previously described[1]. Data were unlinked from possible patient identifiers and each patient was included only once in the database. Results were analyzed using the 2-way contingency table analysis and exact binominal confidence intervals calculation programs from www.statpages.org. [2,3]

The Bioelisa was performed on 14622 sera sent to the laboratory in the period between October 2007 and February 2010. In 1.4% (n=209) the Bioelisa was positive or borderline. Since only 6 out of these 209 samples were tested borderline positive and further work-up was similar to the Bioelisa positive samples, these samples were considered positive in further analysis. The main reasons for syphilis screening were sexually transmitted diseases (STD) screening (n=4519, 31%), screening during pregnancy (n=3169, 22%) and work-up for infertility (n=1168, 8%). In another 7% (n=998) of the cases, syphilis screening was requested for known reasons not previously mentioned (for example suspicion of neurosyphilis), leaving 33% (n=4768) of the patients for whom no clinical data were available. The TPPA, VDRL and FTA

were performed in 99%, 100% and 89% of Bioelisa positive samples, respectively and 30% of positive samples was sent to the reference laboratory. Ultimately, 65% of the Bioelisa positive or borderline samples was reported as syphilis positive. Confirmative assays (e.g. TPPA) were also performed on 55 samples which were Bioelisa negative (TPPA was used in serum/ cerebrospinal fluid to exclude neurosyphilis), resulting in 1 true positive sample.

Taking into account results of all performed diagnostic assays, the specificity of the Bioelisa was 99.5% (95%CI 99.4-99.6%) and positive predictive value (PPV) was 65.1% (95%CI 58.2-71.5%) (table 1, B).[2,3] The overall specificity of 99.5% is very consistent with the specificity (100%) found in the initial study, when taking in account the confidence interval (95%CI was 98.0-100% in the initial study). Moreover, our results further narrow down the confidence interval of specificity, presently 95%CI 99.4-99.6%.

When analyzing the results of the largest patient categories according to available clinical data, the values of specificity were very similar (table 1, B1-3). Since Bioelisa negative samples were not confirmed by additional testing, we have analyzed the data assuming a Bioelisa sensitivity of 95% or 90% and again the specificity of the Bioelisa does not drop below 99.5% (sensitivity analysis, table 1, C1-2). In the absence of an ideal comparison standard we also analyzed the data assuming a comparison standard sensitivity of 90% or 80% and this again did not change specificity significantly (table 1, D1, D2, respectively). This was also true in subgroup analyses (data not shown).

Performing a prospective evaluation is time consuming and costly. It took us over 2 years to gather 137 serologically confirmed syphilis cases. In our previous evaluation study, we have therefore used a selection of archived sera. In the present study, we have compared the results of the previous study with the performance in practice, after implementation of this assay in our laboratory. In the initial evaluation, all syphilis serologically positive samples comprehended 44% of the total number of sera. The prevalence of syphilis in The Netherlands, however, is only 0.2% among pregnant women and 2.3% in men having sex with men.[4] Our current analysis shows that the high specificity found in the initial study, stands up after implementation in a population with a low syphilis prevalence (0.9%). The specificity was similar in all subgroups of patients, and hence differences in PPV are solely due to differences in the prevalence of syphilis, as can be seen in table 1 (B1-3), with PPVs ranging from 21.1% (in the low risk group of pregnancy screening) to 75.0% (in the group without clinical data).

A comparison between both our evaluations is hampered by the fact that in our initial study the TPPA was performed on all samples, whereas in our current analysis -performed after implementation- the TPPA was almost strictly limited to Bioelisa positive samples. This

causes a verification bias compared to the initial study: not all samples were subjected to the reference test.[5] False negative Bioelisa results could have been missed (as was seen in our evaluation). This would, however, not significantly affect the specificity, nor PPV as shown in table 1 (C1-2). In general, ELISA are considered equally sensitive or more sensitive for the detection of *Treponema pallidum* antibodies compared with TPPA or TPHA (*Treponema pallidum* Hemaglutinine Agglutination).[6-9] Diagnostic evaluations are limited since often no ideal comparison standard is available, with every assay having its limitations. This applies for syphilis in particular, since the sensitivity of Syphilis nucleic acid amplification tests in the absence of skin lesions is poor.[10,11] We have therefore analyzed our data assuming a comparison standard sensitivity of 90% or 80% (table 1, D1-2), but again this did not significantly change the specificity in our population.

We confirm the initially reported specificity, and show that this high specificity is valid across diverse patient categories and several assumptions using sensitivity analysis. Our results demonstrate that in screening situations, the positive predictive value is mainly dependent on prevalence. Even with a very high specificity, the high number of non-diseased can result in a substantial number of false positive results. Understanding this phenomenon is important when interpreting a test result.[12] Confirmative assays are therefore essential in the serological diagnosis of syphilis, especially in low prevalence patient groups.

Table 1 Bioelisa evaluation results in a selected sample set and after clinical implementation[2,3]

					Final conclusion						
					Positive		Negative	Total			95% CI
Initial	A. Total	Bioelisa	Positive		145		0	145	PPV	NA	NA
			Negative		0		185	185	NPV	NA	NA
			Total		145		185	330			
				Se	100%	Sp	100%				
				95% CI	97.5-100%	95% CI	98.0-100%		Syphilis prevalence 43.9%		
Re-evaluation	B. Total	Bioelisa	Positive		136		73	209	PPV	65.1%	58.2-71.5%
			Negative		1		14412	14413	NPV	100%	100-100%
			Total		137		14485	14622			
				Se	99.3%	Sp	99.5%				
				95% CI	96.0-100%	95% CI	99.4-99.6%		Syphilis prevalence 0.9%		
	B1. No clinical data	Bioelisa	Positive		78		26	104	PPV	75.0%	65.6-83.0%
			Negative		1		4663	4664	NV	100%	99.9-100%
			Total		79		4689	4768			
				Se	98.7%	Sp	99.4%				
				95% CI	93.5-100%	95% CI	99.2-99.6%		Syphilis prevalence 1.7%		
	B2. STD screening	Bioelisa	Positive		38		26	64	PPV	59.4%	46.4-71.5%
			Negative		0		4455	4455	NPV	100%	99.9-100%
			Total		38		4481	4519			
				Se	100%	Sp	99.4%				
				95% CI	90.8-100%	95% CI	99.2-99.6%		Syphilis prevalence 0.8%		

	B3. Pregnancy	Bioelisa	Positive		3		11	14	PPV	21.4%	4.7-50.8%
			Negative		0		3155	3155	NPV	100%	99.9-100%
			Total		3		3166	3169			
				Se	100%	Sp	99.7%				
				95% CI	29.2-100%	95% CI	99.4-99.8%		Syphilis prevalence 0.1		
Sensitivity analysis	C1. Total: Assuming Bioelisa Se 95%	Bioelisa	Positive		136		73	209	PPV	65.1%	58.2-71.5%
			Negative		7		14406	14413	NPV	100%	99.8-100%
			Total		143		14479	14622			
				Se	95.1%	Sp	99.5%				
				95% CI	90.2-98.0%	95% CI	99.4-99.6%		Syphilis prevalence 1.0%		
	C2. Total: Assuming Bioelisa Se 90%	Bioelisa	Positive		136		73	209	PPV	65.1%	58.2-71.5%
			Negative		14		14399	14413	NPV	100%	99.8-100%
			Total		150		14472	14622			
				Se	90.7%	Sp	99.5%				
				95% CI	84.8-94.8%	95% CI	99.4-99.6%		Syphilis prevalence 1.0%		
	D1. Total: Assuming comparison standard Se 90%	Bioelisa	Positive		143		66	209	PPV	68.4%	61.7-74.7%
			Negative		1		14412	14413	NPV	100%	100-100%
			Total		144		14478	14622			
				Se	99.3%	Sp	99.3%				
				95% CI	96.2-100%	95% CI	99.4-99.7%		Syphilis prevalence 1.0%		
	D2. Total: Assuming comparison standard Se 80%	Bioelisa	Positive		151		58	209	PPV	72.2%	65.7-78.2%
			Negative		1		14412	14413	NPV	100%	100-100%
			Total		152		14470	14622			
				Se	99.3%	Sp	99.6%				
				95% CI	96.4-100%	95% CI	99.5-99.7%		Syphilis prevalence 1.1%		

Abbrevations: Se, sensitivity; Sp, specificity; NPV, negative predictive value; PPV, positive predictive value; STD, sexual transmitted diseases; 95%CI, exact binomial 95% confidence interval; NA, not applicable.

REFERENCES

1. van Dommelen L, Smismans A, Goossens VJ, Damoiseaux J, Bruggeman CA, van Tiel FH, et al. Evaluation of a rapid one-step immunochromatographic test and two immunoenzymatic assays for the detection of anti-*Treponema pallidum* antibodies. Sex Transm Infect 2008;84(4):292-6.
2. Clopper CJ PE. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 1934;26:404-413 (http://statpages.org).
3. Rosner B. Fundamentals of Biostatistics (http://statpages.org). 2006.
4. S.C.M. Trienekens FDHK, I.V.F. van den Broek, H.J. Vriend, E.L.M. Op de Coul, M.G. van Veen, A.I. van Sighem, I. Stirbu-Wagner, M.A.B. van der Sande. Sexually transmitted infections, including HIV, in the Netherlands in 2011: National Institute for Public Health and the Environment, 2012.
5. Lijmer JG, Mol BW, Heisterkamp S, Bonsel GJ, Prins MH, van der Meulen JH, et al. Empirical evidence of design-related bias in studies of diagnostic tests. Jama 1999;282(11):1061-6.
6. Binnicker MJ, Jespersen DJ, Rollins LO. Treponema-specific tests for serodiagnosis of syphilis: comparative evaluation of seven assays. J Clin Microbiol 2011;49(4):1313-7.
7. Maple PA, Ratcliffe D, Smit E. Characterization of *Treponema pallidum* particle agglutination assay-negative sera following screening by treponemal total antibody enzyme immunoassays. Clin Vaccine Immunol 2010;17(11):1718-22.
8. Vulcano F, Milazzo L, Volpi S, Battista MM, Barca A, Hassan HJ, et al. Italian national survey of blood donors: external quality assessment (EQA) of syphilis testing. J Clin Microbiol 2010;48(3):753-7.
9. Sena AC, White BL, Sparling PF. Novel *Treponema pallidum* serologic tests: a paradigm shift in syphilis screening for the 21st century. Clin Infect Dis 2010;51(6):700-8.
10. Heymans R, van der Helm JJ, de Vries HJ, Fennema HS, Coutinho RA, Bruisten SM. Clinical value of *Treponema pallidum* real-time PCR for diagnosis of syphilis. J Clin Microbiol 2010;48(2):497-502.
11. Grange PA, Gressier L, Dion PL, Farhi D, Benhaddou N, Gerhardt P, et al. Evaluation of a PCR test for detection of *treponema pallidum* in swabs and blood. J Clin Microbiol 2012;50(3):546-52.
12. Billings PR, Bernstein MS. Physicians poor at prevalence and positive predictive value. Jama 1985;254(9):1173-4.

8. *Chlamydia trachomatis* DNA stability independent of preservation temperature, type of medium en storage duration.

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Laura van Dommelen, Petra F. G. Wolffs, Frank H. van Tiel
Nicole Dukers, Selma B. Herngreen, Cathrien A. Bruggeman
and Christian J. P. A. Hoebe

We validated the use of stored samples for *Chlamydia trachomatis* research. *C. trachomatis* DNA was detected by real-time PCR in clinical (urine and self-taken vaginal swabs) and spiked samples using six different media, five different time points (up to 2 years), and four different temperature conditions. *C. trachomatis* was detected in all 423 samples, and no clinically relevant degradation impact was detected.

Chlamydia trachomatis is the most prevalent bacterial sexually transmitted microorganism worldwide. Many researchers conveniently use stored samples for their *C. trachomatis* research. In previous decades, the viability of *C. trachomatis* has been extensively explored using culture, and freezing samples appeared to be the most essential step in keeping *C. trachomatis* culturable.[1,2] The package insert of the COBAS TaqMan CT test [3], for instance, states that urine can be stored refrigerated or frozen for at maximum 7 and 30 days, respectively, before being processed. Swabs in transport medium can be stored at room temperature and frozen for, at maximum, 14 and 30 days, respectively, according to the package insert.[3] The effect of different storage conditions on the load of *C. trachomatis* using nucleic acid amplification tests (NAAT), however, has never yet been thoroughly assessed in a clinical trial. We hypothesized that storage would not lead to false-negative NAAT results. Therefore, we assessed the impact of four different temperature conditions, six different types of medium, and five increasing lengths of duration of storage, up to 2 years, on *C. trachomatis* DNA detection.

For this purpose, phosphate-buffered saline (PBS), 2-sucrosephosphate (2-SP) medium, COBAS Amplicor medium (Roche Diagnostics, Mannheim, Germany), and urine samples were spiked with the same amount of *C. trachomatis* serovar D elementary bodies (MyBioSource, San Diego,CA) and were stored at roomtemperature (RT), 4°C, -20°C, and -80°C, in triplicate. Samples were thawed only once, on the day of *C. trachomatis* DNA testing. Furthermore, clinical *C. trachomatis*-positive urine samples, as well as *C. trachomatis*-positive swabs in COBAS Amplicor medium, were collected, pooled, and stored in triplicate at the same four temperatures.

Samples were tested in triplicate on day 0 and after 1, 7, 14, and 30 days and 2 years of storage (136 clinical and 287 spiked samples) for the presence of *C. trachomatis* DNA. DNA was isolated using the Qiagen DNA minikit (Qiagen GmbH, Hilden, Germany). For the real-time PCR targeting the cryptic plasmid as described by Jalal et al.[4], the total PCR volume was adjusted to 50 μl. Also, only the inner primers were used, to avoid a nested PCR setup. For PCR amplification, an ABI 7900 HT real-time PCR machine (Applied Biosystems, Carlsbad, CA) was employed. Approximately 3,000 plasmids were available per PCR (e.g., per 20μl sample used in the PCR).

Generalized linear models were used, controlling for repeated measurements. Models were run separately for the six evaluated modalities of samples. We tested whether the number of PCR cycles needed to detect *C. trachomatis* DNA changed as storage time increased. An increase in the number of cycles needed corresponds to a decrease in *C. trachomatis* DNA detected compared to the amount in the previous sample. An increase of 3.3 PCR cycles corresponds to an approximately 1-log decrease in *C. trachomatis* DNA load. Furthermore, the influence over time of storage temperature, with four categories, was examined for the different media. Due to the large time interval, generalized linear models were only used for analyzing data obtained within the first month. Analyses were conducted using SPSS19 (IBM Corporation, Somers, NY); a P value of <0.05 was considered statistically significant.

C. trachomatis could be detected in all clinical samples and spiked media at all time points and irrespective of the storage temperature (Table 1). For spiked PBS and 2-SP and pooled *C. trachomatis*-positive swabs in COBAS Amplicor medium, the cycle threshold was independent of storage duration and temperature within the first month. For *C. trachomatis* DNA detection in spiked COBAS Amplicor medium, the cycle threshold increased within the first month at-20°C and-80°C (both $P<0.01$), while time trends showed a nonsignificant ($P = 0.09$) decrease at room temperature andstability at 4°C ($P=0.95$). Finally, for spiked urine and for pooled clinical urine samples, the cycle threshold decreased within the first month ($P<0.01$), including all but one (4°C, $P=0.09$) of the studied temperatures, reflecting an increase in *C. trachomatis* DNA load (data not shown). Regarding the results obtained after the 2-year storage interval, several findings are noteworthy (Table 1). The cycle thresholds in the spiked PBS and 2-SP experiments were stable over time. For spiked COBAS Amplicor medium, the cycle threshold, which had increased during the first month, was found to have decreased in the samples frozen for 2 years. In both spiked and clinical urine samples, the cycle threshold had increased after 2 years in the frozen samples, after the initial decrease.

C. trachomatis DNA could be detected in all clinical samples and spiked media tested, implying that none of the conditions had a clinically relevant degrading impact on the available *C. trachomatis* DNA. Nevertheless, several remarkable findings ought to be highlighted.

Although the *C. trachomatis* DNA input in all spiked samples is similar, variation exists in the test results on day 0 (immediately after composing the samples). It is likely that lysis already had started in the 2-SP and COBAS Amplicor samples, which explains the lower cycle thresholds in these samples in comparison with those of the spiked PBS samples. Since the pooled clinical urine and swab samples contained an unknown *C. trachomatis* load, their numbers of PCR cycles needed to detect *C. trachomatis* on day 0 or any other time point cannot be compared directly with the numbers for the spiked samples.

TABLE 1 Cycle threshold values of *Chlamydia trachomatis* DNA in various media at different time points [a]

Specimen	Temp (°C)	CT value [average (SD)] at indicated time point					
		Day(s)					
		0	1	7	14	30	2 yr.
Spiked PBS	RT	27.90 (1.59)	28.50 (0.37)	29.35 (1.90)	27.54 (0.40)	27.67 (0.11)	27.15 (0.59)
	4	29.02 (0.18)	28.76 (0.22)	29.03 (0.04)	28.81 (0.41)	27.88 (0.12)	28.48 (0.36)
	-20	29.58 (0.58)	28.87 (0.63)	31.29 (0.28)	29.58 (0.69)	29.28 (0.33)	29.71 (0.16)
	-80	29.10 (0.81)	28.22 (0.69)	28.00 (2.14)	27.88 (0.97)	28.68 (1.31)	29.30 (0.25)[c]
Spiked 2-SP	RT	24.93 (0.67)	24.03 (0.23)	24.44 (0.73)	25.04 (1.02)	24.41 (0.23)	23.03 (0.44)
	4	24.59 (1.00)	23.42 (0.64)	24.21 (0.97)	24.10 (0.27)	23.80 (0.90)	23.34 (0.73)
	-20	24.82 (0.83)	24.59 (0.84)	25.19 (1.58)	25.39 (0.75)	23.08 (1.32)	23.62 (0.67)
	-80	25.40 (0.41)	24.78 (1.32)	24.23 (1.16)	25.22 (0.49)	24.45 (0.47)	23.92 (0.46)
Spiked COBAS medium	RT	26.00 (0.34)	25.00 (0.15)	24.39 (0.95)	24.42 (0.25)	24.23 (0.43)	24.45 (0.44)
	4	25.30 (0.92)	23.86 (1.27)	28.85 (1.48)	28.96 (0.02)	24.66 (0.96)	25.03 (0.52)
	-20	25.45 (1.00)	24.70 (1.51)	28.77 (0.96)	29.35 (0.72)	28.54 (0.89)	23.99 (1.56)
	-80	25.62 (0.56)	24.24 (0.83)	28.71 (0.20)	29.33 (0.46)	28.97 (0.86)	24.37 (1.24)
Spiked urine	RT	27.85 (1.34)	25.96 (2.53)	24.63 (0.75)	23.34 (0.64)	23.56 (1.05)	22.58 (0.17)
	4	28.78 (2.21)	26.84 (1.88)	23.40 (2.17)	24.19 (0.52)	24.92 (0.35)	24.93 (1.78)
	-20	29.87 (0.65)	26.61 (0.66)	24.31 (0.62)	23.75 (1.37)	23.61 (1.20)	32.56 (1.03)
	-80	29.40 (1.21)	25.84 (0.95)	24.22 (0.61)	24.60 (0.52)	24.69 (0.87)	31.84 (2.45)
Clinical C. trachomatis-positive urine	RT	33.63 (0.75)	33.21 (1.07)	30.70 (0.43)	29.94 (0.19)	29.97 (0.36)	30.37 (0.50)
	4	32.83 (0.87)	33.73 (0.37)	34.17 (0.65)	31.88 (0.83)	30.71 (0.49)	29.97 (0.21)
	-20	33.46 (0.66)	33.48 (1.08)	33.59 (0.94)	32.90 (0.44)	32.60 (0.24)	37.16 (0.61)
	-80	33.81 (0.74)	33.78 (0.18)	33.35 (0.84)	33.00 (0.29)	32.43 (0.26)	36.53 (0.23)
Clinical C. trachomatis-positive swabs in COBAS medium	RT	27.06 (0.48)	26.19 (0.05)	27.12 (0.11)	26.79 (0.37)	27.39 (0.16)	34.11 (0.57)[c]
	4	26.25 (0.41)	26.12 (0.19)	26.79 (0.46)	26.46 (0.38)	26.47 (0.20)	29.89 (NA)[b]
	-20	27.32 (0.36)	25.90 (0.30)	26.25 (0.10)	26.50 (0.20)	26.94 (1.20)	26.81 (NA)[b]
	-80	27.72 (0.49)	25.84 (0.08)	26.08 (0.51)	26.87 (0.67)	26.07 (0.13)[c]	26.34 (NA)[b]

a CT, cycle threshold; PBS, phosphate-buffered saline; 2-SP, 2-sucrose-phosphate medium; COBAS, COBAS Amplicor medium; RT, room temperature.
b Tested singly.
c Tested in duplicate.

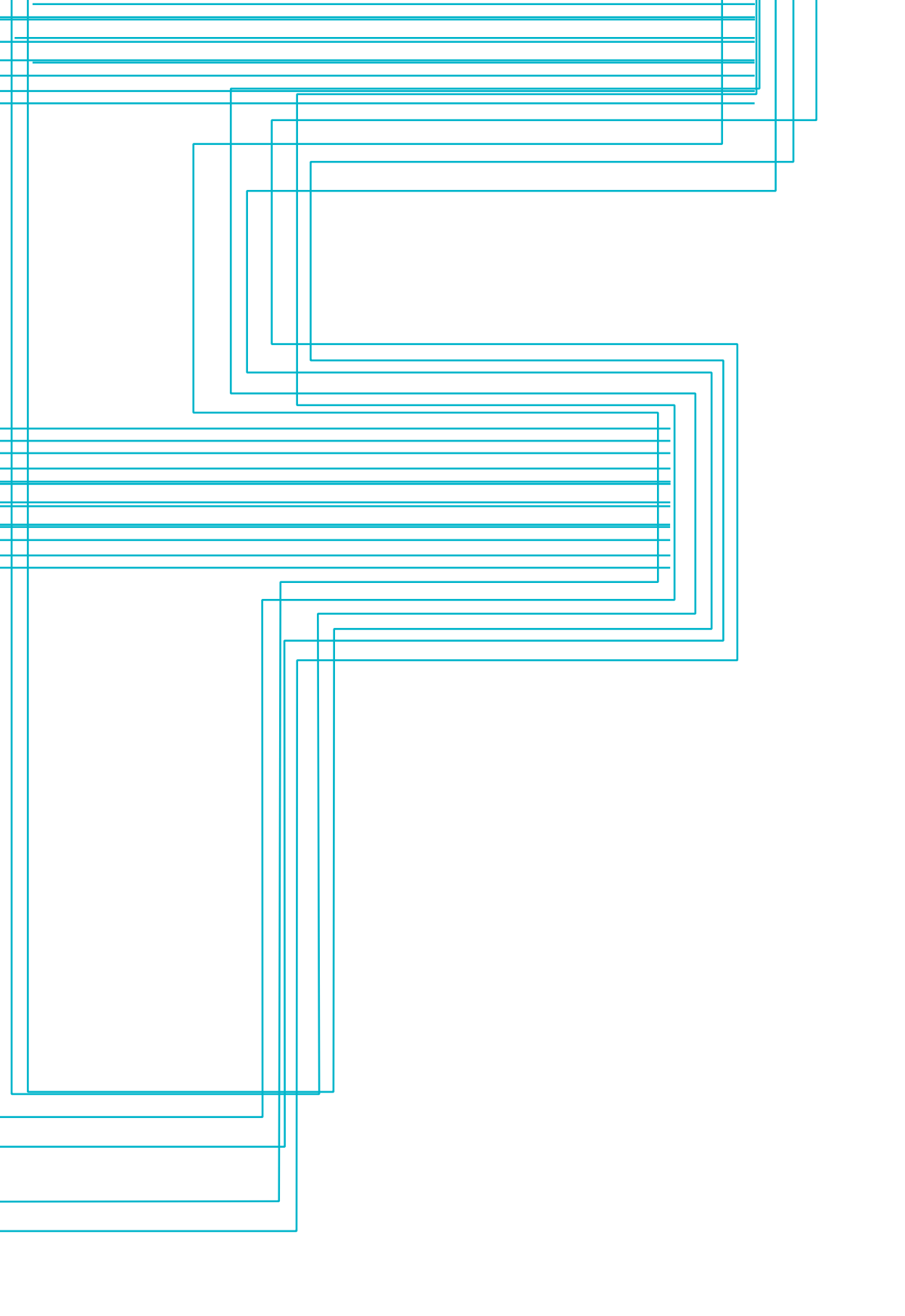
We found a significant decrease in the number of cycles needed over time to detect *C. trachomatis* DNA in the spiked urine samples within the first month. This decrease did not hold for the frozen samples after 2 years of storage. The stability of C. trachomatis DNA in urine samples was previously investigated by Morré et al.[5] Overall, *C. trachomatis* could be detected in all their samples, although initial freezing appeared to impair detection. This could have been due to the subsequent release of cellular DNase, as hypothesized by Morré et al. In contrast, in our study, during which the stored samples were not prefrozen, we were able to detect higher loads of *C. trachomatis* DNA over time in stored urine samples. This might reflect a decrease in the amount of PCR-inhibiting substances known to be present in urine samples.[6] Long-term freezing, however, seems to result in degradation of *C. trachomatis* DNA in urine samples, since the phenomenon was observed for spiked as well as clinical urine samples. As can be seen in Table 1, variations in cycle threshold exist between triplicate measurements in the spiked urine samples (especially at RT and 4°C), which are not present in the clinical urine samples. This could reflect a difference in degrading enzymes and/or inhibiting substances between patients' urine samples. In spiked COBAS Amplicor samples, we detected a significant decrease in C. trachomatis load within the first month. This was not the case in the pooled *C. trachomatis*-positive swabs in COBAS medium nor in any of the other experiments. The decrease in *C. trachomatis* DNA load occurred only in the frozen samples, not in samples stored at 4°C or RT. This finding is remarkable, since if the decrease in *C. trachomatis* DNA load had been the result of an enzymatic process, one would expect this to occur in the samples stored at 4°C or RT, which was not the case. Surprisingly, the *C. trachomatis* DNA load was found to reverse to initial values in these samples after 2 years of frozen storage. The COBAS Amplicor package insert does not recommend storage of swab samples in COBAS medium.

Maass et al. explored the viability of *Chlamydophila pneumoniae* after storage in different media and temperatures.[7] They found a higher survival rate of *C. pneumoniae* when samples were frozen at -75°C than at 4°C or 22°C. Eley et al. used different lyophilized *C. trachomatis* strains which were stored at different temperatures to assess the viability after 1 week and 1 month.[8] Storage temperature affected viability, but recovery was relatively stable between the two time points. A rise in temperature clearly affected viability, with no recovery at the highest temperature (37°C) in the study by Eley et al. Using PCR, *Chlamydia spp.* do not need to be viable and our results indicate that PCR results are significantly less affected. Catsburg et al. described that even *C. trachomatis* DNA preserved on dry vaginal swabs which were stored at -80°C could be detected after 1 year of storage.[9]

Our results demonstrate that storage conditions and duration hardly affect *C. trachomatis* DNA detection by PCR in a negative manner, although frozen urine samples, stored for prolonged periods (more than 2 years), could become *C. trachomatis* negative. Nevertheless, our study does validate the use of stored samples in *C. trachomatis* research. Furthermore, it justifies the use of mailed samples in large screening programs in countries with moderate climate [10] and could be of use in home-based or outreach-based diagnostic testing procedures.

REFERENCES

1. Mahony JB, Chernesky MA. 1985. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. J. Clin. Microbiol. 22:865–867.
2. Tjiam KH, van Heijst BY, de Roo JC, de Beer A, van Joost T, Michel MF, Stolz E. 1984. Survival of *Chlamydia trachomatis* in different transport media and at different temperatures: diagnostic implications. Br. J. Vener. Dis. 60:92–94.
3. Roche Molecular Diagnostics. 2009. COBAS TaqMan CT Test, v2.0, package insert. Roche Molecular Diagnostics, Basel, Switzerland. http: //molecular.roche.com/assays/Pages/COBASTaqManCTTestv20.aspx.
4. Jalal H, Stephen H, Curran MD, Burton J, Bradley M, Carne C. 2006. Development and validation of a rotor-gene real-time PCR assay for detection, identification, and quantification of *Chlamydia trachomatis* in a single reaction. J. Clin. Microbiol. 44:206–213.
5. Morré SA, van Valkengoed IG, de Jong A, Boeke AJ, van Eijk JT, Meijer CJ, van den Brule AJ. 1999. Mailed, home-obtained urine specimens: a reliable screening approach for detecting asymptomatic *Chlamydia trachomatis* infections. J. Clin. Microbiol. 37:976–980.
6. Huggett JF, Novak T, Garson JA, Green C, Morris-Jones SD, Miller RF, Zumla A. 2008. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon. BMC Res. Notes 1:70. doi: 10.1186/1756-0500-1-70.
7. Maass M, Dalhoff K. 1995. Transport and storage conditions for cultural recovery of *Chlamydia* pneumoniae. J. Clin. Microbiol. 33:1793–1796.
8. Eley A, Geary I, Bahador A, Hakimi H. 2006. Effect of storage temperature on survival of *Chlamydia trachomatis* after lyophilization. J. Clin. Microbiol. 44:2577–2578.
9. Catsburg A, van Dommelen L, Smelov V, de Vries HJ, Savitcheva A, Domeika M, Herrmann B, Ouburg S, Hoebe CJ, Nilsson A, Savelkoul PH, Morré SA. 2007. TaqMan assay for Swedish *Chlamydia trachomatis* variant. Emerg. Infect. Dis. 13:1432–1434.
10. Gotz HM, van Bergen JE, Veldhuijzen IK, Hoebe CJ, Broer J, Coenen AJ, de Groot F, Verhooren MJ, van Schaik DT, Richardus JH. 2006. Lessons learned from a population-based *chlamydia* screening pilot. Int. J. STD AIDS 17:826–830.



Discussion and Summary

9. Discussion and Summary

Laura van Dommelen

Someone once said: assumptions are the mother of all mistakes. In clinical practice, lots of things are assumed every day, partly based on clinical experience. In this thesis, several assumptions in STI diagnostics were given a closer look. Is SVS the best sample to be tested for Ct in women or could this be improved by adding urine to detect urinary-tract-only infections more effectively? Is Ct DNA stable when frozen or does storage effect test results after thawing? And does a test evaluation using a selected sample set give reliable results which can be used in clinical practice?

Self-taken vaginal swab, urine or combination in Ct diagnosis?

In women, the sensitivity using NAAT on urine, cervical swabs and self-taken vaginal swabs, has been thoroughly explored. NAAT performs equally well on cervical as on SVS, and the sensitivity is usually slightly higher on both of these sample types than on urine samples.[1-6] There are however women with a presumed isolated urinary tract infection which are missed when using SVS as a single sample. Testing both SVS and urine, however, is not cost effective.[7] It appears appropriate to combine both samples in single test to achieve 100% sensitivity, but this could have several disadvantages. A common problem when using NAAT for instance, is inhibition of the assay due to elements in the sample, especially in urine.[8,9] Moreover, adding urine may unnecessarily dilute the amount of Ct present in a SVS, which could theoretically lead to a negative NAAT.

In chapter 2, we have shown that combining urine and a SVS in a single sample does not result in a higher sensitivity. The Ct detection rate for SVS, FCU and SVS/FCU combination were 94%, 90% and 94%, respectively. If SVS and FCU would be tested in separate assays (2-test algorithm), all Ct-positive clients would have been detected (100%). No significant difference in sensitivity was found between the SVS, urine or the combination sample. Also, no inhibition occurred in any of the samples. This is in line with a recent study by Falk et al[10], although urine samples performed significantly worse in their study The Ct detection rate in urine in their study was 88%, compared to 97%, 97% and 95% in endocervical specimens, SVS and SVS/FCU, respectively.

DNA stability under different storage conditions

Laboratories usually have a biobank with clinical samples. Using these samples to evaluate novel diagnostic assays is an easy way to obtain results quickly. But does testing on stored samples generate the same results as on 'fresh' samples? In case of Ct, data on preserving the bacteria over time usually concern culture methods.[11-14] Before NAAT were available, Ct culture was considered the gold standard. The sensitivity of culture however is low[15] and is therefore not common practice anymore. Samples tested positive with NAAT, do not necessarily contain viable organisms. The overall sensitivity and specificity of NAAT for Ct detection in SVS ranges between 97-99% and 95-100% [2, 16] and between 96-100% and 99-100% for urine, respectively.[17] Package inserts of NAAT usually state storage limitations, but do

not give references. In the evaluation of NAAT, several sample types have been used [18-20], but studies on the validation of the use of stored samples are lacking. What happens to the Ct load when samples are stored for a prolonged period?

In chapter 8, we have shown that fortunately Ct DNA is relatively stable. In this study, variation in samples types, storage temperature and duration of storage, did not result in samples testing negative for Ct. The initial amount of elementary bodies in the spiked samples however, was relatively high (approximately equal to 150.000 EB/ml). Using a qPCR on clinical swab specimens in the Ct POC test study (this thesis), the variation in Ct load in SVS turned out to be broad (data not shown) with a median Ct load of 19410 IFU/ml, which is tenfold lower than in the spiked samples. Theoretically, samples could therefore become negative when stored over longer periods of time when the Ct load is low.

Sample selection vs prospective test evaluation

Another advantage of a sample library, is the ability to select samples which have already tested positive for (antibodies against) the micro-organism you are looking for. Numerous studies have used selected sample collections to evaluate the sensitivity and specificity of a diagnostic assay. But do these results hold in clinical practice were the disease prevalence is totally different? How do data need to be interpreted?

In chapter 6, data have been presented on an evaluation of the Bioelisa Syphilis using archived sera. The performance of the Bioelisa syphilis was excellent with a sensitivity and specificity of both 100%. Since the Bioelisa can be performed on an automated system, it was preferred over the TPPA and was implemented at our laboratory. Automation reduces the number of human errors and requires less hands-on time in the laboratory. The syphilis prevalence in the general (Dutch) population however, was different compared to the prevalence in the initial evaluation (44% in the latter, versus in the general population 2.3% in MSM and 0.2% in pregnant women[21]). Several false positive results were noticed and a retrospective analysis was performed to investigate if the results in the initial evaluation would stand. These results are presented in chapter 7.

When analysing the Bioelisa data available since implementation of the Bioelisa as a syphilis screenings assay, the number of false positive results with the Bioelisa was perceived as high (n=73). Especially taking into account that all these samples had to be sent to a reference laboratory, which is costly. This perception however changed, when we performed a sensitivity analysis. The specificity was shown to be stable under several hypothetical situations, and across diverse patient categories. Finding false positive results can be expected when the disease prevalence is low. Although this is a well known phenomenon, our study is a nice example and can therefore be helpful for clinicians. Confirmatory testing is therefore mandatory in low prevalence settings.

Interpretation of the results in the retrospective analysis of the Bioelisa was limited by the fact that confirmative assays were only performed on a small number of the samples tested by the Bioelisa (e.g. when the Bioelisa was positive). Potentially, a number of false negative Bioelisa results are present in the database. With sensitivity analysis this problem has been addressed, but is far from ideal because such an analysis is a statistical method instead of a laboratory-based method. Even when all samples are tested by two different assays, it is difficult to interpret the data, especially in the absence of a 'gold standard'. Considering Ct NAAT, performance characteristics are not based on comparison with a 'gold standard'. In evaluation studies of syphilis different assays are compared in parallel and if one or more of the assays are positive, the patient is considered infected.[22]

When writing the manuscripts in this thesis, the do's and don'ts in performing discrepancy analysis have been discussed several times. Hagdu et al. addressed the limitations of discrepancy analysis in the Lancet in 1996 and many response articles followed. [23] Although discrepancy analysis is suitable for determining specificity, its use in determining sensitivity is disputed. Taking Ct NAAT evaluations as an example, determining sensitivity is based on assuming a culture specificity is 100% and an equal sensitivity of NAAT in culture positive and culture negative samples. As with all diagnostic assays, both assumptions are probably not true and therefore sensitivity estimates are biased. Green et al. suggest performing additional assays besides the reference test and the test to be evaluated, but this is obviously expensive.[24] Schachter therefore pleaded against 'statistical correctness' since no reasonable alternative is available for discrepancy analysis considering the increased workload and expenses needed for adding a third assay on all samples used in the test evaluation.[25]

CHEAPER, FASTER, BETTER

When entering 'diagnostic assay evaluation infection' in Pubmed, over 35,000 hits are available. The total number of articles published has increased from over a 1000 publications in the year 2000 to already more than 2500 in the year 2010. Increasing technological possibilities and a demanding public (professional and non-professional) result in new assays becoming available every week. Laboratories are under continuous pressure to perform better, faster and cheaper. Also, there is the rise of internet, which makes all products available to virtually everyone and makes people become their own health care provider.

On the other hand, STI diagnostics are unavailable in (parts of) developing countries. Remote clinics may often offer free aid, but cannot afford to offer proper diagnostic assays. Overall, laboratory medicine in developing countries has a low priority when compared to disease prevention and management.[26] Unfortunately, 90% of STI are diagnosed in these countries where patients have no or difficult access to, nor the means to pay for the health care they need.[27] The WHO recommends practising syndromic management, but its sensitivity and specificity are poor.[28,29] In the study by Yin et al. the physician correctly diagnosed Ct infection in only 2.2% of the men and 10.7% of the women with Ct infection.[28] For syphilis, these percentages were 0.0% and 16.1%, respectively. The PPV for physicians diagnosing STI, based on syndromic management was 50%. This means that 50% of the treated individuals were treated unnecessarily. Even more disturbing is the overall sensitivity of 10%, which implies that 90% of the individuals with an STI are missed.

Point-of-care tests

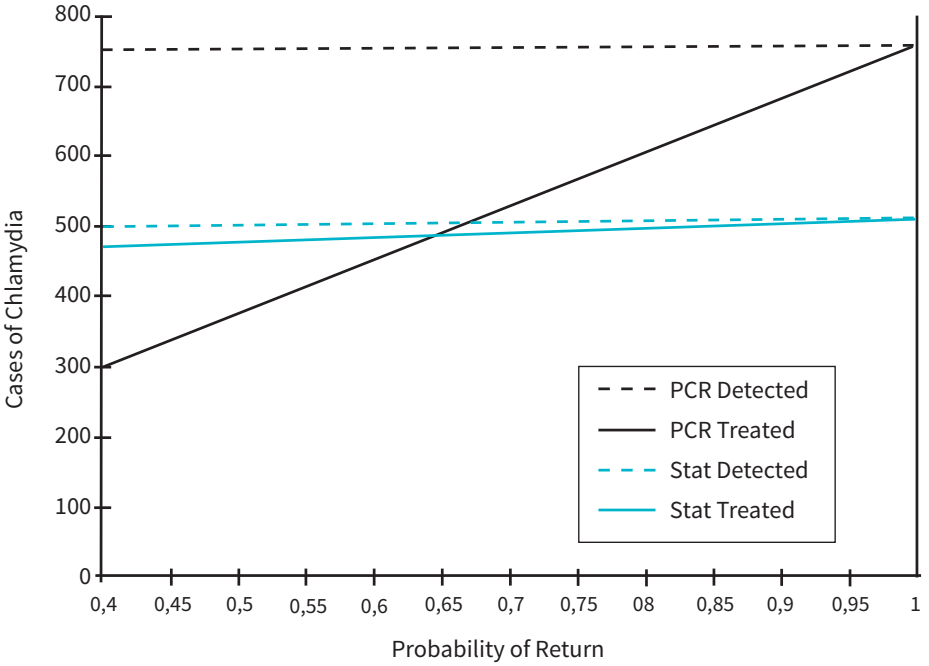
The developments mentioned above, have led to the introduction of point-of-care tests (POCT) to diagnose infections. POCT are well known in infectious diseases diagnostics, for instance to diagnose HIV infection[30] and malaria[31]. The main concern regarding point-of-care tests to diagnose STI, is the potentially low sensitivity (see next paragraphs). Moreover, rapid tests are not the assay of choice when processing large sample volumes considering the hands-on time. The ASSURED criteria (Table 1) have been developed as a standard which a rapid test must meet to be introduced as an STI diagnostic method (www.WHO.int).

Table 1 ASSURED criteria (modified, www.WHO.int)

Test characteristics	Ng/Ct	Syphilis
Affordable	US $ 6-15	US $ 0.19-3
Sensitive	43-65%	85-99%
Specific	98%	93-100%
User-friendly	7-14 steps	3-4 steps
Rapid/robust	30 min./storage at 8-30°C	20 min./storage at 8-30°C
Equipment-free	Yes	Yes
Deliverable	?	?

Several mathematical models have been developed to predict the beneficial effects of introducing a POCT in clinical practice.[32-34] Benefits depend on STI prevalence within a given population, loss to follow-up, test characteristics and costs. Gift et al.[32] developed a cost-effectiveness model, and applied this for different diagnostic strategies, including Ct POCT and Ct POCT/NAAT combination. Endpoint was the number of averted Ct related PID cases. They concluded that the Ct POCT/NAAT strategy is most cost- effective and detects most Ct cases. Results however, are highly dependent on return rate (the percentage of patients returning the clinic to get the test result) as displayed in Figure 1. As can be seen in this figure, diagnosis by Ct POCT results in the treatment of as many Ct positive cases as diagnosis by NAAT, when the return rate is 65%. The Ct prevalence also influences cost-effectiveness: the Ct POC/NAAT combination is only cost-effective if prevalence is above 9%.

Figure 1 Cases of Ct detected and treated as a function of the return rate. [32]



The mathematical POCT model of Vickerman et al.[34] is based on a population of female sex-workers and assumes treatment using syndromic management. These authors predicted that if a hypothetical POCT for Ct and Ng would have a sensitivity of 70% and a specificity of 95%, 37.3% more Ct and Ng cases would be treated and 42.0% more HIV infections would be averted, compared with syndromic management. Moreover, prevalence of Ct and Ng would decrease under these circumstances, and the percentage of incorrectly treated or not treated individuals would decrease. Concerning the costs, the WHO states that the upper limits for the cost effectiveness for interventions are $70 per DALY (disability adjusted life years) saved or $1300 per HIV infection averted. In the model of Vickerman et al., only a POCT with a sensitivity of less than 50% would not be cost effective.

In chapter 5, the benefits of an introduction of a Ct POCT in a STI clinic in a developed country without loss to follow-up was explored. A POCT with a sensitivity of 100% would avert at least eight additional Ct cases (n=772, Ct prevalence 11%) caused by transmission during treatment

delay, between sampling and diagnosis. A POCT could therefore potentially decrease Ct spread significantly, irrespective of cost-effectiveness. In this thesis, four POCT have been evaluated, one for the detection of antibodies against syphilis and three for the detection of Ct antigen. Starting with syphilis, presented in chapter 6, several articles were published on POCT [35-40], showing sensitivities and specificities ranging between 40%-100% and 85%-100%, respectively, concerning different populations. The Biorapid Syphilis however, showed a sensitivity and specificity of 92% and 79%, respectively, in our evaluation using selected samples. The ASSURED criteria use a specificity of at minimum 93% for a syphilis rapid test. The Biorapid Syhilis in our study had a specificity of 79% with a 95% confidence interval between 72%-85%, and is therefore unsuitable for clinical introduction according to these standards. Moreover, the test result was often difficult to read, which makes this test unsuitable for untrained personnel. And, most importantly, the Biorapid Syphilis missed RPR positive samples (e.g. patient with active disease), which is worrisome.

All the above mentioned syphilis POCT detect treponemal antibodies, and can therefore not differentiate between active and latent infection. Using such a POCT may result in significant overtreatment. Recently, a study was published by Castro et al. on the evaluation of a rapid test which simultaneously detects *Treponema pallidum* specific antibodies and the nontreponemal antibodies used to diagnose syphilis. In their study, although most positive samples reacted both in the treponemal and nontreponemal line in the POCT, 17% of the positive samples only reacted in the treponemal line, meaning no active infection was present. This enables the practitioner to distinguish between active and latent syphilis, and helps avoiding overtreatment. The concordance with the reactive line for the RPR and TPPA was 98.4% (if RPR >= 1:2) and 96.5%%, respectively. Field studies are needed to confirm their (promising) results.

Considering the Ct POCT, presented in chapter 5, the performance of all rapid tests were dramatic, with sensitivities ranging between 12% and 27%. One of the Ct POCT was widely available via the internet when the study was initiated (Handilab-C) and all POCT used in the study had a CE-mark (Conformitée Européenne). One would expect a certain level of quality when a product has been marketed, but obviously this is not the case. The Handilab-C has now been withdrawn from the market, but, unfortunately, (non-evaluated) Ct POCT are still available via the internet.

The ASSURED criteria state a minimal sensitivity for Ct POC-tests of 43% in case of the absence of a clinical laboratory. This requirement was obviously not met. Moreover, all rapid tests were difficult to interpreted, even after multiple testing. So even if the results were promising, the test would not be suitable for home-based use. In 2007, Saison et al. published the first data on the *Chlamydia* Rapid Test (CRT) and presented a sensitivity and specificity of 71% and

99% in a high Ct prevalence setting and 87% and 100%, in a low prevalence setting.[41] Several studies followed, with again promising results.[42,43] All studies however, were performed by the same group which also developed the CRT. In 2012, van der Helm et al published the non-manufactured-sponsored study using vaginal swabs and although specificity was high (96%), sensitivity was only 41%.[44] The authors indicate that sensitivity is dependent on Ct load, since the sensitivity raised to 74% in samples with the highest Ct load. This relation was not found in the Ct-POC study in this thesis.

Chlamydia trachomatis typing

Understanding Ct dynamics in vivo and within the human population requires knowledge of every aspect of Ct infection. In chapter 3, we have presented a new NAAT to detect the Swedish variant Ct (swCt). The emergence of a new Ct strain was suspected when an unexpected fall in Ct incidence of 25% was noticed in Halland County, Sweden.[45] Since this new variant was not detected by the most commonly used commercial Ct NAAT (Roche, Basel, Switzerland, and Abbott Laboratories, Abbott Park, IL, USA), a specific assay to detect the swCt was urgently needed. A real-time PCR (TaqMan assay) was developed that specifically detects the swCt variant by using a probe that spans the 377-bp left and right gap border sequences. In our cohort, consisting of samples from The Netherlands and Russia, no swCt was detected. Until now, surprisingly few swCt have been detected outside Sweden/Scandinavia.[46,47] Introduction of a specific (in-house) assay to detect the swCt in 2007 coincided with the beginning of an absolute and relative decline in the number of Ct infected patients in Sweden.[48] At the time of introduction of this new assay the swCt comprised 30% of all Ct infections found. Moreover, a peak in the absolute number of all Ct positive cases was noticed compared to the preceding years (2005-2006). In 2011 the percentage of the swCt was 6% of the total number of Ct infections, suggesting that the improved testing, and consequently improved treatment and partner tracing have helped to reduce the circulation of swCt.

A study by Bjartling et al found that females with swCT infection reported significantly less complaints of painful urination (p=0.02) and abdominal pain (p=0.02) and were less often diagnosed with urethritis (p=0.04), compared to females infected with the wild type.[49] Although these results are interesting, the relations between different Ct strains and clinical symptoms is unclear.[50-52] Recently, the first large study using whole genome sequencing on multiple Ct strains was published.[53] This study showed, that recombination is not rare in Ct. The whole ompA gene, for instance, can be exchanged between different Ct lineages, including between ocular and LGV strains. Exchanging ompA can result in immune evasion, if the patient was recently infected with Ct. Exchange of whole plasmids is thought to be rare. Whole genome

sequencing is still relatively expensive and time consuming. The genetic complexity of Ct, the complexity of the human immune system and variation in other factors (like the vaginal microbiome) make it difficult to find associations if these are actually present but weak.[54]

The usefulness of less extensive genotyping is limited, but it can be useful to detect double infections for instance, which can be clinically relevant. In chapter 4, we have compared the Ct Detection and genoTyping Kit and the COBAS Amplicor CT/NG (Roche Diagnostics Systems, Basel, Switzerland) for their ability to detect Ct in a well described female population consulting a sexually transmitted diseases (STD) clinic. The Ct-DT is directed at two targets (on the cryptic plasmid and Omp1 gene) to detect Ct, whilst the COBAS Amplicor has only one target on the cryptic plasmid. We have shown that the Ct-DT is a sensitive and highly specific assay to detect Ct compared to the COBAS Amplicor CT/NG and can therefore be used in large screening studies. Although recently several papers have been published on Ct strains without a plasmid[55,56], we did not find any additional Ct positive sample with the Ct-DT.

THE WAY FORWARD

As mentioned in the introduction, prevention and control of STI focuses on: education and counseling of persons at risk in order to achieve changes in sexual behaviors; identification of asymptomatically infected persons and of symptomatic persons unlikely to seek diagnostic and treatment services; effective diagnosis and treatment of infected persons; evaluation, treatment, and counseling of sexual partners of persons who are infected with an STI and pre-exposure vaccination of persons at risk for vaccine-preventable STI.[57] Regarding syphilis, the biggest problem to overcome is reaching the people at risk, e.g. in developing countries.[58,59] Obviously in developing countries there is much to gain in the control of Ct spread, but this subject is more complex. Even in developed countries like the Netherlands, an increase in Ct testing and Ct prevalence is noticeable over the last few years, instead of a decrease in prevalence.[21] An overview of the existing literature regarding education and counselling is beyond the scope of this thesis, but current intervention strategies might therefore be successful in certain aspects, but do not result in a decrease in Ct incidence, and therefore are not yet effective enough. Considering identification of infected patient, a recent article by Op de Coul et al. illustrates that the populations at risk for Ct are the most difficult to reach even when STI screening is actively offered.[60] Moreover, large scale screening did not result in a decrease in Ct prevalence.[61]

Still it is important to keep trying to identify and test individuals at risk and finding novel innovative methods to reach populations at risk in addition to regular care by general

practitioners, STI clinics and gynaecologists. STI prevalence in sexual partners is high.[62] Although partner treatment has been shown to be effective in preventing transmission, its practice can be improved in case of Ct.[63,64] Also, Ct re-infection is common in the first 3-6 months after initial infection.[65] The Centers for Disease Control (CDC) guidelines advice to repeat Ct testing after 3 months in case of a positive test. Hoover et al. studied the adherence to repeated testing by analysing laboratory data. Retesting was only performed in 22% of men and 38% of non-pregnant women, while positivity rates in patients who did get tested were 16% and 14%, respectively.[66] Increasing retesting rate could therefore result in a public health gain.

Strategies used in Ct control are based on the information which is currently available. Although much is known about Ct infections, even more is yet unclear. Diagnostic assays can be useful in revealing the whole pathogenesis of Ct infections, which in turn can be used to control Ct spread. For instance why do some females have symptoms and others do not? Studies on the interaction between Ct and the human immune system has already led to some interesting observations.[67-69] Bailey et al. for instance analysed the difference in in vitro lymphoproliferative response to Ct elementary bodies between monozygotic and dizygotic twins and found that genetic differences accounted for 39% of the variation found in lymphoproliferative response.[68] Whole genome analysis has overthrown some well accepted assumptions concerning the Ct genome which shine a new light on the organism itself and its interactions with the environment.[53] This environment is composed of the human cells, but also, in case of females, the microbiome of the vagina. The vaginal microbiome is complex and studying its flora has resulted in an overload in information. It yet needs to be determined how the different micro organisms relate to each other and their environment.[70]

It is also not clear yet if the Ct load is a relevant finding for understanding the pathogenesis and transmission of Ct infection. It may be possible that higher loads are associated with worse symptoms – or even the opposite: massive scarring in case of low load chronic infections. It has been shown that some females shed Ct for longer periods after treatment than other patients[71], but large follow-up studies are needed to determine the clinical relevance of this phenomenon. If large groups of females shed Ct after treatment, does this equal therapy failure? Since determining phenotypic nor genotypic Ct antibiotic resistance is performed routinely in most laboratories, it is not clear whether antibiotic resistance is common in Ct, and can account for prolonged shedding. In conclusion, there is still a lot to gain in Ct control and accurate Ct diagnostic assays remain essential in the much needed research beyond state of the art .

REFERENCES

1. Hoebe CJ, Rademaker CW, Brouwers EE, ter Waarbeek HL, van Bergen JE. Acceptability of self-taken vaginal swabs and first-catch urine samples for the diagnosis of urogenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with an amplified DNA assay in young women attending a public health sexually transmitted disease clinic. Sex Transm Dis 2006;33(8):491-5.
2. Schachter J, Chernesky MA, Willis DE, Fine PM, Martin DH, Fuller D, et al. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results from a multicenter evaluation of the APTIMA assays for both infections. Sex Transm Dis 2005;32(12):725-8.
3. Michel CE, Sonnex C, Carne CA, White JA, Magbanua JP, Nadala EC, Jr., et al. *Chlamydia trachomatis* load at matched anatomic sites: implications for screening strategies. J Clin Microbiol 2007;45(5):1395-402.
4. Skidmore S, Horner P, Herring A, Sell J, Paul I, Thomas J, et al. Vulvovaginal-swab or first-catch urine specimen to detect *Chlamydia trachomatis* in women in a community setting? J Clin Microbiol 2006;44(12):4389-94.
5. Shafer MA, Moncada J, Boyer CB, Betsinger K, Flinn SD, Schachter J. Comparing first-void urine specimens, self-collected vaginal swabs, and endocervical specimens to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by a nucleic acid amplification test. J Clin Microbiol 2003;41(9):4395-9.
6. Schoeman SA, Stewart CM, Booth RA, Smith SD, Wilcox MH, Wilson JD. Assessment of best single sample for finding *chlamydia* in women with and without symptoms: a diagnostic test study. Bmj 2012;345:e8013.
7. Blake DR, Maldeis N, Barnes MR, Hardick A, Quinn TC, Gaydos CA. Cost-effectiveness of screening strategies for *Chlamydia trachomatis* using cervical swabs, urine, and self-obtained vaginal swabs in a sexually transmitted disease clinic setting. Sex Transm Dis 2008;35(7):649-55.
8. Carder C, Mercey D, Benn P. *Chlamydia trachomatis.* Sex Transm Infect 2006;82 Suppl 4:iv10-2.
9. Huggett JF, Novak T, Garson JA, Green C, Morris-Jones SD, Miller RF, et al. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon. BMC Res Notes 2008;1:70.
10. Falk L, Coble BI, Mjornberg PA, Fredlund H. Sampling for *Chlamydia trachomatis* infection - a comparison of vaginal, first-catch urine, combined vaginal and first-catch urine and endocervical sampling. Int J STD AIDS 2010;21(4):283-7.
11. Eley A, Geary I, Bahador A, Hakimi H. Effect of storage temperature on survival of *Chlamydia trachomatis* after lyophilization. J Clin Microbiol 2006;44(7):2577-8.
12. Maass M, Dalhoff K. Transport and storage conditions for cultural recovery of *Chlamydia* pneumoniae. J Clin Microbiol 1995;33(7):1793-6.
13. Mahony JB, Chernesky MA. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. J Clin Microbiol 1985;22(5):865-7.
14. Tjiam KH, van Heijst BY, de Roo JC, de Beer A, van Joost T, Michel MF, et al. Survival of *Chlamydia trachomatis* in different transport media and at different temperatures: diagnostic implications. Br J Vener Dis 1984;60(2):92-4.
15. Livengood CH, 3rd, Wrenn JW. Evaluation of COBAS AMPLICOR (Roche): accuracy in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by coamplification of endocervical specimens. J Clin Microbiol 2001;39(8):2928-32.
16. Van der Pol B. COBAS Amplicor: an automated PCR system for detection of *C. trachomatis* and N. *gonorrhoeae.* Expert Rev Mol Diagn 2002;2(4):379-89.
17. Gaydos CA, Theodore M, Dalesio N, Wood BJ, Quinn TC. Comparison of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* in urine specimens. J Clin Microbiol 2004;42(7):3041-5.
18. Knox J, Tabrizi SN, Miller P, Petoumenos K, Law M, Chen S, et al. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. Sex Transm Dis 2002;29(11):647-54.

19. Chandeying V, Lamlertkittikul S, Skov S. A comparison of first-void urine, self-administered low vaginal swab, self-inserted tampon, and endocervical swab using PCR tests for the detection of infection with *Chlamydia trachomatis*. Sex Health 2004;1(1):51-4.
20. Koumans EH, Black CM, Markowitz LE, Unger E, Pierce A, Sawyer MK, et al. Comparison of methods for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using commercially available nucleic acid amplification tests and a liquid pap smear medium. J Clin Microbiol 2003;41(4):1507-11.
21. S.C.M. Trienekens FDHK, I.V.F. van den Broek, H.J. Vriend, E.L.M. Op de Coul, M.G. van Veen, A.I. van Sighem, I. Stirbu-Wagner, M.A.B. van der Sande. Sexually transmitted infections, including HIV, in the Netherlands in 2011: National Institute for Public Health and the Environment, 2012.
22. Skidmore S, Horner P, Mallinson H. Testing specimens for *Chlamydia trachomatis*. Sex Transm Infect 2006;82(4):272-5.
23. Hadgu A. The discrepancy in discrepant analysis. Lancet 1996;348(9027):592-3.
24. Green TA, Black CM, Johnson RE. Evaluation of bias in diagnostic-test sensitivity and specificity estimates computed by discrepant analysis. J Clin Microbiol 1998;36(2):375-81.
25. Schachter J. Two different worlds we live in. Clin Infect Dis 1998;27(5):1181-5.
26. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. Clin Infect Dis 2006;42(3):377-82.
27. Peeling RW, Mabey D, Herring A, Hook EW, 3rd. Why do we need quality-assured diagnostic tests for sexually transmitted infections? Nat Rev Microbiol 2006;4(12):909-21.
28. Yin YP, Wu Z, Lin C, Guan J, Wen Y, Li L, et al. Syndromic and laboratory diagnosis of sexually transmitted infection: a comparative study in China. Int J STD AIDS 2008;19(6):381-4.
29. Vuylsteke B. Current status of syndromic management of sexually transmitted infections in developing countries. Sex Transm Infect 2004;80(5):333-4.
30. Pant Pai N, Balram B, Shivkumar S, Martinez-Cajas JL, Claessens C, Lambert G, et al. Head-to-head comparison of accuracy of a rapid point-of-care HIV test with oral versus whole-blood specimens: a systematic review and meta-analysis. Lancet Infect Dis 2012;12(5):373-80.
31. McMorrow ML, Aidoo M, Kachur SP. Malaria rapid diagnostic tests in elimination settings--can they find the last parasite? Clin Microbiol Infect 2011;17(11):1624-31.
32. Gift TL, Pate MS, Hook EW, 3rd, Kassler WJ. The rapid test paradox: when fewer cases detected lead to more cases treated: a decision analysis of tests for *Chlamydia trachomatis*. Sex Transm Dis 1999;26(4):232-40.
33. Vickerman P, Watts C, Alary M, Mabey D, Peeling RW. Sensitivity requirements for the point of care diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women. Sex Transm Infect 2003;79(5):363-7.
34. Vickerman P, Watts C, Peeling RW, Mabey D, Alary M. Modelling the cost effectiveness of rapid point of care diagnostic tests for the control of HIV and other sexually transmitted infections among female sex workers. Sex Transm Infect 2006;82(5):403-12.
35. West B, Walraven G, Morison L, Brouwers J, Bailey R. Performance of the rapid plasma reagin and the rapid syphilis screening tests in the diagnosis of syphilis in field conditions in rural Africa. Sex Transm Infect 2002;78(4):282-5.
36. Montoya PJ, Lukehart SA, Brentlinger PE, Blanco AJ, Floriano F, Sairosse J, et al. Comparison of the diagnostic accuracy of a rapid immunochromatographic test and the rapid plasma reagin test for antenatal syphilis screening in Mozambique. Bull World Health Organ 2006;84(2):97-104.
37. Siedner M, Zapitz V, Ishida M, De La Roca R, Klausner JD. Performance of rapid syphilis tests in venous and fingerstick whole blood specimens. Sex Transm Dis 2004;31(9):557-60.
38. Campos PE, Buffardi AL, Chiappe M, Buendia C, Garcia PJ, Carcamo CP, et al. Utility of the Determine Syphilis TP rapid test in commercial sex venues in Peru. Sex Transm Infect 2006;82 Suppl 5:v22-v25.
39. Diaz T, Almeida MG, Georg I, Maia SC, De Souza RV, Markowitz LE. Evaluation of the Determine Rapid Syphilis TP assay using sera. Clin Diagn Lab Immunol 2004;11(1):98-101.

40. Herring AJ, Ballard RC, Pope V, Adegbola RA, Changalucha J, Fitzgerald DW, et al. A multi-centre evaluation of nine rapid, point-of-care syphilis tests using archived sera. Sex Transm Infect 2006;82 Suppl 5:v7-v12.
41. Saison F, Mahilum-Tapay L, Michel CE, Buttress ND, Nadala EC, Jr., Magbanua JP, et al. Prevalence of *Chlamydia trachomatis* infection among low- and high-risk Filipino women and performance of *Chlamydia* rapid tests in resource-limited settings. J Clin Microbiol 2007;45(12):4011-7.
42. Nadala EC, Goh BT, Magbanua JP, Barber P, Swain A, Alexander S, et al. Performance evaluation of a new rapid urine test for *chlamydia* in men: prospective cohort study. Bmj 2009;339:b2655.
43. Mahilum-Tapay L, Laitila V, Wawrzyniak JJ, Lee HH, Alexander S, Ison C, et al. New point of care *Chlamydia* Rapid Test--bridging the gap between diagnosis and treatment: performance evaluation study. Bmj 2007;335(7631):1190-4.
44. van der Helm JJ, Sabajo LO, Grunberg AW, Morre SA, Speksnijder AG, de Vries HJ. Point-of-care test for detection of urogenital *chlamydia* in women shows low sensitivity. A performance evaluation study in two clinics in Suriname. PLoS One 2012;7(2):e32122.
45. Ripa T, Nilsson P. A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill 2006;11(11):E061109 2.
46. Fieser N, Simnacher U, Tausch Y, Werner-Belak S, Ladenburger-Strauss S, von Baum H, et al. *Chlamydia trachomatis* prevalence, genotype distribution and identification of the new Swedish variant in Southern Germany. Infection 2013;41(1):159-66.
47. Shipitsyna E, Hadad R, Ryzhkova O, Savicheva A, Domeika M, Unemo M. First reported case of the Swedish new variant of *Chlamydia trachomatis* (nvCT) in Eastern Europe (Russia), and evaluation of Russian nucleic acid amplification tests regarding their ability to detect nvCT. Acta Derm Venereol 2012;92(3):330-1.
48. Persson K, Hammas B, Janson H, Bjartling C, Dillner J, Dillner L. Decline of the new Swedish variant of *Chlamydia trachomatis* after introduction of appropriate testing. Sex Transm Infect 2012;88(6):451-5.
49. Bjartling C, Osser S, Johnsson A, Persson K. Clinical manifestations and epidemiology of the new genetic variant of *Chlamydia trachomatis*. Sex Transm Dis 2009;36(9):529-35.
50. Pedersen LN, Herrmann B, Moller JK. Typing *Chlamydia trachomatis*: from egg yolk to nanotechnology. FEMS Immunol Med Microbiol 2009;55(2):120-30.
51. Byrne GI. *Chlamydia trachomatis* strains and virulence: rethinking links to infection prevalence and disease severity. J Infect Dis 2010;201 Suppl 2:S126-33.
52. Christerson L, de Vries HJ, Klint M, Herrmann B, Morre SA. Multilocus sequence typing of urogenital *Chlamydia trachomatis* from patients with different degrees of clinical symptoms. Sex Transm Dis 2009;38(6):490-4.
53. Harris SR, Clarke IN, Seth-Smith HM, Solomon AW, Cutcliffe LT, Marsh P, et al. Whole-genome analysis of diverse *Chlamydia trachomatis* strains identifies phylogenetic relationships masked by current clinical typing. Nat Genet 2012;44(4):413-9, S1.
54. Turner K, Clarke I, Timpson N, Horner P. *Chlamydia trachomatis* in the age of the genome: application of molecular genotyping to improve our understanding of the immunopathogenesis of *Chlamydia* genital tract disease. Sex Transm Dis;38(6):495-8.
55. An Q, Radcliffe G, Vassallo R, Buxton D, O'Brien WJ, Pelletier DA, et al. Infection with a plasmid-free variant *Chlamydia* related to *Chlamydia trachomatis* identified by using multiple assays for nucleic acid detection. J Clin Microbiol 1992;30(11):2814-21.
56. Magbanua JP, Goh BT, Michel CE, Aguirre-Andreasen A, Alexander S, Ushiro-Lumb I, et al. *Chlamydia trachomatis* variant not detected by plasmid based nucleic acid amplification tests: molecular characterisation and failure of single dose azithromycin. Sex Transm Infect 2007;83(4):339-43.
57. Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep 2010;59(RR-12):1-110.
58. Global incidence and prevalence of selected curable sexually transmitted infections - 2008 World Health Organization, 2012.

59. Advancing MDG 4, 5 and 6: impact of congenital syphilis elimination: World Health Organization, 2010.
60. Op de Coul EL, Gotz HM, van Bergen JE, Fennema JS, Hoebe CJ, Koekenbier RH, et al. Who participates in the Dutch *Chlamydia* screening? A study on demographic and behavioral correlates of participation and positivity. Sex Transm Dis 2012;39(2):97-103.
61. van den Broek IV, van Bergen JE, Brouwers EE, Fennema JS, Gotz HM, Hoebe CJ, et al. Effectiveness of yearly, register based screening for *chlamydia* in the Netherlands: controlled trial with randomised stepped wedge implementation. Bmj;345:e4316.
62. Khan A, Fortenberry JD, Juliar BE, Tu W, Orr DP, Batteiger BE. The prevalence of *chlamydia*, gonorrhea, and trichomonas in sexual partnerships: implications for partner notification and treatment. Sex Transm Dis 2005;32(4):260-4.
63. Santo I, Azevedo J, Nunes B, Gomes JP, Borrego MJ. Partner notification for *chlamydia trachomatis* urogenital infections: eight years of patient referral experience in the major Portuguese sexually transmitted infections clinic, 2000-07. Int J STD AIDS 2011;22(10):548-51.
64. Forbes G, Clutterbuck DJ. How many cases of *chlamydia*l infection would we miss by not testing partners for infection? Int J STD AIDS 2009;20(4):267-8.
65. Walker J, Tabrizi SN, Fairley CK, Chen MY, Bradshaw CS, Twin J, et al. *Chlamydia trachomatis* incidence and re-infection among young women--behavioural and microbiological characteristics. PLoS One 2012;7(5):e37778.
66. Hoover KW, Tao G, Nye MB, Body BA. Suboptimal adherence to repeat testing recommendations for men and women with positive *Chlamydia* tests in the United States, 2008-2010. Clin Infect Dis 2012;56(1):51-7.
67. Agrawal T, Gupta R, Dutta R, Srivastava P, Bhengraj AR, Salhan S, et al. Protective or pathogenic immune response to genital *chlamydia*l infection in women--a possible role of cytokine secretion profile of cervical mucosal cells. Clin Immunol 2009;130(3):347-54.
68. Bailey RL, Natividad-Sancho A, Fowler A, Peeling RW, Mabey DC, Whittle HC, et al. Host genetic contribution to the cellular immune response to *Chlamydia trachomatis*: Heritability estimate from a Gambian twin study. Drugs Today (Barc) 2009;45 Suppl B:45-50.
69. Morre SA, Karimi O, Ouburg S. *Chlamydia trachomatis*: identification of susceptibility markers for ocular and sexually transmitted infection by immunogenetics. FEMS Immunol Med Microbiol 2009;55(2):140-53.
70. Srinivasan S, Hoffman NG, Morgan MT, Matsen FA, Fiedler TL, Hall RW, et al. Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. PLoS One 2012;7(6):e37818.
71. Dukers-Muijrers NH, Morre SA, Speksnijder A, van der Sande MA, Hoebe CJ. *Chlamydia trachomatis* test-of-cure cannot be based on a single highly sensitive laboratory test taken at least 3 weeks after treatment. PLoS One 2012;7(3):e34108.

10. Samenvatting

Laura van Dommelen

In dit proefschrift worden een aantal aspecten van de *Chlamydia trachomatis* (Ct) en *Treponema pallidum* (Tp) diagnostiek onder de loep genomen met als doel deze te verbeteren in verschillende opzichten.

In hoofdstuk 1 wordt op basis van beschikbare literatuur uiteengezet wat de impact is van seksueel overdraagbare aandoeningen (SOA) in globaal opzicht en gefocust op de Nederlandse setting. Tevens wordt er dieper ingegaan op de ziektebeelden veroorzaakt door Ct en Tp. De WHO schatte dat in 2008 wereldwijd bijna een half miljard mensen besmet zijn geraakt met Tp, *Neisseria gonorrhoeae* (Ng), Ct en *Trichomonas vaginalis* (Tv). Dit zijn allemaal behandelbare infecties.[1] In dit proefschrift ligt de nadruk op Tp, de veroorzaker van syfilis (lues) en Ct. Syfilis tijdens de zwangerschap kan leiden tot foetaal en neonataal overlijden. Dit is een groot probleem in ontwikkelingslanden, terwijl screening en behandeling van syfilis tezamen slechts $1.5 per persoon kost.[2] Ook Ct infecties kunnen zorgen voor gecompliceerde aandoeningen, zoals 'pelvic inflammatory disease' (ontsteking van de baarmoeder en eierstokken). Een ander probleem is dat een infectie met Ct vaak asymptomatisch verloopt. Desondanks kan er sprake kan zijn van een (chronische) infectie welke kan leiden tot onvruchtbaarheid.[3] Het asymptomatisch verloop is tevens een reden waarom Ct zich zo makkelijk verspreidt. Bij seksueel contact raakt gemiddeld 70% van de partners besmet met Ct.[4-6]

Ook in Nederland komen SOA veel voor, met name bij jongeren onder de 25 jaar oud, mannen die seks hebben met mannen (MSM) en bij van oorsprong Surinaamse en Antilliaanse Nederlanders.[7] Ct is de meest voorkomende bacteriële SOA in Nederland. Bij de GGD werd in 2011 gemiddeld 11.5% van de cliënten positief getest voor Ct infectie. Bij Ng lag dit percentage op 3.2%. Syfilis is relatief zeldzaam met slechts 476 gerapporteerde infecties in 2011 en wordt voornamelijk gevonden bij MSM (90% van het totaal aantal infecties).[7] De meeste SOA gerelateerde consulten vinden plaats bij de huisarts (63%) en de overigen bij de GGD.[8] In Nederland wordt er op de SOA polikliniek van de GGD standaard getest op Ct, Ng, syfilis en humaan immunodeficiëntie virus (HIV). Bij jongeren onder 25 jaar zonder risicofactoren wordt er alleen op Ct getest.

Er is veel onderzoek gedaan naar welk materiaal bij vrouwen het meest geschikt is om Ct te diagnosticeren.[9-14] Inmiddels is helder dat een zelf afgenomen vaginale uitstrijk (SVS) even geschikt is als een cervicale uitstrijk genomen door een zorgverlener. Daarnaast kan urine gebruikt worden, maar dit is wat minder gevoelig ten opzichte van eerder genoemde materialen voor het diagnosticeren van een Ct infectie bij vrouwen. Desalniettemin blijft de vraag of er bij vrouwen mogelijk een Ct urethritis (urineweginfectie) kan worden gemist als er enkel op cervicaal of vaginaal materiaal wordt getest. Aangezien het testen van een SVS en urine niet kosteneffectief is[15], hebben wij gekeken of het combineren van een SVS met urine in een enkele test een toegevoegde waarde heeft ten opzichte van een SVS. In hoofdstuk 2 laten we zien dat het combineren van urine met een SVS bij vrouwen niet resulteert in een hogere gevoeligheid: de sensiviteit is in beide gevallen 94%. Een SVS blijft bij vrouwen dus het materiaal van keuze voor het diagnosticeren van een Ct infectie.

Hoofdstuk 3 behandelt een bijzonder deel in de geschiedenis van Ct detectie, namelijk de detectie van de 'Swedish variant' Ct (swCt) of 'new variant' Ct. In Halland County, Zweden, werd in 2006 vermoed dat er een nieuwe Ct stam in omloop was nadat er een 25% afname in incidentie was geconstateerd.[16] Deze stam bleek niet te worden opgepikt door de

commerciële nucleïne zuur amplificatie testen (NAAT) die op dat moment het meest gebruikt werden, vanwege een deletie in het 'cryptic' plasmide, en daarom was een gerichte NAAT voor de betreffende stam nodig. Wij hebben een 'real-time' polymerase ketting reactie (PCR) ontwikkeld die gericht is op het gebied rondom de betreffende deletie. Met gebruikmaking van deze nieuwe NAAT zijn verschillende materialen uit Nederland en Rusland getest, maar er werd geen swCt gedetecteerd. Ook in andere studies wordt, buiten Scandinavië, nauwelijks de swCt gezien.[17,18]

In hoofdstuk 4 hebben we de Ct Detection and genoTyping Kit (Ct-DT; Labo Bio-medical Products B.V., Rijswijk, Nederland) en de COBAS Amplicor CT/NG (Roche Diagnostics Systems, Basel, Zwitserland) vergeleken ten aanzien van de detectie van Ct bij vrouwen. De COBAS Amplicor CT/NG grijpt aan op 1 punt van een mobiel stuk DNA van Ct ('cryptic plasmid'). De Ct-DT daarentegen grijpt aan op 2 verschillende punten in het genetisch materiaal van Ct: de 'cryptic' plasmide en het Omp1 gen. Ook kunnen met de Ct-DT dubbelinfecties met verschillende typen Ct worden gedetecteerd. Dit kan van belang zijn om bijvoorbeeld onderscheidt te kunnen maken tussen een persisterende en een nieuwe Ct infectie. Tevens zouden met de Ct-DT eventuele stammen zonder plasmide kunnen worden aangetoond, welke zijn beschreven in de literatuur.[19,20] Uit onze evaluatie bleek dat de Ct-DT een hele gevoelige test is, vergelijkbaar met de COBAS Amplicor CT/NG, en dat er inderdaad dubbelinfecties worden opgepikt. Er zijn geen Ct stammen zonder plasmide aangetoond in onze studie.

Hoewel binnen de medische microbiologie in sommige gevallen de tijd lijkt stil te staan, gaan anderzijds de ontwikkelingen heel hard. Een voorbeeld van dat laatste zijn de sneltesten of 'point-of-care' testen die meestal binnen een kwartier aan kunnen geven of iemand wel of niet een infectie heeft. In hoofdstuk 5 laten we de resultaten zien van de evaluatie van verschillende Ct sneltesten. Deze waren bij aanvang van de studie (deels) vrij verkrijgbaar via internet en hadden allemaal een CE-markering (Conformitée Européenne). Een goede sneltest zou een bijdrage kunnen leveren aan het verminderen van de verspreiding van Ct, omdat geïnfecteerde patiënten meteen behandeld kunnen worden. Tevens kan een sneltest overal worden uitgevoerd, ook op plekken waar bijvoorbeeld geen elektriciteit is, en dit zou dus een enorme impact kunnen hebben op de diagnostische mogelijkheden in ontwikkelingslanden waar nu geen Ct diagnostiek plaatsvindt. Hoewel niet geheel onverwacht, waren de resultaten helaas dramatisch slecht: de gevoeligheid van de Ct sneltesten liep uiteen tussen de 12% en 27%. Volgens de WHO moet een sneltest minimaal een gevoeligheid hebben van 43% om toegevoegde waarde te hebben in een klinische setting. Tot nu toe is er geen overtuigend bewijs dat er een Ct sneltest is die hier aan voldoet.[21-24]

In hoofdstuk 6 wordt de evaluatie van een syfilis sneltest en een aantal 'enzyme-linked immunoassays' (ELISA) geëvalueerd. Al deze testen detecteren antilichamen tegen Tp en kunnen geen onderscheid maken tussen een actieve, een latente of een reeds behandelde syfilis infectie. Daarvoor is aanvullende diagnostiek vereist (e.g. 'non-treponemale' testen). De sneltest bleek een gevoeligheid van 92% en een specificiteit van 79% en voldoet daarmee niet aan de criteria die de WHO stelt (namelijk een specificiteit van minimaal 93%). In de praktijk bleek het resultaat van de sneltest ook soms moeilijk te interpreteren: er werd enkel een dubieus streepje gezien en geen duidelijke lijn. De ELISA kwamen er in de evaluatie goed uit ten opzichte van de agglutinatie test die voorheen werd gedaan. Het voordeel van een ELISA is de mogelijkheid om deze uit te voeren op een apparaat, hetgeen minder arbeidsintensief en foutgevoelig is dan het handmatig inzetten van een test.

De sera die zijn gebruikt voor de studie beschreven in hoofdstuk 6 komen uit een biobank: een collectie vriezers waarin allerlei patiëntenmateriaal en micro-organismen kunnen worden opgeslagen ten behoeve van wetenschappelijk onderzoek. Bij de evaluatie van een diagnostische test wordt er vaak gebruik gemaakt van opgeslagen materialen omdat dit makkelijker is dan prospectief materiaal te verzamelen. In de in hoofdstuk 6 beschreven evaluatie werd er gebruik gemaakt van sera van patiënten waarvan bekend was of ze wel of geen syfilis hadden (doorgemaakt) en van patiënten die geen syfilis hadden, maar wel een aandoening die zou kunnen storen in de syfilis diagnostiek, zoals de ziekte van Lyme, een HIV infectie of een autoimmuunaandoening. Door de gebruikte strategie, was het percentage syfilis positieve samples in de studie 44%, terwijl de prevalentie onder de Nederlandse bevolking slechts 0.2% is bij zwangeren, tot 2.3% bij MSM.[7]

Na implementatie van de Biolisa Syphilis 3.0 (Biokit SA, Barcelona, Spain), een van de ELISA geëvalueerd in hoofdstuk 6, werden er veel vals positieve resultaten gezien, terwijl dit niet werd gezien in de initiële evaluatie. Daarom is besloten om de verzamelde resultaten na implementatie van de nieuwe test, te vergelijken met de resultaten in de eerste evaluatie en de resultaten daarvan staan in hoofdstuk 7. Bij elke diagnostische test komen vals positieve resultaten voor, bijvoorbeeld door kruisreactiviteit. Het aantal vals positieve resultaten staat los van het aantal echt positieve resultaten ('true positives'). Dit betekent dat bij een lage prevalentie van een ziekte, zoals bij syfilis, de positief voorspellende waarde van een test over het algemeen lager is dan bij een test voor een aandoening die veel voorkomt. Wat onze studie liet zien, was dat de specifiteit van de test die we geïmplementeerd hadden heel stabiel was bij verschillende aannames en bij verschillende patiëntengroepen. De relatief lagere positief voorspellende waarde gevonden bij de herevaluatie is dus inderdaad te wijten aan een lagere syfilis prevalentie in de geteste populatie. De eerder gevonden specificiteit van de test werd bevestigd.

Hoofdstuk 8 behandelt de stabiliteit van Ct DNA. Zoals aangegeven in de voorgaande alinea wordt er bij de evaluatie van diagnostische testen vaak gebruikt gemaakt van materiaal uit een biobank. Met betrekking tot *Chlamydia* spp. is er in het verleden enkel gekeken naar de levensvatbaarheid van het micro organisme (e.g. de mogelijkheid tot opkweken)[25-28], het is echter niet bekend hoe stabiel Ct DNA is als het opgeslagen wordt onder verschillende omstandigheden. Dit kan echter wel de uitkomst van een evaluatie van een NAAT bepalen indien een nieuwe test op reeds langdurig ingevroren materiaal wordt uitgevoerd en wordt vergeleken met een NAAT die op 'vers' materiaal gedaan is. In onze studie opzet bleek dat Ct relatief stabiel in bij verschillende bewaarcondities en voor langere duur (2 jaar). Desalniettemin kan niet uitgesloten worden dat materialen met een lage hoeveelheid Ct DNA na langer bewaren negatief worden, terwijl initieel positief getest.

De bestrijding van SOA is volgens het Centers for Disease Control and Prevention (CDC) gebaseerd op 5 pijlers: educatie en counseling van risicopopulaties om risicogedrag te voorkomen; identificatie van (asymptomatisch) geïnfecteerde personen; adequate diagnostiek en behandeling van SOA; partnerwaarschuwing, behandeling en counseling; vaccinatie van populaties tegen SOA die met vaccinatie voorkomen kunnen worden.[29] T.a.v. syfilis is de grootste uitdaging het bereiken van de populatie die het meeste risico loopt, m.n. in ontwikkelingslanden.[1,2] Bij Ct is het probleem meer complex. Ondanks alle inspanningen stijgt de prevalentie van Ct in Westerse landen, waaronder in Nederland.[7] Het blijkt dat de mensen die het meeste risico lopen op Ct, het moeilijkst te bereiken zijn voor de gezondheidszorg en dat actief screenen op Ct geen effect heeft op de prevalentie.[30,31] Desondanks blijft het belangrijk om de populatie 'at risk' te identificeren, te testen op SOA en te behandelen.

Ondanks dat er reeds heel veel bekend is Ct, is er nog veel wat niet bekend is. Waarom krijgen sommige vrouwen bijvoorbeeld wel klachten en andere vrouwen niet? Het immuunsysteem speelt hierbij een grote rol en het verschil in klachten kan dus mogelijk verklaard worden op basis van ons (humaan) erfelijk materiaal.[32-34] Door 'whole genome sequencing', komen we ook steeds meer te weten over Ct, wat inzicht kan geven in de pathogenese en epidemiologie.[35] Ook de microbiële flora van het menselijk lichaam wordt steeds beter in kaart gebracht en de vraag is welke rol bijvoorbeeld het vaginale microbioom speelt bij een Ct infectie.[36] Naast deze zijn er nog tal van andere onbeantwoorde vragen. Goede diagnostische testen en de juiste interpretatie ervan zijn een belangrijk gereedschap om meer inzicht te krijgen in de verschillende infecties om ze daarmee uiteindelijk te kunnen bestrijden.

REFERENTIES

1. Global incidence and prevalence of selected curable sexually transmitted infections - 2008 World Health Organization, 2012.
2. Advancing MDG 4, 5 and 6: impact of congenital syphilis elimination: World Health Organization, 2010.
3. Carey AJ, Beagley KW. *Chlamydia trachomatis*, a hidden epidemic: effects on female reproduction and options for treatment. Am J Reprod Immunol 2010;63(6):576-86.
4. Markos AR. The concordance of *Chlamydia trachomatis* genital infection between sexual partners, in the era of nucleic acid testing. Sex Health 2005;2(1):23-4.
5. Rogers SM, Miller WC, Turner CF, Ellen J, Zenilman J, Rothman R, et al. Concordance of *chlamydia trachomatis* infections within sexual partnerships. Sex Transm Infect 2008;84(1):23-8.
6. Quinn TC, Gaydos C, Shepherd M, Bobo L, Hook EW, 3rd, Viscidi R, et al. Epidemiologic and microbiologic correlates of *Chlamydia trachomatis* infection in sexual partnerships. Jama 1996;276(21):1737-42.
7. S.C.M. Trienekens FDHK, I.V.F. van den Broek, H.J. Vriend, E.L.M. Op de Coul, M.G. van Veen, A.I. van Sighem, I. Stirbu-Wagner, M.A.B. van der Sande. Sexually transmitted infections, including HIV, in the Netherlands in 2011: National Institute for Public Health and the Environment, 2012.
8. van Bergen JE, Kerssens JJ, Schellevis FG, Sandfort TG, Coenen TJ, Bindels PJ. Prevalence of STI related consultations in general practice: results from the second Dutch National Survey of General Practice. Br J Gen Pract 2006;56(523):104-9.
9. Hoebe CJ, Rademaker CW, Brouwers EE, ter Waarbeek HL, van Bergen JE. Acceptability of self-taken vaginal swabs and first-catch urine samples for the diagnosis of urogenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with an amplified DNA assay in young women attending a public health sexually transmitted disease clinic. Sex Transm Dis 2006;33(8):491-5.
10. Schachter J, Chernesky MA, Willis DE, Fine PM, Martin DH, Fuller D, et al. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results from a multicenter evaluation of the APTIMA assays for both infections. Sex Transm Dis 2005;32(12):725-8.
11. Michel CE, Sonnex C, Carne CA, White JA, Magbanua JP, Nadala EC, Jr., et al. *Chlamydia trachomatis* load at matched anatomic sites: implications for screening strategies. J Clin Microbiol 2007;45(5):1395-402.
12. Skidmore S, Horner P, Herring A, Sell J, Paul I, Thomas J, et al. Vulvovaginal-swab or first-catch urine specimen to detect *Chlamydia trachomatis* in women in a community setting? J Clin Microbiol 2006;44(12):4389-94.
13. Shafer MA, Moncada J, Boyer CB, Betsinger K, Flinn SD, Schachter J. Comparing first-void urine specimens, self-collected vaginal swabs, and endocervical specimens to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by a nucleic acid amplification test. J Clin Microbiol 2003;41(9):4395-9.
14. Schoeman SA, Stewart CM, Booth RA, Smith SD, Wilcox MH, Wilson JD. Assessment of best single sample for finding *chlamydia* in women with and without symptoms: a diagnostic test study. Bmj 2012;345:e8013.
15. Blake DR, Maldeis N, Barnes MR, Hardick A, Quinn TC, Gaydos CA. Cost-effectiveness of screening strategies for *Chlamydia trachomatis* using cervical swabs, urine, and self-obtained vaginal swabs in a sexually transmitted disease clinic setting. Sex Transm Dis 2008;35(7):649-55.
16. Ripa T, Nilsson P. A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill 2006;11(11):E061109 2.
17. Fieser N, Simnacher U, Tausch Y, Werner-Belak S, Ladenburger-Strauss S, von Baum H, et al. *Chlamydia trachomatis* prevalence, genotype distribution and identification of the new Swedish variant in Southern Germany. Infection 2013;41(1):159-66.
18. Shipitsyna E, Hadad R, Ryzhkova O, Savicheva A, Domeika M, Unemo M. First reported case of the Swedish new variant of *Chlamydia trachomatis* (nvCT) in Eastern Europe (Russia), and evaluation of Russian nucleic acid amplification tests regarding their ability to detect nvCT. Acta Derm Venereol 2012;92(3):330-1.

19. An Q, Radcliffe G, Vassallo R, Buxton D, O'Brien WJ, Pelletier DA, et al. Infection with a plasmid-free variant *Chlamydia* related to *Chlamydia trachomatis* identified by using multiple assays for nucleic acid detection. J Clin Microbiol 1992;30(11):2814-21.
20. Magbanua JP, Goh BT, Michel CE, Aguirre-Andreasen A, Alexander S, Ushiro-Lumb I, et al. *Chlamydia trachomatis* variant not detected by plasmid based nucleic acid amplification tests: molecular characterisation and failure of single dose azithromycin. Sex Transm Infect 2007;83(4):339-43.
21. Saison F, Mahilum-Tapay L, Michel CE, Buttress ND, Nadala EC, Jr., Magbanua JP, et al. Prevalence of *Chlamydia trachomatis* infection among low- and high-risk Filipino women and performance of *Chlamydia* rapid tests in resource-limited settings. J Clin Microbiol 2007;45(12):4011-7.
22. Nadala EC, Goh BT, Magbanua JP, Barber P, Swain A, Alexander S, et al. Performance evaluation of a new rapid urine test for *chlamydia* in men: prospective cohort study. Bmj 2009;339:b2655.
23. Mahilum-Tapay L, Laitila V, Wawrzyniak JJ, Lee HH, Alexander S, Ison C, et al. New point of care *Chlamydia* Rapid Test--bridging the gap between diagnosis and treatment: performance evaluation study. Bmj 2007;335(7631):1190-4.
24. van der Helm JJ, Sabajo LO, Grunberg AW, Morre SA, Speksnijder AG, de Vries HJ. Point-of-care test for detection of urogenital *chlamydia* in women shows low sensitivity. A performance evaluation study in two clinics in Suriname. PLoS One 2012;7(2):e32122.
25. Eley A, Geary I, Bahador A, Hakimi H. Effect of storage temperature on survival of *Chlamydia trachomatis* after lyophilization. J Clin Microbiol 2006;44(7):2577-8.
26. Maass M, Dalhoff K. Transport and storage conditions for cultural recovery of *Chlamydia* pneumoniae. J Clin Microbiol 1995;33(7):1793-6.
27. Mahony JB, Chernesky MA. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. J Clin Microbiol 1985;22(5):865-7.
28. Tjiam KH, van Heijst BY, de Roo JC, de Beer A, van Joost T, Michel MF, et al. Survival of *Chlamydia trachomatis* in different transport media and at different temperatures: diagnostic implications. Br J Vener Dis 1984;60(2):92-4.
29. Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep 2010;59(RR-12):1-110.
30. Op de Coul EL, Gotz HM, van Bergen JE, Fennema JS, Hoebe CJ, Koekenbier RH, et al. Who participates in the Dutch *Chlamydia* screening? A study on demographic and behavioral correlates of participation and positivity. Sex Transm Dis 2012;39(2):97-103.
31. van den Broek IV, van Bergen JE, Brouwers EE, Fennema JS, Gotz HM, Hoebe CJ, et al. Effectiveness of yearly, register based screening for *chlamydia* in the Netherlands: controlled trial with randomised stepped wedge implementation. Bmj;345:e4316.
32. Agrawal T, Gupta R, Dutta R, Srivastava P, Bhengraj AR, Salhan S, et al. Protective or pathogenic immune response to genital *chlamydia*l infection in women--a possible role of cytokine secretion profile of cervical mucosal cells. Clin Immunol 2009;130(3):347-54.
33. Bailey RL, Natividad-Sancho A, Fowler A, Peeling RW, Mabey DC, Whittle HC, et al. Host genetic contribution to the cellular immune response to *Chlamydia trachomatis*: Heritability estimate from a Gambian twin study. Drugs Today (Barc) 2009;45 Suppl B:45-50.
34. Morre SA, Karimi O, Ouburg S. *Chlamydia trachomatis*: identification of susceptibility markers for ocular and sexually transmitted infection by immunogenetics. FEMS Immunol Med Microbiol 2009;55(2):140-53.
35. Harris SR, Clarke IN, Seth-Smith HM, Solomon AW, Cutcliffe LT, Marsh P, et al. Whole-genome analysis of diverse *Chlamydia trachomatis* strains identifies phylogenetic relationships masked by current clinical typing. Nat Genet 2012;44(4):413-9, S1.
36. Srinivasan S, Hoffman NG, Morgan MT, Matsen FA, Fiedler TL, Hall RW, et al. Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. PLoS One 2012;7(6):e37818.

Addendum

11. Dankwoord

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AFDELING MEDISCHE MICROBIOLOGIE MUMC

AIOS, analisten & alle andere medewerkers

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VRIENDEN

Hopelijk vanaf nu meer tijd!

Na een paar hele drukke jaren komt er nu hopelijk iets meer lucht en dus tijd voor leuke dingen! En daar gaan we nu meteen mee beginnen! Dank voor al jullie bemoedigende woorden en heerlijke momenten als er wel tijd was voor ontspanning. Ondanks de lage kwantiteit is er de afgelopen jaren geen gebrek geweest aan kwaliteit! Een paar mensen wil ik in het bijzonder noemen. Mascha, onze eerste ontmoeting beloofde veel goeds en het gevoel van toen heeft ons nooit meer losgelaten. Waar je ook bent vandaag, je bent er sowieso bij voor mij! Dorothee, jouw doorzettingsvermogen is indrukwekkend en een inspiratie, maar daarbij ben je natuurlijk een vooral een fantastische partner op de dansvloer hihihi. Birgit, we kennen elkaar inmiddels alweer vijftien jaar en weten elkaar altijd te vinden. Voor jou reis ik graag weer af naar het zuiden! Elske, ik zie je natuurlijk veel te weinig, maar jouw rust, vrolijkheid en humor maakt het altijd weer heerlijk om bij je te zijn! Janneke, Marlinde en alle andere dames van Allicht, samen sta je sterker! Haagse partypeople, er verandert veel, maar met jullie blijft het gelukkig altijd hetzelfde!

FAMILIE

Sjoerd, papa, mama, oma & familie Cappendijk/Jacobs

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Vincent, Willem en Stella

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Met jullie is het leven fantastisch!

12. Co-authors (in alphabetical order)

Brink, A.A.T.P. PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands. Currently: Pathofinders BV, Maastricht, The Netherlands

Brouwers, E.E.H.G. MSc - Public Health Service South Limburg, Geleen, The Netherlands

Bruggeman, C.A. Prof PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

Catsburg, A. – Previously Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands. Currently: Microbiome Ltd, Houten, The Netherlands.

Damoiseaux, J. MD PhD – Department of Immunology, Maastricht University Medical Centre, The Netherlands

Domeika, M - Uppsala University, Uppsala, Sweden

Dukers-Muijrers, N. MD PhD – Public Health Service South Limburg, Geleen, The Netherlands; Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

Goossens, V.J. MD PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

Herrmann, B - Section of Clinical Bacteriology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Herngreen, S.B. – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

Hoebe, C.J.P.A. Prof MD PhD – Public Health Service South Limburg, Geleen, The Netherlands; Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

van Loo, I.H.M. MD PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

Morré, S.A. Prof PhD- Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands; Institute of Public Health Genomics, Department of Genetics and Cell Biology, Maastricht University Medical Centre, The Netherlands

Nilsson A. - Section of Clinical Bacteriology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.

Sander Ouburg, PhD - Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands

Quint, W.G.V. MD PhD - DDL Diagnostic Laboratory, Rijswijk, The Netherlands

Savelkoul, P.H.M. Prof PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands; Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands

Alevtina Savitcheva - D.O. Ott Research Institute of Obstetrics and Gynaecology, St. Petersburg, Russia

Smelov, V. MD - Department of Urology and Andrology, North-Western State Medical University named after I.I. Mechnikov, St. Petersburg, Russia; St. Petersburg State University Outpatient Clinic, St. Petersburg, Russia; Family Planning Center, Pushkin, St. Petersburg, Russia; Outpatient clinic, D.O. Ott Research Institute of Obstetrics and Gynaecology, St. Petersburg; STI clinic "ImmunoBioServis", St. Petersburg, Russia;

Smismans, A. MD PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands. Currently: Klinisch laboratorium, Imelda ziekenhuis, Bonheiden, Belgium

Terporten, P.H.W. – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

Thijs, C MD PhD – Department of Epidemiology, Maastricht University Medical Centre, The Netherlands

van Tiel, F.H. MD PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

de Vries, H.J.C. Prof MD PhD – Epidemiology & Surveillance Department, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM), Bilthoven; Cluster of Infectious Diseases, Public Health Service Amsterdam; Department of Dermatology, Academic Medical Center, Amsterdam, the Netherlands.

Wolffs P.F. PhD – Department of Medical Microbiology, Maastricht University Medical Centre, The Netherlands

13. About the author

Laura van Dommelen was born on November 24th 1978 in the St. Joseph hospital in Eindhoven. After moving to Woudenberg, she attended the Herman Jordan Lyceum in Zeist and finished secondary school in 1997. Because of her interest in tropical medicine, she wanted to attend medical school, but due to pre-selection, she had to wait one year, in which she travelled to Ghana for a wildlife preservation project among other things.

In 1998 she started at the Maastricht University to get her medical degree. During her study, she was active within a sorority ('Allicht'), participated and organised an extra-curricular tropical medicine course, was active in the association for medical students ('Ko-beraad') and did several of her internships abroad (Indonesia, Surinam, Mexico). She always had affinity with microscopy and infectious diseases and therefore choose to do her last internship at the department of medical microbiology at the Maastricht University Medical Centre.

Without any hesitation, she started her residency to become a medical microbiologist. After her registration, she started to work as an all-round medical microbiologist at the Laboratory for Pathology and Medical Microbiology (PAMM) in Veldhoven. Hopefully when you read this paragraph, she has just moved to Sint Michielsgestel with her partner Vincent and their children Willem and Stella.

14. List of (peer reviewed) Publications

1. van Dommelen L, Wolffs PF, van Tiel FH, Dukers N, Herngreen SB, Bruggeman CA, Hoebe CJ. Influence of temperature, medium, and storage duration on *Chlamydia trachomatis* DNA detection by PCR. J Clin Microbiol. 2013 Mar;51(3):990-2

2. Martens RJ, van Dommelen L, Nijziel MR. Fever and back pain. Neth J Med. 2012 Dec;70(10):465, 470

3. van Dommelen L, Stoot JH, Cappendijk VC, Abdul Hamid MA, Stelma FF, Kortbeek LM, van der Giessen J, Oude Lashof AM. The first locally acquired human infection of Echinococcus multilocularis in The Netherlands. J Clin Microbiol. 2012 May;50(5):1818-20

4. van Dommelen L, Dukers-Muijrers N, van Tiel FH, Brouwers EE, Hoebe CJ. Evaluation of one-sample testing of self-obtained vaginal swabs and first catch urine samples separately and in combination for the detection of *Chlamydia trachomatis* by two amplified DNA assays in women visiting a sexually transmitted disease clinic. Sex Transm Dis. 2011 Jun;38(6):533-5

5. van Dommelen L, van Tiel FH, Ouburg S, Brouwers EE, Terporten PH, Savelkoul PH, Morré SA, Bruggeman CA, Hoebe CJ. Alarmingly poor performance in *Chlamydia trachomatis* point-of-care testing. Sex Transm Infect. 2010 Oct;86(5):355-9

6. van Dommelen L, Verbon A, van Doorn HR, Goossens VJ. Acute *hepatitis B virus* infection with simultaneous high HBsAg and high anti-HBs signals in a previously HBV vaccinated HIV-1 positive patient. J Clin Virol. 2010 Mar;47(3):293-6

7. van Dommelen L, Smismans A, Goossens VJ, Damoiseaux J, Bruggeman CA, van Tiel FH, Hoebe CJ. Evaluation of a rapid one-step immunochromatographic test and two immunoenzymatic assays for the detection of anti-*Treponema pallidum* antibodies. Sex Transm Infect. 2008 Aug;84(4):292-6

8. Catsburg A, van Dommelen L, Smelov V, de Vries HJ, Savitcheva A, Domeika M, Herrmann B, Ouburg S, Hoebe CJ, Nilsson A, Savelkoul PH, Morré SA. TaqMan assay for Swedish *Chlamydia trachomatis* variant. Emerg Infect Dis. 2007 Sep;13(9):1432-4

COLOFON

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Someone once said: Assumptions are the mother of all mistakes ...

In clinical practice, lots of things are assumed every day, partly based on clinical experience.
In this thesis, several assumptions in STI diagnostics were given a closer look. Is SVS the best sample to be tested for Ct in women or could this be improved by adding urine to detect urinary-tract-only infections more effectively? Is Ct DNA stable when frozen or does storage effect test results after thawing? And does a test evaluation using a selected sample set give reliable results which can be used in clinical practice?

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Laura van Dommelen
lauravandommelen@yahoo.com

Optimizing *Chlamydia trachomatis* and *Treponema pallidum* diagnostics