# Why do we need quality-assured diagnostic tests for sexually transmitted infections?

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Abstract | The bacterial sexually transmitted infections (STIs) syphilis, gonorrhoea and chlamydia can all be cured with a single dose of antibiotic. Unfortunately, however, these infections often remain undiagnosed as many infected individuals have few if any symptoms. Diagnostic tests with high sensitivity and specificity are available for all three infections but, owing to their expense and the lack of laboratory capacity, most people in developing countries do not have access to these tests. There is a great need for simple, cheap diagnostic tests for STIs that can be performed at the point of care, enabling treatment to be given immediately. It is hoped that recent advances in our understanding of the pathogenesis of these infections, and the availability of the complete genome sequences for each causative organism, will lead to the development of improved point-of-care tests that will reduce the burden of these diseases in developing countries.

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Bacterial sexually transmitted infections (STIs) cause a huge burden of morbidity and mortality that is borne disproportionately by women and infants[1] (FIG. 1). Syphilis causes 30–50% of stillbirths in Africa and kills more than 1 million babies each year worldwide[2,3]. Gonorrhoea (caused by *Neisseria gonorrhoeae*) and genital chlamydia (caused by *Chlamydia trachomatis*) are the main causes of pelvic inflammatory disease, tubal infertility and ectopic pregnancy in women in developing countries. In some African countries, gonococcal ophthalmia affects up to 3% of babies born to infected mothers and is a preventable cause of blindness[4].

Bacterial STIs also facilitate the transmission of HIV[5,6]. Infections such as gonorrhoea and chlamydia increase the shedding of HIV in genital secretions, thereby increasing the infectivity of the virus. Ulcerative STIs, such as syphilis, increase both the infectiousness of HIV and susceptibility to the virus[7,8]. The effect of STIs in enhancing HIV transmission is particularly important in early or concentrated HIV epidemics when the infection is concentrated in high-risk groups, such as sex workers and intravenous drug users, and when most new infections are acquired from a casual partner[9].

Syphilis, gonorrhoea and chlamydia can all be cured with only a single dose of antibiotic, and early treatment prevents complications and stops transmission of the infection. Diagnostic tests are a crucial element of control programmes for these and other STIs. In this review, we outline the clinical manifestations of these three bacterial STIs and how they are diagnosed. We also discuss the pathogenesis of the causative organisms, and the potential for developing improved diagnostics.

## Clinical signs and treatment

***Syphilis.*** The causative organism of syphilis is *Treponema pallidum*. The biology and pathogenesis of *T. pallidum* are discussed in BOX 1. Syphilis is a chronic illness that spans many years and that can be divided into three stages — primary, secondary and tertiary[10–12] (TABLE 1). Primary syphilis is characterized by the appearance of a single painless lesion (the chancre) at the site of inoculation approximately 21 days after infection. The development of multiple primary lesions is less common. Although the primary lesion can occur at any site of exposure to the bacterium, it most often occurs on the genitalia and can be accompanied by regional inflammation of the lymph nodes. The lesion typically resolves spontaneously even without treatment. Resolution of primary syphilis is followed on average 6–8 weeks later by secondary syphilis, and this stage most commonly affects non-genital cutaneous and mucosal sites, although clinical manifestations can occur in virtually any anatomical location or organ. Similar to the primary lesion, secondary lesions also resolve without treatment. The infection then enters a

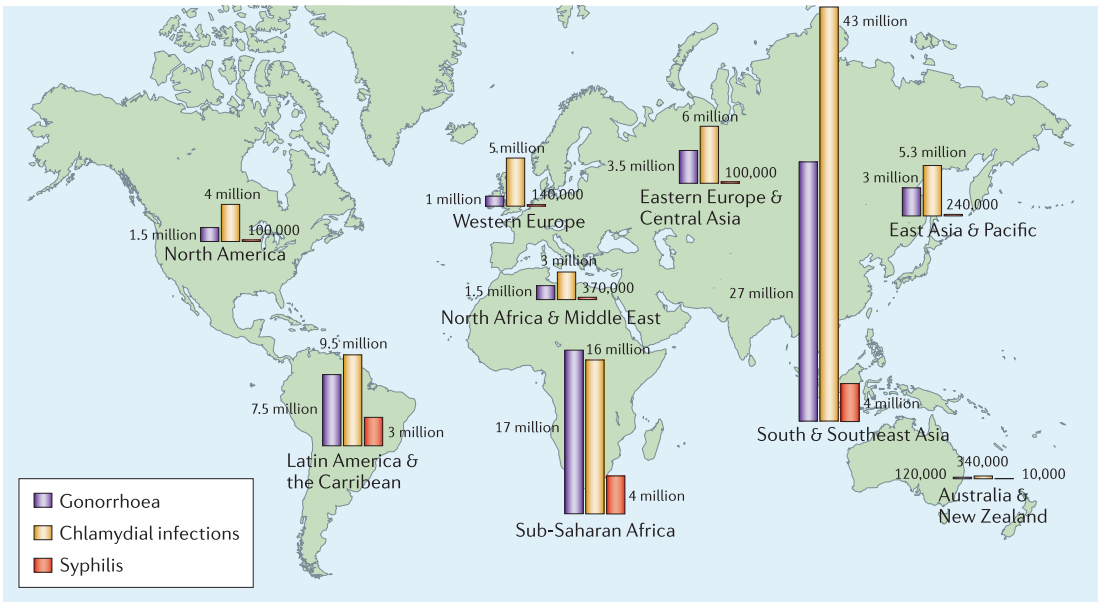



Figure 1 | **Estimated new cases of curable sexually transmitted infections among adults.** The map shows the estimated new cases of gonorrhoea, chlamydia and syphilis worldwide in 1999. Data taken from REF. 1.

latent stage during which there are no clinical symptoms or signs. The sexual transmission of syphilis occurs following contact with infectious lesions during the primary and secondary stages. By contrast, congenital transmission can occur at any stage of infection, including the latent stage.

Many years after the resolution of the primary and secondary stages, a minority of individuals with latent syphilis progress to late (or tertiary) syphilis. Tertiary syphilis causes neurological deficits (known as neurosyphilis), cardiovascular disease and lesions of the skin, bones or viscera (known as gummata). Studies in the pre-antibiotic era showed that 15–40% of untreated infected individuals develop recognizable late-stage complications[12,13].

*T. pallidum* can cross the placenta and infect the fetus, which can lead to stillbirth, or congenital infection that results in neonatal death or life-long sequelae[14–19]. Congenital syphilis is a catastrophic but readily preventable manifestation of the failure of syphilis-control programmes. Almost all pregnancies that occur during maternal primary and secondary syphilis lead to fetal infection, with approximately 30–50% of those pregnancies resulting in fetal death *in utero*, stillbirth or death shortly after delivery[20]. In one study of 24 pregnant women with syphilis, 66% of fetuses either contracted congenital syphilis or *T. pallidum* was detected in the amniotic fluid[21]. The rate of fetal infection was 50%, 67% and 83% for maternal primary, secondary and early latent infection, respectively. The longer the duration of the infection preceding pregnancy, the better the chance of fetal survival and, over the natural course of maternal syphilis, fetal-infection rates progressively decreased, reaching 10% in late latency[20,22].

Since its discovery, penicillin has been the mainstay of treatment for syphilis. Penicillin hastens the resolution of the clinical manifestations in primary and secondary syphilis, prevents or halts the progression of the tertiary stages, and prevents the infection from being transmitted. As *T. pallidum* has a relatively long generation time (30–33 hours), long-acting penicillin preparations such as benzathine penicillin G are the preferred therapy. Unlike many other pathogenic bacteria, at present there is no evidence that *T. pallidum* has developed resistance to penicillin. There is preliminary evidence that a 2 g dose of azithromycin can be used as an oral alternative to injection with penicillin[23].

A recent study has confirmed that treatment with a single dose of benzathine penicillin G is effective in preventing adverse pregnancy outcomes in maternal syphilis[24]. There was a significant association between mothers with a high non-treponemal antibody titre, which is a manifestation of active untreated syphilis, and stillbirth, low birth weight, pre-term birth and intrauterine growth retardation. Another study of 43 women treated for syphilis showed that treatment failure associated with the delivery of a congenitally infected baby correlated with a high maternal non-treponemal antibody titre at treatment and delivery, an early maternal stage of syphilis, and a period between syphilis treatment and delivery of less than 30 days[25].

***Syphilis and HIV.*** In common with other ulcerative STIs, syphilis facilitates the sexual transmission of HIV[5]. One cohort study indicated that primary and secondary syphilis increase HIV viral load and lead to a fall in the $CD4^+$ T-cell count in HIV-positive individuals[8]. Case reports have indicated that syphilis might be more severe, and progress more rapidly to the secondary and tertiary stages with neurological involvement, in HIV-positive individuals, but this hypothesis has not been supported by larger studies[26]. The response to standard penicillin treatment has been shown to be similar in HIV-positive and HIV-negative patients, although HIV infection might affect the serological response to treatment[27].

Box 1 | **The biology and pathogenesis of *Treponema pallidum***

**Biology**

*Treponema pallidum* is a helical, microaerophilic bacterium that measures 6–20 μm by 0.1–0.18 μm (see the figure). It comprises a central protoplasmic cylinder bound by a cytoplasmic membrane, an overlying layer of peptidoglycan and an outer membrane. It moves using two or three flagella, which are found at each end of the organism. The outer membrane of *T. pallidum* does not contain lipopolysaccharide and has few surface-exposed transmembrane proteins. The lack of outer membrane immune targets has led to *T. pallidum* being called a 'stealth pathogen'[80–82].

**Pathogenesis**

The study of *T. pallidum* pathogenesis has been hindered by the fact that the organism cannot be cultured for sustained periods using artificial media. Although several animal models for syphilis have been described, rabbit models most closely resemble human primary infection and the pathogenesis of disseminated infection, however late-stage manifestations have not been documented in any animal model[83]. The *T. pallidum* genome has been sequenced and comprises a circular chromosome of 1.14 Mb that contains 1,041 open reading frames[84]. This small genome strengthens the assumption that *T. pallidum* relies on host biosynthetic pathways for many of its metabolic needs.

Although not much is known about the membrane proteins of *T. pallidum*, these proteins have the potential to be virulence determinants and at least one has been shown to be a porin[85]. Recent studies have identified a family of *T. pallidum* repeat genes, the *tpr* genes, which encode proteins that are homologous to the major surface proteins of *Treponema denticola*, and that mediate attachment to host tissues and function as porins[86]. The Tpr proteins are immunogenic in rabbits and TprK has been shown to be a target for opsonic antibodies. Activated macrophages can phagocytose opsonized treponemes and clear them from the circulation. In a rabbit model, antibodies to variable TprK regions confer protection against homologous, but not heterologous, strains[87,88]. With successive passage, diversity is seen in the *tprK* genes[89]. Antigenic variation through gene conversion in infection has been hypothesized to be another mechanism by which the organism avoids the host immune response, allowing for prolonged infection and persistence.

**Immune response**

*T. pallidum* infection provokes a strong humoral and cell-mediated immune response early in the course of infection. Despite this, without treatment, *T. pallidum* can survive in the human host for several decades and continues to be transmitted or eventually causes organ damage.

Although patients who have been previously treated for syphilis can be re-infected, untreated patients seem to have some immunity to repeated infection. In the 19th century, Colles observed that 'wet nurses' who breastfed infants with congenital syphilis often developed chancres of the nipple, whereas the mothers of the infected infants did not, implying that they were somehow protected from repeated infection. This concept became known as Colles' law.

At all stages of the disease, syphilitic lesions are characterized by pathological vascular changes and local cellular infiltrates that consist of lymphocytes, macrophages and plasma cells. The importance of the cellular immune response in containing the infection, as well as in pathogenesis, is shown by the presence of granulomata that, in the case of gummatous disease, are necrotising. In primary chancres, $CD4^+$ T cells and macrophages predominate, whereas in secondary syphilitic lesions, $CD8^+$ T cells predominate. In lesions of both primary and secondary syphilis, increased expression of the T-helper-1 ($T_H1$) cytokines interleukin-2 (IL-2) and interferon-γ (IFNγ) is seen in both humans and in the rabbit model[82,90,91]. This cell-mediated response peaks in the secondary stage. Increased apoptosis of peripheral blood lymphocytes and $CD4^+$ T cells by the Fas-mediated death pathway in patients with early secondary syphilis might account for the incomplete clearance of *T. pallidum* from lesions, leading to the establishment of chronic infection[92].

**Syphilis and pregnancy**

Evidence from human infection and animal studies indicates that primary syphilis induces a $T_H1$ response in the host, with a gradual shift to a $T_H2$ response as the disease progresses to the secondary stage[93]. The increased production of the inflammatory cytokines IL-2, IFNγ, tumour necrosis factor-α and prostaglandins induced by maternal infection, together with the intense inflammatory responses associated with the activation of macrophages by treponemal lipoproteins, might be responsible for fetal death or pre-term delivery in primary or early secondary syphilis, and for severe growth retardation or some of the other neonatal manifestations of congenital syphilis. Image courtesy of Joyce Ayers, Centers for Disease Control and Prevention.

***Gonorrhoea and chlamydia.*** The clinical manifestations of infection with *C. trachomatis* and *N. gonorrhoeae* are similar. The biology and pathogenesis of these organisms are discussed in BOX 2 and BOX 3. Although there is a considerable degree of overlap, which makes it impossible to identify the causative organism on clinical grounds, the symptoms and signs of *C. trachomatis* infection are usually milder than those of *N. gonorrhoeae* infection. The main clinical manifestations of each infection in men, women and infants, and their complications, are shown in TABLE 2. Both infections are often asymptomatic, especially in women.

In men, dysuria and urethral discharge are the usual presenting symptoms. Complications include

Table 1 | **The clinical manifestations of syphilis**

| Stage of syphilis | Clinical manifestations |
|---|---|
| ***Non-congenital syphilis*** | |
| Primary | Chancre, regional lymphadenopathy |
| Secondary | Maculopapular rash, generalized lymphadenopathy, mucous patches, condylomata lata, ocular, hepatic or neurological involvement |
| Latent | Asymptomatic |
| Tertiary | Cardiovascular, neurological disease, gummata |
| ***Congenital syphilis*** | |
| Early | Fulminant disseminated infection, mucocutaneous lesions, anaemia, hepatosplenomegaly, neurosyphilis |
| Late | Interstitial keratitis, lymphadenopathy, hepatosplenomegaly, bone involvement, condylomata lata, anaemia, Hutchinson's teeth, arthropathy, neurosyphilis |
| ***With HIV co-infection*** | Multiple persistent chancres, ocular involvement and neurosyphilis more common, rapid progression to gummatous disease characterized by lesions of skin, bone and viscera |

epididymitis and urethral stricture, although stricture is rare except in individuals with long-standing untreated infections. Patients, especially men having sex with men, can present with proctitis caused by either organism. This can be severe when infection is caused by the more invasive lymphogranuloma venereum (LGV) strains of *C. trachomatis*.

In women, both organisms primarily infect the endocervix, as the vagina is too acidic to support their replication. Cervical infections are often asymptomatic, but a mucopurulent endocervical discharge can be seen on speculum examination. The main complications are caused by ascending infection of the endometrium and fallopian tubes, which leads to pelvic inflammatory disease. This can cause lower abdominal pain and dyspareunia (pain on intercourse) and, in severe cases, peritonitis. The involvement of the liver capsule can cause perihepatitis, which can present with chronic pain in the right upper quadrant. Ascending infection is more common following cervical trauma, for example as occurs during termination of pregnancy, insertion of an intra-uterine contraceptive device or following delivery. Infection can permanently damage the fallopian tubes, resulting in infertility or ectopic pregnancy. Although uncommon, in both sexes, *N. gonorrhoeae* can cause disseminated infection with involvement of the skin and joints, and *C. trachomatis* infection can cause a reactive arthritis.

Approximately one-third of infants born through an infected cervix will develop purulent conjunctivitis within a few days of birth (known as ophthalmia neonatorum). This is usually more severe when it is caused by *N. gonorrhoeae* than by *C. trachomatis*. Gonococcal ophthalmia can rapidly lead to corneal ulceration or even perforation and hence to blindness. *C. trachomatis* can cause pneumonitis in infected infants, which presents in the first 3 months of life with a characteristic 'staccato' cough.

*N. gonorrhoeae*, unlike *C. trachomatis*, has become resistant to many commonly used antibiotics, including penicillins, tetracycline and, more recently, quinolones. Resistance tends to spread rapidly once it is introduced into a population. Currently, cephalosporins are used for treatment in most parts of the world and high-level resistance of *N. gonorrhoeae* to this antibiotic has not yet arisen. Should high-level resistance to cephalosporins arise, treatment of gonorrhoea would once again become problematic. Worryingly a multiple-drug-resistant strain of *N. gonorrhoeae* with reduced susceptibility to ceftriaxone has been reported in Japan[28].

*C. trachomatis* can be treated with tetracyclines or macrolides given orally for at least 7 days or with a single oral dose of the long-acting azalide azithromycin. As patients infected with *N. gonorrhoeae* are often co-infected with *C. trachomatis*, presumptive treatment for chlamydia is usually given to those diagnosed with gonorrhoea.

## Diagnosis of STIs

The cornerstone of STI control is early diagnosis and treatment, including the treatment of sexual partners. This prevents complications in the treated individuals and also prevents further transmission. Unfortunately, STIs are often asymptomatic, and even in those with clinical signs and symptoms an STI might not be diagnosed. Asymptomatic or undiagnosed infections can only be detected by using screening tests or through notification by an infected partner. It is estimated that 90% of new STI cases occur in developing countries where access to STI laboratory services is limited or absent. Even in settings with access to laboratory-based testing, some patients might find it difficult to return for the results of laboratory tests or for treatment. Therefore, affordable rapid diagnostic tests (RDTs) and point of care (POC) tests that lead to immediate diagnosis and treatment are particularly valuable tools for STI control.

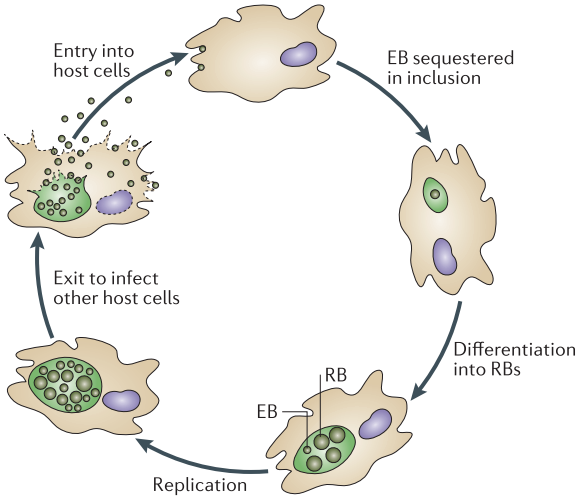
Where access to diagnostics is limited, the WHO has developed algorithms for syndromic case management, in which patients presenting with syndromes that are associated with common STIs are treated for all the probable causes of these syndromes. To be effective, the algorithms must be adapted to accommodate local patterns of disease and antimicrobial susceptibilities. Although syndromic management is inexpensive and is useful in all settings with a relatively modest amount of training, it can lead to unnecessary treatment in many cases, especially for one of the common presenting syndromes, vaginal discharge. Even for symptomatic patients, the use of appropriate diagnostic tests can increase the specificity of the syndromic management algorithms used and therefore reduce unnecessary treatment.

Syndromic management works better in men, in whom the syndrome of urethral discharge is reasonably specific for gonorrhoea[29,30]. By contrast, vaginal discharge is more commonly caused by bacterial vaginosis, candidiasis or infection with *Trichomonas vaginalis* than by infection with *C. trachomatis* or *N. gonorrhoeae*. This can result in unnecessary treatment in 60–98% of cases when syndromic management is used for women presenting with vaginal discharge[31–34]. The amount of

Box 2 | **The biology and pathogenesis of *Chlamydia trachomatis***

**Biology**

As an obligate intracellular organism, *Chlamydia trachomatis* can only replicate inside eukaryotic host cells. It has a unique developmental cycle, with metabolically inert, spore-like elementary bodies (EBs) that infect host cells and develop into metabolically active, replicative reticulate bodies (RBs) within a membrane-bound inclusion. RBs redifferentiate into EBs 24–48 hours after infection and the EBs are eventually released by lysis of the host cell (see the figure).




**Pathogenesis**

*C. trachomatis* infects epithelial cells in the eye and genital tract. The early stage of infection can present with a mucopurulent discharge but infections are often asymptomatic at this stage. In most infected women the infection resolves, but in those with persistent or repeated infections, the infection can spread upwards from the endocervix to the fallopian tubes. Persistent infection eventually leads to fibrosis and scarring. In the eye, this leads to distortion of the eyelid margin, causing the lashes to turn inwards and rub against the cornea, a disease known as trachoma, which is a leading cause of preventable blindness. Similarly, genital chlamydial infections can cause scarring of the fallopian tubes, leading to infertility or ectopic pregnancy as a result of tubal occlusion by scar tissue. With the exception of the lymphogranuloma venereum (LGV) strains, which cause systemic illness and infect regional lymph nodes, *C. trachomatis* infection usually remains confined to mucosal surfaces.

Our understanding of chlamydial pathogenesis has been hindered by the fact that, so far, it has not proved possible to manipulate the organism genetically. However, genome sequencing of several *C. trachomatis* strains has led to significant advances. The genome of *C. trachomatis* serovar D has been sequenced[94]. At 1.04 Mb it encodes only 894 proteins. The genome contains genes that are homologous with those encoding virulence factors in other bacteria, including a cytotoxin gene and genes encoding components of a type III secretion pathway[94]. A conserved chlamydial protease, proteasome-like activity factor (Cpaf), is secreted into the host-cell cytoplasm, where it interferes with the assembly and surface expression of human leukocyte antigen molecules and inhibits apoptosis[95]. Some of the repeated genes revealed by genome sequencing almost certainly function as virulence determinants. A family of polymorphic membrane proteins (Pmps) with structures similar to bacterial autotransporters contains members that are thought to function as adhesins[96,97]. There is also a large group of related proteins (Inc proteins) that have been shown to be located in the inclusion membrane and probably regulate traffic between the inclusion and chlamydial cells.

The lack of a genetic system to investigate gene function in *C. trachomatis* has given considerable impetus to comparative genome analysis. The *C. trachomatis* serovar A genome has been sequenced recently and found to be 99.6% identical to the genome of *C. trachomatis* serovar D[98]. The 'ocular' serovars (serovars A, B, Ba and C) have been shown to differ from the 'genital' serovars (serovars D–K) in that they do not have a functional tryptophan-synthase gene, rendering them more sensitive to the inhibitory activity of interferon-γ (IFN-γ)[99]. Surprisingly, the LGV strains, which cause considerably more acute pathology than the ocular and genital serovars, have only a truncated form of the cytotoxin gene, and the explanation for the different tissue tropism and pathogenesis of LGV strains remains obscure[100].

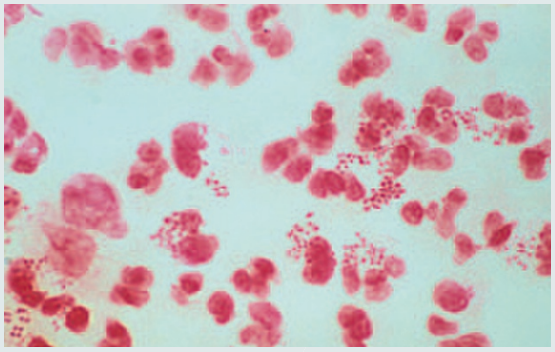
**Immune response**

The rapidly declining age-specific prevalence of ocular *C. trachomatis* infection in trachoma-endemic communities indicates that protective immunity follows infection. A study in The Gambia showed that the duration of untreated ocular infection is shorter in adults than in children[101]. Several trachoma-vaccine trials were conducted in the 1960s using killed whole-organism vaccines, some of which provided a degree of protection. At the same time, studies in primates indicated that vaccination could provoke more severe disease on subsequent challenge, suggesting that much of the damage caused by *C. trachomatis* was immunopathological[102]. A chlamydial heat-shock protein Hsp60, which is homologous to *Escherichia coli* GroEL, has been shown to elicit high antibody titres, which correlates with the severe sequelae of *C. trachomatis* infection in both the eye and genital tract[103,104]. *In vitro* studies have shown that IFN-γ interferes with the chlamydial developmental cycle, leading to persistent infection with continuing release of Hsp60 (REF. 105). However, it remains uncertain whether the immune response to Hsp60 is itself the cause of immunopathological damage, or merely a marker of more severe or prolonged infection.

Studies in trachoma-endemic communities indicate that, in humans, similar to mice, cell-mediated immune responses of the T-helper-1 type are important for the clearance of ocular *C. trachomatis* infection. Current studies are exploring the role of regulatory T cells in limiting the degree of inflammation[106,107]. Studies of host-gene expression in the conjunctival epithelium are starting to identify the molecular pathways that are involved in fibrosis, and matrix metalloproteinase-9 seems to have an important role[108]. Case-control studies have identified polymorphisms in several immune-response genes, which are associated with the severe scarring sequelae of trachoma[109,110].

Box 3 | **The biology and pathogenesis of *Neisseria gonorrhoeae***

**Biology**



*Neisseria gonorrhoeae* is a Gram-negative coccus (see the figure) that is found in pairs in biological samples and is therefore described as a diplococcus. It is a fastidious organism that grows best at 33–35°C on enriched media in an atmosphere of 5% $CO_2$. Unlike *Neisseria meningitidis*, *N. gonorrhoeae* does not have a polysaccharide capsule.

One of the most striking features of *N. gonorrhoeae* is the marked degree of variation between isolates. The source of this variation lies not only in genetic mutation but also in genetic exchange, which can include the exchange of genetic material with other neisserial species. Strains of *N. gonorrhoeae* are naturally competent for DNA uptake and can also exchange genetic material by conjugation. One practical result of this variation is that *N. gonorrhoeae* has proven particularly adept at developing antimicrobial resistance, and resistance genes are located on both plasmids and the bacterial chromosome.

**Pathogenesis**

*N. gonorrhoeae* infects epithelial surfaces in the human genital tract, including the urethra, endocervix and fallopian tubes. It also infects the conjunctiva, and infection can damage the cornea leading to blindness. Rarely, *N. gonorrhoeae* invades the bloodstream, causing disseminated infection. To persist in the male urethra and avoid being washed away by the passage of urine, *N. gonorrhoeae* has developed effective methods to adhere to epithelial cells and can also be found intracellularly[111]. Numerous polymorphonuclear leukocytes (PMNs) are attracted to the site of infection. Most gonococci taken up by PMNs are killed, but up to 2% can survive as they avoid oxidative killing by various mechanisms[111]. Tissue damage is caused by the host inflammatory response to two structural components of the organism: lipooligosaccharide (LOS) and peptidoglycan. Purified LOS and fragments of the cell wall or peptidoglycan can mimic the effects of gonococcal infection in fallopian tube organ cultures, notably in reducing the activity of ciliated epithelial cells. The lipid A portion of LOS seems to be responsible for this effect[112].

Recent studies indicate that different molecular mechanisms are used to initiate infection in different anatomical sites. In the male urethra and in the upper female genital tract, gonococcal interaction with epithelial cells triggers cytokine release and promotes the influx of PMNs. By contrast, gonococcal engagement of complement receptor-3 on epithelial cells of the cervix does not promote inflammation[113].

Our understanding of the pathogenesis of gonococcal infection has been hindered by the lack of a suitable animal model. Studies of experimental urethral infection in male volunteers and in fallopian tube organ cultures have contributed greatly to the field[114,115]. The pili and opacity (Opa) proteins have been shown to be important for the success of infection. Piliated strains and strains that express Opa proteins adhere better to human columnar-epithelial cells, and adherence can be blocked by monoclonal antibodies to these proteins[116,117]. Pili have also been shown to increase the adherence of *N. gonorrhoeae* to human PMNs and to increase resistance to phagocytosis and killing[118].

The genome of *N. gonorrhoeae* strain FA1090 has been sequenced and, although an overview of the genome is still to be published, the sequence has been made available on the internet for over a year (see the *N. gonorrhoeae* genome sequence link in Further Information). The genome is 2.15 Mb in size, and contains approximately 2,870 protein-coding genes, of which more than 60% have been assigned a function. Several research groups have exploited the availability of the genome sequence of *N. gonorrhoeae* strain FA1090 to investigate genetic systems known to be involved in virulence in other bacteria. However, it is clear that a comprehensive understanding of the molecular pathogenesis of a bacterium as genetically resourceful as *N. gonorrhoeae* will require a substantial long-term effort.

**Immune response**

Repeated infection with *N. gonorrhoeae* is common. Human volunteer studies have shown that vaccination with antigens from the pilus provides partial protection against urethral infection with a homologous strain, but no protection against heterologous challenge[119]. Several *N. gonorrhoeae* proteins, including the pilus and Opa proteins, undergo antigenic or phase variation, enabling them to escape the host immune response. There are several copies of the genes that encode the pilus proteins in the *N. gonorrhoeae* genome and these genes contain variable numbers of tandem repeats. Antigenic variation results from recombination events that move a different variant to the expression site[120]. In the case of the Opa proteins, antigenic and phase variation results from differences in the number of pentameric repeats immediately downstream of the AUG start codon, which results in regulation of expression at the level of translation rather than transcription[121]. *N. gonorrhoeae* expresses an IgA protease that inactivates both serum and secretory $IgA_1$, providing another possible mechanism of immune evasion, although experiments in human volunteers have shown that loss of IgA protease does not reduce infectivity[122]. Image courtesy of Norman Jacobs, Centers for Disease Control and Prevention.

Table 2 | **Clinical manifestations of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection**

| Patient group | Uncomplicated infection | Main complications |
|---|---|---|
| Adult males | Urethritis | Epididymitis, proctitis, urethral stricture, disseminated gonococcal infection, reactive arthritis |
| Adult females | Acute urethral syndrome, cervicitis | Pelvic inflammatory disease, tubal infertility, ectopic pregnancy, bartholinitis, perihepatitis |
| Infants | Ophthalmia neonatorum, chlamydial pneumonitis | Corneal ulceration/perforation, blindness |

unnecessary treatment is more pronounced in populations with a lower prevalence of *N. gonorrhoeae* and *C. trachomatis* infection[33,34].

The inappropriate use of antibiotics is a major concern as resistance to the most effective single-dose oral regimens for *N. gonorrhoeae* has become widespread[35]. Furthermore, if one considers the negative social connotations of being diagnosed with an STI, and the evidence that women have experienced violence following STI disclosure to their partners[36], this level of unnecessary treatment is unacceptable because of the possible psychosocial consequences. More specific tests are therefore important in these settings to reduce the amount of unnecessary treatment and to identify asymptomatic infections.

***Diagnosis of syphilis.*** Serological testing is the mainstay of laboratory diagnosis of syphilis as syphilitic lesions can be transient and most infected individuals are asymptomatic[37] (TABLE 3). The first serological test for syphilis, the Wasserman reaction, was described 100 years ago[38]. Non-treponemal tests detect antibodies to a non-treponemal cardiolipin antigen that is present in tissues and crossreacts reliably with *T. pallidum* antigens. Currently the most commonly used assay of this type is the rapid plasma reagin (RPR) test, a flocculation assay in which an antigen–antibody lattice that is formed by the reaction between patient antibodies and a test antigen is detected using carbon particles (FIG. 2). In the early stages of syphilis, in the presence of excess antibody a false-negative result can be obtained (this is called the prozone effect). The non-treponemal antigen detected by RPR and related tests (including the venereal disease research laboratory (VDRL) test and the toluidine red unheated serum test (TRUST)) are non-protein, T-cell-independent antigens that usually induce little if any immunological memory. Therefore, the anti-cardiolipin antibody titre falls when the disease is successfully treated and is typically low or absent in the quiescent, non-infectious latent phase of the disease. Therefore, quantitative non-treponemal tests can be used to monitor the effects of treatment. Unfortunately, diseases such as malaria and leprosy, and some autoimmune conditions or chronic tissue-damaging processes, can lead to the induction of anti-cardiolipin antibodies and can therefore cause false-positive results in non-treponemal serological

Table 3 | **Characteristics of serological tests for syphilis**

| Test characteristic | Non-treponemal tests | | Treponemal tests | | | |
|---|---|---|---|---|---|---|
| | RPR | VDRL | RDT | EIA | TPHA/TPPA | FTA-ABS |
| Specimen | Serum or plasma | Serum or plasma | Serum or whole blood | Serum or plasma | Serum or plasma | Serum or plasma |
| Sensitivity | 86–100% | 78–100% | 84–98% | 82–100% | 85–100% | 70–100% |
| Specificity | 93–98% | 98–100% | 94–98% | 97–100% | 98–100% | 94–100% |
| Ease of use | Easy | Easy | Easy | Moderate | Complex | Complex |
| Level of use | Examination room, on-site laboratory | Examination room, on-site laboratory | Examination room, on-site laboratory | Intermediate laboratory, reference laboratory | Reference laboratory | Reference laboratory |
| Equipment | Rotator, refrigerator | Light microscope, refrigerator | None | Incubator, microwell plate washer and reader | Incubator | Fluorescence microscope |
| Training | Minimal | Minimal | Minimal | Moderate | Extensive | Extensive |
| Average cost | US$ 0.5 | US$ 0.5 | US$ 0.19–3 | US$ 3 | US$ 3 | US$ 3 |
| Comments | Most RPR reagents require refrigeration | Reagents require refrigeration | Most tests can be stored at room temperature for 9–18 months | 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; RDT, rapid diagnostic test; RPR, rapid plasma reagin test; TPHA/TPPA, *Treponema pallidum* haemagglutination assay/*T. pallidum* particle agglutination assay; VDRL, venereal disease research laboratory test.

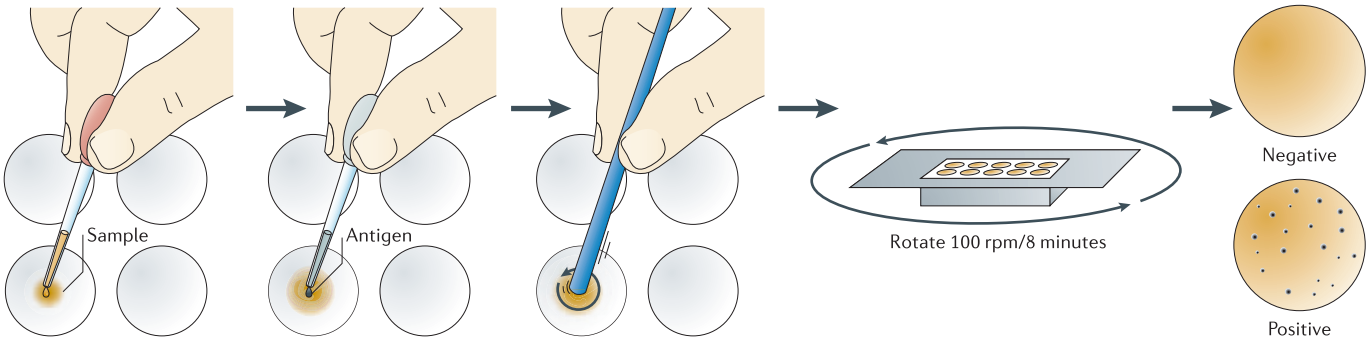



Figure 2 | **The rapid plasma reagin test.** The rapid plasma reagin test is the most commonly used non-treponemal test for the diagnosis of syphilis. The test takes the form of a flocculation assay in which a cardiolipin antigen and the patient's anti-cardiolipin antibodies form an antigen–antibody lattice, which can be visualised when carbon particles are trapped within it.

tests for syphilis. False-positive results are also common in pregnant women, intravenous drug users, people with chronic connective-tissue disease and in old age. More specific tests are therefore required for confirmation of syphilis diagnosis.

*Treponema*-specific tests, such as the *T. pallidum* haemagglutination assay (TPHA), the *T. pallidum* particle agglutination assay (TPPA) and the fluorescent treponemal antibody absorption (FTA-ABS) test are used as confirmatory assays. *Treponema*-specific tests usually remain positive for life even after successful treatment. As the non-treponemal and treponemal tests for syphilis are directed at different antigens, they are often used for syphilis diagnosis in a two-stage process in which non-treponemal tests are used first for screening, followed by 'confirmatory testing' using the treponemal tests to reduce the likelihood of false-positive serological diagnoses. As infected individuals usually retain anti-treponemal antibodies for life even after successful treatment, *Treponema*-specific tests cannot be used to monitor the efficacy of treatment or to distinguish between active and prior infections. Treponemal enzyme immunoassays are also commercially available. They are sensitive and specific and can be used for high-throughput screening[39].

Treponemal RDTs are available and can be used in settings where laboratory services and electricity supplies are unreliable or absent (TABLE 4; FIG. 3). In these circumstances, tests that can be performed using whole blood that does not require further processing are particularly useful. These RDTs have been shown to have reasonable performance characteristics, with sensitivities of 85–99% and specificities of 93–100% when compared with laboratory-based treponemal tests[40–49]. They are easy to use in any setting, and studies have shown that they can be introduced into peripheral settings where testing was not previously possible. The only drawback is that these tests are *Treponema*-specific and so they cannot be used to distinguish between prior treated infection and active infection, making them less useful in settings where a high proportion of infected individuals have been treated previously. Mathematical models developed at a site for prenatal screening in Tanzania showed that screening with RDTs is cost-effective if the price per test does not exceed US$ 0.63 (REF. 50). Currently, eight syphilis RDTs evaluated by the WHO Sexually Transmitted Diseases Diagnostics Initiative (SDI) are included in the WHO Bulk Procurement Scheme at negotiated prices ranging from US$ 0.19 to US$ 1 per test[40].

Table 4 | **Comparison of the rapid plasma reagin test versus the rapid test for syphilis diagnosis**

| | Test | |
|---|---|---|
| **Feature** | **RPR** | **Rapid test** |
| Sample required | Serum or plasma | Whole blood |
| Facilities required | Electricity to run refrigerator, centrifuge and rotator | No additional equipment required |
| Speed | Single test, 8 mins; samples often processed in batches so patients need to return for results | Single test, 15–20 mins; results available immediately to inform treatment |
| Sensitivity/specificity | Can get false-negative results (prozone effect) and false-positive results (owing to other tissue-damaging diseases) | No prozone effect, test is *Treponema* specific |
| Interpretation of results | Can distinguish between active and past infection | Cannot distinguish between active and past infection as once positive, remains positive for life |
| Access to testing | Restricted, as requires electricity and trained personnel | Could be rolled out to primary healthcare settings, as no electricity required; wider access |

RPR, rapid plasma reagin test.

Table 5 | **Performance and operational characteristics of diagnostic tests for chlamydia and gonorrhoea***

| | | | Complexity‡ | | | |
|---|---|---|---|---|---|---|
| **Test** | **Sensitivity§** | **Specificity§** | **Expertise** | **Equipment** | **Time** | **Relative cost‡** |
| Bacterial culture | 60–70%‖ | 99–100% | +++ | +++ | 48 hours | ++ |
| Microscopy (NG only) | Men: 84–95% Women: 50% | ≥95% | ++ | + | 1 hour | + |
| NAHT | 85–90% | 95–99% | +++ | +++ | 4 hours | +++ |
| NAAT | 90–95%¶ | 98–100% | ++++ | ++++ | 4 hours (longer to confirm) | ++++ |
| ***Antigen detection*** | | | | | | |
| EIA | 50–70% | 95–99% | ++ | ++ | 4 hours | ++ |
| RDT | ND | ND | + | None | 30 minutes | ND |

*Data from REFS 51–57, 61, 64, 123; ‡+ denotes minimal requirements for training, equipment and cost; ++++ denotes requirement for highly trained personnel, sophisticated equipment/laboratory facilities and high cost; §Test performance compared to a combined reference standard of bacterial culture or two NAATs; ‖Bacterial culture for NG is close to 100% sensitive under optimal conditions; ¶Sensitivity is lower for urine specimens[124]. EIA, enzyme immunosorbant assay; NAAT, nucleic-acid amplification test; NAHT, nucleic acid hybridization test; ND, not determined; NG, *Neisseria gonorrhoeae*; RDT, rapid diagnostic test.

***Diagnosis of genital gonococcal and chlamydial infection.*** A wide range of tests are available for the laboratory diagnosis of genital gonococcal and chlamydial infection (TABLE 5; FIG. 4). The mean performance characteristics based on published studies, the level of complexity required in terms of training and equipment, the time required to obtain test results and the relative cost of testing are discussed in REFS 51–61.

The reference standard for the laboratory diagnosis of gonococcal infection remains bacterial culture using selective solid media such as modified Thayer-Martin media. Under ideal conditions, bacterial culture has the highest sensitivity and specificity, but its performance is often affected by transport conditions[51,56] and its specificity is lower if confirmation using biochemical tests such as sugar-fermentation tests is not carried out. In men and in infants with ophthalmia neonatorum, microscopy of Gram-stained smears to identify gonococci in urethral or eye swabs is 84–95% sensitive compared with bacterial culture; but in women, microscopy of a smear from a cervical swab detects only ~50% of infections. One advantage of bacterial culture is that antimicrobial-susceptibility tests can be performed to ensure appropriate and effective treatment.

Antigen-detection tests using enzyme immunoassays have been shown to have reasonable specificity, but inadequate sensitivity[51,61]. Nucleic-acid amplification tests (NAATs) usually have good sensitivity compared with culture and antigen-detection tests, but as a result of published reports of crossreactivity with other *Neisseria* species, such as *Neisseria subflava* and *Neisseria cinerea*, the results of some NAATs require confirmation[62,63]. Both laboratory-based enzyme immunoassays and NAATs can be batched for large-scale screening. Also, the high sensitivity of NAATs allows the use of non-invasive specimens such as urine, or minimally invasive specimens such as vaginal swabs, which can be self-collected. The ease of specimen collection outside of traditional clinic settings and the high sensitivity and specificity of NAATs make these tests a useful method for increasing access to screening. The drawbacks are their high cost and the requirement for a sophisticated laboratory and highly trained personnel to perform the tests to avoid false-positive results owing to contamination.

The reference standard for genital chlamydial infection was bacterial culture until the advent of NAATs. As *C. trachomatis* is an obligate intracellular parasite, culture of this pathogen requires a cell-culture facility, a high level of technical expertise and 48–72 hours to obtain a result. The performance of culture is also highly dependent on transport conditions, and maintaining pathogen viability is difficult. Enzyme immunoassays that detect chlamydial antigens became commercially available in the 1980s. These assays are technically easier than culture techniques, do not have stringent requirements for specimen transport and are less expensive as the specimens can be processed in batches. They have comparable sensitivity and reasonable specificity relative to culture and became the test of choice for chlamydia-screening programmes. However, it has now been established that chlamydial NAATs have significantly higher sensitivity than culture, together with excellent specificity. They also allow the use of non-invasive specimens such as urine and vaginal swabs. Several types of NAAT are now commercially available and some offer duplex testing for both chlamydial and gonococcal infections.

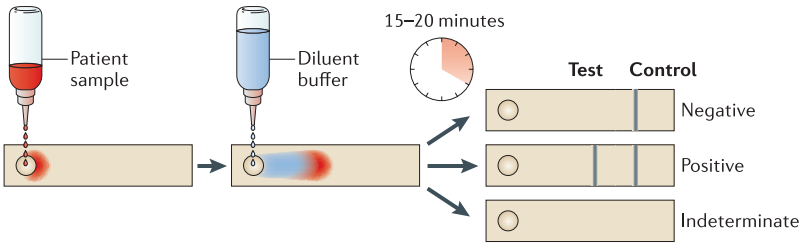



Figure 3 | **The rapid diagnostic test for syphilis.** The specified amount of sample is added to the sample well, followed by the specified amount of diluent buffer. The test result can be read after the time specified in the test instructions has elapsed (usually 15–20 minutes).

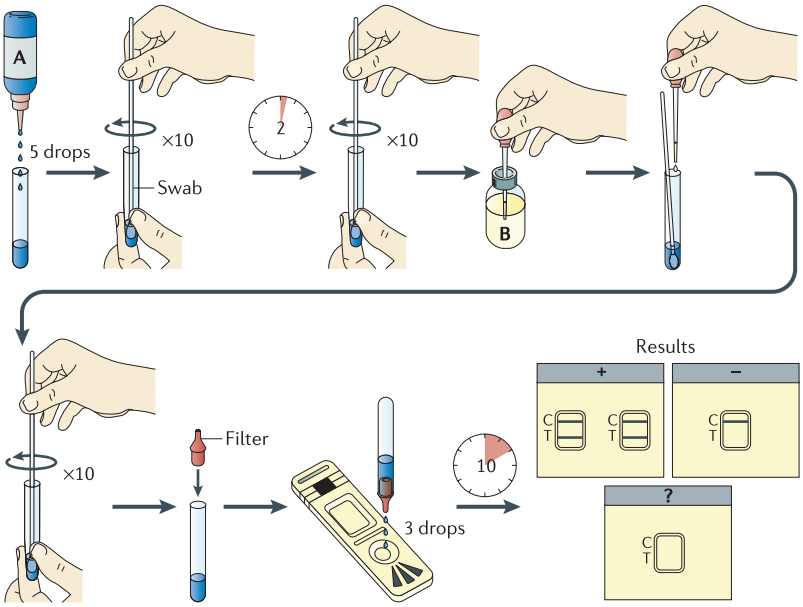



Figure 4 | **Schematic representation of a rapid point-of-care test for chlamydia diagnosis.** The test is an immunoassay in the format of immuno-chromatographic strips (ICSs) encased within a plastic cassette. The test detects antigen using high-affinity antibodies fixed onto nitrocellulose strips. A is a solubilising buffer, which releases the chlamydia bacteria from the host cells on the swab. B is a reagent mixture, which contains the reagents necesssary to visualise the test result.

RDTs and POC tests for the diagnosis of chlamydial and gonococcal infection currently include microscopy performed on a Gram-stained smear of genital discharge from infected patients for gonorrhoea and POC immunoassays for both infections. The POC immunoassays are designed as immuno-chromatographic strips (ICSs), often encased in a plastic cassette. The ICS detects antigen using high-affinity antibodies that are fixed onto nitrocellulose strips. Most of these immunoassays do not require any additional equipment and can give a visual result in 30 minutes. Currently, two main drawbacks are that each ICS costs approximately US$ 5–16 and the processing involves 7–14 steps as the swab must be processed to release the antigen for detection (FIG. 4), which makes it difficult to incorporate these tests into a busy clinic routine. Preliminary evaluation shows that these tests are 50–70% sensitive compared to NAATs but they are highly specific[64,65]. Although their sensitivity is low, RDTs allow patients to be screened and treated in the same clinic visit. The simplicity, rapidity and ease of interpretation of test results make them a good choice for clinics without access to a laboratory, in outreach settings, or for use in rapid situational assessments. But how good do these rapid tests need to be to have an impact?

## The ideal test for STIs

The reasons for the failure to prevent and control STIs in the developing world are complex, but a significant barrier to effective healthcare in Africa is the lack of reliable and good quality laboratory services[66,67]. The 2004 World Development Report cited the lack of access to services and unaffordability of healthcare services as two main reasons why healthcare services fail[68]. Diagnostic testing is particularly crucial for STIs as infected individuals are often asymptomatic or are undiagnosed but nonetheless infectious. Sensitive diagnostic tests are required for early detection to guide treatment, to prevent the development of reproductive sequelae and to interrupt onward transmission.

As >90% of STIs occur in settings where laboratory services are limited or not accessible, there is an urgent need for simple, cheap, POC tests for STIs. These tests should meet the 'ASSURED' criteria (affordable, sensitive, specific and user friendly (simple to perform in a few steps with minimal training)) that have been developed by the WHO SDI (see the SDI link in Further information).

Mathematical modelling has been used to address the question of how good RDTs have to be to be effective diagnostic tests[34,69]. Gift *et al.* found that the proportion of infected patients who are treated following diagnosis with an RDT with 65% sensitivity that does not require a patient to return to the clinic for the results is higher than the proportion of infected patients who are treated following diagnosis with an NAAT with 90% sensitivity that does require patients to return for treatment[70,71]. This is because a significant number of infected individuals fail to return for treatment and also because transmission occurs in the interval between testing and treatment.

***The potential impact of RDTs for syphilis.*** Screening and treatment for syphilis in pregnancy is one of the most cost-effective health interventions available[72,73]. Non-treponemal tests are affordable and, under ideal conditions, have excellent sensitivity for detecting women at risk of adverse pregnancy outcomes associated with syphilis. However, several studies have shown that, when performed in rural health centres and hospitals under less than ideal conditions and when the prevalence of prior treated infection is high, the sensitivity and specificity of non-treponemal tests can be poor[44,74,75]. When the RPR test is performed in a central laboratory rather than a local facility the results can be more reliable but, because the samples are often processed in batches to save costs, overall fewer infected women are treated as many fail to return for their results[76]. The syphilis RDTs evaluated by the SDI have been shown to be both sensitive and specific for the diagnosis of syphilis compared with gold-standard serological tests, even when performed in antenatal or STI clinics, as they are easier to perform and interpret than the RPR test[49]. However, they are usually more expensive than non-treponemal tests. The greatest impact of syphilis RDTs is likely to be in increasing the coverage of antenatal-screening programmes in developing countries where many women are not screened because the RPR test is not available or is performed poorly[67,77].

***The potential impact of RDTs for gonorrhoea and chlamydia.*** POC tests for *N. gonorrhoeae* and *C. trachomatis* could be used either to screen asymptomatic women for these infections or to increase the specificity of syndromic-management algorithms for women presenting

with vaginal discharge by reducing unnecessary treatment. The current reference-standard tests for these infections are based on bacterial culture or nucleic-acid amplification. They are highly sensitive and specific but must be performed in a well-equipped laboratory. An RDT that is less sensitive than the reference standard could have a greater impact in terms of the treatment of cases and prevention of onward transmission in settings where not all women return for the results of the reference-standard test[70,78]. Unfortunately, the POC tests for *N. gonorrhoeae* and *C. trachomatis* that have been evaluated by the SDI have shown poor sensitivity, have been technically difficult to perform and cost more than US$ 5 per test[64,65]. A new POC test for *C. trachomatis* has shown a sensitivity of >80% for the detection of ocular infection compared with PCR and is simpler to perform than other POC tests[79]. This test needs to be evaluated for the diagnosis of sexually transmitted *C. trachomatis* infection.

A modelling study compared the impact and cost effectiveness of RDTs with that of a modified syndromic-management approach for the identification of sex workers in Benin with *N. gonorrhoeae* and *C. trachomatis* infection. This showed that a POC test for both infections that was 70% sensitive and 95% specific would result in treatment of a higher proportion of infected women and a lower proportion of uninfected women than the modified syndromic-management algorithm[69]. Therefore, in this high-risk population, a test with these characteristics that costs US$ 2 would result in a cost effectiveness of US$ 2.9 per *N. gonorrhoeae* or *C. trachomatis* infection averted, and US$ 151 per HIV infection averted, assuming that *N. gonorrhoeae* and *C. trachomatis* infection increase the risk of HIV transmission by five fold.

In summary, the results from these mathematical models have shown that even tests with less than optimal sensitivity and specificity can have a significant impact on disease burden and disease sequelae as long as they fulfil the ASSURED criteria and are widely accessible. Simple syphilis RDTs approach the ASSURED criteria but suffer from a major setback in that they cannot be used to distinguish between active and prior treated infection. There need to be marked improvements in the sensitivity of the simple RDTs for the diagnosis of chlamydia and gonorrhoea.

## Conclusions

In spite of significant advances in laboratory diagnosis, genomics and curative single-dose treatment for syphilis, gonorrhoea and chlamydia, the prevalence of infection and disease burden remain unacceptably high, especially in the developing world. As these infections are often asymptomatic or not easily diagnosed, ASSURED tests are needed to increase screening coverage and improve the specificity of syndromic management. A further reduction in the global burden of disease will only be achieved if new and improved diagnostic tests are developed. We now have an opportunity to use genomics and proteomics to gain a greater insight into the biology and pathogenesis of these sexually transmitted microorganisms and hopefully identify novel host or microbial biomarkers to improve diagnosis and treatment.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**Competing interests statement**
The authors declare no competing financial interests.


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