Syphilis

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Abstract | *Treponema pallidum* subspecies *pallidum* (*T. pallidum*) causes syphilis via sexual exposure or via vertical transmission during pregnancy. *T. pallidum* is renowned for its invasiveness and immune-evasiveness; its clinical manifestations result from local inflammatory responses to replicating spirochaetes and often imitate those of other diseases. The spirochaete has a long latent period during which individuals have no signs or symptoms but can remain infectious. Despite the availability of simple diagnostic tests and the effectiveness of treatment with a single dose of long-acting penicillin, syphilis is re-emerging as a global public health problem, particularly among men who have sex with men (MSM) in high-income and middle-income countries. Syphilis also causes several hundred thousand stillbirths and neonatal deaths every year in developing nations. Although several low-income countries have achieved WHO targets for the elimination of congenital syphilis, an alarming increase in the prevalence of syphilis in HIV-infected MSM serves as a strong reminder of the tenacity of *T. pallidum* as a pathogen. Strong advocacy and community involvement are needed to ensure that syphilis is given a high priority on the global health agenda. More investment is needed in research on the interaction between HIV and syphilis in MSM as well as into improved diagnostics, a better test of cure, intensified public health measures and, ultimately, a vaccine.

Syphilis is a sexually transmitted infection (STI) that can also be transmitted vertically. It is caused by the spirochaete *Treponema pallidum* subspecies *pallidum* (order Spirochaetales) (FIG. 1). Three other organisms within this genus are causes of nonvenereal or endemic treponematoses. *T. pallidum* subspecies *pertenue* is the causative agent of yaws, *T. pallidum* subspecies *endemicum* causes endemic (nonvenereal) syphilis and *T. carateum* causes pinta. These pathogens are morphologically and antigenically indistinguishable. However, they can be differentiated by their age at acquisition, principal mode of transmission, clinical manifestations, capacity for invasion of the central nervous system (CNS) and placenta, and genomic sequences, although the accuracy of these differences remains a subject of debate¹. Analyses based on the mutation rates of genomic sequences suggest that the causative agents of yaws and venereal syphilis diverged several thousand years ago from a common progenitor originating in Africa². These estimates argue against the so-called Columbian hypothesis — the notion that shipmates of Christopher Columbus imported a newly evolved spirochaete causing venereal syphilis from the New World into western Europe in the late 15th century³.

T. pallidum is an obligate human pathogen renowned for its invasiveness and immune-evasiveness^{4–7}; clinical manifestations result from the local inflammatory

response elicited by spirochaetes replicating within tissues^{8–10}. Infected individuals typically follow a disease course divided into primary, secondary, latent and tertiary stages over a period of ≥10 years. Different guidelines define early latency as starting 1–2 years after exposure. Typically, ‘early syphilis’ refers to infections that can be transmitted sexually (including primary, secondary and early latent infections) and is synonymous with active (infectious) syphilis; the WHO defines ‘early syphilis’ as an infection of <2 years duration¹¹, whereas the guidelines from the United States¹² and Europe¹³ define it as an infection <1 year in duration. These differences in definition can affect the interpretation of results and the prescription of therapeutic regimens used in some circumstances.

Owing to its varied and often subtle manifestations, which can mimic other infections, syphilis has earned the names the Great Imitator and Great Mimicker¹⁴. Patients with primary syphilis present with a single ulcer (chancre) or multiple lesions on the genitals or other body sites involved in sexual contact and present regional lymphadenopathy ~3 weeks after infection; these are typically painless and resolve spontaneously. Resolution of primary lesions is followed 6–8 weeks later by secondary manifestations, which can include fever, headache and a maculopapular rash on the flank, shoulders, arm, chest or back and that often involves the palms of the hands and soles of the feet. As signs and

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symptoms subside, patients enter a latent phase, which can last many years. A patient in the first 1–2 years of latency is still considered infectious owing to a 25% risk of secondary syphilis-like relapses¹⁵. Historical literature suggests that 15–40% of untreated individuals will develop tertiary syphilis, which can manifest as destructive cardiac or neurological conditions, severe skin or visceral lesions (gummas) or bony involvement⁹. Recent data suggest that tertiary syphilis is less common today, perhaps owing to the wide use of antibiotics. Numerous case reports and small series suggest that HIV infection increases the risk of neuro-ophthalmological complications in those with syphilis¹⁶. Importantly, neurosyphilis is typically described as a late manifestation but can occur in early syphilis. Indeed, *T. pallidum* can be frequently identified in the cerebral spinal fluid (CSF) of patients with early disease^{9,15,17}. However, the majority of patients with early syphilis who have CSF abnormalities do not demonstrate CNS symptoms and do not require therapy for neurosyphilis¹². Symptomatic manifestations of neurosyphilis include chronic meningitis, meningo-vascular stroke-like syndromes and manifestations common in the neurological forms of tertiary syphilis (namely, tabes dorsalis and general paresis, a progressive dementia mimicking a variety of psychotic syndromes)⁹. Numerous case reports and small series suggest that HIV infection predisposes individuals with syphilis to neuro-ophthalmological complications¹⁶. Cardiovascular syphilis, typically involving the aortic arch and leading to aneurysmal dilatation, usually occurs 10–30 years after the initial infection⁹.

Sexual transmission of syphilis occurs during the first 1–2 years after infection (that is, during primary, secondary and early latent stages of infection)⁹. The risk of mother-to-child transmission (MTCT) is highest in primary and secondary stages, followed by the early latent stage. However, transmission risk continues during the first 4 years after exposure, after which the risk of vertical transmission declines over time¹⁸. The rate of fetal infection depends on the stage of maternal infection, with ~30% of pregnancies resulting in fetal death *in utero*, still-birth (late second and third trimester fetal death) or death shortly after delivery^{19–21}. Infants born to infected mothers are often preterm, of low birthweight or have clinical signs that mimic neonatal sepsis (that is, poor feeding, lethargy, rash, jaundice, hepatosplenomegaly and anaemia).

Given that *T. pallidum* has a long generation time of 30–33 hours²², long-acting penicillin preparations (such as benzathine penicillin G) are the preferred therapies for most patients with syphilis. Since the 1940s (when penicillin became widely available), syphilis prevalence has continued to decline in regions able to appropriately test for and treat the infection. However, syphilis outbreaks continue to occur throughout the world. In particular, with declining AIDS-related mortality related to effective HIV treatment over the past two decades, syphilis has re-emerged in urban settings among men who have sex with men (MSM). High-income and middle-income countries have observed rises in syphilis case rates as well as increased neurosyphilis case rates (such as ocular syphilis) and, in some countries, congenital syphilis. In low-income countries where syphilis prevalence has remained high, MTCT of syphilis continues to be the most common cause of STI-related mortality outside HIV^{23,24}, with perinatal deaths owing to untreated syphilis exceeding those due to HIV or malaria²⁵. Following malaria, syphilis is now the second-leading cause of preventable stillbirths worldwide²⁵.

Syphilis should be an ideal disease for elimination as it has no known animal reservoir, can usually be diagnosed with simple and inexpensive tests and can be cured^{9,16}. Nevertheless, syphilis remains a continuing public health challenge globally²⁶. In this Primer, we describe recent discoveries that have improved our understanding of the biological and genetic structure of the pathogen, novel diagnostic tests and testing approaches that can improve disease detection and current, evidence-based management recommendations. We also draw attention to the call for the global elimination of MTCT of syphilis and HIV, as well as recent successes in eliminating syphilis in low-income and middle-income countries (LMICs), particularly through fundamental public health strategies such as ensuring quality antenatal care that includes testing for syphilis early in pregnancy and providing prompt treatment of women and their partners. We also report on the rising numbers of syphilis cases in MSM and ongoing work supporting improved interventions against syphilis in marginalized populations and, ultimately, the development of an effective vaccine.

Epidemiology

According to the most recent estimation of the WHO, ~17.7 million individuals globally 15–49 years of age had syphilis in 2012, with an estimated 5.6 million new cases every year²⁷ (FIG. 2). The estimated prevalence and incidence of syphilis varies substantially by region or country, with the highest prevalence in Africa and >60% of new cases occurring in LMICs²⁷. The greatest burden of maternal syphilis occurs in Africa, representing >60% of the global estimate^{23,24}.

Prevalence and incidence

In LMICs, the heterosexual spread of syphilis has declined in the general population but remains problematic in some high-risk subpopulations, such as female sex workers (FSWs) and their male clients. A recent study of FSWs in Johannesburg, South Africa, showed that 21%

of participating women had antibodies suggestive of past or current infection, and 3% had an active (infectious) infection²⁸. Another study of FSWs in 14 zones in Sudan showed a high seroprevalence (median 4.1%), with the highest value (8.9%) in the eastern zone of the country²⁹. A large study of >1,000 FSWs in Kampala, Uganda,

showed that 21% were seropositive for syphilis and 10% had an active infection³⁰. Studies in emerging economies, such as China, indicate that syphilis is increasing among 'mobile men with money' (REF. 31). Although syphilis case rates are low in the general population in China, syphilis prevalence is ~5% among FSWs and ~3% among their male clients^{31,32}. The risk of infection varies among FSWs working in different venues, with the highest prevalence (~10%) among street-based FSWs and the lowest prevalence (~2%) among venue-based FSWs³³.

By contrast, higher-income countries have had declining syphilis prevalence among heterosexual men and women. However, a resurgence of syphilis that disproportionately affects MSM has been noted. Syphilis is associated with high-risk sexual behaviours and infection substantially increases in association with HIV transmission and acquisition. Indeed, the numbers and rates of reported cases of syphilis among MSM in the United States and western Europe have been increasing since 1998 (REF. 34). In 2015, the case rate for primary and secondary syphilis among MSM (309 per 100,000) in the United States was 221-fold the rate for women (1.4 per 100,000) and 106-fold the rate for heterosexual men (2.9 per 100,000)³⁵. In Canada, the incidence of syphilis was 300-fold greater among MSM positive for HIV than the reported case rate in the general male population³⁶. Syphilis infection has been associated with certain behavioural and other factors, including incarceration, multiple or anonymous sex partners, sexual activity connected with illicit drug use, seeking sex partners on the Internet and other high-risk sexual network dynamics^{37–41}. Risk factors for syphilis are frequently overlapping⁴⁰. Reports of unusual presentations and rapid progression of syphilis in patients with concurrent HIV infection have led to the hypothesis that infection with or treatment for HIV alters the natural history of syphilis⁴².

MTCT

Adverse birth outcomes caused by fetal exposure to syphilis are preventable if women are screened for syphilis and treated before the end of the second trimester of pregnancy²¹. However, MTCT of syphilis caused such a high rate of perinatal and infant mortality that, in 2007, the WHO and partners launched a global initiative to eliminate it as a public health problem^{43–45}. At the time of the campaign launch, ~1.4 million pregnant women had active syphilis infections, of whom 80% had attended at least one antenatal visit, suggesting missed opportunities for testing and treatment²³. At that time, untreated maternal syphilis infections were estimated to have resulted in >500,000 adverse pregnancy outcomes, including >300,000 perinatal deaths (stillbirths and early neonatal deaths).

Syphilis testing and treatment during pregnancy are highly effective and were included in the Lives Saved Tool for effective maternal–child health interventions⁴⁶. Furthermore, studies have shown that prenatal syphilis screening, testing to support treatment and treatment during pregnancy are highly cost-effective in most countries regardless of disease prevalence or the availability of resources and can even be cost-saving in LMICs

Figure 1 | *Treponema pallidum*. **a** | Like all spirochaetes, *Treponema pallidum* consists of a protoplasmic cylinder and cytoplasmic membrane bounded by a thin peptidoglycan sacculus and outer membrane^{239,240}. Usually described as spiral-shaped, *T. pallidum* is actually a thin planar wave similar to *Borrelia burgdorferi*, the agent that causes Lyme borreliosis²³⁹. The bacterium replicates slowly and poorly tolerates desiccation, elevated temperatures and high oxygen tensions⁵⁵. **b** | Periplasmic flagellar filaments, a defining morphological feature of spirochaetes, originate from nanomotors situated at each pole and wind around the cylinder atop the peptidoglycan, overlapping at the middle of the cell. Force exerted by the rigid filaments against the elastic peptidoglycans deforms the sacculus to create the flat-wave morphology of the spirochaete¹⁰⁰. Panel b is reproduced with permission from American Society for Microbiology (REF. 239); Journal of Bacteriology, volume 191, 7566–7580, doi: 10.1128/JB.01031-09. **c** | Ultrathin section of *T. pallidum* showing the outer and cytoplasmic membranes and flagellar filaments (endoflagella) within the periplasmic space⁹. **d** | Surface rendering of a flagellar motor based on cryo-electron tomograms. Panel d is reproduced with permission from REF. 240, Elsevier. **e** | Darkfield micrograph showing the flat-wave morphology of *T. pallidum*. The arrow and arrowhead indicate segments that are oriented 90° from each other. The different appearances of the helical wave at 90° to the viewer can be explained only by a flat-wave morphology; a corkscrew shape would appear the same from any angle. Panel e is reproduced with permission from American Society for Microbiology (REF. 239); Journal of Bacteriology, volume 191, 7566–7580, doi: 10.1128/JB.01031-09.

Figure 2 | **Incidence of syphilis worldwide.** The WHO estimates of incident cases of syphilis by region in 2012 are shown for the different geographical regions. Data from REF. 27.

with a syphilis prevalence $\geq 3\%$ in pregnant women^{47–50}. In China, where syphilis and HIV prevalence in pregnant women are low but increasing, the integration of prenatal syphilis and HIV screening was shown to be highly cost-effective⁵¹.

Since 2007, an increasing number of countries have implemented regional and national initiatives to prevent MTCT of syphilis⁵², improving guidance documents, using point-of-care (POC) tests as a means of improving access to testing and treatment and integrating behavioural and medical interventions into HIV prevention and control programmes⁵³. By 2012, these efforts had contributed to a reduction in the global number of adverse pregnancy outcomes due to MTCT of syphilis to 350,000, including 210,000 perinatal deaths, and had decreased the rates of maternal and congenital syphilis by 38% and 39%, respectively^{23,24}. In 2015, Cuba became the first country to be validated for having achieved the elimination of MTCT of HIV and syphilis⁵⁴. Subsequently, Thailand, Belarus and four United Kingdom Overseas Territories (Bermuda, the Cayman Islands, Montserrat and Antigua) were validated for the elimination of MTCT of HIV and syphilis, Moldova was validated for the elimination of MTCT of syphilis and Armenia was validated for the elimination of MTCT of HIV. However, these gains were mostly in Asia and the Americas — the maternal prevalence in Africa has remained largely unchanged^{23,24}.

Mechanisms/pathophysiology

Although a local inflammatory response elicited by spirochaetes is thought to be the root cause of all clinical manifestations of syphilis⁹, the mechanisms that cause tissue damage, as well as the host defences that eventually gain a measure of control over the bacterium, are ill-defined. The recalcitrance of *T. pallidum* to *in vitro* culture and the consequent inability to harness genetic

techniques to delineate its virulence determinants remain the primary obstacles to progress⁵⁵. Additionally, the fragility and low protein content of its outer membrane have confounded efforts to characterize surface-exposed molecules^{56,57}. Finally, facile murine models to dissect the host response and the components of protective immunity are also lacking⁵⁸. Outbred rabbits are essential for isolating *T. pallidum* strains from clinical specimens⁵⁹ and for routine propagation in the laboratory⁶⁰. Because rabbits are highly susceptible to *T. pallidum* infection, develop lesions grossly and histopathologically resembling chancres following intradermal inoculation and generate antibody responses similar to those in humans, the rabbit is the model organism of choice for studying endogenous and exogenous protective immunity^{61,62}. However, the rabbit model poorly recapitulates some clinical and immunological facets of the human disease⁶³. Not surprisingly, even in the post-genomics era, our understanding of the pathogenic mechanisms in syphilis lags well behind that of other common bacterial diseases⁶³.

Molecular features

The morphological features of *T. pallidum* are described in FIG. 1. Because of its double-membrane structure, the spirochaete is often described as a Gram-negative bacterium. However, this analogy is phylogenetically, biochemically and ultrastructurally inaccurate^{63,64}. The *T. pallidum* outer membrane lacks lipopolysaccharides⁶⁵ and has a markedly different phospholipid composition than the outer membranes of typical Gram-negative bacteria⁶⁶. Although *T. pallidum* expresses abundant lipoproteins, these molecules reside predominantly below the surface^{5,63,67}. Accordingly, this paucity of surface-exposed pathogen-associated molecular patterns (PAMPs) enables the spirochaete to avoid triggering host innate surveillance mechanisms, facilitating local

replication and early dissemination. Its limited surface antigenicity promotes the evasion of adaptive immune responses (that is, antibody recognition), facilitating persistence^{5,56,68,69}. Collectively, these attributes have earned *T. pallidum* its designation as ‘the stealth pathogen’ (REFS 63,69). Understanding events unfolding at the host–pathogen interface requires a detailed knowledge of the *T. pallidum* repertoire of surface-exposed proteins. However, characterization of the protein constituents of the outer membrane has been, and continues to be, daunting^{8,55,57,63}.

Lipoproteins. In the 1980s, investigators screened *E. coli* recombinant libraries with syphilitic sera and murine monoclonal antibodies based upon the unproven (and, as it turned out, immunologically incorrect) assumption that immunoreactive proteins ought to be surface-exposed in *T. pallidum*⁵⁷. Biochemical and genetic analyses subsequently revealed that most of the antigens identified by these screens are lipoproteins^{70–72} tethered by their N-terminal lipids to the cytoplasmic membrane (hence, the protein moieties are in the periplasmic space)^{67,73–75}. However, convincing evidence now shows that the spirochaete displays small amounts of lipoproteins on its surface that have the potential to enhance infectivity (FIG. 3). For example, TP0751 (also known as pallilysin) is a laminin-binding lipoprotein and zinc-dependent metalloproteinase capable of degrading clots

and the extracellular matrix^{76–78}. Although expressed by *T. pallidum* in minute quantities, surface exposure of TP0751 has been demonstrated by knock-in experiments in *Borrelia burgdorferi* (the spirochaete that causes Lyme borreliosis⁷⁹) and the cultivatable commensal treponeme *Treponema phagedenis*⁸⁰, in opsonophagocytosis assays in *T. pallidum*⁷⁷ and, most recently, in the protection of immunized rabbits against the dissemination of spirochaetes following intradermal challenge⁸¹. The X-ray structure of TP0751, which demonstrates an unusual lipocalin fold, should inform efforts to clarify its multifunctionality⁷⁹. Additionally, the lipoprotein Tpp17 (also known as TP0435) has been shown to be at least partially surface-exposed and can function as a cytoadhesin⁸². The structurally characterized lipoprotein TP0453 attaches to the inner leaflet of the outer membrane via its N-terminal lipids and two amphipathic helices within its protein moiety⁸³.

BamA. With the publication of the *T. pallidum* genome in 1998 (REF. 65), only one protein with sequence relatedness to an outer membrane protein of Gram-negative bacteria was identified: β -barrel assembly machinery A (BamA, also known as TP0326)^{84,85}. BamA has a dual domain architecture consisting of a 16-stranded, outer membrane-inserted, C-terminal β -barrel and five tandem polypeptide transport-associated repeats within the periplasm^{84,85}. The opening of the channel is covered by a

Figure 3 | Molecular architecture of the cell envelope of *Treponema pallidum*. Shown in the outer membrane are TP0751 (as known as pallilysin)^{79,81} and Tpp17 (also known as TP0435)^{82,241}, two surface-exposed lipoproteins; TP0453, a lipoprotein attached to the inner leaflet of the outer membrane⁸³; β -barrel assembly machinery A (BamA, also known as TP0326)^{84,94}; a full-length *T. pallidum* repeat (Tpr) attached by its N-terminal portion to the peptidoglycan^{93,94}; and a generic β -barrel that represents other non-Tpr outer membrane proteins (OMPs) identified by computational mining of the *T. pallidum* genome¹¹². Substrates and nutrients present in high concentration in the extracellular milieu (such as glucose) traverse the outer membrane through porins, such as TprC. At the cytoplasmic membrane, prototypic ABC-like transporters (such as RfuABCD, a riboflavin transporter) use a periplasmic substrate-binding protein (SBP), usually lipoproteins, and components with transmembrane

and ATP-binding domains to bind nutrients that have traversed the outer membrane for transport across the cytoplasmic membrane. The energy coupling factor (ECF)-type ABC transporters use a transmembrane ligand-binding protein in place of a separate periplasmic SBP for binding of ligands (BioMNY is thought to transport biotin)²⁴². Symporter permeases (for example, TP0265) use the chemiosmotic or electrochemical gradient across the cytoplasmic membrane to drive substrate transport²⁴³. The tripartite ATP-independent periplasmic (TRAP)-type transporters also use transmembrane electrochemical gradients to drive substrate transport; the periplasmic component protein TatT (also known as TP0956) likely associates with the SBP TatP (also known as TP0957), which binds ligands (perhaps hydrophobic molecules, such as long chain fatty acids), the uptake of which is probably facilitated by the permease TatQ-M (also known as TP0958)^{244,245}. Figure adapted from REF. 63, Macmillan Publishers Limited.

'dome' comprising three extracellular loops, one of which contains an opsonic target that is sequence-variable among *T. pallidum* strains⁸⁵. BamA is the essential central component of the molecular machine that catalyses the insertion of newly exported outer membrane proteins to the outer membrane⁸⁶.

Tpr proteins. The *T. pallidum* repeat (Tpr) proteins, a 12-member paralogous family with sequence homology to the major outer sheath protein of the oral commensal *Treponema denticola*, were also identified in the *T. pallidum* genomic sequence⁶⁵. Of these, TprK (TP0897) has received the most attention because of its presumed role in immune evasion by the spirochaete^{87,88}; it has been shown to undergo antigenic variation in seven regions believed to be extracellular loops harbouring B cell epitopes^{89–92}. DNA sequence cassettes that correspond to V-region sequences in an area of the *T. pallidum* chromosome located away from the *tprK* gene have been proposed to serve as unidirectional donor sites for the generation of variable regions by nonreciprocal gene conversion⁸⁹. Two other Tpr proteins, TprC and TprI, have met stringent experimental criteria for being classified as rare outer membrane proteins. They form trimeric β -barrels when refolded *in vitro*, cause large increases in permeability upon insertion into liposomes and are surface-exposed opsonic targets in *T. pallidum*^{93,94}. Unlike classic porins, for which the entire polypeptide forms a β -barrel, TprC and TprI are bipartite. As with BamA, the C-terminal domain forms the surface-exposed β -barrel, whereas the N-terminal half anchors the barrel to the peptidoglycan sacculus. These results collectively imply that Tprs serve as functional orthologues of Gram-negative porins, using variations in the substrate specificities of their channel-forming β -barrels, probably along with differential expression, to import the nutritional requirements of the spirochaete into the periplasmic space from blood and body fluids^{95,96}. These proteins also furnish a topological template for efforts to understand how antibody responses to Tprs promote bacterial clearance.

Biosynthetic machinery. *T. pallidum* has evolved to dispense with a vast amount of the biosynthetic machinery found in other bacterial pathogens^{55,63–65}. *T. pallidum* relies on an optimized conventional glycolytic pathway as its primary means for generating ATP. By dispensing with oxidative phosphorylation, the spirochaete has no need for cytochromes and the iron required to synthesize them. Accordingly, the spirochaete maintains a complex, yet parsimonious, assortment of ABC transporters and symporters (totalling ~5% of its 1.14 Mb circular genome) to transfer essential molecules from the periplasmic space to the cytosol (FIG. 3). Whereas many pathogens have highly redundant systems for the uptake of transition metals across the cytoplasmic membrane, *T. pallidum* accomplishes this task with just two ABC transporters (Tro, which imports zinc, manganese and iron, and Znu, which is zinc-specific). A small but powerful arsenal of enzymes neutralizes superoxides and peroxides to fend off host responses to infection. Lastly,

the spirochaete possesses novel and surprisingly intricate mechanisms ostensibly to redirect transcription and fine-tune metabolism in response to environmental cues and nutrient flux⁶³.

Transmission and dissemination

Transmission of venereal syphilis occurs during sexual contact with an actively infectious partner; exudate containing as few as ten organisms can transmit the disease^{8,68}. Spirochaetes directly penetrate mucous membranes or enter through abrasions in skin, which is less heavily keratinized in perigenital and perianal areas than skin elsewhere^{8,68}. To establish infection, *T. pallidum* must adhere to epithelial cells and the extracellular matrix components; *in vitro* binding studies suggest that fibronectin and laminin are key substrates for these interactions^{76,97–99}. Once below the epithelium, organisms multiply locally and begin to disseminate through the lymphatic system and bloodstream. Spirochaetes penetrate the extracellular matrix and intercellular junctions via 'stop and go' movements that coordinate adherence with motility and are powered by front-to-back undulating waves generated by flagellar rotation and presumably assisted by the proteolytic activity of TP0751 (REFS 77,100). *Ex vivo* studies using cultured human umbilical vein endothelial cells (FIG. 4a) suggest that spirochaetes invade tissues using motility to negotiate their way through intercellular junctions: so-called 'interjunctional' penetration^{7,101}. The infection rapidly becomes systemic^{9,16,100}. Profuse spirochaetes within the epidermis and superficial dermis in secondary syphilitic lesions (FIG. 4b) enable tiny abrasions created during sexual activity to transmit infection^{10,102}. Penetration of the blood–brain barrier, occurring in as many as 40% of individuals with untreated early syphilis, can cause devastating neurological complications^{9,16}.

Adaptive immune response and inflammation

Although the paucity of PAMPs in the *T. pallidum* outer membrane enables the bacterium to replicate locally and undergo repeated bouts of dissemination, pathogen sensing in the host is eventually triggered. The organisms are taken up by dendritic cells¹⁰³, which then traffic to draining lymph nodes to present cognate treponemal antigens to naive B cells and T cells. The production of opsonic antibodies markedly enhances the uptake and degradation of spirochaetes by phagocytes (FIG. 4c,d), liberating lipopeptides and other PAMPs for binding to Toll-like receptors lining the interior of the phagosome and antigenic peptides for presentation to locally recruited T cells^{62,104,105}. Activated lesional T cells secrete IFN γ , promoting clearance by macrophages but also bolstering the production of tissue-damaging cytokines, such as tumour necrosis factor and IL-6 (REFS 10,106,107). Immunohistochemical analysis has identified CD4⁺ and CD8⁺ T cells^{10,106,108,109}, natural killer cells¹⁰ and activated macrophages in early syphilitic lesions^{10,109}. Perivascular infiltration of lymphocytes, histiocytes (phagocytic cells in connective tissues) and plasma cells with endothelial cell swelling and proliferation are characteristic histopathological findings in all

stages of syphilis and can progress to frank endarteritis obliterans (leading to the occlusion of arteries and severe clinical manifestations, such as the stroke syndromes of meningovascular syphilis)^{9,110}.

Antibody avoidance

T. pallidum is widely regarded as an extracellular bacterium⁶¹. Thus, a question of paramount importance is why, unlike 'classic' extracellular pathogens, syphilis-causing spirochaetes not only fail to be cleared rapidly but can also replicate and circulate in the midst of a prolific antibody response^{8,68,69}. Immunolabelling, opsonophagocytosis and complement-dependent neutralization assays have shown that *T. pallidum* populations consist of antibody-binding and nonbinding subpopulations; the minority of organisms that bind antibodies do so in minute amounts and with delayed kinetics^{10,111–114}. Accordingly, one can envision a scenario whereby nonbinders replenish the spirochaetes that bind and are cleared⁶³.

Understanding the basis for the heterogeneity of *T. pallidum*'s surface antigenicity is critical to unravelling its strategy for antibody avoidance. The picture emerging from our evolving understanding of the molecular architecture of the spirochaete is multifactorial and probably involves the copious production of antibodies against subsurface lipoprotein 'decoys' (REFS 57, 110); poor target availability due to low copy numbers of outer membrane proteins and surface-exposed lipoproteins^{67,77,82,84,93}; in the case of bipartite outer membrane proteins, limited production of antibodies against surface-exposed epitopes along with the skewed production of antibodies against periplasmic domains^{84,93}; organism-to-organism variation in the levels of expression of outer membrane proteins and outer surface lipoproteins through a variety of mechanisms, including phase variation^{82,92,115,116}; and, in the case of TprK, antigenic variation as a result of intra-genomic

recombination^{89,92,117}. Additionally, the ability of motile spirochaetes to 'outrun' infiltrating phagocytes and reach sequestered locations, including the epidermis, could be an under-appreciated aspect of immune evasion^{10,102}. As infection proceeds, the antibody repertoire possibly broadens and intensifies to the point where the antigen-poor surface of the spirochaete is overwhelmed and its capacity for antigenic variation is exhausted, ushering in the asymptomatic period called latency. Once in the latent state, the organism can survive for years in untreated individuals, establishing niduses of inflammation in skin, bones, the thoracic aorta, the posterior uveal tract and the CNS that set the stage for recrudescence — collectively referred to as tertiary syphilis. How immune containment mechanisms decline and enable the balance to shift back in favour of the pathogen in tertiary syphilis is unclear⁹, although a hyper-intense cellular response to the spirochaete is generally believed to be the cause of the highly destructive lesions of tertiary syphilis⁹.

Congenital infection

Although MTCT of syphilis can occur at the time of delivery, the overwhelming majority of cases are caused by *in utero* transmission. Studies have shown spirochaetes in placental and umbilical cord samples, supporting the transplacental passage of the organism to the fetus, as early as 9–10 weeks of gestation¹¹⁸. Although fetal syphilis infections were thought to not occur before the second trimester, the fetus can indeed be infected very early in pregnancy but may be unable to mount a characteristic immune response until the development of the embryonic immune system at 18–20 weeks of gestation.

Transmission risk is directly related to the stage of syphilis in the pregnant woman (that is, the extent and duration of fetal exposure to spirochetes). Small case series have found the highest MTCT risk in primary and

Figure 4 | *Treponema pallidum* invasion. **a** | Transmission electron micrograph of *Treponema pallidum* (arrowheads) penetrating the junctions between cultured umbilical vein endothelial cells. 'Interjunctional invasion' following attachment to the vascular endothelium is thought to provide *T. pallidum* access to tissue parenchyma during haematogenous dissemination. Part a is reproduced by permission of Oxford University Press (REF. 101); Riley, B.S. et al., Virulent *Treponema pallidum* activates human vascular endothelial cells, The Journal of infectious diseases, 1992, 165, 3, 484–493. **b** | Immunohistochemical staining (using commercial anti-*T. pallidum* antibodies) of a secondary syphilitic skin lesion reveals abundant spirochaetes embedded within a mixed cellular inflammatory infiltrate in the papillary dermis. The inflammatory response elicited by spirochaetes replicating in tissues is widely thought to be the cause of clinical manifestations at all stages of syphilis. Reproduced from REF. 10. **c** | Fluorescence microscopy images showing that human syphilitic serum (HSS) dramatically enhances opsonophagocytosis of *T. pallidum* by purified human peripheral blood monocytes compared with part **d**, which shows normal human serum (NHS). Arrowheads indicate treponemes being degraded within phagolysosomes.

secondary stages, during which transmission probability may be $\geq 80\%$. Systematic reviews assessing women with predominantly asymptomatic infections are consistent in showing that delayed or inadequate treatment results in stillbirth, early neonatal death, prematurity, low birth-weight or congenital infection in infants (more than half of syphilis-exposed fetuses); syphilitic stillbirth was the most commonly observed adverse outcome^{21,45,119}.

Diagnosis, screening and prevention

Syphilis has varied and often subtle manifestations that make clinical diagnosis difficult and can lead to many infections being unrecognized. The classically painless lesions of primary syphilis can be missed, especially in hidden sites of exposure such as the cervix or rectum. The rash (FIG. 5) and other symptoms of secondary syphilis can be faint or mistaken for other conditions. A syphilis diagnosis is often based on a suggestive clinical history and

supportive laboratory^{9,16} (that is, serodiagnostic) test results. Serological testing has become the most common means to diagnose syphilis, whether in people with symptoms of syphilis or in those who have no symptoms but are identified through screening. A limitation of all syphilis serological tests is their inability to distinguish between infection with *T. pallidum* subspecies *pallidum* and the *T. pallidum* subspecies that cause (nonvenereal) yaws, pinta or bejel.

Ensuring the accuracy and reliability of syphilis testing is important, especially in nonspecialized laboratories, where most patient samples are tested¹²⁰. Syphilis-specific quality assurance strategies include the training of technologists on specific techniques as well as implementation of internal quality control systems, test evaluation and interassay standardization of commercially available test kits on a regular basis^{37,120}. It is especially important to provide adequate training and regular external quality assessment or proficiency testing with corrective action to ensure the quality of tests and testing for health care providers who perform rapid tests in clinic-based or outreach settings^{121–124}. Because many parts of the world lack laboratory capacity for making an accurate diagnosis, the requirement for laboratory testing has greatly constrained the control of syphilis and the elimination of congenital syphilis. However, the development of inexpensive, rapid tests that can be performed at the POC has greatly increased access to prenatal screening and diagnosis, even in under-resourced and remote settings.

Definitive diagnosis by direct detection

The choice of method for diagnosing syphilis depends on the stage of disease and the clinical presentation¹²⁵. In patients presenting with primary syphilitic ulcers, condyloma lata (genital lesions of secondary syphilis) or lesions of congenital syphilis, direct detection methods — which include darkfield microscopy, fluorescent antibody staining, immunohistochemistry and PCR — can be used to make a microbiological diagnosis. However, with the exception of PCR, these methods are insensitive and require fresh lesions from which swab or biopsy material can be collected, as well as experienced technologists (TABLE 1).

Microscopy had been used for direct detection and diagnosis since 1920 but is now used infrequently. A 2014 survey of national reference and large clinical laboratories in Latin America and the Caribbean showed that only two of 69 participating facilities, of which half were reference laboratories, still performed darkfield or direct fluorescent antibody staining for *T. pallidum* (DFA-TP)¹²⁶. The most recent European guidelines recommended against DFA-TP testing in clinical settings, and the reagents are no longer available¹³. PCR techniques are increasingly used; however, there is as yet no commercially available or internationally approved PCR test for *T. pallidum*¹³. Species-specific and subspecies-specific *T. pallidum* PCR testing is a developing technology that is still primarily available only in research laboratories^{127,128}, although these tests are anticipated to be more widely available in the near future. A systematic review and meta-analysis concluded that *T. pallidum*

Figure 5 | Clinical presentation of primary, secondary and congenital syphilis. **a** | Primary chancre. **b** | Primary chancre with rash in secondary syphilis. **c** | Secondary syphilis in a pregnant woman who has a palmar rash. **d** | Palmar rash in secondary syphilis. **e** | 3-month-old baby with congenital syphilis showing hepatosplenomegaly and a desquamating rash. The child also presented with nasal discharge. **f** | Typical palmar desquamating rash in a baby with congenital syphilis.

Table 1 | Direct detection methods for *Treponema pallidum*

Method	Sample	Advantages	Disadvantages
Darkfield microscopy	Fresh (<20 minutes) samples from chancres or erosive cutaneous lesions of primary, secondary or congenital syphilis	<ul style="list-style-type: none"> • Diagnosis can be made during the clinical visit by visualization of characteristic motile organisms 	<ul style="list-style-type: none"> • Insensitive: false negatives in ≥30% of samples • Should not be used in oral or rectal specimens due to commensal treponemes • Requires specialized equipment • Labour-intensive expertise is needed • Subjective • Rarely used anymore
Direct fluorescent antibody staining for <i>T. pallidum</i>	Samples from chancres or erosive cutaneous lesions of primary, secondary or congenital syphilis	<ul style="list-style-type: none"> • Can be used for oral lesions • Specific detection of <i>T. pallidum</i> • Samples can be saved or shipped for verification 	<ul style="list-style-type: none"> • Negative test result does not rule out syphilis • Requires specialized equipment and antibody conjugates that are no longer available • Labour-intensive expertise is needed • Subjective
Immunohistochemistry	Skin, mucosal or tissue lesion analysis performed on fixed paraffin-embedded tissues using commercially available treponemal antibody reagents	<ul style="list-style-type: none"> • Samples can be saved or shipped for verification • Can use tissue samples from placenta and umbilical cord • Useful for unusual forms of syphilis if tissue biopsies are obtained when syphilis is not initially suspected 	<ul style="list-style-type: none"> • Negative test result does not rule out syphilis • Requires specialized equipment and stains • Labour-intensive expertise is needed • Subjective
PCR	Skin, mucosal or tissue lesions; not recommended for blood or cerebral spinal fluid as few organisms are present	<ul style="list-style-type: none"> • Can be used to detect syphilis in oral or rectal lesions that are either stored or frozen 	<ul style="list-style-type: none"> • No internationally approved or commercially available tests • High sensitivity and specificity for specimens from genital ulcers • Negative test result does not rule out syphilis • Requires specialized equipment and expertise

PCR testing was more efficient for confirming a diagnosis of syphilis than for excluding a diagnosis in samples from lesions¹²⁹. Recent research indicates that this technology might be helpful for the diagnosis of neurosyphilis via the detection of *T. pallidum* DNA in the CSF of patients with syphilis, particularly among individuals infected with HIV^{130,131}.

Diagnosis using serology

Serodiagnostic tests are the only means for screening asymptomatic individuals and are the most commonly used methods to diagnose patients presenting with signs and symptoms suggestive of syphilis. Serodiagnostic tests for syphilis can be broadly categorized into nontreponemal tests (NTTs) and treponemal tests (TTs).

NTTs. NTTs measure immunoglobulins (IgM and IgG) produced in response to lipoidal material released from the bacterium and/or dying host cells. The most commonly used NTTs — the rapid plasma reagin (RPR) test, the toluidine red unheated serum test (TRUST) and the Venereal Disease Research Laboratory (VDRL) test — are flocculation (precipitation) tests that detect antibodies to a suspension of lecithin (including phosphatidylcholine and phosphatidylethanolamine), cholesterol and cardiolipin. NTTs are useful in detecting active syphilis. However, because individuals with an infection do not become positive until 10–15 days after the onset of the primary lesion, 25–30% of primary syphilis cases may be missed^{132,133} (FIG. 6). Although simple and inexpensive, NTTs must be performed manually on serum, and they rely on a subjective interpretation (TABLE 2). These tests also require trained laboratory personnel and specialized reagents and equipment and, therefore, do not fulfil the

ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to those who need them) criteria for tests that can be used at the POC¹³⁴.

Without treatment, titres peak at 1–2 years after infection and remain positive even in late disease (usually at a low titre). After treatment, titres generally decline and in most immunocompetent individuals become nonreactive within 6 months. However, up to 20% of individuals infected with syphilis show persistently reactive (albeit low-titre) NTT results even after treatment, possibly related to a less-robust pro-inflammatory immune response¹³⁵. These patients are labelled as having a serofast status, which is observed more commonly with treatment for late latent than for early syphilis^{37,136}. Biological false-positive results can occur in ~2–5% of the population, regardless of the NTT test used, although the proportion is difficult to estimate with certainty because it is influenced by the population studied¹³⁷. These low-titre reactions might be of limited duration if related to acute factors (such as febrile illness, immunization or pregnancy) or longer duration if related to chronic conditions (such as autoimmune diseases, hepatitis C infection or leprosy)^{136,138}. By contrast, false-negative results can occur in sera with very high titres (such as sera from patients with secondary syphilis) that are not diluted before testing, a phenomenon known as a prozone effect. Pre-dilution of sera re-establishes the concentration needed for optimal antibody–antigen interaction and avoids this problem.

TTs. In contrast to NTTs, TTs detect antibodies directed against *T. pallidum* proteins and are theoretically highly specific. However, as most individuals infected with

Figure 6 | Serological response to primary and secondary syphilis. Diagnosis of syphilis can be made by measuring a patient's serological response to infection. IgM antibodies against *Treponema pallidum* proteins are the first to appear, followed a few weeks later by IgG antibodies. Both IgM and IgG antibodies can be measured using treponemal tests such as the *T. pallidum* haemagglutination assay (TPHA), *T. pallidum* particle assay (TPPA), fluorescent treponemal antibody absorption assay (FTA-ABS), enzyme immunoassays (EIAs) and chemiluminescence immunoassays (CIAs). IgM and IgG antibodies against proteins that are not specific to *T. pallidum* (nontreponemal antibodies) can be detected using the rapid plasma reagin (RPR) Venereal Disease Research Laboratory (VDRL) or toluidine red unheated serum (TRUST) tests and usually appear 2–3 weeks after treponemal antibodies are detected. With effective treatment (which is arbitrarily shown here as occurring at 6 months), the nontreponemal antibody levels decline, whereas the treponemal antibodies remain high for many years. In ~20% of patients, nontreponemal antibodies persist 6 months after treatment; these individuals are labelled as having a serofast status. Despite repeated treatment, ~11% of patients remain serofast¹⁸⁷. Here, we show early syphilis (including primary, secondary and early latent infections; infectious syphilis) and late syphilis (including late latent and tertiary infections) as being ≤1 year in duration and >1 year in duration, respectively, in line with US and European guidelines. However, the WHO guidelines place this demarcation at 2 years. Beyond primary and secondary syphilis, the pattern of serological response over time is less well defined and is accordingly not shown.

syphilis develop treponemal antibodies that persist throughout life, TTs cannot be used to distinguish an active from a past or previously treated infection and are not useful in evaluating treatment effectiveness. TTs are used as confirmatory assays following a positive NTT result.

TT results become positive 6–14 days after the primary chancre appears (~5 weeks after infection) and, therefore, may be useful to detect early syphilis missed by NTT testing. These tests are usually laboratory-based and include the fluorescent treponemal antibody absorbed (FTA-ABS) test, the microhaemagglutination assay for antibodies to *T. pallidum* (MHA-TP), the *T. pallidum* passive particle agglutination (TPPA) and *T. pallidum* haemagglutination (TPHA) assays (TABLE 2). These tests also require trained personnel in a laboratory setting, are more expensive and technically complex than NTTs and involve specialized reagents and equipment. For these reasons, in the developing world, laboratory-based TTs are not widely available in primary care settings, hence limiting their utility as assays for confirming NTT results.

In recent years, TTs using recombinant *T. pallidum* antigens in enzyme and chemiluminescence immunoassays (EIA and CIA, respectively) have been commercialized. These assays are useful for large-scale screening as they are automated or semi-automated and, because they are read spectrophotometrically, are not subjective^{13,139–142}. In higher-income countries, many health care institutions depend on high-throughput screening and have adopted 'reverse' algorithms that screen with an automated treponemal EIA or CIA and confirm results with an NTT rather than the opposite, traditional approach (FIG. 7). Few studies as yet have addressed the accuracy of these 'reverse testing' algorithms^{40,143}. The traditional and reverse approaches should theoretically produce the same result. However, the reverse algorithm results in the detection of patients with early syphilis (TT-positive, NTT-negative) who would not be detected by the conventional approach¹⁴⁴. As this pattern of serological reactivity occurs in very early primary syphilis, in previously treated disease and late infection, considerable attention should be given to a thorough physical examination of the patient and assessment of previous history and recent sexual risk factors before initiating any treatment and partner notification activities.

Rapid tests. Rapid POC TTs are a recent technology that enable onsite screening and treatment and are particularly useful in settings with limited laboratory capacity. Rapid syphilis tests use a finger-prick whole blood sample and are typically immuno-chromatographic strip-based TT assays using components that can be stored at room temperature, require no equipment and minimal training and give a result in <20 minutes¹⁴⁵ (TABLE 2). Various rapid tests have been evaluated in a range of clinical and community settings and shown to fulfil the ASSURED criteria^{134,146–154}. Like other TTs, most POC diagnostics have the limitation of being unable to distinguish between recent and previously treated syphilis infections and, therefore, could lead to overtreatment. Ideally, patients who test positive to a POC TT would be further evaluated with an NTT to support management decisions; however, this is often not possible in settings with limited laboratory capacity as is the case in many antenatal care clinics and outreach programmes for high-risk populations. Rapid POC tests play an important part when delayed diagnosis is problematic, such as in pregnant women in whom delayed or no treatment poses substantial risks to the fetus that far outweigh the risks of overtreatment for the mother^{45,155}. In nonpregnant individuals who test positive, the recommendations are treatment for those who have no prior history of treatment and referral to have an NTT for those with a prior history¹¹.

At least one test has been developed that enables the simultaneous detection of nontreponemal and treponemal antibodies in a single POC device^{156–158}. Additionally, rapid, dual syphilis and HIV tests are available to screen for HIV and treponemal antibodies using a single lateral-flow immuno-chromatographic strip. These tests are increasingly important tools for the global elimination of MTCT of HIV and syphilis in settings in which laboratory capacity is limited¹⁵⁹.

Table 2 | Serological tests for *Treponema pallidum*

Method	Sample	Advantages	Disadvantages
NTTs			
Venereal Disease Research Laboratory (VDRL) slide test	Serum, plasma or CSF	<ul style="list-style-type: none"> • Can be used to monitor treatment efficacy • Only test suitable for diagnosis of neurosyphilis using CSF • Inexpensive and relatively simple • Results can be available in <15 minutes • Sensitivity of 71–100% depending on disease stage • Specificity of 98% 	<ul style="list-style-type: none"> • False positives due to cross reactivity with acute and chronic conditions • Must be performed manually • Subjective • Requires a microscope • Antigen suspension must be freshly prepared (not commercially available) • Cannot be used on whole blood; requires a centrifuge
Rapid plasma reagin (RPR) or toluidine unheated serum test (TRUST)	Serum or plasma	<ul style="list-style-type: none"> • Can be used to monitor treatment efficacy • Inexpensive and relatively simple compared with VDRL • Does not require a microscope as results can be read by eye • Antigen suspension can be purchased commercially and is stable (does not require fresh preparation) • Results can be available in <15 minutes • Sensitivity of 73–100% depending on the disease stage • Specificity of 98% 	<ul style="list-style-type: none"> • False positives can occur in dusty settings (for example, rural clinics) and due to cross reactivity with acute and chronic conditions • False negatives can occur in early primary syphilis or due to the prozone effect • Must be performed manually • Subjective • Cards cannot be reused • Cannot be used on whole blood; requires a centrifuge
Tts			
Fluorescent treponemal antibody absorption (FTA-ABS) test	Serum, plasma or CSF	<ul style="list-style-type: none"> • Considered the gold standard in many middle-income and low-income countries • Sensitivity of 96–100% depending on disease stage • Specificity of 99% 	<ul style="list-style-type: none"> • Not recommended for the resolution of discordant TT and NTT results due to potentially lower sensitivity and specificity than other TTs^{132,204} • Time consuming, expensive and difficult to read; requires specialized reagents and a microscope • Manual operation • Occasional biological false-positive results during pregnancy or in patients with autoimmune disease
<i>T. pallidum</i> particle agglutination (TPPA)	Serum or plasma	<ul style="list-style-type: none"> • Inexpensive and widely available • Sensitivity of 82–100% depending on disease stage • Specificity of 99% 	<ul style="list-style-type: none"> • Must be performed manually • Subjective to individual interpretation
<i>T. pallidum</i> haemagglutination (TPHA) and micro-haemagglutination assays (MHA-Tp)	Serum or plasma	<ul style="list-style-type: none"> • Inexpensive, although less widely available than TPPA • Sensitivity of 82–100% depending on disease stage • Specificity of 99% 	<ul style="list-style-type: none"> • Must be done manually • Subjective to individual interpretation
Treponemal enzyme immunoassay (EIA)	Serum	<ul style="list-style-type: none"> • Can be automated, especially suitable for high-throughput screening of asymptomatic populations and blood or plasma donors • Sensitivity of 82–100% depending on disease stage • Specificity of 99% 	<ul style="list-style-type: none"> • Expensive and requires laboratory equipment • IgM EIA has low sensitivity in active syphilis, not helpful in staging or evaluating treatment efficacy
Chemiluminescence immunoassay (CIA)	Serum	<ul style="list-style-type: none"> • Can be automated, especially suitable for high-throughput screening of asymptomatic populations and blood or plasma donors • Sensitivity of 82–100% depending on disease stage • Specificity of 99% 	<ul style="list-style-type: none"> • Expensive and requires specialized equipment
Rapid tests			
TT	Whole blood, plasma or serum	<ul style="list-style-type: none"> • Allows a presumptive diagnosis and treatment at the clinical visit • Easy to use; results in <20 minutes • Relatively inexpensive • Median sensitivity of 86%²⁵⁰; pooled sensitivity of 84% (serum test) and 80% (whole blood)²⁵¹ 	<ul style="list-style-type: none"> • Cannot distinguish between new and prior, previously treated infections
Dual TT and NTT	Whole blood, plasma or serum	<ul style="list-style-type: none"> • Rapid, easy to use and enables testing and treatment at the clinical visit • Can distinguish between new and previously treated infections • Overall performance for diagnosis of active infection of 88.3% (range 87.1–89.4%)^{156–158} 	<ul style="list-style-type: none"> • Can be more costly than traditional TTs on a per-test basis
Dual syphilis and HIV tests	Whole blood, plasma or serum	<ul style="list-style-type: none"> • Can detect treponemal and HIV antibodies in the same test using a single finger-prick specimen • HIV component of the test shows 98% agreement with laboratory-based assays • Syphilis component of the test shows 85% agreement with laboratory-based assays 	<ul style="list-style-type: none"> • Lower sensitivity and specificity for syphilis than for HIV

The data on sensitivity and specificity of each test with respect to disease stage are reported in the WHO Manual on Laboratory Diagnosis of Sexually Transmitted Infections, including HIV²⁵². CSF, cerebrospinal fluid; NTT, nontreponemal test; TT, treponemal test.

Tests useful in special situations

Neurosyphilis. The diagnosis of neurosyphilis is challenging. The CSF is frequently abnormal in patients with neurosyphilis, with both pleocytosis (lymphocyte accumulation) and an increased protein concentration. The VDRL assay performed on CSF is considered the gold standard for specificity but is recognized to have limited sensitivity^{160,161}. Other CSF tests, including serological assays, such as the RPR¹⁶², FTA-ABS¹⁶³ and TPHA tests¹⁶⁴, and molecular assays, such as PCR¹⁶⁵, have all been assessed and show differences in their specificity and sensitivity for the diagnosis of neurosyphilis.

Figure 7 | Screening algorithms for syphilis. a | The traditional algorithm begins with a qualitative nontreponemal test (NTT) that is confirmed with a treponemal test (TT). This algorithm has a high positive predictive value when both tests are reactive, although early primary and previously treated infections can be missed owing to the lower sensitivity of NTTs¹³⁶. Importantly, this algorithm is less costly than reverse screening algorithms and does not require highly specialized laboratory equipment, but it is limited by the subjective interpretation of the technologist. Additionally, false-negative NTT results can arise from the prozone effect (when there is an excess of antibody). Finally, because the traditional algorithm is not always followed by a confirmatory TT, previously treated, early untreated and late latent patients can be missed, and biologically false-positive patients can be overtreated. **b** | The reverse screening algorithm uses a TT with recombinant *T. pallidum* antigens in enzyme immunoassay (EIA) or chemiluminescence immunoassay (CIA) formats that, when reactive, is followed by an NTT. This approach is associated with higher initial setup costs and ongoing operational costs than the traditional algorithm, but the algorithm permits treatment of 99% of patients with syphilis, which is higher than the percentage treated on the basis of the traditional algorithm in a low-prevalence setting²⁴⁶. However, this approach is at the expense of serodiagnosis in patients without risk factors and, therefore, who are unlikely to have syphilis, leading to potential overtreatment and any psychosocial consequences associated with a false positive result. Additionally, because TTs are not flocculation assays, false-negative test results due to the prozone effect do not occur. However, in high-risk populations, screening with a TT can result in a high rate of positive results due to previously treated infections, leading to an increase in the clinician workload needed to review cases and determine appropriate management. Some guidelines recommend further evaluation of reactive TT results with a quantitative NTT and, if results of the latter are nonreactive, a second (different) TT to help resolve the discordant results^{143,247,248}. The European Centre for Disease Prevention and Control uses a variation of this approach: a reactive TT immunoassay is followed by a second (different) TT of any kind (that is, not followed by an NTT)²⁴⁹. Ideally, a positive TT result should be supplemented by another TT or an NTT. However, in most developing countries, particularly given the serious consequences of syphilis during pregnancy, treatment is recommended for a patient with a positive TT result. RPR, rapid plasma reagin test; TPHA, *T. pallidum* haemagglutination assay; TPPA, *T. pallidum* particle assay; VDRL, Venereal Disease Research Laboratory test.

Difficulties in the interpretation of CSF pleocytosis in individuals co-infected with HIV and syphilis make it challenging to evaluate the relationship between the two diseases. CSF pleocytosis occurs in individuals with either infection alone^{37,165}; thus, discerning the cause of pleocytosis in individuals with co-infections is not always possible.

Congenital syphilis. Diagnosing congenital syphilis in exposed, asymptomatic infants is another area of testing that can be improved. Because maternal nontreponemal and treponemal IgG antibodies can be transferred from mother to child, treponemal testing of infant serum is difficult to interpret and is not recommended³⁷. An infant with a reactive RPR or VDRL serum titre that is at least fourfold that of the mother is highly suggestive of congenital syphilis, but its absence does not exclude a diagnosis. A clinical examination, reactive infant CSF VDRL assay results, abnormal complete blood count or liver function test results or suggestive long-bone radiographs (that, for example, show retarded ossification or dislocation of epiphyses and radiolucencies (low-density areas)) can support a diagnosis of congenital syphilis. Use of IgM immunoblots is controversial due to the limited availability of tests and inconclusive data thus far on their sensitivity; their use in diagnosing congenital syphilis is recommended in some guidelines^{11,13} but not others³⁷. Maternal syphilis infection is highly correlated with fetal loss; thus, the evaluation of a stillborn infant should include an evaluation of maternal test results for syphilis¹¹.

Screening

The wide availability of effective treatments and resulting decline in syphilis prevalence has led to a low yield of screening in low-prevalence settings; thus, screening in low-risk adults (for example, premarital adults or those admitted to hospital) has been abandoned in most places. However, systematic reviews provide convincing evidence in favour of syphilis screening for pregnant women^{13,166}, adults and adolescents at increased risk of infection^{13,40} and individuals donating blood, blood products or solid organs^{13,167–169}. Several countries also recommend syphilis testing in people with unexplained sudden visual loss, deafness or meningitis as these may be manifestations of early neurosyphilis^{13,37}.

Prenatal screening. Syphilis screening is universally recommended for pregnant women, regardless of previous exposure, because of the high risk of MTCT during pregnancy and the availability of a highly effective preventive intervention against adverse pregnancy outcomes^{11,37,41,46}. Global normative authorities and most national guidelines recommend syphilis screening at the first prenatal visit, ideally during the first trimester^{11,37,41,170}. Some countries recommend that women at high risk have repeat screening in the third trimester and again at delivery to identify new infections³⁷. Women should be tested during each pregnancy, even if they tested negative in a previous pregnancy. When access to prenatal care is not optimal or laboratory capacity is

Box 1 | WHO guidelines for the treatment of syphilis

Early syphilis

- Intramuscular benzathine penicillin G (single dose)
- Or intramuscular procaine penicillin (daily doses for 10–14 days)
- If penicillin-based treatment cannot be used, oral doxycycline (two doses daily for 10–14 days)* or intramuscular ceftriaxone (daily doses for 10–14 days)

Late syphilis

- Intramuscular benzathine penicillin G (weekly doses for 3 weeks)
- Or intramuscular procaine penicillin (daily doses for 20 days)
- If penicillin-based treatment cannot be used, oral doxycycline (daily doses for 30 days)*

Congenital syphilis

- Intravenous aqueous benzyl penicillin (daily doses for 10–15 days)
- Or intramuscular procaine penicillin (daily doses for 10–15 days)

*Contraindicated during pregnancy. From REF. 11

limited, rapid tests have been shown to be beneficial in detecting and treating syphilis in pregnant women¹⁴⁸. Guidelines recommend that after delivery, neonates should not be discharged from the health facility unless the serological status of the mother had been determined at least once during pregnancy and preferably again at delivery^{11,37}.

The importance of universal syphilis screening in pregnancy to prevent perinatal and infant morbidity and mortality is highlighted in the current WHO global initiative to eliminate congenital syphilis^{43,44} and is justified by the continuing high global burden of congenital syphilis, availability of an effective and affordable preventive intervention and wider availability of low cost rapid POC tests that can be used when laboratory capacity is lacking^{23,43,44,46,145}. A systematic review of studies (most of which were conducted in low-income countries) reporting on antenatal programmes initiating or expanding syphilis screening, compared with various local control conditions, found that enhanced screening reduced syphilis-associated adverse birth outcomes by >50%¹⁷¹. Integration of syphilis testing with other prenatal interventions, including HIV testing, has been shown to be cost-effective across settings, even when syphilis prevalence is low^{48–51}. Strategies that enhance screening coverage, such as increased use of rapid POC testing and integrating syphilis and HIV screening, will further support the global elimination of congenital syphilis^{145,172–174}.

Screening at-risk populations. Increased risks of infection can be related to personal or partner behaviours leading to syphilis infection or living in a community with a high syphilis prevalence^{37,40}. In many countries, syphilis testing is recommended for all attendees at STI or sexual health centres and as part of integrated services targeted to high-risk groups (such as HIV testing centres or drug treatment centres)^{13,37}. The optimal screening interval for individuals at an increased risk of infection is not well established; however, some guidelines suggest that MSM or people with HIV show a greater benefit from more frequent screening than others at risk of syphilis infection (for example, testing every 3 months rather than a single annual screening)^{37,40,175,176}.

At-risk communities are often marginalized from care and experience discrimination and stigma when using traditional STI services¹⁷⁷. Innovations in promoting the uptake of testing and developing user-friendly services are important in the control of syphilis in these communities to reduce transmission. Social entrepreneurship and crowdsourcing approaches have been shown as innovative approaches to improve HIV and syphilis testing coverage rates and accelerate linkage to care, two fundamental elements within the cascade of STI service delivery^{178,179}. Studies evaluating other interventions, such as pre-exposure prophylaxis for syphilis, are also underway¹⁸⁰. One future option might be to administer pre-exposure prophylaxis simultaneously for syphilis and HIV¹⁸¹.

Blood-bank screening. Although syphilis was among the first identified infectious risks for blood donation and syphilis transmission through blood has been documented^{182–184}, reports of transfusion-transmitted syphilis have become exceedingly rare over the past 60 years as more countries adopt donor selection processes, universal serological screening of donors and the use of refrigerated products rather than fresh blood components^{183,185}. The survival of *T. pallidum* in different blood components has been shown to vary according to storage conditions, with fresh blood or blood components stored for <5 days being more infectious than blood stored for longer periods¹⁸³. Screening of blood, blood components and solid organs for syphilis remains a recommendation in many countries^{13,169}. Occasional cases of transfusion-transmitted syphilis are still reported in settings with high syphilis prevalence, particularly with the transfusion of fresh blood¹⁶⁷.

Prevention

There is as yet no vaccine against syphilis; the most effective mode of prevention is prompt treatment to avoid continued sexual transmission or MTCT, and the treatment of all sex partners to avoid reinfection. Other prevention modalities against the sexual transmission of syphilis are latex condom use, male circumcision and avoiding sex with infected partners³⁷. Treating exposed sex partners is important to avoid reinfection³⁷.

Management

Important factors in managing syphilis are early detection, prompt treatment with an effective antibiotic regimen and treating sex partners of a person with infectious syphilis (primary, secondary or early latent infections). The WHO guidelines¹¹ (BOX 1) and European guidelines¹³ for the management of early syphilis in adults are the same. The US Centers for Disease Control and Prevention (CDC) guidelines do not suggest procaine penicillin as a treatment but are otherwise identical¹². Patients with late syphilis are no longer infectious. Thus, the objective of treatment is to prevent complications in persons who are asymptomatic (that is, those who have late latent syphilis) or arrest disease development if the patient has manifestations of tertiary disease. Treating late syphilis requires longer courses of antimicrobial therapy than treating early syphilis.

Penicillin

Penicillin has been the mainstay of treatment for syphilis since it first became widely available in the late 1940s. Although its efficacy was never demonstrated in a randomized controlled trial, it was clearly far superior to all previous treatments, and *T. pallidum* resistance to penicillin has never been reported. As *T. pallidum* divides slower than most bacteria, it is necessary to maintain penicillin levels in the blood above the minimum inhibitory concentration for ≥ 10 days; this can be achieved by giving a single intramuscular injection of long-acting benzathine penicillin G (which benefits from not requiring patient adherence to a long-term drug regimen). The first-line treatments for early syphilis recommended by the CDC and European (authored by the International Union Against Sexually Transmitted Infections) guidelines are very similar^{12,13}, as are recommendations for treating exposed sex partners. Patients with late syphilis, or with syphilis of unknown duration, should receive longer courses of treatment (BOX 1). Those with symptoms suggestive of neurosyphilis or ocular involvement should undergo lumbar puncture to confirm or rule out the presence of neurosyphilis, which requires more-intensive treatment. However, CDC and European guidelines define latent syphilis as occurring beginning at 1 year after infection, whereas the WHO defines latent syphilis to occur beginning at 2 years, resulting in some differences in management; that is, a longer treatment duration is recommended for some patients in the United States and Europe.

Given that confirmation or exclusion of the presence of viable *T. pallidum* after treatment is not possible, treatment efficacy is measured indirectly using serology. Being cured is usually defined as reversion to a negative serostatus or a fourfold reduction in the titre from an NTT. However, as noted earlier, a minority of patients remain seropositive, with a less than fourfold reduction in their NTT titre, in spite of almost certainly having been cured and with no evidence of progressive disease — the so-called serofast state¹⁸⁶. The management of these patients depends on taking a careful sexual history to exclude the possibility of reinfection, which can be challenging as patients may not recognize new infections. The serofast state more commonly occurs in patients with late syphilis and low NTT titres and in patients positive for HIV who are not on antiretroviral treatment¹⁸⁷. Because few data are available on long-term clinical outcomes in serofast patients, CDC guidelines recommend continuing clinical follow-up and retreatment if follow-up cannot be ensured¹².

Second-line treatments

Patients who are allergic to penicillin should be treated with doxycycline or ceftriaxone (although an allergy to cephalosporins is more common in those who are allergic to penicillin) with repeat NTT serology as a follow-up. Doxycycline is contraindicated during pregnancy. Two treatment trials of early syphilis in Africa showed that a single oral dose of azithromycin was equivalent to treatment with benzathine penicillin G (REFS 188,189). Unfortunately, strains of *T. pallidum* with a mutation that

confers resistance to azithromycin and other macrolide antibiotics are common in the United States, Europe, China and Australia^{190–194}. A study of HIV-positive patients with syphilis showed that administering azithromycin to prevent opportunistic infections led to better serological outcomes¹⁹⁵. The WHO recommends the use of azithromycin for the treatment of syphilis only in settings where the prevalence of macrolide-resistant *T. pallidum* is known to be very low.

HIV co-infection

In patients with early syphilis, an increased cell count and protein concentration are found more frequently in the CSF of patients with an HIV infection than in patients without an HIV infection, and there is some evidence that early symptomatic neurosyphilis is more common in patients positive for HIV^{196,197}. As single-dose benzathine penicillin G treatment does not reliably lead to treponemicidal levels in the CSF, some experts have suggested that HIV co-infected patients with early syphilis should receive enhanced treatment¹⁹⁸. However, a randomized controlled trial ($n = 541$) showed no significant difference in clinical outcomes between patients receiving a standard or enhanced treatment¹⁵. Notably, the 101 patients infected with HIV enrolled in the trial responded less well serologically, but due to loss at follow-up, the study was underpowered to detect a twofold difference in the standard versus enhanced treatment in patients co-infected with HIV. Furthermore, a large ($n = 573$) prospective observational study in Taiwan found no difference between single-dose benzathine penicillin G and enhanced treatments in a per-protocol analysis¹⁹⁹. However, using a last-observed-carried-forward analysis to account for missing data, the authors concluded that 67.1% of those who received one dose responded serologically compared with the 74.8% response rate in those who received the enhanced treatment, a statistically significant difference ($P = 0.044$)¹⁹⁹. Finally, a retrospective study ($n = 478$) showed no difference in serological response rates at 13 months between those receiving a single-dose of benzathine penicillin G or enhanced treatment²⁰⁰. Given the inconclusive results of these studies, many clinicians continue to offer enhanced therapy to patients with early syphilis and HIV co-infection.

Treatment during pregnancy

Adverse pregnancy outcomes are common in women with syphilis^{45,119}. A study in Tanzania found that of women with latent syphilis who had RPR titres $\geq 1:8$, 25% delivered a stillborn infant, and 33% delivered a live but preterm infant²¹. A second study showed that adverse pregnancy outcomes due to syphilis can be prevented with a single dose of benzathine penicillin G given before 28 weeks of gestation²⁰¹ and that, in this setting in which 5–6% of pregnant women had syphilis, this was one of the most cost-effective interventions available in terms of cost per disability-adjusted life years saved²⁰².

Penicillin is the only antibiotic known to be effective in treating syphilis during pregnancy and preventing adverse birth outcomes. Given that doxycycline

is contraindicated during pregnancy, and macrolides such as azithromycin and erythromycin do not cross the placenta effectively, there are few alternatives to penicillin for the treatment of pregnant women with syphilis who are allergic to penicillin. The CDC recommends desensitization for those who are allergic to penicillin¹².

Congenital syphilis

The WHO recommends that infants with suspected congenital syphilis, including infants who are born to mothers who are seropositive for syphilis and not treated with penicillin >30 days before delivery, should be treated with aqueous benzyl penicillin or procaine penicillin (BOX 1). All infants exposed to syphilis, including infants without signs or symptoms at birth, should be followed closely, ideally with NTT titres. Titres should decline by 3 months of age and be nonreactive by 6 months¹². TTs are not useful in infants due to persistent maternal antibodies.

Neurosyphilis and ocular syphilis

Involvement of the CNS can occur during any stage of syphilis, but there is no evidence supporting a need to deviate from recommended syphilis regimens without the presence of clinical neurological findings (such as ophthalmic or auditory symptoms, cranial nerve palsies, cognitive dysfunction, motor or sensory deficits or signs of meningitis or stroke)²⁰³. With symptoms and tests indicating neurosyphilis, or any suggestion of ocular syphilis regardless of CSF testing, more-intensive treatment is recommended. For example, the CDC recommends that adults with neurosyphilis or ocular syphilis should be treated with high-dose intravenous aqueous crystalline penicillin, or intramuscular procaine penicillin plus probenecid, for 10–14 days²⁰⁴.

Quality of life

Historical reports dating from the 15th century indicate that syphilis was perceived as a dangerous infection and a source of public alarm via fear of contagion and dread of its manifestations, as well as anxiety around its highly toxic 'cures' (heavy metal therapy with mercury, arsenic or bismuth)^{205–207}. Case reports through the 19th century, as well as modern re-evaluations of skeletal remains, support the fact that the disease could cause severe physical stigmata, with individuals having disfiguring rashes, nonhealing ulcerations, painful bony lesions that often involved destruction of the nose and palate, visceral involvement, dementia and other incapacitating neurological complications and early death²⁰⁸. Stigmatization associated with syphilis was also evident, with symptomatic patients quarantined to specialized hospitals, and affected people hiding their symptoms, perhaps fearing societal shunning or the dubiously effective treatment regimens even more than they feared the disease²⁰⁹. Reductions in syphilis prevalence were documented after the introduction of penicillin²¹⁰, and since that time, the most virulent manifestations of the disease have almost vanished; today it is rare to find a patient with tertiary disease²¹¹. Nevertheless, continuing reports emphasize that complications of late syphilis, particularly those

involving the eyes, CNS and cardiovascular system, can cause lifelong disability and even death⁹. For example, the number of cases of ocular syphilis has increased with rising syphilis incidence in many communities²¹², with delayed treatment associated with permanently diminished visual acuity²¹³. Thus, caregivers must be cognizant of the need to screen at-risk patients for latent infection and administer therapy if previous treatment has not been documented.

Few modern studies have addressed quality of life in men and women with syphilis, whether in social, psychological or economic contexts. One study ($n = 250$) showed only a minor effect on patient-reported quality of life at time of treatment and essentially no effect 1 month after treatment²¹⁴. The currently high case rates of syphilis infection and reinfection among MSM in urban centres throughout the world might lend support to the notion that syphilis in the modern era poses a limited impact on quality of life as long as it is detected and treated. However, partner notification studies suggest that STI diagnoses can lead to substantial social stigma, intense embarrassment and fear of retaliation, domestic violence or loss of relationships¹⁷⁷. Public health experts have posited that syphilis is the source of more stigma than other STI diagnoses, although this is difficult to measure with certainty because STI programmes tend to focus contact tracing efforts more strongly on syphilis than on other curable STIs owing to its serious consequences²¹⁵. In one study measuring the level of shame associated with several stigmatizing skin diseases, patients assigned the greatest shame to syphilis — more than to HIV/AIDS, other STIs or several disfiguring skin conditions²¹⁶.

Untreated maternal syphilis results in severe adverse perinatal outcomes, most prominently stillbirth, in at least half of affected pregnant women⁴⁵. Although MTCT of syphilis is clearly linked to a lack of prenatal care, WHO data indicate that globally, whether in wealthy or poor nations, most adverse pregnancy outcomes caused by maternal syphilis are in women who attended prenatal care but were not adequately tested or treated²⁴. This suggests that other factors, such as ineffective health systems, gender inequality, lack of political will to support quality STI and reproductive health services or other structural influences associated with a lack of screening might be at play²¹⁷. Increasing research supports the conclusion that, as for infant loss, a stillbirth can lead to poor mental and other health outcomes for both parents and the wider family, even extending to health care providers. For example, experiencing a stillbirth has been linked to 'unspoken grief' and a variety of psychosocial consequences such as depression, blame, shame, social isolation, problems in future pregnancies and relationship dissolution^{218–220}. In Haiti, pregnancy loss associated with syphilis is so common (maternal prevalence of 6%) that a myth about a werewolf sucking the blood out of the unborn fetus has developed to help women with their loss and suffering²²¹. Economic research suggests that a stillbirth results in substantial direct and indirect costs and can sometimes require more resources than a livebirth²¹⁹.

Box 2 | Major challenges and a call to action wish list

Eliminate mother-to-child transmission of syphilis

- Requires political commitment
- Prenatal syphilis screening to be integrated into mother-to-child transmission elimination programmes for HIV or as a component of an essential diagnostic package for prenatal care
- Develop point-of-care tests with data connectivity or data transmission capability to facilitate automated surveillance and to improve the efficiency of health systems

Address HIV and syphilis co-infection in MSM

- Requires research into potential synergies between the two infections
- Implement scientific and community involvement to reach at-risk populations
- Integrate programmes for HIV, syphilis, hepatitis and other sexually transmitted infections

Develop tests for active infection, neurosyphilis and congenital syphilis

- Identify and validate biomarkers for test development
- Develop a network of clinical sites for rapid validation of new tests

Develop new oral drugs to prevent transmission to fetus and to sex partners

- Provide incentives for drug discovery programmes
- Provide incentives to evaluate drug combinations

Develop vaccines

- Requires research to better understand syphilis pathogenesis
- Requires research to identify vaccine targets and methods for validation

Outlook

With syphilis continuing to be the leading cause of preventable stillbirths in the developing world and re-emerging as a public health threat in developed nations, particularly in MSM co-infected with HIV, the demand for improved diagnostics, prevention strategies and treatments is growing. Here, we describe the most pressing issues and propose a call to action (BOX 2).

Elimination of MTCT of syphilis

The WHO campaign to eradicate yaws, which treated >50 million people with penicillin and reduced the number of cases by ≥95% worldwide between 1952 and 1964, was ultimately unsuccessful. What can we learn from this heroic failure? The yaws eradication campaign was based on clinical examination and serological testing to determine prevalence by community and on mass treatment or selective mass treatment (patients and contacts) of communities with penicillin depending on prevalence. Unfortunately, as the prevalence of yaws fell, it was no longer perceived as an important public health problem worthy of an expensive vertical programme; resources were diverted to other programmes, yaws was forgotten, and it re-emerged²²². To some extent the same is true of syphilis; once penicillin became available, its incidence and prevalence declined in many parts of the world, and it was no longer seen as a public health priority. Although screening of all pregnant women for syphilis has continued to be recommended in most countries, coverage has been low in many regions; for example, WHO estimates that approximately 50% of antenatal clinic attendees in Africa are not currently screened for syphilis²⁴. This low coverage has resulted in a high burden of entirely preventable

stillbirths and neonatal deaths²³. Exacerbating this situation, the WHO has received reports of depleted stocks and shortages of injectable benzathine penicillin G in multiple countries, many with a high burden of maternal and congenital syphilis. In collaboration with international partners, the WHO has spearheaded an initiative to assess the global supply, current and projected demand and production capacity for benzathine penicillin G (REF. 223).

Strong advocacy will be needed to ensure that the control and elimination of syphilis is given a high priority on the global health agenda. Policy makers and funders need to be made aware that syphilis is a leading cause of preventable stillbirths and neonatal death, that these deaths can be prevented with a single dose of penicillin given to the mother before 28 weeks of gestation and that this is one of the most cost-effective health interventions available^{51,202}. Perhaps with this awareness and political will, syphilis MTCT elimination programmes, which have failed to progress in the past 10 years²²⁴, will witness the same success achieved in the MTCT of HIV programmes in Africa. Other developments are occurring that are forging change. For example, the availability of POC tests has led to increased coverage of antenatal screening and treatment for syphilis in many settings¹⁴⁸, and the WHO campaign for the elimination of MTCT of HIV and syphilis has increased the visibility of syphilis on the global health agenda. In 2014, the WHO target for the elimination of MTCT of syphilis was ≤50 cases of congenital syphilis per 100,000 live births. The targeted processes are antenatal care coverage (at least one visit) of ≥95% of pregnant women, syphilis testing coverage for ≥95% of pregnant women and treatment of ≥95% of pregnant women seropositive for syphilis²²⁵. Additionally, the WHO has conducted a systematic review of the performance of rapid, dual HIV and syphilis tests and issued an information note on testing algorithms for dual HIV and syphilis tests²²⁶.

The huge reduction in the number of infants positive for HIV in Africa in recent years, a more difficult undertaking than reducing MTCT of syphilis, is proof of concept that congenital syphilis elimination is achievable. Given that Cuba, Thailand, Belarus, Moldova and Armenia have eliminated MTCT of HIV, syphilis or both, elimination can be achieved with political will and a well-organized health care system. Indeed, inclusion of syphilis and HIV screening with tests for anaemia, diabetes and pre-eclampsia as a package of essential diagnostics for prenatal care should be implemented as a minimum standard to ensure safe and healthy pregnancies worldwide.

The use of POC testing has greatly increased access to screening for pregnant women and has the potential to increase access to screening for high-risk groups such as MSM and FSWs through outreach programmes. However, the quality of testing must be assured given that these tests are conducted outside the laboratory. Strategies to ensure the reliability of POC tests include the use of electronic readers²²⁷ and microfluidic assays powered by smart phones²²⁸ for real-time

monitoring of progress²²⁹ and the routine provision of proficiency testing panels^{121,122}. For example, one study in the Amazon region of Brazil showed that proficiency panels consisting of dried serum tubes that were assessed by each health care worker could be used to monitor the performance of health care workers in remote settings¹²³.

HIV and syphilis co-infection in MSM

In developed countries, the incidence of syphilis in MSM is several hundred times higher than in the general population. Furthermore, the incidence continues to increase as condom use has fallen with increasing use of pre-exposure prophylactic antiretroviral medications for HIV^{42,230}. Indeed, with wider HIV treatment coverage in recent years and HIV no longer considered a 'death sentence', safe sex practices have declined and risk-taking behaviours have increased²³¹. However, the alarming increase in the incidence of syphilis, compared with that of other STIs, in HIV-infected MSM cannot be explained by behavioural factors alone. The frequent co-infection of HIV and syphilis in MSM in many countries has led researchers and policy makers to consider the hypothesis that treatment for HIV may be a double-edged sword that contributes to increased susceptibility to syphilis through impairment of the innate or acquired immunity to *T. pallidum*^{42,232}.

Accordingly, research is urgently needed to understand the underlying causes of this twin epidemic. The involvement of the MSM community is critical in the design and implementation of innovative approaches to promote the uptake of testing and linkage to care, particularly as this community is still stigmatized and marginalized from care in many societies. Although self-testing for HIV and hepatitis C virus infection is now possible using highly sensitive and specific oral tests that are commercially available, syphilis does not elicit sufficient antibody levels for an oral test. Thus, implementation science is needed to integrate and optimize the delivery of a package of HIV, syphilis, hepatitis and other STI screening and treatment strategies and partner notification systems for MSM in different cultural, socioeconomic and political settings.

Better diagnostic tests

Research is needed to identify biomarkers that can more accurately distinguish between past, treated syphilis infections and active infections requiring treatment, can identify patients who have become reinfected and can provide a test of cure. Using current serological tools, a high proportion of patients have been shown to remain serofast after treatment in some settings, and the optimal management of these individuals is uncertain. Additionally, more-accurate diagnostic tests are needed to confirm the diagnosis of congenital syphilis, as serological tests based on IgG antibodies cannot distinguish between infected infants and those with passively acquired maternal antibodies. IgM tests can be highly sensitive in symptomatic infants but have sub-optimal sensitivity in infants who are infected but not symptomatic at birth¹².

The diagnosis of neurosyphilis also remains a challenge, particularly in patients co-infected with HIV, in whom an increased CSF protein concentration or cell count does not necessarily indicate that the patient has neurosyphilis. Promisingly, a rapid POC test has been adapted for the diagnosis of neurosyphilis using CSF²³³; the performance of this test is better in cell-free specimens, requiring the use of a centrifuge. Another promising assay might be the measurement of macrophage migration factor (MIF); measurement of CSF levels of MIF alone was shown to have a sensitivity of 74.42% and a specificity of 67.74% for the diagnosis of neurosyphilis in one study ($n = 43$)²³⁴. By integrating all CSF parameters (pleocytosis, increased protein concentrations and MIF), the sensitivity and specificity would be improved. Additionally, assays of B cell attractant chemokine CXCL13 in the CSF could be used to distinguish the pleocytosis caused by HIV from that caused by neurosyphilis in patients infected with HIV²³⁵.

Better use of existing drugs

With the use of penicillin, many countries still struggle with the fear of injections on the part of patients and the management of anaphylactic shock on the part of the health care providers. Oral regimens that are safe for use during pregnancy and effective in preventing the transmission of syphilis to the fetus are urgently needed. Furthermore, macrolide resistance is correlated with treatment failure in patients with primary syphilis¹⁹¹, lending further urgency to the need to find alternative oral therapies. Incentives for a drug discovery programme for syphilis need to be established and, in the meantime, evaluation of existing drug combinations might be useful as alternatives to reduce the threat of developing resistance.

Vaccine development

Human-challenge studies have shown that people with late latent syphilis are resistant to symptomatic reinfection with heterologous strains of *T. pallidum*, and protective immunity has been induced in rabbits by repeated inoculation with γ -irradiated *T. pallidum*^{236,237}. Accordingly, it should be possible to develop protective vaccines. However, research on virulence determinants of *T. pallidum* and our understanding of protective immunity against *T. pallidum* have been hindered by our inability to culture the bacteria *in vitro*. Genome sequencing of *T. pallidum* directly from clinical samples is now possible, which can overcome this limitation^{92,238}. This advance should enable the understanding of strain variation on a global scale and help to identify outer membrane proteins and other surface antigens as possible vaccine candidates⁸¹. A recent study showed that the immunization of rabbits with the lipoprotein TP0751 prevented the dissemination of *T. pallidum* and, hence, has become a promising vaccine candidate⁸¹. Integrating potential vaccine targets with diagnostic targets in discovery programmes also holds promise in accelerating progress towards the development of improved tools for the control, prevention and, ultimately, elimination of this disease.

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Author contributions

Introduction (R.W.P. and D.M.); Epidemiology (D.M. and X.-S.C.); Mechanisms/pathophysiology (J.D.R.); Diagnosis, screening and prevention (R.W.P., M.L.K., X.-S.C. and A.S.B.); Management (D.M.); Quality of life (M.K. and A.S.B.); Outlook (all authors); overview of the Primer (R.W.P.).

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