ANTIGEN COMBINATION AND METHODS AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to an antigen combination comprising *Treponema pallidum* (Tp) antigens. The present invention also relates to vectors, polynucleotides, host cells and methods for producing such antigen combinations. The present invention also relates to method and uses of such antigen combinations. The present invention also relates to compositions, assays and kits and the methods and uses thereof.

BACKGROUND OF THE INVENTION

The bacterial spirochete *Treponema pallidum* (Tp) causes the sexually transmitted infection known as syphilis in humans with approximately 7 million new infections reported in 2020 (WHO report). Syphilis is transmitted through sexual contact with infectious lesions, via blood transfusion, or from mother to foetus. Mother-to-foetal transmission is the most devastating form of infection causing adverse birth outcomes in 50–80% of cases which include still birth, neonatal death, prematurity, low birth weight and congenitally infected infants. Between 2012 and 2016, the prevalence of active syphilis increased in 78 countries (Korenromp et al., 2018). Syphilis infections are also increasing in men who have sex with men, where the prevalence of syphilis is 7.5% compared to the prevalence in the general population of 0.5% (Tsuboi et al., 2021). There has been a 26% increase in the number of new syphilis infections in 2021 with calls for better diagnosis and treatment to curtail new cases (Harris, 2023).

Tp infection is an invasive infection resulting in local inflammation due to the replication of the spirochete and progresses through defined stages. Primary syphilis can cause a single painless ulcer (chancre) or multiple painless lesions, usually on the genitals but can occur elsewhere, along with lymphadenopathy occurring within 3 weeks of exposure. Lesions may go unnoticed depending on their severity. After 6–8 weeks, this stage resolves and patients progress to secondary syphilis, with symptoms such as fever, rash, and headache. If the infection is undiagnosed, patients can then progress to early latent syphilis which can last for years. During the first 1–2 years, patients are considered infectious as they may relapse into secondary syphilis. Neurological symptoms can occur in early syphilis and Tp can be identified in cerebral spinal fluid. Without treatment, 15–40% of patients will develop tertiary syphilis which destroys cardiac tissue, can cause neurological problems and severe skin or visceral lesions. Neurological manifestations include chronic meningitis, meningovascular stroke-like syndromes and a form of progressive dementia.

For women infected with Tp who do not receive treatment, approximately 30% of pregnancies that occur during the first 4 years after exposure result in foetal death in utero, stillbirth, or death shortly after birth (Peeling et al., 2017). Babies born to infected mothers have low birth weight, are preterm, and display clinical symptoms such as jaundice, lethargy, rash, enlarged spleens and anaemia. Adverse birth outcomes can be prevented if women receive treatment before the end of the second trimester. In 2016, there were an estimated 661,000 cases of congenital syphilis with the highest burden of disease in Africa (57%) with a prevalence of 1.52% compared to the global average of 0.69% (Korenromp et al., 2019). The elimination of congenital syphilis is a high priority in global health strategies.

The treatment of syphilis is simple, generally requiring the use of the commonly available antibiotic penicillin. Diagnosis of syphilis in Australia involves a combination of serology, polymerase chain reaction (PCR) testing of lesions, history and clinical assessment. Blood collected from the suspected case is referred for measurement of syphilis antibody using Chemiluminescent Microparticle Immunoassay (CMIA) or Enzyme Immunoassay (EIA). If reactive, the laboratory will additionally perform a *Treponema pallidum* particle agglutination(haemagglutination) assay (TPPA/TPHA) and rapid plasma reagin (RPR) assay to support the diagnosis of active syphilis. A swab of the ulcer (if present) will be analysed by PCR.

Diagnosis of active syphilis therefore remains challenging, since immunoglobulin (Ig) G antibodies persist even after treatment, meaning that antibody-based tests cannot distinguish between active and past-treated cases. To overcome this limitation, diagnostic assays based on detection of immunoglobulins require distinction of treponemal (TPHA) and non-treponemal (RPR) tests to distinguish active versus past-treated infections. While IgM tests are highly sensitive in symptomatic patients, IgM antibodies are not always detectable in asymptomatic patients. There remains an unmet need for simple, point of care (POC) or near POC assays to diagnose active syphilis infections in resource-constrained settings.

To date, only one commercially available test in Europe and USA combines treponemal and non-treponemal tests: the Dual Path Platform Syphilis Screen and confirm assay (DPP-RDT). A meta-analysis of currently available syphilis rapid diagnostic tests for active case finding reported high sensitivity and specificity for both treponemal and non-treponemal antigens. Overall, the pooled sensitivity and specificity of the treponemal component were 0.93 (95% CI: 0.86 to 0.97) and 0.98 (95% CI: 0.96 to 0.99), respectively. A recent study in Canada using the dual path Syphilis screen and confirm POC test showed sensitivity of 87.5% and specificity of 98.3% (Tsang et al.,

2022). Laboratory evaluation of the DPP Syphilis screen and confirm assay showed sensitivity for the treponemal component of 83.2–85.9% and specificity of 100%, while non treponemal sensitivity was 65.7–69% and specificity was 88.7–89.4 % (Vargas et al., 2022).

There remains an urgent need for simple affordable tests for use at the PCT or at home for the diagnosis of active syphilis.

There remains an urgent need for simple diagnostic tests that can detect active syphilis infection that do not detect past-treated cases of syphilis nor those who have never been infected. Such tests could be used in a pathology laboratory, in clinical settings, or as a rapid point of care test either administered by a health care provider or for self-testing.

SUMMARY OF THE INVENTION

It is against this background that the present inventors have developed a *Treponema pallidum* (Tp) antigen combination suitable for detecting the presence of Tp antibodies in a sample.

The present inventors have developed an antigen combination comprising two or more or a combination of Tp antigens selected from: Tp15 or an antigenic fragment thereof; Tp17 or an antigenic fragment thereof: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof and/or TmpA or an antigenic fragment thereof.

In an aspect, the present application provides an antigen combination comprising three, four, or five Tp antigens selected from the group consisting of: Tp15 or an antigenic fragment thereof; Tp17 or an antigenic fragment thereof; Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and/or TmpA or an antigenic fragment thereof.

In an aspect, the present invention provides an antigen combination comprising the *Treponema pallidum* antigens: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA an antigenic fragment thereof.

In an embodiment, the antigen combination further comprises one or more binding molecule/s. In an embodiment, the binding molecule is selected from: a binding protein, a binding polynucleotide and a small molecule.

In an embodiment, the binding molecule is selected from a: reproductive health binding molecule, a sexual health binding molecule, a women's health binding molecule and a communicable disease binding molecule.

In an embodiment, the binding protein is selected from an antibody and a non-*Treponema pallidum* antigen.

In an embodiment, the binding protein is a HIV antigen or antibody.

In an embodiment, the antigen combination consists of: i) Tp47 or an antigenic fragment thereof; ii) Tp0453 or an antigenic fragment thereof; iii) TmpA or an antigenic fragment thereof; and iv) one or more binding molecules.

In an embodiment, the antigen combination consists of: i) Tp47 or an antigenic fragment thereof; ii) Tp0453 or an antigenic fragment thereof; and iii) TmpA or an antigenic fragment thereof.

In an embodiment, the antigen combination consists of: Tp47, Tp0453 and TmpA.

In an aspect, the present invention provides a vector or polynucleotide encoding one or more antigen(s) in the antigen combination as described herein.

In an aspect, the present invention provides a host cell comprising the vector or polynucleotide as described herein.

In an aspect, the present invention provides a method of producing the antigen combination as described herein, the method comprising expressing the vector or polynucleotide as described herein in a host cell or cell-free expression system.

In an aspect, the present invention provides a lysate or extract from a cell as described herein, wherein the extract comprises one or more of the antigens as described herein.

In an aspect, the present invention provides a composition comprising the antigen combination as described herein.

In an aspect, the present invention provides a method of detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject, the method comprising exposing the sample from the subject to the antigen combination described herein under conditions which enable an antibody-antigen complex to form and detecting the presence or absence of an antibody-antigen complex.

In an aspect, the present invention provides a method of detecting the presence or absence of an active syphilis infection in a sample from a subject, the method comprising exposing the sample from the subject to the antigen combination as described herein under conditions which enable an antibody-antigen complex to form and detecting the presence or absence of an antibody-antigen complex.

In an embodiment, the antibody-antigen complex is an IgA-specific antibody-antigen complex.

In an aspect, the present invention provides a kit for detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject, wherein the assay or kit comprises the antigen combination as described herein.

In an aspect, the present invention provides a kit for detecting an active syphilis infection in a sample from a subject, wherein the kit comprises the antigen combination as described herein.

In an aspect, the present invention provides an assay for detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject comprising the antigen combination as described herein.

In an aspect, the present invention provides an assay for detecting an active syphilis infection in a sample comprising the antigen combination as described herein.

In an aspect, the present invention provides a lateral flow device comprising a solid support that comprises: a detection region comprising the antigen combination as described herein; optionally wherein one or more of the antigens comprises a detectable label; a second detection region configured as a control region; and one or more of: a chromatography matrix, a sample pad, and a wicking pad.

In an aspect, the present invention provides a method of detecting the presence of *Treponema pallidum* antibodies in a sample, the method comprising: 1) contacting the sample with the antigen combination as described herein, to form one or more antibody/antigen complexes; and 2) detecting the antibody/antigen complex; thereby detecting the presence of *Treponema pallidum* antibodies in the sample.

In an aspect, the present invention provides use of the antigen combination as described herein, the kit as described herein, the assay as described herein or the lateral flow device as described herein, for detecting the presence or absence of *Treponema pallidum* antibodies in a sample.

In an aspect, the present invention provides use of the antigen combination as described herein, the kit as described herein, or the assay as described herein, or the lateral flow device as described herein for detecting an active syphilis infection in a subject.

In an aspect, the present invention provides use of the antigen combination as described herein, the kit as described herein, or the assay as described herein, or the lateral flow device as described herein for detecting a syphilis infection in a subject, wherein a past-treated syphilis infection is not detected.

In an aspect, the present invention provides a method of treating an active syphilis infection in a subject, the method comprising: contacting a biological sample from the subject with the antigen combination as described herein; and directly or indirectly detecting the presence of an antibody-antigen complex, wherein the presence of the antibody-antigen complex indicates that a subject has an active syphilis infection; and if

an antibody-antigen complex is detected, administering a treatment for an active syphilis infection.

Any embodiment herein shall be taken to apply *mutatis mutandis* to any other embodiment unless specifically stated otherwise. For instance, as the skilled person would understand examples of Tp antigens outlined above for the antigen combination of the invention equally apply to the compositions, methods, kits, assays, uses, and devices as described herein.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE ACCOMPANING DRAWINGS

Figure 1. Shows a schematic diagram of the syphilis spirochete. Outer membrane, peptidoglycan space and inner membrane are shown. The five proteins examined here are shown in their putative locations.

Figure 2. Describes the putative size of each antigen in *T pallidum*, its location in the bacterium and its function are described. All proteins listed in this table are specific to *T. pallidum*.

Figure 3. Shows an SDS-PAGE gel, run under reducing conditions, shows five purified *T. pallidum* proteins recombinantly expressed in *Escherichia coli* and confirms the expected molecular mass of each protein. The expected molecular size varies slightly from the theoretical size listed in **Figure 2**, due to the addition of protein tags.

Figure 4. Shows a schematic of an example lateral flow assay to detect antibodies to *T. pallidum* antigens. In this test format the patient sample is applied to the sample pad and diffuses laterally along the lateral flow strip, where patient antibodies form a complex

with *T. pallidum* antigens immobilised on the membrane. Unspecific IgA present in the patient sample subsequently binds to the Protein L control line that is immobilised to the membrane. In a second step, buffer is added to the rehydration pad and the gold pad is rehydrated. The gold pad in this example contains anti-IgA gold-conjugate, which upon rehydration diffuses laterally along the strip and binds specifically to any patient IgA antibodies that have formed a complex with *T. pallidum* antigens. Left-over conjugate then binds to the non-treponemal reactive IgA captured by the Protein L stripe.

Figure 5. Example of lateral flow tests in cassettes for the diagnosis of active syphilis. The test result window, sample port and buffer port are visible. The test window of the tests shown here presents different possible outcomes of the test: sample 18 presents as a negative result due to the absence of a test line, whereas samples 19, 20 and 21 are all positive results due to the presence of a visible test line.

Figure 6. Shows the results of lateral flow testing of a panel of sera using single *T. pallidum* antigens striped onto nitrocellulose. **A)** shows the results for people who have never had syphilis infection **B)** shows the results for people with past-treated syphilis and **C)** shows the results for people with confirmed active syphilis. A fixed amount of serum was added to the sample port and 1 drop of buffer added to aid the flow of the sample. Antigen specific IgA present in the sample bound to the striped test line and formed an antigen and antibody complex. After 10 minutes 5 drops of buffer were added to the buffer port to rehydrate the gold particles conjugated to anti-IgA antibody and allowed to diffuse laterally. The gold conjugated anti-IgA bound to the antigen and antibody complex on the test line. The intensity of the test line was measured in a strip reader and the values plotted on the y axis. The name of each *T. pallidum* antigen is shown on the x axis. Samples were determined as positive if the Axxin strip reader detected a test line value >400, which is indicated as a dotted line on each graph.

Figure 7. Shows the results of lateral flow testing of a panel of sera using single *T. pallidum* antigens striped onto nitrocellulose for people with past-treated syphilis to highlight that Tp17 is the worst performing antigen. Samples were determined as positive if the Axxin strip reader detected a test line value >400, which is indicated as a dotted line on each graph.

Figure 8. Shows the sensitivity (the percentage of patients correctly identified as active cases of syphilis), the specificity for past-treated (correctly identified as past-treated case

as not infected), the specificity for never infected (percentage of people never infected correctly identified) and the overall specificity (percentage of people past-treated or never infected correctly identified). The clinician's diagnosis of active syphilis, or past-treated and never infected, was used as the true positive and negative, respectively.

Figure 9. Shows quantitation of data obtained from a lateral flow strip reader of a lateral flow tests' ability to detect IgA antibody levels to individual or two combined *T. pallidum* antigens in three different clinical sample types. In this figure only select samples of the whole clinical sample set are shown. Samples were determined as positive if the Axxin strip reader detected a test line value >400, which is indicated as a dotted line on each graph. **A)** shows select active samples tested on single antigens, as designated in the x-axis. **B)** shows select active samples tested using a combination of two antigens, as designated in the x-axis. Here it is visible that the combination of select antigens improves the ability to correctly identify active syphilis samples compared to single antigens (shown in **A**). **C)** shows select past-treated samples tested on single antigens, as designated in the x-axis. **D)** shows select past-treated samples tested on a combination of two antigens, as designated in the x-axis of the graph. In some cases, the combination of two antigens leads to more misidentification of past-treated cases as active infection, while some combinations decrease misidentification, which are desirable combinations. **E)** shows select samples from never infected individuals tested on single antigens, as designated in the x-axis. **F)** shows select samples from never infected individuals tested on a combination of two antigens, as designated in the x-axis of the graph. It is evident, that the combination of two antigens improves the ability of the test to correctly identify never infected samples, compared to individual antigens (shown in **E**).

Figure 10. Shows the sensitivity and specificity for each individual and combined antigen tested, based on the results shown in **Figure 9**. The specificity is further broken down into the ability of each antigen to correctly identify past-treated individuals and the ability to correctly identify never infected individuals.

Figure 11. Shows the assessment of IgA antibody levels in clinical samples with either active or past-treated syphilis, or never infected using a combination of either **A)** the antigens Tp0453 + Tp047 or **B)** the antigens Tp0453 + TmpA in the test line. Samples were determined as positive if the Axxin strip reader detected a test line value >400, which is indicated as a dotted line on each graph.

Figure 12. Shows the sensitivity and specificity for each antigen calculated based on the test results measured in **Figure 11**. Both double antigens have identical sensitivity, whereas the combination of Tp0453 and Tp47 has higher specificity.

Figure 13. Shows how combining three antigens can result in higher sensitivity for detecting active cases of Syphilis. Tp47, TmpA and Tp0453 were combined at a **A)** 2:1:1 ratio or **B)** 1:1:1 ratio with a constant amount of antigen of 0.45 mg/mL on the test stripe.

Figure 14. Shows an analysis of the data presented in **Figure 13** for sensitivity (percentage of active cases of syphilis correctly identified), specificity for detection of true negatives (percentage of true negatives correctly identified) and specificity for past-treated (PT) (number of past-treated correctly identified). The overall specificity is the sum of past-treated and true negatives correctly identified.

Figure 15. Shows the results of how combining antigens and modifying concentration and excipients improves diagnostic performance. In panel **A)**, the clinical samples were tested at a 2:1:1 ratio in a total volume of 0.45 mg/mL while in panel **B)**, a 2:1:1 ratio was used with 0.7 mg/mL antigen plus 0.5% (w/v) sucrose. The number of false positive samples in the never infected and past-treated groups reduces when more antigen is added in the presence of 0.5% (w/v) sucrose. The number of samples that are classified as a positive remains the same in the active group.

Figure 16. Shows an analysis of the data presented in **Figure 15** for specificity and sensitivity. Increased antigen amount and sucrose does not alter sensitivity, however specificity for both past-treated and never infected samples increased substantially when more antigen was added to the test line in the presence of sucrose.

Figure 17. Shows how slightly increasing total antigen concentration from 0.6 mg/mL to 0.7 mg/mL increases the values seen in the test line for all groups of patients, **A)** never infected **B)** past-treated **C)** active syphilis **D)** mean values of patients from each group.

Figure 18. Shows how slightly decreasing the OD of the gold conjugate from 7.2 to 5.2 decreases the values seen in the test line for all groups of patients, **A)** never infected **B)** past-treated **C)** active syphilis **D)** mean values of patients from each group.

Figure 19. Shows how slightly varying the ratios of the three antigens Tp47, Tp0453 and TmpA in the test line will not significantly impact the test's ability to discriminate active from past-treated samples. **A)** never infected **B)** past-treated **C)** active syphilis **D)** percentage patients recording a positive result from each group.

Figure 20. Shows the results of testing a limited sample panel all with 0.7 mg/mL total antigen but using four different antigen ratios. Panel **A)** shows results for the best performing ratio, while **B–D)** show the results of making one antigen heavily dominant. The table in panel **E)** shows the calculated sensitivity and specificity of each test.

Figure 21. Shows the test lines of past-treated patients (from Tp47:Tp0453:TmpA in a 2:1:1 ratio at a concentration of 0.7 mg/mL + 0.5% (w/v) sucrose) plotted against **A)** number of months since last past-treated infection was diagnosed **C)** number of prior treated infections. Tables showing this data can be seen in **B)** and **D)**.

Figure 22. Shows the results of testing the patient panel on the optimal antigen concentration, combination and ratio (Tp47:Tp0453:TmpA in a 2:1:1 ratio at a total concentration of 0.7 mg/mL + 0.5% (w/v) sucrose) (also referred to as the Burnet test) as compared to the commercial syphilis point of care tests Abbott Bioline 3.0 and Abbott Determine TP. The different columns show sensitivities and specificities when the stated criteria are used to exclude patients from each analysis.

Figure 23. Shows the results of testing the patient panel on the optimal antigen concentration, combination and ratio (Tp47:Tp0453:TmpA in a 2:1:1 ratio at a total concentration of 0.7 mg/mL + 0.5% (w/v) sucrose) when the nine patients who had their blood taken <6 months post last infection or had >2 past infections were excluded from the testing panel. The sensitivities and specificities for this data set can be seen in **Figure 24A**.

Figure 24. Shows the results of testing the optimal antigen concentration, combination and ratio (Tp47:Tp0453:TmpA in a 2:1:1 ratio at a total concentration of 0.7 mg/mL + 0.5% (w/v) sucrose), the Abbott Bioline 3.0 and Abbott Determine TP. **A)** Shows the results when excluding patients who had their blood taken <6 months post last infection or had >2 past infections. This is the same data as presented in the last column of **Figure 22**, but here the individual specificities for the past-treated and never infected patient groups are shown. This shows that the low specificities observed in the Bioline and

Abbott Determine tests are due to inaccurate determination of past-treated patients as positive for syphilis. The same data is used to calculate positive and negative predictive values of the three tests using either **B)** all patients in the sample set **C)** or applying the stated exclusions.

Figure 25. Shows the results of testing on the Burnet test (Tp47:Tp0453:TmpA in a 2:1:1 ratio at a total concentration of 0.7 mg/mL + 0.5% (w/v) sucrose) in tabular form. In this data analysis clinical samples were re-classified based on their THPA and RPR results. These re-classified data were then used to calculate the sensitivity and specificity, as well as then positive and negative predictive values of the test using either **A)** all patients in the sample set **B)** or applying the stated exclusions.

Figure 26. Shows the results of testing on a previously used version of the Syphilis test comprising Tp0453 + Tp15, Tp17, Tp47 antigens (Meridian) and the new Burnet test (Tp47:Tp0453:TmpA in a 2:1:1 ratio at a total concentration of 0.7 mg/mL + 0.5% (w/v) sucrose) in tabular form with **A)** applying the stated exclusion criteria and with **B)** all patients in the sample set. Each table shows the sensitivity and specificity, as well as then positive and negative predictive values of either test and shows that the Burnet test has higher sensitivity and specificity in both cases.

Figure 27. Shows the result of testing the entire panel, **A)** never infected **B)** past-treated **C)** active syphilis, using either anti-IgG gold conjugate and assessing anti-*T. pallidum* IgG antibodies or using the standard anti-IgA gold conjugate for assessing anti-*T. pallidum* IgA levels. The results show that when both tests have similar sensitivities (93 and 95%) using anti-IgG detector leads to higher detection of past-treated samples and thus lower specificity (ability to correctly identify active samples) of the test. As patient anti-Tp IgG levels are significantly higher than IgA levels, conjugate OD and total TP antigen concentration were adjusted till both tests gave similar sensitivities (Conjugate OD4 (IgA) vs OD2 (IgG) and total antigen concentration of 0.7 mg/mL (IgA test) vs 0.28 mg/mL (IgG test)). Both tests antigen lines were comprised of Tp47, Tp0453 and TmpA in a 2:1:1 ratio and included 0.5% (w/v) sucrose.

Figure 28. Assesses the effect HIV status has on the test performance. It shows the results of testing the entire panel on the triple antigen combination, Tp47, Tp0453 and TmpA in a 2:1:1 ratio and including 0.5% sucrose. Panels **A)** to **C)** show never infected, past-treated and active syphilis groups respectively, each stratified by HIV status. Figure **28**

D) shows the specificity and sensitivity of the test, when stratified by HIV status. The results show that HIV status does not have a significant effect on test performance.

Figure 29. Shows the amino acid sequences of Tp15, Tp17 and Tp47, described as the possible constituents of the 48 kDa fusion protein *T. pallidum* p15/p17/p47 (Meridian Cat#R01681). The portions of these proteins that could be identified as present using mass spectrometry are shown in bold, underlined large type. No peptide fragments corresponding to Tp15 were found, whereas one peptide fragment from Tp47 was identified, and the majority of the Tp17 protein was covered.

Figure 30. Shows phylogenetic trees of **A)** Tp15 **B)** TmpA **C)** Tp17 **D)** Tp0453 generated by comparing a representative sample of protein homologues found within the Treponema genus. **E)** shows a phylogenetic tree of Tp47 homologues identified without restriction to the Treponema genus, as within the Treponema genus Tp47 family members are either highly homologous to that from *T. pallidum* (>99% identity) or entirely absent. Protein homologues were retrieved by running BLASTp search using the native antigen sequences as queries (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Figure 31. Shows a diagram of a syphilis test strip that can be used to perform a one-step lateral flow test. The location of the rehydration pad, conjugate pad, sample pad, blood retention pad, nitrocellulose membrane and absorbent pad are shown.

Figure 32. Shows a photo of the fully integrated AtomoRapid ™ Pascal cassette. This is an example of how the two-step syphilis test can be adapted into a one-step test, in this case a fully integrated lateral flow device. The device contains an integrated lancet to prick the finger, an integrated blood collection unit that measures a precise volume of blood in this case 10 µl, and an integrated blister pack containing running buffer that is popped when the button is depressed, popping the blister. The test strip components are enclosed within this device.

Figure 33. Shows the results of assessing patient samples using the one-step format in the AtomoRapid™ Pascal cassette (Tp47:Tp0453:TmpA in a 2:1:1 ratio at a total concentration of 1.4 mg/mL + 0.5% sucrose). Test line intensities were read in a test strip reader (Leelu, Lumos Diagnostics) and values were used to calculate the sensitivity and specificity, as well as the positive and negative predictive values of the test using either

A) all patients in our sample set or **B)** applying the stated exclusions. A test line intensity of >0.1 was considered a positive result giving a demonstrably visible line.

Figure 34. Shows photos from running fresh finger prick blood on the BDI test in a one-step format in AtomoRapid™ Pascal cassette (Tp47:Tp0453:TmpA in a 2:1:1 ratio at a total concentration of 1.3 mg/mL + 0.5% sucrose). The two photos on the left show finger prick blood from two patients designated as having active syphilis by standard serology testing (TPHA positive, RPR>8), while the two patients on the right have never been infected with syphilis (TPHA negative).

Figure 35. Shows the results of running an ELISA measuring the IgA antibodies in select patient serum samples towards Tp47, Tp0453 and TmpA. Individual antigens were used to coat ELISA wells and capture patient antibodies. Bound antibodies were detected with horse radish peroxidase anti-IgA antibody. Serum samples from never infected, past-treated cases (not currently active) and active syphilis cases re shown.

Figure 36. Shows the results of testing for antibodies from select patient samples towards two reportedly antigenic peptide fragments of Tp47 and TmpA. This was assessed in a wet system lateral flow format, with peptides and full- length proteins spotted and dried on nitrocellulose membrane at 0.4 mg/mL and any bound IgA visualised with an anti-IgA colloidal gold conjugate.

Figure 37. Shows the results of testing select past-treated patient samples in lateral flow format. This data is for select past-treated patients, to illustrate that some past-treated patients are only determined as false positives when Tp17 is used in the test line. Antigens were striped in the test lines at 2 mg/mL (Tp15) or 0.2 mg/mL (TmpA, Tp47, Tp17, Tp0453) and patient antibody binding visualised using an anti-IgA gold conjugate. False positive test results are shown in light grey boxes (Axxin reader values >400).

Figure 38. Shows strip photos from testing select patient samples when either a Tp17+Tp47+TmpA test line is used (top panels) or a Tp0453+Tp47+TmpA is used (bottom panels). Both antigen mixes are striped at a total protein concentration of 0.7 mg/mL +0.5% sucrose and samples were tested in a two-step lateral flow format with patient antibody binding visualised by an anti-IgA gold conjugate. The top panel images were collected by a LeeLu reader and the bottom panel images by an Axxin reader. Above each image is an icon showing whether the test is determined as negative (-) or

positive (+) where a positive reader value corresponds to the ability to detect a visual line (by eye positive tests give test line values >0.01 using a LeeLu reader or >400 using an Axxin reader).

Figure 39. Shows the results of lateral flow testing for IgA antibodies from select patient samples towards full length Tp47 and TmpA antigens, as compared to truncated variants of these antigens. All proteins were striped in the test line at an antigen concentration of 0.7 mg/mL + 0.5% sucrose. **A)** shows the results of testing full length Tp47 vs Tp47 N-Trunc (a Tp47 variant with 42 amino acids removed from the N terminus) **B)** shows the results of testing full length TmpA vs TmpA C-Trunc (a TmpA variant with 40 amino acids removed from the C terminus). IgA antibodies bound to the test line were visualised with an anti-IgA gold conjugate and quantified on a Leelu reader.

KEY TO SEQUENCE LISTING

SEQ ID NO:	Name	Sequence
1	Tp47	Amino acid sequence
2	Tp0453	Amino acid sequence
3	TmpA	Amino acid sequence
4	Tp15	Amino acid sequence
5	Tp17	Amino acid sequence
6	Tp47	Nucleotide sequence
7	Tp0453	Nucleotide sequence
8	TmpA	Nucleotide sequence
9	Tp15	Nucleotide sequence
10	Tp17	Nucleotide sequence
11	Tp47-6His (codon optimised)	Nucleotide sequence
12	Tp0453-6His (codon optimised)	Nucleotide sequence
13	TmpA-6His (codon optimised)	Nucleotide sequence
14	Tp15-6His (codon optimised)	Nucleotide sequence
15	Tp17-6His (codon optimised)	Nucleotide sequence
16	Tp47-6His	Amino acid sequence
17	Tp0453-6His	Amino acid sequence
18	TmpA-6His	Amino acid sequence
19	Tp15-6His	Amino acid sequence
20	Tp17-6His	Amino acid sequence

21 to 70	Tp47 fragments	Amino acid sequence
71 to 114	Tp0453 fragments	Amino acid sequence
115 to 160	TmpA fragments	Amino acid sequence
161 to 198	Tp15 fragments	Amino acid sequence
199 to 233	Tp17 fragments	Amino acid sequence
234	TmpA fragment	Amino acid sequence
235	Tp47 fragment	Amino acid sequence
236	N-terminally truncated Tp47-6His	Nucleotide sequence
237	C terminally truncated TmpA-6His	Nucleotide sequence
238	N-terminally truncated Tp47-6His	Amino acid sequence
239	C terminally truncated TmpA-6His	Amino acid sequence
240	N-terminally truncated Tp47s	Nucleotide sequence
241	C terminally truncated TmpA	Nucleotide sequence
242	N-terminally truncated Tp47	Amino acid sequence
243	C terminally truncated TmpA	Amino acid sequence
244	N-terminally truncated Tp47 codon optimised	Nucleotide sequence
245	C terminally truncated TmpA codon optimised	Nucleotide sequence

DETAILED DESCRIPTION

General techniques and definitions

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., enzyme, fermentation, inoculation).

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification the word “comprise”, or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein, the term "about", unless stated to the contrary, refers to +/- 10%, more preferably +/- 5%, even more preferably +/- 1%, of the designated value.

As used herein, the term "subject" is any animal. In an embodiment, the subject is a mammal. In an embodiment, the subject is a human. In an embodiment, the subject is select from a/an: neonate, infant, child, adolescent and adult. In an embodiment, the subject is a neonate. In an embodiment, the subject is an infant. In an embodiment, the subject is a child. In an embodiment, the subject is an adolescent. In an embodiment, the subject is an adult. In an embodiment, the subject has an active syphilis infection. In an embodiment, the subject has congenital syphilis.

As used herein, "recombinant" refers to DNA, proteins, cells, or organisms that are generated from the combination of genetic material from two or more different sources.

As used herein, reference to a "sample" refers to a biological sample of any type of biological material derived form a subject that can analysed, for example, any biological fluid or fraction thereof. For example, the sample is blood serum, urine, tissue, cells, saliva, cell culture, or any fraction thereof, or any biological sample or fraction thereof which may produce or comprise anti-*Treponema pallidum* (anti-Tp) antibodies from a subject as described herein. In an embodiment, the sample is selected from: plasma, serum, whole blood, and finger prick blood. In an embodiment, the sample is whole blood or a fraction thereof. In an embodiment, the sample is plasma. In an embodiment, the sample is serum. In an embodiment, the sample is human plasma. In an embodiment, the sample is human serum. In an embodiment, the sample is finger prick blood or a fraction thereof. In an embodiment, the whole blood is venous blood. In an embodiment, the whole blood is capillary blood. In an embodiment, the blood fraction is venous blood. In an embodiment, the blood fraction is capillary blood.

As used herein, the term "increase" or "increases" or "increased" or "increasing" refers to having a higher or greater level of a given parameter after application of the antigen combination, compositions, methods, uses etc. as described herein compared to the level of a given parameter at baseline. For example, an increase in the ability to accurately determine positive patients refers to a greater or improved ability to accurately determine positive patients compared to the ability previously demonstrated.

As used herein, the term "higher" means great or greater than a comparative e.g. value, for example size, quantity, or intensity, or greater than normal. For example, a higher specificity means a greater or improved specificity, e.g. a greater percentage specificity than the comparator or than normal.

As used herein, the terms “treating” or “treatment” refers to at least partially obtaining a desired therapeutic outcome. In an embodiment, treatment comprises reducing or eliminating at least one symptom of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to ten symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to nine symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to eight symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to seven symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to six symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to five symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to four symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one to three symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one or two symptoms of a specified condition. In an embodiment, treatment comprises reducing or eliminating one symptom of a specified condition.

As used herein, the term “isolated” in the context of an antigen, or antigen combination, or one or more of the antigens of an antigen combination as disclosed herein, or any fragment thereof, or amino acid encoding the foregoing, or nucleic acid encoding the foregoing, shall be taken to mean that the antigen, or antigen combination, or one or more of the antigens of an antigen combination as disclosed herein, or any fragment thereof, or amino acid encoding the foregoing, or nucleic acid encoding the foregoing is substantially removed from its naturally-occurring environment, e.g., the environment in which it was produced (cell, cell secretion, or culture environment). In an embodiment, it is in a heterologous environment and/or that it is substantially free of contaminating agents, e.g., at least about 70% or 75% or 80% or 85% or 90% or 95% or 96% or 97% or 98% or 99% free of contaminating agents. For example, it is about 70 to 100%, or about 75 to 100%, or about 80 to 100%, or about 85 to 100%, or about 90 to 100%, or about 95 to 100%, or about 96to 100%, or about 97 to 100%, or about 98 to 100%, or about 99 to 100% free of contaminating agents.

As used herein, the term “antigen” refers to a molecule or structure containing one or more epitopes that induce, elicit, augment or boost a cellular and/or humoral immune response. Antigens are polypeptides that can also be used as binding molecules, and can be used as an indicator of a disease or infection. Antigens can include, for example, proteins and peptides from a pathogen such as a virus, bacteria, fungus, protozoan, plant or from a tumour. For example, antigens of the present invention include

Tp antigens and non-Tp antigens. In an embodiment, the antigens of the invention are only Tp antigens. In an embodiment, the antigens of the invention are Tp antigens and non-Tp antigens from any one or more pathogens relevant to a condition or disease as described herein.

As used herein, an "antigen combination" refers to a non-naturally occurring collocation, mixture, or specific arrangement of at least three different antigens or fragments thereof, wherein each antigen or antigenic fragment is at least partially distinct or distinguishable from the other antigen(s)/fragment(s) of the combination, and wherein each antigen/fragment may or may not be in direct physical contact with another. For example, an antigen combination can be a mixture of at least three antigens or fragments thereof, regardless of whether other molecules, substrates, or components are also present in the mixture. For example, an antigen combination can be a mixture of three or more antigens or fragments thereof in solution. For example, an antigen combination can be three or more antigens or fragments thereof on a solid support, for example but not limited to on a microchip, in a well plate, or on a nitrocellulose membrane. For example, an antigen combination can be at least three antigens or fragments thereof combined in a single polypeptide, for example, a fusion protein. For example, an antigen combination may be two or more antigens or fragments thereof combined in a single polypeptide, for example, a fusion protein mixed with or in a specific arrangement with one or more additional antigens, fragments thereof, and/or fusion peptides, thereby achieving three or more different antigens. Antigens may be isolated as described herein. In an embodiment, the antigen combination comprises the *Treponema pallidum* antigens: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA or an antigenic fragment thereof. In an embodiment, the antigen combination comprises any combination of fragments and/or full-length Tp 47, TmpA, and Tp0453. In an embodiment, the antigen combination comprises of: Tp47 and/or a Tp47 fragment, Tp0453 and/or a Tp0453 fragment, and TmpA and/or a TmpA fragment. In an embodiment, the antigen combination comprises three or more of the *Treponema pallidum* antigens: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; TmpA or an antigenic fragment thereof, Tp15 or an antigenic fragment thereof, and Tp17 and an antigenic fragment thereof. In an embodiment, the antigen combination comprises the *Treponema pallidum* antigens: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; TmpA or an antigenic fragment thereof, and Tp15 or a antigenic fragment thereof. In an embodiment, the antigen combination comprises the *Treponema pallidum* antigens: Tp47 or an antigenic fragment

thereof; Tp0453 or an antigenic fragment thereof; TmpA or an antigenic fragment thereof, and Tp17 or an antigenic fragment thereof.

In an embodiment, the antigen combination does not comprise Tp17 or an antigenic fragment thereof. In an embodiment, the antigen combination does not comprise Tp17.

Treponema pallidum

Treponema Pallidum, also referred to as *T. pallidum* or "Tp" is a gram-negative rod, comprised of an outer membrane, periplasmic space with endoflagella, peptidoglycan layer and inner membrane which surrounds the cytoplasmic cylinder. There are at least four known subspecies: *T. pallidum pallidum*, *T. pallidum pertenue, T. pallidum carateum* and *T. pallidum endemicum*. The helical structure of *T. pallidum pallidum* allows it to move in a corkscrew motion through viscous mediums such as mucus. *Treponema pallidum* sub sp. *pallidum* has one of the smallest bacterial genomes at 1.14 million base pairs (Mb) and has limited metabolic capabilities. As used herein, reference to Tp is a reference to all *T. pallidum* subspecies.

Syphilis infection is caused by *Treponema pallidum* infection. A syphilis infection develops in stages: primary, secondary, latent and tertiary.

As used herein the term "active syphilis" refers to a period of Tp infection before a subject has been treated to clear the infection, beginning from the time the subject is first infected with Tp, and ending once the infection has been cured by treatment. A subject with active syphilis will generally be considered infectious even if they do not have symptoms. Subjects with active syphilis may also be referred to herein as positive for syphilis. For example, a subject with active syphilis may have been diagnosed by their doctor but not yet received treatment. As the skilled person will be aware, it is common in the field to refer to primary, secondary and latent phases of syphilis infection. As used herein, a subject may have active syphilis in any of these phases, if they have been infected and not treated to clear the infection as described herein.

As used herein, the term "past-treated" refers to subjects who have previously had a syphilis infection for which they have previously received treatment (e.g. penicillin or doxycycline). In an embodiment, the subject is cured by the treatment. In an embodiment, subject has been diagnosed by their doctor for having syphilis and treated by their doctor for syphilis. In an embodiment, a past-treated subject can be classified using a combination of RPR test and TPHA test, subjects are classified as past-treated if they are TPHA positive but have RPR levels that have decreased four-fold or greater post antibiotic treatment.

As used herein the term "never infected" refers to a subject who has not previously contracted a syphilis infection. In an embodiment, the subject has no medical history of contracting a syphilis infection. In an embodiment, the subject has a negative TPHA serological test.

Treponema pallidum antigens

The present invention relates to an antigen combination comprising *T. pallidum* antigens and/or fragments thereof. Suitable *T. pallidum* antigens for the antigen combination, composition, assay, kit, methods or uses as described herein include but are not limited to Tp antigens: Tp47 (Gene ID: 15851864) or an antigenic fragment thereof; Tp0453 (Gene ID: 2611031) or an antigenic fragment thereof; TmpA (Gene ID: 15852058) or an antigenic fragment thereof; Tp15 (Gene ID: 15851469) and Tp17 (Gene ID: 6333547) or an antigenic fragment thereof.

In an embodiment, the antigens of the present invention bind antibodies directed to Tp. For example, binding and subsequent detection of Tp antigens in a sample can indicate presence of Tp infection. For example, Tp antigens can bind to an antibody directed to Tp proteins to form an antigen-antibody complex. For example, detection of a Tp antigen-antibody complex indicates Tp infection.

In some embodiments, one or more of the antigens of the disclosure contain a purification tag. For example, a histidine tag.

As used herein, the terms "antigenic fragment", "antigen fragment", and related terms refer to a portion of an antigen described herein that can stimulate an immune response the same or similar to an antigen as described herein. In an embodiment, that antigen fragment can bind an antibody bound by the parent antigen. For example, an antigen fragment is a portion of a Tp antigen, for example Tp47, Tp;0453, TmpA, Tp17 or Tp15, that is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 and 42 or more amino acids shorter in length than a corresponding full-length Tp antigen. For example, an antigen fragment is a portion of a Tp antigen, for example Tp47, Tp;0453, TmpA, Tp17 or Tp15, that is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 and 42 or more amino acids shorter in length than a corresponding full-length Tp antigen. For example, the antigen fragment is a portion of the Tp antigen as set out in any one of **SEQ ID NOs: 1 to 15**, for example the portion is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 and 42 or more amino acids shorter in length than any one of **SEQ ID NOs: 1 to 15**. For

example, an antigen fragment lacks 1, 2, 3, 4, 5, or more terminal amino acids compared to a full-length antigen. For example, an antigen fragment lacks one or more C terminus amino acids. For example, the antigen fragment lacks 1 to about 40 C terminus amino acids. For example, the antigen fragment lacks 1 to about 38 C terminus amino acids. For example, the antigen fragment lacks 1 to about 35 C terminus amino acids. For example, the antigen fragment lacks 1 to about 30 C terminus amino acids. For example, the antigen fragment lacks 1 to about 25 C terminus amino acids. For example, the antigen fragment lacks 1 to about 20 C terminus amino acids. For example, the antigen fragment lacks 1 to about 15 C terminus amino acids. For example, the antigen fragment lacks 1 to about 10 C terminus amino acids. For example, the antigen fragment lacks 1 to about 5 C terminus amino acids. For example, an antigen fragment lacks 1 or more N terminal amino acids. For example, the antigen fragment lacks 1 to about 42 N terminus amino acids. For example, the antigen fragment lacks 1 to about 38 N terminus amino acids. For example, the antigen fragment lacks 1 to about 35 N terminus amino acids. For example, the antigen fragment lacks 1 to about 30 N terminus amino acids. For example, the antigen fragment lacks 1 to about 25 N terminus amino acids. For example, the antigen fragment lacks 1 to about 20 N terminus amino acids. For example, the antigen fragment lacks 1 to about 15 N terminus amino acids. For example, the antigen fragment lacks 1 to about 10 N terminus amino acids. For example, the antigen fragment lacks 1 to about N C terminus amino acids. For example, the antigen fragment is a portion of a Tp antigen as set forth in any one or more of Tables 3 and 4, and **SEQ ID NOs: 21 to 233**. In an embodiment, the antigen fragment comprises at least 18 amino acids. In an embodiment, the antigen fragment comprises at least 25 amino acids. In an embodiment, the antigen fragment comprises at least 50 amino acids. In an embodiment, the antigen fragment comprises at least 75 amino acids. In an embodiment, the antigen fragment comprises at least 100 amino acids. In an embodiment, the antigen fragment comprises at least 150 amino acids. In an embodiment, the antigen fragment comprises at least 200 amino acids. In an embodiment, the antigen fragment comprises at least 250 amino acids. In an embodiment, the antigen fragment comprises at least 280 amino acids. In an embodiment, the antigen fragment comprises at least 300 amino acids. In an embodiment, the antigen fragment comprises at least 304 amino acids. In an embodiment, the antigen fragment comprises at least 310 amino acids. In an embodiment, the antigen fragment comprises at least 318 amino acids. In an embodiment, the antigen fragment comprises about 18 to about 350 amino acids. In an embodiment, the antigen fragment comprises about 25 to about 350 amino acids. In an embodiment, the antigen fragment comprises about 50 to about 350 amino acids. In an

embodiment, the antigen fragment comprises about 100 to about 350 amino acids. In an embodiment, the antigen fragment comprises about 200 to about 350 amino acids. In an embodiment, the antigen fragment comprises about 300 to about 350 amino acids. In an embodiment, the antigen fragment comprises about 300 to about 325 amino acids. In an embodiment, the antigen fragment comprises about 304 to about 318 amino acids. In an embodiment, the antigen fragment comprises about 18 to about 25 amino acids. In an embodiment, the antigen fragment comprises about 18 to about 30 amino acids. In an embodiment, the antigen fragment comprises about 18 to about 40 amino acids. In an embodiment, the antigen fragment comprises about 18 to about 50 amino acids.

The skilled person will be aware of suitable methods for generating antigen fragments, and of routine methods for testing the antigenicity of fragments. In an embodiment, an antigenic fragment is a portion of an isolated and/or recombinant protein defined herein capable of binding one or more antibody/antibodies and forming an antigen-antibody complex. For example, the portion of an isolated and/or recombinant protein is capable of binding one or more Tp antibody/antibodies and forming an antigen-antibody complex. For example, the portion of an isolated and/or recombinant protein is capable of binding one or more non-Tp antibody/antibodies and forming an antigen-antibody complex. For example, the portion of an isolated and/or recombinant protein is capable of binding one or more HIV antibody/antibodies and forming an antigen-antibody complex. Antigenic fragments can be any size as long as they maintain the defined activity. Examples of antigenic fragments include those comprising or consisting of an amino acid sequence provided in **SEQ ID NOs: 21 to 233**.

In an embodiment, the antigenic fragment has an immunogenic activity of about 20% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of about 30% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of about 40% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of about 50% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of about 60% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of about 70% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of about 80% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of about 90% to 100% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of at least 20% of the activity of the full-length protein. In

an embodiment, the antigenic fragment has an immunogenic activity of at least 30% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of at least 40% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of at least 50% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of at least 60% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of at least 70% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of at least 80% of the activity of the full-length protein. In an embodiment, the antigenic fragment has an immunogenic activity of at least 90% of the activity of the full-length protein. As used herein "immunogenic activity" refers to the immune response produced by an antigen in a subject.

In an embodiment, the antigenic fragment binds a solid support as described herein. In an embodiment, the solid support is a membrane, preferably a nitrocellulose membrane (NCM).

In some embodiments, provided herein is a method of identifying a fragment which binds one or more of Tp47, Tp0453, TmpA, Tp17, or Tp15, the method comprising: a) contacting a candidate fragment or a library of candidate fragments with a sample under conditions which enable an antibody-antigen complex to form, and b) determining whether an antibody-antigen complex has formed, wherein formation of the antibody-antigen complex identifies a fragment which binds one or more of Tp47, Tp0453, TmpA, Tp17, or Tp15. In some embodiments, the sample is a reference sample. In some embodiments, the sample is run in parallel with the combinations of antigens and/or antigenic fragments with known immunogenic activity and antigen binding capacity, for example, to compare the binding of candidate fragments to thereby identify antigenic fragments of suitable binding sensitivity and specificity. In some embodiments, the methods further comprise selecting the fragments. In some embodiments, the method further comprises testing the sensitivity and specificity of any identified fragments, either alone or in combination, in detecting past and/or active syphilis infection, using methods known in the art or described herein.

In some embodiments, provided herein is a method of screening for suitable antigenic fragments which bind one or more of Tp47, Tp0453, TmpA, Tp17, or Tp15, the method comprising replacing one or more of the antigens or antigenic fragments of the invention described herein with a corresponding candidate fragments and performing any of the methods described herein for detecting a syphilis infection. For example, replacing the Tp47 antigen or antigenic fragment described herein with one or more

candidate Tp47 antigen fragments. For example, replacing the Tp0453 antigen or antigenic fragment described herein with one or more candidate Tp0453 antigen fragments. For example, replacing the TmpA antigen or antigenic fragment described herein with one or more candidate TmpA antigen fragments. For example, replacing the Tp17 antigen or antigenic fragment described herein with one or more candidate Tp17 antigen fragments. For example, replacing the Tp15 antigen or antigenic fragment described herein with one or more candidate Tp15 antigen fragments. For example, antigenic fragment candidates are identified using methods known to those skilled in the are and/or described herein, including but not limited to using bioinformatic modelling.

Tp47

As used herein, Tp47 refers to a immunogenic outer-membrane lipoprotein which activates epithelial cells. In an embodiment, Tp47 comprises the amino acid sequence as set forth in **SEQ ID NO: 1**, or an amino acid sequence at least 95% identical thereto, or amino acid sequence at least 96% identical thereto, or amino acid sequence at least 97% identical thereto, or amino acid sequence at least 98% identical thereto, or amino acid sequence at least 99% identical thereto. In an embodiment, Tp47 comprises an amino acid about 95 to 100%, about 96 to 100%, about 97 to 100%, about 98 to 100%, or about 99 to 100% identical to **SEQ ID NO: 1**, or an antigenic fragment thereof. In an embodiment, Tp47 comprises the amino acid sequence set forth in **SEQ ID NO:16**.

In an embodiment, a Tp47 antigenic fragment is a fragment of **SEQ ID NO: 1** comprising immunogenic activity. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 21 to 69**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 21 to 26**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 21**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 22**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 23**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 24**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 25**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 26**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 27**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 28**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 29**.

In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 30**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 31**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 32**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 33**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 34**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 35**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 36**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 37**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 38**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 39**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 40**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 41**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 42**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 43**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 44.** In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 45**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 46**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 47**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 48**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 49**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 50**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 5**1. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 52**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 53.** In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 54**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 5**5. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 56**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 57**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth

in **SEQ ID NO: 58**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 59**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 60**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 6**1. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 62**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 63**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 64**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 65.** In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 66**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 67**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 68**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 69**. In an embodiment, a Tp47 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 70**.

In an embodiment, Tp47 or the fragment thereof is a recombinant antigen.

TP0453

As used herein, Tp0453 refers to a *T. pallidum* outer membrane lipoprotein. Tp0453 is distinct from Tp17, despite some early references which use a different nomenclature system. In an embodiment, Tp0453 comprises the amino acid sequence as set forth in **SEQ ID NO: 2**, or an amino acid sequence at least 95% identical thereto, or amino acid sequence at least 96% identical thereto, or amino acid sequence at least 97% identical thereto, or amino acid sequence at least 98% identical thereto, or amino acid sequence at least 99% identical thereto. In an embodiment, Tp47 comprises an amino acid about 95 to 100%, about 96 to 100%, about 97 to 100%, about 98 to 100%, or about 99 to 100% identical to **SEQ ID NO: 2**, or an antigenic fragment thereof. In an embodiment, Tp0453 comprises the amino acid sequence set forth in **SEQ ID NO:17.**

In an embodiment, a Tp0453 antigenic fragment is a fragment of **SEQ ID NO: 2** comprising immunogenic activity. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 71 to 114**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 71 to 75**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 71**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in

SEQ ID NO: 72. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 73**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 74**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 75**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 76**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 77**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 78**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 79**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 80**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 81**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 82**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 83**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 84**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 85**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 86**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 87**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 88**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 89**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 90**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 91**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 92**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 93**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 94**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 95**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 96**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 97**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 98**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in

SEQ ID NO: 99. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 100**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 101**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 102**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 103**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 104**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 105**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 106**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 107**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 108**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 109**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 110**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 111**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 112**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 113**. In an embodiment, a Tp0453 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 114**.

In an embodiment, Tp0453 or the antigenic fragment thereof is a recombinant antigen.

TmpA

As used herein, TmpA refers to a *T. pallidum* an inner-membrane lipoprotein also known as Tp44.5. In an embodiment, TmpA comprises the amino acid sequence as set forth in **SEQ ID NO: 3**, or an amino acid sequence at least 95% identical thereto, or amino acid sequence at least 96% identical thereto, or amino acid sequence at least 97% identical thereto, or amino acid sequence at least 98% identical thereto, or amino acid sequence at least 99% identical thereto. In an embodiment, Tp 47 comprises an amino acid about 95 to 100%, about 96 to 100%, about 97 to 100%, about 98 to 100%, or about 99 to 100% identical to **SEQ ID NO: 3**, or an antigenic fragment thereof. In an embodiment, TmpA comprises the amino acid sequence set forth in **SEQ ID NO:18**.

In an embodiment, a TmpA antigenic fragment is a fragment of **SEQ ID NO: 3** comprising immunogenic activity. In an embodiment, a TmpA antigen fragment

comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 115 to 160**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 115 to 124**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 115**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 116**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 117**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 118**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 119**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 120**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 121**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 122**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 123**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 124**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 125**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 126**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 127**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 128**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 129**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 130**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 131**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 132**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 133**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 134**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 135**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 136**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 137.** In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 138**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 139**. In an

embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 140**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 141**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 142**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 143**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 144**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 145**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 146**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 147**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 148**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 149**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 150**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 151**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 152**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 153**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 154**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 155**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 156**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 157**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 158**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 159**. In an embodiment, a TmpA antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 160**.

In an embodiment, TmpA or antigenic fragment thereof is a recombinant antigen.

Tp15 and Tp17

As used herein, Tp15 refers to a *T. pallidum* the inner-membrane lipoprotein. In an embodiment, Tp15 comprises the amino acid sequence as set forth in **SEQ ID NO: 4**, or an amino acid sequence at least 95% identical thereto, or amino acid sequence at least 96% identical thereto, or amino acid sequence at least 97% identical thereto, or amino acid sequence at least 98% identical thereto, or amino acid sequence at least 99%

identical thereto. In an embodiment, Tp 47 comprises an amino acid about 95 to 100%, about 96 to 100%, about 97 to 100%, about 98 to 100%, or about 99 to 100% identical to **SEQ ID NO: 4**, or an antigenic fragment thereof. In an embodiment, Tp15 comprises the amino acid sequence set forth in **SEQ ID NO:19**.

In an embodiment, a Tp15 antigenic fragment is a fragment of **SEQ ID NO: 4** comprising immunogenic activity. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 161 to 198**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 161 to 170**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 161**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 162**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 163**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 164**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 165**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 166**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 167**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 168**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 169**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 170**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 171**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 172**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 173**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 174**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 175**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 176**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 177**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 178**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 179**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 180**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 181**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth

in **SEQ ID NO: 182**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 183**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 184**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 185**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 186**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 187**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 188**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 189**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 190**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 191**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 192.** In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 193**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 194**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 195**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 196**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 197**. In an embodiment, a Tp15 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 198**.

In an embodiment, Tp15 or an antigenic fragment is a recombinant antigen.

As used herein, Tp17 refers to a *T. pallidum* inner-membrane lipoprotein. In an embodiment, Tp17 comprises the amino acid sequence as set forth in **SEQ ID NO: 5**, or an amino acid sequence at least 95% identical thereto, or amino acid sequence at least 96% identical thereto, or amino acid sequence at least 97% identical thereto, or amino acid sequence at least 98% identical thereto, or amino acid sequence at least 99% identical thereto. In an embodiment, Tp 47 comprises an amino acid about 95 to 100%, about 96 to 100%, about 97 to 100%, about 98 to 100%, or about 99 to 100% identical to **SEQ ID NO: 5**, or an antigenic fragment thereof. In some embodiments, Tp15 comprises the amino acid sequence set forth in **SEQ ID NO:19.**

In an embodiment, a Tp17 antigenic fragment is a fragment of **SEQ ID NO: 5** comprising immunogenic activity. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 199 to 233**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in any one or more of **SEQ ID NOs: 199 to 202**. In an embodiment, a Tp17 antigen

fragment comprises the amino acid sequence set forth in **SEQ ID NO: 199**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 200**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 201**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 202**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 203**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 204**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 205**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 206**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 207**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 208**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 209**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 210**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 211**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 212**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 213**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 214**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 215**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 216**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 217**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 218**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 219**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 220**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 221**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 222**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 223**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 224**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 225**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 226**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 227**.

In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 228**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 229**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 230**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 231**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 232**. In an embodiment, a Tp17 antigen fragment comprises the amino acid sequence set forth in **SEQ ID NO: 233**.

In an embodiment, Tp17 or an antigenic fragment thereof is a recombinant antigen.

Binding molecule

In an embodiment, the antigen combination as described herein further comprises one or more one or more binding molecule/s. In an embodiment, the binding molecule is a non-Treponemal binding molecule (does not bind an antigen, antibody, or biomarker indicative of *Treponema pallidum*).

As used herein, the term "binds" or "binding" refers to the interaction of a binding molecule with an antigen means that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the antigen. For example, a binding molecule recognises and binds to a specific protein structure rather than to proteins generally. If a binding molecule binds to epitope "A", the presence of a molecule containing epitope "A" (or free, unlabelled "A"), in a reaction containing labelled "A" and the binding molecule, will reduce the amount of labelled "A" bound to the binding molecule.

As used herein, the term "specifically binds" or "specific for X" shall be taken to mean a binding molecule of the disclosure reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular antigen or antigens or cell expressing same than it does with alternative antigens or cells. For example, a molecule that specifically binds to an antigen binds that antigen with greater affinity (e.g., 20-fold or 40-fold or 60-fold or 80-fold to 100-fold or 150-fold or 200-fold greater affinity), avidity, more readily, and/or with greater duration than it binds to other antigens. It is also understood by reading this definition that, for example, a molecule that specifically binds to a first antigen may or may not specifically bind to a second antigen.

In an embodiment, the binding molecule binds an antibody, antigen or biomarker indicative of a disease or condition as described herein.

As used herein, "biomarker" refers to indicators of typical biological processes, pathogenic processes, or pharmacological reactions to therapy. Numerous biomarkers exist, and may be descriptive, prognostic or predictive. Biomarkers include enzymes, antigens and lipids, and can be found in any biological sample taken from a subject. Biomarkers include complex biomarkers or combinations of markers. The skilled person will be aware of suitable biomarkers for monitoring the development of disease, see, for example, Bodaghi et al., (2023); Yang et al., (2022); Califf (2018), Masson et al., (2019). For example, the biomarker is an inflammatory marker. For example, the biomarker is an interferon. For example, the biomarker is a chemokine. For example, the biomarker is a cytokine. For example, the biomarker is a CD antigen. For example, the biomarker is any biochemical biomarker that can be detected by an antibody. For example, the biomarker is alanine transaminase (ALT). For example, the biomarker is blood sugar level. For example, the biomarker is complete blood count. For example, the biomarker is creatinine. For example, the biomarker is C-reactive protein. For example, the biomarker is glial fibrillary acidic protein (GFAP). For example, the biomarker is hematocrit (HCT). For example, the biomarker is Haemoglobin (Hgb). For example, the biomarker is red blood cell count (RBC). For example, the biomarker is thyroid-stimulating hormone (TSH). For example, the biomarker is triglyceride. For example, the biomarker is troponin. For example, the biomarker is ubiquitin carboxy-terminal hydrolase L1 (UCH-L1).

In an embodiment, the disease or condition is selected from one of the following groups: reproductive health, sexual health, women's health, and communicable diseases.

As used herein, a condition or disease relevant to "reproductive health" refers to a condition or disease relating to the reproductive system and to its functions and processes. In an embodiment, reproductive health refers to female reproductive health. In an embodiment, reproductive health refers to male reproductive health.

As used herein, a condition or disease relevant to "sexual health" refers to a condition or disease relating to a sexually transmitted infection, disease, or condition. In an embodiment, sexual health refers to women's and men's sexual health. In an embodiment, sexual health refers to women's sexual health. In an embodiment, sexual health refers to men's sexual health.

As used herein, a condition or disease relevant to "women's health" refers broadly to a condition or disease related to physical and mental health problems that are of exclusive concern for women.

As used herein, a condition or disease relevant to "communicable diseases" refers to a disease that can spread from subject to subject, for example by sharing of bodily fluids, skin contact and close proximity between subjects.

Antigens and antibodies directed to testing for conditions and diseases related to reproductive health, sexual health, women's health, and communicable diseases can be tested for using known antigens and antibodies, as utilised in commercially available kits and assays, for example provided by health providers, of which the skilled person will be aware. For example, HIV antigens and antibodies directed to HIV proteins can be used to detect a disease or condition relevant to any one or more of reproductive health, sexual health, women's health, or a communicable disease.

In an embodiment, the binding molecule is selected from a: reproductive health binding molecule, a sexual health binding molecule, a women's health binding molecule and a communicable disease binding molecule.

In an embodiment, the binding molecule is selected from: a binding protein, binding polynucleotide and a small molecule.

In an embodiment, the binding molecule is a binding protein. In an embodiment, the binding protein is an antibody as described herein. In an embodiment, the binding protein is a non-Tp antigen as described herein.

The terms "protein" and "polypeptide" are generally used interchangeably herein. A polypeptide may be defined by the extent of identity (% identity) of its amino acid sequence to a reference amino acid sequence, or by having a greater % identity to one reference amino acid sequence than to another. The % identity of a polypeptide to a reference amino acid sequence is typically determined by GAP analysis (Needleman and Wunsch, 1970; GCG program) with parameters of a gap creation penalty = 5, and a gap extension penalty = 0.3. In an embodiment, the query sequence is at least 50 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 50 amino acids. In an embodiment, the query sequence is at least 100 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 100 amino acids. In another embodiment, the query sequence is at least 150 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 150 amino acids. In an embodiment, the query sequence is 50 to 500 amino acids in length and the GAP analysis aligns the two sequences over a region of 50 to 500 amino acids. In an embodiment, the query sequence is 100 to 500 amino acids in length and the GAP analysis aligns the two sequences over a region of 100 to 500 amino acids. In another embodiment, the query sequence is 150 to 500 amino acids in length and the GAP analysis aligns the two sequences over a region of 150 to 500 amino acids. Preferably, the GAP analysis aligns

two sequences over their entire length. Preferably, the polypeptide has an antigenic activity of at least 10% of the activity of the reference polypeptide. For example, the polypeptide has an antigenic activity of about 10 to 100% of the activity of the reference polypeptide.

With regard to a defined polypeptide, it will be appreciated that % identity figures higher than those provided herein will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the polypeptide/protein comprises an amino acid sequence which is at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to the relevant nominated SEQ ID NO. For example, it is preferred that the polypeptide/protein comprises an amino acid sequence which is about 90 to 100%, more preferably about 91 to 100%, more preferably about 92 to 100%, more preferably about 93 to 100%, more preferably about 94 to 100%, more preferably about 95 to 100%, more preferably about 96 to 100%, more preferably about 97 to 100%, more preferably about 98 to 100%, more preferably about 99 to 100%, more preferably about 99.1 to 100%, more preferably about 99.2 to 100%, more preferably about 99.3 to 100%, more preferably about 99.4 to 100%, more preferably about 99.5 to 100%, more preferably about 99.6 to 100%, more preferably about 99.7 to 100%, more preferably about 99.8 to 100 %, and even more preferably about 99.9% identical to the relevant nominated SEQ ID NO.

It will be apparent to the skilled person that some modification to the sequence identity of the antigens disclosed herein is contemplated by the present invention, and routine methods will be apparent to the skilled person. For example, amino acid sequence mutants of the polypeptides defined herein can be prepared by introducing appropriate nucleotide changes into a polynucleotide defined herein, or by *in vitro* synthesis of the desired polypeptide. Such mutants include for example, deletions, insertions, or substitutions of residues within the amino acid sequence. A combination of deletions, insertions and substitutions can be made to arrive at the final protein, provided that the final polypeptide product possesses the desired characteristics.

Mutant (altered) polypeptides can be prepared using any technique known in the art, for example, using directed evolution or rational design strategies (see below).

In designing amino acid sequence mutants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series for example, by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting other residues adjacent to the located site.

Amino acid sequence deletions generally range from about 1 to 15 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Substitution mutants have at least one amino acid residue in the polypeptide removed and a different residue inserted in its place. Such conservative substitutions are shown in **Table 1** under the heading of "exemplary substitutions".

Table 1. Exemplary substitutions.

Original Residue	Exemplary Substitutions
Ala (A)	val; leu; ile; gly
Arg (R)	lys
Asn (N)	gln; his
Asp (D)	glu
Cys (C)	ser
Gln (Q)	asn; his
Glu (E)	asp
Gly (G)	pro, ala
His (H)	asn; gln
Ile (I)	leu; val; ala
Leu (L)	ile; val; met; ala; phe
Lys (K)	arg
Met (M)	leu; phe
Phe (F)	leu; val; ala
Pro (P)	gly
Ser (S)	thr
Thr (T)	ser
Trp (W)	tyr
Tyr (Y)	trp; phe

Val (V)	ile; leu; met; phe, ala

In a preferred embodiment a mutant/variant polypeptide has only, or not more than, one or two or three or four conservative amino acid changes when compared to a naturally occurring polypeptide. Details of conservative amino acid changes are provided in **Table 1**. Mutants with desired activity may be engineered using standard procedures in the art such as by performing random mutagenesis, targeted mutagenesis, or saturation mutagenesis on known genes of interest, or by subjecting different genes to DNA shuffling.

In an embodiment, the isolated and/or recombinant protein or antigenic fragment thereof comprises a signal sequence to aid secretion of the protein form a cell or a tag to aid purification of the protein. In an embodiment, the tag is a protein tag. In an embodiment, the protein tag is a FLAG-tag or HIS-tag. The term "FLAG-tagged" or "FLAG-tag" refers to a polypeptide tag that can be added to a protein using recombinant DNA technology i.e. modification of the nucleotide sequence encoding the polypeptide.

In some embodiments, non-Tp antigens are antigens of a pathogen. In some embodiments, non-Tp antigens are antigens that are a biomarker of a disease or condition as described herein. The skilled person will be aware of suitable biomarkers that can be detected by antibodies and aptamers, including but not limited to cytokines, inflammatory markers, interferons, chemokines, lipids and CD antigens. In an embodiment, the biomarker is a lipid. In an embodiment, the biomarker is cardiolipin (detected with an anti-cardiolipin antibody). In an embodiment, the biomarker is not cardiolipin. In some embodiments, suitable biomarkers are any other biochemical biomarker detectable by an antibody. For example, biomarker antigens that are detectable by specific antibody binding. For example, biomarker antigens that are detectable by aptamer. In some embodiments, suitable biomarkers are a protein. For example, an enzyme, an enzyme fragment, a substrate for enzyme activity, or product of enzymatic activity. The skilled person will be aware of suitable methods for detection and/or quantification of proteins and enzymatic reactions, including but not limited to western blot, ELISA, chemiluminescence and mass spectrometry. The skilled person will be aware of suitable assays for measuring and detecting enzymatic activity, including, for example, spectrophotometric, fluorometric, calorimetric, chemiluminescent, light scattering, microscale thermophoresis, radiometric, and chromatographic assays. In some embodiments, biomarkers are detectable by enzyme assay. In some embodiments, biomarkers are lipids, for example, cardiolipin.

In an embodiment, the antigen combination as described herein comprises one or more non-Tp antigens from a non-Tp pathogen or a biomarker relevant to a condition or disease selected from one or more of: reproductive health, sexual health, women's health or a communicable disease. In an embodiment, the non-Tp antigen is a reproductive health antigen. In an embodiment, the non-Tp antigen is a sexual health antigen. In an embodiment, the non-Tp antigen is a women's health antigen. In an embodiment, the non-Tp antigen is a communicable disease antigen. In an embodiment, the non-Tp antigen is HIV. In an embodiment, the non-Tp antigen is cardiolipin.

In an embodiment, the antigen combination as described herein comprises one or more antibodies to a non-Tp pathogen (non-Tp antibodies) or a biomarker, for example, antibodies directed to proteins relevant to a condition or disease selected from one or more of: reproductive health, sexual health, women's health or a communicable disease. In an embodiment, the non-Tp antibody is a reproductive health antibody. In an embodiment, the non-Tp antibody is a sexual health antibody. In an embodiment, the non-Tp antibody is a women's health antibody. In an embodiment, the non-Tp antibody is a communicable disease antibody. In an embodiment, the non-Tp antibody is HIV. In an embodiment, the non-Tp antibody is cardiolipin.

In some embodiments, the non-Tp antibody is an anti-lipid antibody. For example, an anti-cardiolipin antibody.

In some embodiments, assay results from an antigen combination assay are combined with assay results from an assay comprising antibodies to a non-Tp pathogen or biomarker, for example, antibodies directed to proteins relevant to a condition or disease selected from one or more of: reproductive health, sexual health, women's health or a communicable disease, and/or antibodies directed to a lipid, for example, cardiolipin.

In an embodiment, the binding molecule is a binding polynucleotide. In an embodiment, the polynucleotide is DNA or RNA. In an embodiment, the polynucleotide is a mixture of DNA and RNA and/or can contain one or more modified bases or base analogues. For example, a binding polynucleotide is complementary to a single-stranded oligonucleotide or a single 'sense' strand, for example, mRNA, precursorRNA, siRNA, miRNA, pri-miRNA, single stranded DNA overhangs, aptamer, and short sequences of artificial DNA or RNA designed to bind a specific target (probe). As used herein "aptamer" is a single stranded nucleic acid that has a three-dimensional conformation capable of recognising and binding an antigen as described herein.

In an embodiment, the binding molecule is a small molecule which binds an antigen as described herein. As used herein a "small molecule" refers to a chemical compound or molecule having a molecular weight below 2000 Daltons, preferably below

1500 Daltons, more preferably below 1000 Daltons, still more preferably below 750 daltons, yet more preferably below 500 Daltons. In an embodiment, the small molecule is not a polypeptide.

Treponema pallidum antigen combination

In an embodiment, the antigen combination of the present invention comprises individual antigens and/or fragments thereof that are mixed together, or pooled. In an embodiment, the antigen combination of the present invention is mixed or pooled with one or more binding proteins as described herein. In some embodiments, the antigen combination of the present invention comprises individual antigens and/or fragments thereof that are collocated or specifically arranged on the same substrate material. For example, the antigen combination is striped onto a membrane, for example, a nitrocellulose membrane. In some embodiments, the antigen combination is pooled and then applied to a substrate, for example a membrane. In some embodiments, the antigen combination is applied sequentially to a substrate, for example a membrane. In some embodiments, antigens of the antigen combination are arranged on different areas of the same substrate, for example a membrane. It will be apparent to the skilled person that any combination of these approaches is contemplated by the present application. For example, a pool of two antigens is applied to a substrate and an individual antigen is applied to a substrate to produce an antigen combination of three antigens. Similarly, the antigens may be combined in any order, and over any suitable duration.

In some embodiments, an anti-lipid antibody line is striped onto a solid substrate. In some embodiments, an anti-cardiolipin antibody line is striped onto a solid substrate.

In an embodiment, two or more of the antigens and/or fragments thereof disclosed herein are present as fusion proteins. As used herein, the term “fusion protein” is a protein consisting of at least two domains that are encoded by separate genes that have been joined so that they are transcribed and translated as a single unit, producing a single polypeptide. In an embodiment, the fusion protein comprises at least two antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises at least three antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises at least four antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises at least five antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises at least two Tp antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises at least three Tp antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises at least four Tp antigens or fragments thereof

as described herein. In an embodiment, the fusion protein comprises two to five antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises three to five antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises four or five antigens or fragments thereof as described herein. In an embodiment, the fusion protein comprises five antigens or fragments thereof as described herein.

In an aspect, the present invention provides a Tp antigen combination or fusion protein comprising two or more Tp antigens selected from the group comprising Tp15, Tp17, Tp47, Tp0453 and TmpA or fragments thereof. For example, the antigen combination or fusion protein comprises Tp15, Tp17, Tp47 and TmpA or fragments thereof.

In an aspect, the present invention provides an antigen combination, or fusion protein comprising two Tp antigens selected from the group consisting of Tp15, Tp17, Tp47, Tp0453 and TmpA, wherein at least one antigen is TmpA and/or Tp0453, and wherein the antigen combination or fusion protein does not comprise more than two Tp antigens.

In an aspect, the present invention provides an antigen combination, or fusion comprising three or four Tp antigens selected from the group consisting of Tp15, Tp17, Tp47, Tp0453 and TmpA, wherein at least one antigen is Tp0453, and wherein the antigen combination, or fusion protein does not comprise more than four Tp antigens.

In an aspect, the present invention provides an antigen combination comprising the Tp antigens: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA or an antigenic fragment thereof.

In an embodiment, the antigen combination further comprises one or more binding molecules as described herein.

In some embodiments, the antigen combination consists of: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; TmpA or an antigenic fragment thereof; and one or more binding molecules as described herein.

In some embodiments, the antigen combination consists of: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA or an antigenic fragment thereof.

In some embodiments, the antigen combination consists of: Tp47, Tp0453 and TmpA.

In some embodiments, the antigen combination for detection of active syphilis does not include Tp17 or an antigenic fragment thereof.

In some embodiments, the antigen combination for detection of active syphilis consists of: Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA or an antigenic fragment thereof.

In some embodiments, the antigen combination for detection of active syphilis consists of: Tp47, Tp0453 and TmpA.

In some embodiments, the antigen combination for detection of active syphilis consists of: Tp47 or an antigenic fragment thereof, Tp0453 and TmpA.

In some embodiments, the antigen combination for detection of active syphilis consists of: Tp47, Tp0453 or an antigenic fragment thereof and TmpA.

In some embodiments, the antigen combination for detection of active syphilis consists of: Tp47, Tp0453 and TmpA or an antigenic fragment thereof.

In some embodiments, at least two of Tp47, Tp0453 and TmpA are present in a fusion protein, optionally comprising a linker.

In some embodiments, Tp47, Tp0453 and TmpA are present in a fusion protein, optionally comprising a linker.

In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:1:1.

In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:2:1. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:1:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:2:1. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:1:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:2:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:1:1. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:2:1. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:1:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:2:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:1:3. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:2:3. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:1:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:3:1.In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:1:3. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:2:3. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:3:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:2:3. In some embodiments,

Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:1:3. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:2:3. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:3:2. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 3:3:1. In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:3:1.

In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:1:1.

In some embodiments, Tp antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:1:1, wherein the ratio includes variations up to 20% of each ratio. For example, Tp47:Tp0453:TmpA are present in a ratio of about 50:25:25. For example, Tp47:Tp0453:TmpA are present in a ratio of about 40:20:40. For example, Tp47:Tp0453:TmpA are present in a ratio of about 45:23:33.

In some embodiments, the total antigen concentration is between about 0.1 mg/mL to about 2 mg/mL. In some embodiments, the total antigen concentration is between about 0.5 mg/mL to about 1 mg/mL. In some embodiment, the total antigen concentration is about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, or about 2.0 mg/mL. In some embodiments, the total antigen concentration is about 0.1 mg/mL. In some embodiments, the total antigen concentration is about 0.2 mg/mL. In some embodiments, the total antigen concentration is about 0.3 mg/mL. In some embodiments, the total antigen concentration is about 0.4 mg/mL. In some embodiments, the total antigen concentration is about 0.5 mg/mL. In some embodiments, the total antigen concentration is about 0.6 mg/mL. In some embodiments, the total antigen concentration is about 0.7 mg/mL. In some embodiments, the total antigen concentration is about 0.8 mg/mL. In some embodiments, the total antigen concentration is about 0.9 mg/mL. In some embodiments, the total antigen concentration is about 1 mg/mL. In some embodiments, the total antigen concentration is about 1.1 mg/mL. In some embodiments, the total antigen concentration is about 1.2 mg/mL. In some embodiments, the total antigen concentration is about 1.3 mg/mL. In some embodiments, the total antigen concentration is about 1.4 mg/mL. In some embodiments, the total antigen concentration is about 1.5 mg/mL. In some embodiments, the total antigen concentration is about 1.6 mg/mL. In some embodiments, the total antigen concentration is about 1.7 mg/mL. In some embodiments, the total antigen concentration is about 1.8 mg/mL. In some embodiments, the total antigen

concentration is about 1.9 mg/mL. In some embodiments, the total antigen concentration is about 2 mg/mL.

The antigen combination, composition, assay, kit, methods or uses as described herein can be combined with antigens/assays for other, non-*T. pallidum* diseases and conditions. For example, the antigen combination, composition, assay, kit, methods or uses as described herein can be combined with tests for HIV, which is useful in settings where both HIV and syphilis co-infection are prevalent, such as men who have sex with men, sex workers, and in low-resource settings.

For example, the condition is HIV. For example, the antigen combination further comprises one or more of the antigens in **Table 2** to assess for HIV. For example, the antigen combination further comprises HIV antigens gp41, gp120, and p24.

Table 2: Proteins of the Human Immunodeficiency virus and their function. Adapted from German Advisory Committee Blood (Arbeitskreis Blut) doi: 10.1159/000445852.

Gene	Size[a]	Protein	Function
gag		Pr55Gag	precursor of the inner structural proteins
	p24	capsid protein (CA)	formation of conical capsid
	p17	matrix protein (MA)	myristilated protein, forming the inner membrane layer
	p7	nucleoprotein (NC)	formation of the nucleoprotein/RNA complex
	P6		involved in virus particle release
pol		Pr160GagPol	precursor of the viral enzymes
	p10	protease (PR)	proteolytic cleavage of Gag (Pr55) and Gag-Pol (Pr160GagPol) precursor protein; release of structural proteins and viral enzymes
	p51	reverse transcriptase (RT)	transcription of HIV RNA in proviral DNA
	p15 (66)	RNase H	degradation of viral RNA in the viral RNA/DNA replication complex
	p32	integrase (IN)	integration of proviral DNA into the host genome
env		PrGp160	precursor of the envelope proteins SU and TM, cleavage by cellular protease
	gp120	surface glycoprotein (SU)	attachment of virus to the target cell
	gp41	transmembrane protein (TM)	anchorage of gp120, fusion of viral and cell membrane
tat	p14	transactivator protein	activator of transcription of viral genes
rev	p19	RNA splicing regulator	regulates the export of non-spliced and partially spliced viral mRNA

Gene	Size*	Protein	Function
nef	p27	negative regulating factor	myristilated protein, influence on HIV replication, enhancement of infectivity of viral particles, downregulation of CD4 on target cells and HLA cells on target
vif	p23	viral infectivity protein	critical for infectious virus production in vivo
vpr	p15	virus protein r	component of virus particles, interaction with p6, facilitates virus infectivity, effect on the cell cycle
vpu	p16	virus protein unique	efficient virus particle release, control of CD4 degradation, modulates intracellular trafficking
vpx	p15	virus protein x	interaction with p6 in virus particles, involved in early steps of virus replication of HIV-2, component of virus particles
tev	p26	tat/rev protein	Tat-Env-Rev fusion protein, regulates the activity of Tat and Rev in nucleus

In some embodiments, the antigen combination, composition, assay, kit, methods or uses as described herein further comprise an excipient. For example, a functional excipient that improves the sensitivity and/or specificity of the antigen combination for detecting Tp in a sample. In some embodiments, the excipient is any sugar. For example, the excipient is selected from the group comprising sucrose, galactose, maltose, glucose, lactose, and trehalose. In some embodiments, the excipient is sucrose. In some embodiments, the excipient is galactose. In some embodiments, the excipient is maltose. In some embodiment, the excipient is glucose. In some embodiments, the excipient is lactose. In some embodiments, the excipient is trehalose. In some embodiments, the excipient is present in an amount of 0.1 to 1% (w/v). In some embodiments, the excipient is present in an amount of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0% (w/v). In some embodiments, the excipient is present in an amount of 0.5% of the total antigen concentration. For example, the excipient is sucrose and is present in an amount of 0.5% (w/v) of the total antigen concentration.

In some embodiments, the excipient is present in an amount of about 1.0 to 5% (w/v). In some embodiments, the excipient is present in an amount of about 1.2, 1.5, 1.7, 2.0, 2.2, 2.5, 2.7, 3.0, 3.2, 3.5, 2.7, 4.0, 4.2, 4.5, 4.7, or 5.0% (w/v).

Compositions

In an aspect, the present invention provides a composition comprising the antigen combination as described herein.

In an aspect, the present invention provides a composition comprising one or antigens or antigenic fragments of the antigen combination as described herein.

In an embodiment, the composition comprises a single Tp antigen or an antigenic fragment thereof. In an embodiment, the composition comprises two Tp antigens or antigenic fragments thereof. In an embodiment, the composition comprises three Tp antigens or antigenic fragments thereof. In an embodiment, the composition comprises four Tp antigens or antigenic fragments thereof. In an embodiment, the composition comprises five Tp antigens or antigenic fragments thereof. In an embodiment, the composition comprises one or more non-Tp antigens and one or more binding molecules as described herein. In an embodiment, the composition comprises one or more non-Tp antigens or non-Tp antibodies as described herein. In an embodiment, the composition in a form selected from a: solution, powder, liquid, emulsion, lyophilised, dried, frozen, or freeze-dried.

In an embodiment, the composition is a liquid. In an embodiment, the composition is a solution. In an embodiment, the composition is a powder. In an embodiment, the composition is an emulsion. In an embodiment, the composition is lyophilised. In an embodiment, the composition is dried. In an embodiment, the composition is frozen. In an embodiment, the composition is freeze-dried. In an embodiment, the composition is stored in one form and used in another. For example, the composition is stored in solution and is used dried (immobilised), for example on a test strip. For example, the composition is dried, lyophilised, or freeze-dried when stored and used in solution.

Antigen combination sensitivity and specificity

As used herein, “sensitivity” refers to a methods ability to correctly identify those with the disease (true positive rate, i.e. without false negatives).

As used herein “specificity” refers to a methods ability to correctly identify those without the disease (true negative rate, i.e. without false positives).

The ability to correctly diagnose and/or correctly identify patients with syphilis, with active syphilis, and/or correctly classify patients into categories of never infected, past-treated and active infection is a percentage ability and is derived from a combination of the specificity and sensitivity of the test.

In some embodiments, the specificity of the antigen combination as described herein for detecting active syphilis infection in subjects is about 95%.

In some embodiments, the antigen combination as described herein are able to correctly classify between about 51% to about 79% of past-treated syphilis subjects. In some embodiments, the antigen combination as described herein are able to correctly

classify about 58% of past-treated syphilis subjects. In some embodiments, the antigen combination as described herein are able to correctly classify about 71% of past-treated syphilis subjects. In some embodiments, the antigen combination as described herein are able to correctly classify about 80% of past-treated syphilis subjects. In some embodiments, the antigen combination as described herein are able to correctly classify between about 76% and about 100% of subjects.

In some embodiments, the antigen combination as described herein are able to correctly classify subjects with a sensitivity of about 95%. In some embodiments, the antigen combination as described herein are able to correctly classify subjects with a sensitivity of about 97%.

In some embodiments, the antigen combination as described herein are able to correctly classify subjects with a specificity of about 83%. In some embodiments, the antigen combination as described herein are able to correctly classify subjects with a specificity of about 84%. In some embodiments, the antigen combination as described herein are able to correctly classify subjects with a specificity of about 86%. In some embodiments, the antigen combination as described herein are able to correctly classify subjects with a specificity of about 92%.

In some embodiments, the antigen combination as described herein have a sensitivity of up to and including about 100% for syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% for active syphilis infection.

In some embodiments, the antigen combination as described herein have a sensitivity of up to and including 100% for active syphilis infection. In some embodiments, the antigen combination, as described herein have a sensitivity of between about 80% to 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of between about 86% to 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of between about 86% to about 92% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of between about 90% to 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of between about 93% to 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of between about 95% to 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a

sensitivity of between about 97% to 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of between 98% to 100% for active syphilis infection.

In some embodiments, the antigen combination as described herein have a sensitivity of 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% for active syphilis infection.

In some embodiments, the antigen combination as described herein have a sensitivity of about 80% active syphilis infection. In some embodiments, the antigen combination as herein have a sensitivity of about 86% active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of about 90% active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of about 92% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of about 93% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of about 95% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of about 97% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of about 98% for active syphilis infection. In some embodiments, the antigen combination as described herein have a sensitivity of about 100% for active syphilis infection.

In some embodiments, the antigen combination as described herein have a specificity of up to and including 100% for syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% for active syphilis infection.

In some embodiments, the antigen combination as described herein have a specificity of up to and including 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of at least 95% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of at least 94% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of at least 90 % for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of at least 80 % for active syphilis infection.

In some embodiments, the antigen combination as described herein have a specificity of between 51% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 51% and 79% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 55% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 58% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 63% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 76% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 78% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 80% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 83% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 86% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 92% and 100% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of between 94% and 100% for active syphilis infection.

In some embodiments, the antigen combination as described herein have a specificity of about 55% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 58% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 63% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 78% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 80% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 83% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 84% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 86% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 92% for active syphilis infection. In some embodiments, the antigen combination as described herein have a specificity of about 94% for active syphilis

infection. In some embodiments, the antigen combination as described herein have a specificity of about 100% for active syphilis infection.

In some embodiments, the antigen combination as described herein have a specificity of 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94 , 95, 96, 97, 98, 99, or 100% for active syphilis infection.

In some embodiments, the antigen combination described herein is between 50–100% more effective at detecting active syphilis than other reported methods.

In some embodiments, the antigen combination described herein is about 50%, 60%, 70%, 80%, 90% and up to 100% more effective at detecting active syphilis than other reported methods.

In some embodiments, the antigen combination as described herein reduces false positive detection of active syphilis by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and up to 100%.

In some embodiments, the antigen combination as described herein reduces false positive detection of active syphilis by up to 80%.

In some embodiments, the combination of two or more of the antigens described herein reduce the misidentification of past-treated syphilis as active syphilis. In some embodiments, combinations of two or more antibodies described herein reduce false positive active syphilis diagnoses in patients with past-treated syphilis.

In some embodiments, the antigen combination does not detect a past-treated syphilis infection.

In some embodiments, the sensitivity and/or specificity of the antigen combination as described herein for detecting syphilis infection is in subjects never diagnosed with syphilis. In one embodiment, the specificity for detecting syphilis infection in never infected subjects is 100%. In one embodiment, the specificity for detecting syphilis infection in never diagnosed subjects is about 94%.

In some embodiments, the sensitivity and/or specificity of the antigen combination as described herein for detecting syphilis infection is in subjects with past-treated syphilis. In one embodiment, the specificity for detecting syphilis infection in past-treated subjects is about 63%. In one embodiment, the specificity for detecting syphilis infection in past-treated subjects is about 78%. In one embodiment, the sensitivity for detecting syphilis infection in past-treated subjects is about 90%. In one embodiment, the sensitivity for detecting syphilis infection in past-treated subjects is about 84.7%.

In some embodiments, the sensitivity and/or specificity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis.

In some embodiments, the sensitivity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is about 90%. In some embodiments, the sensitivity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is about 93%. In some embodiments, the sensitivity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is about 95%. In some embodiments, the sensitivity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is 100%.

In some embodiments, the specificity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is about 55%. In some embodiments, the specificity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is about 58%. In some embodiments, the specificity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is about 63%. In some embodiments, the specificity of the antigen combination as described herein for detecting syphilis infection is in subjects with active syphilis is about 78%.

In some embodiments, the sensitivity and/or specificity of the antigen combination as described herein for detecting an active syphilis infection is in subjects never diagnosed with syphilis.

In some embodiments, the sensitivity and/or specificity of the antigen combination as described herein for detecting an active syphilis infection is in subjects with past-treated syphilis.

In some embodiments, the sensitivity and/or specificity of the antigen combination as described herein for detecting an active syphilis infection is in subjects with active syphilis.

In some embodiments, the antigen combination as described herein have a sensitivity of about 95%. In some embodiments, the antigen combination as described herein have a sensitivity of about 95% when subjects who are <6 months post-last infection are excluded. In some embodiments, the antigen combination as described herein have a sensitivity of about 94% when subjects who have had more than one past infection are excluded. In some embodiments, the antigen combination as described herein have a sensitivity of about 94% when subjects who have had more than two past infections are excluded. In some embodiments, the antigen combination as described

herein have a sensitivity of about 95% when subjects who are <6 months post-last infection and have had more than two past infections are excluded.

In some embodiments, the antigen combination as described herein have a specificity of about 86%. In some embodiments, the antigen combination as described herein have a specificity of about 90% when subjects who are <6 months post-last infection are excluded. In some embodiments, the antigen combination as described herein have a specificity of about 91% when subjects who have had more than one past infection are excluded. In some embodiments, the antigen combination as described herein have a specificity of about 90% when subjects who have had more than two past infections are excluded. In some embodiments, the antigen combination as described herein have a specificity of about 92% when subjects who are <6 months post-last infection and have had more than two past infections are excluded.

The advantages of the present application include the high sensitivity and/or specificity for syphilis infection, enabling accurate identification of infection status. The advantages of the present application further include the high sensitivity and/or specificity for detecting active syphilis infection in subjects, regardless of any previous history of syphilis, enabling accurate identification of active and latent infection status, thereby enabling immediate treatment appropriate to the stage of syphilis infection, and also enabling immediate appropriate steps to prevent further transmission of infection.

Antibodies

The term “antibody” as used herein includes monoclonal antibodies, bispecific antibodies, fusion diabodies, triabodies, heteroconjugate antibodies, chimeric antibodies including intact molecules as well as fragments thereof, nanobodies and other antibody-like molecules. For example, an antibody binds to the antigen(s) of the disclosure to form an antibody-antigen complex. In an embodiment, the antibody is an antibody fragment.

Antibodies include modifications in a variety of forms including, for example, but not limited to, domain antibodies including either the VH or VL domain, a dimer of the heavy chain variable region (VHH, as described for a camelid), a dimer of the light chain variable region (VLL), Fv fragments containing only the light (VL) and heavy chain (VH) variable regions which may be joined directly or through a linker, or Fd fragments containing the heavy chain variable region and the CH1 domain.

Antibodies can consist of VHH regions, in isolation or multiple VHH domains joined directly with linkers, and then connected to the Fc region comprising CH2 and CH3 domains of an immunoglobulin.

A scFv consisting of the variable regions of the heavy and light chains linked together to form a single-chain antibody (Bird et al., 1988; Huston et al., 1988) and oligomers of scFvs such as diabodies and triabodies are also encompassed by the term "antibody". Also encompassed are fragments of antibodies such as Fab, (Fab')2 and FabFc2 fragments which contain the variable regions and parts of the constant regions. Complementarity determining region (CDR)-grafted antibody fragments and oligomers of antibody fragments are also encompassed. The heavy and light chain components of an Fv may be derived from the same antibody or different antibodies thereby producing a chimeric Fv region. The antibody may be of animal (for example mouse, rabbit or rat) or may be chimeric (Morrison et al., 1984). The antibody may be produced by any method known in the art.

The antibodies may be Fv regions comprising a variable light (VL) and a variable heavy (VH) chain in which the light and heavy chains may be joined directly or through a linker.

As used herein, “variable region” refers to the portions of the light and/or heavy chains of a binding protein as defined herein that specifically binds to an antigen and, for example, includes amino acid sequences of CDRs; i.e., CDR1, CDR2, and CDR3, and framework regions (FRs). For example, the variable region comprises three or four FRs (e.g., FR1, FR2, FR3 and optionally FR4) together with three CDRs. As used herein, the term “complementarity determining regions” (i.e., CDR1, CDR2, and CDR3) refers to the amino acid residues of a binding protein variable region (e.g., a VHH chain) the presence of which are major contributors to specific antigen binding. Each VHH chain of e.g. a camelid-derived binding protein typically has three CDR regions identified as CDR1, CDR2 and CDR3. “Framework regions” are those variable domain residues other than the CDR residues.

There are multiple conventions to define, annotate and describe the CDRs (and by extension FRs) of a binding protein, such as a VHH chain or single domain binding protein. To this end, the length and sequence of specific CDRs of a binding protein can vary depending upon the specific nomenclature, algorithm or the like used to define them. Exemplary conventions to define CDRs include the Kabat definition (which is based on sequence variability and is the most commonly used; See, e.g., Sequences of Proteins of Immunological Interest, Kabat, et al.; National Institutes of Health, Bethesda, Md.; 5th ed.; NIH Publ. No. 91-3242 (1991)), the Chothia definition (which is based on the location of the structural loop regions; See, e.g., Chothia et al., (1987), the AbM definition (which is a compromise between the Kabat and Chothia definitions and is based on Oxford Molecular's AbM antibody modelling software), the IMGT definition

(see, e.g., https://www.imgt.org/IMGTindex/CDR.php) and the method described by Kontermann and Diibel (2010).

In an embodiment, the antibody is a monoclonal antibody. In an embodiment, the antibody is a polyclonal antibody.

In an embodiment, the antibody is an IgA antibody. The term "IgA" or "total IgA" as used herein refers collectively to both subclasses (IgA1 or IgA2) and subtypes "m" "d" or "s" of IgA (overall there are six subtypes dIgA, mIgA, sIgA, dIgA2, mIgA2 and sIgA2 which fall within the two subclasses). IgA is attractive for diagnostic purposes, because it is predominantly made during the acute phase of infection, and high levels of antigen-specific IgA can provide a marker of current infection, with or without the concurrent detection of IgM. In addition, because IgA is the predominant antibody class that is secreted at mucosal epithelial surfaces, its presence is considered as a marker of mucosal immunity. The role of different IgA structural forms as biomarkers for infection, such as specifically dIgA, is not well understood.

In an embodiment, the antibody is a Tp antibody. Anti-Tp antibodies may be produced by the subject as a result of Tp infection, or may be developed synthetically, by means known in the art, for example, by antigen injection into a host organism. Commercially available anti-Tp antibodies include Creative Biolabs VAnt-Wyb212, VAnt-Wyb210, and CABT-NS1525 (directed to Tp47, Tp17, and TmpA respectively.

In an embodiment, the antibody is a non-Tp antibody as described herein.

In some embodiments, the antigen combination, kits, assay, and devices disclosed herein further detect antigen specific IgA.

In some embodiments, the antigen combination is suitable for detecting an active syphilis infection.

In some embodiments, the antigen combination is suitable for distinguishing between an active versus past-treated syphilis infection.

In some embodiments, the antigen combination has lower/improved false positive rate for detecting active versus past-treated syphilis infections. In some embodiments, the antigen combination has lower/improved false positive rate for detecting active versus past-treated syphilis infections than an antigen combination comprising Tp17 or a fragment thereof. In some embodiments, the antigen combination has lower/improved false positive rate for detecting active versus past-treated syphilis infections than an antigen combination comprising Tp17.

In some embodiments, the antigen combination can discriminate between an active syphilis infection and a past-treated syphilis infection.

Detectable label

As used herein, a "detectable label" is a molecular or atomic tag or marker that generates or can be induced to generate an optical or other signal or product that can be detected visually or by using a suitable detector. Detectable labels are well-known in the art and include, for example, metal labels, magnetic labels, beads, fluorescent labels, chemical labels, radionucleotides, coloured particles, quantum dots, fluorescent latex particles, carbon nanoparticles, luminescent label, chemiluminescence based label, liposome based probes, Raman-active tags, a prosthetic group, a contrast agent, an ultrasound agent, and protein labels (Song et al., 2008; Nuntawong et al., 2022; Muyldermans, 2013). In an embodiment, the detectable label is detectable via a smartphone (Zangheri et al., 2015).

In an embodiment, the detectable label is directly detectable, for example, by visual inspection. In an embodiment, the detectable label is indirectly detected, for example, by enzymatic reaction.

In some embodiments, the detectable label is gold. For example, an IgA-specific gold label. In some embodiments, the detectable label is a nanoparticle label. Suitable nanoparticle labels are known in the art, and include labels which have colloidal stability in solution under various conditions and temperatures, susceptibility for detection over a large (and useful) dynamic range, efficiency and reproducibility of conjugation (without the loss of chemical and biological integrity and activity), lack of or very low non-specific binding characteristics (ensuring a high signal-to-noise ratio), commercial availability at low cost, and utilise an easy and scalable conjugation procedure.

In some embodiments, Tp antigen binding proteins and/or binding proteins of a non-Tp condition, for example, HIV, comprising an antibody variable region are conjugated to coloured particles for visual analysis, for example, colloidal gold and/or latex microspheres. For example, the detectable label is colloidal gold. For example, the detectable label is latex. For example, the latex label is tagged with a detector reagent, for example coloured or fluorescent dyes or magnetic or paramagnetic components. For example, the detectable label is a carbon label, for example, carbon nanotubes. Detectable labels may be enzymatically modified to improve sensitivity of the assay. Polymer encapsulation and/or surface blocking may also be used to improve sensitivity, for example by reducing background noise of fluorescent nanoparticle labels such as quantum dots.

In one example, a detectable label is an enzyme. Examples of enzymes useful in the disclosure include, without limitation, alkaline phosphatase and horseradish peroxidase. Alternatively or in addition, the enzyme can be, for example, luciferase. The

enzyme can be linked to the antibody by conventional chemical methods, or it can be expressed together with the antibody as a fusion protein. In one example, the enzyme is horseradish peroxidase.

Radioisotopes useful as detectable labels in the disclosure are well known in the art and can include 3H, 11C, 18F, 35S, 64Cu, 67Ga, 68Ga, 99mTc, 111In, 123I, 124I, 125I, and 131I.

In an embodiment, the detectable label is selected from one or more of: red intense microspheres (Merck e.g. catalogue numbers FR180380637 and FR180380638), cellulose nanobeads, latex beads, alkaline phosphatase, horseradish peroxidase, colloidal gold, gold nanoshells, europium, fluorescent label and a luminescent label. In an embodiment, the detectable label is red intense microspheres. In an embodiment, the detectable label is cellulose nanobeads. In an embodiment, the detectable label is latex beads. In an embodiment, the detectable label is alkaline phosphatase. In an embodiment, the detectable label is horseradish peroxidase. In an embodiment, the detectable label is colloidal gold. In an embodiment, the detectable label is gold nanoshells. In an embodiment, the detectable label is europium. In an embodiment, the detectable label is red fluorescent protein. In an embodiment, the detectable label is green fluorescent protein.

In an embodiment, the fluorescent label is selected from, but not limited to, Green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), fluorescein (FITC), alexa fluor, 5,6-carboxymethyl fluorescein, texas red, nitrobenz-2-oxa-l,3-diazol-4-yl (NBD), coumarin, dansyl chloride, rhodamine, 4'-6-diamidino-2-phenylinodole (DAPI), and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7, fluorescein (5-carboxyfluorescein-N-hydroxysuccinimide ester), and rhodamine (5,6-tetramethyl rhodamine), Emerald, Superfolder GFP, Azami Green, mWasabi, TagGFP, TurboGFP, mNeonGreen, mUKG, AcGFP, ZsGreen, Cloverm Sapphire, T-Sapphire, Enhanced blue fluorescent protein (EBFP), EBFP2, Azurite, TagBFP, mTagBFP, mKalama1, Cyan fluorescent protein (CFP), mCFP, Enhanced cyan fluorescent protein (ECFP), mECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, CyPet, AmCyan1, Midori-Ishi Cyan, TagCFP, mTFP1 (Teal), Yellow fluorescent protein (YFP), Enhanced yellow fluorescent protein (EYFP), Super yellow fluorescent protein (SYFP), Topaz, Venus, Citrine, mCitrine, YPet, TagYFP, TurboYFP, PhiYFP, ZsYellow1, mBanana, Kusabira Orange, Kusabira Orange2, mOrange, mOrange2, dTomato, dTomato-Tandem, Red fluorescent protein (RFP), TurboRFP, TurboFP602, TurboFP635, Tag ref fluorescent protein (RFP), TagRFP-T, DsRed, DsRed2, DsRed-Express (T1), DsRed-Monomer, mTangerine, mKeima-Red, mRuby, mRuby2, mApple, mStrawberry,

AsRed2, mRFP1, JRed, mCherry, mKate2, mKate (TagFP635), HcRed1, mRaspberry, dKeima-Tandem, HcRed-Tandem, mPlum, mNeptune, NirFP, Sirius, TagRFP657, AQ143, Kaede, KikGR1, PX-CFP2, mEos2, IrisFP, mEOS3.2, PSmOrange, PAGFP, Dronpa, Allophycocyanin, GFPuv, R-phycoerythrin (RPE), Peridinin Chlorophyll (PerCP), P3, Katusha, B-phycoerythrin (BPE), mKO, and J-Red. In an embodiment, the fluorescent protein is RFP. In an embodiment, the fluorescent protein is GFP. The absorption and emission maxima, respectively, for these fluors are: FITC (490 nm; 520 nm), Cy3 (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm), Cy5 (652 nm: 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm).

In an embodiment, the luminescent label is selected from: aequorin, firefly luciferase, renilla luciferase, gaussia luciferase, bacterial luciferase and nanoluc.

In an embodiment, the magnetic label is a magnetic or paramagnetic compound, such as, iron, steel, nickel, cobalt, rare earth materials, neodymium-iron-boron, ferrous-chromium-cobalt, nickel-ferrous, cobalt- platinum, or strontium ferrite.

In some embodiments, one or more of the antigens comprise a detectable label.

In some embodiments, the detectable label is selected from a: metal label, magnetic label, bead, colorimetric label, radioactive label, enzymatic label, luminescent label, fluorescent label, antibody, quantum dot, fluorescent latex particle, chemiluminescence based label, liposome-based probe and a Raman-active tag.

In some embodiments, the detectable label is specific to IgA antibodies. In some embodiments, the antibody is an anti-IgA gold.

Production of *Treponema pallidum* antigen combination

A person skilled in the art will appreciate that the antigen or antigen combination as described herein can be produced by a variety of methods, including cell based and cell-free expression systems. In one embodiment, an antigen or antigen combination of the disclosure is produced by culturing a cell under conditions sufficient to produce one or several of the antigens described herein, to produce the antigen combination as described herein.

When using recombinant techniques, the antigens and/or antigen combination of the disclosure can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Where the protein is secreted into the medium, supernatants from such expression systems can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore

Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants. Supernatants can also be used directly for purification.

The present disclosure further provides a polynucleotide encoding an antigen or fusion protein, or a plurality of antigens of the disclosure. For example, the polynucleotide is a recombinant DNA sequence. In one example, a recombinant DNA sequence is a plasmid.

The antigen or antigen combination prepared from the cells or supernatant can be purified using, for example, ion exchange, hydroxyapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, affinity chromatography (e.g., protein A affinity chromatography or protein G chromatography), or any combination of the foregoing. These methods are known in the art and described, for example in WO99/57134 or Zola (1987).

The skilled artisan will also be aware that an antigen or antigen combination of the disclosure can be modified to include a tag to facilitate purification or detection (examples of such are described above). The resulting protein is then purified using methods known in the art, such as affinity purification. For example, a protein comprising a hexa-his tag is purified by contacting a sample comprising the protein with nickel-nitrilotriacetic acid (Ni-NTA) that specifically binds a hexa-His-tag immobilized on a solid or semi-solid support, washing the sample to remove unbound protein, and subsequently eluting the bound protein. Alternatively, or in addition a ligand or antibody that binds to a tag is used in an affinity purification method.

In some embodiments, provided herein is a host cell comprising the vector or polynucleotide of the disclosure. In some embodiments, the host cell is a eukaryotic cell. In some embodiments, the host cell is a bacterial cell.

In some embodiments, provided herein is a method of producing an antigen combination of the disclosure which comprises expressing the vector or polynucleotide disclosed herein in a host cell or cell-free expression system.

In some embodiments, provided herein is a lysate or extract from a cell disclosed herein, wherein the extract comprises one or more of the antigens of the present disclosure.

The present disclosure further provides a polypeptide encoding an antigen or fusion protein, or a plurality of antigens of the disclosure.

In some embodiments, a polynucleotide encoding one or more of the antigens described herein, or the antigen combination as described herein is placed into one or

more expression construct/s, e.g., expression vector(s), which is/are then transfected into a host cell, such as a bacterial cell, a yeast cell, an insect cell, or a mammalian cell, for example bacterial cell, for example an *E. coli* cell. The antigen combination may be produced using one vector or polynucleotide encoding all of the antigens in the combination, or several vectors or polynucleotides encoding one or more of the antigens in the combination. For example, the nucleotides encoding the antigens of the antigen combination are combined in a single polynucleotide. For example, the nucleotides encoding the antigens of the antigen combination are encoded by two or more polynucleotides, for example for expression and subsequent combination and thereby production of the antigen combination disclosed herein.

Exemplary bacterial cells include *E. coli*. Exemplary mammalian cells include simian COS cells, Human Embryonic Kidney (HEK) cells and their derivatives, Chinese Hamster Ovary (CHO) cells, Hela, Human embryonic kidney 293 cells (HEK293), human osteosarcoma U2OS, A549, HT1080, Cath. -a-differentiated cells (CAD), P19, NIH 3T3, L929, N2a, Hep G2 or myeloma cells that do not otherwise produce immunoglobulin protein. Molecular cloning techniques to achieve these ends are known in the art and described, for example in Ausubel (ed, 1988 including all updates until present) or Sambrook et al., (1989). A wide variety of cloning and *in vitro* amplification methods are suitable for the construction of recombinant nucleic acids, as would be suitable for generating the antigens disclosed herein, and thereby the antigen combination disclosed herein. Methods of producing recombinant antigens are also known in the art, as are methods of producing recombinant antibodies. See, for example, Fox and Klass (1989); US4816567; US7923221and US7022500.

In some embodiments, the nucleic acid is operably linked to a promoter. As used herein, the term “promoter” is to be taken in its broadest context and includes the transcriptional regulatory sequences of a genomic gene, including the TATA box or initiator element, which is required for accurate transcription initiation, with or without additional regulatory elements (e.g., upstream activating sequences, transcription factor binding sites, enhancers and silencers) that alter expression of a nucleic acid, e.g., in response to a developmental and/or external stimulus, or in a tissue specific manner. In the present context, the term “promoter” is also used to describe a recombinant, synthetic or fusion nucleic acid, or derivative which confers, activates or enhances the expression of a nucleic acid to which it is operably linked. Exemplary promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or alter the spatial expression and/or temporal expression of said nucleic

acid. As used herein, the term “operably linked to" means positioning a promoter relative to a nucleic acid such that expression of the nucleic acid is controlled by the promoter.

It will be understood that it is possible to improve the expression of a nucleic acid in a host organism or host cell by replacing the nucleotide sequences coding for a particular amino acid (i.e., a codon) with another codon which is better expressed in the host organism (i.e., codon optimisation). One reason that this effect arises is due to the fact that different organisms show preferences for different codons. In some embodiments, a nucleic acid as disclosed herein is modified or optimised such that the nucleotide sequence reflects the codon preference for the particular host cell, preferably mammalian or bacterial cell. In an embodiment, the nucleic acid is codon optimised for mammalian cell culture. In an embodiment, the nucleic acid is codon optimised for bacterial cell culture. Methods of codon optimisation will be apparent to the skilled person. For example, tools for codon optimisation include, for example, GeneArt GeneOptimizer (Thermofisher®) or GenSmart® (GeneScript®).

In some embodiments, the nucleic acid will comprise an N-terminal sequence to aid expression in a host cell.

In an embodiment, the nucleic acid comprises a nucleotide sequence selected from: **SEQ ID NOs: 6** to **10** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises a nucleotide sequence selected from: **SEQ ID NOs: 11** to **15** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 6** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the

sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 7** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 8** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 9** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 10** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence: **SEQ ID NO: 11** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the

sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 12** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 13** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 14** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto. In an embodiment, the nucleic acid comprises the nucleotide sequence **SEQ ID NO: 15** or a codon optimised version thereof, or a sequence at least 70%, at least 80%, at least 85%, at least 90, at least 95%, or at least 98% identical thereto. For example, the sequence is between about 70 to 100% identical thereto. For example, the sequence is between about 80 to 100% identical thereto. For example, the sequence is between about 90 to 100% identical thereto. For example, the sequence is between about 95 to 100% identical thereto. For example, the sequence is between about 98 to 100% identical thereto.

In some embodiments, provided herein is a vector or polynucleotide encoding one or more antigen(s) in the antigen combination of the disclosure.

In an aspect, the present invention provides a vector or polynucleotide encoding three or more antigen(s) in the antigen combination as described herein.

In some embodiments, the vector or polynucleotide as described herein, wherein the vector or polynucleotide encodes at least two of the following: i) Tp47; ii) Tp47

antigenic fragment; iii) Tp0453; iv) Tp0453 antigenic fragment; v) TmpA; vi) TmpA antigenic fragment; and vii) one or more binding proteins.

In some embodiments, the vector or polynucleotide encodes i), iii) and viii); or i), iii), viii) and ivii).

In some embodiments, the vector or polynucleotide encodes Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA or an antigenic fragment thereof.

In some embodiments, the vector or polynucleotide is codon optimised. Methods and resources for codon-optimisation are known to those skilled in the art and include algorithmic models for sequence selection and codon design. The described invention contemplates all motivations for codon optimisation, including but not limited to increasing host suitability, increasing yield, increasing purity, and increasing yield compared to using a non-codon optimised vector or polynucleotide.

In some embodiments, the vector(s) or polynucleotide(s) disclosed herein are a DNA construct.

In some embodiment, different vectors or polynucleotides encode each of the antigens of the present disclosure.

In some embodiments, different vectors or polynucleotides encode each of Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA or an antigenic fragment thereof.

In some embodiment, different vectors or polynucleotides encode each of Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; TmpA or an antigenic fragment thereof; and one or more binding proteins as described herein.

In some embodiments, the antigens of the present application are encoded on two or more vectors.

In some embodiments, the antigens of the present application are encoded on a single vector.

In some embodiments, different vectors or polynucleotides encoding the antigens of the disclosure are expressed in the same cell.

In some embodiments, different vectors or polynucleotides encoding the antigens of the disclosure are expressed in the different cells.

In some embodiments, the vectors or polynucleotides of the disclosure encode a fusion protein comprising one or more of the antigens disclosed herein.

In some embodiments, the antigens of the antigen combination are encoded by a fusion protein.

In some embodiments, the vectors or polynucleotides of the disclosure encode a fusion protein comprising one or more of Tp47 or an antigenic fragment thereof; Tp0453 or an antigenic fragment thereof; and TmpA or an antigenic fragment thereof.

Assays

The antigen combinations and compositions described herein are suitable for *in vitro* use, for example in an assay format known to a person skilled in the art, including as an immunoassay, chromatographic assay or a homogenous assay.

As used herein, the term "assay" is a procedure or method for measuring the biochemical and/or immunological activity of a sample. For example, an assay for detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject.

As used herein, "immunoassay" refers to assays using immunoglobulins or parts thereof that are capable of detecting and quantifying a desired biomarker. The immunoassay may be one of a range of immune assay formats known to the skilled addressee. A wide range of immunoassay techniques are available, such as those described in Wild D. "The Immunoassay Handbook" Nature Publishing Group, 4th Edition, 2013 and subsequent innovations.

In an embodiment, the immunoassay is selected from: electrochemiluminescence (ELICA), enzyme-linked immunosorbent assay (ELISA), chemiluminescent ELISA, fluorescent immunosorbent assay (FIA), a bead-type immunoassay and a particle-based immunoassay (e.g. mesoscale delivery platform (MSD)). Examples of detectable-groups include, for example and without limitation: fluorochromes, enzymes, epitopes for binding a second binding reagent (for example, when the second binding reagent/antibody is a mouse antibody, which is detected by a fluorescently-labelled anti-mouse antibody), for example an antigen or a member of a binding pair, such as biotin. The surface may be a planar surface, such as in the case of a typical grid-type array (for example, but without limitation, 96-welI plates and planar microarrays) or a non-planar surface, as with coated bead array technologies, where each "species" of bead is labelled with, for example, a fluorochrome (such as the Luminex technology described in U. S. Patent Nos. 6,599,331, 6, 592,822 and 6,268,222), or quantum dot technology (for example, as described in U. S. Patent No. 6,306,610).

In some embodiments, patient sample anti-TP antigen IgA antibodies, for example towards TmpA, Tp47 and Tp0453, are detected using a lateral flow format. In some embodiments, patient sample anti-TP antigen IgA antibodies, for example towards TmpA, Tp47 and Tp0453, are detected and/or measured using ELISA.

In some embodiments, the immunoassay is a wet-system immunoassay, for example, a wet-system lateral flow test. As used herein, "wet-system" refers to an immunoassay, such as but not limited to a lateral flow test, in which the test reagents are in solution, and specifically, wherein the visualisation reagent is in solution. For example, a wet system assay uses a gold conjugate diluted in running buffer, for example in PBS, 1% Tergitol. Wet-system assays also use a liquid or wet sample, such as a biological, fluid, for example blood or blood fraction. A system is still a wet-system if some of the test reagents are immobilised or dried down, for example immobilised onto a solid support, as long as a visualisation agent, such as a gold conjugate, is in liquid.In some embodiments, the immunoassay is a dry-system, for example, a dry-system lateral flow test. As used herein, a "dry-system" refers to an immunoassay, such as but not limited to a lateral flow test, in which at least one of the visualisation reagents , for example, a gold conjugate is immobilised. For example, immobilised on a solid support. A system is still a dry system if some of the reagents, for example a running buffer are a liquid, as long as a visualisation agent, such as a gold conjugate, is in dried/solid/immobilised format.

In an embodiment, the immunoassay is performed on an automated platform e.g. a Cobas Immunology Analyzer (Roche) or an Architect immunoassay analyzer (Abbott).

Lateral flow assays and more recently non-lateral flow and microfluidics provide a useful set up for biological assays. Such assays can be qualitative, quantitative or semi quantitative. In microfluidic devices, small volumes of liquid are moved through microchannels generated in, for example, a chip or cartridge. A wide range of detection reagents are available including metal nanoparticles, coloured or luminescent materials. Resonance enhanced adsorption (REA) of bioconjugated metal nanoparticles offers rapid processing times and other advantages. These devices have been combined with barcode technologies to identify the patient and the analyte being tested. Computer software and hardware for assessing input data are encompassed by the present disclosure. Point-of-care devices and arrays and high throughput screening methods are also contemplated. In an embodiment, the assay is a point-of-care device. In an embodiment, the point-of-care device comprises or is accompanies with a lance for obtaining a sample as described herein.

Qualitative assays providing an intermediate or definitive diagnosis require integrated thresholds, gates or windows that permit scoring of samples as likely or not to have a condition. Instrument readers and software are often employed to collate data and process it through a diagnostic algorithm or decision tree.

In an aspect, the present invention provides an assay for detecting the presence or absence of *Treponema pallidum* antibodies in a sample, wherein the assay comprises the antigen combination described herein.

In an embodiment, a bead-type immunoassay is selected from a Luminex LabMAP assay, Bio-Plex Multiplex immunoassay (Bio-Rad). In the bead-type immunoassays, the Luminex LabMAP system can be utilized. The LabMAP system incorporates polystyrene microspheres that are dyed internally with two spectrally distinct fluorochromes. Using precise ratios of these fluorochromes, an array is created consisting of different microsphere sets with specific spectral addresses. Each microsphere set can possess a different reactant on its surface. Because microsphere sets can be distinguished by their spectral addresses, they can be combined, allowing up to 100 different analytes to be measured simultaneously in a single reaction vessel. A third fluorochrome coupled to a reporter molecule quantifies the biomolecular interaction that has occurred at the microsphere surface. Microspheres are interrogated individually in a rapidly flowing fluid stream as they pass by two separate lasers in the Luminex analyser. High-speed digital signal processing classifies the microsphere based on its spectral address and quantifies the reaction on the surface in a few seconds per sample.

In one embodiment, the assay is a homogenous assay, meaning an assay format allowing an assay-measurement by a simple mix and read procedure without the necessity to process samples by separating or washing. Such assays do not include an immunosorbent solid phase step. In one embodiment, the homogenous assay is time-resolved Förster resonance energy transfer (FRET).

In one embodiment, the assay is a flow cytometry-, bead array-, lateral flow-, cartridge-, microfluidic- or immunochromatographic-based method or the like. In one embodiment, the assay is a point-of-care assay. In one embodiment, the point-of-care assay reader is an Axxin AX-2X-type reader, Leelu (Lumos diagnostics), or equivalent or modified device. For example, the device may be modified to include LEDs and filters of the appropriate wavelength for the subject assays.

In one embodiment, the assay detects *T. pallidum*. In an embodiment, the assay further detects a non- *T. pallidum* condition as described herein. In some embodiments, the assay detects active syphilis infection. In some embodiments, the assay detects past-treated or latent syphilis infection. In some embodiments, the assay detects active syphilis and another non-*T. pallidum* condition. In some embodiments, the assay detects active syphilis and HIV.

In some embodiments, the assay is “one-step” assay. As used herein, a one-step assay refers to an assay, for example a lateral flow assay, which requires only one buffer

introduction post sample application. In some embodiments, a one-step test can be read at 15 minutes after the reagents are added. A one-step test may include simultaneous sample and running buffer addition, or may include a time interval between the addition of the sample and the addition of the running buffer. In an embodiment, a one-step assay for detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject is performed when using an AtomoRapid™ Pascal cassette. The assay may comprise the steps of pricking a subject's finger and then reading the displayed result, because the detection, measuring, and/or analysis steps are integrated into the AtomoRapid™ Pascal cassette.

In some embodiments, the assay is a "two-step" assay. As used herein, a two-step assay refers to an assay that requires two or more separate additions of running buffer. In an embodiment, the assay requires two separate additions of running buffer. In an embodiment, the assay requires three separate additions of running buffer.

In some embodiments, the same test performance can be achieved in the one-step format as in the two-step format.

In some embodiments, a one-step assay achieves a sensitivity of 95% and/or a specificity of 86%. In some embodiments, a one-step assay achieves a 95% sensitivity and/or a 92% specificity. In some embodiments, a one-step assay achieves a positive predictive value (PPV) of 83%. In some embodiments, a one-step assay achieves a PPV of 90%. In some embodiments, a one-step assay achieves a negative predictive value (NPV) of 96%.

In some embodiments, assays use fresh samples. In some embodiments, assays use defrosted plasma. In some embodiments, assays use a combination of fresh and defrosted sample. For example, defrosted plasma mixed in about a 50:50 ratio with freshly collected blood cells. For example, defrosted plasma mixed in about a 50:50 ratio with finger prick blood. For example, defrosted plasma mixed in about a 50:50 ratio with serum.

Capture and detection

In an aspect, the present invention provides an assay comprising an antigen combination as described herein.

In an aspect, the present invention provides an assay comprising an antigen combination as described herein immobilised on a solid support.

In an aspect, the present invention provides an assay comprising an antigen combination as described herein, and a binding protein for a non-*T. pallidum* condition as described herein.

In embodiment, the assay as described herein comprises an antigen combination for detecting *T. pallidum* antibodies (detection protein), for example by forming an antigen-antibody complex, and a binding protein for capturing the *T. pallidum* antigen-antibody complex (a capture protein). As used, herein the "detection protein" can be used to directly or indirectly measure the level of *T. pallidum* in a sample. In an embodiment, the detection protein comprises a detectable label. In an embodiment, a detectable label is added to the detection protein. As used herein, a "capture protein" is used to capture *T. pallidum* antigen-antibody complex in the sample. In an embodiment, the capture protein is an antibody. In an embodiment, the capture agent is bound/immobilised to a solid support. The term "immobilised", "immobilisation" is to be understood to involve various methods and techniques to fix proteins onto specific matrices, e.g. as described in WO99/56126 or WO02/26292. For example, immobilisation can serve to stabilise the proteins so that its activity is not reduced or adversely modified by biological, chemical or physical exposure, especially during storage or in single-batch use. In an embodiment, the capture agent is bound to the solid support in the presence of one or more excipients as described herein. In an embodiment, the solid support is a membrane made from a polymer such as nitrocellulose, polyvinylidene fluoride, nylon, polyethersulfone, cellulose, glass fibres, polyester, polypropylene, polytetrafluoroethylene or other similar material. In an embodiment, the membrane is made from nitrocellulose. In an embodiment, the lateral flow device is a microfluidic lateral flow device. In an embodiment, the solid support is a polycarbonate and silicon based support, for example but not limited to polydimethylsiloxane (PDMS).

Lateral flow assays

The present disclosure provides a lateral flow assay for detecting *T. pallidum* in a sample. A lateral flow assay requires a liquid sample, which moves, generally by capillary action, through various zones of polymeric strips, on which molecules for interaction with the analytes are immobilised.

Lateral flow immunoassays, also known as 'immunochromatographic strip tests' operate on the same principles as enzyme-linked immunosorbent assays (ELISA). In essence, these tests run a liquid sample along the surface of a membrane or filter paper with reactive molecules that show a visual positive or negative result depending on the presence of a particular analyte. Lateral flow assays comprise a chromatographic matrix for separation of components of a sample, enabling analysis and/or detection and/or quantification of a sample analyte of interest. Lateral flow assays may comprise several

membranes for detection and or quantification of several different analytes from the same or different samples.

A lateral flow assay device is a device configured to receive a sample or samples at a sample region or sample pad(s), and to provide for the sample to move laterally, via, e.g. wicking, by capillary action from the sample region to a detection region. In some embodiments, the lateral flow assay device further comprises one or more conjugation region(s) or conjugation pad(s), wherein the lateral flow assay device is configured to provide for lateral flow of a sample from a sample region/pad to one or more conjugation region(s)/pad(s) prior to reaching a detection region. In related examples of a lateral flow assay device, a sample region is in contact with a conjugation region and the conjugation region is in contact with one end of a detection region such that the lateral flow assay device is configured to allow a sample to flow from the sample region, to a conjugation region and finally to a detection region. In certain examples of the lateral flow assay device, the device further comprises an absorbent region in contact with a detection region such that the device is configured to allow the flow of a sample from a sample region to a detection region and finally to the absorbent region.

A lateral assay device typically has a backing card onto which an optional sample region, an optional conjugation region, the detection region, and an optional absorbent region are mounted. The backing card provides support for the pads and membranes of the actual assay but are otherwise is not involved in the reaction or flow of the sample and analyte. Backing cards are for example made of polyvinylchloride (PVC). The assembly of pads and solid supports such as membranes on the backing card will typically be in a plastic housing although this is not required. The housing may have at least one opening ("sample port") over the sample pad for application of the sample. For example, one to five openings, for example one to four openings, for example one to three openings, for example one to two openings, for example one opening. A one-step assay may have one sample port. A two-step assay may have two or more sample port/s.

The control and test zones are visible (e.g. via an opening or window) to detect or measure the bound label. The housing prevents the user from applying the sample anywhere except the sample pad. The housing also serves to protect the strip from inadvertent splash onto the membrane. External labelling on the housing can also be used to indicate the position of test and control lines and provide other information. Housings can be obtained as off-the-shelf cassettes or custom-designed to fit around the strip. Internal pins and bars can be used to hold the strip in place relative to the sample port and viewing window. They hold the materials in fluid communication with one another while the test strip is running.

The "sample region", if present, receives the sample upon application and promotes the even distribution of the sample onto the detection region or conjugation region, if present. It may also influence the rate at which liquid enters the detection region, preventing flooding of the device. In addition, the sample region or pad may also comprise additional components such as proteins, detergents, viscosity enhancers and buffer salts in order to process the sample (e.g. separation of sample components, removal of interferences, adjustment of pH, increasing the viscosity, solubilising components and/or preventing non-specific binding between conjugate and analyte or other components or to the reaction membrane).

The "conjugate region" or "conjugation region", if present, comprises a dried and mobilizable composition comprising, for example, the labelled antibody or labelled protein described herein. When sample flows into the conjugation region, the labelled antibody or labelled protein lifts off the conjugate region material, and moves with the sample front into the detection region. If applicable, the conjugation region will also comprise the dried and mobilizable control conjugate. In some embodiments, the conjugate pad comprises a first Tp binding protein. In some embodiments, the Tp binding protein is detectably labelled.

In other examples, the lateral flow assay device does not comprise a separate conjugate region or pad. In such examples, the sample is mixed with a composition comprising the labelled antibody or labelled protein disclosed herein in a separate container, prior to migration along the lateral flow assay device. Such devices may be referred to as lateral flow assay dipsticks. For example, a sample from a subject may be contacted with the composition described herein in a separate container to create a mixed solution, and then a lateral flow assay device comprising a detection region may be dipped into the solution such that it migrates along the detection region to the test and control zones.

The "detection region" is typically a membrane which comprises a test zone and control zone comprising irreversibly bound capture reagents, including an antigen combination of the disclosure, an antibody against the labelled protein or labelled antibody (e.g anti IgA gold; HIV antibody), or a capture reagent such as streptavidin, or a control capture reagent such as Protein L. Nitrocellulose is an exemplary option for the reaction membrane. Nitrocellulose membranes bind proteins (such as antibodies or biotin-binding proteins) electrostatically through interaction of the strong dipole of the nitrate esters with strong dipoles of the peptide bonds within the protein. In some embodiments, the lateral flow assay device includes a test line comprising an immobilised antigen combination of the disclosure. In some embodiments, the assay

device includes a second test line comprising an IgA-specific binding protein, optionally conjugated to a detectable label, for example, anti-IgA gold. In some embodiments, the assay device includes a third test line comprising a binding molecule as described herein, for example, an HIV binding protein or antigen, optionally conjugated to a detectable label. In some embodiments, the assay device includes a third test line comprising a non-Tp condition binding protein or antigen, for example, an HIV binding protein or antigen, optionally conjugated to a detectable label. In some embodiments, the lateral flow assay device comprises a control line comprising an immobilised reagent which binds the Ig light chain, for example, Protein L. In an embodiment, the device is configured to detect IgA antibodies.

The lateral flow assay device may also comprise an "absorbent region" or "absorption region". The absorbent region is placed at the distal end of the detection region and reserves the remaining sample. In some embodiments, the absorbent region is a wicking pad. It wicks the fluid through the membrane and collects the processed liquid. Moreover, it increases the total volume of sample that can enter the detection region.

Suitable materials for a sample region, conjugation region, or a detection region that may be comprised in a lateral flow assay device described herein include, but are not limited to organic or inorganic polymers, and natural and synthetic polymers, including glass fibre, cellulose, nylon, cross-linked dextran, various chromatographic papers and nitrocellulose. It will be appreciated that suitable materials will enable a sample to flow laterally, via capillary action, along the device described herein. In certain examples, the detection region is a nitrocellulose membrane. In some embodiments, a sample region and a conjugation region may be composed of the same material. In certain examples, a lateral flow assay device comprises a sample region in capillary contact with a detection region. Suitable commercially available materials will be known to the skilled person. Commercially available materials may be used for a sample region, conjugation region, and/or detection region that may be comprised in a lateral flow assay device described herein.

The lateral flow assay device may further comprise a sample filter membrane applied to the sample region. The sample filter membrane may be composed of any suitable material including, but not limited to, a hydrophobic material capable of filtering out cells from fluids. Suitable sample membranes will be apparent to the skilled person and may have, for example, a filter size of about 0.22 pm to about 10 pm.

In some embodiments, a sample is applied to the sample region of a lateral flow assay device and the device is incubated. Incubation comprises allowing the device to

remain at a temperature, for example room temperature (e.g. about 20°C to about 25°C), such that the sample flows from the sample region to the detection region. In examples further comprising a conjugation region, incubation comprises allowing a lateral flow device to remain at a temperature, for example room temperature (e.g. about 20°C to about 25°C), such that the sample flows from the sample region to the conjugation region followed by the detection region.

In some embodiments, the lateral flow assay device is incubated after applying a sample to the sample region for about 2 minutes to about 20 minutes, about 2 minutes to about 15 minutes, or about 2 minutes to about 10 minutes. For example, the lateral flow assay device is incubated for about 10 minutes to about 15 minutes after applying a sample to the sample region.

In some embodiments, the lateral flow assay device may further comprise a control component immobilised in the control zone of the detection region. In one example, the detection region of a lateral flow assay device is configured such that the sample flows past a test zone before the control zone. In one example, the assay may further comprise inspection of the signal of a control line to confirm valid operation of a lateral flow assay device. Inspection may comprise visual confirmation of signal on a control line.

In one example, assessing comprises a quantitative measurement of the molecules captured on a test zone and/or control zone. In one example, assessing may comprise semi-quantitative or qualitative assessment of a test zone, e.g. detection of a signal above a pre-determined threshold. Suitable means of assessing a test zone will depend on the signal generated by a test zone. For example, detection may be optical, thermal, magnetic or electrochemical. In one example, assessing may comprise quantitatively or qualitatively measuring the signal from, for example, a fluorescent dye or a colloidal metal. Assessing may be carried out visually. Assessing may be carried out by a smartphone. In certain examples, assessing may comprise use of a portable fluorescence meter. Commercially available devices for measuring a signal from a lateral flow assay device will be familiar to the skilled person. In some embodiments, the detection of Tp and/or a non-Tp condition is undertaken in a point-of-care setting.

Nucleic acid-based lateral flow assays detect DNA, RNA, and/or mRNA, and follow the same principles as lateral flow immunoassays described below, except that they include a first amplification step to amplify the nucleic acid of interest using PCR and the PCR product as attest sample. Colorimetric detection allows detection of PCR product by visual inspection, without the need for additional equipment or skilled personnel. In some embodiments, the detection of HIV nucleic acid by lateral flow assay

is undertaken in a point-of-care setting. In some embodiments, the lateral flow assay device enables quantification of HIV nucleic acid. In some embodiments, quantification of HIV nucleic acid is undertaken in a point-of-care setting.

In an embodiment, the lateral flow device comprises a solid support that comprises: a detection region comprising the antigen combination as described herein; optionally wherein one or more of the antigens comprises a detectable label; a second detection region configured as a control region; and one or more of: a chromatography matrix, a sample pad, and a wicking pad.

In some embodiments, sample collection is integrated into the lateral flow assay device. For example, the patient sample is finger-prick blood and the lateral flow assay device comprises an integrated lancet for pricking the patient finger, thereby collecting the sample. The sample may be collected in a collection unit, and/or may be transferred via a conduit to the sample pad.

In some embodiments, release of assay running buffer is integrated into the lateral flow assay device. In some embodiments, release of assay running buffer is automated.

In some embodiments, the running buffer rehydrates a gold pad. In some embodiments, the gold pad further comprises an anti-human IgA gold conjugate.

In some embodiments, a Tp antigen combination is immobilised on a membrane of the lateral flow assay device. For example, the membrane is a nitrocellulose membrane. For example, the membrane is any other suitable membrane. For example, the antigen combination forms a test line on the membrane.

The gold conjugate and patient antibodies from the applied sample are carried by the running buffer across the strip. Patient antibodies from the sample form a complex with Tp antigens immobilised on a membrane, for example on a membrane test line.

Patient sample antibody complex formation with Tp antigen combination is visualised by, for example, anti-IgA gold conjugate.

In some embodiments, the lateral flow assay device comprises a Protein L control line. Non-syphilis antigen-specific antibodies present in the patient sample bind to the Protein L control line. Protein L and non-Tp antigen combination antibody binding is visualised, for example by anti IgA gold conjugate.

In some embodiments, the test can be read at 30 minutes after the sample and running buffer are applied to the test strip.

In some embodiments, the test can be read at 15 minutes after the sample and running buffer are applied to the test strip.

In some embodiments, a lateral flow assay device, such as a cassette, utilises a one-step assay.

In some embodiments, a lateral flow assay device, such as a cassette, utilises a two-step assay.

In some embodiments, a one-step assay comprises a previously obtained sample. In some embodiments, a one-step assay comprises a non-integrated addition of running buffer, for example, the running buffer is added from a separate running buffer bottle or container.

In some embodiments, the running buffer is integrated into the cassette. For example, can be released to the test strip by pushing down on the test to burst an integrated blister prefilled with running buffer. In some embodiments, test results can be read and/or quantitated by an automated reader. For example, the Lumos LeeLu or Cerberus reader (Lumos, Carlsbad). In some embodiments, test results can be interpreted through visual assessment, for example of the assay, for example, the test strip, or, for example, by plotting the numerical readout of the test line intensities, for example in an automated manner.

In some embodiments, the running buffer comprises one or more of the following: 1 X DPBS, about 2% Synperonic F108, about 1% Tween 20, about 1.8 mg/mL K_2EDTA and a pH of about 7.4. In some embodiments, the running buffer comprises 1 X DPBS. In some embodiments, the running buffer comprises about Synperonic F108. In some embodiments, the running buffer comprises about 2% Synperonic F108. In some embodiments, the running buffer comprises about Tween 20. In some embodiments, the running buffer comprises about 1% Tween 20. In some embodiments, the running buffer comprises about K_2EDTA.In some embodiments, the running buffer comprises about 1.8 mg/mL K_2EDTA.

Microfluidic lateral flow devices

Microfluidic lateral flow devices couple the channels of microfluidic devices with the capillary drive action of lateral flow devices to avoid the need for pumps in microfluidic devices and to improve detection limits of traditional lateral flow devices and/or to enable quantitative measurements. For a recent review of paper-based microfluidic devices, see, for example, Carrell, et al. (2019). Briefly, porous material, such as paper or other e.g. cellulose membrane or for example synthetic polymers with inorganic backbones and organic groups, such as silicones and polydimethylsiloxanes (PDMS), are patterned with microfluidic channel networks and can carry out multiplexed analyses in POC settings and in disposable devices. Accordingly, a microfluidic lateral flow device is especially suited to methods using the antigen combination of the present

invention either alone, or in conjunction with one or more additional diagnostic tests, for example but not limited to tests for HIV.

In some embodiments, the methods, kits, and assays comprise a microfluidic lateral flow device. In some embodiments, provided herein is a solid support for use in a microfluidic lateral flow device comprising the antigen combination described herein. In some embodiments, the solid support is a polycarbonate based support. In some embodiments, the solid support is a silicon based support, for example, polydimethylsiloxane (PDMS).

Lateral Flow in one-step format in AtomoRapid™ Pascal

In some embodiments, a lateral flow assay device of the present application comprises the lateral flow test strips described herein in the AtomoRapid™ Pascal cassette. In some embodiments, AtomoRapid™ Pascal cassette comprises one or more of the following features: the antigen combination striped onto nitrocellulose membranes; anti-IgA (DCNDx) gold conjugate pads(s); membrane laminated together with an absorbent pad and/or a blood retention pad; a sample pad; a conjugate pad; and a gold rehydration pad. In some embodiments, components are laminated together on an adhesive backing card from which test strips for integration into the cassette are formed. For example, the test strips are 4 mm.

Kits

In an aspect, the present invention provides a kit comprising the antigen combination disclosed herein.

In an aspect, the present invention provides a kit comprising a binding protein as described herein.

In an embodiment, the kit comprises a strip, chip or cartridge for use on point-of care device.

In an embodiment, the kit comprises at least one of: a lateral flow assay, ELISA and an Luminex® assay.

In an embodiment, the kit is designed for use by a health care practitioner.

In an embodiment, the kit is a self-test kit designed for home use.

In an embodiment, the kit is contained within an all-in-one device for home use or health care practitioner use. In an embodiment, the kit comprises a cassette with a built-in lance and a blister pack of running buffer. The device is designed for at home or point-of-care test.

In an aspect, the present invention provides a kit for detecting the presence or absence of *Treponema pallidum* antibodies in a sample, wherein the assay comprises the antigen combination disclosed herein.

In an aspect, the present invention provides a kit for detection of Tp antibodies in a sample, the kit comprising using the antigen combination, assay, or device disclosed herein.

In an aspect, provided herein is a kit for performing any of the methods disclosed herein, comprising at least one of the antigen combination, assays, or devices disclosed herein.

In some embodiments, the kit comprises positive and/or negative controls.

In some embodiments, the kit comprises calibration samples.

In some embodiments, the kit comprises one or more of enzyme conjugate, substrate for enzyme conjugate, buffer solution, detectable label, excipient and washing solution.

In some embodiments, the kit comprises an excipient. For example, the excipient is any sugar. In some embodiments, the kit comprises sucrose.

In some embodiments, the kit comprises sucrose, wherein the sucrose is used in an amount of 0.1 to 5% (w/v). In some embodiments, the kit comprises sucrose, wherein the sucrose is used in an amount of 01 to 1%(w/v). In some embodiments, the kit comprises sucrose, wherein the sucrose is used in an amount of 0.5% (w/v).

In some embodiments, the kit comprises a detectable label and the label is an antibody.

In some embodiments, the kit comprises a detectable label and the label is gold.

In some embodiments, the gold is an anti-IgA gold.

Methods and uses

It will be apparent from the description herein that the present disclosure provides various methods for detecting *Treponema pallidum*. It will be apparent from the description herein that the present disclosure provides various methods/ uses for diagnosing/prognosing and/or monitoring conditions/treatments associated with Tp, and optionally a combination of Tp and a non-Tp disease or condition as described herein.

In an aspect, the present invention provides a method of detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject, the method comprising exposing the sample from the subject to the antigen combination as described herein under conditions which enable an antibody-antigen complex to form, and detecting the presence or absence of an antibody-antigen complex.

In an aspect, the present invention provides a method of detecting the presence or absence of an active syphilis infection in a sample from a subject, the method comprising exposing the sample from the subject to the antigen combination as described herein under conditions which enable an antibody-antigen complex to form, and detecting the presence or absence of an antibody-antigen complex.

In an embodiment, the antibody-antigen complex is an IgA-specific antibody-antigen complex. In such embodiment, the antibody binds specifically to IgA immunoglobulin.

In an embodiment, an antigen/antibody complex is detected. A skilled person will be aware of suitable ways to detect an antibody/antigen complex, as known in the art or described herein. In an embodiment, an IgA-specific antibody/antigen complex is detected. For example, an antibody/antigen complex wherein at least one bound antibody specifically binds IgA. For example, preferentially over any other immunoglobulin.

In an aspect, the present invention provides a method of detecting the presence of Tp antibodies in a sample, the method comprising: 1) contacting the sample with the antigen combination as described herein, to form one or more antibody/antigen complexes; and 2) detecting the antibody/antigen complex; thereby detecting the presence of Tp antibodies in the sample.

In an aspect, the present invention provides use of the antigen combination as described herein, the kit as described herein, or the lateral flow device as described herein for detecting the presence or absence of Tp antibodies in a sample.

In an aspect, the present invention provides use of the antigen combination as described herein, the kit as described herein, the assay as described herein or the lateral flow device as described herein for detecting an active syphilis infection in a subject.

In an aspect, the present invention provides a method of treating an active syphilis infection in a subject, the method comprising: i) contacting a biological sample from the subject with the antigen combination as described herein; and ii) directly or indirectly detecting the presence of an antibody-antigen complex, wherein the presence of the antibody-antigen complex indicates that a subject has an active syphilis infection; and iii) administering a treatment for an active syphilis infection. For example, the treatment is administered if an antibody/antigen complex is detected. In an embodiment, the treatment comprises administering an antibiotic. The skilled person will be aware of available and suitable antibiotics in each individual case. In an embodiment, the treatment comprises administering penicillin. In an embodiment, the treatment comprises administering doxycycline. In an embodiment, the treatment comprises administering tetracycline. In an embodiment, the treatment comprises administering azithromycin. In

an embodiment, the treatment comprises administering a cephalosporin. In an embodiment, the treatment comprises administering ceftriaxone. Administration of the treatment can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the treatment may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Administration can be via any suitable route, including, but not limited to parenterally, for example, by intramuscular, subcutaneous, or intravenous or intra-arterial injection, or orally. In an embodiment, the treatment comprises injectable penicillin administered intramuscularly as a single dose. In an embodiment, the treatment comprises oral doxycycline administered twice per day over 14 days. It will be appreciated by the skilled person that any suitable known treatment regime is contemplated by the described invention.

EXAMPLES

Example 1: Methods

Expression and purification of Tp15, Tp17, Tp47 and TmpA

The DNA sequences of Tp15, Tp17, Tp47 and TmpA were codon optimised for expression in *E. coli* and synthesised by GeneArt. The sequence of TmpA was synthesised in a pET151-D vector with a N-terminal His tag, V5-tag and TEV cleavage site. Tp15 and Tp47 were subcloned into the pET151-D vector with the same tags using BamHI/SacI restriction enzyme cloning. Tp17 was subcloned into vector pET28a with a N-terminal His tag and Thrombin cleavage site using BamHI/SacI restriction enzyme cloning.

Tp15, Tp17, Tp47 and TmpA were expressed in *E. coli* BL21 Star(DE3) cells (Invitrogen) in LB supplemented with 100 µg/mL ampicillin (Tp15, Tp47 and TmpA) or 50 µg/mL Kanamycin (Tp17) at 37 °C. Expression was induced by addition of 0.1 mM IPTG at 18 °C overnight. Cells were harvested, resuspended in 50 mM Sodium Phosphate, 200 mM Sodium Chloride, 10 mM Imidazole, pH 7.6, with 1 mM phenylmethylsulfonyl (PMSF) for protease inhibition added, and lysed by sonication (Branson). Proteins were purified from clarified cell lysate by affinity chromatography using HisTrap FF (Cytiva) with 50 mM Sodium Phosphate, 200 mM NaCl, 10 mM imidazole, pH 7.6 and elution with 20–500 mM imidazole gradient. Recombinant protein was further purified by gel-filtration chromatography using a Superose 12 10/30 (Amersham) or Hiload 16/600 Superdex 200pg (Cytiva) pre-equilibrated with 50 mM Sodium Phosphate, 500 mM Sodium Chloride, pH 7.6.

Tp0453 was purchased from Genscript (custom order).

T. pallidum p15/p17/p47, Recomb antigen was purchased from Meridian Biosciences (Cat# R01681) and is referred to herein as "Meridian antigen".

SDS-PAGE gels

SDS-PAGE analysis was conducted using Bolt™ Bis-Tris Plus Mini Protein Gels, 4–12% (Invitrogen) with Bolt MES SDS Running Buffer (Invitrogen). The gels were visualised on ChemiDoc Imaging System (Bio-Rad).

Lateral flow

Lateral flow strips were prepared by striping two test lines across a nitrocellulose membrane (NCM) (Vivid 90, Pall Corporation) using an IsoFlow dispenser (Imagene Technology). For wet system testing, the striped NCM was laminated together with a sample pad (Glass Fibre 1285, Ahlstrom) and absorbent pad (CF6 absorbent pad, GE Healthcare) on an adhesive backing card and cut into 5 mm test strips using a guillotine cutter (Kinbio Shangai Kinbio Tech).

The first test line contains *T. pallidum* antigens, either individually or combined at different concentrations, in the presence or absence of 0.5% (w/v) sucrose. The second test line contains 0.2 mg/mL Protein L (Sigma), which forms a complex with kappa chain IgA antibodies present in the sample and/or a highly specific IgA gold conjugate. Test lines were striped using an IsoFlow dispenser (Imagene Technology) at a dispense rate of 0.100 μL/mm and dried for 2h at 37°C.

Lateral flow in cassettes

For testing the lateral flow test strips in a cassette, nitrocellulose membranes (NCMs) (Vivid90, Cytiva) were striped as outlined above. Anti-IgA (DCNDx) or anti-IgG gold (BBI) was diluted in 10 mM borate, 2 mM EDTA, 0.25% Tween-20, 20% sucrose, 5% trehalose, 1% BSA, 0.35% PEG8000 (1/1000 or 1/2000) and dispensed (1 mL/30 cm) onto a conjugate pad (glass fibre 8951, Ahlstrom) and dried at 37°C overnight. The NCM was then laminated together with a sample pad (glass fibre 8951, Ahlstrom) soaked in 0.5% casein, followed by the conjugate pad and a gold rehydration pad (glass fibre 8951, Ahlstrom) at the bottom of the NCM and absorbent pad (CF6 absorbent pad, GE Healthcare) at the top of the NCM. All components were laminated together on an adhesive backing card and cut into 5 mm test strips using a guillotine cutter (Kinbio Shanghai Kibio Tech). The strips were assembled into a disposable plastic housing (Burnet Institute).

To run an assay, 5 µL of plasma or serum is added to the sample pad of the test strip, followed by 1 drop of running buffer (PBS, 1% Tergitol). The sample and buffer are allowed to diffuse laterally for 10 minutes. After 10 minutes, 5 drops of running buffer are added to the gold rehydration pad and the rehydrated gold allowed to diffuse laterally for 20 minutes. After 20 minutes the test lines are visualised and quantitated using an automated reader, such as the Axxin AX-2X reader (Axxin Ltd, Melbourne).

The intensity of the test line can be assessed visually or using an automated reader such as the Axxin reader (AX-2X, Axxin Ltd Melbourne). The Axxin reader gives a numerical readout for each test line, as well as a photograph of the test strip. The test results were interpreted through visual assessment of the photographed test strips, as well as by plotting the numerical readout of the test line intensities.

Lateral flow in wet system

Early test optimisations were run in wet system assays using free standing strips. To run a wet system assay, strips were assembled as described above, but omitting the conjugate pad and gold rehydration pad. Instead, gold-conjugate was diluted to the desired OD in running buffer (PBS, 1% Tergitol) and 30 µL of this was added to wells of a 96 well plate. Assays were initiated by adding 5 µL of sample to each strip followed by one drop of running buffer and the sample was allowed to diffuse laterally for 10 minutes. Strips were then placed in the 30 µL of diluted conjugate and the gold allowed to diffuse through the strip for 20 minutes. One additional drop of running buffer was added to the strips (to wash out residual gold) and after 10 minutes strips were assessed as above.

Lateral Flow in one-step format in AtomoRapid™ Pascal

For testing the lateral flow test strips in the AtomoRapid™ Pascal cassette, nitrocellulose membranes (Vivid90, Cytiva or Hi-flow90, Millipore) were striped as outlined above, but at higher antigen concentrations (1.3–1.4 mg/mL total protein) than those used in the two-step test (0.65–0.7 mg/mL). Anti-IgA (DCNDx) gold conjugate pads were made as previously described. The nitrocellulose membrane (NCM) was then laminated together with an absorbent pad (CF6, GE Healthcare) at the top of the NCM and a blood retention pad (FR-1(0.35), mdi) at the bottom. A sample pad (glass fibre 8980, Ahlstrom) was added below the blood retention pad, followed by the conjugate pad and a gold rehydration pad (glass fibre 8951, Ahlstrom) at the bottom of the NCM. All components were laminated together on an adhesive backing card and cut into 4 mm

test strips using a guillotine cutter (Kinbio Shanghai Kibio Tech). The strips were assembled into the AtomoRapid™ Pascal cassette.

To run an assay with stored patient samples, defrosted plasma was mixed in a 50:50 ratio with freshly collected blood cells from venous blood draws of never infected human volunteers. Cells were collected by spinning freshly collected blood at 1000 x g for 5 mins and removing the top plasma layer. From this mix, 10 µL of reconstituted blood sample was pipetted into the blood collection unit of the AtomoRapid™ Pascal cassette and applied to the sample pad. Running buffer was then released to the test strip by pushing down on the test to burst an integrated blister prefilled with running buffer. Running buffer consisted of 1 X Dulbecco's phosphate buffered saline (DPBS), 2% Synperonic F108, 1% Tween20, 1.8 mg/mL K_2EDTA, pH 7.4. The test was allowed to sit for 15 minutes, then visualised and the test and control lines quantitated by an automated reader such as the Lumos LeeLu or Cerberus reader (Lumos, Carlsbad) modified to fit the Pascal cassette. The test results were interpreted through visual assessment of the photographed test strips, as well as by plotting the numerical readout of the test line intensities.

To run an assay with fresh finger prick blood, the integrated lancet unit of the AtomoRapid™ Pascal cassette was used to prick the patient or volunteer's finger, and the blood collection channel was placed into the fingerpick blood drop to collect 10 µL of blood via capillary action. This was then applied to the test strip and the test conducted as previously described.

ELISA for IgA antibodies towards T pallidum antigens

Maxisorp plates (96 well, Nunc) were coated overnight at 4–8°C with the indicated TP antigen at 5 µg/mL in carbonate-bicarbonate buffer (Sigma-Aldrich, C3041). The wells were blocked for 1 hr at 37° C 1.5% (w/v) Bovine Serum albumin (BSA) in PBS-T (PBS with 0.05% Tween-20) before incubation with patient serum samples diluted 1:100 in ELISA diluent buffer (0.5% BSA (w/v) in PBS-T) for 1 h. The wells were subsequently incubated with an HRP conjugated anti-human IgA secondary antibody (A0295, Sigma-Aldrich) diluted at 1:5000 in ELISA buffer for 1 h. Bound human IgA was quantified using KPL Sure Blue Reserve TMB Microwell Peroxidase Substrate (Seracare) and the reaction stopped by addition of 0.5 M sulfuric Acid. The optical densities at 620 nm and 450 nm were measured using a Multiskan SkyHigh reader (ThermoFisher) and the OD (450 nm–620 nm) calculated. All incubations were conducted at 37° C. Wells were washed six times with PBS-T after each step.

Spotting and lateral flow testing of biotinylated peptides

Two peptide fragments, one from TmpA (ASGAKEEAEKKAAEQRAL) and one from Tp47(SVLSKQETEDSRGRKKWEKETDPSV) were synthesised with the addition of biotin at the N terminus (Genscript, Australia). To test patient antibody binding in lateral flow template these peptides were resuspended in dimethyl sulfoxide (DMSO) at a concentration of 40 mg/mL, before dilution to 0.4 mg/mL in PBS. Both the peptides and full-length TmpA and Tp47 were spotted in 1 µL volumes onto the middle of 0.4 mm strips of Hi-flow90 nitrocellulose membranes, with the membrane adhered to CF6 absorbent pad at the top of the NCM, and an 8951-glass fibre sample pad at the bottom of the NCM. The strips were dried for 2 hours at 37 °C and then assessed for patient antibody binding using an anti-IgA gold conjugate in wet system format as previously described.

Example 2: Selection of antigens for evaluation as candidate antigens in diagnostic test development

The syphilis spirochete has an outer membrane, peptidoglycan space and inner membrane. The five proteins examined here are shown in their putative locations and are located on the inner surface of the outer membrane, and the inner membrane (**Figure 1**). The spirochete is unusual in that it does not possess lipids in its membrane and presents relatively few antigens to the immune system. These features are believed to contribute to its ability to evade immunity. The putative size of each antigen in *T. pallidum* varies between 15 and 47 kDa. Tp15 is a lipoprotein, Tp47 is a lipoprotein and carboxypeptidase, Tp17 is a periplasmic lipoprotein and has been described as a target for the generation of antibodies. These three proteins are frequently used in syphilis diagnostic tests, sometimes expressed as a single protein Tp15-Tp17-Tp47 in any order. Tp0453 is associated with the inner leaflet of the outer membrane and is the only known outer membrane protein. TmpA, also known as TpN44.5 is a lipoprotein (**Figure 2**).

The five proteins were expressed in *E. coli*. Reducing SDS-PAGE performed on purified *T. pallidum* antigens shows they are highly purified, and their observed molecular mass corresponds to the expected mass. Each antigen was expressed in *E. coli* and purified using nickel affinity chromatography followed by gel filtration (**Figure 3**).

Example 3: An example of a lateral flow assay to detect antibodies to *T. pallidum*

The antigens can be used in a lateral flow assay to detect antibodies in patient blood (serum, plasma, whole venous blood or finger prick blood) towards *T. pallidum* antigens (**Figure 4**). Nitrocellulose membranes on backing cards are striped with *T.*

pallidum proteins (antigens) which is the "test" line. A second line acts as a procedural "control line" which can be an antibody that captures the excess conjugate or another protein that captures excess conjugate such as Protein L. The striped nitrocellulose membrane is then placed in a cassette including a sample pad, a rehydration pad and a conjugate pad containing the desired detector, usually gold-labelled anti-species antibody. In this case, an anti-human IgA antibody is used conjugated to 40 nm gold particles. The sample is applied to the sample pad where the antibodies are allowed to flow along the nitrocellulose membrane towards the *T. pallidum* antigens. If antibodies are present, they bind to the antigen. Running buffer is added to the rehydration pad which reconstitutes the conjugate which travels along the nitrocellulose membrane. If antibodies in the patient sample have been bound to the test line, these will be detected by the anti-IgA-gold-conjugate. The excess gold-conjugate will then bind to the control line telling the operator that the test is valid. The presence of a reactive test and control line indicates the presence of antibodies in the patient sample to *T. pallidum*. The distinguishing feature of this test is the ability of a serological test for trepanomal antibodies to accurately discriminate between active infection and past infection, at least in part due to the inclusion of an anti-IgA antibody (**Figure 4**), and described further below. Assembled tests that have dry components are placed in a plastic cassette for use in diagnostic testing by health care practitioners or at home (**Figure 5**).

Example 4: Evaluating antigens for their ability to detect active syphilis and discriminate people with past-treated syphilis

Antigens were evaluated for their ability to detect active syphilis and discriminate people with past-treated syphilis using clinical samples collected by the Melbourne Sexual Health Centre under Alfred Hospital Ethics approval ID 625/22. Clinical samples collected from 40 cases of active syphilis, 40 cases of past-treated syphilis and 17 people who have never had syphilis were used to evaluate the ability of each antigen individually to detect IgA specific antibodies to syphilis. IgA antibodies were selected based on prior art disclosed in Pham et al., (2020) where a point of care test for the diagnosis of active syphilis was developed based on the detection of IgA antibodies. In this study, the sensitivity for active syphilis was 84.7% and was able to correctly diagnose past-treated syphilis in 71% of cases. The antigens used in this assay were Tp15, Tp17, Tp47; sourced from Fapon, (China) and Tp0453 antigen sourced from Genscript.

Individual antigens were striped on nitrocellulose in the assembled lateral flow tests using these clinical samples (**Figure 6** and **7**). The most critical feature of antigen selection is the ability to discriminate between active and past-treated syphilis. Here it is

a requirement that the smallest number of cases as possible from subjects with past-treated infection are not classified as active syphilis. Examination of the antigens using the past-treated samples shows that the worst performing antigen was Tp17 (**Figure 7**).

Analysis of the panel for sensitivity and specificity was used to further select antigens. The highest specificity (fewest false positives) in those never infected with syphilis was achieved with TmpA, Tp15 and Tp47 showing 100% specificity, and Tp0453 and Tp17 showing 94% specificity (**Figure 8**). The ability of single *T. pallidum* antigens to correctly diagnose active syphilis ranged from 53% to 80% (**Figure 8**) with the best performing antigen being TmpA (**Figure 8**) and worst being Tp15. In past-treated cases, single *T. pallidum* antigens were able to correctly classify patients between 73% and 100%, with the worst performing antigen being Tp17 (**Figure 8**).

Using a limited sample panel of serum samples collected from 21 active cases, 14 past-treated cases, and 2 never infected people, the *T. pallidum* antigens were combined and tested in pairs. Comparison of the results obtained with the single antigen are shown for active syphilis (**Figure 9A**) and compared to paired combinations of antigens (**Figure 9B**). In people with active syphilis, more of the samples tested positive (above 400 units) when two of the antigens were combined. The best combinations were Tp0453+Tp47 > Tp0453+Tp17=Tp0453+TmpA>Tp0453+Tp15=Tp47+TmpA (**Figure 10**).

Comparison of the results obtained with the single antigen are shown for past-treated syphilis (**Figure 9C**) and compared to paired combinations of antigens (**Figure 9D**). The best combinations that correctly identified past-treated cases were Tp47+TmpA=Tp0453+Tp15>Tp0453+Tp47=Tp0453+TmpA>Tp0453+Tp17 (**Figure 10**).

Comparison of the results obtained with the single antigen are shown for never infected people (**Figure 9E**) and compared to paired combinations of antigens (**Figure 9F**). The best combinations for specificity for never infected samples were Tp0453+Tp15=Tp47+TmpA>Tp0453+Tp47=Tp0453+TmpA>Tp0453+Tp17 (**Figure 10**). The selection of best performing paired antigens was done based on achieving the highest sensitivity and ability to discriminate active syphilis from past-treated cases.

Example 5: Combining antigens to improve sensitivity and specificity of detecting active and past-treated syphilis

The full panel of 40 cases of active syphilis, 40 cases of past-treated syphilis and 17 people who have never had syphilis were used for the best performing combinations Tp0453+Tp47 and Tp0453+TmpA (**Figure 11**). Here it can be seen that combining TmpA with Tp0453 increased the number of people incorrectly identified as active

syphilis in the past-treated samples and increased the number of false positives in the never infected group (y-axis>400). This was reflected in a reduced specificity for correctly identifying those with past-treated syphilis of 63% (Tp0453+TmpA) versus 78% for Tp0453+Tp47. False positives increased from 16% for Tp0453+Tp47 to 28% for Tp0453+TmpA (**Figure 12**).

Based on the above performance, the Tp47, TmpA and Tp0453 antigens were combined at different ratios to determine the optimal sensitivity and specificity (**Figure 13**). The sensitivity for detection of active syphilis improved from 90% observed for the double antigen combinations to 95% using a 2:1:1 ratio of the three antigens (**Figure 13A** and **Figure 14**). Using a 1:1:1 ratio also increased sensitivity relative to the double antigen combinations to 93% (**Figure 13B** and **Figure 14**). However, when combining antigens, the specificity for diagnosing past-treated infections correctly dropped from 63% and 78% for Tp0453+TmpA and Tp0453+Tp47, to 58% and 55% for 2:1:1 and 1:1:1 ratio of the three antigens, respectively (**Figure 14**).

Further improvements to the performance of the test in its ability to discriminate active versus past-treated syphilis were achieved by adjusting the antigen concentration and through the addition of excipients. Maintaining a 2:1:1 ratio of Tp47:TmpA:Tp0453 but increasing the amount of antigen added to the test line to 0.7 mg/mL and addition of 0.5% (w/v) sucrose increased the performance of the test by reducing the background signal (**Figure 15**). Comparison of the data using 2:1:1 Tp47:TmpA:Tp0453 at 0.45 mg/mL (**Figure 15A**) and the data of Tp47:TmpA:Tp0453 at 0.7 mg/mL and 0.5% (w/v) sucrose (**Figure 15B**) shows that samples from those who have never had syphilis all now fall well below the visual cut-off of 400 axxin units and improves the specificity of the assay from 94% to 100%, respectively (**Figure 16**). The specificity for accurately classifying past-treated rose from 58% to 80%. The sensitivity of the assay for detection of active syphilis remained unchanged at 95%. This data shows that increasing the amount of protein contained within the test line with addition of 0.5% (w/v) sucrose reduces background and improves diagnostic accuracy.

A total antigen concentration of 0.7 mg/mL comprised of Tp47:Tp0453:TmpA in a ratio of 2:1:1 + 0.5% (w/v) sucrose provides the best sensitivity and specificity for this patient sample set. However, the response of the test line can be tuned with varying the total concentration of antigen (**Figure 17**). As many other features of lateral flow assays can also impact test line signal intensity (e.g. OD of gold-conjugate, membrane identity, volume of sample applied) the exact ideal total antigen concentration may vary substantially if other parameters are varied. This is demonstrated in **Figure 18**, wherein decreasing the OD of the gold in the conjugate pad from the standard OD 7.2 to an OD

of 5.2 decreases the test line strength. Accordingly, suitable antigen concentrations range from between 0.1 mg/mL to 2 mg/mL total antigen. Furthermore, slight variations in antigen ratio (50:25:25 vs 40:20:40 vs 45:23:33, **Figure 19**) will not impact the ability to discriminate past-treated from active patients. When a subset of marginal patients was tested on these antigen ratios the exact percentages of patients identified as active or past-treated varied, but the overall strength of the signal was similar, as an increase in the ability to accurately determine positive patients was always accompanied by an increase in past-treated patients being incorrectly identified as active **Figure 19D**). As can be seen in **Figure 20** tests with more extreme ratio variations, (with one antigen comprising 80% of the test line and the other two antigens each 10%) resulted in compromised sensitivities and specificities as compared to tests with the standard 2:1:1 Tp47:TmpA:Tp0453 ratio. However, all tests still reported sensitivity and specificity of >80% (**Figure 20E**).

To determine if there are patient groups that the test will not be suitable for, the test line responses of all past-treated patients were compared to how many months had passed since their last diagnosis with syphilis (**Figure 21A and B**) and how many prior infections they had experienced (**Figure 21C and D**). The percentage of false positive past-treated patients decreased with time since last infection but increased with the number of prior infections experienced. Determining which factor most contributes to the high IgA levels of false positive patients is complicated using the relatively small sample size and by patient overlap between these two categories. The impact of excluding certain patient subgroups on test sensitivity and specificity can be seen in **Figure 22**. If both patients that have had more than 2 prior infections and are <6 months post last infection are excluded, the patient test panel decreases from 40 patients with active syphilis, 40 past-treated and 17 never infected to 39 active, 32 past-treated and 17 never infected patients. The sensitivity of the test is unchanged at 95%, but the specificity increases from 86% to 92%. The individual patient test line data following this exclusion can be seen in **Figure 23**.

The full patient panel was also tested on two commercial syphilis point-of-care tests: the Abbott Bioline 3.0 and Abbott Determine TP. This data is presented in the first column of **Figure 22**, with the results if patient exclusions applied presented in the indicated columns. Regardless of the patient exclusions both commercial tests showed high sensitivity (100% and 97% respectively), but low specificities (ranging from 37–44% for the Abbot Bioline 3.0 and 28–36% for the Abbot Determine TP depending on patient exclusions). As can be seen in **Figure 24** this is due almost entirely to the tests classifying the majority of past-treated patients as active cases. These tests do not claim

to discriminate between active and inactive syphilis, and the test data presented here supports that they instead discriminate between TPHA positive and TPHA negative patients, as all past-treated patients in the sample set are TPHA positive. As described in the patent background, a TPHA assay is a standard serological test for total patient antibodies towards *T. pallidum* antigens, and patients typically remain TPHA positive for life post any infection with syphilis, even after successful treatment. In **Figure 24,** these results have been used to calculate positive and negative predictive values for each test, with **24B** showing results from the full patient panel and **24C** the results once excluding patients >2 prior infections and <6 months post last infection.

The designation of patients in the sample set used as positive, past-treated and never infected is based on the patient's doctor's diagnosis, performed using a combination of RPR and TPHA serological tests, patient history, PCR results and dark field microscopy. PCR and dark field microscopy specifically examine the patient for the presence of *T. pallidum* and can be used for diagnosis when sores are present in early stages of infection. As previously described, a positive TPHA test indicates that a patient has at some point been infected by *T. pallidum*, while the rapid plasma reagin (RPR) test is a marker of active infection as it measures antibodies present in the blood towards cardiolipins released by damaged cells. While RPR results can show substantial interpatient variability and are ideally monitored over time for an accurate diagnosis, a simplified stratification of patients can be performed using just TPHA and RPR results. With this classification patients are designated as never infected if they are TPHA negative, as past-treated if they are TPHA positive but have RPR levels <8, or with active syphilis infection if they are TPHA positive AND have RPR levels ≥8. It can be seen in **Figure 25** that reclassifying the patient set with this simplified criterion results in a test with 97% sensitivity and 83% specificity for the entire patient panel, or 97% sensitivity and 84% specificity when the previously described patients were excluded from the analysis.

In a previous iteration of this test, published by researchers at the Burnet, a commercial protein described as a fusion of antigenic regions of the *T. pallidum* proteins Tp15, Tp17 and Tp47 was used in the test line, in combination with the full antigenic protein Tp0453. To compare potential performance of this test with the current iteration (Tp47:Tp0453:TmpA in a ratio of 2:1:1 + 0.5% (w/v) sucrose) a similarly described fusion protein was sourced from Meridian and striped with Tp0453 in a 1:1 ratio (**Figure 26**). This test was less sensitive than the best performing Burnet test (64–65% sensitivity depending on patient exclusions vs the Burnet test sensitivity of 95%) and showed a slightly poorer specificity (84–90% vs a Burnet test specificity of 86–92%).

Example 6: Comparison of IgA vs IgG detectors

Both commercial point-of-care tests evaluated here are designed to detect all antibodies towards the tests' *T. pallidum* antigens, regardless of antibody isotype. In contrast, the test conceived of by the present inventors is specific for IgA antibodies. This is accomplished by using a highly specific anti-IgA gold conjugate. As can be seen in **Figure 27**, when the patient panel was evaluated with an anti-IgG detector on tests tuned to give a similar sensitivity to the anti-IgA test (anti-IgA sensitivity of 95% vs anti-IgG sensitivity of 93%), using an anti-IgG detector significantly decreased test specificity (anti-IgA specificity of 92% vs anti-IgG of 63%) as many more past-treated patients were detected as false positivises. Specifically detecting IgA antibodies in patient samples improves the ability to discriminate active versus past-treated syphilis infections, compared to IgG antibodies.

Example 7: No effect of HIV status on test performance

HIV (human immunodeficiency virus) status was also known for 96 of the 97 patients in the panel. The results of testing each patient on nitrocellulose membrane striped with Tp47:Tp0453:TmpA in a ratio of 2:1:1 + 0.5% (w/v) sucrose were compared for HIV positive and HIV negative patients (**Figure 28**). Patients' HIV status has no impact on the test performance, supporting the possible combination of a point-of-care test for active syphilis with a point of care test for other conditions such as HIV.

Example 8: Prediction of Tp15, TmpA and Tp47 antigenic fragments

Prior reports suggest that the sera from *T. pallidum* infected rabbits or humans show reactivity to fragments or domains of Tp15, TmpA and Tp47, with some regions proving immunodominant (Antoni et al., 1996; US9,316,642; Baughn et al 1996). Suggesting that the test as described herein can be recapitulated using fragments of one or more of Tp15, TmpA and Tp47 instead of full-length proteins. **Table 3** shows a sample of predicted antigenic peptides of the five *T. pallidum* proteins, identified either via prediction tools (http://tools.immuneepitope.org/bcell/) or reported in the cited publications. The experimental work presented here was performed with recombinant proteins covering the full length of the mature *T. pallidum* antigens. In some embodiments, of the invention the test comprises fragments of the *T. pallidum* antigens instead of one or more of the full-length proteins.

In **Table 4** shows the sequences selected by six epitope prediction models as likely to be highly antigenic are shown underlined.

The sequences of the 6-His recombinant versions of each *T. pallidum* antigen were inputted into the prediction tool (http://tools.immuneepitope.org/bcell/). This was used to generate scores for potential epitopes within the proteins, based on likely antigenicity using different prediction models. The six models used were (1) Emini Surface Accessibility Prediction (Emini et al., 1978) (2) Parker Hydrophilicity Prediction (Parker et al., 1986) (3) Kolaskar & Tongaonkar Antigenicity (Kolaskar and Tongaonkar 1990) (4) Chou & Fasman Beta-Turn Prediction (Chou and Fasman 1978) (5) Karplus & Schulz Flexibility Prediction (Karplus and Schulz 1985) (6) Bepipred Linear Epitope Prediction 2.0 (Jespersen et al., 1986). Outputs were ranked by highest scoring regions, and the sequences of each protein that encompass the top 20 highest scoring regions (excluding the recombinant protein tags) are shown underlined in **Table 4**.

Table 3: Predicted antigenic peptides of TmpA, Tp47, Tp0453, Tp15 and Tp17.

Protein	Amino acid sequence	Position	Predictive method or Publication
TmpA	KEEAEKK	40–46	• Emini et al., 1978 • Parker et al., 1986
	DGDSANA	142–148	• Parker et al., 1986 • Chou and Fasman 1878
	LKVAVEL	207–213	• Kolaskar and Tongaonkar 1990
	KTKQKAS	272–278	• Parker et al., 1986 • Karplus and Schulz 1985 • Emini et al., 1978
	SDTSSPS	327–333	• Karplus and Schulz 1985 • Chou and Fasman 1978 • Parker et al., 1986
	GGRSPKS	343–349	• Karplus and Schulz 1985 • Chou and Fasman 1978
	(1)ASGAKEEAEKKAAEQRALL (2)EAEKALQSAKTKQKASSDLARSADKSAPLPENAQ	(1) 23–41 (2) 250–283 (3) 277–312	Antoni et al., 1996

	(3)PLPENAQGFSKEPIEVE PLPNDRLNTTQADESAPIPI (4)DESAPIPISDTSSPSRVQ SRGVEDGGRSPKSSMN	(4) 306–339	
Tp47	SKQETED	114–120	• Jespersen et al., 1986 • Emini et al., 1978 • Parker et al., 1986 • Karplus and Schulz 1985
	RKKWEY	124–129	• Jespersen et al., 1986 • Emini et al., 1978
	FVPVAVP	192–198	• Kolaskar and Tongaonkar 1990
	DDASYTN	299–305	• Chou and Fasman 1978 • Parker et al., 1986 • Jespersen et al., 1986
	KGSGPGY	340–346	• Chou and Fasman 1978 • Karplus and Schulz 1985
	DLDAGMFDAVSRATHGH GAFRQQFQYAVEVLGEKV LSKQETEDSRGRKKWEYET DPSVTKMVRASASFQDLG EDGEIKFEAVEGAVALADR ASSFMVDSEEYKITNVKVH GM	63–147	• US9,316,642
Tp0453	SGSYPYA	76–82	• Jespersen et al., 1986 • Chou and Fasman 1978
	HFSCVIF	119–125	• Kolaskar and Tongaonkar 1990
	GSSKRER	126–132	• Emini et al., 1978 • Karplus and Schulz 1985
	NPDRPQL	142–148	• Chou and Fasman 1978 • Emini et al., 1978
	TSEGTSQ	157–163	• Parker et al., 1986

			• Karplus and Schulz 1985
Tp15	LEKGNPE	116–121	• Jespersen et al., 1986 • Emini et al., 1978 • Karplus and Schulz 1985
	VVYDYQH	73–79	• Jespersen et al., 1986 • Emini et al., 1978 • Kolaskar and Tongaonkar 1990
	KSQDADY	85–91	• Parker et al., 1986 • Karplus and Schulz 1985
	ARRGEKE	147–153	• Emini et al., 1978 • Karplus and Schulz 1985
	(1) MVQVVYD (2) DYHRVMY (3) VMYASSG (4) EKAFREL (5) VTGATVS	(1) 53–59 (2) 73–79 (3) 77–83 (4) 87–93 (5) 109–115	Baughn et al., 1996
Tp17	EKKSAP	83–88	• Jespersen et al., 1986 • Emini et al., 1978
	VSSEQSK	110–116	• Emini et al., 1978 • Karplus and Schulz 1985 • Parker et al., 1986
	GAGKPSK	139–145	• Chou and Fasman 1978 • Emini et al., 1978
	APFYVLK	148–154	• Kolaskar and Tongaonkar 1990 • Jespersen et al., 1986

Table 4: Predicted antigenic peptides.

Tp15-6His
BepiPred Linear Epitope Prediction 2.0.
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSSFSSIPNGTYRATYQDFDENGW KDFLEVTFDGGKMVQVVYDYQHKEGRFKSQDADYHRVMYASSGIGPEKAFRELA DALLEKGNPEMVDVVTGATVSSQSFRRLGRALLQSARRGEKEAIISR*

Chou & Fasman Beta-Turn Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSSFSSIPNGTYRATYQDFDENGW KDFLEVTFDGGKMVQVVYDYQHKEGRFKSQDADYHRVMYASSGIGPEKAFRELA DALLEKGNPEMVDVVTGATVSSQSFRRLGRALLQSARRGEKEAIISR*
Emini Surface Accessibility Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSSFSSIPNGTYRATYQDFDENGW KDFLEVTFDGGKMVQVVYDYQHKEGRFKSQDADYHRVMYASSGIGPEKAFRELA DALLEKGNPEMVDVVTGATVSSQSFRRLGRALLQSARRGEKEAIISR*
Karplus & Schulz Flexibility Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSSFSSIPNGTYRATYQDFDENGW KDFLEVTFDGGKMVQVVYDYQHKEGRFKSQDADYHRVMYASSGIGPEKAFRELA DALLEKGNPEMVDVVTGATVSSQSFRRLGRALLQSARRGEKEAIISR*
Kolaskar & Tongaonkar Antigenicity
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSSFSSIPNGTYRATYQDFDENGW KDFLEVTFDGGKMVQVVYDYQHKEGRFKSQDADYHRVMYASSGIGPEKAFRELA DALLEKGNPEMVDVVTGATVSSQSFRRLGRALLQSARRGEKEAIISR*
Parker Hydrophilicity Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSSFSSIPNGTYRATYQDFDENGW KDFLEVTFDGGKMVQVVYDYQHKEGRFKSQDADYHRVMYASSGIGPEKAFRELA DALLEKGNPEMVDVVTGATVSSQSFRRLGRALLQSARRGEKEAIISR*
Tp17-6His
BepiPred Linear Epitope Prediction 2.0.
MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECALKGGIFRGT LPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSPLTYRGTWMVREDGIVELSLVS SEQSKAPHEKELYELIDSNSVRYMGAPGAGKPSKEMAPFYVLKK*
Chou & Fasman Beta-Turn Prediction
MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECALKGGIFRGT

LPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSPLTYRGTWMVREDGIVELSLVS SEQSKAPHEKELYELIDSNSVRYMGAPGAGKPSKEMAPFYVLKK*
Emini Surface Accessibility Prediction
MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECALKGGIFRGT LPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSPLTYRGTWMVREDGIVELSLVS SEQSKAPHEKELYELIDSNSVRYMGAPGAGKPSKEMAPFYVLKK*
Karplus & Schulz Flexibility Prediction MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECALKGGIFRGT LPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSPLTYRGTWMVREDGIVELSLVS SEQSKAPHEKELYELIDSNSVRYMGAPGAGKPSKEMAPFYVLKK*
Kolaskar & Tongaonkar Antigenicity MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECALKGGIFRGT LPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSPLTYRGTWMVREDGIVELSLVS SEQSKAPHEKELYELIDSNSVRYMGAPGAGKPSKEMAPFYVLKK*
Parker Hydrophilicity Prediction MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECALKGGIFRGT LPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSPLTYRGTWMVREDGIVELSLVS SEQSKAPHEKELYELIDSNSVRYMGAPGAGKPSKEMAPFYVLKK*
Tp47-6His
BepiPred Linear Epitope Prediction 2.0.
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSGSSHHETHYGYATLSYADYWA GELGQSRDVLLAGNAEADRAGDLDAGMFDAVSRATHGHGAFRQQFQYAVEVLGE KVLSKQETEDSRGRKKWEYETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVAL ADRASSFMVDSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTN GLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVMLKRADGSEL SHREFIDYVMNFNTVRYDYYGDDASYTNLMASYGTKHSADSWWKTGRVPRISCGI NYGFDRFKGSGPGYYRLTLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGAD AETLMDAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSVR AKVVAQ*

Chou & Fasman Beta-Turn Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSGSSHHETHYGYATLSYADYWA GELGQSRDVLLAGNAEADRAGDLDAGMFDAVSRATHGHGAFRQQFQYAVEVLGE KVLSKQETEDSRGRKKWEYETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVAL ADRASSFMVDSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTN GLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVMLKRADGSEL SHREFIDYVMNFNTVRYDYYGDDASYTNLMASYGTKHSADSWWKTGRVPRISCGI NYGFDRFKGSGPGYYRLTLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGAD AETLMDAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSVR AKVVAQ*
Emini Surface Accessibility Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSGSSHHETHYGYATLSYADYWA GELGQSRDVLLAGNAEADRAGDLDAGMFDAVSRATHGHGAFRQQFQYAVEVLGE KVLSKQETEDSRGRKKWEYETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVAL ADRASSFMVDSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTN GLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVMLKRADGSEL SHREFIDYVMNFNTVRYDYYGDDASYTNLMASYGTKHSADSWWKTGRVPRISCGI NYGFDRFKGSGPGYYRLTLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGAD AETLMDAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSVR AKVVAQ*
Karplus & Schulz Flexibility Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSGSSHHETHYGYATLSYADYWA GELGQSRDVLLAGNAEADRAGDLDAGMFDAVSRATHGHGAFRQQFQYAVEVLGE KVLSKQETEDSRGRKKWEYETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVAL ADRASSFMVDSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTN GLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVMLKRADGSEL SHREFIDYVMNFNTVRYDYYGDDASYTNLMASYGTKHSADSWWKTGRVPRISCGI NYGFDRFKGSGPGYYRLTLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGAD AETLMDAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSVR AKVVAQ*
Kolaskar & Tongaonkar Antigenicity
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSGSSHHETHYGYATLSYADYWA GELGQSRDVLLAGNAEADRAGDLDAGMFDAVSRATHGHGAFRQQFQYAVEVLGE

KVLSKQETEDSRGRKKWEYETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVAL ADRASSFMVDSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTN GLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVMLKRADGSEL SHREFIDYVMNFNTVRYDYYGDDASYTNLMASYGTKHSADSWWKTGRVPRISCGI NYGFDRFKGSGPGYYRLTLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGAD AETLMDAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSVR AKVVAQ*
Parker Hydrophilicity Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSGSSHHETHYGYATLSYADYWA GELGQSRDVLLAGNAEADRAGDLDAGMFDAVSRATHGHGAFRQQFQYAVEVLGE KVLSKQETEDSRGRKKWEYETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVAL ADRASSFMVDSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTN GLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVMLKRADGSEL SHREFIDYVMNFNTVRYDYYGDDASYTNLMASYGTKHSADSWWKTGRVPRISCGI NYGFDRFKGSGPGYYRLTLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGAD AETLMDAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSVR AKVVAQ*
Tp0453-6His
BepiPred Linear Epitope Prediction 2.0.
MHHHHHHWKASVDPLGVVGSGADVYLYFPVAGNENLISRIIENHESKADIKKIVDR TTAVYGAFFARSKEFRLFGSGSYPYAFTNLIFSRSDGWASTKTEHGITYYESEHTDVS IPAPHFSCVIFGSSKRERMSKMLSRLVNPDRPQLPPRFEKECTSEGTSQTVALYIKNG GHFITKLLNFPQLNLPLGAMELYLTARRNEYLYTLSLQLGNAKINFPIQFLISRVLNA HIHVEGDRLIIEDGTISAERLASVISSLYSKKGSS*
Chou & Fasman Beta-Turn Prediction
MHHHHHHWKASVDPLGVVGSGADVYLYFPVAGNENLISRIIENHESKADIKKIVDR TTAVYGAFFARSKEFRLFGSGSYPYAFTNLIFSRSDGWASTKTEHGITYYESEHTDVS IPAPHFSCVIFGSSKRERMSKMLSRLVNPDRPQLPPRFEKECTSEGTSQTVALYIKNG GHFITKLLNFPQLNLPLGAMELYLTARRNEYLYTLSLQLGNAKINFPIQFLISRVLNA HIHVEGDRLIIEDGTISAERLASVISSLYSKKGSS*
Emini Surface Accessibility Prediction

MHHHHHHWKASVDPLGVVGSGADVYLYFPVAGNENLISRIIENHESKADIKKIVDR TTAVYGAFFARSKEFRLFGSGSYPYAFTNLIFSRSDGWASTKTEHGITYYESEHTDVS IPAPHFSCVIFGSSKRERMSKMLSRLVNPDRPQLPPRFEKECTSEGTSQTVALYIKNG GHFITKLLNFPQLNLPLGAMELYLTARRNEYLYTLSLQLGNAKINFPIQFLISRVLNA HIHVEGDRLIIEDGTISAERLASVISSLYSKKGSS*
Karplus & Schulz Flexibility Prediction
MHHHHHHWKASVDPLGVVGSGADVYLYFPVAGNENLISRIIENHESKADIKKIVDR TTAVYGAFFARSKEFRLFGSGSYPYAFTNLIFSRSDGWASTKTEHGITYYESEHTDVS IPAPHFSCVIFGSSKRERMSKMLSRLVNPDRPQLPPRFEKECTSEGTSQTVALYIKNG GHFITKLLNFPQLNLPLGAMELYLTARRNEYLYTLSLQLGNAKINFPIQFLISRVLNA HIHVEGDRLIIEDGTISAERLASVISSLYSKKGSS*
Kolaskar & Tongaonkar Antigenicity
MHHHHHHWKASVDPLGVVGSGADVYLYFPVAGNENLISRIIENHESKADIKKIVDR TTAVYGAFFARSKEFRLFGSGSYPYAFTNLIFSRSDGWASTKTEHGITYYESEHTDVS IPAPHFSCVIFGSSKRERMSKMLSRLVNPDRPQLPPRFEKECTSEGTSQTVALYIKNG GHFITKLLNFPQLNLPLGAMELYLTARRNEYLYTLSLQLGNAKINFPIQFLISRVLNA HIHVEGDRLIIEDGTISAERLASVISSLYSKKGSS*
Parker Hydrophilicity Prediction
MHHHHHHWKASVDPLGVVGSGADVYLYFPVAGNENLISRIIENHESKADIKKIVDR TTAVYGAFFARSKEFRLFGSGSYPYAFTNLIFSRSDGWASTKTEHGITYYESEHTDVS IPAPHFSCVIFGSSKRERMSKMLSRLVNPDRPQLPPRFEKECTSEGTSQTVALYIKNG GHFITKLLNFPQLNLPLGAMELYLTARRNEYLYTLSLQLGNAKINFPIQFLISRVLNA HIHVEGDRLIIEDGTISAERLASVISSLYSKKGSS*
TmpA-6His
BepiPred Linear Epitope Prediction 2.0.
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEKKAAEQRALLVES AHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIEGDLKKAAGVASEA ADKYEILRNRVEVADLQSKIQTHQLAQYDGDSANAAEESWKKALELYETDSAQCL QSTVEALESYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQ YQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTKQKASS

DLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQADESAPIPISDTSSPSRVQSR GVEDGGRSPKSSMNEEGASR*
Chou & Fasman Beta-Turn Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEKKAAEQRALLVES AHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIEGDLKKAAGVASEA ADKYEILRNRVEVADLQSKIQTHQLAQYDGDSANAAEESWKKALELYETDSAQCL QSTVEALESYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQ YQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTKQKASS DLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQADESAPIPISDTSSPSRVQSR GVEDGGRSPKSSMNEEGASR*
Emini Surface Accessibility Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEKKAAEQRALLVES AHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIEGDLKKAAGVASEA ADKYEILRNRVEVADLQSKIQTHQLAQYDGDSANAAEESWKKALELYETDSAQCL QSTVEALESYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQ YQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTKQKASS DLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQADESAPIPISDTSSPSRVQSR GVEDGGRSPKSSMNEEGASR*
Karplus & Schulz Flexibility Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEKKAAEQRALLVES AHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIEGDLKKAAGVASEA ADKYEILRNRVEVADLQSKIQTHQLAQYDGDSANAAEESWKKALELYETDSAQCL QSTVEALESYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQ YQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTKQKASS DLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQADESAPIPISDTSSPSRVQSR GVEDGGRSPKSSMNEEGASR*
Kolaskar & Tongaonkar Antigenicity
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEKKAAEQRALLVES AHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIEGDLKKAAGVASEA ADKYEILRNRVEVADLQSKIQTHQLAQYDGDSANAAEESWKKALELYETDSAQCL QSTVEALESYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQ YQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTKQKASS

DLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQADESAPIPISDTSSPSRVQSR GVEDGGRSPKSSMNEEGASR*
Parker Hydrophilicity Prediction
MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEKKAAEQRALLVES AHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIEGDLKKAAGVASEA ADKYEILRNRVEVADLQSKIQTHQLAQYDGDSANAAEESWKKALELYETDSAQCL QSTVEALESYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQ YQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTKQKASS DLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQADESAPIPISDTSSPSRVQSR GVEDGGRSPKSSMNEEGASR

Example 9: Mass spectrometry analysis

For mass spectrometry analysis, proteins were first run on SDS-PAGE under reducing conditions using Bolt™ Bis-Tris Plus Mini Protein Gels, 4–12% (Invitrogen) with Bolt MES SDS Running Buffer (Invitrogen). The band corresponding to the desired protein was excised from the SDS-PAGE gel using a scalpel. Mass spectrometry was performed using a Fusion (Thermo Scientific) instrument with a Dionex Ultimate 3000 RSLCano Nano LC system. The following mass spectrometry columns were used: Analytical column: Acclaim PepMap RSLC (75 µm x 50 cm, nanoViper,C18, 2 µm, 100Å; Thermo Scientific); Trap column: Acclaim PepMap 100 (100 µm x 2 cm, nanoViper,C18, 5 µm, 100Å; Thermo Scientific). Data was analysed using the Byonic (ProteinMetrics) search engine. Results are shown in **Figure 30**.

Example 10: Interrogation of diversity of Tp antigen protein families

To interrogate the known diversity in the protein families of the five Tp antigens Tp15, TmpA, Tp17, Tp0453 and Tp47, BLASTp searches were conducted using native antigen sequences as queries (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Sayers et al., 2022). All searches were conducted against the non-redundant protein sequences (nr) database, with applying the restriction of limiting searches to the Treponema genus for proteins Tp15, TmpA, Tp17 and Tp0453. This restriction was not in place for Tp47. Sequences retrieved were downloaded and those containing miscalled amino acid residues (i.e. X) or sequences with >99% homology were removed. Phylogenic trees of the sequences were generated using Clustal Omega Multiple Sequence Alignment on default settings (Madeira et al., 2022). Results are shown in **Figure 31**.

Example 11: Demonstration of functioning one-step test compatible with the AtomoRapid™ Pascal cassette

In addition to the previously described 30-minute two-step lateral flow test (shown in **Figure 4**), a 15-minute test, which requires only one buffer introduction post sample application (i.e. a one-step test) has been developed, the schematic of which is shown in **Figure 31**, and the device itself shown in **Figure 32**. In this one-step test format, the patient sample is obtained by pressing the integrated lancet to the finger which pricks the skin releasing finger prick blood. The blood is applied to the sample pad via the blood collection unit. Immediately after sample application, the running buffer button is depressed, releasing buffer which diffuses laterally across the lateral flow strip. This rehydrates a gold pad, which in this example contains an anti-human IgA gold conjugate. The gold conjugate and patient antibodies from the applied sample are carried by the running buffer across the strip, where patient antibodies form a complex with T pallidum antigens immobilised on the membrane test line. This is visualised by the anti-IgA gold conjugate. Non-syphilis antigen-specific antibodies present in the patient sample subsequently bind to the Protein L control line and are also visualised by the anti IgA gold conjugate. The test can be read at 15 minutes after the sample and running buffer are applied to the test strip, and in contrast with the previously described two-step version of this test, there is no timed interval between sample application and buffer addition, and only one introduction of running buffer. The test can also be developed for other cassette types in a one-step format known to those in the art using a separate lancet and/or running buffer bottle and demonstrates the broad applicability of the test to alternative lateral flow devices.

The results of assessing clinical samples collected from 40 cases of active syphilis, 41 cases of past-treated syphilis and 17 people never infected using this test strip in the AtomoRapid™ Pascal cassette can be seen in **Figure 33**. The same test performance can be achieved in the one-step format as in the two-step format, with a sensitivity of 95% and a specificity of 86% when assessing the entire patient set (**Figure 33A**) and a 95% sensitivity/92% specificity when excluding samples with blood taken < 6 months post infection or from patients with > 2 past infections (**Figure 33B**). A positive predictive value (PPV) of 83% and negative predictive value (NPV) of 96% is achieved with no exclusions. When exclusions are applied, the PPV is 90% and NPV is 96%.

The unique combination of treponemal antigens Tp0453, Tp47 and TmpA that differentiates active syphilis infection from past-treated and never infected cases developed here can be adapted to alternative lateral flow test formats and simplified to enhance usability. The antigens and the components of the lateral flow device were

adapted to fit a commercially available integrated device, the AtomoRapid Pascal cassette. Here, the lancet, a blister containing running buffer and a blood collection unit are contained with the lateral flow cassette. Adaptation of the test antigens to this device allowed for reduction of the number of steps, the need for additional timed intervals, and shortened the time to result to 15 minutes. The sensitivity and specificity against the same panel of active, past-treated and never infected patients was identical to that in the traditional cassette format. This demonstrates how the Tp0453, Tp47 and TmpA test antigens can be used in alternative cassette types.

Example 12: Demonstrating that fresh finger prick blood can be used as a sample for the lateral flow test

The lateral flow test can be successfully conducted with stored serum as shown in **Figure 24** or using venous blood as shown in **Figure 33**. To demonstrate that the test can also function with fresh finger prick blood as the sample type, a small number of patients undergoing standard serology testing for syphilis also volunteered to perform a finger prick test using the AtomoRapid™ Pascal cassette and a one-step syphilis lateral flow test strip. Four representative results of these tests can be seen in **Figure 34**, with a control line and a positive test line observable for two patients with active syphilis, and only a control line visible for two patients with negative syphilis serology.

The intended matrix that is applied to the diagnostic test for detection of antibodies associated only with active infection is finger prick blood. Detection of antibodies in finger prick blood samples was successful, and was equally demonstrated using venous blood, plasma, serum, and finger prick blood. For point of care assessment, obtaining finger prick blood can be self-performed or performed by a health care practitioner. In other settings, venous blood, serum or plasma may be the most easily available sample type. Accordingly, Tp0453, Tp47 and TmpA antigens have demonstrated broad applicability across different sample types for diagnosis of active syphilis in different health care and patient settings.

Example 13: Demonstrating the use of anti-IgA ELISA to distinguish patients who are positive, past-treated and never infected for syphilis

In addition to detecting anti-TP antigen IgA antibodies using a lateral flow format, it is possible to measure patient IgA antibodies towards TmpA, Tp47 and Tp0453 using ELISA. An example of this with a small subset of patient samples can be seen in **Figure 35**. IgA antibodies to all three antigens are detectable from the syphilis positive patient

samples, and a pooled positive sample, while negligible IgA antibodies can be detected in the never infected and past-treated patient samples.

The Tp0453, Tp47 and TmpA antigens can also be used in plate based assays and other assay formats known to those in the art for detection of antibodies in cases of active syphilis. Tp0453, Tp47 and TmpA can be applied to enzyme linked immunosorbent assay plates to capture antibodies in patient blood. This assay format may be more amenable to pathology laboratory based testing to diagnose active syphilis and high throughput screening for serosurveillance studies of active syphilis. Bead based assays could also be developed using this antigen combination for luminex and bioplex instruments.

Example 14: Comparison to previously published antigen combinations and fragments

Two peptide fragments, one from TmpA (ASGAKEEAEKKAAEQRAL) and one from Tp47(SVLSKQETEDSRGRKKWEKETDPSV) have been disclosed as antigenic domains of their respective proteins (CN1151171C). To investigate their potential utility for the detection of active syphilis, both synthetic peptides and full length TmpA and Tp47 were spotted and dried on nitrocellulose membrane strips. These tests strips were then assessed for their ability to bind IgA from one never infected and four active syphilis patient samples (**Figure 36A**) in a wet system lateral flow format. While IgA antibodies from most (TmpA, 3/4) or all (Tp47, 4/4) of the active syphilis samples tested readily bound the full-length proteins, no binding towards either Tp47 or TmpA peptides could be detected. Small peptides are well known to bind inefficiently to NCMs, so it is possible that while these peptides are antigenic, they are unsuitable for use in lateral flow as they cannot bind to the NCM.

Additionally, Rodriguez et al., (2023) described an ELISA that measures serum IgA antibodies towards a triple antigen combination of TmpA, Tp17 and Tp47. This assay gave a reported sensitivity of 90.8% for patients with active syphilis infection, a specificity of 98.1% for never infected patients and a specificity of 61.3% for past-treated patients. Assessment of serum IgA towards individual antigens Tp15, Tp17, TmpA, Tp0453 and Tp17 using lateral flow (**Figure 37**), revealed that a subset of past-treated patients displays strong reactivity towards Tp17 and are false-positive results. Therefore, inclusion of Tp17 would substantially reduce the accuracy of a diagnostic test to discriminate between an active syphilis infection versus a past-treated infection.

When plasma is assessed in a two-step lateral flow format using a combination of TmpA, Tp47 and Tp17 triple antigen in the test line (**Figure 38** top panels) all 5 past-

treated samples are reactive and would be false-positive results (**Figure 38**). Using the same total protein antigen combination using our best-performing antigens (**Figure 38** bottom panels, TmpA +Tp47+Tp0453, at a 0.7 mg/ml total antigen + 0.5% sucrose), the same five past-treated samples tested showed no (4/5) or very weak reactivity (1/5). These data confirm that TmpA +Tp47+Tp0453 are substantially improved combination of antigens for discriminating active versus past-treated syphilis infections.

Other manuscripts have described antigenic fragments or alternative antigens for diagnosis of syphilis. TmpA peptide (ASGAKEEAEKKAAEQRAL) and Tp47peptide (SVLSKQETEDSRGRKKWEKETDPSV) described as being antigenic components of TmpA and Tp47 proteins respectively, were examined. Spotting of these antigens onto nitrocellulose membranes and application of patient samples from active syphilis resulted in no binding of anti-treponemal IgA antibodies. This demonstrated that they are not useful reagents for the detection of antibodies to syphilis in lateral flow format. Furthermore, prior art has described an ELISA that measures serum IgA antibodies towards a triple antigen combination of TmpA, Tp17 and Tp47. Analysis of the ability of antibodies in past-treated samples to bind various treponemal antigens was performed, and a subset of these samples showed very strong reactivity to Tp17 suggesting that Tp17 is not a suitable treponemal antigen for discrimination of past-treated (which should have no reactivity) and active syphilis. This was further confirmed when the combination of TmpA, Tp17 and Tp47 was used in a lateral flow assay where 100% of past-treated samples were reactive and would be false-positive results compared to 20% using the combination of Tp47, Tp0453, and TmpA. Therefore, Tp17 is not a useful treponemal antigen for discriminating active and past-treated syphilis and the combination of Tp47, Tp0453, and TmpA is superior.

Example 15: The effect of removing short (40–42 amino acid) regions of Tp antigens Tp47 and TmpA

To determine if the same antigenic response is generated from truncated versions of the TP antigens, variants of Tp47 and TmpA were designed with short amino acid truncations (40–42 amino acids) from either the C or N terminus of the full-length protein. Presented in **Figure 39** is data from testing select never infected and active syphilis patient plasma samples on lateral flow membranes striped with test lines of either full length Tp47 and N-terminally truncated Tp47, and full-length and C-terminally truncated TmpA (all proteins striped at 0.7 mg/mL +0.5% sucrose). Patient antibody binding to the test line was visualised with an anti-IgA gold conjugate and quantified using a LeeLu reader.

Truncating the Tp47 protein by 42 amino acids from the N terminus (Tp47 N-Trunc, **Figure 39A**) resulted in an antigen variant with similar ability to bind IgA antibodies in patients with active syphilis compared to full length Tp47 (test line values of 76–109% for Tp47 N-Trunc vs full length Tp47). IgA recognition of TmpA truncated from the C terminus by 40 amino acids (TmpA C-Trunc, **Figure 39B**) in actively infected syphilis patients was similar to full-length TmpA (64%–120%, positive test line values).

We explored whether deletions of the full-length proteins could also be utilised as antigenic fragments for diagnosis of active syphilis. Removal of 42 amino acids from the N terminus of Tp47 and 40 amino acids from the C terminus of TmpA did not substantially alter the ability of anti-treponemal IgA antibodies to bind the antigens. Therefore, it is possible that further modifications of TmpA and Tp47 are permissible while retaining full antigenic activity, however, it is highly likely that a significant portion of the proteins must be present as the TmpA peptide (ASGAKEEAEKKAAEQRAL) and Tp47peptide (SVLSKQETEDSRGRKKWEKETDPSV) were not reactive.

Table 5: Table of key sequences.

SEQ ID/Name	Sequence
1 Tp47	MKVKYALLSAGALQLLVVGCGSSHHETHYGYATLSYADYWAG ELGQSRDVLLAGNAEADRAGDLDAGMFDAVSRATHGHGAFRQ QFQYAVEVLGEKVLSKQETEDSRGRKKWEYETDPSVTKMVRAS ASFQDLGEDGEIKFEAVEGAVALADRASSFMVDSEEYKITNVKV HGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTNGLKTMLTED SFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVMLKRAD GSELSHREFIDYVMNFNTVRYDYYGDDASYTNLMASYGTKHSA DSWWKTGRVPRISCGINYGFDRFKGSGPGYYRLTLIANGYRDVV ADVRFLPKYEGNIDIGLKGKVLTIGGADAETLMDAAVDVFADGQ PKLVSDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSVRAKVVA Q
2 Tp0453	MIRRRYRGCTQGAWIVSVGMLFASCTSGAWKASVDPLGVVGSG ADVYLYFPVAGNENLISRIIENHESKADIKKIVDRTTAVYGAFFAR SKEFRLFGSGSYPYAFTNLIFSRSDGWASTKTEHGITYYESEHTDV SIPAPHFSCVIFGSSKRERMSKMLSRLVNPDRPQLPPRFEKECTSE GTSQTVALYIKNGGHFITKLLNFPQLNLPLGAMELYLTARRNEYL YTLSLQLGNAKINFPIQFLISRVLNAHIHVEGDRLIIEDGTISAERL ASVISSLYSKKGSS
3 TmpA	MNAHTLVYSGVALACAAMLGSCASGAKEEAEKKAAEQRALLV ESAHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIE GDLKKAAGVASEAADKYEILRNRVEVADLQSKIQTHQLAQYDG DSANAAEESWKKALELYETDSAQCLQSTVEALESYRKVAHEGG RLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQYQEAREA EEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTK QKASSDLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQAD ESAPIPISDTSSPSRVQSRGVEDGGRSPKSSMNEEGASR
4 Tp15	MVKRGRFALCLAVLLGACSFSSIPNGTYRATYQDFDENGWKDFL EVTFDGGKMVQVVYDYQHKEGRFKSQDADYHRVMYASSGIGP

	EKAFRELADALLEKGNPEMVDVVTGATVSSQSFRRLGRALLQSA RRGEKEAIISR
5 Tp17	MKGSVRALCAFLGVGALGSALCVSCTTVCPHAGKAKAEKVECA LKGGIFRGTLPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSP LTYRGTWMVREDGIVELSLVSSEQSKAPHEKELYELIDSNSVRY MGAPGAGKPSKEMAPFYVLKKTKK
6 Tp47	GTGAAAGTGAAATACGCACTACTTTCTGCCGGAGCGCTGCAGT TGTTGGTTGTAGGCTGTGGCTCGTCTCATCATGAGACGCACTA TGGCTATGCGACGCTAAGCTATGCGGACTACTGGGCCGGGGA GTTGGGGCAGAGTAGGGACGTGCTTTTGGCGGGTAATGCCGA GGCGGACCGCGCGGGGGATCTCGACGCAGGCATGTTCGATGC AGTTTCTCGCGCAACCCACGGGCATGGCGCGTTCCGTCAGCAA TTTCAGTACGCGGTTGAGGTATTGGGCGAAAAGGTTCTCTCGA AGCAGGAGACCGAAGACAGCAGGGGAAGAAAAAAGTGGGAG TACGAGACTGACCCAAGCGTTACTAAGATGGTGCGTGCCTCTG CGTCATTTCAGGATTTGGGAGAGGACGGGGAGATTAAGTTTG AAGCAGTCGAGGGTGCAGTAGCGTTGGCGGATCGCGCGAGTT CCTTCATGGTTGACAGCGAGGAATACAAGATTACGAACGTAA AGGTTCACGGTATGAAGTTTGTCCCAGTTGCGGTTCCTCATGA ATTAAAAGGGATTGCAAAGGAGAAGTTTCACTTCGTGGAAGA CTCCCGCGTTACGGAGAATACCAACGGCCTTAAGACAATGCTC ACTGAGGATAGTTTTTCTGCACGTAAGGTAAGCAGCATGGAG AGCCCGCACGACCTTGTGGTAGACACGGTGGGTACCGGTTAC CACAGCCGTTTTGGTTCGGACGCAGAGGCTTCTGTGATGCTGA AAAGGGCTGATGGCTCTGAGCTGTCGCACCGTGAGTTCATCGA CTATGTGATGAACTTCAACACGGTCCGCTACGACTACTACGGT GATGACGCGAGCTACACCAATCTGATGGCGAGTTATGGCACC AAGCACTCTGCTGACTCCTGGTGGAAGACAGGAAGAGTGCCC CGCATTTCGTGTGGTATCAACTATGGGTTCGATCGGTTTAAAG GTTCAGGGCCGGGATACTACAGGCTGACTTTGATTGCGAACG GGTATAGGGACGTAGTTGCTGATGTGCGCTTCCTTCCCAAGTA CGAGGGGAACATCGATATTGGGTTGAAGGGGAAGGTGCTGAC CATAGGGGGCGCGGACGCGGAGACTCTGATGGATGCTGCAGT TGACGTGTTTGCCGATGGACAGCCTAAGCTTGTCAGCGATCAA GCGTGAGCTTGGGGCAGAATGTCCTCTCTGCGGATTTCACTCC CGGCACTGAGTACACGGTTGAGGTTAGGTTCAAGGAATTCGG TTCTGTGCGTGCGAAGGTAGTGGCCCAGTAG
7 Tp0453	ATGCTATTTGCATCGTGCACTTCAGGGGCGTGGAAGGCATCAG TAGATCCGTTGGGGGTTGTGGGATCTGGTGCAGATGTGTACCT GTATTTCCCTGTAGCGGGGAACGAGAATTTGATTTCTCGTATT ATCGAGAACCATGAGTCAAAGGCAGATATTAAAAAAATAGTG GACAGGACTACCGCGGTATACGGTGCTTTTTTTGCCCGATCAA AAGAGTTTCGTTTGTTCGGAAGCGGTTCGTATCCATACGCCTT TACTAATTTGATTTTTTCTCGATCCGATGGCTGGGCATCTACGA AAACGGAACACGGAATCACGTACTATGAAAGTGAACATACGG ACGTTTCGATTCCTGCGCCGCATTTTTCCTGTGTGATTTTTGGT TCCTCCAAAAGGGAGCGGATGAGCAAAATGCTGTCTCGGCTC GTTAACCCCGATCGACCGCAGTTACCGCCTCGCTTTGAAAAAG AATGTACGTCGGAAGGTACGAGCCAGACTGTTGCACTCTATAT AAAAAACGGGGGACACTTTATTACCAAACTGTTGAATTTTCCG CAGCTTAATTTACCACTTGGGGCAATGGAACTGTACTTGACCG CGCGGAGGAATGAGTATCTTTACACGTTGAGCTTGCAGCTGGG GAATGCAAAGATAAATTTTCCCATACAGTTTTTAATCTCTCGT GTGCTTAACGCGCACATTCACGTGGAGGGGGACAGGTTAATT ATTGAAGACGGCACAATTTCTGCTGAGCGTCTTGCGTCGGTGA TCTCGTCGTTGTACTCCAAAAAGGGAAGTTCGTAA

8 TmpA	ATGAGCCGAGGAGGTATCACGTGGGAGGTTAACATCATGAAT GCTCATACGCTTGTGTACTCCGGCGTAGCACTTGCCTGCGCGG CTATGCTCGGCTCCTGTGCCTCGGGCGCCAAGGAGGAAGCTG AAAAGAAGGCTGCAGAGCAGCGTGCGCTTCTGGTCGAGAGTG CGCATGCTGACCGTAGGCTTATGGAGGCGCGTATCGGCGCGC AAGAGTCTGGCGCAGACACCCAGCACCCCGAACTTTTCTCCCA GATTCAGGACGTTGAGCGCCAGTCTACCGACGCCAAGATTGA AGGGGACCTCAAGAAAGCTGCCGGTGTCGCCTCAGAAGCTGC GGATAAGTACGAGATTCTCAGGAACCGAGTTGAAGTTGCTGA CCTACAATCTAAGATCCAGACTCACCAGCTTGCGCAGTACGAC GGGGACAGCGCGAACGCTGCGGAAGAATCGTGGAAGAAGGC ACTTGAATTATACGAGACCGATAGCGCGCAGTGTCTGCAATCC ACCGTCGAAGCGCTCGAGTCGTATCGGAAAGTCGCGCATGAG GGATTCGGCCGCTTACTACCCGATATGAAGGCACGTGCGGGT GCTGCAAAGACGGACGTTGGCGGTCTTAAGGTAGCCGTCGAG TTGCGTCCACAGCTGGAAGAAGCTGACAGCCAATACCAAGAA GCACGTGAAGCTGAAGAGGTAAATGCACGTGCCAAAGCTTTT AGCGGGTACCACCGTGCCCTCGAGATCTACACAGAACTGGGG AAGGTTGTACGCCTGAAGAAGACCGAGGCGGAAAAGGCGCTG CAGTCTGCAAAAACAAAGCAAAAGGCGTCCTCTGACCTTGCG CGGAGTGCGGATAAGAGTGCCCCACTTCCTGAAAACGCTCAG GGTTTCTCAAAGGAGCCGATTGAGGTAGAGCCGCTTCCAAAC GACAGGCTTAACACAACGCAGGCAGATGAGTCTGCGCCGATC CCCATATCTGACACCTCTTCACCTTCTCGCGTGCAGTCTCGGG GTGTTGAAGACGGAGGACGTTCTCCAAAATCCTCTATGAACG AAGAAGGAGCCTCTCGATGA
9 Tp15	ATGGTGAAAAGAGGTCGGTTCGCGCTGTGTCTTGCGGTGTTGC TTGGGGCGTGTTCATTTAGTTCTATCCCGAATGGCACGTACCG GGCGACGTATCAGGATTTTGATGAGAATGGTTGGAAGGACTTT CTCGAGGTTACTTTTGATGGTGGCAAGATGGTGCAGGTGGTTT ACGATTATCAGCATAAAGAAGGGCGGTTTAAGTCCCAGGACG CTGACTACCATCGGGTCATGTATGCATCCTCGGGCATAGGTCC TGAAAAGGCCTTCAGAGAGCTCGCCGATGCTTTGCTTGAAAA GGGTAATCCCGAGATGGTGGATGTGGTCACCGGTGCAACTGTT TCTTCCCAGAGTTTCAGGAGGTTGGGTCGTGCGCTTCTGCAGA GTGCGCGGCGCGGCGAGAAGGAAGCCATTATTAGCAGGTAG
10 Tp17	ATGAAAGGATCTGTCCGCGCGCTGTGCGCGTTCCTTGGTGTTG GAGCGCTCGGTAGCGCTTTGTGTGTCTCGTGCACAACCGTGTG TCCGCACGCCGGGAAGGCCAAAGCGGAAAAGGTAGAGTGCGC GTTGAAGGGAGGTATCTTTCGGGGTACGCTACCTGCGGCCGAT TGCCCGGGAATCGATACGACTGTGACGTTCAACGCGGATGGC ACTGCGCAAAAGGTAGAGCTTGCCCTTGAGAAGAAGTCGGCA CCTTCTCCTCTTACGTATCGCGGTACGTGGATGGTACGTGAAG ACGGAATTGTCGAACTCTCGCTTGTGTCCTCGGAGCAATCGAA GGCACCGCACGAGAAAGAGCTGTACGAGCTGATAGACAGTAA CTCCGTTCGCTACATGGGCGCTCCCGGCGCAGGAAAGCCTTCA AAGGAGATGGCGCCGTTTTACGTGCTCAAAAAAACAAAGAAA TAG
11 Tp47-6His (codon optimised DNA)	ATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTC TCCTCGGTCTCGATTCTACGGAAAACCTGTATTTTCAGGGAAT TGATCCCTTCACCGGATCCGGTAGCTCACATCATGAAACCCAT TATGGTTATGCAACCCTGAGCTATGCCGATTATTGGGCAGGCG AACTGGGTCAGAGCCGTGATGTTCTGCTGGCAGGTAATGCCG AAGCAGATCGTGCCGGTGATCTGGATGCAGGTATGTTTGATGC AGTTAGCCGTGCAACCCATGGTCATGGTGCATTTCGTCAGCAG TTTCAGTATGCAGTTGAAGTTCTGGGCGAAAAAGTTCTGAGCA

	AACAAGAAACCGAAGATAGCCGTGGTCGTAAAAAGTGGGAAT ATGAAACCGATCCGAGCGTTACCAAAATGGTGCGTGCAAGCG CAAGCTTTCAGGATCTGGGAGAAGATGGTGAAATTAAGTTTG AAGCCGTTGAAGGTGCAGTTGCACTGGCCGATCGTGCAAGCA GTTTTATGGTTGATAGCGAAGAGTACAAAATCACCAACGTTAA AGTGCACGGCATGAAATTTGTTCCGGTTGCCGTTCCGCATGAA CTGAAAGGCATTGCAAAAGAAAAATTTCATTTTGTGGAAGAT AGTCGCGTGACCGAAAATACCAATGGTCTGAAAACCATGCTG ACGGAAGATAGCTTTAGCGCACGTAAAGTGAGCAGCATGGAA AGTCCGCATGATCTGGTTGTTGATACCGTTGGCACCGGTTATC ATAGCCGTTTTGGTAGTGATGCGGAAGCAAGCGTTATGCTGAA ACGTGCAGATGGTAGCGAACTGAGTCATCGTGAATTTATCGAT TACGTGATGAACTTTAATACCGTGCGCTATGATTATTATGGTG ATGATGCAAGCTATACCAATCTGATGGCCAGCTATGGCACCA AACATAGCGCAGATAGTTGGTGGAAAACCGGTCGTGTTCCGC GTATTAGCTGTGGTATTAACTATGGTTTCGATCGCTTTAAAGG TAGCGGTCCGGGTTATTATCGTCTGACCCTGATTGCAAATGGT TATCGTGATGTTGTGGCAGATGTTCGTTTTCTGCCGAAATATG AAGGCAACATTGATATCGGTCTGAAAGGTAAAGTGCTGACCA TTGGCGGTGCAGATGCAGAAACCCTGATGGATGCAGCAGTTG ATGTTTTTGCCGATGGTCAGCCGAAACTGGTTAGCGATCAGGC CGTTAGCCTGGGCCAGAATGTTCTGAGCGCAGATTTTACACCG GGTACAGAATATACCGTTGAAGTGCGTTTTAAAGAATTTGGTA GCGTTCGTGCAAAAGTTGTTGCACAGTAA
12 Tp0453 (consensus codons)	[ATGATHMGNMGNMGNTAYMGNGGNTGYACNCARGGNGCNT GGATHGTNWSNGTNGGNATGYTNTTYGCNWSNTGYACNWSN GGNGCNTGGAARGCNWSNGTNGAYCCNYTNGGNGTNGTNGG NWSNGGNGCNGAYGTNTAYYTNTAYTTYCCNGTNGCNGGNAA YGARAAYYTNATHWSNMGNATHATHGARAAYCAYGARWSNA ARGCNGAYATHAARAARATHGTNGAYMGNACNACNGCNGTN TAYGGNGCNTTYTTYGCNMGNWSNAARGARTTYMGNYTNTTY GGNWSNGGNWSNTAYCCNTAYGCNTTYACNAAYYTNATHTTY WSNMGNWSNGAYGGNTGGGCNWSNACNAARACNGARCAYG GNATHACNTAYTAYGARWSNGARCAYACNGAYGTNWSNATH CCNGCNCCNCAYTTYWSNTGYGTNATHTTYGGNWSNWSNAAR MGNGARMGNATGWSNAARATGYTNWSNMGNYTNGTNAAYC CNGAYMGNCCNCARYTNCCNCCNMGNTTYGARAARGARTGY ACNWSNGARGGNACNWSNCARACNGTNGCNYTNTAYATHAA RAAYGGNGGNCAYTTYATHACNAARYTNYTNAAYTTYCCNCA RYTNAAYYTNCCNYTNGGNGCNATGGARYTNTAYYTNACNGC NMGNMGNAAYGARTAYYTNTAYACNYTNWSNYTNCARYTNG GNAAYGCNAARATHAAYTTYCCNATHCARTTYYTNATHWSNM GNGTNYTNAAYGCNCAYATHCAYGTNGARGGNGAYMGNYTN ATHATHGARGAYGGNACNATHWSNGCNGARMGNYTNGCNWS NGTNATHWSNWSNYTNTAYWSNAARAARGGNWSNWSN
13 TmpA-6His (codon optimised DNA)	ATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTC TCCTCGGTCTCGATTCTACGGAAAACCTGTATTTTCAGGGAAT TGATCCCTTCACCGGATCCGCAAGCGGTGCCAAAGAAGAAGC CGAAAAGAAAGCAGCAGAACAGCGTGCACTGCTGGTTGAAAG CGCACATGCAGATCGTCGTCTGATGGAAGCACGTATTGGTGCA CAAGAAAGCGGTGCAGATACCCAGCATCCGGAACTGTTTAGC CAGATTCAGGATGTTGAACGTCAGAGCACCGATGCAAAAATT GAAGGTGATCTGAAAAAGGCAGCCGGTGTTGCAAGCGAAGCA GCCGATAAATATGAAATTCTGCGTAATCGTGTTGAAGTTGCCG ATCTGCAGAGCAAAATTCAGACCCATCAGCTGGCACAGTATG ATGGTGATAGCGCAAATGCAGCAGAAGAAAGCTGGAAGAAA

	GCACTGGAACTGTATGAAACCGATAGCGCACAGTGTCTGCAG AGTACCGTTGAAGCCCTGGAAAGCTATCGTAAAGTTGCACAT GAAGGTTTTGGTCGTCTGCTGCCGGATATGAAAGCACGTGCCG GTGCAGCAAAAACCGATGTTGGTGGTCTGAAAGTTGCAGTTG AACTGCGTCCGCAACTGGAAGAAGCAGATAGCCAGTATCAAG AAGCACGCGAAGCCGAAGAAGTTAACGCACGCGCAAAAGCAT TTAGCGGTTATCATCGTGCACTGGAAATCTATACCGAACTGGG TAAAGTTGTTCGTCTGAAGAAAACCGAAGCAGAAAAAGCACT GCAGAGCGCAAAAACCAAACAGAAAGCAAGCAGCGATCTGG CACGTAGCGCAGATAAAAGCGCACCGCTGCCGGAAAATGCAC AGGGTTTTAGTAAAGAACCGATTGAAGTTGAACCGCTGCCTA ATGATCGTCTGAATACCACACAGGCAGATGAATCAGCACCGA TTCCGATTAGCGATACCAGCAGTCCGAGCCGTGTTCAGAGCCG TGGTGTTGAAGATGGTGGTCGTAGCCCGAAAAGCAGCATGAA TGAAGAAGGTGCAAGCCGTTAA
14. Tp15-6His (codon optimised)	ATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTC TCCTCGGTCTCGATTCTACGGAAAACCTGTATTTTCAGGGAAT TGATCCCTTCACCGGATCCAGCTTTAGCAGCATTCCGAATGGC ACCTATCGTGCAACCTATCAGGATTTTGATGAAAACGGCTGGA AAGATTTTCTGGAAGTTACCTTTGATGGTGGCAAAATGGTTCA GGTGGTTTATGATTATCAGCACAAAGAAGGTCGCTTCAAAAG CCAGGATGCAGATTATCATCGTGTTATGTATGCAAGCAGCGGT ATTGGTCCTGAAAAAGCATTTCGTGAACTGGCAGATGCACTGC TGGAAAAAGGTAATCCGGAAATGGTTGATGTTGTTACCGGTG CAACCGTTAGCAGCCAGAGCTTTCGTCGTCTGGGTCGTGCCCT GCTGCAGAGCGCACGTCGTGGTGAAAAAGAAGCAATTATTAG TCGCTAA
15 Tp17-6His (codon optimised)	ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTG GTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAG CAAATGGGTCGCGGATCCGGTAAAGCAAAAGCAGAAAAAGTT GAATGTGCACTGAAAGGTGGTATTTTTCGTGGCACCCTGCCTG CAGCAGATTGTCCGGGTATTGATACCACCGTGACCTTTAATGC AGATGGCACCGCACAGAAAGTTGAACTGGCCCTGGAAAAGAA AAGCGCACCGAGTCCGCTGACCTATCGCGGTACATGGATGGTT CGTGAAGATGGTATTGTTGAACTGAGCCTGGTTAGCAGCGAA CAGAGCAAAGCACCGCATGAGAAAGAACTGTATGAACTGATT GATAGCAATAGCGTGCGTTATATGGGTGCACCTGGTGCCGGTA AACCGAGCAAAGAAATGGCACCGTTTTATGTTCTGAAAAAGT AA
16 Tp47-6His	MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSGSSHHETHYG YATLSYADYWAGELGQSRDVLLAGNAEADRAGDLDAGMFDAV SRATHGHGAFRQQFQYAVEVLGEKVLSKQETEDSRGRKKWEYE TDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVALADRASSFMV DSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTE NTNGLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSD AEASVMLKRADGSELSHREFIDYVMNFNTVRYDYYGDDASYTN LMASYGTKHSADSWWKTGRVPRISCGINYGFDRFKGSGPGYYRL TLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGADAETLM DAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVRF KEFGSVRAKVVAQ
17 Tp0453-6His	MHHHHHHWKASVDPLGVVGSGADVYLYFPVAGNENLISRIIENH ESKADIKKIVDRTTAVYGAFFARSKEFRLFGSGSYPYAFTNLIFSR SDGWASTKTEHGITYYESEHTDVSIPAPHFSCVIFGSSKRERMSK MLSRLVNPDRPQLPPRFEKECTSEGTSQTVALYIKNGGHFITKLL NFPQLNLPLGAMELYLTARRNEYLYTLSLQLGNAKINFPIQFLISR VLNAHIHVEGDRLIIEDGTISAERLASVISSLYSKKGSS

18 TmpA-6His	MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEK KAAEQRALLVESAHADRRLMEARIGAQESGADTQHPELFSQIQD VERQSTDAKIEGDLKKAAGVASEAADKYEILRNRVEVADLQSKI QTHQLAQYDGDSANAAEESWKKALELYETDSAQCLQSTVEALE SYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEE ADSQYQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTE AEKALQSAKTKQKASSDLARSADKSAPLPENAQGFSKEPIEVEPL PNDRLNTTQADESAPIPISDTSSPSRVQSRGVEDGGRSPKSSMNEE GASR
19 Tp15-6His	MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSSFSSIPNGTYR ATYQDFDENGWKDFLEVTFDGGKMVQVVYDYQHKEGRFKSQD ADYHRVMYASSGIGPEKAFRELADALLEKGNPEMVDVVTGATV SSQSFRRLGRALLQSARRGEKEAIISR
20 Tp17-6His	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKV ECALKGGIFRGTLPAADCPGIDTTVTFNADGTAQKVELALEKKSA PSPLTYRGTWMVREDGIVELSLVSSEQSKAPHEKELYELIDSNSV RYMGAPGAGKPSKEMAPFYVLKK
21 Tp47 fragment	SKQETED
22 Tp47 fragment	RKKWEY
23 Tp47 fragment	FVPVAVP
24 Tp47 fragment	DDASYTN
25 Tp47 fragment	KGSGPGY
26 Tp47 fragment	DLDAGMFDAVSRATHGHGAFRQQFQYAVEVLGEKVLSKQETED SRGRKKWEYETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAV ALADRASSFMVDSEEYKITNVKVHGM
27 Tp47 fragment	DAEASVMLK
28 Tp47 fragment	SSHHE
29 Tp47 fragment	WAGELGQSRDVLLAGNAEADRAGDLDAGM
30 Tp47 fragment	ATHGHGAFRQQ
31 Tp47 fragment	EKVLSKQETEDSRGRKKWEYETDPSVT
32 Tp47 fragment	ASASFQDLGE
33 Tp47 fragment	LKGIAKEKFHFVEDSRVTENTNGLKTMLTEDSFSARKVSSMESPH DLVVDTVGTGYHSRFGSDAEASVMLKRADGSELSHR
34 Tp47 fragment	YYGDDASYTNLMASYGTKHSADSWWKTGRV
35 Tp47 fragment	YGFDRFKGSG
36 Tp47 fragment	LPKYEGNID
37 Tp47 fragment	ADGQPKLVSDQAVSLGQNVLSADFTPG
38 Tp47 fragment	EFGSV
39 Tp47 fragment	GSSHHET
40 Tp47 fragment	RYDYYGDDASYTN
41 Tp47 fragment	ISCGINYG
42 Tp47 fragment	RFKGSGPGYYR
43 Tp47 fragment	SKQETEDSRGRKKWEYETDPS
44 Tp47 fragment	DSEEYK
45 Tp47 fragment	RYDYYGDD
46 Tp47 fragment	PKYEGN
47 Tp47 fragment	ELGQSRDV
48 Tp47 fragment	LSKQETEDSRGRKK
49 Tp47 fragment	VTENTNG
50 Tp47 fragment	KRADGSEL
51 Tp47 fragment	RFKGSGPGY
52 Tp47 fragment	ADGQPKL
53 Tp47 fragment	FQYAVEVLGEKVL
54 Tp47 fragment	VEGAVAL
55 Tp47 fragment	KFVPVAVPHEL

56 Tp47 fragment	SPHDLVVDTVG
57 Tp47 fragment	RDVVADVRFL
58 Tp47 fragment	AAVDVFA
59 Tp47 fragment	LVSDQAV
60 Tp47 fragment	SVRAKVVA
61 Tp47 fragment	GNAEADRAGD
62 Tp47 fragment	SKQETEDSRGRKK
63 Tp47 fragment	EYETDPS
64 Tp47 fragment	DLGEDGE
65 Tp47 fragment	EDSRVTE
66 Tp47 fragment	GSDAEAS
67 Tp47 fragment	KRADGSE
68 Tp47 fragment	GDDASYTN
69 Tp47 fragment	TKHSADS
70 Tp47 fragment	GGADAET
71 Tp0453 fragment	SGSYPYA
72 Tp0453 fragment	HFSCVIF
73 Tp0453 fragment	GSSKRER
74 Tp0453 fragment	NPDRPQL
75 Tp0453 fragment	TSEGTSQ
76 Tp0453 fragment	NHESKADIKKIVD
77 Tp0453 fragment	SGSYPYA
78 Tp0453 fragment	FSRSDGWASTKTEHGITYYESEHTDVSIP
79 Tp0453 fragment	SKRERMSKMLSRLVNPDRPQLPPRFEKECTSEGT
80 Tp0453 fragment	LLNFPQLN
81 Tp0453 fragment	DGTISA
82 Tp0453 fragment	PVAGNEN
83 Tp0453 fragment	LFGSGSYPYA
84 Tp0453 fragment	FSRSDGWAS
85 Tp0453 fragment	VNPDRPQLPP
86 Tp0453 fragment	CTSEGTS
87 Tp0453 fragment	YIKNGGH
88 Tp0453 fragment	SSLYSKKGSS
89 Tp0453 fragment	RSKEFR
90 Tp0453 fragment	STKTEH
91 Tp0453 fragment	TYYESEHTD
92 Tp0453 fragment	GSSKRERMSK
93 Tp0453 fragment	NPDRPQLPPRFEKE
94 Tp0453 fragment	TARRNEYL
95 Tp0453 fragment	NHESKADI
96 Tp0453 fragment	LFGSGSYP
97 Tp0453 fragment	FSRSDGWASTKTEH
98 Tp0453 fragment	IFGSSKRERM
99 Tp0453 fragment	ECTSEGTSQTVA
100 Tp0453 fragment	IKNGGHF
101 Tp0453 fragment	LYSKKGSS
102 Tp0453 fragment	VDPLGVV
103 Tp0453 fragment	ADVYLYFPVAG
104 Tp0453 fragment	VSIPAPHFSCVIFGSS
105 Tp0453 fragment	QTVALYI
106 Tp0453 fragment	YLYTLSL
107 Tp0453 fragment	FLISRVL
108 Tp0453 fragment	LASVISSLYS
109 Tp0453 fragment	ENHESKADIK

110 Tp0453 fragment	ASTKTEHG
111 Tp0453 fragment	YESEHTDVS
112 Tp0453 fragment	GSSKRERMSK
113 Tp0453 fragment	EKECTSEGTSQTV
114 Tp0453 fragment	YSKKGSS
115 TmpA fragment	KEEAEKK
116 TmpA fragment	DGDSANA
117 TmpA fragment	LKVAVEL
118 TmpA fragment	KTKQKAS
119 TmpA fragment	SDTSSPS
120 TmpA fragment	GGRSPKS
121 TmpA fragment	ASGAKEEAEKKAAEQRALL
122 TmpA fragment	EAEKALQSAKTKQKASSDLARSADKSAPLPENAQ
123 TmpA fragment	PLPENAQGFSKEPIEVEPLPNDRLNTTQADESAPIPI
124 TmpA fragment	DESAPIPISDTSSPSRVQSRGVEDGGRSPKSSMN
125 TmpA fragment	ASGAKEEAEKKAAEQRALLV
126 TmpA fragment	HADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIEGDL KKAAGVASEAADKYEILRNRVEVADLQSKIQTHQLAQYDGDSA NAAEESWKKALELYETDSAQCLQSTVEALES
127 TmpA fragment	VAVELRPQLEEADSQYQEAREAEEVNARAKAFSGYH
128 TmpA fragment	KKTEAEKALQSAKTKQKASSDLARSADKSAPLPENAQGFSKEPIE VEPLPNDRLNTTQADESAPIPISDTSSPSRVQSRGVEDGGRSPKSS MNEEG
129 TmpA fragment	AQYDGDSANA
130 TmpA fragment	EPLPNDRLNT
131 TmpA fragment	PISDTSSPSRVQSRGVEDGGRSPKSSMNE
132 TmpA fragment	KEEAEKK
133 TmpA fragment	DTQHPE
134 TmpA fragment	QDVERQSTD
135 TmpA fragment	EESWKK
136 TmpA fragment	RPQLEE
137 TmpA fragment	DSQYQEAR
138 TmpA fragment	RLKKTEAEK
139 TmpA fragment	QSAKTKQKASS
140 TmpA fragment	RSPKSS
141 TmpA fragment	AQESGAD
142 TmpA fragment	SAKTKQKASSD
143 TmpA fragment	ISDTSSPSRVQ
144 TmpA fragment	VEDGGRSPKSSM
145 TmpA fragment	EQRALLVESAH
146 TmpA fragment	VEVADLQ
147 TmpA fragment	SAQCLQSTVE
148 TmpA fragment	VGGLKVAVELRPQL
149 TmpA fragment	LGKVVRLKK
150 TmpA fragment	GAKEEAEKK
151 TmpA fragment	AQESGADTQ
152 TmpA fragment	ERQSTDAK
153 TmpA fragment	YDGDSANA
154 TmpA fragment	AREAEE
155 TmpA fragment	KKTEAEK
156 TmpA fragment	KQKASSD
157 TmpA fragment	NTTQADESA
158 TmpA fragment	SDTSSPSR
159 TmpA fragment	EDGGRSPK

160 TmpA fragment	NEEGASR
161 Tp15 fragment	LEKGNPE
162 Tp15 fragment	VVYDYQH
163 Tp15 fragment	KSQDADY
164 Tp15 fragment	ARRGEKE
165 Tp15 fragment	MVQVVYD
166 Tp15 fragment	DYHRVMY
167 Tp15 fragment	VMYASSG
168 Tp15 fragment	EKAFREL
169 Tp15 fragment	VTGATVS
170 Tp15 fragment	FSSIPN
171 Tp15 fragment	YQDFDENGW
172 Tp15 fragment	DYQHKEGRFKSQDADYHRVMYASSGIGPEK
173 Tp15 fragment	LEKGNPEM
174 Tp15 fragment	RGEKEA
175 Tp15 fragment	SSIPNGTYRA
176 Tp15 fragment	QDFDENGWKD
177 Tp15 fragment	YASSGIGPEK
178 Tp15 fragment	PNGTYRATYQDFDEN
179 Tp15 fragment	YDYQHKEGRFKSQDADYHR
180 Tp15 fragment	EKAFRE
181 Tp15 fragment	EKGNPE
182 Tp15 fragment	SQSFRR
183 Tp15 fragment	RRGEKEA
184 Tp15 fragment	SIPNGT
185 Tp15 fragment	TFDGGKM
186 Tp15 fragment	GRFKSQDAD
187 Tp15 fragment	LEKGNPEM
188 Tp15 fragment	TVSSQSFR
189 Tp15 fragment	ARRGEKEA
190 Tp15 fragment	GKMVQVVYDYQHK
191 Tp15 fragment	DYHRVMYA
192 Tp15 fragment	ELADALLE
193 Tp15 fragment	PEMVDVVTGATVS
194 Tp15 fragment	LGRALLQ
195 Tp15 fragment	QDFDENGWKD
196 Tp15 fragment	EKGNPEM
197 Tp15 fragment	GATVSSQS
198 Tp15 fragment	QSARRGEKE
199 Tp17 fragment	EKKSAP
200 Tp17 fragment	VSSEQSK
201 Tp17 fragment	GAGKPSK
202 Tp17 fragment	APFYVLK
203 Tp17 fragment	AKAEKVECALK
204 Tp17 fragment	GTLPAADCPGIDTTVTFNADGTAQKVELALEKKSAPSPLTYRGT WMVREDGIVELSLVSSEQSKAPHEKELYELIDSNSVRYMGAPGA GKPSKEMAPFYVLKKTKK
205 Tp17 fragment	SSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECALK
206 Tp17 fragment	HHHHHHSSGLVPRGSHMASMTGGQQMGRGSGKAKAEKVECAL K
207 Tp17 fragment	TAQKVELALEKKSAPS
208 Tp17 fragment	LVSSEQSKAPHEKELYEL
209 Tp17 fragment	PGAGKPSKEMAPFY
210 Tp17 fragment	TGGQQMGRGSGKAK

211 Tp17 fragment	PAADCPGIDT
212 Tp17 fragment	KKSAPSPLTYRG
213 Tp17 fragment	DSNSVRY
214 Tp17 fragment	GAPGAGKPSK
215 Tp17 fragment	KAKAEKVE
216 Tp17 fragment	DGTAQK
217 Tp17 fragment	ALEKKSAPS
218 Tp17 fragment	SSEQSKAPHEKELYE
219 Tp17 fragment	AGKPSKEM
220 Tp17 fragment	MGRGSGKAKAE
221 Tp17 fragment	KKSAPSPLT
222 Tp17 fragment	LVSSEQSKAPH
223 Tp17 fragment	GAGKPSKEM
224 Tp17 fragment	EKVECALKG
225 Tp17 fragment	TLPAADCP
226 Tp17 fragment	QKVELALEK
227 Tp17 fragment	GIVELSLVSSE
228 Tp17 fragment	MAPFYVLKK
229 Tp17 fragment	GRGSGKAKAEK
230 Tp17 fragment	NADGTAQ
231 Tp17 fragment	EKKSAPS
232 Tp17 fragment	VSSEQSKAPHEKE
233 Tp17 fragment	GAGKPSKE
234 TmpA fragments	ASGAKEEAEKKAAEQRAL
235 Tp47 fragment	SVLSKQETEDSRGRKKWEKETDPSV
236 N-terminally truncated Tp47-6His	ATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTC TCCTCGGTCTCGATTCTACGGAAAACCTGTATTTTCAGGGAAT TGATCCCTTCACCGGATCCGATCTGGATGCAGGTATGTTTGAT GCAGTTAGCCGTGCAACCCATGGTCATGGTGCATTTCGTCAGC AGTTTCAGTATGCAGTTGAAGTTCTGGGCGAAAAAGTTCTGAG CAAACAAGAAACCGAAGATAGCCGTGGTCGTAAAAAGTGGGA ATATGAAACCGATCCGAGCGTTACCAAAATGGTGCGTGCAAG CGCAAGCTTTCAGGATCTGGGAGAAGATGGTGAAATTAAGTT TGAAGCCGTTGAAGGTGCAGTTGCACTGGCCGATCGTGCAAG CAGTTTTATGGTTGATAGCGAAGAGTACAAAATCACCAACGTT AAAGTGCACGGCATGAAATTTGTTCCGGTTGCCGTTCCGCATG AACTGAAAGGCATTGCAAAAGAAAAATTTCATTTTGTGGAAG ATAGTCGCGTGACCGAAAATACCAATGGTCTGAAAACCATGC TGACGGAAGATAGCTTTAGCGCACGTAAAGTGAGCAGCATGG AAAGTCCGCATGATCTGGTTGTTGATACCGTTGGCACCGGTTA TCATAGCCGTTTTGGTAGTGATGCGGAAGCAAGCGTTATGCTG AAACGTGCAGATGGTAGCGAACTGAGTCATCGTGAATTTATC GATTACGTGATGAACTTTAATACCGTGCGCTATGATTATTATG GTGATGATGCAAGCTATACCAATCTGATGGCCAGCTATGGCAC CAAACATAGCGCAGATAGTTGGTGGAAAACCGGTCGTGTTCC GCGTATTAGCTGTGGTATTAACTATGGTTTCGATCGCTTTAAA GGTAGCGGTCCGGGTTATTATCGTCTGACCCTGATTGCAAATG GTTATCGTGATGTTGTGGCAGATGTTCGTTTTCTGCCGAAATA TGAAGGCAACATTGATATCGGTCTGAAAGGTAAAGTGCTGAC CATTGGCGGTGCAGATGCAGAAACCCTGATGGATGCAGCAGT TGATGTTTTTGCCGATGGTCAGCCGAAACTGGTTAGCGATCAG GCCGTTAGCCTGGGCCAGAATGTTCTGAGCGCAGATTTTACAC CGGGTACAGAATATACCGTTGAAGTGCGTTTTAAAGAATTTGG TAGCGTTCGTGCAAAAGTTGTTGCACAGTAA

237 C terminally truncated TmpA-6His	ATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTC TCCTCGGTCTCGATTCTACGGAAAACCTGTATTTTCAGGGAAT TGATCCCTTCACCGGATCCGCAAGCGGTGCCAAAGAAGAAGC CGAAAAGAAAGCAGCAGAACAGCGTGCACTGCTGGTTGAAAG CGCACATGCAGATCGTCGTCTGATGGAAGCACGTATTGGTGCA CAAGAAAGCGGTGCAGATACCCAGCATCCGGAACTGTTTAGC CAGATTCAGGATGTTGAACGTCAGAGCACCGATGCAAAAATT GAAGGTGATCTGAAAAAGGCAGCCGGTGTTGCAAGCGAAGCA GCCGATAAATATGAAATTCTGCGTAATCGTGTTGAAGTTGCCG ATCTGCAGAGCAAAATTCAGACCCATCAGCTGGCACAGTATG ATGGTGATAGCGCAAATGCAGCAGAAGAAAGCTGGAAGAAA GCACTGGAACTGTATGAAACCGATAGCGCACAGTGTCTGCAG AGTACCGTTGAAGCCCTGGAAAGCTATCGTAAAGTTGCACAT GAAGGTTTTGGTCGTCTGCTGCCGGATATGAAAGCACGTGCCG GTGCAGCAAAAACCGATGTTGGTGGTCTGAAAGTTGCAGTTG AACTGCGTCCGCAACTGGAAGAAGCAGATAGCCAGTATCAAG AAGCACGCGAAGCCGAAGAAGTTAACGCACGCGCAAAAGCAT TTAGCGGTTATCATCGTGCACTGGAAATCTATACCGAACTGGG TAAAGTTGTTCGTCTGAAGAAAACCGAAGCAGAAAAAGCACT GCAGAGCGCAAAAACCAAACAGAAAGCAAGCAGCGATCTGG CACGTAGCGCAGATAAAAGCGCACCGCTGCCGGAAAATGCAC AGGGTTTTAGTAAAGAACCGATTGAAGTTGAACCGCTGCCTA ATGATCGTCTGAATACCACACAGGCA
238 N-terminally truncated Tp47-6His	MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSDLDAGMFDA VSRATHGHGAFRQQFQYAVEVLGEKVLSKQETEDSRGRKKWEY ETDPSVTKMVRASASFQDLGEDGEIKFEAVEGAVALADRASSFM VDSEEYKITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVT ENTNGLKTMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGS DAEASVMLKRADGSELSHREFIDYVMNFNTVRYDYYGDDASYT NLMASYGTKHSADSWWKTGRVPRISCGINYGFDRFKGSGPGYY RLTLIANGYRDVVADVRFLPKYEGNIDIGLKGKVLTIGGADAETL MDAAVDVFADGQPKLVSDQAVSLGQNVLSADFTPGTEYTVEVR FKEFGSVRAKVVAQ
239 C terminally Truncated TmpA-6His	MHHHHHHGKPIPNPLLGLDSTENLYFQGIDPFTGSASGAKEEAEK KAAEQRALLVESAHADRRLMEARIGAQESGADTQHPELFSQIQD VERQSTDAKIEGDLKKAAGVASEAADKYEILRNRVEVADLQSKI QTHQLAQYDGDSANAAEESWKKALELYETDSAQCLQSTVEALE SYRKVAHEGFGRLLPDMKARAGAAKTDVGGLKVAVELRPQLEE ADSQYQEAREAEEVNARAKAFSGYHRALEIYTELGKVVRLKKTE AEKALQSAKTKQKASSDLARSADKSAPLPENAQGFSKEPIEVEPL PNDRLNTTQA
240 N-terminally truncated Tp47	GTGAAAGTGAAATACGCACTACTTTCTGCCGGAGCGCTGCAGT TGTTGGTTGTAGGCTGTGATCTCGACGCAGGCATGTTCGATGC AGTTTCTCGCGCAACCCACGGGCATGGCGCGTTCCGTCAGCAA TTTCAGTACGCGGTTGAGGTATTGGGCGAAAAGGTTCTCTCGA AGCAGGAGACCGAAGACAGCAGGGGAAGAAAAAAGTGGGAG TACGAGACTGACCCAAGCGTTACTAAGATGGTGCGTGCCTCTG CGTCATTTCAGGATTTGGGAGAGGACGGGGAGATTAAGTTTG AAGCAGTCGAGGGTGCAGTAGCGTTGGCGGATCGCGCGAGTT CCTTCATGGTTGACAGCGAGGAATACAAGATTACGAACGTAA AGGTTCACGGTATGAAGTTTGTCCCAGTTGCGGTTCCTCATGA ATTAAAAGGGATTGCAAAGGAGAAGTTTCACTTCGTGGAAGA CTCCCGCGTTACGGAGAATACCAACGGCCTTAAGACAATGCTC ACTGAGGATAGTTTTTCTGCACGTAAGGTAAGCAGCATGGAG AGCCCGCACGACCTTGTGGTAGACACGGTGGGTACCGGTTAC CACAGCCGTTTTGGTTCGGACGCAGAGGCTTCTGTGATGCTGA

	AAAGGGCTGATGGCTCTGAGCTGTCGCACCGTGAGTTCATCGA CTATGTGATGAACTTCAACACGGTCCGCTACGACTACTACGGT GATGACGCGAGCTACACCAATCTGATGGCGAGTTATGGCACC AAGCACTCTGCTGACTCCTGGTGGAAGACAGGAAGAGTGCCC CGCATTTCGTGTGGTATCAACTATGGGTTCGATCGGTTTAAAG GTTCAGGGCCGGGATACTACAGGCTGACTTTGATTGCGAACG GGTATAGGGACGTAGTTGCTGATGTGCGCTTCCTTCCCAAGTA CGAGGGGAACATCGATATTGGGTTGAAGGGGAAGGTGCTGAC CATAGGGGGCGCGGACGCGGAGACTCTGATGGATGCTGCAGT TGACGTGTTTGCCGATGGACAGCCTAAGCTTGTCAGCGATCAA GCGGTGAGCTTGGGGCAGAATGTCCTCTCTGCGGATTTCACTC CCGGCACTGAGTACACGGTTGAGGTTAGGTTCAAGGAATTCG GTTCTGTGCGTGCGAAGGTAGTGGCCCAGTAG
241 C terminally truncated TmpA	ATGAGCCGAGGAGGTATCACGTGGGAGGTTAACATCATGAAT GCTCATACGCTTGTGTACTCCGGCGTAGCACTTGCCTGCGCGG CTATGCTCGGCTCCTGTGCCTCGGGCGCCAAGGAGGAAGCTG AAAAGAAGGCTGCAGAGCAGCGTGCGCTTCTGGTCGAGAGTG CGCATGCTGACCGTAGGCTTATGGAGGCGCGTATCGGCGCGC AAGAGTCTGGCGCAGACACCCAGCACCCCGAACTTTTCTCCCA GATTCAGGACGTTGAGCGCCAGTCTACCGACGCCAAGATTGA AGGGGACCTCAAGAAAGCTGCCGGTGTCGCCTCAGAAGCTGC GGATAAGTACGAGATTCTCAGGAACCGAGTTGAAGTTGCTGA CCTACAATCTAAGATCCAGACTCACCAGCTTGCGCAGTACGAC GGGGACAGCGCGAACGCTGCGGAAGAATCGTGGAAGAAGGC ACTTGAATTATACGAGACCGATAGCGCGCAGTGTCTGCAATCC ACCGTCGAAGCGCTCGAGTCGTATCGGAAAGTCGCGCATGAG GGATTCGGCCGCTTACTACCCGATATGAAGGCACGTGCGGGT GCTGCAAAGACGGACGTTGGCGGTCTTAAGGTAGCCGTCGAG TTGCGTCCACAGCTGGAAGAAGCTGACAGCCAATACCAAGAA GCACGTGAAGCTGAAGAGGTAAATGCACGTGCCAAAGCTTTT AGCGGGTACCACCGTGCCCTCGAGATCTACACAGAACTGGGG AAGGTTGTACGCCTGAAGAAGACCGAGGCGGAAAAGGCGCTG CAGTCTGCAAAAACAAAGCAAAAGGCGTCCTCTGACCTTGCG CGGAGTGCGGATAAGAGTGCCCCACTTCCTGAAAACGCTCAG GGTTTCTCAAAGGAGCCGATTGAGGTAGAGCCGCTTCCAAAC GACAGGCTTAACACAACGCAGGCATGA
242 N-terminally truncated Tp47	MKVKYALLSAGALQLLVVGCEADRAGDLDAGMFDAVSRATHG HGAFRQQFQYAVEVLGEKVLSKQETEDSRGRKKWEYETDPSVT KMVRASASFQDLGEDGEIKFEAVEGAVALADRASSFMVDSEEYK ITNVKVHGMKFVPVAVPHELKGIAKEKFHFVEDSRVTENTNGLK TMLTEDSFSARKVSSMESPHDLVVDTVGTGYHSRFGSDAEASVM LKRADGSELSHREFIDYVMNFNTVRYDYYGDDASYTNLMASYG TKHSADSWWKTGRVPRISCGINYGFDRFKGSGPGYYRLTLIANG YRDVVADVRFLPKYEGNIDIGLKGKVLTIGGADAETLMDAAVD VFADGQPKLVRDQAVSLGQNVLSADFTPGTEYTVEVRFKEFGSV RAKVVAQ
243 C terminally Truncated TmpA	MNAHTLVYSGVALACAAMLGSCASGAKEEAEKKAAEQRALLV ESAHADRRLMEARIGAQESGADTQHPELFSQIQDVERQSTDAKIE GDLKKAAGVASEAADKYEILRNRVEVADLQSKIQTHQLAQYDG DSANAAEESWKKALELYETDSAQCLQSTVEALESYRKVAHEGG RLLPDMKARAGAAKTDVGGLKVAVELRPQLEEADSQYQEAREA EEVNARAKAFSGYHRALEIYTELGKVVRLKKTEAEKALQSAKTK QKASSDLARSADKSAPLPENAQGFSKEPIEVEPLPNDRLNTTQA
244 N-terminally truncated Tp47 codon optimised	ATGGATCTGGATGCAGGTATGTTTGATGCAGTTAGCCGTGCAA CCCATGGTCATGGTGCATTTCGTCAGCAGTTTCAGTATGCAGT TGAAGTTCTGGGCGAAAAAGTTCTGAGCAAACAAGAAACCGA

	AGATAGCCGTGGTCGTAAAAAGTGGGAATATGAAACCGATCC GAGCGTTACCAAAATGGTGCGTGCAAGCGCAAGCTTTCAGGA TCTGGGAGAAGATGGTGAAATTAAGTTTGAAGCCGTTGAAGG TGCAGTTGCACTGGCCGATCGTGCAAGCAGTTTTATGGTTGAT AGCGAAGAGTACAAAATCACCAACGTTAAAGTGCACGGCATG AAATTTGTTCCGGTTGCCGTTCCGCATGAACTGAAAGGCATTG CAAAAGAAAAATTTCATTTTGTGGAAGATAGTCGCGTGACCG AAAATACCAATGGTCTGAAAACCATGCTGACGGAAGATAGCT TTAGCGCACGTAAAGTGAGCAGCATGGAAAGTCCGCATGATC TGGTTGTTGATACCGTTGGCACCGGTTATCATAGCCGTTTTGG TAGTGATGCGGAAGCAAGCGTTATGCTGAAACGTGCAGATGG TAGCGAACTGAGTCATCGTGAATTTATCGATTACGTGATGAAC TTTAATACCGTGCGCTATGATTATTATGGTGATGATGCAAGCT ATACCAATCTGATGGCCAGCTATGGCACCAAACATAGCGCAG ATAGTTGGTGGAAAACCGGTCGTGTTCCGCGTATTAGCTGTGG TATTAACTATGGTTTCGATCGCTTTAAAGGTAGCGGTCCGGGT TATTATCGTCTGACCCTGATTGCAAATGGTTATCGTGATGTTGT GGCAGATGTTCGTTTTCTGCCGAAATATGAAGGCAACATTGAT ATCGGTCTGAAAGGTAAAGTGCTGACCATTGGCGGTGCAGAT GCAGAAACCCTGATGGATGCAGCAGTTGATGTTTTTGCCGATG GTCAGCCGAAACTGGTTAGCGATCAGGCCGTTAGCCTGGGCC AGAATGTTCTGAGCGCAGATTTTACACCGGGTACAGAATATAC CGTTGAAGTGCGTTTTAAAGAATTTGGTAGCGTTCGTGCAAAA GTTGTTGCACAGTAA
245 C terminally truncated TmpA codon optimised	ATGGCAAGCGGTGCCAAAGAAGAAGCCGAAAAGAAAGCAGC AGAACAGCGTGCACTGCTGGTTGAAAGCGCACATGCAGATCG TCGTCTGATGGAAGCACGTATTGGTGCACAAGAAAGCGGTGC AGATACCCAGCATCCGGAACTGTTTAGCCAGATTCAGGATGTT GAACGTCAGAGCACCGATGCAAAAATTGAAGGTGATCTGAAA AAGGCAGCCGGTGTTGCAAGCGAAGCAGCCGATAAATATGAA ATTCTGCGTAATCGTGTTGAAGTTGCCGATCTGCAGAGCAAAA TTCAGACCCATCAGCTGGCACAGTATGATGGTGATAGCGCAA ATGCAGCAGAAGAAAGCTGGAAGAAAGCACTGGAACTGTATG AAACCGATAGCGCACAGTGTCTGCAGAGTACCGTTGAAGCCC TGGAAAGCTATCGTAAAGTTGCACATGAAGGTTTTGGTCGTCT GCTGCCGGATATGAAAGCACGTGCCGGTGCAGCAAAAACCGA TGTTGGTGGTCTGAAAGTTGCAGTTGAACTGCGTCCGCAACTG GAAGAAGCAGATAGCCAGTATCAAGAAGCACGCGAAGCCGA AGAAGTTAACGCACGCGCAAAAGCATTTAGCGGTTATCATCG TGCACTGGAAATCTATACCGAACTGGGTAAAGTTGTTCGTCTG AAGAAAACCGAAGCAGAAAAAGCACTGCAGAGCGCAAAAAC CAAACAGAAAGCAAGCAGCGATCTGGCACGTAGCGCAGATAA AAGCGCACCGCTGCCGGAAAATGCACAGGGTTTTAGTAAAGA ACCGATTGAAGTTGAACCGCTGCCTAATGATCGTCTGAATACC ACACAGGCA

This application claims priority from Australian Provisional Application No. 2024900445 entitled “Antigen combination and methods and uses thereof” filed on 23 February 2024, the entire contents of which are hereby incorporated by reference.

All publications discussed and/or referenced herein are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

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CLAIMS

1. An antigen combination comprising the *Treponema pallidum* antigens:
   i) Tp47 or an antigenic fragment thereof;
   ii) Tp0453 or an antigenic fragment thereof; and
   iii) TmpA or an antigenic fragment thereof.

2. The antigen combination of claim 1, further comprising one or more binding molecule/s.

3. The antigen combination of claim 2, wherein the binding molecule is selected from: a binding protein, a binding polynucleotide and a small molecule.

4. The antigen combination of claim 3, wherein the binding molecule is selected from a: reproductive health binding molecule, a sexual health binding molecule, a women's health binding molecule and a communicable disease binding molecule.

5. The antigen combination of claim 3 or claim 4, wherein the binding protein is an antibody or a non-Treponemal antigen.

6. The antigen combination of claim 1, wherein the antigen combination consists of:
   i) Tp47 or an antigenic fragment thereof;
   ii) Tp0453 or an antigenic fragment thereof; and
   iii) TmpA or an antigenic fragment thereof.

7. The antigen combination of claim 1, wherein the antigen combination consists of: Tp47, Tp0453 and TmpA.

8. The antigen combination of any one of claims 1 to 6, wherein the Tp47 antigenic fragment comprises the amino acid sequence set forth in any one of SEQ ID NOs: 21 to 70.

9. The antigen combination of any one of claims 1 to 6, wherein the Tp0453 antigenic fragment comprises the amino acid sequence set forth in any one of SEQ ID NOs: 71 to 114.

10. The antigen combination of any one of claims 1 to 6, wherein the TmpA antigenic fragment comprises the amino acid sequence set forth in any one of SEQ ID NOs: 115 to 160.

11. The antigen combination of any one of claims 1 to 10, wherein one or more of the antigens comprise a purification tag.

12. The antigen combination of any one of claim 1 to 11, wherein one or more of the antigens comprise a detectable label.

13. The antigen combination of claim 12, wherein the detectable label is selected from a: metal label, magnetic label, bead, colorimetric label, radioactive label, enzymatic label, luminescent label, fluorescent label, antibody, quantum dot, fluorescent latex particle, chemiluminescence based label, liposome-based probe and a Raman-active tag.

14. The antigen combination of any one of claims 1 to 13, wherein at least two of i), ii) and iii) are present in a fusion protein, optionally comprising a linker.

15. The antigen combination of any one of claims 1 to 14, wherein i), ii) and iii) are present in a fusion protein, optionally comprising one or more linker(s).

16. The antigen combination of any one of claims 1 to 15, wherein *Treponema pallidum* antigens Tp47:Tp0453:TmpA are present in a ratio of about 2:1:1.

17. The antigen combination of any one of claims 1 to 16, wherein *Treponema pallidum* antigens Tp47:Tp0453:TmpA are present in a ratio of about 1:1:1.

18. The antigen combination of any one of claims 1 to 17, for use in detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject.

19. The antigen combination of any one of claims 1 to 18, for use in detecting the presence or absence of an active syphilis infection in a sample from a subject.

20. A vector or polynucleotide encoding one or more antigen(s) in the antigen combination of any one of claims 1 to 19.

21. A vector or polynucleotide of claim 20, wherein the vector or polynucleotide encodes at least two of the following:

i) Tp47;

ii) Tp47 antigenic fragment;

iii) Tp0453;

iv) Tp0453 antigenic fragment;

v) TmpA;

vi) TmpA antigenic fragment; and

vii) one or more binding molecule/s.

22. The vector or polynucleotide of claim 21, wherein the vector or polynucleotide encodes i), iii) and v); or i), iii), v) and vii).

23. A host cell comprising the vector or polynucleotide of any one of claims 20 to 22.

24. The host cell of claim 23, which is a eukaryotic cell.

25. The host cell of claim 23, which is a bacterial cell.

26. The host cell of 25, wherein the bacterial cell is *E. coli*.

27. A method of producing an antigen combination of any one of claims 1 to 19 comprises expressing the vector or polynucleotide according to any one of claims 20 to 22 in a host cell or cell-free expression system.

28. A lysate or extract from a cell according to any one of claims 23 to 26, wherein the extract comprises one or more of the antigens of any one of claims 1 to 19.

29. A composition comprising the antigen combination of any one of claims 1 to 19.

30. A solid support comprising the antigen combination of any one of claims 1 to 19.

31. A method of detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject, the method comprising exposing the sample from the subject to the antigen combination of any one of claims 1 to 19 under conditions which enable

an antibody-antigen complex to form, and detecting the presence or absence of an antibody-antigen complex.

32. A method of detecting the presence or absence of an active syphilis infection in a sample from a subject, the method comprising exposing the sample from the subject to the antigen combination of any one of claims 1 to 19 under conditions which enable an antibody-antigen complex to form, and detecting the presence or absence of an antibody-antigen complex.

33. The method of claim 31 or claim 32, wherein the antibody-antigen complex is an IgA-specific antibody-antigen complex.

34. The method of any one of claims 31 to 33, wherein the sensitivity for detecting an active syphilis infection is at least about 95%.

35. The method of any one of claims 31 to 34, wherein the specificity for detecting an active syphilis infection is at least about 68%.

36. The method of any one of claims 31 to 35, wherein the specificity for detecting an active syphilis infection is at least about 80%, or at least about 83%, or at least about 86%, or at least about 89%, or at least about 92%.

37. The method of any one of claims 31 to 36, wherein the specificity for detecting an active syphilis infection is about 86% to about 92%.

38. The method of any one of claims 31 to 37, wherein the specificity for detecting an active syphilis infection from a past-treated syphilis infection is at least 88%.

39. The method of any one of claims 30 to 38, wherein the specificity for detecting an active syphilis infection from never infected is 100%.

40. A kit for detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject, wherein the kit comprises the antigen combination of any one of claims 1 to 19.

41. A kit for detecting an active syphilis infection in a sample from a subject, wherein the kit comprises the antigen combination of any one of claims 1 to 19.

42. The kit of claim 40 or claim 41, wherein the kit detects IgA isotypes of *Treponema pallidum* antibody.

43. The kit of any one of claims 40 to 42, further comprising Protein L.

44. The kit of any one of claims 40 to 43, wherein the total antigen concentration is between about 0.25 mg/mL to 1.5 mg/mL.

45. The kit of any one of claims 40 to 44, further comprising a detectable label.

46. The kit of claim 45, wherein the detectable label is selected from a: metal label, magnetic label, bead, colorimetric label, radioactive label, enzymatic label, luminescent label, fluorescent label, antibody, quantum dot, fluorescent latex particle, chemiluminescence based label, liposome-based probe and a Raman-active tag.

47. The kit of claim 46, wherein the antibody is (an anti-IgA) gold.

48. The kit of any one of claims 45 to 47, wherein the detectable label is specific to IgA antibodies.

49. The kit of any one of claims 49 to 48, wherein the antigen combination is immobilised on a solid support.

50. The kit of claim 49, wherein the antigen combination is immobilised on a solid support in the presence of an excipient.

51. The kit of claim 50, wherein the excipient is selected from: sucrose, trehalose, galactose, maltose, or lactose.

52. The kit of claim 51, wherein the excipient is sucrose.

53. The kit of any one of claims 50 to 52, wherein the excipient is present in amount of about 0.5% (w/v).

54. An assay for detecting the presence or absence of *Treponema pallidum* antibodies in a sample from a subject comprising the antigen combination of any one of claims 1 to 19.

55. An assay for detecting an active syphilis infection in a sample comprising the antigen combination of any one of claims 1 to 19.

56. The assay of claim 54 or claim 55, wherein the assay is a point-of-care assay.

57. The assay of any one of claims 54 to 56, wherein the assay is a one-step assay.

58. The assay of any one of claims 54 to 57, wherein the assay does not detect a past-treated syphilis infection.

59. The kit of any one of claims 40 to 53 or the assay of any one of claims 53 to 55, wherein the kit or assay is suitable for: lateral flow, ELISA, chemiluminescent immunoassay, western blot, and radioimmunoassay.

60. The kit of any one of claims 40 to 53 or claim 59 or the assay of any one of claims 54 to 59, wherein the kit or assay is suitable for lateral flow.

61. A lateral flow device comprising a solid support that comprises:

i) a detection region comprising the antigen combination of any one of claims 1 to 19; optionally wherein one or more of the antigens comprises a detectable label;

ii) a second detection region configured as a control region; and

iii) one or more of: a chromatography matrix, a sample pad, and a wicking pad.

62. The device of claim 61, where in ii) is configured to detect IgA antibodies.

63. The device of claim 61 or claim 62, further comprising a third detection region configured to detect one or more further antigens, antibodies and/or nucleic acids indicative of a non-*Treponema pallidum* condition.

64. Use of the antigen combination of any one of claims 1 to 19, the kit of any one of claims 40 to 53, 59 or 60, the assay of any one of claims 54 to 60, or the lateral flow

device of any one of claims 61 to 63 for detecting the presence or absence of *Treponema pallidum* antibodies in a sample.

65. Use of the antigen combination of any one of claims 1 to 19, the kit of any one of claims 40 to 53, 59 or 60, the assay of any one of claims 54 to 60, or the lateral flow device of any one of claims 61 to 63 for detecting an active syphilis infection in a subject.

66. A method of treating an active syphilis infection in a subject, the method comprising:

i) contacting a biological sample from the subject with the antigen combination of any one of claims 1 to 19; and

ii) directly or indirectly detecting the presence of an antibody-antigen complex, wherein the presence of the antibody-antigen complex indicates that a subject has an active syphilis infection; and

iii) if an antibody-antigen complex is detected, administering a treatment for an active syphilis infection.

67. The method of claim 66, wherein the antibody-antigen complex is an IgA-specific antibody-antigen complex.

68. The method of claim 66 or claim 67, wherein the treatment comprises administering penicillin.

69. The steps, features, integers, compositions and/or compounds disclosed herein or indicated in the specification of this application individually or collectively, and any and all combinations of two or more of said steps or features.