Use of recombinant proteins in the diagnosis of sexually transmitted infections: a systematic review and meta-analysis

Uso de proteínas recombinantes no diagnóstico de infecções sexualmente transmissíveis: uma revisão sistemática e metanálise

Uso de proteínas recombinantes en el diagnóstico de infecciones de transmisión sexual: revisión sistemática y metanálisis

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Abstract
The present systematic review and meta-analysis aimed to investigate the use of recombinant proteins to diagnose STIs and evaluate the performance of the tests identified. The databases, SciELO, PubMed, CAPES Journal Portal, and LILACS, were searched according to the PRISMA protocol. A study was considered eligible if it met previously defined criteria. The risk of study bias was assessed according to the QUADAS-2 protocol. The meta-analysis was performed based on the random-effects model, and heterogeneity was quantified using the I² statistic. A total of 1,355 studies were selected, 30 of which were relevant to the following: human immunodeficiency virus (12/30), Treponema pallidum (10/30), Chlamydia trachomatis (3/30), herpes simplex virus (HSV, 2/30), Trichomonas vaginalis (2/30), and HSV/cytomegalovirus (1/30). The main serological tests were ELISA and
immunocromatography. The risk of bias was low for most included studies. According to each microorganism, the meta-analysis revealed satisfactory sensitivity and specificity for the analyzed tests. The findings reinforce the relevance of diagnostic tests based on recombinant proteins as viable alternatives for production and inclusion in clinical practice. **Keywords**: STIs; Systematic review; Meta-analysis; Diagnosis.

**Resumo**
A presente revisão sistemática e metanálise teve como objetivo investigar o uso de proteínas recombinantes no diagnóstico de IST e avaliar o desempenho dos testes identificados. As bases de dados SciELO, PubMed, Portal de Periódicos CAPES e LILACS foram pesquisadas de acordo com o protocolo PRISMA. Um estudo foi considerado elegível se atendesse a critérios previamente definidos. O risco de viés do estudo foi avaliado de acordo com o protocolo QUADAS-2. A metanálise foi realizada com base no modelo de efeitos aleatórios e a heterogeneidade foi quantificada pela estatística P. Um total de 1.355 estudos foram selecionados, dos quais 30 eram relevantes para: vírus da imunodeficiência humana (12/30), Treponema pallidum (10/30), Chlamydia trachomatis (3/30), vírus herpes simples (HSV, 2/30), Trichomonas vaginalis (2/30) e HSV/citomegalovírus (1/30). Os principais testes sorológicos foram ELISA e imunocromatografia. O risco de viés foi baixo para a maioria dos estudos incluídos. De acordo com cada microorganismo, a metanálise revelou sensibilidade e especificidade satisfatórias para os testes analisados. Os achados reforçam a relevância dos testes diagnósticos baseados em proteínas recombinantes como alternativas viáveis para produção e inclusão na prática clínica. Número de registro PROSPERO: CRD42020206331.

**Palavras-chave**: IST; Revisão sistemática; Meta-análise; Diagnóstico.

**Resumen**

**Palabras clave**: ITS; Revisión sistemática; Metanálisis; Diagnóstico.

**1. Introduction**

Sexually transmitted infections (STIs) are a worldwide public health problem. These infections are caused by several microbial agents transmitted mainly through sexual contact; however, these agents can also be spread through congenital and blood transmission (Spiteri et al., 2019). More than 30 microorganisms can be sexually transmitted. In fact, some bacteria and viruses should be highlighted owing to their high prevalence and/or the severity of the resulting diseases (WHO, 2018). Besides the main bacterial (Treponema pallidum and Chlamydia trachomatis) and viral (hepatitis B, herpes simplex virus [HSV], human immunodeficiency virus [HIV], and human papillomavirus [HPV]) agents (WHO, 2018), mycoplasmas (Mycoplasma hominis and Mycoplasma genitalium) are also a type of bacteria that cause STIs and are related to reproductive disorders (Cina et al., 2019).

Advancements in molecular techniques, such as nucleic acid amplification, have improved diagnosis, mainly due to their good sensitivity and specificity. However, the need for trained professionals and specific equipment, and high costs, still limit their inclusion in clinical routine (Nodjikouambaye et al., 2019). Despite the mentioned methods, new technologies have been introduced in laboratory routines for STI diagnosis based on serological parameters that are good indicators of the individual's infection status and have been considered as viable alternatives for the diagnosis of STIs (Sanchez-Trincado et al., 2017). These new techniques use bioinformatics and recombinant DNA technology for optimization and large-scale production.
of antigens of the agents causing STIs, which are employed to produce antibodies that are used to develop immunodiagnostic tests (Drancourt et al., 2016). With advances in primary studies, systematic reviews and meta-analyses have gained more recognition, mainly in diagnostic test accuracy (Juneyoung Lee, Kyung Won Kim, Sang Hyun Choi & Seong Ho Park, 2015). The objective of these studies is to identify variables that affect diagnostic test accuracy, summarize reliable information on test accuracy, and support the development of future research (Irwig et al., 1994).

For studies on the development of immunodiagnostic tests, antigenic and immunogenic proteins can be produced by recombinant DNA technology using heterologous expression systems (Duarte et al., 2020). Many studies have opted to use prokaryotic systems (Escherichia coli and Bacillus subtilis); however, eukaryotic systems (yeast, insect cells, and mammalian cells) or fully in vitro systems are also used (Tripathi & Shrivastava, 2019). To the best of our knowledge, no available systematic review and meta-analysis provide information on the use and diagnostic accuracy of recombinant proteins to diagnose STIs. To fill this knowledge gap, we reviewed studies on the use of recombinant proteins for the diagnosis of some pathogens and the diagnostic accuracy of the identified tests.

Overall, this systematic review and meta-analysis aimed to investigate the use of recombinant proteins for the diagnosis of STIs as well as the sensibility and specificity of the tests detected.

2. Methodology

2.1 Type of research

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and meta-analysis (PRISMA) guidelines (Page et al., 2021). The protocol for this systematic review was registered in the International Prospective Register of Systematic Review (PROSPERO) database before initiating literature search (CRD42020206331).

2.2 Search strategy

Searches were conducted using the Scientific Electronic Library Online (Scielo), PubMed, Virtual Health Library (VHL), CAPES Journal Portal, and LILACS databases to identify studies published between 2010 and July 2020 that assessed the use of recombinant proteins in the diagnosis of STIs. Published papers registered in these databases were identified using the descriptors: “recombinant protein”, “sexually transmitted infections,” and “diagnosis STI.” Details of the research process are provided in Table 1.

2.3 Eligibility criteria

A study was considered eligible for this systematic review and meta-analysis if it met the following criteria:

(1) used recombinant proteins in the diagnosis of STIs.
(2) included serum and/or plasma biological samples.
(3) were available in the selected databases.
(4) were published between 2010 and 2020; and
(5) were available in English or Portuguese.
### Table 1. Database search strategies.

<table>
<thead>
<tr>
<th>Database</th>
<th>Terms</th>
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<tbody>
<tr>
<td>Scielo</td>
<td>((recombinant protein* OR Chimeric recombinant protein OR Biosynthetic proteins) AND (sexually transmitted infections* OR sexually transmitted disease) AND (STI diagnosis* OR STI diagnostic))</td>
</tr>
<tr>
<td>Pubmed</td>
<td>((recombinant protein* OR Chimeric recombinant protein OR Biosynthetic proteins) AND (sexually transmitted infections* OR sexually transmitted disease) AND (STI diagnosis* OR STI diagnostic))</td>
</tr>
<tr>
<td>Virtual Health Library (VHL)</td>
<td>((recombinant protein* OR Chimeric recombinant protein OR Biosynthetic proteins) AND (sexually transmitted infections* OR sexually transmitted disease) AND (STI diagnosis* OR STI diagnostic))</td>
</tr>
<tr>
<td>CAPES Journal Portal</td>
<td>((recombinant protein* OR Chimeric recombinant protein OR Biosynthetic proteins) AND (sexually transmitted infections* OR sexually transmitted disease) AND (STI diagnosis* OR STI diagnostic))</td>
</tr>
<tr>
<td>LILACS</td>
<td>((recombinant protein* OR Chimeric recombinant protein OR Biosynthetic proteins) AND (sexually transmitted infections* OR sexually transmitted disease) AND (STI diagnosis* OR STI diagnostic))</td>
</tr>
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</table>

Source: Authors.

### 2.4 Study selection

Two reviewers (C.P.G. and W. N.) independently examined article titles and abstracts to identify potentially relevant articles using the Rayyan QCRI platform (http://rayyan.qcri.org/). The eligibility of studies meeting the inclusion criteria in this phase was confirmed by reading the full article. Articles meeting all inclusion criteria were included in the qualitative and quantitative synthesis. Disagreements between the reviewers were resolved by a third reviewer (L.S.C.S.).

Data extraction was performed by two independent reviewers (L.S.C.S. and V.G.) using a standardized form prepared by the authors based on the Prisma checklist of the University of Oxford (Page et al., 2021). The extracted data included information related to publication and design, sample, study location, and year of study. Study authors were contacted by e-mail when the selected studies did not provide all information necessary to calculate sensitivity and specificity.

### 2.5 Risk of bias assessment

Two review authors (L.S.C.S. and C.P.G.) independently assessed the risk of bias of the included studies in the meta-analyses using QUADAS-2 (Quality Assessment for Diagnostic Accuracy Studies). The tool consists of 4 key domains that discuss patient selection, index tests, reference standards, and flow of patients through the study and timing of the index tests and reference standard according to Penny F. Whiting, Anne WS Rutjes, Marie E. Westwood, Susan Mallett, Jonathan J. Deeks, Johannes B. Reitsma, MD, Mariska MG Leeflang, 2011. Disagreements were resolved by a third reviewer (C.T.C.).

### 2.6 Statistical analysis

Data were extracted from eligible studies and arranged in a 2x2 table. The sensitivity, specificity, and their respective confidence intervals (95% CI) were calculated following the random-effects model. As heterogeneity is expected in the meta-analysis of diagnostic accuracy studies, a random-effects model is preferred according to Petra Macaskill, Constantine Gatsonis & Roger Harbord, 2010. The results were presented separately for chlamydia, HIV, herpes, and syphilis. When feasible, a subgroup analysis was conducted.

Spearman correlation analysis was conducted to assess the presence of the threshold effect in each outcome analyzed. The presented threshold effect was considered substantial when the correlation coefficient was 0.6 or higher, according to Kim et al., 2015. A value of 0.5 was added to the empty cells to avoid computational problems, according to Reiman et al., 2017.

Bivariate random-effects meta-analysis was conducted, and summary receiver operating characteristic (SROC) curves were generated for the outcomes with 10 or more studies as described by Petra Macaskill, Constantine Gatsonis & Roger Harbord, 2010. The SROC curves were used to assess heterogeneity by visual inspection. Furthermore, these curves summarize the diagnostic performance as the area under the curve (AUC), values of which can be used to predict accuracy.
according to Junyoung Lee, Kyung Won Kim, Sang Hyun Choi & Seong Ho Park, 2015. Accuracy was considered as follows: fail, 0.50–0.60; poor, 0.61–0.70; fair, 0.71–0.80; good, 0.81–0.90; and excellent, 0.91–1.00, as per (Reiman et al., 2017).

Publication bias was not assessed due to the language restriction, which may lead to a spurious interpretation of bias. Analyses were carried out with R version 4.0.3, the “meta” package version 4.15-1 by (Balduzzi et al., 2019), and the “mada” package version 0.5.10 by Philipp Doebler, 2020.

3. Results

3.1 Selected studies

A total of 3,461 articles were selected from the searched databases. Based on their titles and abstracts, 3,425 (98.9%) were not found to meet the previously defined eligibility criteria and were thus not considered for further analysis. The remaining 36 articles (1.04%) were read in full, 6 of which were excluded based on the exclusion criteria. Therefore, this review was based on the analysis of 30 articles. After data extraction, 9 articles had insufficient information (lack of data) for inclusion in meta-analysis; thus, these studies were excluded. As a result, the meta-analysis was based on 21 articles (Figure 1).

Figure 1. Flowchart for the selection of studies.

![Figure 1. Flowchart for the selection of studies.]

3.2 Characteristics of studies

Most studies were published in 2013 (7/30), followed by 2016 and 2018 (each 4/30). Serum was the main biological sample used to develop tests based on recombinant proteins (25/30). Regarding serological test type, ELISA was the most used assay (20/30), followed by immunochromatography (7/30). Analysis based on STI-related microorganisms revealed that most studies focused on HIV (12/30), followed by T. pallidum (10/30), C. trachomatis (3/30), HSV (2/30), T. vaginalis (2/30), and HSV 1 and 2 and cytomegalovirus (CMV, 1/30). Based on study locations, most investigations were carried out in China (11/30) and the USA (5/30) (Figure 2). Individual and categorized data were extracted based on the study reference, biological sample, microorganism, and antigen of interest (Table 2). There was significant variability among the antigens used for each microorganism in the diagnosis of STIs with recombinant proteins.
Figure 2. General characterization of studies included in the systematic review (n=30). The percentage of studies is shown by a) year of publication; b) test type; c) microorganism of interest; and d) study location.

ICG: Immunochromatographic; ZAF: South Africa; and GBR: United Kingdom. Source: Authors.
| Reference number | Study environment | Test type | Year | Sample used | Target microorganism/| Reference number | Study environment | Test type | Year | Sample used | Target microorganism/ |
|------------------|-------------------|-----------|------|-------------|----------------------|------------------|-------------------|-----------|------|-------------|----------------------|------------------|-------------------|-----------|------|-------------|----------------------|------------------|-------------------|
| (J. F. Alderete, 2017) | USA | ELISA | 2017 | Serum | Trichomonas vaginalis/ ACT-P2 | (Jiang et al., 2016) | China | ELISA | 2016 | Serum | Treponema pallidum/ Flab1, Flab2, Flab3, Tp0463 and Tp1038 | (Xie et al., 2016) | China | ELISA | 2019 | Serum | Treponema pallidum/ Tp0821 |
| (de Haro-Cruz et al., 2019) | Mexico | ELISA | 2016 | Serum | Chlamydia trachomatis/ MOMP | (Tan et al., 2018) | China | ELISA | 2019 | Serum | Treponema pallidum/ TpF1 |
| (Jiang et al., 2016) | China | ELISA | 2017 | Serum | Treponema pallidum/ | (Xie et al., 2016) | China | ELISA | 2016 | Serum | Treponema pallidum/ Flab1, Flab2 and Flab3 |
| (Ma et al., 2016) | China | Immunochromatography | 2016 | Serum or plasma | Herpes simplex virus type 2/ gG2 | (Rikhtegaran Tehrani et al., 2015b) | Iran | ELISA | 2015 | Serum and Plasma | HIV-1/ p24, gp41, and gp120/160 |
| (Lin et al., 2010) | China | Immunochromatography | 2010 | Serum | Treponema pallidum/ TPN17 and TPN47 |
| (Purpia et al., 2010) | South Africa | Immunochromatography | 2010 | Plasma | HIV/p24 |
| (Lin et al., 2011) | China | Immunochromatography | 2011 | Serum | Treponema pallidum/ TPN17 and TPN47 |
| (John F Alderete, 2020) | USA | ELISA | 2020 | Serum | Trichomonas vaginalis/ SOE (AEG) |
| (Pfeilsticker et al., 2013) | USA | ELISA | 2013 | Serum | HIV/ gp41 |
| (Hokynar et al., 2017) | Georgia | ELISA | 2017 | Serum | Chlamydia trachomatis/ Troa and Htra |
| (Yufenyuy & Parekh, 2018) | Georgia | Multiplex assay | 2018 | Plasma | HIV-1 and HIV-2/ p24-gp41 |
| (Granade et al., 2013b) | Georgia | Immunochromatography | 2013 | Serum | HIV/ gp41 |
| (Gallerano et al., 2015) | Austria | Immunochromatography | 2015 | Serum | HIV/ gp120 |
| (Y. Liu et al., 2013) | China | ELISA | 2013 | Serum | Herpes simplex virus type 2 and Cytomegalovirus/ gG2, F150 |
| (Cai et al., 2019) | China | ELISA | 2019 | Serum | HIV/ gp41 |
| (Wesley et al., 2018) | USA | ELISA | 2018 | Serum | Herpes simplex virus type 2/ gG2 |
| (Tiwari et al., 2013) | India | ELISA | 2013 | Serum | HIV 1 and 2 / gp120, gp36, gp41 and rp24 |
| (Moshgabadi et al., 2015b) | Canada | Immunochromatography | 2015 | Serum | HIV 1/ gp41 |

Source: Authors.
3.3 Risk of bias of the included studies

QUADAS-2 analysis (Figure 3) revealed that 86.6% of the articles had a low risk of bias for the patient selection domain, and 13.3% had an unclear description for that domain. According to the index test, 43.3%, 33.3%, and 23.3% of the studies had low, unclear, and probable risks, respectively. The reference standards showed a low risk of bias in 90% and an unclear risk of bias in 10%. Finally, the time courses of the studies were assessed, and 60% of the studies were found to have a low risk of bias, whereas 40% were reported to have an unclear risk. Regarding the applicability of the studies based on the key question of the review, a low risk was observed in 100% of the studies in the three sections (patient selection, index test, and reference standard).

Figure 3. Analysis of the risk of bias and applicability of the studies included in the review according to QUADAS-2: a) Domain and b) applicability.
3.4 Meta-analysis

C. trachomatis

The specificity and sensibility of diagnostic tests based on recombinant antigens for C. trachomatis were assessed by three studies [18–20]. The meta-analyses revealed specificity of 86.4% (95% CI 71.4-94.2; p < 0.01; I² = 86.0%) and sensibility of 52.5% (95% CI 31.3-72.9; p < 0.01; I² = 93.0%) for these tests (Figure 4).

Herpes

Two studies estimated the specificity and sensibility of diagnostic tests based on recombinant antigens for herpes[21,22], and revealed a specificity of 99.6% (95% CI 98.0-99.9; p = 0.58; I² = 0.0%) and sensibility of 97.1% (95% CI 83.5-99.6; p = 0.08; I² = 60.0%) (Figure 5).

HIV

Six studies evaluated the specificity and sensibility of diagnostic tests based on recombinant antigens for HIV [23–28]. The meta-analyses revealed an overall specificity of 99.3% (95% CI 98.8-99.6; p = 0.49; I² = 0.0%) – 99.0% (95% CI 97.4-99.6) for immunochromatography, 99.7% (95% CI 97.9-100.0) for ELISA, and 99.6% (95% CI 98.8-99.8) for multiplex. The overall sensitivity was 98.3% (95% CI 93.8-99.6; p < 0.03; I² = 68.0%) – 95.6% (95% CI 79.4-99.2) for immunochromatography, 99.0% (95% CI 81.3-100.0) for ELISAs, and 99.7% for multiplex assays (95% CI 98.7-99.9) (Figure 6).

T. pallidum

The specificity and sensibility of diagnostic tests based on recombinant antigens for T. pallidum were estimated by ten studies[29–38]. A global specificity of 96.1% (95% CI 92.5-98.0; p < 0.01; I² = 92.0%) was found: 92.2% (95% CI 87.9-95.0) for ELISAs, 98.9% (95% CI 85.1-99.9) for immunochip, 99.9% (95% CI 98.8-100.0) for multiplex, and 99.7% (95% CI 90.7-100.0) for immunochromatography. The overall sensitivity was 95.0% (95% CI 91.1-97.3; p < 0.01; I² = 97.0%) – 93.8% (95% CI 95% 88.7-96.7) for ELISA, 94.1% (95% CI 86.6-97.5) for immunochip, 98.8% (95% CI 96.4-99.6) for multiplex assays, and 99.2% (95% CI 96.5-99.8) for immunochromatography (Figure 7).
Figure 4. Forest plot for studies based on recombinant proteins for the diagnosis of *Chlamydia trachomatis*: (a) Specificity and (b) sensitivity.

Source: Authors.
Figure 5. Forest plot for studies based on recombinant proteins for the diagnosis of Herpes: (a) Specificity and (b) sensitivity. The graph shows estimates of sensitivity and specificity for the studies (with 95% confidence intervals). The studies are ordered according to (a) author, true negative, true negative + false positive; (b) author, true positive, true positive + false negative. Wesley et al. 2018 (a): high risk; Wesley et al. 2018 (b): mixed risk.

Source: Authors.
Figure 6. Forest plot for studies based on recombinant proteins for the diagnosis of HIV: (a) specificity and (b) sensitivity. The graph shows estimates of sensitivity and specificity for the studies (with 95% confidence intervals). The studies are ordered according to (a) author, true negative, true negative + false positive; (b) author, true positive, true positive + false negative.

Source: Authors.
Figure 7. Forest plot for studies based on recombinant proteins for the diagnosis of syphilis (a) specificity and (b) sensitivity. The graph shows estimates of sensitivity and specificity for the studies (with 95% confidence intervals). The studies are ordered according to (a) author, true negative, true negative + false positive; (b) author, true positive, true positive false negative.

Source: Authors.
3.5 Threshold effect and SROC curve

The studies assessing C. trachomatis (r=0.65), HIV (r=0.80) and HSV (r=0.81) revealed a threshold effect in the correlation analysis. However, this effect was not observed in the analysis of syphilis (r=0.23) (data not shown). Visual inspection of the SROC curve did not show heterogeneity. The area under the curve (AUC= 0.976) showed good accuracy in the diagnostic tests for syphilis.

4. Discussion

4.1 Analysis of the STI profiles

Analyses of diagnostic accuracy studies should consider different methodological aspects that can influence research results. Therefore, the quality of the included studies must be assessed. In the present study, the QUADAS-2 tool was used to assess the methodological rigor of the diagnostic accuracy studies included in the review (Penny F. Whiting, Anne WS Rutjes, Marie E. Westwood, Susan Mallett, Jonathan J. Deeks, Johannes B. Reitsma, MD, Mariska MG Leeflang, 2011). Most studies were found to have a low risk of bias in all assessed domains. The tool is well established in the scientific field for assessing diagnostic accuracy studies, as revealed by the study of Lisboa Bastos et al (2020) that assessed the diagnostic accuracy of serological tests for COVID-19. With regard to STI diagnostic studies, QUADAS-2 has already been used, as revealed in the studies of Arshad et al (2019) and Phang Romero Casas et al (2018).

This systematic review provides evidence on the use of recombinant proteins for the development and applicability of STI diagnostic tests. The main microorganisms identified were HIV, T. pallidum, C. trachomatis, HSV 1 and 2, T. vaginalis, and CMV. STIs are caused by several pathogens in addition to those described in the study (Thomas et al., 2019) thereby indicating a deficit in new diagnostic tests based on recombinant proteins that address the other infectious agents. For example, M. genitalium has been associated with STIs, and the infections caused by this pathogen are most often asymptomatic, making treatment difficult and favoring transmission (Cadosch et al., 2020). Furthermore, no diagnostic tests with recombinant proteins have been identified for this pathogen in the literature.

Most included studies sought new diagnostic methods for HIV, an infection with high incidence and prevalence worldwide that require diagnostic screening tests to reduce transmission and establish early treatment (BRASIL, 2020). Globally, approximately 40 million individuals are infected with HIV type 1 or 2. Although HIV-1 is distributed worldwide, HIV-2 is restricted mainly to East Africa but has been detected in Portugal, France, and the United States as well (Bbosa et al., 2019). In general, the African and Asian continents have the highest numbers of infected individuals (Ghosn et al., 2018). In 2019, 41,909 new cases of HIV and 37,308 of acquired immunodeficiency syndrome (AIDS) were reported in Brazil (BRASIL, 2020). Data analysis of the studies identified several antigens that can be used to diagnose these infections. Glycoprotein In the literature, GP160 of the viral envelope is well described to be related to early HIV seroconversion in several serological tests. Studies such as those by Talha et al (2012), Rikhtegaran Tehrani et al (2015a), and Curtis et al (2012) have revealed favorable results for the recombinant expression of this antigen (GP160) for use in diagnostic tests. Other proteins that play an important role in viral assembly and maturation, such as p24, have also been identified in studies by Ma et al (2016), Rikhtegaran Tehrani et al (2015b), Parpia et al (2010), and Yufenyuy and Parekh (2018).

The methodologies of all new tests identified herein are based on serology. The host immune response can be useful for in vitro diagnosis compared to direct pathogen detection methods (Van Den Heuvel et al., 2019). The tests for HIV and other microorganisms included in this review were based on enzyme immunoassays. Most included studies sought to develop ELISAs with recombinant proteins. However, there are issues with the routine use of ELISA tests, such as execution complexity and reaction time (Daskalakis, 2011). In this regard, studies such as that of Ma et al (2016) developed more
practical, faster methods, such as immunochromatographic tests, using the recombinant protein, p24, and obtained satisfactory results for sensitivity (100%) and specificity (98.03%). The search for faster and easier tests was also highlighted in the studies of Granade et al (2013a) and Gallerano et al (2015), both for HIV. Other studies developed immunochromatographic tests for syphilis, as observed in the studies by Lin et al (2010) and Lin et al (2011), which offered promising prospects to include other STI-causing microorganisms eventually. According to the ASSURED criteria (accessible, sensitive, specific, easy to use, robust/fast, equipment-free, and reaching those who need it), fast point-of-care (POC) tests are essential to overcome the challenges of STI control (Peeling et al., 2006). POC tests for syphilis and HIV in prenatal clinics decreased perinatal and infant morbidity and mortality rates in low-income countries (Bump & Salisbury, 2013). The focus of POC testing is to reduce the number of undiagnosed patients and improve the quality of life of individuals through early patient treatment and follow-up (Herbst De Cortina et al., 2016).

Despite the feasibility of a simple and inexpensive diagnostic test, research for new methods of detecting T. pallidum was also identified in this study. The causative agent of syphilis is not only an STI but is also transmitted vertically and is responsible for hundreds of premature births and newborn deaths. Furthermore, it is re-emerging as a global public health problem, especially in developing countries (Peeling et al., 2018). T. pallidum is distributed worldwide, with almost 20 million infected individuals, mainly in African and Asian countries (Peeling et al., 2018). Immunological methods have been widely applied and developed for the diagnosis of T. pallidum based on immunodominant antigens and/or surface or periplasmic antigens, as observed in the studies of Long et al (2012), Jiang et al (2013), Xie et al (2016), Jiang et al (2016), and Runina et al (2018). However, these diagnostic tests have varying sensitivity at different stages of infection. To solve this problem, studies have used chimeric proteins or a broad spectrum of antigens (Smith, Simpson, Morshed, Cowen, Hof, Wetherell, & Cameron, 2013).

Only three studies sought new diagnostic tests using recombinant proteins for C. trachomatis (Smith, Simpson, Morshed, Cowen, Hof, Wetherell, & Cameron, 2013; Rikhtegaran Tehrani et al., 2015b; Xu et al., 2016). Although the most common bacterium in STIs, with higher prevalence in the Americas and Pacific regions WHO, (1981), the diagnosis of C. trachomatis using classic antigens has high cross-reactivity and low sensitivity (K. Shamsur Rahman, a Toni Darville, b Harold C. Wiesenfeld, c Sharon L. Hillier, 2018). Notably, no studies were found on new diagnostic tests using recombinant proteins for important STI-causing pathogens, such as HPV, hepatitis C virus, M. genitalium, M. hominis, and ureaplasmas (causative agents of urethritis and cervicitis). First, HPV is a leading cancer-related infection in both sexes and has a global prevalence of 11.7%, which varies considerably between regions, with a greater concentration in Africa and Oceania (Serrano et al., 2018). It is estimated that most sexually active men and women will become infected by this virus (Ault, 2006). M. genitalium is an important causative agent of non-gonococcal urethritis and an uncommon STI, with a prevalence of approximately 1%. Despite the low prevalence, increasing evidence of macrolide-resistant strains of M. genitalium owing to the widespread use of azithromycin for the treatment of STIs and the limited feasibility of diagnostic tests for this bacterium are of concern (Horner & Martin, 2017).

4.2 Applicability of recombinant proteins

The heterologous production of recombinant antigens is an important tool for the development of prophylactic measures and/or diagnostic tests (AnandaRao et al., 2005). Accordingly, computational biology, which involves mining and extracting information from genomes deposited in databases, is an important area for uncovering important information on antigens of several pathogens (J. Liu et al., 2021). The prediction of antigens from gene sequences that may be good targets in
silico to develop recombinant antigens is a valuable approach for producing sensitive antibodies against different subtypes and populations of a given pathogen (Barbosa et al., 2020).

These technologies and methodologies play a central role in modern immunobiological studies and have been extensively applied for the development of diagnostic tests against STIs (Moshgabadi et al., 2015a). The studies included in this review used E. coli as the expression system (data not shown). E. coli is the preferred host for the initial screening of recombinant protein expression, as this bacterium can be readily manipulated and grown inexpensively and rapidly (Jia & Jeon, 2016). The choice of target antigens is also highly relevant to the successful development of a diagnostic test (Santos Junior et al., 2020). The articles included in this review that evaluated the use of recombinant antigens for developing diagnostic tests to detect different pathogens (T. vaginalis, C. trachomatis, T. pallidum, HIV, and HSV) predominantly employed cloning and expression of antigens located on the outer membrane surface. Such a choice is very interesting when planning diagnostic test development as these antigens can interact directly with the host's immune system, triggering a series of events that lead to a specific immune response (Herbst De Cortina et al., 2016).

4.3 Quantitative analysis

This systematic review and meta-analysis provide information on the accuracy estimates of studies using recombinant proteins for the diagnosis of STIs, such as chlamydia, HIV, herpes, and syphilis. In this regard, two main results should be highlighted. First, the diagnostic tests for chlamydia showed low sensitivity and high specificity, which favor false-negative test results and interfere with diagnosis. Notably, the serological assays for C. trachomatis used for decades may present cross-reactivity with other Chlamydia spp (Persson, 2002), and compromise the sensitivity of results. Accordingly, new assays have been developed with the ability to detect antibodies against various antigens (Sarah C Woodhall et al, 2019). Sarah C Woodhall et al (2019) reported that establishing the performance of serological assays for C. trachomatis is complex due to numerous factors, such as the study population and the determination of assay detection limits.

The second important result of this meta-analysis is related to the diagnostic tests for herpes, HIV, and syphilis that have high sensitivity and specificity. However, the low number of included studies on diagnostic tests with recombinant proteins for these STIs must be noted, which can introduce type I errors and findings due to chance; this may influence the meta-analysis findings, although meta-analyses with few studies provide valid results (Herbison et al., 2011). As sensitivity and specificity are generally correlated measures, analyses that do not consider their correlation can produce inaccurate results due to threshold effects (Junyoung Lee, Kyung Won Kim, Sang Hyun Choi & Seong Ho Park, 2015). Therefore, correlation analyses were conducted to assess the presence of threshold effects in the studies for each STI. The results revealed threshold effects in studies that used diagnostic tests for C. trachomatis, HIV and HSV. Our analyses also detected high heterogeneity between studies, which can have a clinical, methodological, or statistical origin (Jonathan J Deeks, Julian PT Higgins, 2020). The clinical origin refers to the variability of the participants and is always present in meta-analyses. The methodological conditions are among the characteristics inherent to the sample or the diagnostic tests. Moreover, statistical origin refers to the variability between the measures of clinical effect, methodology, or both (Borges, 2016).

The change in sensitivity and specificity values according to the threshold effect and accuracy of the diagnostic tests included in this review was assessed using the SROC curve (Ault, 2006). This curve could only be obtained for syphilis due to the low number of included studies on diagnostic tests with recombinant proteins for chlamydia, HIV, and herpes. The curve analysis did not reveal heterogeneity between the studies, corroborating the result found in the correlation analysis. Overall, diagnostic tests with recombinant proteins for syphilis can be used to correctly discriminate health and disease status due to their high accuracy (Borges, 2016).
5. Conclusion

The present systematic review and meta-analysis collected important data to discuss the current scenario of STIs, with a special emphasis on new diagnostic methods. The promising test sensitivity and specificity data of studies using recombinant proteins further reinforce the importance of new biotechnological processes for developing diagnostic tests. Other issues addressed in this review, such as the lack of studies for other STI-causing pathogens, highlight the need for studies on other microorganisms of clinical and epidemiological importance. In addition, the difficulty of performing quantitative analyses for diagnostic accuracy based on the high heterogeneity found suggests the need for new research that aim to identify the causes of the heterogeneity found in the present meta-analysis. Moreover, the systematization of data can facilitate decision-making regarding the types of tests produced and the STIs analyzed. Although the World Health Organization considers POC laboratory tests as powerful tools for STI management and control, the appropriate choice for diagnosis remains a difficult task due to the high number of STIs and the variety of potential available tests. Finally, studies, such as the current one, provide scientific knowledge for future studies, such as the development of diagnostic methods for other microorganisms that cause STIs, which aim to contribute to a better quality of life and organization in health services related to the prevention and treatment of STIs.

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