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Tools for the Diagnosis of Herpes Simplex Virus 1/2: A Systematic Review of Studies Published Between 2012-2018

Zeeshaan Arshad, Abrar Alturkistani, David Brindley, Ching Lam, Kimberley Foley, Edward Meinert

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Abstract

Background: Herpes simplex viruses (HSV) 1 and 2 are common infections affecting the global population. HSV 1 is the most common type estimated to affect 67% of the global population. HSV can have rare, but severe manifestations such as encephalitis and neonatal herpes necessitating the use of reliable and accurate diagnostic tools for the detection of the viruses. Currently used HSV diagnostic tools require highly specialized skills, availability of a laboratory setting and may lack sensitivity. More recent HSV diagnostic tools are numerous and need to be identified and compared in a systematic way to be able to make the best decision about which diagnostic tool to use. Diagnosis of HSV is essential for prompt treatment with antivirals. To select the best test for a patient, knowledge of the performance and limitations of each test are critical.

Objective: This systematic review summarizes recent study articles evaluating HSV-1 and HSV-2 diagnostic tools.

Methods: Following the PRISMA guidelines, selection criteria, data extraction and data analysis were determined prior to commencement of the study. Studies assessing the specificity/sensitivity of an HSV 1 or HSV 2 diagnostic tool published between 2012 and 2018 were included. Quality assessment of included studies was performed using the quality assessment of diagnostic accuracy studies (QUADAS-2) tool.

Results: Searches of the PubMed database yielded 264 studies. Eleven studies included eleven molecular assays, and eight studies included 19 different serological assays for the detection of either HSV 1, HSV 2 or both types of the virus. A greater proportion of tools based on molecular assays are being developed by commercial entities. Studies that tested molecular assays mostly focused on cutaneous and mucosal HSV infections (n=13); two studies focused on ocular disease, while a single one on central nervous system manifestations. The Simplexa HSV-1/2 Direct is currently the only FDA approved device for use on cerebrospinal fluid. No tools focused on prenatal screening. We also present performance metrics of tests for benchmarking of future technology. Most of the included studies had a high risk of bias rating in half of the QUADAS-2 tool risk of bias domains.

Conclusions: The landscape of diagnostic tools for HSV-1 and HSV-2 infections is rapidly moving away from laboratory based and culture methods that have long been considered the gold standard technique. The majority of tools study cutaneous and mucosal HSV infections (n=13); two tests focused on ocular disease, while a single one on CNS manifestations. No diagnostic tools included in our systematic review are currently suitable for use as prenatal tools, however. The incidence of acute infections is rising, and since these infections present the greatest risk to unborn fetuses further work needs to be done to develop diagnostic tools to detect primary infection in expectant mothers to prevent vertical transfer. This will assist in lowering the rate of neonatal herpes, which can be a life threatening condition. We believe this can only be achieved through prenatal screening for primary infection and subsequent medical intervention. Clinical Trial: N/A

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Systematic Review

Tools for the Diagnosis of Herpes Simplex Virus 1/2: A Systematic Review of Studies Published Between 2012-2018

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Abstract

Background: Herpes simplex viruses (HSV) 1 and 2 are common infections affecting the global population. HSV 1 is the most common type estimated to affect 67% of the global population. HSV can have rare, but severe manifestations such as encephalitis and neonatal herpes necessitating the use of reliable and accurate diagnostic tools for the detection of the viruses. Currently used HSV diagnostic tools require highly specialized skills, availability of a laboratory setting and may lack sensitivity. More recent HSV diagnostic tools are numerous and need to be identified and compared in a systematic way to be able to make the best decision about which diagnostic tool to use. Diagnosis of HSV is essential for prompt treatment with antivirals. To select the best test for a patient, knowledge of the performance and limitations of each test are critical. This systematic review summarizes recent study articles evaluating HSV-1 and HSV-2 diagnostic tools.

Methods: Following the PRISMA guidelines, selection criteria, data extraction and data analysis were determined prior to commencement of the study. Studies assessing the specificity/sensitivity of an HSV 1 or HSV 2 diagnostic tool published between 2012 and 2018 were included. Quality assessment of included studies was performed using the quality assessment of diagnostic accuracy studies (QUADAS-2) tool.

Results: Searches of the PubMed database yielded 264 studies. Eleven studies included eleven molecular assays, and eight studies included 19 different serological assays for the detection of either HSV 1, HSV 2 or both types of the virus. A greater proportion of tools based on molecular assays are being developed by commercial entities. Studies that tested molecular assays mostly focused on cutaneous and mucosal HSV infections (n=13); two studies focused on ocular disease, while a single one on central nervous system manifestations. The Simplexa HSV-1/2 Direct is currently the only FDA approved device for use on cerebrospinal fluid. No tools focused on prenatal screening. We also present performance metrics of tests for benchmarking of future technology. Most of the included studies had a high risk of bias rating in half of the QUADAS-2 tool risk of bias domains.

Discussion: Use of serologic tests to diagnose genital lesions is inappropriate as positive results may be due to chronic infection, while negative results may overlook recent infection. The incidence of acute infections is rising. As these infections present the greatest risk to fetuses, work needs to be done to prevent vertical transfer. Prenatal screening for primary infection and subsequent medical intervention will assist in lowering the rate of neonatal herpes. In conclusion, HSV diagnosis is moving away from culture-based methods to serology based or PCR based methods. Sensitive, rapid and efficient HSV diagnostic tools should be adopted for prevention of acute infections and neonatal herpes.

Introduction

Herpes simplex virus 1 (HSV-1) and Herpes simplex virus 2 (HSV-2) are DNA viruses that belong to a subfamily of the Herpesviridae family called Alphaherpesvirinae [1]. They are the causative agents in a wide range of human diseases that include oral and genital mucocutaneous lesions, and some rare, but life-threatening conditions such as fulminate encephalitis [2].

HSV is a very common condition with HSV 1 estimated to affect 67% of the global population while HSV 2 is estimated to affect 11% of the global population [2]. The prevalence can be even higher in low and middle income countries or among certain patient sub-populations. For instance, HSV-2 prevalence in sub-saharan countries is estimated to be as high as 53.7% among individuals aged 15-25 years old [3]. HSV-2 prevalence can be used as a biomarker for human immunodeficiency virus (HIV) epidemic due to its high association among patients having an HSV-2 infection [4].

One of the most severe manifestations of HSV is encephalitis, which can have a mortality rate up to 97% [5]. Encephalitis is a rare manifestation, which in developed countries, could result in about 4-5 cases/million population/year [6,7]. HSV infections are most common among women of reproductive age, which can increases the risk of virus transmission from mother to the child during birth resulting in neonatal herpes [4]. Neonatal herpes is also one of the severe manifestations of the disease and can cause long-term health complications requiring appropriate and reliable identification of the disease.

In addition to the prevalence and potential complications, lesions caused by HSV-1 and/or HSV-2 are nonspecific and have variable presentations. Chronic carriage necessitate the use of different laboratory testing methods appropriate to each case [8]. Currently, viral culture is the most commonly used method of diagnosis and is considered the gold standard method [8]. This method is limited, however, by the need of a laboratory setting, aseptic technique and variable accuracy dependent on disease stage [8]. It also takes up to seven days to get the viral culture results back during which time the infection may have developed further. The viral culture will have a lowered sensitivity after the first 48 of appearance of the symptoms and is best if administered as soon as the symptoms appear [9]. Although Antigens specific to HSV-1 and HSV-2 can also be readily detected by direct immunofluorescence (IF) assay; these methods have found to lack sensitivity in non-symptomatic patients [8].

Other methods for HSV diagnosis include serological tests which use blood sample to check for the disease “antibodies” [9]. These tests can take between 1 day to 3 weeks [9]. Serological tests are suitable to detect the subtype of the HSV virus (HSV1 or HSV 2) and can detect asymptomatic patients [10]. Serology based tests can be used to confirm a clinical diagnosis of HSV since it is a more reliable method than clinical diagnosis [10]. It has been argued that serology based tests are more “direct” and “economical” compared to viral cultures [10].

More modern methods of infection detection include techniques that detect viral DNA such as PCR. These methods use molecular based assays and are widely used for detection of infectious disease [11]. Such methods have been found to be more sensitive, more rapid, and require less stringent conditions in terms of collection and [11,12]. Type-specific diagnosis are also possible using antigen detection techniques such as enzyme immunoassays targeting the glycoprotein G antigen [8].

To select the most appropriate test for a patient a thorough knowledge of the performance and limitations of available tests is critical to inform clinical decision-making and the development of future technologies. Previous systematic review regarding HSV diagnostic tools, reviewed only commercially available tests used to diagnose only one type of the virus (HSV 2) and only reviewed tests used in Sub-Saharan Africa [13]. Another review looked at HSV diagnostic tools but limited its population only to pregnant women and neonates [14]. This systematic review

summarizes recent study articles evaluating HSV-1 and HSV-2 diagnostic tools. We will evaluate the study characteristics; the performance of the various tests included in the studies, and finally discuss their limitations and strengths. We will then suggest areas in which there is unmet medical need.

Methods

Selection criteria, data extraction, and methods of analysis were decided prior to the study in line with the PRISMA guidelines [15].

Eligibility Criteria

We selected only original articles published between 2012-2018. We used the PICO (Population, Intervention, Comparator, Outcome) framework to highlight the review's inclusion and exclusion criteria (Table 1). The population included both animal and human populations of all sex and age groups. Patients with multimorbidities were also included including patients with Hepatitis, and/or HIV due to the possibility that HSV co-exists with diseases such HIV and Hepatitis in some patient populations [16,17]. The intervention under study included diagnostic tools to detect all types HSV-1 or HSV-2 infections including oral, genital, ocular or central nervous system (CNS) related infections. The elements in the PICO were determined as a result of a preliminary search in Pubmed prior to the formulation of the review's eligibility criteria. To help compare studies and their outcomes, we only included studies that reported sensitivity and/or specificity as performance indicators of the diagnostic tools [18]. We excluded book chapters, reviews and reports and articles not in the English language. To capture the most up to date evidence, we restricted the search to the years 2012-2018 (Multimedia Appendix 1).

Table 1. Review inclusion and exclusion criteria reported using the PICO (Population, Intervention, Comparator, Outcome) framework.

Population	All animal and human populations including individuals of all ages and both sexes such as neonates, infants, children, adolescents, and pregnant women. Individuals with multimorbidity will also be included such as patient co-infected with HSV and HIV, or HSV and Hepatitis C.
Intervention	All diagnostic tests for HSV-1 or HSV-2 used for anytype of the infection such as oral, anogenital, ocular or central nervous system infections. Diagnostic tests that detect other viruses in addition to HSV-1 and HSV-2 will not be included.
Comparator	Not specified.
Outcome	Sensitivity and/or specificity as performance measured of the diagnostic test.
Dates	Database searches will be limited to the dates: 17/04/2012-31/12/2018

Search Strategy and Sources of Information

The complete search strategy used to identify studies is detailed in Multimedia Appendix 1. PubMed (Medline) was searched from 01/01/2012-31/12/2018. We only searched the PubMed database because most of the studies concerning diagnostic tools are indexed in Medline [19]. We also searched the bibliographies of relevant articles and reviews that we

identified in our initial scoping searches.

Study Selection

Studies were selected first by removing duplicates from the initially identified studies then manually screening the titles and abstracts of the studies against the eligibility criteria and finally performing full-text reading of eligible studies. ZA and AA completed study screenings. Study records were managed using Mendeley Desktop (v1.16.3) software.

Data Extraction

Data extraction sheets were developed prior to the data extraction. Data extraction was completed separately by the study authors ZA and AA to increase accuracy. Items collected in the data extraction sheets were identified and finalized through iterative discussions with the study authors.

The data extraction sheets collected information on:

1. General study information including country of the study, HSV subtype, sample size, study design (retrospective or prospective), the name of the diagnostic test under study as well as the comparison tools.

2. Methodological details including sample collection method, transportation/ storage method, study aims, conclusions.

After identification of the diagnostic test under study, the tests were categorized as either: serology-based HSV detection assays, molecular assays for detection of HSV, or culture-based methods.

3. Tables for each method were then created that summarized the performance of each test. Although sensitivity and specificity were our primary performance metrics: agreement (total, positive and negative), kappa, AROC (area under receiver operator curve), Negative Predictive Value (NPV), and Positive Predictive Value (PPV) were also recorded. Cost and time to run the test were also extracted. In addition, the FDA and EMA status of each included diagnostic tool were ascertained through search of the respective organizations websites [20,21]. For studies that reported analytical performance and clinical performance, only the clinical performance results were reported in this review.

Risk of Bias in Studies

The risk of bias of included studies was assessed using the quality assessment of diagnostic accuracy studies (QUADAS-2) tool [22].

Results

Search Results and Study Inclusion

We identified 264 records from the Pubmed search and an additional 30 through the bibliography search. There were no duplicated in the records identified, therefore all 294

records titles and abstracts were screened for eligibility. 235 articles were excluded based on the eligibility criteria. Full-text reading of the 59 screened articles resulted in a total of 19 full-text studies for inclusion in this systematic review (Figure 1). We screened the abstracts of 264 articles for relevance and found 59 records that could not be excluded based purely on reviewing the abstracts and titles and that were assessed as full texts. We subsequently excluded 40 articles because, after deeper examination, there was no performance metric measured (n=8), article did not include a diagnostic tool (n = 9), study described viruses other than HSV-1 or HSV-2 (n = 11), sensitivity and/ or specificity were not measured (n=8), or the article was a review (n = 4).



Figure 1. PRISMA Flow search summary. Search of the Pubmed database returned 264 records.

An additional 30 were found through bibliography search. 59 full-text articles were reviewed, resulting in 19 articles included in this systematic review. Flow diagram adapted from Moher et al. [23]

Study Characteristics

Summaries of the 19 included studies can be found in Tables 2-3. The majority of studies were prospective in nature (63%); less than 40% of the studies relied on archived samples (Table 2). The number of studies over the last six years has grown, reaching its highest in 2017: from five studies in 2012 to eleven in 2017 (Table 2). This growth declined in 2018, with only one study was found to be focused on HSV1 or 2 diagnostic tools. Although since the search was completed on December 31, 2018, there may be other 2018 studies that were yet to be published on the day of the search. Sample sizes within studies ranged from 60 in Al-Shobaili et al. [24] and Loughman et al. [25] to 3408 in Patel et al. [26] with a median sample size being of 179 patients (Table 2). Studies investigated diagnostic tools for HSV-1/2 (n=10), HSV-2 only (n=7), or HSV-1 only (n=2) (in one study the viral subtype could not be ascertained [27] so it was labelled as HSV-1/2). 58% of tests were molecular assays, with remainder using serology based HSV detection methods. No studies used a culture-based method as their primary method; studies did use them as a comparison method (Table 2). In terms of disease state: two studies investigated patients with ocular disease [27,28], one for CNS infection [29], with the remainder for patients with suspected mucosal and/ or cutaneous lesions (Table 2). Given the heterogeneous nature of the studies included within this review in terms of sample type, collection, and patient demographics it was not possible to draw statistical inferences or perform a meta-analysis.

Table 2. Summary of Included Studies. Table summarizes study location, herpes subtype, sample size, study design (retrospective or prospective), and the name of the diagnostic test under study as well as the tools it will be compared to.

Study ID	Country	Study Design	Herpes 1/2	Sample Size	Disease	Test	Test Type	Comparison
Al-Shobaili et al. [24]	Saudi Arabia	Prospective	HSV-2	60 - 35 males - females	Genital Ulcer Disease (Genital)	HerpeSelect Express Rapid Test (Focus Technologies Inc., Cypress, CA, United States)	Immunochromatographic (Serology-based HSV detection assay)	HerpeSelect 2 IgGELISA(Focus Technologies Inc., Cypress, CA, United States) Kalon HSV-2 IgG ELISA assay (Kalon Biological Ltd, Guilford, United Kingdom) MAb-EIA
Barrado et al. [28]	Spain	Prospective	HSV-1	188	Suspicion of herpetic Epithelial keratitis and non-herpetic corneal ulcer (Ocular)	Real-time singleplex PCR	Molecular assays for detection of HSV	Cell Culture-csPCR, csCC, cswPCR, csWCC
Burton et al. [30]	USA	Retrospective	HSV-2	84	Veterans with hepatitis C and HSV-2 co-	Focus Herpeselect Hsv-2 Igg	Serology-based HSV detection	Biokit HSV-2 rapid assay (Biokit USA,

					infection	(Focus Technologies Inc., Cypress, CA, United States)	assay	Lexington, MA)
Gitman et al. [31]	USA	Prospective	HSV-1 HSV-2	171	Dermal, genital, ocular, mouth	Simplexa HSV 1 & 2 PCR (Focus Diagnostics, Cypress, CA, United States)	Molecular assays for detection of HSV	Cell culture (Diagnostic Hybrids, Athens, USA) Cytospin-enhanced direct fluorescent antibody (DFA) Real-time TaqMan PCR (LDT HSV PCR)
Granato et al. [32]	USA	Prospective	HSV-1 HSV-2	1,351	Cutaneous and mucocutaneous herpes infection (Cutaneous)	AmpliVue HSV 1 and 2 Assay (Quidel, San Diego, CA)	Molecular assays for detection of HSV	ELVIS HSV ID and D3 Typing System (Quidel DHI, Athens, OH)
Hobbs et al. [33]	Kenya	Retrospective	HSV-2	198	Not specified	HERPES Select 2 (Focus Diagnostics, Cypress, CA) Kelon ELISA (Kalon Biological, Guildford, UK)	Serology-based HSV detection assay	Same tests but with serum sample
Lang et al. [34]	Canada	Prospective	HSV-1 HSV-2	276	Anogenital, oral	Viper HSV-Qx assay (BD Molecular Diagnostics)	Molecular assays for detection of HSV	LightCycler 2.0 platform (HSV-LC) (Roche Diagnostics, Basel, Switzerland)
Lee et al. [35]	Singapore	Retrospective	HSV-1 HSV-2	117	Not specified	Luminex ARIES HSV 1&2 Assay (Luminex Corp., Austen, TX, USA)	Molecular assays for detection of HSV	Förster resonance energy transfer (FRET)-based PCR assay and FTD Neuro 9 assay (Fast Track Diagnostics, Junglinster, Luxembourg)

Lierman n et al. [36]	Germany	Retrospectiv e	HSV-1 HSV-2	263	Not specified	Orgentec ELISA Anti-HSV-2 IgM (Mainz. Germany) Orgentec ELISA Anti-HSV-1 IgM (Mainz. Germany) Orgentec ELISA Anti-HSV-1/2 IgM (Mainz. Germany) Serion ELISA classic HSV 2 IgM IgM (Würzburg, Germany) Serion ELISA classic HSV 1 IgM (Würzburg, Germany) Serion ELISA classic HSV 1 + 2 IgM IgM (Würzburg, Germany) Orgentec ELISA Anti-HSV-2 IgG IgG (Mainz. Germany) Orgentec ELISA Anti-HSV-1 IgG (Mainz. Germany) Serion ELISA classic HSV 2 IgG IgM (Würzburg, Germany) Serion ELISA classic HSV 1 IgG IgM (Würzburg, Germany) Serion ELISA classic HSV 1 + 2 IgG IgM (Würzburg, Germany)	Serology-based HSV detection assay	HerpeSelect 1 ELISA IgG (Focus Diagnostics, Cypress, CA, United States) HerpeSelect 2 ELISA IgG (Focus Diagnostics, Cypress, CA, United States) Immunoblot assay recomLine HSV-1 & HSV-2 IgG (Mikrogen, Neuried, Germany)
Liu et al. [37]	China	Prospective	HSV-2	318	Not specified	$gG_{321580His}$ ELISA test	Serology-based HSV detection assay	HerpeSelect 2 ELISA IgG (Focus Diagnostics, Cypress, CA, United States)
Loughm an et al. [25]	France	Retrospectiv e	HSV-2	60 - 42 fe ma	Not specified	HSV-2 biochip test using reagents from the	Serology-based HSV-2 immunoassay with biochip	LIAISON® HSV-2 Type Specific IgG chemilumines

				les		HerpeSelect® 2 IgG kit (Focus Diagnostics, CA, USA; product EL0920G)		cent immunoassay test (DiaSorin, MN, USA)
Binnicker et al. [29]	USA	Prospective	HSV-1 HSV-2	100	Central nervous system infection due to herpes simplex virus (CNS)	Simplexa HSV-1/2 Direct (Focus Diagnostics, Cypress, CA)	Molecular assays for detection of HSV	Roche HSV-1/2 ASR(Roche Diagnostics, Indianapolis, USA)
Miller et al. [38]	USA	Prospective	HSV-1 HSV-2	179	Genital Herpes	IDbox HSV 1/2 assay (GenturaDx, USA)	Molecular assays for detection of HSV	Culture MultiCode HSV PCR
Parra-Sánchez et al. [39]	Spain	Prospective	HSV-1 HSV-2	283	Various mucosal and cutaneous lesions, CSF, urinary	HSV OligoGen kit (Operon-Immuno&Molecular Diagnostics, Zaragoza, Spain)	Molecular assays for detection of HSV	Roche LightCycler HSVóQual Kit assay (Roche Diagnostics, Basel, Switzerland)
Patel et al. [26]	South Africa and Zambia	Retrospective	HSV-2	3408	HSV-2/HIV-1 co-infected individuals and HIV-uninfected heterosexual partners	Kalon Herpes Simplex Virus Type 2 IgG ELISA (Kalon Biological Ltd, Surrey, United Kingdom)	Serology-based HSV detection assay	Washington HSV western blot
Van Der Pol et al. [40]	USA	Prospective	HSV-1 HSV-2	508	Anogenital Infections (Anogenital)	BD ProbeTec HSV Qx (HSVQx) system (BD Diagnostics, Sparks, USA)	Molecular assays for detection of HSV	Elvis culture system (San Diego, USA) Laboratory-developed PCR assay
Shevlin and Morrow [41]	USA	Retrospective	HSV-2	100	Not specified	Uni-Gold™ HSV-2 Rapid (Trinity Biotech, Ireland)	Serology-based HSV detection assay	Washington HSV Western blot
Shoji et al. [27]	Japan	Prospective	Viral subtype could not be ascertained	59 + 23 healthy volunteers	Herpes simplex keratitis (Ocular)	HSV DNA (by PCR) and HSV specific sIgA antibody levels (ELIZA) in tears.	Serology-based HSV detection assay AND Molecular assays for detection of HSV	N/A Control patients?
Tong et al. [42]	USA	Prospective	HSV-1 HSV-2	176	Genital and oral lesions (Oral and Genital)	IsoGlow HSV Typing assay (BioHelix Corp., Beverly, USA	Molecular assays for detection of HSV	IsoAmp HSV (BioHelix Corp., Beverly, USA) ELVIS Shell Vial Assay ((Diagnostic Hybrids, Athens, USA)

The methodological characteristics of included studies are presented in Table 3. Basic demographic information was reported in 13 studies. In studies that reported participant gender (n=11), between 30%-92% of study participants were male. Participant age ranged from a 5 months old to 85 years old (Table 3).

Table 3. Methodological Characteristics of Included Studies. Table summarizes study aims, conclusions, and demographic data.

Study ID	Patient Demographic Data	Aims/ Rationale	Conclusions
Al-Shobaili et al. [24]	58% male Mean age 37 °(SD 13.6) years	To evaluate a point of care test (Herpes Select Express rapid test) for more rapid turn around of results in a non-laboratory setting.	The HerpeSelect Express rapid test has adequate sensitivity and specificity for confirming HSV-2 infection in patients with genitourinary disease. The test is good at diagnosing high risk individuals.
Barrado et al. [28]	52.2% male Mean age 56.9 (SD 19.7) years	Assess if conjunctival swabs samples were equivalent to corneal scrapings to diagnose herpetic keratitis.	Conjunctival swabs may serve as supplemental method for diagnosis of typical HK despite limited sensitivity when the collection of corneal scrapings would not be feasible. PCR must be considered gold standard for diagnosis of typical HK.
Burton et al. [30]	92% male 67% african america – 33% white 97% heterosexual	To assess type-specific tests for HSV-2 in patients with chronic hepatitis C infection	In veterans with chronic hepatitis C infection, HerpeSelect1 HSV-2 index values between 1.1 and 2.89 should be confirmed with an alternate test for HSV-2 infection. Only effective within certain range.
Gitman et al. [31]	26.4% male 148 adult 23 pediatric	Compare the performance, time to result, and cost of the Simplexa HSV1&2direct PCR with those of conventional	Simplexa HSV 1 & 2 direct PCR is expensive but required the least training had the lowest hands-on time and fastest assay time (75 min, versus 3 h by LDTHSVPCR), detected most positives, considered an internal control, and provided the HSV type.
Granato et al. [32]	N/A	To evaluate the performance of Amplivue HSV1+2 assay compared to ELVIS HSV IDD3 in detecting HSV1-2 from clinical specimens.	The results of this study show that the AmpliVue HSV 1/2 assay was more sensitive than ELVIS culture for detecting HSV-1 and HSV-2 in a wide variety of cutaneous and mucocutaneous specimens
Hobbs et al. [33]	198 adult males and females 18 samples used for Kalon assay 178 samples used for the Focus assay	To evaluate and validate the use of Focus and Kalon ELISAs in detecting HSV-2 antibodies from dried blood sample as part of an HIV prevention trial.	The use of dried blood spots cab reduce time and provide an effective way to test for HSV-2 in resource-limited settings. The study found that the use of dried blood spots with the Kalon assay did not perform well and that the use of dried blood spots with the Focus assay resulted in comparable sensitivity and specificity to the use of serum sample.
Lang et al. [34]	N/A	Compare HSV-Q and real-time HSV PCR in terms of accuracy and cost. Devise approved for anogenital infections, this study investigates its use on lesions from other anatomical locations.	The HSV-Qx assay was found to be highly sensitive and accurate. Gray zone may be required for specimens with values falling between 50 and 800 maximum relative fluorescence units. HSV-Qx has a lower cost per specimen ($22) compared to that of HSV-LC ($34). Samples lying near the positivity cut off should be retested.
Lee at al. [35]	N/A	To evaluate the analytical and clinical performance of the Luminex ARIES assay in detecting HSV1 and HSV2 compared to an approved assay for the HSV testing for the diagnosis of meningitis/encephalitis. .	The analytical performance results of the assay showed that the assay had lower sensitivity than the comparator. On the other hand, the clinical performance results of the assay showed that it had comparable performance to the routinely used assay. Compared to two other routinely used assay, the ARIES assay required the shortest amount of hands-on and assay time and was the least labor-intensive. The study concluded that Luminex ARIES assay can be used for successful detection of HSV 1 and HSV 2 for skin and genital infections, meningitis, and encephalitis.
Liermann et al. [36]	Healthy children between 5 months and 3 years of age, healthy voluntary blood donors aged 18 to 65 years or hospitalized	Evaluate the HSV type-common and type-specific IgG and IgM enzyme-linked immunosorbent assays for the diagnosis of acute and latent HSV infections.	HSV type-common IgM ELISAs can be useful to confirm acute newly acquired HSV infections, the use of single-type IgM ELISAs on the basis of whole-virus antigen is dispensable.

	patients between 14 and 70 years of age.		
Liu et al. [37]	64% male Mean age 35.9 (SD 4.52) years for males Mean age 30.7 (SD 4.65) years for females	Need for a convenient, high-quality, rapid and inexpensive domestic serodiagnostic kit that differentiates between HSV-1 and HSV-2.	The study indicates that $gG_{321-580H}$ is has a high diagnostic potential for HSV-2 virus serodiagnosis in humans.
Loughman et al. [25]	30% male Median age of 40.5 (15-85)	To validate the HSV-2 biochip assay as a point of care test to be able to use it in resource-limited settings.	Membrane-touch biochip requires some improvements such as expanded calibration before it can be used as a rapid diagnosis tool. The tool was validated and performs comparable to other standard diagnostic method. The study concluded that the assay could potentially as a rapid, point-of-care test for HSV-2.
Binnicker et al. [29]	N/A	Investigates a more rapid, cheaper method of detecting HSV in CSF fluid that only uses a small amount of fluid. This makes it suitable for use in neonates.	The Simplexa HSV-1/2 assays demonstrated a combined sensitivity and specificity of 96.2% and 97.9%, respectively. Assay does not require nucleic acid extraction. Results are available in 60 min. The Simplexa assay requires only 50 ml of CSF.
Miller et al. [38]	N/A	New system designed to maximize sensitivity. This study assesses whether this is achieved. Is fully automated.	Assay shows acceptable clinical performance characteristics and demonstrates promise for further development of this fully automated platform for detection of pathogen nucleic acid in clinical laboratories
Parra-Sánchez et al. [39]	42.2% male Median age of 30.5 (Q1–Q3 24.0–39.0) years	Evaluate a new assay, the HSV OligoGen Kit for the detection of HSV in several types of clinical samples	Detection of HSV-1 and HSV-2 in clinical samples by HOK was not significantly different from detection by LCHK assay ($P \geq 0.8$, t test). Statistical data obtained in this study confirms the usefulness and reliable results of this new assay from a variety of specimens.
Patel et al. [26]	51% male Median Age (IQR) of 32.0 (27-39) HIV Positive – 50%	Evaluate an on site HSV tool in a population of HIV infected and non-infected patients and to evaluate the precision of the Kalon HSV-2 IgG ELISA.	In populations with optimal diagnostic accuracy, Kalon is a reliable stand-alone method for on-site HSV-2 IgG antibody detection. Kalon can be utilized in resource limited settings, enhancing the feasibility to monitor the epidemic and assess intervention efforts.
Van Der Pol et al. [40]	34% male Median age – 25 with a range of 17 to 71	Results of a multicenter study evaluating the performance of a recently FDA-approved, commercially available, type-specific nucleic acid amplification test that allows type specific HSV discrimination.	Assay performs as well as the other assays on a fully automated system that provides results within a few hours rather than many days No differences in test performance based on gender, clinic type, location of the lesion, or type of lesion were observed.
Shevlin and Morrow [41]	35% male (estimate) Pediatric sera (age <18 years) were not included	Evaluation of a point of care and inexpensive device to detect anti-HSV anybody in serum or blood.	UHR is a reliable, low-cost alternative to other point-of-care HSV-2 diagnostic tests. Showed both sensitivity and specificity in a small group of adults.
Shoji et al. [27]	51% male	Assesses whether the combined utilization of IgAantibody (HSV-sIgA) levels in tears, and real-time PCR can improve diagnostic ability.	The combination of laboratory detection of HSV DNA by real-time PCR and of HSV-sIgA by ELISA using tear samples enables higher reliability in diagnosing the subgroups of HSK.
Tong et al. [42]	N/A	Substantial market need for a low-cost, point-of-care HSV typing assay. Such a device is assessed within this study.	Both formats of the IsoGlow HSV typing assay had sensitivities comparable to that of the FDA–cleared IsoAmp HSV test and specificity for the two types of HSV comparable to that of ELVIS HSV Turn around time of around one hour.

Diagnostic Test Performance

Molecular Assays

Molecular assays for detection of HSV are summarized in Table 4. A total of 11 diagnostic tools were investigated within the 11 studies (including one study that used both molecular and serological methods in conjunction [27], out of which six have received FDA approval (Table 4). Some FDA approved tools had an approval for only some components of the test, or certain uses of the test only. For example, the Viper HSV-Qx assay [34] and the BD ProbeTec HSV Qx (HSVQx) system [40] were only approved for anogenital lesions, Luminex ARIES HSV 1&2 Assay [35] was only approved for cutaneous of mucocutaneous lesions only. In eight studies samples were collected using mucosal or cutaneous swabs (Table 4) In the remaining three studies: one collected samples through corneal scrapings or conjunctival swabs, one collected CSF, and one tears (for ocular manifestations) (Table 4).

Table 4. Molecular Assays for Detection of HSV. Table summarizes the different molecular assays studied within the 10 included studies. Information includes regulatory status, collection/ storage/ transport method, and performance.

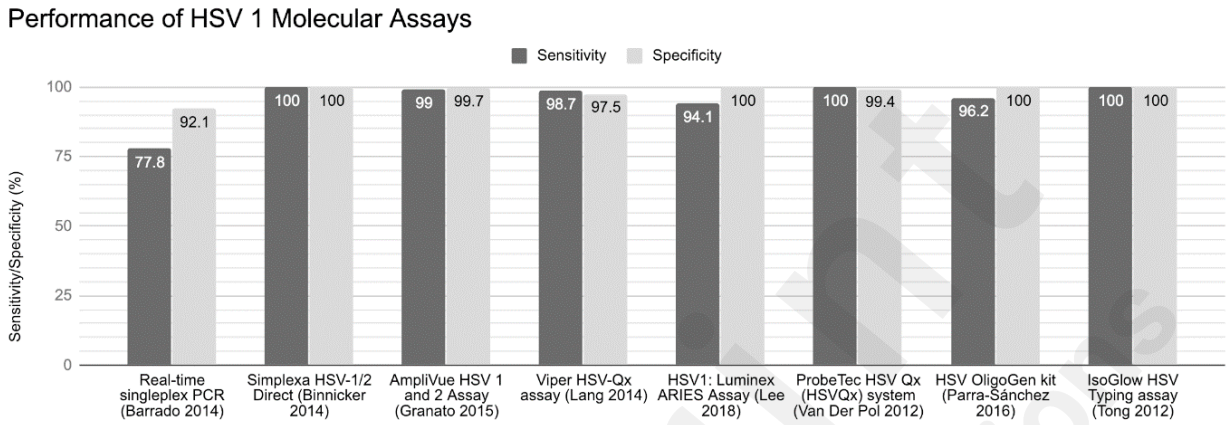
Test	Study ID	Manufacturer	FDA Status	EMA Status	Sample Type	Collection Method
Real-time singleplex PCR	Barrado et al. [28]	University Hospital Madrid, Spain (academic)	No	No	Corneal scrapings and conjunctival swabs	Corneal scrapings by a platinumKimura spatula using as viral transport medium UTM (Univer-sal transport medium, Copan Diagnostic, Inc.). Conjunctival swabs obtained by polyester swabs
Simplexa HSV-1/2 Direct	Gitman et al. [31]	Focus Diagnostics, Cypress, CA, USA	Yes	Unclear	Dermal, Ocular, Mouth	Swabs were collected from local clinics, doctors' offices, and inpatient wards in 3ml ofM4 viral transport medium (VTM) (Remel, Lenexa, KS)
Simplexa HSV-1/2 Direct	Binnicker et al. [29]	Focus Diagnostics, Cypress, CA	Yes	Unclear	Cerebro spinal fluid	Unknown how CSF collected.
AmpliVue HSV 1 and 2 Assay	Granato et al. [32]	Quidel, San Diego, CA	Yes	Unclear	Cutaneous and mucocutaneous swabs	All specimens were collected on swabs, transported to the laboratory in viral transport medium (VTM)
Viper HSV-Qx assay	Lang et al. [34]	BD Diagnostics, Sparks, USA	Yes (only for anogenital lesions)	Unclear	Anogenital and oral swabs	N/A
Luminex ARIES HSV 1&2 Assay	Lee et al. [35]	Luminex Corp., Austen, TX, USA	Yes (only for cutaneous or mucocutaneous lesion samples)	Unclear	Lesion swab samples	Lesion swab samples were collected in clinic and de-identified for the study
BD ProbeTec HSV Qx (HSVQx) system.	Van Der Pol et al. [40]	BD Diagnostics, Sparks, USA	Yes (only for anogenital lesions)		Anogenital swabs	Sampled using a polyester swab, which was placed directly into universal viral transport medium (UVT) (Becton, Dickinson [BD], Sparks,

						MD), and then using a second, foam-tipped swab, which was placed directly into the BDQx liquid wet-swab transport medium (Qx WS).
IDbox HSV 1/2 assay*	Miller et al. [38]	GenturaDx, Hayward, USA	No	No	Genital swabs	Swabs collected in 3mL of universal transport medium, transported to labs, aliquoted samples stored in -70 degrees Celsius
HSV OligoGen kit	Parra-Sánchez et al. [39]	Operon-Immuno&Molecular Diagnostics, Zaragoza, Spain	No	No	Various swabs). 110 ulcer specimens, 48 urine, 48 endocervical, 43 cerebral spinal fluids, 4 urethral and 3 pharyngeal swabs	Cobas PCR media Urine Sample kit or cobas PCR media Female swab sample kit (Roche Diagnostics GmbH, Mannheim, Germany). 110 ulcer specimens, 48 urine, 48 endocervical, 43 cerebral spinal fluids, 4 urethral and 3 pharyngeal swabs
HSV DNA (by PCR) and HSV specific sIgA antibody levels (ELIZA)*	Shoji et al. [27]	School of Medicine, Tokyo, Japan (academic)	No	No	Tears	Using Schirmer strips (Schirmer Tear Production Measuring Strips; Showa-Yakuhinkako, Tokyo, Japan)
IsoGlow HSV Typing assay	Tong et al. [42]	BioHelix Corp., Beverly, USA	No	Unclear	Swabs	Clinical swabs suspended in vial transport medium were collected from the Cleveland Clinic (Cleveland, OH). The samples were shipped on ice for overnight delivery, and were aliquoted on receipt. Some were placed at -80°C for long-term storage, and some were placed at -20°C for short-term storage or near-term testing.

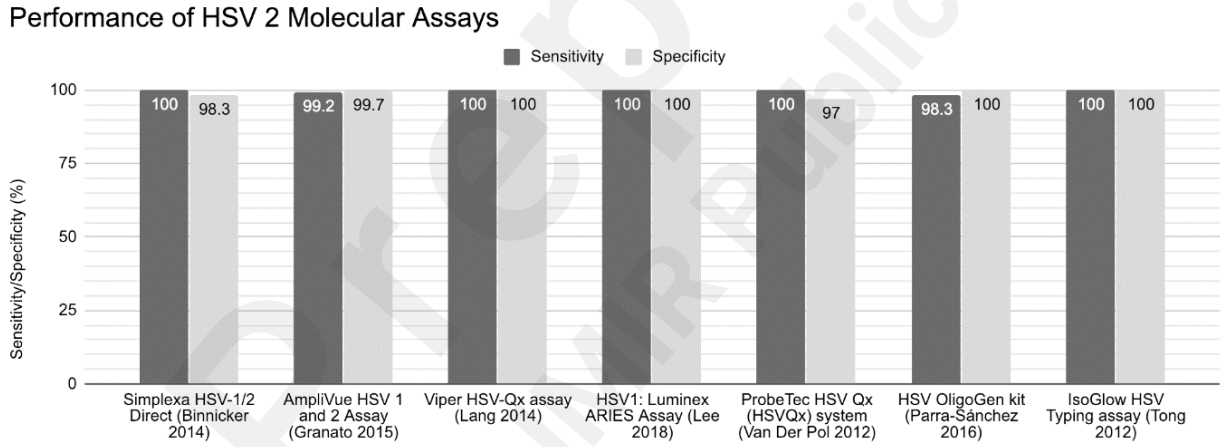
The performance metrics of the various molecular tests are summarized for comparison to future technologies summarized in Figure 2. One study [35] reported positive percent agreement (PPA), negative percent agreement (NPA) instead of sensitivity and specificity which is equivalent to the measures sensitivity and specificity measures in the absence of a standard [43]. Sensitivities for molecular methods ranged from 76.9 [27] to 100% (for a number of tools) (Figure 2). Shoji et al. [27] was the single study that used both a serological and molecular technique in conjunction using tears as a sample material. The study reported the lowest specificity of 82.6% using their ELIZA and PCR techniques. The second worst sensitivity was indicated by a real-time singleplex PCR tested in an academic center by Barrado et al. [28]. Here, a sensitivity of 77.8% using corneal scrapings was found [28]. All other values were above 90% (Table 4; Figure 2). Gitman et al. [31] aimed to compare cell culture, DFA, and a laboratory-developed real-time TaqMan PCR (LDT HSV PCR) for the detection of HSV in dermal,

genital, ocular, mouth, or other swab samples [31]. Conventional culture was found to have a sensitivity and specificity of 87.9 (0.768–0.943) and 99.1 (0.945–1.000), respectively. The difference when using direct immunofluorescence, TaqMan PCR, or Simplexa direct PCR did not improve these metrics [31].

A.



B.



C.

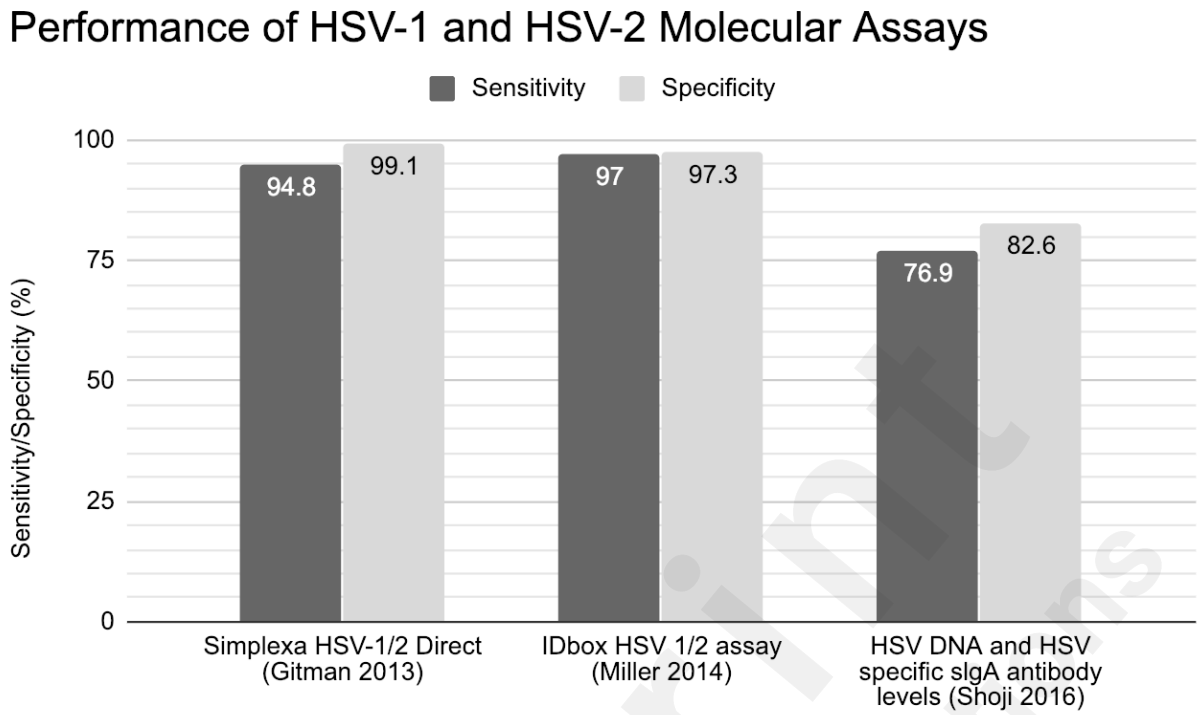
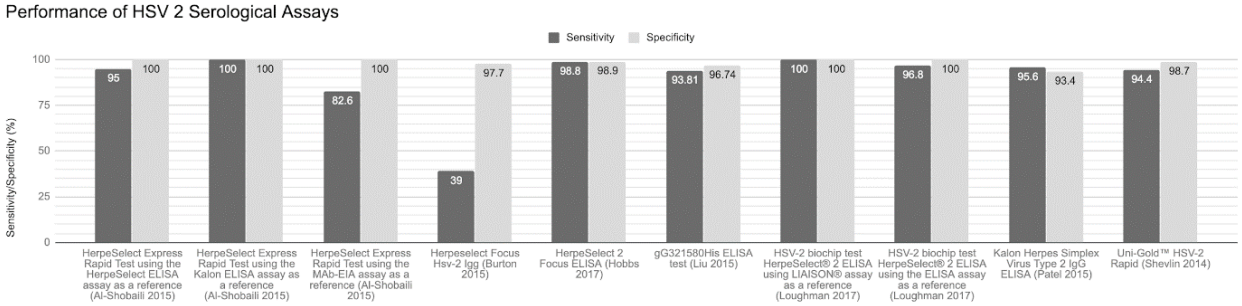


Figure 2. Sensitivity and specificity of Molecular Based Diagnostics. Due to heterogeneous nature of studies included in this systematic review, it is not possible to draw strict conclusions with regards to performance metrics. This figure, therefore, aims solely to act as a means of visually displaying the sensitivity and specificity results of each study.

Serological Assays

Serological assays for detection of HSV are summarized in Table 5. A total of 19 serological diagnostic tools were investigated within 8 studies (plus one study [27] that used both molecular and serological methods in conjunction - detailed in Table 4). Four of the serological diagnostic tools have received FDA approval (Table 5) of which HERPES Select 2 [33] was approved for use with serum only, and the test used by Loughman et al. [25] only used reagents from the FDA approved HerpeSelect® 2 IgG kit. All of the studies in this category used serum samples, and only one study also tested the use of dried blood spots instead [33]. The performance metrics of the various tests are summarized for comparison to future technologies (Table 5, Figure 3). Sensitivity for serological tools ranged from 39% in hepatitis co-infected individuals using the Herpeselect Hsv-2 Igg [30] tool to 100% (for a number of tools) (Figure 3). All others were equal to or above 80% (Table 5; Figure 3). The study by Liermann et al. [36] investigated the detection of primary infection, and concluded that its HSV IgM serology tool should not be used to make decisions for antiviral treatment in HSV.

A.



B.

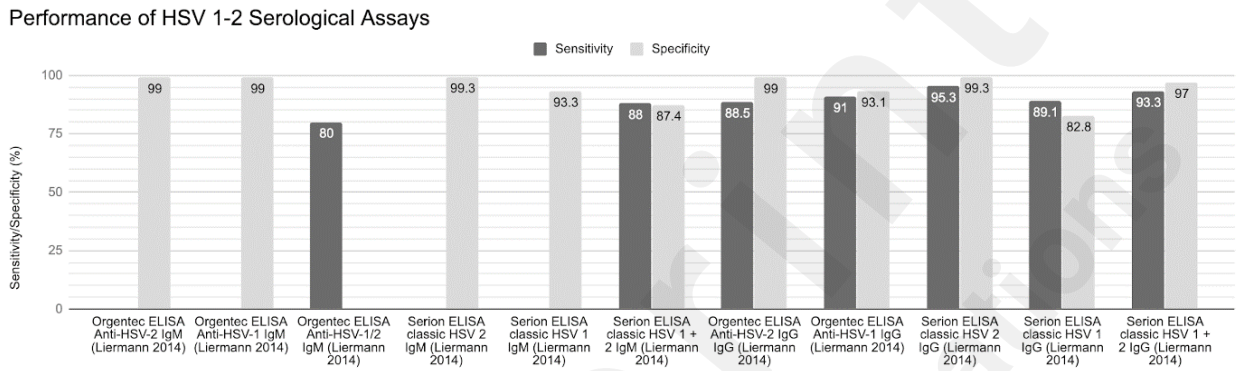


Figure 3. Sensitivity and specificity of Molecular Based Diagnostics. Due to heterogeneous nature of studies included in this systematic review, it is not possible to draw strict conclusions with regards to performance metrics. This figure, therefore, aims solely to act as a means of visually displaying the sensitivity and specificity results of each study.

Table 5. Serological Assays for Detection of HSV. Table summarizes the different serological assays studied within the 6 included studies. Information includes regulatory status, collection/ storage/ transport method, and performance.

	Study ID	Manufacturer	FDA Status	EMA Status	Sample Type	Collection Method	Transportation/ Storage Method	Performance % (95% CI)						
								Sensitivity	Specificity	PPV	NPV	Agreement	Disconrdant Results	Other Metric
HerpeSelect Express Rapid Test	Al-Shobaili et al. [24]	Focus Technologies Inc., Cypress, CA, United States	Yes	Unclear	Serum	Blood samples collected in pyrogen-free tubes under aseptic conditions. Allowed to be clotte	Frozen in aliquots at –20°C until use.	95 when compared to HerpeSelect ELISA 100 when compared to Kalon	100 when compared to HerpeSelect ELISA 100 when compared to Kalon	100 when compared to HerpeSelect ELISA 100 when compared to MAb-EIA	97.56 when compared to HerpeSelect ELISA 100 when compared to Kalon	98.3 when compared to HerpeSelect ELISA 100 when compared to Kalon ELIS	Potentially five	Able to deal with 5 equivocal results

						d and centrifuged.		ELISA 82.6 when compared to MAb-EIA	ELISA 100 when compared to MAb-EIA		ELISA 90.24 when compared to MAb-EIA	A 93.3 when compared to MAb-EIA			
Herpeselect Focus Hsv-2 Igg	Burton et al. [30]	Focus Technologies Inc., Cypress, CA, United States	Yes	Unclear	Serum	Archived samples	N/A	39.0 (24.2 – 55.5)	97.7 (87.7 – 99.6)	94.1 (71.2 – 99.0)	62.7 (50.0 – 74.2)	N/A	N/A	N/A	
HerpeSelect 2 Focus ELISA	Hobbs et al. [33]	Focus Diagnostics, Cypress, CA, United States	Yes (for use with serum only)	Unclear	Serum (dried blood spots)	Blood samples obtained through venipuncture and stored in EDTA containing tubes. Dried blood spot samples were prepared by spotting EDTA blood onto S&S 903 filter paper (Schle	Dried blood spots eluted with phosphate buffered saline at 4°C for 18–24 hours	98.8 (92.7 - 99.9)	98.9 (93.4 - 99.9)	N/A	N/A	Unweighted Kappa - 0.96 (0.913-0.999)	N/A	N/A	

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						icher & Schuell, Dassel, Germany), dried in a biosafety cabinet for 18-24 hours, and stored at 4°C until use									
Kelon ELISA	Hobbs et al. [33]	Kalon Biological, Guildford, UK	No	Unclear	Serum (dried blood spots)	Blood samples obtained through venipuncture and stored in EDTA containing tubes. Dried blood spot samples were prepared by spotting EDTA blood onto S&S 903 filter paper (Schleicher & Schuell, Dassel, Germany), dried in a biosafety cabinet for 18-24 hours,	Dried blood spots diluted with a kit diluent	< 9.1 (0-25.9)	100 (64.6-100)	N/A	N/A	Unweighted Kappa < 0.068 (0-0.369)	N/A	N/A	

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						and stored at 4°C until use									
Orgentec ELISA Anti-HSV-2 IgM	Lierman et al. [36]	Orgentec, Mainz. Germany	No	No	Serum	N/A	Stored in aliquots at -20	Not calculated	99.0 (93.8 – 99.9)	N/A	N/A	N/A	Cannot quantify discrepant results	N/A	
Orgentec ELISA Anti-HSV-1 IgM	Lierman et al. [36]	Orgentec, Mainz. Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	Not calculated	99.0 (93.8 – 99.9)	N/A	N/A	N/A	Cannot quantify discrepant results	N/A	
Orgentec ELISA Anti-HSV-1/2 IgM	Lierman et al. [36]	Orgentec, Mainz. Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	80.0 (58.7 – 92.4) (Cut-off 12.5 U/ml :))	Not tested	N/A	N/A	N/A	Cannot quantify discrepant results	N/A	
Serion ELISA classic HSV 2 IgM	Lierman et al. [36]	Serion, Würzburg, Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	Not calculated	99.3 (95.8 – 100.0) to 100.0 (96.9 – 100.0)	N/A	N/A	N/A	Cannot quantify discrepant results	N/A	
Serion ELISA classic HSV 1 IgM	Lierman et al. [36]	Serion, Würzburg, Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	Not calculated	93.3 (87.8 – 96.6) to 96.0 (91.2 – 98.4)	N/A	N/A	N/A	Cannot quantify discrepant results	N/A	
Serion ELISA classic HSV 1 + 2 IgM	Lierman et al. [36]	Serion, Würzburg, Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	88.0 (67.7 – 96.8) to 96.0 (77.7 – 100.0)	87.4 (80.8 – 92.1) to 94.7 (89.5 – 97.5)	N/A	N/A	Kappa - 0.96 (0.88 – 1.00)	Cannot quantify discrepant results	N/A	
Orgentec ELISA Anti-HSV-2 IgG IgG	Lierman et al. [36]	Orgentec, Mainz. Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	88.5 (79.4 – 94.1) to 95.4 (88.0 – 98.5)	99.0 (93.8 – 99.9)	N/A	N/A	Kappa - 0.94 (0.90 – 0.99)	Cannot quantify discrepant results	N/A	
Orgentec ELISA Anti-HSV-1 IgG	Lierman et al. [36]	Orgentec, Mainz. Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	91.0 (83.2 – 95.5) to 96.0 (89.5 –	93.1 (85.0 – 97.2) to 100.0 (94.7 –	N/A	N/A	Kappa - 0.95 (0.91 – 1.00)	Cannot quantify discrepant results	N/A	

								98.7)	100.0)						
Serion ELISA classic HSV 2 IgG	Lierman et al. [36]	Serion, Würzburg, Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	95.3 (87.7 – 98.5) to 97.6 (91.0 – 99.6)	99.3 (95.8 – 100) to 100.0 (96.9 – 100.0)	N/A	N/A	Kappa - 0.98 (0.96 – 1.00)	Cannot quantify discrepant results	N/A	
Serion ELISA classic HSV 1 IgG	Lierman et al. [36]	Serion, Würzburg, Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	89.1 (81.0 – 94.2) to 98.0 (92.3 – 99.7)	82.8 (75.1 – 88.6) to 97.0 (92.0 – 99.0)	N/A	N/A	Kappa - 0.93 (0.88 – 0.98)	Cannot quantify discrepant results	N/A	
Serion ELISA classic HSV 1 + 2 IgG	Lierman et al. [36]	Serion, Würzburg, Germany	No	No	Serum	N/A	N/A Stored in aliquots at -20	93.3 (87.4 – 96.7) to 95.6 (90.2 – 98.2)	97.0 (90.8 – 99.2) to 99.0 (93.8 – 99.9)	N/A	N/A	Kappa - 0.94 (0.89 – 0.98)	Cannot quantify discrepant results	N/A	
$gG_{321580\ His}$ ELISA test	Liu et al. [37]	Hangzhou Center for Disease Control and Prevention, Hangzhou, China (academic)	No	No	Serum	N/A	Sera were frozen and stored at –70℃	93.81	96.74	N/A	N/A	N/A	N/A	Overall accuracy - 94.65 % No false identification of HSV-1.	
HSV-2 biochip test HerpeSelect® 2 ELISA	Loughman et al. [25]	Focus Diagnostics, CA, USA	Only some components of the test	Unclear	Serum	N/A		Using LIAISON® assay as reference: 100 Using ELISA assay as reference: 96.8	Using LIAISON® assay as reference: 100 Using ELISA assay as reference: 100	N/A	N/A	N/A			
Kalon Herpes Simplex Virus Type 2 IgG ELISA	Patel et al. [26]	Kalon Biological Ltd, Surrey, United Kingdom	No	No	Serum	N/A	Serum samples were consented for long term storage in the	95.6 (93.4, 97.3) (cut-off = 1.1)	93.4 (86.9 - 97.3) (cut-off= 1.1)	N/A	N/A	N/A	Of the 16 indeterminate samples by UW-WB, 10 (62.5	AUC - 0.95 (0.92 - 0.97) (cut-off = 1.1)	

							US and were stored at -80						%) were positive by Kalon. No indeterminate samples by UW-WB or Kalon had symptoms of GUD in their medical history (past 3 months) or had physical presentation of GUD.		
Uni-Gold™ HSV-2 Rapid	Shevlin and Morrow [41]	Trinity Biotech, Ireland	No	No	Serum	N/A	Frozen at -20C	94.4	98.7	94.4	98.7	98	Uni-Gold™ HSV-2 Rapid gave a negative but valid test result for 3 sera that had nonspecific banding that could not be identified definitively as being related to HSV by UWWB	One false seronegative and one false seropositive result occurred.	
HSV-sIgA by enzyme-linked immunosorbent assay (ELISA)	Shoji et al. [27]	Division of Ophthalmology, Department of Visual Sciences, Nihon	Unclear		Serum (tears)	Serum samples collected using the Schirmer I method	HSV antigens added to wells with tear samples and incubated at room temper	49.2	82.6						

		University School of Medicine				from the lower fornix	ature for 1 hour								

Cost

Gitman et al. [31] carried out the most comprehensive cost analysis: comparing the performance, time to result, and cost of the Simplexa HSV-1 and HSV-2 direct PCR with those of conventional cell culture, DFA, and a laboratory-developed real-time TaqMan PCR (LDT HSV PCR). The results of this are displayed in Table 6 (table was taken directly from the corresponding article [31]).

Table 6. Comparison of Estimated Costs per Reportable Result and Expertise Required. Table adapted from Gitman et al. [31].

Test	Assay time	Frequency of testing	Cost ($)			Expertise
			Materials and reagents	Labor	Total	
Cytospin-DFA	90 min	On demand	5.14	8.78	13.92	Mid-high
Conventional culture	1–7 days	Examined once a day	5.40	11.94	17.94	Mid-high
Simplexa direct PCR	75 min	Once a day	39.54	2.69	42.23	Mid
LDT HSV PCR with extraction	3 h	Once a day	19.96	5.07	25.03	High

Gitman et al. [31] concluded that the Simplexa HSV 1 & 2 direct PCR was the most expensive but required the least training of the assays used, had the lowest hands-on time and fastest assay time (75 min, versus 3 h by LDT HSV PCR), and provided the HSV type. The Simplexa direct PCR is an FDA approved device (Table 4), and such could be used to benchmark developing technologies.

The cost to run per specimen was also compared between the Viper platform and real-time PRC in the study by Lang et al. [34]. Compared to the HSV-LC system, the Viper instrument was found to be $12 cheaper to run per specimen ($22 versus $34). In addition, the platform is fully automated [34]. Although they did not carry out a formal cost analysis, the study by Binnicker et al. [29] compared time to run for real-time PCR assay and the commercially available Simplexa HSV-1/2 Direct (Focus Diagnostics, Cypress, USA) using CSF samples. In addition, Lee et al. [35] has reported that the automated ARIES assay takes only 2 hours/12 samples and has the fastest turnaround time compared to other in-house assays. In addition to avoiding the need for nucleic acid extraction, the Simplexa devices run time was quoted as 60 minutes in comparison to four hours by conventional methods. A separate tool, the HSVQx assay was found to produce results in a “few hours”, compared to “many days” for conventional culture techniques [40]. Tong et al. [42] also demonstrated a turnaround time of one hour using their portable device, although it was not fully automated (requires hands on time of five minutes).

Sample Type and Patient Sub-Populations

Binnicker et al. [29] found that the Simplexa HSV-1/2 assays demonstrated a combined HSV-1 and HSV-2 sensitivity and specificity of 96.2% and 97.9%, respectively. Critically, the results are available in 60 min and the test only requires 50ul of CSF [29].

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Risk of Bias in Individual Studies

Most of the studies received high risk of bias in the patient selection and in the index test domains of the QUADAS tool (Table 7; Figure 4). High risk of bias in the patient selection domain was mostly due to the lack of randomization in recruiting patients and the use of case-control design in selecting participants and/or recruited patients with "confirmed diagnosis." Only few studies included patients with suspected disease and no pretested samples [40,41] or recruited a random sample [26]. High risk of bias in the index test category was mostly due to the index tests results being interpreted with knowledge of the reference test results; most of the studies used the reference test first, and after knowing the results, performed the index test. Because all of the studies used the gold standard or an approved and validated reference tests, the reference standard mostly received low risk of bias (Figure 4). Studies that received high bias rating in the flow and timing domain, used different reference standards for different patient groups, and did not include all patients or samples in the analysis [27,36]. Studies that received low risk of bias rating in general, recruited a random sample [26], blinded study conductors to the results of the reference and index tests [26,29,36], de-identified previously collected samples [41] or conducted reference and index tests simultaneously [34]. All of the studies received low risk of bias in the applicability concerns domains of the QUADAS tool due to the reviews' inclusion criteria being inclusive of a wide range of patients, index tests and references standards (Table 7).

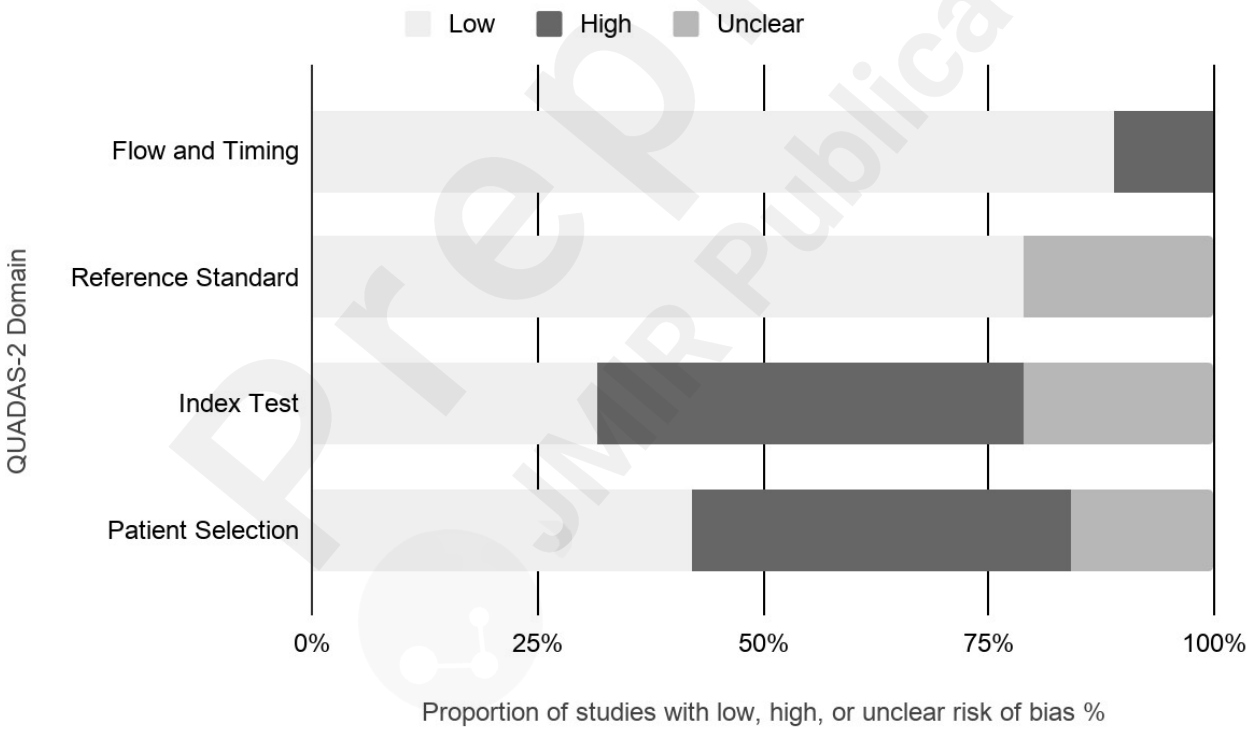


Figure 4. Graphical display for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool results.

Table 7. Tabular presentation for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool results. Table format adapted from table 2 in Whiting et al. [22].

Study	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard

Al-Shobaili et al. [24]	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Barrado et al. [28]	Low risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk
Burton et al. [30]	High risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Gitman et al. [31]	Low risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk
Granato et al. [32]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hobbs et al. [33]	High risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk
Lang et al. [34]	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Lee et al. [35]	Low risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk
Liermann et al. [36]	Unclear risk	Low risk	Low risk	High risk	Low risk	Low risk	Low risk
Liu et al. [37]	High risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Loughman et al. [25]	High risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk
Binnicker et al. [29]	High risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Miller et al. [38]	High risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk
Parra-Sánchez et al. [39]	High risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk
Patel et al. [26]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Van Der Pol et al. [40]	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk
Shevlin and Morrow [41]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shoji et al. [27]	High risk	High risk	Unclear risk	High risk	Low risk	Low risk	Low risk
Tong et al. [42]	Unclear risk	High risk	Low risk	Low risk	Low risk	Low risk	Low risk

Discussion

To select the most appropriate test for a patient a thorough knowledge of the performance and limitations of available tests is critical to inform clinical decision-making and the development of future technologies that may penetrate innovation gaps. This systematic review aimed to summarize recent study articles evaluating HSV-1 and HSV-2 diagnostic tools. We evaluated the study characteristics and the performance of the various tests included in the studies; here we conclude by discussing the various strengths and limitations of various tools, and how they may relate to the development of future diagnostics.

Performance

It is evident that further work needs to be done with regards to tools that work better with different sample types. It also appears that tools developed in academic centers perform worse. This may be because they are at an earlier stage of the developmental pathways, and thus not

nearing approval or commercialization. Overall, tests were more specific than sensitive, and it appears that serological tests perform worse.

Culture and laboratory based methods have become the gold standard choice for the diagnosis of HSV-1 and HSV-2 infection. However, translational effort is focused on developing alternate techniques based on serological and molecular assays as evidenced by the absence of novel culture-based methods in this review.

Specimen quality and adequate transport and handling to maintain infectivity are essential while using culture methods. Due to the enveloped nature of the HSV-1 and HSV-2 virus, specimens collected using a swab must be transferred to suitable viral transport media with the addition of antimicrobials. Using standard culture methods, results are usually available within five days; although in some cases the result may take up to two weeks[4]. Such issues are largely mitigated when using molecular methods. The use of an antigen detection system such as Herpchek (DuPont, Wilmington, DE) can decrease turnaround time [44]. Shell vial culture also has a decreased average turnaround time, with high sensitivity achieved within 24 h [45]. In Tong et al. [42], the IsoGlow HSV Typing assay (NAAT based technique) was compared to the ELVIS Shell Vial Assay where it displayed similar performance, while having the advantages that it was more rapid, portable, and does not require laboratory equipment. Another limitation of traditional culture methods is that viral subtyping requires the additional step of using HSV-1 and -2 monoclonal antibodies[46,47]. Similarly Lee et al. [35], found that the ARIES assay required the least amount of time when compared to two other routinely used assays and the least technical skills and knowledge [35]. The enzyme-linked virus-inducible system (ELVIS; Diagnostic Hybrids, Inc., USA) enables faster turnaround than standard culture or shell vial methods, with similar performance characteristics [45]. High sensitivities using culture based methods are hindered by highly variable specimen quality, dependency on stage of the lesion as well as primary versus recurrent infection [47].

Turnaround Time and Cost

In HSV infections, expecially when it results in CNS related infections, rapid turn-around is essential for treatment [48,49]. Rapid turnaround is important for providing the treatment to patient as early as possible. In the case of HSV, which has no cure, antivirals are the only treatment available to manage the symptoms of the disease [50]. Antivirals are recommended to be administered within the first 48-72 hours of the appearance of symptoms for best results [51], which is a very short time interval that requires a rapid turnaround time in case the provider has to wait for the diagnostic test results before prescribing the medication. Even more importantly, is the throughput time (i.e. how many tests can be ran using the system within certain amount of time) of a diagnostic test. In most cases, a confirmed diagnostic is necessary to provide antiviral treatment for HSV since prolonged use of antivirals can lead to antiviral resistance [50]. According to Lee et al. [35], the ARIES assay's throughput time was reported as 12 samples in 2 hours. This throughput time is rapid compared to 3-7 days of processing time needed for viral culture [52].

Automation increases turnaround time, improve reproducibility of the test, and reduce the chance of human error [53]. Although not quantified, capital would be saved due to the automated nature and more rapid turnaround of such devices. Automated diagnostic tests may only require minimal technical knowledge reducing the likelihood or "economically wasteful misclassification" due to limited knowledge of the lab technician [53]. Rapid turnaround time is

important for most infectious or viral diseases. For example, for respiratory viruses, diagnostic test should be provided at the point-of-care (possible with results ready in less than 1.6 hours) to ensure the best outcomes for the patient [54].

Sample Type and Patient Sub-Populations

HIV status of an individual does not appear to affect the performance of HSV ELISAs [55]. This is an important point because of the high rate of co-infection and increased risk of transmission and acquisition of HIV by HSV-positive individuals [56,57]. Among other common sexually transmitted diseases, only co-infection with Neisseria gonorrhoeae has been shown to significantly reduce HSV immunoassay performance [57]. However, the specificity of immunoassays can differ based on population demographics based on the findings of a meta-analysis conducted on studies from Africa [13].

While HSV can rarely be cultured from the CSF, molecular assays can yield results in a matter of hours, which can be important when dealing with possible HSV encephalitis or meningitis. Currently, the only device approved for use for HSV virus detection in cerebrospinal fluid is the Simplexa HSV-1/2 Direct (Focus Diagnostics, Cypress, USA) (Table 4). The latter is important for the use of the test in the neonatal population. Neonates immune system has a limited ability in fighting off infections, hence resulting in mortality rates exceeding 80% from HSV infection [58]. A PCR-based HSV detection system from CSF is critical for this patient population. Given this information, it is concerning that greatest incidence of HSV infections occurs in women of reproductive age increasing the risk of maternal transmission of the virus to the fetus or neonate [59,60]. This is a major public health concern in both developed and developing countries: a three year study in Canada found a neonatal HSV incidence of 5.9 per 100,000 live births and a case fatality rate of 15.5% [61]. Prevention is challenging given the asymptomatic nature of the disease in its latent phase [61], and current guidelines recommend delivery by cesarean section. This is because 85% of vertical transmissions occur during the vaginal delivery where the fetus comes into contact with infected genital secretions [61]. A 33-year study in the New York, USA concluded that deaths from HSV have increased over the time period (incidence 0.82 deaths per 100 000 live births), despite deaths from HIV and syphilis reducing [62]. They stated this is due to an increasing number of pregnant women having no immunity to HSV type 1 putting them at increased risk of contracting the disease during pregnancy [62]. Interestingly, neonatal HSV-1 infection in developing countries is rare with the vast majority of infections being caused by HSV-2, which carries a worse prognosis. In addition, acute infection is associated with a higher transmission rate in part due to lack of maternal to fetal antibody transfer in the third trimester [60]. A test that can accurately diagnose acute (not just latent infection) is urgently required as current methods may not detect early infection. The study by Liermann et al. [36] was the only study to investigate detection of primary infection, and concluded that its HSV IgM serology tool should not be used to make decisions for antiviral treatment in HSV [36]. Thus, no diagnostic tools included in this systematic review have addressed this unmet medical need.

The use of serologic tests to diagnose suspected genital lesions are inappropriate because positive results may be due to chronic infection, while negative results may overlook recent infection.

Risk of Bias of the Included Studies

A common source of bias in diagnostic tool studies occurs when studies recruit patients based on their disease status. This often leads to overestimation of the results and may exaggerate diagnostic accuracy [22]. All of the studies included in this review used sample populations that had a higher than average prevalence of HSV infection. As a result, these sensitivities may be overestimated.

Limitations

The studies included within this systematic review employed heterogeneous methods while also using different patient populations. For this reason a statistical comparison of results was not conducted, and instead the results displayed in a qualitative manner in order to benchmark future technology. Although a PRISMA compliant search was searching the PubMed database, it is possible that some studies may have been inadvertently missed.

Conclusions

The landscape of diagnostic tools for HSV-1 and HSV-2 infections is rapidly moving away from laboratory based and culture methods that have long been considered the gold standard technique. The majority of tools study cutaneous and mucosal HSV infections (n=13); two tests focused on ocular disease, while a single one on CNS manifestations. No diagnostic tools included in our systematic review are currently suitable for use as prenatal tools, however. The incidence of acute infections is rising, and since these infections present the greatest risk to unborn fetuses further work needs to be done to develop diagnostic tools to detect primary infection in expectant mothers to prevent vertical transfer. This will assist in lowering the rate of neonatal herpes, which can be a life threatening condition. We believe this can only be achieved through prenatal screening for primary infection and subsequent medical intervention.

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Conflicts of Interest

None declared.

Multimedia Appendix 1: Search Terms

Multimedia Appendix 2: PRISMA 2009 Checklist

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