Diagnostic accuracy of dried blood spots for serology of vaccine-preventable diseases: a systematic review

ABSTRACT

Introduction: Venous serum and plasma are optimal specimens for serological testing but may be logistically infeasible. Dried blood spots (DBS) are a feasible alternative, provided results are adequately sensitive and specific. We aimed to assess the diagnostic accuracy of DBS to measure IgG and IgM antibodies for vaccine-preventable diseases and compare test validity of DBS with venous blood.

Areas covered: In October 2020, we searched seven databases for peer-reviewed studies assessing the diagnostic accuracy of DBS specimens compared with serum in detecting antibodies to VPDs in humans. We extracted data and assessed risk of bias in all included studies. We calculated sensitivity and specificity with 95% confidence intervals for each index-reference test comparison. We narratively synthesized the identified evidence on diagnostic accuracy and blood collection and processing methods for DBS. Studies on measles and rubella IgG and IgM were the most frequently identified and reported generally high sensitivity and specificity.

Expert opinion: Lack of standardization in collection, storage, and testing methods limited systematic comparison across studies. Our findings indicate a need for additional validation studies on the diagnostic accuracy of DBS to expand their use in serological surveillance. We recommend practical considerations to improve standardized reporting for DBS validation studies.

Keywords: capillary blood, diagnostic accuracy, dried blood spots, fingerprick, serology, serosurveillance, venous blood

Article highlights:

- Serological surveys, the systematic collection of blood from a target population and testing for pathogen-specific antibodies, are potentially the best way to identify susceptible populations.
- Venous serum and plasma are regarded as the gold standard specimens for measuring IgG and IgM antibodies for vaccine-preventable diseases (VPDs), but the collection, transport, processing, and storage of venous blood samples are particularly challenging in remote and low-resource settings.
- Studies of the diagnostic accuracy of DBS have been conducted but their methods and results have been highly variable.
- We identified 28 studies that compared DBS with a reference specimen, usually serum. Most studies examined serology for measles, rubella, or dengue.
- We observed wide variation in risk of bias and applicability of included studies; for most studies, the risk of bias and applicability were unclear due to lack of reported information.
- Few studies reported whether appropriate measures were taken to ensure sample quality.
- Lack of standardization in collection, storage, and testing methods limited systematic comparison across studies. Our findings indicate a need for additional validation studies on the diagnostic accuracy of DBS to enable their expanded use in serological surveillance.

1. INTRODUCTION

Immunization programs are increasingly committed to identifying susceptible populations and tailoring vaccination strategies to reach them[1-3]. Serological surveys, the systematic collection of blood from a target population and testing for pathogen-specific antibodies, are potentially the best way to identify susceptible populations [4]. Venous serum and plasma are regarded as the gold standard specimens for measuring IgG and IgM antibodies for vaccine-preventable diseases (VPDs), but the collection, transport, processing, and storage of venous blood samples are particularly challenging in remote and low-resource settings [5-8]. These low-resource settings with the greatest challenges in venous blood collection are also the settings where less is known about susceptibility and burden of disease [9]. Dried blood spots (DBS) are a feasible alternative to venous blood in these settings provided that the tradeoff between feasibility and diagnostic accuracy is acceptable for the goals of the analysis. Fingerprick, earlobe, or heelprick capillary blood samples can be easily collected by minimally trained staff and dried on filter paper at room temperature, eliminating the need for centrifugation, processing within 48 hours, and strict temperature and storage requirements that are needed for venous blood [5-8]. However, to improve feasibility and convenience, alternative specimens like DBS may sacrifice sensitivity and/or specificity.

Studies of the diagnostic accuracy of DBS have been conducted but their methods and results have been highly variable [5-8]. In 2014, Smit et al. systematically reviewed studies of DBS use for diagnosis of tropical diseases and found that the diagnostic accuracy of DBS was similar to that of gold standard samples [9]. However, the review excluded multiple VPDs and did not specifically focus on IgG and IgM antibodies. DBS are frequently used for serosurveillance, but only a limited number of peer-reviewed studies have validated DBS.

We conducted a systematic review of the accuracy of DBS in serological testing of VPDs in humans. Specifically, we assessed the validity of DBS specimens in measuring the presence and concentration of IgG and IgM antibodies.

2. METHODS

2.1 Overview

We conducted a large systematic review of the accuracy and reliability of oral fluid and capillary blood specimens in measuring antibodies to all World Health Organization (WHO) pre-qualified VPDs as of October 2020. This analysis focuses on the use of DBS in measuring the presence and concentration of IgM and IgG antibodies to VPDs. The overall methods are described below and conform with Cochrane methods for conducting systematic reviews of diagnostic test accuracy studies [10]. The review is reported in accordance with the recent Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement for diagnostic test accuracy studies (PRISMA-DTA) (Appendix Table A1 includes a completed checklist) [11,12]. The review protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD #42018094855) [13].

2.2 Eligibility Criteria

We included studies that assessed the diagnostic accuracy of DBS specimens compared with venous blood in detecting antibodies to VPDs in humans. Only VPDs with a vaccine pre-qualified by the WHO were eligible and included: cholera, dengue, diphtheria, *Haemophilus influenzae* type b (Hib), hepatitis A, hepatitis B, herpes zoster virus (shingles), human papilloma virus (HPV), influenza, Japanese encephalitis, measles (rubeola), meningococcal disease (*Neisseria meningitidis*) mumps, pertussis (*Bordetella pertussis*, whooping cough), pneumococcus (*Streptococcus pneumoniae*), polio (poliomyelitis), rabies, rotavirus, rubella (German measles), tetanus, tick-borne encephalitis, tuberculosis (*Mycobacterium tuberculosis*), typhoid fever (*Salmonella typhi*), varicella, and yellow fever. We only included studies that explicitly reported measurement of IgM and/or IgG antibodies.

2.3 Search Strategy

MG, an informationist at the Johns Hopkins Welch Medical Library, iteratively developed and conducted the search after input from the rest of the team. The search was created in Medline (accessed via PubMed) (Appendix – Table A2) and adapted for the following databases: Embase, Biosis, PASCAL Biomed, Ovid's Global Health, Global Index Medicus, IndMed, CINAHL, and Web of Science Core Collection. We used a combination of controlled vocabulary and keyword terms for the concepts of (1) DBS and (2) VPDs. We did not limit our search by publication date. We searched for articles published in English. The search was current as of October 31, 2020.

2.4 Screening

Citations identified in the search were downloaded and duplicates were removed before being uploaded into Covidence (Melbourne, Australia) [14]. Two investigators independently screened each abstract. All potentially relevant abstracts were then screened as full texts independently. Each screening discrepancy was resolved through discussion between the two investigators or through arbitration by a third investigator.

2.5 Data Extraction

A data extraction form was developed in Covidence and pilot tested with five eligible full-text articles. Two investigators then independently extracted the data for each study, resolving discrepancies through discussion or arbitration by a third investigator.

From each study, we extracted information regarding capillary or venous blood collection and processing methods for DBS. When the same study reported data for multiple index tests, populations, thresholds, or time-frames, these results were extracted and analyzed separately.

2.6 Risk of Bias Assessment

After an initial pilot test with five included studies, two investigators assessed the risk of bias in each included study independently using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool [15,16]. Discrepancies were resolved through discussion. We categorized studies as being of high methodological quality if there was "low risk of bias" and "low concern" in all QUADAS-2 domains. This tool comprises four domains related to risk of bias (patient selection, index test, reference standard, and flow and timing) and three related to applicability (patient selection, index test, and reference test).

2.7 Statistical Analysis

Our primary diagnostic accuracy measures of interest were sensitivity and specificity. Each index test was compared with venous blood. For studies that reported sensitivity and specificity of DBS compared with venous blood, we extracted the sensitivity and specificity metrics directly. For studies that did not report these metrics, we extracted data to generate the four cell values of a diagnostic 2×2 table. We then calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), with 95% confidence intervals for each index-reference test comparison. When needed, we emailed the study authors to either obtain the necessary 2×2 table cell values or to confirm our calculated estimates of the metrics. We conducted all analyses using Stata version 14 (College Station, Texas) and RevMan 5.3 (Copenhagen, Denmark).

3. RESULTS

3.1 Identification of Studies

Our search yielded 8,396 unique records (Figure 1). We included a total of 28 studies that compared DBS with a reference specimen for measuring IgG or IgM pathogen-specific antibodies. Most studies (70%)

used capillary DBS as the index specimen and the remaining 30% used venous DBS, typically analyzed as a secondary specimen when venous blood was collected.

3.2 Study Characteristics

We identified 12 studies examining serology for measles, 10 for rubella, eight for dengue, three for HPV, three for cholera, and one each for mumps, hepatitis A, and hepatitis B (Tables 1-3). Some studies tested for multiple antigens. We did not identify any studies examining diagnostic accuracy of DBS for meningococcal disease, pneumococcal disease, pertussis, polio, rotavirus, or varicella. Nineteen studies (68%) were designed to assess diagnostic accuracy. Other study designs focused on surveillance, outbreak investigations, or programmatic impact. All included studies used the same assay for both index and reference test specimens.

Among the 12 studies evaluating measles serology, four studies examined IgM and eight examined IgG (Table 1). Sample sizes ranged from 5 to 1153 (median 119), with most studies conducted in Africa or Europe. The included measles studies used DBS samples from populations of all ages but primarily from children and adolescents. The included measles studies mostly used indirect enzyme-linked immunosorbent assay (EIA; primarily Enzygnost Marburg, Germany) for both index and reference test specimens. None of the included measles studies used plaque reduction neutralization (PRN) assays as the reference test.

Among the 10 studies of rubella serology, two assayed IgM and eight IgG antibodies (Table 2). Sample sizes ranged from 47 to 590 (median 180), with most conducted in the United States or Europe among populations of all ages. The included rubella studies used indirect EIA, primarily Enzygnost (Marburg, Germany).

Among the nine studies on dengue, three assayed IgM and three IgG antibodies (Table 3). Sample sizes ranged from 43 to 288 (median 104), with most conducted in Latin America and Asia among populations of all ages. The included dengue studies used capture EIA.

All three studies on HPV assayed IgG antibodies (Table 3). Sample sizes for these studies included 46 vaccinated and 103 unvaccinated people, 96 people, and 985 people, with studies conducted in the UK or Mongolia among healthy adults. The included HPV studies used multiplex assays as well as indirect or capture EIA.

Among the three studies on cholera, one assayed IgM, one IgG, and one both (Table 3). Sample sizes for these studies included 15 people, 15 people, and 34 people, with studies conducted in South Sudan or Bangladesh among adult populations. The cholera studies used indirect EIA.

The three studies evaluating other antigens included those for mumps, hepatitis A, and hepatitis B (Table 3). The studies on mumps and hepatitis A assayed IgG antibodies, whereas the study on hepatitis B assayed both IgM and IgG antibodies. Sample sizes included 292 people for mumps, 65 for hepatitis A, and 389 for hepatitis B, with the studies conducted in India or Europe primarily among healthy populations.

Studies focused on HPV, cholera, hepatitis A, and hepatitis B measured and reported only average antibody concentrations, so sensitivity and specificity values were unavailable or could not be calculated.

Among all studies in this review, 19% included fewer than 20 participants, while 63% included fewer than 100 participants.

3.3 Quality of Reporting in Studies

We observed wide variation in reporting quality (Tables 1–3). We were able to retrieve or calculate sensitivity and specificity values for only 46% of studies (n=13). Studies focused on measles, rubella, and dengue typically either reported 2×2 tables or provided data that enabled us to generate 2×2 tables.

Only 55% of studies using capillary blood and 57% of studies using venous blood reported the diameter of the DBS. Only 50% of studies using capillary blood and 41% of studies using venous blood reported whether the specimen volume was considered when determining volume of buffer added (Table 4).

Of the 13 DBS studies that reported dilution procedures, few sufficiently and explicitly reported the complete elution and dilution protocols. As an example of clear reporting of assumptions, De Swart et al. (2001) reported their assumption that DBS containing 25 µl of blood contains 10 µl of serum, so they treated each sample as a 1:50 dilution of the serum [17].

While most studies reported storage temperature, the duration of storage, use of desiccants, and humidity conditions were not consistently documented. One major concern surrounding the use of DBS on filter paper is dryness and subsequent degradation. Twenty-two studies reported the type of filter paper (Table 4), with seven using Whatman 903 papers. Only nine studies described the amount of time the filter paper was dried. Only eight studies reported whether desiccant packets were used, and few discussed the protocol for keeping the DBS dry. Eleven studies reported whether the DBS were kept in separate packaging, such as plastic bags, to protect the specimens and prevent exposure to moisture.

3.4 Risk of Bias and Applicability

We observed wide variation in risk of bias and applicability of included studies (Appendix, Figure A1). For most studies, the risk of bias and applicability concerns were unclear due to lack of reported information preventing us from making an assessment. Twenty-one studies (75%) had at least one domain presenting low risk of bias, while 18 studies (64%) had at least one domain presenting high risk of bias.

3.5 Diagnostic Accuracy

Of the nine studies on measles IgG serology, seven reported diagnostic accuracy and six documented both sensitivity and specificity values greater than 90% for DBS (Table 1). The seventh study was a small study in Italy with high sensitivity (96%) but 75% specificity with wide confidence intervals. Studies on measles IgG diagnostic accuracy represented all ages, were conducted across multiple regions, and included healthy populations, recently vaccinated children, and populations with suspected or recent measles infections. Measles vaccine use and time since measles vaccination varied by study and were not

consistently reported. Of the four studies on measles IgM serology, three published diagnostic accuracy estimates, all of which reported sensitivity and specificity of DBS greater than 90% (Table 1). Correlation coefficients, which quantify the strength of the relationship of quantitative antibody results, were high for measles IgG and IgM, when reported.

Of the eight studies on rubella IgG serology, four reported diagnostic accuracy estimates, with sensitivity and specificity of DBS 98% to 100%, with high measures of correlation (Table 2). Of the two studies on rubella IgM serology, sensitivity and specificity estimates for DBS were between 97% and 100%, with high measures of correlation. For other antigens, sensitivity and specificity values for DBS were above 80%, but the number and comparability of studies were limited (Table 3). Diagnostic accuracy was not reported for antigens without a known correlate of protection or accepted cutoff for the assay such as cholera and HPV. Measures of correlation, kappa, or the area under the receiver operating characteristic (ROC) curve were reported for studies of dengue, HPV, cholera, and hepatitis A serology as measures of agreement between DBS and the reference specimen; these measures showed moderate correlation between DBS and the reference specimen.

4. DISCUSSION

Serological surveillance is increasingly used to monitor susceptibility to VPDs at the population level. This systematic review demonstrates variable evidence on diagnostic accuracy using DBS to measure antibodies against VPDs. Differences in assays used and study population characteristics, such as age groups, hindered comparability across studies. DBS remains the most logistically feasible specimen in low-resource settings, but questions remain about its diagnostic accuracy and how best to standardize its use. Our systematic review indicates that studies of the diagnostic accuracy of measles IgG, measles IgM, and rubella IgG were most common. Of the seven studies reporting diagnostic accuracy of measles IgG, the sensitivity and specificity of DBS was greater than 90% in six. All three studies reporting the diagnostic accuracy for measles IgM reported sensitivity and specificity greater than 90%. For rubella IgG (eight

studies) and IgM (two studies), both sensitivity and specificity were at least 97% and 98%, respectively. These results indicate that DBS may be a feasible alternative to venous blood in some settings, but there remains an insufficient number of studies validating the diagnostic accuracy.

DBS are widely used as index specimens in low-resource settings, despite limited peer-reviewed evidence of their diagnostic accuracy. The use of DBS overcomes specimen collection challenges in the field by shifting the complexity of sample processing to the lab. The included studies generally showed good performance of DBS compared with venous blood in the detection of IgM and IgG antibodies for different VPDs. There is more evidence on diagnostic accuracy for DBS for measles and rubella than other antigens. We did not identify studies addressing diagnostic accuracy of DBS for meningococcal disease, pneumococcal disease, pertussis, polio, rotavirus, or varicella; however, DBS are currently used to measure antibodies against these pathogens [18-24]. We also observed that few studies reported diagnostic accuracy data for antigens other than measles and rubella; this could be due to challenges in identifying correlates of protection that can be easily quantified [25].

The collection of DBS on filter paper is considered a convenient and affordable alternative to serological surveillance with venous blood [9,26]. However, variations in DBS collection methods, filter paper used, and storage conditions could affect antibody recovery. Several included studies failed to report the type of filter paper used in sample collection, limiting our ability to make comparisons among studies. Additionally, there is a difference between using DBS for clinical diagnosis and for serosurveillance research; for clinical diagnosis, the use of DBS would require extensive validation studies and published guidelines from authorities like the World Health Organization. For research studies examining seroprevalence, this validation of DBS can be conducted internally as part of the study. For the purposes of this systematic review, we focused on the latter case.

While DBS samples do not have the same processing, centrifugation, and storage requirements as serum samples, specific storage and temperature conditions are needed to ensure diagnostic accuracy of DBS. High humidity conditions, particularly prevalent in tropical environments, have been shown to reduce

recovery of antibodies from DBS [27]. Exposure to moisture can result in the degradation of antibodies, fungal contamination, and subsequent loss of diagnostic accuracy compared with serum. The temperature and duration of storage can also contribute to antibody degradation. A study in India found that measles and rubella IgG concentration in DBS were stable compared with sera for at least 90 days when stored at 4˚C and for as long as 30 days at ambient temperature [28].

In the absence of large validation studies for DBS, it is crucial for individual studies to validate DBS compared with the reference specimen. To ensure comparability between DBS and the reference standard, researchers should either optimize the DBS assay [29] or use a correction factor to adjust the results post hoc [30]. Most studies included in this review reported optimizing the assay.

We suggest that study teams implement a checklist of practical items (Figure 2) concerning the collection, storage, transportation, and processing of DBS specimens to improve diagnostic accuracy. The most recently published reporting guidelines, Standards for Reporting of Diagnostic Accuracy (STARD) [31], should be followed when reporting diagnostic accuracy studies; our practical recommendations are in consonance with the STARD checklist. Authors should document details on collection, storage, and processing of samples as described in Smit et al. 2014 [9], wherever feasible.

4.1 Limitations

This systematic review highlights the lack of standardization in the testing and reporting of diagnostic accuracy of DBS samples for serology of VPDs, which made it difficult to compare results across studies. Many studies, particularly in low- and middle-income settings, had small sample sizes. Several studies only included serologically positive participants, likely due to the study population, preventing researchers from evaluating specificity. As such, the study population for many included studies may not be representative of the target population. This may also indicate substantial reporting bias in these studies if equivocal results were excluded. There is also a risk of publication bias if diagnostic accuracy and

validation studies were conducted but not published. Lastly, we limited our search to English-language papers.

5. CONCLUSION

The findings of this systematic review begin to pinpoint the diagnostic accuracy of DBS samples, particularly for measles and rubella, but also indicate a need for additional validation studies on the diagnostic accuracy of DBS to enable their expanded use in serological surveillance. In addition to better validating DBS specimens, investigators should more consistently report DBS results to improve our understanding of the diagnostic accuracy. DBS specimens are logistically feasible and highly promising for serosurveillance, but substantial improvements in terms of validation remain to be made.

6. EXPERT OPINION

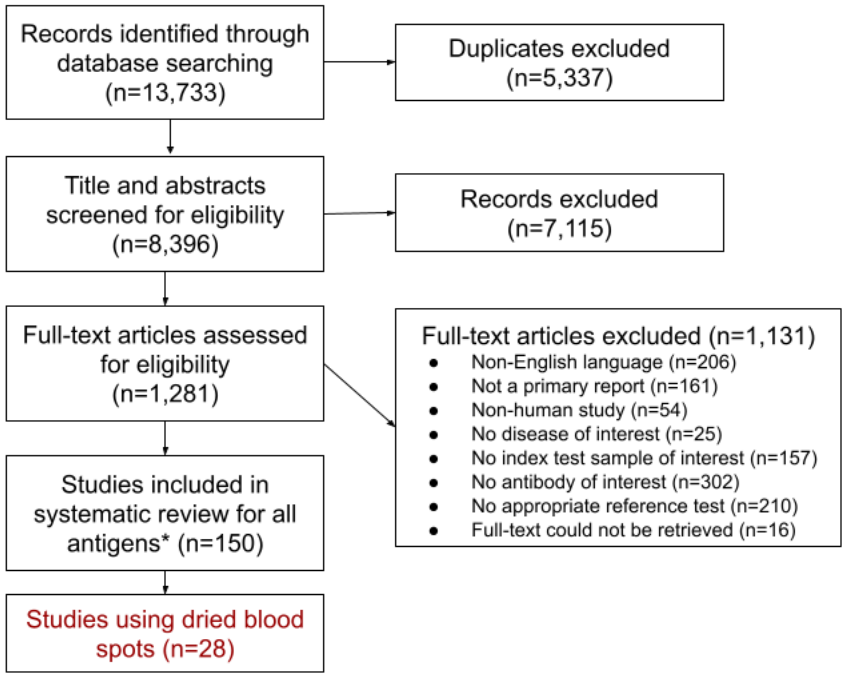
Venous serum and plasma are the gold-standard specimens for serological testing but often may not be feasible or convenient due to well-documented challenges with the collection, transport, processing, and storage of venous blood. DBS remain the most logistically feasible alternative specimen in the field in low-resource settings, but questions remain about their diagnostic accuracy and how best to standardize their use. DBS are already widely used as index specimens in low-resource settings, despite limited evidence of diagnostic accuracy in the peer-reviewed literature. However, regardless of the widespread usage of DBS in serosurveillance for the diagnosis of VPDs, our findings indicate an urgent need for additional validation studies of the diagnostic accuracy of DBS to enable their expanded use in serological surveillance. Our systematic review identifies only 28 studies that examined the diagnostic accuracy of IgG or IgM for VPDs. This review demonstrates that studies of the diagnostic accuracy of measles IgG, measles IgM, and rubella IgG generally demonstrate sensitivity and specificity higher than 90%, suggesting that DBS may be a feasible alternative to venous blood in some settings. The already widespread use of DBS indicates the operational and technical feasibility of this specimen in clinical practice, but additional validation is needed to assess the diagnostic accuracy of DBS compared with venous blood. Evidence is particularly lacking for VPDs other than measles, rubella, and dengue, suggesting that there is room for improvement for less-studied VPDs that continue to contribute to the burden of disease in many settings.

This review highlights the lack of standardization in the testing and reporting of diagnostic accuracy of DBS samples for serology of vaccine-preventable diseases, as very few studies reported whether appropriate measures were taken to ensure sample quality. In addition to the need for more published validation studies, key areas for improvement in studies validating the diagnostic accuracy of DBS include the need for larger sample sizes, the inclusion of both serologically positive and negative participants to better represent the target population, the reporting of sample storage and processing procedures to ensure replicability, and the reporting of negative or equivocal results. Along with DBS, the use of saliva and oral fluid as alternative specimens also present important opportunities for reducing inequities in

serosurveillance. The advancement of the serosurveillance field may be limited by the fact that DBS are most widely utilized in low-resource settings that may lack the capacity to conduct high-quality validation studies—but these same settings are often those most in need of stronger surveillance programs for infectious disease.

A significant amount of surveillance research thus far has been conducted using DBS, likely due to the logistical feasibility of storage, transport, and processing compared to serum specimens. Improved data on the diagnostic accuracy of DBS for VPD diagnosis has the potential to drastically widen the serosurveillance field, improve opportunities for serosurveillance in hard-to-reach or especially vulnerable communities, and narrow gaps in surveillance between low- and high-resource settings. Future research will require investigators to more consistently report DBS results and better validate DBS usage in order to improve our understanding of the diagnostic accuracy of these specimens. DBS specimens are logistically feasible and highly promising for serological surveillance, but substantial improvements still need to be made in terms of validation before the serosurveillance field can evolve further.

Figure 1: PRISMA diagram showing study selection process for studies of dried blood spots



* We undertook a systematic review to determine the accuracy and reliability of both oral fluid and capillary blood specimens in measuring antibodies to licensed VPDs. The analysis reported in this article is restricted to the use of dried blood spots in measuring the presence and concentration of IgM and IgG antibodies to VPDs in humans.

Table 1: Summary of studies evaluating dried blood spots for detection of antibodies to measles virus

Study	Country	Sample size	Study population	Index and reference assay	Sensitivity % (95% CI)	Specificity % (95% CI)	Other indicators of test performance (95% CI, p-value)	Notes
Antibody detected: Measles-specific IgM								
Uzicanin 2011[32]	Uganda	513	8m–12y hospitalized, mostly suspected infection	Indirect EIA (Enzygnost)	99 (98, 100)	94 (80, 99)	κ = 0.81 (0.72, 0.92)	High incidence setting
Helfand 2001[33]	US	119	All ages, healthy population	Indirect EIA (in-house)	100 (94, 100)	100 (94, 100)	R^2 = 0.99	–
DeSwart 2001[17]	Sudan	117	Infants, suspected infection	Capture EIA (in-house)	91 (84, 96)	100 (84, 100)	–	No effect of 5-month storage on results
Van Binnendijk 2003[34]	Netherlands	5	Children with suspected infection and healthy contacts	Indirect EIA (in-house)	NE	NE	–	No 2×2 values reported
Antibody detected: Measles-specific IgG								
Riddell 2003[35]	Australia	1153	School age, both suspected infection and healthy population	Indirect EIA (Enzygnost)	96 (94, 98)	92 (74, 99)	–	DBS highly correlated with serum. No effect of 6-month storage on results
Hayford 2019[30]	Zambia	590	9m–15y, healthy population	Indirect EIA (Enzygnost)	NE	NE	–	No 2×2 values reported
Cilleruelo 2019[36]	Spain	292	Mothers >18y, infants, healthy population	Indirect EIA (Enzygnost)	NE	NE	–	No 2×2 values reported. High loss to follow-up
Chakravarti 2003[37]	India	165	1–2y, healthy population	Indirect EIA (Melotec)	100 (97, 100)	90 (73, 98)	R^2 = 0.93 (p=0.001)	–
Helfand 2001[33]	US	117	All ages, healthy population	Indirect EIA (in-house)	96 (91, 99)	100 (16, 100)	R^2 = 0.77	–
Colson 2015[29]	Mexico	50	8–22m, healthy population	Indirect EIA (Human Diagnostics Worldwide)	100 (77, 100)	97 (83, 100)	R^2 = 0.92 (0.84, 1.0)	–
Wassilak 1982[38]	US	44	Children, suspected infection	Hemagglutination inhibition	100 (79, 100)	96 (82, 100)	–	DBS highly correlated with serum
Novello 1996[39]	Italy	27	2–12y, healthy population	Indirect EIA (Enzygnost)	96 (78, 100)	75 (19, 99)	–	–

NR, not reported; NE, not estimable; CI, confidence interval; IgG, immunoglobulin G; IgM, immunoglobulin M; EIA, enzyme-linked immunosorbent assay; R^2, coefficient of determination; κ, Kappa.

Table 2: Summary of studies evaluating dried blood spots for detection of antibodies to rubella virus

Study	Country	Sample size	Study population	Index and reference assay	Sensitivity % (95% CI)	Specificity % (95% CI)	Other indicators of test performance (95% CI, p-value)	Notes
Antibody detected: Rubella-specific IgM								
Helfand 2007[40]	Peru	273	> 8m, suspected infection	Indirect EIA (Enzygnost)	97 (93, 99)	98 (90, 100)	R^2 = 0.91	–
Helfand 2001[33]	US	87	All ages, healthy population	Capture EIA (in-house)	100 (82, 100)	100 (94, 100)	R^2 = 0.92	–
Antibody detected: Rubella-specific IgG								
Hayford 2019[30]	Zambia	590	9m–15y, healthy population	Indirect EIA (Enzygnost)	NE	NE	–	No 2×2 values reported
Vicente 2016[41]	Sao Tome and Principe	316	2–35y, healthy population	Indirect EIA (Serion)	NE	NE	–	No 2×2 values reported. High incidence setting
Cilleruelo 2019[36]	Spain	292	Mothers >18y, infants, healthy population	Indirect EIA (Enzygnost)	NE	NE	–	No 2×2 values reported. High loss to follow-up
Helfand 2007[40]	Peru	273	> 8m, suspected infection	Indirect EIA (Enzygnost)	98 (93, 100)	99 (95, 100)	R^2 = 0.94	–
Helfand 2001[33]	US	84	All ages, healthy population	Capture EIA (in-house)	100 (94, 100)	100 (77, 100)	R^2 = 0.94	–
Hardelid 2008a[42]	UK	79	Ages NR, healthy population	Indirect EIA (Enzygnost)	100 (95, 100)	NE	R^2 = 0.93	Specificity not estimable because no true negatives reported
Hardelid 2008b[42]	UK	73	Ages NR, healthy population	Indirect EIA (Diesse)	100 (91, 100)	100 (82, 100)	R^2 = 0.93	–
Vejtorp 1981[43]	Denmark	47	> 18y, healthy population	Capture EIA (in-house)	NE	NE	R^2 = 0.96	No 2×2 values reported. DBS highly correlated with serum

NR, not reported; NE, not estimable; CI, confidence interval; IgG, immunoglobulin G; IgM, immunoglobulin M; EIA, enzyme-linked immunosorbent assay; R^2, coefficient of determination.

Table 3: Summary of studies evaluating dried blood spots for detection of antibodies to mumps, dengue, HPV, cholera, and hepatitis B

Study	Country	Sample size	Study population	Antibody detected	Index and reference assay	Sensitivity % (95% CI)	Specificity % (95% CI)	Other indicators of test performance (95% CI, p-value)	Notes
Disease of interest: Mumps									
Cilleruelo 2019[36]	Spain	292	Mothers >18y, infants, healthy population	IgG	Indirect EIA (Enzygnost)	NE	NE	–	No 2×2 values reported. High loss to follow-up. IgG detection not synonymous with protection due to reinfection risk
Disease of interest: Dengue									
Balmaseda 2008b[44]	Nicaragua	276	8m–12y, suspected infection	IgM	Capture EIA (in-house)	59 (44, 73)	81 (75, 86)	AUC = 0.911	Risk of cross-reactivity with other flaviviruses. Other markers (IgA, IgG) also measured
Matheus 2007[45]	French Guiana	130	Age NR, suspected infection	IgM	Capture MACELISA (in-house)	82 (71, 90)	91 (80, 97)	–	–
Tran 2006a[46]	Vietnam	110	Age NR, suspected infection	IgM	Capture EIA (Focus Diagnostics)	NE	NE	$\kappa = 0.191$	No 2×2 values reported. DBS correlated poorly with serum. Substantial inter-laboratory variation. Risk of cross-reactivity with other flaviviruses
Chakravarti 2013[47]	India	104	2–60y, suspected infection	IgM	Capture EIA (in-house)	97 (89, 100)	90 (74, 98)	$\kappa = 0.89$	Dengue NS1 antigen can detect infection earlier than IgM
Matheus 2008[48]	Paraguay	44	Age NR, suspected infection	IgM	Capture MACELISA (in-house)	NE	NE	–	No 2×2 values reported for IgM. Small sample size
Anders 2012a[49]	Cambodia	44 (enrollment); 43 (discharge)	6–39y, suspected infection	IgM	Capture MACELISA (in-house)	100 (3, 100); 100 (89, 100)	100 (92, 100); 75 (35, 97)	–	Venous blood samples spotted on filter paper. Substantial inter-site variation
Balmaseda 2008a[44]	Nicaragua	288	8m–12y, suspected infection	IgG	Capture EIA (in-house)	86 (73, 94)	92 (88, 96)	AUC = 0.911	Risk of cross-reactivity with other flaviviruses. Other markers (IgA, IgG) also measured
Tran 2006b[46]	Vietnam	110	Age NR, suspected infection	IgG	Capture EIA (Focus Diagnostics)	NE	NE	–	–
Anders 2012b[49]	Cambodia	44 (enrollment); 43 (discharge)	6–39y, suspected infection	IgG	Capture GACELISA (in-house)	100 (3, 100); 96 (80, 100)	100 (92, 100); 93 (68, 100)	–	–

Disease of interest: Human papillomavirus									
Waterboer 2011[50]	Mongolia	985	16–63y, healthy population	IgG	Multiplex	82 (76-87)	95 (93-96)	R^2 = 0.85, κ = 0.77 (0.72, 0.81)	Venous blood samples spotted on filter paper. Extrapolated predefined serum cutoff values to DBS
Bhatia 2019[51]	UK	96	21–59y healthy population	IgG	Indirect EIA (in-house)	NE	NE	Avidity Index 35% (95% CI 25%–45%)	No 2×2 values reported. Used WHO international standard serum to calibrate assay for DBS
Louie 2018[52]	UK	46 (vaccinated); 103 (unvaccinated)	20–28y, healthy population	IgG	Capture EIA (in-house)	NE; NE	NE; NE	AUC = 0.958	No 2×2 values reported. Self-reported vaccination status
Disease of interest: Cholera									
Bhuiyan 2019[53]	Bangladesh	34	18–59y, suspected infection	IgG; IgM	Indirect EIA (in-house)	NE; NE	NE; NE	ICC IgG = 0.7, IgM = 0.7	Adapted vibriocidal assay procedure to ELISA using DBS eluates
Iyer 2016a[54]	South Sudan	15	Ages NR, healthy population	IgM	Indirect EIA (in-house)	NE; NE	NE; NE	AUC = 0.68 (p<0.001)	Adapted vibriocidal assay procedure to ELISA using DBS eluates
Iyer 2016b[54]	South Sudan	15	Ages NR, healthy population	IgG	Indirect EIA (in-house)	NE; NE	NE; NE	AUC = 0.64 (p<0.001)	–
Disease of interest: Hepatitis A									
Chitambar 2000[55]	India	49 (Filter paper stored for 10 days); 16 (Filter paper stored for 2 months)	Ages NR, healthy population	IgG	Capture EIA (in-house)	NE; NE	NE; NE	AUC = 0.49 (p<0.001)	No 2×2 values reported. No effect of 2-month storage on results
Disease of interest: Hepatitis B									
Van Loo 2015[56]	Netherlands	389	Ages NR, suspected infection	Anti-HBcore IgM and IgG combined	Direct EIA (Roche)	NE; NE	NE; NE	–	No 2×2 values reported for anti-HBcore IgM and IgG. 90% sensitivity, 99% specificity reported for HBsAg with DBS. Low quality for 18% of samples

NR, not reported; NE, not estimable; CI, confidence interval; IgG, immunoglobulin G; IgM, immunoglobulin M; EIA, enzyme-linked immunosorbent assay; MACELISA, Immunoglobulin M antibody capture enzyme-linked immunosorbent assay; GACELISA, Immunoglobulin G antibody capture enzyme-linked immunosorbent assay. R^2, coefficient of determination; κ, Kappa; AUC, area under curve (receiver operating characteristic); ICC, intra-class correlation coefficient.

Table 4: Summary of studies reporting practical aspects in evaluating the use of DBS

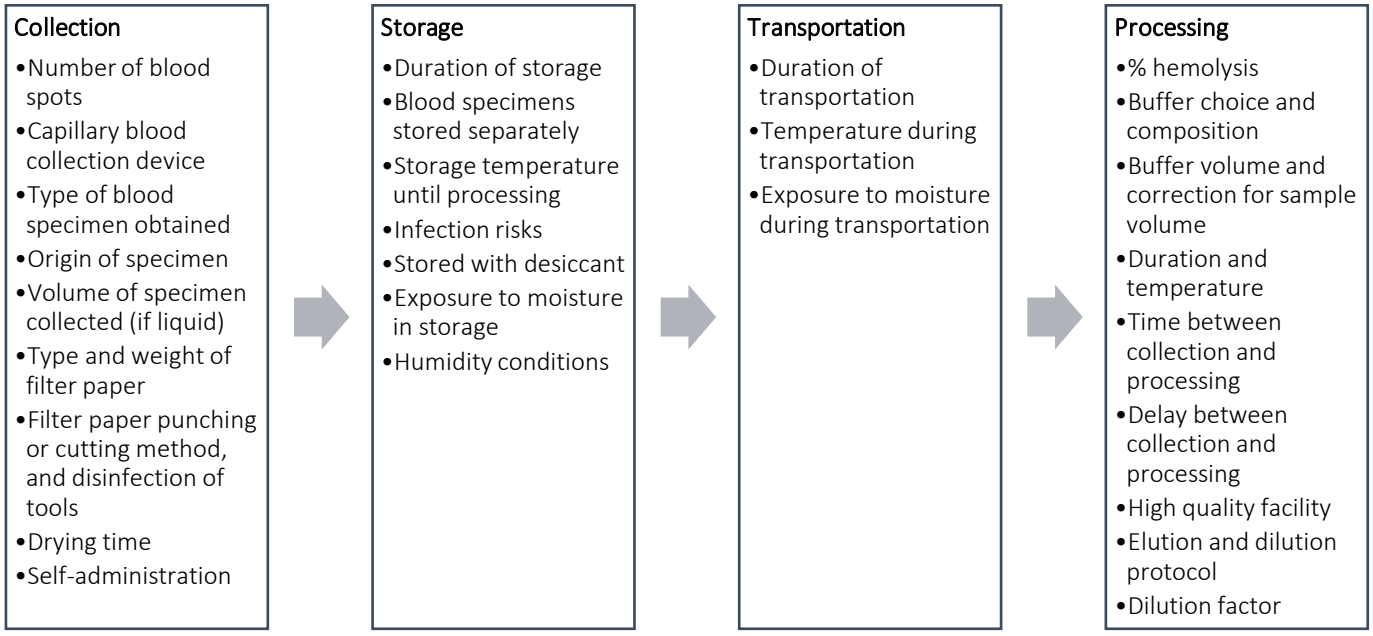
Study	Antigen(s) evaluated	Source of specimen	Origin of specimen	Type of blood spotted	Filter paper	Diameter of DBS	Paper punched or cut	Drying time	Stored with desiccant	Duration of storage	Storage temperature
Anders 2012[49]	Dengue	Venous	–	Serum	Whatman No. 3	NR	NR	Overnight	Yes	NR	4˚C
Balmaseda 2008[18]	Dengue	Venous	–	Serum	Whatman No. 3	NR	Cut	2h	No	12m	4˚C
Bhatia 2019[24]	HPV	Venous	–	Serum	Guthrie	NR	NR	24h	No	NR	4˚C
Bhuiyan 2019[26]	Cholera	Venous	–	Serum	Whatman 903	NR	Punched	2h	Yes	1-6m	-80˚C
Chakravarti 2003[7]	Measles	Capillary	Finger or heel	Whole blood	Whatman No. 3	20mm	NR	Overnight	Yes	NR	-2˚C
Chakravarti 2013[47]	Dengue	Capillary	Finger	Whole blood	Whatman No. 3	15mm	NR	NR	Yes	NR	4˚C
Chitambar 2000[55]	Hepatitis A	Capillary	Finger	Whole blood	Whatman No. 1	12mm	NR	NR	No	10d	RT
Cilleruelo 2019[10]	Measles, rubella, mumps	Capillary	Heel	Serum	Whatman 903	3mm	Cut	NR	No	NR	NR
Colson 2015[9]	Measles	Capillary	Finger or heel	Whole blood	Whatman 903	6mm	Punched	1h	Yes	15-20d	10-12˚C
DeSwart 2001[1]	Measles	Venous	–	Whole blood	Whatman No. 3	10mm	Cut	NR	No	1-2y	-70˚C
Hardelid 2008[14]	Rubella	Venous	–	Serum	Whatman 903	5mm	Punched	Overnight	No	NR	-20˚C
Hayford 2019[11]	Measles, rubella	Capillary	Finger	Whole blood	Whatman 903	NR	Cut	8h	Yes	NR	-20˚C
Helfand 2001[2]	Measles, rubella	Capillary	Finger	Serum	Whatman 903	0.25in	Punched	Overnight	Yes	NR	-20˚C
Helfand 2007[12]	Rubella	Capillary	Finger	Serum	Whatman 903	NR	NR	4h	Yes	NR	-20˚C
Iyer 2016a[25]	Cholera	Capillary	Finger	Serum	Whatman 903	6mm	Punched	NR	Yes	9m	-20˚C
Louie 2018[23]	HPV	Capillary	Finger	Whole blood	Whatman 903	12mm	Punched	4-24h	No	NR	-80˚C
Matheus 2007[17]	Dengue	Capillary	Finger	Whole blood	Whatman 903	NR	Cut	NR	No	NR	RT
Matheus 2008[19]	Dengue	Capillary	Finger	Whole blood	Whatman 903	NR	Cut	NR	No	NR	-80˚C
Novello 1996[6]	Measles	Capillary	Finger	Whole blood	Serono 60011	5mm	Punched	NR	No	NR	-20˚C
Riddell 2003[8]	Measles	Venous	–	Whole blood	Whatman 903	13mm	NR	NR	No	6m	4˚C

Tran 2006a[16]	Dengue	Venous	–	Serum	Whatman 903	6mm	Punched	NR	Yes	NR	-20˚C
Uzicanin 2011[4]	Measles	Capillary	Finger or heel	Whole blood	Whatman 903	13mm	NR	4h	Yes	3-15m	-20˚C
Van Binnendijk 2003[3 *]	Measles	Capillary	Finger	Whole blood	NR	NR	NR	NR	NR	NR	NR
Van Loo 2015[28]	Hepatitis B	Venous	–	Serum	NR	NR	NR	NR	NR	NR	NR
Vejtorp 1981[13]	Rubella	Capillary	Earlobe	Whole blood	Frisenette AGF 818	13mm	Punched	NR	No	NR	-20˚C
Vicente 2016[15]	Rubella	Capillary	NR	Serum	Guthrie	NR	NR	NR	NR	NR	NR
Wassilak 1982[5]	Measles	Capillary	Finger or heel	Whole blood	Ropaco No. 1023	12mm	Cut	NR	No	6w	RT or 4-8˚C
Waterboer 2011[22]	HPV	Venous	–	Whole blood	Whatman 903	2.85mm	Punched	NR	No	1m	-20˚C

* Indicates specimens were processed as described in DeSwart 2001.

DBS, dried blood spot. NR, not reported. RT, room temperature. HPV, human papillomavirus.

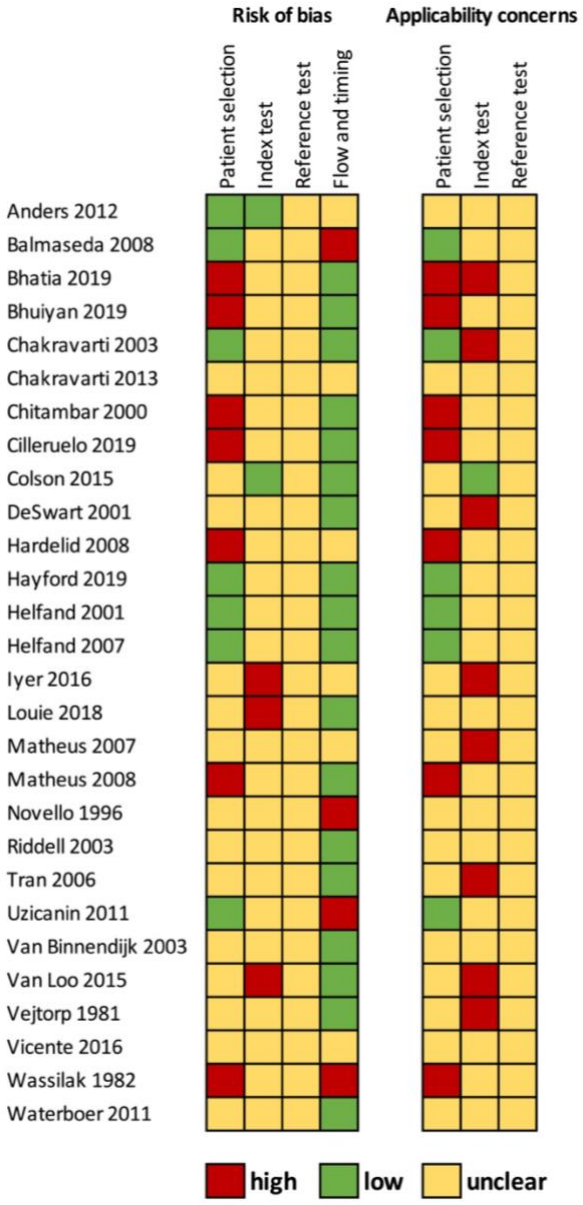
Figure 2: Practical checklist for the use of dried blood spot assays



Note: This practical checklist was developed based on the Standards for Reporting of Diagnostic Accuracy (STARD).

APPENDIX

Figure A1: Risk of bias and applicability concerns for included DBS studies according to QUADAS-2



QUADAS, Quality of Diagnostic Accuracy Studies

Table A1: PRISMA-DTA Checklist

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
TITLE / ABSTRACT			
Title	1	Identify the report as a systematic review (+/- meta-analysis) of diagnostic test accuracy (DTA) studies.	2
Abstract	2	Abstract: See PRISMA-DTA for abstracts.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Clinical role of index test	D1	State the scientific and clinical background, including the intended use and clinical role of the index test, and if applicable, the rationale for minimally acceptable test accuracy (or minimum difference in accuracy for comparative design).	4
Objectives	4	Provide an explicit statement of question(s) being addressed in terms of participants, index test(s), and target condition(s).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (participants, setting, index test(s), reference standard(s), target condition(s), and study design) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full search strategies for all electronic databases and other sources searched, including any limits used, such that they could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Definitions for data extraction	11	Provide definitions used in data extraction and classifications of target condition(s), index test(s), reference standard(s) and other characteristics (e.g. study design, clinical setting).	6
Risk of bias and applicability	12	Describe methods used for assessing risk of bias in individual studies and concerns regarding the applicability to the review question.	7
Diagnostic accuracy measures	13	State the principal diagnostic accuracy measure(s) reported (e.g. sensitivity, specificity) and state the unit of assessment (e.g. per-patient, per-lesion).	7

Synthesis of results	14	Describe methods of handling data, combining results of studies and describing variability between studies. This could include, but is not limited to: a) handling of multiple definitions of target condition. b) handling of multiple thresholds of test positivity, c) handling multiple index test readers, d) handling of indeterminate test results, e) grouping and comparing tests, f) handling of different reference standards	7
Meta-analysis	D2	Report the statistical methods used for meta-analyses, if performed.	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Provide numbers of studies screened, assessed for eligibility, included in the review (and included in meta-analysis, if applicable) with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each included study provide citations and present key characteristics including: a) participant characteristics (presentation, prior testing), b) clinical setting, c) study design, d) target condition definition, e) index test, f) reference standard, g) sample size, h) funding sources	8
Risk of bias and applicability	19	Present evaluation of risk of bias and concerns regarding applicability for each study.	10
Results of individual studies	20	For each analysis in each study (e.g. unique combination of index test, reference standard, and positivity threshold) report 2x2 data (TP, FP, FN, TN) with estimates of diagnostic accuracy and confidence intervals, ideally with a forest or receiver operator characteristic (ROC) plot.	16-20
Synthesis of results	21	Describe test accuracy, including variability; if meta-analysis was done, include results and confidence intervals.	10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression; analysis of index test: failure rates, proportion of inconclusive results, adverse events).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence.	11
Limitations	25	Discuss limitations from included studies (e.g. risk of bias and concerns regarding applicability) and from the review process (e.g. incomplete retrieval of identified research).	13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence. Discuss implications for future research and clinical practice (e.g. the intended use and clinical role of the index test).	13
FUNDING			
Funding	27	For the systematic review, describe the sources of funding and other support and the role of the funders.	14

Adapted From: McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, The PRISMA-DTA Group (2018). Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. JAMA. 2018 Jan 23;319(4):388-396. doi: 10.1001/jama.2017.19163.

Table A2: PRISMA-DTA Abstract Checklist

Section/topic	#	PRISMA-DTA for Abstracts Checklist item	Reported on page #
TITLE and PURPOSE			
Title	1	Identify the report as a systematic review (+/- meta-analysis) of diagnostic test accuracy (DTA) studies.	2
Objectives	2	Indicate the research question, including components such as participants, index test, and target conditions.	2
METHODS			
Eligibility criteria	3	Include study characteristics used as criteria for eligibility.	2
Information sources	4	List the key databases searched and the search dates.	2
Risk of bias & applicability	5	Indicate the methods of assessing risk of bias and applicability.	2
Synthesis of results	A1	Indicate the methods for the data synthesis.	2
RESULTS			
Included studies	6	Indicate the number and type of included studies and the participants and relevant characteristics of the studies (including the reference standard).	2
Synthesis of results	7	Include the results for the analysis of diagnostic accuracy, preferably indicating the number of studies and participants. Describe test accuracy including variability; if meta-analysis was done, include summary results and confidence intervals.	2
DISCUSSION			
Strengths and limitations	9	Provide a brief summary of the strengths and limitations of the evidence	3
Interpretation	10	Provide a general interpretation of the results and the important implications.	3
OTHER			
Funding	11	Indicate the primary source of funding for the review.	3
Registration	12	Provide the registration number and the registry name	3

Adapted From: McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, The PRISMA-DTA Group (2018). Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. JAMA. 2018 Jan 23;319(4):388-396. doi: 10.1001/jama.2017.19163.

Table A3:

#1	"Measles"[Mesh] OR "Cholera"[Mesh] OR "Neisseria meningitidis"[Mesh] OR "Meningococcal Infections"[Mesh] OR "Meningitis, Meningococcal"[Mesh] OR "Influenza, Human"[Mesh] OR "Diphtheria"[Mesh] OR "Mumps"[Mesh] OR "Tetanus"[Mesh] OR "Tetanus Toxoid"[Mesh] OR "Hepatitis A"[Mesh] OR "Hepatitis B"[Mesh] OR "Whooping Cough"[Mesh] OR "Tuberculosis"[Mesh] OR "Pneumococcal Infections"[Mesh] OR "Meningitis, Pneumococcal"[Mesh] OR "Pneumonia, Pneumococcal"[Mesh] OR "Typhoid Fever"[Mesh] OR "haemophilus influenzae type b"[mesh] OR "Poliomyelitis"[Mesh] OR "Encephalitis, Tick-Borne"[Mesh] OR "Encephalitis Viruses, Tick-Borne"[Mesh] OR "Rabies"[Mesh] OR "Rabies virus"[Mesh] OR "Herpesvirus 3, Human"[Mesh] OR "Chickenpox"[Mesh] OR "Herpes Zoster"[Mesh] OR "Papillomaviridae"[Mesh] OR "Rotavirus"[Mesh] OR "Rotavirus Infections"[Mesh] OR "Gastroenteritis"[Mesh] OR "Yellow Fever"[Mesh] OR "Yellow fever virus"[Mesh] OR "Encephalitis, Japanese"[Mesh] OR "Dengue"[Mesh] OR "Dengue"[Mesh] OR "Dengue Virus"[Mesh] OR "Smallpox"[Mesh] OR "Influenza A Virus, H1N1 Subtype"[Mesh]
#2	"Measles"[all fields] OR Rubeola[all fields] OR "morbilli"[all fields] OR "Rubella"[Mesh] OR "Rubella"[all fields] OR "Rubellas"[all fields] OR "epidemic roseola"[all fields] OR "german measles"[all fields] OR "Cholera"[all fields] OR "Choleras"[all fields] OR cholera[all fields] OR OR "Meningococcal"[all fields] OR "meningococci"[all fields] OR "Meningococcus"[all fields] OR "Micrococcus intracellularis"[all fields] OR "Neisseria weichselbaumi"[all fields] OR "meningitides"[all fields] OR "Meningitis"[all fields] OR ((human[all fields] OR humans[all fields]) AND (Influenzas[all fields] OR influenza[all fields] OR flu[all fields])) OR "bronchitis epidemica"[all fields] OR "epidemic bronchitis"[all fields] OR "Diphtheria"[all fields] OR "Diphtherias"[all fields] OR "Mumps"[all fields] OR "Parotitis"[all fields] OR "parodotis"[all fields] OR "Tetanus"[all fields] OR "Hepatitis A"[all fields] OR "hep A"[all fields] OR "paraditis"[all fields] OR "Hepatitis B"[all fields] OR "Whooping Cough"[all fields] OR "Pertussis"[all fields] OR "Pertusses"[all fields] OR "Tuberculosis"[all fields] OR Tuberculoses[all fields] OR "Kochs Disease"[all fields] OR "Koch's Disease"[all fields] OR "Koch Disease"[all fields] OR "parotiditis"[all fields] OR "Pneumococcal"[all fields] OR "pneumoniae Infections"[all fields] OR "pneumoniae Infection"[all fields] OR "Streptococcus pneumonia"[all fields] OR "S.pneumoniae"[all fields] OR "Typhoid"[all fields] OR "Enteric Fever"[all fields] OR "Enteric Fevers"[all fields] OR "typhi"[all fields] OR "enterica infection"[all fields] OR "Typhus"[all fields] OR "haemophilus influenzae type b"[all fields] OR "hib"[all fields] OR "h influenzae"[all fields] OR "hemophilus influenzae"[all fields] OR "Poliomyelitis"[all fields] OR "polio"[all fields] or "poliovirus"[all fields] OR "polios"[all fields] OR "Infantile Paralysis"[all fields] OR "Tick-Borne Encephalitis"[all fields] OR "TickBorne Encephalitis"[all fields] OR "Tick-Borne Encephalitides"[all fields] OR "TickBorne Encephalitides"[all fields] OR "Powassan Encephalitis"[all fields] OR "Powassan Encephalitides"[all fields] OR "Louping Ill Encephalitides"[all fields] OR "Louping Ill Encephalitis"[all fields] OR "Spring-Summer Encephalitis"[all fields] OR "Encephalitis"[all fields] OR "European Encephalitis"[all fields] OR "Hemorrhagic Fever Virus"[all fields] OR "Langat virus"[all fields] OR "Hemorrhagic Fever Virus"[all fields] OR "Al-Khurma virus"[all fields] OR "AlKhurma virus"[all fields] OR "Hubert disease"[all fields] OR "lyssavirus"[all fields] OR "lytta"[all fields] OR "Hydrophobia"[all fields] OR "Lyssa"[all fields] OR "Lyssas"[all fields] OR "Human Herpesvirus 3"[all fields] OR "Chickenpox"[all fields] OR "Chicken pox"[all fields] OR "Herpes zoster"[all fields] OR "HHV-3"[all fields] OR "Varicella-Zoster"[all fields] OR "Herpesvirus 3"[all fields] OR "VZ Virus"[all fields] OR "VZ Viruses"[all fields] OR "Herpesvirus Varicella"[all fields] OR "varicella"[all fields] OR "Herpes Zoster"[all fields] OR "Zona"[all fields] OR Zoster[all fields] OR Shingles[all fields] OR "Papillomaviridae"[all fields] OR "Human Papilloma Virus"[all fields] OR "Human Papilloma Viruses"[all fields] OR "Human Papillomavirus"[all fields] OR HPV[all fields] OR "Rotavirus"[all fields] OR "Rotaviruses"[all fields] OR "Neonatal Calf Diarrhea Virus"[all fields] OR "Gastroenteritis"[all fields] OR

	"Gastroenteritides"[all fields] OR "Yellow Fever"[all fields] OR "Yellow Fevers"[all fields] OR "Japanese B Viral Encephalitis"[all fields] OR "Japanese B Encephalitis"[all fields] OR "Japanese Encephalitis"[all fields] OR "Breakbone Fever"[all fields] OR "Smallpox"[all fields] OR "Variola"[all fields] OR "Variolas"[all fields] OR "Alastrim"[all fields] OR "H1N1"[all fields] OR "swine flu"[all fields]
#3	#1 OR #2
#4	"saliva"[mesh] OR "Dried Blood Spot Testing"[Mesh]
#5	"oral fluid"[all fields] OR "oral fluids"[all fields] OR "saliva" [all fields] OR "salivas"[all fields] OR "capillary sample"[all fields] OR "capillary samples"[all fields] OR "Fingerstick"[all fields] OR "Finger stick"[all fields] OR "Heelstick"[all fields] OR "Heel stick"[all fields] OR "Dried Blood Spot"[all fields] OR DBS[all fields] OR "filter paper blood"[all fields] OR "Heel prick" [all fields] OR "capillary blood" [all fields] OR "finger prick" [all fields] OR "fingerprick" [all fields] OR "Mucosal fluids"[all fields] OR "mucosal fluid"[all fields] OR "Gingival crevicular fluid"[all fields] OR "gingival fluid"[all fields]
#6	#4 OR #5
#10	#3 AND #6

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