Review Article: An Overview of the Clinical Use of Filter Paper in the Diagnosis of Tropical Diseases

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Abstract. Tropical infectious diseases diagnosis and surveillance are often hampered by difficulties of sample collection and transportation. Filter paper potentially provides a useful medium to help overcome such problems. We reviewed the literature on the use of filter paper, focusing on the evaluation of nucleic acid and serological assays for diagnosis of infectious diseases using dried blood spots (DBS) compared with recognized gold standards. We reviewed 296 eligible studies and included 101 studies evaluating DBS and 192 studies on other aspects of filter paper use. We also discuss the use of filter paper with other body fluids and for tropical veterinary medicine. In general, DBS perform with sensitivities and specificities similar or only slightly inferior to gold standard sample types. However, important problems were revealed with the uncritical use of DBS, inappropriate statistical analysis, and lack of standardized methodology. DBS have great potential to empower healthcare workers by making laboratory-based diagnostic tests more readily accessible, but additional and more rigorous research is needed.

INTRODUCTION

When performing diagnostic or epidemiological surveys, particularly in remote areas in resource-poor settings, the facilities for processing blood and maintaining frozen samples frequently do not exist. This finding is especially true for neglected tropical diseases, because they are frequently in populations remote from sophisticated diagnostic facilities. Dried blood spots (DBS) provide a potentially useful and inexpensive means of overcoming these difficulties. Samples, such as finger-prick blood, are easily and quickly collected onto filter paper and shipped at room temperature (even by post). However, blood sample volumes on filter paper are inevitably small, and therefore, rigorous assay validation must be performed to achieve optimum sensitivity and specificity.

Filter paper was first used as a scientific tool in 1815 by the Swedish chemist Jöns Berzelius. In the 1940s, Heatley described the use of filter paper for incorporating antimicrobial solutions in Oxford, giving rise to antibiotic susceptibility disc testing.[1] To overcome the difficulties in collecting blood for standard diagnostic tests under field conditions in Cuba, Chediak[2] developed a method of identifying syphilis from blood dried on a glass slide in 1932. However, it was Zimmermann[3] at the start of World War II in Germany who adapted the method by Chediak[2] by drying finger- or ear-prick blood on strips of filter paper to diagnose syphilis using the microscopic agglutination test. In 1950, Joe[4] in Leiden, The Netherlands received feces dried onto filter paper by post from Indonesia and was able to detect *Shigella*, and in 1961, Anderson and others[5] published methods for detecting *Schistosoma* antibodies in DBS sent from endemic areas up to 3 months after collection. Robert Guthrie is widely credited as being the first to use blood dried on filter paper (so-called Guthrie cards) to diagnose phenylketonuria in neonates in 1963.[6] Since then filter paper has

become a commonly used method of storing and transporting diverse specimen types from humans, animals, and plants. Almost all types of human body fluids (from blood to saliva and feces to breast milk) have been stored on filter paper for a diverse range of biochemical assays (e.g., newborn screening), screening for genetic mutations, determination of metabolites by mass spectrometry, therapeutic drug monitoring, and detection of nucleic acids, antigens, and serological markers for infectious disease diagnosis. The recent call for the use of DBS in diagnostics platforms for the integrated mapping, monitoring, and surveillance of seven neglected tropical diseases and the World Health Organization (WHO/Joint United Nations Programme on HIV/AIDS (UNAIDS) Treatment 2.0 initiative to achieve and sustain universal access to treatment highlights the need for review of the methodology of DBS preparation, storage, and elution to ensure best practice.[7]

Some aspects of the use of DBS in infectious diseases have been reviewed,[8–17] such as for epidemiological studies,[15] human immunodeficiency virus (HIV) detection and monitoring,[9–12] virology[17] and drug assays.[18] However, there are no recent clinically orientated overviews of the use of DBS for the diagnosis and surveillance of infectious disease.

There are important problems with uncritical use of DBS, inappropriate statistical analysis, and lack of standardization of terminology and methodology. We, therefore, reviewed the literature on the use of filter papers and focused on evaluation of DBS assays compared with recognized gold standards for the diagnosis and/or surveillance of infectious diseases for both nucleic acid amplification tests (NAATs) and serological assays. Statistical analysis of the studies included in this review was not performed, because most of the papers cited used different assays, settings, and reference methods, suggesting that a meta-analysis would not provide meaningful information. We discuss key issues in the preparation, processing, and storage of DBS and briefly review the use of filter paper with samples other than blood. Filter paper specimens are also used for veterinary health, with some overlap with human health. We, therefore, briefly summarize this parallel work, particularly for livestock diseases with significant economic impact. We highlight key difficulties encountered in using DBS, discuss

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the heterogeneity in terminology and methodology used, and
suggest improvements in these areas (Box 1).

RESULTS

Figure 1 depicts the process of study selection for inclusion in
the review. In total, 4,011 potential references were identified, of
which 101 references evaluated DBS against a recognized gold
standard and 192 references assessed the practical aspects of
filter paper use, non-whole blood samples, and veterinary health.

HIV 1 and 2 and HTLV 1. Efforts to make HIV testing
more accessible in rural areas in developing countries, where
> 90% of new HIV infections occur, are critical for controlling
the disease.[19] DBS have the potential to provide simple,
robust, and affordable options to collect whole blood for
screening, quality control of point-of-care tests, HIV viral
load measurements, and drug resistance testing in environ-
ments where traditional venous blood collection/transport
cannot be performed.[9,11,12,20] Twenty-four studies examined
the use of DBS for detection of HIV compared with serum or
plasma; 12 studies evaluated serological assays, and 12 studies
evaluated NAATs (Supplemental Table A).

Serological assays using DBS samples were evaluated
in 13 diverse countries, thereby probably representing all
HIV-1 subtypes, using third generation enzyme-linked immu-
nosorbent assays (ELISAs) that detect antibodies, fourth
generation ELISAs that detect antibodies and antigens, and
specific antigen tests (p24). The p24 antigen tests are used
as an alternative to NAATs to detect infection in infants
(Table 1). Only one study examined detection of HIV-2
using DBS against serum, reporting sensitivity and speci-
ficity of 87.5% and 100%, respectively.[21]

DBS have been evaluated for the detection of HIV-1 with
diverse NAATs in 11 countries. Although HIV is an RNA
virus, proviral HIV-1 DNA detection is commonly used for
infant diagnosis. Six studies evaluated the Roche Amplicor and
Roche Cobas Taqman (Basel, Switzerland) assays on DBS,
giving sensitivities and specificities between 97% and 100%
and between 99.6% and 100%, respectively.[20,22–26]

Most HIV viral load assays use quantitative reverse tran-
scriptase polymerase chain reaction (PCR), which requires
large quantities of plasma (100–600 µL) to transcribe RNA into
DNA before amplification. Other than extracellular HIV-1
RNA amplified from plasma samples, DBS contain whole
blood and therefore, intracellular HIV-1 RNA and HIV-1 pro-
viral DNA. As a result, when HIV-1 viral load assays are used
with DBS, both HIV-1 RNA and HIV-1 DNA will be ampli-
fied, making it potentially more sensitive than HIV-1 DNA
plasma assays. This finding has implications for early detection
of HIV but also, potential overestimation of viral load.

Three studies evaluated the Roche and Abbott (Abbott
Park, North Chicago, IL) NAATs to detect HIV-1 RNA and
DNA in DBS versus whole blood.[26–28] The bioMerieux
(Craponne, France) HIV-1 RNA assay cannot amplify HIV-1
DNA. False positive results by quantitative NAATs are a con-
cern when used for qualitative purposes, but these assays
remain a promising alternative for infant diagnosis.[20,29] Indeed,
the WHO recommends testing infants for HIV DNA, HIV
RNA, or the ultrasensitive p24 antigen on plasma or DBS
samples given that the sensitivity and specificity of DBS
are > 98%.[30] Two papers examined the possibility of detect-
ing human T-lymphotropic virus type I (HTLV-1) serologically
or by in-house NAATs.[31,32] Both studies showed good
performance compared with plasma but had relatively small
sample sizes.[31,32]

Hepatitis viruses. Eight studies evaluated the use of DBS
for the diagnosis of hepatitis viruses (Supplemental Table B).
Three studies evaluated DBS hepatitis C (HCV) serology
against serum or plasma, finding high sensitivity and specific-
ity (> 98%).[33–35] Two studies investigated DBS for hepatitis
A (HAV) serology and reported sensitivities > 90% and spec-
ificity approaching 100%.[36,37] DBS were also used success-
fully to detect the humoral response to HAV vaccination.[37]
Only two studies have examined the use of DBS samples for
hepatitis B (HBV) serology, yielding different performances
for three serological HBV assay types, with sensitivities rang-
ing from 78% (for anti-HBs) to 97% (for HBs-Ag).[38,39] The
inclusion of combined HCV, HBV, and HIV diagnoses on
one DBS could be a potentially cost-effective way to expand
screening in resource-poor and remote populations.

The detection of HCV and hepatitis E virus by NAATs
seems promising, but more evaluations are needed before
conclusions can be drawn. More evaluation of the optimal
storage DBS conditions for HCV NAAT is required, because
studies have given conflicting results.[35,40]

Flaviviruses. Capture or sandwich ELISAs are used to
serologically diagnose acute dengue (immunoglobulin M
[IgM] and IgG antibodies and nonstructural protein 1 [NS1]
antigen) and in surveillance and outbreak investigations.
Five studies comparing dengue antibody ELISAs using DBS
and serum reported high sensitivities (> 86%) and specificities
(> 89%)[41–45] (Table 2). One study reported poor correlation
of DBS with serum results,[44] but the statistical analysis was
inappropriate.[46] Antibody titers determined from DBS were
more variable and lower than those titers from sera, suggest-
ing a limited role in the diagnostic confirmation of acute
dengue. All studies concluded that DBS IgG determination
could be used successfully for seroprevalence studies.

Dengue nucleic acid detection from DBS was also highly
sensitive (> 90.7%) compared with serum. The 100% specific-
ity reported by Prado and others[47] may reflect the nature of
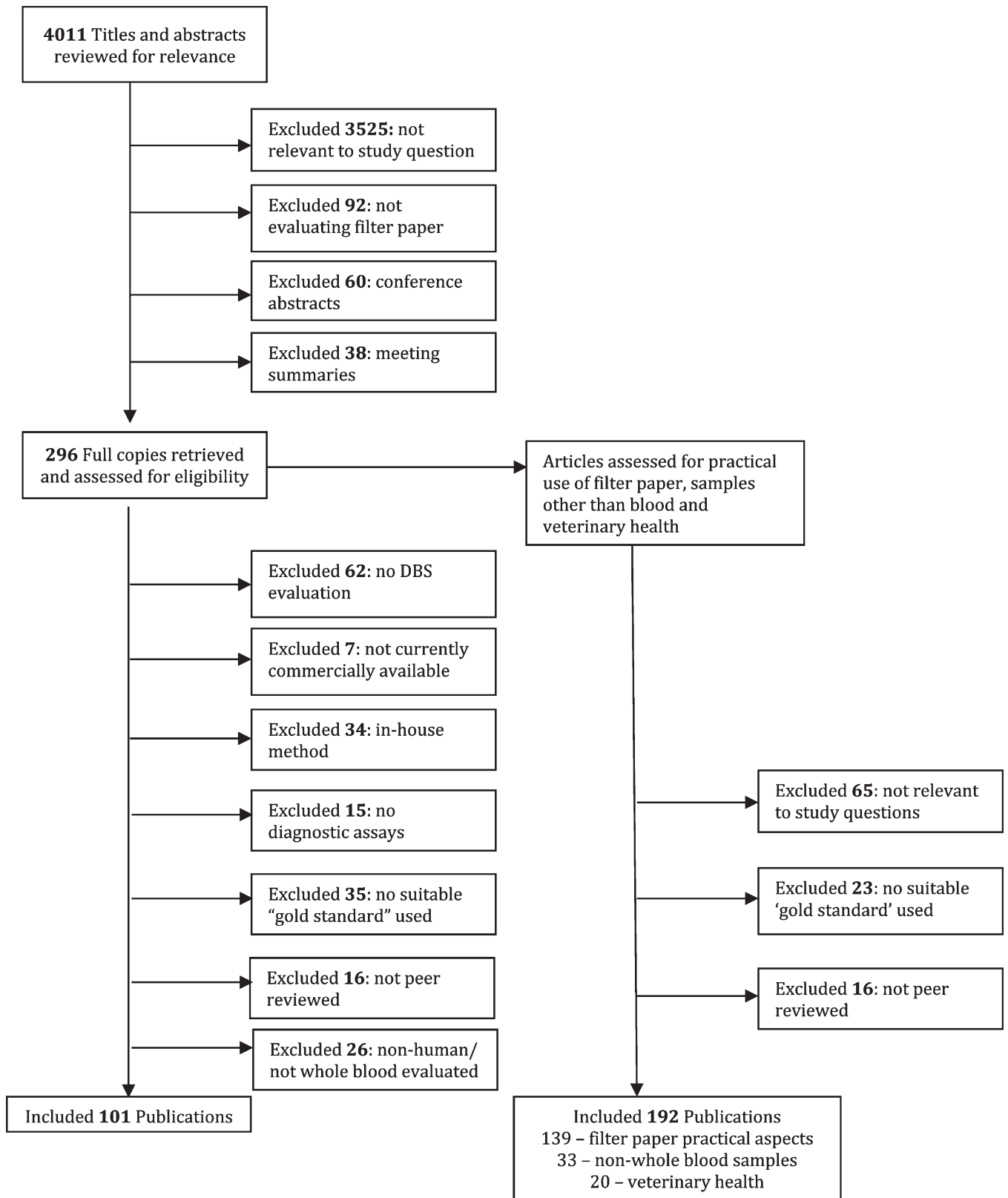
FIGURE 1.   Selection of reports included in the analysis.

the samples, which were prepared by spiking whole blood with dengue virus. Consistent with the period of highest viremia, sensitivity was highest on day 1 of infection and fell rapidly by day 4. Matheus and others[43] found that dengue RNA could still be detected in dried capillary blood samples from a small number of patients 12 days after infection, whereas corresponding venous samples were negative. Dengue RNA on DBS could be detected after storage at 37°C for

TABLE 1
Summary of studies evaluating serological and NAAT diagnosis of HIV comparing DBS with whole blood (DNA) and serum/plasma (RNA)

Assay type	HIV-1 detection	No. of studies	Sensitivity (%)	Specificity (%)	Refs.
Serology	Ab/Ag	7	100	98.7–100	21, 145, 150–154
Serology	Ag (p24)	5	84–98.8	98–100	146, 147, 155–157
Serology	Western blot	1	92	100	145
NAAT	DNA	6	97–100	99.6–100	20, 22–26
NAAT	RNA	6	99.2–100	95.6–100	22, 28, 29, 158–160
NAAT	DNA and RNA	3	99.7–100	100	24, 26, 27

1 year.[47] It is important to note that the virus may remain viable and confers an infective risk during at least the first 48 hours after spotting on untreated filter paper.[47]

Other viruses. In a seroprevalence study of chikungunya virus, IgG was successfully detected in DBS with 97.9% sensitivity compared with serum.[48] Although IgM was not fully evaluated on DBS, it seemed to give similar results to those from sera.[48]

Three studies evaluated measles antibody (IgM or IgG) detection using DBS.[49–51] Uzicanin and others[51] showed that the sensitivity of DBS compared with serum increased for IgM from 95.7% for samples collected from days 1 to 6 of the

TABLE 2
Summary of studies evaluating DBS for Flavivirus and chikungunya diagnosis

Disease, assay type and country	Ref.	Number of samples/filter paper type	Test	Sensitivity (%)	Specificity (%)	Notes
Dengue serology						
Puerto Rico	41	NR/unspecified filter paper	In-house IgM and IgG ELISA	97 IgM; 96 IgG	97 IgM; 91 IgG	IgM results are for weak positives (OD = 0.2–0.35).
Vietnam	44	781 patients/ Whatman 903	Dengue fever IgM and IgG ELISA (Focus Diagnostics)	NR	NR	DBS correlated poorly with serum, particularly for acute 1° and acute 2° dengue infection. However, correlation was inappropriate for analysis.[46] Limited role of IgM from DBS for diagnostic confirmation of dengue cases. IgG was useful for seroprevalence studies. No effect of 1 month storage on results.
Cuba	45	189 patients/ Whatman 2992	In-house ultramicro-ELISA	92.1	98.6	
French Guiana	43	130 patients/ Whatman paper	In-house ELISA IgM	89	94	IgM stable at room temperature for 1 month and at 4°C for > 2 months.
Nicaragua	42	169 patients/ Whatman No.3	In-house ELISA IgM, IgA, and IgG	96 IgM; 93 IgA; 86 IgG	89 IgM; 89 IgA; 92 IgG	Detecting IgM or IgA is useful for acute dengue diagnosis. IgG is optimal for dengue incidence surveillance. Danger of cross-reactivity of IgG with other flaviviruses.
Dengue NAAT						
Cuba	47	52 samples/ Nobuto paper	In-house PCR	93	100	Samples prepared with blood spiked with dengue virus. Lower limit of detection for dengue serotype 2 than 3. RNA stable at 37°C for 1 year. Risk of viral infectivity from paper for 48 hours at room temperature.
French Guiana	45	130 patients/ Whatman paper	In-house PCR	90.7	82.9	Serotyping also performed. Sensitivity and specificity were highest during the first 4 days of infection, falling rapidly thereafter. However, virus still detectable in 27% up to day 12 in capillary but not venous samples.
Japanese B encephalitis virus serology						
Thailand	161	243 patients/ Nobuto paper	In-house ELISA and in-house HI	72 and 26/38 and 33 during epidemic and non-epidemic periods	NR	ELISA and HI tests were compared with serum. ELISA was more sensitive during epidemic periods. Newer commercially available assays are available but have so far not been evaluated on DBS.
Chikungunya serology						
La Reunion	48	144 patients/ Whatman 903	IgG ELISA (National Arbovirus Reference Laboratory, Lyon, France)	97.9	100	Seroprevalence study. IgM also detected with similar OD thresholds as sera, but no independent quality control performed.

HI = hemagglutination inhibition; NR = not recorded; OD = optical density.

illness to 100% when samples were collected 1 week after the appearance of the rash.[51]

We found only one study evaluating the use of DBS for Epstein–Barr virus (EBV) serology. Interestingly, this study compared venous and capillary blood spotted on two different filter paper types (Whatman 903 and No. 3) for ELISA (EBNA1 plus VCA-p18) and found similar sensitivities of 75–80% and specificities of 97–100% compared with plasma.[52] For the detection of cytomegalovirus (CMV), a serological assay and an NAAT test were evaluated between plasma and DBS. The NAAT was 100% sensitive and specific, whereas the serological assay had lower sensitivity and specificity (both were > 93%) (Supplemental Table C).[53,54] At 4°C DBS storage, measles antibody and EBV IgA and IgG were stable for at least 24 weeks.[49,52]

Malaria. For the diagnosis and speciation of malaria, we found no evaluations of commercially available DBS assays using PCR in peer-reviewed journals. Two studies compared PCR on DBS against liquid whole blood and found a lower sensitivity, particularly for samples with low parasitaemia[55,56] (Table 3). DBS PCR compared with microscopy achieves comparable performance or in some studies, is more sensitive.[57] However, DBS PCR has a lower sensitivity than PCR

on whole blood. Because both DBS PCR and microscopy may miss low-level parasitemia that whole-blood PCR detects, DBS PCR seems to have a higher specificity than whole-blood PCR. This result is because of the imperfect nature of the gold standard of microscopy.[56,58] Based on 10 papers included in this review, malaria detection using the nested PCR on DBS by Snounou and others[59] seemed to be a suitable alternative to microscopy. DBS are also commonly used for detection of malaria resistance molecular markers.[60]

Parasites. Non-malarial parasites cause many neglected tropical diseases afflicting hundreds of millions of people, predominantly in resource-poor regions with limited access to diagnostic facilities.[61] The potential use of filter paper to aid diagnosis and understanding of the epidemiology of these diseases is, thus, very attractive. The mapping of lymphatic filariasis and monitoring of elimination programs provide an ideal role for DBS. Three recent studies evaluated serological tests for *Wuchereria bancrofti* Og4C3 antigen on DBS compared with serum, giving sensitivities of > 93% and specificities of 82–100%[62–64] (Table 4). An early study performed in Ghana reported a lower sensitivity (50%),[65] possibly because of a difference in strain type (most other studies were performed in Asia), an assay cutoff that was set too high, or

TABLE 3
Summary of studies evaluating DBS for malaria (malaria NAAT assays)

Country	Ref.	Sample size/ filter paper	Assay	Pf	Po	Pv	Pm	Unknown	Sensitivity (%)	Specificity (%)	Reference test	Notes
Thailand	[162]	56 samples/ Whatman 903	In-house					✓	94.6	NR	Thin/thick blood smear	
Malaysia	[163]	166 patients/ Whatman 3MM	In-house (adjusted)	✓		✓			97.4	NR	Thick blood smear	
Malaysia	[164]	129 patients/ Whatman 3MM	In-house	✓	✓	✓	✓		NR	NR		Limit of detection: 6 parasites/μL.
Singapore	[165]	52 patients/ Whatman No.1	In-house	✓		✓			100	100	Thin/thick blood smear	Limit of detection: 4 parasites/μL.
Malaysia, Myanmar, Thailand	[166]	81 patients/ Isocode cards	In-house	✓	✓	✓			94.1 (Pf); 100 (Pv)	100 (Pf); 99.1 (Pv)	Thin/thick blood smear	1 of 1 Po samples detected.
Thailand	[58]	136 patients/ Whatman 3MM	Multiplex PCR	✓		✓			100 (Pf); 92.7 (Pv)	100 (Pf); 100 (Pv)	Consensus of three PCR assays	Specificity of all three assays lower (93.8–97%) compared with microscopy. Microscopy had 90.7–92.5% sensitivity and 91.5–100% specificity.
			Nested PCR	✓		✓			100 (Pf); 100 (Pv)	99 (Pf); 100 (Pv)		
			RT-PCR	✓		✓			100 (Pf); 100 (Pv)	100 (Pf); 100 (Pv)		
Saudi Arabia	[55]	118 patients/ Whatman paper	In-house					✓	73	NR	Thin/thick blood smear	Several microscopy-negative samples were positive on DBS PCR.
Thailand, Zimbabwe	[141]	156 patients/ FTA card	In-house	✓					97.8	100	Thin/thick blood smear	Limit of detection: 10 copies/reaction.
Iran	[56]	75 patients/ DNA Banking Card	In-house	✓		✓			97	100	Thin/thick blood smear	Whole blood was more sensitive but less specific than DBS compared with microscopy (100% sensitivity, 95.2% specificity).
Kenya	[57]	356 patients/ Whatman 3MM	In-house	✓					100	79	Thin/thick blood smear	Low specificity potentially caused by insufficient microscopy expertise.[57]

Pf = *Plasmodium falciparum*; Pm = *P. malariae*; Po = *P. ovale*; Pv = *P. vivax*; RT-PCR = real time PCR; NR = not reported.

TABLE 4
Summary of studies evaluating DBS for parasites other than malaria

Disease, assay type, and country	Ref.	Number of samples/filter paper type	Test	Sensitivity (%)	Specificity (%)	Notes
Lymphatic filariasis: *Wb, Bspp* serology and dipstick antibody test India	[167]	94 patients/ Whatman 903	In-house EIA	92	77	
Ghana	[65]	1,808 patients/ Og4C3 paper	Og4C3 ELISA (*Wb*; Tropical Biotechnology)	50.3	96.4	
Sri Lanka	[62]	60 patients/ Nobotu 1	Og4C3 ELISA (*Wb*)	97	NR	
India	[63]	30 patients/ Whatman No.3	Og4C3 ELISA (*Wb*)	76.6–93.3	100	Time of the day at which samples are collected impacts sensitivity.
India, Egypt, Haiti, Kenya, Papua New Guinea, Sri Lanka	[64]	188 patients/ Whatman No.3	Og4C3 ELISA (*Wb*)	NR	NR	
Egypt	[66]	81 samples/filter paper (Tropical Biotechnology)	Filariasis (*Wb* and *Bspp*) CELISA (Cellabs)	91 (*Wb*); 98 (*Bspp*)	NR	Based on a panel of known positives.
Uganda	[67]	66 patients/ Whatman 3MM	Brugia Rapid (Reszon Diagnostics)	79	NR	Significant cross-reactivity with other filarial infections.[66,67]
Lymphatic filariasis: *B. malayi*, NAAT Indonesia	[68]	36 patients/ Whatman 3MM	In-house PCR and ELISA combination	86	NR	PCR-ELISA produced comparable results compared with DNA Detection Test Strips (Roche, Germany).
Malaysia	[69]	21 patients/ Whatman 3MM	In-house PCR	NR	NR	
Mansonelliasis NAAT Brazil	[168]	12 patients/ Whatman paper	In-house PCR	NR	NR	PCR was able to distinguish between *O. volvulus*, *M. ozzardi*, and *M. perstans*.
***Loa loa* filariasis NAAT** Cameroon	[70]	68 patients/ NR	In-house PCR	96	NR	High specificity. No cross-reactivity with other filarial species. Limit of detection 1 microfilaria/20 μL whole blood (as DBS).
HAT serology (card agglutination test) Sudan	[71]	100 patients/ NR	Micro-CATT (ITM Antwerp)	91	NR	
Central African Republic, Ivory Coast	[72]	940 patients/ Whatman No.4	Micro-CATT (ITM Antwerp)	89.4–95.5	95.5–96.6	Truc and others[72] report rapid drop in sensitivity (67.8%) after 3 days without strict humidity control of paper. Ranges reported by Truc and others[72] reflect testing at two different sites.
Chagas disease serology Brazil	[74]	6,222 patients/ Whatman No.1	In-house ELISA, IF, and HA	ELISA, 78.1; IF, 69.2; HA, 64.6	ELISA, 99.7; IF, 99.4; HA, 99.6	
Brazil	[73]	24 patients/ NR	Chagas Stat-Pak (ICT; Chembio Diagnostic Systems)	100	100	Chagas Stat-Pak performed on small sample size. More sensitive and specific than large-scale evaluation with serum.
Echinococcosis serology Argentina	[76]	479 patients/ Whatman No.1	In-house ELISA	NR	NR	Coltorti and others[76] report sensitivity of DBS to be similar to serum.
Uruguay	[78]	1,149 patients/ Whatman No.1	In-house ELISA	NR	NR	
China	[77]	2,482 patients/ Whatman No.1	In-house ELISA	96	87	

TABLE 4
Continued

Disease, assay type, and country	Ref.	Number of samples/filter paper type	Test	Sensitivity (%)	Specificity (%)	Notes
Visceral leishmaniasis NAAT Portugal	75	24 patients/ Whatman No.2	In-house PCR	71–75	NR	15/20 positive for patients not on treatment and 17/24 if patients on treatment included. Useful as an initial screening tool.
Fascioliasis serology Bolivia	79	68 patients/ Whatman No.1	In-house ELISA	NR	NR	Samples missed on DBS had the lowest ELISA readings. Samples stored for 10 years at 4°C were successfully detected.
Giardiasis serology Saudi Arabia	84	147 patients/ Whatman No.4	In-house ELISA	72–96	39–98	al-Tukhi and others[84] reported ranges that depended on ELISA OD reading and final eluate dilution. Guimaraes and Sogayar[83] had a high rate of false positives with ELISA.
Brazil	83	133 patients/ Whatman No.1	In-house IF; in-house ELISA	82; 72	70; 39	
Cysticercosis serology Brazil	80	151 patients/ Whatman No.4	Qualicode Cysticercosis ELISA kit (Immunetics Inc.)	80	NR	Good agreement between serum and DBS. May be a useful initial screening test. Fall in sensitivity if filter paper was not frozen after 1 week storage. Ranges were caused by samples being processed at two sites using two methods.
Mexico	81	305 patients/ Whatman No. 311	In-house ELISA	39–66	87–96	
Toxoplasmosis serology (latex agglutination) United Kingdom	82	273 patients/ Whatman 903	Eiken Toxoreagent Latex Agglutination	98.8	100	

Bssp = Brugia malayi and B. timori; CATT = Card Agglutination Test for Trypanosomiasis; HAT = Human African Trypanosomiasis; IF = Immunofluorescence; *Wb = Wucheraria bancrofti*.

insufficient blood volume spotted onto filter paper. The CELISA (Cellabs Pty Ltd, Manly, Australia) (*W. bancrofti* and *Brugia* spp.) and Brugia Rapid (Reszon Diagnostics, Selangor, Malaysia) (*Brugia* spp.) tests performed on DBS eluate and compared with serum or plasma proved reasonably sensitive (71–98%).[66,67] Nucleic acid testing was evaluated for DBS versus microscopy for Brugian filariasis and *Loa loa* and seems sensitive, particularly for the latter at 96%.[68–70] African and American trypanosomiases have both been successfully diagnosed on DBS with high sensitivity and specificity,[71–74] but the sample size for *Trypanosoma cruzi* was relatively small.[73] Strict control of humidity by storing DBS in sealed plastic bags with silica gel immediately after drying may have been a key factor, resulting in the higher sensitivity reported in the work by Chappuis and others[71] compared with the work by Truc and others.[72]

PCR testing on DBS for visceral leishmaniasis (*Leishmania infantum*) in immunocompromised patients before therapy was evaluated against bone marrow microscopy in a small series of patients, yielding a sensitivity of 75%.[75] PCR on DBS was significantly more sensitive than microscopy and culture of peripheral blood. Campino and others[75] suggest a possible role for PCR on DBS as an initial screening test, potentially avoiding more invasive bone marrow aspiration. Seroprevalence studies for echinococcosis, fascioliasis, cysticercosis, and toxoplasmosis performed well on DBS.[76–82] However, antibodies to cysticercosis decreased rapidly when stored on filter paper.[81] Detection of exposure to giardiasis suffered from low specificity, possibly reflecting cross-reactivity or long-term persistence of antibodies.[83,84]

Bacteria. There have been few studies evaluating the use of filter paper to diagnose or determine the seroprevalence of bacterial infections compared with viruses and parasites (Table 5).

The success of using both serum and DBS to screen for leprosy is dependent on the bacillary burden, with multibacillary patients more readily identified.[85–87] The commercially available Serodia Leprae particle agglutination test (Fujirebio, Tokyo, Japan) using DBS had 97.5% concordance with serum for patients of any bacillary burden.[88] Interestingly, the sensitivity of capillary DBS taken from skin smear sites, such as the earlobe, was slightly but significantly higher compared with venous DBS and serum. This result may reflect a higher concentration of antibodies at the site of infection compared with circulating antibodies.[85]

Brucella antibodies were eluted from filter paper with difficulty, and correlation coefficients with serum were modest.[89] However, correlation coefficients are not valid statistical tests for comparison of diagnostic methods.[46] Serological tests for other bacterial pathogens, including syphilis, yaws, leptospirosis, and some rickettsial diseases, performed well on DBS and could be stored successfully for sufficient periods of time to allow transport to a laboratory for analysis.[90–95]

PRACTICAL ASPECTS AND IMPLICATIONS
OF USING DBS SAMPLES COMPARED
WITH TRADITIONAL METHODS

Some of the key neglected but practical aspects that should be taken into account when using DBS samples are discussed below (Figure 2).

TABLE 5
Summary of studies evaluating DBS for bacteria

Disease, assay type, and country	Ref.	Number of samples/ filter paper type	Test	Sensitivity (%)	Specificity (%)	Notes
Leprosy serology						
French Polynesia	86	168 patients/ Whatman No.1	In-house ELISA	Multibacillary 96; Paucibacillary 29	Multibacillary 96; Paucibacillary 96	
India	87	94 patients/ Whatman No.3	In-house ELISA	Multibacillary 97; Paucibacillary 73	Multibacillary 100; Paucibacillary 100	Based on a cutoff of 1:40 (OD)
India	88	81 patients/ Whatman No.3	MLPA (Fujirebio); in-house ELISA	67.7 (MLPA); 76.9 (ELISA)	98.7 (MLPA); 83.4 (ELISA);	
Nepal	85	200 patients/ NR	In-house ELISA	NR	NR	Earlobe capillary blood more sensitive than serum or finger-prick blood[85]
Orientia tsutsugamushi and *Rickettsia typhi* (scrub typhus and murine typhus)						
Laos	93	53 scrub typhus patients; 53 murine typhus patients/ Whatman 903	In-house ELISA	95 IgM and 90 IgG; 91 IgM and 82 IgG	88 IgM and 100 IgG; 100 IgM and 100 IgG	Lower antibody titers with DBS; storage at room temperature for 1 month did not affect antibody titers[93,94]
Coxiella burnetii, *Bartonella quintana* and *Rickettsia conorii* serology						
France	94	94 patients/ Fischer Scientific paper[94]	In-house ELISA	100	100	
Leptospirosis serology (MAT)						
La Reunion	90	52 patients/ Whatman 903	MAT	100	100	DBS samples showed lower antibody titers compared with serum
Syphilis serology						
United States	91	1,098 patients/ Whatman 903	In-house ELISA	96	94	
Tanzania	92	1,037 patients/ Whatman 903	Serodia TPPA (Fujirebio)	98.3	100	
Yaws serology						
Papua New Guinea	95	70 patients/ Whatman 903	TPHA–Serodia TP kit (Fujirebio)	96.5	100	Results unaffected by up to 2 months storage
Brucella serology						
Spain	89	160 patients/ Whatman 2992	*Brucella* ELISA (Virotech System Diagnostika)	NR	NR	Pearson correlation coefficient: $r = 0.8$ for IgM and IgG; time-consuming extraction method

MAT = microscopic agglutination test; MLPA = *Mycobacterium leprae* particle agglutination; TPHA = *Treponema pallidum*-specific hemagglutination test; TPPA = *Treponema pallidum* particle agglutination test.

Filter paper. There are many different filter paper brands available consisting of 100% cellulose, and they vary in thickness and pore size. Although many manufacturers produce cards, only two brands are US Food and Drug Administration (FDA) -approved for human whole-blood collection (Whatman 903 and PerkinElmer [Beaconsfield, UK] 226 filter papers). For the Centers for Disease Control and Prevention (CDC) newborn screening quality assurance program, each lot is checked to ensure that the relationship between spot size and whole-blood volume varies minimally.[96] When comparing 903 and 226 filter papers, < 4–5% difference was detected for analytes used for neonatal screening.[96] FTA Elute and FTA (Whatman; GE Healthcare, Buckinghamshire, UK) are treated filter papers that lyse cells and inactivate antibodies, viruses, and bacteria but allow NAAT assays. Assays should not be transferred between paper types without additional evaluation.

Sample collection and storage recommendations. Manufacturers' recommendations as well as the protocols presented by Mei and others[14] and the US CDC[97] provide useful guides. The WHO guidelines for HIV drug resistance testing with DBS and others contain a more detailed description of how to collect DBS samples (particularly for RNA viruses).[96,98,99] A number of studies also examined HIV DNA and RNA storage conditions when validating DBS methods.[100–103] For serology, specific collection and storage recommendations have been produced by the CDC.[97]

Collecting finger- or heel-prick blood with DBS is a fast and convenient method that requires minimal training. After the DBS sample has been dried for at least 3 hours, it should be stored in a zipped bag with desiccant to reduce humidity damage. If DBS are stored in freezers, ensure that they are dried thoroughly after being brought to room temperature to avoid condensation inside the bag. The effect of long-term
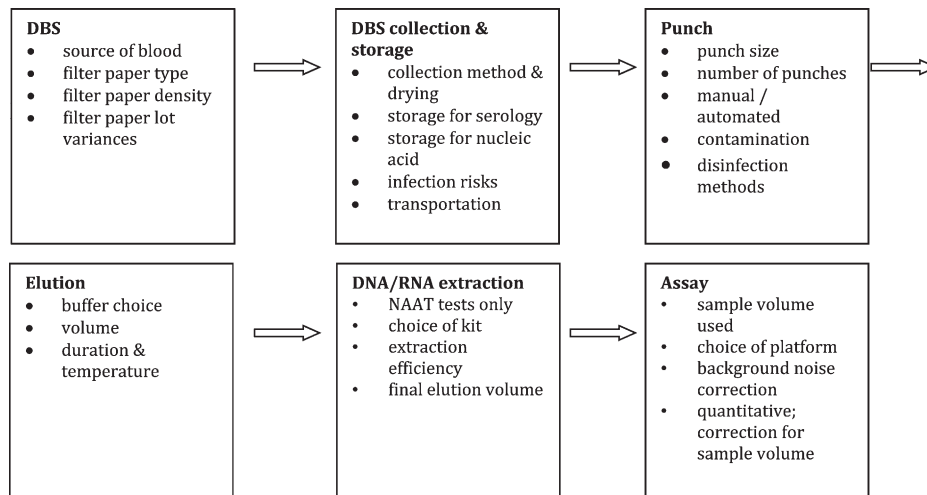
FIGURE 2.   Practical aspects and implications of using DBS. NA = nucleic acid.

storage at different temperatures on diagnostic accuracy of DBS has been investigated for only a few pathogens with variable results (e.g., HCV with poor/uncertain stability[35,40] versus dengue, EBV, and measles with better stability).[47,49,52] Standardization of experimental methods for assessing DBS stability would help considerably.

Recording the quality and integrity of filter paper samples on arrival at the laboratory is essential, as they can vary because of incorrect blood sampling or environmental factors, such as humidity, contamination, and mold overgrowth.

The presence of nucleic acids or antibodies in venous and capillary blood may vary for different pathogens. Two studies suggest that dengue virus capillary viremia may be more prolonged than venous viremia,[43,104] suggesting that it would be important, in an evaluation of NS1 assays and NAATs, that both DBS and liquid blood samples are compared using capillary blood.

Biosafety issues. Because DBS contain dried blood, regardless of the pathogen being investigated, the samples should be processed as potentially infectious material, and health and safety regulations should be followed. However, safety and packaging requirements are simpler than for liquid blood, and DBS can be shipped as non-regulated, exempt materials.[105] However, although it is believed that bacteria and viruses have reduced activity when stored as DBS samples, group A streptococci could still be cultured after elution of DBS samples, and dengue virus is still viable after 48 hours on DBS at room temperature.[47,106,107] FTA paper carries the advantage of inactivating highly pathogenic organisms to allow safe transportation, with reported complete inactivation of highly pathogenic Avian Influenza Virus (AIV) 1 hour after adsorption onto FTA paper.[108] However, more evaluation of the potential infectiousness of different pathogens on DBS is needed.

Contamination risks. Manual or automated punch devices, such as handheld office punches or automated machines (like the devices used for neonatal screening), are suitable for removing paper discs from DBS. There is a potential risk of carryover contamination that can be avoided by cleaning the punch device with bleach or related products and punching sterile blank paper between samples. Recently, perforated filter paper cards have become available (Whatman and PerkinElmer),

allowing the spots to be removed with a pipette tip, obviating the need for punching machines and reducing contamination risks.

Selecting an assay. For quantitative assays, adjusting the cutoff for DBS samples compared with whole blood or serum may improve sensitivity and/or specificity, depending on the required balance between them.[34] Assays that use a relatively small quantity of plasma/serum that is first diluted with sample buffer are more suitable for DBS samples than assays requiring large quantities. Attempts to keep DBS elution comparable with serum/plasma according to the manufacturer's recommendations will greatly improve the chances that results of assays on DBS and standard samples will have comparable accuracy. The quantity of serum in whole blood dried on filter paper is difficult to determine but essential for protocol development. Factors, such as hematocrit, blood volume per spot, and filter paper characteristics, contribute to different extraction yields of a DBS sample.[109]

Certain pathogens, such as HIV, are present in large quantities in whole blood (up to 10^4 copies per drop), whereas others, such as *Salmonella enterica* serovar Typhi and *Orientia tsutsugamushi*, are present at very low density. DBS as an alternative to standard samples is only possible if the pathogen is present in sufficient numbers for nucleic acid amplification.

Reporting DBS evaluation studies. The Standards for Reporting of Diagnostic Accuracy (STARD) guidelines[110] are an important starting point for assessing DBS evaluations. Many studies evaluating filter paper do not include full details on the paper type or processing, key information regarding reference standards, and use of appropriate statistical tests. In Table 6, we propose additional points to the current STARD checklist to address these issues.

USE OF FILTER PAPER FOR SAMPLES OTHER THAN WHOLE BLOOD

Whole blood is the most practical sample to collect on filter paper; however, many reference assays have used other samples types (e.g., serum or plasma), and some diseases are preferably diagnosed using other specimen types (Supplemental Table D).

Evaluation of dried serum spots to detect HAV antibodies showed a sensitivity and specificity of 100% compared with liquid serum,[111] and HIV ELISA had a sensitivity of 83%.[112]

TABLE 6
Additional suggested Standards for Reporting of Diagnostic Accuracy (STARD) checklist points for DBS evaluation

Concerns when using DBS	STARD checklist adjustments for DBS evaluations
Inconsistency in terminology	Make use of clear terminology (i.e., DBS, dried serum spots, dried urine spots, etc. or dried "sample type" spots).
Unclear or not reporting filter paper sample collection method	Sample collection: state the filter paper brand and weight used, which and how fluids were obtained and spotted onto filter paper, and the drying period before storage.
Unclear reporting of reference method and sample	Report the index sample and its collection, storage, and transportation details; provide detailed rationale for discordances in methods between index and reference test.
Unclear or not reporting storage and time between collecting and analyzing samples	Sample processing: state the time and storage conditions (humidity control and temperature) in the field, during transportation, and in the laboratory, preferably in a tabled manner.
Unclear or not reporting punch method and punch disinfection procedure	Report punching method with reference to source or manufacturer and punch disinfection procedure if used.
Unclear or not reporting how quantitative data was obtained from filter paper samples	For quantitative or numerical test results, indicate the calculation methods and rationale of the index and reference standard.
Unclear or not reporting the biological variability of samples and mean difference between index and reference sample	For quantitative test outcomes, report the mean and range of results for index and reference test.
Unclear or not reporting of diagnostic accuracy of quantitative test outcomes.	For quantitative test outcomes, estimates of diagnostic accuracy and measures of statistical uncertainty (e.g., 95% confidence intervals) by quantitative grouped ranges (e.g., 1,000–5,000 copies/mL).

NAATs of dried serum spots perform very well for HAV (92.3% and 100%) and HCV (100% and 100%) sensitivity and specificity, respectively, versus liquid serum.[111,113] Both hepatitis viruses showed a 10-fold fall in viral load after storage for 4 weeks on paper at room temperature.[111,113]

Three studies used dried plasma spots and one study used dried breast milk spots compared with liquid plasma for HIV quantitative PCR.[114–116] HIV RNA on filter paper was stable at room temperature for > 1 year. Dried buffy coat spots may be used as a substrate to detect HIV proviral DNA. When dried on filter paper and compared with liquid samples, there was 100% concordance between results.[117]

Although bone marrow is a difficult sample to obtain, it is the most sensitive substrate for diagnosis of visceral leishmaniasis. In one small study, 34 of 35 patients suspected of having the disease on clinical grounds were positive by NAAT on dried bone marrow spots. This test was more sensitive than bone marrow microscopy.[118]

Cutaneous and mucocutaneous samples may be scraped, aspirated, or directly impressed onto filter paper to diagnose leishmaniasis and using slit skin smears, leprosy. The sensitivity of PCR on lesions impressed onto paper for leishmaniasis ranged from 92.3% to 100% and specificity was 100% compared with PCR on tissue samples[119,120]; parasite speciation was also possible. *Mycobacterium leprae* was detected by PCR from slit skin smears on filter paper (60%) in patients with known leprosy as frequently as from slit skin smears stored in ethanol (58%).[121]

Sputum and saliva have been more widely examined. Only 67% of serologically positive measles patients were positive by PCR on dried saliva spots, which were inferior to whole-saliva and throat swabs.[122] Detection of malaria DNA in dried saliva and dried urine spots was less sensitive than blood microscopy.[123] Dried induced sputum and bronchoalveolar lavage fluid spots to identify *Pneumocystis jirovecii* by PCR had reported sensitivity of 67% and 90–91%, respectively, compared with microscopic examination of liquid samples.[124] Dried cervical smear fluid spots were evaluated for detection of Human Papilloma Virus by PCR. Concordance of 94–100% was reported in two of three studies compared with PCR directly on smear or cytobrush samples.[125–127]

Dried cerebrospinal fluid (CSF) spots in children with meningitis were assayed by PCR for *Streptococcus pneumoniae* and *Haemophilus influenzae* with a sensitivity of 92% and 70% and specificity of 99% and 100%, respectively, compared with direct CSF PCR.[128] The detection of cysticercosis antibodies was less successful, ranging from 52% to 63%, compared with neat CSF depending on the type of filter paper used to store CSF.[81]

Both stool and urine have been stored on filter paper. *Vibrio cholerae* could be cultured from dried stool spots after 14 days if humid conditions were maintained[129] and was equivalent to standard transport medium. Viral enteric pathogens, including Norovirus, Rotavirus, and Adenovirus serotypes 40 and 41, were detected by NAAT from dried stool spots on chromatography paper, with good concordance with enzyme immunoassay (EIA) performed directly on stool.[130–132] Pre-treating the paper with sodium dodecyl sulfate (SDS)/ethylenediaminetetraacetic acid (EDTA) inactivated the virus, allowing safe handling of the paper. CMV is readily detected in urine in viremic patients. Dried urine spots were reported to have 90% concordance with PCR on DNA extracted directly from urine.[133]

USE OF FILTER PAPER IN TROPICAL VETERINARY HEALTH

Filter paper has been widely used as a specimen substrate in tropical veterinary health in both livestock and wildlife diseases. Several zoonotic diseases discussed above, including echinococcosis, brucellosis, and trypanosomiasis,[134] are also important causes of mortality in other mammals. However, non-zoonotic diseases are responsible for about one-half of livestock losses worldwide.[134] Poultry, swine, and cattle suffer the greatest burden of disease, with viruses and parasites being the major causes. Early warning systems are needed to detect highly pathogenic organisms, such as AIV. The difficulties of traditional sample collection methods, discussed above for humans, are equally applicable in the veterinary setting. Filter paper has played a key role in circumventing many of these challenges for veterinary medicine. Smith and Burgoyne[135] discuss the problems likely to be faced with the

use of filter paper (FTA) with veterinary samples. Leishmaniasis is an important zoonosis with reservoirs in canids; however, serological studies among dogs using filter paper compared with serum have given relatively poor sensitivity of 22.2% or agreement of 68.8% ($k = 0.234$).[136,137]

DISCUSSION

Over the last 50 years, filter paper has gained an increasingly important role as a substrate for the diagnosis and surveillance of infectious diseases. Recently, this role has gone beyond diagnosis to include detection of markers of resistance, detailed genetic or serological analysis, and monitoring of therapeutic interventions, including drug levels, vaccine-induced responses, and viral loads.

Almost any clinical sample may be stored on filter paper for subsequent analysis, although finger-prick blood is the most convenient and widely used. Point-of-care tests are increasingly providing a key role in diagnosing and surveying infectious diseases in remote settings, and affordable microfluidics devices based on paper to diagnose infectious diseases are promising tools.[138]

Viruses, particularly HIV, have been most frequently targeted with filter paper diagnostics. Serological tests perform very well, with seven studies reporting sensitivity and specificity close to 100%. NAAT performance is more variable because of the greater instability of nucleic acids, but mostly, it reached similar diagnostic accuracy. Infant diagnoses using both RNA and DNA are feasible; however, RNA tests tend to suffer from reduced specificity. Hepatitis viruses, many of the Herpes virus family, measles, and rubella also perform well with serological tests, with sensitivities and specificities of > 90%. NAATs seem promising, although more evaluations are needed, particularly for HCV and HEV. Dengue serology performed on DBS is clearly suitable for seroprevalence studies, although it is less clear for the diagnosis of acute primary and acute secondary infections. Dengue serotyping is epidemiologically important and can also be successfully performed from DBS.[43,139]

DBS also play a key role in the diagnosis of parasitic infections. Detection of malaria by PCR using in-house methods is generally superior to microscopy. Most studies report sensitivities of > 94% and specificities of > 99%.[56,58,140,141] Because of the prevalence of filariasis in remote settings, filter paper has been used in the diagnosis and investigation of epidemiology and response to eradication programs. Using commercially available assays, sensitivities of > 90% may be achieved.[62,66] Leishmaniasis, cysticercosis, and giardiasis have proved to be less promising in the few studies that have evaluated DBS compared with a recognized gold standard.[75,81,83] Serological tests for leptospirosis, treponemal infections, and some rickettsia have yielded excellent results,[90,92,94] whereas others, such as brucellosis, have been less successful.[89]

The selection of pathogens that may perform well on filter paper is dependent on several important factors, crucially the presence and quantity of serological markers and nucleic acids in the blood at the time of sample collection, their stability on filter paper, and the elution method that maximizes test performance with DBS.

There are several key advantages of using filter paper over the traditional specimens of whole blood or serum. Many of the pathogens discussed above are most common in remote and resource-poor settings with limited access to advanced diagnostic facilities. Filter paper obviates the need for a cold chain to preserve specimens in transport to a central laboratory, thus enormously increasing the accessibility of these tests. Filter paper is generally cheap (although some of the treated papers, such as FTA, are very expensive), requires only a small sample volume, and needs minimal technical expertise to perform. These factors are likely to make sample collection more acceptable to the patient and less of a burden for the health system, and they will probably increase testing uptake.[142] Filter paper is easily and safely delivered using almost any existing transportation network. Recent advances in chemically pre-treated cards have provided increased safety in handling and transporting samples.[108] Filter paper has been used with multiplex serological and NAATs to diagnose combinations of Hepatitis B, C, and HIV,[143,144] increasing the diagnostic potential of a single DBS.

There are, however, important difficulties and limitations in the evaluation of filter papers as diagnostic tools. A great variety of terminology has been used, and studies evaluating the same pathogen often use different methodologies encompassing almost every stage of the process from filter paper selection to final assay procedures, making comparison vexed. Some studies have used DBS without justifying that the method is accurate against a reference standard. Many filter paper varieties have been used (products are not always clearly labeled with the paper weight in grams per meter^2), and sample volumes will vary; therefore, care is required when moving techniques between paper types. A consensus document on terminology and methodology would be invaluable for advancing the field of filter paper diagnostics. Surprisingly, there have been no cost-effectiveness analyses of the use of filter paper for infectious disease diagnostics.

Human and animal health are inextricably linked, but there has been very little, if any, collaboration between scientists and health workers interested in human and non-human health and filter paper diagnostics. More One Health collaboration on these techniques would benefit both fields.

High temperatures and humidity over prolonged periods severely reduce test sensitivity, particularly for NAATs, although this finding seems to vary between pathogens.[35,47,145–147] Inevitably, the volume of blood per spot will be less than the volume of a whole-blood sample collected by venipuncture. DBS containing whole blood may also influence NAATs or serological assays because of the presence of inhibitors. They can, however, be overcome by DBS-specific protocols.[148,149] Although some guidelines exist, there is an urgent need for more robust standardized protocols for sampling, storage, processing, and evaluating filter paper techniques. Of the studies reported in this review, 42% of them were not prospective, real-life evaluations; such studies would provide a stronger evidence base to support recommendations. Additionally, most studies used pipettes to spot venous blood onto filter paper, giving a greater consistency in blood volume than direct application of blood to paper. However, this consistency is unlikely to be achieved with field samples. A number of studies did not report sensitivity and specificity, and several studies inappropriately used correlation coefficients.[46] The inclusion of additional reported items to improve accuracy and completeness of filter paper studies could greatly improve consistency and clinical use of the results (Table 6).

Our review has important limitations. We only included studies published in English, excluded related subjects, such as filter paper assays of drug resistance and viral loads, excluded in-house assays for those diseases with well-recognized commercially available assays, and did not do a detailed assessment of veterinary use of filter paper (this assessment would require a literature review in its own right).

This work is a first attempt to summarize the subject of filter paper diagnostics in tropical diseases. We highlight the many advantages that filter paper offers over traditional samples and discuss the associated limitations and difficulties. Consensus should be reached regarding the methodology and terminology used to better advance this important diagnostic tool. Filter paper has been shown to be a valuable asset in increasing accessibility, making affordable, robust, sensitive, and specific diagnostic testing available to patients in remote settings. Its use in surveillance of neglected tropical diseases targeted for elimination and potentially, veterinary pathogens makes DBS an important tool in international health.

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