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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, Chediak² developed a method of identifying syphilis from blood dried on a glass slide in 1932. However, it was Zimmermann³ at the start of World War II in Germany who adapted the method by Chediak³ by drying finger- or ear-prick blood on strips of filter paper to diagnose syphilis using the microscopic agglutination test. In 1950, Joe⁴ 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 others⁵ 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 virology¹² 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
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. 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 environments where traditional venous blood collection/transport cannot be performed. 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 immunosorbent 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 specificity of 87.5% and 100%, respectively.

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.

Most HIV viral load assays use quantitative reverse transcriptase 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 proviral DNA. As a result, when HIV-1 viral load assays are used with DBS, both HIV-1 RNA and HIV-1 DNA will be amplified, 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. The bioMérieux (Craponne, France) HIV-1 RNA assay cannot amplify HIV-1 DNA. False positive results by quantitative NAATs are a concern when used for qualitative purposes, but these assays remain a promising alternative for infant diagnosis. 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%. Two papers examined the possibility of detecting human T-lymphotropic virus type I (HTLV-1) serologically or by in-house NAATs. Both studies showed good performance compared with plasma but had relatively small sample sizes.

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 specificity (>98%). Two studies investigated DBS for hepatitis A (HAV) serology and reported sensitivities >90% and specificity approaching 100%,. DBS were also used successfully to detect the humoral response to HAV vaccination. 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 ranging from 78% (for anti-HBs) to 97% (for HBs-Ag). 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.

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%) (Table 2). One study reported poor correlation of DBS with serum results, but the statistical analysis was inappropriate. Antibody titers determined from DBS were more variable and lower than those titers from sera, suggesting 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% specificity reported by Prado and others may reflect the nature of
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\textsuperscript{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
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>
<thead>
<tr>
<th>Disease, assay type and country</th>
<th>Ref.</th>
<th>Number of samples/filter paper type</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>41</td>
<td>NR/unspecified filter paper</td>
<td>In-house IgM and IgG ELISA</td>
<td>97 IgM; 96 IgG</td>
<td>97 IgM; 91 IgG</td>
<td>IgM results are for weak positives (OD = 0.2–0.35).</td>
</tr>
<tr>
<td>Vietnam</td>
<td>44</td>
<td>781 patients/Whatman 903</td>
<td>Dengue fever IgM and IgG ELISA (Focus Diagnostics)</td>
<td>NR</td>
<td>NR</td>
<td>DBS correlated poorly with serum, particularly for acute 1(^{st}) and acute 2(^{nd}) 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.</td>
</tr>
<tr>
<td>Cuba</td>
<td>45</td>
<td>189 patients/Whatman 2992</td>
<td>In-house ultramicro-ELISA</td>
<td>92.1</td>
<td>98.6</td>
<td>IgM stable at room temperature for 1 month and at 4(^\circ)C for ≥ 2 months. 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.</td>
</tr>
<tr>
<td>French Guiana</td>
<td>43</td>
<td>130 patients/Whatman paper</td>
<td>In-house ELISA IgM</td>
<td>89</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Nicaragua</td>
<td>42</td>
<td>169 patients/Whatman No.3</td>
<td>In-house ELISA IgM, IgA, and IgG</td>
<td>96 IgM; 93 IgA; 86 IgG</td>
<td>89 IgM; 89 IgA; 92 IgG</td>
<td></td>
</tr>
<tr>
<td>Dengue NAAT</td>
<td></td>
<td></td>
<td>In-house PCR</td>
<td>93</td>
<td>100</td>
<td>Samples prepared with blood spiked with dengue virus. Lower limit of detection for dengue serotype 2 than 3. RNA stable at 37(^\circ)C for 1 year. Risk of viral infectivity from paper for 48 hours at room temperature. 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.</td>
</tr>
<tr>
<td>Cuba</td>
<td>47</td>
<td>52 samples/Nobuto paper</td>
<td>In-house PCR</td>
<td>90.7</td>
<td>82.9</td>
<td></td>
</tr>
<tr>
<td>French Guiana</td>
<td>45</td>
<td>130 patients/Whatman paper</td>
<td>In-house PCR</td>
<td>90.7</td>
<td>82.9</td>
<td></td>
</tr>
<tr>
<td>Japanese B encephalitis virus serology</td>
<td>161</td>
<td>243 patients/Nobuto paper</td>
<td>In-house ELISA and in-house HI</td>
<td>72 and 26/38 and 33 during epidemic and non-epidemic periods</td>
<td>NR</td>
<td>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.</td>
</tr>
<tr>
<td>Chikungunya serology</td>
<td>48</td>
<td>144 patients/Whatman 903</td>
<td>IgG ELISA (National Arbovirus Reference Laboratory, Lyon, France)</td>
<td>97.9</td>
<td>100</td>
<td>Seroprevalence study. IgM also detected with similar OD thresholds as sera, but no independent quality control performed.</td>
</tr>
</tbody>
</table>

HI = hemagglutination inhibition; NR = not recorded; OD = optical density.
illness to 100% when samples were collected 1 week after
the appearance of the rash.54

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 dif-
terent 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 stor-
age, 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 parasitaemia55,56
(Table 3). DBS PCR compared with microscopy achieves
comparable performance or in some studies, is more sensi-
tive.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 others59 seemed to be a suit-
able 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 com-
pared with serum, giving sensitivities of >93% and specifici-
ities 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 per-
formed in Asia), an assay cutoff that was set too high, or

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

PF = Plasmodium falciparum; Po = P. malariae; Pv = P. ovale; RT-PCR = real time PCR; NR = not reported.
<table>
<thead>
<tr>
<th>Disease, assay type, and country</th>
<th>Ref.</th>
<th>Number of samples/filter paper type</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphatic filariasis:</strong> Wb, Bspp serology and dipstick antibody test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>167</td>
<td>94 patients/ Whatman 903</td>
<td>In-house EIA</td>
<td>92</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Ghana</td>
<td>65</td>
<td>1,808 patients/ Og4C3 paper</td>
<td>Og4C3 ELISA (Wb; Tropical Biotechnology)</td>
<td>50.3</td>
<td>96.4</td>
<td></td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>62</td>
<td>60 patients/ Nobotu 1</td>
<td>Og4C3 ELISA (Wb)</td>
<td>97</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>64</td>
<td>188 patients/ Whatman No.3</td>
<td>Og4C3 ELISA (Wb)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>India, Egypt, Haiti, Kenya, Papua New Guinea, Sri Lanka, Egypt</td>
<td>66</td>
<td>81 samples/filter paper (Tropical Biotechnology)</td>
<td>Filariasis (Wb and Bspp) CELISA (Cellabs)</td>
<td>91 (Wb); 98 (Bspp)</td>
<td>NR</td>
<td>Based on a panel of known positives.</td>
</tr>
<tr>
<td>Uganda</td>
<td>67</td>
<td>66 patients/ Whatman 3MM</td>
<td>Brugia Rapid (Reszon Diagnostics)</td>
<td>79</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td><strong>Lymphatic filariasis:</strong> B. malayi, NAAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indonesia</td>
<td>68</td>
<td>36 patients/ Whatman 3MM</td>
<td>In-house PCR and ELISA combination</td>
<td>86</td>
<td>NR</td>
<td>PCR-ELISA produced comparable results compared with DNA Detection Test Strips (Roche, Germany).</td>
</tr>
<tr>
<td>Malaysia</td>
<td>69</td>
<td>21 patients/ Whatman 3MM</td>
<td>In-house PCR</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td><strong>Mansonelliasis NAAT</strong></td>
<td>168</td>
<td>12 patients/ Whatman paper</td>
<td>In-house PCR</td>
<td>NR</td>
<td>NR</td>
<td>PCR was able to distinguish between O. volvulus, M. ozzardi, and M. perstans.</td>
</tr>
<tr>
<td>Cameroon</td>
<td>70</td>
<td>68 patients/ NR</td>
<td>In-house PCR</td>
<td>96</td>
<td>NR</td>
<td>High specificity. No cross-reactivity with other filarial species. Limit of detection 1 microfilaria/20 µL whole blood (as DBS).</td>
</tr>
<tr>
<td><strong>HAT serology (card agglutination test)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sudan</td>
<td>71</td>
<td>100 patients/ NR</td>
<td>Micro-CATT (ITM Antwerp)</td>
<td>91</td>
<td>NR</td>
<td>Truc and others report rapid drop in sensitivity (67.8%) after 3 days without strict humidity control of paper. Ranges reported by Truc and others reflect testing at two different sites.</td>
</tr>
<tr>
<td>Central African Republic, Ivory Coast</td>
<td>72</td>
<td>940 patients/ Whatman No.4</td>
<td>Micro-CATT (ITM Antwerp)</td>
<td>89.4–95.5</td>
<td>95.5–96.6</td>
<td></td>
</tr>
<tr>
<td><strong>Chagas disease</strong> serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>74</td>
<td>6,222 patients/ Whatman No.1</td>
<td>In-house ELISA, IF, and HA</td>
<td>ELISA, 78.1; IF, 69.2; HA, 64.6</td>
<td>ELISA, 99.7; IF, 99.4; HA, 99.6</td>
<td>Chagas Stat-Pak performed on small sample size. More sensitive and specific than large-scale evaluation with serum.</td>
</tr>
<tr>
<td>Brazil</td>
<td>73</td>
<td>24 patients/ NR</td>
<td>Chagas Stat-Pak (ICT; Chembio Diagnostic Systems)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Echinococcosis</strong> serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>76</td>
<td>479 patients/ Whatman No.1</td>
<td>In-house ELISA</td>
<td>NR</td>
<td>NR</td>
<td>Coltorti and others report sensitivity of DBS to be similar to serum.</td>
</tr>
<tr>
<td>Uruguay</td>
<td>78</td>
<td>1,149 patients/ Whatman No.1</td>
<td>In-house ELISA</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>77</td>
<td>2,482 patients/ Whatman No.1</td>
<td>In-house ELISA</td>
<td>96</td>
<td>87</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
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%). Nucleic acid testing was evaluated for DBS versus microscopy for Brugian filariasis and Loa loa and seems sensitive, particularly for the latter at 96%, but the sample size for African and American trypanosomiasis have both been successfully diagnosed on DBS with high sensitivity and specificity, but the sample size for *Trypanosoma cruzi* was relatively small. 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 Truc and others compared with the work by Chappuis and others.

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%. PCR on DBS was significantly more sensitive than microscopy and culture of peripheral blood. Campino and others 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. However, antibodies to cysticercosis decreased rapidly when stored on filter paper. Detection of exposure to giardiasis suffered from low specificity, possibly reflecting cross-reactivity or long-term persistence of antibodies.

**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. 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. 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.

**Brucella** antibodies were eluted from filter paper with difficulty, and correlation coefficients with serum were modest. However, correlation coefficients are not valid statistical tests for comparison of diagnostic methods. 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.

**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).
There are many different filter paper brands 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. When comparing 903 and 226 filter papers, < 4-5% difference was detected for analytes used for neonatal screening. 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 and the US CDC 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). A number of studies also examined HIV DNA and RNA storage conditions when validating DBS methods. For serology, specific collection and storage recommendations have been produced by the CDC. 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 storage can be minimized by storing DBS samples at lower temperatures, such as -20°C or lower. For long-term storage, DBS samples can be successfully stored at -20°C or lower, with minimal impact on antibody titers. However, it is important to check the stability of each assay and each type of filter paper to ensure accurate results.
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 versus dengue, EBV, and measles with better stability). 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, 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. 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. 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. 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. 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.

Certain pathogens, such as HIV, are present in large quantities in whole blood (up to 10^7 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 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 sample 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, and HIV ELISA had a sensitivity of 83%.
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 buccal 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 samples119,120; parasite speciation was also possible. *Mycobacterium leprae* was detected by PCR from slit skin smears stored in ethanol (58%).121

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 zoontic diseases discussed above, including echinococcosis, brucellosis, and trypanosomiasis,134 are also important causes of mortality in other mammals. However, non-zoontic 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 Burgoyne135 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% \( (k = 0.234) \).\textsuperscript{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.\textsuperscript{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.\textsuperscript{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%.\textsuperscript{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.\textsuperscript{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.\textsuperscript{75,81,83} Serological tests for leptospirosis, treponemal infections, and some rickettsias have yielded excellent results,\textsuperscript{90,92,94} whereas others, such as brucellosis, have been less successful.\textsuperscript{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.\textsuperscript{142} Filter paper is easily and safely delivered using almost any existing transportation network. Recent advances in chemically pre-treated cards have provided increased size in handling and transporting samples.\textsuperscript{108}

Filter paper has been used with multiplex serological and NAATs to diagnose combinations of Hepatitis B, C, and HIV,\textsuperscript{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\textsuperscript{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.\textsuperscript{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.\textsuperscript{146,149} 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.\textsuperscript{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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FILTER PAPER DIAGNOSIS OF TROPICAL DISEASES

207


