

Leader

# The use of the dried blood spot sample in epidemiological studies

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The concept that capillary whole blood, obtained by heel or fingerprick and blotted on to a filter paper (Guthrie card), could be used to screen for metabolic disease in large populations of neonates was introduced in Scotland by Guthrie and Susie in 1963.<sup>1</sup> Neonatal screening for phenylketonuria became nationwide in 1969/70. Since then, Guthrie card samples from two to nine day old babies have been collected routinely in over 20 countries to screen for phenylketonuria and more recently for congenital hypothyroidism and sickle cell disorders.<sup>2-4</sup> The detection of markers of disease, such as medium chain acyl CoA dehydrogenase (MCAD deficiency), human chorionic gonadotrophin (hCG) in Down syndrome, and glycated haemoglobin in insulin dependent diabetes, and the estimation of drug levels have been also been investigated.<sup>5-8</sup> The limitations of sensitivity and specificity when screening such small volumes of blood (equivalent to 5-10 µl) restricted the use of dried blood spots (DBS) for many years. However, recent advances such as the production of monoclonal antibodies, expression of synthetic proteins, and the introduction of the polymerase chain reaction (PCR) have overcome many of these problems, allowing the potential of a vast bank of stored DBS to be realised by biochemists, geneticists, and microbiologists.

Blood obtained from neonates contains IgG antibodies that are primarily of maternal origin. This has enabled extensive studies to be performed on neonatal DBS to estimate the

seroprevalence of infections in women of childbearing age (table 1). Similarly, DBS obtained from children and adults have been used for the diagnosis of infections and for sentinel surveys in many parts of the world (tables 2 and 3).

In this review we concentrate on the benefits and applications of DBS technology with specific reference to the field of microbiology.

### The dried blood spot sample

Peripheral blood is collected by piercing the skin of either a finger or heel with a Sterilette™ and blotted onto high quality filter paper, for example a Guthrie card (S&S 903, Schleicher and Scheull UK). The use of S&S 2992, Whatman grade 1 or 3 papers has also been reported.<sup>52, 53</sup> The blood spot should then be allowed to air dry and ideally stored in low gas permeable bags that contain desiccant to reduce humidity. Samples can be tested immediately, stored at ambient temperature even under tropical conditions,<sup>54</sup> or frozen at -20°C, where the level of IgG antibodies may remain stable for several years.<sup>55-57</sup>

Before testing in the laboratory a disc of dried blood typically 3.0-5.5 mm in diameter, equivalent to 1-4 µl of serum, is punched either manually or with an automated machine into the wells of a flat bottomed microtitre plate. The blood is eluted out in phosphate buffered saline containing 0.05% Tween 80 and 0.005% sodium azide, overnight at 4°C (figs 1-3). The resultant plate containing the eluates forms the "master" from which dilutions can be made for subsequent testing.

### Advantages of DBS

#### REDUCTION OF RISKS

The collection of capillary blood on filter paper has significant advantages over venepuncture, particularly when bleeding neonates; it is simple to perform, requires minimal training, and does not involve the risks associated with the use and disposal of needles and syringes.

The DBS represents a low infectious hazard as some viruses such as HIV-1 and -2, human T cell leukaemia/lymphoma virus (HTLV) -I and -II, and hepatitis C virus (HCV) that are known to be present in serum or plasma lose infectivity owing to disruption of their envelope on drying.<sup>58</sup> However, virus in high titre may

Table 1 Anonymous surveys based on dried blood spot sample testing

Agent	Country	Assay	Year/reference
HIV	USA	ELISA and western blot	92 <sup>9</sup>
		As above	94 <sup>10</sup>
	Scotland England	GPA and western blot	93 <sup>11</sup>
		GPA and western blot GACPAT and western blot	97 <sup>12</sup>
HTLV-I	England	GPA, ELISA and immunoblot	95 <sup>13</sup>
	South Africa	GPA and immunoblot	96 <sup>14</sup>
HCV	England	"In house" ELISA, GPA, and RIBA	97 <sup>15</sup>
	Pakistan	As above	98 unpublished
	South Africa	As above	98 unpublished
<i>Toxoplasma gondii</i>	England	Latex agglutination test	92 <sup>16</sup>
	Denmark	ELISA	95 <sup>17</sup>
	Ireland	Latex agglutination test	98 <sup>18</sup>

ELISA, enzyme linked immunosorbent assay; GACPAT, IgG capture gelatin particle adherence test; GPA, gelatin particle agglutination; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV, human T cell leukaemia/lymphoma virus; RIBA, recombinant immunoblot assay.

Table 2 Dried blood spot sample protocols and sentinel surveys based on the use of blood spots collected on filter paper

Agent	Study	Assay type	Country of survey	Year/ref
<b>Viral</b>				
HBV and HAV	Prevalence of markers	RIA, RPHA	Saudi Arabia	78 <sup>19</sup>
HBV	Epidemiology	RIA	Italy	81 <sup>20</sup>
Rubella		ELISA	Denmark	81 <sup>21</sup>
HBV and HAV	WHO field studies	RIA	Sao Tome	85 <sup>22</sup>
Measles	Vaccine study	HAI	Guinea-Bissau	94 <sup>23</sup>
	Vaccine study	ELISA	Italy	98 <sup>24</sup>
Measles, mumps and rubella	MMR vaccine study	ELISA	Italy	94 <sup>25</sup>
				98 <sup>26</sup>
HAV	Prevalence study	ELISA	Spain	95 <sup>27</sup>
<b>Bacterial</b>				
<i>Mycobacterium leprae</i>	Epidemiological study	PA	India	92 <sup>28</sup>
Diphtheria and tetanus antitoxin	Immunity study and vaccine monitoring	ELISA	Australia	94 <sup>29</sup>
		ToBI	Vietnam	96 <sup>30</sup>
<b>Protozoan</b>				
<i>Trypanosoma cruzii</i>	Chagas disease	IFAT, HA, ELISA	Brazil	90 <sup>31</sup>
<b>Helminthic</b>				
<i>Echinococcus granulosus</i>	Hydatidiosis	ELISA	Argentina	88 <sup>32</sup>
		ELISA	Kenya	93 <sup>33</sup>

ELISA, enzyme linked immunosorbent assay; GPA, gelatin particle agglutination; HAV, hepatitis A virus; HBV, hepatitis B virus; HIA, haemagglutination inhibition assay; IFAT, immunofluorescence antibody test; PA, particle agglutination; RIA, radioimmunoassay; RPHA, reverse passive haemagglutination; ToBI, toxin binding inhibition test.

remain viable on a surface for several days and hepatitis B virus (HBV) may remain infectious for at least seven days.<sup>59</sup>

Risks associated with shipping are minimised as DBS cannot leak or be broken in transit and there is no requirement for carriage on dry ice. Handling of potentially infected material is also reduced as the need to centrifuge and separate sera from blood clots is eliminated.

#### ECONOMIC ADVANTAGES

Collection and processing of DBS is considerably cheaper than samples collected by venepuncture. Sterilettes are available at a one third of the price of sterile disposable needles and syringes in the United Kingdom.<sup>52</sup> DBS can be readily shipped in sealed envelopes to reference centres, whereas sera need to be packed in break-proof containers. Transport of

frozen sera may require the use of dry ice or liquid nitrogen, requiring further specialised handling and adding considerable weight to the item.

The majority of the diagnostic techniques, for example particle agglutination tests and enzyme immunoassays (EIA) which have been used to screen for antibodies in DBS, are simple and generally require little capital expenditure. The use of the agglutination test is particularly cost-effective as the assay can be performed in the absence of an electricity supply. This is ideally suited to countries with limited health budgets.

#### SURVEILLANCE

Large scale epidemiological surveys can be performed rapidly, enabling strategic decisions to be made about targeting at risk populations.

Table 3 Dried blood spot sample based protocols specific for the serodiagnosis of infection

Agent	Disease	Assay type	Country	Year/ref
<b>Viral</b>				
Measles	Measles	HAI	USA	84 <sup>34</sup>
Dengue	Dengue fever	ELISA	Brazil	91 <sup>35</sup>
		ELISA	Thailand	94 <sup>36</sup>
Rubella	Rubella	HI, SPIHIT	Thailand	91 <sup>37</sup>
<b>Bacterial</b>				
<i>Mycobacterium leprae</i>	Leprosy	RIA	India	90 <sup>38</sup>
		ELISA	Polynesia	91 <sup>39</sup>
		PA	India	92 <sup>28</sup>
<i>Helicobacter pylori</i>	Dyspepsia	ELISA	Australia	91 <sup>40</sup>
<i>Brucella sp</i>	Human brucellosis	IgM-ELISA	Spain	95 <sup>41</sup>
<i>Treponema pallidum</i>	Syphilis	GPA	England	98 <sup>42</sup>
<b>Protozoan</b>				
<i>Plasmodium falciparum</i>	Malaria	ELISA	Venezuela	89 <sup>43</sup>
<i>Toxoplasma gondii</i>	Congenital toxoplasmosis	ELISA	USA	92 <sup>44</sup>
		Dye test	England	94 <sup>45</sup>
		ELISA	Denmark	95 <sup>17</sup>
<i>Giardia lamblia</i>	Giardia	ELISA	Saudi Arabia	93 <sup>46</sup>
<i>Leishmania spp</i>	Kala-azar	Dot-ELISA	India	93 <sup>47</sup>
	Leishmaniasis	PA	India	95 <sup>48</sup>
<i>Trypanosoma cruzi</i>	Chagas disease	IFAT, HAI, ELISA	Brazil	95 <sup>49</sup>
<b>Helminthic</b>				
<i>Schistosoma haematobium</i>	Bilharzia	ELISA	Somalia	88 <sup>50</sup>
<i>Brugia malayi</i>	Filariasis	IgG <sub>4</sub> -ELISA	Indonesia	96 <sup>51</sup>

ELISA, enzyme linked immunosorbent assay; GPA, gelatin particle agglutination; HAI, haemagglutination inhibition assay; IFAT, immunofluorescence antibody test; PA, particle agglutination; RIA, radioimmunoassay; SPIHIT, solid phase immunosorbent haemagglutination inhibition test.

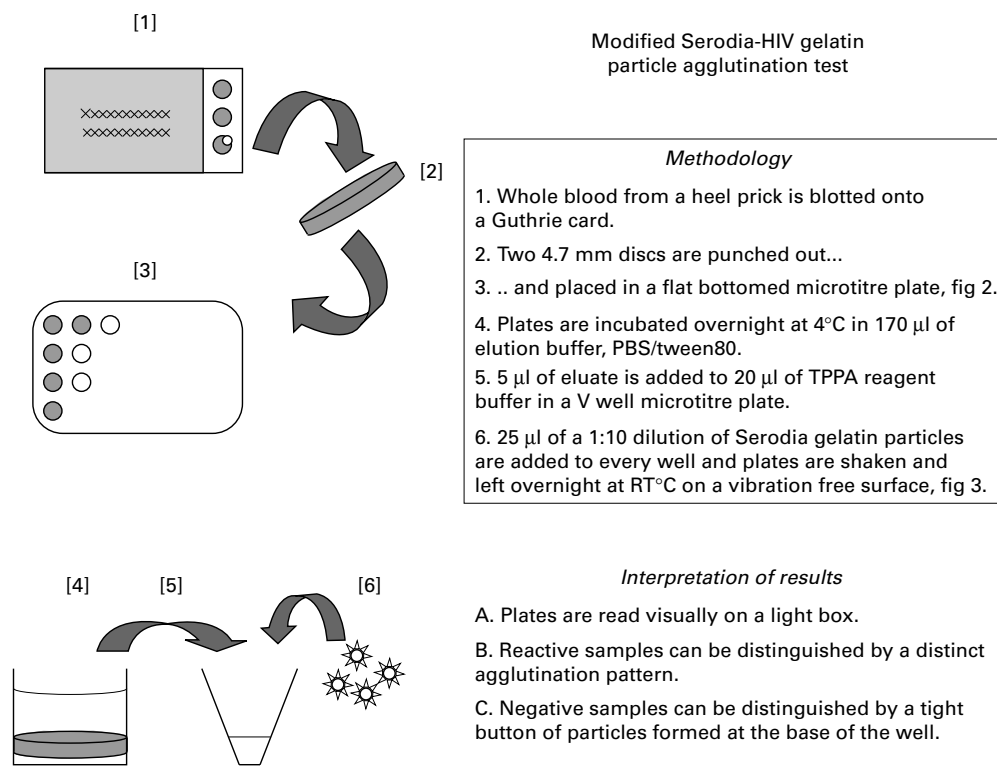


Figure 1 Procedure for processing dried blood spot samples. RT, room temperature.

This may be particularly valuable for monitoring herd immunity and the extent of vaccine coverage at a time when the eradication of several viruses—for example, poliomyelitis, measles, mumps, and rubella—is in prospect in many parts of the world. The Guthrie card or neonatal DBS provides information on the prevalence of antibody to a pathogen in women of childbearing age, thus giving a more representative indication of the impact within a community than screening blood donors, who

are often unrepresentative. Furthermore, within the United Kingdom, coverage of the Guthrie card programme for metabolic screening approaches 100%,<sup>2</sup> providing a resource that has almost complete coverage of a specific target population, a rare event in epidemiological studies.

#### Application of DBS technology

Following the historical use of DBS to study the prevalence of HBV,<sup>19</sup> measles,<sup>34</sup> and

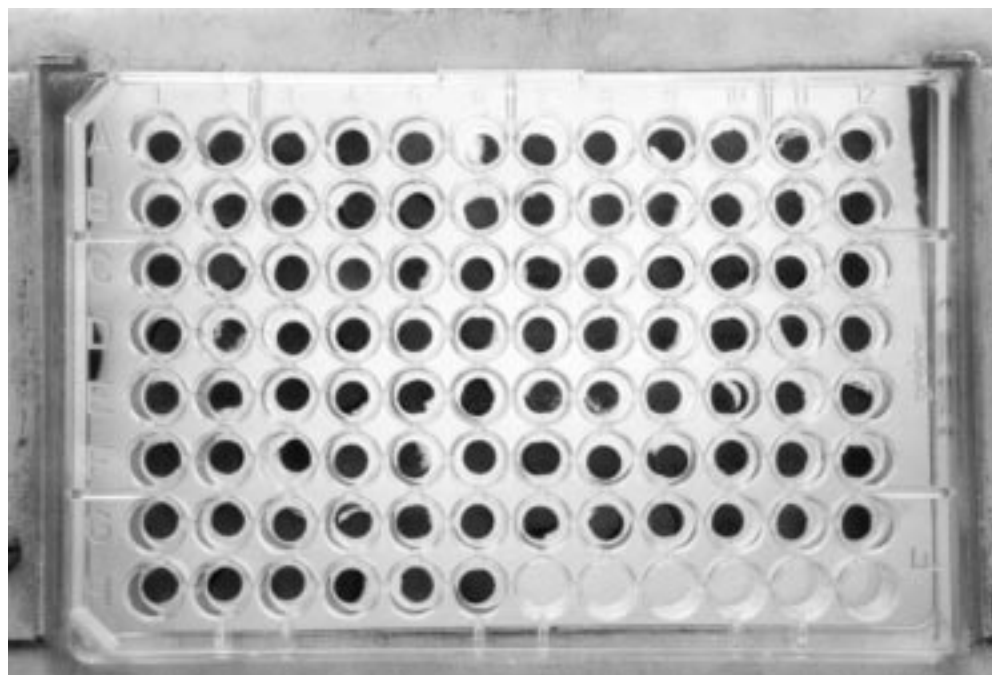


Figure 2 Two dried blood spot samples are punched into the first 90 wells of a flat bottomed plate.

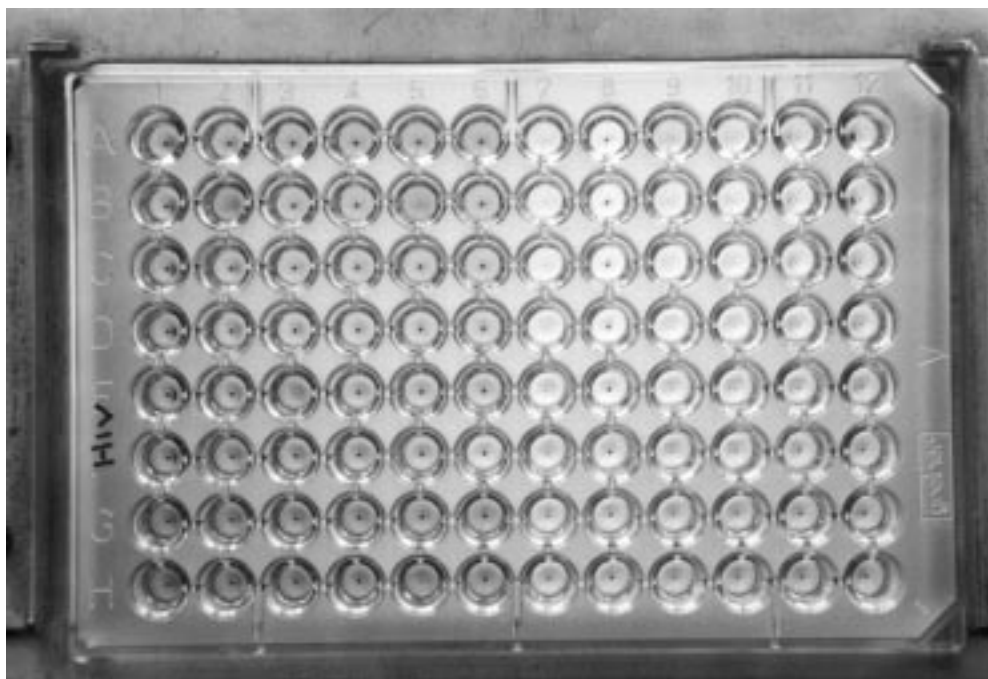


Figure 3 The modified Serodia HIV GPA test. Agglutination in wells B2, E3, B5, and H5 indicates reactivity to HIV-1 specific gelatin particles. In the absence or reactive particles settle at the bottom of the V well. Columns 7–12: initial reactive samples are titrated out by double dilution to end point; column 7: positive serum control (1:64); column 8: negative serum control (< 1:5); column 9: sample B2 (1:64); column 10: sample E3 (1:64); column 11: sample B5 (1:16); column 12: sample H5 (1:8).

rubella,<sup>21</sup> the feasibility of using similar technology for sentinel surveillance of HIV in the developing world was evaluated.<sup>60 61</sup> At the same time the need for an inexpensive and effective means for monitoring the extent of HIV in Europe<sup>62 63</sup> and the USA<sup>64</sup> was being increasingly recognised.

#### SURVEILLANCE OF HIV

Initial doubts over the sensitivity and specificity of DBS based compared with serological assays were dispelled by parallel testing of DBS and sera<sup>55 65 66</sup> which showed 99–100% concordance. Furthermore extensive studies proved that concerns about the presence of haem in the eluate and adverse storage, for example in tropical conditions, causing false reactions were unfounded.<sup>54 55 67 68</sup>

The prevalence of antibodies to HIV-1 in women of childbearing age based on the testing of anonymous DBS obtained from neonates was first reported in the USA in 1988.<sup>64</sup> Laboratories at the Centers for Disease Control, Atlanta, extended the newborn screening quality assurance programme to include test panels of anti-HIV positive and negative DBS, which were distributed throughout the USA and overseas.<sup>69</sup> Subsequently in 1990, after a successful pilot study, anonymous neonatal testing was introduced in the United Kingdom and a similar quality control programme was made available by the Central Public Health laboratory in London.<sup>70</sup>

Since the inception of anonymous screening at Great Ormond Street Hospital for Children and the Institute of Child Health, over 1.2 million DBS have been tested enabling accurate data to be obtained about the prevalence of

HIV-1 in mothers in the Thames region over an eight year period.<sup>12</sup>

#### HUMAN T CELL LEUKAEMIA/LYMPHOMA VIRUS

HTLV-I has been recognised as the causal agent of adult T cell leukaemia, tropical spastic paraparesis, myelopathy, and uveitis. Transmission may occur by several means including breast feeding and through contaminated blood. In order to assess the extent of HTLV-I infection in women of child bearing age in London, a modified gelatin particle assay costing 10 p per test was used to screen over 10 000 DBS obtained from neonates.<sup>13</sup> The prevalence rate was 0.05%, lower than that reported (0.21% to 0.27%) in antenatal surveys from other London districts but higher than the rates reported in blood donors (0.004% to 5%), reviewed in 1996.<sup>71</sup> The technique has also been successfully applied to screen mothers and children in South Africa.<sup>14</sup>

#### TOXOPLASMA GONDII

*Toxoplasma gondii* is recognised to be a major cause of congenital infection, which may result in mental retardation and late onset chorioretinitis. However, the prevalence of infection varies widely in different European countries, ranging from less than 15% in Scandinavia and England to more than 70% in France.<sup>72</sup> This has led to differing opinions about the need for antenatal screening.

A modified latex test costing less than 5 p per test has been used for extensive surveys to determine the maternal prevalence of antibodies in London. The results of screening 12 902 neonatal DBS showed the rate in United Kingdom born women in inner London was

12.5%,<sup>16</sup> similar to the rate of 13.3% obtained when 3768 sera from a comparable group were screened by EIA.<sup>72</sup> Recently, neonatal Guthrie cards were used to screen 16 035 specimens collected during 1995, representing 33% of the live births registered in Eire that year. The results showed a prevalence ranging from 20.3% in urban Dublin to 45% in a rural area, Louth, and an overall prevalence of 25%.<sup>18</sup> This compares with a prevalence of 31% in sera collected between 1980 and 1986 from women born in Eire.<sup>72</sup>

#### DETECTION OF NUCLEIC ACID IN DBS

The potential for the use of DBS for the detection of nucleic acid was first realised in 1987, when DNA was released from the filter paper by microextraction for the purposes of newborn screening.<sup>73</sup> The molecular analysis of DNA obtained from neonates has enabled detection of genetic mutations responsible for, among others, cystic fibrosis,<sup>74</sup> oncogenesis,<sup>76</sup> markers of type 1 diabetes,<sup>77</sup> and  $\alpha$  thalassaemia.<sup>78</sup>

The stability of DNA has made it possible to apply PCR to detect proviral HIV DNA sequences several months after sample collection, even when DBS had been held at room temperature.<sup>79</sup> Recent studies have shown that HIV DNA and RNA can be recovered after five years without refrigeration,<sup>80</sup> which may provide the potential for retrospective surveys and the use of stored Guthrie cards as DNA banks.<sup>81</sup>

An unexpected benefit of storage is that inhibitors of PCR such as protein, haemoglobin, and iron seem to become increasingly resistant to elution, whereas "fixation" of nucleic acid to the matrix of the filter paper seems to aid stability and does not impair its elution.<sup>82</sup>

The ability to detect HIV proviral DNA in DBS obtained from neonates has been used in several retrospective studies to estimate rates of vertical transmission.<sup>83-88</sup> However, it has been shown that there are some limitations to the use of Guthrie cards. For example, proviral DNA could only be detected in 50% of infected children aged less than 10 days, the time at which samples are generally collected from neonates in the United Kingdom. In contrast, virtually all infected children were identified when DBS obtained from infants aged one month were tested.<sup>54</sup> Amplification of DNA has enabled sequences encoding the *gag* and *env* regions to be studied, providing valuable epidemiological data about the geographical distribution of HIV subtypes.<sup>88</sup> Quantification of HIV-1 RNA using kit based amplification technology in the form of NASBA and Amplicor-HIV has been described using dried plasma spots collected on filter paper.<sup>80</sup> PCR methods have also been developed to detect proviral sequences from DBS obtained from neonates infected with cytomegalovirus<sup>90</sup> and patients infected with HTLV-I.<sup>91</sup>

#### Concluding remarks

Significant increases in the sensitivity and specificity of diagnostic tests have allowed the

detection of microquantities of antibodies in saliva, dried blood spot samples, and urine. These samples herald a move away from invasive techniques and the need to obtain large volumes of blood. Dried blood spot samples have an advantage over saliva in that the quality of the sample is less variable and transport and storage is comparatively simple. Application of the techniques described above is ideally suited to surveillance in countries with limited resources and in those participating in large anonymous epidemiological surveys. The ease of transport can ensure effective quality control measures are put in place, highlighted by the success of the quality assurance programme at the Centers for Disease Control, Atlanta, in the shipment of anti-HIV-1 positive and negative DBS to international participants involved in newborn anonymous HIV screening programmes.<sup>69</sup>

Guthrie cards have been collected and stored for many years and provide the potential to perform numerous serological and genetic studies. This has stimulated debate as to who should have access to this material and how the use of such a unique resource can be maximised.

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