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Current and future applications of dried blood spots in viral disease management

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ABSTRACT

Almost five decades after their first application in diagnostics, dried blood spot (DBS) cards remain to be of key interest in many research areas and clinical applications. The advantages of sample stability during transport and storage, can now be combined with the high sensitivity of novel diagnostic techniques for the measurement and analysis of nucleic acids, proteins and small molecules which may overcome the limitations of the small samples sizes in DBS cards. Here we present a survey of the literature on the use of DBS cards for diagnosis, monitoring and epidemiological studies of virus infections other than HIV, including CMV, HBV, HCV, HAV, HEV, HTLV, EBV, HSV, measles-, rubella- and dengue-virus. The minimal invasiveness of sampling and the relative ease of handling and storing DBS cards is expected to offer additional opportunities to measure and analyze biomarkers of viral disease in resource poor settings or when limited amount of blood can be obtained. Large retrospective studies of virus infections in newborns using stored DBS cards have already been undertaken for screening of congenital infections. In addition, DBS cards have been used prospectively for prevalence studies, outbreak surveillance, mass screening for viral infections, follow-up of chronic infection and its treatment in resource-limited areas. We do not expect that current wet sampling techniques of plasma or serum will be replaced by DBS sampling but it allows extension of sampling in persons and settings that are currently difficult to access or that lack suitable storage facilities. In conclusion, DBS card sampling and storage will aid adequate outbreak management of existing and emerging viral diseases.

Contents

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1. Introduction

Dried blood spot (DBS) cards have successfully been used in neonatal screening for almost half a century (Guthrie and Susi, 1963). Initially they were used for metabolic disease screening in neonates. With time and the development of new techniques, the disease range and possible applications of DBS have greatly been expanded. Traditionally, plasma and serum samples prepared from comparatively large quantities (typically 1–6 ml for adults) of whole blood after venipuncture are standard biological substrates for laboratory diagnosis, epidemiological surveys and therapy monitoring of viral infections. To prevent degradation, these samples normally need to be processed in the laboratory on the same day, and the products need to be kept frozen or refrigerated from isolation onwards. DBS samples are being tested as an alternative method of sampling for laboratory diagnosis, epidemiological studies and therapy monitoring of viral infections. DBS card samples can be prepared from a few drops of blood, typically 15–200 μl, obtained from a capillary blood stab, i.e. a finger prick (Turner and Holman, 1978). Thus, DBS cards also offer the opportunity to collect specimens from young children in whom venipuncture is difficult to perform and in whom sampling of even small blood volumes may be too demanding. Furthermore, taking a capillary blood stab requires little clinical training. Logistics and storage of DBS cards provides some important advantages over the traditionally used liquid forms of whole blood, serum and plasma (Parker and Cubitt, 1999). Viral RNA and DNA, antibodies or other proteins and antiviral drugs or their metabolites remain stable in DBS cards for relatively long periods when stored with desiccant in closed bags at ambient temperature, compared to wet plasma or serum stored under the same conditions. The cards are small and light and easily to transport safely.

A common protocol for all purposes comprises a minimum of 2 h drying of the blood spot, storage in plastic zip lock bags with desiccant. A maximum storage of 2 weeks at room temperature (RT) is recommended. Freezing at −20 °C is recommended when DBS cards are stored for more than 2 weeks (Mei et al., 2001; Therrell et al., 1996). Therefore, the cards can be sent by regular mail to a laboratory. Punched out disks from DBS cards normally contain the equivalent of 3.1–12.4 μl blood depending on the punch size (3–6 mm) (Corso et al., 2010). Increasing the number of disks is being used to enlarge the sample volume. A disadvantage of DBS samples compared with plasma or serum samples is the presence of erythrocytes and thus large amounts of hemoglobin that may interfere in PCR assays (Condorelli et al., 1994). A point that has received little attention in the literature is the safety. Some viruses remain infectious for long periods in dried splashes on surfaces and are likely to remain infectious in DBS (Rutula, 2008). This point has been addressed for dengue virus (Prado et al., 2005) and needs further attention for other viruses in future studies.

Recently, excellent reviews have been published on the use of DBS for diagnosis and therapy monitoring of HIV infection (Bertagnolio et al., 2010; Hamers et al., 2009; Johannessen et al., 2009). Here we present a review of the published literature on the use of DBS cards for diagnosis, monitoring and epidemiology of viral infections other than HIV. Our review summarizes data on the performance of DBS card assays compared to serum or plasma assays. We also provide data on the stability of various markers in DBS cards upon storage. In addition we will give a non-exhaustive overview of current applications of DBS cards. Finally, future perspectives on DBS cards use will be addressed.

1.1. Methods

For this review we identified relevant studies on DBS in PubMed (last search performed on the first of March in 2011). The search terms used were: dry or dried, in combination with blood or fluid or serum and spot. The selection criteria were limited by research on humans and the English language. The abstracts of 901 articles were studied for suitability. Studies on metabolic disease and HIV only were removed. Furthermore studies that only reported seroprevalence without analytical validation data were excluded. In addition, studies with analytical validation data that compared less than 10 positive samples were not taken into account. Finally, 44 studies fulfilled the criteria, comparing the performance of DBS card-based assays with the golden standard. These studies are included in the Table 1 with technical details on DBS sample recovery, signal detection and performance and stability of the DBS sample under various storage conditions.

2. Application of dried blood spot samples

2.1. Neonatal screening and vertical transmission

2.1.1. Cytomegalovirus (CMV)

Until today the exact disease burden of congenital CMV infection remains unclear. The reason for this is that 90% of the cases with congenital CMV infection are asymptomatic at birth (Syggelou et al., 2010). This does not necessarily mean that these infections may not lead to sequelae. It has been estimated over 10% children infected may develop sensory hearing loss (Pass, 2005). Neonatal screening for CMV may in theory allow for early intervention. The gold standard for diagnosis of congenital CMV infection is a positive CMV culture from urine collected during the first 3 weeks of life. This technique, however, is not suitable for large-scale screening. The DBS techniques for the detection of CMV DNA extracted from neonatal heel prick cards collected for routine metabolic screening are an alternative tool to screen for neonatal CMV infection and have already been applied in several studies. However, as discussed below some technical difficulties still remain. Since this review focuses on the technical aspects of DBS techniques, we will not further address the ramifications of the options for intervention.

Studies have shown that the specificity for detection of congenital CMV infection approaches 100% when the CMV-DNA PCR of dried blood stored on neonatal heel prick cards is negative (see Table 1). It should be noted however that in one international quality assessment program 9% of the laboratories reported false positive results for detection of CMV DNA in DBS (Barbi et al., 2008).

The sensitivity of a positive CMV DNA PCR from DBS to detect congenital CMV infection varies considerably between studies. Excellent sensitivities of 99–100% were reported in studies that included a relative high proportion of subjects who were symptomatic at birth (Barbi et al., 1996, 2000; Scanga et al., 2006; Vauloup-Fellous et al., 2007). Whereas lower sensitivities ranging from 34% to 82% were reported when the groups of congenitally infected children studied had distributions of symptomatic and asymptomatic cases more representative of the general population (Boppana et al., 2010; Johansson et al., 1997; Soetens et al., 2008; Yamamoto et al., 2001). This may be due to the fact that whole blood CMV DNA concentrations are on average higher in neonates with signs and symptoms of CMV disease than those who are asymptomatic

Table 1
Overview of DBS card usage in detection and diagnostics of viral infections other than HIV.

Virus	Target molecule	Specimen on filter paper (brand name)	Deviation from consensus storage condition	Disk diameter Extraction method Optimal elution	Assay (brand name)	Comparison to golden standard sensitivity%; specificity% Correlation coefficient Limit of detection (LOD)	Target degradation during storage	Reference
CMV	CMV DNA UL 55 gene UL 69 gene	Capillary blood (NR)	Storage at RT for up to 24 months stored side by side	10 mm QiaAMP DNA blood mini kit (Qiagen)	qPCR (in house)	Sens 72–74.2%; spec NR LOD 1500 copies/ml whole blood	80% Degradation of CMV DNA during 2 years of RT storage	Atkinson et al. (2009)
CMV	CMV DNA UL 55 gene Major IE gene	Capillary blood, spiked venous blood (NR)	RT and NR	3 × 3 mm 45 µl MEM (triplicate testing) 1 × 3 mm 35 µl MEM (triplicate testing) Heat shock	Nested PCR (in house) Nested PCR (CMV Early Oligo Mix, Bioline Amplimedical)	Sens 100%; spec 100% LOD 4000 copies/ml whole blood	NR	Binda et al. (2004)
CMV	CMV DNA UL 83 gene	Capillary blood (NR)	NR	NR 2 ml lysis buffer (Biomerieux) NucliSens easy MAG (Biomerieux) 30 min RT	qPCR (NR)	Sens 100%; spec NR LOD 6600 copies/ml	Detection of CMV DNA after 11,5 y storage	Boudewyns et al. (2009)
CMV	CMV DNA UL 123 gene UL 55 gene	Spiked venous blood (NR)	NR	3 mm-whole area of card (50 µl) 200 µl TES Phenol–chloroform QIAmp blood mini kit EasyMag (Biomerieux) 25 µl cell medium Heat extraction	qPCR Nested PCR qPCR, nested PCR qPCR Nested PCR qPCR, nested PCR	200 copies/ml whole blood, whole card 200 copies/ml whole blood, 3 × 3 mm 400 copies/ml whole blood, whole card 20.000 copies/ml whole blood, 3 × 3 mm 20.000 copies/ml whole blood, 2 × 3 mm 20.000 copies/ml whole blood, whole card	NR	Gohring et al. (2010)
CMV	CMV DNA UL 83 gene	Spiked venous blood (903 Whatman)	Storage at RT, up to 3 months	3.2 mm 35 µl MEM Heat extraction 3 × 3.2 mm 125 µl MEM Heat extraction 300 µl ATL buffer QIAamp DNA investigator kit (Qiagen) NR 300 µl ATL buffer QIAamp Investigator BioRobot kit NR 250 µl Lysis buffer MagNA Pure LC (Roche) NR	qPCR	Single testing sens 73%; spec 100% Testing 10^4–10^5 copies/ml ($n = 3$) sens 100% Single testing sens 67%; spec 100% Testing 10^4–10^5 copies/ml ($n = 3$) sens 100% Single testing sens 71%; spec 100% Testing 10^4–10^5 copies/ml ($n = 3$) sens 100% Single testing sens 67%; spec 100% Testing 10^3–10^4 copies/ml ($n = 3$) sens 100% Single testing sens 62%; spec 100% Testing 10^4–10^5 copies/ml ($n = 3$) sens 100% Single testing sens 54%; spec		de Vries et al. (2009)

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Table 1 (*continued*)

Virus	Target molecule	Specimen on filter paper (brand name)	Deviation from consensus storage condition	Disk diameter Extraction method Optimal elution	Assay (brand name)	Comparison to golden standard sensitivity%; specificity% Correlation coefficient Limit of detection (LOD)	Target degradation during storage	Reference
				200 µl buffer ATL QIAsymphony DNA mini kit (Qiagen) NR 2 ml NucliSens easyMag lyisis buffer NucliSens easyMAG Extraction Kit NR 220 µl PBS Dynabeads Silane viral NA kit		100% Testing 10^4–10^5 copies/ml ($n = 3$) sens 100% Single testing sens 53%; spec 100% Testing 10^4–10^5 copies/ml ($n = 3$) sens 100% Single testing sens 32% Testing 10^4–10^5 copies/ml ($n = 3$) sens 57%		
CMV	CMV DNA UL 55 gene	Capillary blood (NR)	NR Storage 15 days up to 4 years	3 × 3 mm 25 µl MEM heat extraction	Nested PCR (in house)	Sens 100%; spec 99%	NR	Barbi et al. (2000)
CMV	CMV DNA UL 123 gene	Capillary blood (NR)	NR	6 × 3 mm 45 µl water Heat extraction 45 µl MEM Heat extraction	Nested PCR (in house)	Sens 58%; spec NR Sens 63%; spec NR	NR	Barbi et al. (1996)
	UL 55 gene			45 µl water Heat extraction 45 µl MEM Heat extraction		Sens 89%; spec NR Sens 100%; spec 100%		
CMV	CMV DNA US 8 gene UL 75 gene UL 83 gene	Capillary blood (903 S&S)	Storage at RT, median storage time 73 months	10 mm (whole single spot) 400 µl TES Phenol–chloroform EasyMAG automated extraction platform (bioMérieux)	Nested PCR (in house) qPCR (in house) Nested PCR (in house) qPCR (in house)	Sens 66%; spec 100%; LOD 2000 copies/ml whole blood Sens 73%; spec 100% Sens 82%, spec 100% Sens 45%; spec 100% LOD 9400 copies/ml whole blood	Detection of CMV DNA after 10,8 y storage	Soetens et al. (2008)
CMV	CMV DNA UL 55 gene UL 122 gene	Capillary blood (903 Whatman)	Storage at RT, mean storage time of 14.6 days	2 × 3 mm 2 ml lysis buffer Qiagen M48 robotic system	qPCR single primer (in house) qPCR 2 primers (in house)	Sens 28.3%; spec 99.9% 250 copies/ml whole blood Sens 34%; spec 99.9%; LOD 50 copies/ml whole blood	NR	Boppana et al. (2010)
CMV	CMV DNA UL 54 gene	Capillary blood (903 S&S)	NR	10 mm 400 µl QIAamp buffer QIAamp DNA micro kit	qPCR (in house)	Sens 100%; spec 100% LOD 1600 copies/ml whole blood	NR	Scanga et al. (2006)
HSV	Anti-HSV-1 IgG, HSV-2 IgG	Venous blood (3 Whatman)	No plastic bags or desiccant	6 mm 150 µl PBS o/n 4 °C	EIA (Focus)	Sens 93–100%; spec 100% depending on equivocal results Correlation coefficient 0.80 Concordance HSV-1 95.4% HSV-2 100%	Stable >1 yr at −20 °C abd 30 days at RT 15% decrease in mean index value	Hogrefe et al. (2002)
HBV	HBV-DNA HBV core gene Pre core mutant Pre-core region	Capillary or venous blood (903 S&S)	No desiccant	5 mm 200 µl H_2O QIAamp (Qiagen) o/n 37 °C	qPCR (in-house) Nested PCR	Correlation coefficient HBV-DNA 0.96 Concordance G1896A 100% LOD 100–10^8 copies/ml LOD: 2000 copies/ml	Stable HBV DNA levels after 3 weeks −20 °C storage	Jardi et al. (2004)

	GenotypeS gene sequenceYMDD motif				RFLP Line probe assay (Inno-Lipa Innogenetics)	Concordance genotyping 90% Concordance YMDD identity 97%		
HBV	Anti-HBs Ig, anti-HBc Ig, HBsAg	Capillary blood (Whatman)	Consensus protocol	6 mm 500 µl PBS o/n RT	EIA (Abbott-Murex Biotech)	Sens 100%; spec 100%	NR	Komas et al. (2010)
HBV	HBsAg	Venous blood (3 Whatman)	No desiccant, storage at RT	25 µl 50 µl PBS o/n 4 °C	EIA (Shantest)	Sens 79%; spec 89%	NR	Forbi et al. (2010)
HBV	Anti-HBc Ig	Capillary blood or serum (903 S&S)	NR	2 × 3 mm 200 µl PBS + Tween 0.5% o/n 4 °C	Haemagglutination (CORECELL, Green Cross)	Sens 79%; spec 100%	NR	Tappin et al. (1998)
HBV	HBV-DNA	Capillary blood and serum mixed in blood cells (3 MM Whatman or BA85 S&S)	No desiccant	7 mm (Whatman) NR (S&S) 75 µl H_2O 10′ microwave 650W	qPCR (in house)	Concordance 93% 10^1–10^4 copies/5 µl depending on PCR protocol	Stable for 5 months at 37 °C	Gupta et al. (1992)
HBV	HBsAg	Venous and capillary blood (BFC 180 Whatman)	No plastic bag or desiccant, storage at 30–33 °C up to 4 weeks	2x6 mm 200 µl PBS o/n	Immuno-chromotography (Abbott)	Sens 96%; spec100%	Stable for at least 4 weeks under humid conditions	Mendy et al. (2005)
HCV	Anti-HCV Ig, HCV-RNA and genotype	Capillary blood (903 Whatman)	Consensus protocol	6 mm 200 µl diluent o/n	EIA (Ortho HCV v3), Immunoblot (Innogenetics)	Sens 99%; spec 98%	HCV-RNA stable for at least 6 days at −20 °C. HCV-RNA stable for less than 3–6 days at RT	Tuaillon et al. (2010)
				2x6 mm 400 µl PBS + Tween 0.05% + BSA 10% Cobas ampliprep NA 2 h 4 °C	qPCR (Cobas Taqman HCV Roche) Sequencing (TRUGENE HCV 5′NC Kit, Siemens	PCR sens 97%; spec N.R qPCR sens 89%; spec NR Sens 100%; spec NR		
HCV	Anti-HCV Ig	Venous blood (903 S&S)	Consensus protocol	6 mm 200 µl PBS Tween 0.05% ± dried milk 0.3% o/n 4 °C	EIA (Monolisa, anti-HCV PLUS v2, Bio-Rad)	Sens 100%; spec 100%	NR	Croom et al. (2006)
HCV	HCV-RNA and genotype	Venous blood (903 S&S)	NR	NR 9 ml GITC lysis buffer 2hrs RT Silica based extraction (Boom technology, Organon)	PCR (in-house) LIPA (Bayer)	Sens > 99%; spec > 95% Concordance genotyping 100%	HCV-RNA stable for at least 11 months at RT	Solmone et al. (2002)
				500 µl H_2O 400 µl target capture reagent 1 h 60 °C TMA extraction protocol	TMA (Bayer)	Sens >99%; spec >95% LOD: 24.000 IU/ml		
HCV	Anti-HCV IgG, c22-3, c200 and NS 5	Capillary blood serum spiked in blood (NR)	No desiccant, storage at 4 °C	5.5 mm 100 µl PBS + Tween 0.05% o/n 4 °C	IgG ELISA (in-house) Immunoblot (RIBA v3)	Concordance 99.8% Concordance 98.9%	NR	Parker et al. (1997)
HCV	Anti-HCV Ig	Venous blood (NR)	No plastic bag or desiccant, storage at 4 °C Storage duration not reported	3 mm 100 µl PBS + Tween 0.05% o/n 4 °C	EIA (Monolisa Sanofi Pasteur)	Sens 97.2–100%; spec 87.5–100% depending on cut off	NR	McCarron et al. (1999)
HCV	HCV-RNA NCR region	Venous blood (3 Whatman)	No transportation or storage	3 × 3 mm QIAamp DNA mini kit (Qiagen)	PCR (SYBR green Qiagen)	Sens 94%; spec 100% LOD: spiked blood 2500 copies/ml	NR	De Crignis et al. (2010)

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Table 1 (*continued*)

Virus	Target molecule	Specimen on filter paper (brand name)	Deviation from consensus storage condition	Disk diameter Extraction method Optimal elution	Assay (brand name)	Comparison to golden standard sensitivity%; specificity% Correlation coefficient Limit of detection (LOD)	Target degradation during storage	Reference
EBV	Anti-EBV p18 VCA IgG	Capillary blood (903 S&S)	No plastic bag or desiccant	2,5 mm 250 μl assay diluent buffer o/n RT	EIA (Diasorin)	Correlation coefficient 0.97	Stable >8 weeks at 4 °C and RT >2 years −20 °C and up to 6 freeze/thaw cycles	McDade et al. (2000)
HTLV	HTLV proviral DNA pX gene	Venous whole blood (NR)	−4, 20, 25 °C	3 × 6 mm 100 μl H_2O 15′ heat	PCR (in house)	Sens 100%; spec 100% LOD: 8–10 copies of pX/0.29–1.0 μg DNA	Proviral DNA stable for at least 7 weeks at 25 °C	Noda et al. (1993)
HTLV	Anti-HTLV Ig	Serum and plasma spiked (simulated) blood samples (NR)	No desiccant, storage at 4 °C	5.5 mm 100 μl PBS + Tween 0.05% o/n 4 °C	Agglutination test (Serodia HTLV-I, Fujirebio)	Sens 100%; spec NR	NR	Parker et al. (1995)
					EIA (Select-HTLV, Biostat Diagnostics)	Sens 100%; spec NR LOD: serodia titer >1:10 can be detected		
					WB (HTLV-BLOT 2.3, Diagnostic Biotechnology)	Sens 92%; spec NR		
MV	Anti-MV Ig IgM, N specific IgM, MV RNA NP gene	Capillary blood (903 S&S)	No plastic bag or desiccant, storage up to 33 months at 4 °C	1 or 2 × 6 mm 100 μl PBS 2% FBS o/n 4 °C	In-house capture IgM sandwich ELISA	NR	IgM stable for one year when stored at 4 °C	El Mubarak et al. (2004)
				220 μl PBS + Tween 0.5% + dried milk 5% o/n 4 °C, 250 μl H_2O + lysis buffer	Indirect IgM ELISA (Dade) PCR and Sanger sequencing			
MV	Anti-MV IgM	Venous blood (903 S&S)	No desiccant, storage up to 24 months at 4 °C	2 × 6 mm 220 μl PBS + Tween 0.5% + dried milk o/n 4 °C	EIA (Dade)	Sens 100%; spec 97.1%, kappa value 0.93	IgM stable at 4 °C up to 6 months	Riddell et al. (2002)
MV	Anti-MV IgM and IgG MV nucleoprotein	Capillary blood (903 S&S)	Time between collection and storage at −20 °C not specified	2 × 6.4 mm 1 ml H_2O + Tween 5% + dried milk 5% + timerosol 1%	Capture IGM EIA (NR)	Concordance 94% Correlation coefficient 0.99	NR	Helfand et al. (2001)
				1,5 ml H_2O + Tween 5% + dried milk 5% + timerosol 1% o/n 4 °C	Indirect IgG EIA	Concordance 93% Correlation coefficient 0.77		
MV	Total anti-MV IgM, N specific IgM, MV RNA N-gene	Capillary and venous blood (3 Whatman) MV infected cells in blood (2992 S&S)	Storage for 5 months at RT after −70 °C storage	10 mm 500 μl PBS 2% FBS o/n 4 °C	Sandwich ELISA (in-house) Capture ELISA	IgM sens 95%; spec 96%	NR	De Swart et al. (2001)
					PCR	PCR sens 48%; spec 100% combined sens 99%; spec 96% LOD > 10 cells/25 μl		
HAV	Anti-HAV-Ig total	Capillary blood (2992 S&S)	Storage at 4 °C up to 90 days	NR 1 ml NaCl 0.115% + BSA 1.5% 12 h RT	EIA/ELISA (IMx, Abbott)	Sens 91%; spec 99%	NR	Gil et al. (1997)
HAV	Anti-HAV-Ig total	Capillary blood (1 Whatman)	Storage at 4 °C no desiccant	2x25 mm 500 μl PBS + Tween 0.2% + BSA 5% o/n 4–8 °C	Micro EIA (Abbott) ELISA (Sorin)	Sens 100%; spec 98% Sens 89.6%; spec 97.5%	NR	De Almeida et al. (1999)

HEV	HEV-RNA	Capillary blood (Whatman and Isocode stix, S&S)	Storage at 28–40 °C	NR 220 µl PBS + Tween Magna pure extraction method	qPCR (in-house)	Concordance 90.6%	NR	Merens et al. (2009)
Rubella	Anti-rubella Ig total and IgM	Venous blood (1 and 3 Whatman)	Samples in envelope, storage up to 48 days at RT	27 mm (No. 1) 21 mm (No. 3) 800 µl kaolin 25% suspension o/n 4 °C	Haemagglutination inhibition	Ig total sens 99%; spec 100% IgM sens 100%; spec NR	Ig total stable >4 weeks at RT IgM stable for up to 38 days at RT	Punnarugsa et al. (1991)
Rubella	Anti-rubella IgM and IgG	Capillary blood (903 S&S)	Time between collection and storage at −20 °C not specified	6.4 mm IgG 272 µl Wampole dilution buffer IgM 1 ml Captia dilution buffer o/n 4 °C	IgG indirect EIA (Wampole) IgM capture EIA (Captia, Sanofi)	Concordance 94% (IgM) 93% (IgG). Correlation coefficient 0.92 (IgM) and 0.94 (IgG)	NR	Helfand et al. (2001)
Rubella	Anti-rubella IgM and IgG	Capillary blood (903 S&S and Whatman (NR))	Consensus protocol	2 × 6.4 mm (IgM) 1 × 6.4 mm (IgG) 125 µl Dade dilution buffer/disk 1 h RT	Capture IgM (sanofi) Indirect IgG (Wampole)	IgM sens 98%; spec 97% IgG sens 99% spec 98% Correlation coefficient 0.91 (IgM) and 0.94 (IgG)	NR	Helfand et al. (2007)
Rubella	Anti-rubella IgM	Venous blood and serum mixed with erythrocytes (903 S&S)	No desiccant, storage at 4 °C for 24 months	2 × 6 mm 220 µl PBS + Tween 0.5% + dried milk 5% o/n 4 °C	EIA (Dade Behring)	Sens 93–97% depending on interpretation equivocal results; spec 100%	NR	Karapanagiotidis et al. (2005)
Rubella	Anti-rubella IgG	Capillary blood (Whatman)	Storage at 4 °C	5 mm 50 µl PBS + Tween 0.05% 30' RT	EIA (in house)	Concordance 99.1%	Slight (NR) decrease in titer after 15 days at RT and 4 months at +4 °C	Condorelli et al. (1994)
Rubella	Anti-rubella IgG	Serum spiked in blood (903 S&S)	Consensus protocol	5 mm 100 µl PBS + Tween o/n 4 °C	EIA (Diesse) EIA Dade Behring	Sens 97.4%; spec 90.5% Concordance 91.8% Agreement Ig titers (<2SD) 91.8% Sens 98.6%; spec NR Concordance 91.1% Agreement in titers 96.2%	NR	Hardelid et al. (2008)
Mumps	Anti-Mumps virus IgG	Capillary blood (Whatman)	Storage at 4 °C	5 mm 50 µl PBS + Tween 0.05% 30' RT	EIA (in house)	Concordance 96.0%	NR	Condorelli et al. (1994)
Dengue	Anti-dengue IgM, IgA, IgG	Venous blood (3 Whatman)	No desiccant, storage at 4 °C	6 mm 200 µl PBS + Tween o/n	Capture EIA (NR)	IgM sens 96%; spec 89% IgA sens 93%; spec 89% IgG sens 86%; spec 92%	NR	Balmaseda et al. (2008)
Dengue	Anti-dengue IgM dengue RNA	Capillary blood (S&S)	Storage at 90% humidity	NR 400 µl PBS Tween 0.5% 5% dried milk 30' RT TRIzol (Invitrogen)	EIA (in house) Semi-nested PCR	IgM sens 89%; spec 94% RNA sens 82%; spec 91%	NR	Matheus et al. (2007)
Dengue	Dengue RNA (serotype 2, 3)	Virus spiked in venous blood (Nobuto, Toyo Roshi Kaisha)	No desiccant, stored at −70, 4 °C and RT	Rectangle of 5 × 30 mm = 100 µl blood 400 µl H_2O 30' 37 °C TRIzol (invitrogen)	Nested PCR (in house)	Sens 100%; spec 93%	RNA stable >9 weeks at RT	Prado et al. (2005)
Dengue	Anti-dengue IgM	Capillary blood (2992 S&S)	Storage at 4 °C	3 or 5 mm 70 µl 15 mmol/l Tris–HCl, + Tween 0.05%, pH 7.8 30 or 60' at RT	Capture UMELISA	Sens 92%; spec 99%, Correlation coefficient 0.95	IgM stable >30 days at RT and >2 months at 4 °C	De la Herrera et al. (2006)

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Table 1 (*continued*)

Virus	Target molecule	Specimen on filter paper (brand name)	Deviation from consensus storage condition	Disk diameter Extraction method Optimal elution	Assay (brand name)	Comparison to golden standard sensitivity%; specificity% Correlation coefficient Limit of detection (LOD)	Target degradation during storage	Reference
Dengue	Anti-dengue IgM and IgG	Venous blood. (903 S&S)	Storage at RT	2 × 6 mm 500 μl PBS 2% FBS o/n 4 °C	EIA (Focus)	Concordance Acute primary: 33.0% Acute secondary: 58.9% Past dengue: 66.7% No dengue 81.0%	Stable for at least 166 days when stored at RT	Tran et al. (2006)

Consensus storage:
Dried blood spot card in zip-lock plastic bag with desiccant. Storage at RT for a maximum of 2 weeks, prolonged storage at −20 °C

ATL: animal tissue lysis
DBS: dried blood spot
EDTA: ethylenediaminetetraacetic acid
(M)EIA: (micro) enzyme immunoassay
FBS: fetal bovine serum
HTLV: human T-lymphotropic virus
HBsAg: hepatitis B surface antigen
IE: immediate early (CMV)
Ig: immunoglobulin
LOD: limit of detection
MEM: modified Eagle's medium
MV: measles virus
NCR: non-coding region
NP: nucleoprotein
NR: not reported
N-gene: nucleoprotein-gene

Sensitivity expresses the proportion of individuals with disease, who are correctly identified by DBS sampling vs. golden standard, i.e. virus culture from urine or saliva for CMV or serum/plasma technology for other viruses.
Specificity expresses the proportion of non-infected individuals identified as such using dried blood spot sampling.
The concordance combines the percentages of correctly identified infected and non-infected individuals
NS: non structural
o/n: overnight
PBS: phosphate buffered saline
(q)PCR: (quantitative)polymerase chain reaction
RFLP: restriction fragment length polymorphism
RT: room temperature
S&S: Schleicher and Schuell
Sens: sensitivity of test compared to serum or plasma
S-gene: encode for the surface antigen
Spec: specificity of test compared to serum or plasma
TES: buffer containing Tris–HCl, EDTA and NaCl
Tris: tris(hydroxymethyl) aminomethane
UL: unique long region (CMV)
US: unique short region (CMV)
VCA: viral capsid antigen
WB: Western blot

(Boppana et al., 2005; Ross et al., 2009). Furthermore, the limits of CMV DNA detection in neonatal heel prick cards may not be sufficient to detect all cases of congenital CMV infection (de Vries et al., 2009). Boppana et al. hypothesized that the low sensitivity (34%) found in their study may be due to the absence of CMV DNA in the blood of a proportion of the congenitally CMV-infected children (Boppana et al., 2005, 2010; Kimberlin et al., 2008; Ross et al., 2009), and concluded that neonatal heel prick cards are inappropriate samples to screen for congenital CMV infection (Boppana et al., 2010). Others however, have argued that non-viremic neonates are at lower risk for late-onset sequelae than viremic neonates and that the sensitivity of DBS testing is therefore higher for detection of congenital CMV infected neonates who are at risk for CMV-related sequelae than for detection of congenital CMV infection in general (de Vries et al., 2009; Dollard and Schleiss, 2010). More studies are needed to clarify this issue.

Limits of detection of CMV DNA in DBS cards depend on the nucleic acid extraction, amount of DBS card used and PCR method used (de Vries et al., 2009; Gohring et al., 2010) (see Table 1). Barbi et al. developed an algorithm to improve the sensitivity of their technique (Barbi et al., 2000). From each subject, three DBS samples were tested for CMV DNA. At least 2/3 positive PCRs in the first run were needed to count a case as positive. If only 1/3 PCRs was positive in the first run, at least one positive PCR was needed in a second run of triplicate analyses to count a case as positive. This approach improved the sensitivity from 81.9% to 100%. An external quality assessment in which 27 laboratories participated for CMV DNA detection in DBS cards showed that correct positive results were reported by 91% of the laboratories for DBS containing 88,000 copies/ml, 59% for DBS containing 9400 copies/ml and 12% for DBS containing 730 copies/ml (Barbi et al., 2008). Thus, standard operational procedures and international consensus are needed if this technique is to be implemented in national screenings programs.

2.1.2. Other viruses

Nested PCR was used to amplify herpes simplex virus (HSV-1) and HSV-2 DNA in 7 neonatal heel prick cards from 28 children with verified neonatal HSV infection. The virus was only detected 5 days before and 6 days after the onset of symptoms, which gives a limited diagnostic test window (Lewensohn-Fuchs et al., 2003).

Anti-HBc antibody detection in neonatal heel prick cards was used as a hallmark of hepatitis B virus (HBV) infection of the mother. This was considered as a risk factor for congenital infection (Tappin et al., 1998). Guthrie cards from 14,000 neonates were screened for the presence of maternal anti-HBc antibodies, with a sensitivity of 79% and a specificity of 100%. Gupta et al. used another approach to assess the risk of vertical transmission, by testing DBS samples from capillary blood of HBV surface antigen (HBsAg) seropositive mothers for HBV DNA as a marker of an active viral infection (Gupta et al., 1992).

Neonatal heel prick cards used for screening purposes can, on the other hand, be used to determine anti-MV immunoglobulin (Ig)G, which gives insight in the antibody levels of the adult population at that time (Ibrahim et al., 2006).

2.2. Chronic disease monitoring and treatment

2.2.1. Hepatitis B virus (HBV)

Differentiating between stages of HBV infection is established by the investigation of HBV-related biological markers. HBV-DNA and HBeAg (extracellular core) and HBsAg antigens are the first serum markers detectable in acute infection. If the virus is cleared these markers are no longer detectable. Chronic HBV infection is defined by the presence of HBsAg for at least 6 months.

An early study reported lower sensitivity for detection of anti-HBV antibodies when DBS cards were used instead of serum. HBV antigen levels in most patients appeared to be sufficiently high to compensate the lower sensitivity of the assay when DBS cards are used (Villa et al., 1981). More recently, DBS cards have been used to study the prevalence of HBV infection in endemic areas and in specific groups at risk for infection, such as prisoners (Brugal et al., 2009; Komas et al., 2010; Lukacs et al., 2005; Mahfoud et al., 2010; Vallejo et al., 2008). Together with liver enzyme sampling it can be a useful tool to diagnose liver function in resource-limited settings (Mendy et al., 2005). In The Republic of The Gambia, DBS cards have been implemented successfully for routine sampling in patients with liver disease (Mendy et al., 2005). However, a study performed in Nigeria reported a low sensitivity (78.6%) and specificity (88.6%) for HBsAg detection in DBS cards compared to serum samples, thus warranting further investigation before DBS testing can be implemented (Forbi et al., 2010).

DBS cards have also been used for HBV-DNA quantification and HBV genotyping. Using DBS cards the sensitivity of HBV-DNA quantification was approximately one log lower than serum samples (Jardi et al., 2004). HBV pre-core mutants (G1896A) were detected in HBeAg-negative chronic hepatitis and lamivudine resistance of HBV polymerase was evidenced by mutations in the YMDD locus of HBV polymerase, thus offering the possibility to change therapy.

2.2.2. Hepatitis C virus (HCV)

HCV infection may result in acute and chronic hepatitis. Acute HCV infection can be cleared spontaneously, but it leads to chronic infection in 60–80% of the cases. Acute HCV infection is characterized by elevated levels of liver enzymes in blood and large fluctuations in HCV RNA levels. Patients with a chronic infection have sustained high HCV RNA levels (McGovern et al., 2009). Quantification of HCV RNA can also predict the success of therapy beforehand (Chevaliez et al., 2007). DBS cards have been used for detection of anti-HCV antibodies, qualitative and quantitative HCV-RNA detection and for HCV genotyping. DBS-based genotyping may be of particular use in HCV diagnostics and treatment management as genotype 1 is more resistant to pegylated interferon plus ribavirin treatment and thus require prolonged treatment compared to genotypes 2 and 3 (Rosen, 2011). Anti-HCV antibodies appear to be stable in DBS cards stored at RT for at least 4 weeks (Lukacs et al., 2005). Unexpectedly, one report noted that anti-HCV negative DBS cards tested positive for anti-HCV antibodies after storage for 6 days at room temperature (Tuaillon et al., 2010).

In contrast to anti-HCV antibodies, the stability of HCV-RNA is a problem when DBS cards are stored at room temperature. Tuaillon et al. reported significant HCV RNA degradation within 3–6 days and Abe et al. reported a 10-fold reduction in HCV RNA titers after 4 weeks (Abe and Konomi, 1998; Tuaillon et al., 2010), thus warranting immediate storage at −20 °C.

2.2.3. Other viruses

Antibodies (IgG) directed against Epstein–Barr virus (EBV) and herpes simplex viruses (HSV) were tested successfully in eluates of DBS cards, with high correlation coefficients with serum samples in seroprevalence studies (Hogrefe et al., 2002; McDade et al., 2000). DBS and dried serum spot (DSS) cards have been used to detect proviral DNA and antibodies against human T lymphotropic virus (HTLV). Detection of proviral DNA by PCR in paired DBS cards and peripheral blood leukocyte samples showed an excellent concordance and similar detection limits being found (Noda et al., 1993). DBS cards also appeared to be as a reliable tool for serological diagnosis of HTLV (Das et al., 1996; Parker et al., 1995). Both viral DNA and antibodies against these viruses were found to be stable in DBS cards after storage for longer periods at

room temperature. In addition, EBV-specific antibodies were shown stable after 6 freeze thaw cycles (McDade et al., 2000). These viruses are mainly studied for epidemiological surveys.

2.3. Acute infections; outbreak surveillance, vaccine coverage

2.3.1. Measles virus (MV)

DBS cards have been used for detection of anti-MV antibodies, detection of MV RNA, and for MV phylogenetic analysis in epidemiological studies and during MV outbreaks. Furthermore, DBS cards can be used to assess the success rate of vaccination programs by examining seroconversion rates. Ibrahim et al. demonstrated that DBS cards could be used to monitor trends in antibody levels during childhood (Ibrahim et al., 2006). De Swart et al. showed that DBS sampling provides excellent opportunities for phylogenetic analysis of MV during outbreaks. A combined test of IgM detection and RT-PCR was found to have a sensitivity and specificity of 99% and 96% respectively, compared to plasma samples (De Swart et al., 2001). Katz et al. simulated MV-infected blood samples by spiking MV into PBMC and reported a detection limit of 100 infected PBMC per DBS (Katz et al., 2002). DBS sampling is not considered as a replacement for standard virus isolation techniques, but can be effective in surveillance testing (El Mubarak et al., 2004; Katz et al., 2002). The WHO steering committee on research related to measles and rubella vaccines recommended that DBS sampling should only be used in areas that do not have access to conventional specimen collection and recommended further optimization of elution techniques and further development of RT-PCR for use with DBS (Muller et al., 2007).

2.3.2. Hepatitis A virus (HAV)

DBS cards have been successfully used for serological diagnosis of HAV and for quantification of HAV-RNA and phylogenetic analysis. Gil and de Almeida reported a sensitivity of 91.3% and 89.6%, respectively, for serological diagnosis of HAV using DBS cards (de Almeida et al., 1999; Gil et al., 1997). More recently, Desbois et al. reported 100% sensitivity, however, their results could have been influenced by exclusion of sera with values around the cut off of the assay and the use of DSS cards instead of DBS cards (Desbois et al., 2009).

2.3.3. Hepatitis E virus (HEV)

Merens et al. tested the feasibility of DBS sampling for HEV-RNA amplification during a field study on the HEV epidemic in refugee camps in Darfur and Chad in 2004 (Merens et al., 2009). They found a concordance of 90.6% for HEV-RNA detection in DBS and serum, and concluded that DBS samples are accurate and reliable tools for field studies of HEV outbreaks in developing countries.

2.3.4. Rubella virus

DBS cards have been used for detection of anti-rubella antibodies (IgM and IgG) for the surveillance of rubella outbreaks and immunization programs. Several studies reported a high concordance (>90%) for IgG, IgM, or total Ig detection using DBS cards compared to serum samples (Hardelid et al., 2008; Helfand et al., 2001, 2007) (see Table 1). Anti-rubella antibodies appear to be stable in DBS for at least 4 weeks at room temperature (Punnarugsa and Mungmee, 1991). Hefland et al. obtained paired sera collected by venipuncture and DBS cards from capillary blood collected from 273 persons with clinical suspected rubella virus infection during an outbreak in Peru. IgM and IgG were detectable in DBS cards from capillary blood in 34% and 26% of the persons sampled within 1 day after onset of rash. This number increased for both Ig subtypes to 86% in persons sampled 28 days after onset of rash. Similar results were obtained when serum was used for diagnosis (Helfand et al., 2007). In a conference report the WHO Steering Committee recommended further research into the reliability of alternative sampling including DBS cards in detecting rubella antibodies and RNA by RT-PCR (Muller et al., 2007).

2.3.5. Dengue virus

Infection by dengue virus causes clinical manifestations ranging from asymptomatic infection, self-limiting dengue fever and dengue hemorrhagic fever to lethal dengue shock syndrome. Secondary infection with dengue virus of another subtype is associated with a more severe disease outcome (Vaughn et al., 2000). Commonly used diagnostic techniques for dengue virus seroprevalence include (capture or sandwich) ELISA for IgM and IgG. Dengue virus isolation or subtype specific PCR in tissue, blood or other bodily fluids are used to confirm active dengue infection. In addition, serum conversion of IgM, a $\geqslant$ 4-fold rise of IgG, a $\geqslant$ 4-fold rise in plaque reduction neutralization test or IgM in cerebrospinal fluid are considered as evidence for active dengue infection (CDC, 2009) . DBS sampling for diagnostics gave various results in both outbreak and clinical setting. Several groups found high sensitivities (>82%) and specificities (>91%) between DBS cards and serum sampling for dengue virus (Balmaseda et al., 2008; Herrera et al., 2006; Prado et al., 2005; Ruangturakit et al., 1994). All studies agreed on the feasibility of DBS for seroprevalence studies using anti dengue IgG. However, low concordance was found for IgM antibodies in acute primary or acute secondary dengue virus infection, limiting its value for diagnostic classification of clinical outcome (Tran et al., 2006).

3. Current and future applications of DBS cards

DBS cards already have a proven track record in neonatal screening for inborn errors of metabolism. DBS-card based sampling now has the potential to become a powerful diagnostic tool in research and clinical fields of virology in addition to conventional laboratory techniques. Implementation of DBS techniques will, however, depend on the use of robust consensus protocols for sampling, storage and processing. Furthermore, an increase of the clinician's awareness of possibilities, as well as limits, is crucial for the establishment of optimal DBS sampling technology for virus research and diagnostics in different settings.

DBS card sampling still faces technical imperfections that need attention, especially for large scale screening studies. Although applying a drop of blood to a DBS card seems straightforward clear instructions are needed to obtain reliable and reproducible spots. Preferably blood should be captured with a capillary, because this allows for accurate and reproducible sampling, but this may not always be achievable in field settings. The reproducibility of the blood volume collected is also a point of concern. The amount of sample recovered from a punch of a DBS card varies with the size of the spot, which is influenced by the viscosity, due to variations in the hematocrit values (Ht) (Denniff and Spooner, 2010; Mei et al., 2001). Ht is the percentage of whole blood occupied by red blood cells. Especially in young children quality controls correcting for Ht levels may be required because their Ht values may differ from ranges that are common in adults (Pandya et al., 2011). Hemoglobin that is present in DBS samples in relatively large amounts may negatively influence PCR amplification. This has been addressed by testing various elution protocols (Condorelli et al., 1994).

Processing of the samples with a puncher and eluting the specimens is laborious and requires thorough cleaning to avoid contamination by carry over (Barbi et al., 1996). Automation of punching with extensive cleaning is one approach to solve this problem, however these machines add substantially to the relative low costs of DBS card processing. Other developments in DBS tech-

nology include direct sampling of the DBS card in situ without separate elution and sample preparation through ambient ionization-MS (for review see Corso et al., 2010).

Efforts should be undertaken to make DBS sampling possible at a wider scale. For a start samples should be processed according to the consensus protocol (Bertagnolio et al., 2010; Mei et al., 2001), although storage conditions should be investigated as part of the development and validation of a particular assay/test. The longer a sample can be stored at ambient temperature, the simpler and cheaper the process will be. We feel that the establishment and maintenance of designated local centers in a large geographical area specializing in DBS lab techniques together with the development of a robust logistic system should be encouraged. Besides collecting and storing DBS cards, the centers can meet the requirements for sensitive read out on the DBS eluates that comprise 1–2 log less material than standard "wet" blood or plasma samples.

Currently, the use of DBS cards has been shown a feasible tool for diagnosis and monitoring of chronic diseases in resource-limited settings. This has already become common use for HIV infection, especially at the level of plasma viral load measurement and genotyping of resistance (Bertagnolio et al., 2010; Johannessen et al., 2009). The monitoring of chronic HBV infection through the measurement of viral load in DBS samples is sensitive enough, allowing clinical application and genotypic resistance analysis, thus improving treatment possibilities (Mendy et al., 2005). This application may therefore be extended to the diagnostics of chronic infection in endemic areas even when these are resource-limited (Mendy et al., 2005; Villa et al., 1981). By contrast, HCV RNA shows rapid degradation in DBS cards, even when these are stored at room temperature for two weeks or less (Tuaillon et al., 2010), which may limit their use for diagnosis of HCV infection in settings where cold storage is not continuously available.

For CMV DNA detection in DBS from neonatal heel pricks, results were obtained after more than 10 years of storage (Boudewyns et al., 2009; Soetens et al., 2008). These cards, however, have usually not been stored under optimal conditions, according to the recommended standard protocol. Given the stability of CMV DNA in DBS cards, degradation may be limited, but this should be verified and results should be interpreted with caution. In particular, because these studies used qualitative PCR instead of quantitative PCR and thus the exact amount of degradation of CMV DNA in DBS remains to be determined. More importantly, false negative results may be due to the low or absent viral load in DBS cards, when virus can be cultured from urine (Boppana et al., 2010). Retrospective studies for large scale monitoring of congenital CMV infection have been performed. However there is no consensus whether this translates into treatment of neonatal CMV infection.

Treatment in chronic virus infections can also be monitored with DBS cards. Therapeutic drug monitoring for dose optimization and for therapy adherence has been performed using DBS cards (Corso et al., 2010; Hooff et al., 2011; Meesters et al., 2011). A DBS-based assay for cellular biomarkers would be of great value for clinicians in judging the success of therapy. Although efforts have been undertaken, e.g. CD4 T cell counts in HIV infection, (Mwaba et al., 2003), no validated assay is available yet (Johannessen et al., 2009).

Also for other viruses (HEV, HAV, measles virus and rubella virus) DBS sampling is considered as reliable with a concordance of >90% (De Swart et al., 2001; Desbois et al., 2009; Hardelid et al., 2008; Helfand et al., 2001, 2007; Merens et al., 2009). In general, antibodies appear to be stable for prolonged periods of time when stored at room temperature (see Table 1). In these acute virus infections DBS card sampling has more practical use in outbreak surveillance and management (Ibrahim et al., 2006). Phylogenic analyses of viral sequences help to identify sources of new outbreaks and subsequent spreading of viruses. In remote areas DBS sampling seems to be the only practical approach for such surveillance. Besides DBS sampling for virus sequences, DBS sampling for vaccine-induced antibodies may help to monitor vaccine coverage programs (El Mubarak et al., 2004; Melgaco et al., 2011). Performing large screening efforts with the use of DBS card sampling should be done with caution. Gathering and storing information may be appealing as novel developments can be tested in future. However, without a perspective of improving public health, this may be counter-productive as reluctance to giving samples without a clear sense may arise, both with the public, the scientists and the physicians.

DBS cards based technology has also been used to develop anonymous self-test kits. Self-tests for HIV and other viral diseases such as dengue or HCV are widely available on the internet at low prices. Lack of medical follow up and mental support after a positive self-test may affect the well being of the patient. The chances of false positive results are problematic especially in communities with low prevalence; e.g. HIV estimated seroprevalence in The Netherlands is 0,1% and the specificity of HIV self tests ranges from 98–99.9%, rendering the majority of positive test results false. By contrast in areas with high prevalence of HIV infection, a 99% sensitivity of DBS card testing will give many false negative results, offering a false sense of security.

We expect that future use of DBS cards for diagnostics, surveillance and management of viral disease will expand. In remote areas, the possibility to perform diagnostic tests can contribute to public and individual health. Benefits of DBS cards can also be envisaged in clinical practice of neonates, where current sampling techniques are too demanding for the youngest. DBS card sampling using tens of microliters of blood decreases the burden of sampling while opening up new diagnostic possibilities (Pandya et al., 2011). For adults, DBS sampling in general practice may have economical and logistic benefits via blood sampling on DBS cards during consultation, avoiding extra visits to laboratory facilities.

In conclusion DBS sampling offers new opportunities in the diagnostics and treatment of viral disease, provided that uniform and robust protocols are implemented and a clear perspective for treatment of individual and/or public health is within reach.

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