Alternative sampling in infectious diseases: Investigating and leveraging the use of dried blood spots and the **cobas**® Plasma Separation Card

Diagnosis of infectious diseases (such as viral or bacterial infections) is usually done by testing blood plasma or serum samples collected through venipuncture to detect pathogenic antigens, nucleic acids, or host antibodies specific to a certain pathogen. This process can be difficult for various reasons, especially in resource-limited settings.

This white paper examines the applications, clinical performance, and practical application of dried blood spots and the **cobas**® Plasma Separation Card (PSC), focusing on their use with Elecsys® immunoassays for identifying several infectious diseases.

It should be noted that the **cobas**® Plasma Separation Card is currently not an approved specimen type for Elecsys® immunoassays.

Executive Summary

- Several infectious diseases are diagnosed by directly detecting the pathogen in human plasma or serum (e.g., antigens or nucleic acids) or indirectly by testing for pathogen-specific antibodies produced by the host.
- Dried blood spots (DBS) and the cobas® Plasma Separation Card (PSC) are alternative sampling methods to collecting plasma or serum samples; they offer a less invasive and more patient-friendly approach.
- They can be prepared using capillary blood, as well as venous blood, potentially avoiding challenges associated with the latter (e.g., the need for phlebotomists).
- Using DBS/PSC also eliminates the need for cold-chain transportation, as these sample types are stable at ambient temperatures; this can help increase access to diagnostic testing in remote and underserved areas.
- DBS/PSC are suitable for serological testing and nucleic acid testing (NAT) and hold several potential applications in diagnosing infectious diseases, including national screening programs and large epidemiological studies.
- Preparing DBS/PSC with capillary blood may also improve access to testing among individuals with poor venous access and marginalized populations (e.g., people who inject drugs [PWID], inmates, and people living with human immunodeficiency virus [PLHIV]), while also offering the potential to reduce screening costs and support outreach programs.
- Published studies show that various Roche immunoassays (e.g., Elecsys® HIV combi PT, Elecsys® HIV Duo, Elecsys® HBsAg II, Elecsys® Anti-HCV II, Elecsys® Anti-HBc II, Elecsys® Syphilis, Elecsys® Anti-SARS-CoV-2, and Elecsys® Anti-SARS-CoV-2 S) perform well clinically when used with DBS/PSC, compared to plasma/serum samples.
- Although some quality issues and limitations have been reported with DBS/PSC, the existing evidence supports their use with immunoassays for viral hepatitis, HIV, syphilis, and SARS-CoV-2, especially for large-scale population screenings and serological surveys in resource-limited settings.
- Future potential applications of DBS/PSC include diagnosing a broader range of infectious diseases (e.g., tuberculosis, rubella, measles virus, dengue, toxoplasmosis) and conducting biochemical analyses.
- The World Health Organization (WHO) and certain national and international guidelines recommend using DBS/PSC in specific settings. However, updates to policies and guidelines are necessary to optimize the use of DBS/PSC for serological testing of infectious diseases.

Contents

Introduction

Several infectious diseases are diagnosed through direct detection of the pathogen in human plasma or serum (i.e., antigens or nucleic acids) or indirect detection by testing for pathogen-specific antibodies produced by the host.[1] Collection of venous blood samples can be challenging due to the need for trained phlebotomists, poor venous access among some patient populations, and the infrastructure required to store and transport samples.[2] These challenges are particularly evident in resource-limited settings, where there can be high rates of morbidity and mortality due to infectious diseases, and among marginalized, high-risk populations with limited access to healthcare.[1,3,4]

DBS are a specimen type consisting of whole blood spotted and dried onto filter paper,[5,6] DBS offer several benefits that can help to overcome the obstacles associated with traditional sample collection methods for serological testing.[7] Collection of DBS eliminates the need for venipuncture, as cards can be prepared by spotting with capillary blood obtained using a sterile lancet to prick the fingertip or heel. As such, DBS have greater acceptability among patients, with the possibility of peer-or self-collection of samples, as opposed to requiring phlebotomists.[4,8] This may present greater opportunities to test patients with poor venous access (e.g., PWID and elderly patients) and to offer screening for individuals who otherwise may not be tested (e.g., prison population, men who have sex with men [MSM]),[1,9] DBS are stable at room temperature and therefore do not require temperature-controlled storage and transportation. These features facilitate greater opportunities for biological sampling in remote and low-resource settings, in addition to enhancing opportunities for large-scale and repetitive sampling.[1,5,10,11] DBS offer the potential for cost savings by obviating the need for trained personnel and cold-chain storage. Use of DBS may also help to reduce the need for follow-up appointments, as explored in more detail below.[1] However, DBS have limited stability for extended storage at ambient temperatures.[12,13] Additionally, DBS contain potentially interfering whole-blood components, including red blood cells, lymphocytes, and other substances [1,14]

The **cobas**® Plasma Separation Card (PSC) is a sample collection device that is based on a similar premise to DBS. However, the PSC features a porous membrane that selectively allows only plasma to pass through, collecting it on a layer of polyester fleece.[14] This process creates a dried plasma spot from a whole blood sample.[14] The fleece is infused with an RNA-stabilizing reagent, which helps to preserve the samples across a range of temperatures, humidity levels, and storage conditions.[14] The PSC includes three distinct sample collection spots, enabling repeat testing or the option for additional tests to be conducted.[15] This device offers numerous advantages over DBS: it generates a dried plasma specimen, which minimizes the impact of blood cell-associated components and is more closely aligned with the "gold standard" specimen type used in many diagnostic tests. Additionally, it allows for extended transportation times and long-term storage,[14] making it easier to conduct further or confirmatory testing when needed.[15]

Roche's Elecsys® immunoassay portfolio includes assays for the *in vitro* qualitative detection of antigens (e.g., HBsAg) or antibodies against particular antigens (e.g., SARS-CoV-2 nucleocapsid [N] protein), in addition to immunoassays that allow the quantitation of antigens (e.g., Elecsys® HBsAg II quant II) or antibodies (e.g., against the SARS-CoV-2 spike [S] protein).

The purpose of this white paper is to explore the applications, clinical performance, and practical use of DBS and PSC, with a focus on use with Elecsys® immunoassays for the detection of hepatitis viruses (i.e., hepatitis B virus [HBV], hepatitis C virus [HCV]), and sexually transmitted infections (STIs), including human immunodeficiency virus (HIV) and syphilis, and potential new applications, including other infectious diseases. *However, it should be noted that the PSC is currently not an approved specimen type for the Elecsys® assays.*

Applications in infectious disease diagnosis

There is a range of applications for DBS and PSC in the diagnosis of infectious diseases. **Table 1** provides an overview of the publications relating to the use of DBS or PSC in combination with Roche Elecsys® immunoassays or Roche assays for nucleic acid testing (NAT), while **Table 2** presents a summary of each of these studies; both tables are presented according to disease indication. The clinical performance of these immunoassays, when used in combination with DBS/PSC, is considered to be a trade-off between the inherent assay performance and its accessibility in the field, with the latter being affected by cost, infrastructure requirements, and acceptability.[4]

Diagnosis of several types of infectious diseases, including viral hepatitis and HIV, uses a sequential approach that involves initial serological screening for antibodies or antigens, followed by confirmatory NAT in antibody/antigen-positive individuals. In a reflex testing approach, the same specimen, typically frozen serum/plasma, is used for initial and supplemental confirmatory testing. This helps to simplify screening and mitigates the need for a second screening visit, thereby facilitating increased confirmatory testing and linkage to care for infected patients.[1,16,17]

Viral hepatitis testing

The three main serologic markers used to determine HBV infection status are HBsAg (indicates HBV infection, either acute or chronic), antibody to HBsAg (anti-HBs; indicates immunity against HBV after recovery from HBV infection or vaccination), and total antibody to hepatitis B core antigen (anti-HBc; develops in all HBV infections, resolved or current, and typically persists for life).[18] Updated guidance recommends testing for HBsAg, anti-HBs, and total anti-HBc.[18] Testing for HBV DNA, after identifying a person with HBV infection, can provide information on the level of viral replication and infectivity to help guide clinical management.[18] Additionally, because hepatitis delta virus (HDV) requires the presence of HBV to propagate, anti-HDV testing is also recommended in HBsAg-positive individuals.[17,52]

Diagnosis of HCV infection is based on initial testing for the presence of antibodies to HCV (anti-HCV), followed by confirmatory HCV RNA or HCV core antigen testing of antibody-positive individuals to identify those with active infection.[16,19,20] The suitability of PSC for HCV and HBV reflex testing was demonstrated in a study by Martínez-Campreciós et al., which showed high sensitivity and correlation with plasma samples (e.g., anti-HCV sensitivity/specificity: 98.7%/100%; **Table 2**).[21]

DBS represent an alternative specimen matrix that can increase access to serological testing and NAT methods for HBV and HCV, as well as other infectious diseases.[1] The WHO recommends use of DBS for serological (HBsAg and HCV antibodies) and virological (HBV DNA and HCV RNA) testing in regions that lack infrastructure or expertise for venous blood collection, where rapid diagnostic tests are not available, and in people with poor venous access (e.g., PWID).[19,22]

Table 1. Overview of publications on Roche Elecsys® immunoassays and **cobas**® molecular assays used with DBS or PSC samples

Indication	Assay	Analyte	References	
			DBS	PSC
HIV	Elecsys® HIV combi PT	HIV-1 p24 antigen, antibodies to HIV-1 and HIV-2	6, 26, 27	
	Elecsys® HIV Duo	HIV-1 p24 antigen, antibodies to HIV-1 and HIV-2	28	
	COBAS® AmpliPrep/**COBAS**® TaqMan HIV-1	HIV-1 RNA	26	
	cobas® HIV-1	HIV-1 RNA		Data on file
HBV	Elecsys® HBsAg II	Hepatitis B surface antigen (HBsAg)	6, 9, 28–30	8, 15, 21
	Elecsys® Anti-HBc II	Total antibodies to hepatitis B core antigen (HBc)	9, 29, 30	21
	cobas® HBV	HBV DNA		8, 21, data on file
HCV	Elecsys® Anti-HCV II	Antibodies to hepatitis C virus (HCV)	9, 28–32	1, 8, 15, 21
	cobas® HCV	HCV RNA	33	1, 8, 21, 33, data on file
	cobas® HCV GT	HCV genotypes 1-6 and GT1 subtypes a and b		1
Syphilis	Elecsys® Syphilis	Total antibodies to *Treponema pallidum*	28	
COVID-19	Elecsys® Anti-SARS-CoV-2	Total antibodies to the SARS-CoV-2 nucleocapsid protein	3, 5, 11, 34–37	
	Elecsys® Anti-SARS-CoV-2 S	Total antibodies to the SARS-CoV-2 spike protein receptor-binding domain	7, 13, 38	
HIV, HCV, and HBV	**cobas**® MPX	HIV-1 Group M RNA, HIV-1 Group O RNA, HIV-2 RNA, HCV RNA, HBV DNA	31	

Table 2. Overview of studies on Roche Elecsys® immunoassays and **cobas**® molecular assays used with DBS or PSC specimens

Study description	Study aims	Study population	Assay	Performance/study outcome
HIV				
HIV PSC - LoD study (data on file)	To determine if sensitivity improvements could be achieved for the **cobas**® HIV-1 test when performed in combination with PSC samples (current LoD: 790 copies/mL; desired LoD: <200 copies/mL).	Whole-blood samples spiked with HIV-1M[a]	**cobas**® HIV-1	No indication that LoD can be improved.
Study with OKAPI Project, Kinshasa (DRC)[26]	To identify cases of HIV misdiagnosis by comparing the results of rapid diagnostic tests using venous blood with results from DBS samples tested on centralized HIV testing platforms.	365 DBS samples were prepared using venous blood samples from 363 individuals and shipped to Spain; included people with a new HIV positive (n=123) or indeterminate (n=23) result, known HIV-positive patients (n=157), and a negative control group (n=62)	Elecsys® HIV Combi PT, VIDAS® HIV Duo Quick, Geenius™ HIV 1/2 confirmatory assay, COBAS® AmpliPrep/COBAS® TaqMan HIV-1	**Elecsys® HIV combi PT** Sensitivity: 100% Specificity: 98.9%
HIV early infection study, France[27]	To assess the effectiveness of HIV fourth-generation immunoassays in detecting acute HIV infections compared to rapid diagnostic tests using dried serum samples (DSS).[c]	39 archived serum samples from newly diagnosed HIV infected people collected at early phases of infection	Elecsys® HIV combi PT, Liaison® XL Murex HIV Ab/Ag	**Elecsys® HIV combi PT** **Liaison® XL Murex HIV Ab/Ag** Sensitivity: 87.2%
Various (HIV/HBV/HCV/syphilis)				
Feasibility study, Netherlands[6]	To evaluate the acceptability and feasibility of self-collected DBS and to compare test results for HIV, HBV, and syphilis screening from DBS with those from blood drawn by venipuncture.	217 samples from MSM	Elecsys® HIV combi PT, Elecsys® HBsAg II, bioelisa SYPHILIS 3.0	**Elecsys® HIV Ag/Ab** Sensitivity: 100% Specificity: 100% **Elecsys® HBsAg** Sensitivity: 90% Specificity: 99% **bioelisa SYPHILIS 3.0** Sensitivity: 90% Specificity: 99%
Validation study, New Zealand[28]	To validate a DBS method for the serological screening of HIV, syphilis, HCV, and HBV on the Roche **cobas**® system.	250 paired DBS and serum samples and 116 unpaired DBS samples from 366 unique patients from two laboratories	Elecsys® HIV Duo, Elecsys® HBsAg II, Elecsys® Anti-HCV II, Elecsys® Syphilis	**Elecsys® HIV Duo** Sensitivity: 100% Specificity: 100% **Elecsys® HBsAg** Sensitivity: 95,9% Specificity: 100% **Elecsys® Anti-HCV II** Sensitivity: 84% Specificity: 100% **Elecsys® Syphilis** Sensitivity: 68.3% Specificity: 100%
HBV/HCV				
FIND Study, Cameroon, Rwanda, Georgia, and Greece[33]	To assess the performance of three centralized HCV RNA assays using samples collected with **cobas**® PSC and DBS.	Samples from 946 participants enrolled at four sites located in Cameroon, Rwanda, Georgia, and Greece	**cobas**® HCV	**cobas® HCV with DBS/PSC**[d] Sensitivity: 96.9%/97.3% Specificity: 99.8%/95.9%

Study description	Study aims	Study population	Assay	Performance/study outcome
HBV and HCV Internal Study - Prospective Collection (data on file)	To evaluate the performance of HBV DNA and HCV RNA detection using **cobas®** PSC or DBS samples compared to plasma.	Prospective patient samples	**cobas®** HCV, **cobas®** HBV	A strong correlation was observed between PSC and plasma samples for both HBV DNA and HCV RNA. The reproducibility of DBS was better than that of PSC, and although the correlation was strong, it was still lower than that of PSC.
Cross-sectional study, Germany[31]	Cross-sectional study in two German states to evaluate progress in eliminating HCV among PWID. Participants were recruited from low-threshold drug services and opioid agonist treatment (OAT) clinics, and a capillary blood sample was collected as DBS from each person on site.	Between June 2021 and April 2022, 668 PWID were recruited in Berlin (n=155) and Bavaria (n=513).	Elecsys® Anti-HCV II, **cobas®** MPX	**Elecsys® Anti-HCV II and cobas® MPX** assays were successfully used to monitor the prevalence of viraemic and resolved HCV infections in PWID in Germany.
Cross-sectional study, Brazil[29]	To evaluate an automated assay for detecting HBV and HCV markers in DBS samples from PLHIV, those with coagulopathies, and CKD patients.	Paired serum and DBS samples from 430 individuals, including PLHIV, coagulopathies, and CKD	Elecsys® HBsAg II, Elecsys® Anti-HBc II, Elecsys® Anti-HCV II	In COAG/CKD/HIV populations: **Elecsys® HBsAg II** Sensitivity: 100%/100%/85% Specificity: 100%/99.6%/94.7% **Elecsys® Anti-HBc II** Sensitivity: 81.3%/79.6%/97.2% Specificity: 100%/97.1%/88.9% **Elecsys® Anti-HCV II** Sensitivity: 83.3%/93.5%/100% Specificity: 96.3%/99.2%/100%
Prospective, observational validation study, Spain and subsequent pilot study, Angola[21]	To correlate serologic HBV, HCV, and HDV status and reflex the respective viral load testing using PSC samples from capillary blood versus conventional plasma samples.	Patients with chronic viral hepatitis, Spain (n=201). Non-consecutive volunteers from outpatient clinics, Angola (n=93)	Elecsys® HBsAg II, Elecsys® Anti-HBc II, Elecsys® Anti-HCV II, Liaison Anti-HDV, **cobas®** HBV, **cobas®** HCV	**Elecsys® HBsAg II** Sensitivity: 98.4% Specificity: 96.2% **Elecsys® Anti-HBc II** Sensitivity: 95.9% Specificity: 100% **Elecsys® Anti-HCV II** Sensitivity: 98.7% Specificity: 100% **Liaison XL Murex Anti-HDV** Sensitivity: 84.6% Specificity: 100% **cobas® HBV** Sensitivity: 53.8% Specificity: 100% **cobas® HCV** Sensitivity: 100% Specificity: 100%
Cohort study, Kinshasa (DRC)[32]	To analyse HCV diagnostic assays based on DBS specimens collected in Kinshasa.	DBS samples from 270 patients attending hospitals in Kinshasa as part of the OKAPI project, which studied HIV infection	Elecsys® Anti-HCV II, Vidas anti-HCV, INNO-LIA HCV Score	**Elecsys® Anti-HCV II** Sensitivity: 100% Specificity: 100% **Vidas anti-HCV** Sensitivity: 61.5% Specificity: 89.4% **INNO-LIA HCV Score** Sensitivity: 100% Specificity: 100%

Study description	Study aims	Study population	Assay	Performance/study outcome
Prospective study, Italy[15]	To evaluate the performance of PSC in collecting and preparing blood samples for testing HCV and HBV serological markers.	334 consecutive patients referred for HCV/HBV testing	Elecsys® HBsAg II, Elecsys® Anti-HBs II, Elecsys® Anti-HCV II	**Elecsys® HBsAg II** Sensitivity: 100% **Elecsys® Anti-HBs** Sensitivity (>10 IU/mL cutoff): 53% Sensitivity (>2.6 IU/mL cutoff): 100% **Elecsys® Anti-HCV II** Sensitivity: 91%
Prospective case-control study, Spain[1]	To compare the performance of PSC with plasma and serum obtained from venipuncture for serologic and virologic diagnosis of HCV.	DBS and paired plasma or serum samples (101 anti-HCV Ab-positive and 50 Ab-negative patients)	Elecsys® Anti-HCV II, **cobas®** HCV, **cobas®** HCV GT	**Elecsys® Anti-HCV II** Sensitivity: 92.8% Specificity: 100% **cobas® HCV (>1,000 IU/mL)** Sensitivity: 94.9% Specificity: 100% **cobas® HCV GT** 95.5% concordance in genotyping between plasma and PSC samples
Cross-sectional study, Cameroon and Uganda[8]	To evaluate the feasibility and acceptability of PSC sampling for viral hepatitis B and C screening among PLHIV in Cameroon and Uganda.	192 PLHIV in Cameroon (n=104) and Uganda (n=88)	Elecsys® HBsAg II, Elecsys® Anti-HCV II, **cobas®** HCV, **cobas®** HBV	First study evaluating the feasibility and acceptability of using PSC for viral hepatitis screening among PLHIV in Cameroon and Uganda.
Cost analysis study, Pakistan[39]	To evaluate the cost-effectiveness of a reference laboratory-based confirmatory testing method compared to a molecular POC confirmatory approach for screening the general population for HCV in Pakistan.	General testing population for chronic HCV in Pakistan	SD Biosensor Standard Q HCV Ab, **cobas®** HCV, GeneXpert® HCV	Demonstrates the utility of the PSC sampling approach to address the challenges associated with rural population testing.
Pilot study, Sheffield, UK[9]	To evaluate a model of HBV and HCV testing using DBS sampling in an outreach setting.	229 Chinese subjects recruited from September 2009 to June 2012	Elecsys® HBsAg II, Elecsys® Anti-HBc II, Elecsys® Anti-HCV II	**Elecsys® HBsAg II** Sensitivity: 100% **Elecsys® Anti-HBc II** Sensitivity: 99% **Elecsys® Anti-HCV II** Sensitivity: 100%
COVID-19				
Cohort study, USA[34]	To assess the use of the Elecsys® Anti-SARS-CoV-2 assay with capillary DBS for high-throughput testing on the Roche **cobas®** 6000 system.	30 paired donor samples. An additional cohort (n=50), including COVID-19 convalescent cases, was evaluated for at-home collection for mail transport and stability studies.	Elecsys® Anti-SARS-CoV-2	Agreement between DBS and serum results: 98.9% **Self-collected DBS** Sensitivity: 71.4% Specificity: 100% Accuracy: 86.7%
Head-to-head, real-world comparison study, Canada[7]	To evaluate the Elecsys® Anti-SARS-CoV-2 S assay using DBS prepared from plasma-spiked RBC, and to compare results from matched plasma and DBS collected by finger prick from individuals with a PCR-confirmed positive history of COVID-19.	Residual plasma samples from PCR-confirmed positive SARS-CoV-2 patients (n=24, plasma collected 5-39 days after swab collection for PCR testing) and COVID-19-negative patients (n=21, pre-COVID-19 plasma samples or samples from PCR-confirmed negative patients) were used for spiking RBCs. Matched plasma and DBS were also collected from 52 PCR-positive confirmed patients, 90 days or more after diagnosis, and from 11 healthy controls.	Elecsys® Anti-SARS-CoV-2 S	Sensitivity: 98% Specificity: 100%

Study description	Study aims	Study population	Assay	Performance/study outcome
Feasibility study, Germany[13]	To investigate the possibility of combining the qualitative anti-N DBS Roche Elecsys® protocol with a quantitative anti-S measurement.	Randomly selected population-based COVID-19 cohort study (KoCo19) in Munich, Germany, which is part of the Europe-wide Consortium ORCHESTRA. Anonymised DBS cards from colleagues and other study participants were also included.	Elecsys® Anti-SARS-CoV-2 S	Sensitivity: 96.6% Specificity: 97.8%
Proof-of-concept study[35]	To explore the possibility of using the automated Elecsys® Anti-SARS-CoV-2 immunoassay with a volumetric DBS device.	DBS prepared using volunteers' blood samples collected in EDTA tubes (n=20).	Elecsys® Anti-SARS-CoV-2	Linearity and correlation were satisfactory between volumetric DBS and plasma. A cut-off value was suggested and should be validated with more samples.
Pilot study, UK[36]	To compare antibody detection in DBS with paired plasma samples.	195 participants from a COVID-19 serodiagnosis study of keyworkers (EDSAB-HOME)	Elecsys® Anti-SARS-CoV-2	Sensitivity: 89% Specificity: 100%
Performance study, Germany[3]	To assess the performance of self-sampled DBS for antibody detection using a semi-automated protocol.	Samples from three independent datasets: 1) 100 DBS/venous sample pairs, including 50 positive samples 2) 10,247 employees from the German company Deutsche Post DHL group 3) Home-sampled cohort (n=4,465)	Elecsys® Anti-SARS-CoV-2	Sensitivity: 99.2% Specificity: 98.7%
Comparative study, Germany[11]	To compare test results from DBS specimens with conventionally collected venous blood samples.	434 venous whole blood and DBS samples collected from volunteers; 72 blood samples from SARS-CoV-2 PCR-confirmed hospital patients	Elecsys® Anti-SARS-CoV-2	Demonstrated that anti-SARS-CoV-2 antibodies can be detected in DBS using both manual and automated spot extraction and ECLIA. The linear correlation between serum and the corresponding reconstituted DBS values was highly significant ($P < 0.001$). A significant difference was observed between DBS and Mitra-tip-based results (~4-fold).
Multi-site, multi-assay comparative study, Canada[5]	To determine the performance of 10 commercial and two in-house developed tests for SARS-CoV-2 antibodies using a well-characterised SARS-CoV-2 DBS panel sent to laboratories across Canada.	SARS-CoV-2 antibody-positive plasma was collected from COVID-19 convalescent donors at Mount Sinai Hospital in Toronto, Canada. SARS-CoV-2 negative plasma was obtained from healthy donors at the National Microbiology Laboratory in Winnipeg, Canada. A panel of 10 unique SARS-CoV-2 antibody-positive DBS cards was prepared, and 10 negative DBS cards were directly spotted from EDTA whole blood to evaluate the performance of commercial and in-house serological tests.	Elecsys® Anti-SARS-CoV-2	Sensitivity: 100% Specificity: 100%
Feasibility study, Sweden[37]	To determine whether a quantitative DBS device (Capitainer® qDBS 10 mL) could be used in combination with the Elecsys® Anti-SARS-CoV-2 S immunoassay to monitor anti-S antibodies.	Study 1: 14 volunteers who received two doses of the Comirnaty® (Pfizer) vaccine Study 2: 200 patients with unknown COVID-19 infection/vaccination status from a clinical chemistry department in Stockholm	Elecsys® Anti-SARS-CoV-2	Demonstrated that 10 µL DBS can be reliably used to quantify anti-S antibodies with an automated immunoassay. DBS results were comparable to plasma results, although there was approximately a 50-fold difference that remained stable across plasma values ranging from 20 to 20,000 U/mL

Study description	Study aims	Study population	Assay	Performance/study outcome
Cross-sectional study, Kinshasa, DRC[38]	Primary: To determine SARS-CoV-2 seroprevalence among patients attending a reference hospital in Kinshasa. Secondary: To evaluate the analytic performance of two serological testing platforms (Elecsys® and VirClia®) and the feasibility of DBS as samples to analyse SARS-CoV-2 seroprevalence.	DBS venipuncture samples from patients positive for COVID-19 and pre-pandemic DBS samples obtained in 2017 from participants in the Congolese OKAPI cohort[26] as negative controls	Elecsys® Anti-SARS-CoV-2, Elecsys® Anti-SARS-CoV-2 S, COVID-19 VIRCLIA® IgG MONOTEST, COVID-19 VIRCLIA® IgM+IgA MONOTEST	**Elecsys® Anti-SARS-CoV-2** Sensitivity: 91.4% Specificity: 82.1% **Elecsys® Anti-SARS-CoV-2 S** Sensitivity: 96% Specificity: 75.8% **COVID-19 VIRCLIA IgG** Sensitivity: 96.8% Specificity: 61.1% **COVID-19 VIRCLIA IgM+IgA** Sensitivity: 12.3% Specificity: 96.2%

[a] Whole-blood samples were spiked with a high concentration of HIV-1M (the most prevalent group of HIV), and 200 µL (instead of 140 µL) of spiked sample were applied to PSC, Two or three spots were added per extraction tube (adjusting SPER volume as needed) and 1 mL of extract was transferred into a secondary tube, Analyses were performed of C_t shifts for target (detects improvements) and for QS (indicates inhibition), and the potential to reduce LoD by calculations based on CT shifts was estimated.

[b] A prospective cohort study designed to evaluate factors associated with changes in HIV knowledge and sexual behaviours after 6 and 12 months of follow-up.[26]

[c] Prepared by spotting 50 µL of serum onto Whatman 903 Protein Saver Card (Whatman GmbH, Dassel, Germany) and drying at ambient temperature for at least 3 hours.[27]

[d] Results for samples tested on the **cobas®** 6800[33]

[e] When the Roche cutoff value, optimised for plasma, was applied to the DBS eluates.[36]

[f] SARS-CoV-2 serological status was considered a true positive (reference standard) if it was reactive or borderline in at least one ECLIA test (platform A; noncompetitive ECLIA performed on **cobas®** e 411 analyser) and one CLIA-based assay (platform B; noncompetitive CLIA conducted on VIRCLIA instrument), Samples with two positive assays in one platform and none on the other were interpreted as negative due to the validation criteria developed with pre-pandemic and COVID-19 samples. This approach was conceived to increase specificity whilst maintaining high enough sensitivity.[38]

Use of DBS for HBV and HCV screening is also recommended in some national and international guidelines, including those from EASL, the American Association for the Study of Liver Diseases (AASLD), and the Infectious Diseases Society of America (IDSA),[16,20,23-25]

Several studies comparing serum/plasma and DBS specimens have shown comparable results for the detection of HBsAg, with sensitivities >90% (range: 90-100%) and specificity >99% (99-100%).[6,9,28,30] Sensitivity and specificity for anti-HBc of 86,5% and 97.8%, respectively, were observed in one DBS study,[30] and a sensitivity of 99% in another study.[9] The authors of the first study suggested that the low sensitivity for anti-HBc (86,5%) may be due to co-infections (e.g., HIV).[30] However, a study focused on at-risk populations reported sensitivities in individuals with coagulopathies/ individuals with chronic kidney disease (CKD)/PLHIV of 81,3%/79,6%/97,2%, respectively, for anti-HBc; 100%/100%/85,0% for HBsAg, and 83,3%/93,5%/100% for anti-HCV,[29] Anti-HCV sensitivities were >95% in three other DBS studies,[9,30,32,] while a further study reported a sensitivity of 84%;[28] anti-HCV specificities of 100% have been reported from DBS studies.[28,30,32]

The comparability of PSC to plasma samples for serological testing of HBV (sensitivity: 98,4%; specificity: 96,2%) and HCV (sensitivity: 98,7%; specificity: 100%) was demonstrated in a study by Martinez-Campreciós et al.[21] In a separate study in Italy, sensitivities of 100% and 91% were reported for HBsAg and anti-HCV results, respectively; however, sensitivities of 100% and 53% were reported for anti-HBs using thresholds of >2,6 IU/L and >10 IU/L, respectively.[15] Results of a prospective case-control study in Spain also showed that PSC represents a suitable specimen type for diagnosis of HCV (anti-HCV sensitivity/specificity: 92,8%/100%), Additionally, the study reported the performance of PSC to be comparable to paired plasma or serum samples for detection of viral loads over 1,000 IU/mL (sensitivity: 95%; specificity: 100%).[1] A multicentre study that enrolled participants in Cameroon, Rwanda, Georgia, and Greece reported sensitivities of 96.9% and 97.3%, and specificities of 99,8% and 95,9%, for PSC and DBS, respectively, when used in combination with **cobas®** HCV.[33]

HIV

The WHO advises that while plasma specimens are the preferred sample type for HIV viral load testing, DBS using venous or capillary whole blood can be used in settings with logistical and infrastructural barriers.[40]

Studies have demonstrated good clinical performance in immunoassays for the detection of HIV antigen/antibodies using DBS with the Elecsys® HIV Combi PT and Elecsys® HIV Duo assay (**Table 2**).[6,26,28] A feasibility study in the Netherlands, assessing self-collected DBS versus routinely collected serum samples for screening multiple infections, including HIV, demonstrated sensitivity and specificity values of 100% for HIV Ag/antibody (Ab).[6] Similar performance (sensitivity and specificity values of 100%) was reported from a study in New Zealand comparing paired DBS and serum samples.[28] In a study based on archived serum-spotted specimens from patients with suspected early HIV infection in Burkina Faso, a sensitivity of

87.2% was reported with the Elecsys® HIV combi PT assay compared with rapid diagnostic testing. The findings demonstrate that DBS provide a useful approach for diagnosing early HIV infections in hard-to-reach populations and those living in remote areas.[27] A study in the Democratic Republic of the Congo (DRC) showed that the Elecsys® HIV combi PT assay had sensitivity and specificity of 100% and 98,9%, respectively, when using DBS compared with the results of rapid diagnostic tests using venous blood samples, The authors noted the value of DBS testing in low-income countries for confirming or ruling out HIV infection, which can help to reduce unnecessary treatment.[26]

Syphilis

A study to validate a DBS approach for serological screening of syphilis reported a sensitivity of 68.3% and a specificity of 100% compared with paired serum samples.[28] The authors suggested that the low sensitivity of the Elecsys® Syphilis assay might be attributable to sample dilution during the elution step, which increased the limit of detection (LoD), in particular, for those samples containing low levels of antibodies as a result of treatment.[28]

SARS-CoV-2

During the COVID-19 pandemic, serological assays played a key role in providing population-level prevalence estimates to inform public health responses, in addition to monitoring humoral immune responses to COVID-19 vaccination, However, the pandemic highlighted that large-scale serological testing can be challenging due to costs associated with venous blood collection and the need for trained medical personnel to collect samples,[13]

Several studies demonstrated the utility of DBS for detection of antibodies to the SARS-CoV-2 virus; sensitivities of 71-100% and specificity of 99-100% have been reported for antibodies to the N protein,[3,5,34,36] and sensitivities of 96-98% and specificities of 76-100% for antibodies to the S protein (**Table 2**).[7,13,38]

Advantages of DBS/PCS in immunoassays

Sample collection, storage, and transportation

Collection, storage, and transport of serum or plasma samples derived from venous blood can be challenging in remote and under-resourced settings, and among marginalised populations (e.g., those in prison and PWID).[1]

By contrast, collection of DBS does not necessitate venipuncture, as samples may be collected using capillary blood; as such, DBS have greater acceptability among patients.[4] Additionally, sampling of DBS requires no professional training and could be performed by individuals in their own home by peers, or themselves.[34] Indeed, studies have shown that home collection of DBS is feasible without the need for medical training.[13] A study in the Netherlands reported no difference in the quality between self-collected and healthcare worker-collected DBS, highlighting the value in increasing testing access for difficult-to-reach populations (e.g., STI testing in MSM).[6]

Venipuncture is also not essential for PSC, as samples may be prepared using either capillary or venous blood. Acceptability among patients has been demonstrated, with a study in Cameroon and Uganda reporting PSC to be an acceptable viral hepatitis screening tool among 67% (129/192) of participants.[8] It should be noted that training is needed to ensure appropriate sample collection and preparation of the PSC;[8] published data regarding self-collection are not available.

Use of DBS and PSC mitigates the need for cold-chain storage and transportation, as samples are stable under harsh environmental conditions.[36] Additionally, DBS and PSC samples are safer and easier to transport, with lower potential for leakage or breakage than other sample types,[9] and it has been suggested that the biohazard risk is reduced as many viruses lose infectivity upon sample drying.[34] Consequently, authors of several studies propose using regular mail as a convenient way to transport DBS for central laboratory testing.[1,6,34]

Stability

The stability of DBS and PSC represents a key benefit of these alternative collection approaches, which can help to address logistical and cost challenges associated with storing and transporting blood samples.[41] Fontaine et al. reported that antibodies, as assessed by Elecsys® assays, remained stable in DBS at ambient temperature for at least 30 days,[34] and a study by Castelletti et al, demonstrated that Elecsys® assay results were unaffected by storage of DBS in the freezer, in the fridge, or at room temperature for 11 days, However, the authors noted a significant deterioration in antibodies in DBS stored for 11 days at 37°C or in direct sunlight, as well as those stored wet at room temperature.[13] A study assessing the long-term stability of HBsAg, anti-HBc, and anti-HCV in DBS reported reduced antigen/ antibody detection in DBS stored at temperatures of 4-37°C for up to 14 days. The authors proposed that long-term storage is suitable at -20°C or -70°C.[12] Meanwhile, serological testing has been reported using PSC stored for up to 21 days before processing,[1] and HIV-1 RNA was reported to be stable on PSC at 45°C for 3 weeks.[14]

Large-scale testing and serosurveillance studies

There are numerous challenges to conducting large-scale serosurveillance studies, including the availability of trained personnel, access to rural and remote communities, access to laboratory testing, and the need for cold-chain storage and transportation of biological specimens.[3,5,13] Additionally, in under-resourced settings and for large-scale epidemiological studies, use of rapid point-of-care (POC) diagnostic platforms (e.g., GeneXpert) may be

unfeasible due to high costs and/or the lack of appropriately trained personnel, making centralized testing at reference laboratories a more feasible alternative.[1]

The potential to mitigate these challenges with DBS was explored in a study conducted in Canada, a country with geography-related logistical challenges (e.g., remote communities). The study showed that when used in combination with DBS, the Elecsys® Anti-SARS-CoV-2 test had a sensitivity of 100% and a specificity of 100% compared with plasma samples.[5] Similarly, a cross-sectional study on the impact of COVID-19 in Kinshasa, DRC, demonstrated good clinical performance for the Elecsys® Anti-SARS-CoV-2 test compared with other assay platforms (sensitivity: 96.0%; specificity: 75,8%) when used with DBS in a resource-limited setting.[38]

Roche **cobas**® e immunochemistry analysers required to run Elecsys® immunoassays are widely used around the world and provide access to high-throughput screening.[3] Greater efficiencies in the workflow may also be obtained with automated methods for the extraction of antibodies from DBS; for instance, using a semi-automated protocol with self-sampled DBS, Beyerl et al. reported testing of 300 samples per hour [3]

Cost

A study in Pakistan evaluating the cost-effectiveness of approaches for confirmatory NAT to screen the general population for HCV demonstrated the potential utility of PSC as an alternative sampling approach to address the challenges associated with rural population testing.[39] Another study, conducted in Zambia, evaluated the cost and impact of DBS compared with PSC for viral load testing in resource-limited settings. By modelling different specimen type scenarios (plasma alone; DBS or PSC alone; and DBS or PSC in combination with plasma), the authors found that the plasma plus PSC scenario correctly classified the most significant number of viral load results, while plasma plus DBS represented the lowest average cost scenario. Allowing for the full and partial adoption of dried specimens increased viral load testing access by 19% (N=965,587).[41]

DBS/PSC offer potential cost savings by obviating the need for trained personnel and cold-chain storage during transport. They also present cost savings by facilitating reflex testing, thereby mitigating the need for follow-up appointments for confirmatory diagnostic testing.[1] Importantly, the use of DBS/PSC may facilitate the implementation of scalable screening programmes that can help to increase access to testing and increase the opportunity for diagnosis and treatment of infectious cases (e.g., HCV), thereby mitigating the potential for onward transmission [15]

Reflex testing

As discussed above with regard to diagnosis, reflex testing is a recommended approach for the diagnosis of infectious disease; the **cobas**® PSC has three spots per card, which provides utility for different tests and additional/confirmatory tests.[15] Similarly, commercially available DBS have several spots per card and may be stored for use on further testing, if required.[36]

Results of a study by Martinez-Campreciós et al. demonstrated the suitability of PSC for HCV and HBV reflex testing; for instance, sensitivity/ specificity for anti-HCV and HCV RNA compared with plasma from venous blood were 98.7%/100% and 100%/100%, respectively (**Table 2**).[21] A study in the Netherlands assessed the feasibility of using self-collected DBS for multiple screening tests. Among the self-collected specimens, sensitivity/ specificity was 100%/100% for HIV Ag/Ab, 90%/99% for HBsAg, and 90%/99% for anti-treponemal antibodies (syphilis).[6]

Limitations

Usability/quality

A study comparing SARS-CoV-2 antibody detection in DBS prepared by phlebotomists with no prior training in collecting DBS versus paired plasma samples suggested that DBS sample quality can vary by phlebotomist.[36] Quality issues were also reported from a real-world study evaluating the use of PSC with capillary blood collected by trained nurses, for HBV and HCV screening among PLHIV in Cameroon and Uganda. The authors noted that 75% (143/192) of PSC tests had at least one spot sample filled and were viable for analysis (99% [87/88] were correctly filled in Uganda and 53.4% [56/104] in Cameroon); this indicates that one in four PSC tests were either incompletely loaded or were unsuitable for analysis-a finding that suggests greater awareness is needed about effective sample collection techniques.[8] Notably, a different study that demonstrated good clinical performance for the immunoassays conducted (i.e., Elecsys® HIV combi PT sensitivity/specificity: 100%/100%; Elecsys® HBsAg II sensitivity/specificity: 90%/99%), reported no difference in specimen quality between self- and healthcare worker-collected DBS.[6] DBS contain potentially interfering substances due to the collection of whole blood, while PSC, by virtue of a porous membrane that allows separation of plasma from whole blood, minimises the potential of these interfering substances.[1,14] However, one study with PSC reported clogging of the membrane and restricted plasma recovery as a result of a highly viscous whole-blood sample.[1] There are also reported differences in analyte abundance between capillary and venous systems.[42]

Variation in DBS results may be associated with the biological variation of haematocrit values among the population, and the fact that blood can be diluted to a varying degree with tissue fluid during capillary blood sampling.[3]

Reports indicate that elution from DBS can be influenced by several parameters, including the number of circles spotted with blood, the punch size, the number of punches, and the elution buffer volume.[7]

As discussed in the above subsection on Stability, reports indicated that DBS are not suitable for the long-term storage of samples at ambient temperatures.[12,13] There are no data available on the stability of PSC for serology testing other than those described in Velasquez et al.[1]

As DBS/PSC have more prolonged sample stability, they are often used to facilitate access to centralised laboratory testing among remote and under-resourced communities. A perceived disadvantage of this approach is the lack of timely results for a same-visit diagnosis to inform clinical decision-making.[43]

Limit of detection

The LoD of serology tests (and NATs) with DBS and PSC, compared with plasma or serum, may be affected by the lower amount of starting material and dilution of the sample during reconstitution.[1,28,37] This is reported to particularly affect samples with lower antibody levels and may impact assay volume and cut-off index.[28]

Excellent correlation has been reported between quantitative plasma and DBS antibody levels using Elecsys® assays (e.g., Elecsys® Anti-SARS-CoV-2 S), with a predictable decrease in signal.[7] A pilot study by Mulchandani et al. estimated the LoD of the Elecsys® Anti-SARS-CoV-2 assay to be approximately 30 times lower with plasma samples compared with DBS.[36] Increased LoD was observed for HCV testing using DBS[28] and using PSC,[1] McAuliffe et al. noted the potential for using optimised assay cut-offs for DBS to perform comparably with serum for serological testing.[28] Although good correlation has been demonstrated between DBS and plasma, an internal Roche study using these sample matrices combined with the **cobas**® HCV and **cobas**® HBV tests found a higher correlation between PSC and plasma than between DBS and plasma (data on file). The same study showed that the reproducibility of DBS was higher than that of PSC.

In a PSC study for reflex viral load testing in people with chronic hepatitis, Martinez-Campreciós et al. reported low sensitivity for detecting viral loads <1000 IU/mL as a key limitation. The study found viral loads for HBV, HCV, and HDV correlated well, but a 1-log difference was observed, between PSC and plasma samples.[21] Similarly, Velasquez-Orozco et al. reported a 2.65-log difference in viral load measurements between plasma and DBS.[1] A study investigating whether LoD improvements could be achieved for the **cobas**® HIV-1 test when used in combination with PSC samples indicated that LoD could not be improved (data on file).

Case studies and real-world examples

Several studies have explored the use of DBS for immunodiagnosis of infectious diseases, including regions with limited healthcare infrastructure (**Table 2**). A study using DBS from patients in Kinshasa, DRC, showed that Elecsys® HIV combi PT immunoassays had sensitivity and specificity of 100% and 98.9%, respectively, for detecting acute HIV infections when using DBS.[26] Another study demonstrated a sensitivity of 87.2% for the Elecsys® HIV Combi PT compared with serum specimens and rapid diagnostic testing, for detecting acute HIV infections, when used in combination with dried serum samples.[27]

Studies conducted in Europe, South America, and Africa have also demonstrated the clinical utility of DBS specimens for viral hepatitis testing.[9,29,30,32] For instance, sensitivity and specificity of 100% were reported for the Elecsys® Anti-HCV II assay, compared with comparator immunoassays, in a study conducted in sub-Saharan Africa.[32] DBS have also been used with Elecsys® Anti-HCV II as a means of assessing progress in eliminating HCV infections among PWID.[31] DBS sampling is also suitable for studies in outreach settings, as demonstrated in an HBV testing pilot

project (sensitivity for HBsAg/anti-HBc/anti-HCV: 100%/99%/100%) and may help to provide a more patient-centred screening model to increase access for individuals who otherwise may not be tested.[9]

Satisfactory clinical performance (sensitivity: 96.0%; specificity: 75.8%) has also been reported for the Elecsys® Anti-SARS-CoV-2 S immunoassay in combination with DBS from venipuncture and finger-prick blood.[38] High concordance was demonstrated in a real-world, head-to-head study comparing Elecsys® Anti-SARS-CoV-2 S assay results from matched plasma and finger-prick-collected DBS from individuals with a polymerase chain reaction (PCR)-confirmed positive history of COVID-19 (sensitivity: 98%; specificity: 100%).[7]

Similarly, PSC samples presented a feasible and acceptable matrix for viral hepatitis testing in a real-world study evaluating PSC use with capillary blood for testing of HBsAg or anti-HCV among PLHIV in Cameroon and Uganda.[8] A pilot study in Angola also reported that PSC with capillary blood provided a suitable approach for hepatitis screening using Elecsys® Anti-HCV II (sensitivity: 98.7%; specificity: 100%), Elecsys® HBsAg II (sensitivity: 98.4%; specificity: 96.2%), and Anti-HBc II (sensitivity: 95.9%; specificity: 100%),[21] while a study in Europe demonstrated utility of PSC with venous blood for hepatitis screening using the same serology tests.[15] Another study in Africa demonstrated the suitability of the PSC for HCV testing with Elecsys® Anti-HCV II (sensitivity: 92.8%; specificity: 100%).[1]

Future directions

DBS/PSC may be suitable for use on an expanded repertoire of infectious diseases (such as tuberculosis, rubella, measles, and human T-lymphotropic virus type 1)[44-46] and represent a suitable specimen type for surveillance of vector-borne diseases (such as dengue, toxoplasmosis, Chagas disease, and malaria).[47-50]

Additionally, the opportunity to self-collect DBS/PSC, in combination with self-collected specimens for other STIs (e.g., chlamydia and gonorrhoea), may provide an opportunity in outreach settings for more comprehensive STI testing of difficult-to-reach high-risk populations (e.g., MSM).[6] This community-based approach has wide-ranging potential across a range of infectious diseases, although cases of self-sampling use require further validation prior to adoption into clinical practice.

Biochemical analyses present another potential opportunity; for instance, DBS samples are currently used for assessing glucose and for screening newborns for inherited metabolic disorders.[14] A study assessing a PSC from another manufacturer (Telimmune DUO plasma separation cards) suggested that PSC may provide a suitable alternative to plasma for lipidomic analysis; however, comparability with standard plasma samples was reported to be lipid- and lipid class-dependent.[51]

Conclusions

Use of DBS and PSC as alternative sample types to serum/plasma allows *in vitro* diagnostic tests to be performed without venipuncture. This less invasive approach may have greater acceptability among patients and allow for sample collection without the need for trained personnel.[4,8] DBS and PSC are stable at ambient temperature and may be easily transported to central laboratories, obviating the need for costly cold-chain transport.

According to existing literature, DBS/PSC are suitable for serological testing and NAT, and may be used for national screening programmes, to expand access to testing in remote and under-resourced settings, and in large epidemiological surveillance studies.[36] DBS/PSC sampling has been successfully used for large-scale population screening,[5,38] and for conducting serological surveys in resource-poor settings, due to the requirement for minimal equipment and training, and easy storage and transportation.[8,32,38]

There are long-term advantages to be realised in adopting DBS/PSC. These sample types can increase access to testing in remote settings and among marginalised patient populations, and they can reduce costs associated with screening programmes. Studies have shown that DBS represent a valid and acceptable alternative to serum specimens for testing of difficult-to-reach populations (e.g., PWID) and have wide potential utility in an outreach setting.[6,28]

Use of DBS/PSC can help to facilitate implementation of scalable screening programmes, which can improve access, increase the opportunity for diagnosis and treatment, and mitigate onward transmission.[15] Existing guidelines and policies related to serological testing of infectious diseases should be re-evaluated and updated to ensure the effective use of DBS/PSC specimens across applications.

Acknowledgements

COBAS and ELECSYS are trademarks of Roche. All other product names and trademarks are the property of their respective owners,

For inquiries, please contact:

Peter Ramge, PhD, PharmD
Senior Global Medical Affairs Manager Infectious Diseases | Global Medical Affairs Lead
Roche Diagnostics Solutions, Clinical Development and Medical Affairs, Rotkreuz, Switzerland
Email: peter.ramge@roche.com
Website: www.diagnostics.roche.com

Abbreviations

AASLD	American Association for the Study of Liver Diseases
Ab	Antibody
Ag	Antigen
anti-HBc	Antibodies to hepatitis B core antigen
anti-HBs	Antibodies to HBsAg
anti-HCV	Antibodies to hepatitis C virus
CKD	Chronic kidney disease
CLIA	Chemiluminescent immunoassays
COAG	Coagulopathy
COVID-19	Coronavirus disease 2019
DBS	Dried blood spots
DRC	Democratic Republic of the Congo
EASL	European Association for the Study of the Liver
ECLIA	Electrochemiluminescence immunoassay
EDTA	Ethylenediaminetetraacetic acid
GT	Genotyping
HBc	Hepatitis B core antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDV	Hepatitis Delta virus
HIV	Human immunodeficiency virus
HTLV	Human T-cell lymphotropic virus
IDSA	Infectious Diseases Society of America
LoD	Limit of detection
MPX	Multiplex
MSM	Men who have sex with men
N antigen	Nucleocapsid antigen
NAT	Nucleic acid testing
OKAPI	Observational Kinshasa AIDS Prevention Initiative
PCR	Polymerase chain reaction
POC	Point-of-care
PLHIV	People living with HIV
PSC	Plasma Separation Card
PT	Pre-treatment
PWID	People who inject drugs
qDBS	Quantitative dried blood spot sampling
QS	Quantitation standard
RBC	Red blood cell
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
S protein	Spike protein
SPER	Specimen Pre-Extraction Reagent
STI	Sexually transmitted infection
WHO	World Health Organization

References

1. Velásquez-Orozco F, Rando-Segura A, Martínez-Campreciós J, et al. Utility of the **cobas**® Plasma Separation Card as a sample collection device for serological and virological diagnosis of hepatitis C virus infection. Diagnostics. 2021;11(3):473.
2. Bajis S, Maher L, Treloar C, et al. Acceptability and preferences of point-of-care finger-stick whole-blood and venepuncture hepatitis C virus testing among people who inject drugs in Australia. Int J Drug Policy. 2018;61:23–30.
3. Beyerl J, Rubio-Acero R, Castelletti N, et al. A dried blood spot protocol for high throughput analysis of SARS-CoV-2 serology based on the Roche Elecsys anti-N assay. EBioMedicine. 2021;70:103502.
4. Tuaillon E, Kania D, Pisoni A, et al. Dried blood spot tests for the diagnosis and therapeutic monitoring of HIV and viral hepatitis B and C. Front Microbiol. 2020;11:373.
5. Cholette F, Mesa C, Harris A, et al. Dried blood spot specimens for SARS-CoV-2 antibody testing: A multi-site, multi-assay comparison. PLoS One. 2021;16(12):e0261003.
6. van Loo IHM, Dukers-Muijrers N, Heuts R, et al. Screening for HIV, hepatitis B and syphilis on dried blood spots: A promising method to better reach hidden high-risk populations with self-collected sampling. PLoS One. 2017;12(10):e0186722.
7. Brinc D, Biondi MJ, Li D, et al. Evaluation of dried blood spot testing for SARS-CoV-2 serology using a quantitative commercial assay. Viruses. 2021;13(6):962.
8. Picchio CA, Nicolàs A, Ayemfouo Fofou IV, et al. Acceptability and feasibility of the Plasma Separation Card for an integrated model of care for HBV and HCV screening among people attending HIV clinics in Cameroon and Uganda. J Epidemiol Glob Health. 2024;14(3):827–38.
9. Vedio AB, Ellam H, Rayner F, et al. Hepatitis B: report of prevalence and access to healthcare among Chinese residents in Sheffield UK. J Infect Public Health. 2013;6(6):448–55.
10. Cholette F, Périnet S, Neufeld B, et al. Validity of dried blood spot testing for sexually transmitted and blood-borne infections: A narrative systematic review. PLOS Glob Public Health. 2024;4(6):e0003320.
11. Knoop A, Geyer H, Lerch O, et al. Detection of anti-SARS-CoV-2 antibodies in dried blood spots utilizing manual or automated spot extraction and electrochemiluminescence immunoassay (ECLIA). Anal Sci Adv. 2021;2(9-10):440–6.
12. McAllister G, Shepherd S, Templeton K, et al. Long-term stability of HBsAg, anti-HBc, and anti-HCV in dried blood spot samples and eluates. J Clin Virol. 2015;71:10–7.
13. Castelletti N, Paunovic I, Rubio-Acero R, et al. A dried blood spot protocol for high-throughput quantitative analysis of SARS-CoV-2 RBD serology based on the Roche Elecsys system. Microbiol Spectr. 2024;12(4):e0288523
14. Carmona S, Seiverth B, Magubane D, et al. Separation of plasma from whole blood by use of the cobas Plasma Separation Card: A compelling alternative to dried blood spots for quantification of HIV-1 viral load. J Clin Microbiol. 2019;57(4):e01336–18.
15. Colucci G, Renteria SU, Ceriotti F, Lampertico P. Clinical evaluation of plasma separation cards as a tool to collect, store, and test blood samples for hepatitis b and c serological markers. Clin Chem. 2021;68(1):214–7.
16. EASL recommendations on treatment of hepatitis C: Final update of the series. J Hepatol. 2020;73(5):1170–1218.
17. Palom A, Rando-Segura A, Vico J, et al. Implementation of anti-HDV reflex testing among HBsAg-positive individuals increases testing for hepatitis D. JHEP Rep. 2022;4(10):100547.
18. Conners EE, Panagiotakopoulos L, Hofmeister MG, et al. Screening and Testing for Hepatitis B Virus Infection: CDC Recommendations – United States, 2023. MMWR Recomm Rep. 2023;72(1):1–25.
19. World Health Organization (WHO). Updated recommendations on treatment of adolescents and children with chronic HCV infection, and HCV simplified service delivery and diagnostics. 2022. Available at: https://iris.who.int/bitstream/handle/10665/363590/9789240052734-eng.pdf. Accessed May 2025.
20. Bhattacharya D, Aronsohn A, Price J, Lo Re V. Hepatitis C Guidance 2023 Update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. Clin Infect Dis. 2023:ciad319.
21. Martínez-Campreciós J, Rando-Segura A, Buti M, et al. Reflex viral load testing in dried blood spots generated by plasma separation card allows the screening and diagnosis of chronic viral hepatitis. J Virol Methods. 2021;289:114039.
22. World Health Organization (WHO). Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection. March 2024. Available at: https://iris.who.int/bitstream/handle/10665/376353/9789240090903-eng.pdf. Accessed May 2025.
23. Public Health Wales. Dried blood spot testing for hepatitis C, hepatitis B and HIV: Information for substance misuse services. Available at: https://phw.nhs.wales/topics/ substance-misuse-drugs-alcohol/evidence/drugs/drugs-evidence-documents/dried-blood-spot-testing-for-hepatitis-c-hepatitis-b-and-hiv/. Accessed May 2025.
24. Public Health England. PHE national free dried blood spot (DBS) testing service for infants born to hepatitis B positive mothers. Available at: https://assets.publishing.service.gov.uk/media/5ba3c268e5274a55e18d2bb1/PHE_factsheet_Free_dried_blood_spot_testing__.pdf. Accessed May 2025.
25. Ghany MG, Morgan TR. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America recommendations for testing, managing, and treating hepatitis C virus infection. Hepatology. 2020;71(2):686–721.
26. Barquín D, Ndarabu A, Carlos S, et al. HIV-1 diagnosis using dried blood spots from patients in Kinshasa, DRC: a tool to detect misdiagnosis and achieve World Health Organization 2030 targets. Int J Infect Dis. 2021;111:253–60.
27. Kania D, Truong TN, Montoya A, et al. Performances of fourth generation HIV antigen/antibody assays on filter paper for detection of early HIV infections. J Clin Virol. 2015;62:92–7.

28. McAuliffe G, Gerber A, Chhibber A, et al. Evaluating the sensitivity and specificity of dried blood spots for serological testing of HIV, syphilis, hepatitis B and C Elecsys assays on the Roche Cobas system. Pathology. 2023;55(7):1000–3.
29. Flores GL, Barbosa JR, Cruz HM, et al. Dried blood spot sampling as an alternative for the improvement of hepatitis B and C diagnosis in key populations. World J Hepatol. 2021;13(4):504–14.
30. Villar LM, Cruz HM, Deodato RM, et al. Usefulness of automated assays for detecting hepatitis B and C markers in dried blood spot samples. BMC Research Notes. 2019;12(1):523.
31. Steffen G, Krings A, Guttmann S, et al. Progress and challenges in the elimination of hepatitis C among people who inject drugs in Germany: results of a pilot study for a national monitoring system, 10 years after the first data collection. Harm Reduct J. 2024;21(1):222.
32. Carrasco T, Barquín D, Ndarabu A, et al. HCV diagnosis and sequencing using dried blood spots from patients in Kinshasa (DRC): A tool to achieve WHO 2030 targets. Diagnostics (Basel). 2021;11(3):522.
33. Malobela A, Amougou M, Ilioupoulos P, et al. Diagnostic accuracy of dried blood spot and plasma separation card samples for testing hepatitis C virus RNA. Poster presented at the International Liver Congress, 3–26 June 2021 (Virtual meeting).
34. Fontaine E, Saez C. Analysis of SARS-CoV-2 antibodies from dried blood spot samples with the Roche Elecsys Immunochemistry method. Pract Lab Med. 2021;25:e00234.
35. Marchand A, Roulland I, Semence F, Ericsson M. Adaptation of Elecsys(®) anti-severe acute respiratory syndrome coronavirus-2 immunoassay to dried blood spots: proof of concept. Bioanalysis. 2021;13(3):161–7.
36. Mulchandani R, Brown B, Brooks T, et al. Use of dried blood spot samples for SARS-CoV-2 antibody detection using the Roche Elecsys® high throughput immunoassay. J Clin Virol. 2021;136:104739.
37. Marchand A, Roulland I, Semence F, et al. Use of quantitative dried blood spots to evaluate the post-vaccination level of neutralizing antibodies against SARS-CoV-2. Life (Basel). 2021;11(11).
38. de Aguirre PM, Carlos S, Pina-Sánchez M, et al. High pre-Delta and early-Omicron SARS-CoV-2 seroprevalence detected in dried blood samples from Kinshasa (D.R. Congo). J Med Virol. 2024;96(3):e29529.
39. Babigumira JB, Karichu JK, Clark S, et al. Assessing the potential cost-effectiveness of centralised versus point-of-care testing for hepatitis C virus in Pakistan: a model-based comparison. BMJ Open. 2023;13(5):e066770.
40. World Health Organization (WHO). What's new in treatment monitoring: viral load and CD4 testing. Available at: https://www.who.int/publications/i/item/WHO-HIV-2017.22. Accessed May 2025.
41. Nichols BE, Girdwood SJ, Shibemba A, et al. Cost and impact of dried blood spot versus plasma separation card for scale-up of viral load testing in resource-limited settings. Clin Infect Dis. 2020;70(6):1014–20.
42. Lim MD. Dried blood spots for global health diagnostics and surveillance: Opportunities and challenges. Am J Trop Med Hyg. 2018;99(2):256–65.
43. Campbell S, Anderson N, Babady E, et al. Centralized vs decentralized molecular infectious disease testing. Clin Chem. 2021;67(5):713–9.
44. Helfand RF, Cabezas C, Abernathy E, et al. Dried blood spots versus sera for detection of rubella virus-specific immunoglobulin M (IgM) and IgG in samples collected during a rubella outbreak in Peru. Clin Vaccine Immunol. 2007;14(11):1522–5.
45. Malsagova K, Kopylov A, Stepanov A, et al. Dried blood spot in laboratory: Directions and prospects. Diagnostics (Basel). 2020;10(4).
46. Parker SP, Taylor MB, Ades AE, Cubitt WD, Peckham C. Use of dried blood spots for the detection and confirmation of HTLV-I specific antibodies for epidemiological purposes. J Clin Pathol. 1995;48(10):904–7.
47. Maldonado-Rodríguez A, Rojas-Montes O, Vazquez-Rosales G, et al. Serum Dried Samples to Detect Dengue Antibodies: A Field Study. Biomed Res Int. 2017;2017:7215259.
48. Hegazy MM, Hegazy MK, Azab MS, Nabih N. Validation of dried blood spots in monitoring toxoplasmosis. Pathog Glob Health. 2020;114(5):242–50.
49. Corran PH, Cook J, Lynch C, et al. Dried blood spots as a source of anti-malarial antibodies for epidemiological studies. Malar J. 2008;7:195.
50. Silgado A, Gual-Gonzalez L, Sánchez-Montalvá A, et al. Analytical evaluation of dried blood spot and rapid diagnostic test as a new strategy for serological community screening for chronic Chagas disease. Front Cell Infect Microbiol. 2021;11:736630.
51. Bishop LM, Fiehn O. Comprehensive lipidomic profiling by plasma separation cards. Anal Bioanal Chem. 2023;415(1):193–201.
52. Cornberg M, et al. EASL Clinical Practice Guidelines on the management of hepatitis B virus infection J Hepatol. 2025;83(2):502 – 583.

Appendix

Methodology

Setup and materials

Capillary blood may be collected by fingerstick using a disposable contact-activated lancet.[1] Venous blood is collected in tubes containing anticoagulants, such as EDTA.

DBS samples may be collected using commercially available DBS cards comprising Whatman paper contained within a rigid frame for handling and labelling; each DBS card typically contains several pre-printed circles for the collection of whole blood and any predefined spots (Figure 1A).[2,3] Alternatively, DBS can be prepared using filter paper without any predefined spots.

The PSC has three specimen areas, each comprising a porous membrane that allows plasma to be separated from whole blood (**Figure 1B**). The plasma is collected on an underlying polyester fleece, which has an RNA-stabilising reagent that stabilises the samples over a range of temperatures, humidity levels, and storage conditions.[4]

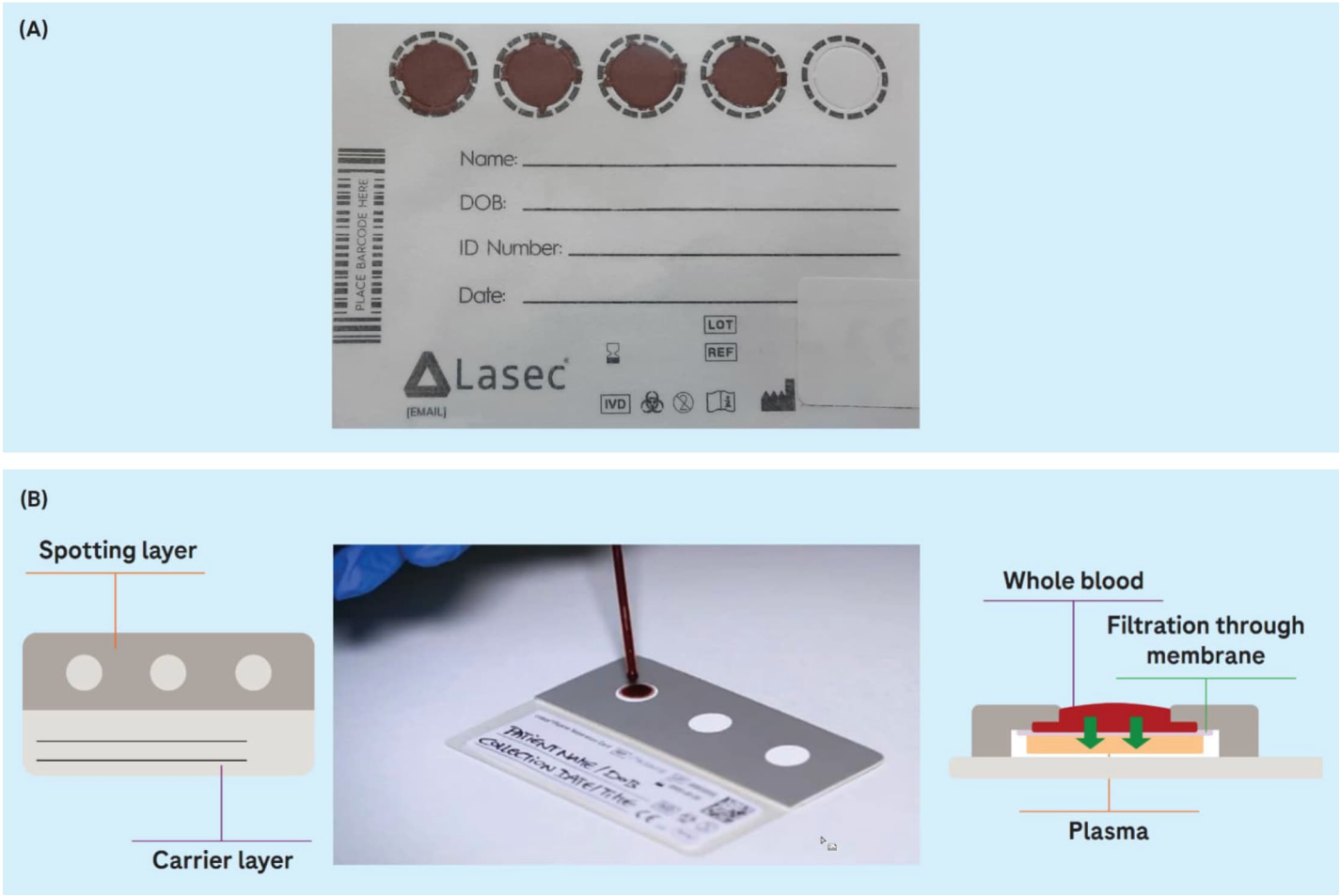
Sample collection

DBS (50–70 µL blood per spot) and PSC (140 µl blood per spot) are prepared by spotting whole-blood samples obtained from finger pricks or venous blood.[2,5]

Sample storage

DBS may be stored together with a desiccant pack in suitable plastic bags at room temperature until analysis.

Figure 1. (A) Example DBS card showing five pre-printed circles for blood spot collection, and **(B)** PSC showing three distinct sample spots and the filtration membrane that facilitates generation of dried plasma from whole blood (image reproduced from Carmona et al.[14] with no changes, according to CC BY 4,0 license).



DBS, dried blood spot; PSC, Plasma Separation Card,

The PSC should reach the centralised testing facility within 28 days from sample collection at 18-45°C and at <85% relative humidity.[6]

Sample preparation

Preparation of DBS for analysis may be performed manually or using a card extraction robot.[7] DBS are extracted from filter paper by punching out the spot sample and eluting the blood into a solution.[7]

PSC are currently validated solely for HIV and HCV NAT (i.e., **cobas**® HIV and HCV tests), A suggested protocol for processing PSC for subsequent virological testing includes the following steps: individual dried spots are removed from PSC and incubated at 56°C with 950 µL of Specimen Pre-Extraction Reagent for 10 minutes at 1,000 rpm on a pre-heated thermoshaker; the entire eluate is transferred to a new tube, leaving the PSC paper in the primary tube.[5]

A suggested protocol for processing PSC for subsequent serological testing includes the following steps: spots are removed from PSC and incubated overnight (>8 hours) at 37°C in 600 µL of universal diluent dedicated to Elecsys® serology assays; samples are then centrifuged at 1,300 rpm for 10 minutes, before transferring the eluate to a new tube, leaving the PSC paper in the primary tube.[5]

Central lab testing

Remote and under-resourced areas often lack skilled healthcare workers and the infrastructure necessary to conduct diagnostic testing. DBS can help to overcome the challenges in settings where there is a paucity of facilities or expertise to take venous blood specimens or where transport of blood specimens is challenging. As cold-chain transport is not needed for DBS/PSC, transport of specimens to central laboratories is made easier. Collection of DBS/PSC can be performed outside of the traditional healthcare setting by capillary puncture, and transported at room temperature to centralised testing facilities, where high-throughput screening may be performed using Roche **cobas**® e immunochemistry analyser.[2,8]

References

1. Fontaine E, Saez C, Analysis of SARS-CoV-2 antibodies from dried blood spot samples with the Roche Elecsys Immunochemistry method, *Pract Lab Med.* 2021;25:e00234.
2. Tuaillon E, Kania D, Pisoni A, et al, dried blood spot tests for the diagnosis and therapeutic monitoring of HIV and viral hepatitis B and C, *Front Microbiol.* 2020;11:373.
3. Lim MD, Dried blood spots for global health diagnostics and surveillance: Opportunities and challenges, *Am J Trop Med Hyg.* 2018;99(2):256-65.
4. Carmona S, Seiverth B, Magubane D, et al, Separation of plasma from whole blood by use of the cobas Plasma Separation Card: A compelling alternative to dried blood spots for quantification of HIV-1 viral load, *J Clin Microbiol.* 2019;57(4):e01336-18.
5. Velasquez-Orozco F, Rando- Segura A, Martinez-Camprecios J, et al, Utility of the Cobas® Plasma Separation Card as a sample collection device for serological and virological diagnosis of hepatitis C virus infection, *Diagnostics* 2021;11(3):473.
6. Roche Diagnostics Ltd, cobas® Plasma Separation Card: Method sheet, Available at: https://elabdoc-prod,roche,com/eLD/api/downloads/6932b75f- b7d5-ec11-1591-005056a71a5d?countryIsoCode=be. Accessed May 2025.
7. Knoop A, Geyer H, Lerch O, Rubio A, Schrader Y, Thevis M, Detection of anti-SARS-CoV-2 antibodies in dried blood spots utilizing manual or automated spot extraction and electrochemiluminescence immunoassay (ECLIA), Anal Sci Adv. 2021;2(9-10):440-6.
8. Beyerl J, Rubio-Acero R, Castelletti N, et al, A dried blood spot protocol for high throughput analysis of SARS-CoV-2 serology based on the Roche Elecsys anti-N assay, EBioMedicine. 2021;70:103502.

Roche Diagnostics International Ltd
diagnostics.roche.com