Featuring a Review from the Rice Optical Spectroscopy and Imaging Laboratory, Dr. Rebecca Richards-Kortum, Rice University, Texas, USA.

Point-of-care diagnostics to improve maternal and neonatal health in low-resource settings

We review technologies to diagnose the leading causes of maternal and neonatal mortality, with a focus on identifying key gaps in development where new technology could improve health outcomes at the point of care.

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CRITICAL REVIEW

of these facilities lack sophisticated laboratory infrastructure and do not have the resources to transport clinical specimens to central laboratories. Where available, point-of-care (POC) diagnostics can provide a solution to this challenge. However, as shown in Tables 1 and 2, only a limited number of POC diagnostic tools are available for use at health centers and health posts to detect the conditions that account for the majority of maternal and neonatal deaths.[6,7] For many of these conditions, early detection and rapid initiation of treatment is key to reducing morbidity and mortality and achieving SDG three.[8,9]

Currently available diagnostic tools often face barriers to implementation at the POC. Many diagnostic techniques can only be performed in laboratory facilities with access to constant power, water, and trained staff. For example, polymerase chain reaction, a standard method for diagnosing HIV in neonates, requires the use of expensive thermocycling equipment and highly trained technicians. Additionally, reagents used in many diagnostic tests have special storage or transportation requirements, such as cold transportation of antibodies used in ELISA testing to detect biomarkers of many diseases. Consumables, such as test strips or specialized cartridges, can be difficult to supply and lead to higher per-test costs. Instrumentation cost and associated maintenance costs also prevent some diagnostic technologies from being implemented in low-resource settings. The time-to-result associated with some tests limits their utility in both low- and high-resource settings. For example, bacterial culture is the gold standard to diagnosis sepsis, but the technique requires 24 to 48 hours to complete,[10] preventing diagnosis-directed treatment during the effective treatment window.[11] Finally, insufficient human resources can limit the efficacy of diagnostics for some conditions that require continuous monitoring, such as neonatal hypothermia. While low-cost thermometers exist to measure a neonate's temperature, the human resources required for constant monitoring present a barrier. All of these barriers must be considered when developing a useful POC test for low-resource settings that can be appropriately implemented.

To address the shortcomings of existing diagnostics for low-resource settings, the WHO introduced a list of criteria for the ideal point-of-care test, known as ASSURED (affordable, sensitive, specific, user-friendly, robust & rapid, equipment-free, and deliverable).[12] Diagnostic tests targeted for use at the POC in low-resource settings should be designed with these criteria in mind to minimize barriers for successful implementation. However, there has been some criticism of the ASSURED criteria as being subjective and not

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Table 1 Causes of maternal mortality globally with commercially available diagnostic tools. Global mortality column represents annual mortality rates. Level of health system indicates the level at which commercially available diagnostics can be deployed, taking into account the need for electrical power, refrigeration, consumable reagents, device and consumable costs, and necessary human resources for use.[375]

Cause of death	Global mortality[6]	Condition	Level of health system		
			Reference lab	Health center	Health post
Pre-existing conditions complicated by pregnancy	683 000	HIV diagnosis	■	■	■
		HIV monitoring	■		
		Malaria	■	■	■
		Diabetes	■	■	*
		Anemia	■	■	*
Postpartum hemorrhage	661 000	Hemorrhage	■	■	**
Pre-eclampsia	343 000	Hypertension	■	■	***
		Proteinuria	■	■	****
Bacterial infections	261 000	PROM	■	■	
		Sepsis	■		
		Leukocytosis	■	■	

* Available at health posts but limited by a lack of affordable consumables. ** Technology exists for measuring blood loss but not for predicting those at risk. *** Available at health posts but limited by a lack of human resources. **** Available at health posts but limited by a lack of sensitivity.

Table 2 Causes of neonatal mortality globally with commercially available diagnostic tools. Global mortality column represents annual mortality rates. Level of health system indicates the level at which commercially available diagnostics can be deployed, taking into account the need for electrical power, refrigeration, consumable reagents, device and consumable costs, and necessary human resources for use.[375]

Causes of death	Global mortality[7]	Condition	Level of health system		
			Reference lab	Health center	Health post
Premature birth complications	965 000	Hypothermia	■	■	**
		Jaundice	■		
		Hypoglycemia	■	■	*
Intrapartum complications	662 000	Birth asphyxia	■		
Sepsis	421 000	Bacterial infections	■		
		Leukocytosis	■	■	
Congenital abnormalities	276 000		■		
Neonatal pneumonia	136 000	Pneumonia	■	■	
Other disorders	232 000	HIV	■		
		Congenital syphilis	■	■	

* Available at health posts but limited by a lack of affordable consumables. ** Available at health posts but limited by a lack of human resources.

sufficiently comprehensive for new technologies. Additionally, meeting all of the ASSURED criteria does not necessarily mean that a technology is appropriate for use at the POC. As Pai *et al.* stated in 2012, "… the technology as such does not define a POC test nor determine its use at the POC. Rather, it is the successful use at the POC that defines a diagnostic process as POC testing,"[13] underscoring the importance of testing technologies at the POC and performing rigorous usability studies in the field. It is important to consider the final context in which a technology will be used during the design process and to determine how this context will affect the definitions used for and the relative importance of each of the ASSURED criteria. For example, the use of smartphones in POC diagnostics, which has previously been reviewed,[14] has allowed for implementation of detection methods that previously required inaccessible equipment, changing our understanding of the criterion "equipment-free". Despite these shortcomings, the ASSURED criteria are frequently used in discussing the ability of a newly developed technology to be deployed at the POC.

When coupled with effective treatment strategies, low-cost POC diagnostics that can be administered in low-resource settings have the potential to reduce both neonatal and maternal mortality. Tables 3 and 4 summarize representative diagnostic technologies that are commercially available to meet maternal and neonatal health needs, respectively, highlighting a need to develop further commercialized technologies that reduce per-test cost, improve accuracy, and move away from reliance on power and benchtop analyzers.

Here, we review key innovations in POC diagnostic tools to detect the leading causes of maternal and neonatal death in low-resource settings. We review both commercially available technologies and technologies that are currently in development. We begin by reviewing diagnostic formats,

Table 3 Representative commercially available technologies available to diagnose the leading causes of maternal mortality. Notably, many of the listed technologies are lacking robust usability, scalability, and field performance studies

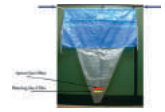
Condition	Representative technology	Analyte measured	Core technology	Input sample	Diagnostic performance	Cost	Power[a]	Health system use level	Advantages	Limitations	Image
HIV diagnosis	*Alere Determine HIV-1/2 Ag/Ab Combo*[376,377]	HIV-1/2 antibodies and free HIV-1 p24 antigen	Lateral flow	Whole blood	Sensitivity: 99.9% Specificity: 98.3–99.9%[b376]	$18.22	None	Heatlh post	Results in 20 minutes; approved for fingerstick use; excellent sensitivity	Unsuitable for acute infection and treatment monitoring	
HIV monitoring	*Xpert HIV-1 Viral Load*[24,378,379]	HIV RNA	Automated microbiology analyzer	Whole blood	R^2 = 0.92; limit of detection 40 cp mL^{-1} [bd378]	$17 000 instrument $9.98 per test	Mod	Reference lab	Minimal hands-on time; 92 minute run time; high throughput; integrated sample preparation	High cost	
Malaria	*SD BIOLINE Malaria Ag Pf*[60,380,381]	*Plasmodium falciparum* histidine-rich protein II (*Pf*HRP-II)	Lateral flow	Whole blood	Sensitivity: 95% Specificity: 97.1%[b60]	$0.70	None	Health post	Low-cost; minimal training requirements; results in 30 minutes; excellent sensitivity	Counterfeit tests on market; limit-of-detection unsuitable for submicroscopic infections	
Diabetes	*LifeScan OneTouch Ultra 2*[98,382]	Glucose	Lateral flow with reader	Whole blood	AUC: 0.778 95% CI: 0.726–0.829[b97]	$19.99 instrument $1.45–1.60 per test	Low	Health post	Widely available; integrated sample processing	High per-test cost	
Anemia	*HemoCue*[383–385]	Hemoglobin	Microfluidics	Whole blood	R^2 = 0.85–0.995 AUC: 0.96[b383]	$449 instrument $1.53–$1.86 per test	Low	Health post	Integrated sample processing	High per-test cost	
Hemorrhage	*BRASS-V drape*[146,148,386]	Blood loss	Blood collection and volume measurement	Whole blood	R = 0.928[c148]	$4 per test	None	Health post	Accurate; minimal training requirements	High per-test cost	
Pre-eclampsia	*Alere Triage PlGF*[185,186,387]	Placental growth factor	Lateral flow with reader	Plasma	Sensitivity: 77% Specificity: 95%[b186]	$1750 instrument $50 per test	Mod	Health center	Results in 20 minutes	High per test cost; requires plasma separation from whole blood	
Bacterial Infection	*Blood Culture*[388–391]	Bacterial load	Laboratory microbiology	Sterile whole blood	Sensitivity: 73%[b388]	$28	High	Reference lab		Prone to contamination and false positives	

Table 3 (*continued*)

Condition	Representative technology	Analyte measured	Core technology	Input sample	Diagnostic performance	Cost	Power[a]	Health system use level	Advantages	Limitations	Image
PROM	*Amnisure ROM*[205,219,392]	Placental alpha-microglobulin 1 (PAMG-1)	Lateral flow	Vaginal swab	Sensitivity: 96.8% Specificity: 98.3%[b205]	$60 per test	None	Health center	Excellent sensitivity, minimal training requirements, results in 10 minutes	High per test cost, blood contamination can give false positives	

[a] Power can be battery based, with low indicating small, easily replaceable batteries, moderate indicating larger, more expensive batteries, and high indicating dependency on electrical power grid. [b] Reported diagnostic performance is independently verified. [c] Diagnostic performance is manufacturer reported. [d] Reported diagnostic performance was achieved using plasma samples. HIV Diagnosis image provided courtesy Alere Inc. HIV Monitoring image provided courtesy of Cepheid. Malaria image provided courtesy Alere Inc. Hemorrhage image reproduced from ref. 386 under the terms of Creative Commons Attribution License. Pre-eclampsia image provided courtesy Alere Inc. Bacterial Infection image reproduced from ref. 391 under the terms of Creative Commons Attribution License. PROM image provided courtesy of Qiagen.

including types of biomarkers detected in many diagnostic tests. We then focus on technologies that are available or in development to address maternal and neonatal health needs. The included figures illustrate the form and/or function of selected, representative diagnostic technologies. Finally, we discuss key unmet needs in maternal and neonatal health where further innovation in POC diagnostics is desired.

Diagnostic format

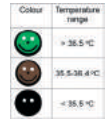
In high-resource settings, diagnostic tests are typically performed in laboratory settings by highly-trained technicians using expensive equipment. Because of this, field-appropriate benchtop analyzers have been developed to miniaturize and simplify some of the technologies found in lab instruments. Although these devices are not suitable for bedside use, they can be used in some low-resource settings that have consistent power and lab technicians. To detect biomarkers quickly and accurately at the bedside, equipment-free POC tests have been developed; one of the most common formats for these tests is the lateral flow assay (LFA), sometimes referred to as a dipstick test[15] (Fig. 1). While the per-test cost of LFAs is often higher than that of high-throughput laboratory instruments, the lack of instrumentation cost makes these tests extremely attractive for use in low-resource settings.[15,16] LFAs generally meet all of the ASSURED criteria and are appropriate for use at health centers.[15] However, one of the challenges of developing LFAs is in achieving clinically relevant sensitivity and specificity.

Choice of biomarker can greatly affect sensitivity and specificity of a test; therefore, biomarker selection is one of the most important considerations in the development of any diagnostic. Proteomics, metabolomics, and genomics research identifies biomarkers associated with various maternal and neonatal diseases, and verified disease biomarkers can be targeted in point-of-care diagnostics.[17] Here, we briefly introduce the most commonly targeted biomarkers for diagnostics, including patient antibodies, other proteins and small molecules, and pathogen nucleic acid before discussing diagnostic technologies available for maternal and neonatal health in low-resource settings.

Antibody detection

In response to a pathogenic threat, IgM antibodies are produced quickly, while IgG antibodies are produced later and circulate in the bloodstream longer.[18,19] Most antibody-based diagnostics use recombinant proteins to detect both types of antibodies in a patient sample in order to estimate infection duration and level of exposure.[18,19] However, IgG antibodies are inappropriate for detection of many neonatal conditions because maternal antibodies can be transferred to the fetus during pregnancy and persist for 12–18 months after birth.[20–22] IgM antibodies can detect acute infection in neonates.[23] Additionally, some antibody-based tests are unsuitable for determining treatment efficacy, as antibody levels remain elevated longer than other biomarkers.[18,19]

Table 4 Representative commercially available technologies available to diagnose the leading causes of neonatal mortality. Notably, many of the listed technologies are lacking robust usability, scalability, and field performance studies

Condition	Representative technology	Analyte measured	Core technology	Input sample	Diagnostic performance	Cost	Power[a]	Health system use level	Advantages	Limitations	Image
Hypothermia	*ThermoSpot*[252,254]	Body temperature	Trans-cutaneous sensor	N/A	Sensitivity: 88% Specificity: 97%[b252]	$0.11	None	Health post	Low cost; minimal training requirements; noninvasive	Can fall off of neonate; only provides temperature range	
Jaundice	*BiliChek*[393–395]	Bilirubin	Trans-cutaneous Optical sensor	N/A	R^2 = 0.86 *When TSB > 14 mg dL^{-1}:* AUC: 0.9595% CI: 0.92–0.97[b393]	$3900 instrument $7 per test	Low	Health center	Noninvasive	High cost; may require additional confirmatory lab tests	
Hypoglycemia	*Nova StatStrip*[276,396]	Glucose	Lateral flow with reader	Whole blood	Sensitivity: 90–100% Specificity: 97.7–99.4%[b276]	$250 instrument $0.30 per test	Low	Health post	Accurate with neonatal samples; integrated sample preparation	Higher instrument cost than other glucometers	
Birth asphyxia	*Moyo*[301,397]	Fetal heart rate	Trans-cutaneous Doppler sensor	N/A	Accuracy: ±5 bpm in the range of 50–200 bpm[c301]	$198	Low	Heatlh center	Noninvasive; portable	Not as sensitive for asphyxia as gold standard	
Sepsis	*Blood Culture*[388–391]	Bacterial load	Laboratory microbiology	Sterile whole blood	Sensitivity: 73%[b388]	$28	High	Reference lab		Prone to contamination and false positives	
HIV	*Alere™ q HIV-1/2 Detect*[328,398]	HIV RNA	Automated microbiology analyzer	Whole blood	Sensitivity: 99.3%, Specificity: 100.0%[b328]	$25 000 instrument, $25.00 per test	Mod	Reference lab	High diagnostic performance; integrated sample preparation	Maximum of 8 samples per day, high cost	
Congenital syphilis	*SD Bioline Syphilis 3.0*[344,399,400]	Antibodies to *Treponema pallidum*	Lateral flow	Whole blood	Sensitivity: 84.5% Specificity: 97.9%[b344]	$0.90	Low	Health post	Low-cost; minimal training requirements, results in 20 minutes	Only used to diagnose mother during pregnancy	
Pneumonia	*Counting Beads*[321]	Respiratory rate	Clinical observation	N/A	Sensitivity for fast breathing: 68%[b321]	Provided by International Organizations	Low	Heatlh post	Power-based equipment-free; minimal training requirements	Requires training and significant time by staff	

[a] Power can be battery based, with low indicating small, easily replaceable batteries, moderate indicating larger, more expensive batteries, and high indicating dependency on electrical power grid. [b] Reported diagnostic performance is independently verified. [c] Diagnostic performance is manufacturer reported. Hypothermia image reproduced from ref. 254 under the terms of Creative Commons Attribution License. TSB: Total serum bilirubin. Jaundice provided courtesy of Philips. Hypoglycemia image provided courtesy of Nova Biomedical. Birth Asphyxia figure from Laerdal Global Health AS. All rights reserved. Sepsis image reproduced from ref. 391 under the terms of Creative Commons Attribution License. HIV image provided courtesy Alere Inc. Congenital Syphilis image provided courtesy Alere Inc. Pneumonia image reproduced from ref. 321 under the terms of Creative Commons Attribution License.

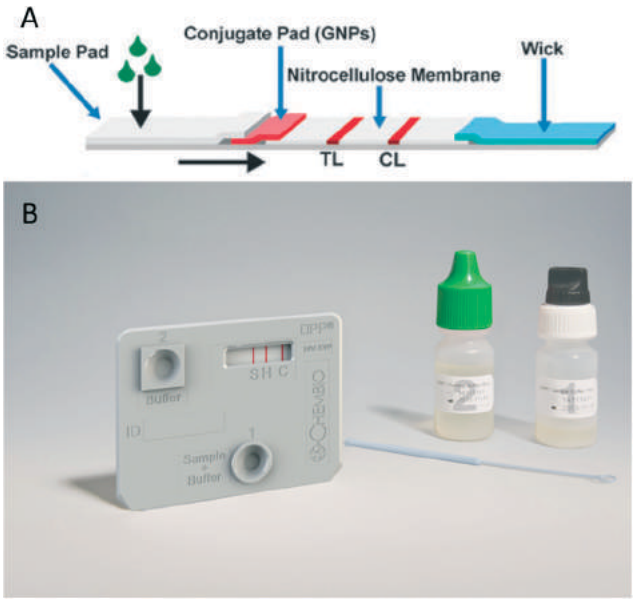


Fig. 1 Some examples of lateral flow tests to diagnose neonatal and maternal conditions. (A) Generalized depiction of a lateral flow device with a sample pad, conjugate pad, nitrocellulose membrane, and wicking pad. Figure reproduced from ref. 401 with the permission of The Royal Society of Chemistry.[401] (B) The ChemBio DPP HIV-Syphilis Assay detects antibodies for both syphilis (first "S" line) and HIV (second "H" line) from a drop of whole blood. The patented Dual Path Platform enables separate delivery of sample and detection reagents and improves sensitivity. Figure from Chembio Diagnostic Systems, Inc. All rights reserved.

Protein and small molecule biomarker detection

Many proteins and small molecules already present in bodily fluids deviate from normal physiological levels in response to disease. Therefore, protein- and small molecule-based tests need to be optimized to detect biomarker levels that differentiate between normal and diseased states. Analyte selection is important to consider because the choice of a biomarker that is modulated in multiple conditions may result in poor diagnostic specificity for a given condition. Proteins and small molecules are often present at concentrations that can be detected without the need for amplification.[15]

Nucleic acid detection

For conditions that have low pathogen concentrations, require viral load measurement, or need high specificity against related viruses, nucleic acid testing is the standard of care. Nucleic acid tests (NATs) detect specific encoded sequences within the genetic material of pathogens. NATs may detect and amplify very few copies of the target nucleic acid, making them highly sensitive and specific. Unlike antibody tests, NATs can detect a pathogen as soon as it is present. NATs typically accomplish three goals: sample preparation, amplification of target, and detection of amplicon. Real-time PCR has allowed amplification and detection to be performed simultaneously, and several completely automated platforms have been developed for use at the POC.[24]

Sample preparation. Sample preparation can be a challenge in detecting any biomarker, but it provides a significant challenge for NATs, as nucleic acid amplification is inhibited by components of bodily fluids.[25,26] Sample preparation for POC tests typically consists of collection, separation, extraction, and concentration of nucleic acids. Commercialized paper-based sample collection and plasma separation technologies are commonly used for sample collection.[27] Novel approaches to sample preparation, including microfluidic separation techniques[28,29] and extraction and concentration techniques,[29] have been previously reviewed. However, challenges remain before unprocessed blood or other clinical samples can be applied directly to amplification and detection assays at the POC.

Amplification of target. The standard method for amplification of genomic material is polymerase chain reaction (PCR), which requires expensive thermal cycling equipment, reliable electricity, trained personnel to prepare the reactions, and several hours to complete. To perform amplification at the POC, promising technologies employ isothermal enzymatic amplification methods that require a shorter incubation period and eliminate the need for thermal cycling.[30]

Detection of amplicon. Nucleic acid amplicons may be detected either during the amplification process (real-time detection) or at the end of the reaction (endpoint detection). The presence of amplified nucleic acids is measured via an optical or electrical signal. Real-time detection uses fluorescently labeled probes to bind to the amplicon and instrumentation to quantify the signal.[31] For end-point detection, amplification products may be tagged with reporter molecules to create a visible signal indicating the presence of the amplicon and be detected in equipment-free formats.[31] Real-time detection techniques are commonly used in quantitative assays and are highly sensitive and specific, while end-point detection typically requires fewer resources and may suffice for less sensitive applications.[24]

The biomarker types and detection schemes discussed above each have advantages and disadvantages, and the most appropriate test for use at the POC depends on the condition and available infrastructure. We now discuss in detail technologies for each leading cause of maternal and neonatal mortality as well as areas for potential diagnostic development.

Diagnostics for maternal health

Pre-existing conditions complicated by pregnancy

HIV. HIV is a virus that attacks the body's immune system, most notably CD4 white blood cells that fight off infections. In 2014, an estimated 1.5 million women living with HIV gave birth, and HIV-positive women are 6–8 times more likely to die giving birth than those who are HIV negative.[32] POC devices that make maternal HIV diagnosis as rapid and simple as possible are of high importance and have been the target of much funding and research over the past 20 years.[33] With effective diagnosis, women with HIV can receive

appropriate prophylactic strategies to reduce viral load, both for their health and the health of the neonate. Ninety percent of HIV infections in children are due to mother-to-child transmission.[32] However, the prevention of mother-to-child-transmission (PMTCT) of HIV is one of the great public health successes of the past 20 years.[33] The vertical transmission rate for HIV-positive pregnant women with no intervention is 25–42%, but this rate has been reduced to 1% or less when all prophylactic strategies are implemented.[33] Precautions including antiretroviral therapy (ART) during pregnancy, labor and delivery, and the postnatal period to the infant, as well as elective cesarean delivery, have contributed to this success.

HIV is now widely diagnosed in adults using affordable antibody dipstick tests. However, antibody concentrations reach a peak concentration during the early stage of HIV infection and decrease after acute infection.[34] Thus, antibody/antigen tests are useful for screening but not treatment monitoring. To diagnose and confirm treatment failure, the WHO strongly recommends viral load monitoring.[35] Current guidelines encourage viral load testing at six and 12 months after initiating ART, and every 12 months thereafter if the patient is stable on ART. Virological failure is defined by two consecutive viral load measurements exceeding 1000 copies per mL within a 3-month interval, with adherence support between measurements, after at least six months of a new ART regimen. Despite these recommendations, where viral load testing is not available, CD4 count and clinical symptoms are still used to diagnose treatment failure.[24] Here we discuss antibody/antigen tests as well as CD4 testing for HIV; solutions for point-of-care viral load testing are discussed in the Neonatal HIV section.

Antibody detection. Antibody assays to diagnose HIV infection in adults detect antibodies against the HIV viral envelope proteins gp41 and gp36. Developed tests include the ChemBio sure check HIV 1/2,[36] the UniGold Recombigen HIV 1/2,[37] the VIKIA HIV 1/2,[38] the OraQuick in-home test,[39] the INSTI,[40] and the Alere Determine HIV 1/2 Ab + Ag.[41] The mChip is a microfluidic ELISA that detects antibodies against HIV viral envelope proteins as well as syphilis.[42] While first-generation HIV tests were able to detect antibodies about a month after infection, second- and third-generation tests improved the sensitivity to allow earlier detection. Fourth-generation tests now screen for the p24 antigen in addition to antibodies, allowing detection as soon as 18 days after infection, although the p24 antigen portion has shown variable performance thus far.[43] Antibody/antigen tests are the easiest and most affordable option for HIV diagnosis and have seen success with use in clinical settings. These technologies meet ASSURED criteria and are available in many low-resource settings.

In late 2016, the WHO issued a strong recommendation that HIV self-testing should be offered as an additional approach to HIV testing services.[44] This recommendation was based on studies indicating that compared to standard HIV testing, HIV self-testing can result in identifying an equivalent or greater proportion of HIV-positive people. As of July 2017, there is one WHO prequalified HIV self-test, the OraQuick® HIV Self-Test (OraSure Technologies Inc.).[45] An additional four HIV self-testing products are on the market and have been registered and approved by a founding member of the Global Harmonization Task Force: the autotest VIH® (AAZ Labs), the BioSURE HIV Self Test (BioSURE), the OraQuick® In-Home HIV Test (OraSure Technologies Inc.), and the INSTI HIV Self Test (bioLytical Laboratories).[45] The per-test cost of these self-tests vary widely based on implementation setting and change rapidly; for example, the WHO prequalified OraQuick® HIV Self-Test has a retail price of $9.50 in low and middle-income countries, but a June 2017 agreement between OraSure and the Bill & Melinda Gates Foundation will offer the test to public-sector buyers in 50 countries for $2.00. Retail prices for the other four products on the market currently range from $22–48 per test, though several are also available in select instances at under $10. Finally, four HIV self-tests are available in some private-sector markets, while ten self-tests that use whole blood, oral fluid, or urine are currently in the pipeline.[45]

Viral load testing. Several sample-to-answer NATs are on the market and in the pipeline for viral load testing. The SAMBA II distinguishes between viral loads above and below 1000 copies per mL in 90 minutes. In a study conducted in London, Malawi, and Uganda, the SAMBA semi-Q was 97.3% concordant with the gold standard test, the Roche TaqMan v2.[46] Although easy to use, the system requires electricity and benchtop equipment, limiting its use to settings with significant infrastructure. In addition, it is only used for monitoring and not for diagnosis and is not currently able to detect HIV-2. Fig. 2 illustrates the ease-of-use afforded by a rapid sample-to-answer system, including sample collection, automated analysis, and reporting of results. The LIAT Analyzer (Roche), shown in Fig. 2, is a portable reverse-transcriptase PCR (RT-PCR) system that uses whole blood as a sample. Recent studies have validated a very low limit of detection of 57 copies per mL.[47] The system detects multiple HIV subtypes as well as HIV-2. However, sample preprocessing is required. Another technology that is highly effective but still costly is the GeneXpert HIV-1 Viral Load (Cepheid), a NAT that detects HIV-1 viral load in a less than two hours with only one minute of hands-on time. While this test provides quick results and requires minimal training to run, its unit cost of $17 000 USD and POC per-test cost of $9.98 limit its utility.[48] Other viral load tests currently in the pipeline include the EOSCAPE-HIV Rapid RNA Assay (Wave 80 Biosciences), the TrueLab Real Time micro PCR (MolBio Diagnostics), the Bioluminescent Assay in Real Time technology (Lumora), the ExaVir Load, and the NWGHF Savanna HIV VL test, though none of these have yet been validated.[49]

CD4 count. Although viral load testing has been shown to be more accurate at indicating therapy failure than CD4 testing, many low-resource environments still rely on CD4 lymphocyte counting to monitor HIV treatment efficacy.[50]

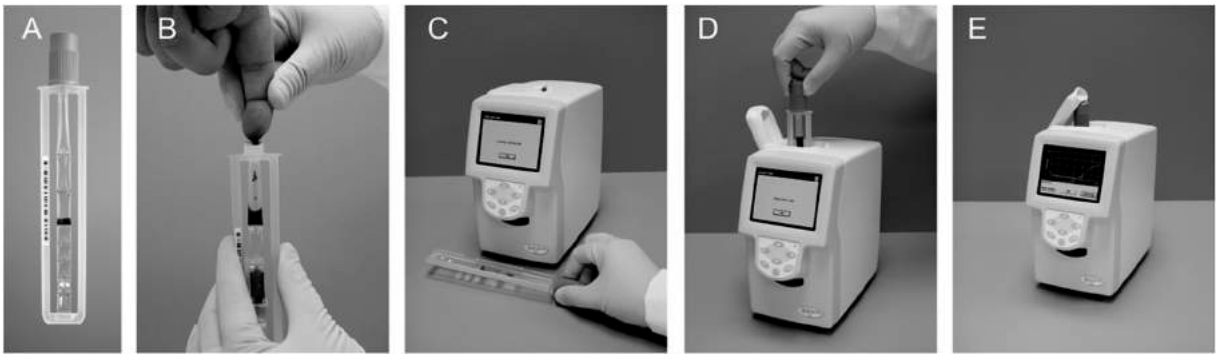


Fig. 2 The complete workflow of the Liat Analyzer. The Liat Analyzer is an example of an automated sample-to-answer NAT platform that performs nucleic acid extraction, purification, reverse transcription, PCR amplification and real-time detection. A sample such as whole blood (shown) or plasma, is collected directly into a Liat Tube (A and B). After the tube is capped, the analyzer scans the tube barcode (C), and the tube is inserted into the analyzer (D). Then, the analyzer automatically performs all the nucleic acid testing steps and reports results in 1 hour (E). The mechanism for measurement and assay components are not depicted here. Reproduced from S. Tanriverdi, L. Chen and S. Chen, "A Rapid and Automated Sample-to-Result HIV Load Test for Near-Patient Application." *J. Infect. Dis.*, 2010, **201**(s1), S52–S58, by permission of Oxford University Press.[47]

Similar to antibody detection, a CD4 lymphocyte count indicates the state of an HIV patient's immune system. A low CD4 count indicates a severe level of infection and correlates with poor clinical outcomes in the first year of infection. However, this amount can be affected by other conditions, and thus cannot be used as a direct measure of HIV. Additionally, baseline CD4 counts have not been shown to be indicative of mortality after 5 years of treatment.[51] However, CD4 counts are still used as a clinical measure of immune system function and progression to AIDS, especially in areas that do not have access to viral load testing.

Commercially available and in-the-pipeline CD4 testing platforms vary widely in terms of their throughput, infrastructure requirements, and cost. Although there is no official gold standard for CD4 counting, many experts consider the high-throughput BD FACSCalibur™ (BD Biosciences) system to be a suitable reference. This platform uses flow cytometry, a technology able to take quantitative measurements of multiple features of large numbers of cells.[24] The instrumentation for this platform costs $75 000, and thus is only suitable for central and national reference laboratories. The most widely used platform in low-resource settings is the BD FACSCount™ system (BD Biosciences). The FACSCount™ uses a whole blood sample, and can calculate both CD4 count and CD4 percentage, which is useful for young children. This system has been used for over a decade, and its performance has been well-validated by several independent studies.[52,53] With a $30 000 instrumentation cost and a per-test cost of $3.50–10.00, this system is well suited to district hospital settings. Two additional and similar medium-throughput CD4 platforms are the Aquios CL™ (Beckman Coulter) and the Apogee Auto40 Flow Cytometer (Apogee).

The flow cytometers described above are not suitable for true POC testing due to high costs and staffing requirements. Thus, other methods of CD4 counting have been developed, and several less expensive technologies for low-throughput POC CD4 testing are on the market. The imaging-based Pima™ Analyser (Alere) introduces fluorescently labeled antibodies to a sample, then acquires and analyzes fluorescence images in an imaging chamber. This system is able to service only three tests per hour, but it is suitable for all levels of healthcare and has been shown to positively impact patient retention and ART initiation.[54] The CyFlow® CD4 miniPOC (Sysmex Partec) is an alternative imaging-based CD4 platform that is compact, rugged, and can be operated with a battery pack for up to 5 hours. Finally, the BD FACSPresto™ (BD Biosciences) is another imaging-based technology that has been WHO prequalified since 2014. This instrument costs less than $10 000 and each test costs less than $10, making the FACSPresto™ a more affordable option than flow cytometry for some resource-limited settings.

While the CyFlow® CD4 miniPOC can be powered temporarily with a battery pack, all of the fully quantitative CD4 tests described above currently require continuous electricity, and costs remain high.[55] Large CD4 equipment also suffers from high infrastructure requirements, while smaller analyzers are limited by low throughput. For example, while a Pima™ CD4 test takes only 20 minutes, the system can only process one sample at a time, leading to long waits for patients.[56,57] The only rapid, disposable, and equipment-free CD4 test currently available is the Visitect® CD4, a semiquantitative test developed by the Burnet Institute and currently licensed to Omega Diagnostics Ltd (United Kingdom). The Visitect® CD4 uses a simple lateral flow device to capture the CD4 protein on T-cells rather than directly measuring CD4 cells, and shows a readout that indicates whether a patient's fingerprick sample contains lymphocytes above or below a threshold of 350 cells per uL.[57] An automatic battery-powered reader interprets results in less than 40 minutes, and Omega Diagnostics has also developed a smartphone application for the assay.

Finally, detection of cells in a microfluidic channel has been difficult due to the high shear stress generated in flow-based ELISA, but alternative microfluidic platforms for CD4 testing are being studied in several academic groups. One approach moves captured CD4 lymphocytes through different aqueous phases as opposed to moving the aqueous phases

across the substrate, allowing cells to be captured without experiencing high shear stress.[58] It also integrates image processing using a smartphone, lowering the equipment requirements of the system. A second novel strategy carefully optimizes shear stress for lymphocyte capture, and employs only electrical methods to interrogate whole blood samples. This eliminates the need for image-based detection and makes the system more robust to environmental challenges.[59] This microfluidic device was shown to be at least as accurate as the Pima CD4 and, with modifications to the capture chamber, could provide over twice the testing throughput of the Pima CD4.

As of 2016, the WHO supports stopping routine CD4 count testing where viral load testing is available, limiting the use of these tests to prioritizing patients for urgent linkage to care.[35] Further, ART initiation is now recommended for all adult and adolescent patients regardless of CD4 cell counts and disease stage. These recommendations indicate a decreasing importance of CD4 testing and a shift towards viral load testing as the central focus of HIV care. Accordingly, a number of CD4 technologies that were on the market or in the pipeline within the last five years have been discontinued. These include the Daktari™ CD4 Counter (Daktari Diagnostics Inc.), the MBio CD4 System (MBio Diagnostics Inc.), and the CD4 Test (Zyomyx Inc.). Daktari appears to have shifted its focus towards development of a viral load test in accordance with market shift, MBio Diagnostics is no longer marketing their CD4 system, and Zyomyx Inc. appears to no longer be in business.[24] Despite this shift in priorities, CD4 cell count remains the best indicator of a patient's immune status, clinical status, and risk of opportunistic infections, and it supports the management of patients with advanced HIV disease. Furthermore, viral load testing for treatment monitoring is still inaccessible to a large portion of those affected by HIV. Therefore, innovative and cost-effective POC solutions for CD4 testing are still needed.

Malaria. Malaria, a mosquito-borne parasitic disease, affects between 150 and 300 million people per year.[60] While malaria infection typically leads to flu-like symptoms, pregnancy increases susceptibility to infection and severity of disease.[61,62] Complications from malaria, such as anemia, hypertension, and low birth weight, cause 10 000 maternal deaths and 200 000 neonatal deaths in Africa annually.[62] In the absence of pregnancy, malaria is typically detected with blood smear microscopy or LFAs. Blood smear microscopy is a widely used method of diagnosing malaria in which trained technicians look for parasites within a blood sample using a microscope. Benefits include the ability to detect multiple species of parasite along with parasitemia, or level of infection, although appropriate training and staffing is required. Additionally, many malaria LFAs available on the market are appropriate for use at a bedside or rural health center, and over 314 million rapid tests were sold in 2014.[60] Some LFAs detect only *Plasmodium falciparum*, the parasite species with the most severe outcomes, while other LFAs detect multiple species including *P. vivax*, *P. malariae*, and *P. ovale*. The WHO selection criteria for malaria LFAs include a sensitivity of 75% at 200 parasites per μL in all transmission settings, a false positive rate less than 10%, and an invalid rate less than 5%.[63] The product should also be thermally stable, able to easily store, and easy-to-use. A list of reviewed LFAs that meet these criteria was recently published by the WHO.[63–66]

However, these tests may perform differently in cases of malaria in pregnancy. Malaria caused by *Plasmodium falciparum* is more difficult to diagnose during pregnancy because the parasites sequester in the placenta and therefore can have low concentrations in peripheral maternal blood.[62] The number of parasites in the blood varies depending on treatment with antimalarial drugs, whether it is a mother's first pregnancy, and other complicating factors.[61,62] It is unclear from recent studies whether low parasite concentrations are associated with adverse maternal or neonatal outcomes.[62,67–73] If future studies indicate little correlation between submicroscopic infections and adverse outcomes, then commonly used malaria diagnostics like blood smear microscopy or LFAs could be used for testing pregnant women at the POC. However, if future studies do show a correlation between submicroscopic infections (<50 parasites per μL) and adverse outcomes, more complex and sensitive diagnostics will be needed to diagnose malaria in pregnancy.[62,74,75]

To detect submicroscopic malaria infections, several commercially available or in-the-pipeline NATs have been previously reviewed, although not validated for malaria in pregnancy.[76–82] The Nanomal (QuantuMDx), Accutas (Aquila Diagnostic Systems Inc.), and DiscoGnosis LabDisk system (IMTEK) all require minimal infrastructure and sample preparation and can be used at the POC. However, cost may be prohibitive for low-resource settings, with the lowest estimated per-test cost at $2–4 and high equipment costs.[82] Other promising inexpensive malaria NATs are being developed which integrate sample preparation to reduce potential contamination, do not require cold chain storage, and have limits of detection appropriate for highly sensitive testing.[76,79,81] In addition, two commercially available imaging-based tests, the Rapid Assessment of Malaria (RAM) Device (Disease Diagnostic Group Inc.) and Magneto-optical Device (MOD) (Meditopian LLC), detect submicroscopic infections in under one minute for less than $1 per test, with minimal sample preparation and rechargeable batteries to reduce infrastructure requirements, although the equipment cost for RAM is high.[82] Finally, a few highly sensitive protein-based methods for submicroscopic malaria detection have also been reviewed.[76,82–85] These tests are low-cost, easy-to-use, and do not require electricity to run, making them suitable for use at the POC. While several of the above tests may be appropriate for submicroscopic detection in low-resource settings, their diagnostic performance for malaria in pregnancy has yet to be fully validated.

Gestational diabetes. Diabetes is a metabolic disease in which a patient experiences prolonged periods with high

blood sugar levels. Diabetes developed over the course of a pregnancy is known as gestational diabetes. Gestational diabetes affects an estimated 10–25% of all pregnancies globally[86,87] and can lead to serious maternal and neonatal health consequences, including pre-eclampsia, infections, obstructed labor, postpartum hemorrhage, preterm births, stillbirths, congenital anomalies, birth injuries, and death.[88,89]

The WHO criteria for gestational diabetes diagnosis includes one or more of the following: fasting plasma glucose between 92–125 mg dL^{-1}, venous plasma glucose one hour after ingestion of 75 g oral glucose load above 180 mg dL^{-1}, or venous plasma glucose two hours after ingestion of 75 g oral glucose load between 153–199 mg dL^{-1}.[86] The use of this diagnostic criteria requires the patient to present for the test after fasting overnight and to stay for 1–2 hours after ingesting glucose, a process known as an oral glucose tolerance test. Bhavadharini *et al.* report one of the greatest patient-related barriers to screening and diagnosis of gestational diabetes in low- and middle-income countries to be patients coming for checkups in the fasting state.[90]

Diabetes can alternatively be diagnosed through the measurement of glycated hemoglobin (HbA_{1C}), a measure of the 3 month average glucose concentration in the patient's blood. HbA_{1C} testing circumvents the need for an oral glucose tolerance test. However, the utility of HbA_{1C} tests in screening for gestational diabetes is still being investigated, as HbA_{1C} levels are higher during pregnancy. Recent studies have shown very low sensitivity (7–81% depending on chosen cutoff value) in HbA_{1C} tests for gestational diabetes screening and suggest confirmatory screenings with oral glucose tolerance tests.[91–93] HbA_{1C} tests are currently not readily available worldwide, are unaffordable in low- and middle-income countries, and have the potential to be adversely affected by hemoglobinopathies.[94] Because of the limitations of HbA_{1C} testing for gestational diabetes, only direct glucose measurement is discussed here.

Gold standard glucose tests are run on clinical chemistry analyzers and test the glucose in plasma. Plasma glucose tests generally require centrifugation to separate red blood cells from plasma due to glycolysis by red blood cells, a process that rapidly degrades glucose in a blood sample. However, plasma separation by centrifugation is not always available in resource-limited settings. A more detailed discussion of low-resource centrifugation methods can be found in the Hematocrit section.

Glucometers intended for self-monitoring of blood glucose (SMBG) are considered one of the founding technologies of the POC testing era,[95] and SMBG is the largest market segment of POC testing.[96] POC glucometers have been shown to be effective for gestational diabetes screening when venous plasma glucose measurements are not available.[97] Generally, POC glucometers generate an enzymatic reaction with glucose and measure the output through photometric or amperometric detection. Commonly used enzymes include glucose oxidase (GOX) and glucose-1-dehydrogenase (GDH), and GDH modified with pyrroloquinoline quinone (GDH-PQQ) has been used recently as well. More comprehensive descriptions of the enzymatic reactions and detection methods have previously been described,[98] and multiple groups have previously reviewed commercialized glucometers.[96,98]

While glucometers are relatively inexpensive and testing requires no sample preparation, the cost of compatible test strips can be prohibitive in low-resource settings (approximately $1.50 per test).[99,100] Additional limitations of commercially available glucometers include designs that are not robust to humidity and temperature fluctuations[101] as well as inaccurate conversions from whole blood glucose values to plasma glucose values.[86] Conversion to plasma glucose values is required for comparison to diagnostic cutoffs; many glucometers perform this conversion by increasing the whole blood glucose value by roughly 11%, though varying hematocrit levels will determine the exact patient-specific conversion. Accurate plasma correlation remains a challenge.[102]

Several reviews discuss recent glucose sensing innovations that attempt to circumvent issues faced by commercially available POC glucometers. Approaches include electrochemical detection,[103] sensors based on carbon nanomaterials[104] and nanostructured metal-oxides,[105] nonenzymatic sensors,[106] non-invasive monitoring technology,[107,108] and emerging technology more generally.[109]

Anemia. Anemia, a condition characterized by insufficient hemoglobin leading to diminished oxygen carrying capacity in the blood, affects an estimated 32 million pregnant women worldwide.[110] Severe anemia is strongly associated with maternal mortality, and progress toward decreasing morbidity and mortality associated with maternal anemia has been slow over the last 20 years.[111] Anemia can be diagnosed by measuring the amount of hemoglobin, the oxygen-carrying protein in red blood cells (RBCs), or hematocrit, the fraction of RBCs in the blood. RBCs are often counted as part of a complete blood count (CBC). Hemoglobin concentration, hematocrit, and RBC counts are generally correlated, though the relationship may be altered in the presence of some hematological disorders, including hemoglobinopathies.[112] An important consideration for developing blood count tests is the variability of hemoglobin, WBCs, and platelets observed from one fingerprick drop to the next; drop volumes used should be sufficiently large for clinical correlation to well-mixed venous blood.[113] For more information on blood counts, see the section on Bacterial infections and puerperal sepsis.

Hemoglobin and hematocrit measures can be integrated into diagnostic tests for additional analytes in order to increase accuracy or to provide additional relevant diagnostic information. For example, as described in the Gestational diabetes section, glucometers often convert whole blood glucose values to plasma glucose values inaccurately; direct hematocrit measurement incorporated into whole blood glucose measurement can allow for more accurate plasma glucose reporting. Additionally, when clinically relevant, anemia diagnostics can be multiplexed with detection of additional analytes, such as HIV antibodies as demonstrated by Guo *et al.*[114] Here, we discuss a variety of approaches toward hemoglobin and hematocrit detection.

Hemoglobin. The gold standard of hemoglobin diagnosis, which is used in hematology analyzers in high-resource settings, relies on the conversion of hemoglobin to cyanmethemoglobin, a stable molecule that absorbs light at 540 nm.[115] Absorption measurements require a spectrometer, limiting the use of this method at the POC. Other methods have been developed to quantify hemoglobin for low-resource settings. The WHO Haemoglobin Color Scale (HCS) is a semi-qualitative method of hemoglobin measurement. A drop of blood is applied to paper and is compared to a color scale by visual interpretation. The WHO HCS has a very low per-test cost (approximately $0.02 per test in Malawi) but suffers from low accuracy (sensitivity between 76–96%, specificity between 33–86%[116]), particularly with inadequate training or lighting,[117] and especially in cases of severe anemia.[118] In the Sahli method of anemia detection, hemoglobin is converted to acid hematin and compared visually to a solid glass color standard. The Sahli method has been considered the standard practice in many low-resource settings for decades. Per-test cost is reportedly higher than the WHO HCS, but sensitivity and specificity are both reported to be 85%.[116] Commercially, the HemoCue system has been developed to measure hemoglobin for POC applications with higher accuracy than the WHO HCS or Sahli method, with reported sensitivity and specificity of 85–100% and 94%, respectively.[116] Blood is drawn into a plastic cuvette, hemoglobin is converted to azidemethemolobin, and the sample is inserted into a spectrophotometric reader for an absorbance measurement.[116] The HemoCue system was found to be the most appropriate hemoglobin measurement device in Malawi, though the per-test cost remains prohibitively high for widespread use (approximately $1.00 per cuvette in Malawi).[115,119] A more in-depth look at several features of the commercially available technologies as well as a couple of technologies in development can be found in the PATH landscape report on anemia.[116]

More recently, the development of an alternative paper-based, colorimetric hemoglobin test was reported.[120] In this approach, hemoglobin is converted to cyanmethemoglobin with Drabkin's reagent, and the sample is applied to a microfluidic paper-based device, allowed to dry for 25 minutes, and imaged on a flatbed scanner. The use of a scanner circumvents decreases in accuracy due to ambient light conditions. The reported 95% limits of agreement between the paper-based assay and the reference assay were 1.30 and 1.18 g dL^{-1}, and the test performed with relatively high quantitative accuracy (R^2 = 0.96). However, there is a need for sample pre-processing and a cost associated with the flatbed scanner (reported as $44 for a refurbished scanner). Tyburski *et al.* developed a fully disposable hemoglobin color scale test. The device has two components: a sample tube that collects 5 μL of blood after a fingerprick and a component that is preloaded with a color-changing reagent. The group reports an optional smartphone quantification application, as well. In laboratory evaluation, the device performed with high sensitivity and specificity by visual interpretation in cases of both severe (<7 g dL^{-1}) and mild (<11 g dL^{-1}) anemia (sensitivity: 90% and 90.2%, specificity: 94.6% and 83.7%, respectively), but relatively low quantitative accuracy (R^2 = 0.864). The per-test cost (US $0.50) is also much higher than the WHO HCS, which may prove to be prohibitive in low-resource settings.[121]

Other groups have developed alternative systems that aim to bring per-test cost down while maintaining high accuracy. A low cost spectrophotometric hemoglobin detection system was developed with the use of chromatography paper as the matrix for sample deposition and hemoglobin measurement, shown in Fig. 3. Absorbance at two wavelengths (528 and 656 nm) is used to calculate hemoglobin concentrations. Ninety-five percent of samples tested with this system were within 2 g dL^{-1} of HemoCue readings, and the per-test cost (<US $0.01 per test) is projected to be significantly cheaper than the HemoCue system.[115,122]

Hematocrit. Hematocrit can also be used for anemia diagnosis. Traditionally, commercially available centrifuges, such as the Zipocrit Hematocrit Centrifuge (LW Scientific) are used in conjunction with capillary tubes loaded with a patient's blood and sealed with wax. Centrifugation results in

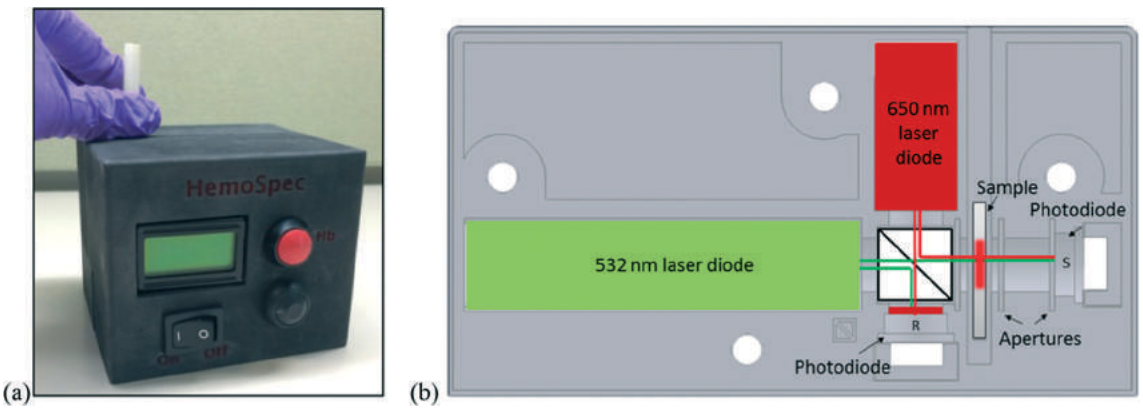


Fig. 3 Photo (a) and optical diagram (b) emphasizing the ease of use of HemoSpec, a portable device that optically measures hemoglobin concentration from blood spotted onto chromatography paper. The form, user interface, and included optical components are shown, but sample loading is not depicted. © 2014 IEEE. Reprinted, with permission, from M. Bond, J. Mvula, E. Molyneux and R. Richards-Kortum, presented in part at 2014 IEEE Healthcare Innovation Conference (HIC), 2014.[122]

layers of packed RBCs, WBCs, and plasma; the height of the RBC layer can be compared to standardized charts that quantitatively produce hematocrit values for a given capillary tube fill height. While this method is cheap and relatively simple, it requires a centrifuge, which is expensive. Several approaches have recently been shown to lower the cost and complexity of centrifuges. Thompson *et al.* reported the development of a rotation-driven microdevice (RDM) for hematocrit determinations. The RDM is estimated to cost less than US $0.50, runs 12 samples at once, accepts less than 3 μL of whole blood, and produces a result in less than 8 minutes. Rotation is generated by a CD player motor, and a cell phone is used for quantification. Laboratory samples were comparable to clinical lab determinations, though extensive characterization has not yet been reported.[123] Drawing inspiration from the whirligig, an ancient toy that generates high centrifugal force, Bhamla *et al.* developed the human-powered "paperfuge". The paperfuge has an estimated cost of US $0.20, is lightweight, and can separate plasma from whole blood in less than 90 seconds with comparable performance to a commercial centrifuge.[124] The paperfuge cost is insignificant compared to a commercial centrifuge, but data on usability and robustness of the paperfuge is necessary to understand potential clinical utility. The paperfuge is among other innovative approaches toward hand-powered centrifuge development,[125] including repurposed salad spinners[126] and egg beaters.[127] Unconventional approaches for centrifugation may improve access to hematocrit measurement, but the clinical utility of these approaches must be demonstrated. Of note, centrifugation is useful in many diagnostic assays beyond hematocrit measurement.[125]

In addition to rotational separation, technologies have been developed to quantify hematocrit through impedance spectroscopy[128,129] and paper-based plasma separation.[130] Impedance spectroscopy utilizes the principle that higher hematocrit levels will increase the current flow path between reference and working electrodes. With impedance spectroscopy, results could be achieved very quickly, and the reported cost is less than US $12. Additionally, it is unclear whether sample pre-processing is required and whether non-RBC components of blood would falsely elevate hematocrit measurements. Further evaluation of complex samples and of clinical utility is required.[129] The paper-based approach utilizes the natural fluid-wicking properties of paper to differentially separate blood cells from plasma. The paper-based devices are low-cost (US $0.03), though time-to-results (30 min) is much longer than with centrifugation. The upper hematocrit limit is 57%, which is below the range of some neonatal blood samples, limiting the utility of the device in its current form. The quantitative performance and reproducibility of this paper-based test has yet to be established.[130] Other methods of plasma separation have been previously reviewed.[28]

Hemorrhage. Hemorrhage (severe bleeding) is the leading single cause of maternal mortality, accounting for 27.1% of maternal deaths worldwide, and 659 000 women die from hemorrhage per year in low-resource settings.[6] Researchers have identified known risk factors such as Caesarian sections and prolonged third stage of labor, and preventative guidelines have been established.[6,131] However, disease burden is still high, and low-resource diagnostics are needed to supplement the preventative guidelines. A bleeding disorder such as HELLP (hemolysis, elevated liver enzymes, low platelets) or von Willebrand disease may be a causative factor for some women who develop hemorrhage.[132] Several platelet aggregation assays with or without Ristocetin have been developed to diagnose these conditions, either prior to hemorrhage or at its onset.[133,134] Typically, in the presence of large amounts of blood loss or drop in blood pressure, complete blood counts and coagulation assays are performed.[132,135] High resource settings particularly quantify change in peripartum hemoglobin, since it accounts for internal hematomas and hemolysis as well as external blood loss.[136] Most of these tests require high infrastructure and are performed at central hospitals. For more on platelet aggregation and complete blood counts in low-resource settings, see the White blood cell count and differential section. LFAs for coagulation have also been developed by assessing how far 100 μL of blood travels on nitrocellulose.[137]

POC devices have been developed to measure the amount of blood lost during hemorrhage for diagnosis and management. Quantifying blood loss is important for preventing hypoxia, heart attack, organ failure, and death.[136] Typically, in low-resource settings, blood loss is estimated visually, is highly variable depending on staff training, and can be underestimated by as much as 75%.[138] The use of bedpans, blood collection pads, and gravimetric sponges allow for more accurate blood loss estimates.[136,139] Hemorrhage is diagnosed as blood loss over 500 mL, and many pads or cloths, including "Quaiyum's mat" developed in Bangladesh[140] (approximately $0.50 per mat)[141] and a kanga system in Tanzania ($6–7 for two kanga),[142,143] utilize this value to absorb only 500 mL. Other devices like Kelly's pad in India (about $25)[139,144,145] and BRASS-V drape in Nigeria (about $4 per sterilized drape)[139,146–148] funnel blood to accumulate and measure it. The SAPHE mat has multiple squares of super absorbent polymer, each holding 50 mL of blood, and blood loss is estimated by counting filled squares.[149] The SAPHE pad has a 0.96 Pearson's correlation for volume blood loss, but at $0.50 to $2.50 per pad, it is not a cost-effective strategy, given the cost of misoprostol and other uterine contraction agents to prevent hemorrhage is as low as $1. Kelly's pad can be washed and sterilized, while most other pads or cloths must be decontaminated and disposed increasing the per-use cost. An area for further innovation is the development of a more quantitative blood loss measurement technique for an extremely low per-use cost.

In addition to these blood loss techniques, a rapid diagnostic test for fibrinogen was developed to predict the severity of hemorrhage during intrapartum complications.[150,151] Fibrinogen is a protein essential for coagulation and has a 99.3% specificity for predicting severe hemorrhage.[150]

However, the assay has a sensitivity of only 12.4%. The test may be useful for triaging, but a more sensitive biomarker is needed for accurate diagnosis.

Pre-eclampsia. Pre-eclampsia is the second largest direct cause of maternal mortality worldwide, second only to hemorrhage. The WHO estimates that 14% of maternal deaths in low-resource settings, about 341 000 per year, are caused by pre-eclampsia.[6] Pre-eclampsia is a disorder characterized by high blood pressure and proteinuria, or excess protein in a 24 hour urine sample, and it can lead to severe morbidities such as seizure, placental abruption, hemorrhage, and preterm delivery. A test for proteinuria is the gold standard for pre-eclampsia, but blood pressure, blood-based biomarker tests, and algorithms can also be used for diagnosis.[152,153] However, the diagnostic accuracy of these methods is debated.[154] A 2005 survey in the United Kingdom claims that 45% of women with eclampsia displayed neither proteinuria nor high blood pressure in the week leading up to seizure.[154,155] Additionally, while dipsticks can be used in low-resource settings to determine proteinuria with a tetrabromophenol blue colorimetric agent, urine dipsticks are often unavailable in many low-resource settings. Urine dipsticks for proteinuria do not detect key misfolded biomarkers associated with pre-eclampsia,[156] vary with urine concentration,[154] and can have sensitivities for pre-eclampsia as low as 55%.[157] Because proteinuria is not reliably seen in all pre-eclampsia cases and the dipsticks have low sensitivity for detecting proteinuria, dipsticks are often not used when available.[154,156,158–160] As such, alternative methods to diagnose pre-eclampsia in low-resource settings, including low-cost blood pressure devices and both blood- and urine-based POC diagnostics, are discussed here.

Blood pressure monitors. Many low resource settings do not measure blood pressure because of a lack of appropriate measuring devices or lack of trained healthcare workers.[154,158,161–163] For example, 36% of health centers providing antenatal care in Malawi did not have blood pressure measurement equipment according to the Malawi Demographic Health Survey 2013–2014.[154,164] However, a few blood pressure monitors have recently been developed for low-resource settings. Most notably, the Omron HEM-SOLAR and Microlife 3AS1-2 have been validated for use with pregnant women in low-resource settings.[154,162,165] Omron HEM-SOLAR, a semi-automated device that uses solar energy to recharge, is slightly more accurate (cost about $30),[162,166–168] whereas the Microlife 3AS1-2 is relatively cost-effective ($19) and easy to use by untrained workers.[154,167,169–171] The Microlife 3AS1-2 can be manually inflated, requires rechargeable batteries, and uses a "traffic light" system to alert healthcare workers of abnormal pressures, which has received positive feedback from both trained and untrained users.[169] The Nissei DS-400 (Nissei Japan Precision Instruments) has similar features of manual inflation and rechargeable batteries, although the cost is slightly higher ($30),[172] and the test has only been validated on pregnant women in high-resource settings.[173] The LifeSource One-Step Monitor measures blood pressure accurately with automatic inflation and rechargeable batteries, but the price is greater than other POC blood pressure monitors.[174–178] Additionally, the LifeSource One-Step Monitor needs to be evaluated in a pregnant population.[178] Other low-cost blood pressure monitors are in development and may be appropriate for use at the POC.[154,179,180] However, as discussed above, the diagnostic accuracy of blood pressure for pre-eclampsia is debated.[154]

Blood-based POC diagnostics for pre-eclampsia. Several other biomarkers are also associated with increased risk for pre-eclampsia, and some of these biomarkers have been integrated into benchtop diagnostics or LFAs.[181,182] Multiple benchtop readers have been developed to measure serum-based biomarkers of pre-eclampsia, specifically placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and glycosylated fibronectin (GlyFn).[183,184] These biomarkers have all shown promise for diagnosis of pre-eclampsia in the third trimester with area under the receiver operator curves (AUROCs) of 0.94 for PlGF, 0.96 for sFlt-1, 0.98 for sFlt-1/PlGF ratio, and 0.99 for GlyFn.[184]

The Triage PlGF test (Alere Inc) uses fluorescence immunoassay detection and the Triage MeterPro POC analyzer to quantitatively determine PlGF levels in blood plasma samples and returns a result in 15 minutes. Sensitivity and specificity of the Alere Triage PlGF test vary depending on the cutoff value used; between 20 and 34 weeks of gestation, the test has a 96% sensitivity and 56% specificity for pre-eclampsia using a cutoff value of <100 pg mL^{-1} and a 63% sensitivity and 90% specificity using a cutoff value of <12 pg mL^{-1}.[185] When gestational age is known, a cutoff of <5th percentile for normal gestational-age dependent range improves test performance with 100% sensitivity and 96% specificity for early onset pre-eclampsia and 77% sensitivity and 95% specificity for all gestational ages.[186] However, with all cutoff values, sensitivity dramatically decreases after week 34 of gestation.[185]

The Elecsys immunoassay sFlt-1/PlGF ratio test (Roche Diagnostics Limited) measures the relative amount of PlGF to sFlt-1 in serum samples using two separate electrochemiluminescence immunoassays with the Roche Elecsys benchtop analyzer. Similar to the Alere Triage test, Roche Elecsys performance varies on the designated cut-off value during weeks 24 through 36 of gestation. At a ratio of 23, the test has a 92% sensitivity and 81% specificity, while at a ratio of 85, the test has a 56% sensitivity and 97% specificity.[185] Two additional tests have been developed, the DELFIA Xpress PlGF 1-2-3 test (PerkinElmer), which quantitatively measures PlGF levels in serum samples using a solid-phase, two-site fluoroimmunometric sandwich assay, and the BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio test (Thermo Fisher Scientific GmbH) which detects sFLT-1/PlGF ratio with two immunofluorescent sandwich assays.[183,185,187] However, both of the latter tests must be validated in a clinical setting to determine sensitivity and specificity for pre-eclampsia diagnosis.[185]

Lastly, glycosylated fibronectin is a promising biomarker, and DiabetOmics currently employs a cassette reader for a

fluorescent immunoassay with high sensitivity and specificity for pre-eclampsia (97% and 93%, respectively) in the third trimester.[182,184] DiabetOmics test is only available in select markets, but the company has licensed the clinical test to Becton, Dickinson and Company for integration into their BD Veritor device,[188] which is costly for low-resource settings.[189] Alternatively, an equipment-free GlyFn test is possible, with a cutoff value of 176.4 μg mL^{-1}, although this test needs to be validated for clinical use.[184]

The detection of PlGF and sFlt-1 at the POC demonstrate an area for further innovation. The tests discussed above, including the Triage PlGF (Alere Inc), Elecsys sFlt1/PlGF (Roche Diagnostics Limited), and the DELFIA Xpress PIGF 1-2-3 (PerkinElmer), all have high per-test costs (>$40).[185] Additionally, the clinically relevant detection ranges of PlGF (pg mL^{-1}) and sFlt-1 (ng mL^{-1}) are currently not detectable by tests appropriate for low resource settings such as LFAs. Recent innovations in developing a paper-based ELISA platform have demonstrated limits of protein detection at 100 pg mL^{-1},[83] which could be applied for detection of these protein targets.

Urine-based POC diagnostics for pre-eclampsia. While urine samples are easy to obtain and require little or no sample preparation, limitations to the commercially available dipsticks still exist, as discussed above, due to limitations in tetrabromophenol blue detection of proteinuria. Two new low-cost tests (<$0.10) for proteinuria include a proteinuria pen developed by researchers at John Hopkins and a proteinuria stamp developed by Diagnostics for All, although both are undergoing test validation and not commercially available.[154] Both devices add colorimetric reagent followed by a drop of urine to paper-based platform, and a color change occurs in the presence of proteinuria. However, these tests will likely have performances equivalent to dipstick tests for proteinuria, as they function in a similar manner.[160]

A recent review article lists many additional promising biomarkers for pre-eclampsia, but notes that only a few of them have POC tests.[182] Urine-based rapid diagnostic tests have been developed for pre-eclampsia using urinary adipsin and amyloid aggregates (GestVision).[152,156,182,190] However, urinary adipsin as a biomarker has a poor specificity (70%) unless combined with diastolic blood pressure readings.[182] In contrast, a GestVision diagnostic uses Congo Red Dot (CRD) to detect protein misfolding in urine, which can predict pre-eclampsia before symptoms appear.[152,156] Recent articles have shown that this protein misfolding may have greater predictive value for pre-eclampsia than other biomarkers such as PlGF and sFlt-1,[156,182] and the GestVision test uses amyloidophilic Congo Red dye that binds to misfolded, unfolded, or amyloid-like proteins to detect misfolded proteins quickly.[191] The test is appropriate for low-resource settings due to its rapid time-to-result (<30 minutes), low cost ($0.30), high sensitivity (86%), and high specificity (85%). A healthcare worker simply spots urine onto nitrocellulose and then adds CRD to note misfolding. Additionally, while the test primarily benefits from high diagnostic performance during the third trimester, recent reports suggest it may also predict pre-eclampsia as early as the first trimester. A prospective cohort found that in the first trimester, CRD predicts 33.3%, 16.1%, and 20% of early, late, and all pre-eclampsia cases, respectively.[152,191] The GestVision CRD test shows great promise for use at the POC in low-resource settings, but it is not yet commercially available as the company is performing final validation studies before releasing the product.

Bacterial infections and puerperal sepsis. Puerperal sepsis is a leading cause of maternal death, with an estimated 5 million cases and 62 000 deaths worldwide annually. Puerperal sepsis is an infection of the genital tract occurring between the time of rupture of the amniotic membranes and the 42nd day postpartum. The infection must be accompanied by fever and at least one of the following: pelvic pain, abnormal vaginal discharge, discharge odor, and delayed reduction in size of the uterus.[192] In high-resource areas, puerperal sepsis accounts for 2.1% of all maternal deaths, while in low-resource settings it is estimated to cause at least 11.6%. Further, the relative risk of mortality due to puerperal sepsis is much higher than other causes of maternal death, with a 10% mortality rate in high-income countries, and a 33% mortality rate in low-income countries.[193]

Protein-based tests for sepsis. Procalcitonin (PCT) has been demonstrated to be a consistent marker of inflammation caused by severe infections in both neonates and pregnant women[194] and can be useful in assessing both infection severity and response to antibiotic treatment.[195] BioMerieux's VIDAS B.R.A.H.M.S. PCT system has demonstrated consistent measurement of PCT in both reference standards and in clinical samples.[196] The VIDAS system is a benchtop reader system that performs enzyme-linked fluorescent immunoassay and reports a detection limit of 0.09 ng mL^{-1} within 20 minutes.[196] However, recent meta-analysis data has shown varied results in the reported sensitivity and specificity of PCT to diagnose sepsis.[197] Presepsin, on the other hand, has been demonstrated as a specific marker of sepsis that is induced by the phagocystosis of bacteria rather than the presence of inflammation, an important consideration when distinguishing sepsis from other causes of inflammation.[198] Mitsubishi Chemical Corporation recently introduced the PATHFAST Presepsin for the rapid detection of presepsin. The PATHFAST system uses a chemiluminescent enzyme immunoassay to quantitatively measure presepsin in whole blood via a benchtop analyzer. The results of the PATHFAST system have shown a strong correlation of measured signal with presepsin concentration across a wide range of concentrations in both whole blood and plasma samples. Additionally, the system has been used to demonstrate a significant increase in presepsin levels in septic patients.[199] Despite the progress in development of these benchtop analyzers, both of these biomarkers have clinically relevant limits of detection that have potential to be detected in an equipment-free, LFA format. Developing more POC-friendly LFAs for these biomarkers would allow detection in health posts where the infrastructure required for the VIDAS B.R.A.H.M.S. PCT system

and PATHFAST Presepsin is not available, thereby allowing earlier detection of suspected sepsis cases.

In addition, blood-based LFAs have been developed to detect bacterial infections and sepsis using C-reactive protein (CRP).[200] However, the concentration of CRP is very high in the blood, and LFAs to detect CRP suffer from the Hook effect, in which excess protein present in the sample binds to capture and detection antibodies separately. This prevents the formation of a sandwich at the detection line and creates a false negative result. In samples with high concentrations of target protein, the sample must be diluted prior to detection, adding user steps, or alternative techniques such as competitive assays must be used to overcome the Hook effect. For example, a near-infrared (NIR) LFA by SRI International utilizes a competitive assay, with CRP spotted onto nitrocellulose.[201] Any CRP present in the blood of a neonate or mother binds to the NIR antibodies, and the antibodies do not bind to the CRP on the nitrocellulose. In this case, a lack of signal represents a positive test result. Antibodies conjugated to NIR dye improve the signal-to-background ratio and are detected with an emission scanner, limiting the readout of this assay in low-resource settings. As an alternative to the SRI International LFA, the InfectCheck CRP LFA detects CRP in a semi-quantitative ladder assay with multiple detection lines, allowing for rapid assessment of CRP level in a POC setting, although the assay does lack specificity.[202]

Protein-based tests for PROM. Premature rupture of membranes (PROM), or breakage of the amniotic sac prior to labor, is associated with an increased risk of puerperal sepsis,[193] as well as increased risk of preterm delivery, neonatal sepsis, and other neonatal complications.[203] An array of biomarkers has been identified as promising candidates to predict PROM and preterm delivery, and from those biomarkers, several effective vaginal swab-based POC diagnostics have been developed for diagnosing PROM; they include the Amnisure ROM test (AmniSure),[203–205] Actim PROM (Medix Biochimic),[203,205,206] AMNI Check (MAST Diagnostica),[203,207] ROM Plus (Clinical Innovations),[208] AmnioQuick Duo+ (Meridian Healthcare),[209] QuickLine IL-6 (Milenia),[210] and Lactate Pro (Arkray).[203,211] All of these tests work rapidly with high sensitivity and specificity.

The two most commonly reviewed tests are the Amnisure ROM test, which detects the biomarker placental alpha-microglobulin 1 (PAMG-1), and the Actim PROM tests which identifies the presence of Insulin like growth factor binding protein 1 (IGFBP-1).[205,212] Many studies note that the two tests are comparable in performance, running in 5–10 minutes with a pooled sensitivity and specificity of 96.8% and 98.3% for Amnisure ROM and 92.1% and 90.5% for Actim PROM.[205] Amnisure ROM is slightly more accurate in the absence of significant blood since PAMG-1 has a 1000 to 10 000 fold difference between amniotic fluid and normal cervicovaginal secretions.[203] However, PAMG-1 levels in maternal blood can cause false positives for Amnisure ROM, while Actim PROM is less susceptible to false positives from blood contamination. Not only are IGFBP-1 levels in blood lower than the Actim PROM limit-of-detection, but IGFBP-1 largely exists in a phosphorylated form in blood with a lower affinity for the Actim PROM antibody.[205]

Two other common PROM LFAs, AmnioQuick Duo+ and ROM Plus, test both IGFBP-1 for detection of PROM in the first trimester, along with the protein alpha-fetoprotein (AFP) which significantly decreases in the third trimester.[213] AmnioQuick Duo+ works in 10 minutes with 94.1% sensitivity and 87.5% specificity, and ROM Plus uses a combination of monoclonal and polyclonal antibodies with 99% sensitivity and 91% specificity in 5 to 20 minutes.[208,214] Both tests are susceptible to false positives from blood contamination due to the AFP, and all women with hemorrhage were excluded from diagnostic analysis.[213] The Lactate Pro test determines the amount of lactate in vaginal fluid using an electrochemical strip powered by a battery.[215] Results appear in one minute with 85% sensitivity and 91% specificity. Although the Lactate Pro does require consumable batteries and lactate is not the most sensitive PROM biomarker, with a per-test cost of $1 to $3, it is relatively low cost compared to other PROM tests.[203,211,216–218]

While these LFAs are easy to use at the POC, issues with specificity in the presence of blood and cost have limited their uptake this far. The Amnisure ROM test, Actim PROM, and ROM plus test all have a high per-test costs (>$30).[219–222] An area for further innovation is to create a truly low-cost method for diagnosing PROM in low-resource settings. Urea and creatinine have shown promise in multiple studies for highly sensitive and specific detection of PROM with close to 100% sensitivity and 100% specificity, although the optimal cutoff values have varied between studies.[203,216,217,223–229] In these studies, the urea and creatinine are measured from vaginal washes, where 3 to 5 mL of saline is injected into the posterior vaginal fornix and subsequently aspirated with the same syringe.[226] Urea is detected with a spectrometer and creatinine with an enzymatic based assay, but a ladder-based LFA for creatinine was developed in the laboratory which runs within 20 minutes and has 90% agreement with the traditional Jaffe method.[230] Such technologies show promise for an affordable POC PROM test appropriate for low-resource settings.

Additionally, a few diagnostics can predict the risk of preterm delivery while the membrane is still intact through cervical and vaginal swab-based tests. The QuikCheck fFN test (Hologic) and Actim Partus (Medix Biochemic) predict preterm labor for women with intact membranes by measuring concentrations of fetal fibronectin (fFN) and the phosphorylated isoform of insulin-like growth factor binding protein 1 (phIGFBP-1), respectively. The QuikCheck rapid test runs in 10 minutes with 90% sensitivity and 64.8% specificity for preterm birth within 7 days.[231] As a biomarker, fFN is not specific to preterm labor, which contributes to the low specificity of the QuikCheck test. In addition, the QuikChek test may be less reproducible than its US-based counterpart, the Rapid fFN test with the TLI_{IQ} System (Hologic); the Hologic system includes internal controls and a reader to interpret test

results, and quantitative fFN values can provide more information, especially for preterm risk at different gestational ages.[231–234] Contamination of the vaginal swab by other fluids such as blood or semen may also affect results.[231–233] Likewise, the Actim Partus test (Medix Biochimic) detects phIGFBP-1, a biomarker released from the decidua and potentially signifies labor, with 78.3% sensitivity and 89.3% specificity for preterm labor within 7 days.[231] Both the QuikCheck fFN and Actim Partus test have a per-test cost that is prohibitive in low-resource settings.[232] Other biomarkers such as matrix metalloproteinase-8 have been developed into a rapid test (SK Pharma Co, Ltd) for preterm delivery with high specificity (>97%) over a range of gestational ages but poor sensitivity.[235] Areas for potential development include creating rapid tests with new biomarkers or a combination of the above biomarkers, which can provide both high sensitivity and specificity over a range of gestational ages, as well as a reduced per-test cost for effective use in low-resource settings.

Bacterial culture at the point of care. Biomarker and nucleic acid tests can detect the presence of bacteria. However, they may cause false positives after an infection has resolved, since bacterial DNA can persist in a patient's blood stream after the bacteria are rendered non-viable by the immune system or antibiotic treatment.[236,237] Additionally, many protein biomarkers of infection are elevated in a number of inflammatory conditions further creating potential for false positives in these cases.[238] Due to these limitations, some groups have begun working on techniques to perform a POC bacterial culture. These techniques aim to be faster than traditional culture, which can have a diagnostic turnaround time of 24–48 hours.[236] Rather than growing bacteria as in traditional culture, POC tests aim to detect bacterial viability as well as drug sensitivity. Thus, these platforms are amenable to detecting not only viable bacteria in a sample, but also rapid screening for antibiotic resistance.[239–241]

Funes-Huacca *et al.* reported in 2012 a portable, self-contained culture device for bacteria. While their device is capable of culturing bacteria with similar detection limits to standard culture techniques, they also show rapid color change resulting from paper impregnated with a viability dye, resazurin. When viable bacteria are added to the paper and plastic device, the paper pad changes from blue to red, indicating the presence of live bacteria in less than 5 hours. The color change is visible by eye and can be quantified as number of colony forming units (CFU) with a hand-held reader, shown in Fig. 4. The device demonstrated the ability to detect concentrations down to 10 CFU mL^{-1}, however these lower CFU concentrations required longer overnight incubation times.[239] Additionally, methods using metabolic monitoring have been proposed to rapidly detect bacteria. In these techniques, rather than detecting the bacteria, small volumes of sample are monitored using an oxygen-sensitive fluorophore. Any viable bacteria present in a sample metabolize oxygen, and fluorescence intensity decreases. Because of the small sample volume used, changes in fluorescence can be detected in less than one hour; however, the device has only been tested in a lab with *E. coli* concentrations from 10^4 to 10^8 CFU mL^{-1}.[240] Roche recently procured a technology known as Smarticles: small particles with an embedded genetic sequence. When added to a sample, the particles specifically bind to the surface of any living target bacteria present, and its embedded genetic sequence is inserted into the bacterial cell, creating a luminescent response. This technique has the ability to be multiplexed with a panel of Smarticles specific to various bacterial species, each encoding a different luminescent response, but the approach has yet to be demonstrated in a clinical setting.[237,241]

Each of these techniques also has the potential to be used to rapidly assess antibiotic resistance. Because the outputs of each test are only responsive to living bacteria, comparing the response of antibiotic-free samples to those of samples that have been impregnated with antibiotics can quickly assess if the bacteria respond to the antibiotic through a quenched output.[239–242]

To date, there are no POC-appropriate technologies to detect bacterial infections and perform strain differentiation and antibiotic resistance testing. Further, devices based on the principle of bacterial culture are limited by sample collection challenges. First, due to low bacteremia loads in many septic patients, a larger volume of blood is required in order to collect sufficient bacteria for culture detection. Second, these devices require a sterile blood draw in order to avoid sample contamination. As such, POC detection of bacterial infections presents an opportunity for further development.

White blood cell count and differential. A WBC count with differential is frequently used in diagnosing bacterial and viral infections. In addition, a WBC count can be used to assess disease severity and monitor the effectiveness of a treatment regimen.[243] The differential provides important information in distinguishing viral, bacterial, fungal, and parasitic infections, in addition to providing information about inflammation severity and autoimmune diseases.[244] As noted in the section on Anemia, drop-to-drop variability of WBCs, hemoglobin, and platelets must be accounted for in WBC count test development.[113]

HemoCue recently developed the HemoCue WBC DIFF, a portable imaging system for performing a 5-part WBC count and differential at the POC. Similar to HemoCue's hemoglobin test, blood is drawn into a cuvette that is then inserted into the device for measurement.[245] However, the per-test cost for the cuvettes is high ($3.12 per cuvette, CliaWaived.com price, February 2017).

In addition to HemoCue's commercially available test, several in-development approaches have been reported. Majors *et al.* developed a digital fluorescence microscopy system to perform a three-part WBC differential that has been demonstrated in laboratory settings. The low-cost microscope is fabricated from plastic components by 3D printing and diamond turning techniques. Whole blood samples of less than 15 μL are added in a novel disposable cartridge design, removing the need for sample processing.[246] Acquired images are analyzed with an automated algorithm to report the white blood

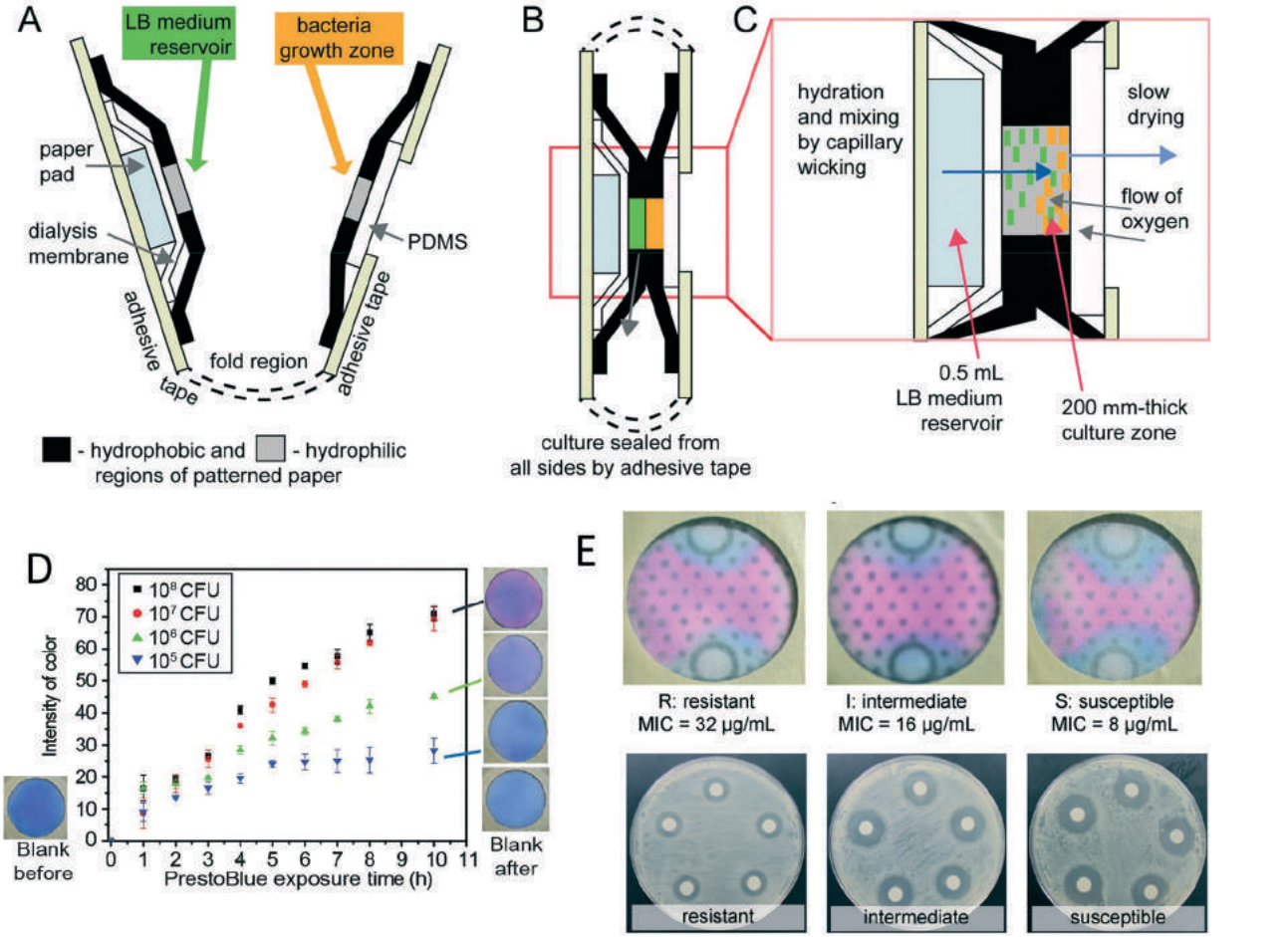


Fig. 4 Schematic of paper-based bacterial culture device. This work illustrates a novel, low-cost platform for performing bacterial culture at the POC. (A) Devices are fabricated out of wax patterned paper, tape, PDMS, and a dialysis membrane. (B) When the device is folded shut, the lysogeny broth (LB) medium reservoir is brought into contact with the bacterial growth zone for bacterial culture. Because of the dialysis membrane and oxygen flow through the PDMS (C), bacteria are able to grow within the device. (D) When the device is impregnated with viability dye resazurin (PrestoBlue™), a visual color change occurs during incubation, allowing quantification of bacterial load within the sample. Reproduced from ref. 239 with permission of the Royal Society of Chemistry. (E) Antibiotics can be added to the paper disks to allow for antibiotic susceptibility testing, in which growth will be stunted surrounding the antibiotic areas in susceptible bacterial strains, whereas it will not be in antibiotic resistant strains. Reproduced from ref. 242 with permission of the Royal Society of Chemistry. The images presented here depict the platform components, assay overview, and selected results but do not provide information on workflow and use of the platform.

cell count and 3-part differential, which differentiates lymphocytes, monocytes, and granulocytes. All samples measured in this study fell within 20% of the gold standard measurements, falling just outside the required accuracy of ±15% for CLIA waiver; the projected cost of the microscope when produced in quantities of 10 000 is approximately $613 with a per-test cost of less than $0.25, shown in Fig. 5 (top).[247] Similarly, Smith *et al.* developed a CBC measurement system for use with sub-microliter volumes of blood. In this method, a blood sample is stained and loaded into a low-cost microscope, and an automated cell counting analysis is performed. The system is able to count RBCs, WBCs, platelets, granulocytes, lymphocytes, and monocytes (Fig. 5, bottom).[248] Using slightly larger volumes of blood (10 μL), a cell phone-based WBC, RBC, and hemoglobin measurement system has been demonstrated. LEDs are used for excitation of the sample, and the cell phone camera is modified and used for emission detection. Automated cell counting is then performed directly on the cell phone.[249] Small-volume tests must ensure drop-to-drop variability does not hinder diagnostic performance.[113]

Diagnostics for neonatal health

Premature birth complications

Hypothermia. Neonatal hypothermia, defined as body temperature below 36.5 °C, is a significant threat to neonatal survival and is also strongly correlated with premature birth, birth asphyxia, and infection.[250] Traditionally, an axillary, rectal, or skin (forehead, abdomen, or foot) temperature is used to define and diagnose hypothermia in neonates. However, low nursing staff-to-patient ratios prohibit regular temperature monitoring, and thermometers that continuously and automatically measure lower temperatures to detect

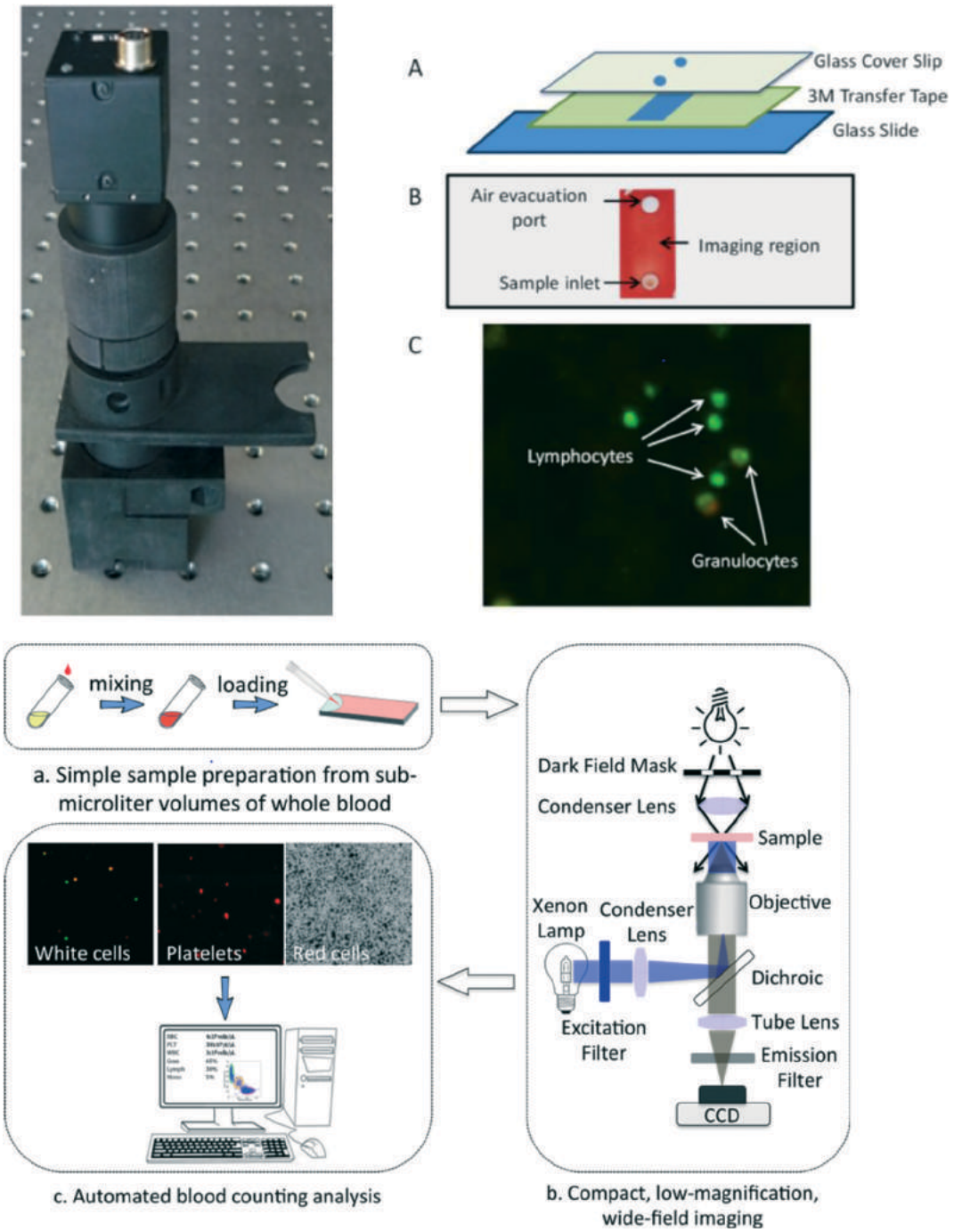


Fig. 5 Technologies in development for performing a WBC count. All-plastic microscope (Top left) used with disposable slides (Top right: A and B) for complete blood count measurements. Sample diagnostic images obtained of lymphocytes and granulocytes are shown (Top right: C). The chosen images depict the size of the microscope, the relatively small number of components required to build the system and slides, and a representative image produced by the system. However, the figure does not illustrate the workflow, including sample loading. (Bottom) Method of imaging and analyzing blood count diagnostics using sub-microliter volumes of blood. The images presented here show the workflow and representative images produced by the system, but do not provide information on the size or form of the microscope. Figure permissions: (Top left) figure reprinted from ref. 247 with permission from the Optical Society of America; (Top right) © 2014 IEEE. Reprinted, with permission, from C. E. Majors, M. E. Pawlowski, T. Tkaczyk and R. R. Richards-Kortum, presented in part at 2014 IEEE Healthcare Innovation Conference (HIC), Seattle, WA, 2014.[246] Figure reproduced from ref. 248 with permission from the Royal Society of Chemistry.

hypothermia are expensive and difficult to obtain.[251] When low temperatures are sensed, skin-to-skin contact, swaddling, or placement in a warmer must be initiated quickly. In cases where the mother requires attention following delivery, skin-to-skin contact cannot be immediately initiated. Therefore, to ensure that other warming methods are employed when

necessary, continuous temperature monitoring devices are needed for unstable and low-birth-weight infants in low-resource settings. Several promising continuous monitoring technologies are described.

The ThermoSpot is a liquid crystal thermometer in the form of a 12 mm diameter disk that is stuck to the skin via an adhesive. When placed in the axilla or on the upper abdomen, the liquid crystal disk turns green for temperatures above 36.5 °C, brown for temperatures 35.5–36.4 °C, and black for temperatures below 35.5 °C. ThermoSpot has shown a sensitivity of 88–100% in community and hospital settings for detecting hypothermia in neonates, costs only $0.11 per spot, and has been used successfully by illiterate users.[252,253] One drawback to the ThermoSpot is rashes due to the adhesive. In a study involving 43 mothers, 16% reported rashes on their newborns. However, in all of these cases, transparent tape had been used to apply a non-adherent ThermoSpot, and mothers were still willing to use ThermoSpot.[254]

Recently, Blue Spark Technologies developed the TempTraq, a thin, disposable, flexible and battery-powered temperature skin patch for continuous monitoring of skin temperature. An initial study in adults showed no significant difference between TempTraq measurements and oral and axillary measurements (p = 0.25, 0.33), and plans are in place to test the device in a pediatric population.[255] The device is commercially available as a 24 hour, single-use monitor, but the high cost of $19.99 currently prevents its accessibility in low-resource settings.

The Bempu Bracelet is a temperature-monitoring bracelet made of a silicone band and a thermistor metal cup. The bracelet blinks blue light when the neonate is normothermic, and sounds an alarm with flashing red light when the neonate is hypothermic. In a recent study, the Bempu bracelet achieved a sensitivity and specificity of 98.5% and 95%, respectively, when compared to axillary temperatures in a sample size of 2424 alarm events.[256] The Bempu is commercially available but, at a $29.76 purchase price at the time of publication, is not affordable in low-resource settings. In addition, its current battery lasts for one month and is non-replaceable.

Hypothermia is closely associated with severe infection, prematurity, and asphyxia, necessitating a reliable monitoring strategy for neonates at risk.[250] While ThermoSpot, Temptraq, and the Bempu bracelet, are all easy to interpret, the TempTraq only lasts 24 hours and is cost-prohibitive for low-resource settings. ThermoSpot and the Bempu bracelet show promise as technologies that can be taken home and used by a caregiver to monitor neonates in the first weeks of life. However, the Bempu must be offered at an affordable price and should be equipped with a rechargeable battery to allow reuse. Also, the qualitative results of the ThermoSpot and Bempu bracelet may be useful to an untrained caregiver, but staff may require a more exact temperature reading in a hospital setting. In conclusion, a device that is usable for at-home caregivers but also provides precise temperature information to nurses that is inexpensive, reusable, and easily visible in a crowded ward would be ideal for use in neonatal intensive care units and in Kangaroo Mother Care.

Jaundice. Over 60% of all newborns develop neonatal jaundice, most commonly due to elevated bilirubin levels. While many cases are benign, development of neonatal jaundice into severe hyperbilirubinemia is associated with severe morbidities and mortality. Severe hyberbilirubinemia is preventable with early diagnosis, continued monitoring, and treatment with phototherapy.[257] Traditional laboratory approaches use either chemical determination, such as the diazo method, or spectrophotometry on blood samples to measure total serum bilirubin concentration.

Additionally, several non-invasive transcutaneous devices for measuring bilirubin have been developed and are commercially available. Skin reflectance is measured and normalized based on skin tone and hemoglobin, and reflected light at 455 nm and 575 nm is detected to calculate serum bilirubin.[258] Because the accuracy of transcutaneous bilirubinometry can be affected by various factors, including prematurity, use during phototherapy,[259] and skin color,[260,261] there is debate on whether transcutaneous bilirubinometers can fully replace serum bilirubin testing for guiding treatment decisions.[259,260,262,263] Guidelines for the management of late-preterm and term infants with hyperbilirubinemia in low-resource settings outline that the use of transcutaneous bilirubinometry is appropriate, but confirmatory serum bilirubin measurements should be used when transcutaneous measurements are above 12 mg dL^{-1} because of increased accuracy in serum bilirubin tests.[264] Several benchtop laboratory bilirubin instruments and transcutaneous bilirubinometers have been previously described by Carceller-Blanchard *et al.*[265]

BiliStick is a newer development that aims to bring serum bilirubin measurements closer to the POC. Whole blood is applied to a plasma-separating membrane, and reflectance of blue and green light is measured from the plasma on the nitrocellulose membrane by a handheld reader. The difference in wavelength measurements is used to determine bilirubin concentration due to the signature absorbance spectrum of bilirubin in plasma. Initial clinical studies have shown 95% of BiliStick measurements falling within −2.22 mg dL^{-1} and +3.43 mg dL^{-1} of the values measured by the reference laboratory. Further, BiliStick measurements had a mean bias of 0.6 mg dL^{-1} and a correlation level of 0.961 compared to standard measurements. The cost was estimated to be less than US $160 for the instrument and a few cents for each test strip.[266] In a follow-up clinical study, BiliStick was shown to have comparable diagnostic performance to the JM-103 transcutaneous bilirubinometer. Compared to clinical laboratory results, the limits of agreement were −5.8 to +3.3 mg dL^{-1} and −5.4 to +6.0 mg dL^{-1} for BiliStick and JM-103, respectively.[267]

Hypoglycemia. Hypoglycemia is common among neonates and can lead to neurodevelopmental disorders, visual and hearing impairments, and disorders of the central nervous system, among other morbidities.[268,269] Blood glucose monitors for adults are prevalent and well-developed;[98,270,271] see

the Gestational diabetes section of this review for more information. However, most commercially available POC glucometers are not optimized in the neonatal blood glucose range, which is lower than the adult glucose range. Plasma glucose values should be above 30 mg dL^{-1} in the first 24 hours of life and above 45 mg dL^{-1} for the remainder of the neonatal period. Devices therefore need to be accurate below 30 mg dL^{-1} and above 180 mg dL^{-1}, which are important values for clinical decision-making. These values lie at the limits of accurate detection for most POC glucose meters. The International Organization of Standardization (ISO) guideline ISO 15197-2013, which defines requirements for POC glucometer accuracy, outlines that 95% of samples have to fall within ±15 mg dL^{-1} for blood glucose values <100 mg dL^{-1} and within ±15% for blood glucose values >100 mg dL^{-1}.[272] Further, high hematocrit levels in neonates can interfere with commercial blood glucose meters optimized for adults, leading to low glucose readings.[273]

The Nova StatStrip, a commercially available glucometer, has been optimized for use with neonatal samples. The StatStrip utilizes a modified glucose oxidase-based amperometric test system and is able to correct for hematocrit levels in samples of 1.2 μL.[274] Studies have found the StatStrip to correlate well with laboratory-based equipment in neonatal patient samples.[275,276] The StatStrip, like other glucometers, has relatively expensive test strips and an above-average meter cost (around US $250). Devices such as the Elite XL and the EML105 have been designed for use with neonatal samples, but they have not been found reliable in diagnosing neonatal hypoglycemia.[273]

While a POC glucometer capable of use with neonatal samples is important, pain caused by continual heel sticks in neonates has been associated with long-term morbidities.[277] Due to the need for frequent glucose monitoring in certain neonatal populations (*e.g.* premature neonates and infants of diabetic mothers), it would be beneficial to move toward non-invasive neonatal glucose monitoring. Research to create transdermal glucose monitors has not yet resulted in a clinical use for adults, and there are no devices for use with neonates.[273] Many groups have worked toward developing continuous glucose monitors,[278–281] but the need for continuous glucose monitoring in neonates is debated.[282] The need for and approaches toward achieving accurate and noninvasive glucose monitoring in neonates has previously been reviewed.[283]

Multianalyte detection and monitoring. Multianalyte systems detect small molecules in blood; three commercially available systems include the Abbott i-Stat, Abaxis Piccolo® xpress, and Alere epoc® Reader.[284] The cartridges in all three systems provide the capability to integrate sample preparation and to measure many analytes within one test run. The Abbott i-Stat uses electrochemical methods within disposable cartridges to detect a wide range of small molecules, including glucose, carbon dioxide, oxygen, potassium, chloride, sodium, lactate dehydrogenase, hematocrit, and more. 65–100 μL of blood is drawn into the cartridges without preprocessing. The detection of specific analytes includes potentiometry, amperometry, and conductive measurement, in some cases following an enzymatic reaction with the analyte of interest.[285] Abaxis designed a compact disk-based approach, which utilizes centrifugal and capillary forces for sample preparation and analyte analysis.[286] Alere designed a chip that utilizes electroosmotic flow and pneumatic pumps for fluid actuation with the capability for high degrees of multiplexing.[284] The system is comprised of sensor containing test cards, a wireless card reader, and a mechanism for wireless transmission of data to a computer.[287] The systems presented here have prohibitively high per-test costs for use in resource-limited settings (the Abbott i-Stat has a per test cost of approximately $25), but provide solutions for emergency care in higher-resource settings.

Multianalyte blood chemistry diagnostics continue to incorporate new technology developments to decrease cost. Microfluidic paper-based analytical devices (μPADs) developed by the Whitesides group are low-cost, easy-to-use platforms for bioanalysis. μPADs have shown great promise in multiplexing bioanalytical tests for urinalysis semi-quantitatively[288] and quantitatively.[289] μPADs for quantitative glucose, cholesterol, lactate, and alcohol testing in human blood or urine were designed to be compatible with commercial glucometers.[290] Furthermore, the Whitesides group developed the universal Mobile Electrochemical Detector (uMED), a POC technology that expands the functionality of glucometers, which only perform amperometry, to include more capabilities found in a benchtop potentiostat, including cyclic voltammetry, square wave voltammetry, and potentiometry.[291] The developments of μPADs and uMED increase the sophistication of POC electrochemistry in an accessible format for resource-limited settings. The number of tests that can be multiplexed into one assay has not yet matched the capabilities of the commercially available cartridge-based systems, which may be acceptable depending on the diagnostic context. Additional work toward the development of paper-based electroanalytical devices for medical diagnostics has been previously reviewed by Maxwell *et al.*[292]

Birth asphyxia. Birth asphyxia is the third leading cause of neonatal mortality, following pre-term complications and infections, and results in 662 000 deaths per year.[293,294] Birth asphyxia is characterized by a lack of oxygen supply to the neonate and can lead to mortality or lifelong morbidities, including severe organ damage, cognitive impairment, neurodegenerative diseases, epilepsies, and other chronic illnesses.[295,296] Asphyxia is associated with prolonged labor, maternal infections, pre-eclampsia, hypertension, hemorrhage, prematurity, multiple births, and certain medications given to the mother.[295,296]

In high resource settings, asphyxia is predicted before birth using cardiotocography, which monitors fetal heartbeats and uterine contractions.[297,298] In low resource settings, abnormal fetal heart rate can be detected, although this alone is not as sensitive as cardiotocography.[9,299] The company Laerdal developed a fetal heart rate monitor, Moyo, for low-

resource settings; Moyo is sold for less than $200.[300,301] This device may help indicate birth asphxyia prior to birth and allow for more timely obstetric responses. Additionally, Jhiego has developed the e-Partogram, a handheld device to assess progression of labor. Similarly to Moyo, this device can allow for rapid detection of complications in labor and allow earlier referrals to address prolonged or obstructed labor.[302]

After birth, asphyxia is diagnosed immediately by testing the pH of the umbilical cord blood.[297] A lack of oxygen and increase in carbon dioxide leads to metabolic acidosis, which results in low pH in cases of asphyxia.[298] In addition to pH, the base excess of umbilical cord blood provides an indication of prolonged asphyxia, and the APGAR score determines severity.[297,298,303,304] APGAR is a symptomatic-based scoring system based on appearance, pulse, grimace, activity, and respiration.[296] A pH value less than 7, a base deficit greater than 12 mmol L^{-1}, and an APGAR score from 0–3 indicate asphyxia.[298] In low-resource settings, the APGAR symptomatic-based scoring system is most commonly used to diagnose asphyxia; however, APGAR scores and other symptomatic-based algorithms are not as sensitive as conventional methods, and delayed assessment or understaffing can result in lack of diagnosis.[305–308] Recently, lactate testing for severity of asphyxia has been shown to be a valid biomarker as well but has not yet been integrated into standard clinical practice.[296,304]

These symptomatic-based algorithms are used for diagnosis in low-resource settings because few quantitative diagnostics for asphyxia are appropriate for the point-of-care. While several POC blood analyte monitors detect pH and lactate with handheld devices, most have not been validated for diagnosing asphyxia. A recent review lists several of these devices, along with need for consumables, parameters tested, and amount of blood required.[309] Chin *et al.* also provide a useful review of POC microdevices for clinical chemistry.[310] Additional tests capable of measuring pH and lactate have been previously discussed in the section on Multianalyte detection and monitoring.

A few paper-based tests have also been developed for measuring pH and lactate, including paper-fluidic electrochemical pH and lactate strips, although these tests require consumable batteries.[211,311] Most equipment-free colorimetric pH strips use urine or saliva as a sample, not blood, and thus cannot be used for umbilical cord blood testing. In low-resource settings, low-cost quantitative tools to document birth asphyxia could be helpful in monitoring and evaluating efforts to improve quality of care during labor and delivery to reduce birth asphyxia. Potential solutions include the device-based and paper-based pH and lactate diagnostics mentioned above. However, clinical validation of these tests in cases of asphyxia at the POC is necessary to determine their diagnostic value.

Sepsis. Neonatal sepsis, a systematic infection occurring within the first 28 days of life, is a leading cause of neonatal morbidity and mortality.[10] Each year, an estimated 421 000 infants die within the first month of life due to neonatal sepsis.[7] This accounts for over 5% of childhood mortality[293] and 15% of neonatal mortality.[312] Further, 99% of all neonatal sepsis deaths occur in developing regions[11] where there is a lack of adequate sepsis diagnostics. The immaturity of the neonatal immune system, particularly in that of premature neonates, puts them at high risk of sepsis and can complicate diagnosis.[10] Fortunately, many of the diagnostic techniques discussed above in the Bacterial infections and puerperal sepsis section are also applicable to neonatal sepsis. Both CRP and PCT are well-studied and characterized over the course of a neonatal bacterial infection. CRP has demonstrated a sensitivity range from 41 to 96% and a specificity range from 72 to 100%, with a value to 10 mg L^{-1} as the most commonly reported diagnostic cutoff. In contrast, PCT has reported sensitivities ranging from 62 to 100% and specificities ranging from 82 to 96% (ref. 313–315) and a diagnostic cut off of 0.1 ng mL^{-1}.[10] Appropriate methods to detect both of these biomarkers have been previously described. WBC counts and differentials are used in typical screening for neonatal sepsis, but have shown limited success in identifying the septic infants. Of most promise for the rapid detection of neonatal sepsis are the POC bacterial culture discussed above and molecular testing methods to detect bacterial DNA. However, to date the detection of bacterial DNA in blood has been largely limited to PCR performed in centralized laboratories.[10]

Pneumonia. Neonatal pneumonia, an infection of the lungs, is a leading cause of neonatal mortality world-wide and is the leading cause of respiratory failure in neonates.[316] Neonatal pneumonia can be classified as early-onset, in which the infection is transmitted to the neonate during birth by aspiration of infected amniotic fluid or colonization of the birth canal, and late-onset, in which the disease in brought on by a hospital acquired infection.[317] The clinical presentation of pneumonia is often non-specific, complicating diagnosis, and traditional diagnostic criteria of neonatal pneumonia has been based on the combination of chest radiographic findings[316] and clinical presentation of sepsis.[317] However, guidelines have been in place to detect pneumonia based on rapid breathing since the 1970s,[318] and the WHO has set the respiratory rate threshold indicating neonatal pneumonia as greater than 60 breaths per minute.[319]

To measure respiratory rate in low-resource settings, manual counting of breaths has been shown to be a reliable measurement of respiratory rate in neonates.[320] To assist community health workers in this counting, a number of international health organizations have undergone studies of using beads and small timers as counting aids to measure respiratory rate in children. In this technique, community health workers of varying levels of literacy and numeracy count the number of breaths taken by a child or neonate over the course of one minute with the use of color-coded beads, shown in Fig. 6. One bead is counted per breath, and if the community health worker reaches the red beads during the one-minute period, the child or neonate is classified as having fast breathing (different strands of color-coded beads are used for measuring children in different age classifications). When measured by primarily illiterate community health workers in Uganda and

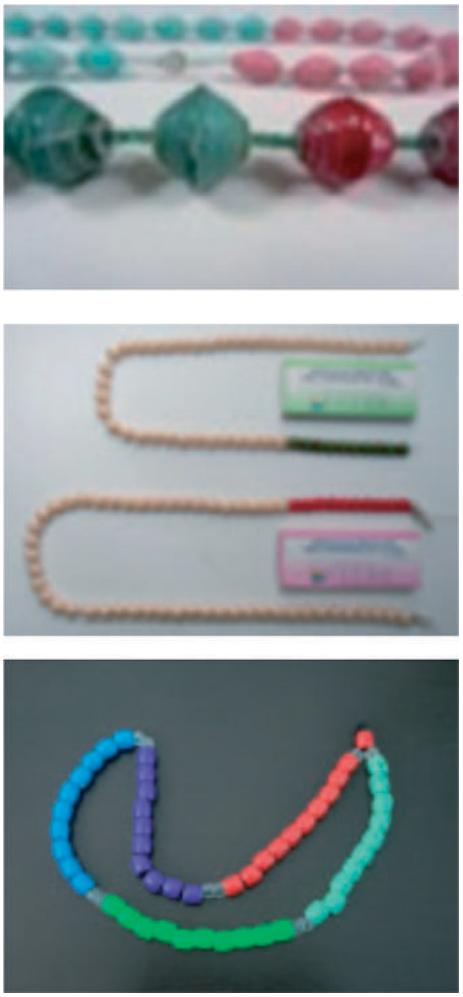


Fig. 6 Counting beads used by various international organizations. (Top) Bead strands used by Save the Children, which employs two age-specific, color-coded strands that can be distinguished by bead size and colors. (Middle) Bead strands used by the Malaria Consortium and the International Rescue Committee, which also employs two age-specific, color-coded strands that are distinguished by colored beads that match the age specific amoxicillin packaging. (Bottom) Bead strand used by UNICEF that is non-specific for ages 0–5 years and each color is made up of 10 beads for ease of counting. Reprinted from ref. 321 under the terms of Creative Commons Attribution License.

South Sudan, the rate of correctly classifying fast breathing in children increased from 27% to 68% when using the counting beads.[321] While counting techniques have shown great improvements in the ability to accurately detect fast breathing, automated devices that decrease the time burden on health care workers could further improve pneumonia detection. Some wearable, continuous infant monitoring devices have been developed (for example, the Rest Devices' Mimo and Snuza® Hero^{MD}), but these devices remain prohibitively expensive for use in low-resource settings, as the per device costs range from $150–300.[322]

HIV. Although the mother-to-child-transmission (MTCT) rate of HIV has declined from 25–42% to 1% or less in settings where a full array of prophylactic strategies can be implemented, pediatric HIV infection remains an ongoing epidemic in less advantaged settings.[33] Although new pediatric HIV infections decreased by over 50% in the 21 UNAIDS Global Plan countries in sub-Saharan Africa from 2009 to 2015, an estimated 110 000 cases were still reported in 2015, most of them from MTCT.[324] In the absence of diagnosis and treatment, 50% of HIV-infected infants will die before their second birthday. Antibody based tests used for adult populations are unsuitable for neonates because maternal antibodies may persist for 12–18 months after birth.[324] As such, the standard of care for early infant diagnostics (EID) of HIV is nucleic acid testing, and should be accomplished within 8 weeks of birth to ensure prompt treatment and infant survival.[325] However, in 2015, only 51% of HIV-exposed infants in 21 priority African countries received a virological test within the first two months of life due to lack of diagnostic tools in low-resource settings.[324] Unlike the quantitative NATs used for viral load monitoring, qualitative nucleic acid testing (a yes/no test for the presence of HIV virus) is sufficient for EID.[35] Here we describe approaches toward making EID more accessible. In addition to the technologies described below, quantitative viral load technologies discussed earlier may also be used.

Although NATs are the most sensitive and specific method available for diagnosing and monitoring infectious disease, no commercially available NAT currently meets the ASSURED criteria. So far, all NAT tests require some form of instrumentation, failing to meet the 'equipment-free' and 'deliverable' criteria. Two laboratory platforms are currently used for EID: the Roche Molecular Diagnostics COBAS® HIV-1 Qualitative Test and the Abbott RealTime Qualitative HIV-1 Test.[24] Both of these instruments accept either plasma or dried blood spots as samples, and perform real-time PCR to identify the presence of HIV-1 virus. Most often, 4-5 drops of whole blood are spotted onto filter paper cards to create dried blood spot specimens, which are then transferred to a reference laboratory.[326] Although these platforms are well-validated and have excellent sensitivity and specificity, they have extremely high costs and infrastructure requirements. Access to this type of testing is a critical barrier that limits access to ART in HIV-infected infants in low-resource settings.[324] Where available, the time required to receive test outcomes results in a high loss-to-follow-up and low ART initiation rates.[33,323]

Sample-to-answer NATs for EID requiring fewer resources than the gold standard include the SAMBA platform (Diagnostics for the Real World Ltd), the Alere™ q HIV 1/2 Detect (Alere™), and the GeneXpert® System (Cepheid).[24] These technologies may be suitable for district hospitals, but cannot be employed in more remote settings. The SAMBA platform has several assays, one of which is a fully automated sample-to-answer system that accepts whole blood for EID and has been validated in several countries including Kenya, Uganda, and Zimbabwe. The Alere™ q HIV-1/2 Detect amplifies HIV-1 and HIV-2 RNA in 52 minutes from 25 μL of blood. A 2014 study on 827 infant samples from primary health clinics in Mozambique reported 98.5% sensitivity and

99.9% specificity when compared to the Roche Cobas Taqman/Ampliprep instrument.[327] This device recently received WHO prequalification, making it available for public sector procurement.[328,329] Alere has several antibody tests available for HIV diagnostics, but the q HIV-1/2 Detect is their only test currently suitable for EID. The GeneXpert® System performs sample preparation, amplification, and detection all in a single cartridge. It is simple to operate, and has been powered successfully in mobile laboratories as well as with solar panels. A more portable version of the GeneXpert® was recently released; known as the GeneXpert Omni®, this system is highly portable at only 1 kg, designed for rugged conditions, and can be powered on a rechargeable battery.[24] Finally, an emerging approach toward EID detects the p24 antigen in a lateral flow assay. The LYNX HIV p24 Antigen Assay (Northwestern Global Health Foundation) runs in less than 50 minutes. It has shown a low sensitivity of 71.9% compared to laboratory-based NATs, but may provide test results to up to 81% more patients compared to laboratory-based testing.[326] This test is expected to cost $700–2000.

Promising approaches in development toward equipment-free EID employ isothermal nucleic acid amplification technologies.[330] These approaches use enzymes to perform the strand separation that would normally be achieved by heating, allowing these reactions to be incubated in a heat block. The amplicon can then be detected on a lateral flow strip or by a low-cost fluorescence reader. Several proof-of-concept studies have been performed to perform isothermal reactions in paper, incubate the reaction using body heat, or detect amplified HIV DNA on lateral flow strips.[331–337] However, the complexities of biological samples and the need for high sensitivity again limit the current usefulness of these technologies. In a thorough review by Craw & Balachandran,[330] the authors conclude that although several isothermal nucleic acid amplification techniques have been extensively validated, the limiting factor is the integration of upstream sample preparation and nucleic acid extraction with downstream detection techniques.[330]

Because sample preparation remains a barrier for many commercial devices, several groups have begun investigating low-cost, simple-to-use solutions for sample preparation and integration with NATs. For example, Rodriguez *et al.* developed a device made entirely of paper and plastic with polyethersulfone (PES) filter paper used as a sample port. An absorbent pad makes contact with the bottom of the PES sample port, and a lateral flow detection strip is initially separated from the PES sample port by a hydrophobic barrier. DNA is precipitated onto the PES sample port and washed. The absorbent pad and the hydrophobic barrier to the lateral flow detection strip are then removed, which could potentially introduce contamination, but allows for elution of the immobilized DNA down the strip. This assay was validated with HPV DNA from cervical swab samples; amplification is not required in this assay due to the abundance of HPV DNA in the swab.[338] The multiplexable autonomous disposable nucleic acid amplification test (MAD NAAT) is another fully integrated sample-to-answer nucleic acid testing platform. The device accepts a nasal swab sample and produces a lateral flow result in 60 minutes, though in some samples, detection time was noted to be as fast as five minutes. The MAD NAAT is comprised of a reusable plastic housing, which facilitates heating and reagent dispensing, as well as disposable components, including fluid storage components, shown in Fig. 7. Timed reagent dispensing is accomplished through melting wax barriers.[339]

Technological innovation in the field of POC HIV diagnostics is still urgently needed, particularly with enclosed sample-to-answer viral load tests and with POC genotypic resistance tests. HIV-1 has a high rate of mutation, and the

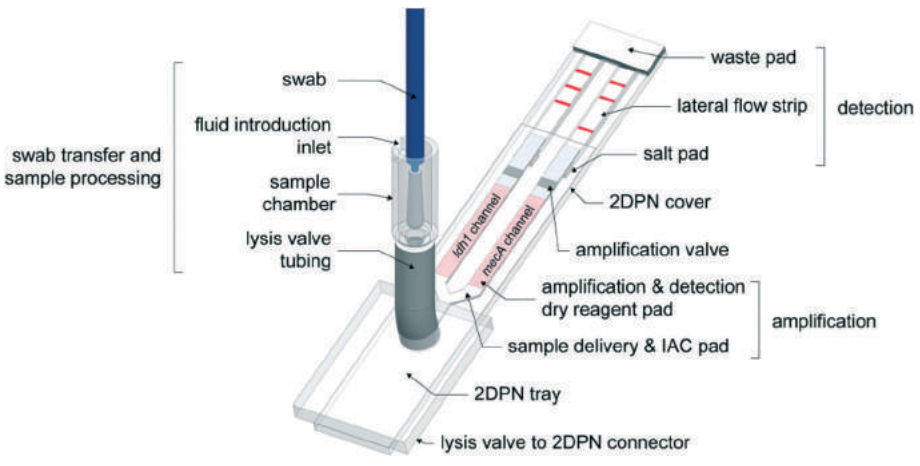


Fig. 7 MAD NAAT is a fully integrated sample-to-answer nucleic acid testing platform. The sample processing, amplification, and detection schemes are depicted. Reagent delivery is timed by melting wax barriers (valves). The components shown here are housed in a reusable plastic cassette with heaters included, which are not depicted here, but can be seen in the original source along with figures showing user workflow and timing. The representative image shown here shows the novel integration of multiple sample preparation steps for nucleic acid testing through the MAD NAAT's reagent delivery scheme. Figure reproduced from ref. 339 with permission from the Royal Society of Chemistry.

WHO reports that levels of HIV drug resistance in countries scaling up ART have been slowly increasing. In some areas, including East Africa, resistance rates to non-nucleoside reverse transcriptase inhibitors (NRTIs) are above 10%. Furthermore, between 10% and 30% of people receiving a first-line ART regimen will develop virological failure at some point during their treatment.[402] Where resistance testing is not available, WHO guidelines recommend reliance on viral load monitoring to inform treatment switches. Specifically, an immediate adherence intervention is recommended when virological failure is detected, followed by a repeated viral load test three months later.[35] If the second viral load test confirms virological failure, a switch to second-line ART is recommended. Despite this recommendation, in practice, HIV care providers often do not switch patients immediately, due to concerns that virological failure resulted from non-adherence.[403] Excluding non-adherence as a cause of high viral load is challenging; existing adherence measurement tools rely on self-reporting measures which are inaccurate. Therefore, POC genotypic resistance tests are urgently needed to determine the cause of virological failure and empower healthcare providers to make informed treatment decisions. Meanwhile, viral load testing platforms should continue to be made cheaper and more accessible. Innovative and cost-effective diagnostic assays may bridge the treatment gap between neonates and adults, reduce the time between infection and treatment of HIV, and control the spread of drug resistance.[340]

Congenital syphilis. Syphilis is a sexually transmitted bacterial infection (*Treponema pallidum*) that can be transmitted from mother to child *in utero*.[341] If syphilis is diagnosed while a woman is pregnant and she is appropriately treated early in gestation with penicillin, there is little risk of congenital syphilis in the neonate.[342] However, syphilis left untreated in a pregnant woman leads to increased rates of transmission to the neonate and more adverse outcomes, including stillbirth, neonatal death, and neonatal morbidities such as visceral or neurologic damage.[341,342] As such, rapid and inexpensive diagnostics in maternal populations are necessary to decrease mortality and morbidity associated with congenital syphilis. If a mother infected with syphilis does not receive appropriate treatment while pregnant, the neonate must undergo more rigorous diagnostic and monitoring to determine if they are infected, including testing of long bone radiographical examination to identify characteristic bone lesions and cerebrospinal fluid testing.[342]

Antibody-based syphilis tests for detection in pregnant women. Syphilis is detected in a pregnant woman using both treponemal and nontreponemal antibody-based LFA tests, where treponemal tests detect antibodies generated directly against the causative bacterial agent *Treponema pallidum* and nontreponemal tests detect antibodies associated with the host response to the infection. Treponemal tests use *Treponema pallidum* antigens spotted onto the paper membrane to target antibodies, but these tests cannot distinguish between past and present infections.[343–345] The most commonly reviewed LFAs for treponemal syphilis include Determine (Alere), SD Bioline (Standard Diagnostics Inc.), Syphicheck (Qualpro), and Visitect (Omega).[343–345] In general, these tests meet the ASSURED criteria, as they are specific (>95% in whole blood), low-cost (<$1), user-friendly, rapid (<15 minutes), robust, and equipment-free.[344,346] However, sensitivity varies depending on the test and sample type with whole blood sensitivities of 86.3%, 84.5%, 74.5%, and 74.26% for Determine, SD Bioline, Syphicheck, and Visitect respectively.[344] On the other hand, diagnostics for nontreponemal syphilis detect anti-cardiolipin antibodies or other non-specific antibodies generated against host reactions to *Treponema pallidum*. Known as the rapid plasma reagin (RPR) test, this test is inexpensive ($0.15–0.23 per test) and quick (<10 minutes),[346] but it detects biomarkers that are present in many other diseases, including autoimmune diseases, and therefore may produce false positive results.[343–345] A few diagnostics, such as the DPP Screen and Confirm assay (ChemBio), multiplex both types of syphilis screens onto the same strip.[343–345] The DPP Screen and Confirm assay (ChemBio) is reported to have reduced sensitivity compared to some treponemal-only tests like Determine (Alere) with plasma sensitivities of 89.8% and 97.3% respectively.[343] However, the multiplexed treponemal and nontreponemal antibodies can help distinguish past and active infections.[343] Additionally, in a recent pilot study in Rwanda, a microfluidic-based syphilis and HIV multiplexed diagnostic detected treponemal syphilis with an AUC of 0.90 and nontreponemal syphilis with an AUC of 0.92.[347] This device costs $34 per test, but is easy-to-use and appropriate for use at the POC with a smartphone to power the microfluidic component and provide quantitative readout.

Syphilis tests for detection in neonates. Screening for syphilis in newborns is difficult, since IgG antibodies transferred from mother to child can circulate in an infant for 15 months after birth.[348] While IgM antibodies do not cross the placenta, the CDC does not recommend IgM tests due to poor test performance, as commercially available IgM tests like Capita Syphilis-M EIA (Trinity BioTech) have sensitivities ranging from 64% in early latent infection to 93% in primary infection.[342,348,349] Instead, nontreponemal tests that detect both IgG and IgM antibodies are used for diagnosting neonates, although they have a high false positive rate.[348]

Other methods for diagnosing congenital syphilis include darkfield microscopy and nucleic acid testing, which both have high sensitivities. However, darkfield microscopy and real-time PCR machines like Rotor-Gene (Corbett Research) and the iCycler (Bio-Rad) are inappropriate for use at the POC in low-resource settings due to infrastructure requirements, need for trained personnel, and high costs.[350] Other low-cost and easy-to-use methods of nucleic acid amplification could be applied to the detection of congenital syphilis at the POC. Likewise, new methods for detecting congenital syphilis in a neonate at the POC in the absence of diagnosis and treatment in the mother are needed to rapidly screen neonates and initiate appropriate treatment.

Emerging infections

Zika virus

While Zika virus (ZIKV) is not a leading cause of maternal or neonatal mortality, it has lately been recognized as an emerging threat to both neonatal and maternal health and has been the subject of accelerated diagnostic innovation. Zika virus is a mosquito-borne flavivirus with a relatively mild clinical manifestation resembling dengue fever and chikungunya, including fever, headache, myalgia, and rash.[351] However, Zika virus drew attention in early 2015 with a widespread outbreak in Brazil that illuminated the relationship between prenatal infection and poor pregnancy outcomes, including congenital Zika transmission to the neonate and microcephaly.[352] Because of the non-specific nature of the clinical manifestation, differential diagnosis can be difficult in pregnant women,[351] leading to the rapid development of new diagnostics for Zika virus.

Antibody-based Zika tests. To differentiate between acute and past Zika infection, the Biocan Zika test uses a combination of the ZIKV NS1 and envelope proteins to detect both IgG and IgM antibodies from whole blood, achieving results from a whole blood sample within 10 minutes.[19] The test also claims 99.5% specificity, but validation studies need to verify this claim. Typically, serological cross-reactivity between flaviviruses such as Zika, dengue, West Nile, and yellow fever limits the specificity of these antibody-based tests, and the Center for Disease Control (CDC) recommends a plaque reduction neutralization test for diagnosing Zika in order to avoid this limitation.[19]

NATs for Zika. Because of the cross-reactivity issues present in antibody-based tests, NATs present an emerging solution to distinguish between flaviviruses. The CDC developed a Zika virus assay comprised of two one-step real-time RT-PCR reactions detecting the premembrane gene and envelope gene. The specificity of the assay was confirmed by testing with RNA from a variety of other flaviviruses, and no cross-reactivity was seen. Additionally, the analytical sensitivity was shown to be as low as 100 copies. This assay was used demonstrate a relatively short duration of detectable viremia following the onset of clinical symptoms (less than 3 days). It was also used to demonstrate detection in samples other than plasma and serum, including saliva, urine, and amniotic fluid.[353] This RT-PCR assay demonstrates the potential for NATs to provide rapid and specific diagnosis of Zika virus, but lacks development into a POC-friendly test format.

Recently, several portable, low-cost platforms for molecular diagnosis of Zika have been demonstrated. Chan *et al.* developed a platform that utilizes reverse transcriptase recombinase polymerase amplification to perform RT-PCR isothermally from urine samples. A modified 3D printer was used for magnetic particle-based nucleic acid extraction, and the included heating unit within the printer can be used to heat samples for the RPA reaction. Fluorescence monitoring was performed using a smartphone camera for nucleic acid quantification following amplification, and clinical relevant sensitivity was demonstrated (5 plaque-forming units per mL).[354] Song *et al.* developed an instrument-free POC platform for the molecular detection of Zika virus. In their platform, reverse-transcription loop-mediated isothermal amplification is performed in a disposable cassette that is chemically heated in a reaction cup without the need for electrical power. This platform has demonstrated a limit of detection of 5 plaque-forming units from oral samples, and the authors report a per test cost of $2.00.[355] Additionally, Pardee *et al.* demonstrated a test that can identify single-base differences between viral strains of Zika using only a drop of blood applied to paper discs. This test employs isothermal RNA amplification, toehold switch RNA sensors, and CRISPR/Cas-9 technology to achieve a sample-to-answer result in less than three hours.[356] The toe-hold switches use a synthetic biology technique that resembles a RNA hairpin; the ribosome binding site (RBS) is contained in the loop of the hairpin, and a trigger RNA sequence unfolds the hairpin structure to expose the RBS and allow translation to a protein structure to occur. This technique has demonstrated excellent orthogonality when tested against similar flaviviruses and is illustrated in Fig. 8. The test was demonstrated using a handheld reader with an estimated cost of $250 and an estimated per-test cost of $0.10–1.00.[357]

There is a great need for further development of diagnostic tests for Zika virus that have been field validated. Currently available diagnostic methods are limited to laboratory settings, preventing the efficient monitoring and diagnosis of Zika at the POC. Further, tests that multiplex Zika with other common flaviviruses such as dengue and chikungunya have the potential to be of great utility when distinguishing flaviviruses at the POC.[353]

Discussion

While great progress has been made to decrease maternal and neonatal mortality, there are still major gaps in the availability of POC tools that aim to diagnose the leading causes of maternal and neonatal death in low-resource settings, as shown in Tables 1 and 2. Only two maternal conditions, HIV and malaria, have readily available POC diagnostics that do not face major barriers for effective implementation at the bedside. Hemorrhage, pre-eclampsia, and bacterial infections combined account for over 50% of global maternal mortality, yet there are no available POC diagnostics to identify women at risk for developing these conditions.[6] Furthermore, no neonatal conditions discussed here have POC diagnostics that are appropriate for use at a health post. The only diagnostic tools available for these and other conditions face numerous barriers to being implemented at the POC, including infrastructure requirements, supply chain concerns, consumable use, per-test and instrument costs, time-to-results, and human resource requirements. Many of these challenges to implementation are summarized in Tables 3 and 4. New developments should focus on making existing technologies less dependent on staffing and infrastructure and bridging

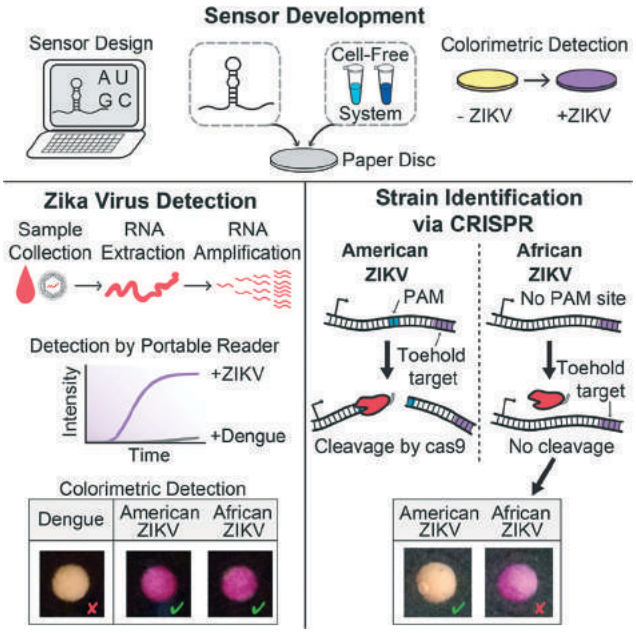


Fig. 8 A novel detection technique for Zika virus that incorporates toe-hold switches and CRISPR-Cas9 technology capable of detecting single-nucleotide differences in RNA strands. (Top) Sensors for specific RNA strands are developed using a novel detection technique known as toe-hold. In short, these toe-hold switches (shown as the RNA hairpin complex) only unfold in the presence of the target RNA strand, revealing a ribosome binding site; this, in combination with a cell-free system dried into paper discs, results in the translation of proteins that cause a visual color change. (Bottom left) The toe-hold switches are combined with an isothermal RNA amplification technique to detect this color change in samples with clinically relevant RNA levels. (Bottom right) CRISPR-Cas9 is used to detect strain mutations; in the American ZIKV strain, the target RNA contains a PAM site (protospacer adjacent motif) generated by the strain mutation at which CRISPR-Cas9 binds and cleaves the target RNA, preventing the downstream translation of color-change proteins. As such, color change will only occur in the African ZIKV strain. This figure provides an overview of the molecular detection components of the system, but does not show the size of the system, required equipment, or workflow to perform the assay. Figure reprinted from Cell, 165, K. Pardee, A. A. Green, M. K. Takahashi, D. Braff, G. Lambert, J. W. Lee, T. Ferrante, D. Ma, N. Donghia, M. Fan, N. M. Daringer, I. Bosch, D. M. Dudley, D. H. O'Connor, L. Gehrke and J. J. Collins, "Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components," 1255–1266, Copyright (2016), with permission from Elsevier.[356]

the gaps that currently exist, especially in biomarker validation, monitoring technologies, and sample preparation. Finally, affordable platform technologies and devices that link diagnostic results with clinical action have the potential to connect more people with improved health care while using fewer resources.

With advancements in proteomics and metabolomics, several biomarkers have been identified as potential targets for future maternal and neonatal POC diagnostics. Panels of biomarkers have been listed for preterm labor,[358] pre-eclampsia,[152,181–183,187,359–364] PROM,[203] congenital syphilis,[365] bacterial infection,[313,366,367] and inflammation markers associated with neonatal pneumonia.[313,366,367] Many are currently performed in high-resource platforms such as Western Blots, ELISAs, or benchtop analyzers, and have potential for development as RDTs.[183,187] Protein biomarkers such as PCT, presepsin, and PlGF/sFlt-1 currently require small benchtop readers, but have clinically relevant limits of detection that suggest they could be implemented in microfluidic and LFA platforms. Potential biomarkers should be evaluated for sensitivity and specificity of diagnosing a certain disease before development of POC-friendly diagnostics that meet ASSURED criteria. These biomarkers present a major opportunity to move detection away from centralized laboratory facilities and towards implementation at the bedside.

In some cases, uptake of POC diagnostics is limited by a lack of human resources rather than a lack of infrastructure. Here, effective and automatic monitoring technologies are needed to allow effective implementation. For example, appropriate technologies to measure temperature and respiratory rate of preterm babies exist, but healthcare providers must manually evaluate neonates on a regular basis; inadequate staffing levels often lead to infrequent monitoring. While 37% of global health care providers work in the Americas to serve 10% of the global disease burden, only 4% of global health care providers work in sub-Saharan Africa to serve 25% of the global disease burden.[368] Low-resource settings would benefit greatly from affordable, robust technologies that constantly monitor temperature or analyte values and alert healthcare providers when values are no longer within the normal range.

A third major gap in development exists in the lack of adequate sample preparation techniques. The technical requirements of preanalytical procedures to extract and concentrate a biomarker prevent many in-development tests, most notably NATs,[29,31] from being implemented into POC clinical settings. Existing preanalytical procedures are also a major source of errors in laboratory diagnostics.[369] Therefore, there is a need for further research into streamlined and integrated sample preparation modules that allow tests to accept unprocessed patient samples and produce diagnostic results with minimal user steps. Well-designed sample preparation techniques can increase the sensitivity and specificity of existing POC diagnostics tools, reduce human error associated with existing techniques, and allow access to platform technologies that use nucleic acid detection for applications such as neonatal HIV.

Given the existing health system constraints, novel technology platforms should be designed strategically in order to best implement them in low-resource settings. For example, assays that require more resources should be equipped to handle multiple disease targets. Platform technologies, such as GeneXpert, have the potential to revolutionize POC disease testing by detecting numerous disease targets with interchangeable test cartridges that contain pre-dried reagents and a code containing identifying information. Although high-throughput platform technologies may only be suitable for large, high-resource laboratories, they may have a more significant impact on disease burden than multiple tests that require fewer resources but also report with lower sensitivity.

Another strategy to use existing resources efficiently at the POC is multiplexing. However, there have been significant challenges in implementing multiplexed assays at the POC, most notably a decrease in analyte sensitivity as additional test targets are added. For example, a platform to detect the presence of three intestinal protozoa using isothermal amplification and lateral flow detection was demonstrated in 2016; however, the limit of detection was approximately an order of magnitude higher for each target than it was when detected in a singleplex assay.[370] Additionally, many commercially available multiplexed NATs, such as GeneXpert, are only capable of a low-throughput of samples, limiting their usability in populations with high burdens of disease.[371] There is an urgent need to continue progress towards developing platforms capable of multiplexing disease targets into a single test without sacrificing test sensitivity.[372]

To overcome barriers to access, technologies themselves should help strengthen linkage to care after diagnosis whenever possible. For example, recent developments in smartphone usage at the POC suggest that smartphones could be used to connect test results to electronic medical records systems.[14] Also, barcodes are commonly used in platform technologies to identify kits, reagents, and expiration dates, reducing the level of training required of healthcare workers and the chance of user error.[24] Finally, the recent use of quick response (QR) codes in lateral flow strips indicates that QR codes could be used to communicate test results among healthcare workers, to transfer data in ways similar to barcodes, or to act as anti-counterfeit measures for diseases that face many counterfeit tests, such as malaria.[373] Imaging platforms that allow smartphones to image a printed code could be useful for a wide range of applications including mobile health initiatives and surveillance efforts for other infectious diseases.

The past few decades have witnessed major declines in child and maternal mortality and progress in the fight against HIV and malaria in developing countries. In 2014, the number of new HIV infections had decreased 20% since the peak of the global epidemic in 1998.[34] Due to the cooperation of several international agencies, significant funding, and the timely innovation of sensitive and specific point-of-care diagnostic tools, 1.2 million new HIV child infections have been avoided in the 21 most affected countries in sub-Saharan Africa, and over 2 million more pregnant women have started receiving lifesaving antiretroviral therapy.[324] Similar interventions allowed malaria rapid diagnostic test sales to increase from 46 million in 2008 to 314 million in 2014, and have contributed to an estimated 60% worldwide decrease in malaria mortality rates over the past 16 years.[374] The successes of adult HIV and malaria diagnostics illustrate the potential for bringing effective, inexpensive, and lifesaving technologies to the POC. However, progress in other areas is still urgently needed. With strategic design, new technologies will continue to expand access to quality diagnostic tools to the areas most affected by the leading causes of maternal and neonatal mortality.

Conflicts of interest

There are no conflicts to declare.

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References

1 World Health Organization, *Health in 2015: From MDGs to SDGs*, 2015.
2 World Health Organization, UNICEF, UNFPA, World Bank Group and UN, *Trends in Maternal Mortality: 1990 to 2015*, 2015.
3 UNICEF, *Levels & Trends in Child Mortality; Report 2015*, 2015.
4 L. De Bernis, M. V. Kinney, W. Stones, P. Ten Hoope-Bender, D. Vivio, S. H. Leisher, Z. A. Bhutta, M. Gülmezoglu, M. Mathai, J. M. Belizán, L. Franco, L. McDougall, J. Zeitlin, A. Malata, K. E. Dickson and J. E. Lawn, *Lancet*, 2016, **387**, 703–716.
5 United Nations General Assembly, *Transforming our world: the 2030 Agenda for Sustainable Development*, 2015.
6 L. Say, D. Chou, A. Gemmill, Ö. Tunçalp, A.-B. Moller, J. Daniels, A. M. Gülmezoglu, M. Temmerman and L. Alkema, *Lancet Glob. Health*, 2014, **2**, e323–e333.
7 L. Liu, S. Oza, D. Hogan, J. Perin, I. Rudan, J. E. Lawn, S. Cousens, C. Mathers and R. E. Black, *Lancet*, 2015, **385**, 430–440.
8 R. E. Black, R. Laxminarayan, M. Temmerman and N. Walker, in *Climate Change 2013 – The Physical Science Basis*, ed. Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, 2016, pp. 1–30.
9 Institute of Medicine (US) Committee on Improving Birth Outcomes, *Improving Birth Outcomes: Meeting the Challenge in the Developing World*, National Academies Press, Washington, D.C., 2003.
10 K. A. Simonsen, A. L. Anderson-Berry, S. F. Delair and H. D. Davies, *Clin. Microbiol. Rev.*, 2014, **27**, 21–47.
11 H. A. Ganatra, B. J. Stoll and A. K. M. Zaidi, *Clin. Perinatol.*, 2010, **37**, 501–523.
12 R. W. Peeling, K. K. Holmes, D. Mabey and A. Ronald, *Sex Transm. Infect.*, 2006, **82**, v1–v6.
13 N. P. Pai, C. Vadnais, C. Denkinger, N. Engel and M. Pai, *PLoS Med.*, 2012, **9**(9), e1001306.
14 X. Xu, A. Akay, H. Wei, S. Wang, B. Pingguan-Murphy, B. E. Erlandsson, X. Li, W. Lee, J. Hu, L. Wang and F. Xu, *Proc. IEEE*, 2015, **103**, 236–247.

15 *Lateral Flow Immunoassay*, ed. R. Wong and H. Tse, Humana Press, Totowa, NJ, 2009.
16 A. St John and C. P. Price, *Clin. Biochem. Rev.*, 2013, **34**, 61–74.
17 E. Drucker and K. Krapfenbauer, *EPMA J.*, 2013, **4**, 7.
18 R. N. Charrel, I. Leparc-Goffart, S. Pas, X. de Lamballerie, M. Koopmans and C. Reusken, *Bull. W. H. O.*, 2016, **94**, 574–584D.
19 I. B. Rabe, J. E. Staples, J. Villanueva, K. B. Hummel, J. A. Johnson, L. Rose, S. Hills, A. Wasley, M. Fischer and A. M. Powers, *MMWR Morb. Mortal. Wkly. Rep.*, 2016, **65**, 543–546.
20 D. A. Anderson, S. M. Crowe and M. Garcia, *Curr. HIV/AIDS Rep.*, 2011, **8**, 31–37.
21 P. A. Moncada and J. G. Montoya, *Expert Rev. Anti-Infect. Ther.*, 2012, **10**, 815–828.
22 E. C. Neto, R. Rubin, J. Schulte and R. Giugliani, *Emerging Infect. Dis.*, 2004, **10**, 1069–1073.
23 J. E. Bennett, R. Dolin and M. J. Blaser, *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*, Elsevier Health Sciences, 8th edn, 2014.
24 UNITAID, *HIV/AIDS Diagnostic Technology Landscape*, 5th edn, 2015.
25 C. Schrader, A. Schielke, L. Ellerbroek and R. Johne, *J. Appl. Microbiol.*, 2012, **113**, 1014–1026.
26 S. Kersting, V. Rausch, F. Bier and M. von Nickisch-Rosenegk, *Malar. J.*, 2014, **13**, 99.
27 R. H. Tang, H. Yang, J. R. Choi, Y. Gong, S. S. Feng, B. Pingguan-Murphy, Q. S. Huang, J. L. Shi, Q. B. Mei and F. Xu, *Crit. Rev. Biotechnol.*, 2017, **37**, 411–428.
28 M. Kersaudy-Kerhoas and E. Sollier, *Lab Chip*, 2013, **13**, 3323.
29 F. Cui, M. Rhee, A. Singh and A. Tripathi, *Annu. Rev. Biomed. Eng.*, 2015, **17**, 267–286.
30 J. Li and J. Macdonald, *Biosens. Bioelectron.*, 2015, **64**, 196–211.
31 A. Niemz, T. M. Ferguson and D. S. Boyle, *Trends Biotechnol.*, 2011, **29**, 240–250.
32 Elizabeth Glaser Pediatric AIDS Foundation, *Maternal, newborn and child health: The Foundation to a Successful Response to Pediatric HIV*, 2017.
33 S. A. Hurst, K. E. Appelgren and A. P. Kourtis, *Expert Rev. Anti-Infect. Ther.*, 2015, **13**, 169–181.
34 M. K. Haleyur Giri Setty and I. K. Hewlett, *AIDS Res. Treat.*, 2014, **2014**, 1–20.
35 World Health Organization, *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection*, 2016.
36 M. Jaspard, G. Le Moal, M. Saberan-Roncato, D. Plainchamp, A. Langlois, P. Camps, A. Guigon, L. Hocqueloux and T. Prazuck, *PLoS One*, 2014, **9**, e101148.
37 H. Shafiee, M. Jahangir, F. Inci, S. Wang, R. B. M. Willenbrecht, F. F. Giguel, A. M. N. Tsibris, D. R. Kuritzkes and U. Demirci, *Small*, 2013, **9**, 2553–2563.
38 C. Gomes and J. M. Azevedo-Pereira, *J. Virol. Methods*, 2011, **173**, 353–356.
39 N. E. Rosenberg, G. Kamanga, S. Phiri, D. Nsona, A. Pettifor, S. E. Rutstein, D. Kamwendo, I. F. Hoffman, M. Keating, L. B. Brown, B. Ndalama, S. A. Fiscus, S. Congdon, M. S. Cohen and W. C. Miller, *J. Infect. Dis.*, 2012, **205**, 528–534.
40 A. E. Singh, B. Lee, J. Fenton and J. Preiksaitis, *Expert Opin. Med. Diagn.*, 2013, **7**, 299–308.
41 M. Brauer, J. C. De Villiers and S. H. Mayaphi, *J. Virol. Methods*, 2013, **189**, 180–183.
42 C. D. Chin, T. Laksanasopin, Y. K. Cheung, D. Steinmiller, V. Linder, H. Parsa, J. Wang, H. Moore, R. Rouse, G. Umviligihozo, E. Karita, L. Mwambarangwe, S. L. Braunstein, J. van de Wijgert, R. Sahabo, J. E. Justman, W. El-Sadr and S. K. Sia, *Nat. Med.*, 2011, **17**, 1015–1019.
43 Y. T. Duong, Y. Mavengere, H. Patel, C. Moore, J. Manjengwa, D. Sibandze, C. Rasberry, C. Mlambo, Z. Li, L. Emel, N. Bock, J. Moore, R. Nkambule, J. Justman, J. Reed, G. Bicego, D. L. Ellenberger, J. N. Nkengasong and B. S. Parekh, *J. Clin. Microbiol.*, 2014, **52**, 3743–3748.
44 World Health Organization, *Guidelines on HIV self-testing and partner notification: supplement to consolidated guidelines on HIV testing services*, 2016.
45 UNITAID, *HIV rapid diagnostics for self-testing*, 3rd edn, 2017.
46 H. H. Lee, M. A. Dineva, Y. L. Chua, A. V. Ritchie, I. Ushiro-Lumb and C. A. Wisniewski, *J. Infect. Dis.*, 2010, **201**, S65–S72.
47 S. Tanriverdi, L. Chen and S. Chen, *J. Infect. Dis.*, 2010, **201**, S52–S58.
48 K. Schnippel, G. Meyer-Rath, L. Long, W. MacLeod, I. Sanne, W. S. Stevens and S. Rosen, *Trop. Med. Int. Health*, 2012, **17**, 1142–1151.
49 H. Shafiee, S. Wang, F. Inci, M. Toy, T. J. Henrich, D. R. Kuritzkes and U. Demirci, *Annu. Rev. Med.*, 2015, **66**, 387–405.
50 C. J. Hoffmann, J. Maritz and G. U. van Zyl, *Trop. Med. Int. Health*, 2016, **21**, 219–223.
51 M. T. May, J.-J. Vehreschild, A. Trickey, N. Obel, P. Reiss, F. Bonnet, M. Mary-Krause, H. Samji, M. Cavassini, M. J. Gill, L. C. Shepherd, H. M. Crane, A. d'Arminio Monforte, G. A. Burkholder, M. M. Johnson, P. Sobrino-Vegas, P. Domingo, R. Zangerle, A. C. Justice, T. R. Sterling, J. M. Miró and J. A. C. Sterne, *Clin. Infect. Dis.*, 2016, **62**, 1571–1577.
52 A. Lopez, I. Caragol, J. Candeias, N. Villamor, P. Echaniz, F. Ortuno, A. Sempere, K. Strauss and A. Orfao, *Cytometry*, 1999, **38**, 231–237.
53 K. Strauss, I. Hannet, S. Engels, A. Shiba, D. M. Ward, S. Ullery, M. G. Jinguji, J. Valinsky, D. Barnett, A. Orfao and L. Kestens, *Cytometry*, 1996, **26**, 52–59.
54 I. V. Jani, N. E. Sitoe, E. R. Alfai, P. L. Chongo, J. I. Quevedo, B. M. Rocha, J. D. Lehe and T. F. Peter, *Lancet*, 2011, **378**, 1572–1579.
55 W. Stevens, N. Gous, N. Ford and L. E. Scott, *BMC Med.*, 2014, **12**, 173.
56 L. Scott, *SAMJ*, 2013, **103**, 883–884.
57 M. T. Glynn, D. J. Kinahan and J. Ducrée, *Lab Chip*, 2013, **13**, 2731.

58 S. Wang, S. Tasoglu, P. Z. Chen, M. Chen, R. Akbas, S. Wach, C. I. Ozdemir, U. A. Gurkan, F. F. Giguel, D. R. Kuritzkes and U. Demirci, *Sci. Rep.*, 2014, **4**, 3796.
59 N. N. Watkins, U. Hassan, G. Damhorst, H. Ni, A. Vaid, W. Rodriguez and R. Bashir, *Sci. Transl. Med.*, 2013, **5**, 214ra170.
60 World Health Organization, *Malaria Rapid Diagnostic Test Performance: Summary results of WHO product testing of malaria RDTs: rounds 1-6 (2008–2015)*, 2015.
61 C. J. Uneke, *Yale J. Biol. Med.*, 2007, **80**, 39–50.
62 M. Fried, A. Muehlenbachs and P. E. Duffy, *Expert Rev. Anti-Infect. Ther.*, 2012, **10**, 1177–1187.
63 Global Malaria Programme, *Recommended selection criteria for procurement of malaria rapid diagnostic tests*, 2016.
64 World Health Organization, *Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: round 6 (2014–2015)*, 2015.
65 World Health Organization, *World Health Statistics: 2016*, 2016.
66 M. L. Wilson, *Clin. Infect. Dis.*, 2012, **54**, 1637–1641.
67 J. E. Williams, M. Cairns, F. Njie, S. Laryea Quaye, T. Awine, A. Oduro, H. Tagbor, K. Bojang, P. Magnussen, F. O. ter Kuile, A. Woukeu, P. Milligan, D. Chandramohan and B. Greenwood, *Clin. Infect. Dis.*, 2016, **62**, 837–844.
68 L. M. Cohee, L. Kalilani-Phiri, S. Boudova, S. Joshi, R. Mukadam, K. B. Seydel, P. Mawindo, P. Thesing, S. Kamiza, K. Makwakwa, A. Muehlenbachs, T. E. Taylor and M. K. Laufer, *Malar. J.*, 2014, **13**, 274.
69 A.-M. Rantala, S. M. Taylor, P. A. Trottman, M. Luntamo, B. Mbewe, K. Maleta, T. Kulmala, P. Ashorn and S. R. Meshnick, *Malar. J.*, 2010, **9**, 269.
70 A. Mayor, L. Moro, R. Aguilar, A. Bardaji, P. Cistero, E. Serra-Casas, B. Sigauque, P. L. Alonso, J. Ordi and C. Menendez, *Clin. Infect. Dis.*, 2012, **54**, 1561–1568.
71 F. Saute, C. Menendez, A. Mayor, J. Aponte, X. Gomez-Olive, M. Dgedge and P. Alonso, *Trop. Med. Int. Health*, 2002, **7**, 19–28.
72 F. P. Mockenhaupt, B. Rong, H. Till, T. A. Eggelte, S. Beck, C. Gyasi-Sarpong, W. N. Thompson and U. Bienzle, *Trop. Med. Int. Health*, 2000, **5**, 167–173.
73 F. P. Mockenhaupt, G. Bedu-Addo, C. von Gaertner, R. Boyé, K. Fricke, I. Hannibal, F. Karakaya, M. Schaller, U. Ulmen, P. A. Acquah, E. Dietz, T. A. Eggelte and U. Bienzle, *Malar. J.*, 2006, **5**, 119.
74 B. Tegegne, S. Getie, W. Lemma, A. N. Mohon and D. R. Pillai, *Malar. J.*, 2017, **16**, 34.
75 D. J. Kyabayinze, J. K. Tibenderana, M. Nassali, L. K. Tumwine, C. Riches, M. Montague, H. Counihan, P. Hamade, J.-P. Van Geertruyden and S. Meek, *Malar. J.*, 2011, **10**, 306.
76 E. C. Oriero, J.-P. Van Geertruyden, D. C. Nwakanma, U. D'Alessandro and J. Jacobs, *Expert Rev. Mol. Diagn.*, 2015, **15**, 1419–1426.
77 M. Safavieh, M. K. Kanakasabapathy, F. Tarlan, M. U. Ahmed, M. Zourob, W. Asghar and H. Shafiee, *ACS Biomater. Sci. Eng.*, 2016, **2**, 278–294.
78 M. C. Giuffrida and G. Spoto, *Biosens. Bioelectron.*, 2017, **90**, 174–186.
79 E. C. Oriero, J. Jacobs, J.-P. Van Geertruyden, D. Nwakanma and U. D'Alessandro, *J. Antimicrob. Chemother.*, 2015, **70**, 2–13.
80 J. M. Roth, D. A. Korevaar, M. M. G. Leeflang and P. F. Mens, *Crit. Rev. Clin. Lab. Sci.*, 2016, **53**, 87–105.
81 S. Britton, Q. Cheng and J. S. McCarthy, *Malar. J.*, 2016, **15**, 88.
82 UNITAID, *Malaria Diagnostics Technology and Market Landscape*, 3rd edn, 2016.
83 B. D. Grant, C. A. Smith, K. Karvonen and R. Richards-Kortum, *Anal. Chem.*, 2016, **88**, 2553–2557.
84 W. S. Bauer, D. W. Kimmel, N. M. Adams, L. E. Gibson, T. F. Scherr, K. A. Richardson, J. A. Conrad, H. K. Matakala, F. R. Haselton and D. W. Wright, *Analyst*, 2017, **142**, 1569–1580.
85 C. E. Anderson, K. G. Shah and P. Yager, *Methods Enzymol.*, 2017, 383–411.
86 World Health Organization, *Global Report on Diabetes*, 2016.
87 Y. Zhu and C. Zhang, *Curr. Diabetes Rep.*, 2016, **16**, 7.
88 D. R. Coustan, *Clin. Chem.*, 2013, **59**, 1310–1321.
89 L. Kanguru, N. Bezawada, J. Hussein and J. Bell, *Glob. Health Action*, 2014, **7**, 23987.
90 B. Bhavadharini, R. Uma, P. Saravanan and V. Mohan, *Clin. Diabetes Endocrinol.*, 2016, **2**, 13.
91 P. B. Renz, G. Cavagnolli, L. S. Weinert, S. P. Silveiro and J. L. Camargo, *PLoS One*, 2015, **10**, e0135989.
92 A. Khalafallah, E. Phuah, A. M. Al-Barazan, I. Nikakis, A. Radford, W. Clarkson, C. Trevett, T. Brain, V. Gebski and A. Corbould, *BMJ Open*, 2016, **6**, e011059.
93 M. Ye, Y. Liu, X. Cao, F. Yao, B. Liu, Y. Li, Z. Wang and H. Xiao, *Diabetes Res. Clin. Pract.*, 2016, **114**, 43–49.
94 World Health Organization, *Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus*, 2011.
95 P. K. Drain, E. P. Hyle, F. Noubary, K. A. Freedberg, D. Wilson, W. R. Bishai, W. Rodriguez and I. V. Bassett, *Lancet Infect. Dis.*, 2014, **14**, 239–249.
96 A. St John and C. P. Price, *Clin. Biochem. Rev*, 2014, **35**, 155–167.
97 B. Bhavadharini, M. M. Mahalakshmi, K. Maheswari, G. Kalaiyarasi, R. M. Anjana, M. Deepa, H. Ranjani, M. Priya, R. Uma, S. Usha, S. D. Pastakia, B. Malanda, A. Belton, R. Unnikrishnan, A. Kayal and V. Mohan, *Acta Diabetol.*, 2016, **53**, 91–97.
98 A. Rebel, M. A. Rice and B. G. Fahy, *J. Diabetes Sci. Technol.*, 2012, **6**, 396–411.
99 World Health Organization, *Medical Devices: Managing the Mismatch*, 2010.
100 S. Mendis, I. Al Bashir, L. Dissanayake, C. Varghese, I. Fadhil, E. Marhe, B. Sambo, F. Mehta, H. Elsayad, I. Sow, M. Algoe, H. Tennakoon, L. D. Truong, L. T. T. Lan, D. Huiuinato, N. Hewageegana, N. A. W. Fahal, G. Mebrhatu, G. Tshering and O. Chestnov, *Int. J. Hypertens.*, 2012, **2012**, 1–7.

101 B. H. Weigl and J. K. Drake, *Point of Care*, 2013, **12**, 33–40.
102 M. Vučić Lovrenčić, V. Radišić Biljak, S. Božičević, E. Pape-Medvidović and S. Ljubić, *Int. J. Endocrinol.*, 2013, **2013**, 1–6.
103 C. Chen, Q. Xie, D. Yang, H. Xiao, Y. Fu, Y. Tan and S. Yao, *RSC Adv.*, 2013, **3**, 4473.
104 Z. Zhu, L. Garcia-Gancedo, A. J. Flewitt, H. Xie, F. Moussy and W. I. Milne, *Sensors*, 2012, **12**, 5996–6022.
105 M. M. Rahman, A. J. S. Ahammad, J.-H. Jin, S. J. Ahn and J.-J. Lee, *Sensors*, 2010, **10**, 4855–4886.
106 K. Tian, M. Prestgard and A. Tiwari, *Mater. Sci. Eng., C*, 2014, **41**, 100–118.
107 A. J. Bandodkar and J. Wang, *Trends Biotechnol.*, 2014, **32**, 363–371.
108 S. K. Vashist, *Anal. Chim. Acta*, 2012, **750**, 16–27.
109 N. S. Oliver, C. Toumazou, A. E. G. Cass and D. G. Johnston, *Diabetic Med.*, 2009, **26**, 197–210.
110 G. A. Stevens, M. M. Finucane, L. M. De-Regil, C. J. Paciorek, S. R. Flaxman, F. Branca, J. P. Peña-Rosas, Z. A. Bhutta and M. Ezzati, *Lancet Glob. Health*, 2013, **1**, e16–e25.
111 S. New and M. Wirth, *BJOG*, 2015, **122**, 166–169.
112 E. Kohne, *Dtsch. Arztebl. Int.*, 2011, **108**, 532–540.
113 M. M. Bond and R. R. Richards-Kortum, *Am. J. Clin. Pathol.*, 2015, **144**, 885–894.
114 T. W.-A. Guo, R. Patnaik, K. Kuhlmann, A. J. Rai and S. K. Sia, *Lab Chip*, 2015, **15**, 3514–3520.
115 M. Bond, C. Elguea, J. S. Yan, M. Pawlowski, J. Williams, A. Wahed, M. Oden, T. S. Tkaczyk and R. Richards-Kortum, *Lab Chip*, 2013, **13**, 2381.
116 PATH, *Maternal anemia rapid landscape analysis document*, 2012.
117 L. G. T. Darshana and D. I. Uluwaduge, *Anemia*, 2014, **2014**, 1–4.
118 H. Marn and J. A. Critchley, *Lancet Glob. Health*, 2016, **4**, e251–e265.
119 A. Medina Lara, C. Mundy, J. Kandulu, L. Chisuwo and I. Bates, *J. Clin. Pathol.*, 2005, **58**, 56–60.
120 X. Yang, N. Z. Piety, S. M. Vignes, M. S. Benton, J. Kanter and S. S. Shevkoplyas, *Clin. Chem.*, 2013, **59**, 1506–1513.
121 E. A. Tyburski, S. E. Gillespie, W. A. Stoy, R. G. Mannino, A. J. Weiss, A. F. Siu, R. H. Bulloch, K. Thota, A. Cardenas, W. Session, H. J. Khoury, S. O'Connor, S. T. Bunting, J. Boudreaux, C. R. Forest, M. Gaddh, T. Leong, L. A. Lyon and W. A. Lam, *J. Clin. Invest.*, 2014, **124**, 4387–4394.
122 M. Bond, J. Mvula, E. Molyneux and R. Richards-Kortum, presented in part at *2014 IEEE Healthcare Innovation Conference (HIC)*, 2014.
123 B. L. Thompson, R. J. Gilbert, M. Mejia, N. Shukla, D. M. Haverstick, G. T. Garner and J. P. Landers, *Anal. Chim. Acta*, 2016, **924**, 1–8.
124 M. S. Bhamla, B. Benson, C. Chai, G. Katsikis, A. Johri and M. Prakash, *Nat. Biomed. Eng.*, 2017, **1**, 9.
125 M. Bond and R. Richards-Kortum, *Nat. Biomed. Eng.*, 2017, **1**, 17.
126 J. Brown, L. Theis, L. Kerr, N. Zakhidova, K. O'Connor, M. Uthman, Z. M. Oden and R. Richards-Kortum, *Am. J. Trop. Med. Hyg.*, 2011, **85**, 327–332.
127 A. P. Wong, M. Gupta, S. S. Shevkoplyas and G. M. Whitesides, *Lab Chip*, 2008, **8**, 2032–2037.
128 D. Trebbels, D. Hradetzky and R. Zengerle, presented in part at *2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 2009.
129 J. Punter-Villagrasa, J. Cid, C. Páez-Avilés, I. Rodríguez-Villarreal, E. Juanola-Feliu, J. Colomer-Farrarons and P. Miribel-Català, *Sensors*, 2015, **15**, 4564–4577.
130 S. B. Berry, S. C. Fernandes, A. Rajaratnam, N. S. DeChiara and C. R. Mace, *Lab Chip*, 2016, **16**, 3689–3694.
131 S. Ngwenya, *Int. J. Women's Health*, 2016, **8**, 647–650.
132 A. James, D. L. Cooper and M. J. Paidas, *Int. J. Women's Health*, 2015, **7**, 873–881.
133 S. Just, *Semin. Thromb. Hemostasis*, 2016, **43**, 075–091.
134 M. Ettel, M. A. Nardi and L. McVoy, *Int. J. Lab. Hematol.*, 2017, **39**, 286–292.
135 Royal College of Obstetricians and Gynaecologists, *Prevention and management of postpartum haemorrhage*, 2009.
136 E. C. Atukunda, G. R. Mugyenyi, C. Obua, E. B. Atuhumuza, N. Musinguzi, Y. F. Tornes, A. G. Agaba and M. J. Siedner, *PLoS One*, 2016, **11**, e0152408.
137 H. Li, D. Han, G. M. Pauletti and A. J. Steckl, *Lab Chip*, 2014, **14**, 4035–4041.
138 A. Hancock, A. D. Weeks and D. T. Lavender, *BMC Pregnancy Childbirth*, 2015, **15**, 230.
139 PATH, *Blood Loss Measurement*, 2013.
140 N. Prata, M. A. Quaiyum, P. Passano, S. Bell, D. D. Bohl, S. Hossain, A. J. Azmi and M. Begum, *Soc. Sci. Med.*, 2012, **75**, 2021–2027.
141 The Economist Newspaper Limited, Econ., 2013.
142 N. Prata, G. Mbaruku and M. Campbell, *Int. J. Gynecol. Obstet.*, 2005, **89**, 49–50.
143 S. A. Spangler, *Med. Anthropol. Q.*, 2011, **25**, 479–498.
144 *Kelly's Pad*, http://www.healthklin.com/Kellys-Pad, (accessed March 2017).
145 *Kelly's Pad*, http://maternova.org/kellys-pad, (accessed March 2017).
146 *Calibrated obstetric drape (case of 100)*, https://maternova.net/products/calibrated-obstetric-drape, (accessed March 2017).
147 T. Lertbunnaphong, N. Lapthanapat, J. Leetheeragul, P. Hakularb and A. Ownon, *Singapore Med. J.*, 2015, **57**, 325–328.
148 A. Patel, S. S. Goudar, S. E. Geller, B. S. Kodkany, S. A. Edlavitch, K. Wagh, S. S. Patted, V. A. Naik, N. Moss and R. J. Derman, *Int. J. Gynecol. Obstet.*, 2006, **93**, 220–224.
149 L. Wilcox, C. Ramprasad, A. Gutierrez, M. Oden, R. Richards-Kortum, H. Sangi-Haghpeykar and M. Gandhi, *Matern. Child Health J.*, 2017, **21**, 516–523.
150 M. Cortet, C. Deneux-Tharaux, C. Dupont, C. Colin, R.-C. Rudigoz, M.-H. Bouvier-Colle and C. Huissoud, *Br. J. Anaesth.*, 2012, **108**, 984–989.
151 M. M. Dudek, T. L. Lindahl and A. J. Killard, *Anal. Chem.*, 2010, **82**, 2029–2035.
152 O. Nwanodi, *Health Care Curr. Rev.*, 2016, **4**, 26.

153 National Institute for Health and Care Excellence, *PlGF-based testing to help diagnose suspected pre-eclampsia (Triage PlGF test, Elecsys immunoassay sFlt-1/PlGF ratio, DELFIA Xpress PlGF 1-2-3 test, and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio)*, 2016.
154 *The FIGO textbook of pregnancy hypertension: An evidence-based guide to monitoring, prevention and management*, ed. L. A. Magee, P. Von Dadelszen, W. Stones and M. Mathai, The Global Library of Women's Medicine, London, 2016.
155 M. Knight, *Obstetric Anesthesia Digest*, 2008, **28**, 145–146.
156 I. A. Buhimschi, U. A. Nayeri, G. Zhao, L. L. Shook, A. Pensalfini, E. F. Funai, I. M. Bernstein, C. G. Glabe and C. S. Buhimschi, *Sci. Transl. Med.*, 2014, **6**, 245ra92.
157 K. M. Khashia, M. J. Willett and R. M. Elgawly, *J. Obstet. Gynaecol.*, 2007, **27**, 388–389.
158 T. M. Herrick, C. M. Harner-Jay, A. M. Levisay, P. S. Coffey, M. J. Free and P. D. LaBarre, *BMC Pregnancy Childbirth*, 2014, **14**, 10.
159 K. O. Osungbade and O. K. Ige, *J. Pregnancy*, 2011, **2011**, 1–6.
160 *Proteinuria testing: Technology Solutions Landscape*, http://sites.path.org/mnhtech/assessment/preeclampsia-and-eclampsia/proteinuria/technology-solution/, (accessed March 2017).
161 J. Villar, L. Say, A. Shennan, M. Lindheimer, L. Duley, A. Conde-Agudelo and M. Merialdi, *Int. J. Gynecol. Obstet.*, 2004, **85**, S28–S41.
162 E. C. Baker, N. Hezelgrave, S. M. Magesa, S. Edmonds, A. de Greeff and A. Shennan, *Trop. Doct.*, 2012, **42**, 101–103.
163 B. Oiyemhonlan, E. Udofia and D. Punguyire, *Afr. J. Reprod. Health*, 2013, **17**, 129–140.
164 Malawi Ministry of Health and ICF International, *Malawi Service Provision Assessment 2013-14*, Lilongwe, Malawi, 2014.
165 A. de Greef, D. Ashraf, L. Saad, N. L. Hezelgrave and A. H. Shennan, *Arch. Dis. Child. Fetal Neonatal Ed.*, 2011, **96**, Fa105.
166 G. Parati, M. O. Kilama, A. Faini, E. Facelli, K. Ochen, C. Opira, S. Mendis, J. Wang, N. Atkins and E. O'Brien, *Hypertension*, 2010, **56**, 1047–1053.
167 P. V. Kewalbansing, A. R. P. P. Ishwardat, L. M. Brewster, G. Oehlers and G. A. van Montfrans, *Blood Press. Monit.*, 2013, **18**, 78–84.
168 D. Carey, *Omron HEM-Solar(HEM-4500-SOLE)*, UBM, 2011.
169 A. de Greeff, H. Nathan, N. Stafford, B. Liu and A. H. Shennan, *Blood Press. Monit.*, 2008, **13**, 342–348.
170 H. L. Nathan, A. de Greeff, N. L. Hezelgrave, L. C. Chappell and A. H. Shennan, *Blood Press. Monit.*, 2015, **20**, 299–302.
171 H. Nathan, A. El Ayadi, N. Hezelgrave, P. Seed, E. Butrick, S. Miller, A. Briley, S. Bewley and A. Shennan, *BJOG*, 2015, **122**, 268–275.
172 *Nissei DS-400 Semi-Automatic Upper Arm BPM*, http://www.white-medical.co.uk/Nissei-DS-400-Semi-Automatic-Upper-Arm-BPM, (accessed March 2017).
173 A. de Greeff and A. H. Shennan, *Trop. Doct.*, 2015, **45**, 168–173.
174 E. Casiglia, V. Tikhonoff, F. Albertini and P. Palatini, *Hypertension*, 2016, **68**, 896–903.
175 A. N. Rogoza, T. S. Pavlova and M. V. Sergeeva, *Blood Press. Monit.*, 2000, **5**, 227–231.
176 P. D. Davis, J. L. Dennis and R. Railton, *J. Hum. Hypertens.*, 2005, **19**, 197–203.
177 *LifeSource UA-767 Blood Pressure Monitor Review*, http://bloodpressuremonitorguide.com/lifesource-ua-767-review, (accessed July 2007).
178 A&D Medical, *Advanced/Deluxe One Step Auto-Inflation: Instruction Guide- Model UA-767 Plus*, 2009.
179 M. Golparvar, H. Naddafnia and M. Saghaei, *Anesth. Analg.*, 2002, **95**, 1686–1690.
180 C. Arteta, J. S. Domingos, M. A. F. Pimentel, M. D. Santos, C. Chiffot, D. Springer, A. Raghu and G. D. Clifford, in *Wireless Mobile Communication and Healthcare, MobiHealth 2011, Lecture Notes of the Institute for Computer Sciences, Social Informatics and Telecommunications Engineering*, ed. K. S. Nikita, J. C. Lin, D. I. Fotiadis and M. T. Arredondo Waldmeyer, Springer, Berlin, Heidelberg, 2012, vol. 83, pp. 335–342.
181 PATH, *Candidate blood based biomarkers for preeclampsia testing*, 2014.
182 N. Acestor, J. Goett, A. Lee, T. M. Herrick, S. M. Engelbrecht, C. M. Harner-Jay, B. J. Howell and B. H. Weigl, *Clin. Chem. Lab. Med.*, 2016, **54**, 17–27.
183 L. B. Andersen, B. Frederiksen-Møller, K. Work Havelund, R. Dechend, J. S. Jørgensen, B. L. Jensen, J. Nielsen, S. Lykkedegn, T. Barington and H. T. Christesen, *J. Am. Soc. Hypertens.*, 2015, **9**, 86–96.
184 J. Rasanen, M. J. Quinn, A. Laurie, E. Bean, C. T. Roberts, S. R. Nagalla and M. G. Gravett, *Am. J. Obstet. Gynecol.*, 2015, **212**, 82.e1.
185 G. K. Frampton, J. Jones, M. Rose and L. Payne, *Health Technol. Assess.*, 2016, **20**, 1–160.
186 S. J. Benton, Y. Hu, F. Xie, K. Kupfer, S.-W. Lee, L. A. Magee and P. von Dadelszen, *Am. J. Obstet. Gynecol.*, 2011, **205**, 469.
187 Diagnostics Assessment Programme, *Pre-eclampsia: The Triage PlGF test, Elecsys immunoassay sFlt-1/PlGF ratio, DELFIA Xpress PlGF 1-2-3 test and BRAHMS sFlt-1 Kryptor/PlGF plus Kryptor PE ratio to aid the assessment of suspected pre-eclampsia*, 2015.
188 T. Kirkpatrick and M. N. Dolecki, *BD Licenses Technology to Advance Testing for Preeclampsia, Gestational Diabetes: Tests from DiabetOmics to be Integrated into BD VeritorTM Point-of-Care Diagnostic Platform*, https://www.bd.com/press/2016/BD-Licenses-Technology-to-Advance-Testing-for-Preeclampsia-Gestational-Diabetes.aspx, (accessed March 2017).
189 *BD Veritor™ System for the Rapid Detection of Flu A+B*, https://www.fishersci.com/shop/products/bd-veritor-system-the-rapid-detection-flu-a-b-3/p-4322335, (accessed March 2017).
190 T. Wang, R. Zhou, L. Gao, Y. Wang, C. Song, Y. Gong, J. Jia, W. Xiong, L. Dai, L. Zhang and H. Hu, *Hypertension*, 2014, **64**, 846–851.

191 M. Sammar, A. Syngelaki, A. Sharabi-Nov, K. Nicolaides and H. Meiri, *Fetal Diagn. Ther.*, 2017, **41**, 23–31.
192 A. C. Seale, M. Mwaniki, C. R. Newton and J. A. Berkley, *Lancet Infect. Dis.*, 2009, **9**, 428–438.
193 N. Arulkumaran and M. Singer, *Best Pract. Res. Clin. Obstet. Gynaecol.*, 2013, **27**, 893–902.
194 M. Meisner, *Clin. Chim. Acta*, 2002, **323**, 17–29.
195 M. Meisner, K. Tschaikowsky, T. Palmaers and J. Schmidt, *Crit. Care*, 1999, **3**, 45.
196 K.-E. Kim and J.-Y. Han, *Korean J. Lab. Med.*, 2010, **30**, 153.
197 B. M. P. Tang, G. D. Eslick, J. C. Craig and A. S. McLean, *Lancet. Infect. Dis.*, 2007, **7**, 210–217.
198 Q. Zou, W. Wen and X.-C. Zhang, *World J. Emerg. Med.*, 2014, **5**, 16–19.
199 Y. Okamura and H. Yokoi, *Clin. Chim. Acta*, 2011, **412**, 2157–2161.
200 J. Volanakis, *Mol. Immunol.*, 2001, **38**, 189–197.
201 C. Swanson and A. D'Andrea, *Clin. Chem.*, 2013, **59**, 641–648.
202 W. Leung, C. P. Chan, T. H. Rainer, M. Ip, G. W. H. Cautherley, R. Renneberg and J. Immunol, *Methods*, 2008, **336**, 30–36.
203 A. B. Caughey, J. N. Robinson and E. R. Norwitz, *Rev. Obstet. Gynecol.*, 2008, **1**, 11–22.
204 S. Chhabra, N. Kumar and P. Kalra, *J. Basic Clin. Reprod. Sci.*, 2015, **4**, 29.
205 M. Palacio, M. Kühnert, R. Berger, C. L. Larios and L. Marcellin, *BMC Pregnancy Childbirth*, 2014, **14**, 183.
206 E.-M. Rutanen, T. H. Kärkkäinen, J. Lehtovirta, J. T. Uotila, M. K. Hinkula and A.-L. Hartikainen, *Clin. Chim. Acta*, 1996, **253**, 91–101.
207 P. G. Morris and K. Jain, *J. Obstet. Gynaecol.*, 1998, **18**, 33–36.
208 L. C. Rogers, L. Scott and J. E. Block, *Clin. Med. Insights: Reprod. Health*, 2016, **10**, 15.
209 G. U. Eleje, E. C. Ezugwu, A. C. Eke, J. I. Ikechebelu, C. C. Obiora, N. O. Ojiegbe, I. U. Ezebialu, C. O. Ezeama, B. O. Nwosu, G. O. Udigwe, C. I. Okafor and F. O. Ezugwu, *J. Perinat. Med.*, 2017, **45**(1), 105–112.
210 P. Chaemsaithong, R. Romero, S. J. Korzeniewski, A. Martinez-Varea, Z. Dong, B. H. Yoon, S. S. Hassan, T. Chaiworapongsa and L. Yeo, *J. Matern.-Fetal Neonat. Med.*, 2016, **29**, 349–359.
211 E. Wiberg-Itzel, H. Pettersson, S. Cnattingius and L. Nordstrom, *BJOG*, 2006, **113**, 1426–1430.
212 D. Liang, H. Qi, X. Luo, X. Xiao and X. Jia, *J. Obstet. Gynaecol. Res.*, 2014, **40**, 1555–1560.
213 I. A. Abdelazim, M. M. Al-Sherbeeny, M. E. M. Ibrahim, A. A. Fahmy, N. H. Rabei and A. A. Aziz Khalifa, *Acta Medica International*, 2016, **3**, 69–74.
214 R. W. McQuivey and J. E. Block, *Med. Devices: Evidence Res.*, 2016, **6**, 69–74.
215 E. Wiberg-Itzel, S. Cnattingius and L. Nordström, *BJOG*, 2005, **112**, 754–758.
216 A. El-Messidi and A. Cameron, *J. Obstet. Gynaecol. Can.*, 2010, **32**, 561–569.
217 V. Desai Shyam and P. Tank, *Handbook on Preterm Prelabor Rupture of Membranes in a Low Resource Setting*, Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 1st edn, 2012.
218 *Lactate Pro 2 Test Strips 25/Box*, http://www.habdirect.co.uk/lactate-pro-2-test-strips, (accessed January 2017).
219 *AmniSure ROM Test (Rupture of [fetal] Membranes test)*, https://www.qiagen.com/us/shop/sample-technologies/protein/protein-preparation/amnisure-rom-test-10-min-us/#orderinginformation, (accessed January 2017).
220 *AmniSure® ROM Test – box of 10 tests*, http://www.medshop.co.nz/Amnisure, (accessed January 2017).
221 South Eastern Sydney Local Health District Obstetric Clinical Guidelines Group, *Actim PROM: Qualitative diagnosis of preterm premature rupture of membranes guideline*, Sydney, Australia, 2009.
222 *ROM Plus Fetal Membranes Rupture Test*, http://www.superiormedical.com/product/rom-plus-fetal-membranes-rupture-test/, (accessed January 2017).
223 O. M. Osman and M. Elghazaly, *Open J. Obstet. Gynecol.*, 2014, **4**, 967–972.
224 H. Kafali and C. Öksüzler, *Arch. Gynecol. Obstet.*, 2007, **275**, 157–160.
225 A. M. M. Mohamed and W. A. I. Mostafa, *Kasr Al-Aini Journal of Obstetrics and Gynecology*, 2011, **2**, 41–47.
226 A. Hanfy, *Med. J. Cairo Univ.*, 2010, **78**, 313–317.
227 M. S. Zanjani and L. Haghighi, *J. Obstet. Gynaecol. Res.*, 2012, **38**, 505–508.
228 M. Tigga and S. Malik, *Int. J. Reprod. Contraception, Obstet. Gynecol.*, 2015, **4**, 1070–1075.
229 C. Gezer, A. Ekin, C. Golbasi, C. Kocahakimoglu, U. Bozkurt, A. Dogan, U. Solmaz, H. Golbasi and C. E. Taner, *J. Matern.-Fetal Neonat. Med.*, 2017, **30**, 772–778.
230 K.-K. Fung, C. P.-Y. Chan and R. Renneberg, *Anal. Bioanal. Chem.*, 2009, **393**, 1281–1287.
231 M. M. C. Bruijn, J. Y. Vis, F. F. Wilms, M. A. Oudijk, A. Kwee, M. M. Porath, G. Oei, H. C. J. Scheepers, M. E. A. Spaanderman, K. W. M. Bloemenkamp, M. C. Haak, A. C. Bolte, F. P. H. A. Vandenbussche, M. D. Woiski, C. J. Bax, J. M. J. Cornette, J. J. Duvekot, B. W. A. Nij Bijvank, J. van Eyck, M. T. M. Franssen, K. M. Sollie, J. A. M. van der Post, P. M. M. Bossuyt, B. C. Opmeer, M. Kok, B. W. J. Mol and G.-J. van Baaren, *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2016, **206**, 220–224.
232 P. Corabian, *The Actim™ Partus versus the TLiIQ® System as rapid response tests to aid in diagnosing preterm labour in sympotmatic women*, Edmonton, AB, Canada, 2008.
233 D. S. Abbott, S. K. Radford, P. T. Seed, R. M. Tribe and A. H. Shennan, *Am. J. Obstet. Gynecol.*, 2013, **208**, 122.
234 M. Bruijn, J. Vis, F. Wilms, M. Oudijk, A. Kwee, M. Porath, G. Oei, H. Scheepers, M. Spaanderman, K. Bloemenkamp, M. Haak, A. Bolte, F. Vandenbussche, M. Woiski, C. Bax, J. Cornette, J. Duvekot, B. Nij Bijvanck, J. van Eyck, M. Franssen, K. Sollie, J. van der Post, P. Bossuyt, B. Opmeer, M. Kok, B. Mol and G.-J. van Baaren, *BJOG*, 2016, **123**, 1965–1971.

235 J. K. Nien, B. H. Yoon, J. Espinoza, J. P. Kusanovic, O. Erez, E. Soto, K. Richani, R. Gomez, S. Hassan, M. Mazor, S. Edwin, R. Bahado-Singh and R. Romero, *Am. J. Obstet. Gynecol.*, 2006, **195**, 1025–1030.
236 M. Klouche and U. Schröder, *Clin. Chem. Lab. Med.*, 2008, **46**, 888–908.
237 S. Aellen, Y.-A. Que, B. Guignard, M. Haenni and P. Moreillon, *Antimicrob. Agents Chemother.*, 2006, **50**, 1913–1920.
238 M. Singer, C. S. Deutschman, C. W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G. R. Bernard, J.-D. Chiche, C. M. Coopersmith, R. S. Hotchkiss, M. M. Levy, J. C. Marshall, G. S. Martin, S. M. Opal, G. D. Rubenfeld, T. van der Poll, J. Vincent and D. C. Angus, *JAMA*, 2016, **315**, 801.
239 M. Funes-Huacca, A. Wu, E. Szepesvari, P. Rajendran, N. Kwan-Wong, A. Razgulin, Y. Shen, J. Kagira, R. Campbell and R. Derda, *Lab Chip*, 2012, **12**, 4269.
240 S. Ayyash, Wen-I Wu and P. Ravi Selvaganapathy, presented in part at *2014 IEEE Healthcare Innovation Conference (HIC)*, 2014.
241 *Smarticles Technology*, https://www.rochemicrobiologytests.com/healthcare-associated-infections/innovative-solutions.html, (accessed June 2016).
242 F. Deiss, M. E. Funes-Huacca, J. Bal, K. F. Tjhung and R. Derda, *Lab Chip*, 2014, **14**, 167–171.
243 B. Houwen, *Lab. Hematol.*, 2001, **7**, 89–100.
244 R. J. Laudicina and Y. Simonian, in *Clinical Laboratory Hematology*, ed. S. B. McKenzie and J. L. Williams, Pearson, Boston, 2nd edn, 2010, pp. 104–145.
245 H. Russcher, N. Van Deursen, T. Ermens and R. De Jonge, *Ned. Tijdschr. Klin. Chem. Laboratoriumgeneeskd.*, 2013, **38**, 140–141.
246 C. E. Majors, M. E. Pawlowski, T. Tkaczyk and R. R. Richards-Kortum, presented in part at *2014 IEEE Healthcare Innovation Conference (HIC)*, Seattle, WA, 2014.
247 A. Forcucci, M. E. Pawlowski, C. Majors, R. Richards-Kortum and T. S. Tkaczyk, *Biomed. Opt. Express*, 2015, **6**, 4433.
248 Z. J. Smith, T. Gao, K. Chu, S. M. Lane, D. L. Matthews, D. M. Dwyre, J. Hood, K. Tatsukawa, L. Heifetz and S. Wachsmann-Hogiu, *Lab Chip*, 2014, **14**, 3029–3036.
249 H. Zhu, I. Sencan, J. Wong, S. Dimitrov, D. Tseng, K. Nagashima and A. Ozcan, *Lab Chip*, 2013, **13**, 1282.
250 K. Lunze, D. E. Bloom, D. T. Jamison and D. H. Hamer, *BMC Med.*, 2013, **11**, 24.
251 N. Kennedy, L. Gondwe and D. C. Morley, *Lancet*, 2000, **355**, 1364.
252 D. A. Green, *Arch. Dis. Child. Fetal Neonatal Ed.*, 2006, **91**, F96–F98.
253 R. K. Pejaver, R. Nisarga and B. Gowda, *Indian J. Pediatr.*, 2004, **71**, 795–796.
254 T. B. Mole, N. Kennedy, N. Ndoya and A. Emond, *PLoS One*, 2012, **7**, e45823.
255 K. Stoner and J. Frisone, *Use of a Skin Patch Device to Obtain Temperature Measurements (Adult Study)*, 2015.
256 V. Tanigasalam, V. Bhat and B. Adhisivam, *Novel method of detecting hypothermia in low birth weight neonates in resource limited settings using wristband (Bempu Bracelet) device, a descriptive study*, Pondicherry, 2016.
257 C. Greco, G. Arnolda, N.-Y. Boo, I. F. Iskander, A. A. Okolo, R. Rohsiswatmo, S. M. Shapiro, J. Watchko, R. P. Wennberg, C. Tiribelli and C. D. Coda Zabetta, *Neonatology*, 2016, **110**, 172–180.
258 World Health Organization, *Bilirubinometer*, 2011.
259 A. Rubio, C. Epiard, M. Gebus, M. Deiber, S. Samperiz, C. Genty, A. Ego and T. Debillon, *Neonatology*, 2017, **111**, 1–7.
260 S. Rylance, J. Yan and E. Molyneux, *Paediatr. Int. Child Health*, 2014, **34**, 101–107.
261 B. O. Olusanya, D. O. Imosemi and A. A. Emokpae, *Pediatrics*, 2016, **138**, e20160907.
262 L. Murli, A. Thukral, M. J. Sankar, S. Vishnubhatla, A. K. Deorari, V. K. Paul, A. Sakariah, Dolma and R. Agarwal, *J. Perinatol.*, 2017, **37**, 182–187.
263 M. Mansouri, A. Mahmoodnejad, R. T. Sarvestani and F. Gharibi, *Int. J. Pediatr.*, 2015, **3**, 633–641.
264 B. O. Olusanya, T. A. Ogunlesi, P. Kumar, N.-Y. Boo, I. F. Iskander, M. F. B. de Almeida, Y. E. Vaucher and T. M. Slusher, *BMC Pediatr.*, 2015, **15**, 39.
265 A. Carceller-Blanchard, J. Cousineau and E. E. Delvin, *Clin. Biochem.*, 2009, **42**, 143–149.
266 C. D. Coda Zabetta, I. F. Iskander, C. Greco, C. Bellarosa, S. Demarini, C. Tiribelli and R. P. Wennberg, *Neonatology*, 2013, **103**, 177–181.
267 C. Greco, I. F. Iskander, D. M. Akmal, S. Z. El Houchi, D. A. Khairy, G. Bedogni, R. P. Wennberg, C. Tiribelli and C. D. C. Zabetta, *J. Perinatol.*, 2017, DOI: 10.1038/jp.2017.94.
268 J. Su and L. Wang, *Transl. Pediatr.*, 2012, **1**, 108–115.
269 C. B. Sweet, S. Grayson and M. Polak, *J. Pediatr. Pharmacol. Ther.*, 2013, **18**, 199–208.
270 R. M. Bergenstal, *Insulin*, 2008, **3**, 5–14.
271 C. Tack, H. Pohlmeier, T. Behnke, V. Schmid, M. Grenningloh, T. Forst and A. Pfützner, *Diabetes Technol. Ther.*, 2012, **14**, 330–337.
272 D. C. Klonoff, *Diabetes Spectr.*, 2014, **27**, 174–179.
273 K. Beardsall, *Early Hum. Dev.*, 2010, **86**, 263–267.
274 S. Vanavanan, P. Santanirand, U. Chaichanajarernkul, A. Chittamma, J. A. DuBois, T. Shirey and M. Heinz, *Clin. Biochem.*, 2010, **43**, 186–192.
275 M. G. Lockyer, K. Fu, R. M. Edwards, L. Collymore, J. Thomas, T. Hill and S. Devaraj, *Clin. Biochem.*, 2014, **47**, 840–843.
276 T. Makaya, A. Memmott and P. Bustani, *J. Paediatr. Child Health*, 2012, **48**, 342–346.
277 M. Ranger, C. M. Y. Chau, A. Garg, T. S. Woodward, M. F. Beg, B. Bjornson, K. Poskitt, K. Fitzpatrick, A. R. Synnes, S. P. Miller and R. E. Grunau, *PLoS One*, 2013, **8**, e76702.
278 K. Beardsall, *Arch. Dis. Child. Fetal Neonatal Ed.*, 2005, **90**, F307–F310.
279 V. Lodwig, B. Kulzer, O. Schnell and L. Heinemann, *J. Diabetes Sci. Technol.*, 2014, **8**, 397–402.

280 D. DeSalvo and B. Buckingham, *Curr. Diab. Rep.*, 2013, **13**, 657–662.
281 D. Rodbard, *Diabetes Technol. Ther.*, 2016, **18**, S2-3–S2-13.
282 D. L. Harris, M. R. Battin, P. J. Weston and J. E. Harding, *J. Pediatr.*, 2010, **157**, 198–202.e1.
283 H. C. Woo, L. Tolosa, D. El-Metwally and R. M. Viscardi, *Arch. Dis. Child. Fetal Neonatal Ed.*, 2014, **99**, F153–F157.
284 C. D. Chin, V. Linder and S. K. Sia, *Lab Chip*, 2012, **12**, 2118.
285 C. Papadea, J. Foster, S. Grant, S. A. Ballard, J. C. Cate, W. M. Southgate and D. M. Purohit, *Ann. Clin. Lab. Sci.*, 2002, **32**, 231–243.
286 R. Gorkin, J. Park, J. Siegrist, M. Amasia, B. S. Lee, J.-M. Park, J. Kim, H. Kim, M. Madou and Y.-K. Cho, *Lab Chip*, 2010, **10**, 1758.
287 J. H. Nichols, A. Rajadhyaksha and M. Rodriguez, *Point Care J. Near-Patient Test. Technol.*, 2008, **7**, 7–11.
288 A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3–10.
289 Z. Nie, C. A. Nijhuis, J. Gong, X. Chen, A. Kumachev, A. W. Martinez, M. Narovlyansky and G. M. Whitesides, *Lab Chip*, 2010, **10**, 477–483.
290 Z. Nie, F. Deiss, X. Liu, O. Akbulut and G. M. Whitesides, *Lab Chip*, 2010, **10**, 3163.
291 A. Nemiroski, D. C. Christodouleas, J. W. Hennek, A. A. Kumar, E. J. Maxwell, M. T. Fernandez-Abedul and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 11984–11989.
292 E. J. Maxwell, A. D. Mazzeo and G. M. Whitesides, *MRS Bull.*, 2013, **38**, 309–314.
293 L. Liu, H. L. Johnson, S. Cousens, J. Perin, S. Scott, J. E. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, C. Mathers and R. E. Black, *Lancet*, 2012, **379**, 2151–2161.
294 *Newborn death and illness*, http://www.who.int/pmnch/media/press_materials/fs/fs_newborndealth_illness/en/, (accessed March 2017).
295 B. Chikuse, E. Chirwa, A. Maluwa, A. Malata and J. Odland, *Open J. Nurs.*, 2012, **2**, 351–357.
296 O. Golubnitschaja, K. Yeghiazaryan, M. Cebioglu, M. Morelli and M. Herrera-Marschitz, *EPMA J.*, 2011, **2**, 197–210.
297 G. Bogdanovic, A. Babovic, M. Rizvanovic, D. Ljuca, G. Grgic and J. DjuranovicMilicic, *Med. Arch.*, 2014, **68**, 102.
298 E. Pirnareva and M. Tsankova, *Ultrasound Obstet. Gynecol.*, 2016, **48**, 322.
299 P. Chaturvedi and N. Shah, *Indian J. Pediatr.*, 1991, **58**, 63–67.
300 *MOYO- Improved Fetal Heart Rate Monitoring for Safer Births*, https://savinglivesatbirth.net/summaries/294, (accessed March 2017).
301 *Moyo Fetal Heart Rate Monitor*, http://www.laerdalglobalhealth.com/doc/2555/Moyo-Fetal-Heart-Rate-Monitor, (accessed March 2017).
302 Jhpiego, *Addressing Labor Complications: A Low-Cost Solution for a Persistent Global Health Challenge|Childbirth*, Baltimore, 2011.
303 L. C. Gilstrap, K. J. Leveno, J. Burris, M. L. Williams and B. B. Little, *Am. J. Obstet. Gynecol.*, 1989, **161**, 825–830.
304 G. L. Malin, R. K. Morris and K. S. Khan, *BMJ*, 2010, **340**, c1471.
305 D. Ghosh, O. N. Bhakoo, A. Narang and K. Dhall, *J. Trop. Pediatr.*, 1997, **43**, 108–111.
306 S. N. Wall, A. C. Lee, S. Niermeyer, M. English, W. J. Keenan, W. Carlo, Z. A. Bhutta, A. Bang, I. Narayanan, I. Ariawan and J. E. Lawn, *Int. J. Gynecol. Obstet.*, 2009, **107**, S47–S64.
307 World Health Organization, *Guidelines on Basic Newborn Resuscitation*, 2012.
308 A. Leach, T. F. McArdle, W. A. Banya, O. Krubally, A. M. Greenwood, C. Rands, R. Adegbola, A. de Francisco and B. M. Greenwood, *Ann. Trop. Paediatr.*, 1999, **19**, 33–43.
309 L. J. Stoot, N. A. Cairns, F. Cull, J. J. Taylor, J. D. Jeffrey, F. Morin, J. W. Mandelman, T. D. Clark and S. J. Cooke, *Conserv. Physiol.*, 2014, **2**, cou011.
310 C. D. Chin, S. Y. Chin, T. Laksanasopin and S. K. Sia, in *Point-of-Care Diagnostics on a Chip*, ed. D. Issadore and R. M. Westervelt, Springer-Verlag, Berlin, Heidelberg, 2013, pp. 3–21.
311 J. Yang, T. J. Kwak, X. Zhang, R. McClain, W.-J. Chang and S. Gunasekaran, *ACS Sens.*, 2016, **1**, 1235–1243.
312 D. You, L. Hug, Y. Chen, H. Newby, T. Wardlaw, C. Mathers, T. Boerma, D. Hogan, J. Ho, E. Suzuki, F. Pelletier, K. Andreev, P. Gerland, D. Gu, N. Li, C. Sawyer, G. Bay, T. Miller, D. J. Faijer and K. Hill, *Levels & Trends in Child Mortality: Report 2014*, 2014.
313 M. Meem, J. K. Modak, R. Mortuza, M. Morshed, M. S. Islam and S. K. Saha, *J. Glob. Health*, 2011, **1**, 201–209.
314 I. Bersani, C. Auriti, M. P. Ronchetti, G. Prencipe, D. Gazzolo and A. Dotta, *BioMed Res. Int.*, 2015, **2015**, 1–10.
315 E. Kocabaş, A. Sarikçioğlu, N. Aksaray, G. Seydaoğlu, Y. Seyhun and A. Yaman, *Turk. J. Pediatr.*, 2007, **49**, 7–20.
316 J. Liu, F. Liu, Y. Liu, H.-W. Wang and Z.-C. Feng, *Chest*, 2014, **146**, 383–388.
317 T. Duke, *Arch. Dis. Child. Fetal Neonatal Ed.*, 2005, **90**, F211–F219.
318 E. A. F. Simoes, T. Cherian, J. Chow, S. A. Shahid-Salles, R. Laxminarayan and T. J. John, *Acute Respiratory Infections in Children*, 2006.
319 S. C. Redd, R. Vreuls, M. Metsing, P. H. Mohobane, E. Patrick and M. Moteetee, *Bull. World Health Organ.*, 1994, **72**, 113–118.
320 S. Singhi, A. K. Bhalla, A. Bhandari and A. Narang, *Ann. Trop. Paediatr.*, 2003, **23**, 135–138.
321 A. C. Noordam, Y. Barbera Lainez, S. Sadruddin, P. M. van Heck, A. O. Chono, G. L. Acaye, V. Lara, A. Nanyonjo, C. Ocan and K. Kallander, *Health Policy Plan.*, 2015, **30**, 696–704.
322 D. King, *BMJ*, 2014, **349**, g6639.
323 World Health Organization, *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach*, 2013.

324 UNAIDS, *On the fast track to an AIDS-free generation*, 2016.
325 G. B. Woelk, M. P. Kieffer, D. Walker, D. Mpofu and R. Machekano, *Trials*, 2016, **17**, 88.
326 B. Meggi, T. Bollinger, N. Mabunda, A. Vubil, O. Tobaiwa, J. I. Quevedo, O. Loquiha, L. Vojnov, T. F. Peter and I. V. Jani, *PLoS One*, 2017, **12**, e0169497.
327 I. V. Jani, B. Meggi, N. Mabunda, A. Vubil, N. E. Sitoe, O. Tobaiwa, J. I. Quevedo, J. D. Lehe, O. Loquiha, L. Vojnov and T. F. Peter, *JAIDS, J. Acquired Immune Defic. Syndr.*, 2014, **67**, e1–e4.
328 World Health Organization, *WHO Prequalification of In Vitro Diagnostics PUBLIC REPORT*, 2016.
329 J. Lustig and J. Cunningham, *Alere q HIV-1/2 Detect Point-of-Care Molecular HIV Assay Receives WHO Prequalification*, 2016.
330 P. Craw and W. Balachandran, *Lab Chip*, 2012, **12**, 2469.
331 B. A. Rohrman and R. R. Richards-Kortum, *Lab Chip*, 2012, **12**, 3082–3088.
332 B. A. Rohrman, V. Leautaud, E. Molyneux and R. R. Richards-Kortum, *PLoS One*, 2012, **7**, e45611.
333 N. M. Rodriguez, J. C. Linnes, A. Fan, C. K. Ellenson, N. R. Pollock and C. M. Klapperich, *Anal. Chem.*, 2015, **87**, 7872–7879.
334 J. R. Choi, J. Hu, R. Tang, Y. Gong, S. Feng, H. Ren, T. Wen, X. Li, W. A. B. Wan Abas, B. Pingguan-Murphy and F. Xu, *Lab Chip*, 2016, **16**, 611–621.
335 L. Lillis, D. Lehman, M. C. Singhal, J. Cantera, J. Singleton, P. Labarre, A. Toyama, O. Piepenburg, M. Parker, R. Wood, J. Overbaugh and D. S. Boyle, *PLoS One*, 2014, **9**, e108189.
336 Z. A. Crannell, B. Rohrman and R. Richards-Kortum, *PLoS One*, 2014, **9**, e112146.
337 Z. A. Crannell, B. Rohrman and R. Richards-Kortum, *Anal. Chem.*, 2014, **86**, 5615–5619.
338 N. M. Rodriguez, W. S. Wong, L. Liu, R. Dewar and C. M. Klapperich, *Lab Chip*, 2016, **16**, 753–763.
339 L. K. Lafleur, J. D. Bishop, E. K. Heiniger, R. P. Gallagher, M. D. Wheeler, P. Kauffman, X. Zhang, E. C. Kline, J. R. Buser, S. Kumar, S. A. Byrnes, N. M. J. Vermeulen, N. K. Scarr, Y. Belousov, W. Mahoney, B. J. Toley, P. D. Ladd, B. R. Lutz and P. Yager, *Lab Chip*, 2016, **16**, 3777–3787.
340 M. Mauk, J. Song, H. H. Bau, R. Gross, F. D. Bushman, R. G. Collman and C. Liu, *Lab Chip*, 2017, **17**, 382–394.
341 N. S. Wijesooriya, R. W. Rochat, M. L. Kamb, P. Turlapati, M. Temmerman, N. Broutet and L. M. Newman, *Lancet Glob. Heal.*, 2016, **4**, e525–e533.
342 S. R. Arnold and E. L. Ford-Jones, *Paediatr. Child Health*, 2000, **5**, 463–469.
343 L. M. Causer, J. M. Kaldor, C. K. Fairley, B. Donovan, T. Karapanagiotidis, D. E. Leslie, P. W. Robertson, A. M. McNulty, D. Anderson, H. Wand, D. P. Conway, I. Denham, C. Ryan and R. J. Guy, *PLoS One*, 2014, **9**, e91504.
344 Y. Jafari, R. W. Peeling, S. Shivkumar, C. Claessens, L. Joseph and N. P. Pai, *PLoS One*, 2013, **8**, e54695.
345 N. S. Kay, R. W. Peeling and D. C. Mabey, *Expert Rev. Anti-Infect. Ther.*, 2014, **12**, 63–73.
346 L. Kleutsch, S. A. Harvey and W. Rennie, *Rapid syphilis tests in Tanzania: A long road to adoption, Case Study.*, Bethesda, MD, 2009.
347 T. Laksanasopin, T. W. Guo, S. Nayak, A. A. Sridhara, S. Xie, O. O. Olowookere, P. Cadinu, F. Meng, N. H. Chee, J. Kim, C. D. Chin, E. Munyazesa, P. Mugwaneza, A. J. Rai, V. Mugisha, A. R. Castro, D. Steinmiller, V. Linder, J. E. Justman, S. Nsanzimana and S. K. Sia, *Sci. Transl. Med.*, 2015, **7**, 273re1.
348 M. G. Morshed and A. E. Singh, *Clin. Vaccine Immunol.*, 2015, **22**, 137–147.
349 *CDC, Congenital Syphilis*, https://www.cdc.gov/std/tg2015/congenital.htm, (accessed July 2017).
350 A. G. Koek, S. M. Bruisten, M. Dierdorp, A. P. van Dam and K. Templeton, *Clin. Microbiol. Infect.*, 2006, **12**, 1233–1236.
351 J. Mlakar, M. Korva, N. Tul, M. Popović, M. Poljšak-Prijatelj, J. Mraz, M. Kolenc, K. Resman Rus, T. Vesnaver Vipotnik, V. Fabjan Vodušek, A. Vizjak, J. Pižem, M. Petrovec and T. Avšič Županc, *N. Engl. J. Med.*, 2016, **374**, 951–958.
352 S. A. Rasmussen, D. J. Jamieson, M. A. Honein and L. R. Petersen, *N. Engl. J. Med.*, 2016, **374**, 1981–1987.
353 J. J. Waggoner and A. Pinsky, *J. Clin. Microbiol.*, 2016, **54**, 860–867.
354 K. Chan, S. C. Weaver, P.-Y. Wong, S. Lie, E. Wang, M. Guerbois, S. P. Vayugundla and S. Wong, *Sci. Rep.*, 2016, **6**, 38223.
355 J. Song, M. G. Mauk, B. A. Hackett, S. Cherry, H. H. Bau and C. Liu, *Anal. Chem.*, 2016, **88**, 7289–7294.
356 K. Pardee, A. A. Green, M. K. Takahashi, D. Braff, G. Lambert, J. W. Lee, T. Ferrante, D. Ma, N. Donghia, M. Fan, N. M. Daringer, I. Bosch, D. M. Dudley, D. H. O'Connor, L. Gehrke and J. J. Collins, *Cell*, 2016, **165**, 1255–1266.
357 K. Pardee, A. A. Green, T. Ferrante, D. E. Cameron, A. DaleyKeyser, P. Yin and J. J. Collins, *Cell*, 2014, **159**, 940–954.
358 Y. J. Heng, S. Liong, M. Permezel, G. E. Rice, M. K. W. Di Quinzio and H. M. Georgiou, *Front. Physiol.*, 2015, **6**, 151.
359 L. M. Dusse, D. R. A. Rios, M. B. Pinheiro, A. J. Cooper and B. A. Lwaleed, *Clin. Chim. Acta*, 2011, **412**, 17–21.
360 R. Kazmi, A. Cooper and B. Lwaleed, *Semin. Thromb. Hemostasis*, 2011, **37**, 131–136.
361 L. C. Kenny, D. I. Broadhurst, W. Dunn, M. Brown, R. A. North, L. McCowan, C. Roberts, G. J. S. Cooper, D. B. Kell and P. N. Baker, *Hypertension*, 2010, **56**, 741–749.
362 R. T. Blankley, C. Fisher, M. Westwood, R. North, P. N. Baker, M. J. Walker, A. Williamson, A. D. Whetton, W. Lin, L. McCowan, C. T. Roberts, G. J. S. Cooper, R. D. Unwin and J. E. Myers, *Mol. Cell. Proteomics*, 2013, **12**, 3148–3159.
363 L. Dawonauth, L. Rademacher, A. D. L'Omelette, S. Jankee, M. Y. Lee Kwai Yan, R. B. Jeeawoody and T. W. Rademacher, *J. Reprod. Immunol.*, 2014, **101–102**, 148–152.
364 P. J. Williams, K. Gumaa, M. Scioscia, C. W. Redman and T. W. Rademacher, *Hypertension*, 2007, **49**, 84–89.
365 A. Marangoni, C. Foschi, M. G. Capretti, P. Nardini, M. Compri, L. T. Corvaglia, G. Faldella and R. Cevenini, *Clin. Vaccine Immunol.*, 2016, **23**, 410–416.

366 Y. Lubell, S. D. Blacksell, S. Dunachie, A. Tanganuchitcharnchai, T. Althaus, W. Watthanaworawit, D. H. Paris, M. Mayxay, T. J. Peto, A. M. Dondorp, N. J. White, N. P. J. Day, F. Nosten, P. N. Newton and P. Turner, *BMC Infect. Dis.*, 2015, **15**, 511.
367 M. Mussap, E. Puxeddu, M. Puddu, G. Ottonello, F. Coghe, P. Comite, F. Cibecchini and V. Fanos, *Clin. Chim. Acta*, 2015, **451**, 65–70.
368 C. Castillo-Laborde, *Hum. Resour. Health*, 2011, **9**, 4.
369 M. Plebani, *Clin. Biochem. Rev.*, 2012, **33**, 85–88.
370 Z. Crannell, A. Castellanos-Gonzalez, G. Nair, R. Mejia, A. C. White and R. Richards-Kortum, *Anal. Chem.*, 2016, **88**, 1610–1616.
371 A. Zumla, J. A. Al-Tawfiq, V. I. Enne, M. Kidd, C. Drosten, J. Breuer, M. A. Muller, D. Hui, M. Maeurer, M. Bates, P. Mwaba, R. Al-Hakeem, G. Gray, P. Gautret, A. A. Al-Rabeeah, Z. A. Memish and V. Gant, *Lancet*, 2014, **14**, 1123–1135.
372 C. Dincer, R. Bruch, A. Kling, P. S. Dittrich and G. A. Urban, *Trends Biotechnol.*, 2017, **35**, 728–742.
373 T. F. Scherr, S. Gupta, D. W. Wright and F. R. Haselton, *Lab Chip*, 2017, **17**(7), 1314–1322.
374 *WHO | Fact Sheet: World Malaria Day 2016*, http://www.who.int/malaria/media/world-malaria-day-2016/en/, (accessed March 2017).
375 World Health Organization, *Interagency list of medical devices for essential interventions for reproductive, maternal, newborn and child health*, 2015.
376 CDC, *Alere Determine™ HIV-1/2 Ag/Ab Combo Information Sheet for Testing Programs*, 2016.
377 M. Hoenigl, J. Graff-Zivin and S. J. Little, *Clin. Infect. Dis.*, 2016, **62**, 501–511.
378 S. Moyo, T. Mohammed, K. E. Wirth, M. Prague, K. Bennett, M. P. Holme, L. Mupfumi, P. Sebogodi, N. O. Moraka, C. Boleo, C. N. Maphorisa, B. Seraise, S. Gaseitsiwe, R. M. Musonda, E. van Widenfelt, K. M. Powis, T. Gaolathe, E. J. Tchetgen Tchetgen, J. M. Makhema, M. Essex, S. Lockman and V. Novitsky, *J. Clin. Microbiol.*, 2016, **54**, 3050–3055.
379 *Cepheid | GeneXpert IV*, http://www.cepheid.com/en/cepheid-solutions-uk/systems/genexpert-systems/genexpert-iv, (accessed April 2017).
380 *SD Bioline Malaria Ag RDT Series*, http://www.standardia.com/en/home/product/Rapid_Diagnostic_Test/Malaria_Ag_Pf-HRP2.html, (accessed February 2017).
381 *A guide for the selection of malaria RDTs to be purchased with 3DF grants*, http://www.threediseasesfund.org/images/stories/pdf/procurement/SOPs_and_Guidelines/Malaria-RDTs-Guideline-3DF-v1.0.pdf, (accessed March 2017).
382 *OneTouch Ultra®2 Meter*, http://www.shoponetouch.com/product/35580, (accessed March 2017).
383 F. Sanchis-Gomar, J. Cortell-Ballester, H. Pareja-Galeano, G. Banfi and G. Lippi, *J. Lab. Autom.*, 2013, **18**, 198–205.
384 *CLIA waived: HemoCue*, http://www.cliawaived.com/web/HemoCue.htm, (accessed March 2017).
385 *Hemoglobin test – HemoCue® Hb 201+ System*, http://www.hemocue.us/en-us/solutions/hematology/hemocue-hb-201plus-system, (accessed March 2017).
386 A. Boopathi, S. R. Nayak, A. Rao and B. Rao, *Open J. Obstet. Gynecol.*, 2014, **4**, 666–671.
387 Alere, *The Alere Triage System*, Jouy en Josas, France, 2013.
388 A. Lee, S. Mirrett, L. B. Reller and M. P. Weinstein, *J. Clin. Microbiol.*, 2007, **45**, 3546–3548.
389 P. E. Marik, *Crit. Care*, 2014, **18**, 529.
390 J. D. Schuur and M. Lin, *Blood Culture Testing: Send Samples Selectively to Lower Costs, Medico-Legal Risk*, http://www.acepnow.com/article/blood-culture-testing-send-samples-selectively-lower-costs-medico-legal-risk/, (accessed March 2017).
391 M. J. Ali, A. Ayyar, S. R. Motukupally, S. Sharma and M. N. Naik, *J. Ophthalmic Inflammation Infect.*, 2014, **4**, 27.
392 *AmniSure ROM Test Overview*, http://www.amnisure.com/care/test, (accessed March 2017).
393 F. Raimondi, S. Lama, F. Landolfo, M. Sellitto, A. C. Borrelli, R. Maffucci, P. Milite and L. Capasso, *BMC Pediatr.*, 2012, **12**, 625.
394 A. Robertson, S. Kazmierczak and P. Vos, *J. Perinatol.*, 2002, **22**, 12–14.
395 *Philips BiliChek System*, http://www.usa.philips.com/healthcare/product/HC989805644871/bilichek-bilirubinometer, (accessed April 2017).
396 *Nova Biomedical: StatStrip*, http://www.novabio.us/statstrip-glu/, (accessed April 2017).
397 *Moyo-Fetal Heart Rate Monitor*, http://designawards.core77.com/Commercial-Equipment/49815/Moyo-Fetal-Heart-Rate-Monitor, (accessed March 2017).
398 Elizabeth Glaser Pediatric AIDS Foundation and UNITAID, *Point-of-Care Early Infant Diagnosis*, 2016.
399 World Health Organization, *Diagnostics Evaluation Series No. 1- Laboratory-based evaluation of rapid syphilis diagnostics*, 2003.
400 *SD BIOLINE Syphilis 3.0*, http://www.standardia.com/en/home/product/Rapid_Diagnostic_Test/SyphilisTest.html, (accessed March 2017).
401 R. Wilson, *Chem. Soc. Rev.*, 2008, **37**, 2028.
402 S.-Y. Rhee, M. R. Jordan, E. Raizes, A. Chua, N. Parkin, R. Kantor, G. U. Van Zyl, I. Mukui, M. C. Hosseinipour, L. M. Frenkel, N. Ndembi, R. L. Hamers, T. F. Rinke de Wit, C. L. Wallis, R. K. Gupta, J. Fokam, C. Zeh, J. M. Schapiro, S. Carmona, D. Katzenstein, M. Tang, A. F. Aghokeng, T. De Oliveira, A. M. J. Wensing, J. E. Gallant, M. A. Wainberg, D. D. Richman, J. E. Fitzgibbon, M. Schito, S. Bertagnolio, C. Yang and R. W. Shafer, *PLoS One*, 2015, **10**, e0145772.
403 V. Johnston, K. L. Fielding, S. Charalambous, G. Churchyard, A. Phillips and A. D. Grant, *J. Acquired Immune Defic. Syndr.*, 2012, **61**, 370–380.