EXPERT REVIEWS

Point-of-care diagnostics, a major opportunity for change in traditional diagnostic approaches: potential and limitations

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'Point-of-care' (POC) diagnostics are a powerful emerging healthcare approach. They can rapidly provide statistically significant results, are simple to use, do not require specialized equipment and are cost-effective. For these reasons, they have the potential to play a major role in revolutionizing the diagnosis, initiation and monitoring of treatment of major global diseases. This review focuses on antibody-based POC devices that target four major global diseases: cardiovascular diseases, prostate cancer, HIV infection and tuberculosis. The key statistics and pathology of each disease is described in detail, followed by an in-depth discussion on emerging POC devices that target each disease, highlighting their potential and limitations.

KEYWORDS: acute myocardial infarction • antibody • biosensor • cardiovascular disease • HIV • mHealth • POC • prostate cancer • TB

Point-of-care diagnostic devices

'Point-of-care' (POC) and 'Near-Patient' devices are terms that describe diagnostic devices that can be used at the immediate healthcare location, be it at the patient's bedside, in an emergency department or in the field. Over recent years, research has focused on the development of POC tests (POCTs), as it is generally agreed that the ideal POCT can offer significant advantages over traditional central laboratory-based tests.

WHO developed a set of guidelines for ideal diagnostic tests under the acronym ASSURED, that is, affordable, sensitive, specific, user-friendly, rapid, equipment-free, delivered to those who need it [1]. POCTs are ideally relatively inexpensive and cheap to manufacture [2], can be operated by users with minimal laboratory or medical training and require minimal handling steps, thus, reducing operational errors [2–4]. They rapidly provide analytical results, thus, decreasing patient turnaround time (TAT). This can shorten the interval between patient-admission and therapeutic response and treatment, thereby improving patient convenience and therapeutic outcomes [2–4]. Testing can be performed at the point of need and, hence, may obviate the need for certain central laboratory tests, minimizing problems that may be encountered with sample transport and identification [2,4]. They require only small whole-blood or saliva samples and employ ready-to-use reagents [2]. They can preferably use non-pretreated blood samples, which removes/reduces the need for specialized sample preparation, although this may not be possible with all targets [2,3]. They can be a prerequisite for early detection of life-threatening conditions and for effective and rapid directing of patients through the most appropriate treatment path [2]. Miniaturization reduces reagent consumption, enhances device portability and allows for total analysis to be performed, that is, can process all sample handling steps and measurements within the device. Ideally, this should lead to the lowering of the costs of the tests [3]. Such test platforms

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Box 1. Characteristics of an ideal 'point-of-care' test.

ASSURED

- A: Affordable – Cheap to manufacture, with inexpensive reagents and are disposable.
- SS: Sensitive and specific – Provide statistically significant results with high levels of sensitivity and specificity.
- U: User-friendly – 'Ease-of-use' with minimal manual handling/addition steps.
- R: Rapid – Shorten turnaround time; improve therapeutic outcomes and patient convenience.
- E: Equipment-free – Can use non-pretreated samples and can be performed at the point of need. Thus, need for central laboratory testing may be removed.
- D: Delivered to those who need it – Can be used in 'resource-limited' settings.

can be used in 'resource-limited' settings with minimally trained personnel, insufficient good quality water, high temperatures and humidity and unreliable electricity supplies [5]. These characteristics are summarized in detail in Box 1.

Biosensors are key devices employed in many of the commonest forms of POCT, combining a biological recognition element (such as an antibody or lectin) with a transduction element (a physical or chemical signaling device that relays the biological signal) [6,7]. The biological recognition element is responsible for specific interaction with its corresponding target ligand. The biomolecular interactions (e.g., between an immobilized antibody and its free target antigen) are converted into digital signals, which are translated into a readout that can be interpreted by the user [7,8]. The properties of the transduction element and the quality of the biological recognition element are critical for the sensitivity and specificity of these devices [8]. There are numerous biosensor formats, some of which are described in the section 'Common biosensor formats'.

Common biosensor formats

Piezoelectric biosensors

These detect changes in mass that occur as a result of a biomolecular interaction between two molecules, such as between an antigen and an antibody. Some piezoelectric sensors utilize a piezoelectric quartz crystal, which emits a mass-dependent resonant frequency. Hence, adsorbate recognition leads to a change in resonant frequency, which can be detected by computer software [3,7].

Optical biosensors

The detection method of many optical biosensors relies on surface plasmon resonance (SPR). SPR is a phenomenon that occurs when visible or near-infrared light is shone onto a metallic (e.g., gold) surface via a hemispherical prism at an angle related to the refractive index (RI). At the critical angle, photons from the light source penetrate through the metallic film and the resultant oscillation of free electrons leads to the generation of surface plasmons that resonate and absorb light. The specific wavelength at which this occurs is related to the RI near the gold surface and the mass on the metallic surface. Changes in mass (e.g., as a result of a biomolecular interaction between a surface-immobilized ligand and a free analyte) causes the resonance wavelength to shift and introduces a change in the RI. This RI change affects the angle at which light is reflected off the surface. Angle changes are picked up by a detector and computer software relates these angle changes to their associated mass changes [8,9]. While SPR-based optical biosensors are among the most common optical biosensors, others require interferometry, optical waveguides and optical ring resonator-based biosensors. The reader is referred to two excellent reviews in which these are described in detail [10,11].

Impedimetric biosensors

The basis for impedimetric biosensors is the impedance of flow of electrons in an AC circuit due to redox reactions and molecular interactions at the surface of the electrode [12]. Using this principle, molecular interactions, for example, between an antibody and its antigen, can be characterized. These platforms can also facilitate detection and quantification of microorganisms via metabolic redox reactions. For example, an increase in the metabolism of viable microbial biomass leads to an increase in conductance and capacitance and a decrease in impedance [8].

Conductometric biosensors

In such platforms, the transduction element is a conductive polymer, such as polyaniline, polypyrrole or polyacetylene [8]. These biosensors have numerous important advantages over other electrochemical transducers, for example, the reference electrode is relatively technologically simple, they are light-insensitive, amenable to miniaturization and standard inexpensive thin-film technology can be applied in their production [13].

Lateral flow immunoassay

Lateral flow immunoassays (LFIAs) are prefabricated strips of a carrier material (often nitrocellulose, but also nylon, polyethylene and fused silica) containing dried reagents that are rehydrated and activated by the addition of a test fluid sample. In many LFIAs, nanoparticle-labeled antibodies (or fluorescent probe-labeled antibodies in fluorescent immunoassays) specific to the target are dried in the conjugate pad. In the test zone, a second target-specific antibody is immobilized. In the control zone, an anti-species antibody is immobilized. A sample is added via the sample pad and subsequently migrates into the conjugate pad. When the target analyte is present in a sample, it will be bound first by the nanoparticle-labeled antibody in the conjugate pad. Capillary forces will draw the fluid to the test zone where the target will be bound by the second target-specific antibody and a colored signal will form on the test line. The fluid will then flow over the control zone, where the anti-species antibodies will bind the nanoparticle-labeled antibody, resulting in the formation of a colored signal on the control line. The absence of a signal at the test line indicates the

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absence of the target analyte (negative result), while the absence of a signal at the control line indicates a fault with the test (invalid result) (Figure 1). For further information on LFIA and other lateral flow assay formats, the reader is referred to the following reviews [14,15].

In this review article, four main diseases will be the focus, that is, cardiovascular disease (CVD), prostate cancer (PCa), HIV-derived diseases and tuberculosis (TB). These diseases were chosen as they represent some of the major causes of death in the developed and developing worlds. The disease statistics and pathology will be discussed followed by a discussion on emerging antibody-based POC diagnostic devices that target these diseases. The advantages and disadvantages of these devices will be outlined and it will be discussed how they can benefit patient diagnosis and treatment.

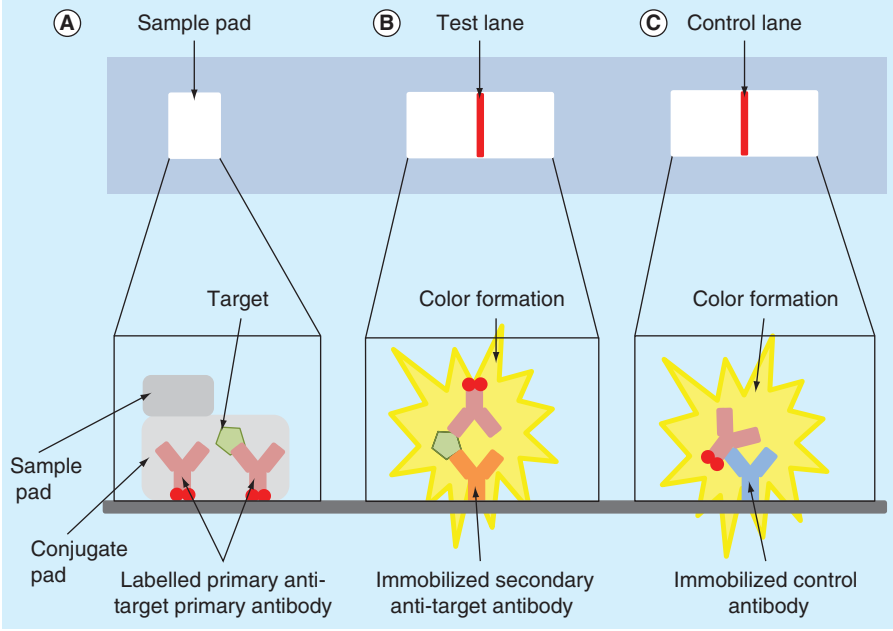

Figure 1. Illustration of lateral flow immunoassay. (A) Sample is added to sample pad and migrates into conjugate pad. The target is bound by labeled anti-target primary antibody (usually mouse, rabbit or goat IgG). Capillary forces carry the sample to the test lane and control lane. **(B)** An immobilized secondary anti-target antibody binds the target at another epitope and, thus, a color forms due to the immobilization of labeled primary antibody. **(C)** In the control lane, immobilized control antibodies (usually anti-mouse/rabbit/goat IgG) bind the labeled primary antibody, which leads to a color formation.

Cardiovascular disease

CVD statistics & pathology

Globally, CVDs have greater mortality rates than any other cause of death. It was estimated that, in 2010 in the USA, approximately 780,000 people died from CVDs, the leading cause of death that year [16,17]. CVDs account for almost half of all deaths in the western world and place an overall financial burden of approximately €192 billion per year in the EU [18]. Different forms of coronary heart disease include coronary atherosclerosis and acute myocardial infarction (AMI). Coronary atherosclerosis is the thickening of arteries due to the formation of atherosclerotic plaques [19]. The fissuring and disruption of these plaques can initiate intraluminal thrombosis, occluding the blood flow to the myocardium [19–21]. This in turn can lead to the death of cardiac myocytes, that is, AMI, and can reduce left-ventricular function, affecting the patient's quality of life and can cause premature mortality [21].

AMI biomarkers

The death of myocytes can lead to the release of specific cardiac biomarkers into the blood, that is, biological analytes that can be detected at elevated levels after the onset of myocardial damage or during the course of CVD. Ideally, they should be absent from non-myocardial tissue and present only in cardiac tissue [18]. Cardiac troponins I and T (cTnI and cTnT), cardiac myoglobin (cMb) and creatine kinase-myocardial bound (CK-MB) are cardiac biomarkers routinely used in clinical practice, while C-reactive protein (CRP), heart-type fatty acid binding protein (H-FABP), carbonic anhydrase III, N-terminal pro-brain natriuretic peptide, D-dimer and ischemia-modified albumin (IMA) are emerging biomarkers that show promise for AMI diagnosis [18,20,22–24]. Currently, in clinical settings, patients are diagnosed based on two of the three following conditions: characteristic chest pain, changes in diagnostic ECG and elevation of blood biochemical markers. However, as ECG has unsatisfactory diagnostic sensitivity (35–60%) [23,25] and the therapeutic window for AMI treatment is within 6 h of symptom onset, there is a demand for diagnostic tests that can quantify cardiac-specific biomarkers with a rapid TAT.

Troponins such as cTnI and cTnT reach peak levels relatively quickly (4–6 h) and remain in circulation for 24–48 h [3]. They are considered as the gold standard cardiac biomarkers. AMI can be diagnosed based on a falling or rising pattern of the cardiac troponin concentrations, with a minimum of one sample above the 99th percentile, with a level of imprecision of 10% [22,25]. However, the main issue regarding the use of cardiac troponins is the poor sensitivity of diagnosis during the early hours of symptom onset.

In a study by Stengaard *et al.*, a strong correlation was shown between a cTnT level above 50 ng/l (measured using POCT) and mortality. However, the sensitivity of detection of AMI patients at 60–120 and >120 min after symptom onset was 38% (95% CI: 25–52%) and 52% (95% CI: 40–65%), respectively, which is unacceptably low [26]. This is due to the low levels of cTn in circulation during the first few hours of AMI and, thus, at this early stage, cTn measurement is insufficient for AMI diagnosis [23]. cMb is released into circulation as early as 1–3 h after the onset of AMI symptoms and has high

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sensitivity [18,20], however, it is not an absolutely cardiac-specific biomarker [23]. H-FABP shows promise as an early indicator for AMI, as it can be detected in blood within 1 h of symptom onset, it reaches peak values at 3–6 h and it may be elevated in the absence of cTnI increase [22,27]. However, as a stand-alone biomarker, the sensitivity and specificity of H-FABP in early AMI symptom onset was found to be 84% (95% CI: 76–90%) and 84% (95% CI: 76–89%), respectively, which in the case of a high mortality disease such as AMI, a 16% false-negative test result is unacceptably high [28].

The multi-marker cardiac panel approach appears to have greater promise for accurate diagnosis of AMI. The combined measurement of troponin and H-FABP had a pooled sensitivity of 91% (95% CI: 88–92%), specificity of 82% (95% CI: 80–84%) and a pooled negative predictive value (NPV) of 0.95 (95% CI: 0.94–0.96) [27]. In another report, the combined use of H-FABP and cTnI had a sensitivity and specificity of 83.3 and 92.2%, respectively. However, this lack of concordance could also be an indicator of the non-standardized reference tests used for AMI [23].

As was already mentioned, IMA is an US FDA-approved biomarker that shows promise in the diagnosis and triage of acute coronary syndrome (ACS) patients, as it is cardiac ischemia-specific, rises within minutes of symptom onset, remains elevated for several hours and may be present in the absence of other AMI biomarkers [29]. In a meta-analysis, it was found that, in low-risk emergency department patients, a negative IMA coupled with non-diagnostic ECG and negative troponin (called a negative triple prediction test) had a sensitivity and NPV for ACS of 94.4 and 97.1%, respectively [24]. In another study, it was found that combined measurement of IMA and cTnI had a sensitivity, specificity, PPV and NPV of 96, 93, 93.33 and 96.26%, respectively [30]. Such testing could potentially rule out low-risk ACS patients in the early hours of symptom onset with high confidence, thus saving on treatment costs (it was estimated that approximately US$2900 is saved with every patient discharged on the basis of a negative IMA value [29]) and freeing up physicians to test and treat higher-risk patients. A multi-marker POCT for IMA and cTnI could be of great value in this situation. However, an issue with IMA testing using immunoassay-based detection is that the alteration of the albumin molecule is transient. This hampers the feasibility of an immunoassay-based detection method, as the IMA-specific epitope is not permanently available [31]. Thus, this issue will make it more difficult to develop an immunoassay-based multi-marker device for IMA and cTn. An alternative would be to incorporate the standard albumin cobalt binding assay with POC cTnI testing and ECG monitoring, but further work is clearly required in this area.

A study by Tomonaga *et al*. found that a triple marker POCT (Roche Cardiac Reader) that measured cTnT, N-terminal pro-brain natriuretic peptide and D-Dimer had a sensitivity, specificity and NPV of 90, 92 and 99%, respectively. However, this study could have been limited due to the low patient number enrolled [32]. Another study used the Triage Cardiac Panel POC device (Biosite) for the quantitation of cTnI, CK-MB and cMb and determined the sensitivity, specificity, NPV and PPV to be 98% (95% CI: 95.7–100.2%), 99.8% (95% CI: 99.6–99.9%), 99.9% (95% CI: 99.9–100.0%) and 92.4% (95% CI: 88.2–96.5%), respectively (Table 1). The measurement of cTnI alone had a comparatively lower sensitivity and PPV (68.2% [95% CI: 60.7–75.7%] and 89.4% [95% CI: 83.5–95.1%], respectively) [33]. However, an issue was previously noted about the Triage Cardiac Panel, which was the occurrence of false-negative results due to use of a certain lot, which was later recalled [34]. Also, other studies disagree that the incorporation of cMb and CK-MB measurement add significant value to the use of troponin measurement alone [25,35–37]. Thus, it is not evident as to what is the gold standard combination of cardiac markers and more work is required in this respect. However, the authors believe that with the discovery/approval of new biomarkers one could foresee new POCT platforms that can detect diagnostically valuable combinations of the relevant biomarkers. Importantly, POCT could provide statistically significant results with a rapid TAT and, thus, significantly improving AMI diagnosis and treatment.

POCTs for other acute diseases such as venous thromboembolism, pulmonary embolism, acute lower respiratory tract infections are available but are not addressed in this article. The reader is referred to the following references [38–43].

Prostate cancer

PCa statistics

Cancer is the main cause of death in developed countries and the second leading cause of death in economically developing countries [44]. In the USA, PCa is the most frequently diagnosed cancer in men after skin cancer [45] and is the second leading cause of mortality after lung cancer. In the USA alone, it is estimated that approximately 240,000 new PCa cases will be diagnosed and approximately 30,000 men will die of the disease per annum [46]. Prostatic adenocarcinoma is an aggressive malignant growth which often spreads from the prostate to other parts of the body, such as the lymph nodes and the bones, if not successfully treated at an early stage [47]. Sathiakumar *et al*. reported that PCa patients with bone metastasis but no skeletal-related events (SREs) and those with bone metastasis and SREs had 6.6-times (95% CI: 6.4–6.9) and 10.2-times (95% CI: 9.8–10.7) greater hazard risk, respectively, compared with PCa patients without bone metastasis [48]. Jayasekera *et al*. reported that the healthcare cost for patients with SREs was on average US$29,696 (95% CI: US $24,730–$34,662) higher than for those without SREs [49].

Prostate-specific antigen (PSA) has remained the main biomarker for the detection and diagnosis of PCa for decades [50]. It is accepted that the concentration of PSA in healthy individuals in blood is typically <4.0 ng/ml [6] but studies have shown that patients with a PSA level below the threshold can also have PCa. For this reason, there is considerable controversy as to the value of PSA as a PCa biomarker. A similar or greater predictive value was observed with a PSA concentration of

Table 1. Characteristics of some commercially available acute myocardial infarction point-of-care diagnostics.

Technology	Application	Sensitivity	Specificity	PPV (95% CI)	NPV (95% CI)	Cutoff	Advantages	Disadvantages	Ref.
i-STAT cTnI (Abbott) (POC)	Miniaturized ELISA format for cTnI quantification in whole-blood	100%	98.6%	86.7% (83.5–89.4%)	100% (97.8–100%)	cTnI = 0.1 ng/ml			[129]
		43% (34–52%)	85% (82–87%)	33% (26–41%)	89% (87–92%)	cTnI = 0.08 ng/ml		Suboptimal clinical performance	[130]
		–	–	–	–		TAT of less than 10 min. Ease-of-use. Does not require highly trained staff		[158]
Stratus CS (Siemens) (POC)	cTnI quantification in whole-blood	54% (44–64%)	78% (74–81%)	25% (19–31%)	93% (90–95%)	cTnI = 0.07 ng/ml			[39]
Cardiac Reader® (Roche Diagnostics) (POC)	Multiplex cardiac panel that quantifies cTnT, NT-proBNP and D-dimer in whole-blood	90%	92%	–	99%	cTnT = 0.1 ng/ml NT-proBNP = 125 pg/ml D-dimer = 0.5 µg/ml	8–12 min TAT	These data could be skewed due to small sample size	[32]
Triage Cardiac Panel (Biosite) (POC)	Fluorescent immunoassay that quantifies cTnI, CK-MB and cMb	98% (95.7–100.2%)	99.8% (99.6–99.9%)	92.4% (88.2–96.5%)	99.9% (99.9–100.0%)	cTnI = 0.4 ng/ml	~15 min TAT. Requires a few drops of EDTA-anti-coagulated whole-blood or plasma. Ease-of-use		[33]
								A class 1 recall of a certain lot of this test occurred in 2009, due to the occurrence of false-negative test results	[46]

EDTA: Ethylenediaminetetraacetic acid; NPV: Negative predictive value (proportion of negative results that are true negatives); POC: Point-of-care; PPV: Positive predictive value (proportion of positive results that are true positive results).

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2.5 ng/ml [50]. Depending on the particular country's regulations, a positive PSA measurement is found with PSA concentrations above a cut-off value of 2.5 or 4.0 ng/ml. Hence, in order to facilitate earlier detection of recurrence, assays that are capable of detecting serum PSA in pg/ml levels are required [6,51]. However, a known problem associated with the use of PSA is that it is found at elevated levels both in individuals with PCa and also in those with benign prostatic hyperplasia (BPH), as it leaks from damaged prostate epithelial cells into the circulation [6,51,52]. It is a major worry that PSA screening may result in overdiagnosis and overtreatment of PCa patients [9,53,54], and those who are diagnosed with PCa that are at low-to-intermediate risk may die of comorbidities as a result of treatment, such as radical prostatectomy [53]. Thus, due to the heterogeneity of PCa, it is broadly agreed that PSA alone is insufficient for use as a diagnostic marker for PCa, whereas a multi-marker panel is required for optimal PCa diagnosis [9,53]. It should also be noted that, recently, the US Preventative Services Task Force has recommended against the use of PSA in PCa screening in the USA [55] due to its lack of sensitivity and specificity [54]. It was suggested that a useful alternate means to discriminate PCa and BPH is the ratio of free PSA (fPSA) to total PSA (i.e., %fPSA) [6,51] as it was found that a cut-off value of ≤25% fPSA could detect 95% of PCa cases. However, drawbacks associated with the use of %fPSA alone include the occurrence of false negatives and the variability of reliability [54]. Perhaps an effective alternative are the isoforms of PSA, especially -2proPSA (p2PSA), which have proven promising for sparing men from unnecessary biopsies. For PSA in the range of 2–10 ng/ml, the Prostate Health Index (PHI = (p2PSA/fPSA) × $PSA^{1/2}$), at a sensitivity of 80%, had a specificity of 45%, which was significantly greater than that of %fPSA alone (37%). It was also found that a higher PHI value was associated with a 4.7- and 1.61-fold increased risk of PCa and Gleason ≥7 disease on biopsy, respectively [56]. Another study found that at 90% specificity, the sensitivities of PHI (42.9%) and % p2PSA (38.8%) were significantly higher than that of %fPSA (20%) [57]. However, while these figures are promising, it is evident that when using PHI and %p2PSA, one must compromise sensitivity for specificity or vice versa. The best balance of sensitivity and specificity (64.8 and 71.3%, respectively) was found at a PHI threshold of 40, however, this is still less than optimal for accurate and reliable PCa diagnosis [58].

Prostate cancer antigen 3 (PCA3) is a PCa biomarker that is upregulated 66-fold compared with benign prostate tissue and, thus, it may be useful in discriminating PCa patients from those with BPH [59,60]. Mucin-1 is an overexpressed oncoprotein found in prostate and other carcinomas [61], while PSA α_1-antichymotrypsin (PSA-ACT) is a complex of PSA and α_1-antichymotrypsin and it represents a major form of PSA, found at >85% when PSA levels exceed 1000 ng/ml. PSA-ACT is also found at higher levels in PCa patients versus BPH patients and may be a useful discriminating biomarker [62]. Other emerging PCa biomarkers that have been found to be associated with aggressive cancers include E-cadherin, enhancer of Zeste homolog 2 and hepsin [52]. Assays that could detect and quantify combinations of these biomarkers would have significantly greater predictive power for a patient's clinical status over the detection of PSA alone and would, therefore, contribute to improving assessment of patient clinical status and would guide physicians as to which treatment regime would provide the best possible quality of life for the patient.

PCa diagnostics

As mentioned above, PCA3 is a potentially useful biomarker for discriminating BPH patients from PCa patients. The Progensa PCA3 assay quantifies PCA3 and PSA RNA in urine samples and generates a PCA3 score (s-PCA3) (the ratio of PCA3/PSA RNA multiplied by 1000) [60,63]. The positivity of biopsies in patients with positive s-PCA3 is notably increased (45.2%) compared with those analyzed only with PSA and s-PCA3 shared a strong correlation with the Gleason score, while a PCA3 score less than 25 is correlated with a decreased likelihood of PCa [60,64]. However, in a review by Vlaeminck-Guillem *et al.*, it was found that in seven studies that used FDA-approved Progensa assays, the sensitivity and specificity ranged from 53 to 69% and 71 to 85%, respectively [65]. While the specificity appears to be greater over the use of PSA and PHI, the occurrence of false positives would range from 15 to 29%, which is still unacceptably high. It was noted that, in older men, PCa is not a fast-progressing disease and many men will die with the disease rather than from the disease. However, this level of false-positives would likely lead to high occurrence of unnecessary and overtreatment and, as a result, would lead to greater patient anxiety and discomfort and may, potentially, lead to other complications.

While large, automated commercial systems boast advantages such as high-throughput analysis (e.g., the UniCel® DxI 800 Immunoassy system, Beckman Coulter that can measure total PSA, fPSA and p2PSA in patient samples, with a declared throughput of 400 samples per hour [66]) with low detection limits of 5–50 pg/ml, an important disadvantage with these systems is that they are found only in dedicated laboratories. Testing using such systems inevitably requires sample transportation, which can result in delays in processing and reporting of results which can have an extended TAT [6]. Also, the cost of such a system may be prohibitively high for many clinical and hospital settings. POCT is ideal for on-site testing of patient samples and, with rapid TAT, results can be delivered quickly to patients, relieving anxiety. POCT can also potentially be used for large-scale monitoring of PCa occurrence via testing in GP offices or walk-in clinics and for the monitoring of disease progression during cancer treatment, while also offering a cost-effective alternative to laboratory-based testing.

The issue with PCa is not that tests are not sensitive enough; it is that PCa biomarkers are not simultaneously sufficiently sensitive and specific (Table 2). However, as mentioned above, assays that can measure combinations of PCa biomarkers could improve this. Some POC-ready devices that are in development include a multiplexed, antibody-functionalized, silicon-nanowire

Table 2. Characteristics of some prostate cancer diagnostic assays.

Technology/ measurement	Application	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cutoff	Advantages	Disadvantages	Ref.
Progensa PCA3 assay (Laboratory-based)	Nucleic acid amplification test for the quantification of PCA3 mRNA in urine samples	95.4% (84.2–99.4%)	30.9% (25.2–37.1%)	19.3% (14.2–25.2%)	97.5% (91.2–99.7%)	PCA3 = 20	–	–	[131]
		90.6%	27.9%	31.9%	88.9%	PCA3 ≥20	–	–	[132]
		71.9%	41.8%	31.5%	80.0%	PCA ≥35	–	–	
		82.3%	54.8%	51.9%	84.0%	PCA3 = 20	–	–	[133]
		66.5%	71.6%	58.1%	78.3%	PCA3 = 35	–	–	
		77.7%	50.0%	54.0%	75.0%	PCA3 = 35	–	–	[134]
		68.9%	66.7%	60.0%	73.0%	PCA3 = 60	–	–	
Prostate health index (PHI) (Beckman Coulter) (Laboratory-based)	$PHI = \left(\frac{p2PSA}{fPSA}\right) \times \sqrt{PSA}$	64.2% (51.5–75.5%)	63.2% (55.5–70.4%)	40.6% (31.2–49.9%)	81.8% (75.2–88.4%)	PHI = 41	–	Suboptimal clinical sensitivity, specificity and PPV	[135]
		66.3% (60.2–72.0%)	66.3% (55.5–76.0%)	–	–	PHI ≥ 44	–		[136]
		62.9% (56.7–68.7%)	62.3% (57.2–67.2%)	53.5% (48.0–59.1%)	70.8% (66.0–75.7%)	PHI ≥ 41.5	–		[137]
%fPSA (f/t PSA) (Laboratory-based)	Measurement of the ratio of free to total PSA	65%†	60%†	–	–	% fPSA = 10	–	Poor clinical sensitivity and specificity	[138]
		63%‡	58%‡			% fPSA = 7	–		
		94%†	13%†	–	–	% fPSA = 20	–		[139]
S2,3PSA (Laboratory-based)	Measurement of PCa-specific aberrant glycosylation of PSA	90.6%	64.2%	66.5%	89.7%	MFI = 1130	Superior specificity over PSA and %fPSA measurement	Based on ELISA format and, hence, requires numerous reagent handling, washing and incubation steps. Suboptimal clinical specificity	[140]
MRSI combined with MRI (Laboratory-based)		58%	93%	–	–	–	–	–	[141]

†In the PSA range 4–10 ng/ml.
‡In the PSA range 4–20 ng/ml.
MFI: Mean fluorescent intensity; MRSI: Magnetic resonance imaging spectroscopy; NPV: Negative predictive value; PCa: Prostate cancer; PCA3: Prostate cancer antigen 3; fPSA: free PSA; PHI: Prostate Health Index; PPV: Positive predictive value; PSA: Prostate-specific antigen; S2,3PSA: α2,3-Linked sialyation of free PSA.

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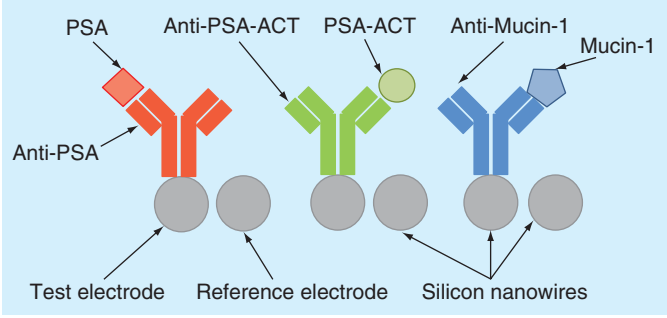

Figure 2. Multiplexed biosensor for the detection of PCa-specific biomarkers. The binding of antigens to wire-immobilized antibodies results in a change in conductance in the electrode. This change in signal is relayed to computer software, which analyzes the binding event.
PSA: Prostate-specific antigen; PSA-ACT: PSA-α1-antichymotrypsin.
Information sourced from [67].

field effect sensor for the quantification of PSA, PSA-ACT complex and mucin-1 at femtomolar sensitivity (Figure 2) [67]; a fluorescence immunochromatography assay that can detect PSA as low as 1 ng/ml in whole-blood samples [51] and an ultra-sensitive, multiplexed assay for the detection of PSA and IL-8, another cancer biomarker [68].

It is clear that, at the time of writing, there appears to be no ideal PCa biomarker for the diagnosis of PCa patients with both high sensitivity and specificity. At this moment in time, the most pressing concern for improving the case for PCa diagnosis and disease management is not the improvement of detection platforms and assays (the multiplex assay developed by Zheng *et al.* [67]. (Figure 2), Wan *et al.* [68] and Chin *et al.* [69]. [discussed below] are notable examples of excellent technology) but rather the discovery and validation of new and better PCa-specific biomarkers. Nevertheless, multi-marker assays for combinations of biomarkers (such as those discussed above) have not yet been developed and/or clinically tested and it is worth investigating if combinations of PCa biomarkers can improve the accuracy of PCa diagnosis.

HIV-related diseases

HIV statistics

HIV-1 is a retrovirus that can infect $CD4^+$ T-lymphocytes via their CD4 receptor. Infection and replication of HIV in $CD4^+$ T-lymphocytes lead to cell death and ultimately to CD4 depletion and chronic immune activation. This in turn leads to the stress and fatigue of homeostatic T-cell responses and progressive immunodeficiency. The long-term effect of HIV is manifested in the form of full immunodeficiency, that is, AIDS, during which patients are mortally susceptible to secondary infection [70]. According to WHO, HIV and TB are the infectious diseases with the highest global mortality rates [3]. It was estimated that in 2012, approximately 35.3 million people were living with HIV and approximately 1.6 million people died from AIDS [71]. Annually, 5–10% of global HIV infections occur from transfusions of contaminated blood. The safety of blood donations could be improved prior to transfusion through use of HIV POCT, as it has been found that donor screening has greatly reduced transmission of HIV by transfusion [3,72]. The key strategy for control of HIV and TB infections is early diagnosis and initiation of treatment and, hence, the use of HIV POCT could aid the early detection of HIV in patients, especially in developing countries where populations are faced with challenges such as weak healthcare infrastructure and ease-of-access to effective and timely medical care [4].

Currently, there are few approved HIV biomarkers available and most biomarkers are still under investigation. Examples of HIV biomarkers include plasma viral HIV RNA, p24 antigen (HIV viral coat protein), $CD4^+$ T-lymphocyte cell counts, antibodies against p24, gp120 and gp41, CRP, human leukocyte antigens and soluble Toll-like receptors, among others. However, the clinical utility of all these biomarkers has not yet been investigated, so the value of these biomarkers in HIV diagnosis is hard to determine [73].

Another challenge for HIV diagnosis is the existence of the acute HIV infection (AHI) phase, which is characterized by viral replication and shedding but occurs before detectable antibodies appear (~22 days after infection) [74–76]. Individuals in the AHI phase are maximally infectious, as viral load peaks in blood and genital secretions, the immune system has not yet generated binding antibodies and the patients' HIV status is often unknown [74,75]. Thus, the AHI phase affords an important opportunity for HIV prevention, as most HIV-infected individuals take measures to reduce their risk of further transmission upon learning of their infection.

HIV POCT

The current state of inexpensive alternatives to lab-based HIV testing was previously reviewed [77]. However, the issue with these tests is that, although they are more cost-effective over lab-based tests, they are not yet suitable for use in resource-limited settings. This review will give an outline to emerging POC that are in development and that are potentially suitable for use in 'resource-limited' settings.

In 'resource-limited' settings such as developing countries and rural settings, there is a need for a low-cost diagnostic device for detecting HIV, TB, syphilis and other infectious diseases. POCT can facilitate 'on-site' testing and hence, higher rates of treatment of such diseases can be achieved. The area of microfluidics holds promise for the development of low-cost POCT platforms as it allows for sophisticated methods of sample processing, signal amplification and detection, fluid-handling and miniaturization [69]. There is a demand for a device that encompasses each of these components. ELISA is considered the gold standard for detection and validation of protein-based biomarkers [69,78]. A miniaturized ELISA device called the mChip was developed [69]. This is a mobile microfluidic chip for immunoassay on protein markers and mirrors the sample processing and detection steps of ELISA. The device is heterogeneous (i.e., can be used with whole-blood samples), easily manufacturable, allows delivery of multiple reagents, uses

an optical method of detection and it does not require large bench-top instrumentation. A 20-min analysis can carry out multiplex detection of biomarkers in just several microliters of whole-blood, plasma or serum, matching the performance of lab-based immunoassays. This device facilitated the detection of anti-HIV and anti-syphilis antibodies in unprocessed whole-blood samples and also quantifies the generated signal, obviating the need for subjective user-interpretation, which is often required with qualitative tests. Hence, the device is accessible to users with minimal medical or laboratory training and, as it does not require external instrumentation or electricity, it can be operated in 'resource-limited' settings. A potential drawback of this device is the susceptibility of wet reagents to degradation due to temperature fluctuations, but the most susceptible reagents (gold-labeled antibodies and silver-development reagents) were shown to remain stable between 15 and 25°C for more than 6 months [69]. An additional potential drawback is associated with the cost of such gold-labeled antibodies and silver-development reagents, which could be prohibitively high for use in developing or rural locations. While this device boasts numerous advantages, this significant disadvantage should be factored into consideration before such a device is introduced into 'resource-limited' settings.

In clinical applications, WHO recommends that ART should be initiated in all individuals with a CD4 count of >350 and ≤500 cells/mm^3 [79]. Thus, it is imperative that HIV POCT be able to achieve such sensitivity so as to be able to guide physicians on the most appropriate treatment regime. It was found that CD4$^+$ T-lymphocyte counts by flow cytometry are performed an insufficient number of times per year in developing countries, generally twice per year, compared with four-times per year in developed countries. This problem is heightened by the fact that flow cytometers are often prohibitively expensive for use in 'resource-limited' settings. Thus, there is a requirement for a low-cost CD4$^+$ POCT for the diagnosis and long-term monitoring of disease state in HIV-infected individuals that will provide physicians with information on the most appropriate treatment regime [3–5]. Such a device, developed by Moon *et al.* [80], uses lenseless shadow imaging techniques to detect and enumerate captured, label-free CD4$^+$ T lymphocytes using a charge-coupled device sensor. It required only approximately 10 µl of fingerprick-derived whole-blood. Another microfluidic device, reported by Glynn *et al.* separates magnetically labeled CD4$^+$ cells from 4 µl of blood sample. The CD4$^+$ cell count can be estimated by bright-field microscopy or optical fluorescence measurement and the test produces a linear response over the entire range of medically relevant CD4$^+$ cell concentrations. The device is also easy-to-use and is inexpensive to manufacture [81]. Either of these devices could be of great use in 'amplification-free' quantification of CD4$^+$ T-lymphocyte counts at the POC.

Third-generation anti-HIV immunoassays are currently on the market, which detect antibodies (IgM/IgG) to HIV-1 and HIV-2 and allow for detection of HIV as early as 22 days after infection [4,74]. However, due to the presence of the AHI phase, there is a window during which such third-generation immunoassays would generate false-negative results for the detection of HIV antibodies [74,76,82]. HIV RNA can be identified during this phase through use of nucleic acid amplification testing (NAAT). However, while NAAT boasts advantages such as improving overall HIV case detection [74], there are associated problems, including the occurrence of false negatives when NAAT was used to analyze patients with long-standing HIV infection, increased cost of HIV-testing (as antibody testing is prerequisite for NAAT) and the need for sophisticated laboratory equipment [74,76,83]. Thus, NAAT may prove prohibitively expensive for use in 'resource-limited' settings and outreach settings. These problems may be addressed by recently FDA-approved Determine™ HIV-1/2 Ag/Ab Rapid Test (Alere, Waltham, MA, USA).

The Determine HIV-1/2 Ag/Ab Rapid Test (Combo RT) is a POCT that detects anti-HIV-1 and anti-HIV-2 antibodies as well as the p24 antigen. During the AHI phase, while anti-HIV antibodies are not being produced, p24 antigen may be released into the circulation, so this device has the potential of diagnosing patients during the AHI phase [84]. During investigation it was found that the Combo RT achieved a sensitivity of 75.8% for early HIV infection and appears to be a good screening assay for use in outreach settings [76]. While its sensitivity was lower than that of lab-based fourth-generation assays, its specificity was greater and the rapid test is disposable, amenable for 'near-patient' testing and has a TAT of 20 min [85].

However, numerous reports have indicated that the Determine Combo RT does not achieve high enough sensitivity to reliably detect AHI HIV. The p24 antigen component of the test has reported sensitivities of 0–10% [86–89]. Thus, until the sensitivity of such POCTs can be improved, the diagnosis of patients with AHI HIV must be the burden of laboratory-based testing, such as detection of HIV RNA using NAAT or detection of anti-HIV-1/2 antibodies and HIV p24 antigen using dedicated, high-throughput and highly sensitive lab analyzers (Table 3). However, as mentioned previously, the issue with laboratory-based tests is the high cost associated with the requirement for dedicated laboratory personnel and infrastructure and the extended TAT due to sample transport.

Tuberculosis

TB statistics & pathology

TB is a disease caused by infection of *Mycobacterium tuberculosis*. It was reported by WHO that, in 2011, TB led to 8.7 million cases and 1.4 million deaths [90] and WHO also predicts the trend for the total number of new annual cases to increase to 10 million by 2015 [91]. Approximately one-third of the global population is latently infected with *M. tuberculosis*, which can create a reservoir for future TB disease. In approximately 90% of those infected, the immune system is capable of permanently containing the infection, and these individuals will not develop the disease. However, in the approximately 10% that are unable to permanently contain the infection, active TB disease usually develops after a latent interval that can range

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Table 3. Characteristics of some HIV diagnostic assays.

Technology	Application	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cutoff	Advantages	Disadvantages	Ref.
Pima™ (Alere) (POC)	$CD4^+$ cell enumeration via fixed volume cytometry	93.3% (77.9–99.2)	96.0% (92.5–98.1)	–	–	CD4 = 200	TAT of 20 min. The POCT was highly accepted among patients. 25 µl of venous or fingerstick whole-blood. No sample processing. Disposable cartridges	Occasionally underestimates $CD4^+$ counts relative to laboratory tests	[142]
		94.8% (88.3–98.3)	88.0% (81.9–92.6)	–	–	CD4 = 350			
		98.6 (95.0–99.8)	70.5% (61.2–78.8)	–	–	CD4 = 500			
		–	–	–	–		Reduction in time to test patients can increase ART initiation and improve patient survival		[143]
		88.6% (84.8–92.4%)	87.5% (84.9–90.1%)	74.4% (69.6–79.2%)	94.9% (93.1–96.7%)	CD4 <350 cells/µl	Monitoring of patients receiving ART. Ease of use	At CD4 counts >350 cells/µl, negatively biased CD4 estimates and lower sensitivity relative to FACSCalibur™ (Beckton Dickinson) laboratory-based flow cytometer were observed	[144]
		96.1% (94.4–97.8%)	83.0% (79.2–86.7%)	88.3% (85.6–91.0%)	94.1% (91.6–96.6%)	CD4 <500 cells/µl			
		–	–	–	–			Unable to estimate CD4% in children between ages 2 and 6	[145]
INSTI HIV-1/HIV-2 Antibody Test (bioLytical Laboratories, Richmond, Canada) (POC)	Flow-through immunoassay for the qualitative detection of anti-HIV-1 and anti-HIV-2 antibodies in human EDTA-whole-blood, fingerstick blood, serum or plasma	100.0% (39.8–100.0%)	99.8% (99.3–100.0%)	100.0% (99.6–100.0%)	66.7% (22.3–95.7)				[146]
		–	–	–	–		TAT of less than 1 min Requires 50 µl fingerstick blood		[147]
		100%	99.9%	100%	96%				[148]
Anti-p24 dipstick lateral flow assay (POC)	Semi-quantitative detection of p24 antigen in plasma samples	95.8% (88–99%)	99.4% (98–100%)				TAT of ~40 min. Requires ~25 µl plasma. Signal measurement by scanner eliminates user-misinterpretation	Current data on other field trials is unavailable. Thus, it is uncertain as to the use of this test for AHI HIV patient testing	[149]

AHI: Acute HIV infection; EDTA: Ethylenediaminetetraacetic acid; EIA: Enzyme immunoassay; NPV: Negative predictive value; POCT: Point-of-care tests; PPV: Positive predictive value; S/CO: Signal/Cutoff; TAT: Turnaround time.

Table 3. Characteristics of some HIV diagnostic assays (cont.).

Technology	Application	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cutoff	Advantages	Disadvantages	Ref.
Architect HIV Ab/Ag Combo (Abbot) (Laboratory-based)	Automated chemiluminescent immunoassay for detection of p24 antigen and antibodies to HIV-1 and HIV-2	100% (96.3–100%)	97.6% (92.6–99.3%)			S/CO >1	Has potential for diagnosis of patients with AHI HIV	Laboratory-based test, thus, requires trained personnel, established laboratory infrastructure, etc.	[150]
Elecsys® HIV combi PT (Roche) (Laboratory-based)	Automated immunoassay for detection of p24 antigen and antibodies to HIV-1 and HIV-2	100%	99.86%			S/CO ≥1	Has potential for diagnosis of patients with AHI HIV	Laboratory-based test, thus, requires trained personnel, established laboratory infrastructure, etc.	[151]
Cobas TaqMan HIV-1 V2.0 (Roche) (Laboratory-based)	*In vitro* nucleic acid assay for detection of HIV-1 in plasma and serum	97%	83%	91%	94%	HIV RNA = 800 copies/ml	Can potentially diagnoses patients with AHI HIV	High cost relative to EIA and western blot. Requires trained laboratory personnel and established laboratory infrastructure	[152]
NucliSENS EasyQ HIV-1 V2.0 (bioMerieux) (Laboratory-based)	*In vitro* nucleic acid assay for detection of HIV-1 in plasma and serum	89%	98%	98%	90%	HIV RNA = 800 copies/ml	Can potentially diagnoses patients with AHI HIV	High cost relative to EIA and western blot. Requires trained laboratory personnel and established laboratory infrastructure	[152]

AHI: Acute HIV infection; EDTA: Ethylenediaminetetraacetic acid; EIA: Enzyme immunoassay; NPV: Negative predictive value; POCT: Point-of-care tests; PPV: Positive predictive value; S/CO: Signal/Cutoff; TAT: Turnaround time.

from weeks to decades [91–93]. There are a number of environmental factors that can contribute to susceptibility and reactivation of *M. tuberculosis* including malnutrition, overcrowding, poverty and stress [91] and 90% of TB patients live in low- and middle-income countries [94]. Primary infection usually occurs in the lungs and leads to the development of tuberculous granulomas (areas of inflammation), which are formed by the immune system as a means to contain the infection. These granulomas vary in their morphology: solid granulomas are composed of organized macrophages and lymphocytes without necrosis; and caseous granulomas are composed of macrophages and lymphocytes surrounding a caseous necrosis (a form of coagulative necrosis). The latter are associated with high bacterial transmission. The areas of the lungs that are directly exposed to a necrotic granuloma can develop cavitary lesions and represent the erosion of the airway [92].

TB biomarkers

Promising biomarkers for TB include IFN-γ-inducible protein (IP)-10 and monocyte chemotactic protein (MCP)-2, two proinflammatory chemokines. It was suggested that these proteins could be used in a multiplex device to improve IFN-γ release assay tests such as the Quanti-FERON TB® Gold In-Tube (Cellestis Carnegie, Australia) and the T-SPOT.TB® (Oxford Immunotech, Abingdon, UK), as these tests often have differing, indeterminate results [95], especially in HIV-infected individuals [96] and those that are immunocompromised (as this can impair sensitivity) [97,98]. IP-10 and MCP-2 have been shown to be expressed in significantly higher amounts in TB individuals than IFN-γ relative to controls [99,100]. It was suggested that a combined test for these two biomarkers could increase the TB detection rates 81–90% without significantly affecting the rate of false positives. The agreement between these tests was also high (>89%) [99]. A disadvantage associated with the use of IP-10, however, is it inability to distinguish active and latent TB. Also, because it is a marker for inflammation, it can be found in raised levels in individuals with autoimmune disease,

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allergy and acute coronary syndrome. This highlights that IP-10 is too non-specific to be used as a single biomarker, and thus it must be used in conjunction with other biomarkers [101].

Other potential biomarkers for TB include MCP-1, MCP-3 and IL-1 receptor antagonist, as these biomarkers were found in significantly higher amounts in patients relative to controls. However, there is evidence indicating to their lower specificity compared with IP-10 and MCP-2 [98]. Nevertheless, a panel of these biomarkers may serve as a powerful tool for the diagnosis of TB, as it has been previously shown that combinations of disease-specific biomarkers can improve the overall diagnostic performance [102–106], though this must be verified for TB. Also, while it is still early in the investigations, these have been evidence suggesting the advantage of using IL-15 (a cytokine that is involved in the upregulation of antimicrobial protein cathelicidin) and MCP-1 for discerning active and latent infections; when combined, these biomarkers identified 83 and 88% of active and latent TB, respectively. However, further investigation with larger study groups is required to further validate these findings [100].

Another promising biomarker for differentiating active from latent TB is *Mtb*-specific TNF-α. This is calculated by subtracting background TNF-α of controls from TNF-α secreted by *Mtb*-specific antigen-stimulated cells (i.e., cells exposed to *M. tuberculosis*). *Mtb*-specific TNF-α levels were found significantly elevated in active TB patients versus non-TB patients and, at a cutoff value of 136.9 pg/ml *Mtb*-specific TNF-α, active and latent TB could be differentiated with sensitivity and specificity of 72 and 91%, respectively [107].

While it is still early into investigations, it was reported that during paradoxical TB-associated immune reconstitution inflammatory syndrome (i.e., a worsening of patient symptoms of a known disease due to alterations in immune responses to pre-existing antigens or pathogens), there were lower median concentrations of MCP-1 and IL-10 and a higher ratio of CRP:IL-10 relative to controls. Though this study is limited due to a small patient cohort size, this information may provide another potential route for diagnosis of TB that is unbeknownst to patients and warrants further investigation [108].

A study by Shekhawat *et al.* found that host heat shock proteins [Hsp(s)] (Hsp 25, Hsp 70 and Hsp 90) were elevated in the cell lysates of TB-infected monocytes relative to non-infected monocytes. The roles of these Hsps include cell protection from apoptosis, cytosolic chaperones and proinflammatory effectors. They also observed that *M. tuberculosis* Hsp 16, 65 and 71 were increased in monocyte cell lysate after 48 h exposure to the bacteria. These bacterial Hsp(s) are highly immunogenic and have a likely role in production of proinflammatory cytokines [109]. While studies in this area are ongoing, it is believed that these Hsp(s) have potential as serving as biomarkers for TB disease.

As with the other diseases discussed in this article, there does not appear to be any single ideal biomarker that can accurately and confidently diagnose TB or distinguish latent from active TB. Thus, it seems imperative that multi-marker approaches be investigated further.

TB POC diagnostics

Less than 20% of the 8.7 million patients suffering with TB per year receive a microbiologically confirmed diagnosis. It is generally agreed that by making simple, 'user-friendly' and inexpensive detection tools, lives could be saved and the overall patient and health system costs could be reduced. While sputum smear microscopy is considered the gold standard for pulmonary TB diagnosis and is simple and relatively inexpensive, it requires highly trained microscopists, high-quality microscopes, multiple sputum examinations and exacting quality control. In high-prevalence settings, specificity of microscopy is >95% but sensitivity can range between 20 and 80% [3,94]. Mycobacterial culture is an alternative method of TB detection that can overcome the issue of low sensitivity. However, this method has a number of disadvantages that make it unsuitable for use in POCT: results take weeks to acquire; it needs dedicated equipment and trained personnel; and has additional costs [94]. NAATs (e.g., the Xpert® MTB/RIF assay, Cepheid Inc.; Table 4) hold promise for the development of a TB POCT that can be robust and simple to use outside the laboratory, as they can diagnose multidrug-resistant TB and HIV-associated TB with great sensitivity and specificity. However, the major drawback with the use of molecular amplification techniques is the cost of equipment and personnel, which may be prohibitively high for many laboratories in the developing world [3,94]. Immunochromatographic tests are suitable for use in 'resource-limited' setting as they are inexpensive and do not require specialized equipment or highly trained personnel, making them a promising means to address the issue of low detection rate of TB-infected patients [94]. However, WHO evaluated 19 commercially available TB immunological diagnostic assays and, while it was found that 12 were considered 'easy-to-use', none of the tests outperformed traditional microscopy, with overall sensitivity ranging from 1 to 60% and specificity of <80%. However, if the test performance could match or surpass that of microscopy, these POCTs would be appropriate for use in 'near-patient' settings in developing countries [94]. Thus, until this time comes, microscopy will remain the most feasible means of TB screening and diagnosis, but the challenges of its associated cost and limited accessibility will persist. A solution to these problems may be presented in the form of mobile phone diagnostics.

TB and malaria are endemic in many developing areas [110,111] and the number of cases detected by microscopy in laboratories in these areas, which are often overworked and underfunded, can be as low as 20–35% and result TAT can take 3–6 months for patients in countries with a high TB burden. This extended TAT can lead to increased disease transmission and exacerbates the patients' disease state [111]. While access to healthcare and testing is limited in developing countries, mobile phone networks are widespread in these areas. A large portion of modern phones come equipped with a digital camera and are capable of tremendous computational power [110,112]. It has also been shown that mobile phone cameras are capable of capturing images from the eyepiece of a standard microscope [110]. Thus, the area of mobile phone diagnostics, called mHealth, could

Table 4. Characteristics of some tuberculosis diagnostic assays.

Technology	Application	Sensitivity (95% CI)	Specificity (95% CI)	Advantages	Disadvantages	Ref.
Xpert® MTB/RIF (Cepheid Inc.) (Laboratory-based)	NAAT for the detection of TB and RIF-resistant TB	83% (77.2–88.0%)	95% (92.9–96.6%)	Can allow for same-day diagnosis and treatment initiation	Lower sensitivity in patients co-infected with HIV. Requires trained nursing staff and lab infrastructure in order to be used at POC. Expensive	[124]
		90.4% (89.2–91.4%)[†] 94.1% (91.6–96.0%)[‡] 80.4% (75.0–85.1%)[§] 81.7% (77.0–85.8%)[¶]	98.4% (98.0–98.7%)[†] 97.0% (96.0–97.7%)[‡] 86.1% (83.5–88.4%)[§] 98.0% (96.6–98.9%)[¶]			[153]
QuantiFERON TB Gold In-tube (Cellestis) (Laboratory-based)	Uses ELISA to measure T-cell-based IFN-γ response to MTB antigens in whole-blood	0.76 (0.65–0.74)	97.7% (96–99%)	Relative to the tuberculin skin test; testing requires only one patient visit; there is a reduced risk for adverse effects, as this test is *ex vivo*	Requires laboratory infrastructure and trained personnel. Drawn blood must be handled carefully to maintain lymphocytes. High material costs	[154]
		0.79 (0.75–0.82)[#] 0.57 (0.52–0.61)[††]	0.97 (0.96–0.98)[#] 0.85 (0.82–0.88)[††]			[155]
T-SPOT.TB (Oxford Immunotec) (Laboratory-based)	Uses Elispot to measure IFN-γ production by peripheral mononuclear cells after induction with MTB antigens	0.88 (0.81–0.95)	92.5% (86–99%)	Relative to the tuberculin skin test; testing requires only one patient visit, as this test is *ex vivo*, there is a reduced risk for adverse effects	Requires laboratory infrastructure and trained personnel. Drawn blood must be handled carefully to maintain lymphocytes. High material costs	[154]
		0.67 (0.62–0.73)[#] 0.61 (0.57–0.65)[††]	0.98 (0.96–0.99)[#] 0.93 (0.87–0.96)[††]			[155]
Anda-TB IgG (Anda Biologics) (Laboratory-based)	ELISA format for the detection of antibodies specific to A60 MTB antigen	76% (63–87%)[‡‡] 59% (10–96%)[§§]	92% (74–98%)[‡‡] 91% (79–96%)[§§]			[156]
		83.3% (63.5–94.0%)	72.0% (67.2–74.6%)		TAT of ~2 h. Requires numerous reagent handling steps, wash and incubation steps. Requires laboratory infrastructure, spectrophotometer and trained laboratory personnel	[157]

[†]In pulmonary TB.
[‡]For detecting RIF resistance.
[§]For extrapulmonary TB.
[¶]For HIV co-infection.
[#]High-income countries.
[††]Low-income countries.
[‡‡]In smear-positive patients.
[§§]In smear-negative patients.
MTB: *Mycobacterium tuberculosis*; NAAT: Nucleic acid amplification test; POC: Point-of-care; RIF: Rifampin; TAT: Turnaround time; TB: Tuberculosis.

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hold promise for the diagnosis of infectious diseases in 'resource-limited' areas, as it could take diagnostic testing out of overworked and underfunded laboratories and bring it to the patients in need. mHealth could also allow for repeat 'on-site' testing of patient samples and reduce the TAT of results. Such a mobile phone diagnostic device was reported [110].

Breslauer *et al*. developed a mobile phone microscope attachment that is capable of high-resolution brightfield and fluorescent imaging [110]. This attachment facilitated the capture of fluorescent images of TB in Auramine O-stained sputum smears, while imaging processing software allowed for the labeling and counting of TB cells in a captured image. It was shown elsewhere that mHealth could identify *Schistosoma haematobium* eggs in urine samples [113] *Giardia lamblia* cysts could be visualized in PBS [114] and malaria biomarker, *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) could be detected with a low limit of detection in human serum [115]. Another mobile phone-based POCT uses oligonucleotide probes specific to *M. tuberculosis* that are coupled to gold nanoparticles. The presence of target DNA is indicated by a color change from blue to red, which can be scanned and processed by a generic smart phone camera and Red Green Blue (RGB) analysis software [116].

Such mobile phone-based diagnostics could provide populations in developing and rural settings with remote access to digital record keeping, automated sample analysis and expert diagnosis, while in-build phone GPS would enhance epidemiological disease monitoring. mHealth is also not limited to infectious diseases but can also assess patients' blood oxygen saturation and pulse rate [117,118] and glucose, lactate and ethanol levels [119], among various other functions.

As mentioned earlier in this review, a multiplex test that can simultaneously quantify levels of combinations of TB biomarkers in patient samples could serve as an effective means for diagnosis of patients with TB or for discriminating latent from active TB. It has been shown previously that multiplexed, colorimetric detection of multiple targets associated with infectious diseases can be achieved at high sensitivity using gold and silver nanoparticles [120,121] and it is not hard to believe that such technology could be integrated into a multiplex TB test. An example of a multiplex POC-ready device was developed by Li *et al*. This lateral-flow device uses gold-nanoparticle-labeled antibodies to detect *Staphylococcus aureus* and *Pseudomonas aeruginosa* in unprocessed sputum samples. The test has a TAT of <5 min, a specificity of 100% and a detection range of 500–5000 CFU/ml [122].

Fu *et al*. have shown that two-dimensional paper network (2DPN) lateral flow tests can allow for low high sensitivity detection of biomarkers and can be expanded to allow for multiplexed detection. They have shown that 2DPN can have approximately fourfold limit of detection (LOD) improvement compared with standard lateral flow assays due to enhanced rinse and amplification steps [123]. Such a test has potential to accommodate TB-specific antibodies to allow for the multiplexed detection of TB-specific biomarkers.

Also, while multiplex assays designed specifically for the purpose of measuring combinations of the above TB biomarkers have not yet been developed, the multiplex technologies discussed previously [67–69] may provide excellent platforms for this development, as the core biosensor platforms could be maintained while exchanging the biological recognition element for ones specific for TB biomarkers (e.g., antibodies specific for IP-10 and MCP-2). Such flexible assays could someday serve as highly useful platforms for the detection of a broad range of disease biomarkers.

Expert commentary

It is the authors' opinion that the area of POCT diagnostics holds major potential for revolutionizing healthcare in both the developed and developing worlds. However, in some aspects, notably the issue of disease-specific biomarkers and their approval, POCT are currently limited. For example, in the case of PCa, there was so much focus on POCTs for PSA, a non-cancer-specific biomarker, but comparatively little focus on approving new biomarkers or developing assays for multiplex detection of existing biomarkers. Approving new biomarkers and developing multiplex assays is, in the authors' opinion, key to improving the case for diagnosis of both PCa and AMI.

While there are a range of biomarkers available for diagnosis of later-stage HIV and the performance of later-stage HIV POCT is impressive (especially in the case of the INSTI HIV-1/HIV-2 Antibody Test), few are available for AHI. NAATs and lab-based immunoassays still present the only feasible means to diagnose AHI, as sensitivity of rapid POC immunoassays is poor; however, incorporation of these lab tests into resource-limited settings may prove difficult. Nevertheless, as was shown by Theron *et al*. [124], it is feasible to incorporate NAATs into these settings, but there still remains a requirement for trained personnel. It may also be a necessity that manufacturers of such NAATs allow for a cost-reduction to facilitate the purchase and wider distribution of these tests in developing countries. It will also be necessary to identify AHI phase-specific protein biomarkers that can be detected by immunoassay techniques, as these assays have proven to be more amenable to miniaturization and incorporation in POCT than most NAAT.

mHealth is another avenue for improving the case for diagnosis of infectious diseases in the developing world, and is, in the authors' opinion, excellent and innovative use of available and extremely valuable resources in otherwise resource-poor settings. mHealth offers the potential of access for a significant proportion of the population to readily-available healthcare solutions, which is an extremely exciting prospect.

Five-year view

In regards to AMI, it is likely that new assay platforms will come to the fore, incorporating different combinations of available biomarkers, such as troponins, H-FABP, cMb and may include IMA.

Perhaps within the next 5 years, information will emerge as to the usefulness of multiplex PCa assays in a clinical setting. Also, the authors are skeptical that the issue of poor specificity will be fully resolved, due to persistence with the use of PSA.

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However, it is likely that specificity will be improved with the inclusion of PCa-specific biomarker measurement. Perhaps also, multiplex PCa POCT will begin to emerge within the next 5 years, as, in the authors' own research group, extensive work is being done in this area.

It is likely that the area of microfluidics will allow for improved CD4 cell counting in resource-limited settings. Already, lab-on-a-chip technologies are emerging for the enumeration of $CD4^+$ cells and viral loads in resource-limited settings [81,125,126] and one can imagine that further developments in this area will emerge in the next 5 years. A literature search for POC HIV nuclear amplification techniques returns promising results, which suggest that POC detection of AHI may be feasible within the next 5–10 years [127]. It is feasible that the clinical utility of HIV biomarkers such as human leukocyte antigens and soluble Toll-like receptors will also come to fruition in this time frame.

It needs to be verified that 'multi-marker' approaches for TB can improve overall clinical utility. If this is achieved it is likely that multiplex TB POCT will begin to emerge, but it is difficult to see this occurring in as short a time frame as 5 years; perhaps within the next decade this will come to pass. It is likely that additional effort will be expended on the approval of MCP-2 and IP-10, as they seem to be the most likely candidates for future TB biomarkers, given their good diagnostic performance. Clinical studies will likely emerge using these biomarkers and will give an indication of their clinical performance. After this, it can be expected that assays for the measurement of MCP-2 and IP-10 will be thoroughly investigated and developed.

It is also highly likely that mHealth technology will be of great use for analyzing results of TB immunochromatographic tests to eliminate subjective user-interpretation (as is the case with the Deki Reader [128]).

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Key issues

- A plethora of acute myocardial infarction (AMI) biomarkers are available, each with its own advantages and clinical utility, but no definitive evidence as to the ideal biomarker of choice is available. The multiplex measurement of AMI biomarkers appears to be the most viable means to improve the case for AMI diagnosis, as a significant increase in clinical accuracy was observed using this approach. However, it has still not been evaluated as to which biomarker combination can decisively diagnose AMI with high accuracy and confidence. While point-of-care tests (POCTs) are available for multi-marker detection, the critical flaw of inappropriate/suboptimal biomarkers identifications hampers performance.
- An overreliance on prostate-specific antigen, a non-prostate cancer (PCa)-specific biomarker, for PCa diagnosis can often lead to overdiagnosis and overtreatment. New emerging biomarker assays and measurements, such as prostate cancer antigen 3 and Prostate Health Index, are improving the case for discriminating between benign and cancerous diseases, however, the issue of lack of specificity has persisted. As with AMI, there is a requirement for determining a biomarker combination that can improve the overall clinical performance and for the approval of new biomarkers. Once this has been determined, POCTs can be developed, as currently there is a reliance on laboratory-based testing, which can have a long turnaround time and are considerably more expensive to perform. POCT in PCa diagnosis could have profound impact on large-scale disease monitoring, monitoring of disease state during treatment and providing a cost-effective alternative to laboratory-based testing.
- POCT is currently providing effective means for diagnosing patients with later-stage HIV in 'resource-limited' and outreach settings. However, POCT is lacking in the diagnosis of acute HIV infection (AHI) phase HIV patients, due to a lack of sensitivity to AHI biomarkers. Efforts to introduce laboratory-based issue with laboratory-based tests is the high cost associated and immunoassays into the POC may address the immediate need for more sensitive AHI diagnosis, but the issue of cost will persist. Thus, there is a requirement for the identification of AHI-specific biomarkers and the development of highly sensitive, low-cost assays that can detect such biomarkers in resource-limited settings.
- Tuberculosis (TB) biomarkers are emerging and are demonstrating significant potential in improving TB case detection and diagnosis. Promising biomarkers such as IFN-γ-inducible protein-10 and monocyte chemotactic protein-2 are claimed to significantly increase TB case detection, however, they are unable to discriminate latent and active disease. Other emerging biomarkers such as *Mtb*-specific TNF-α may address this need, but regulatory approval must first be acquired before such biomarkers can be put to use. At the time of writing, the case for approved immunological TB POCTs was poor, as these assays were severely deficient of sensitivity and specificity. The area of mHealth is showing potential for the identification of microorganisms in human samples and may be able to address the need for improved TB case detection; however, no data have emerged as to the clinical utility of such testing.

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