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# Point of Care Diagnostics: Status and Future

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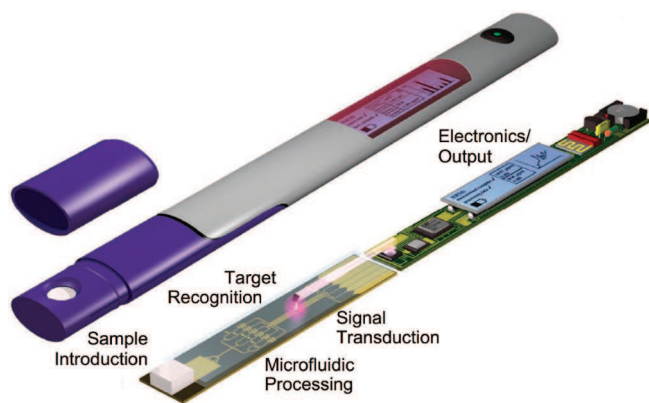
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## ■ INTRODUCTION

We review point-of-care (POC) diagnostics, *in vitro* diagnostic (IVD) tests that do not involve the use of laboratory staff and facilities to provide the result. The analytical “targets” include proteins, nucleic acids, metabolites, drugs, dissolved ions and gases, human cells, and microbes. Samples are blood, saliva, urine, or other bodily fluids or (semi)solids. Whether used “near-patient” in a hospital, clinic, or doctor’s office, or administered at home to maintain health, manage disease, or monitor therapy, or in the field to test the safety of water, food, or compliance with laws and regulations, these tests accept a sample with little or no pre-preparation and provide a result, the “answer”, in seconds to hours.<sup>1</sup> The tests require only elementary instruction to use and some detect multiple analytes or markers.<sup>2</sup> Interpretation may be as simple as viewing a stripe or spot of color on a strip of paper or polymer; increasingly, however, readers ranging from hand-held devices to benchtop instruments read the analytical test, provide a comprehensible result, and if necessary, control and operate the sample-containing platform that executes the analytical process.

An idealized concept of a POC device is shown in Figure 1. These devices are challenged by small sample volumes (hundreds of nanoliters to ~1 mL) of complex biological media with femtomolar to millimolar concentrations of analytes.<sup>3</sup> The devices



**Figure 1.** Idealized POC device. Adapted with permission from ref 6a. Copyright 2011 John Wiley & Sons, Inc.

should be inexpensive disposable chips or cartridges that include microfluidic features to provide or control sample preparation, flow rate, mixing with reagents, reaction time associated with binding events, filtration of nonanalytical components of the sample, separation of interfering agents and of multiple analytes, and an effective measurement capability.<sup>4</sup>

POC diagnostics have been extensively reviewed in recent years, from the points of view of both use<sup>5</sup> and development.<sup>6</sup> The reviews have included coverage of micrototal analysis systems ( $\mu$ TAS),<sup>7</sup> miniaturized isothermal nucleic acid amplification<sup>8</sup> and molecular biological techniques for gene assay,<sup>9</sup> current and anticipated technology for POC infection diagnosis,<sup>10</sup> and microfluidic-based systems leading toward point-of-care detection of nucleic acids and proteins,<sup>11</sup> including multiplexing and label-free methods.<sup>12</sup> Developments in this area include not only technology but also reliable measurement targets, which in some important areas remain elusive: progress toward viable point-of-care protein biomarker measurements for cancer detection and diagnostics has been reviewed.<sup>13</sup>

We review here the present status of POC diagnostics, emphasizing in particular the past 4 years, then extrapolate their progress into the future. Included are IVD tests for biochemical targets of all sorts relevant to human health, diagnosis, and therapy, as enumerated above. We begin with an overview of the different classes of bioanalytical targets. Then, after setting the context using the well-established glucose and pregnancy POC tests, recent progress in key enabling technologies is reviewed, including traditional and advanced lateral flow approaches, printing and laminating technologies, a range of microfluidic advances, progress in surface chemistry and the control of nonspecific binding, and developments in labeled and label-free detection approaches. A number of specific innovative examples, in both commercial products and academic POC research, are presented, including assays based on binding to proteins, nucleic acids, and aptamers, with separate sections devoted to blood chemistry, coagulation, and whole cells. We close with trends and future perspectives.

**Why POC Diagnostics?** *Time* POC measurements provide results rapidly, where needed, and often with major time savings: samples do not travel to a laboratory to await the attention of a skilled technician; results do not wait to be transmitted and collected. Rather, the doctor, nurse, care-giver, patient, or consumer initiates the test and receives the results on the spot. Inevitably this saves time, but speed must not be traded for accuracy or reliability.

*Patient Responsibility and Compliance.* In primary care settings, patients are supervised by a medical team responsible for administering medications and monitoring responses. Although often administered by medical professionals, POC tests are also widely self-administered, making patients far more responsible for managing their own condition(s).<sup>14</sup> Concern over loss of professional control and potential for incorrect interpretation of results<sup>15</sup> necessitate management systems to ensure that patients receive reliable devices and readout training, including everything from device maintenance to finger-stick sampling procedures, along with general information on their medical conditions and where to turn for support.<sup>16</sup>

In-home POC testing reduces the frequency of hospital visits, travel expenses, and lost work time.<sup>17</sup> The success of glucose meters and pregnancy tests has motivated more people to opt for self-testing,<sup>18</sup> conferring increased responsibility to maintain their own medical records and notify their physicians should abnormal results arise.<sup>19</sup> This situation can be tenuous, particularly if the patient's mental or physical faculties are compromised. The advent of "telemedicine" or "telehealth",<sup>14</sup> the provision of health services over long distances via telecommunications, is addressing this challenge by giving healthcare professionals partial control over patient self-testing and data management.<sup>20</sup>

Empowering individuals to do their own tests can improve patient compliance (adherence to diagnosis and treatment regimens).<sup>21</sup> A recent study of the cost effectiveness of POC testing reveals significant increases in testing regularity and adherence to prescribed medications,<sup>22</sup> as well as improvements in clinical outcomes.<sup>23</sup> Near-patient testing in diabetic clinics results in greater patient satisfaction, accompanied by better understanding of medical results<sup>24</sup> and improved long-term prognosis relative to a dearth of testing.<sup>21</sup>

POC testing is well established to monitor blood coagulation time in conjunction with administration of the anticoagulant warfarin: weekly self-monitoring of oral anticoagulation coupled with self-dosing provides more effective anticoagulation therapy than conventional testing.<sup>25</sup> Similarly, the quality of anticoagulation therapy, managed initially in the traditional hospital setting then switched to self-management, deteriorated when patients returned to clinical management.<sup>26</sup>

*Cost.* POC diagnostic cost parameters differ from those of conventional laboratory analysis. Readers (instruments) are smaller and more specialized than laboratory systems, so they cost less but do only one or a few different tests. Samples do not directly contact the reader, hence self-cleaning subsystems are not needed. The POC chip, strip, or cartridge, a consumable that contains the sample but is not designed for cleaning or reuse, may include fluidics, on-board reagents and dyes, optics, electrodes, even thermal control. Relative to a blood-draw tube, the POC device's greater complexity and functionality make it more costly, so tests sold in large volume derive most of their revenue from the consumable.

POC tests can indirectly, sometimes drastically, lower medical costs: sample mislabeling and mishandling, along with misdirection of results, are less likely. Results are provided more quickly, enabling more effective treatment of rapidly progressing afflictions, even making a life-or-death difference with some infections. Rapid POC results can obviate hospital admissions, e.g., when a suspected myocardial infarct is determined rapidly to be indigestion. On the other hand, sophisticated test cartridges cost more than basic sample tubes, and when the cost of a large laboratory system can be amortized over hundreds of thousands of samples, cost per test can be lower.

## ■ DIAGNOSTIC TARGETS

**Proteins.** Proteins, including enzymes, antibodies, and some hormones, are common targets for POC diagnostics. An early POC device developed in 1957, the urinalysis dipstick measures urinary protein using paper strips impregnated with a pH indicator dye.<sup>27</sup> Diabetics and kidney-disease patients were enabled to test their urine at home, providing a previously unrealizable degree of feedback to manage their own health. Today, products such as the Clinitek Status analyzer by Siemens Healthcare Diagnostics allow semiquantitative electronic readout of urine dipsticks.

Modern POC devices utilize immunoassay technology, which includes antigen–antibody binding, whether the antibody is the assay target or the means to capture it. These assays target disease-specific protein markers such as glycosylated hemoglobin (HbA1c) for diabetics, C-reactive protein (CRP) for inflammation including cardiovascular disease, D-dimer for thrombosis, troponin I or T for cardiac damage, prostate-specific antigen (PSA) for this common cancer, and bacterial and viral infection-related markers such as human immunodeficiency virus (HIV), influenza, chlamydia, and hepatitis.<sup>5b</sup> Commercial POC devices that detect antibodies developed by the host in response to infection include OraQuick ADVANCE Rapid Antibody Test for HIV, QuickVue Influenza AB, and Accustrip Strep A for group A *Streptococcus*.

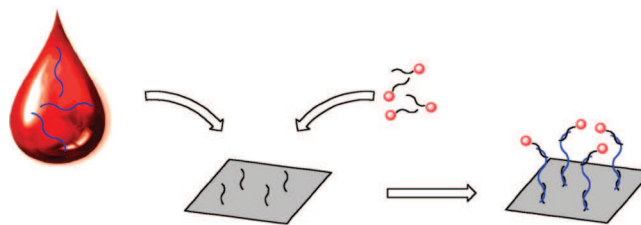
The best-known home POC protein-detection device, the pregnancy test kit measures the pregnancy hormone human chorionic gonadotropin (hCG). The test's key component, the lateral-flow test strip, is described in the Lateral Flow Assays section.

**Metabolites and Other Small Molecules.** Metabolites are products of chemical processes, metabolism, that generate energy, process nutrients or wastes, or break down and renew body tissues.<sup>28</sup> Because of similarities in their physiological transport and detection approaches for POC assays, we treat them here together with simple ionic blood chemicals ( $H^+$ ,  $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $HCO_3^-$ , etc.) and small-molecule organic species, including nonprotein hormones (e.g., epinephrine, cortisol, and peptide hormones). Levels of metabolites, hormones, and blood-borne chemicals are often diagnostic indicators of disease. The current panel of metabolites most often targeted by POC diagnostics includes glucose, cholesterol, triglycerides, creatinine, lactate, ammonia, and urea.<sup>29</sup> One of the early and best-known clinical POC analyzers is the i-STAT hand-held system (Abbott Point of Care, Princeton, NJ) for blood chemistry.<sup>30</sup>

The best-known metabolite, glucose, enables diagnosis and management of diabetes mellitus, an endocrine disorder afflicting more than 125 million people worldwide;<sup>14</sup> glucose biosensors account for approximately 85% of the entire biosensor market.<sup>31</sup> Diabetic complications are controllable with tight regulation of glucose levels, prompting the development of POC glucose sensors in the 1990s.<sup>32</sup> Most diabetic patients now regulate their condition at home using hand-held glucose meters that analyze a small capillary blood sample.<sup>5a</sup>

Screening cholesterol,<sup>6b</sup> triglycerides, and other plasma lipids is an important component in the management of cardiovascular disease, a leading cause of mortality worldwide.<sup>33</sup> Stroke and diabetes are also linked to high cholesterol, bolstering its importance as a POC diagnostic target.

Creatinine, a byproduct of kidney function, is produced at a constant rate in healthy individuals; its level is diagnostic for defective renal function through estimation of the glomerular filtration rate.<sup>34</sup> Lactate measurements are often performed in the emergency room to provide valuable information about tissue



**Figure 2.** Idealized nucleic acid assay: RNA in a drop of blood is captured by complementary probe strands on the device surface, then labeled by a 2nd complementary strand introduced from solution.

perfusion and the presence of ischemia or hypoxia:<sup>35</sup> elevated lactate often suggests inadequate blood oxygenation.

Renal dysfunction, liver disease, and asthma can often be detected through the measurement of urea and ammonia levels;<sup>36</sup> high bloodstream ammonia levels are related to slow conversion to urea due to liver impairment.<sup>37</sup> Uremia or kidney failure can lead to high levels of breath ammonia,<sup>38</sup> which is well suited to noninvasive breath-test-based POC diagnostics.<sup>39</sup>

Other metabolites of interest include those produced as a result of recreational drug use or illegal substance abuse. The effects of nicotine are well-known, with an astounding 4.9 million deaths per annum; self-testing for nicotine metabolites was suggested by Barnfather et al. as a motivational factor in smoking cessation.<sup>40</sup> POC diagnostics are very popular for the detection of the metabolites of illegal drugs such as cocaine, opiates, and cannabis in workplace or prison settings.<sup>41</sup>

**Nucleic Acids.** Nucleic acid diagnostics, often referred to as “molecular diagnostics” (in seeming oblivion to the molecular nature of proteins and metabolites), measure DNA or various types of RNA in order to assay particular genomic or genetic details of a patient or to assay nucleic acid sequences unique to invading pathogens. PCR (polymerase chain reaction) and numerous other methods of selectively copying (“amplifying”) preselected nucleic acid sequences are often part of such assays, rendering them at once exceptionally sensitive (to a few copies of the target sequence), highly selective (only predefined target sequences are amplified), but also more expensive, cumbersome, and time-consuming than other POC assays.

In POC diagnostic devices, sample volumes are often measured in microliters, and little or no user manipulation should be necessary. Assay design is therefore more challenging than standard DNA microarray-based assays<sup>42</sup> and ideally involves just two specific binding events once any sample preprocessing has occurred: target nucleic acid from the sample is specifically captured on a substrate surface, usually through hybridization with a complementary, surface-bound “probe” DNA, and the captured target is then detected through a second hybridization event between its free end and a complementary, labeled oligonucleotide (normally short, 10–15 bases) (Figure 2). Measurement sensitivity is often controlled by the hybridization efficiency of both events and by the level of background signal in the absence of target. Binding kinetics and target specificity can be modulated by various factors, such as ionic strength, reaction temperature, and probe density. Recent work has shown that low probe densities can lead to higher hybridization efficiencies and more rapid binding kinetics<sup>43</sup> at the expense of a smaller measured signal that may be more susceptible to background interference.

The time to obtain a test result is a differentiating factor for nucleic acid tests. For example, current tests for infecting viruses,

bacteria, and fungi include culturing of organisms,<sup>44</sup> various forms of immunoassay,<sup>45</sup> and nucleic acid testing.<sup>46</sup> Culturing microbes requires several hours to more than a week, rendering it broadly inappropriate for POC testing. Immunoassay is accomplished in minutes to an hour or more (depending on incubation times); the time for nucleic acid assay is similar, including demonstrated best examples that take only 12 min.<sup>47</sup>

Direct detection of viruses, bacteria, or fungi by POC immunoassay often suffers from inadequate limits of detection (LODs), which can be addressed by targeting the rRNA molecules present in high copy numbers (hundreds to thousands) in each cell,<sup>48</sup> often with subtyping (strain-specific) data as a bonus. New RNA targets have been recently identified as appropriate markers for various infectious bacteria, an excellent example being tmRNA, present in all bacterial phyla,<sup>49</sup> now well documented as a molecular diagnostic target.<sup>50</sup>

Improvements and new approaches in POC technologies will enable multiparameter assays that focus beyond identification of individual microbial pathogens to allow multiple antibiotic resistance determinations.<sup>51</sup>

**Human Cells.** The identification and enumeration of specific human cells (and animal cells for veterinary diagnostics) in blood and other samples is a promising and rapidly expanding field in POC diagnostics. In addition to basic blood cell counting, it has been widely recognized that POC cell-assay-based devices could implement diagnostic and prognostic testing for infectious diseases, cancers, inflammatory responses, and hematological parameters,<sup>52</sup> and this vision is beginning to be realized.

The assay of cells in POC format is straightforward in principle: target cells are captured or localized using antibodies, proteins, or aptamers; labeled according to the mode of detection; then enumerated. The presence and numbers of specific receptors on cell surfaces can be assayed by selective immunolabeling, and interactions with particular solution or surface antigens or proteins can be assessed via binding; automated counting of cells on device surfaces is often straightforward.<sup>53</sup>

Whole-cell POC assays will not supplant the clinical laboratory flow cytometer, which can assay 10 or more receptors on each of thousands of individual cells per second, also providing cell morphological information via light scattering. However, these bulky, expensive, complex instruments are limited to well-financed central testing laboratories, providing an opportunity for POC cell tests to fill a number of specialized niches.

**Microbes/Pathogens.** Microbes, viruses, and parasites are an important POC analytical target, particularly those that cause infectious disease:<sup>54</sup> by enabling treatment with the proper antimicrobial agent, rapid identification of the causative pathogen of a serious infection can save significant treatment cost, reduce suffering, help stem the spread of disease, and save lives. Because species and strain identification are required, pathogens are often diagnosed using nucleic acid identification;<sup>8b</sup> in some cases they can be diagnosed (as in tuberculosis<sup>55</sup>) via the specific antibodies that are present in an infected host. Nonetheless, a rapid screen to differentiate, for example, bacterial from viral infection, or simply to detect a pathogen or a piece of it directly can be an important diagnostic step. In some cases, bacterial assay can use antibody capture of whole or fragmented organisms or toxins they produce (as in staphylococcal enterotoxin B<sup>56</sup>), rather than more complex genetic analysis.

Common POC tests for microbes or fragments thereof, along with their applications and performance, have been reviewed by Clerc and Greub.<sup>54</sup> For example, group A *Streptococcus pyogenes*

is detected by selective binding of its carbohydrate antigen; an immunochromatographic (lateral flow) assay that detects the C-polysaccharide common to the cell walls of all pneumococcal serotypes of *Streptococcus pneumoniae*, approved by the FDA in 1999, gives a result in 15 min. The malaria parasite is also detected by an immunochromatographic assay of a particular antigen; each species of the parasite has its own assay.

A laboratory demonstration of immunomagnetic preconcentration/localization and detection of salmonella and *Staphylococcus aureus* using fluorescent assay has been reported.<sup>57</sup> Viruses have also been detected: an influenza rapid POC test sensitive to swine lineage A(H1N1) influenza viruses has been evaluated.<sup>58</sup>

**Drugs and Food Safety.** Recreational drug abuse and doping in competitive sports are on the rise and constitute significant social problems worldwide. The past decade has seen a revolution in the development of tests using alternative specimens for drug analysis: tests utilizing sweat, saliva, and meconium (in infants) have been cleared by the U.S. FDA.<sup>59</sup> Oral fluid offers significant promise when detection of relatively recent use of drugs is sought in a noninvasive manner.<sup>60</sup> Technological advances do allow on-site detection of drugs, but there are technical issues in relation to collection of oral fluid and in the variability of drug concentrations in this fluid. One of the successful commercial examples of an on-site test for oral fluid drugs of abuse determination is Oratect. It utilizes a colloidal gold-particle-based lateral flow immunoassay and combines sample collection and drug testing in a single device.<sup>61</sup>

Doping has become an issue in elite sport and sensitive detection assays that enable the identification of small organic compounds on the microscale are required. Sports drug testing approaches can utilize paper, thin-layer, or gas chromatography methods to reveal the presence of prohibited substances such as strychnine, pervitine, captagone, or benzedrine in doping test specimens.<sup>62</sup> Most sport-doping drugs are also used to treat various diseases such as hypertension (beta-blockers), bronchial congestion (theophylline), congestive heart failure (diuretics), anemia (erythropoietin); many anabolics (steroidal hormones), growth hormone, peptides, stimulants, and narcotics are legal only when prescribed. A brief historical overview and the development of detection methods for illegal substances used in sports, including the very few rapid POC-like approaches, are interestingly summarized in a review by Catlin et al.<sup>63</sup>

The challenge in developing drug detection devices is not only short detection times but also the regulation of the tests to ensure that they provide the right answers. Existing drug testing methods often lack sufficient sensitivity or specificity to the diversified list of drugs of abuse. As is the case with all immunoassays, the probe antibody regulates specificity. Because of the similarity of chemical structures within drug classes (e.g., opiates), commercially available antibodies tend to exhibit high cross-reactivity in immunoassays. As a result, immunoassay-based devices could provide a practical means to analyze high quantities of specimens when the majority are expected to test negative. However, confirmatory chemical analysis (e.g., gas chromatography/mass spectrometry, HPLC, etc.) in a laboratory could be required for any specimens that screen positive.

In the food industry, the deliberate contamination of food materials with low-quality, cheap, nonedible, or toxic substances is referred to as adulteration. Some adulterants are dangerous to health and can even cause death: a recent infamous example is melamine that was the source of a Chinese milk scandal in 2008. Fortunately, the first on-site adulterant detection devices are now

commercially available. Typically dipstick devices, they offer an advantage over spot tests because an adulteration check can be performed at the collection site.<sup>64</sup> Intect 7 (Branan Medical Corporation), a dipstick covered with seven dry reagent pads, tests for creatinine, specific gravity, pH, nitrite, glutaraldehyde, bleach, and pyridinium chlorochromate.<sup>61b,65</sup> Overall, there are more than 50 common adulterants that are classified based on their chemical, bacterial, or fungal origins.

## ■ CURRENT CONTEXT OF POC ASSAYS

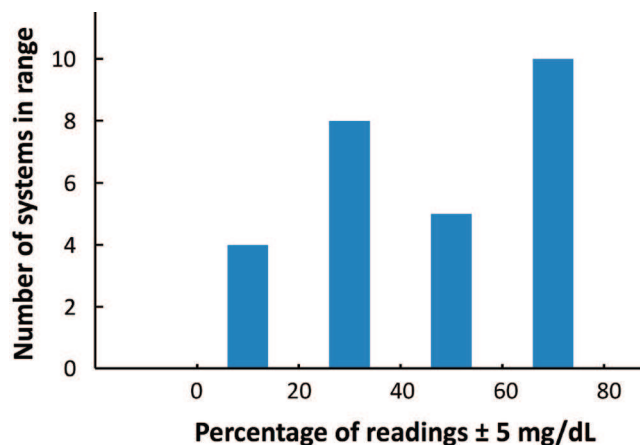
**POC Glucose Assays.** Glucose measurement is the well-established leader in commercial volume for point-of-care testing: glucose test strips for home use are manufactured on an astonishing scale, approaching  $10^{10}$ /year, with single production lines making devices at a rate of  $10^6$ /h using printing and laminating technology. The science and technology of these devices have recently been described comprehensively.<sup>31,66</sup>

The majority of commercial self-test glucose measurement systems are now electrochemical, based on redox-couple-mediated enzymatic oxidation of glucose with either glucose oxidase or glucose dehydrogenase (GDH), the latter having the advantage of faster enzyme kinetics. Several different forms of GDH have been used. Pyrroloquinolinequinone-GDH is not specific to glucose, resulting in a recent warning about the use of devices based on this enzyme for people on certain medications.<sup>67</sup> Advances in recent years have included the reduction in time-to-result to as little as 5 s and necessary blood volume to as little as 300 nL, demonstrating that submicroliter fluidic devices can be reproducibly mass-manufactured and applied.

Glucose detection with these devices relies on measurement of a catalytic electrochemical current; traditionally-measured electrochemical signals are influenced by enzyme reaction kinetics, mediator concentration, and sample viscosity (through diffusion coefficients). Recent commercial developments have aimed to eliminate these influences, such that the precision of measurement is determined solely by manufacturing dimensional tolerances, an example being the microcoulometer described by Heller and Feldman.<sup>66b</sup> Calibration of strips is by statistical sampling, with the results (encoded for example on a barcode) used to set the reader gain and offset for strips from a particular batch.

Evaluation of the analytical and clinical performance<sup>68</sup> of these devices is a complex question, the result depending not only on the performance of the device but also on the training of the person using it, temperature, atmospheric pressure, the way the sample is taken, and characteristics of the blood, particularly hematocrit<sup>69</sup> (red cell content). The current analytical performance requirement is that 95% of individual glucose results fall within  $\pm 15$  mg/dL of a reference measurement at glucose concentrations  $< 75$  mg/dL, and within  $\pm 20\%$  at glucose concentrations  $> 75$  mg/dL. Freckmann et al.<sup>70</sup> performed a laboratory evaluation of the system accuracy of 27 blood glucose monitoring systems using two different reference methods; just 16 of the 27 systems satisfied the performance requirements. For the tighter performance requirement deemed necessary for tight glycemic control,<sup>71</sup>  $\pm 5$  mg/dL for glucose concentration  $< 75$  mg/dL, the systems varied widely in performance (Figure 3): just 4 of the 27 systems had 75% or better of their results within this range.

Some variability no doubt reflects limitations of the error-compensation algorithms meant to deal with sample variability. Unfortunately, it is not possible to identify from the literature

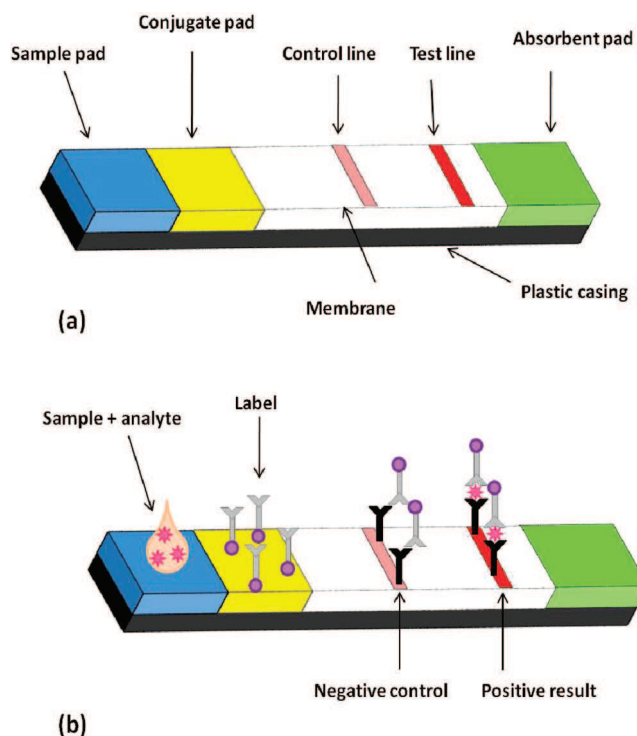


**Figure 3.** Performance of commercial self-test glucose measurement systems, determined under controlled laboratory conditions. Compiled from data tabulated in ref 70.

what specific design variables contribute most. However, a notable design assumption is that, during the filling of the device and in any pause period before the measurement is initiated, glucose in the sample volume is completely converted to gluconolactone as a consequence of a high concentration of enzyme and mediator dispersed into the sample, so that the amount of reduced mediator corresponds to the amount of glucose initially present. By inserting a carbon fiber microelectrode into the sample space in two particular commercial devices, Burt and Unwin<sup>72</sup> measured directly the variations of mediator concentration at different positions within the strip during a glucose determination. They demonstrated unambiguously that the assumption that the enzyme reaction proceeds to completion throughout the entire sample is incorrect.

**Lateral Flow Assays.** The lateral-flow assay (LFA) or lateral-flow immunochromatographic assay, introduced in 1988 by Unipath, is the commonest commercially available POC diagnostic format.<sup>73</sup> Today, POC LFA devices for pregnancy (using hCG levels) and ovulation confirmation, screening for infectious diseases and drugs of abuse, and for measurement of protein markers in blood to aid rapid clinical diagnostics of life-threatening events such as heart attack, stroke, and deep-vein thrombosis are manufactured in very large numbers:  $> 10^7$ /year for pregnancy alone.

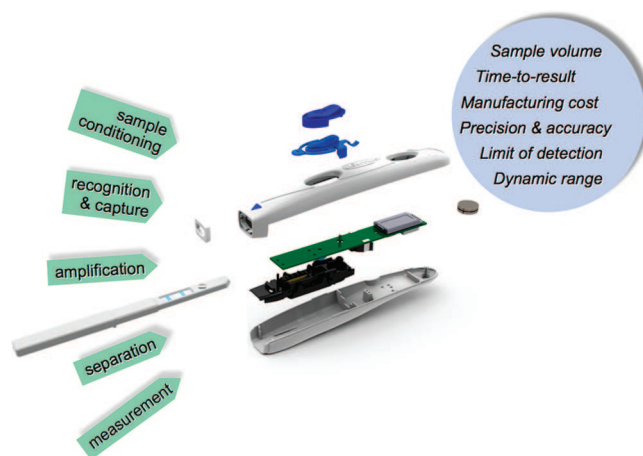
The LFA device incorporates porous membranes, antibodies, and a visible signal-generating system; it depends upon fluid migration or flow technology as outlined in Figure 4.<sup>6b,73</sup> Generation of a response signal begins when a particulate label (commonly colloidal gold or dyed polystyrene or latex spheres), detectable optically at concentration as low as order  $10^{-9}$  M, is dispersed by flow of sample into a volume containing analyte that specifically binds to the label through an adsorbed antibody or nucleic acid fragment. Capture of the analyte by the label takes place during incubation, after which the analyte-decorated label is itself immobilized into a smaller volume. The concentration of captured label in the detection zone thus depends upon the flow rate of the fluid past the capture zone and the average number of bound analyte molecules/particle, itself dependent on the incubation time and kinetics of the capture reaction, which in turn depends on the fraction of the particle surface covered by active binding reagent.<sup>74</sup> With a suitably configured system, LODs of  $10^{-12}$  M are relatively easily obtained.



**Figure 4.** (a) Typical lateral flow immunoassay format. (b) Sample is applied to the sample pad. Analyte present in the sample binds to the antibody-conjugated label, then binds to the test line to return a positive result. If the analyte is absent, the label binds to the control line, generating a negative control result. Adapted from ref 7a [<http://dx.doi.org/10.1039/b820557b>] by permission of The Royal Society of Chemistry and from ref 264, copyright 2010 American Chemical Society.

**Limitations of “Traditional” POC Approaches.** POC devices with single-use test cartridges raise issues about errors and interferences, but there has been relatively little published work on sources of error in POC device designs nor discussion of how errors might be affected by system configuration or particular unit operations implemented within the device. The myriad processes and their impacts are summarized by Figure 5.

Recently, comparison of POC results with those from laboratory clinical analyzers was reported for cardiac markers, particularly cardiac troponin I (cTnI), a challenging analytical task since it requires reliable classification of whole-blood samples near a cutoff of 80 ng/L ( $\sim 3$  pM)<sup>75</sup> of an antigen that requires multiple antibody pairs targeted at different epitopes.<sup>76</sup> Our analysis of the literature indicates that critical issues arise in the dispersion of dried reagents into the sample, in the mixing of reagents and sample, and in the control of incubation time. Furthermore, autoantibodies, heterophilic antibodies, and rheumatoid factor are potentially all present at variable concentrations in blood samples and can cause both positive and negative interference in the commonly used “sandwich” immunoassays for cTnI.<sup>73,77,78</sup> Most modern immunoassays therefore contain materials that block heterophilic antibodies.<sup>77b</sup> In POC test devices, the necessary reagents must be contained within the device, usually being deposited in dried form within a reagent pad or on the base of a channel. The efficacy with which interferences are handled therefore depends upon the efficacy with which the blocking reagents are distributed through the sample, the concentration uniformity of blocking reagent achieved, and control



**Figure 5.** In POC microanalytical systems, unit operations (left-hand side) and overall system specifications (right-hand side) interact such that decisions on one element affect all others. This pregnancy-test device includes optical measurement with digital readout of the LFA-based single-use stick located at left. Device image courtesy of Unipath, Ltd.

of time of incubation with blocking reagent. Furthermore, POC devices necessarily feature filters to remove blood cells, raising the possibility that interactions of blood and filter material, influenced by nonanalyte blood components that vary between samples, can cause variable and unanticipated adsorption of analyte.

The devices studied feature either variants on LFA—on a nitrocellulose strip as in the RAMP (see the Recent Innovations section) design,<sup>79</sup> in a radial configuration as in the dendrimer-enhanced radial-partition immunoassay<sup>80</sup> implemented in the Stratus-CS design, or in a microfluidic channel as in the Biacore design—or a microfluidic implementation of an enzyme-linked immunosorbent assay with electrochemical detection in the i-STAT design (see the Blood Chemistry section for a description of this technology). Clinical evaluation of POC devices for cardiac markers has focused on reliable risk stratification, which has two components: definition of a cutoff level for some biomedical marker above which the risk of a specified condition is considered sufficient for further action and the precision of measurement methods near the cutoff level, which determines the probability of both false-positive and false-negative results. These two parameters are not independent: definition of a cutoff level requires a measurement (with associated probability estimate) to be correlated with a clinical outcome. The evaluation of POC devices has therefore involved two different aspects: comparison of results with reference measurements conducted on laboratory analyzers used to define the cutoff and correlation of clinical outcome with measurement results.

The clinical consequences of variability in analytical results have been assessed: according to Venge et al.,<sup>81</sup> laboratory assays identified significantly more people with elevated cTnI levels than did POC tests (i-STAT and Stratus-CS) and, as a consequence, identified significantly more of the people in the study who subsequently died of cardiovascular disease (80–90%) than did the POC assays (50–60%). They concluded that “the clinical judgment of the patient with suspected myocardial ischemia should not solely rely on results from POC assays.” This illustrates the difficulties of measurement in POC devices where results from single-use cartridges depend on batch sampling and comparison between different cartridges for calibration and where

unit operations (particularly mixing, incubation timing, sample normalization, and rinsing) may not be as well controlled as in a laboratory machine. Over the range 0–17 pM cTnI, both POC devices had roughly constant standard errors of estimate, ~2 pM (50 ng/L), relative to the laboratory analyzer.<sup>81</sup> Both also had very small offset error but also a large number of outliers, which dominated the regression and contributed to the poorer clinical predictive value of the POC results relative to the laboratory analyzers.

In some contrast, Apple et al.<sup>82</sup> reported participation by three different hospitals in a patient specimen and analytical validation study ( $n = 186$ ) for the i-STAT cTnI assay, yielding a regression slope between the POC device and laboratory analyzer for whole blood of 0.9 from 1 pM to 1.5 nM. The results on the clinical samples indicated a tendency to outliers, with the i-STAT indicating low values relative to the laboratory analyzer. However, the standard error of estimate relative to the laboratory analyzer over the range 2–5 pM, assessed using spiked, pooled whole-blood samples, was very small, ~0.5 pM. There was no bias between whole blood and plasma measurements, both made on the i-STAT.

Bock et al.<sup>75</sup> compared cTnI measured by the i-STAT device with that measured by a clinical laboratory analyzer for 557 specimens that initially tested positive by i-STAT. The study covered a very wide concentration range, up to 2 nM. The scatter in correlation was marked at high concentrations; the correlation slope, also dominated by high-concentration values, deviated significantly from unity (~0.6). Over the narrower concentration range 0–2 pM, near the clinical cutoff, Venge et al.<sup>81</sup> show a correlation coefficient of 0.74. Bock et al.<sup>75</sup> reported that the i-STAT cTnI test gave generally reliable patient classifications. However, they also noted that some 6% of values in the range significant for clinical decision-making, 2–4 pM, recorded as negatives by i-STAT, were recorded as positives by the laboratory analyzer. Again, this work highlights the importance of outliers in the results.

Apple et al. concluded that the i-STAT was a satisfactory risk stratification tool (29 positives).<sup>83</sup> Similarly, Lee-Lewandrowski et al. concluded that the RAMP device was a satisfactory risk stratification tool.<sup>84</sup> Examination of the results, as well as those of Wu et al.,<sup>85</sup> shows the importance of outliers. As noted above, there are many aspects of the design of the current POC devices that can give rise to such outliers, including the effects of reagent dispersion, mixing, timing control and, in lateral flow devices, fortuitous cancellation of errors.

## ■ ENABLING TECHNOLOGIES

**Printing and Laminating.** In LFA devices, capture agents are printed as lines at specific locations on the device membrane, followed by a drying process.<sup>73</sup> In the traditional contact-printing mode, a dispenser tip is dragged across the surface of a membrane like a pen while a pump delivers a defined volume of liquid. This method can deform the membrane, causing line width variation. Noncontact printing using tools such as inkjet printers or micro-solenoid-valve-controlled print heads has become more common:<sup>86</sup> it provides accurate control of dispensing volume, accurate positioning, and flexibility in printing patterns. However, clogging of the dispenser tip can be a problem, since the dispensing solution can contain complex protein stabilizers and/or particles such as micrometer-sized beads. Proper process optimization and online vision-based inspection are usually required for the manufacturing process.

It is common for capture DNA to be robotically spotted, in effect a form of printing, onto the solid substrate in predetermined spatial locations. The spots, with volumes as low as 1 nL, typically dry very quickly; spotting variability upon drying is not fully understood and it is very likely a situation of kinetic control, which can be a significant source of the signal variation.<sup>91</sup>

LFA devices and many microfluidic POC devices are fabricated using standard film lamination techniques. In the LFA device, the absorbent pads and porous membranes are traditionally laminated to a plastic sheet for structural strength using pressure sensitive adhesive (PSA); the stack is then cut into test strips that are placed in plastic housings.

In recent years, lamination of polymer layers has been used for the fabrication of a new generation of microfluidics-based POC devices because of its versatility and low cost.<sup>87</sup> In one approach, microfluidic channels are formed in a PSA or thermo-bond (“hot melt”) adhesive sheet using cutting instruments such as a CO<sub>2</sub> laser, metal-blade die cutter, or computer-driven vinyl cutter. The PSA is then laminated between two plastic sheets, with the PSA material forming the sidewalls of the fluidic channel and the PSA thickness defining the channel thickness.<sup>88</sup> Fluidic channels 50 μm tall with variation of less than 5% can be achieved.<sup>66b</sup> This is important because accurate volume control is needed for most quantitative assays. PSA also allows bonding of different materials, such as polycarbonate or nylon filters and cellulose membranes to a plastic sheet.<sup>89</sup> Three-dimensional microfluidic structures can also be formed using this approach by laminating multiple plastic layers with via-holes together using (patterned) PSA films. For example, recent reports demonstrate a fully integrated immunoassay card with dry reagent storage, conjugate pad, and microfluidic channels, enabling quantitative assay with the POC cartridge.<sup>90</sup> The cartridge carries dry reagents that are reconstituted upon use, eliminating the need for refrigerated storage conditions.

A unique new approach uses paper laminated with PSA to form 3D fluidic networks for POC devices, claimed to be suited by virtue of their low cost to applications in the developing world.<sup>92</sup> Narrow hydrophilic conduit pads are defined in cellulose paper by hydrophobic borders formed with various techniques including photolithography, etching, and wax printing. Various assay reagents are printed in the microchannels on layers of paper; the paper layers are then laminated together using PSA with via-holes filled with cellulose powder to wick liquid between paper layers. Sample is introduced at one end of the paper pad and wicks into the hydrophilic fluidic channels. Multiple functionalities including sample preparation and multiplexing have been achieved with this platform.

Typical PSA-based lamination approaches have significant limitations: channel widths are often larger than 400 μm and the side walls are not particularly smooth (on a micrometer scale) due to the limited resolution of most cutting tools. Adhesive material compatibility and thermal compatibility can also be problematic. Direct thermal lamination (bonding) of plastic films and monoliths to one another can address these limitations.<sup>93</sup> Thin plastic films of poly(methylmethacrylate) (PMMA), polystyrene, and cyclo olefin polymer or copolymer (such as Zeonor or Topas) can be laminated to injection-molded or hot-embossed microstructures using heated roller laminators;<sup>92</sup> solvent vapor bonding is feasible as well.<sup>94</sup> These approaches produce sealed microfluidic channels with well-defined walls and tight dimensional control.

**Microfluidic Technologies and Approaches: “Unit Operations” for POC Devices.** Microfluidics has been a significant



component of recent research activity in POC diagnostics. Enabling technologies around microfluidics have been reviewed recently,<sup>95</sup> including centrifugal microfluidics,<sup>96</sup> integration of functionality into polymer-based microfluidic devices produced by high-volume micromolding techniques,<sup>97</sup> unconventional low-cost fabrication and patterning techniques for point of care diagnostics,<sup>87</sup> rapid device prototyping,<sup>98</sup> flexible<sup>99</sup> and thermoplastic<sup>100</sup> substrates, and laser-printer toner and paper-based fabrication techniques.<sup>101</sup> Microfluidic whole-blood immunoassay methods,<sup>3b</sup> including methods of cell and particle separation<sup>102</sup> and methods directed at cardiac marker measurement<sup>103</sup> have been reviewed, as has urine analysis<sup>104</sup> and the emerging field of salivary diagnostics,<sup>105</sup> with discussion of prospective POC applications.

*Pumping and Valving.* Micropumps and microvalves enable precise control of sample, buffer, and reagent flow and delivery. They are necessary for many next-generation POC devices that integrate features such as sample preparation, complex assays that include incubation, mixing, or separation steps and more quantitative outputs.<sup>52b,106</sup> Fluidic pumps and valves can either be integrated into the disposable POC device or into the detection instrument, provided steps are taken to avoid their contamination by diagnostic samples. Both approaches increase cost and can add challenges to device manufacturing and introduce new modes of potential malfunction,<sup>107</sup> so the added performance must be at least commensurate with these drawbacks.

To date, most common POC devices, most notably LFA test strips, rely on capillary-force-driven, passive fluidic flow. LFA strips move samples and analytes using the wetting properties of capillaries in porous substrate materials or integrated wicking pads<sup>86</sup> or, more recently, arrays of microfabricated pillars or posts.<sup>108</sup> Their advantages include simplicity in design, compact form, low cost, disposability, absence of moving parts, and no need for external power. Their limitations are variations in flow rate due to sample viscosity variations, site temperature, changes in the surface properties of the device over time, and poor batch-to-batch reproducibility of substrate materials such as nitrocellulose. To control the flow rate precisely, Cesaro-Tadic et al. used Peltier elements to modulate the evaporation rate at the end of a capillary network.<sup>109</sup> In microfluidic devices, rates of capillary-pressure-driven flow can also be manipulated by controlling channel geometry, using integrated microstructures, and via surface chemistry, as reviewed in detail by Eijkel et al.<sup>110</sup> Recent work by Gervais et al. demonstrated a one-step immunoassay with a fully integrated microfluidic device consisting of a sample collector, delay valves, flow resistors, and capillary pump.<sup>111</sup> Wang et al. also demonstrated a quantitative multiplexed protein barcode-readout assay including on-chip plasma purification from whole blood samples, all with capillary force generated via absorbent paper.<sup>3a</sup>

External pumps, such as diaphragm pumps, peristaltic pumps, and syringe pumps, are common in research-based POC devices.<sup>89b</sup> They offer the advantage of precise flow rate control, but integration of a pump into the detection instrument can limit home care or field use.<sup>112</sup> Miniaturized peristaltic pumps ([http://www.takasago-elec.com/products\\_pump/peristaltic/](http://www.takasago-elec.com/products_pump/peristaltic/)) provide a much smaller version of this laboratory workhorse, with its advantage of tubing being the only wetted material but without the capability to replace the tubing as in larger pumps. Microfabricated reciprocal and rotary displacement pumps<sup>113</sup> can be integrated directly into fluidic cartridges and offer advantages of compact size and large flow rate and pressure ranges; however, their complexity, cost, and power needs can be an issue for POC applications.



**Figure 6.** Abaxis Piccolo Blood Analyzer and CD platform. Reprinted with permission from Abaxis, Inc.

Low-cost pumping mechanisms that can be built into POC devices without electrical power or moving parts are discussed in a pair of excellent reviews.<sup>52b,112</sup> They include human-powered on-chip finger pumps, chemically induced pressure/vacuum pumps, and spring-based pumps.<sup>114</sup> Commercial diagnostic products based on such mechanisms include POC cards by Micronics.<sup>115</sup>

Poly(dimethylsiloxane) (PDMS)-based fluidic networks connected to arrays of external pneumatic valves, extensively developed by Quake and colleagues, can provide both valving and peristaltic pumping to control on-chip liquid flow precisely.<sup>116</sup> While the instruments required to control the networks and the need for a pneumatic pressure source limit the use of such devices for POC applications,<sup>117</sup> these devices may be suitable for benchtop diagnostic applications, such as DNA analysis.<sup>118</sup> Recently, Lee et al. demonstrated incorporation of silicone tubing in an injection-molded plastic device for use as a micropinch valve for RT-PCR-based HIV detection.<sup>119</sup> Zhou et al. (at Rheonix, Ithaca, NY) also reported fabrication and characterization of micropumps through pneumatic actuation of thin polystyrene film.<sup>120</sup>

Spinning CD-like fluidic disks transport samples and reagents by the interplay of centrifugal, capillary, and Coriolis forces; their application in POC and POC-relevant applications has been detailed.<sup>4c,96,121</sup> This technology has been used for clinical analysis for some 4 decades;<sup>96,122</sup> currently, Abaxis, Inc. sells the Piccolo portable clinical chemistry system (Figure 6) for on-site patient testing of multichemistry panels in 100  $\mu$ L of whole blood, serum, or plasma (<http://www.piccololxpress.com/>).

Fluids can be pumped toward the rim of the disk at a wide range of flow rates through control of the spin speed, channel dimensions and surface energy (contact angle), and various geometric details, with temporary capillary “stop valves” (created where a channel abruptly widens or enters a chamber) opened to fluid passage simply by increasing rotational velocity. Steigert et al. demonstrated a system with integrated sample preparation including separating plasma from whole blood, mixing, metering of liquid, and integrated signal enhancement. Rapid detection of glucose, hemoglobin, and alcohol in human whole blood was reported.<sup>123</sup> New developments in the field include integration of the fluidic CD platform with other technologies, such as carbon electrodes for dielectrophoresis,<sup>124</sup> and reports of new immunoassay-based assays from whole blood.<sup>125</sup>

Fluid droplet-based (or “digital”) microfluidics has many (potential) applications in POC diagnostics.<sup>126</sup> Droplets moved by the electrowetting-on-dielectric (EWOD) method are particularly promising, offering electrically controlled liquid movement and adaptability to a range of different bioassays.<sup>127</sup> Droplet

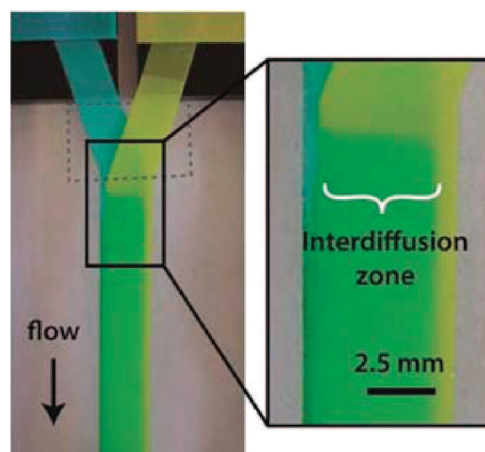
generation, mixing, sorting, and splitting are controlled by a network of electrodes covered with a dielectric coating. Sista et al. demonstrated rapid immunoassay and on-chip extraction and PCR using whole-blood samples with a hand-held instrument and disposable chips.<sup>47</sup> There are, however, some limitations on the use of the device for whole blood samples due to protein surface adsorption, which was avoided by encasing whole blood sample droplets in silicone oil. Companies such as Advanced Liquid Logic are developing POC products based on such technologies.

Electrokinetically based approaches for fluidic movement and separation of charged species have been used many years for a range of applications, including diagnostic assays,<sup>128</sup> with explicit consideration of suitability for POC application of electroosmotic pumping, capillary electrophoresis, electrochromatography, or electrokinetically controlled immunoassay.<sup>7b,129</sup> In addition to traditional DC-powered systems that require high voltages (tens to hundreds of volts, easily supplied by small, high-efficiency power supplies), recent developments in AC approaches such as electrothermal flow and AC electroosmosis also offer potential for POC applications.<sup>107</sup> Sample salt concentration can affect the flow rate and, if high enough, can prevent electrokinetically based approaches, but Huang et al. demonstrated low-power AC-based electroosmotic pumps that move fluid in a high-salt DNA solution without passing current through the salty fluid.<sup>130</sup>

**Mixing.** Resuspension of dried reagents, sample dilution, and reaction of multiple reagents in POC devices often require rapid and efficient mixing. However, mixing in microfluidic platforms is difficult because Reynolds numbers are low ( $<1$ ) so that flow is laminar and mixing is dominated by diffusion unless special measures are taken; efficient micromixing can be achieved through a number of active and passive mixing mechanisms.<sup>131</sup> In active mixing, external driving forces such as acoustic waves, magnetic beads coupled to moving permanent magnets, or actuated air bubbles enhance mixing of samples. In passive mixing, liquids are driven through microstructures designed to increase contact area between the different streams and to speed diffusive or induce chaotic mixing. For example, structures such as the staggered herringbone<sup>132</sup> or modified Tesla structure, which divides a flow into two streams that collide from opposite directions,<sup>133</sup> have been demonstrated to enhance mixing efficiency. There are some excellent recent reviews on mixing in microfluidic platforms in the context of POC devices.<sup>6a,52b,131,134</sup> Some recent advances in mixer design relevant to POC devices are presented here.

Lien et al. presented a membrane-based micromixer relying on air actuation to expand and compress a series of chambers, creating gentle mixing in fluidic channels as part of a platform for leukocyte purification, DNA extraction, and genotyping from whole blood.<sup>135</sup> Air-induced actuation of multilayer PDMS chambers is also used for mixing in urine analysis devices as reported by Lin et al.<sup>118</sup> In acoustic mixing, Ahmed et al. introduced a new mechanism using an external piezoelectric transducer to oscillate bubbles trapped along the side walls of microfluidic channels to produce rapid mixing.<sup>136</sup> Recently, Nath et al. demonstrated a mixing component by direct integration of a PZT (lead zirconate titanate) disk with a laser-cut PSA-based mixing chamber to achieve rapid mixing using trapped air bubbles.<sup>137</sup>

POC platforms that utilize electrical connections or centrifugal forces for pumping, separation, and detection often utilize platform-specific mixing strategies. For example, systems that use electroosmotically driven flow can enhance mixing by introducing restrictions in the microchannel and using an AC electric field



**Figure 7.** Demonstration of mixing in a flat Y-geometry paper mixer. Reproduced from ref 148 [<http://dx.doi.org/10.1039/c004821f>] by permission of The Royal Society of Chemistry.

to generate alternating thin crescent-shaped layers of the two fluids that significantly increase the contact area between the two streams.<sup>134a</sup> In droplet-based microfluidic platforms, mixing is achieved easily by merging individual droplets.<sup>47,127,138</sup> Unique approaches to solving mixing issues in CD platforms using Coriolis-force-induced mixing, rapid oscillation of the disk, and magnetic beads have been reviewed in detail.<sup>4c,96</sup>

Recent advances in passive mixing focus on design optimization to enhance mixing efficiency in short fluidic channel lengths.<sup>139</sup> Tofeberg et al. presented a new design that combines both splitting and recombining liquid streams with microfabricated structures in the channels to enhance mixing.<sup>140</sup> Tsai et al. demonstrated the use of a combination of baffles and a curved channel to enhance mixing;<sup>141</sup> Long et al. presented a 3D vortex micromixer consisting of a single mini-cylinder mixing chamber.<sup>142</sup> Improvement in mixing efficiency also can be achieved through modification of channel surface energy: Swickrath et al. demonstrated high-efficiency passive mixing by a checkerboard pattern of hydrophobic and hydrophilic regions in a microfluidic channel.<sup>143</sup> Photopatterned porous polymer monoliths in channels<sup>144</sup> are another approach to enhance mixing and improve overall chemical reaction rates. Micromixers with passive intestine-inspired serpentine structures were integrated with capillary electrophoresis for pathogen DNA detection at the single-cell level.<sup>145</sup> Choi et al. reported a disposable agglutination device for clinical diagnostics using a serpentine micromixer.<sup>146</sup> The use of micromixers containing a combination of nozzles and 3D pillars reportedly shortened DNA ligation time from 4 h to 5 min.<sup>147</sup>

Mixing mechanisms that are simple, requiring few or no moving parts, are particularly important for in-home POC devices and developing-world applications. Mixing was demonstrated on a paper-based POC platform by simply stacking porous paper strips on top of each other in a flat Y-geometry mixer (Figure 7).<sup>148</sup> Cotton threads with knots used for routing and mixing were demonstrated for potential urine and blood analysis applications.<sup>149</sup>

**Separation.** The genesis of modern microfluidic and lab-on-a-chip devices is closely linked to separations of bio/chemical species on chip, in particular using electrophoresis. LFA, a dominant POC technology, is often referred to as lateral-flow immunochromatographic assay, emphasizing its separations functionality. Separation is important for POC devices: it can increase

target purity, in the process improving LODs and removing interfering agents, background and potential false positives, by separating them from the analytical target prior to detection. Separation is also increasingly critical to POC methods as detection of multiple analytes in one device gains importance (see the Trends, Unmet Needs, and Perspectives section).

Several reviews have described the present state of chip-based separations methods in the context of the components, or “unit operations”, of microfluidic devices.<sup>7a,6a,118,134b,150</sup> Recent developments in electrophoretic separations on microfluidic devices have also been reviewed;<sup>151</sup> Hou and Herr reviewed lab-on-chip affinity-based electrokinetic separations for quantitation of proteins and integration of preparatory functions needed for subsequent analyses of biological samples.<sup>7b</sup> The use of microfluidic methods to separate cells has been reviewed as well.<sup>152</sup> Immuno separations are an inherent component of LFA, which has been reviewed recently.<sup>5c,73,153</sup>

Separation methods applied to POC devices and POC-precursor microfluidic devices include capillary electrophoresis (CE), dielectrophoresis (DEP), isoelectric focusing, various types of liquid (electro)chromatography including micellar electrokinetic chromatography (MEC), optical fields including tweezers and various particle-motivating fields, magnetic motivation and capture, acoustic waves and fields, size-based filtration (using filters, nanostructures, and microstructures), and various combinations of flow, diffusion, and sedimentation-based phenomena, the last of these particularly in centrifugal devices. Some of these methods are better suited to separating molecules, others to particles (including cells), and some work for both.

Separation is based on one or more parameters including charge, polarizability/dielectric properties (at AC or optical frequencies), pK/pH of minimum charge, mass, size, magnetic properties, and physical/chemical/immuno binding interactions. Immuno- or chemical binding is often used to selectively tether the analyte to a particle or label, which may then enable its separation. Common separation metrics include resolution, efficiency, purity, and throughput.

Two processes closely related to separations, because they use many of the same phenomena to implement differential affinity, are treated in the next section, Sample Preparation. Here we summarize some relevant recent advances in separations, a number of which are essentially efforts to integrate established separation methods with various fluidic components or unit operations rather than the development of new separation methods per se.

Jung et al. reported a single-cell-level, multipathogen detection device using a DNA barcode assay. It includes target pathogen magnetic separation as well as capillary electrophoretic separation of DNA barcodes, with a total analysis time of 30 min.<sup>145</sup>

The use of an ion-permeable membrane for chip-based electrophoretic preconcentration followed by CE separation of cancer marker proteins was described by Nge et al.<sup>154</sup> A negatively charged membrane, photopolymerized near the injection intersection of a chip-based CE separation structure, concentrated the target components: cancer markers  $\alpha$ -fetoprotein and heat-shock protein 90 were concentrated over 10-fold in 1 min, then separated by CE.

In a system designed to detect biological toxins using chip-based immunoassays, polymeric gels with large pores were located adjacent to a size-exclusion membrane in order to electrophoretically separate the antibody–analyte complex from the excess antibody prior to detection.<sup>1</sup>

Laurell and colleagues reviewed the transition of acoustic standing-wave techniques from the macro to the micro scale, describing different particle separation modes and surveying potential applications in the medical clinic.<sup>155</sup>

*Reagent Storage.* To make the leap from lab-on-a-chip to practical POC devices, an often-overlooked necessity is the means to store reagents for extended time periods on or in the device.<sup>118</sup> Reagents, including “fragile” molecules like enzymes and antibodies, can be stored in the wet or dry state. The latter is often preferred in those cases where drying (or lyophilization) does not cause total and unrecoverable loss of activity, because reagents that are successfully dried typically exhibit improved stability relative to those stored wet.<sup>52b,112,156</sup> The importance of such storage in the context of global health diagnostics was cited by Yager.<sup>89b</sup>

In many regards, on-chip storage of dry reagents is well developed: LFA strips are dry and include reagents, typically at least one type of antibody and often two, and other reagents as well. Glucose sensors include dried glucose oxidase and electron-transfer catalysts. There is not, however, a single best process for freeze-drying, lyophilizing, or otherwise depositing and drying reagents in a form from which they are readily reconstituted; the successful approach often depends upon details of the reagents, the sample, and the assay. The addition of sugars, trehalose being a favorite, is a widely utilized method to improve bioreagent stability and retention of activity.<sup>52b,112,156</sup> Immobilization of reagents on beads can facilitate storage of reagents in the dry form while removing a solution spotting step from the manufacturing process. For example, protein G beads dried with 80% sucrose were shown to be stable for at least 1 month of storage at 45 °C by McKenzie et al.<sup>157</sup>

An approach reminiscent of LFA methods for on-chip reagents was reported by Stevens et al.<sup>89a</sup> in their microfluidic device implementation of a flow-through membrane immunoassay with on-card dry reagent storage. It utilized both a porous membrane patterned with capture molecules and a fibrous pad containing an anhydrous analyte label; unlike LFA strips, this device relies upon an external pumping and imaging instrument to deliver sample and rehydrated reagent at controlled flow rates, thereby producing more quantitative results. With developing-world applications in mind, the malarial antigen *Plasmodium falciparum* histidine-rich protein II was stored and reconstituted; gold–antibody conjugates were dried in sugar matrices, retaining 80–96% of their activity after 60 days of storage at elevated temperatures. The integrated system gave a respectable detection limit in the subnanomolar range in under 9 min.

A plasma fibrinogen assay was implemented on a polymer micropillar-based LFA platform by drop-casting a mixture of bovine thrombin and the surfactant Triton X-100 onto the dextran-coated platform and air drying.<sup>158</sup> The on-chip thrombin successfully stimulated generation of fibrin from fibrinogen in plasma, leading to clot formation, but chips had to be stored at 4 °C to ensure reagent stability. Using the same dextran-coated pillar-based platform, an assay for CRP was implemented by immobilizing  $\alpha$ CRP antibody in 1% trehalose solution in a line across the chip using a multipass nanodroplet spotting approach to reduce band broadening.<sup>108</sup> This pillar platform was also used for an interferon- $\gamma$  LFA assay via deposition and drying of anti-interferon- $\gamma$  capture antibodies and rabbit antimouse antibodies for the target and control lines, respectively.<sup>159</sup>

Despite an ever-expanding menu of success in storing biological reagents onboard POC devices, fluid in excess of that

contained in a blood, saliva, or urine sample is often required for more complex assays. Large fluid volumes require off-chip storage, but small volumes can be stored within the device with appropriate sealing and release methods. Blister pack technology, well developed by the pharmaceutical industry, has long been a part of POC technology and has recently been reported as a component of lab-on-chip systems.<sup>160</sup> Caution must be exercised when implementing liquid storage using polymer films, many of which have significant permeability to water vapor; PDMS is among the very worst in this regard. Some fluorocarbons and cyclic olefin (co)polymers are significantly better, and most any polymer can be rendered impermeable by vacuum deposition of a thin film of metal such as aluminum. Garcia-Cordero et al. reported long-duration fluid storage using integrated CD-laser-openable thin-film cyclo olefin polymer valves.<sup>161</sup>

**Sample Preparation.** Sample preparation, a necessary analytical step in POC devices prior to analyte measurement, encompasses sample concentration, diffusion, filtration, purification, and fractionation of analytes from analytically noisy background matrices. Although large numbers of POC devices accommodate unprocessed blood samples, the range of assays that can be performed is limited by a lack of well-developed on-chip sample preparation methodologies.<sup>162</sup> Blood, plasma, serum, urine, saliva, and other exudates are all targeted in the development of rapid microfluidic-based diagnostics,<sup>89b</sup> and, according to the details of the assay and measurement to be performed, pre-preparation of the sample may be necessary.

**Phase Extraction and Sample Concentration.** The separation or concentration of analytes based on their physical and chemical properties can improve detection sensitivity in microfluidic formats.<sup>104</sup> Reviews published on sample-concentration techniques<sup>104,163</sup> include developments in liquid-liquid-phase extraction, solid-phase extraction (SPE), isotachopheresis (ITP), immunoconcentration, dialysis, and many more. Lin et al. reviewed many purification and enrichment techniques used in microfluidic urine analysis.<sup>104</sup> Sikanen et al. developed a droplet-membrane-droplet-extraction system to extract acidic analytes from urine, followed by capillary electrophoresis and laser-induced fluorescence detection on-chip.<sup>164</sup> The use of metal ions to separate urea from urea-rich protein samples has also been explored.<sup>165</sup> A five-layer microfluidic system based on diffusion, followed by facilitative diffusion using metal ions such as  $Mn^{2+}$ ,  $Zn^{2+}$ , and  $Fe^{3+}$  allowed for efficient urea removal. Another droplet-based microfluidic system for the passive isolation of T lymphoma cells used PEG droplets that completely encapsulated dextran droplets within a microfluidic channel, partitioning the cells into the PEG phase as they remained in the aqueous droplet.<sup>166</sup>

Capture and purification of RNA via its affinity for  $SiO_2$ , using silica beads immobilized in polymer microfluidic devices, was accomplished for viral RNA from mammalian cells infected with influenza-A (H1N1)<sup>167</sup> and for *E. coli* RNA with pathogen-specific response in under 3 min from the RNA of 100 bacteria using real-time NASBA (nucleic acid sequence-based amplification) for specificity and amplification.<sup>50c</sup> The application of solid-phase extraction to separate and detect psychotropic drugs in plasma samples in a microfluidic format was evaluated.<sup>168</sup> Microfluidic HPLC separation of proteins and peptides has also been established using both methacrylate and styrene-based monoliths.<sup>169</sup>

**Cell Selection and Sorting.** Accurate, fast, and affordable analysis of whole blood is important in clinical diagnostics; however, most analyses require the pre-separation of red blood

cells from plasma due to the interference caused by the cellular components of blood with some measurement techniques, e.g., absorbance and fluorescence. Centrifugal microfluidics offers on-chip autonomy: red blood cells can be separated from plasma using differences in cell density and centrifugally-driven sedimentation.<sup>96,117,121c,125</sup> Plasma separation as part of a centrifugal whole-blood immunoassay system has been reported as well.<sup>125</sup>

Diffusion phenomena have been used in combination with flow in microfluidics to separate smaller, faster-diffusing molecules from larger ones that diffuse more slowly, for example, to extract small molecules from whole blood.<sup>170</sup> A recent paper revisited the concepts of the so-called H-filter and T-sensor technologies and their potential use in size-based extraction of molecules from complex mixtures.<sup>148</sup>

Toner and Irimia summarized the unique requirements and challenges of blood cell separation in the context of whole-blood diagnostic devices.<sup>52</sup> An integrated microfluidic blood analysis system that allows for the separation of plasma from whole blood samples (<5  $\mu L$  in volume) using channels and gravitational sedimentation of red and white cells into filtering trenches (99.9% separation efficiency) on a PDMS chip was recently reported.<sup>171</sup> Separation of plasma from whole blood by a “skimming” process was reported in an integrated device using barcodes for multiplexed protein analysis from microliter blood samples.<sup>172</sup> Continuous enrichment of platelets from diluted whole blood using DEP exploited the fact that platelets are significantly smaller than other blood cells.<sup>173</sup>

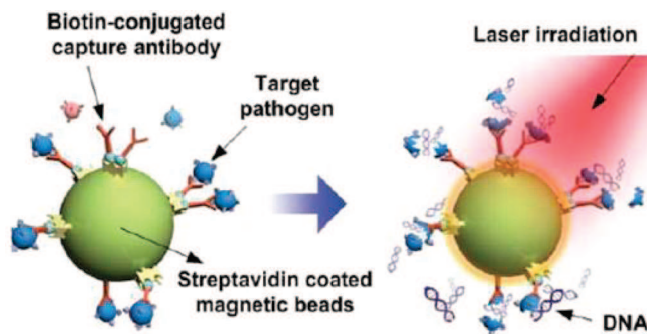
Cell selection also encompasses the use of antibodies to capture target cells in miniaturized devices. Shah et al. reported the specific binding of CD8+ T-lymphocytes on an EWOD platform using antibody-conjugated magnetic beads. Fluid movement controlled by electric signals allowed for close contact between cells and magnetic beads in the droplet, allowing for high binding efficiencies.<sup>174</sup> Magnetic beads coated with antibody have been used in the capture and separation of *Salmonella* and *Staphylococcus aureus*<sup>57</sup> with an effective enrichment factor of ~700–1600, depending on the starting concentrations and ratios of the two cell types. Though significant, this ratio illustrates the challenge of detecting a rare target in the presence of a very common one: a 1000-fold advantage in many cases is inadequate.

**Cell Lysis.** The disruption of cells can be achieved on-chip by a variety of different mechanisms including mechanical or chemical lysis. Vandeventer et al. recently reported on the efficiency of the OmniLyse, a small battery-operated disposable bead blender for lysis of thick-walled *Bacillus* spores and *Mycobacterium* cells in nucleic-acid testing.<sup>175</sup>

A combined chemical/mechanical method for lysis of bacterial cells was reported, where bacterial samples are sheared as they pass through a porous polymer monolith containing detergent lytic conditions, resulting in a concentrated DNA eluent for PCR diagnostics.<sup>176</sup> Stachowiak et al. used thermochemical lysis, a combination of lysis buffer and high temperatures, for protein in their autonomous microfluidic chip for protein profile-based detection of *Bacillus subtilis* cells and spores.<sup>177</sup>

Electroporation allows for the disruption of cell membranes and is used for lysing cells in microfluidic devices. Bao et al.<sup>178</sup> used electroporation to release calcein and protein kinase from cells in a PDMS microfluidic chip.

Cell lysis by means of a low-voltage electrical device on chip was demonstrated by Lee et al.<sup>179</sup> and another group explored



**Figure 8.** Magnetic beads conjugated to target-specific antibody mixed with sample solution. Target pathogens are selectively captured on the magnetic beads and laser irradiation (808 nm, 1.5 W) for 30 s enables DNA extraction from captured pathogens. Reproduced from ref 181 [<http://dx.doi.org/10.1039/b616115d>] by permission of The Royal Society of Chemistry.

the electrolysis of saline solution to generate hydroxide ions ( $\text{OH}^-$ ) at the cathode of the electrochemical device as alkaline lytic agents.<sup>180</sup> In another innovative approach, laser irradiation of magnetic particles (Figure 8) was used for rapid lysis in the extraction of DNA from *E. coli* and hepatitis B virus.<sup>181</sup>

Cell lysis has been performed using an optically induced electrical field in a microfluidic chip.<sup>182</sup> Fibroblasts and oral cancer cells were exposed to an external electric field, which induced a transmembrane potential causing disruption of the cell membrane.

**Nucleic Acid Purification.** In conventional PCR diagnostics, sample preparation steps such as DNA extraction are time-consuming procedures requiring experienced personnel. Recent developments to overcome sample preprocessing include the incorporation of sample purification steps on-chip for DNA extraction from whole blood. An integrated microfluidic platform that incorporates rapid leukocyte purification, genomic DNA extraction, and gene analysis has been reported.<sup>135</sup> Magnetic beads coated with CD15/45 allowed for purification and concentration of leukocytes; DNA was then extracted using surface-charge-switchable DNA-specific magnetic beads in the lysis solution; finally, PCR amplification yielded a turnaround time of 20 min.<sup>135</sup> The use of silica-coated magnetic beads to extract DNA in a microfluidic chip has been reported. DNA binding affinity is high in the presence of high salt concentrations; the captured DNA is released when the salt concentration is reduced.<sup>183</sup> A chip without complex DNA purification steps was introduced by Manage et al.: unprocessed genomic and viral DNA from a whole blood sample was amplified on a three-layer glass chip containing a PDMS membrane for pumping, which interfaced with a miniaturized PCR instrument.<sup>184</sup>

**Protein Preparation.**<sup>154</sup> Martino et al. investigated the use of microdroplet technology to analyze intracellular proteins:<sup>185</sup> cells were introduced onto the microchip and electrically lysed, followed by incubation with antibody-labeled beads in water-in-oil droplets. Protein binding to the beads was then monitored fluorescently within the droplets. Isoelectric focusing has been incorporated into a microfluidic device for the separation of proteins suspended in a microvolume droplet between two Pd electrodes using pH gradients created by electrolysis of buffers with low voltage.<sup>186</sup> The anode fraction was found to be depleted of high-*pI* proteins and the cathode fraction of low-*pI* fractions, indicating this technique's utility in the purification of proteins in small-volume samples.

**Surface Chemistry and Device Substrates.** Despite the important technological innovations described above, considerable progress is required to realize the selective, high-affinity, high-binding-capacity analyte capture methods necessary to push the performance of POC diagnostic devices forward and so enable new applications. Nonspecific adsorption of proteins and other compounds is a critical problem: typically, it effectively controls the background response, directly regulating the device LOD.

Recent trends in the development of immunoassays and biosensor surfaces favor polymer materials over glass, mainly for reasons of cost,<sup>187</sup> although in some cases, e.g., whole-blood assays, where silica surfaces trigger rapid clotting, for biochemical compatibility as well. Although existing chemical methods to prepare biomolecule microarrays on glass can and have been modified to suit polymer substrates, new immobilization strategies have been developed to adapt and often to take advantage of the specific physicochemical properties of the plastic materials.<sup>4a,188</sup>

**Physical Adsorption.** Random interfacial adsorption of biomolecules represents by far the most common immobilization technique in the POC device industry. Molecules typically adsorb on surfaces through ionic bonds, hydrophobic, polar, or electrostatic interactions.<sup>189</sup> While this is the simplest method for capturing biorecognition elements, different antibodies raised against the same antigen can show very heterogeneous binding affinity, undergoing structural changes and exhibiting inadequate orientation of binding epitopes upon adsorption.

The emergence of surface-specific analytical systems has enabled the study of the effects of several factors such as pH, salt concentration, and surface excess of antibodies on the binding capacity for its specific antigen.<sup>190</sup> It is generally accepted that when the activity of physically adsorbed biomolecules is low, this is due to structural unfolding associated with interfacial adsorption. Williams and co-workers reported that the main factor affecting the antibody–antigen binding is related to the surface packing density.<sup>191</sup> Apart from random orientation, loss of activity, and low binding density, an additional drawback of adsorption-based attachment is low bond strength: physically adsorbed proteins may be removed by some buffers or washing detergents during assays.

**Bioaffinity Attachment.** The most common bioaffinity immobilization reaction is based on the specific binding of biotin to avidin and streptavidin. This approach exploits the strongest noncovalent bond found in nature ( $K_d = 1.3 \times 10^{-15}$  M).<sup>192</sup> Biotin-functionalized (“biotinylated”) molecules can be captured using the appropriate (strept)avidin conjugates even in complex media and under harsh conditions during assays.<sup>193</sup> The protein maintains its structural integrity and activity even in 8 M urea or 3 M guanidinium chloride. One of the very few disadvantages of using (strept)avidin is its propensity to bind nonspecifically with compounds other than biotin due to its high carbohydrate content and high isoelectric point. The use of the (strept)avidin–biotin concept in assays has been reviewed extensively.<sup>194</sup>

Other popular affinity immobilization techniques, some of which are highlighted in a pair of recent reviews,<sup>195</sup> include recombinant proteins with genetically engineered histidine-tag systems for site-specific attachment, protein A/protein G-mediated immobilization for specific interactions with  $F_c$  fragments of IgG, and immobilization of glycoproteins through their carbohydrate moiety, which is typically not involved in specific activity.

Efforts have been made to produce artificial ligand–receptor pairs that mimic naturally occurring affinity systems, such as the

(strept)avidin–biotin interaction. For example, Hwang et al. and Rekharsky et al. showed an approach for protein immobilization on gold surfaces based on strong noncovalent interactions between a host cucurbit[7]uril with a hydrophobic cavity and ferrocenemethylammonium or adamantylammonium ions: very good host–guest affinity was demonstrated with high binding constants (up to  $10^{15} \text{ M}^{-1}$ ) and good specificity in aqueous conditions.<sup>196</sup> Other potential strategies based on reversible host–guest interactions exploiting the use of polylysine and cyclodextrin derivatives,<sup>197</sup> supramolecular interactions between an adamantane unit and  $\beta$ -cyclodextrin,<sup>198</sup> and other hydrophobic interactions<sup>199</sup> have been reported.

**Covalent Attachment.** Proteins can be coupled to surfaces by a range of chemical reactions between an appropriately functionalized solid support and many of the complementary functional groups in the amino acid side chains. The most common methods for covalent attachment of proteins to surfaces include the use of amine groups of the lysine residues, carboxy groups of aspartic and glutamic acids, or thiol groups of cysteine residues. Typical examples of compatible groups on the surfaces and the functional group they react with and the related surface performance issues for both nucleic acid and protein microarrays are summarized in several reviews.<sup>42,195</sup> Unfortunately, because of a relatively high abundance of both amine and carboxy groups on the surfaces of proteins, this strategy can lead to problems related to increased heterogeneity and restricted flexibility owing to multipoint attachment.

**Substrate Materials.** Many current and emerging POC platforms are based on substrates made from organic polymers rather than silica.<sup>200</sup> Thermoplastics and new polymeric materials including derivatives of polyacrylates, polystyrenes, polyethylenes, and cyclo olefin (co)polymers (COPs, COCs) stand out as excellent candidates for micrototal analysis system platforms and POC diagnostic devices. Their structures can be selected or modulated to provide excellent optical, thermal, chemical, and biocompatibility properties.<sup>201,202</sup> Plastics are used as structural materials for fluidic chips and cartridges made by methods such as molding, embossing/imprinting,<sup>201,203</sup> etching/micromilling, laser ablation,<sup>204</sup> or die cutting. For the commonly used materials, the pristine polymer surfaces are relatively inert, hydrophobic in nature, and do not possess groups suitable for reactions with capture biomolecules. They are functionalized, therefore, by chemical methods including photografting, oxygen plasma, or UV/ozone treatment, all resulting in formation of a thin, oxidized, hydrophilic layer that can adsorb proteins and oligonucleotides.<sup>188c,205</sup> Such surface films, however, contain many highly reactive and unstable oxidized species that are readily quenched by any impurities and gases/vapors present in air. Moreover, such prepared surfaces provide a low degree of flexibility of the functional groups, and flexibility may be needed for proper orientation of captured biomolecules. A typical solution is to functionalize plastic substrates with organic compounds that can cross-link, polymerize, and form a film from one to a few hundred nanometers in thickness.<sup>206</sup> Because most polymeric substrates are characterized by low chemical resistance to many nonpolar organic solvents and relatively low melting temperatures, the choice of chemical reactions suitable for functionalization of their surfaces is limited.

In general, substrates modified with a range of organosilanes have suitable physicochemical properties for immobilizing proteins and other biologically relevant molecules. The organosilane precursor covalently couples to the surface via formation of

Si–O–Si bonds with the functional group extending from the surface.<sup>207</sup> A number of articles have been published on this subject, most of them addressing the effect of different deposition conditions such as reaction temperature, incubation time, concentration, role of solvent, catalyst, adsorbed water, or curing.<sup>206c,208</sup> The most common techniques rely on activation of the disposable substrate by plasma or UV/ozone followed by immersion into an aqueous or organic solution of the organosilane. Such methods may suffer from a lack of reproducibility in film quality, often due to the fact that organosilanes with multiple Si–X moieties (X = Cl, Br, methoxy, ethoxy, propoxy) tend to polymerize in the presence of even trace quantities of water. The necessity of eliminating water from nonaqueous solvents, along with the solvent waste generated, makes this technique less attractive when bulk quantities of coated substrates are required.

Coatings produced by chemical vapor deposition (CVD) techniques are generally performed at elevated temperatures<sup>208b</sup> and therefore are more suitable for silica-based materials than plastics. Significant advances have been made in the development of a one-step process of surface functionalization based on plasma-enhanced chemical vapor deposition (PECVD). This technique has a number of advantages over multistep, wet chemical methods or conventional CVD: it can be used to coat a large number of substrates in a single batch, it avoids direct contact with solvents, reducing chemical waste, and importantly it operates at room temperature. Also, no major limitations have been observed in the preparation of homogeneous coatings on curved or patterned surfaces or inside microfluidic channels.<sup>209</sup> This method makes it possible to provide better-defined structure as well as the desired chemical functionality using one material on the device surface.<sup>210</sup>

An issue of particular importance to POC devices, due to the increasing prevalence of microfluidic structures, is loss of sample analyte by nonspecific adsorption on channel walls.<sup>188b,211</sup> The manufacturing methods and cost constraints required for making microfluidic systems beyond laboratory volumes constrain the materials that can be used, which in turn constrains the nature of the surfaces that can be engineered, both to minimize nonspecific adsorption and to maximize specific binding activity for target capture. Plasma polymerization is an industrially scalable method for surface layers with a variety of different chemical functionalities, and its application to biochip preparation has recently been reviewed.<sup>212</sup> Alternating current plasma polymerization has been used to deposit poly(ethylene glycol) (PEG)-like coatings from an 18-crown-6 precursor, and the nonfouling characteristics of these coatings have been demonstrated.<sup>213</sup> The applicability of low-energy plasma-enhanced chemical vapor deposition to prepare surfaces with high specific binding activity and low nonspecific adsorption has been demonstrated.<sup>188c,206</sup> Significant protein analyte adsorption onto untreated PMMA surfaces has been demonstrated, and a low-energy PECVD method of making a PEG-like coating from diethylene glycol dimethyl ether precursor proved effective as a means of minimizing such analyte loss.<sup>188b</sup>

**Detection.** In annual manufactured volume, electrochemical (billions of glucose strips, millions of blood chemistry cartridges) and optical (tens of millions of LFA devices for pregnancy and other tests; millions of fluorescence-based assays for cardiac and other disease markers) technologies are the clear leaders. The glucose technology (see above) is an example of label-free, indirect detection: the amperometrically detected product is a proportionate surrogate for glucose concentration in the blood

sample. LFA pregnancy tests are labeled assays: antibody-based binding of gold nanoparticles produces a colored line if sufficient hCG is present in the urine sample.

Detection has been reviewed as one component of POC or “towards POC” devices.<sup>6a,11a,106,214</sup> Some reviews with substantial detection sections are more specifically focused on immunoassay and protein/biomarker-based diagnostics;<sup>3b,103,118,215</sup> nucleic acid testing for infectious disease;<sup>8b</sup> cancer diagnostics;<sup>216</sup> lateral flow<sup>153</sup> and centrifugal<sup>96</sup> platforms; and for global health and limited-resource settings, where instrumental complexity should be minimized.<sup>52b,112</sup>

*Electrochemical Detection.* Electrochemical detection methods include amperometric, potentiometric (including self-amplified ion-sensitive field-effect transistors, ISFETs), and impedimetric<sup>215,217</sup> measurements. The first, used for glucose assays, is most common and typically generates current in proportion to the concentration of the detected species; all three methods are used by the modern version of the i-STAT chemistry analyzer, depending on the target analyte.<sup>52b</sup>

While some analytes are electroactive and can be measured directly without labeling,<sup>218</sup> electrochemical detection often utilizes tagging for analyte specificity with either an electroactive species or an enzyme that converts an electrochemically silent species into electroactive one; this approach also provides signal amplification of multiple orders of magnitude, with detection limits below 1 pM readily accessible. For example, Hoegger et al. reported an electrochemically measured ELISA determination of the folic acid content of food products.<sup>219</sup> Staphylococcal enterotoxin B was captured with specific antibodies and detected via the enzymatic production of an electrochemical signal from a second specific antibody labeled with horseradish peroxidase.<sup>220</sup>

Label-free impedimetric sensing was demonstrated for detection of salmonella at the 1000 cfu/mL level using antibody binding to capture the bacteria on electrodes.<sup>221</sup> Myoglobin, indicative of cardiac damage, was detected at 100 ng/mL via conductivity changes resulting from its antibody-based capture,<sup>222</sup> and similar specific capture of the stroke marker neuron-specific enolase, without addition of a label, was reported at the 0.5 pg/mL level.<sup>223</sup> Such impedance changes can be measured using microfabricated electrodes or, in a device that is more a “bio-resistor” than an electrochemical device, via binding to carbon nanowires.<sup>224</sup>

Assays based on nanoparticles bearing DNA barcodes<sup>225</sup> have been used to specifically detect proteins with electrochemical transduction,<sup>226</sup> and a disposable electrochemical immunodiagnostic device based on nanoparticle probes, stripping voltammetry, and LFA technology was reported.<sup>227</sup> An amperometric immunosensor system was developed, with onboard reagent storage, for the detection of the breast cancer markers carcinoembryonic antigen and cancer antigen 15-3; it was demonstrated for the analysis of patient serum samples.<sup>228</sup> A thrombin-generation amperometric assay in plasma and whole blood has been reported.<sup>229</sup> The use of electrochemical detection means for LFAs in general has been explored.<sup>230</sup>

*Optical Detection.* Optical detection methods used for POC applications include fluorescence<sup>111</sup> with such variants as Förster resonance energy transfer (FRET<sup>231</sup>) and up-converting phosphor technology;<sup>232</sup> (chemi)luminescence;<sup>57,233</sup> absorbance (colorimetry); surface-plasmon resonance (SPR); and various categories of light scattering: Rayleigh (particles much smaller than wavelength), Mie (particles comparable to wavelength; shape-dependent), geometric (particles larger than wavelength),

resonant (wavelength overlaps an electronic transition of the particle), and Raman (vibrational quanta added to or subtracted from the excitation wavelength). Absorbance is by far the commonest due to its use in LFAs based on gold or polymer (nano)particles, while fluorescence is used for the broadest range of different types of POC assays<sup>111</sup> for reasons of sensitivity and, more recently, the ready availability of a range of different colors of efficient fluorophores, including quantum dots,<sup>231a,234</sup> quantum-dot barcodes,<sup>235</sup> and fluorescent nanoparticles,<sup>236</sup> providing improved limits of detection, in some cases reaching single-particle LODs, and enabling multitarget multiplexing. A recent review by Myers and Lee surveys those recent innovative optical detection techniques that meet such POC-relevant criteria as reasonable cost, ruggedness, and ease of integration with fluidic technologies.<sup>237</sup>

A recent variant of fluorescence, so-called supercritical angle fluorescence (SAF), detects only fluorescence emitted in close proximity to a fluorophore-supporting optically transparent chip surface.<sup>238</sup> This method provides substantial enhancement of fluorescence collection efficiency while rejecting background from unbound fluorophores or impurities, as it confines the fluorescence detection volume to material within about one wavelength of the chip surface. An imaging SAF scanner to detect multiple assays on one chip was developed and demonstrated using 200 nm-diameter fluorescent beads.

Major recent advances in the variety, quantum efficiency, output power, and affordability of light-emitting diodes and diode-pumped solid-state lasers (e.g., as in laser pointers and DVD players) is bringing a wide range of excitation wavelengths to compact POC readers without requiring major compromises to LODs that would be imposed by low-intensity light sources. Electroluminescence excitation has also been explored, in one example for detection of botulinum neurotoxin A.<sup>239</sup> In another example of utilization of recent technological development, colorimetric and fluorescent readers utilizing smartphone integrated cameras have been reported.<sup>240</sup>

Most optical methods are based on labeling the analyte by attaching a chromophore, fluorophore, or particle<sup>241</sup> (dye-containing, semiconductor/quantum dot, noble metal,<sup>231b</sup> or scattering) to an antibody or nucleic acid strand that confers specific recognition. The use of gold nanoparticles in molecular diagnostics was reviewed by Radwan and Azzazy.<sup>231b</sup> SPR is an exception, requiring only specific adsorption of the analyte onto an optically appropriate, target-selective surface; examples are reported below in the Label-Free section.

Nanoparticles including quantum dots are finding increasing application. For example, determination of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels was recently reported.<sup>234</sup> Quantum dots were employed on-chip for CD4+ T-cell counting in a POC application.<sup>160</sup> Aptamers were tethered to gold nanoparticles as part of an LFA-like dry-reagent assay strip to detect thrombin.<sup>242</sup>

Thermal-lens microscopy (TLM), an alternative to fluorescence detection, also benefits from dye labeling. TLM detection was integrated into a miniaturized ELISA device including all optical, electronic, and fluidic components necessary to provide an LOD of 2 ng/mL for total IgE measurement.<sup>243</sup>

*Magnetic Detection.* Magnetic particles are a promising technology for POC diagnostics because they can be used to preconcentrate and localize analytes and, although the beads may have different optimal sizes or compositions according to their function, they can also be used as a labeling technology for detection

without the requirements of fluors for optical transparency. Spurred by advances in memory devices, magnetic particle detection technology has evolved rapidly,<sup>244</sup> the most promising and sensitive methods now using the giant magnetoresistance (GMR) effect with detectors based on so-called spin valve (SV) or magnetic tunnel junction (MTJ) methods.<sup>245</sup> The particles must be in close proximity to the detector for good sensitivity, placing some constraints on fluidic device design. Wang and Li review GMR SV sensors for magnetic nanotags for biosensing, showing from their own results that as few as 14 monodisperse 16 nm superparamagnetic ferrite nanoparticles can be detected by submicrometer SV sensors at room temperature.<sup>244</sup> Tang et al. reported on the feasibility of siloxane-coated CoFe<sub>2</sub>O<sub>4</sub> nanoparticles as the basis for a GMR-SV biosensor, with the goal of single-molecule detection.<sup>246</sup>

Philips Research (Eindhoven, NL) reported the development of a compact biosensor platform to detect biomolecules with superparamagnetic particle labels using GMR sensors with integrated field-generating wires.<sup>247</sup> The silicon detection chip is packaged in a disposable cartridge that integrates electrical connections for readout and fluidic subsystem. Philips recently reported sensitive detection of amplified DNA on this system using a miniaturized detection platform suitable for POC application: using various tag-antibody combinations specific for individual genes, they demonstrated multianalyte detection of several antibiotic resistance-associated genes of the pathogen salmonella.<sup>248</sup>

**Label-Free Methods.** Methods appropriate for label-free POC detection include SPR, devices based on mechanical transduction, and direct electrochemical and optical transduction for analytes possessing suitable characteristics. Wang et al.<sup>249</sup> developed a magnetic nanoparticle-enhanced biosensor in conjunction with SPR: a sensor surface was modified with antibodies to capture the target analyte,  $\beta$  hCG. Another microfluidic device based on SPR was developed by Nilsson et al.<sup>250</sup> for the detection of influenza, where recombinant hemagglutinin proteins were immobilized on the chip surface and a change in the SPR response was detected upon binding of the target. An immunoassay incorporating an electro-microchip, gold nanoparticle detection, and silver enhancement for signal amplification was documented by Su et al.<sup>251</sup> High detection sensitivities were shown with both IgG and protein A immobilized on the chip surface.

Mechanical transducers for POC applications oscillate or resonate, including micro- and nanocantilevers (reviewed by Waggoner and Craighead for environmental and biological applications<sup>252</sup>), as well as various acoustic wave devices such as the quartz-crystal microbalance (QCM) and a range of devices in the surface acoustic wave family (reviewed by Rocha-Gaso et al. for biosensor applications<sup>253</sup>). Operating characteristics such as frequency and signal attenuation for piezoelectric devices or resistance and amplitude for piezoresistive (silicon) devices are affected by the mass and mechanical properties of molecules and materials linked to their oscillating surfaces: like SPR, they require only an immobilized selective recognition layer. Nonetheless, “mass tags”, dense particles (typically Au) that bind selectively to the target, can significantly enhance sensitivity.

So-called “bond rupture sensors,” reviewed by Hirst et al., use acoustic energy to rupture bonds between immobilized capture antibodies and target microbes. Because the energy and frequency of added energy can be adjusted, nonspecifically and specifically captured particles can be desorbed differentially according to the

bond strengths and masses, providing a unique discrimination mechanism.<sup>254</sup>

The use of aptamers as biorecognition moieties tethered to QCM biosensors,<sup>255</sup> as well as in cantilever-based biosensors, has been described.<sup>256</sup> Silicon microcantilevers are challenged when operating in liquids due to damping of their motion, but Ricciardi et al. report better performance for microplates than microbeams, with a Q factor of 140 determined for a device that senses the tumor marker angiopoietin-1.<sup>257</sup>

**Enabling Multiplexed Assays.** Detection of multiple analytes with a single POC test is well established in select cases: e.g., for blood chemistry (the i-STAT device) and for cardiac damage markers (Biosite’s Triage system). Multiplexed detection is a positive differentiating feature in many cases and is a necessity in others: many assays are unreliable without a positive control, and some require a negative control as well.

Multiplexing can be achieved spatially: detection of different analytes occurs in different locations on a substrate, e.g., by patterning spots of different selective-capture antibodies or nucleic acid oligomers; by separation prior to detection, e.g., by capillary electrophoresis followed by fluorescence detection; or by differentiable labels, e.g., different emission-wavelength fluors or particles, spectrally distinct Raman tags, or distinguishable nanobarcode. One well-developed tool (from Luminex, Inc.) is based upon a series of defined ratios of two different dyes dissolved in polymer beads: the antibody or capture oligomer for a given target is immobilized on beads with a particular dye ratio, and readout in a cytometry-like fashion includes identifying the bead type according to the ratio of the two dyes (excited by a common wavelength) and quantifying the bound target using a different excitation wavelength for a fluorescent label. This method was used in a feasibility demonstration of a POC multiplexed saliva-based biochip test for acute myocardial infarction.<sup>258</sup> Other multiplexed POC examples are presented in the Trends, Unmet Needs, and Perspectives section.

## ■ RECENT INNOVATION

**Lateral Flow Assay Technologies.** The limitations of LFAs were discussed above, and efforts to address the critical issues of error and accuracy should target control of the sample volume into which the label is dispersed, uniformity of dispersion, and flow rate, which is the main determinant of contact and incubation times. In fact, a fortuitous cancellation of errors can occur because the flow rate determines the total volume of label-containing sample that passes over the measurement zone within the fixed measurement time while it also determines the contact time of sample with label before the capture step (a higher flow rate means a shorter incubation time and hence smaller capture efficiency). Thus, a simple strip design can give satisfactory semiquantitative results, a feature exploited by the recently developed “digital” pregnancy test system wherein measurement of optical density on the capture zone<sup>259</sup> is used to estimate the hCG concentration<sup>260</sup> in the sample, providing both an indication of pregnancy and an estimate of the time since implantation<sup>67</sup> (<http://www.clearblue.com/uk/new-clearblue-digital-pregnancy-test-with-conception-indicator.php>).

Different commercial designs for quantitative LFA utilize different implementation strategies to improve measurements. In the case of cardiac marker detection using the Rapid Analyte Measurement Platform (RAMP, from Response Biomedical Corp., BC, Canada; [www.responsebio.com](http://www.responsebio.com)), the sample is mixed



and incubated with label and buffer in a separate tube before application to the strip, significantly improving this aspect of the test. A ratiometric measurement of the intensity of the test line relative to a control line is also made, small variations in the flow rate being expected to affect the two lines similarly.

Materials are another area of recent development. Standard LFA materials—nitrocellulose, polyester, rayon—suffer from varying degrees of nonspecific binding, brittleness, and sensitivity to humidity.<sup>261</sup> One study of more reliable alternatives implemented a coagulation assay on glass and microstructured COP lateral flow platforms.<sup>262</sup> As microclots formed around the pillars, fluorescently labeled fibrinogen was incorporated into the clot. The sensitive detection of fluorescence on this device was correlated to the heparin concentration in blood samples.

A recent advance in traditional LFA devices, driven by the demands of increasingly quantitative POC applications, is integration of new materials with 3D pore structures having well-controlled pore-size distributions that enable more consistent protein binding and control of fluid flow rates. For example, Whatman's Fusion 5 can act as a sample pad, conjugate release pad, and membrane for test and control lines, consolidating the various materials previously used in LFA devices into a single material, generally simplifying assay and manufacturing process development.<sup>86</sup> Capture lines are formed by printing 2  $\mu\text{m}$  latex beads conjugated with capture agents. The beads are confined in the porous material and form capture lines with the sample and labeled conjugate mixture.

Challenges with nonspecific adsorption and batch-to-batch reproducibility of nitrocellulose and other traditional LFA device substrates have driven the development of injection-molded plastic chips with well-defined microfabricated pillars as an alternative to porous membranes.<sup>108</sup> This is the basis of the 4Cast-Chip developed by Amic AB (Uppsala, Sweden); the technology was subsequently purchased by Johnson & Johnson/Ortho Clinical Diagnostics. The molded cylindrical pillars, typically a few tens of micrometers in diameter and with similar spacing, are made hydrophilic by dextran coating to drive capillary flow of the sample and conjugate. The pillars also allow covalent linkage of capture proteins for a fluorescence-based assay.

Traditional LFA labels, colored or fluorescent particles including colloidal gold and latex or polystyrene beads,<sup>73,261</sup> have on occasion been replaced with carbon or selenium.<sup>7a</sup> More recent advances include the use of quantum dots, paramagnetic particles, upconverting phosphors, and electrochemiluminescent labels.<sup>6b,153</sup> Carbon nanostrings, elongated nanoparticles containing smaller spherical particles, were used for the ultrasensitive detection of DNA in a lateral-flow hybridization assay.<sup>263</sup>

A sensitive LFA for the detection of trichloropyridinol, a metabolite marker for exposure to pesticides, was developed using quantum dots, advantageous in this assay for their higher brightness, resistance to photobleaching, and simultaneous excitation of multiple fluorescence colors.<sup>264</sup> Quantum dots have also been employed in the quantitative detection of ceruloplasmin, a biomarker for cardiovascular disease and lung cancer.<sup>265</sup> Ceruloplasmin was combined with quantum-dot-labeled antinitrotyrosine and captured by anticeruloplasmin antibodies in the test zone of the LFA to produce a fluorescence signal. A LFA for protein biomarker detection using a portable fluorescence system with quantum dot labels was reported.<sup>265</sup>

Song and Knotts reported an LFA using time-resolved (fluorescence) measurement of bright, long-lifetime europium-containing phosphorescent nanoparticles conjugated to a monoclonal

antibody for CRP (an inflammatory marker) in serum over a 0.2–200 ng/mL working range.<sup>266</sup> Their portable reader prototype detects 2.5 ng of immobilized phosphorescent particles with a 1000-fold dynamic range.

Upconverting phosphor technology (UPT) has been used to label targets in LFAs.<sup>232,267</sup> UPT uses rare-earth-doped ceramic (nano)particles, often coated with silica for improved bio/chemical compatibility and ease of functionalization, that absorb near-infrared light and emit a visible signal. This approach minimizes background signal relative to conventional fluorescence labeling. Two studies adapted UPT for increased sensitivity in the detection of infectious diseases in saliva<sup>232,267</sup> and a third integrated UPT with microfabricated COP pillar-based fluidic devices, instead of the typical nitrocellulose support matrix, to detect the cytokine interferon- $\gamma$  with a 3 pM LOD.<sup>159</sup>

Detection in LFAs is no longer limited to optical measurements. One study detected prostate-specific antigen (PSA) using an electrochemical transducer: immunochemical events on the assay strip were detected as shifts in capacitance.<sup>269</sup>

Development of an LFA/ELISA system based on superparamagnetic nanobeads as labels, linked to a monoclonal antibody, for rapid detection of cTnI, was recently reported by Xu et al.<sup>270</sup> Magnetic detection measured cTnI binding to the test zone; the LOD was 10 pg/mL.

Commercially available lateral flow assays using fluorescent labels and FRET for increased sensitivity include the RAMP (see above) and the Triage Cardiac Panel from Biosite, Inc. (San Diego, CA). Both enable sensitive detection of cardiac proteins such as myoglobin and cTnI in whole blood or plasma.<sup>6b,18</sup>

Digital readout LFA systems now integrate the means to calculate and display analyte concentrations, offering quantitative data not available from standard visual color-change readouts. Examples include Metrika's (Sunnyvale, CA) Digital Response (DRx) for measuring hemoglobin levels<sup>6b</sup> and the Clearblue Easy digital pregnancy test system described above.

**Proteins. Antibodies.** Most POC diagnostics are based on affinity techniques, with the target analyte captured by antibodies.<sup>271</sup> An extensive array of antibody-based POC devices currently exists, as outlined under Diagnostic Targets. Recent developments in the area of antibody-based POC diagnostics include research into a broad panel of disease markers, novel detection methodologies, and new microfluidic formats.

Commercial antibody-based POC devices have most commonly used traditional LFA technology. In addition to ultrahigh-volume pregnancy and glucose tests, LFA tests have been developed for markers of celiac disease: CeliacSure from GlutenPro (Mississauga, ON, Canada); BioCard Celiac Disease from Ani Biotech (Vantaa, Finland). Raivio et al.<sup>272</sup> reported that whole-blood POC devices compare favorably with conventional serological central lab-based tests in their comparison study of the BioCard Celiac Disease microfluidic device. For the diagnosis of hepatitis C, Lee et al.<sup>273</sup> evaluated the use of the OraQuick Rapid HCV Antibody Test from OraSure Technologies, Inc. (Bethlehem, PA). This technology is an indirect immunoassay method in an LFA device format and can be used with venous/capillary blood, serum, plasma, and oral fluid samples.<sup>273</sup>

HIV diagnostics continue to be an active area in antibody-based testing. The Dual Path Platform (DPP) chromatographic immunoassay, developed for HIV and syphilis (Chembio Diagnostic Systems, Medford, NY; www.chembio.com), combines a specific antibody-binding protein conjugated to colloidal gold dye particles and HIV 1/2 antigens bound to the membrane solid

phase. The INSTI HIV1/HIV2 rapid antibody test (bioLytical, Richmond, BC, Canada; www.biolytical.com) detects HIV antibodies in blood, plasma, or serum by sample application to the on-chip membrane, which contains HIV-specific proteins; HIV antibodies cause a color change of the membrane spot.

Commercial devices using latex agglutination technologies include the Prolex-Blue from Pro-Lab Diagnostics (Richmond Hill, ON, Canada), which allows for serogrouping of  $\beta$ -hemolytic streptococci. Well-known commercially available immunofiltration devices include the Nycocard and Afinion systems from Axis-Shield (Oslo, Norway) for blood and urine testing of CRP, D-dimer, HbA1c, U-albumin, and creatinine.<sup>6b</sup> These devices work on the principle of filtration through porous membranes containing immobilized antibodies that detect the target analyte.

Companies working on the development of POC devices incorporating immunoassay technology include Vivacta (Kent, U.K.; www.vivacta.com), who have brought POC devices to the market for the detection of thyroid-stimulating hormone (TSH) and cardiac markers including troponin and brain natriuretic peptide (BNP); Chirus (Watford, U.K.; www.chirus.com), who have developed the DXpress reader for measuring cardiac and pregnancy biomarkers as well as drugs of abuse; and Philips (Best, The Netherlands), who supply the Magnotech device for cardiac and sepsis markers (<http://www.business-sites.philips.com/magnotech>). POC optical immunoassays are also available from ThermoBioStar (Louisville, CO; www.thermofisher.com) for the detection of *Neisseria gonorrhoea* and group B *Streptococcus*. Immunoassay cardiac marker POC detection-based products launched in the past 3 years include Cardio 3 (Alere International, Waltham, MA), a triple-analyte test for troponin I, BNP, and CK-MB (creatinine kinase MB).

Blood testing has seen several recent POC advances. Verax Biomedical (Worcester, MA; www.verax.biomedical.com) developed the platelet PGD test to address bacterial contamination of red blood cells: a preprepared platelet sample is applied to the plastic chip with a colored line appearing in the presence of Gram-positive or Gram-negative bacteria. Instrumentation Laboratory (Bedford, MA) received FDA approval in 2011 for infant bilirubin (tBili) testing, an immunoassay performed on their GEM Premier 4000 critical care analyzer. The ABORhCard (Micronics, Seattle, WA; www.micronics.net) contains anti-A, anti-B, and anti-D antibodies in its microfluidic channels for blood type (A/B/O and Rh factor) determination upon blood sample application.

Rapid diagnostics for monitoring protein levels, enzymatic activities, and modifications of mitochondrial proteins in mitochondrial disease have been reported by Marusich et al.<sup>274</sup> This device has a lateral flow dipstick immunoassay format with four spatially separated two-site immunocapture assays, with one monoclonal antibody (mAb) specific for the target protein on the capture zone, while the second labeled mAb specific for a different epitope on the same protein is introduced with the sample. Unlike mass spectrometry, this approach can be adapted into functional POC tests in the characterization and diagnosis of disease.

A microfluidic electrochemical immunoassay for urinary hippuric acid (HA) was integrated with a PDMS chip attached to a glass substrate with patterned electrodes.<sup>275</sup> The chip contains a chamber to store antibodies that bind to HA antigens. Unbound antigens enter the reaction chamber, resulting in a redox reaction on the electrode surface that correlates with the concentration of HA.<sup>275</sup> Another novel microfluidic format includes the development

of a portable disk-based fully automated ELISA based on colorimetric detection through integrated photodiodes and LEDs for infectious disease detection.<sup>125</sup>

**Protein Expression and Purification.** To characterize specific gene products, it is necessary to express and purify recombinant proteins in a variety of expression hosts quickly and efficiently. Such recombinant proteins are then required for the production of antibodies, development of functional assays, identification of interacting proteins, and characterization of their native structures. The need for fast and efficient preparation of active proteins and the relevant determination of the optimal conditions for expression and purification of recombinant proteins is mainly fueled by the pharmaceutical industry, but progress in this area is beneficial to POC test development. A POC device for quantification of recombinant proteins was described by Enomoto et al.<sup>276</sup> They reported a novel double-epitope tag approach, composed of 19 amino acids, that provides a rapid method to detect recombinant proteins via a homogeneous sandwich immunoassay.

**Nucleic Acids.** Nucleic acid assays are the fastest growing component of biomedical diagnostics, replacing or complementing culture-based, biochemical, and immunological assays in microbiology laboratories and, very recently, at the point of care. Detection of viruses and other pathogenic microorganisms, mutations causing human genetic disorders, cancer, hypertension, and other lifestyle-related diseases widely rely upon genetic testing. In this section we present advances in amplification (replication) of nucleic acid targets, the synthetic nucleic acids known as aptamers, and two rapidly growing application areas: infectious diseases and food safety.

The most commonly used technique for gene amplification, necessary in many cases to keep sample sizes reasonable and to reduce the complexity of the analytical task, is PCR with optical detection. The PCR process is complex, requiring a thermal cycler for the reaction; if multiple targets are to be detected in one reaction, electrophoresis and fluorescent labeling separates the amplification products and renders them detectable. Only a few years ago, this combination of techniques appeared inappropriate for applications requiring a small footprint and ease of use, but significant effort has been invested in the development of compact, cost-effective methods to detect nucleic acids.

A recent report on real-time PCR assays for influenza A and B viruses describes the determination of type (A or B) and subtype (H1, H3, or H5) using a single-step/single-reaction vessel format.<sup>277</sup> Archived reference strains were compared to uncultured primary clinical samples (throat swab/nasal wash). The A- and B-specific assays detected all 16 influenza type-A viruses and both currently circulating influenza type-B lineages (Yamagata and Victoria). The assay has a detection threshold of approximately 100 target molecules. These assays are said to be appropriate for field deployment for POC screening during a pandemic influenza outbreak.

An application of a quadruple-allele dipstick assay for the simultaneous visual genotyping of the two key components of the innate immune system, responsible for initiating an inflammatory response against microbial pathogens, was recently presented by Litos et al.<sup>278</sup> The method involves PCR amplification of the region spanning the two polymorphic sites, followed by a single primer-extension reaction for all four alleles, requiring only minutes.

Tomita et al.<sup>279</sup> employed an alternative amplification method to classical PCR. Their detection system, using loop-mediated

isothermal amplification (LAMP), allows for visualization of substantial alteration of the fluorescence during the one-step amplification reaction, requiring some 30–60 min. Another sensitive amplification technique, isothermal rolling circle amplification (RCA), was reported by Stougaard et al. to provide single-molecule detection and quantitative results. RCA has enabled the detection of biomarkers at the attomolar concentration level.<sup>280</sup>

In DNA microarrays, optical methods based on fluorescence detection are the standard for quantifying hybridization between surface-immobilized probes and fluorophore-labeled analytical targets. Electrochemical detection techniques are emerging that can replace physically bulky optical instrumentation in support of portable devices for POC applications. Defever et al.<sup>281</sup> described proof-of-principle real-time PCR using cyclic voltammetry to indirectly monitor the amplified DNA product generated in the PCR reaction solution after each PCR cycle. The design requires the addition of only a minute amount of redox catalyst to the PCR mixture; rapid detection is claimed despite poorer sensitivity than optically based real-time PCR (the exponential amplification provided by PCR can often compensate for limitations in sensitivity). An electrochemical DNA microarray system has been available for several years from Combimatrix (<http://www.combimatrix.com/>).

Multiplexed and specific detection of ferrocene-conjugated DNA targets, as well as real-time monitoring of hybridization on an active electrochemical biosensor array, was reported by Levine et al.<sup>282</sup> This approach, based on fully integrated standard complementary metal-oxide-semiconductor (CMOS) technology, is a potential basis for portable DNA diagnostic platforms.

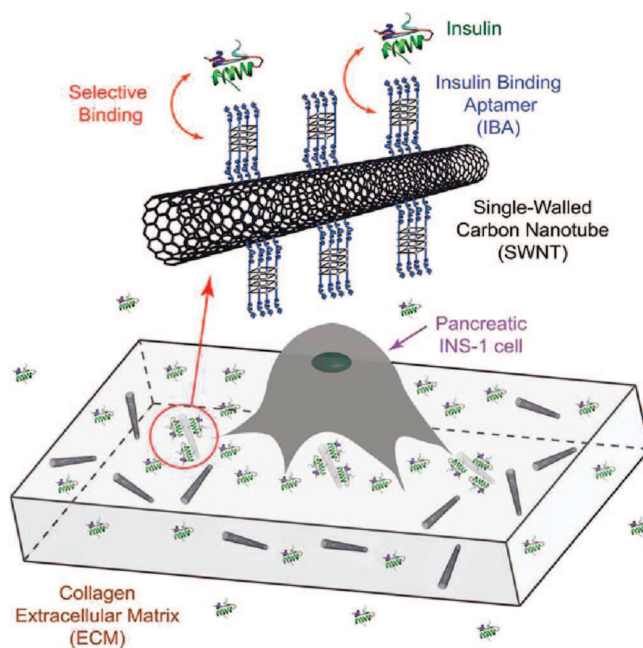
An interesting overview of the technologies of colorimetric biosensors based on DNA-nanoparticle (NP) aggregation assays was presented by Sato et al.<sup>283</sup> The authors compared two types of DNA-NP aggregation assays: aggregation based on the cross-linking of gold NPs and a more novel noncross-linking system.

**Aptamers.** Aptamers, a synthetic alternative to biologically derived antibodies, are single-stranded oligonucleotides that can be DNA or RNA or can be peptide-based; they are synthesized in a combinatorial variety of base sequences, then screened to discover those that bind specifically to target molecules.<sup>11</sup> Aptamers have desirable properties including increased stability (relative to many antibodies), ease of production (relative to biological production of antibodies), ease of manipulation, and ease of modification.<sup>242,268</sup> The application of aptamers to biosensors was recently reviewed in depth by Iliuk et al.<sup>269</sup>

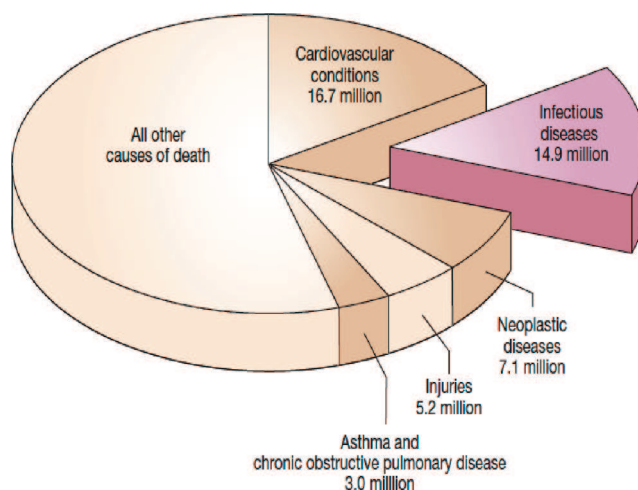
Cass and Zhang describe how the access of a solution-phase redox species, or the impedance at an electrode, is modulated by the binding of a target nucleic acid oligomer to an immobilized selective capture aptamer to produce an analytical signal.<sup>271</sup> Similar strategies were used to detect platelet-derived growth factor<sup>284</sup> and thrombin<sup>285</sup> using aptamer probes. The electrochemical detection of bacteria using carbon nanotubes coated with aptamers has also been reported.<sup>286</sup>

Optical biosensors are appropriate for use in conjunction with aptamers, which are easily labeled with fluorophores and chromophores. Xu et al.<sup>242</sup> presented aptamer-functionalized gold nanoparticles in a dry reagent lateral flow biosensor for thrombin analysis, with excellent selectivity due to the lack of interference from casein, IgG, or IgM, which often interfere with antibody-based assays.

Novel optical biosensor platforms using near-infrared fluorescent single-walled carbon nanotubes (SWNTs), functionalized with aptamer DNA for the real-time detection of cell-signaling



**Figure 9.** Optical nanosensor for insulin measurement from pancreatic cells. SWNTs are functionalized with insulin-binding aptamer DNA to recognize target insulin, which is optically detected in the near IR via quenching. Reprinted from ref 287. Copyright 2011 American Chemical Society.



**Figure 10.** More than 25% of total annual deaths are caused by infectious diseases, with respiratory infections and HIV/AIDS being responsible for the majority of the deaths. Published by the World Health Organization and reprinted from ref 288 by permission from Macmillan Publishers Ltd: *Nature* 2004, 430, 242–249, copyright 2004.

molecules, such as insulin released from pancreatic cells, have been reported (Figure 9).<sup>287</sup>

**Infectious Diseases and Food/Water Safety.** New and re-emerging infectious diseases, including pandemic viruses and drug-resistant bacteria, represent serious health and global security threats: more than 25% of 57 million annual deaths worldwide are related directly to infectious diseases (see Figure 10), not including the millions of deaths due to past infections.<sup>288</sup> Toxicity

and infections caused by foodborne pathogens also represent an increasing public health problem, and diagnostic tests in multiplex format are needed for the rapid identification of food contamination caused by such microbial species as *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. (40 000 cases/year in the United States alone).<sup>289</sup>

Most state-of-the-art diagnostic devices suitable for pathogens rely on PCR-based procedures.<sup>290</sup> They are typically sensitive and accurate; in particular, real-time PCR (qPCR), in addition to being an automated high-throughput-compatible technique, allows quantification of foodborne pathogens.<sup>291</sup> Other promising pathogen-detection techniques include an isothermal target-and-probe amplification method based on a combination of isothermal chain amplification and FRET cycling probe technology<sup>289</sup> as well as automated DEP-facilitated image analysis<sup>292</sup> that targets single-cell LODs.

**Blood Chemistry.** The principles of the i-STAT device, an electrochemically based POC blood chemistry analyzer with cartridges consumed by the millions each year, have been described by Lauks<sup>30</sup> and in the patent literature.<sup>293</sup> It features an electrochemical cell in a microchannel, with buffer and reagent pouch and diaphragm pump, operated by a plunger incorporated in the reader. Air bubbles separate reagents, sample, and wash solutions. An enzyme label (e.g., alkaline phosphatase) converts an electrochemically inactive substrate (e.g., *p*-aminophenol phosphate) to an electrochemically active one (e.g., *p*-aminophenol). An interesting aspect of the design, not discussed in the literature, is the use of convective effects in the meniscus of a moving fluid to promote mixing and dispersion of reagents dried into the channel. A recent description in the patent literature reveals significant improvements in device design: Lowe et al.<sup>294</sup> describe a system in which a step in a fluidic channel is used to form an interface between a whole blood sample and a buffer, stored in a pouch on the test cartridge. Antibody-sensitized magnetic beads, dried onto the walls of the inlet channel, are incubated with the sample by agitation using a motor-driven magnet. After the incubation period, the beads are drawn into the buffer, further washed by agitation, and then moved to the detection zone. An electrochemical scheme using horseradish peroxidase, coupled to the oxidation of glucose, acts as amplifier and signal generator.

**Coagulation Markers.** Coagulation testing has joined the leaders in POC testing due to the well-defined need for anticoagulation monitoring<sup>295</sup> after such common procedures as hip surgery. Devices include clot-based assays that measure the physical clotting time of blood and clotting-factor assays that allow for more accurate diagnoses of clotting factor deficiencies using immunoassay.

Conventional clot-based assays have been on the market for many years, including prothrombin (PT), activated partial thromboplastin time (aPTT), activated clotting time (ACT), and thrombin clotting time (TCT) assays and have been reviewed in detail.<sup>18,296</sup> The most common commercial coagulation POC format is the HemoSense INRatio system (HemoSense Inc.) for PT/INR (international normalized ratio) home-testing, which uses electrochemical impedance to measure the onset of clotting via the interaction of a blood sample with a PT activator.<sup>295</sup> One of the cartridges for the i-STAT analyzer measures PT/INR amperometrically: thrombin generated during clotting cleaves an electrochemical substrate. Many POC coagulation devices use mechanical or optical detection or a combination of both such as the Hemochron Signature (ITC), GEM PCL Plus system (Instrumentation Laboratory) or the Thrombotrack Solo

(Axis-Shield). These systems can execute a range of clot-based tests using the appropriate clotting reagent (PT/aPTT/TT) and measuring such parameters as the physical changes that occur during clot formation or the increasing occlusion of light as clotting progresses.<sup>296a</sup>

Hereditary thrombotic disorders resulting in clotting factor deficiencies cannot be detected using conventional clot-based tests, which has led to the development of more specific factor assays, now trending toward immunoassays. Assay targets include D-dimers and fibrinogen degradation products (FDPs), with increasing interest in developing POC devices that target coagulation markers such as protein C, protein S, antithrombin, and factor V Leidin. Numerous POC devices for measuring D-dimer exist on the market. The CARDIAC D-dimer assay from Roche Diagnostics (Indianapolis, IN) is a quantitative POC device where two D-dimer recognition antibodies form a sandwich complex with the D-dimer, which is taken up by a streptavidin line in the detection zone via the biotinylated antibody. This gold-labeled antibody results in a color change and a red signal line is quantitatively recorded by the reader.<sup>297</sup>

The SimpliRED D-dimer assay from BB International (Cardiff, U.K.) is a whole-blood agglutination assay where the on-chip reagent causes the red blood cells to visibly coalesce ([www.d-dimer.co.uk](http://www.d-dimer.co.uk)). Immunoturbidimetry is used in the MiniQuant D-dimer assay from Kordia (Leiden, The Netherlands; [www.kordia.nl](http://www.kordia.nl)), in which antibody particles agglutinate, increase turbidity, and cause changes in light scattering. The AQT90 Flex D-dimer assay from Radiometer (Brønshøj, Denmark; [www.radiometer.com](http://www.radiometer.com)) uses time-resolved fluorescence detection.<sup>298</sup>

In the laboratory, whole-blood aggregometry is considered the gold standard for platelet function testing. POC devices that assess platelet function have been reviewed extensively.<sup>296a,b,299</sup> Two commonly used POC platelet function assays include the PFA-100 (Siemens Medical Solutions, Malvern, PA), which uses shear to induce platelet adhesion, activation, and aggregation,<sup>296b</sup> while the Plateletworks device from Helena Laboratories (Beaumont, TX) uses an impedance-based cell counter.<sup>296a,300</sup> Thromboelastography (TEG), a global test of hemostasis, generates information about clot strength and stability.<sup>301</sup> TEG is now being adapted for mapping platelets through platelet inhibition and activation, as with the TEG Platelet Mapping System from Hemoscope Corporation (Niles, IL).<sup>296a,300</sup>

In the research arena, a rapid POC device for measuring plasma fibrinogen concentration based on a single-use lateral flow microfluidic chip has been developed.<sup>158</sup> As a plasma sample comes into contact with a thrombin-coated polymeric microstructured LFA device, flow is arrested and the distance traveled by the sample can be correlated with fibrinogen concentration.

An immunosensor for the detection of FDPs using quartz crystal microbalance technology combined with latex agglutination was reported by Aizawa.<sup>302</sup> Thuerlemann et al.<sup>229</sup> have developed an amperometric test strip for the detection of thrombin, whereby cleavage of the substrate is detected electrochemically. This approach could be used for factor V Leidin and activated protein C resistance screening in plasma and whole blood samples. More recently, an aptamer-based polymer microfluidic device for measuring thrombin was developed incorporating the use of magnetic beads and quantum dots.<sup>303</sup>

**Whole Cells.** Cell-based analyses implemented in miniaturized devices suitable for POC application now range from red and white blood cell counts to platelet function and platelet-protein interaction assays in whole blood. VanBerkel et al.

recently reported a microfluidic device for three-part differential leukocyte count (granulocyte/lymphocyte/monocyte) plus erythrocyte (red cell) and thrombocyte (platelet) counts in human blood; data were corroborated with clinical laboratory analyses.<sup>304</sup> Neutrophil (the most abundant white cell) migration is a key phenomenon in the immune response to bacterial infection, and assays have been developed using less than 10  $\mu\text{L}$  of whole blood to perform chemotaxis under the influence of competing chemokines.<sup>305</sup>

Platelet function is critical to hemostasis, and platelets are implicated in everything from cardiovascular disease to the hematogenous spread of cancer. Platelet function analysis, including POC methods, was surveyed in 2005,<sup>299d</sup> and platelet function is now assayed clinically using the commercial Verify-Now POC system,<sup>306</sup> which infers function from platelet aggregation measurements. A sufficient volume of clinical results has been amassed for concerns over limitations of this approach to be expressed.<sup>299c</sup>

The microfluidic means to directly assess platelet function in whole blood via dynamic interactions with immobilized proteins under conditions of arterial shear flow have been reported recently.<sup>307</sup> Platelet activation statistics have been assayed (and correlated with antiplatelet drug effects) from their binding occupancy on arrays of platelet-sized surface spots of proteins and antibodies.<sup>53</sup>

Counting T lymphocytes (particularly CD4+, in some cases supplemented by CD3+ or CD8+, cells) is an effective means to stage and monitor HIV-infected patients.<sup>308</sup> A label-free POC-appropriate CD4-cell-counting device using microfluidics<sup>309</sup> aims to keep costs low enough for developing world applications. Integration of semiconductor quantum dots into nanobiochip systems for enumeration of CD4+ T-cell counts in a POC-type device has been reported as well.<sup>160</sup>

Assaying the small numbers of circulating tumor cells (CTCs), sometimes just a few per milliliter of blood, that are present in early stages of metastasis or cancer recurrence is particularly challenging. Although point-of-care measurement is not a prerequisite for such assays, microfluidic technologies are nonetheless being applied to this needle-in-a-haystack problem, with limited preliminary success. Aptamers have been used for capture and gold nanoparticles for labeling to detect cancer cells in just 15 min,<sup>310</sup> although a minimum of 800 cancer cells must be captured for detection. Arrays of crescent-shaped capture structures were employed to isolate CTCs from blood, at a very realistic cell density of 1–3 CTCs/mL, with impressive capture efficiencies for three cancer cell types.<sup>311</sup>

The use of microfluidics for cell separations of all sorts was recently reviewed.<sup>152</sup> In a recent application of so-called digital microfluidics, a droplet-based device was used to isolate human T lymphoma cells.<sup>166</sup> A diagnostic device for bovine mastitis was demonstrated using a compact-disk-format centrifugal platform to separate white cells from whole milk samples by centrifugal sedimentation.<sup>312</sup>

## ■ TRENDS, UNMET NEEDS, PERSPECTIVES

**Glucose.** The trends in glucose POC devices are toward higher accuracy and minimally- or noninvasive devices directed at continuous monitoring. Advances in electrochemical device technology and the technology for protective membranes have been significant.<sup>66b</sup> Noninvasive measurement has progressed based on advances using infrared or Raman<sup>313</sup> spectroscopy. While it is relatively simple to measure a noninvasive signal that

correlates with in vivo glucose concentrations, it is considerably more difficult to construct glucose calibration models that prospectively provide accurate glucose concentrations in human subjects:<sup>314</sup> calibration requires a significant number of invasive measurements covering a range of glucose concentrations and needs to account for many confounding variables. Caduff et al. demonstrated some success using nonspecific measurements of dielectric response in three frequency ranges (kilohertz to gigahertz) and optical reflectance at three different wavelengths.<sup>315</sup> Progress has been significant toward autonomous closed-loop control of insulin levels—effectively, an artificial pancreas combining continuous glucose sensing with an insulin pump—and a system from Medtronic is undergoing testing, but challenges remain.<sup>316</sup>

**Global Health and the Developing World.** The development of POC diagnostic devices for limited-resource settings, including the developing world, is a very active area<sup>52b,112,317</sup> due to the remarkable need and substantial funding from public and private sources: the U.S. Global Health Initiative, the Bill & Melinda Gates Foundation (Seattle), the Program for Appropriate Technology in Health (PATH; Seattle), the UK Department for International Development, the Foundation for Innovative New Diagnostics (FIND; Geneva), and the European Union's Seventh Framework Programme (FP7) are some of the major initiatives. Infectious diseases have attracted particular attention and activity in the context of global health POC diagnostics, including HIV, tuberculosis (TB), and malaria.

The current status<sup>318</sup> and emerging issues<sup>319</sup> relevant to tackling HIV through robust diagnostics in the developing world have been reviewed. The status of research and barriers for development of point-of-care tests for infectious diseases prevalent in developing countries<sup>317c,320</sup> and particularly for active TB have been reviewed.<sup>55</sup> Studies of the antigens used for TB diagnosis have been analyzed,<sup>321</sup> with a conclusion that POC tests are urgently needed, particularly where TB is endemic. To do this, a number of very specific challenges must be overcome: robustness, storage, cost, and ease-of-use. The context in which the diagnostics must operate, appropriate diagnostic technologies already in distribution, opportunities for innovation, adaptation, and cost reduction, along with some emerging technologies that promise to address this challenge, have been reviewed.<sup>317b</sup> One issue, highlighted by Peeling and Mabey, is that substandard tests have undermined confidence in the use of POC devices; they conclude that appropriate systems for quality control of POC tests needs to be developed if they are to achieve their maximum potential.<sup>322</sup> In this context, system design is clearly important, and one principle is, arguably, that people are more reliable than machines in limited-resource environments: the operation of this idea can be seen in the system presented by Chin et al.,<sup>323</sup> which used silver enhancement of captured gold colloid-labeled antibodies, where the device itself is very simple, no more than a set of channels carrying antigen characteristic of specific diseases (HIV and other sexually transmitted diseases) and a simple optical reader, but the flow control is done with a hand-operated syringe. Reagents (which hence define specific aspects of the technique) are chosen for their stability, are stored separately from the chip, and flow sequentially through the chip separated by air bubbles.

Stimulated in part by a drive to overcome issues of cost and to improve performance of simple devices designed for use in limited-resource economies, there has been much recent work on assay configurations that use inexpensive substrates and readily available reader devices such as camera phones.

Breslauer et al. describe mobile phone-based clinical microscopy.<sup>324</sup> Weigl et al. have reviewed progress toward development of disposable, low-cost, easy-to-use microfluidics-based diagnostics that require no instrument at all. They present examples of microfluidic functional elements, including mixers, separators, and detectors, as well as complete microfluidic devices, that function entirely without any moving parts or external power sources.<sup>112</sup> In a similar vein, a lab-on-a-tube (LOT) device for POC measurement of multiple analytes was described.<sup>218b</sup> It uses passive capillary force or active suction to avoid the need for a pump or injection components, making it in some sense a microfabricated implementation of the passive LFA approach. Evolving low-cost technologies for the control and measurement electronics associated with POC devices is quite relevant.<sup>325</sup>

Paper, one of the original substrates conceived for lateral flow assays but subsequently displaced by porous nitrocellulose on account of the control that this material offers over flow and protein adsorption,<sup>326</sup> has experienced a resurgence of interest in academic research.<sup>327</sup> Patterning is easily achieved by impregnating the paper with a hydrophobic material by printing,<sup>327f</sup> and hence structures for precise control of the flow can be realized.<sup>328</sup> One of the central questions in bioactive paper development is the impact of the properties of the cellulosic support on both immobilization and functionality of biomolecules. A recent review on inkjet printing of biomolecules for bioactive paper applications reports progress made in understanding factors that affect the activity of biomolecules physically immobilized on cellulosic supports.<sup>329</sup> Paper-based microfluidic devices have been combined with optical detection using a mobile phone camera for telemedicine.<sup>317a</sup> The use of multiple colorimetric indicators to extend the dynamic range has been reported.<sup>330</sup> Channel networks can be set up to enable the automated sequential delivery of multiple reagents to a detection region with a single user-activation step, for example, to implement signal enhancement with comparable ease of use to conventional lateral flow tests.<sup>331</sup> 96- and 384-Microzone plates fabricated in paper as alternatives to conventional multiwell plates fabricated in molded polymers have been illustrated; quantitative colorimetric correlation using a scanner or camera to image the zones has been demonstrated.<sup>332</sup> Electrochemical detection and measurement on paper-based devices, using simple apparatus similar to the reader for a commercial glucose device, has also been demonstrated as an alternative low-cost technology.<sup>218a,333</sup> Impedance measurements in fluidic devices have been implemented using a portable music player and a laptop soundcard.<sup>334</sup>

Thread has been explored as a means for low-cost device construction,<sup>149a,335</sup> with fluid moving through the thread by capillary action. Networks can be constructed by weaving, and if the thread is sewn through a hydrophobic material, complex fluidic structures can be constructed and devices incorporated into bandages, for example. Colorimetric assays have been demonstrated on thread-based devices to detect ketones, nitrite, protein, glucose in artificial urine and alkaline phosphatase in artificial plasma.<sup>149a</sup> "Switches" that control when or where flow can occur, or allow the mixing of multiple fluids, have been successfully prototyped from multifilament threads, plastic films, and household adhesive.<sup>335b</sup>

**Personalized Medicine and Home Testing.** Personalized medicine tailors healthcare to individuals, using their genotypic and phenotypic details to complement generic guidelines derived from large-cohort studies, e.g., for choices of medication and dosage. Driving this trend are the potential for better healthcare

for the patient at lower cost, along with the salvaging of thousands of orphan drugs that are safe and effective only for some of the population, who can typically be identified by genotyping. For some drugs, personalization may be as simple as adjusting the dosage to a patient's own metabolism as well as current physiological status: home POC measurement of clotting time, for example, keeps a patient's anticoagulant level in the important window between spontaneous internal clotting and a dangerous propensity to bleed. Huckle concludes that anticoagulant therapy monitoring is now joining diabetes and pregnancy as a significant POC success.<sup>336</sup>

Antiplatelet drugs, including aspirin and Plavix, are intended to diminish the risk of vascular occlusion. These agents are not uniformly effective for all patients and efficacy may vary with a patient's health status, suggesting a role for POC platelet function testing to personalize dosage or choice of drug.<sup>307,337</sup>

Selected infectious disease testing could save time and money through POC home testing. The home market for POC HIV,<sup>338</sup> chlamydia, and other sexually-transmitted disease (STD) tests is significant and growing. Streptococcal pharyngitis ("strep throat") home diagnosis in children would save many doctor visits, as would reliable differentiation between rhinoviral and upper respiratory bacterial infection, an important ancillary benefit being to diminish untold numbers of prescriptions for antibiotics, written inappropriately and usually under patient-exerted duress, for a common cold with no proven bacterial component. This practice currently causes problematic antibiotic resistance.

As the average population of the developed world ages, healthcare and well being will increase their economic and social importance. Significant markets for home testing could emerge for everything from cardiovascular health to osteoporosis to a range of nutritional deficiencies, once the right combinations of biomarkers and affordable technologies are developed.

**Technology Trends.** Low-cost polymer substrates, paper and thread microfluidics, and low-cost readers using smart phones were discussed above. Digital/droplet microfluidic devices are actively researched and just beginning to reach the POC. The line between "traditional" (e.g., porous nitrocellulose) lateral flow assay devices and microfluidic platforms is blurring; in most cases, clever design of the platform will result, for high-volume manufacture, in the onboard reagents and packaging costing more than the fluidic consumable.

While PCR remains a workhorse for nucleic acid amplification, the trend in diagnostics research is to consider a range of related techniques as well, including RCA, LAMP, NASBA, MDA, TMA, SDA, and LCA (rolling-circle amplification, loop-mediated isothermal amplification, nucleic acid sequence-based amplification, multiple-displacement amplification, transcription-mediated amplification, strand-displacement amplification, and ligase chain reaction, respectively), as well as cleavase Invader. Importantly, many of these methods are isothermal and operate at lower temperatures than PCR, making their integration with microfluidic technologies more straightforward.

In detection, new optical methods exploit phenomena including up-conversion, high-brightness nanoparticles, total-internal-reflection fluorescence (TIRF), SAF, FRET, and a range of plasmon-based effects. In many cases, nanoparticles are the reporter for optical detection. The above technologies enhance signal, reduce background, or both; combined with low-cost, compact high-intensity solid-state light sources, this drives POC LODs ever lower. The trend now is for either nonspecific response or limitations of

antibody–antigen binding affinity to determine the LOD, rather than the performance of the detection system.

The significant trend in selective recognition is the use of aptamers in place of antibodies for the specific binding of proteins. Despite a fairly small number of high-target-specificity successes, this technology has generated considerable activity because it replaces highly complex biomolecules produced by living organisms and often varying from batch to batch and vendor to vendor, with synthetically generated reagents.

A number of POC-suitable applications of nanobiotechnology are surveyed by Jain.<sup>339</sup> Nanotechnology for membranes, filters, and sieves has been reviewed as well.<sup>110</sup>

**Multiplexing.** Detecting multiple analytes in a single POC test is an important trend: many of the most promising new POC opportunities are in multianalyte tests or panels, including cancer markers, cardiac health, and infectious disease. Testing for infection-causing pathogens in a blood sample is a particularly important future POC application: it could replace laboratory culture-based analysis requiring hours to days for organism growth, the results of which sometimes arrive too late for a patient with a bloodstream infection that leads to septic shock and death. To identify the causative pathogen and thus select the proper antibiotic, genetically specific testing for ~8–30 different microbial strains should provide identification for 80–90% of all serious infections. Quantum-dot barcodes are one approach that has been studied for multiplexed infectious disease diagnostics.<sup>340</sup>

Sexually transmitted infectious diseases are a challenge in both low-resource and developed environments. A single POC device that includes the commonest of the STDs will save time and money, and rapid results can help reduce the spread of STDs by ensuring that patients learn their status and how to treat it before leaving the clinic or hospital.

Monitoring cancer treatment efficacy or checking for recurrence usually requires tracking multiple biomarkers. The determination of cancer markers in serum and saliva using quantum dot bioconjugate labels has been reported.<sup>234</sup> A fluorophore-based biobarcode amplification protein assay has been researched,<sup>225</sup> and multiantigen fluorescence immunoassays also have been demonstrated using a microfluidic device to provide 100 separate assay chambers.<sup>341</sup>

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**Leanne F. Harris** graduated from Trinity College Dublin in 2001 with a primary degree in Natural Sciences, specializing in Zoology. From there she completed her M.Sc. in University College Dublin in 2002 and received her Ph.D. in Biological Sciences from the Dublin Institute of Technology (DIT) in 2006. Her Ph.D. focused on the development of immunodiagnosics and molecular assays for pathogen detection and control in shellfish. She subsequently lectured on the Biomedical B.Sc. and the Medical and Molecular Cytology M.Sc. courses throughout 2006. From DIT, she moved to DCU in 2007 and began working as a postdoctoral researcher in the Biomedical Diagnostics Institute under the supervision of Prof. Anthony J. Killard. Her work for the last 4 years has involved the development of point-of-care coagulation-based assays and devices using impedimetric, colorimetric, and fluorometric techniques. Her research interests and expertise range from biological assay development and clinical testing to the development of miniaturized biosensors, device fabrication, and reagent printing methodologies.

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