

# Chapter 1

## Point-of-Care Diagnostics

Rapid tests that are low-cost and portable are the first line of defence in healthcare systems. Dipstick and lateral-flow are the two universal assay formats as they are lightweight and compact, and provide qualitative results without external instrumentation. However, existing formats have limitations in the quantification of analyte concentrations. Hence, the demand for sample preparation, improved sensitivity and user-interface has challenged the commercial products. Recently, capabilities, sensors and readout devices were expanded to multiplexable assays platforms, which might transcend the capabilities of existing design format of diagnostic tests. This chapter outlines the evolution of diagnostic devices and current trends in the development of qualitative and quantitative sensing devices for applications in healthcare, veterinary medicine, environmental monitoring and food safety. The chapter also discusses design parameters for diagnostics, their functionalisation to increase the capabilities and the performance, emerging sensing platforms and readout technologies. The factors which limit the emerging rapid diagnostics to become commercial products are also discussed.

The life expectancy has grown worldwide, which also increased the healthcare spending [1, 2]. For example, 720 million people will be aged 65 or older by 2020. Currently four in five people over the age of 75 take at least one prescribed medicine, and this trend is set to increase [3]. For some of the medications, large pharma find it the hard to recover R&D costs while the pressure from the regulatory agencies also increased for the use of new drugs as the first line of defence based on efficacy and cost. Hence, the global healthcare trends and ever increasing pressure from regulatory agencies put a strong case for the development of diagnostics for healthcare monitoring as well as the evaluation of drug efficacies in clinical trials. All these considerations parallels governments' and insurance companies' interests in obtaining the best performance possible and treatment benefits they support. These trends are behind the driving force for the development of diagnostics that can reduce the healthcare costs by developing effective drugs and identifying diseases and conditions at an early stage.

The major stumbling block in monitoring and controlling diseases/contaminations remains delivering simple, low-cost and robust diagnostic tests [4, 5]. In the developing world, the basic healthcare infrastructure and trained healthcare personnel are

limited [6, 7]. Other trends include increase in healthcare associated infections [8, 9], preservation of the life span of cost-effective drugs [10], increase in spurious/counterfeit medicines [11], and mitigating the epidemic-to-pandemic transitions of infectious diseases [12]. In terms of impact, low-cost diagnostics will reach underserved communities. Low-cost diagnostics that can allow local communities in developing regions to improve healthcare [13], environmental safety [14], animal health [15, 16], and food quality [17] will play key roles in the United Nation's Post-2015 Millennium Development Goals.

In the developing world, medical diagnostics for poverty-related conditions are outdated. In the absence of the diagnostic equipment, healthcare personnel make their decisions based on symptoms. WHO's Integrated Management of Childhood Illness (IMCI) is a diagnosis guideline based on signs and symptoms with minimum or no instrumentation [18, 19]. However, such systematic guidelines have limitations in (i) distinguishing asymptomatic diseases, (ii) detecting multiple infections, (iii) identifying the disease window period, and (iv) quantifying concentrations of target analytes. Eventually, the development and delivery of affordable testing technologies can enable local communities that lack access to technical and human resources present in urban areas. The deficiency in diagnostics and healthcare resources can have an irreversible negative effect on developing economies. Such an economic impact is pronounced for non-infectious as well as infectious diseases. For example, the Ebola virus epidemic in West Africa has overwhelmed the healthcare systems of Liberia, Sierra Leone, Guinea and their neighbouring countries since 2013. Such outbreaks have a profound impact on the development of emerging economies. Currently, it is difficult to identify Ebola because its symptoms such as fever are generic, also seen in commonly occurring diseases such as malaria and typhoid fever. Existing laboratory techniques are based on transcription polymerase chain reaction (RT-PCR) and quantitative PCR; however, they are not portable and affordable in the developing world. Other rapid tests suffer from sensitivity and selectivity, which may put the entire healthcare system at risk by misdiagnosing patients. Yet, recent Ebola epidemic is an example of many ongoing difficulties in healthcare systems and highlights the dire need for the development of low-cost rapid diagnostics.

While the main application of rapid tests is in medical diagnostics, such assays are also necessary for veterinary testing, environmental monitoring and food quality testing. Low-cost diagnostics would allow farmers and entrepreneurs in the developing world to assess the quality of their products and mitigate potential risks due to enterohemorrhagic strain of *E. coli* (O157:H7) and *Salmonella* in undercooked meat and poultry products [17]. Low-cost diagnostics are also required in testing water supplies. Inadequate environmental monitoring capabilities were highlighted by the cholera epidemic in Haiti after the earthquake in 2010 [20–24]. Although the cholera epidemic took most people by surprise, it wasn't totally unexpected since Haiti ranked last out of 147 countries surveyed in the 2002 Water Poverty Index [25]. Due to globalisation, such epidemics also concern the developed nations. The development of low-cost, rapid quantitative diagnostics will aid in screening large regions and populations. For the development of rapid

diagnostics, WHO has outlined a set of criteria corresponding to the acronym ASSURED: Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free and Deliverable to those who need it [10]. For the developing world, the trend towards low-cost is a priority; however, clinically useful sensitivity and specificity from rapid diagnostics assays are also required. Operating with low-volume samples without manual manipulation, being portable and functioning without sharps such as needles are considered desirable characteristics of rapid assays. Hence, the development of robust diagnostic tests can enable individuals and local communities to monitor their health condition and timely mitigate the spread of diseases.

## 1.1 The Development of Rapid Diagnostics

Historical assay formats for point-of-care testing are dipsticks and lateral flow devices. In the 1950s, the earliest paper-based strip test emerged for the quantification of the concentration of glucose in urine, and this product was marketed in the 1960s [26]. The principle of operation of the assay was based on glucose oxidase, and developed colours were compared to a reference chart for interpretation. Today, commercial urinalysis strip tests are adapted for a wide range of analytes. Traditionally, these tests are semi-quantitatively read with a colorimetric chart or with detection equipment such as CLINITEK Status<sup>®</sup> + Analyzer (Siemens) or Urisys 1100<sup>®</sup> Urine Analyzer (Roche). In the 1950s, parallel to the development of strip tests, latex agglutination assays and radioimmunoassays were also developed [27, 28]. Since the 1970s, nitrocellulose matrices have been used as a substrate for molecular detection [29–31]. In the 1980s, serological lateral-flow tests emerged [32]. The most notable example is the human pregnancy test, which was derived from the development of hCG beta-subunit radioimmunoassay [33]. Since then, commercial rapid lateral flow assays expanded beyond clinical diagnostics to veterinary, food, environmental applications, bio-defence and drug abuse (Table 1.1). Rapid diagnostic tests often have lower specificity and sensitivity than their laboratory bench counterparts. The majority of these tests are simple and provide yes/no answer, where response time is critical to the user.

The commercial rapid tests have various geometries and configurations with/out housing units (Fig. 1.1). Figure 1.1d shows a multiplexed lateral-flow assay, which allows the analyses of a single sample simultaneously. Lateral-flow immunoassays have two major configurations: Direct (i.e. double antibody sandwich assays) and competitive (i.e. inhibitive) formats. The assay format typically consists of a number of segments: sample pad, conjugate pad, reaction membrane and an absorbent pad (Fig. 1.1e). These segments are supported by a backing card and enclosed in a plastic cassette (housing). Another format in rapid diagnostics is flow-through (vertical), which is relatively more complex than lateral flow format, and its execution requires (i) sample placement, (ii) washing and (iii) addition of analyte-colloidal gold conjugates [68] (Fig. 1.1f). Most of the commercial assays require sample preparation.

**Table 1.1** Rapid diagnostic tests in the market

Test <sup>a</sup>	Application
Urinalysis	Metabolic disorders: Human chorionic gonadotropin (pregnancy) [34], (pH, glucose, protein, ketone, leukocytes, nitrite, blood, urobilinogen, bilirubin, specific gravity) [35–39], albumin to creatinine ratio [40, 41], and ascorbic acid [37, 39]
	Drug abuse <sup>b</sup> : Alcohol, amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, ketamine, methamphetamines, methadone, morphine/opiates, oxycodone, phencyclidine, propoxyphene, 9-tetrahydrocannabinol (marijuana), and tricyclic antidepressants [42–45]
Immunoassays	Infectious diseases: <i>C. difficile</i> , <i>Cytomegalovirus</i> , dengue fever, <i>E. coli</i> , enterics, epstein barr virus, mononucleosis, giardiasis, herpes simplex virus, HIV, Lyme disease, malaria, measles, <i>S. aureus</i> , methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), mumps, rubella, syphilis, toxoplasmosis, tuberculosis, varicella zoster, West Nile virus, hepatitis B/C, Chagas disease, <i>chlamydia</i> , cholera, hantavirus, leishmaniasis, leptospirosis, <i>Listeria</i> , and <i>H. pylori</i> [46–50]
	Respiratory diseases: Influenza (flu), Legionnaire’s disease, pneumonia, respiratory syncytial virus, streptococcal pharyngitis, and <i>Streptococcus pneumoniae</i> [46–48, 50]
	Cardiovascular condition: acute kidney injury, acute coronary syndrome, cyslipidemia, heart failure, oral anticoagulation, shortness of breath, and venous thrombosis [46]
	Oncology: Bladder, colon cancer, and colorectal cancer [46, 48]
	Women’s health: Osteoporosis, ovulation, and preeclampsia [46]
Veterinary diagnostics	Canine: Blood, urobilinogen, bilirubin, protein, nitrite, ketones, glucose, pH, density, leukocytes, heartworm ( <i>Dirofilaria immitis</i> ), parvovirus, <i>Giardia</i> , Lyme disease, distemper virus, <i>coronavirus</i> , <i>Ehrlichia</i> , <i>Leishmania</i> , adenovirus, rotavirus, pancreatic lipase, relaxin, blood group typing, <i>Borrelia</i> , <i>Brucella</i> , c-reactive protein, <i>Leptospira</i> , progesterone, rabies, rheumatoid factor, and vaccination status [51–54]
	Feline: Blood, urobilinogen, bilirubin, protein, nitrite, ketones, glucose, pH, density, leukocytes, immunodeficiency virus, leukemia virus, heartworm ( <i>Dirofilaria immitis</i> ), <i>Ehrlichia</i> , <i>Leishmania</i> , <i>Giardia</i> , parvovirus, infectious peritonitis, <i>Toxoplasma gondii</i> , relaxin, blood group typing, <i>Borrelia</i> , chlamydia, coronavirus, panleukopenia, progesterone, and vaccination status [51, 53, 54]
	Bovine: Alpha toxin, <i>Brucella</i> , <i>Chlamydophila</i> , <i>Clostridium perfringens</i> , coronavirus, <i>Cryptosporidium</i> , rotavirus, <i>E. coli</i> K99, <i>Crypto-sporidium parvum</i> , epsilon toxin, foot-and-mouth disease virus, IgG, <i>Leptospira</i> , <i>Mycobacterium bovis</i> , <i>Neospora</i> , progesterone, rabies, parainfluenza-3, and rotavirus [53]
	Swine: Aujeszky’s disease, <i>Clostridium perfringens</i> , <i>Cryptosporidium</i> , epidemic diarrhoea virus, rotavirus, alpha toxin, foot-and-mouth disease virus, progesterone, and transmissible gastroenteritis virus [53]

(continued)

**Table 1.1** (continued)

Test <sup>a</sup>	Application
	Equine: <i>Borrelia</i> , rotavirus, <i>Clostridium perfringens</i> , IgG, pregnant mare serum gonadotropin, progesterone, tetanus, and troponin [53]
	Avian: Influenza, <i>Chlamydophila</i> , infectious bursal disease, and Newcastle disease [53]
	Small ruminants: Foot-and-mouth disease virus [53]
Food and beverage safety tests	Mycotoxins: Aflatoxins, deoxynivalenol (vomitoxin), fumonisins, zearalenone, ochratoxin, T-2 and HT-2 toxin, patulin, ergot alkaloids) [55–58]
	Food pathogens: <i>E.Coli</i> O157:H7, <i>Listeria</i> , <i>Staphylococcus aureus</i> , and <i>Salmonella Enteritidis</i> [55–58]
	Food allergens: Almond, brazil nut, casein, cashew/pistachio, coconut, hazelnut, lupin, macadamia nut, mustard, peanut, sesame, soy, walnut, whole egg, $\beta$ -lactoglobulin, total milk, crustacea, and gliadin/gluten [55, 56]
	Genetically modified organisms: Bulk grain, seed and leaf, toasted meal, and corn Comb 7 Traits [55]
	Veterinary drug residues: Chloramphenicol, nitrofurantol AMOZ/AOZ/AHD, clenbuterol, ractopamine, beta agonists, dexamethasone, ciprofloxacin, quinolones, $\beta$ -Lactam antibiotics, flunixin, aminoglycoside, amphenicol, enrofloxacin, macrolide, sulphona-mide, tetracycline, and melamine [55–57]
	Beverage: Methanol contamination, acetic acid, citric acid, D-glucose, D-fructose, lactose, milk (lactic acid, urea), and wine (acetic acid, total acidity, glucose, fructose, L-lactic acid, L-malic acid, and sucrose) [56]
	Pesticide residues: Organophosphates, thiophosphates, and carbamates [56]
	Seafood analysis: Amnesic shellfish poisoning (ASP), marine biotoxins (okadaic acid), histamine, and sulphite residues [56]
Species identification: Pork, horse, beef, fish, goat, poultry, rabbit and sheep [56]	
Environmental monitoring devices	Water testing: Algae, alkalinity, aluminium, ammonia, arsenic, bleach, boron, bromine, cadmium, calcium hardness, carbon dioxide, chelant, chloride, chlorine, chromate, chromium, conductivity, copper, cyanuric acid, cyanide, detergents, dissolved oxygen, faecal streptococci, <i>E.Coli</i> and faecal coliforms, filming amine, fluoride, formaldehyde, glutaraldehyde, hardness, hydrazine, hydrogen peroxide, iodine, iron, lead, magnesium, manganese, molybdate/molybdenum, morpholine, nickel, nitrate, nitrite, oil in water, organophosphate, dissolved oxygen, ozone, quaternary ammonium compounds, peracetic acid, pH, phosphate, phosphonate, polyphosphates, polyquat, potassium, <i>Pseudomonas aeruginosa</i> , salinity, silica, sulphate, sulphide, sulphite, turbidity, total dissolved solids, tannin/lignin, tolacide PS biocide, and zinc [59–64]
	Soil: Humus, organic matter, pH, and plant tissue macronutrient (texture) [60]

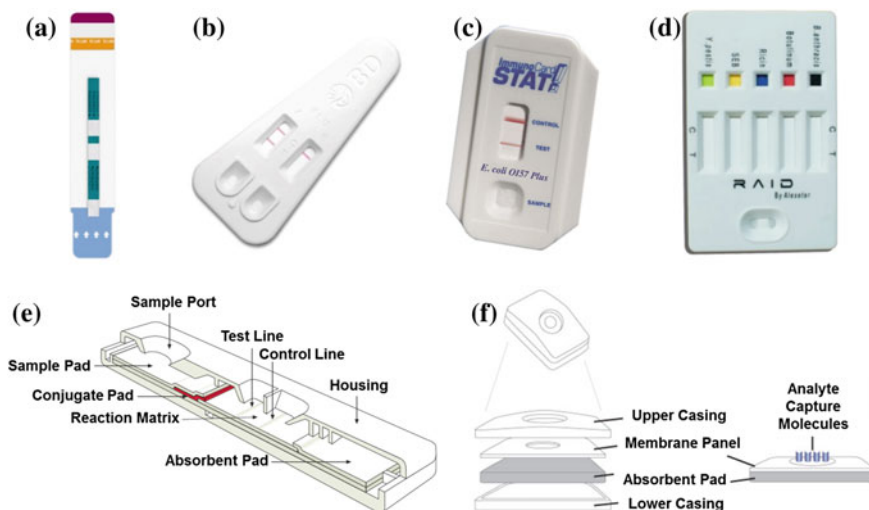
(continued)

**Table 1.1** (continued)

Test <sup>a</sup>	Application
Biothreat detection	Anthrax, plague, tularaemia, ricin, botulinum toxin, <i>Staphylococcal enterotoxin B</i> , orthopox, <i>Brucella</i> , abrin, biological warfare simulants, nerve (G&V series), Category A-C biothreat agents, and blister (HD) agents, acids, bases, aldehydes and oxidisers [65–67]

<sup>a</sup> Require minimal sample preparation

<sup>b</sup> Urine collection and detection are often integrated in one cup



**Fig. 1.1** Lateral-flow point-of-care assays in the market and their cassette formats. **a** Determine™ TB LAM Ag test.® (Alere), **b** Directigen™ EZ Flu A + B (Beckton Dickinson), **c** ImmunoCard STAT!® E. coli O157 Plus (Meridian Bioscience), **d** A multiplex lateral-flow assay. RAID™ 5 for biological threat detection (Alexeter Technologies), **e** Schematic of the lateral-flow assay, and **f** flow-through assay. Adapted from Ref. [69] with permission from The Royal Society of Chemistry

Typical approaches include the removal of contaminants from the samples to improve selectivity and sensitivity, and decrease the turnaround time. Additionally, the sample might require further processing to improve the signal-to-noise ratio. Sample preparation suffers from inhomogeneity, interfering agents, inhibitors, and it requires increasing the viscosity of samples such as whole blood and food samples. Increasing the sensitivity requires tedious sample preparation steps for low concentrations of target molecules or cells. The ideal sample preparation step should be cost-effective and is potentially executed in a single step. The desired outcome of the sample preparation is concentrating the target analyte(s) and reducing the background noise due to matrix interferences. Lateral-flow tests are low-cost, lightweight, portable, but the growing demand for higher sensitivity is challenging its current format [68]. For

example, cardiac markers, biothreat and single cell detection require sensitivities that are not achievable with the existing assays. For these applications, lateral-flow tests must evolve and incorporate novel materials and labelling approaches. Progress in detection/quantification technologies, readout devices and manufacturing techniques will increase the reproducibility and sensitivity of lateral-flow assays. However, all these new advances must maintain the ultimate attractiveness of rapid tests: Sample-to-answer in a single step.

## 1.2 Sensing Mechanisms

A wide array of sensing mechanisms has been proposed for point-of-care diagnostic devices. Applications for such sensors include medical diagnostics, veterinary testing, environmental monitoring and food pathogen testing (Table 1.2). Sensing mechanisms demonstrated, with the exception of molecular dyes, required handheld readers. Excluding other types of sensors can be attributed to high costs, powering requirements and incompatibility with point-of-care assays.

### 1.2.1 Colorimetric Reagents

Molecular and enzymatic dyes are the simplest and most commonly used detection methods, which are semi-quantitatively interpreted by a colour reference chart. Urinalysis test strips such as Multistix (Siemens) and Chemstrip (Roche) are based on colorimetric reagents (Table 1.3). In advanced assays such as paper-based microfluidic devices, multiple detection zones are employed to capture different analytes within the same assay. Such multiplexed assays can be fabricated by printing wax channel-shaped patterns on paper/nitrocellulose, followed by heat treatment to form hydrophobic barriers in the matrix. The patterned regions can be spotted with enzymes, acid-base indicators or dyes in confined zones. Usually, a single inlet wicks the sample, which is distributed to the confined regions to react with the immobilised reagents. pH-, glucose- and protein- sensitive reagents have been used in paper-based microfluidic devices [70, 73]. In the glucose assay, a positive result is observed when the colour shifts from clear to brown due to the enzymatic oxidation of iodide to iodine. Similarly, a positive result in protein assays is interpreted from a colour change of tetrabromophenol blue from yellow to blue [70, 73]. Up to date, multiplex colorimetric assays included medical diagnostics [81, 87, 90, 91], environmental tests [76, 83, 84, 86, 89], and food quality tests [77, 88] (Fig. 1.2a). A disadvantage of colorimetric sensors is the inhomogeneity of the colour distribution and the coffee ring effect in the detection zones, and thus, the judgment of the final colour is challenging by eye [73]. In addition, colorimetric sensors are interfered by the background colour of the paper or the sample. For example, blood assays require

**Table 1.2** Rapid diagnostics: sensing mechanisms, their dynamic ranges and sensitivity/ detection limits

Molecular dyes or enzymatic reactions			
Analyte	Dynamic range	Sensitivity/ detection Limit	Reference
Bovine serum albumin	0.38–75 $\mu\text{M}$	0.75 $\mu\text{M}$	[70, 71]
Glucose	2.5–500 mM	5 mM	[70, 72–75]
pH	5–9 pH units	0.5 pH units	[73]
Human serum albumin	0.46–46 $\mu\text{M}$	0.8 $\mu\text{M}$	[73]
Bendiocarb	<2 $\mu\text{M}$	~ 1 nm	[76]
Carbaryl	<2 $\mu\text{M}$	~ 10 nm	[76]
Paraoxon	<10 $\mu\text{M}$	~ 1 nm	[76]
Malathion	<10 $\mu\text{M}$	~ 10 nm	[76]
Aflatoxin B1	<40 $\mu\text{M}$	~ 30 nm	[77]
Antibodies to the HIV-1 envelope antigen gp41	1:1–1:100 dilution of HIV in serum	54 fmol/zone (rabbit IgG)	[78]
Lactate	1–25 mM	1 mM	[74]
Uric acid	0.1–7 mM	0.1 mM	[74, 75]
Red cell antigens A, B, and D	Qualitative	N/A	[79, 80]
Urinary acetoacetate	5–16 mM	0.5 mM	[81]
Salivary nitrite	5 $\mu\text{M}$ –2 mM	5 $\mu\text{M}$	[81]
Total iron	50 $\mu\text{M}$ –1 mM	44 $\mu\text{M}$	[82]
Hg(II), Ag(I), Cu(II), Cd(II), Pb(II), Cr(VI), Ni(II)	5.4–120.0 ppm	0.001; 0.002; 0.020; 0.020; 0.140; 0.150; 0.230 ppm	[83]
Organic solvents	5–100 % (v/v)	5 % (v/v)	[84]
Hydrogen peroxide	0.1–10 ppm	0.4 ppm	[85]
Volatile organic compounds	Qualitative	Qualitative	[86]
<i>E. coli</i> strain K12 ER2738	~ 10 <sup>5</sup> – ~ 10 <sup>9</sup> colony forming unit	~ 10 <sup>5</sup> CFU	[87]
<i>E. coli</i> O157:H7, <i>Salmonella Typhimurium</i> , and <i>L. monocytogenes</i>	10–10 <sup>3</sup> cfu/cm <sup>2</sup>	10 cfu/cm <sup>2</sup>	[88]
Particulate metal (Fe)	1.5–15 $\mu\text{g}$	1.5 $\mu\text{g}$	[89]
Particulate metal (Ni)	1–15 $\mu\text{g}$	1 $\mu\text{g}$	[89]
Particulate metal (Cu)	1–15 $\mu\text{g}$	1 $\mu\text{g}$	[89]
Alkaline phosphatase	<500 U/L	~ 15 U/L	[90]
Aspartate aminotransferase	<300 U/L	~ 44 U/L	[90]
Aspartate aminotransferase	50–200 U/L	~ 84 U/L	[91]
Alanine aminotransferase	50–200 U/L	~ 53 U/L	[91]
Airborne particulate matter	10–100 pmol min <sup>-1</sup> $\mu\text{g}^{-1}$	0.32–0.65 ng	[92]
Reactive phosphate	0.2–10 mg L <sup>-1</sup>	0.05 mg L <sup>-1</sup>	[93]

(continued)



**Table 1.2** (continued)

Molecular dyes or enzymatic reactions			
Analyte	Dynamic range	Sensitivity/ detection Limit	Reference
<i>Electrochemical sensing</i>			
Glucose	<100 mM	0.21 mM	[94–97]
Lactate	<50 mM	0.36 mM	[94, 95]
Uric acid	<35 mM	1.38 mM	[94, 98]
Ascorbic acid	0.05–0.4 mM	0.02 mmol L <sup>-1</sup>	[98]
Cholesterol	20–200 mg/dL <sup>-1</sup>	13 mg/dL <sup>-1</sup>	[95]
Ethanol	0.1–3 mM	0.2 mM	[95]
Pb(II)	<100 ppb	1.0 ppb	[96, 99, 100]
Cd(II)	10–100 ppb	2.3 ppb	[100]
Cancer and tumour markers (AFP, CA 125-153, CEA)	<100 ng/mL or U/mL	10 <sup>-4</sup> ng mL <sup>-1</sup> , 3.7 × 10 <sup>-5</sup> and 2.6 × 10 <sup>-5</sup> U mL <sup>-1</sup> , 2.0 × 10 <sup>-5</sup> ng mL <sup>-1</sup>	[101–104]
pH	4–10	0.01 pH units	[105]
K <sup>+</sup> ions	10 <sup>-5</sup> –10 <sup>-1</sup> M	4.1 × 10 <sup>-6</sup> M	[105]
NH <sub>4</sub> <sup>+</sup>	10 <sup>-5</sup> –10 <sup>-1</sup> M	7.2 × 10 <sup>-6</sup> M	[105]
Dopamine	<100 μM	56.6 ± 1.1 mV/unit of pH	[106]
Paracetamol	0.05–2.00 mmol L <sup>-1</sup>	25 μmol L <sup>-1</sup>	[107]
4-aminophenol	0.05–2.00 mmol L <sup>-1</sup>	10 μmol L <sup>-1</sup>	[107]
1-butanethiol	2–200 μM	0.5 μM	[108]
<i>Nanoparticles</i>			
DNase I	10 <sup>-5</sup> –10 <sup>-1</sup> unit/μL	10 <sup>-5</sup> unit/μL	[109]
Adenosine	<250 μM	11.8 μM	[110]
Human IgG	<5 mg/L	10 μg/L	[111]
<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i>	0.5–10 × 10 <sup>3</sup> CFU/mL	500–5,000 CFU/ml	[112]
Glucose	0.5–100 mM	0.5 mM	[113]
Hg(II)	5–75 ppm	0.12 ppm	[114]
PfHRP2	5–40 ng/mL	2.9 ng/mL	[115]
Goat IgG	0.05–1 μg/mL	20 ng mL <sup>-1</sup>	[116]
Prostate-specific antigen	0.05–100 μg/L	~360.2 ng/L	[117]
Cu <sup>2+</sup> ions	7.8–62.8 μM	7.8 nM	[118]
HIV DNA	10–10 <sup>5</sup> HIV <i>gag</i> DNA	10 copies	[119]
<i>Mycobacterium tuberculosis</i>	< 30 μg mL <sup>-1</sup>	10 μg mL <sup>-1</sup>	[120]
Immunoglobulin E	0.05–5 pmol	50 fmol	[121]
Cd <sup>2+</sup> ions	10 ppb	0.1–0.4 ppb	[122]

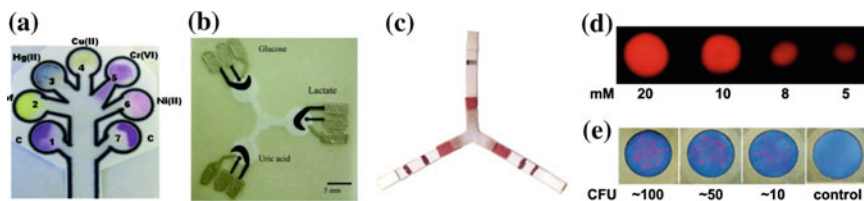
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**Table 1.2** (continued)

Molecular dyes or enzymatic reactions			
Analyte	Dynamic range	Sensitivity/ detection Limit	Reference
<i>Electrochemiluminescence</i>			
2-(dibutylamino)-ethanol	3 $\mu\text{M}$ –10 mM	0.9 $\mu\text{M}$	[123]
Nicotinamide adenine dinucleotide	0.2–20.0 mM	72 $\mu\text{M}$	[123]
Tumour markers (AFP, CA125, CA199, CEA)	5–100 ng or U $\text{mL}^{-1}$	0.15 ng $\text{mL}^{-1}$ , 0.6 and 0.17 U $\text{mL}^{-1}$ , 0.5 ng $\text{mL}^{-1}$	[103, 124, 125]
Carcinoembryonic antigens	0.01–10 ng $\text{mL}^{-1}$	1 fg $\text{mL}^{-1}$	[126]
Dopamine	1 $\mu\text{M}$ –10 mM	1 $\mu\text{M}$	[127]
Pb <sup>2+</sup> ions	<2 $\mu\text{M}$	10 pM	[128]
Hg <sup>2+</sup> ions	<2 $\mu\text{M}$	0.2 nM	[128]
Enzo[a]-pyrene (B[a]P)	0.15–12.5 $\mu\text{M}$	~ 150 nM	[129]
<i>Chemiluminescence</i>			
Glucose	2.5–50 mM $\text{L}^{-1}$	0.14 mmol $\text{L}^{-1}$	[130]
Uric acid	2.5–50 mM $\text{L}^{-1}$	0.52 mmol $\text{L}^{-1}$	[130, 131]
Tumour markers (AFP, CA153, CA199, CEA)	<150 mM ng or U $\text{mL}^{-1}$	1.0 ng $\text{mL}^{-1}$ , 0.4, and 0.06 U $\text{mL}^{-1}$ and 0.02 ng $\text{mL}^{-1}$	[102, 132]
<i>Fluorescence</i>			
Fluorescein isothiocyanate-labeled bovine serum albumin	0.1–100 pmol	125 fmol	[133]
DNA	10–10,000 pM	100 pM	[134]
Nucleic acids	0.08–5.00 $\mu\text{M}$	1,200 input templates	[135]
Lung cancer associated miRNA	10 nM–10 $\mu\text{M}$	~ 100 nM	[136]
Nucleic acid hybridisation	1–5 pmol	300 fmol	[137]
Asian soybean rust	0.0032–3.2 mg/mL	2.2 ng/mL	[138]
<i>Dual electrochemical/colorimetric sensing</i>			
Au(III)	1–200 ppm	1 ppm	[139]
<i>Bacterial whole-cell</i>			
Bacterial quorum signalling molecules– <i>N</i> -acylhomoserine lactones	10 <sup>-9</sup> –10 <sup>-4</sup> M	10 <sup>8</sup> M	[140]

**Table 1.3** The principles of colorimetric reactions in commercial urinalysis tests

Assay	Reaction	Range	Detection limit [144]
Glucose	$\text{Glucose} + \text{O}_2 \xrightarrow{\text{glucose oxidase}} \text{Gluconolactone} + \text{H}_2\text{O}_2$ $\text{Chromogen} + \text{H}_2\text{O}_2 \xrightarrow{\text{peroxidase}} \text{H}_2\text{O} + \text{dye}$	5.5–55	2.2 mmol/L
Protein	$\text{Tetrabromophenol blue (pH 3, yellow)} \xrightarrow{\text{protein}} \text{tetrabromophenol blue (>pH 4.6, blue)}$	30–300	6 mg/dL albumin
Blood	$\text{haemoglobin peroxide} \longrightarrow \text{oxidised chromogen} + \text{H}_2\text{O}_2$	10–80	5–10 erythrocytes/L
Leukocytes	$\text{H}_2\text{O}_2 + \text{chromogen} \xrightarrow{\text{granulocytic esterase}} \text{Indoxyl or pyrrole carbonic acid ester} \longrightarrow \text{Indoxyl or pyrrole}$ $\text{Indoxyl or pyrrole} + \text{diazonium salt} \longrightarrow \text{dye (purple)}$	15–125	10–25 leukocytes/ $\mu\text{L}$
Nitrite	$\text{Nitrite} + \text{p-arsanilic acid} \longrightarrow \text{diazonium compound}$ $3\text{-Hydroxy-1,2,3,4-tetrahydrobenz-(h)-quinoline} + \text{diazonium compound} \longrightarrow \text{pink colour}$	Qualitative	11 $\mu\text{mol/L}$
pH	$\text{HInd (acid form)} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{Ind}^- \text{ (conjugate base of the indicator)}$	4–9	0.25 pH units
Ketone	$\text{Acetoacetic acid} + \text{Na nitroprusside} + \text{glycine} \xrightarrow{\text{alkaline}} \text{violet to purple colour}$	0.5–4.0	5 mg/dL
Urobilinogen	$\text{p-diethylaminobenzaldehyde} + \text{urobilinogen} \longrightarrow \text{azo compound (red)}$	17–200 $\mu\text{mol/L}$	7 mol/L
Bilirubin	$\text{Bilirubin} + \text{diazide} \xrightarrow{\text{acid}} \text{azobilirubin}$	++++	9 $\mu\text{mol/L}$



**Fig. 1.2** Sensors utilised for point-of-care testing, **a** Colorimetric detection of heavy metals, **b** Electrochemical sensing of glucose, lactate and uric acid, **c** Antibody conjugated gold NP detection of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, **d** ECL emission from a paper-based device at different analyte concentrations, **e** Fluorescent sensing for measuring bacterial growth. Adapted from Ref. [69] with permission from The Royal Society of Chemistry

either usage of serum/plasma samples or a red blood cell filter. Despite its drawbacks, colorimetric sensing is widely used, and it can also be quantified by a handheld reader [141, 142] or a smartphone camera [72, 143].

### 1.2.2 Electrochemical Sensors

Electrochemistry and ion-selective electrodes have been explored widely due to their well-known principle of operation and maturity. Traditionally, electrochemical sensors have three electrodes: a counter electrode, a working electrode, and a reference electrode. In paper-based assays, carbon ink was used for the counter and the working electrode, whereas silver/silver chloride ink was used for the fabrication of the reference electrode. The reaction zones comprised this multiple-electrode mechanism (Fig. 1.2b). Electrochemical sensors allowed the detection of glucose [94, 95], lactate [94, 95], uric acid [94], cholesterol [95], tumour markers [101], dopamine [106] and drugs [107]. Other studies described heavy metal sensors for environmental monitoring [94, 96, 99, 100]. As compared to colorimetric reagents, the fabrication of electrochemical sensors required an additional deposition step of conductive inks on the paper matrix. All these electrodes and electronic wires were screen printed using graphite and silver inks, respectively. When the sample was introduced to the device, it was wicked up into the sensing zones and the amperometric measurement was initiated via a glucometer. Alternatively, gold can be sputtered on the paper matrix through a shadow mask for the deposition of electrodes [107]. Such electrodes can be characterised by cyclic and square wave voltammetry, and chronoamperometry. In contrast to colorimetric reagents, electrochemical sensors respond within seconds and have sensitivities down to nM [145]. Electrochemical detection is also independent of the ambient light and is less prone to interference from the colour/deteriorations of substrate. Although the attributes of electrochemical detection such as maturity and suitability for miniaturisation are attractive, the requirement for a readout device increases its complexity and the cost per test.

### 1.2.3 Colloidal Nanoparticles (NPs)

Functionalised colloidal gold and monodisperse latex are the historical sensing reagents for lateral-flow assays, which do not require a readout device for qualitative results [146]. Lateral-flow tests employ antibody-conjugated gold NPs, which have extinction coefficients that are higher than common organic dyes. Inkjet printers have been used to deposit the NPs for multiplex detection on filter paper [111]. Other NP-based assay studies focused on improving the sensitivity of current lateral-flow tests by using paper network platforms. The 2D nitrocellulose-based networks enabled multistep processes to amplify the signal in immunoassays to improve the limit of detection [115, 147]. These cards contained reagents stored dry and the assay was activated by wicking the sample in a single-user step. They were capable of multistep processes such as delivering rinse buffers and signal amplification reagents to the capture zones. These devices were demonstrated by using porous materials such as nitrocellulose and cellulose depending on the sensing application. Other 2D paper networks involved integrating the inlets of a number of lateral flow assays [112] (Fig. 1.2c), adopting folding techniques [119] and microplate paper platforms [120]. NP-based detection has been demonstrated with metabolites [109, 111, 113, 117, 121, 147], bacterial agents [112] in disease diagnosis such as HIV [119], malaria [115], tuberculosis [120] and in environmental monitoring [114, 118]. To multiplex the assay, monodisperse latex can be coupled with fluorescent and coloured dyes, and para/magnetic components. For example, conjugated with dark dyes, monodisperse latex particles exhibit high contrast on nitrocellulose or paper. Additionally, latex particles can be utilised with different colours or fluorescent dyes.

### 1.2.4 Chemiluminescence (CL)

CL is based on the emission of light generated by a chemical reaction. In the presence of reactants A (luminol) and B ( $\text{H}_2\text{O}_2$ ), and a catalyst or excited intermediate (3-aminophthalate), light is produced along with side products. Peroxidase catalyses the oxidation of luminol to 3-aminophthalate, and the decay of the excited state ( $\diamond$ ) to a lower energy level results in light emission, which can be enhanced by using phenol derivatives such as *p*-iodophenol. A typical example of CL is the glow stick, which is based on the reaction of peroxide with a phenyl oxalate ester ( $[\text{A}] + [\text{B}] \rightarrow [\diamond] \rightarrow [\text{products}] + \text{light}$ ). In rapid diagnostics, CL has been explored for the detection of glucose and uric acid [130] and tumour markers [132, 148]. Glucose and uric acid assays were based on oxidase reactions coupled with chemiluminescence reactions of a rhodanine derivative with the generated  $\text{H}_2\text{O}_2$  in an acidic medium [130]. Uric acid was determined through a chemiluminescence reaction between the rhodanine derivative (3-*p*-nitrylphenyl-5-(40-methyl-20-sulphonophenylazo) rhodanine) and

H<sub>2</sub>O<sub>2</sub> [131]. Studies on the detection of tumour markers involved fabricating sandwich-type immunoassays with a typical luminol-H<sub>2</sub>O<sub>2</sub> chemiluminescence system catalysed by Ag<sup>0</sup> NPs [132]. Detection of tumour markers was also achieved by sandwich CL-ELISA with antibodies that were covalently immobilised on a chitosan modified paper zone through glutaraldehyde cross-linking [148]. Correlating the concentration of the analyte and the peak intensity of the emitted light allowed quantitative analysis.

### 1.2.5 Electrochemiluminescence (ECL)

This sensing mechanism is based on luminescence generated by electrochemical reactions. When electrochemically generated intermediates undergo exergonic reactions, they result in an electronically excited state. This state emits light upon relaxation to a lower level state, and therefore it enables readouts without the requirement for a photodetector. ECL has the advantages of both luminescence and electrochemistry. An ECL sensor based on orange luminescence was demonstrated through the detection of 2-(dibutylamino)-ethanol (DBAE) and nicotinamide adenine dinucleotide by readouts of luminescence [123]. The principle of ECL sensor was based on Ru (bpy)<sub>3</sub><sup>2+</sup> and DBAE. At the electrode, the amine was oxidised and formed a radical cation [DBAE]<sup>•+</sup>, followed by deprotonation to create a DBAE<sup>•</sup> radical, which reduced Ru (bpy)<sub>3</sub><sup>3+</sup> to an excited state. Eventually, Ru (bpy)<sub>3</sub><sup>2+</sup> emitted light at 620 nm while relaxing to the ground state [149]. This mechanism served as a coreactant that was oxidised solely by the electrode. In ECL, the electrochemical potential initiated and controlled the chemiluminescence reaction. The electrodes were printed using screen printing and adding an ECL active luminophore followed by drying. The substrates were laminated onto a Zensor screen-printed electrode using an office laminator. The cyclic voltammetry of Ru (bpy)<sub>3</sub><sup>2+</sup> was used to characterise the electrodes. After the lamination step, an incision was made in the laminate layer. After the introduction of the sample to the assay, the potential was stepped from 0 to 1.15 V for a short period to initiate the ECL. The initiation can be achieved by shifting the potential to a level more positive than the oxidation potential of the ruthenium complex. Chronoamperometry was adopted to generate ECL since it provided control over the reaction time (Fig. 1.2d). The ECL readouts were taken with a camera phone housing to block the ambient light. ECL was connected to the mobile phone battery to obtain a short pulse of low voltage. In addition to these drawbacks, ECL required a photomultiplier tube, which was costly in a miniaturised form. The data was analysed based on the red pixel intensity of the ECL emission, which was correlated with a calibration curve, hence the analyte concentration. Other applications to date included sensing tumour markers [103, 124, 126] and ions [128].

### ***1.2.6 Fluorescence***

This detection mechanism was first demonstrated on paper microzone plates. Although paper-based plates are known [150–153], they have recently been suggested for quantitative fluorescence measurements. These paper plates require low sample volumes; 12.5 pmol of fluorescein isothiocyanate-labeled bovine serum albumin generated a relative fluorescence of  $2,700 \pm 850$  a.u. [133]. Although concentration sensitivity and mass sensitivity were comparable with plastic plates, the average relative standard deviation for the replicates of all concentrations was higher. This limitation was attributed to light scattering on the cellulose fibres and the influence of the index of refraction between cellulose and air. Although paper-based plates were suggested as alternatives to conventional plastic multiwell plates, their interpretation required a microplate reader. Other notable studies adopting fluorescent sensing included paper strips comprising DNA-conjugated microgels (MG) for DNA detection [134]. Sensing DNA was accomplished by: (i) targeting DNA promoted ligation of a DNA primer to the MG-bound DNA, (ii) rolling circle amplification (RCA) between the primer and a circle DNA, and (iii) hybridisation of the RCA products and a fluorescent DNA probe. Another study reported a portable device for the growth of bacteria or the amplification of bacteriophages. A fluorescent mCherry reporter was used to quantify the growth of bacteria and the concentration of arabinose [87] (Fig. 1.2e). Another paper-based platform involving fluorescent sensing described the use of non-enzymatic nucleic acid circuits based on strand exchange reactions for the detection of target sequences [135]. Overall, although fluorescence sensing brings new capabilities to point-of-care diagnostics, the feasibility requires reduction in cost and miniaturisation of fluorescence readers.

### ***1.2.7 Genetically-Engineered Cells***

Bacterial quorum signalling (QS) molecules, *N*-acyl homoserine lactones (AHLs), have been used as a sensing mechanism [140]. The bacterial cell-based sensing system comprised two main components: (i) AHL-mediated QS regulatory system as recognition elements, and (ii)  $\beta$ -galactosidase as the reporter enzyme. The bacterial cells were inoculated on paper by liquid drying. The paper strip biosensor detected low concentrations (0.1 nM) of AHLs in saliva. The advent of synthetic biology will accelerate the development of whole-cell based biosensors.

## **1.3 Next Generation Diagnostics**

To expand the current capabilities of point-of-care diagnostics, the materials, sensors and readout devices need to evolve. Extending beyond strip tests and lateral-flow design to multiplexed miniaturised assay configurations will put the

existing diagnostics in a context that will allow differentiation of conditions with similar symptoms and improve the treatment options. Biofunctional materials and synthesis of reversibly responsive compounds could lead to reusable tests, which might be applicable to conditions where frequent measurements are required. The control over structural design parameters, advances in deposition of materials through printing will play greater roles in the realisation of the new generation diagnostics. Studies on substrate-protein/enzyme interactions, surface energy, release characteristics, assay decay will gain momentum in the realisation of diagnostics beyond R&D. Optimisation of capillary flow parameters in lateral-flow devices and advanced microfluidic devices will allow construction of assays with improved control and sensitivity. To date, limited studies in microfluidic platforms have employed unprocessed samples. Incorporation of sample preparation should not be overlooked. Furthermore, the performance of assay after long-term storage, and time-consuming sensor response in colorimetric test require further investigations. Sensing and detection technologies will also evolve. The search for sensing, quantification and readout within a single equipment-free assay will play a greater role in future diagnostics. These attributes may include user-friendly, fool-proof, text/quantity-reporting capabilities and unexplored sensing mechanisms such as paramagnetic particles, quantum dots, coloured latex particles, and genetically engineered whole cells based on synthetic biology, and other novel materials including graphene, plasmonic materials, and printable gratings [154–159]. In improving the sensitivity and providing a user-friendly interface, bioinspired photonic structures, colloidal crystal arrays, diffraction gratings and holographic sensors can offer newer capabilities and readout approaches [160–165]. Some of these sensing and readout mechanisms might not require physically blocking or shaping the substrate for multiplexing. Although significant time has been devoted to quantification with smartphones/handheld readers, equipment-free approaches should not be overlooked. The use of external readers is a barrier for existing assays, yet this requirement will be a greater challenge in adopting newer sensing platforms. The fast-growing mobile phone market in the developing world has made camera phones a potential platform for quantitatively reading diagnostic assays, and this may standardise the readout devices with improved connectivity [166, 167]. Novel approaches towards instrument-free quantification of analytes will be important contributions to the field. Additionally, the trends show that the microfluidic assay formats will be exploited further by the *in vitro* diagnostics industry [168–170]. All these advances will lead to multiplexed diagnostics that are capable of identifying the specific etiological agent that causes a particular syndrome, which is a goal that has not been achieved yet. Such assays can explore less utilised clinical samples such as tear fluid with contact lens sensors [171].

Existing prototypes need to be transformed into highly reproducible diagnostic devices. Having performance data does not always yield efficacy after deployment. Possible small-scale trials should experiment with the feasibility and cost-effectiveness after scaling up. The ultimate test in the realisation of diagnostics depends on the acceptance from experts in the commercial diagnostics industry. Today, the rapid diagnostics business is based on a standard lateral flow format, involving the



use of nitrocellulose as the reaction matrix. The lateral-flow format is the only ubiquitous, universally applicable platform that can be utilised for simple, qualitative, low cost point-of-care applications, while also having enough capability to be functionalised for highly sensitive, fully quantified, multiplexed assays. Hence, the industrial partners are seeking technologies that have improved capabilities, sensitivity, specificity, and compatibility with the existing manufacturing processes. Only low cost is not enough to achieve market penetration in diagnostics. The value of the low-cost and multiplexed diagnostics will be realised upon reaching communities, where they are needed the most.

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