Chapter 1
Introduction to In Vitro Diagnostic Devices

1.1 Overview

Healthcare investment keeps on increasing substantially in recent years [1, 2]. Such investment has also focused on fighting major diseases, enabled by the novel invention of cost-effective and valid drug development for treatment and side effect reduction, along with improved vector control. In addition, the demand for diagnostics that is essential in determining prognosis, identifying disease stages, monitoring treatment, and assessing the spreading as health services has expanded [3].

Molecular-based diagnostics is critical for prevention, identification, and treatment of disease. Current laboratory analyses support correct diagnosis in over 70 % of all diseases and can be used to aid the continuous monitoring of drug therapy [4]. Traditional diagnosis system in central laboratory is therefore a vital component in the clinic and in local general practice. However, classic diagnostic technologies are not completely well suited to meeting the expanded testing requirement because they rely on complicated sample purification and sophisticated instruments which are labor-intensive, timely, and expensive and require of well-trained operators. One of the main challenges for industry is to develop fast, relatively accurate, easy-to-use, and inexpensive devices. For example, microscopy observation requires less infrastructure and is more widely available based on the simplicity and low cost; however, the accuracy is somehow questionable and underutilized (e.g., smear tests for tuberculosis, malaria, and schistosomiasis) [5–7]. As a result, it not only increases the cost and inconvenience of health care but also causes patients to leave the medical system before the diagnostic result is obtained [8]. Faster and more accurate diagnostic tests that require minimum laboratory equipment and operation training play an important role in expanding health care in resource-constrained settings [9, 10].

In addition to the improved efficiency in laboratory diagnostics, there has been a trend toward a more decentralized diagnostics which occurs directly at patients'

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bedside, in outpatient clinics, or at the sites of accidents, so-called point-of-care (POC) systems [11]. The concept of POC testing is mainly for the patient, so short turnaround time, minimum sample preparation and reagent storage and transferring, user-friendly analytical instruments, and digital or visible quantitative or semiquantitative single readout is required [4, 12, 13]. It is clear that on-site or minimum sample preparation and on-chip storage limit the delays that caused by transport and preparation of clinical samples. As a result, shorter turnaround time leads to rapid clinical decision-making and may save fatal consequences. No previous knowledge in sample analysis should be required, so elders can perform the tests at home with minimum training to improve health outcome [14].

The first POC device was urine dipstick test, which was developed in 1957 to measure urinary protein [15]. Glucose meters for diabetic monitoring and lateral-flow devices for pregnancy tests are currently the most widely used devices in POC molecular diagnostics. They are excellent examples of POC tests; however, they are still not applicable if highly sensitive and high-throughput quantitative measurements are required.

In recent decades, some technologies have emerged that fulfill these requirements. Lateral-flow immunoassay (LFIA) devices, for example, which were originally proposed in the 1980s, remain popular largely because of their design simplicity.

Plotz and Singer invented the latex agglutination assay in 1956, from which the technical basis for the LFIA was derived [16]. Plate-based immunoassay was being developed at the same time. The radioimmunoassay was designed by Berson and Yalow in the 1950s [17]. The enzyme immunoassay, which replaced radioisotopes with enzymes, cut down reaction times, and provided higher specificities than a radioimmunoassay, was developed in the 1960s. The fundamental principles of the LFIA continued to be refined through the 1980s and were firmly established during the ensuing years [18, 19]. Since that time, at least another 500 patents have been filed on various aspects of the technology. Several patents have even been formatted by companies such as Becton Dickinson & Co. and Unilever and Carter Wallace.

The chief application driving the early development of solid-phase, rapid-test technology was the human pregnancy test, which was symbolic of continued historical interest in urine testing for medical diagnostic purposes. This particular testing application made great strides in the 1970s, as a result of improvements in antibody generation technologies and significant gains in understanding the biology and detection of human chorionic gonadotropin (hCG), derived largely from the work performed by Vaitukaitis and colleagues [20]. However, to entirely evolve the lateral-flow test, considerable enabling technologies were still required. Many of these technologies, such as nitrocellulose membrane manufacturing, antibody generation, and processing equipment, were developed throughout the 1990s. The purpose of this article is to introduce readers with basic information regarding the LFIA approach.

1.2 Structure

Figure 1.1 displays the key elements of a LFIA. This assay consists of several components, often segmented parts made of different materials. When a test is run, appropriately conditioned sample is added to the proximal end of the strip, the absorbent pad. The treated sample then migrates to the conjugate pad, where an appropriate reagent has been immobilized. The labeled reagent on the conjugate pad can be colloidal gold, or a colored, fluorescent, or paramagnetic latex particle. These specific biological components can be either antigen or antibody depending on the assay format. Next, the sample remobilizes the dried reagent, and particle interaction ensues. Sample and reagent then migrate to the next segment of the strip, the reaction matrix. The reaction matrix is a porous membrane, upon which a final specific biological component has been immobilized. These biological components are usually proteins, either antibody or antigen. They have been bound onto the specific lines of the membrane being used. As the sample and reagent reach this line, they are captured by the applied proteins, and excess liquid moves past this point and is taken up by the absorbent pad. The result is the detectable absence or presence of the test line, read by eyes or by other instruments.

The LFIA may be of two different types: (1) direct (sandwich, Fig. 1.2a) or (2) competitive (inhibition, Fig. 1.2b). Both types can accommodate qualitative, semi-quantitative, and fully quantitative determinations. Direct assay is usually used when testing for larger analytes with multiple antigenic sites, such as hCG, dengue antigen, or human immunodeficiency virus (HIV). A positive result is indicated by the presence of a test line. The conjugated particles also reach and are captured at the control line. The control line typically comprises a species–specific anti-immunoglobulin antibody, specific for the antibody in the conjugate pad. Competitive assay is usually used when testing for small molecules with single antigenic determinants that cannot bind to antibodies on a test line simultaneously. In such cases, a positive result is indicated by the absence of a test line, but a control line may still form.

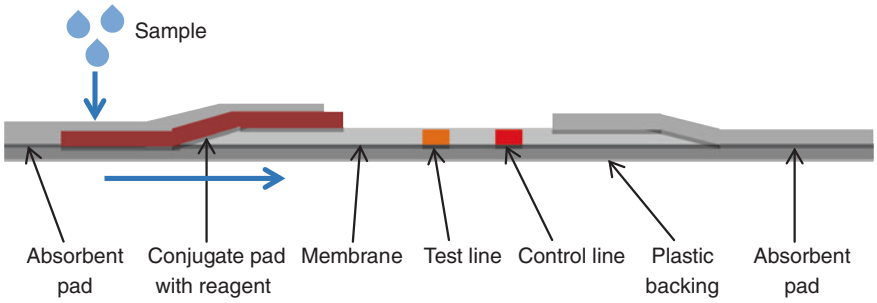

Fig. 1.1 Typical structure of a LFIA strip

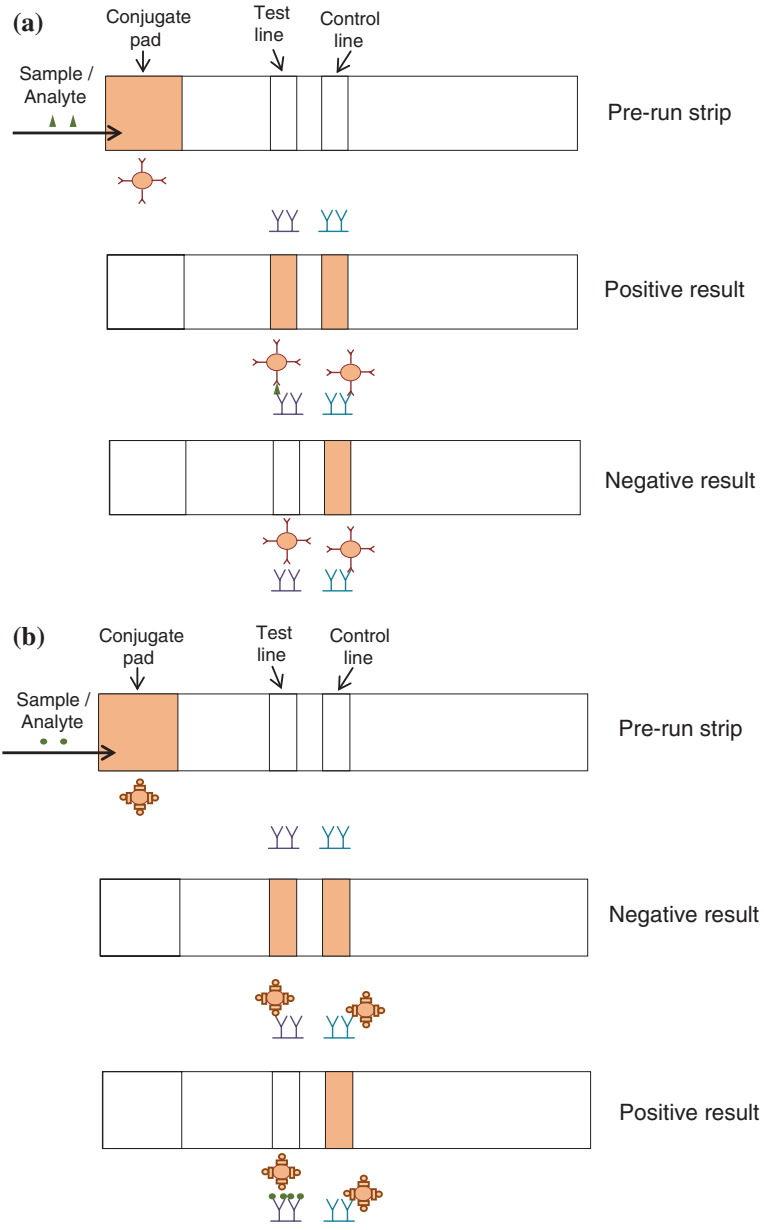

Fig. 1.2 **a** Direct solid-phase immunoassay. **b** Competitive solid-phase immunoassay

1.3 Advantages

LFIAs represent a well-established and very appropriate technology when applied to a wide variety of in vitro diagnostics (IVD) or field-use applications. The advantages of the LFIA are well known:

a. Technology is mature.
b. Manufacture is relatively easy: Equipment and processes are already developed and available.
c. They can be scalable to high-volume production.
d. They can be stored for 12–24 months, often without refrigeration.
e. They are easy to use, requiring minimal operator-dependent steps and interpretation.
f. They can handle small volumes of multiple sample types.
g. They can be integrated with onboard electronics, reader systems, and information systems.
h. They have high sensitivity, specificity, and good stability.
i. Development and approval are relatively low cost and require a short timeline.
j. They are already present and accepted by the market: Minimal education is required for users and regulators.

1.4 Antibody

Although the physical components of the lateral-flow test strip and construction techniques play a major role, the most critical part of the LFIA is the appropriate antibody to provide antigen recognition. If we chose inappropriate antibody, it would not have ability to recognize the target antigen. Much time is spent determining the most suitable antibody for specific assays. Many scientists have spent a great deal of time figuring out the suitable antibodies to fit the assay.

The LFIA is particularly demanding in terms of the mass of the reagent used to drive the antibody and antigen interaction. When an antibody is used in a sandwich-type assay, they are applied at a ratio of 1–3 μg per cm across the width of the nitrocellulose strip, in a line 1 mm wide and with a relatively shallow bed volume of 0.13 mm. This results in an antibody concentration of 10–30 μg per square cm, which is 25–100 times that used in an enzyme-linked immunosorbent assay (ELISA), which can typically require a maximum concentration of 300 ng per square cm [21].

Antibody and antigen affinity also plays an important role in the assay. Consider a typical lateral-flow test strip with antibody immobilized on a test line of 0.5–1.0 mm wide. Antigen flowing up the strip has a flow rate in the range of 0.16–0.66 mm per second, depending on the flow rate of the nitrocellulose membrane selected [22]. Antigen thus spends between 1–6 s on the line where it can interact with the immobilized antibody. Flow speed is actually faster at the

initiation of the flow, since the flow rates decrease proportionately to the square of the distance traveled, a steady flow rate is achieved, and the entire nitrocellulose bed volume becomes saturated.

Antibodies applicable for LFIA are available from many commercial sources [19]. Frequently, these antibodies can be obtained for competitive assay, such as hormones, therapeutic drugs, and drugs of abuse. Similarly, suitable antibodies are purchasable for sandwich assay tests to diagnose pregnancy (hCG), infectious disease (HIV, hepatitis B), cardiac markers (troponin C, creatinine kinase-MB, myoglobin), or malignancies (prostate-specific antigen).

1.5 Labels

Some labels have been successfully commercialized and others appear promising. The development of labels for LFIA has matured hand-in-hand with advances in detection methodology and instrumentation. Sensitive assays with fluorescent and luminescent labels have been used in recent years. The ideal labels for lateral-flow strips have the following characteristics:

a. They can be detected by multiple methods on a large and useful dynamic range.
b. When sample and reagent conjugate, their biological and chemical quality and activity are not be changed.
c. The lack of non-specific binding characteristic such as high signal-to-noise ratio under buffer, salt, or detergent conditions.
d. High stability under various temperatures.
e. They are typically available at low cost.
f. The procedure of conjugating is easy and scalable.
g. They are capable of being used for multianalyte detection.

Liposomes can be used as a vehicle for membrane-based assays in vertical and lateral-flow test strips (e.g., test for malarial antigen from Becton Dickinson) [20]. Because of their ability to encapsulate very high concentrations of signal-generating molecules within their cores, liposomes can improve LFIA sensitivity to 2–3 orders. Lipoproteins, glycolipids, and various other lipid-containing compounds can be incorporated directly into the bilayer. In addition, different chemically active groups can be incorporated onto the lipid surface with controlled surface density for covalent coupling to biological or chemical compounds [23].

Colloidal carbon particles can serve as a label in sol particle immunoassays [24]. They have been reported since the 1970s [25]. Their advantages include good stability and high color comparison on a membrane. They are quite easy to conjugate, and a bottle of carbon particles may consequently last for millions of tests.

Colloidal gold has been widely used in immunoassays for large molecules such as for the detection of hormones (pregnancy, fertility), virus (HIV, hepatitis B and C), and bacteria (*Streptococcus suis* serotype 2). It may be the most widely used label today [26]. Determination via colloidal gold-based immunoassay can

be completed rapidly in a single step [27]. When an antibody labeled with colloidal gold particles is combined with the corresponding antigen, the colored immunoreactant can be visually detected. This user-friendly format possesses several advantages, including rapid reaction time, long-term stability over a wide range of climates, and low cost. These characteristics make it ideally suited for on-site testing by untrained personnel.

A variety of other labels have been used for specific applications. For instance, a portable fluorescence biosensor with rapid and ultrasensitive response for protein biomarker has been created using quantum dots and a LFIA. The superior signal brightness and high photostability of quantum dots are combined with the promising advantages of a lateral-flow test strip, resulting in high sensitivity, selectivity, and speed for protein detection [28]. Also, more recent reporter up-converting phosphor technology has been applied to DNA (hybridization) assays for the detection of specific nucleic acid sequences. This methodology is sensitive and provides a rapid alternative for more elaborate gel electrophoresis and Southern blotting [29].

1.6 Membranes

While a LFIA test strip may include elegant chemical complexity, the common core of all such tests is the nitrocellulose membrane, which for several reasons is the most significant test component [30–32]. First, it is the surface upon which the critical immune complexes form. Second, it is the surface upon which the signal is detected, either visually or electronically. Third, it has been the most difficult material to manufacture consistently.

One of the key membrane performance parameters is protein binding. It is essential to the function of the membrane in a lateral-flow test strip. The membrane usually adsorbs more than 100 μg of IgG per cm^2. At the concentrations of capture reagents typically applied to the membrane, there is fivefold to tenfold more binding capacity than necessary. Adsorptive capacity decreases with the molecular weight of the protein [33]. To maximize adsorption, antibodies and other proteins should be applied to the membrane in buffers that are preferably free of salt, surfactants, and sugars. The buffer should also be at a low concentration so that crystals dried in the membrane are not of sufficient abundance to occlude the pores.

Another key membrane performance parameter is membrane blocking. Blocking prevents non-specific binding of the detector particle and analyte, but is not absolutely essential to LFIA strips. There are many test strips on the market that do not use a blocking agent; however, blocking agents are required for some tests because of the nature of the particular sample and antibody system [34]. Two blocking agents must be used: one blocking agents dissolves upon addition of the sample and moves along the strip with sample, and the other is applied directly to the membrane by spraying on a fixed amount of blocking solution or dipping the membrane into a reservoir of blocking solution.

The final membrane performance parameter to consider is membrane storage capacity. Storage capacity and condition vary depending on the stage of the test strip manufacturing process. Up until the point that reagent is going to be applied, the membrane can be stored under ambient conditions (15–30 °C, 20–80 % relative humidity). A condensing atmosphere should be avoided, as liquid in the pores can cause redistribution of mobile components, such as the surfactant. When a membrane is being prepared for application of the capture reagents, it should be allowed to equilibrate to the humidity of the dispensing room. Humidity from the air hydrates the surface of the nitrocellulose and improves the absorption of the capture reagent solutions. If possible, assembly of the test strips should take place in a dry room.

1.7 Application

LFIA is well established as a valuable tool in food, medical, environmental, veterinary, agricultural, and industrial diagnostics. Sometimes it is used as a rapid screening tool and backed up by more complex and time-consuming assays. Figure 1.3 lists the market segments in which LFIAs are already in production or are known to be in development.

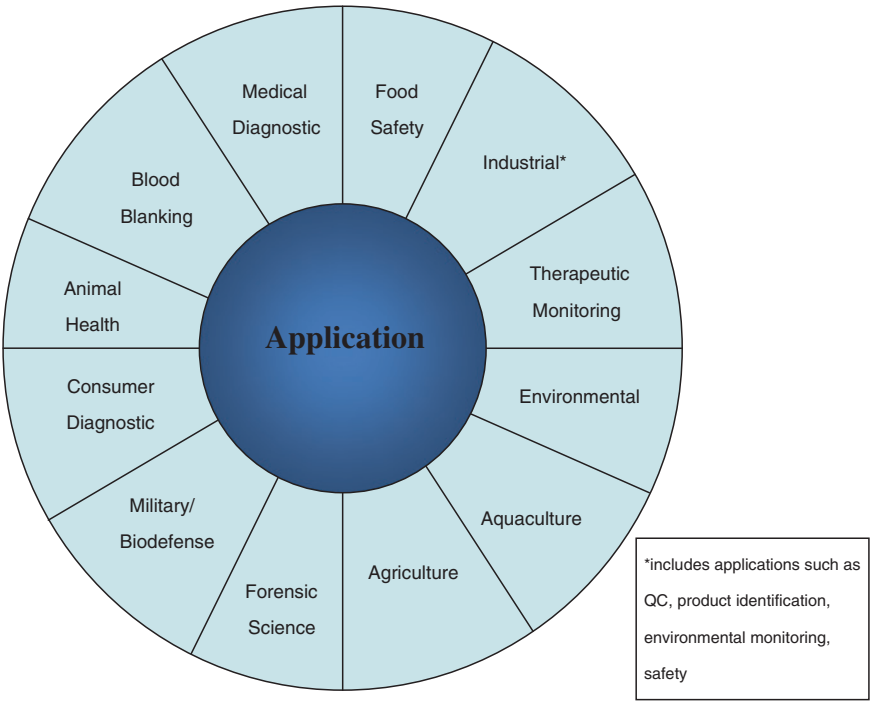

Fig. 1.3 Market segments for LFIA and other point-of-care or field-use technologies

LFIA has well-established formats for POC testing. The first paper-based diabetes dipstick test was created in the 1950s to quantify glucose in urine [35]. Semiquantitative results could be determined by comparing urine-treated test strips to a color-coded chart to determine glucose concentration. Today, commercial urinalysis dipsticks have been widely adapted for a number of analyses. In the 1980s, serological lateral-flow tests started to emerge, particularly for human pregnancy tests. This process was derived from the development of the hCG beta-subunit radioimmunoassay [36, 37].

The majority of these tests come in different sizes, shapes, and configurations. These assays are available without (Fig. 1.4a, b) or with housing units (Fig. 1.4c–f). Nowadays, multiplexing of rapid tests is becoming fairly common as illustrated in Fig. 1.4g–i, which illustrates a lateral-flow format that separates each single lateral-flow test strip into multiple channels. The assay is multiplexed in the sense that a single sample is analyzed simultaneously, but in reality, the test strips are still separate reactions occurring independently of the other reactions [38, 39].

On-chip reagent storage for long-term test and transportation is well developed for IVD. For example, LFIA strips adopt dried gold nanoparticles (AuNPs)-conjugated antibodies regents at conjugation pad for rapid pregnancy, drug abuse, and other diagnostic tests. A plasma fibrinogen assay was implemented on a polymeric micropillar-based LFIA platform by drop-casting bovine thrombin and the surfactant Triton X-100 on the dextran-coated platform [40]. This pillar structure can also be used for an interferon-γ LFIA assay [41].

One of the major application for IVD test is the detection of the metabolites of illegal drugs such as Δ9-tetrahydrocannabinol (THC), amphetamines, benzodiazepines, cocaine, morphine, heroin, opiates, and cannabis in workplace or prison settings. The presence of addictive drugs in the body fluids including blood, urine, sweat, and saliva is monitored to detect and prevent drug abuse, illicit trafficking or driving under the influence of drug (DUID) that is getting more attention worldwide [42, 43]. Furthermore, continuous concern about recreational drug abuse and doping in competitive sports still attracts social attention [44, 45]. The prohibited substances such as strychnine, pervitin, captagon, or Benzedrine are the target molecules for detection.

Oral fluid has been demonstrated as an adequate alternative matrix for drugs identifying and quantifying tests in workplace, clinical treatment, drug rehabilitation center, criminal justice, and DUID settings [46]. The drug tests using oral fluid instead of blood and urine possess various advantages such as inexpensive, rapid, infection risk is lower than for blood sample, and noninvasive of sample collection, which can be easily observed to avoid the need for private facilities and same-sex collectors and decrease adulteration. In addition, oral fluid better reflects recent drug use and reflects free plasma concentrations, providing a better correlation with pharmacodynamic effects.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS) are the most delegated equipment performing high accurate analysis of multiple compounds in a limited oral fluid volume. However, the complex sample preparation using liquid–liquid

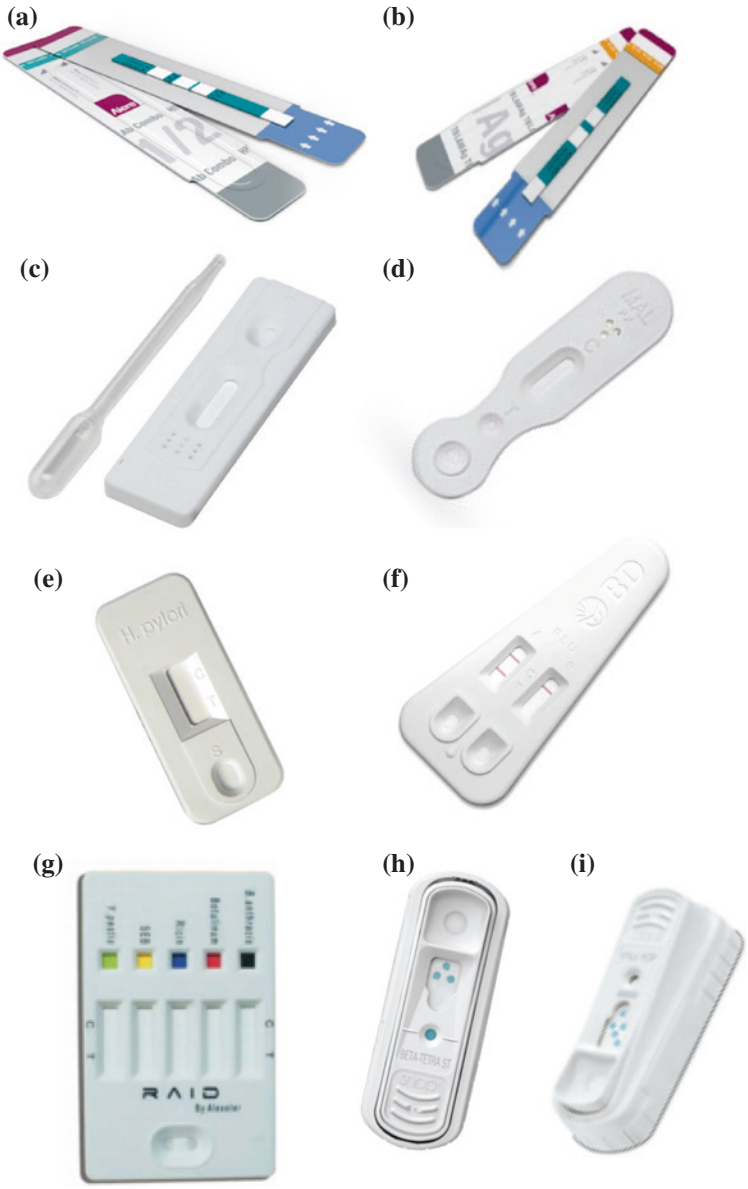

Fig. 1.4 Commercial LFIA tests. **a** Determine™ HIV 1/2 Ag/Ab Combo. © 2013 Alere. All rights reserved. **b** Determine™ TB-LAM Ag test © 2013 Alere. All rights reserved. **c** One Step LH Ovulation Rapid Test © 2010 Accu Plus Medical. All rights reserved. **d** Clearview® Malaria P.f. Test © 2013 Alere. All rights reserved. **e** ICON HP © Beckman Coulter, Inc. All rights reserved. **f** BD™ EZ Flu A + B Test © Becton Dickinson. **g** RAID™ 5 © Alexeter Technologies. **h** SNAPduo™ Beta-Tetra ST Test © 2013 IDEXX Laboratories, Inc. (https://www.idexx.com/small-animal-health/index.html; accessed 10/15/2014). **i** SNAP® Heartworm RT Test © 2012 IDEXX Laboratories, Inc. (https://www.idexx.com/small-animal-health/index.html; accessed 10/15/2014)

extraction or solid-state extraction, time-consuming detection processes, bulky size of equipment, and power sources requirement confined the possibility of on-site tests. There are some commercial portable oral fluid test devices that have been developed and available on market providing satisfactory detection ability to achieve the requirement of detection limit of certain drugs. One of the successful commercial examples for on-site drug test is Oratect. It is a LFIA-based test utilizing AuNPs for colorimetric sensing. In order to collect oral fluidic samples, sample collector is combined in a single device [47].

A number of strategies are available for the detection of nucleic acids in lateral-flow systems [48–50]. The capture of nucleic acids can be performed in an antibody-dependent or antibody-independent way. For example, in an antibody-dependent system, an anti-biotin antibody immobilized on the surface of nitrocellulose is used to capture biotin- and carboxyfluorescein (FAM)-bearing oligonucleotides in RPA amplicons [51]. Binding is subsequently detected using an anti-FAM-colloidal gold conjugate. An antibody-independent alternative utilizes streptavidin as the binding agent. Immobilization of oligonucleotide probes directly onto membranes is also possible using oligonucleotides linked to carrier proteins.

When considering the worldwide market applicability of diagnostics, a socioeconomic division is often applied. Cardiac and other chronic diseases in the expanding middle classes of emerging economies are growing, as are the incidences of previously geographically limited infectious diseases (e.g., malaria, dengue), emerging diseases (e.g., H5N1 influenza), and heretofore well-controlled diseases (e.g., TB in First World countries) in developed countries. At least 30 previously unknown disease agents have been identified since 1973, including HIV, Ebola, hepatitis C, and SARS. In chronic diseases, there remains significant growth, particularly in the areas of inflammation, cardiac markers, and cancer, with a myriad of new labels in development in the search for improved diagnostic and prognostic indicators.

In the past 3–5 years, food safety issues and concerns for public health have led to more stringent legislation in food safety requirements. Legislation has produced increased demand for pathogen and toxin tests in just about every segment of the food production industry. There is a growing demand from food companies for quicker testing to facilitate more rapid release of finished goods and thus reduce inventories. A driver in the demand for rapid and LFIA tests in food production is the adoption of hazard analysis and critical control point (HAACP) regulations that prescribe test procedures throughout the manufacturing process.

1.8 Conclusion

LFIA technology is rapidly being developed. Market needs lead to the improvements in performance and utility and open doors to a vast array of new application areas. With the integration of new reading, labeling, sample handling, and

device designs comes a requirement for a new approach to system development and manufacturing. The development of highly sensitive and reproducible/quantitative next-generation point-of-need diagnostic assays requires a different, more multidisciplinary approach than has been the case with standard LFIAs.

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