REVIEW

Development and application of lateral flow test strip technology for detection of infectious agents and chemical contaminants: a review

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Abstract Recent progress in the laboratory has been a result of improvements in rapid analytical techniques. An update of the applications of lateral flow tests (also called immunochromatographic assay or test strip) is presented in this review manuscrit. We emphasized the description of this technology in the detection of a variety of biological agents and chemical contaminants (e.g. veterinary drugs, toxins and pesticides). It includes outstanding data, such as sample treatment, sensitivity, specificity, accuracy and reproducibility. Lateral flow tests provide advantages in simplicity and rapidity when compared to the conventional detection methods.

Keywords Biological agents · Chemical contaminants · Lateral flow tests · Detection methods

Abbreviations

AIV	Avian Inflenza Virus
CFU	Colony-forming unit
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbant assay
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance Liquid Chromatography
ICA	Immunochromatographic assay
LFT	Lateral flow test strip
NALFIA	Nucleic acid lateral flow immunoassay
NALFT	Nucleic acid lateral flow test strip
RT-PCR	Reverse transcription-polymerase chain reaction
WHO	World Health Organisation

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Introduction

In the last decade, advances in technologies have driven a new era in the analysis of both biological agents and chemical contaminants. For instance, various new diagnostic tools avalaible for detecting biothreat agents are based on genetic techniques (e.g. RT-PCR, hybridization and microarrays) and immunosensor techniques [1, 2]. For monitoring residue contaminants such as veterinary drugs, an analytical strategy has been recommended using two differents methods. This strategy comprises: (i) screening with a first method optimized to prevent false negative results, with a high sample throughput (e.g. ELISA), an acceptable percentage of false positive results and low cost, and (ii) confirmation with an independent second method optimized to prevent false positive results [3–5]. Confirmatory methods are generally separative techniques coupled with various detectors such as HPLC and GC–MS. These analytical techniques have been described and reviewed extensively in the literature [6–13]. Chromatography methods are sensitive and specific, but suffer from being time consuming, laborious and multi-complex. In addition, these technologies are unaffordable to the farmers and some laboratories in the developing countries. There have been therefore emergent needs for developing highly accurate, rapid and cheap analytical tools. To acheive this goal, many attempts have been focused on the development of rapid point-of-care (POC) testing such as lateral flow tests. Immunochromatographic assay (ICA), lateral flow tests

(LFT) or test strips has been well-established diagnostic tool in laboratory. This technology offers additional advantages when compared to the conventional detection methods: rapid, simple and cost-effective. The format in LFT is similar to ELISA, and the base substrate is nitrocellulose membrane in which is immobilized capture binding protein, usually an antibody or antigen. Labels such as latex, colloidal gold, carbon, and recently up-converting phosphorus technology have been employed in LFT development [14–18]. The first application of strip assay was the pregnancy test with the detection of human chorionic gonadotropin (HGC) [19]. The speed observation of results directly by the naked-eyes and the utilization of a membrane strip as the immunosorbent provided an analytical platform that permits one-step, rapid and low-cost analysis. To date, this technology has reached many fields of research such as veterinary diagnostic, food monitoring and environment. Recently, a review paper describing LFT, its strengths, weaknesses, opportunities and threats has been presented by Posthuma-Trumpie et al. [20]. Here, we aimed to present awide range of reports published in these last five years and regarding the application of LFT for the control of pathogen bacteria and related toxins, infectious viruses, veterinary drug residues, mycotoxins and pesticides. Outstanding results from these studies were presented and discussed with illustrations and tables. The principle and assay formats on LFT development are firstly emphasized in this review manuscrit.

LFT principles and assay formats

A simple LFT device consists of four sections (sample pad, conjugate pad, nitrocellulose membrane and absorbent pad) which are laminated into a sheet of plastic backing oderly. There are various possible formats depending on the type of target analyte [21]. The two kinds of format frequently used are competitive assay and sandwich assay. Competitive format is employed most often when testing analytes with low molecular weight or presenting single antigenic determinant [22]. In a competitive format (Fig. 1a), an analyte-protein conjugate (e.g. N-sulfanilyl-4-aminobenzoic acid-Bovine Serum Albumin (BSA)) coated on the test zone of a nitrocellulose membrane captures a labeled anti-analyte monoclonal antibody complex, allowing colour particle (e.g. colloidal gold) to concentrate and form a visible line on the test zone. Another specific antibody coated on the control line allows the capture of the excess antibody complex. One band colour will therefore be visible in the control zone regardless of the presence of target analytes (e.g. sulfonamides), confirming correct test development. Conversely, a negative sample will result to the formation of two band colours visible (test line and

control line) [17]. On the other hand, sandwish assay format (Fig. 1b) is employed to test analytes presenting several epitopes such as viral protein. The system in sandwish assay can employ two different antibodies (polyclonal and monoclonal) that bound distinct epitopes of the analyte: a labeled polyclonal antibody is placed in a dehydrated state onto a glass-fiber membrane (conjugate pad) to serve as detector reagent and a monoclonal antibody specific to the analyte is sprayed at the test line of the nitrocellulose membrane to serve as capture reagent. An additional antibody specific to the detection antibody could be used to produce a control signal. When a sample extract is applied to sample pad, the liquid migrates up by capillary force and the detector reagent is then released. Some of the analyte (e.g. AIV) bind to the detection antibody and some remain free in the solution. Subsequently, the mixture passes through the capture zone and both unbound analytes and bound analytes bind to the capture antibody. The response in the capture zone (test line) is directly proportional to the amount of analyte in the sample [23, 24]. Two disadvantages can be noticed in sandwish assay format [22]: (1) signal generation on the test line may be compromised when the concentration of target analyte exceeds a certain critical value; (2) the target analytes must be able to bind simultaneously to both the detection antibody and the immobilized capture antibody.

Recently, new advances have been made to develop cost-effective and rapid nucleic acid testings based on lateral flow platform [25–28]. Nucleic acids can be captured on lateral flow test strips either in an antibody-dependent or antibody-independent manner. Antibody-dependent format also called "nucleic acid lateral flow immunoassay (NALFIA)" employs an antibody capture line and a labelled amplicon or oligonucleotide probe of complementary sequence to the amplicon. In this case (Fig. 2a), the detection of an amplified double-stranded nucleic acid sequence specific to a target organism can be achieved by using primer with two different tags (e.g. biotin and fluorescence isothiocyanate). Recognition of the analyte is done by binding to a tag-specific antibody (anti-fluorescein antibody) sprayed previously on a nitrocellulose membrane and gold nanoparticles labelled avidin is used as reported enabling the visualisation [29]. Alternatively, antibody-independent format "nucleic acid lateral flow test strip (NALFT)" is based on the use of two binding partners such as biotinylated probe or amplicon, and a streptavidin, which present high affinity and irreversible linkage. Two convenient approaches are possible in such format: (1) the immobilization of the oligonucleotide capture probe to the nitrocellulose membrane through passive adsorption (Fig. 2c) or (2) via passive absorption using BSA oligonucleotide conjugate capture probe (Fig. 2b). The responses are directly proportional to the amount of analytes [30].
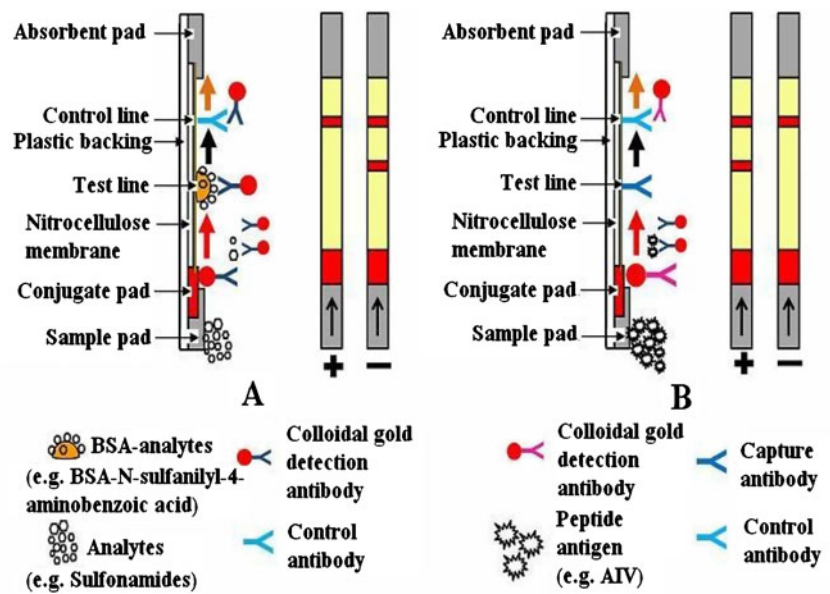
Applications for the detection of infectious agents

Bacteria are causative agents of foodborne illiness in 60% of cases requiring hospitalization [31]. Outbreak of food contaminations caused by *Salmonella, Listeria, Campylobacter, Clostridium* and *Escheriachia co*li have been reported elsewhere [32]. LFT has been confirmed to be a rapid and sensitive method in the detection of food borne pathogens. Other remarkable studies included the detection of bacterial toxins and zoonotic viruses such as Avian Influenza (AIV). The Tables 1 and 2 summarize the published reports on LFT applications in this field.

Bacteria and related toxins

Listeria

The genus *Listeria* (*L.*) comprises six recognized species: *L. monocytogenes, L. ivanovii ssp. ivanovii* and *londoniensis, L. innocua, L. welshimeri, L. seeligeri,* and *L. grayi.* [33]. Blaskoza et al. [26] proposed a NALFI to detect various strains of *Listeria* spp and *Listeria monocytogenes.* The assay procedure involved an enrichment step (24 h), isolation of template DNA and a PCR amplification step of specific sequences (3.5 h), and the detection of amplified product by NALFIA (5–15 min). The NALFIA employed carbon neutravidin conjugate and a capture antibody. The test adapted to a tube format allowed the detection of less than 10 cells of *L. monocytogenes/ Listeria spp* in 25 mL of milk within 28 h (24 h for cultivation, 4 h for PCR and NALFIA). The assay was then compared with mircobial standard methods for the analysis of 24 real samples prepared from different types of food or food-related samples. Of the 24 samples

analysed, 15 contained *Listeria spp.* and 5 were positive for *L. monocytogenes.* All samples tested by the microbiological standard method gave identical results as for the *Listeria*-NALFIA method. Indeed, commercially avalaible test for specific detection of *Listeria monocytogenes* allowed a detection time ranging 37–54 h, whereas the reference method as proposed by International Organization of Standardization (ISO) took 5–7 days. The developed assay is a helpful tool for routine diagnostic since the result analyses can be obtained within 28 h from receipt.

Escherichia coli

Escherichia coli (*E. coli*) are Gram-negative bacteria known as normal inhabitants of the human and animal gut. The bacterium *E.coli* is the etiological agent of a whole range of water and food borne diseases [34]. In host,

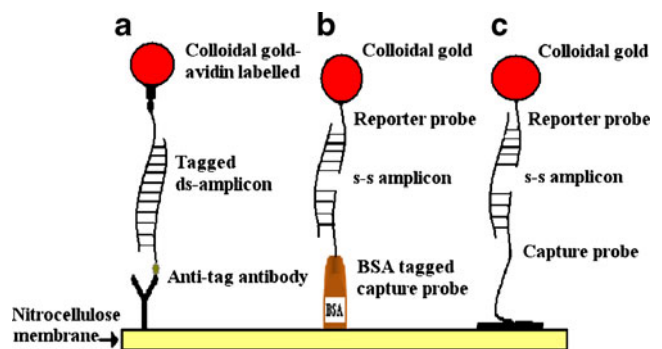Fig. 2 Schematic representation of three detection principles in nucleic acid lateral flow test strip method; (**a**) Nucleic acid lateral flow immunoassay; (**b**) Nucleic acid lateral flow using bovine serum albumin-oligonucleotide conjugate capture probe (**c**) Nucleic acid lateral flow test strip with oligonucleotide capture probe immobilized on the nitrocellulose membrane via passive adsorption

Table 1 Summary of studies on lateral flow test strip for the detection of pathogen bacteria and related toxins

Analytes	Assay formats	Labels	Sensitivities	Samples	References
Listeria spp and Listeria monocytogenes	Sandwich NALFIA	Carbon black	10 CFU. 25 mL^{-1}	Dairy products,nutrition for children and biofilms	[26]
Escherichia coli recombinat protein EspB	Sandwish ICA	Colloidal gold	4 ng. mL^{-1}	Sera and stools	[38]
Escherichia coli O157: H7 lipopolysaccharide	Sandwish ICA	Colloidal gold	1.8×10^5 CFU. mL^{-1}	Pork, bovine feces swine feces	[39]
Escherichia coli	Sandwich ICA	Colloidal gold	10^5 CFU. mL^{-1}	milk powder, flour, starch, etc	[40]
Bacillus anthracis spores	Sandwish based immunomagnetic separation	Magnetic beads	10^5–10^7 CFU. mL^{-1}	Water and dairy products	[44]
Bacillus anthracis spores	Sandwich hybridization LFM	COOH-dye microsphere	250 amol (equivalent of 1.5×10^8 dnaR89 molecules)	PBS	[27]
Staphylococcus aureus Protein A	Sandwish ICA	Colloidal gold	<25 CFU. g^{-1} (93.0–100%)	pork, beef, fried chicken	[49]
Salmonella enteritidis	Sandwish ICA	Colloidal gold	10^{7}CFU. mL^{-1}	Raw eggs	[53]
Campylobacter jejuni and Campylobacter coli	Sandwish ICA	Colloidal gold	1.8×10^4 to 8.2×10^5 CFU/ml.4×10^5 to 4.6×10^{6}CFU. mL^{-1}	Human stools	[57]
Streptococcus suis serotypes 2	Sandwish ICA	Colloidal gold	1.0×10^{6}CFU. mL^{-1}	PBS	[61]
Swine antibodies against Streptococcus suis serotypes 2	Sandwish ICA	Colloidal gold	86.3% compared with reference ELISA	sera	[62]
Yersinia pestis	Sandwish ICA	Up-converting phosphor technology	10^{4}CFU. mL^{-1}	PBS and lung tissues from mouse	[18]
Botulism neurotoxin D	Sandwish ICA	Colloidal gold	50 pg. mL^{-1}	Faecal	[63]
Botulism neurotoxin B	Sandwish ICA	Colloidal gold coupled with silver enhancement	50 pg. mL^{-1}–50 ng.mL^{-1}	Urine, PBS	[64]
Botulism neurotoxin A	Sandwish ICA	Colloidal gold coupled with silver enhancements	1–50 ng.mL^{-1}	PBS	[65]
Staphylococcus aureus enterotoxin B	Sandwish ICA	Colloidal gold coupled with silver enhancements	10 pg. mL^{-1}–100 ng. mL^{-1}	PBS, human urine, serum and cow milk powder	[66]
Staphylococcus aureus enterotoxin B	Sandwish ICA	fluorescent immunoliposomes	0.02–0.6 pg. mL^{-1}	Tap water and surface water, apple juice, raw milk, ham and cheese	[67]

CFU = Colony -forming units

Table 2 Summary of studies on lateral flow test strip for the detection of infectious viruses

Analytes	Assay formats	Labels	Samples	Sensitivities	References
AIV H9	Sandwish ICA	Colloidal gold	Chicken sera and eggs allantoic fluids	0.25 units	[23]
IgG antibodies against AIV subtypes (H5, H7 and H9)	Indirect ICA	Colloidal gold	Chicken sera	1:1024 dilutions	[24]
AIV H5N1	Sandwish ICA	Colloidal gold	Chicken allantoid fluid	1:1000 dilutions	[72]
AIV type A and B	Sandwish ICA	Colloidal gold	Tracheal bronchoalveolar, and swabs specimens	94.4% agreement compared with viral culture method	[74]
AIV type A and B	Sandwish ICA	Colloidal gold	Nasal swab and pharyngeal swab specimens	93.3% agreement compared to viral isolation method	[75]
Hepatite B surface antigen (HbsAg)	Sandwish ICA kits		Human sera	97.5–99.2% agreement compared to PCR	[77]
Hepatite B surface antigen and anti-HBs	Sandwish ICA (commercial kits)		Sera	97–98.5% agreement compared to chemiluminescence immunoassay method	[78]
Hepatite B surface antigen	Sandwish	Europium chelate-loaded silica	Sera	0.03 µg L^{-1}	[79]
Western Nile virus IgM detection	Sandwish ICA (Commercial kits, "Rapid WN ^{TM}")		Sera	92.9% agreement compared to four public health laboratory-developed screening tests	[84]
Western Nile virus IgM detection	Sandwish ICA (commercial kits, "Rapid WN ^{TM}")	Colloidal gold	Sera and plasma specimens	100% agreement compared to reference ELISA method	[85]
PRRSV specific antibodies	Sandwish assay ICA	Colloidal gold	Swine sera	96% agreement compared stantard indirect ELISA and an immunoperoxidase monolayer assay	[86]
PRRSV specific antibodies	Sandwish ICA	Colloidal gold	Swine sera	93.2–98.7% agreement compared to reference ELISA and immunofluorescence assay	[87]
Norovirus genotypes II/3 and genotypes II/4	Sandwish ICA	Latex	Stool	84.1% agreement compared to RT-PCR	[16]
Group A rotavirus and norovirus Genotypes II.4 variant	Sandwish ICA (commercial Kits)		Stool	73.7–87.8% agreement compared with PCR	[88]
Porcine Rotavirus (PRV)	Sandwish ICA	Green microparticles	Stool	1000 plaque-forming units of PRV/ml, and 100% agreement compared to PCR method	[89]
Human Adenovirus	Sandwish ICA (commercial Kit)		Stool	91–95% compared with standard isolation method and PCR	[90]
Surface antigen of Human Rotavirus and Adenovirus	Sandwish ICA (commercial kits)		Stool	22–75% compared with nested PCR method	[91]
Group A rotaviruses	Sandwish ICA (commercial kits)		Fecal	70–98.8% agreement compared with ELISA	[92]
Recombinant nucleoprotein of Rabies virus	Sandwish ICA (commercial kits)	Colloidal gold	Rabid-brain	95.5% compared with PCR method	[149]
Yellow Head Virus (p20 structural protein)	Sandwish ICA	Colloidal gold	Shrimp	1:1200 dilution of sample	[150]

enterohemorrhagic and enteropathogenic *E. coli* strains cause diarrhea through attaching and effacing lesions (A/E) on the intestinal cell surfaces. The genes encoding the A/E phenotype are in the locus of enterocyte effacement, a pathogenicity island in the chromosome. This locus contains genes encoding secreted proteins (EspA, EspB and EspD) which are involved in the A/E lesions [35–37]. An ICA against EspB of enterohemorrhagic and enteropathogenic *E. coli* strains has been described by Nakasone et al. [38]. The test based on double sandwich format used an affinity-purified anti-EspB polyclonal antibody conjugated with colloidal gold, and EspB was purified as recombinant (rEspB). The study comprised the identification of 71 *E.coli* strains collected from different countries and stool samples supplemented with rEspB. Preparation of stool samples included the suspension of 0.1 g in 1 mL of PBS, which was followed by addition of 960 µg. mL^{-1} of rEspB. In the assay of rEspB, 100 µL of rEspB solution was added to the sample pad of the test strip. For the bacterial strains, all strains were cultured in 2.0 mL of Dulbecco modified essential medium (DMEM) in shaking test tubes at 37 °C for 8 h. The test strip was then soaked in the culture supernatant obtained through centrifugation at 3000 rpm for 15 min. With stool samples, stool solution was centrifuged at 3000 rpm for 10 min, and the test strip was soaked in the supernatanant. The developed ICA allowed the detection of rEspB produced at 4 ng. mL^{-1} within 10 min. The detection limit of EspB in stool sample was up to 60 ng. mL^{-1}. However, the test was not able to detect EspB at 100 ng. mL^{-1} when it was directly soaked in stool solution without obtaining a clear supernatant by centrifugation. With the 71 *E. coli* strains, 33 of 34 strains harboring the *eae* gene (locus of enterocyte effacement gene) encoding intimin were positive with the test strip, and all 40 of the eae-negative strains were negative. The results of the ICA sensitivity and specificity were 96.9% and 100%, respectively. An ICA against *E. coli* 0157 in enriched samples (raw beef, pork, bovine feces and swine feces) was described by Jung et al. [39]. This test based on sandwich format employed a murine monoclonal antibody to *E. coli* O157: H7 lipopolysaccharide conjugated with colloidal gold. Using 10 fold diluted *E coli* O157:H7, with a range of 1.8×10^7 to 1.8 CFU. mL^{-1} in enriched raw beef, the detection limit was as low as 1.8×10^5 CFU. mL^{-1} without enrichment and 1.8 CFU. mL^{-1} after enrichment. In the specificity study, 48 pure-cultured bacteria (32 *E. coli* strains and 16 non-*E. coli* strains) were tested by the ICA. Three strains revealed positive signal to *E. coli* O157:H7 by the ICA and the other 29 *E. coli* serotypes were negative. Among the non-*E coli* strains, only *Citrobacter amalonaticus* yielded a positive signal. The specificity of the test strip was somewhat higher with pork samples (98.8%) than with bovine feces samples (87.9%) and swine feces samples

(93.4%). The assay was then compared with traditional culture procedure for the analysis of various samples enriched with *E coli* O157:H7. The agreement rates between the two methods were 100 %.

Wang et al. [40] developed an ICA to detect *E. coli* O157:H7 in various samples including milk powder, flour, starch, etc. The sensitivity of the test was estimated to be 1×10^5 CFU. mL^{-1}, and the detection time was less than 15 min. In the specificity study, no-cross-reactivity was found toward 30 strains of 24 species in Enterobacteriaceae (non-O157 *E. coli*, *Salmonella*, *Shigella*, *Proteus*, *Citrobacter*, *Enterobacter*, *Serratia* and *Yersinia*), *Staphylococus*, *Listeria*, *Aeromonas* and *Vibrios*.

Bacillus anthracis

Bacillus anthracis is the causative agents of anthrax, an infectious disease caused by spore forming of *Bacillus anthrax* [41, 42]. The anthrax incidents of 2001 [43] and terrorism events around the world highlight concerns about unconventional terrorist attacks, including the purposeful contamination of food and its products. A combination of immunomagnetic separation and a LFT was described by Fisher et al. [44] for the detection of *Bacillus anthrax* spore in water and dairy products. Immunocapture of *Bacillus anthracis* spore was carried out with anti-spore antibodies coupled with COOH–magnetic beads for 30 min. The resulting complex was washed twice with PBS, and the spores were eluted with 95% (v/v) formamide-10 mmol. L^{-1} of EDTA for 30 seconde in a micro wave oven. Then, eluted spores (supernatant) were diluted and then applied directly on the LFT. To evaluate the sensitivity limit, a range of *B. anthracis* spore concentrations (10^5–10^8 CFU. mL^{-1}) was examined. *Bacillus anthracis* spore recovery was approximatively 95% when 10^5–10^7 CFU. mL^{-1} of the bacteria spores were inoculated in 1 ml of milk or water samples. The nonspecific binding of the immunomagnetic separation test with other Bacilli species (*B. subtilis, B. cereus, Bacillus niger, and Bacillus licheniformis*) was also evaluated by using 10^6–10^7 spores inoculated in 1 mL of milk or water. The results from this study showed that the recovery of *B. anthracis* by the test strip was above 90% and lower than 10% for all other species. The detection time was achieved within 10 min. Another study conducted by Carter et al. [27] evaluated a lateral flow microarray (LFM) using an isothermal amplification strategy for the detection of *Bacillus anthracis*. In this study, a DNA oligonucleotide (dnaR89) composed of sequence derived from a region of the plcR gene of *B. Anthracis* has been used to provide a readily available and quantifiable target for LFM assay development and optimization. The test device was based on hybridization sandwish format, and it comprised a plastic housing designed to carry a conjugate

release pad (COOH-dyed microsphere linked with a target detection probe) and a LFM membrane with immobilised capture probe. The LFM platform provided rapid detection of as little as 250 amol of target dnaR89 (equivalent of 1.5×10^8 molecules). This limit of detection is sensitively similar to other reported fluorescence and chemiluminescence microarray detection strategies [45, 46]. The authors concluded that the developed LFM reduced sample volume to 10 μL, and this low volume was very convenient since it could help to reduce the consumption of reagents during sample analyses.

Staphylococcus aureus

Staphylococcus aureus is a non-motile, spherical, Gram-positive microscopic bacterium. Food poisoning can be also caused by the consumption of food containing staphylococcal enterotoxins. For the toxic levels of enterotoxin to occur, extensive multiplication (growth) of staphylococci cells generally needs to have taken place in the food [47, 48]. An ICA was developed by Huang et al. [49] for detecting Staphylococcus aureus. The developed assay was based on sandwich format using anti-protein A (specific product of S. Aureus, 99%) IgG with two distinct specificities. The study set-up comprised the identification of the production of protein A by 51 strains and an inoculation experiment. After comparison with the conventional method [50], the sensitivity and specificity of the test were 100 and 93.0–100% for 28 Staphylococcus aureus strains and 23 non-Staphylococcus aureus strains, respectively. A total of eleven processed foods (pork, beef, and fried chicken) artificially inoculated with S. aureus at level <25 CFU. g^{-1} yielded positive results by the test strip. The test was however not as sensitive as the conventional culture procedures in detecting S. aureus in raw foods.

Salmonella

Salmonella are genuses of Gram-negative that are usually associated with animals, both wild and domestic. Food contaminations by Salmonella strains can infect humans by causing severe gastroenteritis [51, 52]. Seo et al. [53] presented an ICA to detect Salmonella enteritidis in raw eggs. The test strip device manufactured by Neogen, Lansing, MI was based on sandwish format. The sample pretreatments comprised three steps: (1) disinfection in 70 % of isopropanol, (2) homogenization of egg contents (10 eggs) for 30 secondes under a stomacher and (3) panel dilutions followed by extraction (centrifugation at 10 000 × g) of the antigen with a mixture of fatty acids or phosphate buffer saline (PBS). The detection limit of the test kit increased more than tenfold up to 10^6–10^5CFU. mL^{-1} in whole egg contents using the acid extraction technique compared with tenfold PBS dilution.

The minimum concentration of Salmonella enteritidis to generate a positive band on the test panel was approximately 10^7CFU. mL^{-1} in pure culture, and no cross-reactivity was observed with two other Salmonella serovars, Salmonella Kentucky, Salmonella typhimurium in pure culture.

Campylobacter

Campylobacter are aerobic Gram-negative, motile helical bacteria. Campylobacter species differ from other foodborne pathogens in that they do not multiply within the food. Most frequently, poultry and cattle are the sources of human infection*.[1] Some species including Campylobacter jejuni, Campylobacter coli, Campylobacter lari and Campylobacter upsaliens have been reported to be associated with human gastroenteritis [54–56]. Kawatsu et al. [57] reported a rapid and simple ICA to detect Campylobacter jejuni and Campylobacter coli in human stool samples. The ICA method used a monoclonal antibody against cell surface protein (15-kDa) of Campylobacter jejuni. The study comprised two steps as follows: (1) the identification of cell extracts of Campylobacter (cell suspension of 86 C. jejuni strains, 27 C. coli strains, and strains of the 4 other Campylobacter species) and 26 non-Campylobacter species; (2) and the testing of 222 stool specimens obtained from different patients with acute gastroenteritis. For detecting Campylobacter species from cell extracts and stool samples by the ICA, a suspension of test strains at various cell concentrations were prepared by using PBS. The detection limit of Campylobacter jejuni and Campylobacter coli by the test strip ranged 1.8×10^4 to 8.2×10^5CFU. mL^{-1} and 1.4×10^5 to 4.6×10^6CFU. mL^{-1}, respectively. A detection limit of 5.3×10^5 and 5.0×10^5CFU. mL^{-1} of cell suspensions was obtained with other Campylobacter species including C. lari and C. Upsaliensis. Of the 86 C. jejuni strains and 27 C. coli strains tested, all yielded positive results with the ICA. Negative results were obtained with the other non-Campylobacter species. In the analysis of stool samples, the ICA showed a sensitivity of 84.8% which was comparable to those of some other commercial available enzyme immunoassays (ProSpecT Campylobacter microplate assay; Alexon-Trend, Ramsey, MN).

Streptococcus suis

Streptococcus suis (S. Suis) is a Gram-negative bacterium recognized as an important occupational zoonosis in human [58, 59]. Among the thirty-five serotypes of S. suis characterized, the serotype 2 (SS2) has been identified as the most common and the most pathogenic to both humans

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and pigs [60]. Recently, Ju et al. [61] proposed a colloidal gold-based ICA to detect *S. suis* serotypes 2. In this study, 15 serotypes of *S. suis* and 6 other pathogenic bacteria (*Streptococcus pyogenes* GAS-M15, GAS-M16, *Staphylococcus aureus, Escherichia coli, Salmonella paratyphi A, Clostridium perfringens* and *Listeria monocytogenes*) were collected to evaluate the specificity of the test strip. The preparation of samples comprised the centrifugation of cell culture suspensions ($>10^8$ CFU. mL^{-1}) at $8000 \times g$ for 10 min at 4 °C, which was followed by serial dilution in PBS. Then, 100 µL of diluted sample was applied to the sample pad of the test strip. The sensitivity of the assay to detect SS2 was estimated to be 1.0×10^6 CFU. mL^{-1}. The coupling of the anti-SS2 polyclonal antibodies with colloidal gold particles resulted in high specificity in the detection of the 15 differents *S. suis* serotypes. Only the SS2 and serotype 1/2 showed positive results in the cross-reactivitity study. The authors stated that the capsules of these two serotypes share some common sugar residues or antigenic determinants.

In another study, Yang et al. [62] described an ICA for the detection of swine antibodies against SS2. The test strips were used to detect antibodies in 14 sera from pigs that had survived challenge with *S.suis* serotype 2 and hyperimmune sera raised against *S. suis* serotype 2 and against other four types of pathogen bacteria (*Escherichia coli, Salmonella enterica serovar Typhi, Pasteuralla multocida*, and *Haemophilus parasuis*). The sensitivity and specificity of the ICA were determined by testing 20 hyperimmune sera 226 clinal sera diluted in 20 mM PBS. The specificity and sensitivity of the test strip compared to ELISA as reference test were 97.1% and 86.3%, respectively. Interestingly, an excellent agreement rate was obtained between the results of bacterial isolation and the ICA. The strip developed in this study would potentially be of great value in seroepidemiological surveys of streptococcosis associated with SS2.

Yersinia pestis

Yersinia pestis (*Y.pestis*) belongs to the family of *Enterobacteriaceae*. This bacterium is known as the ethiological agent of plague. An ICA combined with up-converting phosphorus technology (UPT)–based biosensor was proposed by Yan et al. [18] for the detection of *Y.pestis* in diltution buffer and lung tissue samples from mouse. The test strip was based on sandwish format, and UPT particles (400 nm) were conjugated to antibody against F1 antigen of *Y.pestis*. The detection limit of the test strip was 10^4 CFU. mL^{-1}, which was considered below the level of *Y. pestis* that may be found in the blood of mice or human infected with *Y. pestis*. The specificity of the assay was 100%, which was further demonstrated by showing no cross-reactivity to three strains of *Yersinia pseudotuberculosis,* three strains of

Yersinia enterocolitica, two strains of *Escheriachia coli* and one strain of *Salmonella choleraesuis*. The test strip was compared with plague counting method to analyse 51 lung tissue samples from mouse infected experimentally, and satisfactory correlation ($r^2=0.92$) was observed between the two assays.

This study demonstrates the feasibility of combining a UPT-based biosensor with ICA to create a rapid, on-site testing method for quantitative detection of *Y. pestis*.

Botulism neurotoxin

Botulinum neurotoxins (BoNT) produced by the bacteria *Clostridium botulinum* are the most potent poisonus that causes paratical illness in human. ICA had offered a suitable tool for BoNT detection. Klewitz et al. [63] reported an ICA against botulism neurotoxin D (BoNT/D). The test was based on double sandwish format using a gold-anti BoNT/D monoclonal antibody conjugates (detector reagent) and an anti-BoNT/D chicken polyclonal antibody. Feacal samples or standard samples spiked with various concentrations of BoNT/D were treated in 28 mM gelatine-phosphate buffer, vortexed and stored at temperature ranging 5–8 °C for overnight. 2.0 µg. mL^{-1} of polyclonal chicken anti-BoNT/D IgG and 0.35 µg. mL^{-1} of gold conjuagted monoclonal antibody was added to the sample extracts and incubated at 37 °C for 3 hours. Then, 50 µL of pre-treated samples was applied to the test strip. The results indicated that the binding affinity of the investigated antibodies for BoNT/D and the kinetics of the complex formation were the most determining factors for the test time. Signal intensity of the background signal (no specific interaction) with blank samples increased with the increase of sample volume, the incubation time and the amount of gold conjugated antibody that flows through the test line zone. Optimal conditions were found by applying an incubation time at 2–4 hours. Importantly, the higher the average signal intensity of the blank sample (long incubation time), the lower the determination level as far as no BoNT/D could be determined. The lower limit of determination of BoNT/D by the test strip was 50 pg. mL^{-1}. This concentration was found very satisfactory compared to the mouse bioassay method (50 pg. mL^{-1}). Another study conducted by Chiao et al. [64] developed an ICA against Botulism neurotoxin B (BoNT/B). The detection limit was 50 ng/ml in both PBS standard sample and diluted urine samples (1:1 in PBS). Using a silver enhancement technique, the detection limit was improved at 50 pg. mL^{-1}. The test strip showed no cross-reactivity to BoNT/A and BoNT/E at 1 µg. mL^{-1}. Authors of the same group have also investigated an ICA against BoNT/A [65]. The assay was based on sandwich format using monoclonal antibodies (MAbs) of two distinct specificities. The assay allowed a detection of 50 ng. mL^{-1} of BoNT/A with an assay time of

less than 10 min. Interestingly, a detection limit of 1 ng. mL^{-1} was obtained when using silver enhancement. The ICA also showed no cross-reactivity to type B neurotoxin (BoNT/B) and type E neurotoxin (BoNT/E).

Staphylococcus enterotoxin B

Staphylococcus aureus enterotoxin B (SEB) is a highly heat resistant enteric toxin with a potential as a biothreat agent. A rapid and sensitive method for the detection of staphylococcal enterotoxins is needed for food safety and food defense monitoring. An ICA against SEB was presented by Shyu et al. [66]. The detection limit of the SEB toxin in PBS was achieved at 1 ng. mL^{-1} within 5–10 min. Using a silver enhancer reagent, the sensitivity of the assay was improved to 10 pg. mL^{-1}. Simulated samples (SEB toxin diluted in human urine, serum and cow milk powder) were also analysed by the SEB test strips. The detection limit of SEB in the simulated urine and milk samples was 10 ng. mL^{-1}, and it was 100 ng. mL^{-1} in serum samples.

An interesting study using fluorescent immunoliposome as label has been described by Khreich et al. [67] for the detection of SEB in tap water, surface water, apple juice, raw milk, ham and cheese. The ICA was based on sandwish immunoassay using labeled monoclonal antibodies (mAbs) that binds simultaneously SEB molecules. Labeling of the mAbs was performed using N-hydroxysuccinimidyl ester of biotin as previously described [68]. At the first set of this study, different labels (colloidal gold, fluorescent microsphere, Dextrane rhodamine, dye microsphere and SRB/liposomes) were compared with the main goal to optimize the sensitivity of the test strip. With the colorimetric labels, different labeling methods allowed a detection of SEB close to 0.5 ng. mL^{-1}. Colloidal gold appeared twice as sensitive as the dye microspheres. Labeling with fluorescent microspheres showed better sensitivity than either colorimeter label when measuring the fluorescence. The same results were not found for dextran/rhodamine used as label, which showed a limit of detection close to 0.625 ng. mL^{-1}. Eye-detection provided a result similar to that of colloidal gold for fluorescent immunoliposome labeling. However, when analyzing fluorescence of the wet strips, no signal was obtained on the test strips. To solve this problem, an air-drying treatment (5 min) of the membrane was performed to allow the diffusion of the fluorescence label. This treatment resulted in a strong increase in sensitivity with limit of detection close to 0.02 ng. mL^{-1} (Fig. 3). Following a five serial dilution processes, the sensitivity of the ICA method to detect SEB was achieved at 0.125 ng. mL^{-1} in cheese, apple juice, tap and surface water samples. The detection limit with milk samples using three times dilution was only 0.06 ng. mL^{-1}. The ICA presented in this study showed no cross-reaction with *Staphylococcus enterotoxin* A.
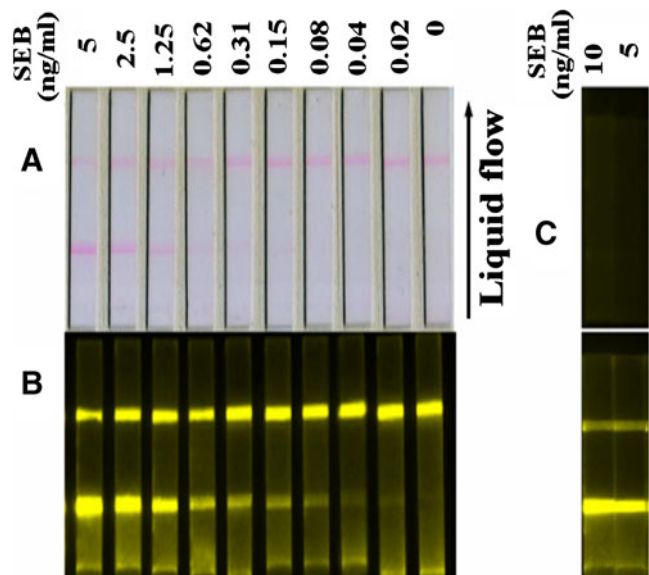

Fig. 3 Comparative detection of *Staphylococcus aureus* enterotoxin B (SEB) using colorimetry and fluorescence detection The same strips corresponding to serial dilutions of SEB (5 to 0.02 ng. mL^{-1}) assayed with the immunochromatographic test using SRB-encapsulated immunoliposomes as tracer were successively analyzed by colorimetric detection (**A**; scanned image obtained with an Epson expression 1640XL scanner) and fluorescent detection (**B**; pictures obtained with Kodak station 2000 MM). The control and test lines are on the top and the bottom of the strips, respectively. The effect of air-drying on the fluorescence of the immunoliposomes is illustrated (**C**; pictures obtained with Kodak Station 2000 MM before and after air-drying), with two strips, corresponding to 10 and 5 ng. mL^{-1} of SEB (reproduced with permission from Khreich et al. [67])

Viruses

Influenza virus

Avian influenza viruses (AIV) are classified in the family *Orthomyxoviridae*, genus *Influenza virus* A. The surface is covered by two types of glycoprotein projections: rodshaped trimers of haemagglutinin (HA) and mushroomshaped tetramers of neuraminidase (NA). Low pathogenic avian influenza virus such as H5 and H7 act as progenitor viruses of highly pathogenic variants [69]. A rapid and accurate detection method is urgently needed because of the increased risk concerns for pandemic influenza caused by either naturally occurring strains or altered strains [70, 71]. An ICA for the detection of H9 Subtype AIV was proposed by Peng et al. [23]. The assay consisted of a colloidal gold anti-hemagglutin monoclonal antibody conjugate (detection antibody) and an anti-Nucleocapside protein (NP) monoclonal antibody used as a precipitation reagent on the test line of a nitrocellulose membrane. In this study, several samples of allantoic fluids harvested from H9N2-infected eggs at 64 units of HA and allantoic fluids from unmanipulated eggs with standard antigens of AIV (H9,

H5 and H7) and five common pathogen viruses in poultry (Newcastle disease virus (NDV)), infectious bronchitis virus (IBV), infectious bursal disease virus (IBDV), infectious laryngotracheitis virus (ILTV) and fowl adenovirus (FAV)) were characterized by the test strips. Results of the assay compared with HA and HI tests indicated higher specificity for the H9AIV detection. The sensitivity was determined to be 0.25 units of HA with an assay time of less than 10 min. Storage of the prepared test strips at 4 °C for 12 months and at room temperature for 6 months did not alter the sensitivity performance. However, continual storage at room temperature for 9 months reduced the sensitivity by 50%. Results from the clinal study demonstrated that the test strips could be used for the rapid, on-site, and early detection of H9AIV. Another similar study has been conducted by the same authors [24] to detect IgG antibodies against the nucleocapsid protein of AIV subtypes (H5, H7 and H9) in chicken sera. In the experimental set-up, the test strips allowed the detection of anti-AIV IgG at 1:1024 dilutions of antisera within 15 min. Sensitivity of test system was similar to that of hemagglutination inhibition (HI) method but it was significantly higher than agar gel immunodiffusion assay (AGID). In the validation study, 166 clinical serum samples were analysed by the three methods. The ratio of the positive number of the test strip to the positive number of HI method was 88.17%, and that of negative rate was 79.45%. With AGID analyses, the positive and negative rates to the strip were 48.39 % and 93.15%, respectively. The ICA also showed no cross-reaction to NDV, IBDV and NDV.

The key to differentiating subtypes of AIV with the immune colloidal gold test strip method has been based on the production of H5 subtype-specific mAb. An ICA strip for rapid detection of AIV H5N1 was proposed by Shangjin et al. [72]. The test strip detected H5N1 at a dilution level of less than 1:1000 in chicken allantoid fluid. The specificity was then determined by using H5N1 chicken embryonic culture, normal chicken allantoises fluid, standard antigens of AIV (H1–H14 subtypes), and antigens of NDV, IBDV, IBV, AILV and Marek's disease virus. The results of the test strip were positive for allantoic fluid of H5N1 and the standard antigen of AIV-H5 but were negative for the other antigens. In the clinical study, cloacal swabs from 483 birds suspected of having AIV-H5 infection were treated with 3 ml of PBS (10,000 U. mL^{-1} of penicillin, 10 mg. mL^{-1} of streptomycin, 5,000 U. mL^{-1} of streptomycin) for 1 hour at room temperature. After centrifugation at $1,000 \times g$ for 10 min, the supernatant was inoculated into the allantoic cavities of 9–11-day-old embryonic eggs, which were followed by incubation at 37 °C for 7 days. Interestingly, the results of analyses by the test strip with the allantoic fluid of each embroynic eggs agreed with those of HA/HI test, RT-PCR,

and ELISA methods. The high stability after storage at 4 °C for 15 months renders the test strip as a suitable tool for the specific diagnosis and epidemiological investigation of AIV H5.

To differenciate the type A and B of AIV, most available methods did not show reliable performance [73]. Andrea et al. [74] compared the performance of a new lateral flow ICA membrane (Xpect FluA/B; Remel Inc., Lenexa, Kans.) with reference standard viral culture method for the rapid differentiation of influenza A and B in 400 respiratory samples from children and adults. The specimens collected in this study were nasal washes (59.75%), nasopharyngeal swabs (30.5%), throat swabs (7.5%), tracheal aspirates (1%), sputum (0.75%) and bronchoalveolar lavage (0.5%). The overall sensitivity of the test strip to detect both types of influenza virus was slightly higher (95.2 versus 94.4%) at the 30 min reading than the 15 min reading. With viral culture method, the mean duration of time that elapsed until viral cultures were detected as positive was 4.43 ± 2.87 days, and 87.6% of cultures were positve within 7 days. The specificity of the rapid assay was 100% for all specimen types. Sensitivity was 100% in throat swabs, 96.1% in nasal washes, and 87.9% nasopharyngeal swabs. The new ICA could be performed to screen persons during a pandemic or other event affecting large numbers of people because. Indeed, the results from this study have been generalized with 95% confidence to a population of at least 1,000,000.

Ikematsu et al. [75] evaluated the performance of an ICA kits (Capilia FluA, B; Becton-Dickinson Japan) for rapid diagnosis of Influenza. The study comprised 2008 cases of influenza diagnosed at 52 clinics in Japan. Nasal swab or pharyngeal swab specimens were collected from influenza-like illness patients. The ICA kits displayed 93.3% sensitivity compared with the results of virus isolation and 84.1% when compared with the results of serological diagnosis.

Hepatitis B virus

Hepatitis B virus (HBV) is the ethiological agent of Hepatitis B diseases, a chronic infection which cause liver cancer in human [76]. The development of sensitive and rapid tests for the detection of HBV infection is also urgently needed in clinical laboratory. To date, majority of rapid tests for detection of Hepatite B surface antigen (HbsAg) are based on ICA principle. A comparison of ICA performance with quantitative PCR method for rapid diagnostic of HbsAg in human sera was presented by Ansari et al. [77]. The study comprised the analyses of 240 sample sera using devices or strips from six companies: ACON (Acon laboratories Inc., San Diego, USA), Atlas Medical (Williams Jams House, Cambridge, UK), Dima (Geseeschaft fur Diagnostika mbH, Germany), Cortez

(Cortez Diagnostics Inc., Calabasas, USA), Blue Cross (Blue Cross Inc., China) and Intec (Intec Products Inc., Xiaman, China). The results of comparison showed that both the sensitivity and the specificity of the test strips ranged 97.5–99.2%. Among the six test strips or device tests, Intec and Blue Cross showed higher sensitivity than Acon Atlas, Dima and Cortez strips. Also, Acon and DIMA had higher specificity than others. Another study conducted by Whang DH Um [78] attempted to compare two differents ICA kits (Daewoong, Genedia) with chemiluminescence immunoassay method (kits from ADVIA Centaur and ARCHITECT) and enzyme immunoassay for the detection of HBsAg and anti-HBs. The diagnostic performances of the two ICA kits for HBsAg were more than 97%. The sensitivity and specificity of both of the ICA kits for HBsAg and anti-HBs ranged 97–98.5% and 74–96%, respectively. In another experiment carried out by Xia et al. [79], an attempt has been made to detect HBs surface antigen by using Europium chelate-loaded silica as label. In this study, antibodies against the HBs surface antigen were covalently conjugated onto europium silica with dextran as a linker. Due the apparent number of europium chelates per nanoparticle (6.86×10^5), Europium chelate-loaded silica nanoparticles present large fluorescence emission (large Stokes shift, long emission wavelength ($\lambda em=615$ nm)). As a result, this property offers improved signal intensity and quantitative discrimination of HBs surface antigen. Results showed a detection limit of 0.03 μg. L^{-1}, which was 100 times lower than the colloidal gold-based ICA and lower than ELISA. To determine the reliability of the assay, a total of 286 clinical samples were analysed by the developed test strip and a quantitative ELISA. A satisfactory agreement rate was then obtained for the qualitative analysis by the test strip and ELISA.

Western Nile virus

Western Nile virus (WNV) is classified as member of the *Flaviviridae* family, and it is closely related to Japanese encephalitis virus, eastern Asia; Kunjin virus, Australia and Southeast Asia; and St. Louis encephalitis virus, North and South America [80, 81]. WNV is transimitted to humans by mosquitoes, and its infections have been reported elsewhere [82]. Immunoglobulin M (IgM) antibody in serum and cerebral spinal fluid (CSF) specimens are considered to be as the preferred marker for the diagnostic of WNV infection [83]. Two reports [84, 85] regarding the development of ICA for WNN IgM detections were found in the litterature. Both the two studies used a commercially rapid lateral flow test "Rapid WN ™". The study carried out by Anthony et al. [84] was an inter-laboratory study (five laboratories) in which the performance of a Rapid WN™ test was compared to four public health laboratory-developed screening tests: WNV IgM Capture DxSelectTM (Focus

Diagnostics, Cypress, CA, USA), WNV IgM Capture ELISA (PanBio, Qld., Australia), WNV-specific IgM antibody-capture (MAC), Centers for Disease Control and Prevention (CDC), WNV-specific IgM micro-immunoassay (CDC). The results of analysis from 125 serum specimens demonstrated that the Rapid WN™ presented a 92.9% sensitivity (range of 88.9–100%), a 95.3% specificity (range of 90.9–100%), a positive predictive values of 96.3% (range of 94.7–100%), and a negative predictive value of 91.0% (range of 83.3–100%) as compared to the predicate screening tests.

Porcine reproductive and respiratory syndrome virus

Porcine reproductive and respiratory syndrome virus (PRRSV) is a member of *Arteriviridae* family. PRRSV has become presently a major economic problem for swine industries worldwide particularly in Asia. ICA has been demonstrated to be very suitable tool for the on-site detection of antibodies or antigens for PRRSV. In a study conducted by Cui et al. [86], the test strip used *Escherichia coli*-expressed viral recombinant membrane protein antigen in combination with recombinant nucleocapsid protein as capture protein for the detection of antibodies against PRRSV. Detection of PRRSV by the test strip was compared with detection by a standard indirect ELISA and an immunoperoxidase monolayer assay (IPMA). Results from this study showed that the test was able to detect anti-PRRSV antibodies in both naturally infected swine sera and experimentally infected swine sera with high sensitivity, specificity and accuracy (typically greater than 96%). Importantly, for screening antibody to PRRSV in sera from piglets infected experimentally and in sera from commercial swine herds, the assay performed as well as ELISA and better than IPMA. Another similar study conducted by Lyoo et al. [87] used a simple pen-side assay (BioSign™ PRRSV) for rapid detection of PRRSV antibody based on ICA. The assay also used *Escherichia coli*-expressed viral nucleocapsid protein antigen for detection of antibodies against PRRSV in swine sera. The results were compared with commercially available antibody ELISA (HerdChek® PRRS ELISA) and an indirect immunofluorescence. The sensitivities and specificities of the test strip ranged 93.2–98.7% and 98.5–99.2%, respectively.

Rotavirus, adenovirus and norovirus

Rotavirus, adenovirus and norovirus are causative agents of viral gastroenteritis in human. ICA has become one of the representative methods in the rapid diagnosis of these viruses. Takanashi et al. [16] reported an ICA test against the prevaling genotypes (genotypes II/3 and genotypes II/4) of norovirus (NoV). The test was based on sandwish assay

format using polyclonal antibodies against recombinant virus-like particles (rVLPs). The rVLPs are known to be morphologically and antigenically similar to the native NoV. The test strip has been evaluated for cross-reactivity through analysis of 15 genotypes of rVLPs and 10 genotypes of NoV, and for the detection of natural viruses in 107 stool samples collected from children with diarrhea in comparison to RT-PCR. In the prospective assessment, the assay showed agreement rate of 84.1%, sensitivity of 69.8% and specificity of 93.7%. With the analysis of genotypes II/3, the detection limit of rVLP was 3.0×10^{-3} ng. μL^{-1}, and that of viral load was 3.5×10^{7} copies.g^{-1} of stool, whereas the ICA for GII/4 gave the values for rVLP of 7.5×10^{-3} ng. μL^{-1} and for viral load of 4.6×10^{6} copies.g^{-1} of stool. No cross-reactivity was observed with other enteric viral pathogens, such as group A rotavirus, sapovirus, and adenovirus. The results from this study indicated the potential applicability of the ICA method to screen stool samples for NoV infection. A prospective study has been also conducted by Nguyen et al. [88] using two ICA kits ("Eik en' Rota kit", SA Scientific, USA; Novovirus IC-1 stick, Immuno-Probe, Japan) for detection of group A rotavirus and norovirus Genotypes II. The results of ICA were compared with PCR as reference method. The sensitivity, specificity and agreement rate between the ICA and PCR ranged 73.7–87.8%, 93.3–100% and 89.4–95.2%, respectively.

In another study, an ICA for porcine rotavirus (PRV) detection in swine stool samples was developed by Kang et al. [89]. The assay was based on a sandwich immunotest configuration using coloured green microparticles (Fishers, Indiana, USA) as label. Two PRV-specific mAb-1 and mAb-2 were employed, such that one mAb-1 coated microparticles acting as a capture protein, and the other mAb-2 was fixed as a probe protein on the nitrocellulose membrane, respectively. The specificity of the ICA for PRV was examined using normal porcine stool spiked with transmissible gastroenteritis virus (TGEV) or porcine epidemic diarrhea virus (PEDV). Purified PRV, TEGV or PEDV stool specimens was diluted in 20-fold with 50 mMborate buffer, and then 150–200 µL of the diluted specimen was applied to the sample pad. With the clinical study, the panel consisted of nine stool specimens obtained from animals manifesting the symptoms of diarrhea, which were analyzed by RNA-PCR test as PRV-positive, and five PRV-negative specimens. Results from this study showed that the test strip was capable to detect1000 plaque-forming units of PRV/mL in less than 5 min. The binding capacity was demonstrated by specific recognition of PRV only but not other porcine diarrhea viruses. In the clinical trial, an excellent concordance (100%) was found between the test strip and RNA PCR methods. Finaly, the authors suggested a potential application of the three methods (PRV, TGEV and PEDV) for the on-site, rapid screening of porcine

diarrhea cases. Another study by Fujimoto et al. [90] evaluated a bedside ICA kit for human adenovirus (HadV) in patients. The kits employed in this study was the SAS Adeno Test (SA Scientific, Inc., San Antonio, Tex.), which was sold under the name Check Ad (AZWELL, Osaka, Japan). Patients were diagnosed by clinical manifestation of pharyngoconjunctival fever ($n=38$) or exudative tonsillitis ($n=100$). The ICA kit showed 95% of sensitivity (116 of 122 patients) with HAdV isolation (isolation) as the standard and 91% sensitivity (116 of 128 patients) with PCR as the standard.

In the tropical areas, an evaluation of Rotavirus and Adenovirus ICA kits was described by Weitzel et al. [91]. The ICA kits (Rida Quick rotavirus/adenovirus Combi test) used labeled monoclonal antibodies against surface antigens of rotavirus and adenoviruses. The study comprised the analyses of 238 stool samples using Rida Quick rotavirus/adenovirus Combi test (R-Biopharm AG, Darmstadt, Germany) and nested PCR. Stool samples were derived from children who were enrolled in a comprehensive clinicoepidemiological study on childhood diarrhea in Ghana. The rate of sensitivities and specificities of the rapid test compared with PCR method were 75% and 95% for rotavirus and 22% and 84% for adenovirus, respectively.

An interesting study conducted by Bon et al. [92] evaluated the performance of seven commercially ICA tests for the rapid detection of group A rotaviruses in fecal samples. The test strips employed in this work were the Rota Strip quick test (Cypress Diagnostics, Belgium), Rotascreen (Microgen, UK), VIKIA Rota/Adeno (bioMérieux, French), Diarlex MB with centrifugation or filtration (Orion Diagnostica, Finland), Combo Rota/Adeno (All Diag, (Strasbourg) French) and Rota/Adeno Combi Stick (biomedical diagnostics, French). When using ELISA as standard test, the detection rate of Rotavirus in 80 fecal samples by the ICA kits ranged 70–98.8%. It was important to notice that the high detection rate (>90%) was obtained with three kits: Rota Strip (Cypress Diagnostics), Rotascreen (Microgen), and VIKIA Rota/Adeno (bioMérieux). The results of specificity tests were also compared to ELISA and RT-PCR in the analysis of 100 negative stool samples, and no false positive was observed with any assay.

The technical features of these studies showed that LFT is a suitable tool in clinical laboratory. LFT did not require expensive equipment and laborious procedure.

Applications for the detection of chemical contaminants

Chemical contaminants such as veterinary drug residues, mycotoxins and pesticides usually cause adulteration problems in international food tradepatterns [93]. A huge

investment in time and effort is being therefore placed on food monitoring contaminant by regulatory and industrial laboratories [94]. Up to now, there is an emergent need in rapid detection methods amenable to POC applications. As illustrated in Table 3, ICA had known increasing interests for controlling chemical contaminants in foods, water and environment. These reports published recently have proved the success of test strip application in this field.

Veterinary drugs

Sulphonamides

Sulphonamides (SAs) are broad-spectrum antimicrobials used in both humans and animals. Residues in food are of concern because of the potential carcinogenic nature of these compounds [95]. To protect consumers, the maximun residue limits (MRLs) for the total sulphonamide concentrations in edibles tissues have been established at 100 μg. kg^{-1} in EU and China. Wang et al. [17] proposed a simple and rapid extraction method for the detection of four sulphonamides (sulfamonomethoxine (SMM), sulfamethoxydiazine, (SMD) sulfadimethoxine (SDM) and sulfadiazine (SDZ)) in eggs and chicken muscles by ICA. In this study, the extractions of the sulphonamides from matrice samples were realized by ethyl acetate. Briefly, muscle or egg samples homogenized in ethyl acetate (2 mL. g^{-1}) were vortexed for 3 min and then centrifuged at 2000 g for 10 min. Resultant supernatants (300 μL) were evaporated to dryness in a 60 °C water bath under a gentle flow of nitrogen. The residues obtained were then resuspended in 150 μLof phosphate buffer (pH7.4) for analysis by ICA test strip. With that method, recoveries of SMM, SMD and SDM ranged 85–95.6% in both chicken muscle and eggs samples spiked at concentration 20–100 ng. g^{-1}. On the other hand, low recovery was obtained with SDZ (44.8–60.9%). The differences of recovery have been explained by the authors. Indeed, the sensitivity limits of the test strip were 20 ng. mL^{-1} for three sulphonamides (sulfamonomethoxine, sulfamethoxydiazine, and sulfadimethoxine) and 40 ng. mL^{-1} for sulfadiazine. Using HPLC as confirmatory method, the validation of the ICA test was achieved by analysing SMM in 27 egg samples and 28 chicken muscle samples from animal experiment. The result of comparison indicated that the differences between the two methods were from 0.8 to 11.2% for egg samples and from 2.2 to 34% for chicken muscle samples for the quantitative detection. The agreement rates between test strips and HPLC were 100%. In the study conducted by Zhang et al. [96], a sensitivity limit at 15 ng. mL^{-1} was obtained when screening SMM in swine serum, and the recovery in sera spiked at 20 ng. mL^{-1} and 30 ng. mL^{-1} ranged 94–103%. Wang et al. [97] presented a combination of ICA test and ELISA method to detect sulfamethazine residues in milk, pig

muscle and fish. To obtain a more sensitive immunoassay, the authors investigated the influence of various analytical parameters including the incubation time, matrix effects, buffer components and immunoreagent concentrations. Pre-treatment of milk samples was performed with acetone, and that of pig muscle and fish samples was realized in methanol. Removal of the matrix effects was achieved at 10–20 fold dilution in PBS (30 mmol. L^{-1}, pH7.2) containing 0.05% Tween. The ICA presented in this study detected efficiently SMZ at concentration 20 μg.kg^{-1} in milk, pig muscle and fish. The detection time was accomplished in less than 10 min.

Results from these studies proved the reliabilityof LFT technology for analysing sulphonamides in food producing animals. Nevertheless, there are still some limitations to find a novel test strip that could detect simultaneously all sulphonamide compounds.

Anabolic steroids

In the EU, the use of steroid and β-agonist to enhance animal growth is prohibited according to the council directive 96/22/EC [98]. A set of minimum performance characteristics that have to be fulfilled by the methods to be used for growth promoting compounds has been defined by the EU and laid down in pertinent Commission Decisions [99]. Monitoring anabolic steroids in meat-producing animals is therefore a challenging task. It implies the development of rapid and sensitive analytical method able to detect and identify sub-μg.kg^{-1} residue levels in complex biological matrices such as meat, urine, or milk. An ICA test for screening 19-nortestosterone (19-NT) in swine urine was reported by Liqiang et al. [100]. The test based on competitive assay format employed a colloidal gold-polyclonal antibody (pAb) conjugate against 19-NT (detector reagent) and 19-NT-ovalbumin conjugate (capture reagent) immobilized on a nitrocellulose membrane. The detection limit of the test strip to 19-NT was 200 ng.mL^{-1} within 10 min. Results of the paralel analysis of urine samples spiked with 19-NT at 0, 100, 200, 400, 800 and 1600 ng. mL^{-1} showed good agreement rate between LC/MS/MS method and the ICA. However, significant cross-reactivities were observed with five other anabolic steroid including 19-norandrostendione (30%), 19-norandrostandione (30%), trenbolone (10%), 17α–trenbolone (10%), 17 α–19–nortestosterone (10%). In our opinion, the developed ICA can not be applied for specific detection of 19-nortestosterone, and it would be more convenient to develop a monoclonal antibody against 19-nortestosterone. Geertruida et al. [101] proposed a test strip against progesterone (PA). The authors investigated the influence of several aspects such as the kinetic interaction of antibody-conjugate with various capture antigens. The influence of several commonly used blocking agents (BSA,

Table 3 Overview of studies on lateral flow test strip for the detection of veterinary drug residues, mycotoxins and pesticides

Analytes	Assay formats	Labels	Samples	Sensitivities	References
Veterinary drugs					
Sulfamonomethoxine Sulfamethoxydiazine, Sulfadimethoxine and Sulfadiazine	Competitive ICA coupled with a strip reader device (qantitative and semi-quantitative)	Colloidal gold	Eggs and chicken muscles	20–40 ng. mL^{-1}	[17]
Sulfamonomethoxine	Competitive ICA	Colloidal gold	Swine sera	15 ng. mL^{-1}	[96]
Sulfamethazine	Competitive ICA	Colloidal gold	milk, pig muscle and fish	20 µg. kg^{-1}	[97]
19-nortestosterone	Competitive ICA	Colloidal gold	Swin urine	200 ng. mL^{-1}	[100]
Progesterone	Competitive ICA	Colloidal gold	bovine milk	0.6–1.2 µg. L^{-1}	[101]
Medroxyprogesterone acetate residue	Competitive ICA	Colloidal gold	Swine urine and liver	5–10 µg. kg^{-1}	[102]
Hexoesterol	Competitive ICA	Colloidal gold	Swine urine and liver	20 µg. kg^{-1}	[103]
Salbutamol	Competitive ICA	Colloidal gold	PBS	80 ng. mL^{-1}	[105]
Clenbuterol	Competitive ICA coupled with a scanner divice	Colloidal gold	Swine urine	0.1–1 ng. mL^{-1}	[106]
Clenbuterol	Competitive ICA	Colloidal gold	Swine urine	3 ng. mL^{-1}	[107]
Clenbuterol and Ractopamine	Competitive ICA	Colloidal gold	Swine urine	0.34–0.55 ng. mL^{-1}	[108]
Chloramphenicol	Competitive ICA	Colloidal gold	Aquaculture tissues	10 ng. g^{-1}	[110]
Kanamycin and Trobramycin	Competitive ICA	Colloidal gold	PBS, Meat, liver, Kidney	5–100 ng. mL^{-1}	[112]
Euroflaxin	Competitive ICA	Colloidal gold	chicken tissues	0.134 µg. kg^{-1}	[113]
Norfloxacin, pefloxacin, Enrofloxacin, ciprofloxacin, norfloxacin, flumequine, pefloxacin, ofloxacin, lomefloxacin, enoxacin, danofloxacin, amifloxacin, oxolinic acid and marbofloxacin	Competitive ICA	Colloidal gold	chicken muscle and chicken liver	50 ng. mL^{-1}	[114]
Mycotoxins					
Deoxynivanelol and Zearalenone	Competitive ICA	Colloidal gold	Wheat	100–1500 µg. kg^{-1}	[117]
Deoxynivanelol	Competitive ICAwith two indicator ranges	Colloidal gold	Bread swheat and Pig fed	250–500 µg. kg^{-1} and 1000–2000 µg. kg^{-1}	[118]
Deoxynivanelol	Competitive ICA	Colloidal gold	Wheat and maize	50 ng. mL^{-1}	[119]
Aflatoxin B1	Competitive ICA coupled with a photometric strip reader	Colloidal gold	Rice, corn and wheat	0.05–2.5 ng. mL^{-1}	[121]
Aflatoxin B2	Competitive ICA	Magnetic nanogold microsphere	Peanut, Hazelnut,Pistacia, Almond	0.9 ng. mL^{-1}	[122]
Total B fumonisins (B1, B2 and B3)	Competitive ICA	Colloidal gold	Maize	4,000 µg. kg^{-1}	[124]
Ochratoxin	Competitive ICA	Colloidal gold	Coffee	5 ng. mL^{-1}	[126]
Ochratoxin	Competitive ICA	Colloidal gold	Barley, wheat, oat, corn, rice, raisin and coffee	1 ng. mL^{-1}	[127]
Ochratoxin	Competitive ICA	Colloidal gold	Corn and wheat	10 ng. mL^{-1}	[128]
T-2 Toxin	Competitive ICA (semi-quantitative)	Colloidal gold	Wheat and oat	100 µg. kg^{-1}	[148]
Pesticides					
Methiocarb	Competitive ICA (semi-quantitative)	Carbon black	Water	0.5 ng. mL^{-1}	[134]

Analyte	Method	Label	Matrix	Detection limit	Ref.
Methamidophos	Competitive ICA	Colloidal gold	Green vegetables	1.0 μg. mL^{-1}	[135]
Carbofuran and triazophos	Competitive ICA	Colloidal gold	Water	4–32 μg. L^{-1}	[136]
Atrazine	Competitive ICA dipstick coupled with a portable calorimeter	Colloidal gold	Water	1.0 ng. mL^{-1}	[137]
Endosulfan, Cypermethrin, Deltamethrin and Fenvalerate	Competitive ICA dipstick	Colloidal gold	Dilution in PBS containing 0.05 % Tween	800–1800 μg. L^{-1}	[138]
2,4-Dichlorophenoxyacetic Acid and Simazine	Competitive ICA	Colloidal gold	Water	12 ng. mL^{-1}	[139]
Thiabendazole and Methiocarb	Competitive ICA	Carbon black	Fruit juices	0.005–0.5 mg. kg^{-1}	[140]
Insecticide carbaryl	Competitive ICA	Colloidal gold	Rice and barley	50–100 μg. L^{-1}	[141]
N-methylcarbamate	Competitive ICA	Colloidal gold	Water	0.25 mg. mL^{-1}	[144]
Bromoxynil	Competitive ICA	Colloidal gold	Water	0.06 mg. L^{-1}	[143]

OVA, β-Lactoglobulin, lactoferrin, casein hydrosylate, polyvinylpyrrolidone (30 kDa), poly-methyl-vinylether-10-maleic anhydride and teleostan gelatin) has been assessed in this study. Results showed that blocking agents like casein hydrolysate, polyvinylpyrrolidone and poly (methylvinylether-10-maleic anhydride) affected the performance of the assay. The addition of OVA, lactoferrin or β-Lactoglobulin as blocking agents was found detrimental in progesterone-specific ICA test. Indeed, the overall charge of lactoferrin in the incubation buffer was positive instead of negative, as was the case for the other blocking agents. The authors did not explain how these charge differences contributed to the absence of signal in the blank sample. Finally, BSA provided the most superior blocking agents, and a half-maximal inhibition concentration (IC$_{50}$) at 0.6–1.2 μg. mL^{-1} was obtained for progesterone detection in bovine milk by the ICA. A validation study using a reference method should be performed before routine use.

Tieming et al. [102] proposed a colloidal gold–based ICA for the detection of medroxyprogesterone acetate (MPA) residue. The reliability of the test was determined through HPLC analysis of uncontaminated samples spiked with MPA at concentrations 1, 5, l0, 20 and 50 mg. kg^{-1}. The detection limit was 5 μg. kg^{-1} in standard solution and 10 μg. kg^{-1} in spiked swine urine and liver samples, and good correlation was found between the two methods. In our opinion, the developed ICA test was not very specific to medoxyprogesterone because of the high cross-reactivities values found with other structurally related compounds including Megestrol acetate (48%), Melengestrol acetate (31%), Chlormadinone acetate (43%), Estradiol (21%), 17β-estradiol 3-benzoate (16%), Progesterone (18%) and Pregnenolone (8%). The same authors [103] developed a similar competitve ICA for detecting hexoestrol residues in swine urine and liver. The reliability of the assay has been determined throughout analyses of negative samples spiked with hexosterol at concentrations ranging 1–50 μg. kg^{-1}. Results of analyses, which were confirmed by HPLC method, indicated a detection limit of 20 μg. kg^{-1} in both swine urine and liver. The method developed in this study provided a preliminary semi-quantitative result. The main limitation of the study was that authors did not investigate the cross-reactivity of the test strip with other anabolic steroids.

β-agonists

β-agonists are growth-promoting drugs with the potential for illegal use in livestock, and human toxicity has resulted from consumption of contaminated animal food products [104] . Therefore, a simple and qualitative screening method is needed to achieve the effective surveillance of

the illegal use of β-agonists. An ICA against Salbutamol (SALB) was described by Khamta et al. [105]. The test strip was constructed by using SALB-BSA conjugate, goat anti rabbit IgG, and anti-SALB-colloidal gold conjugate (SALB-CGC) as test line, a control line, and a detector, respectively. The sensitivity of the assay was estimated to be at 80 ng. mL^{-1} of SALB in PBS. In the cross-reactivity study, clenbuterol and chloramphenicol were used to probe probable interference with the test strip. With clenbuterol, the test line intensity slightly faded when assaying with standard solution at concentration 100 ng. mL^{-1}. In contrast, chloramphenicol showed no competition with anti-SABL-CGC conjugate. An additional experiment using a reference method such as GC-MS should be performed to validate the results from this study.

An ICA for the detection of β-adrenergic agonist Clenbuterol (CL) residues was proposed by Zhang et al. [106]. The detection of CL by the ICA test in spiked urine samples was estimated about 1 ng. mL^{-1} by eye measurement and as low as 0.1 ng. mL^{-1} when using a scanner device. In the specificity study, the IC_{50} of CL was calculated to be 1.78 ± 0.17 ng. mL^{-1} while the IC_{50} values of the other adrenergic agonists, including Salbuterol, Bromobuterol, Cimbuterol, Terbutaline, Epinephine, Norepinephrine, Isoprenaline and Ractopamine, were all greater than 1000 ng. mL^{-1}. Moreover, a paralel analysis between a GC-MS method and the ICA was performed for the identification and quantitation of CL in urine samples from pigs. In the experiment set-up, six animals were dosed with Clenbuterol hydrochlorede (5 mg. kg^{-1} in feed) for 21 days, and the urine samples were collected at 0.5, 1, 2, 4, 8, 16 days after the final dose. Result analyses of clenbuterol by ICA test strip were closely aligned with GC-MS method. The quantitative analysis was introduced to the test strip format assay by scanning the relative optical density of the test capture lines, and the format of competitive lateral flow analysis agreed with the mathematical model proposed by Qian et al. [22]. The test strip could be used as suitable tool for Clenbuterol residues detection at ppb level in swine urine. Lai et al. [107] also presented an ICA against CL. The assay allowed a detection limit at 3 ng. mL^{-1} within 5 min. Finally, a validation study was carried out by analysing 210 urines samples, and the test strip compared to GC-MS gave a false positive rate of 4.4%, a false negative rate of 0 % while the overall relative accuracy obtained was 96.7%. The test strip also showed no cross-reaction with tulobuterol, terbutaline, salbutamol, ritodrine and fenoterol at concentration 100 ng. mL^{-1}. The CL test strip can be stored at room temperature storage for 12 months.

More recently, a test strip for simultaneous detection of clenbuterol (CL) and ractopamine (RAC) was developed by Zhang et al. [108]. The multianalyte test of CL and RAC was based on competitive principle using colloidal gold-labeled anti-CL and anti-RAC polyclonal antibody and two capture test lines coated with BSA-CL and BSA-RAC. In the experiment protocol of sensitivity tests, a total of 20 blank urine samples obtained from different animals were analyzed in triplicate for both CL and RAC by using the CL and RAC simultaneous test strips with a scanner. The results from visual evaluation of the lateral-flow tests of spiked swine urine samples showed that the cut-off values of CL and RAC were 1.0 and 1.0 ng. mL^{-1}, respectively. The limit of detection was 0.34 ng. mL^{-1} for CL and 0.55 ng. mL^{-1} for RAC in swine urine samples. With the cross-reactivity study, the anti-CL polyclonal antibody exhibited cross-reations with salbutamol (2.24%) and terbutaline (0.56%). The anti-RAC polyclonal antibody showed cross-reactivity with isoxsuprine (0.284%) and ritodrine (1.420%). Interestingly, low cross-reactivity (<0.071%) was observed with other β-agonists including Bamethane, Cimbuterol, Cimetro, Fenoterol, Isoproterenol, Salmeterol, Zilpaterol and Ractopamine. In the validation study, the correlation coefficient for the test strips analysis and GC-MS analysis of CL, RAC in 30 unknown swine urine samples was 0.94 and 0.93, respectively. Moreover, the authors have examined the effect of temperature by running spiked swine urine samples with CL and RAC standards of 0, 0.1, 0.5, and 1.0 ng. mL^{-1} at 4, 15, 25, and 35 °C. The results indicated that the determination of test strips by visual observation was affected especially at the lower temperature 15–4 °C. In the other hand, the authors found no obviously changes of values (B/Bo) at various examined temperature comparing with at 25 °C. The developed test strips method was reliable for the CL and RAC detection in swine urine samples. Of particular importance was the fact that anti-CL pAb did not cross-react with RAC and anti-RAC pAb did not cross-react with CL. The method could offer advantages of ease of sample preparation and high throughput. These works demonstrate the potential application of ICA technique to screen β-agonists in food producing animals.

Chloramphenicol

The use of chloramphenicol (CAP) in food producing animals is banned because of its associated serious side effects in human reported elsewhere [109]. Li et al. [110] proposed a colloidal gold-based ICA to detect CAP residues in aquaculture tissues. Fish samples were spiked with CAP at concentrations 10, 20, 30, 40 and 50 ng. g^{-1}, and they were analyzed by the ICA and LC/MS/MS. The results indicated good correlation ($r^2=0.97$) between the data obtained from both the two methods. The detection limit of CAP in aquaculture tissues was 10 ng. g^{-1}. In this study, the ICA method provided only a preliminary, semi-quantitative result, however it could be used to judge

whether a contaminated fish sample contains CAP at concentration exceding the detection limit.

Aminoglucosides

The aminoglycosides are broad-spectrum antibiotics that have bactericidal activity against some gram-positive and many gram-negative organisms. Their clinical use has been limited by the EU because of the side effects of nephrotoxicity and ototoxicity [111]. Therefore, there is a need for developing analytical methods to screen rapidly the presence of aminoglycoside residues in the edible tissues of livestock. A colloidal gold-based ICA for rapid detection of kanamycin and Trobramycin in swine tissues was described by Chen et al. [112]. The test showed a visual detection limit at 5 ng. mL^{-1} in PBS, 50 µg. kg^{-1} in meat or liver, and 100 µg. kg^{-1} in kidney. The detection time of the assay took only 5–10 min. For suitability, the results from this study have to be validated by using a confirmatory method.

Fluoroquinolone

Fluroquinolonones (FQs) are also used as antimicrobial drugs in animal farmers. A colloidal gold-based ICA test strip for the detection of euroflaxin in chicken tissues was described by Zhao et al. [113]. The method showed a detection limit at 0.138 µg. kg^{-1} of euroflaxin as measured in a strip scanner, and the IC$_{50}$ was 0.935 µg. kg^{-1}. Recoveries of the assay with tissue samples spiked at 10, 20 and 30 µg. kg^{-1} ranged 85.3–96% with an acceptable coeficient of variations at 4.5–7.91%. Parallel analysis of muscle samples from animal experiments showed satisfactory results between the test strip and LC-MS method. The test had the advantages of simplicity and speed of performance (requires only 5 min), and it could be a useful screening method for quantitative, semiquantitative, or qualitative detection of euroflaxin residues in chicken muscles.

More recently, Zhu et al. [114] presented a novel ICA to detect several FQs. The test strip used a monoclonal antibody with broad specificity to twelve FQs including norfloxacin, pefloxacin, enrofloxacin, ciprofloxacin, norfloxacin, flumequine, pefloxacin, ofloxacin, lomefloxacin, enoxacin, danofloxacin, amifloxacin, oxolinic acid and marbofloxacin. The detection limits of FQs in spiked chicken muscle and chicken liver samples were 25 ng. mL^{-1} for norfloxacin and pefloxacin. For the other FQs, the lowest detection limit was 50 ng. mL^{-1}. The whole process involving sample preparation and detection can be finished within 10 min. The developed ICA method can be potentially used as a screening tool for the determination of FQ residues in a large amount of samples on site.

However, a confirmatory method is required to validate the obtained data

Mycotoxins

Mycotoxins are small toxic metabolises produced by fungi (e.g. *Aspergillus* and *Fusarium*) mostly by saprophytic moulds growing on a variety of foodstuffs including that of animal feeds and also by many plant pathogens [11]. Due to the health risks for humans and animals, many authorities had addressed the mycotoxin problems by adopting regulatory limits. In instance, for the EU, regulations or action levels have been established to limit the level of some mycotoxins in human foods and animal feeds [115]. New rapid methods such as ICA are still needed to achieve higher sensitivity detection of mycotoxin in agriculture food products [116].

Deoxynivanelol and Zearalenone

Deoxynivanelol (DON) and Zearalenone (ZEA) are secondary metabolites produced by several species of *Fusarium* fungi, in particular *Fusarium graminearum* and *Fusarium culmorum*.

Kolosova et al. [117] presented a colloidal gold-based ICA to detect simultaneously DON and ZEA in wheat sample. Wheat samples were firstly spiked with various concentrations of ZEA/DON mixture (0–2000 µg. kg^{-1}) prepared in methanol, and they were incubated overnight in dark at room temperature. Then, extractions were achieved with 15 mL of methanol-water (80/20, v/v) by using vigorous manual shaking for 3–5 min, and 1 mL of clear supernatant was diluted with 1.4 mL of PBS for analysis by the test strip. Spike series were prepared on different days and were analyzed several times (n=10). After optimization of the test strip components, cut-off values of 1500 and 100 µg.kg^{-1} were obtained for DON and ZEA, respectively. The assay time for the result visualization was less than 10 min. This test strip provided qualitative detection of DON and ZEA in wheat samples, and it also had practical advantage of rapidity and simplicity. In our opinion, an additional experiment using a reference method is required to validate the assay. Authors of the same group [118] developed an ICA against deoxynivanelol. In this study, two test strips with indicator ranges of 250–500 and 1000–2000 µg. kg^{-1}were designed. Indeed, the two designs allowed determining not only the presence/absence of the toxin but also the possible range of DON concentration in wheat and pig fed samples. Validity of the tests was also confirmed under analysis of naturally contaminated wheat samples (n=20) by ELISA and LC–MS/MS. Results obtained with the test strips were in a good agreement with the results of the LC–MS/MS method. The test strip offered

a potential as a reliable, rapid and cost-effective on-site screening. Another study conducted by Xu et al. [119] evaluated an ICA to detect DON in wheat and maize samples. A total of 32 natural samples (16 wheat and 16 maize samples) were spiked with various concentrations of DON (0–1500 µg. kg^{-1}) and were analyzed by the ICA, ELISA and GC/MS methods. Sample pretreatment procedures were achieved as follows: 5 g of ground sample was weighed into a 250 mL glass beaker, and then mixed with 100 mL of PBS containing 0.5% Tween-20 and 0.05% (w/v) anion surfactant. Subsequently, 100 µL of resulting filtrate was pipetted onto the sample pad of the strip for analysis. The detection limit of the assay was achieved at 50 ng/ml within 10 min, and the recoveries by GC and ELISA ranged from 82.2% to 91.5% and from 80% to 90%, respectively. Of particular importance in this experimental set-up was the investigation of the potential use of PBS and deionized water for the extraction of DON from sample instead of acetonitrile as extraction solvent. Moreover, several parameters including pH, capture reagents (e.g. DON–OVA, DON–BSA and DON–CBSA), amount of antibody, size and quality of colloidal gold and nitrocellulose membranes were optimized. With that method, the detection limit of the assay was achieved at 50 ng. mL^{-1} with an assay time of less than 10 min. This sensitivity was judged sufficient to detect DON at the maximum residue limit of 1 mg. kg^{-1} proposed for legislation in China. The method was therefore suitable for use as rapid screening test for DON.

Aflatoxin

Aflatoxins (AFT) are a group of widely researched mycotoxins that are produced by fungi *A. flavus* and *A. parasiticus*. The four major types of AFT are B$_1$, B$_2$, G$_1$, and G$_2$. Among these AFT, B$_1$ and G$_1$ had been considered as potential human carcinogens by the International Agency for Research on Cancer [120]. An ICA for the detection of AFT B$_1$ in foods was proposed by Xiulan et al. [121]. The assay used gold-labeled polyclonal antibodies as detector reagent against AFT B$_1$, and AFT B$_1$-BSA as capture reagents. In the first step of the study, the efficacy of the test strip was evaluated through analysis of AFT B$_1$ in food sample extracts (rice, corn and wheat flour). Briefly, 50 g of finely ground samples were blended with 250 mL methanol/water (6:4, v/v) containing 4 g of NaCl at high-speed in a blender for 2 min and followed by filtration. The solution was then diluted six times with PBS to a final methanol concentration of 10%. Different levels of AFT B$_1$ (2, 5, 10, 20, 50 ng.mL^{-1}) were added to the blank extract for analysis by the ICA. With this method, the limit of detection of AFT B$_1$ by visual observation was 2.5 ng. mL^{-1}, and it ranged 0.05–0.150 ng. mL^{-1} when using photometric strip reader. The analytical recoveries for AFB$_1$ in sample extract (rice,

corn, and wheat) spiked at 2–50 µg.kg^{-1} were from 80.79–110.56% with mean coeficient of variations ranging 6.27–14.63%. Validation of the assay was carried out by analysing naturally contaminated samples including rice, corn and wheat (n=67), and the results showed good correlation (r^2=0.93) between the ICA and a competitive ELISA method. In all assay, the analysis of AFT B$_1$ in one sample was completed within 10 min.

More recently, a novel membrane-based lateral-flow immunodipstick assay was developed by Tang et al. [122] for the fast screening of aflatoxin B$_2$ (AFT B$_2$) in food samples. In this study, the detector reagent consisted of magnetic nanogold microspheres (MnGMs) with nano-Fe$_2$O$_3$ particles as core and gold nanoparticles as shell, and bio-functionalized with monoclonal anti-AFT B$_2$ antibodies. Manually spotted AFT B$_2$–bovine serum albumin conjugates (AFT B$_2$–BSA) and goat anti-mouse IgG on nitrocellulose membrane had been used as test and control lines, respectively. Result showed that the cutoff value of conventional strip with pure gold nanoparticles as detection reagent was 3.1 ng. mL^{-1} AFT B$_2$, while the cutoff value with MnGMs as detection reagent was 3 times lower at 0.9 ng. mL^{-1} AFT B$_2$ (Fig. 4). The reliability of the assay was evaluated in an interlaboratory study with the analysis of spiked peanut samples and naturally contaminated samples (n=24). HPLC has been used as reference test. The assay showed sensitivity rate of 100% and a specificity rate of 75%. This study demonstrates a new approach to develop higher sensitive ICA which makes use of bio-functionalized
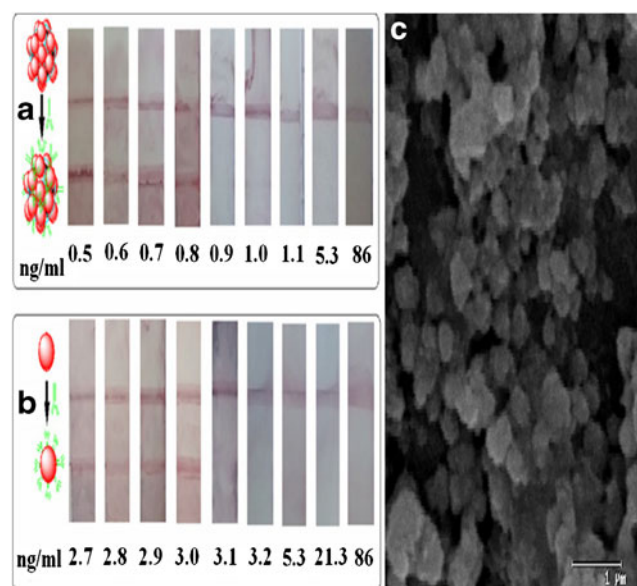

Fig. 4 Lateral-flow test immunodipsticks using different detector reagents for aflatoxin B$_2$ (AFT B$_2$): (**a**) anti-AFT B$_2$–MnGMs, (**b**) anti-AFT B$_2$–nanogold particles and (**c**) electron microscopy (SEM) image of the prepared anti-AFT B$_2$–MnGMs. The immobilized amount of AFT B$_2$–BSA of 1.0 µg.cm^{-1} was identical in the two immunodipstick formats (reproduced with permission from Tang et al. [122])

magnetic nanogold microspheres as detection reagent. The developed method would be convenient for use to detect other toxins and residue contaminants.

Fumonisins

Fumonisins are group of toxins namely produced by *Fusarium* species that grow in several agricultural commodities. Currently, 28 structural fumonisin analogs are known, and the most abundant analogue in nature is fumonisin B_1 (FB_1), followed by fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3) [123]. In the EU commission, recommendations for maximun levels of fumonisin toxins in feed are found in the guidance EC 2006/576/EC [115]. ICA has gained increased interests in the rapid analysis of fumonisins in feeds. Recently, Molinelli et al. [124] reported a test strip for the quantitative determination of total B fumonisins (FB_1, FB_2 and FB_3) within 4 min at concentration range up to 4,000 µg.kg^{-1} in maize samples. In this study, methanol/water (60:40, 70:30 or 80:20, v/v) and water extractions were selected as methods for the extraction of total fumonisins from maize. The recoveries of the assay ranged 79.8–80.3%, as confirmed by LC-MS/MS. In all analysis, the limit of detection of the test strip to total fumonisin B was 199 µg. kg^{-1}. Low cross-reactivity (<3%) was observed to other mycotoxins including zearalenone, T-2 toxin, HT-2 toxin, deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, neosolaniol, and nivalenol.

Ochratoxin

Ochratoxin (OTA) is produced by some *Aspergillus* and *Penicillium* species. Food contamination due to OTA has been reported in a variety of food items [125]. Since, the International Agency for Research on Cancer (IARC) has classified OTA as a possible carcinogen (group 2B) [120], many countries had set limits on OTA levels in food, typically between 1 and 10 ppb depending on the type and quality of the foodstuffs. Wang et al. [126] developed a colloidal gold-based ICA against OTA. The test strip showed a detection limit at 5 ng. mL^{-1} for OTA, and it can be completed within 10 min. Results of OTA analyses in coffee samples by the test strip and a competitive direct ELISA indicated a good agreement between the two methods. A more sensitive and rapid ICA for the detection OTA in food samples (barley, wheat, oat, corn, rice, raisins and coffee) was proposed by Wang et al. [127]. The detection limit was 1 ng. mL^{-1} with an assay time of less than 10 min.

The handling of pure toxin in traditional test strips may pose a toxicity risk to strip manufacturers, users, and the environment. For this purpose, Lai et al. [128] developed a

test stip to detect OTA by using a mimotpe peptide. In this study, two kinds of lateral flow strips (OTA–BSA and mimotope peptide in the nitrocellulose membrane) were evaluated for screening OTA in corn and wheat samples. In the sample pretreatment precedures, 5 g of ground sample was added into a container and extracted with 20 mL of 10% methanol–PBS solution (pH7.4). After a vigourus shaking at 5 min, the extract was filtered and then diluted at two folds with PBS for analysis by the two test strips. Results of analysis showed that the two kinds of lateral flow strips had the same detection limit at 10 ng. mL^{-1}, and the assay time was accomplished within 10 min. This study demonstrates the convenient use of nontoxic chemical as an immunochemical reagent which could replace toxic compounds in test strip development to enhance laboratory and environmental safety.

Pesticides

Pesticides contribute significantly to the contamination of the environment due to their extended use in modern agricultural production. Because of the toxicity risks to human and the ecosystem, legislations have been established on the hazard assessment to control pesticides in water [129, 130]. As mentioned previously, conventional methods available (e.g. HPLC) for monitoring pesticide contaminants are complex and time-consuming. There has therefore a great demand for developing rapid and reliable analytical techniques to control multiple pesticides in foods, agriculture and environmental samples. For this purpose, several cheminulescence based detection strategies have
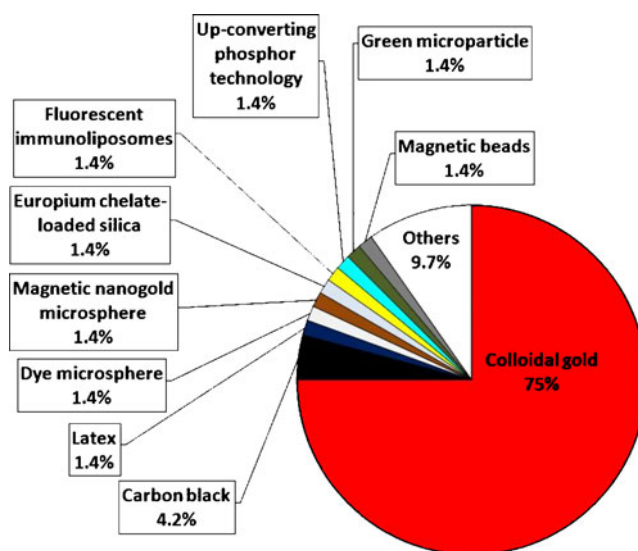

Fig. 5 Overview of study using colored particles as label in lateral flow tests for the detection of pathogen agents and chemical contaminants. Data were obtained from a literature survey. The total number of published reports considered in this figure was seventy two (see Tables 1, 2 and 3)

been proposed in the literature [131–133]. Amongst the modern immunochemical techniques avalaible, ICA had known successful applications [134–144]. Up to now, researches in ICA methods have included the development of more extended formats that permit a one-site detection of multiple pesticides. Guo et al. [136] proposed two gold-based ICAs to detect simultaneously carbofuran and triazophos in water samples (tap water, surface water and groundwater). In thi study, one test strip format employed a bispecific monoclonal antibody against both carbofuran and triazophos. The second test format was based on two different monoclonal antibodies raised specifically against the two pesticides. Gold-monoclonal antibody conjugates were applied on the conjugate pads of the developed test strips to serve as detector reagents. The capture reagents consisted of two test lines (T_1 and T_2) coated with carbofuran-ovalbumin conjugate and triazophos-ovalbumin conjugate, respectively. Water samples were treated by centrifugation (3000 × g for 10 min), and the supernatants were spiked with carbofuran or triazophos at concentrations ranging 0–132 μg. mL^{-1}. The visual detection limits of the pesticides by the two ICA formats were from 4 μg. mL^{-1} to 62 μg. mL^{-1}. The strip showed low cross-reaction to other structurally related pesticide compounds including carbosulfan, carbofuran phenol, isoprocarb, chlorpyrifos, parathion and diazinon. Kranthi et al. [138] also investigated a single test strip format for one-site verification of active ingredients in commercial insectide formulations of endosulfan and three pyrethryroids (cypermethrin, deltamethrin and fenvalerate). Colloidal gold were conjugated with two antisera raised against endosulfan and cypermethrin. The developed test strip consisted of two capture test lines coated separately with endosulfan-BSA conjugate or cypermethrin-BSA conjugate. Finally, the test strip was designed to allow the detection of standardized concentrations of endosulfan (1800 mg. mL^{-1}), cypermethrin (800 mg. mL^{-1}), deltamethrin (1000 mg. mL^{-1}) and fenvalerate (1400 mg. mL^{-1}). These concentrations were found sufficients to inhibit colour development on the test lines. The test strip developed here is very useful for the farmers, and it also could have potential applications in environmental testing.

Conclusions and outlook

A variety of analytical methods available for detecting pathogen organisms or hazardous chemicals related to food safety, human health and environment suffers from being time-consuming, too expensive and too complicated to use. This review paper was intended to provide an overview of recent published reports on LFT. Major advantages found on LFT are low-cost, speed, portable, do not require complicated equipment and technical expertise, which are

critical parameters for POC. However, this technology still needs further improvements. For example, we have found a reduced number of applications in multiresidue analysis. Future researches can be therefore placed on the development of novel formats with more capture test lines to detect several analytes in complex samples. An option is to use generic antibodies, and such applications have been oberved in ELISA methods [145, 146]. Analysis of complex samples like milk, blood/urine, soil or groundwater is another subject matter. Because of their differences in origin and chemical property, these matrices require rigorous treatments (centrifugation, serial dilution or filtration, pH adjustment, etc) to prevent any doubt during analysis. Beside, the choice of appropriate reporter becomes a critical parameter in LFT development. In our literature survey, we found that seventy five percent of published reports on LFT employed colloidal gold particle as label (Fig. 5). Only a study in which fluorescent immunoliposome was used as label for the detection of SEB [67] investigated the alternative use of other types of label including fluorescent microsphere, Dextrane rhodamine and dye microsphere. Fluorescent immunoliposome allowed a fifteen-fold increase in sensitivity compared with that for visual detection of colored labels. Up-converting phosphorus technology used as label may be 10–100 fold more sensitive than colloidal gold or colored latex beads [15]. Europium chelate-loaded silica nanoparticles have also proven great potential to improve sensitivity in LFT at one hundred times compared to colloidal gold [79]. These new findings could complement the use of silver intensification [147] to improve sensitivity of LFT for controlling bio-terrorism agents and infectious viruses. On the other hand, a new approach for monitoring chemical contaminants can be placed on the use of bio-functionalized magnetic nanogold microspheres [122].

Quality control is essentially establishing adequate performance characteristics (sensitivity, specificity, negative predictive value, positive predictive value, cross reactivity, etc.) of a given test. With the globalization, new generations of analytical devices have to be accurate, speed, simple and cost-effective. Lateral flow test strip technology could provide a promising approach for this purpose.

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