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Immunological techniques: immunochromotography, enzyme-linked immunofluorescent assays and agglutination techniques

C.L. Baylis, Campden and Chorleywood Food Research Association, UK

10.1 Introduction

Developments and technological advances in the field of microbiological test methods have resulted in the availability of a wide range of commercial kits and new techniques for the microbiologist. More recently there has been a gradual adoption of molecular biology methods into food testing, although lack of knowledge of these methods, the perceived greater costs associated with their use compared with traditional culture methods and lack of universal acceptance, currently restricts the widespread uptake of molecular methods.

By comparison immunological methods which rely on the interaction between specific antibodies to selectively capture, label and detect antigens associated with the target organism are widely used and accepted for the detection and confirmation of specific microorganisms and certain toxins. The antigen of interest may be a cellular component of the target organism, such as a specific lippopolysaccharide on the outer cell wall, a protein on the flagella of certain motile bacteria, or a product or toxin produced by the organism during growth. The choice of antigen is particularly important as this will determine the specificity of the test.

Antigens present on flagella can provide greater specificity, whilst some somatic antigens can be shared by a wide range of bacteria besides the target organism. A good example of such an antigen is the somatic O-antigen of *E. coli* O157. Although detection of this antigen provides a convenient and relatively specific method for detecting or confirming this pathogen, other bacteria share

similar lipopolysaccharide structures, the most common being *Escherichia hermanii* and *Citrobacter freundii*. However, somatic antibody cross-reactions have also reported for some strains of *Yersinia enterocolitica* and *Brucella* species (Borczyk *et al.*, 1987; Perry and Bundle, 1990; Bettelheim *et al.*, 1993). Consequently, rapid immunological tests, although relatively specific, cannot be used alone to confirm the presence of a particular organism. For this reason, results from immunological detection methods are regarded as presumptive until they have been confirmed by conventional cultural isolation and subsequent confirmation of the target organism. Moreover, whilst immunological methods such as agglutination tests have become an integral part of some confirmation procedures, for example those used for *Salmonella* and *E. coli* O157, these too require supplementary characterisation tests such as biochemical tests to be performed to fully identify and confirm the presence of the target organism.

In addition to the target antigen, the choice and type of antibodies used can markedly affect the specificity of the method and selection requires careful consideration by those developing immunology-based kits. Whilst optimising binding conditions for monoclonal antibodies is relatively straightforward, this can be more difficult for polyclonal antibodies which have a variety of epitopes which may each require different binding conditions. However, the choice and number of antibodies used will depend on the target analyte and the required specificity needed for the test. It should be remembered that a certain degree of method specificity is also provided by the growth media and conditions used before detection or isolation. Although certain organisms may potentially cross-react with the chosen antibodies, the careful choice of selective agents and growth conditions can effectively reduce the risk of false positive results. Moreover, during confirmation procedures, complementary characterisation tests should be used to eliminate non-target organisms.

Probably the most widely used methods for detection of specific microorganisms in foods are those based on the enzyme-linked immunosorbent assay (ELISA) technique which is explained in more detail in Chapter 11. In addition, magnetic beads coated with antibodies have become popular for the separation of the target organism from food homogenates or enrichments containing interfering food particles and competitor organisms using the immunomagnetic separation (IMS) technique. This technique is commonly used to aid the detection of *E. coli* O157 in foods and has become an integral part of conventional and some rapid methods for this pathogen (Scotter *et al.*, 2000; Baylis *et al.*, 2001; Chapman *et al.*, 2001).

Consequently, the widespread use and acceptance of immunology-based methods has resulted in a plethora of commercial test kits for the detection of the common foodborne bacteria in foods including those belonging to the genera *Salmonella*, *Listeria* and *Campylobacter*, and more recently the specific detection of *E. coli* O157:H7. For the subsequent confirmation of suspect isolates there is also a wide range of agglutination tests available which again rely on detection of a particular antigen by monoclonal or polyclonal antibodies. In addition to the common foodborne pathogens, there are kits available for the

detection and confirmation of less common bacteria, protozoans and specific toxins. Many of these kits are currently aimed at the clinical microbiology market, but with time and greater demand some may ultimately find their way into the food microbiology sector.

However, despite the advances and developments in microbiological methods and the higher specificity and rapidity of results offered by many end-point detection systems, their dependence on conventional cultural enrichment still remains the most important limiting feature of the majority of microbiological methods. The minimum limit of detection of many methods, including those based on immunological or molecular approaches, is 10^4 $\log_{10}$ cfu per ml (Jay, 2000) and a particular pathogen in a food is likely to be found in low numbers. It is therefore still necessary to increase the level of the target organism using enrichment techniques.

Moreover, the organism of interest is not likely to be homogeneously distributed throughout the food and is likely to be present together with higher numbers of other closely-related microorganisms which may retard or totally inhibit the growth of the organism of interest (De Medici *et al.*, 1998; Baylis *et al.*, 2000) resulting in a negative assay result. These competitor organisms may also give rise to cross-reactions with the antibodies used in some detection systems and confirmation tests, resulting in false positive results. Furthermore, cells in a food may have sustained sublethal injury from either the treatments used in food processing or from the effects of intrinsic factors such as low pH, high salt content, and the effect of preservatives or other inhibitory properties associated with the food concerned. Such damaged cells are often susceptible to the selective agents used in culture media and commonly exhibit extended lag phases and are less likely to grow rapidly, even under optimum growth conditions (Stephens *et al.*, 1997; Blackburn and McCarthy, 2000). Selective enrichment, which is often required to inhibit or restrict the growth of competitor organisms, may also have a detrimental effect on the target organism. To overcome this, a pre-enrichment step is often necessary, but this adds additional time to the method.

Having enriched the target organism to detectable levels, the microbiologist is faced with a vast array of rapid detection methods. As mentioned previously, the ELISA technique, but especially the microtitre plate format, has become an extremely popular method for routinely screening food samples following enrichment. Traditionally these assays, although rapid to perform, are labour intensive and rely on multiple stages for the washing of microtitre plates and addition of reagents prior to reading the result. Typically these assays take between 1–2 hours, depending on the format of the test and number and length of incubation steps. The introduction of automated plate washers and readers has reduced the hands-on time associated with performing many of the tasks required for these tests, but assay times still remain relatively long because of the multiple stages required. Fully automated systems based on the enzyme-linked immunofluorescent assay technique (ELFA), which are covered later in this chapter, also provide reduced hands-on time whilst retaining the rapidity

and specificity of the traditional ELISA techniques. By comparison immunochromatography techniques such as lateral flow devices (see Section 10.2) may require no instrumentation and results are often available within 15–20 minutes following the addition of a portion of enriched sample to the device. Consequently these tests have become an attractive alternative to the traditional ELISA test.

10.2 Immunochromatography: lateral flow devices

The introduction of membrane-based assays which rely on the immunochromatography principle has had a major impact on rapid end-point testing. This is particularly true in clinical diagnosis where immunochromatographic devices are routinely used for the detection of a wide range of analytes including specific components of urine, blood and other biological fluids, hormones, therapeutic drugs and drugs of abuse, viruses and other agents of disease and infection. This technology has now been applied to food testing and other non-clinical applications, resulting in a wide range of commercial kits for the detection of antibiotic residues, hormones and common foodborne pathogens.

The most common format for immunochromatographic devices is the lateral flow or dipstick design. Although commercial assays are available in a diverse assortment of housings and designs, lateral flow devices generally share the same common composition and performance characteristics. A lateral flow device typically comprises a porous membrane, typically nitrocellulose, onto which is immobilised a capture protein for the target analyte. In most devices, it is common for the capture protein to be an antibody which specifically binds and captures a particular antigen if present in the sample (Fig. 10.1).

A portion of sample is applied to the sample pad, often composed of paper, through an inlet in the housing, commonly referred to as the sample window. Below the sample pad is the conjugate pad, commonly composed of glass fibre, which is attached to the membrane. This pad contains a dried conjugate comprising particles adsorbed with antibodies or antigens specific to the analyte being detected. For some commercial kits, the conjugate contains gold particles, for example the visual immunoprecipitate (VIP) range of tests from BioControl Inc, Reveal tests from Neogen Inc, the Immunocard STAT! *E. coli* O157:H7 test from Meridian Diagnostics and the Singlepath range of LFDs from Merck. By comparison, with other commercial kits such as the *Listeria* Rapid Test (Oxoid Ltd), the conjugate contains coloured latex particles. When a sample is applied to the sample pad, the liquid migrates by capillary diffusion through the conjugate pad, re-hydrating the conjugate. Specific interaction between the conjugate and the sample analyte occurs, resulting in the formation of a complex which proceeds to move onto the membrane and migrate towards the capture binding protein where it becomes immobilised. This process, which is dependent on the flow rate of the fluid through the microporous membrane, typically takes 15–20 minutes for many commercial devices, and produces a distinct band or

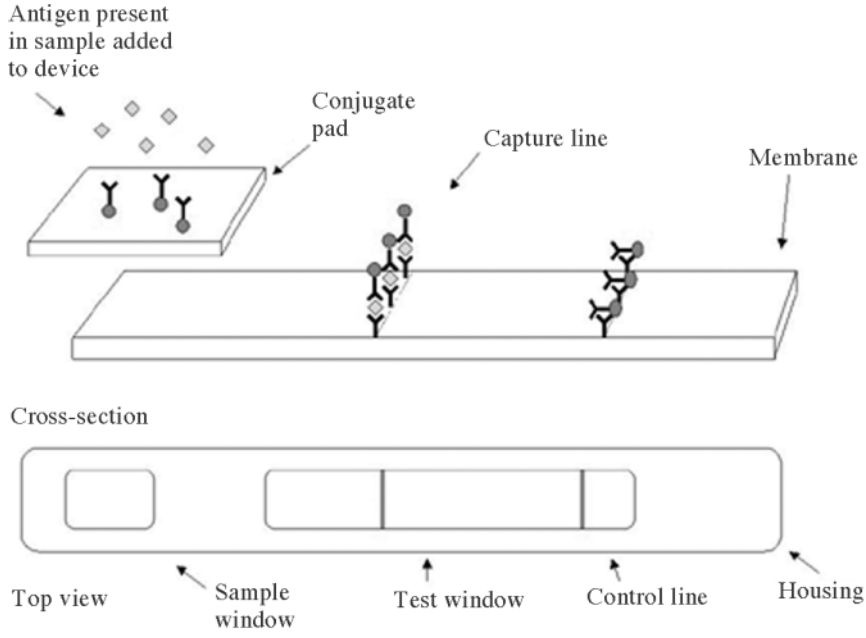

Fig. 10.1 Components of a typical lateral flow device.

line in the test window of the device, which is indicative of a positive result for the analyte of interest. Excess conjugate continues to migrate up the membrane where it is immobilised at another point on the membrane, producing a second line, commonly referred to as the control line, which indicates that the test is complete and the device has functioned properly (Fig. 10.2). In the absence of the target analyte, i.e. a negative result, only the control line will appear.

Whilst the principle of lateral flow devices appears straightforward and relatively simple, in reality there are a number of problems that manufacturers of these devices must consider and overcome if these devices are to give the desired sensitivity and performance. The many interactions and problems associated with nitrocellulose membranes are covered in detail elsewhere (Jones, 1999a, 1999b). Whilst it may not be necessary for the user to concern themselves with these aspects of lateral flow devices, an appreciation of the potential causes of poor performance or unexpected results can be valuable when using these devices for the first time or with different food matrices.

Among the important forces involved in protein binding to nitrocellulose membranes are hydrophobic and electrostatic interactions and hydrogen bonding. Each of these must be carefully considered by developers of LFDs in order to optimise binding of proteins to a particular membrane. The wicking movement of a sample through the membrane is the result of capillary action, which is a function of cohesion forces. To achieve lateral flow and wicking of the liquid through the membrane, the differential between the surface energies of

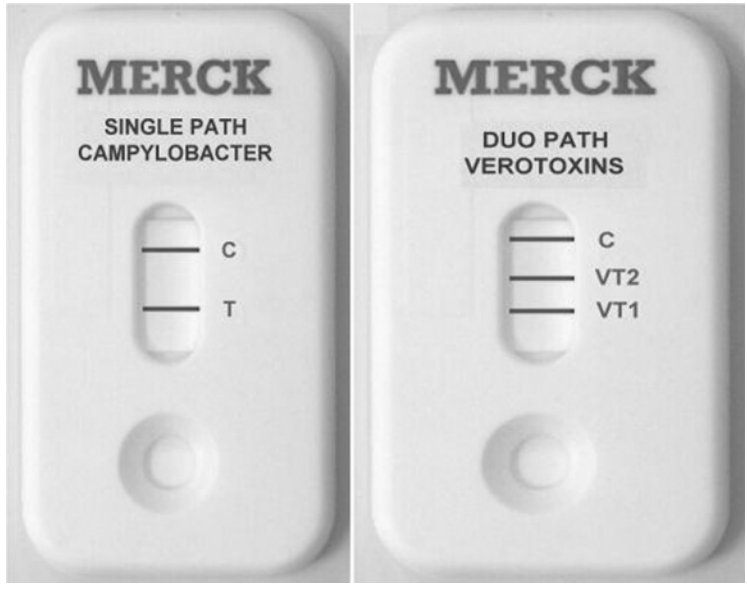

Fig. 10.2 Example of a lateral flow device showing test and control lines (Courtesy Merck KGaA).

the solid substrate and the fluid sample needs to be overcome without mechanical assistance. To improve the flow of fluid through the membrane, the surface energy of a fluid must be decreased. Optimum wicking is achieved by reducing the surface tension of a fluid so that it is less than the surface energy of the surface to be wetted (Meathrel *et al.*, 2001). Manufacturers achieve this by using physical and chemical treatment of plastics. Surfactants can also be used to reduce the surface energy of a fluid. For this purpose they are sometimes used to reduce the surface energy of the fluid directly or they are incorporated into adhesives and coatings used in the construction of these devices.

For certain LFD tests or target proteins, it may be necessary to dilute the sample in a buffer before applying it to the device. For some tests this is required to ensure good solubility of the protein of interest or to maintain it in a stable form when presenting it to the device. If proteins in the sample precipitate before being applied to the membrane the amount of protein available for attachment to the membrane can be substantially reduced or the pores of the membrane can become clogged. Consequently, the performance of the device can be greatly reduced and results could be unreliable. The use of a buffer with such devices can overcome these problems, although many popular LFDs used for food testing enable food homogenates to be applied directly to the device.

Although there are many commercial LFDs used for clinical applications, e.g. the ImmunoCard STAT! (Meridian Diagnostics Inc.), there are some commercially available LFDs that have been subjected to independent evaluations or collaborative studies to compare their performance against other similar devices or against conventional culture methods for the detection of specific

Table 10.1 Examples of commercially available detection tests based on immunochromatography (lateral flow devices)

Kit name	Manufacturer	Target analyte
VIP *Salmonella*	BioControl Inc	*Salmonella*
VIP *Listeria*		*Listeria* spp
VIP EHEC		*E. coli* O157
Reveal *Salmonella*	Neogen Corp	*Salmonella*
Reveal *Listeria*		*Listeria* spp
Reveal *E. coli* O157:H7		*E. coli* O157
Singlepath Salmonella	Merck KGaA	*Salmonella*
Singlepath Listeria		*Listeria* spp
Singlepath E. coli O157		*E. coli* O157
Singlepath Campylobacter		*Campylobacter*
Duopath Verotoxins		VT1 and VT2*
Listeria Rapid test (Clearview)	Oxoid Ltd	Listeria spp (excluding *L. murrayi*)
NOW™ *E. coli* O157 and O157:H7	Binax Inc	*E. coli* O157
E. coli O157 Path-Stik	Celsis Ltd	*E. coli* O157:H7
Salmonella Path-Stick		*Salmonella*
ImmunoCard STAT	Meridian Diagnostics	*E. coli* O157

* VT1 and VT2 refer to verocytotoxins (Shiga toxins).

pathogens in foods. Examples of commercially available LFDs used for foods are presented in Table 10.1 and details of specific kits are given below. In addition, there have been some commercial kits that have been included in validation and collaborative studies for food testing under the scheme operated by the Association of Official Analytical Chemists (AOAC) in the United States. LFDs approved for use with foods and specific sample types include the popular VIP (BioControl Inc.) range of kits for the detection of *E. coli* O157:H7 in selected foods (Feldsine *et al.*, 1997a, b) *Listeria monocytogenes* in selected foods (Feldsine *et al.*, 1997c), and from environmental samples (Feldsine *et al.*, 2002) and *Salmonella* in foods (Feldsine *et al.*, 2000). A second commercial LFD subjected to collaborative studies under the AOAC scheme is the Reveal test for *E. coli* O157:H7, manufactured by Neogen Corp. In addition to the LFD which is used for end-point detection, the manufacturers of this device have developed two enrichment media to selectively increase the amount of target organisms in the food sample to detectable levels. The first is an eight-hour test which has been subjected to collaborative study for detection of *E. coli* O157:H7 in raw ground beef, beef cubes and lettuce rinse (Bird and Hoerner, 2001b) and a 20-hour test for detection of this pathogen in selected foods and environmental swabs (Bird and Hoerner, 2001a). Other commercial LFDs approved by the AOAC for food testing include the PATH-STIK *Salmonella* test (Celsis), Reveal

Salmonella test (Neogen Corp.) and *Listeria* Rapid test (Oxoid Ltd). These tests have all received performance-tested status by the AOAC after independent laboratory validation of the performance claims. As well as the AOAC scheme, there have been independent studies performed to compare the performance of LFDs against each other, against conventional culture methods and against other rapid methods.

In a recent independent study (Chapman *et al.*, 2001), both Reveal 8 h and 20 h test and VIP test were compared with a polymerase chain reaction (PCR) method and a conventional culture method incorporating IMS, for the detection of *E. coli* O157 in various naturally contaminated meats and meat products. It was reported that both LFDs were simple and rapid to use, giving results within 24 h. However, the sensitivity of both devices was less than that of the conventional culture method. In particular, the Reveal 8 h test performed poorly. This was attributed to the short incubation period and incubation temperature of 43ºC which may have been insufficient to recover stressed cells to a detectable level by the Reveal test. In food enrichments containing high levels of closely-related competitor organisms, growth of the target organisms can be severely retarded (Baylis *et al.*, 2000).

In a previous study (Power *et al.*, 2000), the Reveal test (Neogen Corp.) and SafePath microwell ELISA method (SafePath Laboratories) were compared with a cultural method for the detection of *E. coli* O157 in bovine faeces and on meat carcasses. The latter method, which incorporated IMS, was regarded as the reference method. For the detection of *E. coli* O157:H7 on beef carcasses, the study revealed that both LFD and ELISA showed similar high specificity, being 99 per cent and 100 per cent, respectively. However, the sensitivity of both was low at 50 per cent, although this was based on low numbers of contaminated carcass samples. However, for individual faecal samples, the ELISA showed a much higher sensitivity (70 per cent) compared with the LFD (46 per cent). The authors concluded that both the ELISA and LFD showed promise as rapid carcass monitoring tests although more field testing would be necessary to estimate their sensitivity.

For the detection of *Listeria* species in foods, the *Listeria* Rapid test (Oxoid Ltd), launched in 1994, has been reported to give comparable performance to conventional culture methods in a number of studies and trials (Roberts, 1994). The kit relies on the detection of 'B' flagella antigen which is common among *Listeria* species with the exception of *L. grayi* (formerly *L. grayi* subsp *grayi* and *murrayi*). In the device one of the monoclonal antibodies raised against flagellin protein is bound to blue-dyed polystyrene latex particles. The principle of the test is similar to the general format described above and shown in Fig 10.1. If the target antigen is present in the food sample, the antibody-antigen complex will migrate up the membrane and become immobilised by antibodies on the membrane, forming a blue line. Excess latex labelled antibodies continue to migrate up the membrane where they meet another line of immobilised polyclonal antibodies raised against mouse IgG, resulting in a second line that shows that the test has performed correctly.

In two independent evaluations (Barbuti *et al.*, 1995; Jones *et al.*, 1995), the sensitivity of the test was reported to be between 10^3 and 10^6 cfu/ml, although this can be influenced by the serotype and strain as well as the growth conditions prior to using the device. Whilst this test is not designed to detect *Listeria grayi* which lacks the target antigen, conditions such as medium and incubation temperature may affect flagella formation and consequently reduce the yield of target antigen, resulting in false negative results (Jones *et al.*, 1995). False negative results may also occur in certain foods containing low levels of stressed cells that may also fail to yield detectable levels of antigen after enrichment (Barbuti *et al.*, 1995). However, this same situation can occur with any test that relies on multiplication of the target organism and the provision of sufficient amounts of target, be it antigen, DNA or cells, that will be detected by the test device. For the majority of foods, the Listeria Rapid Test has shown good correlation with traditional culture methods but with the advantage of reducing the time for a presumptive positive or negative result from 96 to 48 h.

10.3 Enzyme-linked fluorescent assays (ELFA)

Whilst LFDs provide simplicity and rapidity of results without the need for expensive equipment to perform and read the tests, traditional ELISA tests using microtitre plates remain popular and have become universally accepted methods for detection of pathogens in foods. More recently there has been a move towards greater automation of ELISA tests, which not only reduces the hands-on time but also improves the reproducibility and standardisation of each step of the assay. Whilst many ELISA-based methods rely on chromogenic substrates for end-point detection of the target antigen, enzyme-linked fluorescent assays (ELFA) employ fluorescence for end-point detection.

A good example of commercial assays based on the ELFA principle are those developed by bioMérieux to run on their automated instrument the Vitek Immuno Diagnostic Assay System (VIDAS). In addition to producing assays for the clinical market, bioMérieux also produces a range of assays for the food microbiology sector which are performed on the VIDAS. These include assays for *Salmonella*, *Campylobacter*, *Listeria* species, *Listeria monocytogenes*, *E. coli* O157 and staphylococcal enterotoxins (A, B, C1, C2, C3, D and E). All of these assays are based on the ELFA principle and the steps described below are all performed automatically by the VIDAS instrument.

In the same way that conventional ELISA tests are based on the specific binding of the target antigens to a capture antibody, ELFAs use the same principle except that the second antibody is conjugated to an enzyme which produces a fluorescent reaction upon addition of the enzyme substrate. Assays that are performed on the VIDAS typically comprise two parts. The first of these is a pipette tip-like disposable device, termed the solid phase receptacle (SPR), which is coated with antibodies specific to the target antigen. The SPR serves as both a pipette for the assay and as a solid phase onto which the antibodies are

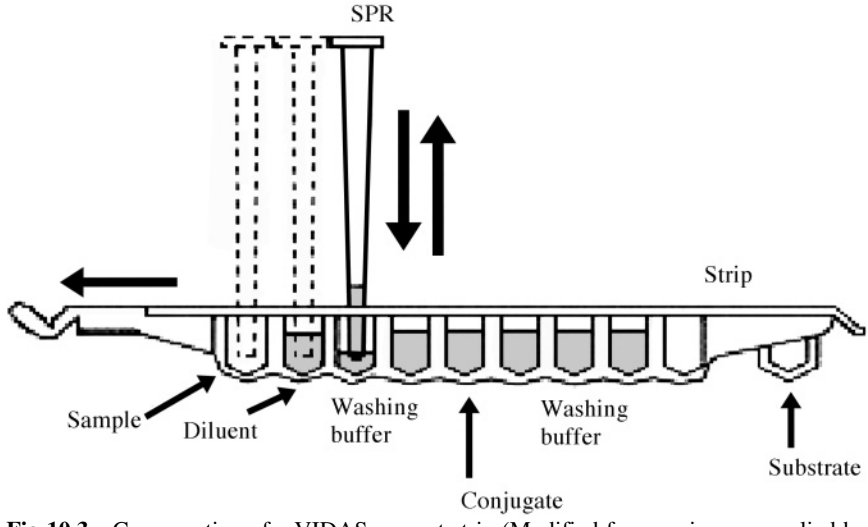

Fig 10.3 Cross-section of a VIDAS reagent strip (Modified from an image supplied by bioMérieux).

coated. The second is the reagent strip, comprising a polypropylene strip consisting of ten wells covered with a foil seal (Figs 10.3 and 10.4). All the reagents necessary for the assay are contained within specific wells in the strip. These include wash solutions, conjugate comprising of alkaline phosphatase labelled antibodies, which can be polycolonal or monoclonal depending on the assay, and the substrate 4-methyl-umbelliferyl phosphate.

Following cultural enrichment of the sample in appropriate liquid media, an aliquot of boiled enrichment sample is placed into the first well of the reagent strip, which is placed in the VIDAS instrument. During the assay, the sample is

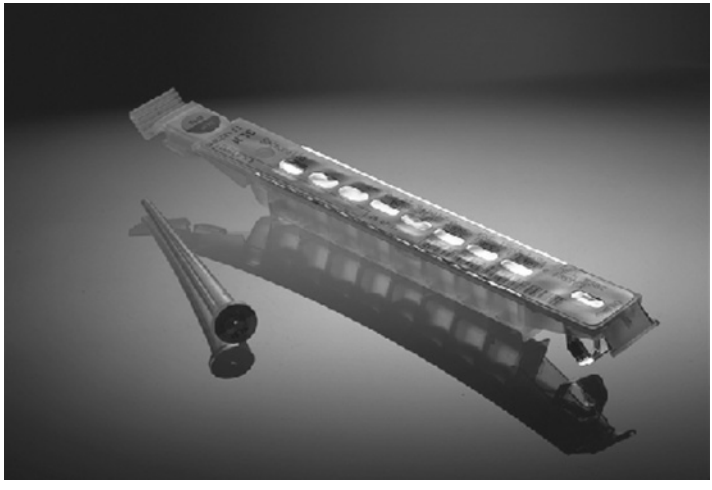

Fig. 10.4 Example of a VIDAS reagent strip (Courtesy bioMérieux).

cycled in and out of the SPR for a specific length of time. If the target antigen is present in the sample it will bind to the antibodies coated to the interior of the SPR. Unbound sample components are removed by subsequent washing steps. The conjugate, which consists of antibodies labelled with alkaline phosphatase also binds to the antigen portion of the immune complex whereas unbound conjugate is removed by a final wash step. The fluorogenic substrate used by the assay, 4-methyl-umbelliferyl phosphate, is converted to the fluorescent product 4-methyl-umbelliferone by bound enzyme remaining on the SPR. The last well in the strip is an optically clear curvette in which intensity of fluorescence is measured by the optical scanner in the instrument.

As with other immunoassays, any positive results for pathogens are regarded as presumptive and require cultural isolation and subsequent confirmation of the target organism. In previous studies, the VIDAS assays have been compared with conventional culture methods, including those incorporating IMS as well as other rapid methods for the detection of *E. coli* O157 (Vernozy-Rozand *et al.*, 1997; Grif *et al.*, 1998; Vernozy-Rozand *et al.*, 1998), *Listeria* spp (Gangar *et al.*, 2000; Kerdahi and Istafanos, 2000), *L. monocytogenes* (Vaz-Velho *et al.*, 2000) and *Salmonella* (Curiale *et al.*, 1997).

In addition to the conventional assays that can be performed on the VIDAS, the *Salmonella* and *E. coli* O157 assays can both be used with an additional stage incorporating immunoconcentration. These procedures are termed immunoconcentration *Salmonella* (ICS) and immunoconcentration *E. coli* (ICE), respectively. Whilst similar in principle to manual IMS techniques, where the target organism is concentrated before screening with a rapid method such as an ELISA, or plating onto an isolation media, VIDAS immuno-concentration techniques provide a fully automated method of concentrating target organisms.

Conventional ELFA for *Salmonella* detection using the VIDAS typically involves enrichment followed by the assay and end-point detection of the target antigen. The immunoconcentration assays both use a SPR device for the solid phase and a strip comprising of wells containing the necessary reagents for this procedure. However, unlike the conventional ELFA run on the VIDAS, the sample is not heat treated and living cells instead are captured by specific antibodies coated on the inside of the SPR. After different washing stages to remove other organisms non-specifically absorbed onto the SPR, a novel release process then enables the captured cells to be released and concentrated in solution in the first well in the strip. This process typically takes 40 min and the solution containing the concentrated cells can either be collected and used for isolation on appropriate media, or it can be used for detection using the ELFA strip.

For *Salmonella* detection, the method requires pre-enrichment followed by selective enrichment, which typically takes 48 h prior to performing the assay. The ICS procedure replaces the selective enrichment stage so the assay can be performed after 16–24 h pre-enrichment, giving presumptive results on the second day. Alternatively selective media can be inspected for presumptive

Salmonella colonies on the second day after using the ICS plating technique. For *E. coli* O157, the ICE is used to aid confirmation of positive samples according to the conventional ELFA. This automated immunoconcentration procedure has the additional benefit of reducing test times but also of improving the specific concentration and isolation of target organisms (De Medici *et al.*, 1998; Vernozy-Rozand *et al.*, 2002).

10.4 Agglutination tests

Owing to the specificity provided by antibody-antigen reactions, immunological methods have become increasingly popular for the detection of foodborne pathogens and toxins. Another application exploiting this interaction between antigen and antibody is serological confirmation and the use of agglutination tests. The term agglutination is used to describe the reaction of an antigen with its corresponding antibody *in vivo* producing macroscopic clumping. This reaction is generally rapid, does not dissociate and shows high affinity.

Agglutination tests have been used extensively in clinical diagnosis of infection and for serological classification of bacteria for many years. As far back as 1896, techniques for testing patient serum for *Salmonella* antibodies in cases of typhoid fever were developed using agglutination of *Salmonella* O and H antigens to aid diagnosis. In addition to this application, which also extends to the diagnosis of other clinical infections, the use of antigenic typing schemes has become another important aspect of epidemiology and classification in microbiology. Probably the most well known of these typing schemes is the Kauffmann-White Antigenic Scheme used for identifying salmonellae.

Traditionally serotypes of *Salmonella* are defined based on the antigenic structure of the somatic or cell wall (O) heat-stable antigens followed by the flagella (H) heat-labile antigens which are usually associated with motility. Whilst one somatic antigen may be common to a group of salmonella strains and thus identify a serogroup, other antigens may be shared among several serogroups. Furthermore, with a serogroup there will be specific antigens that together with the flagella (H) antigens enable a *Salmonella* to be identified to a particular serotype based on the combinations of O and H antigens present. Using this approach, over 2300 serotypes of *Salmonella* have been identified. However, with the increased use of genomics and improvements in molecular biology techniques, it has been realised that *Salmonella* serotypes are not different species, but that they all belong to two DNA hybridisation groups. Consequently, the nomenclature of *Salmonella* has again changed and now all of the *Salmonella* serovars belong to two species, namely *S. bongori* which contains 18 serovars and *S. enterica* which contains the remaining 2300+ serovars divided among six subspecies (Threlfall *et al.*, 1999),

Typically a laboratory would initially use polyvalent antisera that cover the *Salmonella* O Groups Poly A through to Poly G. If agglutination occurs, individual *Salmonella* O Group factor antisera would be used to determine to

which specific serogroup the isolate belongs. Cross-reactions with *Salmonella* O factor antisera are possible because serogroups may share non-major group antigens. Furthermore, cross-reactions with organisms outside the genus can occur and for this reason both morphological and biochemical identification should be used to support any serological identification. Flagella (H) antigens, including analysis of phase 1 and phase 2 antigens, are also identified using a combination of polyvalent and single complex H antisera.

However, to fully serotype and identify *Salmonella* requires time, expertise and a complete range of antisera to be kept by the laboratory. Consequently, whilst some laboratories may identify *Salmonella* biochemically and confirm the presence of O and H antigens using polyvalent antisera, or even use antisera to identify a particular serogroup, it is more common for such isolates to be sent to a specialist laboratory for complete identification involving full serotyping, phage typing or the use of genetic typing methods.

Compared with specialist laboratories performing serology on routine isolates, food testing laboratories will need to confirm the identity of a suspect isolate quickly to enable appropriate action to be taken should contamination of a food product be suspected. Confirmation tests, particularly biochemical and basic serological identification or confirmation of a particular antigen, have become an integral part of some methods, in particular those for *Salmonella* and *E. coli* O157:H7. Although traditional antisera are available, in recent years there has been an increase in the introduction of rapid agglutination tests that are more convenient to use and easier to interpret. More recently, the use of blood cells or latex particles labelled with antibodies are particularly common formats, which not only allow for easy interpretation of clumping, but require less antigenic material to yield visible precipitation compared to conventional agglutination tests using antisera. Although many kits have primarily been developed for clinical diagnosis, many can be and have been adopted for identification of food isolates.

10.4.1 Agglutination tests for confirmation of bacteria

Agglutination tests for Staphylococcus aureus

Probably one of the most common foodborne organisms identified by commercial agglutination tests is *Staphylococcus aureus*. Traditionally, the tube coagulase test which involves confirmation of free coagulase using rabbit plasma is regarded as the 'gold standard' for the identification of *S. aureus*. This test is labour and materials intensive and requires regular readings up to 4 h, but it may require up to 24 h to obtain a positive result, which is characterised by clotting of plasma in the tube. Although conventional antiserum is available, owing to the complex antigenic structure of *S. aureus*, they are not commonly used in food testing laboratories for *S. aureus* identification. For epidemiological studies, typing with bacteriophages has proved to be a more useful approach. Food microbiologists on the other hand rely predominantly on confirmation of coagulase or heat stable nuclease (thermonuclease), but to distinguish *S. aureus*

from other coagulase positive staphylococci, notably *S. hyicus* and *S. intermedius*, the coagulase test must also be accompanied by biochemical identification. Furthermore, although uncommon, some strains of free-coagulase negative *S. aureus* have also been reported (Luijendijk *et al.*, 1996) which further supports the importance of not relying on one test, but instead, using a combination of tests to aid with the identification and confirmation of suspect isolates.

Whilst the tube coagulase test may be used to detect free coagulase, a rapid version, the slide test, detects bound coagulase or fibrinogen affinity factor (clumping factor). This test is favoured because it is quick and easy to perform, involving emulsification of a colony in water on a slide, adding plasma and observing clumping which should occur within seconds. However, this technique is not recommended for colonies directly from selective media, particularly media containing high concentrations of salt which can give rise to autoagglutination and false positive results. Furthermore, if isolates autoagglutinate, these and those giving rise to negative results by slide agglutination test, generally require re-testing using the tube test. Consequently, commercial agglutination tests, which are simple and easy to use and interpret, and can provide results within seconds of testing a colony directly from an isolation medium, have proved popular alternatives to traditional tube and slide tests for routine identification of *S. aureus*.

Two of the most common targets for commercial agglutination kits used for the identification of *S. aureus* are clumping factor and protein A. Some kits may detect clumping factor alone and others may also detect protein A. In clinical microbiology, identification of methicillin-resistant *S. aureus* (MRSA) is of particular importance. However, many MRSA strains possess capsular polysaccharide which can mask other cell components such as clumping factor and protein A. Consequently, many of these strains can give rise to negative results using agglutination tests targeted specifically at these or other cell surface antigens (Hsueh *et al.*, 1999). To overcome this problem some of the earlier commercial agglutination kits have been modified whilst newer ones already include additional targets, such as specific surface antigens and polysaccharides, to improve detection of MRSA strains (Smole *et al.*, 1998).

Some of the earlier tests consisted of erythrocytes sensitised with fibrinogen for the detection of clumping factor. Subsequent commercial kits have employed coated latex particles and/or sensitised sheep erythrocytes for the simultaneous detection of clumping factor and protein A. For example, the Slidex Staph kit (bioMérieux) is an agglutination test based on a combination of latex and haemagglutination. The reagent contains blood cells sensitised with fibrinogen to detect bound coagulase and latex particles sensitised with specific monoclonal antibodies to detect protein A by the Fc fragment of IgG, as well as antigens on the bacterial surface. In contrast the BBL Staphyloslide kit (Beckton Dickenson) is a test based only on haemagglutination and detects the activity of the cell wall polypeptide clumping factor, which binds to fibrinogen sensitised sheep erythrocytes, resulting in visual clumping.

Whilst *S. aureus* produces both free and bound coagulase, some other species of coagulase negative staphylococci have been found to produce clumping factor. These include *S. lugdunensis* and *S. schleiferi*, which although negative by traditional coagulase tube test, has been reported to give rise to positive results with agglutination tests that detect clumping factor (Personne *et al.*, 1997; Zbinden *et al.*, 1997; van Griethuysen *et al.*, 2001). Moreover, even agglutination tests that detect polysaccharides and other surface antigens can give rise to positive results from coagulase negative species, for example *S. haemolyticus* (van Griethuysen *et al.*, 2001) and *S. epidermidis* (Blake and Metcalfe, 2001).

Agglutination tests for Escherichia coli *O157*

Another example of a foodborne pathogen which is routinely screened for and identified with the aid of agglutination tests is *E. coli* O157. As with *S. aureus* there is a plethora of commercial agglutination tests available to the microbiologist to aid with screening of suspect isolates. However, these agglutination tests also have limitations and require supplementary tests to be performed if an accurate positive identification is to be obtained.

Screening foods for *E. coli* O157 commonly involves enrichment in a suitable selective medium which increases the target organism to levels that can be detected by a suitable end-point test or isolation on a suitable plating medium. Fortunately, the O antigen of the *E. coli* O157 serogroup provides a specific and convenient target for immunological methods. This can involve detection of the O157 antigen using ELISA, ELFA and LFD tests and the incorporation of IMS to increase recovery of cells from foods which may contain high levels of closely-related background flora. Following plating onto suitable selective plating media, it is usual to screen a selection of colonies exhibiting typical colony morphology for the presence of O157 antigen. Subsequent identification then usually requires biochemical identification to establish that the isolate is *E. coli*, although *E. coli* O157:H7/H- do not ferment sorbitol rapidly, if at all, unlike typical strains of *E. coli*. Furthermore, the majority of *E. coli* O157 strains lack β-glucuronidase activity, which, again, is atypical for most strains of *E. coli* and is often a useful characteristic used to differentiate *E. coli* O157 from other *E. coli* strains.

As discussed previously, antibodies used for the detection of O157 antigen can cross-react with the lipopolysaccharide (LPS) of other bacteria. Consequently, certain immunoassays can give rise to false positive results and commercial latex agglutination tests can also yield positive results with bacteria besides *E. coli* O157. In one evaluation of three commercial latex agglutination test kits cross-reactions with strains of *Citrobacter freundii* and *Salmonella* O group N were reported (Sowers *et al.*, 1996). In addition to these, *Escherichia hermanii* is another bacterium that exhibits serological cross-reactivity with O157 (Borczyk *et al.*, 1987; Perry and Bundle, 1990). Moreover, like *E. coli* O157 it too does not ferment sorbitol rapidly, if at all and therefore gives rise to similar colony morphology to *E. coli* O157 on sorbitol MacConkey medium,

which is routinely used for the isolation of *E. coli* O157. It also shares the absence of β-glucuronidase activity and therefore resembles presumptive *E. coli* O157 even further. However, biochemical tests can help to distinguish these bacteria, in particular, the ability of *E. hermanii* to ferment rhamnose.

Once a laboratory has confirmed the presence of O157 antigen the isolate is still presumptive until the results from supporting tests such as the biochemical profile confirms the isolate to be *E. coli*. In addition the isolate will require testing to confirm its ability to produce verocytotoxins or the possession of the toxin genes and associated virulence determinants. These tests, together with full typing, including serology, phage typing and toxin typing, would normally be performed by a specialist laboratory. Furthermore, owing to the potential for severe infection and the low infective dose by these bacteria, including reports of laboratory acquired infection, strains of verocytotoxin-producing *E. coli* (VTEC) have been re-categorised to Hazard Group 3. This therefore requires that all work with known cultures of VTEC and samples highly likely to contain these organisms must be conducted in a Containment Level 3 Laboratory.

Agglutination tests for Salmonella

In routine laboratories, *Salmonella* confirmation generally involves confirmation of *Salmonella* somatic O and flagella H antigens supported by biochemical confirmation that the isolate is *Salmonella*. In some laboratories the isolate may be serotyped to a particular O Group but full serology is often performed by a specialist laboratory. The basic serology is often performed using polyvalent antisera. However, there are some commercial latex agglutination kits that can be used to aid with the screening and presumptive identification of suspect isolates.

For screening purposes the *Salmonella* Latex kit (Oxoid Ltd) can provide a useful test to confirm isolates for the presence of *Salmonella* antigen. The test is based on latex particles coated with polyclonal antibodies targeted against flagella H antigens which are detected by agglutination. In addition to this test, others have been developed for screening isolates from clinical samples and for detection of particular *Salmonella* serotypes. For example a latex particle agglutination test has been developed to specifically identify cultured *Salmonella* Enteritidis (Thorns *et al.*, 1994). This test is based on the use of two monoclonal antibody-coated latex reagents, one of which detected the recently discovered SEF14 fimbriae expressed predominantly by *S.* Enteritidis and *S.* Dublin organisms, while the second reagent detected the H′p′ antigen of *S.* Dublin flagella. In a series of field trials 141 out of 142 strains of *S.* Enteritidis from 18 phage types were correctly identified by the latex test. A further 175 *Salmonella* isolates representing 35 serotypes were tested and only two false positives (*S.* Dublin) in the latex test were recorded. This test represents one of the first rapid serotype specific tests to be developed for *S.* Enteritidis and highlights the potential advantage of using fimbrial antigens as novel diagnostic antigens of the future.

In addition to using latex agglutination tests for screening purposes, one commercial kit (Spectate), developed by R-Biopharm Rhône Ltd, also establishes which *Salmonella* serogroup the isolate belongs to, using a combination of coloured latex particles. The kit consists of two reagents each being a mixture of red, blue and green latex particles. In the first reagent the red latex particles have an antibody attached which is specific to group B salmonellas and in the second reagent the red latex is specific to the *Salmonella* Vi antigen. With two reagents and three coloured latex particles targeted at different antigens, this test is able to identify serogroups B, C, D, E or G and the Vi antigen. This test can be used directly on colonies and also on broth cultures, although the latter requires heat treatment to remove any capsular material that could potentially mask any underlying antigens from detection. Although limited to a narrow range of serogroups, this test has been shown to provide a useful confirmation step using heat treated aliquots remaining from samples tested by ELISA (Cheesbrough and Donnelly, 1996).

In addition to the bacteria mentioned above, latex agglutination tests have been developed for a wide range of organisms of clinical importance. For the food microbiologist rapid latex agglutination tests offer a convenient and quick method of screening suspect isolates. However, in addition to using latex agglutination as a method of screening and confirming certain organisms, techniques have also been developed for detection of a range of important toxins in foods.

10.4.2 Agglutination tests for the detection of toxins

Although it is important to be able to detect and confirm the presence of a particular toxin-producing pathogen in a food, it may on some occasions be more appropriate to detect the toxin. This is especially true if the food being tested has undergone cooking and the toxin responsible for food poisoning is heat stable. A good example is *S. aureus* enterotoxins (SET) which can remain active in a food even after heat treatment, even though the numbers of viable cells of *S. aureus* have been substantially reduced or completely destroyed. In this situation, an enumeration test for *S. aureus* would not reveal the risk associated with the food. If a microbiologist does suspect previous contamination, either because the food has been implicated in a case of food poisoning showing characteristic symptoms associated with toxins, or if viable *S. aureus* cells are present in the food post cooking, detection of the toxins would be an important test to perform.

Furthermore, confirmation of a particular toxin also provides further evidence of the disease causing potential of an isolate and in some cases the test may be an integral part of the confirmation procedure. An example would be confirmation of the toxins produced by verocytotoxin producing *E. coli* including *E. coli* O157. This group of pathogenic *E. coli* are characterised by their ability to produce two distinct toxins termed verocytotoxins (VT1 and VT2), which are otherwise known by the term shiga toxins (ST1 and ST2). In

this situation these pathogens cause disease by infection and although the toxins play a role in pathogenesis, the toxins alone are not responsible for the symptoms, unlike toxins produced by other organsisms such as *S. aureus*, *Bacillus cereus* and *Clostridium botulinum*.

Recently it has become increasingly popular to detect specific toxin genes in a culture or an enriched food sample using molecular techniques such as the polymerase chain reaction (PCR). However, there is a range of commercial kits that use latex particles coated with antibodies to detect toxins. Unlike traditional agglutination tests which rely on the interaction between soluble antibody and a particulate antigen such as a bacterial cell, resulting in visual clumping, these tests rely on antibody attached to an inert carrier such as latex particles. The latex particles in this technique are described as passive because they do not play an active role in the antibody-antigen reaction. If the target antibody is present in solution it will bind to the antibodies attached to the latex particles, resulting in the formation of a lattice structure due to cross-linking of particles, which is visible to the naked eye as agglutination. The test is performed in V-well microtitre plates and results are available after 20 to 24 h incubation at room temperature. If the target antigen is absent or below the detection limit of the test, the lattice structure does not form and the coated latex particles collect in the base of the well resulting in a tight 'button' of latex particles.

Oxoid Ltd provides a range of RPLA kits for the detection of different toxins. Kits that are appropriate for testing foods include the SET-RPLA kit that detects staphylococcal enterotoxins A, B, C and D, and the BCET-RPLA which detects *Bacillus cereus* enterotoxin (diarrhoeal type). The VTEC-RPLA, which detects VT1 and VT2 individually, is another kit that can be used on sample filtrates or more commonly on cultures to confirm VT. A similar kit (VTEC Screen) produced by Denka Seiken, Japan detects both VT combined and can be used to test food samples directly following enrichment in appropriate media and subsequent toxin extraction procedures.

These kits provide rapid and convenient methods of detecting toxins, which for *B. cereus* diarrhoeal toxin and VT traditionally rely on cell cytotoxicity assays. However, whilst RPLA provides a rapid and convenient test format, cell cytotoxicity assays are generally more sensitive, requiring less toxin for cell death compared with the amount required for a positive RPLA result. This finding has been reported for both the BCET RPLA test (Fletcher and Logan, 1999) and VTEC Screen (Chart *et al.*, 2001). However, for VT testing, the RPLA tests provide a rapid and easy to use alternative to the Vero cell assay. For the rapid screening and identification of VTEC the VTEC-RPLA (Oxoid Ltd) has been combined successfully with a plating method for the detection of entrohaemolysin (Beutin *et al.*, 1996). More recently, the VTEC Screen (Denka Seiken) has been used with a method of identifying heat-labile enterotoxin-producing *E. coli* to enable rapid screening for VTEC and provision of results within a working day (Bettelheim, 2001).

10.5 Future trends

Over the past three decades immunological methods have become widely accepted and used for detection of specific pathogens in foods. The ELISA has become a common format for detection methods and there are now a plethora of commercial kits available to choose from. In recent years, greater automation of ELISA tests and fully automated systems such as VIDAS have become increasingly popular. With automation comes the added benefit of reducing hands-on time and freeing up staff for other duties or enabling fewer staff to run high numbers of tests. By comparison immunochromatography methods, particularly LFDs, have gained in popularity because of their simple format which requires no additional equipment to perform or interpret these tests. This is particularly attractive to smaller laboratories or those that perform pathogen testing infrequently. The use of immunological methods for confirmation of isolates is not a new concept either, and, in addition, the use of labelled antibodies, particularly latex particles, has become a widespread format for rapid agglutination tests.

However, whilst antigen–antibody reactions provide a relatively high degree of specificity, there can be other organisms besides the target organism that share similar antigenic structures. Consequently both detection methods and agglutination tests can give rise to false positive results. For this reason, samples giving positive results with detection tests are still regarded as presumptive and are confirmed using conventional culture procedures. Agglutination tests are used together with other characterisation tests to aid identification of suspect isolates. More recently there has been a larger uptake of molecular methods which offer greater specificity and rapidity compared with many immunological methods. Lack of knowledge of these techniques, the need for trained staff and the expense of these tests still restrict their uptake. Moreover, tests such as PCR still require sufficient numbers of target cells to obtain a positive result, which still makes them dependent on cultural enrichment, and the food matrix interference can affect these tests.

Despite molecular techniques becoming more user-friendly, there are still advances with tests that rely on antigen–antibody reactions. To start with, many manufacturers have increased the range of organisms detected by a particular assay. More recently, Merck KGaA has increased its Singlepath range of LFDs to include pathogenic *Campylobacter* species and they have just developed an LFD called Duopath that detects both verocytotoxins VT1 and VT2 (Fig. 10.2). In addition to increasing the range of target analytes detected, researchers have also looked at ways of combining lateral flow with other technologies. These include combining lateral flow immunoassay with amperometry and hydrogen peroxide consumption to enable the sensitive detection of low numbers of microorganims (Cowell *et al.*, 2002) and detection of the protozoan *Cryptosporidium parvum* using lateral flow chromatography combined with reverse transcription PCR (Kozwich *et al.*, 2000). There has also been a move towards improving the sensitivity and

interpretation of lateral flow devices using paramagnetic particles instead of latex particles or colloidal gold (LaBorde and O'Farrell, 2002).

Perhaps the one area of biology that has been advanced further by molecular biology than any other is identification and classification of microorganisms. Although immunological methods such as agglutination tests are useful for screening and aiding identification of isolates, molecular methods such as PCR and nucleic acid probes provide greater discrimination and accuracy. Moreover, the increased activity in the field of genomics and bioinformatics has had a huge impact on the understanding of microbial evolution and classification. DNA and microarrays have become increasingly popular for screening isolates for virulence determinants and specific genes which can aid clinical diagnosis, and show great potential for improving the knowledge of the role of some organisms in pathogenesis. Agglutination tests, however, remain popular and one example of a change to the conventional format is the Dryspot range from Oxoid Ltd, which includes tests for *S. aureus* and *E. coli* O157. Whereas traditional agglutination tests rely on the antibodies and antigen being in suspension, the Dryspot range comprises sensitive blue latex reagents dried onto the surface of specially designed reaction cards or test sticks. The advantage of this format is the reduced risk of splashes and these kits have improved shelf-life stability and can be stored at room temperature. Despite this move towards molecular methods many techniques such as pulse field gel electrophoresis and ribotyping are still confined to specialist laboratories. Therefore, easy to use and rapid agglutination tests will remain popular tests in many routine food testing laboratories for the foreseeable future.

10.6 Sources of further information and advice

Publications

- Baylis, C. L. (2000) Catalogue of Rapid Methods (Review No.1) Campden & Chorleywood Food Research Association.

Comprehensive listing of rapid methods and test kits, including their validation status, test times and manufacturers details, including web site addresses.

- (1994) *Rapid Methods and Automation in Microbiology and Immunology* (R.C. Spencer, E.P. Wright, S. W. B. Newsom, eds) Intercept Ltd, Andover.
- *IVD Technology*

Monthly journal for those interested in the development and manufacture of rapid methods and diagnostics tests. Many useful articles on LFDs, microarrays and biosensors. For direct access to the journal online and for additional information see www.devicelink.com/ivdt.

Useful web sites

- www. AOAC.org

Full details on the Institute and its validation schemes, including listings of approved kits

- www.devicelink.com/ivdt

Information and news for those interested in the development and manufacture of rapid methods, including LFDs, microarrays, DNA chip technology and other diagnostics technologies.

Web site addresses for rapid method companies
BioControl Systems Inc.: www.rapidmethods.com
bioMérieux: www.biomerieux.com
Binax Inc: www.binax.com
Becton Dickinson: www.bd.com
Celsis Ltd: www.celsis.com
Denka Seiken Co. Ltd: www.denka-seiken.co.jp
Merck KGaA: www.merck.de/microbiology
Neogen Corp: www.neogen.com
Oxoid Ltd: www.oxoid.com
R-Biopharm Rhône Ltd: www.r-biopharmrhone.com

10.7 References

BARBUTI, S., PANCINI, E. and DELLAPINA, G. (1995). 'Use of an immunochromatographic method for rapid determination of *Listeria* in meat products'. *Industria Conserve* **70**, 398–403.

BAYLIS, C. L., HEUVELINK, A., DE BOER, E. and HOFSTRA, H. (2001). Practical considerations and difficulties associated with the detection of verocytotoxin-producing *Escherichia coli* (VTEC) in foods. In *Verocytotoxigenic E. coli*. G. Duffy, P. Garvey and D. A. McDowell. Trumbull, Food & Nutrition Press, Inc**:** pp. 57–89.

BAYLIS, C. L., MACPHEE, S. and BETTS, R. P. (2000). 'Comparison of two commercial preparations of buffered peptone water for the recovery and growth of *Salmonella* from foods'. *Journal of Applied Microbiology* **89**, 501–10.

BETTELHEIM, K. A. (2001). 'Development of a rapid method for the detection of verocytotoxin-producing *Escherichia coli* (VTEC)'. *Letters in Applied Microbiology* **33**(1), 31–5.

BETTELHEIM, K. A., EVANGELIDIS, H., PEARCE, J. L., SOWERS, E. and STROCKBINE, N. A. (1993). 'Isolation of a *Citrobacter freundii* strain which carries the *Escherichia coli* O157 antigen'. *Journal of Clinical Microbiology* **31**(3), 760–1.

BEUTIN, L., ZIMMERMANN, S. and GLEIER, K. (1996). 'Rapid detection and isolation of shiga-like toxin (verocytotoxin)-producing *Escherichia coli* by direct testing of individual enterohemolytic colonies from washed sheep blood agar plates in the VTEC-RPLA assay'. *Journal of Clinical Microbiology* **34**(11), 2812–14.

BIRD, C. B. and HOERNER, R. J. (2001a). 'Comparison of the Reveal 20–hour method and the BAM culture method for the detection of *Escherichia coli* O157:H7 in selected foods and environmental swabs:collaborative study'. *Journal of AOAC International* **84**(3), 737–51.

BIRD, C. B. and HOERNER, R. J. (2001b). 'Reveal 8–Hour test system for detection of *Escherichia coli* O157:H7 in raw ground beef, raw beef cubes, and lettuce rinse: collaborative study'. *Journal of AOAC International* **84**(3), 719–36.

BLACKBURN, C. W. and MCCARTHY, J. D. (2000). 'Modifications to methods for the enumeration and detection of injured *Escherichia coli* O157:H7 in foods'. *International Journal of Food Microbiology* **55**(1–3), 285–90.

BLAKE, J. E. and METCALFE, M. A. (2001). 'A shared noncapsular antigen is responsible for false-positive reactions by *Staphylococcus epidermidis* in commercial agglutination tests for *Staphylococcus aureus*'. *Journal of Clinical Microbiology* **39**(2), 544–50.

BORCZYK, A. A., LIOR, H. and CIEBIN, B. (1987). 'False positive identifications of *Escherichia coli* O157 in foods'. *International Journal of Food Microbiology* **4**(4), 347–9.

CHAPMAN, P. A., ELLIN, M. and ASHTON, R. (2001). 'A comparison of immunomagnetic separation and culture, Reveal™ and VIP™ for the detection of *E. coli* O157 in enrichment cultures of naturally-contaminated raw beef, lamb and mixed meat products'. *Letters in Applied Microbiology* **32**(3), 171–5.

CHART, H., WILLSHAW, G. A. and CHEASTY, T. (2001). 'Evaluation of a reversed passive latex agglutination test for the detection of Verocytotoxin (VT) expressed by strains of VT-producing *Escherichia coli*'. *Letters in Applied Microbiology* **32**(6), 370–4.

CHEESBROUGH, S. and DONNELLY, C. (1996). 'The use of a rapid Salmonella latex serogrouping test (Spectate) to assist in the confirmation of ELISA-based rapid Salmonella screening tests'. *Letters in Applied Microbiology* **22**(5), 378–80.

COWELL, D. C., HART, J. P., LUXTON, R., NELSON, S. and REYNOLDS, D. (2002). *Rapid Detection and Quantification of Microorganisms using Lateral Flow and Biosensor Technologies*. World Congress on Biosensors, Kyoto, Japan.

CURIALE, M. S., GANGAR, V. and GRAVENS, C. (1997). 'VIDAS enzyme-linked fluorescent immunoassay for detection of *Salmonella* in foods: collaborative study'. *Journal of AOAC International* **80**(3), 491–504.

DE MEDICI, D., PEZZOTTI, G., MARFOGLIA, C., CACIOLO, D., FOSCHI, G. and OREFICE, L. (1998). 'Comparison between ICS-Vidas, MSRV and standard cultural method for *Salmonella* recovery in poultry meat'. *International Journal of Food Microbiology* **45**(3), 205–10.

FELDSINE, P. T., FALBO-NELSON, M. T., BRUNELLE, S. L. and FORGEY, R. L. (1997a). 'Visual immunoprecipitate assay (VIP) for detection of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 in selected foods: collaborative study'. *Journal of AOAC International* **80**(3), 517–29.

FELDSINE, P. T., FORGEY, R. L., FALBO-NELSON, M. T. and BRUNELLE, S. L. (1997b). '*Escherichia coli* O157:H7 visual immunoprecipitate assay: a comparative validation study'. *Journal of AOAC International* **80**(1), 43–8.

FELDSINE, P. T., LIENAU, A. H., FORGEY, R. L. and CALHOON, R. D. (1997c). 'Visual immunoprecipitate assay (VIP) for *Listeria monocytogenes* and related *Listeria* species detection in selected foods: collaborative study'. *Journal of AOAC International* **80**(4), 791–805.

FELDSINE, P. T., LIENAU, A. H., LEUNG, S. C. and MUI, L. A. (2002). 'Method extension study to validate applicability of AOAC Official Method 997.03 visual immunoprecipitate assay (VIP) for *Listeria monocytogenes* and related *Listeria* spp. from environmental surfaces: collaborative study'. *Journal of AOAC International* **85**(2), 470–8.

FELDSINE, P. T., MUI, L. A., FORGEY, R. L. and KERR, D. E. (2000). 'Equivalence of Visual Immunoprecipitate Assay (VIP) for *Salmonella* for the detection of motile and nonmotile *Salmonella* in all foods to AOAC culture method: collaborative study'. *Journal of AOAC International* **83**(4), 888–902.

FLETCHER, P. and LOGAN, N. A. (1999). 'Improved cytotoxicity assay for *Bacillus cereus* diarrhoeal enterotoxin'. *Letters in Applied Microbiology* **28**(5), 394–400.

GANGAR, V., CURIALE, M. S., D'ONORIO, A., SCHULTZ, A., JOHNSON, R. L. and ATRACHE, V. (2000). 'VIDAS enzyme-linked immunofluorescent assay for detection of *Listeria* in foods: collaborative study'. *Journal of AOAC International* **83**(4), 903–18.

GRIF, K., DIERICH, M. P. and ALLERBERGER, F. (1998). 'Dynabeads plus 3M Petrifilm HEC versus Vitek Immunodiagnostic Assay System for detection of *E. coli* O157 in minced meat'. *Letters in Applied Microbiology* **26**(3), 199–204.

HSUEH, P. R., TENG, L. J., YANG, P. C., PAN, H. J., CHEN, Y. C., WANG, L. H., HO, S. W. and LUH, K. T. (1999). 'Dissemination of two methicillin-resistant *Staphylococcus aureus* clones exhibiting negative staphylase reactions in intensive care units'. *Journal of Clinical Microbiology* **37**(3), 504–9.

JAY, J. M. (2000). *Modern Food Microbiology*. Gaithersburg, Aspen Publishers, Inc.

JONES, K. D. (1999a). 'Troubleshooting protein binding in nitrocellulose membranes. Part 1: Principles'. *IVD Technology* **5**(2), 32–41.

JONES, K. D. (1999b). 'Troubleshooting protein binding in nitrocellulose membranes. Part 2: Common problems'. *IVD Technology* **5**(3), 26–35.

JONES, K. L., MACPHEE, S., TURNER, A. J. and BETTS, R. P. (1995). An evaluation of the Oxoid Listeria rapid test (incorporating Clearview) for the detection of *Listeria* from foods, Campden & Chorleywood Food Research Association, R&D Report 19.

KERDAHI, K. F. and ISTAFANOS, P. F. (2000). 'Rapid determination of *Listeria monocytogenes* by automated enzyme-linked immunoassay and nonradioactive DNA probe'. *Journal of AOAC International* **83**(1), 86–8.

KOZWICH, D., JOHANSEN, K. A., LANDAU, K., ROEHL, C. A., WORONOFF, S. and ROEHL, P. A. (2000). 'Development of a novel, rapid integrated *Cryptosporidium parvum* detection assay'. *Applied and Environmental Microbiology* **66**(7), 2711–17.

LABORDE, R. T. and O'FARRELL, B. (2002). 'Paramagnetic-particle detection in lateral flow assays'. *IVD Technology* **8**(3), 36–41.

LUIJENDIJK, A., VAN BELKUM, A., VERBRUGH, H. and KLUYTMANS, J. (1996). 'Comparison of five tests for identification of *Staphylococcus aureus* from clinical samples'. *Journal of Clinical Microbiology* **34**(9), 2267–9.

MEATHREL, W. G., HAND SNR, H. M. and SU, L. H. (2001). 'The effects of hydrophilic adhesives on sample flow'. *IVD Technology* **7**(6), 56–66.

PERRY, M. B. and BUNDLE, D. R. (1990). 'Antigenic relationships of the lipopolysaccharides of *Escherichia hermannii* strains with those of *Escherichia coli* O157:H7, *Brucella melitensis*, and *Brucella abortus*'. *Infection and Immunity* **58**(5), 1391–5.

PERSONNE, P., BES, M., LINA, G., VANDENESCH, F., BRUN, Y. and ETIENNE, J. (1997). 'Comparative performances of six agglutination kits assessed by using typical and atypical strains of *Staphylococcus aureus*'. *Journal of Clinical Microbiology* **35**(5), 1138–40.

POWER, C. A., JOHNSON, R. P., MCEWEN, S. A., MCNAB, W. B., GRIFFITHS, M. W., USBORNE, W. R. and DE GRANDIS, S. A. (2000). 'Evaluation of the Reveal and SafePath rapid *Escherichia coli* O157 detection tests for use on bovine feces and carcasses'. *Journal of Food Protection* **63**(7), 860–6.

ROBERTS, P. (1994). 'An improved cultural/immunoassay for the detection of *Listeria*

species in foods and environmental samples'. *Microbiology Europe* **2**(5), 18–21.

SCOTTER, S., ALDRIDGE, M. and CAPPS, K. (2000). 'Validation of a method for the detection of *E. coli* O157:H7 in foods'. *Food Control* **11**, 85–95.

SMOLE, S. C., ARONSON, E., DURBIN, A., BRECHER, S. M. and ARBEIT, R. D. (1998). 'Sensitivity and specificity of an improved rapid latex agglutination test for identification of methicillin-sensitive and -resistant *Staphylococcus aureus* isolates'. *Journal of Clinical Microbiology* **36**(4), 1109–12.

SOWERS, E. G., WELLS, J. G. and STROCKBINE, N. A. (1996). 'Evaluation of commercial latex reagents for identification of O157 and H7 antigens of *Escherichia coli*'. *Journal of Clinical Microbiology* **34**(5), 1286–9.

STEPHENS, P. J., JOYNSON, J. A., DAVIES, K. W. and HOLBROOK, R. (1997). 'The use of an automated growth analyser to measure recovery times of single heat-injured *Salmonella* cells'. *Journal of Applied Microbiology* **83**, 446–55.

THORNS, C. J., MCLAREN, I. M. and SOJKA, M. G. (1994). 'The use of latex particle agglutination to specifically detect *Salmonella enteritidis*'. *International Journal of Food Microbiology* **21**(1–2), 47–53.

THRELFALL, J., WARD, L. and OLD, D. (1999). 'Changing the nomenclature of salmonella'. *Communicable Disease and Public Health* **2**(3), 156–7.

VAN GRIETHUYSEN, A., BES, M., ETIENNE, J., ZBINDEN, R. and KLUYTMANS, J. (2001). 'International multicenter evaluation of latex agglutination tests for identification of *Staphylococcus aureus*'. *Journal of Clinical Microbiology* **39**(1), 86–9.

VAZ-VELHO, M., DUARTE, G. and GIBBS, P. (2000). 'Evaluation of mini-VIDAS rapid test for detection of *Listeria monocytogenes* from production lines of fresh to cold-smoked fish'. *Journal of Microbiological Methods* **40**(2), 147–51.

VERNOZY-ROZAND, C., MAZUY, C., RAY-GUENIOT, S., BOUTRAND-LOEI, S., MEYRAND, A. and RICHARD, Y. (1997). 'Detection of *Escherichia coli* O157 in French food samples using an immunomagnetic separation method and the VIDAS E. coli O157'. *Letters in Applied Microbiol*ogy **25**(6), 442–6.

VERNOZY-ROZAND, C., MAZUY, C., RAY-GUENIOT, S., BOUTRAND-LOEI, S., MEYRAND, A. and RICHARD, Y. (1998). 'Evaluation of the VIDAS methodology for detection of *Escherichia coli* O157 in food samples'. *Journal of Food Protection* **61**(7), 917–20.

VERNOZY-ROZAND, C., RAY-GUENIOT, S., RAGOT, C., BAVAI, C., MAZUY, C., MONTET, M. P., BOUVET, J. and RICHARD, Y. (2002). 'Prevalence of *Escherichia coli* O157:H7 in industrial minced beef'. *Letters in Applied Microbiology* **35**(1), 7–11.

ZBINDEN, R., MÜLLER, F., BRUN, F. and VON GRAEVENITZ, A. (1997). 'Detection of clumping factor-positive *Staphylococcus lugdunensis* by staphaurex plus'. *Journal of Microbiological Methods* **31**(1–2), 95–8.