11

Immunological techniques: ELISA

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11.1 Introduction

Conventional cultural methods used for detecting microorganisms in foods are well established, simple, inexpensive and can be used in both quantitative or qualitative testing. However, there are some disadvantages to using conventional methods, particularly when applied to pathogen detection. These methods rely on the growth of the target microorganisms in one or more nutrient media, detection of growth by visual assessment and confirmation of the presence of a pathogen, usually involving a combination of biochemical and serological tests. The various stages can be extremely labour intensive, interpretation of results subjective and for some pathogens, the total test time can be many days. A typical protocol for the detection of *Salmonella* in foods involves incubation of the food homogenate in a non-selective pre-enrichment broth (20 h), transfer and incubation in two separate selective broths (24 h), isolation on selective agars (24 or 48 h), followed by visual assessment and purification of presumptive positive colonies on non-selective agars (24 h). Shorter test times and reduced labour requirements can be achieved by adopting alternative or rapid methods. In recent years, there have been remarkable advances in rapid methodology, and improvements in the performance, quality and commercial availability of these methods has led to them being widely adopted and accepted. Presently, rapid methods cannot completely replace conventional methods and in pathogen detection they are usually adopted to replace one stage of the total isolation, detection or identification procedure, resulting in conventional and rapid methods being used in combination.

The term 'rapid methods' encompasses numerous technologies including those based on microscopy, measurement of adenosine triphosphate (ATP),

monitoring of metabolic activity by electrical measurement, nucleic acids and immunology. Also referred to as immunoassays or antibody-based, immunological methods form the basis of a range of tests that can be applied to the detection of foodborne pathogens, determination of the species of origin of meat and identifying chemical contaminants and toxins.

The enzyme-linked immunosorbent assay (ELISA) is a widely used immunological-based assay for the detection of foodborne pathogens. The technique is designed to replace the detection or isolation stage on agar; it is relatively easy to perform, can be applied to a range of pathogens, can be semi-automated and gives a rapid result. The scope of an ELISA can be designed to detect a genus, species or serotype. However, a positive result obtained by an ELISA is presumptive and must be confirmed using conventional tests. The greatest advantage of this technique therefore is in negative screening and including an ELISA test in a microbiological detection procedure has potential to greatly increase the number of samples per day which can be screened for the presence of a particular pathogen, assuming there are acceptable levels of false negative results. In this chapter, an overview of the principles of the technique, examples of different types of ELISA, examples of commercially-available kits and some advantages and disadvantages of using the technique are discussed.

11.2 The basic principles of an ELISA

11.2.1 Antibodies and antigens

All immunological techniques utilise the highly specific binding reaction between antibodies and antigens, and this interaction is also the key to an ELISA. Antibodies are proteins produced by the white blood cells of animals that have been invaded by a foreign molecule or microorganism. Antibodies attach to areas on the invading foreign body referred to as antigens. This attachment is highly specific and immunological tests exploit this phenomenon to detect specific microorganisms, proteins or toxins. Two types of antibody can be used in an ELISA; monoclonal and polyclonal. If the invading foreign body is a large molecule, such as a protein or microorganism, there can be many different antigenic sites and polyclonal antibodies are produced during the immune response of the host. Monoclonal antibodies are produced using tissue culture techniques, procured from a single antigenic site, using a single white blood cell.

11.2.2 Labels

In order to determine if any binding between antibody and antigen has taken place, a system for visualising or measuring the interaction is required. To achieve this, a ‘label’ is attached to the antibody. Labels can be of various types including fluorescent agents, luminescent chemicals, radioisotopes or enzymes. In an ELISA, the term ‘enzyme-linked’ indicates the labelling system is enzyme

mediated. In most systems, the enzyme catalyses the conversion of a colourless substrate, resulting in a coloured product. The endpoint of the test can then easily be visualised by eye or by a spectrophotometer, depending on the type of test. Typical enzyme-substrate complexes used in ELISAs include alkaline phosphatase (enzyme) with para-nitrophenyl phosphate (substrate) and horseradish peroxidase (enzyme) with tetramethylbenzidine (substrate); both complexes produce a yellow coloured product.

11.2.3 Solid supports

The reactions that take place during an ELISA require some form of physical, solid support. In the acronym ELISA, the term 'immunosorbent', implies that antibodies are absorbed onto a surface. Microtitre plates are most commonly used primarily because they are convenient, cheap to produce and allow for high numbers of tests per assay, having 96 wells per plate. Other solid supports used include paper membranes and polystyrene dipsticks.

11.3 ELISA formats

The first stage of an ELISA involves coating a solid support with specific antibodies or antigens. If the assay uses a static support, e.g. microtitre plate, the test sample and various reagents are added to the well in a series of multiple stages throughout the procedure. With dipstick formats, the principle of coating a solid phase with antibodies to the target antigen is again used, however, with these tests, the solid phase is designed to be transferable. The format is often a paddle or elongated stick; hence the term 'dipstick'. With these devices, it is possible to transfer any captured antigens into different media to enhance growth thereby increasing target cell numbers. When transferred to a solution of substrate, the enzyme label, attached to the dipstick, causes it to change colour.

11.3.1 Sandwich ELISAs

The sandwich ELISA has the simplest format and is most commonly used in commercially-available kits. The word 'sandwich' indicates that the assay uses two antibodies which trap or sandwich the target antigen. During the procedure, the antigen is first captured then detected. The capture antibody, specific to the target antigen, is attached to the surface of the solid support, e.g. microtitre well. An enriched food sample is added to the well and if the target antigen is present, it will bind to the antibodies. After a washing procedure, to remove food debris and unbound material, a second 'detection antibody' is added to the well. This antibody has an enzyme label attached. Again, the antibody will bind to the target antigen creating the 'antibody sandwich'. More washing procedures are carried out to remove any unbound antibodies, followed by the addition of a colourless substrate which the enzyme converts to a coloured product. Finally, a

stop solution is added to prevent any further enzyme activity and any change in colour is measured. The total test time for a sandwich ELISA is typically between two and three hours. It is possible to use this format in quantitative tests by calibrating the concentration of antigen against colour intensity. In the detection of foodborne pathogens, however, the sandwich ELISA is usually used for qualitative purposes, indicating the presence or absence of a pathogen. All sandwich ELISAs require an enriched food sample as a starting point. The enrichment procedure depends on the food type and pathogen being detected. The main stages involved in a sandwich ELISA are shown in Fig. 11.1.

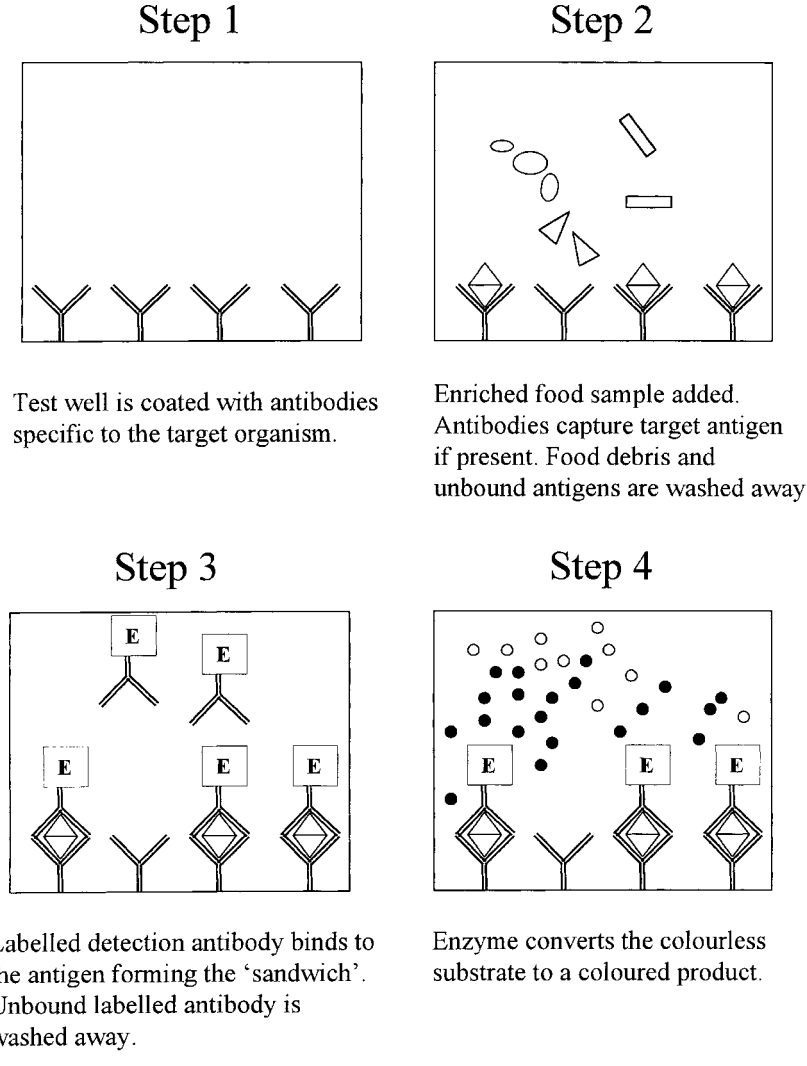

Fig. 11.1 The main steps of a sandwich ELISA.

11.3.2 Indirect sandwich ELISA

In the indirect sandwich ELISA, the detection antibody does not carry the enzyme label. These antibodies carry a marker molecule which will specifically bind to another molecule, e.g. a protein. Biotin is often used as a marker with avidin as the protein binding site. The result is a biotin-avidin, enzyme labelled antibody. This enzyme will again catalyse the conversion of a colourless substrate to a coloured product.

11.3.3 Competitive ELISA

In this format, the test well is coated with antigen, rather than antibody. The sample and labelled antibodies are then added to the test well simultaneously. If target antigens are not present in the sample, the labelled antibodies will bind to the antigen coated on the wells. When the substrate is added, the enzyme will catalyse a colour change as in the sandwich ELISA. However, if target antigens are present in the sample, the antibodies will bind with them, in addition to those attached to the well. During the washing stage, any antibody-antigen complexes in solution are removed. Therefore, any change in colour at the end of the test is due solely to the antibody-antigen complex on the surface of the well. The intensity of the endpoint colour will therefore be low. If the level of antigen in the sample is high, more antibodies will bind in solution, resulting in fewer antibodies available to bind to the surface of the well. In these circumstances, the endpoint will be an extremely weak colour or colourless.

The competitive ELISA gives a reverse result to the sandwich, in that a coloured product indicates a negative result and a colourless (or weak coloured) product indicates a positive result. Competitive ELISAs can be direct or indirect, as in the sandwich format and can also be used in quantitative tests by calibration of antigen concentration against colour intensity. This type of ELISA is often used to detect small molecules that may not easily be detected using the sandwich format, which requires two binding sites. The main stages involved in the competitive ELISA are given in Fig. 11.2.

11.4 Commercially-available ELISAs

Numerous ELISA kits for the detection of a wide range of foodborne pathogens, including *Salmonella* spp., *Listeria* spp., *Escherichia coli* O157:H7, *Campylobacter* spp., staphylococcal enterotoxins, *Bacillus* diarrhoeal enterotoxin, verocytotoxins and *Clostridium botulinum* neurotoxins are now commercially available. The format of the kits varies but most are based on the sandwich ELISA using microtitre plates as the solid support. The design of the plates usually includes removable wells, allowing the user the flexibility to test for a desired number of samples without the need to use a complete 96 well plate each assay. Kits are typically provided with the solid support pre-coated with capture antibodies against a specific pathogen, wash concentrates, freeze-dried

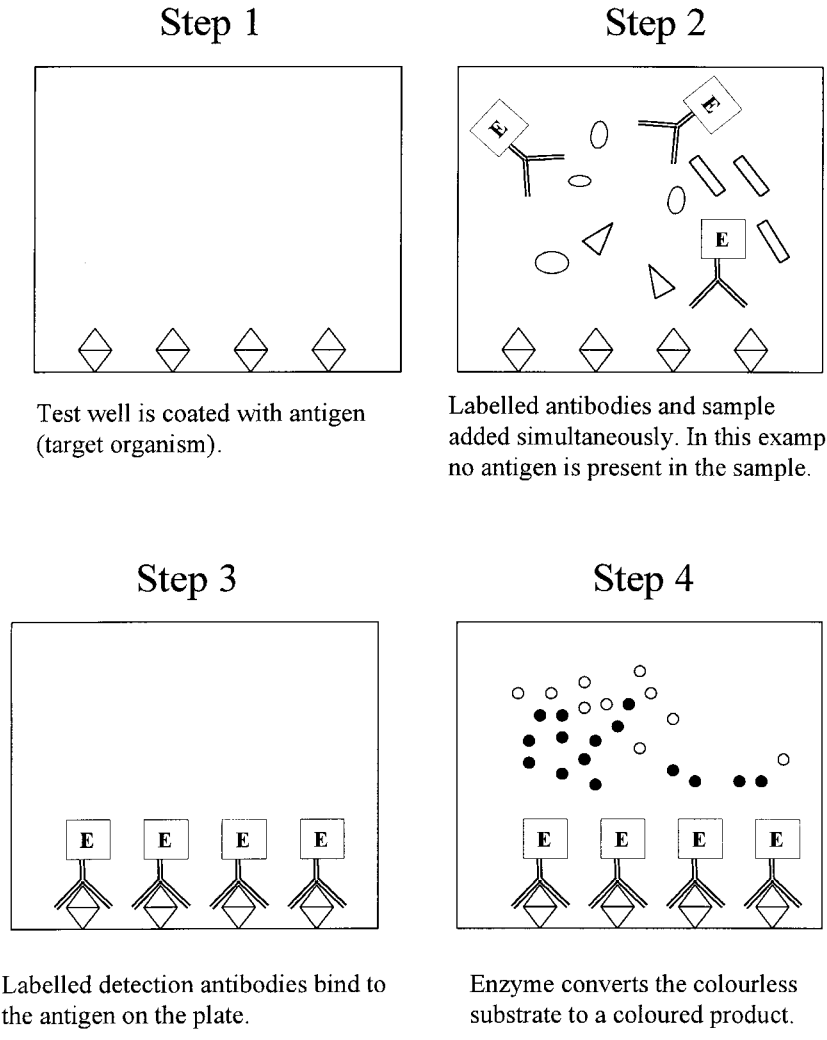

Fig. 11.2 The main steps of a competitive ELISA.

labelled detection antibodies (also referred to as conjugate), freeze-dried substrate, various diluents together with positive and negative controls. The endpoint of some systems can be measured by eye, assessing the degree of colour change against a colour chart, while others require the use of a spectrophotometer or microtitre plate reader. The washing steps are crucial in an ELISA and some manufacturers recommend the use of an automated microtitre plate washer. Few kits are suitable for manual washing (e.g. using a wash bottle) and in those that are, this stage needs to be carried out with great care.

The automation of ELISAs has developed rapidly in recent years. Some systems are simply automated versions of the standard microtitre plate format, incorporating robotics for dispensing reagents and automated washing and reading

procedures. These types of systems require minimal manual input and are most suitable for high throughput screening. Other manufacturers have developed novel formats, and some have incorporated an additional immunological-based stage to increase the specificity of the test and reduce incubation time during the enrichment procedure. This additional stage is known as immunoconcentration or immunocapture and is a means of separating or capturing bacterial cells in culture. The principle is simple and, as in all immunological-based methods, involves antibodies binding to target antigens. A solid support is required and one frequently used is magnetic beads. The beads are coated with capture antibodies, mixed and incubated with the test culture, then collected by application of a magnetic field. Any target antigen binds onto the antibodies on the beads, resulting in them being concentrated and separated from the food matrix and competitive flora. Antigen in this concentrated format is then used as the starting point for an ELISA. Immunoconcentration is also incorporated into certain dipstick assays, with the solid support being the antibody coated dipstick itself. This also acts as the solid support during the ELISA.

From the numerous commercial kits available, information on three is provided in this section. These are manufactured by TECRA, bioMérieux and Foss Electric. Each manufacturer produces kits for detection of more than one pathogen. Therefore, for the purposes of this chapter, the procedure for the detection of *Salmonella* is described for each. It should be noted that these methods were chosen purely as examples of typical kits with quite different formats and protocols, and not as any form of endorsement of one kit over another. A list of other commercially-available pathogen detection ELISA kits is provided in Table 11.1. Each kit listed offers certain advantages and disadvantages, depending on the individual user requirements.

11.4.1 TECRA UNIQUE™

The TECRA UNIQUE™, is an example of a manual-based, dipstick format sandwich ELISA, that incorporates an immunocapture stage. The UNIQUE™ kits are presently available for detection of *Salmonella* spp. and *Listeria* spp. Other kits, including those with a microtitre plate format, are available from this company and full details can be obtained from the manufacturer (Table 11.1). The main stages of the UNIQUE™ *Salmonella* are outlined below:

- A food sample is incubated in a pre-enrichment broth, for 16 h at 37ºC.
- An aliquot of pre-enriched sample is then transferred to the first of six 'test tubes', housed in a self-contained module. Simultaneously, an antibody-coated, 'paddle-shaped' dipstick, is added to the first test tube. During a short incubation period (20 min), any salmonellae present in the sample are captured onto the surface of the dipstick. The dipstick is then transferred into tube two, which contains a wash solution.
- The dipstick is washed by inverting the module several times. It is then transferred to tube three, which contains a nutrient medium.

Table 11.1 Partial list of commercially-available ELISA for the detection of foodborne pathogens

Organism or toxin	Trade name	Manufacturer
Bacillus cereus diarrhoeal enterotoxin	*Bacillus* diarrhoeal enterotoxin	TECRA
Campylobacter	EiaFoss	Foss Electric
	Camplylobacter VIA	TECRA
Clostridium botulinum neurotoxin	BoNT	Rhône-diagnostics
Escherichia coli O157	EHEC-TeK	bioMérieux
	Assurance	BioControl
	E. coli O157 VIA	TECRA
	Premier O157	Meridian
	E. coli O157:H7	Binax
	E. coli Rapitest	Microgen
	Transia card	Transia
Shiga toxin	Premier EHEC	Meridian
	Ridascreen Verotoxin	R-Biopharm
	E. coli ST	Oxoid
Listeria	*Listeria*-TeK	bioMérieux
	Listeria VIA	TECRA
	UNIQUE *Listeria*	TECRA
	Assurance	BioControl
	Transia Listeria	Transia
	Pathalert	Merck
	EiaFoss	Foss Electric
Salmonella	*Salmonella*-TeK	bioMérieux
	Salmonella VIA	TECRA
	UNIQUE *Salmonella*	TECRA
	EQUATE	Binax
	BacTrace	KPL
	LOCATE	Rhône-diagnostics
	Assurance	BioControl
	Salmonella	GEM Biomedical
	Transia	Transia
	Bioline	Bioline
Staphylococcus aureus staphylococcal enterotoxin	*S. aur*eus VIA	TECRA
	SET-EIA	Toxin Technology
	Staphylococcal Enterotoxin VIA	TECRA
	Transia SE	Transia
	RIDASCREEN	R-Biopharm

Adapted from Bacteriological Analytical Manual, Appendix 1 (2001)

- During a four-hour incubation in tube three, any captured salmonellae multiply. After incubation, the dipstick is transferred to tube four.
- In tube four, the dipstick is incubated for 30 min in a solution of labelled detection antibodies. This is followed by transfer to a further wash solution in tube five.

- The dipstick is again washed by inverting the module several times then transferred to a solution of substrate in tube six.
- In tube six, after a 10 min incubation period, a presumptive positive result is expressed as a purple colour on the lower half of the dipstick. Negative and positive controls are built-in on the upper and lower ends of the dipstick.

The total test time for this method is 22 hours. Any presumptive positive results must be confirmed as *Salmonella* using conventional methodology. All stages of the test are contained in the module, are extremely easy to perform and require minimal manual manipulations. However, this format is designed for low sample numbers and would not be suitable for high sample throughput. The UNIQUE™ *Salmonella* test has been evaluated extensively against a variety of food types and overall results indicate at least equivalence to standard conventional methods (Hughes *et al*., 2001; de Paula *et al*., 2002).

11.4.2 Salmonella-Tek

The Salmonella-Tek is manufactured by bioMérieux and uses a sandwich ELISA with microtitre plate format. This system can be used with or without an immunocapture stage, using magnetic beads (Dynabead®). Two different enrichment protocols are recommended for use with the Salmonella-Tek; one for samples expected to have low levels of competitive flora (e.g. processed foods) and one for samples with high levels of competitors (e.g. raw foods). Both procedures are shown in Fig. 11.3. The endpoint of both enrichment protocols is heat treated M broths (with novobiocin) and this is regarded as the starting point for the ELISA. The main stages of the Salmonella-Tek ELISA are outlined below.

- An aliquot (100 μl) of an enriched food sample (see Fig. 11.3) is added to a test well on the microtitre plate. This is then incubated for 30 min at 37ºC to allow any antigen to bind to the antibodies coated onto the surface of the well.
- The wells are then washed six times in a wash solution followed by addition of 100 μl of labelled detection antibodies.
- The plate is then incubated for a further 30 min at 37ºC to allow these antibodies to also bind with the antigen.
- After incubation, a further six washes are carried out.
- After the second washing procedure, 100 μl of substrate is added and the plate is incubated at 20–25ºC for 30 min.
- After incubation, 100 μl of a stop solution is added to each well and any colour change is measured using a microtitre plate reader.

Negative and positive controls are provided with the kit and three wells must be allocated for their use, each time an ELISA is carried out.

The test time for the ELISA is approximately two hours but the total test time, including the enrichment procedure, is approximately 46 and 52 hours for

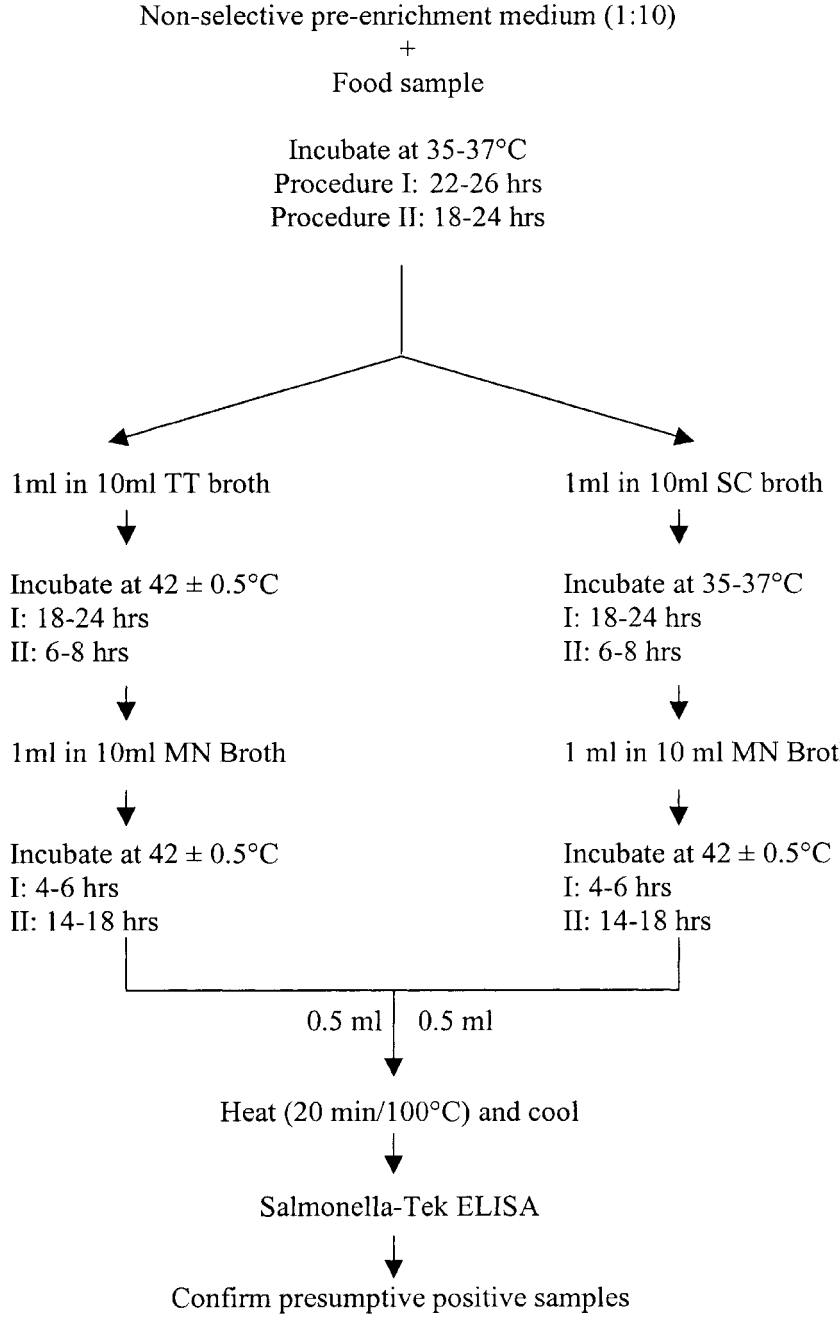

Fig. 11.3 Procedures recommended for use with the Salmonella-Tek ELISA.

processed and raw foods, respectively. Any positive Salmonella-Tek ELISA results are presumptive and must be confirmed using standard methods.

The total test time for the Salmonella-Tek can be reduced by including an immunocapture stage using Dynabeads®. The trade name of the combined immunocapture and ELISA system is Salmonella Capture-Tek™. The starting point for this is a pre-enriched food sample which has been incubated in a suitable pre-enrichment broth, for a minimum of 16 h at 37ºC. An aliquot (1 ml) of this is mixed in a capture tube with a solution of Dynabeads® for 10 min. During this period, any salmonellae present are captured by the antibodies coated onto the surface of the beads. The capture tube is placed into a magnetic particle concentrator allowing the beads to be immobilised and washed. The beads are then incubated in M-broth for five hours before being heat treated. The heated M-broth (containing the beads) is then used as the starting point for the Salmonella-Tek ELISA. By including this immunocapture stage, the total test time is reduced to approximately 24 hours for both processed and raw foods. Again, any positive results are presumptive and require confirmation. bioMérieux also manufacture kits for the detection of *Listeria* spp. (Listeria-Tek) and *E. coli* O157 (EHEC-Tek).

The Salmonella-Tek and Salmonella Capture-Tek have both shown good correlation with conventional methods having been evaluated against a range of food types by different workers (Chapman and Siddons, 1996; Eckner *et al*., 1994; Tveld and Notermans, 1992). These ELISAs are comparatively labour intensive, having numerous pipetting stages and washing steps. However, the Salmonella-Tek is suitable for high sample throughput and can be semi-automated.

11.4.3 EiaFoss

The EiaFoss, manufactured by Foss Electric, is a fully automated ELISA system that incorporates an immunocapture stage using magnetic beads. Kits are available for the detection of *Salmonella* spp., *Listeria* spp., *E. coli* O157 and *Campylobacter* spp. The EiaFoss is novel in that the magnetic beads used for the immunocapture of the target antigen, also act as the solid support stage for the ELISA. All of the reactions take place inside an 'assay tube' and all transfers of reagents, washing steps, immobilisation of magnetic beads and reading of endpoints are carried out automatically by the EiaFoss analyser. The starting point of the EiaFoss Salmonella is an enriched food sample. Two EiaFoss Salmonella enrichment broths are available (SEB I and SEB II) the use of which depends on the food type. The incubation period is also dependent on the type of food, most foods requiring 24 h (at 37ºC), but for those which could contain injured cells or inhibitory substances, e.g. spices, 48 hours would be required. The test time of the automated ELISA is two hours resulting in a total test time of 24 hours for most foods. As with other ELISA kits, any positive results require confirmation using conventional tests.

This system has been thoroughly evaluated against a variety of foods and results have been shown to correlate well with conventional methods (Kleiner

and Weisswange, 2000; Masso and Oliva, 1997; Krusell and Skovgaard, 1993). Manual manipulation is minimal with the EiaFoss and as the system can test between one and 27 samples in each assay (or 108 per working day), it would be suitable for low and high sample numbers. The EiaFoss analyser can also be used with the *Listeria*, *E. coli* O157 and *Campylobacter* kits.

11.5 Advantages and disadvantages in using ELISAs

11.5.1 Sensitivity

The sensitivity of most ELISAs is approximately 10^5 cells/ml. This relatively low sensitivity level is one of the main disadvantages to using these types of assays. A requirement for the food microbiologist is often to detect a single pathogen in a 25 g sample of food. It is therefore necessary to enrich the food sample prior to a detection method such as an ELISA. This enrichment stage is crucial and the performance of the ELISA is dependent on it. Not only is it necessary for the increase in target cell numbers, but also to reduce unwanted competitive flora, dilute any inhibitory substances that may be present in the food matrix and allow for the resuscitation of any injured cells. Cell injury can occur as a result of processes such as heating, freezing, low pH and chemical preservation. The ability of methods to detect sublethally injured cells is an important consideration in all pathogen methodology, but particularly in *E. coli* O157 and *Campylobacter* detection methods due to the potential low infectious dose of these organisms, thought to be as low as <10 organisms for *E. coli* O157 (Willshaw *et al*., 1994) and 500 for *C. jejuni* (Kothary and Babu, 2001).

Some ELISA protocols recommend the use of direct selective enrichment. Inoculating injured cells directly into these conditions has frequently been shown to prevent or inhibit the recovery of low levels of injured cells and result in poor performance of an ELISA, i.e. produce false negative results (Huang *et al*., 1999; Blackburn and McCarthy, 2000). The performance can be improved by including a pre-enrichment stage to provide optimal conditions for the recovery of injured cells. An investigation (McCarthy *et al*., 1999) into the detection of *E. coli* O157 from processed foods using the EHEC-Tek found a ten-fold increase in positive ELISA results when a pre-enrichment stage was included in the protocol. These presumptive positives were later confirmed as *E. coli* O157.

A disadvantage of including a pre-enrichment stage is the increase in total test time, but it is vitally important that appropriate culture procedures are adopted for specific food types and ELISAs when testing for pathogens. This may require modifications to the protocol suggested by the kit manufacturer.

11.5.2 Specificity

An ELISA should have a requisite specificity of a low false negative rate and an acceptable false positive rate. The antibodies used should have high affinity for a

specific antigen and have low cross-reactivity to other microorganisms or proteins. Non-specific reactions can be caused by competing flora, especially those antigenically closely-related to the target organism. As mentioned earlier, two types of antibody can be used in ELISAs: polyclonal and monoclonal. One disadvantage to using polyclonal antibodies is that they are derived from either rabbit or goat serum and there can be variability in the immune response of different animals. In some cases, a range of antibodies can be produced with different specificities. Monoclonal antibodies are more specific and target a single antigenic site. It is not always evident what type of antibody an ELISA kit uses and in some cases, it is necessary to obtain this information from the manufacturer.

Other non-specific reactions can be caused by components of the food sample itself. For example, foods with endogenous peroxidases have been reported to cause false positive reactions when tested for staphylococcal enterotoxins. The endogenous peroxidase imitates the action of the peroxidase (enzyme) label attached to the detection antibodies. Various types of seafood have also been reported to cause false positive reactions in some staphylococcal enterotoxin ELISAs (Park *et al*., 1993). Some false positive reactions can be rectified by treating the food before an ELISA is carried out, e.g. by exposure to chemicals or heat. Sodium azide has been reported to inactivate endogenous peroxidase and heating at 70C has been shown to reduce the number of false positives associated with seafood (Park *et al*., 1994). However, certain pre-treatments can also affect the performance of the ELISA and the possibility of this happening must be considered before a pre-treatment stage is used. Sodium azide has also been reported to reduce the amount of staphylococcal enterotoxin detected by some ELISA kits by as much as 20–30 per cent (Park *et al*., 1994).

11.5.3 Test time and costs

Incorporating an ELISA in pathogen detection methodology can reduce the time required to reach a negative result from 4–5 days (using conventional methodology) to 24–30 hours. Most ELISAs themselves are rapid, taking typically two to three hours to complete, but the requirement for enrichment adds 24–48 hours to the total test time, depending on the food type and pathogen being detected. Despite this, the reduction in analysis time achieved by using an ELISA is still significant.

The cost of an ELISA will undoubtedly be higher than an equivalent conventional test. The cost of equipment maintenance, service contracts, technical support and the cost in the event of equipment failure should also be taken into account. However, all benefits gained need to be included in any cost comparison exercise. These can include reduced labour requirements, greater sample throughput, faster product release and reduced requirements for storage of ingredients or product.

11.6 Future trends

An ideal pathogen detection method would have high sensitivity, high specificity and give accurate and rapid results, preferably within a working day. Presently, no single rapid method is capable of achieving this and it may only be possible by a combination of the various technologies used in rapid methods. Numerous stages are involved in isolating and confirming the presence of a pathogen in a food sample. These include sampling procedures, resuscitation of injured cells, pre-enrichment, selective enrichment, detection and identification. The ELISA is designed to be used at the detection stage and, compared to conventional methods, it is faster and less subjective. The ELISA has been extensively researched, developed and improved and there is now a wide range of systems in use for detection of many foodborne pathogens. Commercial availability, automation, streamlining and simplifying protocols have all contributed to this type of assay being widely accepted.

One of the main disadvantages of an ELISA is low sensitivity levels and the challenges for the future lie in this area. There is a need to obtain target pathogens, in a physiologically uninjured state and at or above detection levels of the ELISA as rapidly as possible. In addition, competitive flora need to be eliminated or be as low as possible to prevent non-specific reactions and any inhibitory substances removed. The most commonly applied method for achieving these requirements is conventional, selective enrichment. If a pre-enrichment stage is included to allow for resuscitation of injured cells, this can take up to 48 hours. Alternative approaches to conventional enrichment include separation and concentration using immunocapture techniques, novel or shortened enrichment procedures or amplification of the target cells using DNA-based techniques.

Immunocapture techniques are being incorporated into certain ELISAs now but the point of application and automation of this stage could be further developed. Using immunocapture techniques, it is possible to have a protocol that has a pre-enrichment stage to allow the recovery of injured cells, concentrated target cells to inoculate into a selective enrichment broth, thus needing a shorter incubation time, with minimal impact on the total test time. This is basically the procedure in the TECRA UNIQUE^{TM} assays where application of immunocapture at the pre-enriched culture stage has proved successful in these systems (Hughes *et al*., 2001). Unfortunately, this system is only suitable for low sample numbers. Immunocapture using magnetic beads has also proved to be extremely successful and beads coated with various antibodies, including those specific for *Salmonella* spp., *Listeria* spp. and *Escherichia coli* O157, are available from a number of manufacturers, e.g. Dynal, Denka and LabM. Immunomagnetic beads have been used extensively in conventional *E. coli* O157 methodology and these have shown to increase sensitivity and reduce total test time (Bennett *et al*., 1996; Ogden *et al*., 2000). The use of immunomagnetic separation has now been incorporated in the UK Public Health Laboratory Service standard method for E. coli O157 isolation (Anon, 1998).

One drawback with immunomagnetic separation is when performed manually, it is very labour intensive requiring numerous pipetting and transfer stages.

Two examples of novel enrichment procedures that could be used with ELISA are the universal pre-enrichment broth (UP), developed by Bailey and Cox (1992) and the Oxoid Simple Pre-enrichment and Rapid Isolation New Technology (SPRINT), manufactured by Oxoid. The UP was originally developed for simultaneous detection of *Salmonella* spp. and *Listeria* spp. in foods. The medium is highly buffered, low in carbohydrates and allows for the resuscitation and multiplication of sublethally heat-injured cells. Investigations into the use of UP as a pre-enrichment broth for culturing heat-injured *E. coli* O157:H7, in addition to *Salmonella* spp. and *Listeria* spp., found the broth allowed growth of low levels ($\leq$ 125 cfu/sample) of each pathogen to at least 10^4 cfu/ml within 24 h incubation at 37ºC (Zhao and Doyle, 2001). This study also emphasised the importance of the incubation time. An incubation period of six hours was insufficient to produce cell populations at (or above) the detection levels of an immunoassay such as ELISA, although some manufacturers advocate this incubation time in their enrichment protocols.

The SPRINT is a more recent development in enhanced enrichment culture and is available for use with *Salmonella* spp. The principles of the system are based on traditional techniques, allowing a pre-enrichment in Buffered Peptone Water (BPW) followed by selective enrichment in Rappaport-Vassiliadis (RV), in one 24-hour step. The formulation of the BPW is designed to enhance recovery of injured cells before addition of the selective agent after six hours' incubation, by means of timed release capsules. The SPRINT has been shown to improve significantly the rate of detection of low numbers of injured salmonellae in ice cream and milk powder, after 24 hours' enrichment (Baylis *et al*., 2000).

Amplification of specific DNA sequences of the target pathogen is possible by use of the Polymerase Chain Reaction (PCR). This technique is described in full in Chapter 12, but in summary, a PCR cycle starts by denaturing the double-stranded DNA from the target cells, hybridisation of each strand with primer oligonucleotides and primer extension by the action of DNA polymerase. The product acts as the template for subsequent cycles, therefore, the number of DNA copies doubles after every cycle. Methods using a combination of PCR and ELISA are currently in use, where the ELISA detects the PCR product. This has proved to be a successful synergy and could be exploited further. The ELISA format is suitable for large-scale screening, automation and has potential to increase the sensitivity of the PCR assay (Ritzler and Altwegg, 1996; Knight *et al*., 1999). When used to detect PCR product from Shiga toxin-producing *E. coli*, the ELISA has shown to increase the sensitivity of the PCR test by up to 100-fold (Ge *et al*., 2002). However, one drawback to this approach is that as PCR targets the nucleic acid of an organism, there is no differentiation between viable and non-viable cells. Another disadvantage could be cost, as PCR techniques are costly in their own right.

The choice of ELISA depends on a number of factors including sample throughput, cost, technical skills required, levels of hands-on time needed and

level of automation available. Some ELISAs may not be suitable for use with some types of food and may not be capable of detecting specific pathogens. It is always advisable to gather as much validation data as possible regarding potentially suitable ELISAs and evaluate the system using in-house facilities and the food type(s) intended for use. Any alternative method, including ELISA, should demonstrate at least equal performance with an equivalent standard conventional method.

11.7 References and further reading

ANON (1998) Detection of *Escherichia coli* O157 by Immunomagnetic Bead Separation F17, Public Health Laboratory Service, Standard Methods for Food Products.

BAILEY J S and COX N A (1992) Universal pre-enrichment broth for the simultaneous detection of *Salmonella* and *Listeria* in foods, *Journal of Food Protection*, **55**(4) 256–9.

BAYLIS C L, MACPHEE S and BETTS R P (2000) Comparison of methods for the recovery and detection of low levels of *Salmonella* in ice cream and milk powder, *Letters in Applied Microbiology*, **30**(4) 320–4.

BENNETT A R, MACPHEE S and BETTS R P (1996) The isolation and detection of *Escherichia coli* O157 by use of immunomagnetic separation and immunoassay procedures, *Letters in Applied Microbiology*, **22**(3) 237–43.

BETTS R and BLACKBURN C (2002) Detecting pathogens in food, in Blackburn C D and McClure P J, *Foodborne Pathogens*, Woodhead Publishing Ltd., 13–52.

BLACKBURN C D 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.

CHAPMAN P A and SIDDONS C A (1996) Evaluation of a commercial enzyme immunoassay (EHEC-Tek) for detecting *Escherichia coli* O157 in beef and beef products, *Food Microbiology*, **13**(2) 175–82.

DE BOER E and BEUMER R B (1999) Methodology for detection and typing of foodborne microorganisms, *International Journal of Food Microbiology*, **50**(1-2) 119–30.

DE PAULA A M R, GELLI D S, LANDGRAF M, DESTRO M and FRANCO B (2002) Detection of *Salmonella* in food using Tecra Salmonella VIA and Tecra Salmonella UNIQUE rapid immunoassay and a cultural procedure, *Journal of Food Protection*, **65**(3) 552–5.

ECKNER K F, DUSTMAN W A, CURIALE M S, FLOWERS R S and ROBISON B J (1994) Elevated temperature, colorimetric, monoclonal, enzyme-linked-immunosorbant-assay for rapid screening of *Salmonella* in foods – collaborative study, *Journal of AOAC International*, **77**(2) 374–94.

GE B, ZHAO S, HALL R and MENG J (2002) A PCR-ELISA for detecting Shiga toxin-producing *Escherichia coli*, *Microbes and Infection*, **4**(3) 285–90.

HUANG H, GARCIA M M, BROOKS, B W, NIELSEN K and NG S (1999) Evaluation of culture enrichment procedures for use with *Salmonella* detection immunoassay, *International Journal of Food Microbiology*, **51**(2-3) 85–94.

HUGHES D, DAILIANIS A E, HILL L, MCINTYRE D A and ANDERSON A (2001) TECRA® Unique™ test for rapid detection of *Salmonella* in food: collaborative study, *Journal of AOAC International*, **84**(2) 416–29.

KLEINER U and WEISSWANGE H (2000) *Salmonella* from minced meat Rapid detection of using the EiaFoss system, *Fleischwirtschaft*, **80**(9) 140–1.

KNIGHT J C, MCGUIRE W, KORTOK M M and KWIATKOWSKI D (1999) Accuracy of genotyping of single-nucleotide polymorphisms by PCR-ELISA allele-specific oligonucleotide hybridisation typing and by amplification refractory mutation system, *Clinical Chemistry*, **45**(10) 1860–3.

KOTHARY M H and BABU U S (2001) Infective dose of foodborne pathogens in volunteers: A review, *Journal of Food Safety*, **21**(1) 49–73.

KRUSELL L and SKOVGAARD N (1993) Evaluation of a new semiautomated screening method for the detection of *Salmonella* in foods within 24 h, *International Journal of Food Microbiology*, **20**(3) 123–30.

MCCARTHY J, HALL, S J and BLACKBURN C D (1999) Potential problems associated with rapid methods used for the detection of *E. coli* O157:H7 in food, in Tuijtelaars A C J, Samson R A, Rombouts F M and Notermans S, *Food Microbiology and Food Safety into the Next Millennium*, Foundation Food Micro'99, TNO Nutrition and Food Research Institute, The Netherlands, pp. 565–8.

MASSO R and OLIVA J (1997) Technical evaluation of an automated analyser for the detection of *Salmonella enterica* in fresh meat products, *Food Control*, **8**(2) 99–103.

OGDEN I D, MACRAE M, HEPBURN N F and STRACHAN N J C (2000) Improved isolation of *Escherichia coli* O157 using large enrichment volumes for immunomagnetic separation, *Letters in Applied Microbiology*, **31**(4) 338–41.

PATEL P D (2000), Salmonella Detection by Enzyme Immunoassays, in Robinson R K, Batt, C A and Patel P D, *Encyclopedia of Food Microbiology*, Academic Press, 1956–7.

PARK C E, AKHTAR M and RAYMAN M K (1993) Simple solutions to false-positive staphylococcal enterotoxin assays with seafood tested with an enzyme-linked immunosorbent assay kit (TECRA), *Applied and Environmental Microbiology* **59** (7) 2210–13.

PARK C E, AKHTAR M and RAYMAN M K (1994) Evaluation of a commercial enzyme immunoassay kit (RIDASCREEN) for detection of staphylococcal-enterotoxins A, B, C, D and E in foods. *Applied and Environmental Microbiology* **60**(2) 677–81.

RITZLER M and ALTWEGG M (1996) Sensitivity and specificity of a commercially available enzyme-linked immunoassay for the detection of polymerase chain reaction amplified DNA, *Journal of Microbiological Methods*, **27**(2-3) 233–8.

STEPHENS P J and JOYNSON J A (1998) Direct inoculation into media containing bile salts and antibiotics is unsuitable for the detection of acid/salt stressed *Escherichia coli* O157:H7, *Letters in Applied Microbiology*, **27**(3) 147–51.

TVELD P H I and NOTERMANS S (1992) Use of reference materials (spray-dried milk artificially contaminated with *Salmonella typhimurium*) to validate detection methods for *Salmonella*, *Journal of Food Protection* **55**(11) 855–8.

WILLSHAW G A, THIRLWELL J, JONES A P, PARRY S, SALMON R L and HICKEY M (1994) Verocytotoxin producing *Escherichia coli* O157 in beefburgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain, *Letters in Applied Microbiology*, **19** (5) 304–7.

ZHAO T and DOYLE M P (2001) Evaluation of Universal Pre-enrichment Broth for Growth of Heat-Injured Pathogens, *Journal of Food Protection*, 64 (11) 1751–5.

Appendix Manufacturers of ELISA kits

BioControl Systems Inc., 12822 SE 32nd Street Bellevue, WA 98005, USA. Info@rapidmethods.com

Bioline ApS, Fredericiavej 414, 7080 Børkop, Denmark. www.bioline.dk

bioMérieux SA Chemin de l'Orme, 69280 Marcy L'Étoile, France. www.biomerieux.com

Binax Inc., Portland, Maine 04103, USA. www.binax.com

Foss Electric, Slangerupgrade 69, Post box 260, DK-3400, Hillerød, Denmark. www.foss.dk

GEM Biomedical Inc., 925 Sherman Avenue, Hamden, CT 06514, USA.

KPL Inc., Kirkrgaard and Perry Laboratories, 2 Cessna Court, Gaithersburg, MD 20879-4174, USA. www.kpl.com

Merck & Co., Inc., Whitehouse Station, New Jersey, USA. www.merck.com

Meridian Diagnostics Inc, 3471 River Hills Drive, Cincinnati, OH 45244, USA.

Microgen Bioproducts Inc., 1, Admiralty Way, Camberley, Surrey GU15 3DT, UK. www.microgenbioproducts.com

Oxoid Ltd., Wade Road, Basingstoke, Hants. RG24 8PW, UK. www.oxoid.com

TECRA International Pty Ltd, 13 Rodborough Road, Frenchs Forest, NSW 2086, Australia. www.tecra.net

Toxin Technology Inc., 7165 Curtis Avenue, Sarasota, FL 34231, USA. www.toxintechnology.com

Transia GmbH, Dieselstr 9a, D-61239, Ober-Mörlen, Germany. www.transia.de

Rhône-diagnostics Technologies Ltd., West of Scotland Science Park, Unit 3.06 Kelvin Campus, Glasgow, Scotland, G20 OSP, UK www.rhone-diagnostics.co.uk

R-Biopharm AG, Dolivostrasse 10, 64293 Darmstadt, Germany. www.r-biopharm.com