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ADVANTAGES AND DISADVANTAGES OF THE USE OF IMMUNODETECTION TECHNIQUES FOR THE ENUMERATION OF MICROORGANISMS AND TOXINS IN WATER

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ABSTRACT

The application of immunological techniques to the analytical challenges presented by water pollution and its tremendous expansion during recent decades are reviewed. Examples of the immunodetection methods evaluated for their advantages and disadvantages in the water field include the use of enzyme-linked immunosorbent assays (ELISA) and radio-immuno-assays (RIA) for the direct detection of viruses from water concentrates; cyto-immuno-labelling and immunofluorescence techniques specific for rota and hepatitis A viruses; and the use of ELISA and immunofluorescence for the detection of bacteria (*Legionella*, faecal coliforms) and protozoan parasites (*Giardia, Cryptosporidium*). The production and use of monoclonal antibodies against algal toxins are also evaluated.

The advantages to be gained by utilizing these techniques in the water field are numerous. In general, they simplify the detection method, shorten detection time and are less labour intensive than other conventional methods. They also provide a tool for the detection of pollutants that otherwise could not be identified. However, many disadvantages are associated with utilising immunological techniques. False positives are often reported due to reactions with nonspecific matter in the water sample or cross-reactivity with a wide range of organisms. These methods are also unable to indicate the viability of organisms. The successful use of immunodetection techniques in the water field often depends on their combination with conventional culturing methods and/or microscopic observation.

KEYWORDS

Immunodetection techniques, monoclonal antibodies, water, microorganisms, toxins.

INTRODUCTION

The methods used for the detection of health-related microorganisms (pathogenic and indicator organisms) and toxins in water provide a useful tool for the control of water quality and therefore indirectly have a great effect on the spread of waterborne diseases in the population. As most of these microorganisms and toxins are known to endanger human health at very low concentrations,

methods for their detection in water require high sensitivity. Methods must have a very low detection limit, often in a very large volume of water samples, as specified in the water quality guidelines or standards. In addition to high sensitivity, high specificity for the enumeration of specific microorganisms or toxins, or microorganism and toxin ranges, is also required. Such detection methods should also be rapid, simple to perform and cost-effective.

Immuno-diagnostic tools provide the medical field with highly sensitive and specific methods for the detection of many pathogenic microorganisms. Since the phenomenon of bacterial agglutination was first discovered in 1896, the precipitin reaction is widely used by microbiologists in many fields of research and technology applications. By 1960 the first radio-immuno-assay (RIA) was developed. This was followed by the solid phase enzyme-linked immunosorbent assay (ELISA). Although the ELISA often replaced the use of RIA, both immuno-assays are widely used. Both RIA and ELISA are based, as are many other types of immuno-assays, on the interaction between a labelled antibody and an antigen or *vice versa*. While this interaction provides specificity, labelling offers both higher sensitivity by signal amplification and quantification of the extent of the reaction. Other techniques make use of either fluorescent or gold conjugates and are used as a basis for immuno-histology or cyto-immuno-assays. The development of monoclonal antibody production has provided the user of immuno-assays with a tool of further increased specificity and sensitivity.

The use of immunodetection assays in the water field is extensive. Although rarely used in the routine analyses of indicator organisms, many immuno-assays are utilised for the identification and enumeration of pathogens (bacteria, viruses and protozoan parasites). Clinical immuno-diagnostic assays form the basis of many of these assays, but direct application in the water field is often inappropriate. Immunodetection methods for microorganisms or toxins in water require greater specificity and sensitivity than are provided in many clinical diagnostic assays. This study presents the immuno-assays used in the water field, and discusses their advantages and disadvantages. Examples of immuno-assays used for the detection of specific microorganisms in water are reviewed.

IMMUNOASSAYS AND THEIR USES IN THE WATER FIELD

Agglutination. Agglutination of bacteria by a specific anti-serum results from the presence of antibodies directed against antigens making up the cell surface capsule or flagella. A classical application of bacterial agglutination is the detection of *Salmonella* serotypes. Hundreds of *Salmonella* serotypes have been identified using this agglutination method (Lüderitz *et al.*, 1966). Bacterial agglutination is also a recommended standard method for identification of *Salmonella* spp in water following selective enumeration and completion of biochemical tests (APHA,AWWA, and WPCF, 1989).

Latex agglutination is a variation of the bacterial agglutination test, utilising a solid matrix which is used in the water field for the detection of rota virus. The method was found to be insensitive in comparison to other immuno-assays (Genthe *et al.*, 1991).

Radio immuno assay (RIA). RIA is based on the binding of a highly radioactive tracer antigen to a low concentration of highly specific antibody. The tracer antigen is labelled with a radioisotope (often I^{125} or I^{131}) of a very high specific activity (10μCi/μg for a molecular weight of 20 000). Antigen molecules present in the test sample compete with the tracer antigen for binding to the antibody. This method can provide a sensitive measure for very low concentrations of antigen even in the presence of many impurities (Yalow, 1980). In addition to competitive RIA in solution, solid phase RIA is widely used. The antibodies are bound or covalently linked to plastic (microtitre plates) or ligands (sepharose beads). The intrinsic affinity of the antibody-antigen binding is increased due to the solid-liquid phase interaction providing a greater sensitivity. Limitations of RIA concern the affinity and concentration of the antibody, and the ratio of antibody to antigen.

In the water field, RIA is used primarily for the detection of viruses (notably hepatitis A) from concentrated water samples. Solid-phase RIA allows for a greater number of assays to be carried out simultaneously and increases the assay sensitivity. It was reported that 10^5 to 10^6 virus particles per mℓ can be detected using RIA (Rao and Melnick, 1986). A variation on RIA for the detection of Hepatitis A virus is radio-immunofocussing in which viruses in a plaque are reacted with a radio-labelled antibody and an autoradiogram is developed (Rao and Melnick, 1986).

Enzyme-linked-immuno-assay (ELISA). In principle ELISA is very similar to RIA. However, the labelling of either the antibody or the antigen is achieved using an enzyme rather than a radioisotope and all ELISAs are solid-phase. The colour reaction resulting from the enzyme-substrate interaction can be observed either visually or by optical density. Most ELISAs employ colour formation in solution. Enzymes most widely used include horse radish peroxidase, alkaline phosphatase and β galactosidase. The ELISA provides a robust, versatile and simple technique which utilises stable and often readily available reagents. The sensitivity of the ELISA is equal to that of RIA. The use of solid-phase interactions and the introduction of amplifying stages, such as secondary-species-specific immunoglobulins, provides for even higher levels of sensitivity. Assay limitations are similar to those mentioned for RIA and include the specificity of antibody used, concentration of both antibody and antigen and type of reaction solution utilised. However, the solid-phase nature of ELISA results in problems associated with accuracy and reproducibility due to factors concerning the binding of either antibody or antigen to the solid matrix. Blocking with proteins such as bovine serum albumen (BSA) may alleviate the problem to a certain extent. The choice of enzyme and its substrate is also critical. The sensitivity of the assay can be greatly increased by the use of the enzyme cascade reaction (Catty and Raykundalia, 1989). As the ELISA measures bound complexes, the protocol of washing the solid phase is critical. False data may be obtained if any washing error takes place.

The ELISA technique is very versatile and has been employed in numerous configurations for many fields of applications (Paul, 1984: Catty and Raykundalia, 1989). These configurations may give clear qualitative data but the quantisation of the assay is often troublesome. Bunch *et al.* (1990) have developed a statistical design for ELISA protocols aimed at increasing accuracy and efficacy.

ELISA techniques are widely used in the medical field for the detection of agents of waterborne diseases, especially for the detection of viruses in stool specimens. Commercial kits are available for a number of viruses such as rotavirus (Rotazyme) and adenovirus type 40 and 41 (Adenoclone). Several of these clinically orientated kits have been adapted and evaluated in the water field, including the rotavirus and the adenovirus assays (Rao and Melnick, 1986; Gerba and Rose, 1990; Genthe *et al.*, 1991). Sensitivity was measured as detection limits of 10^4 to 10^5 viral particles, which yields difficulties in examining environmental water samples directly.

Immuno-fluorescent techniques (IF). Covalent binding of fluorescent dyes to an antibody without significant effect on their reactivity toward a specific antigen was first demonstrated in 1950 (Johnson, 1989). The method combined the high specificity provided by the immuno-reaction with the high topographic precision of microscopy. The temporary nature of radiation emission has presented a problem in the past since the emission ceases almost immediately after the withdrawal of the exciting radiation source. This shortcoming is partially overcome by addition of a retarding agent which allows for "permanent " preparations. IF provides the same sensitivity as immuno-histochemical methods utilising enzymes but is more rapid as no substrate incubation time is needed. The major disadvantage is the unstable nature of the fluorochrome. The specificity of the antibody is, as for all other immuno-assays, the major limiting factor. In comparison to ELISA and RIA, the microscopy using IF techniques provides an additional tool of confirmation. Counterstaining allows for structure analyses of the tissue or microorganism studied. Another advantage of IF is that the use of antibodies of different target specificity and different fluorochrome labels provides simultaneous identification of a number of different antigens. The sensitivity of the IF technique is affected by the choice of fluorochrome and light source, preparation of the biological material, and fixation and staining procedures.

Immunoflourescence is widely used for the detection of microorganisms in water. *Legionella* bacteria (Grabow *et al,* 1991), the protozoan parasites *Giardia* (Sauch,1985) and *Cryptosporidium* (Ongerth and Stibbs, 1987), and certain viruses such as rotavirus (Smith and Gerba, 1982) can also be detected in water using IF techniques.

Immune electron microscopy- immunogold. Immune electron microscopy (IEM) is the principle conventional technique for the detection of non-culturable viruses and their antibodies in clinical specimens (Lin *et al.*, 1991). The IEM technique depends on the morphological identification of individual virus particles aggregated by the use of specific antibodies. Such identification is sometimes difficult under poor staining conditions and with crude specimens. Recently colloidal gold-labelled antibodies provided for a quantitative assay have been utilised for IEM for the detection of rotavirus, enteroviruses and Norwalk virus (Lin *et al.*, 1991).

IEM and IEM-gold are seldom used for the detection of microorganisms in water due to the cost of equipment, expertise and labour-intensiveness required for sample preparation. Certain laboratories make use of this method for research purposes when equipment is readily available.

Immuno enzyme assay (IEA). Although IF has been successfully utilised since 1950, there has been an accelerated interest in the use of IEA in recent years. The latter method can give a simple and stable solution to immuno-histochemistry. The advantages of this method are numerous: there is no need for a fluorescent microscope (a light microscope is sufficient); the preparation is permanent; it is easier to visualise the background; and staining may also produce a preparation suitable for IEM. The main disadvantage of IEA is poor sensitivity in comparison to IF. Increased sensitivity can be partially achieved by a number of amplification steps. However, the amplification steps often increase nonspecific binding and decrease signal-to-noise ratio. Other disadvantages include assaying time and endogenous enzyme activity. The time required for IEA is longer than that required for IF since even the shortest direct IEA requires an extra step for substrate reaction. The substrate reaction may also result in unspecific stain localization. The presence of endogenous enzyme in the biological matter which is of equal specificity to substrate used in the assay is another problem. The blocking of such endogenous enzyme activity sometimes alleviates the problem but often results in the complete or partial destruction of the biological preparation to be examined.

Of the enzymes used in IEAs, horseradish peroxidase is the most popular preparation, although alkaline phosphatase and recently glucose oxidase are also used. Immunoperoxidase (IP) often makes use of the substrate diamino benzidine (DAB) which produces a permanent colour reaction. Other preparations are alcohol liable and are less stable. However, until recently, DAB was a suspected carcinogen which reduced its safety of use. As in other assays, specificity of the primary antibody is of great importance. In some preparations unspecific attachment of the primary specific antibodies to highly charged areas of the preparation resulted in increased unspecific binding. However, the use of non-immune serum or BSA may reduce this type of unspecific binding. The preparation of biological matter and staining is also of great importance. Staining can be carried out directly or by the use of secondary conjugated antibodies of peroxidase anti-peroxidase preparation (PAP) or avidin-biotin preparation which will increase the sensitivity. In the latter case possible reaction with endogenous biotin must be kept in mind (Johnson *et al.*, 1989).

In the medical field IEA or IP is mostly utilised for tissue analysis. Most of its practical application in the water field lies in the detection of viable viruses after infection of cell cultures (Genthe *et al.*, 1991). Rao and Melnick (1986) found IP to be 100 -1000 times more sensitive than IF for the detection of viruses in cell cultures. Another use of IP in water is the detection of viable colonies of *Legionella* without the necessity of microscopy (Bérubé *et al* ., 1989).

Monoclonal Antibodies (MAbs). The relatively new technology of MAb production has contributed immensely to advancement in the use of immunodetection techniques in many fields of research. MAb preparations should provide an invariant, stable and continuous source of antibodies of reproducible specificity and affinity. A high percentage of the specific antibody should theoretically

bind only to a single antigenic determinate. All these characteristics are greatly lacking when using polyclonal antibodies. MAbs are widely employed in ELISA, RIA and especially in IF techniques and are rapidly replacing polyclonal antibodies in many immunoassays utilised in the water field.

The unique attribute of a MAb is its ability to recognise one epitope on a complex antigenic structure. However, this may give rise to a score of unexpected cross-reactivities with unrelated molecules. A number of MAbs have been shown to react with proteins or other molecules across the species line. This can be explained by the presence of identical epitope structures in unrelated molecules and even in unrelated organisms. It is therefore recommended that identification should not be done solely on reactivity with one MAb and that other means of characterisation should be used (Kearney, 1984).

Counter immunoelectrophoresis. Continuous and discontinuous counter immunoelectrophoresis techniques can take between 30 and 120 min and are used for clinical detection of many bacteria including identification of *Vibrio cholerae* strains. Cell fragments and toxins can also be identified using this method. This method utilises either the entire bacterial cell, or extracts of antigens from the bacterial cell wall (Grant, 1978). The assay is highly sensitive (up to ng of antigen) and selective (specific antigen can be detected in the presence of a number of other antigens). However, the assay requires specific equipment and expertise and is rarely utilised in the water field.

Neutralisation. The fundamental observation of neutralisation of viral or toxin activity after reaction with specific antibodies can be clinically used for the identification of antibodies or antigens in a clinical specimen(Grant, 1978). This method is used for identification of bacterial toxins and viruses in medical research but is rarely utilised in the water field.

THE USE OF IMMUNODETECTION ASSAYS IN WATER

Rotavirus. A variety of immunodetection methods for rotavirus in environmental water samples is well documented in the literature. These assays include the use of ELISA, IF and IP for the isolation of rotavirus from fresh and drinking water (Smith and Gerba, 1982), seawater (Goyal and Gerba, 1983), sewage (Hejkal *et al.*, 1984) treated water and activated sludge (Rao *et al.*, 1987). The effect of disinfection on human rotaviruses in water has been studied using immuno-techniques (Harakeh and Butler, 1984).

Only limited comparative studies on the efficacy of various immunodetection techniques for rotavirus in water have been reported. Guttmanbass *et al.* (1987) compared the sensitivity and detection limits of ELISA and IF for rotavirus. Comparison of cytoimmunolabelling techniques, utilising IP with a commercially available ELISA and a latex agglutination kit, indicated that the cytoimmunolabelling method was 10^5 times more sensitive for the detection of rotavirus than the other two methods. This cytoimmunolabelling technique was successfully utilised for the detection of rotaviruses in river water, seawater and wastewater effluents (Genthe *et al.*, 1991). The sensitivity of IF assays was found to be comparable to or even higher than that of other immuno-techniques for the detection of viruses in water (Rao *et al.*, 1987). The cytoimmunolabelling techniques using IF and EIA were shown by Smith and Gerba (1982) to detect as few as 10 infectious viral units per litre of water sample.

Giardia cysts and Cryptosporidium oocysts. Immunodetection techniques were initially developed for the detection of these protozoan parasites in stool specimens (Stibbs *et al.*,1989; Arrowood and Sterling, 1989).

Protozoan parasites can be detected in concentrated water samples and sewage utilising direct microscopy (APHA, AWWA,and WPCF, 1989). However, direct microscopy requires extensive expertise, is labour-intensive and accurate microscopic identification may be greatly affected by any distortion of cyst morphology or/and the presence of other contaminants in the water sample.

Many studies have indicated the presence of *Giardia* cysts and *Cryptosporidium* oocysts in surface and drinking water, sewage and treated effluents and shellfish utilising an IF method which is becoming the method of choice (Gilmour *et al.*, 1991; Rose *et al.*, 1988; Hibler and Hancock, 1990; Kfir *et al.*, unpublished data). The removal of *Giardia* cysts and *Cryptosporidium* oocysts by sewage and water purification processes and the effect of chlorine disinfections and storage of water samples on cyst viability have also been evaluated using immuno-techniques (LeChevallier *et al.*, 1991).

Polyclonal antibodies (rabbit and guinea pig serum) have been used in IF assays for the detection of *Giardia* cysts (Riggs *et al.*, 1988; Suach, 1985) and *Cryptosporidium* oocysts (Ongerth and Stibbs, 1987). The *Giardia* IF assay was found to be faster and easier than direct microscopy. The anti-Giardia polyclonal antibodies were found to react with *Giardia* cysts of a wide range of origins while the anti-*Cryptosporidium* antibodies were found to be highly specific.

The Meridian Diagnostic kit (hydrofluor-combo) provides indirect IF methods for the simultaneous detection of *Giardia* cysts and *Cryptosporidium* oocysts, using FITC-conjugated secondary anti-murine antibody, in environmental water samples. The MAb detecting *Giardia* is pan-specific; the antibody to *Cryptosporidium* is specific to *Cryptosporidium parvum* (Arrowood and Sterling, 1989). The use of the Meridian kit MAbs was found to increase the sensitivity (12 times more sensitive than direct microscopy) and accuracy of the tests and to shorten the assay time by using the same method for the enumeration of both protozoan parasites (Rose *et al.*, 1988). The MAbs from the Meridian kit have been used in many studies for the detection of *Giardia* cysts and *Cryptosporidium* oocysts in water (Le Chevallier *et al.*, 1991; Rose, 1988; Vesey and Slade, 1991; Rodda *et al.*, 1991,1992). Detection limits reported by these studies were greatly affected by sample concentration and elution techniques. Other factors affecting sensitivity limits of the assay are water quality parameters such as high turbidity or organic matter, algae, free living protozoa and even alum and other coagulates (Hibler, 1988).

Legionella. Reported detection methods for *Legionella* in water include direct or indirect IF, ELISA, immunoblot using IP, and immuno-diffusion with rabbit antisera (Ouchterlony and immuno-electrophoresis).

A qualitative method for the enumeration of *Legionella* bacteria in water has been described by Grabow *et al.*, (1991a). This method is based on the combination of selective culturing of bacteria and a direct IF technique. Quantitation is achieved by using the most probable number method. The assay makes use of a commercially available FITC- conjugated rabbit anti-*Legionella* polyclonal antibody reagent specific to *Legionella pnemophila* serotype 1-6 and *L. micadadeie* (Zeus Technology Inc.). Expertise in the morphology and staining characteristic of *Legionella* is necessary in order to avoid false positives. This assay has been successfully utilised for the detection of *Legionella* in a variety of water samples including a large number of cooling towers, potable water, mine water used for cooling systems and the evaluation of biocide efficacy (Grabow *et al.*, 1991a; Grabow *et al.*, 1991b; Augoustinos *et al.*, 1992). This assay provides a simple, relatively rapid and quantitative tool for the enumeration of *Legionella* in water. It provides information on bacterial viability and shows greater specificity than IF techniques which are carried out directly on water samples. Another example of a rapid and simple method for the detection of *Legionella* from water is a direct ELISA (BOET Ltd). In comparison to other direct assays this is a simple and rapid technique, but the lack of microscopic examination may result in false positives as unspecific binding may cause colour formation. As in other direct assays viability data is unavailable.

A combination of selective culturing of *Legionella* with an indirect immunoblot assay on growing colonies utilising MAbs specific to *L.pnemophila* serotype 1-8 and a secondary antibody conjugated to peroxidase has been reported. The stained colonies can be counted and the assay provides an easy and rapid quantitative measure of viable bacteria (Bérubé *et al.*, 1989). The assay has a number of limitations. Possible cross-reactivity of the MAb cannot be identified as no microscopic observation of bacterial cell morphology is carried out. Binding to *Pseudomonas fluorescence*

colonies due to cross-reactions and to *Staphylococcus aureus* due to the presence of protein A have been reported. Another inherent problem is the endogenous peroxidase activity in other bacterial species, although the assay provides for the removal of such activity by incubation of the membranes with 3% hydrogen peroxide. Evaluation of this method has indicated irreversible damage of colony structure even at lower percentages of hydrogen peroxide and in certain cases a complete detachment of the colonies from the membrane (Grabow and Kfir, unpublished data).

Escherichia coli and Enterobacteriaceae. *E. coli* is a normal inhabitant of the digestive tract and is shed in human faeces. It is often used as an indicator organism for faecal pollution of water and is included in many water quality standards and guidelines. Methodology for the recovery of *E.coli* involves the selective recovery of faecal coliforms in accordance with physiological and biochemical reactions, heat resistance and colony morphology. Confirmation of *E.coli* bacteria is, however, a lengthy procedure.

In recent years attempts have been made to use ELISA technique, employing MAbs specific to either the enterobacterial common antigen (ECA) or other lipopolysaccharide extracts of the bacterial wall for the detection of *E.coli*. Obst *et al.* (1989) addressed the use of MAbs against the ECA for the detection of Enterobacteriaceae in drinking water. A sandwich ELISA was practised. The study indicated the need for pre-incubation of water samples in order to increase bacterial numbers, as the assay could not detect a single bacterium per 100mℓ of sample. However, the pre-incubation was also found to reduce sensitivity, possibly due to destruction of the ECA in the acidic conditions formed in the media. Although this MAb was specific to Enterobacteriaceae some cross-reactivity to *Plesiomonas shigelloides* was discussed.

Kfir *et al.* (1993) produced MAbs against *E.coli* and *Klebsiella oxytoca* bacteria isolated from polluted water. The MAbs were raised in BALB/C mice which were immunized with heat-killed bacteria. Although a number of clones producing antibodies against faecal bacteria were obtained, the specificity was not high. A commercial MAb anti-*E. coli* was evaluated and found to cross-react in the same manner.

Cyanoginosin (Microcystin). Anti-toxin sera is widely used for the detection of Gram-negative bacterial toxins (Grant, 1978). However, detection of low molecular weight toxin is a more complex problem. Cyanoginosin (microcystin) is a cyclic polypeptide with a molecular weight of 909 daltons, isolated from *Microcystis aeruginosa*. Toxin production by the alga is variable and inducible by some unapparent environmental or internal signals. It is an hepato-endotoxin which is highly toxic (LD_{50} of 0.05-0.2 μg/g mouse body weight), is structurally stable, is known to withstand a variety of water treatments (with the exception of activated carbon) and is highly thermostable. A number of cyanoginosin variants which vary in their toxicity have been isolated (Kfir *et al.*, 1986). High performance liquid chromatography can be used for toxin identification but is labour-intensive and expensive and cannot indicate toxic variants. Immunological methods were therefore investigated.

There is no recorded evidence of natural production of anti-cyanoginosin antibodies. The toxin is probably not a functioning immunogen due to its small molecular weight,and the production of a hapten-carrier complex prior to animal immunisation is reported in all studies. Both muramyl dipeptide- polylysine and ethylenediamine-bovine serum albumin (EDA-BSA) were used as carriers and the production of both MAbs and polyclonal antibodies is reported (Kfir *et al*, 1986 and Chu *et al.*, 1989). The MAb produced by Kfir *et al.* (1986) showed specificity to all known variants of cyanoginosin and in addition some non-specific binding. The polyclonal antibodies produced by Chu *et al.* (1989)were introduced into a direct-competitive and indirect ELISA and a RIA. Only partial cross-reactivity between toxin variants had been reported. No specificity studies addressing cross-reactivity with other microorganisms or non-specific binding to debris which may occur in environmental water samples have been reported. RIA was found less sensitive than the two ELISAs tested and the direct ELISA was recommended for field study as it is less time consuming than the indirect assay. In a more recent study 83% direct recovery of toxin from a concentration of 1-20 ng/mℓ was reported but interference to the successful application of this ELISA technique for analysis of dried algal matter was reported (Chu *et al.*, 1990).

In recent years an increased use of immuno-assays for the detection of chemical toxicants such as pesticides and chemical carcinogens in environmental samples has been reported. These assays require an initial production of a hapten-carrier complex followed by labour-intensive production of MAbs specific to the target chemical, but will shorten analytical chemical analysis significantly (Vanderlaan *et al.*, 1988).

DISCUSSION AND CONCLUSIONS

Immunodetection techniques provide valuable tools for the detection of microorganisms and toxins in water. The advantages to be gained by utilising these techniques in the water field are numerous. In general, they simplify the detection method, shorten detection time and are less labour intensive than other conventional methods. They also provide a tool for the detection of pollutants that otherwise could not be identified. However, many disadvantages are also associated with utilising immunological techniques. Although these techniques provide high specificity, the antibody has to be thoroughly examined for possible non-specific reactions or cross-reactions. The affinity and specificity of an antibody is the backbone of all immunodetection techniques. The water field is characterised by the presence of many unspecific pollutants and even related organisms which tend to appear in the same sample, hence the specificity of the antibody is of the greatest importance. False positives are often reported due to reactions with non-specific matter in the water sample or cross-reactivity with a wide range of organisms. Highly sensitive immunodetection techniques cannot provide only detection of single microorganisms in water and often require pre-concentration and amplification steps. These methods are also unable to indicate the viability of the organisms tested if used directly.

The successful use of immunodetection techniques in the water field often depends on their combination with conventional culturing methods and/or microscopic observation. The combination of microscopy, and where possible, culturing techniques, with immunoassays provides methods which have high accuracy, high sensitivity and high specificity and which allow the determination of viability. Immuno-fluorescence techniques, which are often used in combination with pre-culturing methods, are the most utilised methods in the water field despite the requirement for an epi-fluorescence microscope. Monoclonal antibodies are replacing polyclonal antibodies in many immunoassays used in the water field. They provide a continuous source of antibodies of identical specificity and sensitivity which will not vary from preparation to preparation, providing the water field with a continuous point of reference and quality assurance by using an identical tool for all water samples tested in many laboratories across the world. These MAbs need to be thoroughly examined for their affinity and specificity before inclusion in any immunodetection technique in the water field.

The use of immunodetection techniques for the detection of toxicants such as pesticides in water may offer a rapid and relatively cost-effective alternative to many of the chemical analytical tools used at present.

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