Immunologic detection of bacterial antigens and antibodies

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Early diagnosis and prompt institution of appropriate antibiotic therapy are required for the optimal treatment of infectious diseases. Traditionally, the laboratory has isolated and identified the specific etiologic agent and when possible, determined its antimicrobial susceptibility profile. If cultural microbiology was not successful, then the identification of the organism was often made by observing the appearance of specific antibodies. While the detection of antibodies is still important in the diagnosis of disease, the process is time consuming in that, for optimal results, the serum sample should be collected 2-3 weeks apart. A variety of immunologic techniques which may be used to detect and identify the antigens are now available. Immunodiffusion and electrophoresis can be used to detect and identify specific microbial antigens in body fluids. Non-traditional approaches to immunologic detection of antibodies and antigens have become popular in clinical microbiology laboratories because the techniques are rapid, cost effective, and provide diagnostic answers in a time frame which is of considerable importance in the treatment of the patient. These tests which are routinely available include counter-immunoelectrophoresis, agglutination, latex agglutination inhibition, coagglutination, radiomunoassay, enzyme-linked immunosorbant assay and fluorescent immunoassay.

COUNTERIMMUNOELECTROPHORESIS (CIE)

Counterimmunoelectrophoresis (CIE) was originally described in 1959 by Bussard and was first used clinically for the detection of “Australia antigen” (HbsAg). Radioimmunoassay using CIE for HbsAg but CIE has become a valuable immunologic tool for detecting both microbial antibodies and antigens. CIE is a rapid precipitation reaction in which the reactants are driven by an electric current and is usually performed on slides. Vincent and Bell* described the use of the tube precipitin reaction for the detection of meningococcal antigen in CSF. Dochez and Avery* identified the capsular polysaccharide of the pneumococcus in patients with meningitis as early as 1917. The precipitin reaction is a function of the precipitation of antibody and soluble antigen at the equivalence point. The reaction may take up to 18 hours. CIE, on the other hand, combines the advantages of immunodiffusion and electrophoresis. One protein is placed in a well on the cathodic side of a solid support and the antibody is placed on the anodic side. The antigen, if negatively charged, migrates toward the anode and the antibody which usually has a weak negative charge also migrates toward the anode. Positively charged buffer counteracts this movement, so the molecule to the cathode. This is called endosmosic flow. If conditions of voltage, current, and time are correct, a precipitate forms between the two wells after as little as 30 minutes of electrophoresis. There are a number of variables which must be controlled for reliable reproducible results. CIE is to be a reliable reproducible method in the clinical laboratory:

- a) The quality of antisera may be the single most important variable in CIE.
- b) Buffer - The buffer in CIE includes agarose, cellulose acetate. Noble agar and bacteriological agar. For most applications, agarose is the gel of choice. This is a neutral-linear polysaccharide, water soluble, and will form a gel at 0.1%. The gel is placed on glass, plastic, or mylar film. The author prefers mylar film as it can be cut to size for the particular application, stained, and entered into the permanent record.
- c) Support systems - Immunologic reactions occur in gels. Several supports have been used for CIE including agarose, cellulose acetate. Noble agar and bacteriological agar. For most applications, agarose is the gel of choice. This is a neutral-linear polysaccharide, water soluble, and will form a gel at 0.1%. The gel is placed on glass, plastic, or mylar film. The author prefers mylar film as it can be cut to size for the particular application, stained, and entered into the permanent record.
- d) Electrophoresis chamber - The chamber provides a physical support for the gel. It contains buffer, and it maintains the voltage and current to the gel matrix.
Clinical applications

The ability of latex agglutination (LA) to detect cryptococcal polysaccharide in serum or CSF has been well documented and is more sensitive than demonstration of the presence of Cryptococcus neoformans by India Ink. Rheumatoid factor may invalidate the test because of non-specific agglutination.

Newman et al. detected H. influenzae capsular antigen by LA in 277 of 308 CSF cases, and this was confirmed by the addition of anti-H. influenzae meningitis. Kadok et al. studied 95 patients with purulent meningitis and 63 controls. The incidence of meningitis, however, was not tested.

With the exception of one urine specimen, none of the controls had a positive LA while 62/95 CSF specimens, 10/14 sera, and 11/17 urine specimens were positive by LA. Although these investigations have produced many false positive agglutinations, it is now appreciated that LA is useful for detecting H. influenzae in CSF specimens greater than 2 mg/ml.

In a similar study, Leenon and Hervier reported that 25/33 CSF specimens using A. meningitidis (ACD) by LA. They reported that LA was at least as sensitive as the conventional microbiological method. In a study performed by Leenon for H. influenzae and A. meningitidis, the detection was not as high as that reported by LA. Leenon and Hervier further observed that LA was found to be more sensitive than CIE, especially in urine.

For type specific antigens, however, LA was found to be less sensitive than LA. The authors stated that there was a greater risk of false positive tests with LA than with CIE.

Leenon and Kayhyti compared CIE, LA, and RIA for detection of H. influenzae. They detected 95.3% of 308 CSF specimens containing H. influenzae and 95.5% for CIE, especially in urine.

A latex agglutination inhibition (LAI) card test is commercially available for detection of meningococcal antigen in serum and CSF. The test is based on the formation of antibody complexes between the meningococcal antigen and antibody in the serum and CSF of patients with meningococcal meningitis. A LAI test, therefore, is useful for the early detection of meningococcal meningitis.

In a study by Standifer et al., they found that when measuring ganciclovir at concentrations >2 μg/ml by LAI, the C.V. was 5.1% to 11.5% for the freeze-dried and freeze-dried to 12% for LAI. For those samples containing >2.0 μg/ml, the C.V. IAI ranged from 0-55%. The LAI test was found to be significantly more sensitive than that of ALP in the diagnosis of meningococcal meningitis. The LAI test was found to be more sensitive than CIE for detecting meningococcal antigen in CSF. They observed that the LAI test was not as sensitive as that of ALP for detecting meningococcal antigen in CSF. They observed that the LAI test was not as sensitive as that of ALP for detecting meningococcal antigen in CSF.

Clinical application

Coagglutination (Coag) reagents can be potentially used to detect any antigen that can be detected by CIE, LA, RIA, and ELISA. The reagent is coated on tubes and is used similar to a latex reagent.

ENZYME IMMUNOASSAYS

The search for methodology that has the advantages of RIAB and few of the disadvantages culminated in the discovery of the homogenous enzyme immunoassay technique. Engwald and Perlman® first used the enzyme linked immunosorbent assay (ELISA) to measure rabies IgG.

ELISA has wide application in clinical microbiology. The concept is similar to RIA. An antigen or an antibody is bound to a solid support, either a plastic tube, tray, or nylon and the binding of the test antigen or antibody is measured.

Immunochemistry

The principal use of RIA is in endocrinology for the measurement of hormones. The reagents are coated on tubes and separation of the phases is as easy as deconating the fluid in the tube.

Although microbiological research employs RIA widely, it is rarely used routinely in clinical microbiology laboratories except for the determination of mycoplasma antibodies in body fluids. kits are currently available for detecting antibodies to Staphylococcus aureus, Enterococcus, and Clostridium perfringens. The test is performed by blocking the antigen with a specific antibody. After equilibrium between the bound and unbound antigen is reached, the bound antigen is separated from the unbound by centrifugation or precipitation. In some applications, the antibody is coated on tubes and separation of the phases is as easy as deconating the fluid in the tube.

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Microbes and Cosmetics

Almost a quarter of a century ago Baker referred to bacterias as "that unwanted cosmetic ingredient", when he described several examples of cosmetic products which had been run by microorganisms. At this time, the cosmetic industry was aware of the potential problems attendant upon the ever-increasing production of cosmetics. Because products which contain a wide range of ingredients, many of which are highly antimicrobial, are used on the skin, the ingredients can be easily contaminated by microorganisms. Microbial contamination is a major problem for the cosmetic industry, and the ability of microorganisms to cause infections is well documented. The role of microorganisms in the production of cosmetic products has led to the development of a wide range of microbiological techniques for the detection and identification of microorganisms. These techniques are based on the understanding of the biology of microorganisms and the use of various methods for their detection.


document

Pseudomonas CN Supplementation

When added to Pseudomonas Agar Base CM595 this supplement enhances the appearance of Ps. aeruginosa and provides enhanced pigment production Right supplement without plate, left plate with supplement.

Pseudomonas CFC Supplementation

This supplement added to Pseudomonas Agar Base CM595 gives a specific medium for the isolation of psychrophilic pseudomonads from chilled foods and pharmaceuticals, in particular Ps. capsici. Left plate without supplement right plate with supplement.

Table 1. Examples of cosmetic ingredients which are assimilated by organisms.

<table>
<thead>
<tr>
<th>Example of Cosmetic Ingredient</th>
<th>Organism Assimilated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Silicones</td>
<td>E. coli</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>S. enterica</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>Polyalcoholic alcohol</td>
<td>P. putida</td>
</tr>
<tr>
<td>Glycerol</td>
<td>E. coli</td>
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</tbody>
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In contrast to the infections from pharmaceuticals clodey, Kallings

wide range of pharmaceutical preparations not officially required to be sterile, such as tablets, ointments, hand creams, baby creams, liquid medicines, ear and nose drops, baby powders and suncosmetics. Not only were all of these preparations found to be contaminated but severe eye disorders (including enucleation) were reported following the use of contaminated commercion ointment while contaminated thyroid tablets were shown to cause pseudomonas cellulosis in over 200 people. The publication of these findings marked the beginning of a concern for the potential for the spread of pseudomonas cellulosis, and a microorganism which is potentially dangerous is pseudomonas cellulosis. Pseudomonas cellulosis is a disease caused by pseudomonas cellulosis, which is a Gram-negative organism, and is characterized by the formation of pseudomonas cellulosis.
et al there is little evidence of contaminated cosmetics causing problems. Where products have been alleged to result in adverse reactions examination has not indicated high microbial counts or the presence of pathogens. This, of course, is not an unexpected state of affairs, because in a situation where cosmetics are applied to the intact skin of healthy individuals, it is, perhaps, only those preparations used to make up the eyes which present a significant hazard. However, it would be unduly complacent to tolerate potentially pathogenic organisms in cosmetics on the grounds that no serious infections had been recorded to date.

The development of microbial standards

A reasonable approach when proposing any standards for cosmetics and toiletries is that they should distinguish between the preparations which have a microbial flora quantitatively similar to that of the environment and those which contain numbers of organisms exceeding the normal environmental contamination. Guidance issued by the Council of the Society of Cosmetic Scientists of Great Britain (1970)* on the hygienic manufacture and preservation of toiletries and cosmetics recommended that “relevant known pathogenic microorganisms be absent from cosmetics and that those preparations intended for use on newborn infants, for direct instillation into the conjunctiva or for use on broken skin, should be sterile at the time of manufacture”. This type of recommendation, which essentially demands the absence of pathogens from cosmetics, can be a source of controversy in respect of the named pathogenic organisms. The genera proposed by the Society of Cosmetic Scientists were Clostridium, Salmonella, Pseudomonas, Escherichia, Klebsiella, Proteus, Streplococci, Shigella and coagulase-positive Staphylococci. An alternative type of standard is one which determines the maximum number of organisms permitted in a given volume or weight of a cosmetic product. Thus, guidelines published by the Cosmetic, Toiletry and Fragrance Association (CTFA) of America in 1973 proposed a general numerical limit for all cosmetics of 1,000 organisms per gram and 500 organisms per gram for baby products and those for opthalmic use.

Acknowledgements

Tables 1 and 2 are reproduced by permission of the Editor of Soap, Perfumery and Cosmetics, United Trade Press, London.

References