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Utilization of the *Staphylococcus aureus* Protein 'A' and the *Streptococcus* spp. Protein 'G' in Immunolabelled Techniques

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1. Introduction

Immunolabelled techniques (ILTs) depend on linking of a specific antibody to a labelling material. The so-produced conjugate is utilized to detect or assay a specific reactant against which the antibody, in the conjugate, is directed. Many labelling materials have been conjugated to specific antibodies. The most commonly used labels included enzymes, avidin-biotin, radioactive materials, fluorescin dyes, ferritin, gold and possibly others.

The specific conjugates could be used to:

a. Detect antibodies in sera of humans or different animal species.

b. Detect antigens such as viruses, bacteria, parasites ..etc.

c. Detect physiological materials such enzymes, hormones, cytokines etc..

d. Detect other materials from plants and insects.

e. Detect pharmaceutical products.

For antibody detection in a specific animal species, the conjugate to be used for that purpose, usually consists of the labelling material (e.g. an enzyme) linked to antibodies against the immunoglobulins of that particular animal species. This necessitates the use of a conjugate against each animal species. However, when for example conducting a serological survey, against a specific disease, in various species of animals including man, the ideal situation, in this case , is to use a single conjugate against all the animal species to be tested. This dilemma was resolved, to a greater extent, by employment of the *Staphylococcus aureus* Protein 'A' and the *Streptococcus* spp. Protein 'G' in conjugates used in ILTs.

2. PA in Immuno-labelled techniques

Protein 'A' (PA) is a novel cell wall protein of the *S. aureus* that binds the Fc portion, of most mammalian IgG molecules (Forsgren & Sjoquist 1966). This binding is not an antigen-antibody reaction, because it does not involve the antigen binding fraction (FAB) of the IgG molecule. This property gave PA special novelty in being a natural universal reagent. So, it is now widely utilized in ILTs.

PA is 40-60 KD. It can bind four molecules of IgG . Optimum binding occurs at pH 8.2. It is stable in the pH range of 1.0 – 12.0. *In vivo* PA disrupts opsonization and phagocytosis in...
blood of an infected host by the *S.aureus* bacteria. So, it is regarded as a potential microbial surface component recognizing adhesive matrix molecules (MSCRAMM).

By virtue of its ability to bind to the Fc portion of most mammalian IgG molecules, PA has been extensively utilized in the different fields of ILTs. It was linked to enzymes such as horse radish peroxidase (Engval 1978) and alkaline phosphatase (e.g. Alnaeem & Abuelzein 2008; Dubois-Dalcq et al 1977; Crowther & Abuelzein 1980), to fluorescin dye (e.g. Chetie et al 1974), to radioactive materials (e.g. Langone 1978; Colombatti & Hilgers 1979; Crowther & Abuelzein 1980), to gold (e.g. Faulk & Taylor 1971), to avidin-biotin (e.g. Hsu & Raine 1981). Accordingly, PA conjugates have been exploited in various areas of ILTs such as ELISA, Radioimmunoassay (RIA), Immuno-histochemistry (IHC), Immuno-electron microscopy (IEM), as an immunological probe to identify cell surface markers (e.g. markers on T and B lymphocytes), to precipitate antigen-antibody complexes without the use of antispecies antibodies, and in other areas of research.

The major value of the use of PA in ILTs is that, it replaced the use of antispecies conjugates against each mammalian species.

### 2.1 PA – ELISA

ELISA, in which antispecies conjugates had been used, was applied with great success in many vital fields of biological sciences such as medicine, veterinary medicine, plant research, entomology, biotechnology, general microbiology and possibly in other fields where ILTs are applicable. Commercial antispecies conjugates are available in the market. However, a problem is always faced when there is a need to examine several animal species, especially those in the wild (e.g. antelopes, skunks, mongoose, hedgehogs, bats etc.). Usually no commercial conjugates are available for these animal species. So, the requirement for one conjugate to cover these species would be ideal. To overcome this obstacle, scientists thought of the use of PA & PG conjugated to enzymes to examine a wide range of IgGs of domestic and wild mammals. As a result, PA - ELISA has found its way in various applications of research. Presently, commercial Kits utilizing PA conjugates in ELISA systems are readily available; and can be employed in the different fields of biological research.

#### 2.1.1 PA – ELISA in the medical field

In the medical field PA-ELISA has been applied in laboratory diagnosis and research. It was applied for the detection of antibodies, against viral (e.g. Madore & Baumgarten 1979; Al-Nakib 1981; Schountz et al 2007), bacterial (e.g. Anson & al 1984; Fuquay et al 1986; Chaud et al 1988; Considine et al 1986; Jagannath & Sehgal 1989; Stobel et al 2002; Nielsen et al 2004) and parasitic diseases (e.g. Mohammed et al 1985; Gandhi et al 1987; Felix de Lima et al 2005) in humans and other mammals. These mammals may act as reservoirs of human diseases or may show overt clinical signs of a zoonotic disease e.g. Rift Valley Fever (RVF); (Madani 2005) or Brucella infection (e.g. Chaud et al 1988; Jagannath & Sehgal 1989).

#### 2.1.1.1 PA – ELISA in veterinary research

PA – ELISA found wide application in veterinary research because of the multiple species of mammals dealt with in the veterinary profession. The technique was applied for the detection of antibodies against viral (Crowther & Abuelzein 1980; Du Plessis et al 1990; Inoshima et al 1999; Smith et al 2004; Schountz et al 2007), bacterial (e.g. Lawman et al 1984; Chand et al 1988; Nielsen et al 2004) and parasitic (e.g. Lima et al 2004) diseases.
2.1.1.2 PA-ELISA in other areas of research

PA-ELISA was applied in plant virology to detect Bacaulovirus (Brown et al 1982). Beside its application in immunodiagnosis, PA has potential applications in immunotherapy and affinity purification of monoclonal antibodies (MABs) (e.g. Considine et al 1986).

2.2 PA – Radioimmunoassay (PA-RIA)

PA-RIA was applied in the different fields of biological sciences (e.g. Dorval et al 1975; Langone 1978; Enzmann 1978; Crowther & Abue lzein 1980). However, its uses in many laboratories was; and so predominantly replaced by the handy ELISA technique.

2.3 PA in immuno-histochemistry

PA has been utilized with great success and versatility in IHC. It was readily conjugated to enzymes such as peroxidase (e.g. Dubois-Dalcq et al 1977;) to avidin- biotin (e.g. Su Ming & Raine 1981) to ferritin (e.g. Templeton & Douglas 1978) to gold (e.g. Roth & Heitz 1989), to fluorescein isothiocyanate (e.g. Notani et al 1979); and used to detect various antigens in tissues (e.g. Alnaeem & Abuelzein 2008; Abuelzein & ElNaeem 2009) either under light or electron microscopes. The advantages of using PA in IHC over other analogous techniques have been well documented ( e.g. Dubois-Dalcq et al 1977; Su Ming & Raine 1981; Roth & Heitz 1989). Beside its versatility in being used against a wide range of animal species, the non-specific reaction experienced with other analogous techniques was not a problem when using PA- IHC . So, PA-IHC was found to be a valuable tool for localization of antigens in tissues.

2.4 PA in Immuno-Electron Microscopy (PA – IEM)

PA conjugated to enzymes (e.g.Dubois-Dalcq 1977), ferritin (e.g. Bach et al 1977), colloidal gold (e.g. Horisberger & Clerc 1985), avidin-biotin (e.g. Hsu & Raine 1981) has been used with great success in immuno-electron microscopy. The technique found successful applications in diagnosis and research in virology (e.g. Wendelschafer et al 1976; Shukla & Gough 1979) , bacteriology (e.g. Van Laere et al 1985) and other areas of research such as histopathology (e.g. Roth & Heitz 1989), andrology (e.g. Schrader et al 2005) and haematology (e.g. Bachi et al 2006).

3. Streptococcus spp. protein G in immuno-labelled techniques

Streptococcus spp. Protein G is a cell wall protein from Streptococcus spp. (Kronvall 1973; Bjorck & Kronvall 1984). Its gene structure and protein binding properties were described by Sjobring et al (1991). Like PA it binds to the Fc portion of IgGs from many mammalian species over a wide range of pH from 4.0-8.0. However, it has a broader range of reactivity than PA. A novel property of PG, is its ability to react with human IgG3. The significance of this, is that IgG3 is over expressed in several autoimmune diseases, representing up to 45% of the autoimmune antibodies. This property has nominated PG to be a candidate of choice for applications in autoimmunity studies and diagnosis.

Due to binding of the native PG molecule to albumin, which is a major component of serum, PG application was rather limited as compared to PA. However, by removal of the binding site from the recombinant forms, PG is now applied with success to various areas of research.
Like PA, PG has been linked to enzymes (e.g. Inoshima et al 1999; Kramsky et al 2003; Stobbe et al 2002; Vansnick et al 2005) & gold (e.g. Taatjes et al 1987) and used in analogous techniques similar to those employed for PA conjugates such as ELISA, IHC & IEM; but to a limited extent.

4. Discussion

The aim of this overview was to throw some light for researchers, to consider the use of PA & PG conjugates where-ever feasible. The merits of using these two types of conjugates over the antispecies ones were discussed above. However, with the emergence of new pathogens in the different geographical regions of the world, researchers find themselves in a situation whereby they should examine various wild mammals for presence of antibodies against the emerging disease. In such situations they need sensitive immunolabelled methods. For these methods antispecies conjugates are required. Such conjugates against these wild mammals are not usually available in the market. So, the need for PA & PG conjugates becomes vital. On the other hand, the use of PG conjugates, for example, can be vital also in some diagnostic situations, such as in the case of autoimmune diseases.

Before using PA or PG conjugates in wild mammals, the reaction of their IgGs against PA & PG conjugates should be assessed, (Inoshima et al 1999).

The choice of using either type of conjugate, PA or PG, depends on the species of animal(s) to be tested. This is because the intensity of reactions of PA & PG with the different species of mammals vary (Inoshima et al 1999; Crowther & Abuelzein 1980). To over come this, some authors used a conjugate combination of both PA & PG (Nielsen et al 2004).

Of the merits of using PA & PG conjugates in ILTs is that the non-specific reactions in the tests are greatly reduced or completely cut down (Crowther & Abuelzein 1980; Alnaeem & Abuelzein 2008; Abuelzein & Alnaeem 2009).

In conclusion, and because of the above mentioned merits, it is recommended that PA & PG are to be used where –ever feasible.

5. References


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The book is coined to provide a professional insight into the different trends of immunoassay and related techniques. It encompasses 22 chapters which are grouped into two sections. The first section consists of articles dealing with emerging uni-and-multiplex immunolabelled methods employed in the various areas of research. The second section includes review articles which introduce the researchers to some immunolabelled techniques which are of vital significance such as the use of the conjugates of the Staphylococcus aureus protein "A" and the Streptococcus Spps. protein "G" in immunolabelled assay systems, the use of bead-based assays and an overview on the laboratory assay systems. The book provides technological innovations that are expected to provide an efficient channel for developments in immunolabelled and related techniques. It is also most useful for researchers and post-graduate students, in all fields, where immunolabelled techniques are applicable.

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