ISSN 1757- 5958

amc technical briefs

Editor: Michael Thompson Analytical Methods Committee AMCTB No 44 February 2010

Immunoanalysis – Part 1: What are antibodies?

Antibodies are widely used as the basis of a number of rapid screening assays used within the clinical chemistry and food/environmental (for example, food allergens and GMOs) sectors. This Technical Brief is a general introduction to antibodies (in relation to protein analysis) and will be followed by others dealing with various aspects of immuno-chemical analysis.

The role of antibodies in *in-vivo* and *in-vitro* protein detection

Antibodies are proteins called gamma globulins (or immunoglobulins). They are produced by the immune system of vertebrates in response to exposure to foreign bodies (known as antigens or immunogens), such as specific proteins, carbohydrates, DNA, lipids and viral/bacterial toxins. In mammals, the antibodies are produced by a subclass of white blood cells known as the B lymphocytes which develop in adult bone marrow or foetal liver. Antibodies circulate the bloodstream and permeate other body fluids, enabling their selective binding to the immunogen, which in turn assists another class of white blood cells, called phagocytes, to engulf and destroy the immunogen. Hence, the ability of antibodies to bind their immunogen targets selectively is a key feature of the immune response. This selective mode of binding between the antibody and its target can be also utilised for diagnostic purposes, that is, for *in-vitro* immunoassays such as the enzyme-linked immunosorbent assay (ELISA). This technique has broad applicability for the detection of any protein of interest for diagnostic purposes, if the appropriate antibodies that bind the protein are available.

Structure

The basic antibody structure (Figure 1) comprises four polypeptide chains: two heavy chains and two light chains held together by disulphide bonds to form a Y-shaped molecule.

The hinge region of the molecule allows a variable distance between the two antigen binding sites. The variable regions of the heavy and light chains form the two identical antigen binding sites.

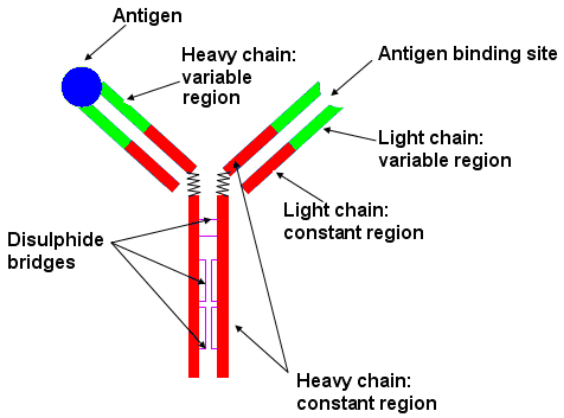

Figure 1. The basic structure of an antibody.

Both the heavy and light chains contain both constant and variable regions. The amino-terminal ends of both types of polypeptide chains comprise a variable sequence, whereas the carboxyl termini of the heavy and light chains have a constant amino acid sequence. Each light chain comprises approximately 110 amino acids within both the variable region and the constant region. The amino-terminal variable region of a heavy chain comprises roughly 110 amino acid residues, whereas the constant region varies from 330 to 440 residues, depending on the class of antibody.

In mammals, there are five distinct classes of antibody (IgA, IgD, IgE, IgG and IgM); each having its own type of heavy chain. These classes of antibody may be found in various body fluids, with a differing temporal response and abundance. IgA (Figure 2) and IgM (Figure 3) are oligomers of the basic unit for four polypeptide chains; IgA is a dimer, whereas IgM is a pentamer. IgAs are generally found in secretory fluids such as tears, saliva, milk, respiratory and intestinal secretions.

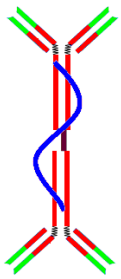

Figure 2. A dimeric IgA molecule.

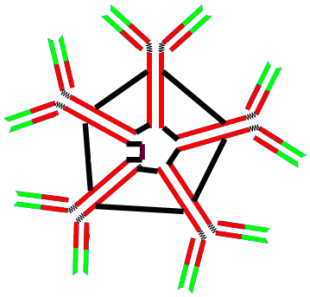

Figure 3. A pentameric IgM molecule.

After the initial exposure to an antigen, IgM is produced by the immune system as the primary response. The monomeric IgGs are the major class of antibodies produced following the secondary challenge of the same foreign protein. IgGs are produced in greater abundance than the IgMs. The IgD population is also expressed during the early immune response but constitutes less than 1 % of the total immunoglobulin in serum. IgDs are the most labile class of antibodies with the tendency to spontaneous proteolysis. The function of circulating IgD is unknown. IgEs are produced during inflammation and allergic reactions. From the diagnostics perspective, the IgGs are the most useful class of immunoglobulins.

In vitro protein detection

Labelled antibodies are employed in techniques, called immunoassays, for detecting antigens. These techniques are popular as they are cost-effective methods for quickly detecting protein analytes. For immunoassays, the production and purification of antibodies is necessary. Purification may be achieved using: gel filtration to isolate the molecules of a particular size; ion exchange chromatography to isolate the appropriate antibody molecules by their positive charge in a neutral pH buffer; ammonium sulphate precipitation of the gamma globulins; and, generally, affinity chromatography.

Polyclonal antibodies in immunoassays

The immune response produces a mixed population of antibodies (of different classes) that exhibit distinct variable regions (even within each class); these are called polyclonal antibodies. Hence, even within one subclass of antibodies (for example, IgGs) the collection of immunoglobulins bind to numerous different recognition sites (or epitopes) on the protein. For *in vitro* use, polyclonal antibodies are purified from the serum of a suitable mammal (for example, rabbit, goat or sheep), that has been injected with the antigen. For constructing diagnostic immunoassays, the advantage of using polyclonal antibodies is that a knowledge of the precise orientation of an analyte is not a prerequisite for the successful construction of an assay.

Production of monoclonal antibodies

Monoclonal antibodies are derived from clonal expansion of a single hybridoma cell line tailored to recognise a single epitope with high immunogenicity. The technique was pioneered by Georges Kohler and Cesar Milstein in 1975, and involves “priming” the B lymphocytes to produce antibodies, by introducing the protein of foreign origin into the host animal, normally a mouse. As the host immune system recognises the protein as a “foreign body”, the B cells produce antibodies against it. A sample of B cells is then extracted from the spleen of the mouse and added to a culture of cancer cells, known as myeloma cells. The fusion of the B cell and myeloma cell is termed a hybridoma. The antibodies from each hybridoma line will recognise a single epitope. The binding constants can be measured to select the most immunogenic antibodies, and the IgG subclass selected by purification. The selected hybridoma may be cultured indefinitely. This gives monoclonal antibodies an obvious advantage over polyclonal antibodies where the finite stock of antibodies enriched from the animal serum may be easily consumed, and batch-to-batch variability may be an issue. Though it is preferable to use a monoclonal antibody in an assay, as it provides greater selectivity for the cognate target than a polyclonal antibody, monoclonal antibodies are more expensive given their laborious method of synthesis.

Other uses of antibodies

This Technical Brief focuses on the properties, production and potential uses of antibodies, a more detailed discussion of their application in analytical chemistry will appear in future AMC Technical Briefs.

Further Reading

- E Harlow and D Lane. *Antibodies: A Laboratory Manual.* Cold Spring Habor Laboratory Press, USA; (1988); ISBN-10: 0879693142
- I Roitt, J Brostoff and D Male. *Immunology.* Mosby International Limited, UK; 5th edition (1998); ISBN 0 7234 2918 9.

This Technical Brief was drafted by Dr Susan Pang (LGC Ltd.) on behalf of the Analytical Methods Committee.

CPD Certification I certify that I have studied this document as a contribution to Continuing Professional Development.

Name…….....
Signature…….…….Date…….

Name of supervisor……..
Signature…….…….Date……