Serology techniques

I. NATURE OF AG-AB REACTIONS

A. Lock and Key Concept - The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains. X-Ray crystallography studies of antigens and antibodies interacting shows that the antigenic determinant nestles in a cleft formed by the combining site of the antibody as illustrated in Figure 1. Thus, Ag-Ab reactions are one of a key (*i.e.* the Ag), which fits into a lock (*i.e.* the Ab).

B. Non-covalent Bonds - The bonds that hold the Ag in the antibody-combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds. Multiple bonding between the Ag and the Ab ensures that the Ag will be bound tightly to the Ab.

C. Reversible - Since Ag-Ab reactions occur via non-covalent bonds they are by their nature reversible.

II. AFFINITY AND AVIDITY

Affinity - Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody (Fig. 6.)

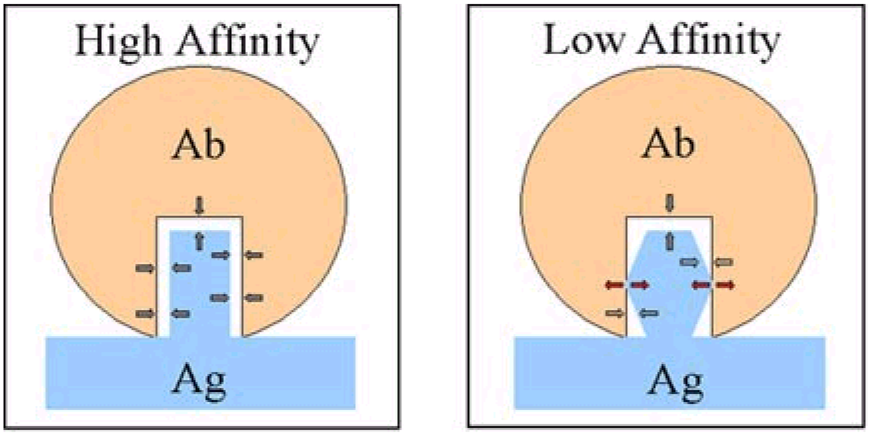

Figure 6 High and low Affinity.

-Most antibodies have a high affinity for their antigens.

Avidity - Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies.

Avidity is influenced by both the valence of the antibody and the valence of the antigen..

Affinity	**Avidity**
The strength of binding between a single antigenic determinant and an individual antibody combining site	The overall strength of binding between multivalent Ag's and Ab's.

III. SPECIFICITY AND CROSS REACTIVITY

A-Specificity -

•The ability of an individual antibody-combining site to react with only one antigenic determinant.

•The ability of a population of antibody molecules to react with only one antigen.

B-Cross reactivity –

•The ability of an individual Ab combining site to react with more than one antigenic determinant.

•The ability of a population of Ab molecules to react with more than one Ag(Fig. 7)

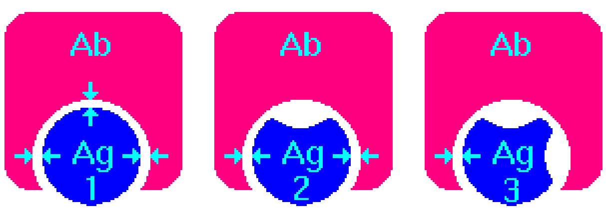

Figure 7. Crossreactivity.

-Crossreactivity can occur when two (or more) antigens share similar structural features.

Example: Consider three different antigens, (Ag1, Ag2 &Ag3). Antibody produced in response to Ag1 is very specific and would, therefore, have a large affinity constant (K) when combining with Ag1. However, Ag2 is similar in shape to Ag1 and is capable of interacting with anti-Ag1 antibody via two of three sites. The interaction between Ab and Ag2 is not as strong as the interaction between Ab and Ag 1 (i.e. K is much smaller) but is still strong enough to allow binding. Hence, Ag1 and Ag2 are said to *cross-react*. Ag3, in contrast, cannot interact very well with anti-Ag1 antibody and would have a K value so low that significant binding would not occur. Ag3, therefore, would not cross-react with Ag1.

-Crossreactivity also forms the basis for several diagnostic tests. For example, infection with *Treponema pallidum* (syphilis) causes the production of antibodies that cross-react with a substance found in cardiac muscle, cardiolipin.

Since it is much easier to obtain pure cardiolipin than pure *Treponemal* antigens, this cross-reaction is used to test for syphilis (Wassermann test). Likewise, antibodies produced against certain *Rickettsia* cross-react with antigens from *Proteus*. Since the latter are much easier to obtain, they can be used to test for the former.