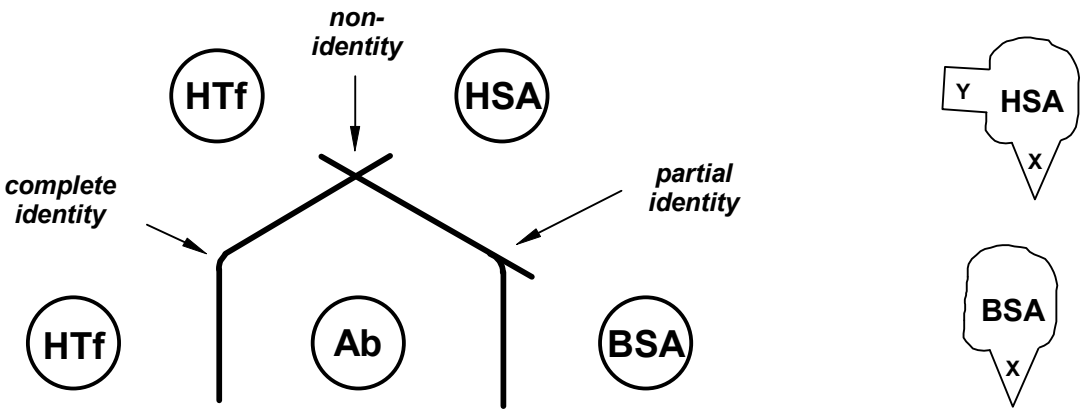
OUCHTERLONY ANALYSIS

The Ouchterlony assay was widely used in myriad research and clinical contexts for many years after its development in 1948, although it has now been largely superceded by other assays which are more sensitive and suitable for quantitation, including Radioimmunoassay (RIA) and Enzyme-Linked Immunosorbent Assay (ELISA). Nevertheless, this technique provides a very powerful tool for illustrating and clarifying the principles of antibody heterogeneity and specificity

In the Ouchterlony assay, solutions of an antigen and an antibody are placed in nearby wells cut out of a thin layer of agarose, and allowed to stand for a few hours or a day or two. During that time they diffuse toward each other, and where they meet they will form a *visible line of precipitation*.

The pattern in which adjacent lines cross one another yields considerable information about the antigenic relationships between different antigens. Let's illustrate this with a pattern generated by a *rabbit antiserum made against whole human serum*, using three purified protein antigens as targets, namely HSA (*human serum albumin*), BSA (*bovine serum albumin*) and HTf (*human transferrin*). The well labelled "Ab" contains the antiserum, and the resulting pattern is shown below.



Several conclusions can be drawn from the pattern of precipitation shown here:

1) The antiserum (in the well labelled "Ab") contains antibodies against *all three antigens*, since each one shows a precipitin line.

2) The lines produced by the two adjacent wells containing HTf join completely, in a pattern of **complete identity,** or simply "*identity*." This indicates that the antigens in the two wells (which in this case we know are exactly the same) are *antigenically indistinguishable* by this antiserum. We can't tell from the pattern whether the antiserum is detecting just one epitope or twenty, but we do know it is detecting *all* of them in both wells.

3) The two antigens HSA and HTf show a pattern of **non-identity** - the precipitin lines cross each other without joining at all. This indicates the

two antigens are *antigenically unrelated,* they have *no* epitopes in common which are recognized by this antiserum. This is not unexpected, since these two molecules are not related in structure or function.

4) The two antigens HSA and BSA show a pattern of **partial identity** - the lines join together, but not completely; there is a "spur" of the HSA line over the BSA line. This indicates that the two antigens are related, but not identical, with respect to this antiserum. More specifically, it means that there are at least *two epitopes* recognized by the antibody on HSA, one of which is also present on BSA. This is illustrated in the two cartoons on the right, showing HSA as bearing two epitopes ("X" and "Y"), while BSA bears only one of the two ("X").

We can be more precise by stating that there are two *classes* of epitopes that the antiserum detects on HSA, and only one of the two classes is present on BSA. That is, what is shown as epitope "X" in the cartoons might actually be, say, eight separate epitopes which are all present on *both* HSA and BSA, while what is indicated as "Y" might be three epitopes present *only* on HSA.