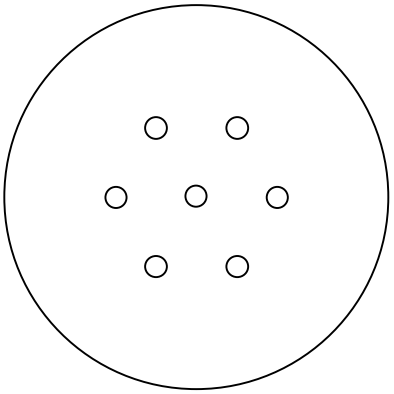
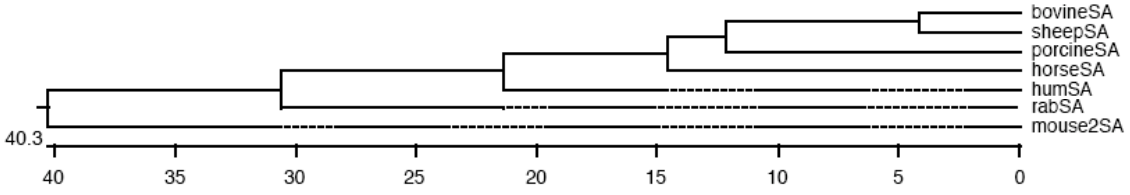
Using antigen-antiserum interactions in scientific problem solving

You keep a menagerie and are worried about West Nile Fever Virus (WNFV) infiltrating the ranks. You withdraw blood from three animals (either a cow, a horse and a sheep or a cow, a pig, and a sheep—groups to be assigned on Wed.) and let it coagulate. You then take the serum and put it in tubes labeled A, B, and C. A problem arises before you get around to testing for WNFV--the neighbor's goat eats the sheet that said which tube is which. You have available: antiserum from rabbits that recognizes bovine (cow) serum proteins (albumin, immunoglobulins etc.) and a phylogenic tree based on relatedness of serum albumin proteins. In your lab you also have double-diffusion plates (six wells for antigen around a single well for antiserum, each of which holds 12 μl of solution—see figure below), saline solution, and pipettors.



Phylogenetic tree based on sequence of serum albumin proteins
Length of lines is proportional to the time since the species diverged.



Questions to consider:

- How will you set up two plates to help you figure out which sample belongs to which animal?
- What patterns do you expect to see that will allow you to distinguish among the animals?

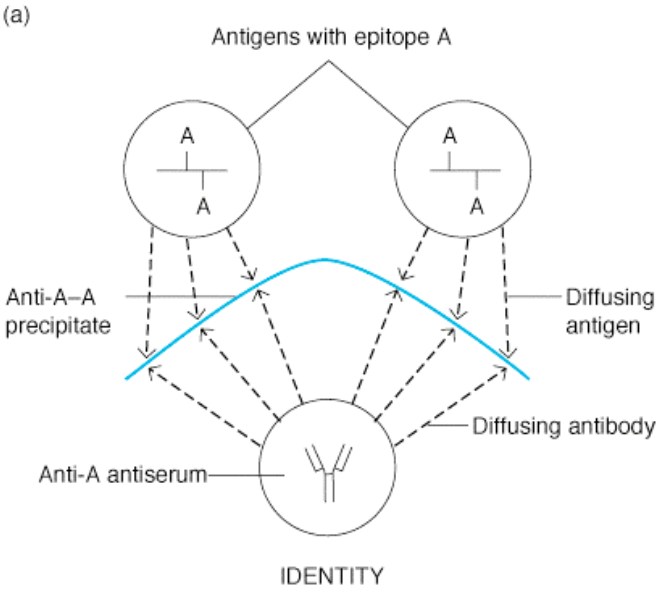
Ouchterlony double diffusion assay

Here's an explanation of double diffusion assays by Rod Langman at the Salk from a lecture he gave in 1999 (back when Kuby had a figure explaining these assays).

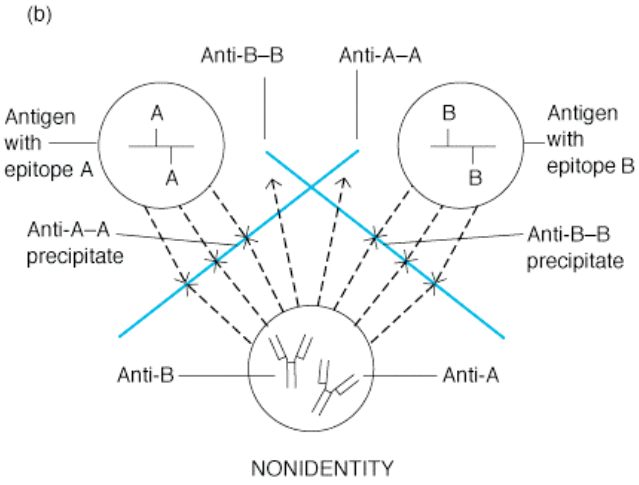
" Here I have to explain that the Ouchterlony double diffusion method is remarkably useful in sorting out relationships between antigens, epitopes and antisera.

The interpretation of the gels is simply based on the formation of lines of precipitation between antigen and antibody. These lines are impermeable to the reacting species. Only antibody that does not react with the antigen in the line can diffuse through.

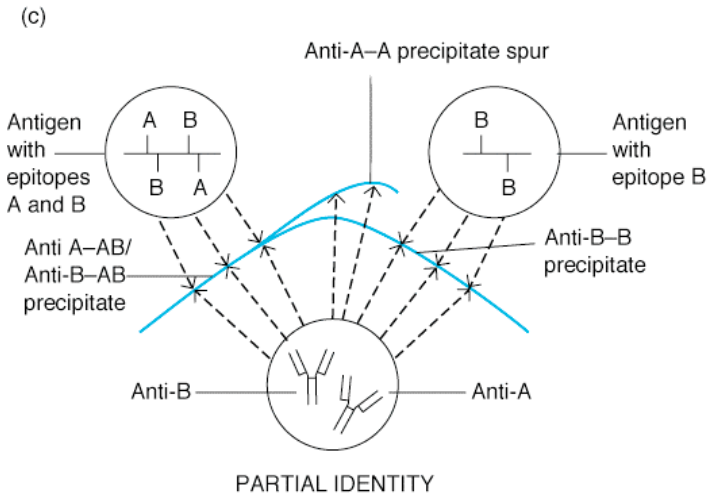
Here in Figure 6-8(a) the two antigen wells have the same antigen and antibody cannot diffuse through the line of precipitation.



Here in figure 6-8(b) there are two different antigens and the antibodies in the center well react with both, but the independence of the two lines indicate that the two antigens share not common epitopes.



Here in figure 6-8(c) there is a line of identity in the same curved shape as seen in (a) and in addition a "spur" linked to the left well which shows there is an antibody that can react with the left and NOT the right. " (Langman, 1999)



Langman, Rod (1999) BICD140 Immunology course: Lecture 11 http://www.cig.salk.edu/bicd_140_W99/lecture11.htm [Sept. 22, 2008]

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