

## Problems

Because of chemicals can be obtained from different companies or different lot numbers, their reactivity declines over time, and various other problems that can arise with these highly sensitive assays. The methods that we have listed for diluting the IgM anti-bodies, ExtrAvidin Peroxidase and ExtrAvidin Alkaline Phosphatase may need to be adjusted. If you are having problems (i.e. are not getting the color development that you expected or your color development is declining over time), running a checkerboard titration plate by making a plate full of one of the standard concentrations and then varying the concentrations of one of these antibodies across the rows and another antibody down the columns may give you a better dilution factor (see section 28-6.4 in the reference below for further details). Keeping and referring to the dilution factors found in the materials accompanying the chemicals may help you determine the concentrations for your titration experiment.

It is a good idea to make several plates full of the standard curve dried to the wells when you have a good sample that you are using as your standard, this will help in solving problems with the antibodies, problems when your standards are getting too old, and can help you calibrate a new standard sample.

Further details about these procedures may be found in:

**Wright, S. F. 1994. Serology and conjugation of antibodies. Chapter 28. Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. SSSA Book Series, No. 5. Soil Sci. Soc. Am., Madison, WI.**