Titration of reagents in the Zika IgM ELISA

When new reagents are received from either CDC or commercial sources, they may come with suggested working dilutions in the package insert. **These dilutions should be independently verified in your laboratory and the working dilutions should be adjusted as necessary.**

Before starting a test in your lab, make sure that you have the type of microtiter plate that is listed in the instructions (using different plates can cause problems), make sure that your buffers are made up fresh and correctly (check the pH's) and make sure none of the commercial reagents (especially TMB) have expired.

The best approach is to **perform a test using the control sera only** (ie no proficiency samples) **using the dilutions suggested on the packing insert.** Desirable results are where the positive control has an OD 450 of approximately 1.0, and the negative control has an OD of approximately 0.05-0.1. The negative should always be less than 0.2; the positive is likely to vary but should be >0.5.

If the results are in the desirable range, then no further adjustment is necessary. If the initial test does not yield desirable results, you should proceed with titration of the reagents.

Titrate the reagents one at a time in the following order: coating antibody, conjugate, antigen, positive control.

Titration procedure

Titrate the reagents using a dilution series one at a time in the following order: coating antibody, conjugate, antigen, positive control.

Coating antibody

Note: KPL goat anti-human IgM catalog #01-10-03 has given consistent results for years when used at 1:2000; therefore there should be no need to titrate it.

If however, there is a need, prepare 8 columns for the following: In duplicate, positive control on viral antigen; negative control on viral antigen; positive control on normal antigen; negative control on normal antigen. If you are using a different coating antibody than listed above, titrate

starting at a dilution of 1:500 in coating buffer. Add 150 µl of the starting dilution to the top wells and dilute serially down the plate for 6x2-fold dilutions (75 ul/well from the previous well + 75 ul coating buffer to make 2-fold dilutions). Run the ELISA according to the protocol using suggested working dilutions for the positive control, antigen, and conjugate. Using the results, choose a working coating antibody dilution that yields an OD reading of between 0.80 and 1.0 for the positive control serum on the viral antigen, and one that yields an OD of around 0.1 for the negative control serum. The positive control on the normal antigen should be well below that of the positive control on the viral antigen. Always use the **negative control** serum at 1:400. **Normal antigen** should always be used at the same dilution as the viral antigen.

Conjugate

With the coating antibody at the dilution determined above, follow the protocol using the working dilutions suggested for the positive control and antigen. Prepare 8 columns for the following: In duplicate, positive control on viral antigen; positive control on normal antigen; negative control on viral antigen; negative control on normal antigen. Prepare conjugate in blocking buffer at a 4-fold smaller dilution than the suggested working dilution. If no guidance is available use 1:500 as a starting point. Add 100 ul of the starting dilution to the top wells. Add 50 ul blocking buffer to all the other wells and serially dilute down the plate using 2-fold dilutions. For the conjugate working dilution, choose one that yields an OD of 0.8-1.0 for the positive control on the viral antigen, and one that yields an OD of around 0.1 for the negative control. The positive control on the normal antigen should be well below that of the positive control on the viral antigen.

Antigen

Using the coating antibody and conjugate at the dilutions determined above, the positive control used at the suggested working dilution, and the negative control at 1:400, follow the protocol to the antigen step. Prepare 8 columns for the following: In duplicate, positive control on viral antigen; negative control on viral antigen; positive control on normal antigen; negative control on normal antigen. Prepare viral and normal antigen in wash buffer at a 4-fold smaller dilution than the suggested working dilution of the viral antigen. Place 100 ul of the viral antigen in the top well of two positive and two negative control columns, and place 100 ul of the normal antigen in the top well of two positive and two negative control columns. Add 50 ul of wash buffer in the other wells. Serially dilute the viral and normal antigens down the plate using 2-fold dilutions. For the antigen working dilution, choose one that yields an OD of 0.8-1.0 for the positive control on the viral antigen, and one that yields an OD of around 0.1 for the negative control. The positive

control on the negative antigen should be well below that of the positive control on the viral antigen.

Positive control

If positive control *serum* is being used (ie a patient sample), it can be titrated to conserve undiluted stock or can be run at the same dilution as the test sera (1:400 in wash buffer). If the chimeric flavivirus positive control is being used, it is usually useful at 1:3000, but it can be titrated if necessary. Proceed with the protocol up to the serum addition step using all previously determined dilutions of other reagents so that the positive control can be titrated on the viral and negative antigens in duplicate. Start the positive control *serum* dilution series at 1: 100 (chimeric start at 1:750). Add 100 µl of diluted antibody to the top wells, 50 ul to the other wells, and serially dilute 2-fold down the plate. Finish the procedure and determine the optimum dilution as above.

Run the positive control in triplicate using the standard diagnostic protocol and the dilutions of the reagents determined above to make sure that the test is running correctly.