

## **ImmunoComb II (HIV 1 & 2 BiSpot)**

This outline is not intended to replace the product insert or your standard operating procedure (SOP).

### **Procedure**

1. Check the expiration date. Do not use expired kits.
2. Remove test kits and samples from the refrigerator. Incubate the developer plate in a 37°C incubator for 20 minutes or allow to stand at room temperature for at least 3 hours.
3. Mix the reagents in the developer plate by shaking. Do not remove the foil from the top of the developer plate, puncture the foiling using the perforator included in the kit.
4. Remove the aluminum pouch from the comb. All or part of a comb can be used at one time. However batching samples and running an entire comb in a single run is recommended.
5. Label each comb tooth with the appropriate identification.

### **Antigen-Antibody Reaction**

6. Pipet 50µl of sample into each test well of row A. Positive and negative controls are included in each the test kit and should be run on each comb.
7. Insert the teeth of the comb in row A. Withdraw and insert the comb in the wells several times to mix. Incubate the comb in row A for 10 minutes. Towards the end of the incubation, perforate the foil over row B.
8. At the end of the incubation period removed the comb from row A, absorb the liquid at the bottom of the comb teeth by touching the teeth to clean, absorbent paper. Do not touch the front surface of the teeth. This should be done each time the comb is removed from a reaction row.

### **First Wash**

9. Insert the comb into the wells of row B. Wash by agitating the comb up and down vigorously for 10 seconds. Repeat this several times over the 2 minute incubation period. Remove the comb from row B and blot the tips of the teeth as described at the end of step 8.

### **Binding of Conjugate**

10. Insert the comb into row C. Mix several times and incubate 10 minutes. Remove comb and blot tips.

### **Second Wash**

11. Insert the comb into row D, agitate repeatedly for 2 minutes. Remove the comb and blot teeth.

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### Third Wash

12. Insert the comb into row E, agitate repeatedly for 2 minutes. Remove the comb and blot teeth.

### Color Reaction

13. Insert the comb into row F, mix by pulling the comb in and out of the wells several times. Incubate 10 minutes, mixing every couple of minutes.

### Stop Reaction

14. Insert the comb row E, incubate 1 minute. Remove the comb, dispose of the developing plate and allow the comb to air dry.

### Test validation:

The **positive control** must produce three spots on the comb.

The **negative control** must produce an upper spot (internal control) and no others.

Each **sample** tested must produce an upper spot.

If any of these three conditions are not met the test results are invalid and the specimens and controls should be retested.

### Interpretation of Test Results (Samples):

The sole appearance of the upper spot indicates the specimen is negative for antibodies to HIV-1 or HIV-2.

Development of the middle spot indicates the presence of antibodies to HIV-2.

Development of the lower spot indicates the presence of antibodies to HIV-1.

High antibody titers to HIV-1 or HIV-2 can result in the development of a faint secondary spot. Co-infection with both HIV-1 and HIV-2 can result in the development of both the middle and lower spots with equal intensity.

The color developed on the comb is stable so the combs may be stored for later documentation.