SAS™ RSV*Alert* FAQ

1. **What are the most common causes of False Negatives?**
   - Applying specimen drop wise with an unqualified pipette delivery system may cause the following:
     - Too much specimen may be added to sample well which may cause flooding of the test and cause the antigen to flow around the test line area.
     - Too little specimen may be applied to the sample well. This will cause the background not to clear. Specimens near the minimal detectable threshold may not be observed.
   - Specimen antigen concentrations below the minimal detectable threshold of the SAS™ RSV*Alert* test
   - Inadequate specimen collection, such as over dilution
   - Improper specimen handling or transport
   - Specimens transported in unqualified transport media

   A negative test result does not rule out the presence of RSV. The results from the SAS™ RSV*Alert* test should be used in conjunction with other clinical findings to establish diagnosis.

2. **What are the common causes of Faint Lines?**
   - The addition of too much sample
   - Sample is near the cutoff value
   - Sample has a matrix affect

3. **I have placed 3-4 drops of the SAS™ RSV Control onto the test device and did not get a result.**
   It is recommended by the PI of the RSV controls to use the controls as a NP wash or aspirate specimens. The PI from the RSV kit recommends pipetting 150 µl from the specimen. The RSV Controls should be treated as a specimen; 150 µl from each bottle should be pipetted into the test device.

4. **The SAS™ RSV*Alert* PI states that I can use 3-4 drops of specimen after treating with extraction buffer; can I use a transfer pipette on untreated sample and apply 3-4 drops to the SAS™ RSV*Alert* test?**
   No, the treated sample is combined with an extraction buffer, which changes the density of the sample. The filtered tips provided with the extraction tubes are calibrated to deliver approximately 150 µl of sample in 3-4 drops when the specimen is combined with extraction buffer.
   At this time, untreated specimens should be delivered with a calibrated pipette capable of delivering 150 µl. The use of dropper pipettes may cause either flooding of the test or insufficient specimen to be added to the test.

5. **I have 2 bottles of RSV Extraction Buffer, should I use it with all patient specimens?**
   No, the RSV Extraction Buffer should only be used with bloody or mucoid specimens.
   Most patient NP washes and aspirate specimens can be applied directly to the SAS™ RSV*Alert* test.

6. **Specimen does not flow into cassette. How do I treat the patient specimen?**
   Repeat test by following PI instructions for mucoid or bloody specimens. Place 250 µl of specimen into extraction tube provided. Add 2 drops of RSV Extraction Buffer to specimen. Insert filter cap, mix and dispense 3-4 drops. Note: Mix vigorously with sonicator or tube vortex to break up heavily mucoid specimens.

7. **I do not have access to a sonicator to treat bloody or mucoid specimens, what is my alternative?**
   You can use a laboratory vortex as substitute. Specimens should be vortexed vigorously in conjunction with the extraction buffer.
   Alternatively, vigorous agitation by hand may be used.

8. **Can the SAS™ RSV*Alert* Test detect both viable and nonviable RSV viruses?**
   Yes, the SAS™ RSV*Alert* test can detect both viable and nonviable RSV viruses.

9. **Will the SAS™ RSV*Alert* Test detect other viruses (i.e. Influenza, Adenovirus, etc.) that are associated with respiratory infections?**
   No, the SAS™ RSV*Alert* Test is not intended for confirmation of a respiratory infection caused by etiological agents other than RSV.

10. **The Respiratory Screen DFA I am using is positive, but the SAS™ RSV*Alert* Test is negative, which test is correct?**
    A Respiratory Screen DFA is not specific for a single virus causing respiratory infections. It usually consists of a mixture of reagents, which detect Influenza A and B, Adenovirus, Pneumovirus and RSV. The SAS™ RSV*Alert* Test is specific for RSV antigens only.

11. **The RSV EIA I am using is positive for RSV and the SAS™ RSV*Alert* test is negative, which result is correct?**
    - It is recommended that all negative SAS™ RSV*Alert* results be confirmed by cell culture or equivalent method. Cell culture is the currect Gold Standard for RSV detection.
    - A direct comparison of an EIA and SAS™ RSV*Alert* test cannot be made due to following:
        - EIA uses washing to remove non-specific binding antigens, longer incubation times and enzyme kinetics. Enzyme kinetics enhances detection of low-level antigen concentrations.
        - SAS™ RSV*Alert* test is a particle based assay. In low-level antigen concentrations, insufficient antibody antigen sandwich binding may occur making a line undetectable by the human eye.

12. **The PI recommends that all negatives should be confirmed by cell culture. We do not currently use any additional means to confirm negatives; can I still use the test?**
    This is a recommendation that is given by the FDA. Each lab should follow its own established guidelines to determine if negatives should be confirmed by cell culture.

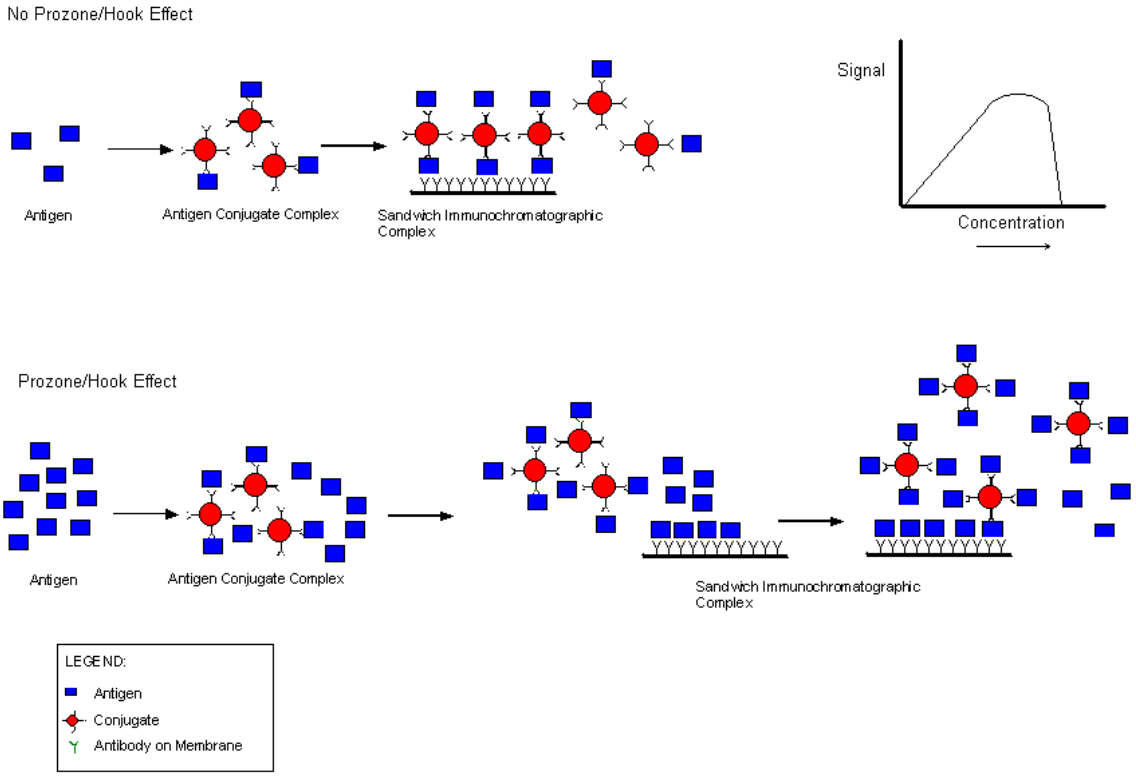
13. **What is the Prozone or Hook Effect?**
    Prozone or Hook Effect is the condition by which large quantities of antigen in an immunoassay system impair antigen-antibody binding, resulting in low antigen determination.

Prozone/Hook Effect FAQ

1. **What is a Prozone/Hook Effect?**

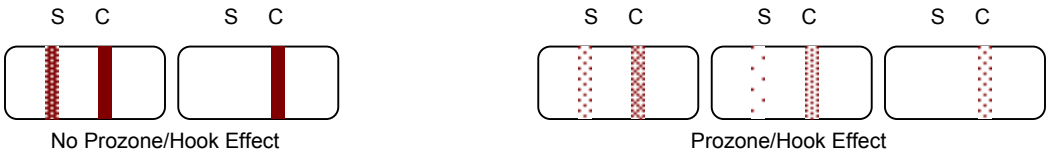
Prozone/Hook Effect occurs when excess antigen binds the antibody at the test line, therefore not allowing the binding of the conjugate-antigen colored complex to the antibody on the test line (See Drawing Below). Light specimen and control lines characterize an assay having a Prozone/Hook Effect.

This phenomenon is mainly found in immunoassays in which three components (antigen, conjugate, and capture antibody) are incubated together. Dilution of the antigen sample with saline or PBS will enhance the signal intensity.



2. How do you recognize a Prozone/Hook Effect in an immunochromatographic assay?

Prozone/Hook Effect is characterized by either a light or non-existent test (specimen) line and a light control line. See illustration below.



3. Can you correct for Hook Effect?

Yes, simply dilute the sample 1:2 or 1:4 with PBS or Saline, and perform the assay once again.

4. Are references available to read more on Prozone/Hook Effect?

Yes, please see list below.

1. Butch AW. [Letter]. Clin Chem 2000: 1720-1721.
2. Dahlmann N et al. "Hook Effect" In a Patient with a Gonadotropin-Secreting Tumor [Letter]. Clin Chem 1990; 36: 168.
3. Diamandis EP and Christopoulos TK. Immunassay. New York, pp. 230-231
4. Ermens AM, et al. Dilution Protocols for Detection of Hook Effects/Prozone Phenomenon [Letter]. Clin Chem 2000; 46: 1719-1720.
5. Hussa RO. The Clinical Marker hCG. West Port, Conn. , 1987.
6. Wu JT, Christensen SE. Effect of different test designs of immunoassays on "hook effect" of CA 19-9 measurement. J. Clin Lab Anal 1191; 5:228-32.