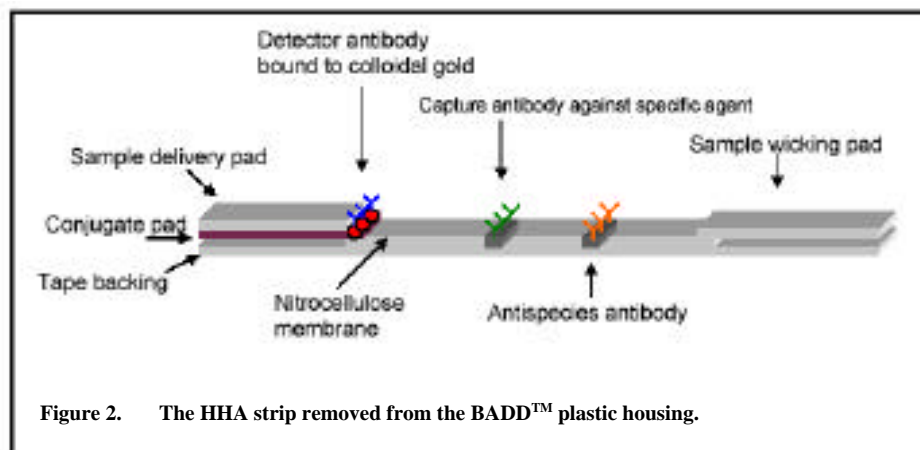
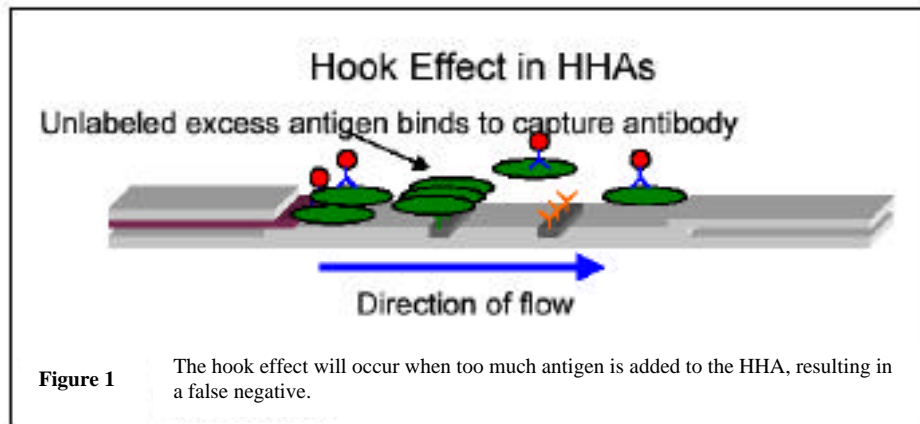


## HOOK EFFECT

**Understanding what hook effect is and why Advnt's test devices are best for protecting you from this serious problem.**

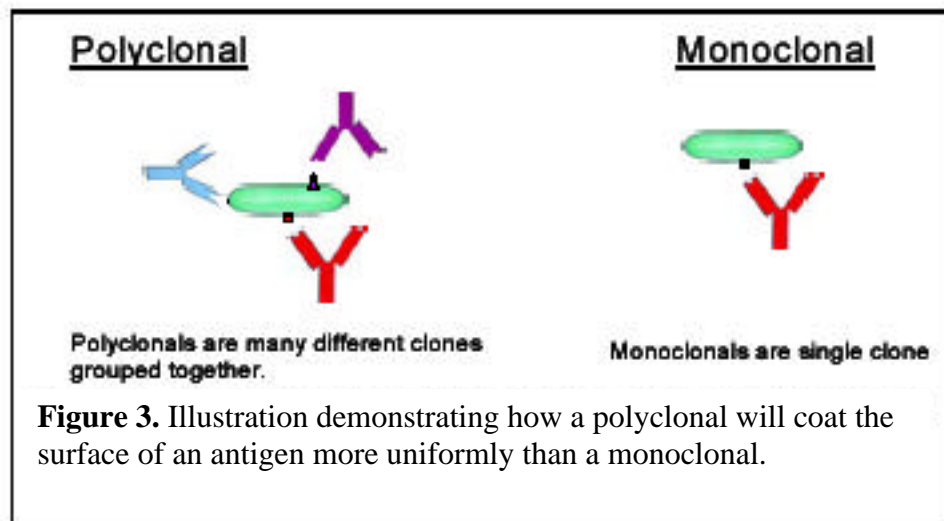
**UNDERSTANDING HOOK EFFECT:** The hook effect (**Figure 1**) occurs when too much antigen (*for example anthrax*) is added to the Hand-Held Assay (HHA), which then results in a false negative. What occurs is the amount of antigen (*anthrax*) exceeds the finite amount of colloidal gold antibody, or colored labeling material. The excess unlabeled antigen (*anthrax*) migrates across the membrane more rapidly than the heavier color-labeled antigen (*anthrax*), thus saturating or binding all the binding sites on the capture antibodies. When the color-labeled antigen arrives, there are no binding sites remaining, so it simply continues on to the wicking pad at the end of the test device. (**Figure 2**) Since no binding sites were available, the colored antigen cannot create the colored test line that would represent a positive result. This, in turn, presents the user with a false negative result (hook effect) even though anthrax is present. A false negative is a serious concern for those of you on the front line. Its effects could be catastrophic. The Advnt test does not produce hook effect.



## WHY the Advnt's product line DOES NOT create the Hook Effect phenomenon.

HHA's exploit the exquisite sensitivity and specificity of antibodies to detect and differentiate microorganisms. These antibodies are able to physically grab on to a portion of an antigen with their antigen-binding site. Two categories of antibodies are typically used in immunoassays:

- Polyclonal antibodies (PAB's) - Polyclonals represent a population of many antibodies which bind to numerous different antigens (epitopes) (Figure 3). Polyclonal antibodies are typically used for immunoassays because of their ease-of-production and their superior sensitivity. What makes polyclonal antibody assays more sensitive is that they can cover the surface of a complex antigen, such as a microorganism, more uniformly, thus improving the detection capability. The more binding sites available when the antigen flows through the device, the better chance that the color-labeled antigen will have something to bind to, providing the user with a positive result when (*in this case*) anthrax is present. The Advnt product line utilizes these same polyclonal antibodies in each of our test products.
- Monoclonal antibodies (MAB's) - Monoclonals represent a single type of antibody, which bind to only one specific antigen (epitope) (Figure 3). Careful screening and selection of a monoclonal antibody can achieve a high degree of sensitivity and specificity against a specific biological agent. However, monoclonal antibodies can bind to only one type of epitope on the surface of the cell, increasing the possibility of reducing the level of coating. The potential then exists to give up a certain level of sensitivity, and when too much antigen is introduced, the possibility of a hook effect increases.



## Why the Advnt's product line works better:

1) There are several critical parts in making a lateral flow diagnostic test. One of them is the development and selection of the antibodies used in the test. The Advnt product line has years of research and development behind each assay. We spent a considerable amount of time and money developing a method to screen for reagents that gave highly reactive and VERY SPECIFIC reactivity. This was an arduous process that included screening for what the antibodies were to bind to and what the antibodies were not to bind to. The results were reagents far superior to anything on the market to date. It is for this reason that organizations, such as the Department of Defense, use our reagents in their own systems... Reagents are the first key ingredients to produce a superior test.

2) The next critical part of developing our tests was to select a combination of materials that synergistically work well together for the particular test. These materials were selected by screening for performance, and after years of research, we feel we have developed the most robust test on the market today... regardless of cost of materials.

3) The selection of the label material is crucial to the sensitivity of our assay. We spent years developing our own in-house method to produce a colloidal gold material superior to anything on the market...a gold that provides little or no cross-reactivity, better performance, and better stability.

4) While we have access to some of the best monoclonals in the world for BioWarfare agents, none of our current products use them. Why? First to avoid the hook effect. A hook effect is a phenomenon whereby too much target material is present such that all of the antigen binding sites are full, and therefore the antigen cannot effectively cross-line the labeled antibody to the capture antibody. By using affinity-purified polyclonal antibodies, we are able to target more of the antigen, thereby giving us higher sensitivity levels, and since a polyclonal antibody prep contains thousands of different epitope binding antibodies that bind at different constants (Kd), the swamping of the system becomes nearly impossible. This selection of using a polyclonal for labeling and binding dramatically reduces the likelihood of a false negative event from occurring, but we also use 1-2 micrograms of antibody per test. More antibodies mean more binding sites present which translates to a further reduction in the potential development of a hook effect. Thus, like other products on the market, you will not have to take another step to dilute the material to avoid a hook effect, which can add to operator error, contamination, and test complexity.

5) One of the most exciting attributes of our tests is the fact that we DO NOT require a reader or other electronic device to interpret results. We spent years optimizing each test to be read by the naked eye at the least concentration reasonably possible. Although a reader may provide even better sensitivity with our own tests, we do not feel one is needed for the stated intended use. Additionally, if a reader or electronic device gets a positive hit, or even if it receives a false positive, the reader will then need to be immediately removed for decontamination. Readers and electronic devices are expensive; they increase the complexity of the test, require maintenance and are another source for error. The Advnt tests were designed to be simple, easy-to-use, cost-effective, disposable and robust.

We hope that these explanations will help you and your team better understand Hand-Held Assays. Should you have any other questions, please let us know. We are always eager to help. Thanks for your continued interest.