

Translational Medicine Tutorial

Reducing Errors in Immunoassay Testing

Interference Blocker Can Be Used for Both ELISA and Lateral Flow Formats

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Immunoassays used for human in vitro diagnostics (IVD) often use animal-sourced antibodies to recognize specific disease markers. A small percentage of individuals, however, may have antibodies in their blood that could react with the animal antibodies in the diagnostic assay and thereby interfere with detection of the disease markers causing a false positive result. Such an interfering antibody is termed a heterophilic antibody (HA).

Due to the wide use of mouse monoclonal antibodies in diagnostic applications, the most well-known HA interfer-

ence is a result of HAMA (human anti-mouse antibodies). Rheumatoid factor (RF), an autoantibody that reacts with the patient's own immunoglobulin (Ig), may also cross-react with animal Ig

resulting in RF interference, which is similar to HA/HAMA interference.

Much like HAMA, HA to other animals such as goat (HAGA), sheep (HASA), and rabbit (HARA) may cause false results, especially when antibodies originating from such animals are used in immunoassays.

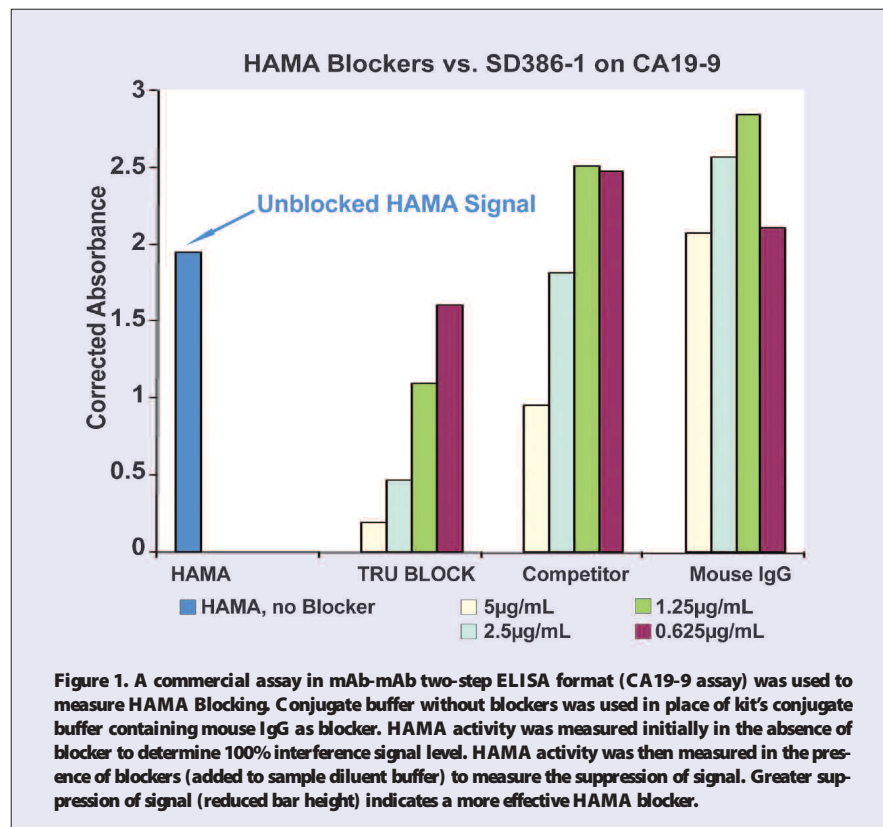
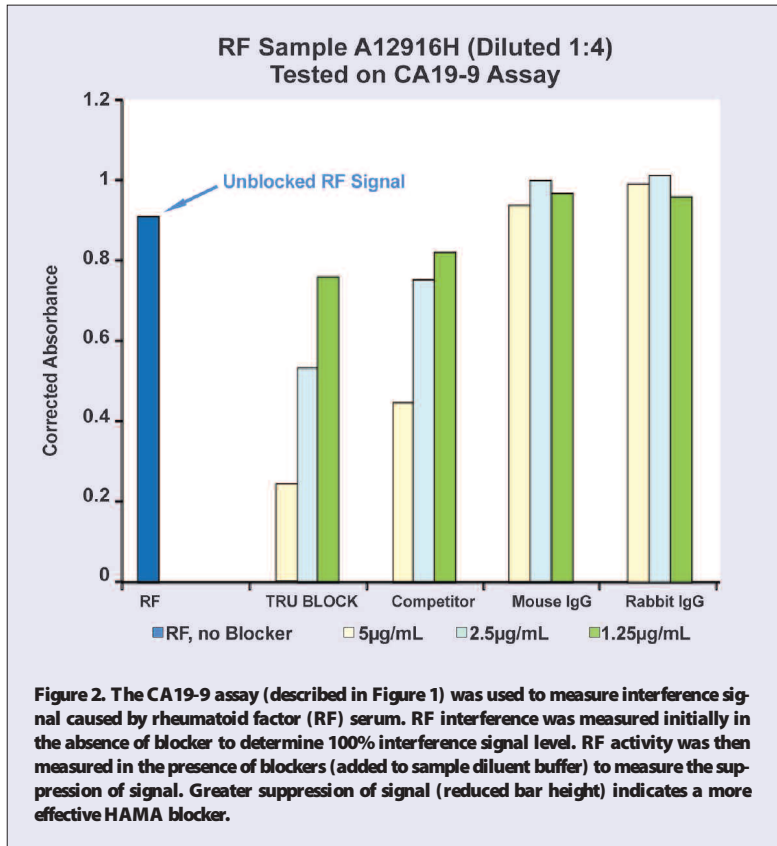


Figure 1. A commercial assay in mAb-mAb two-step ELISA format (CA19-9 assay) was used to measure HAMA Blocking. Conjugate buffer without blockers was used in place of kit's conjugate buffer containing mouse IgG as blocker. HAMA activity was measured initially in the absence of blocker to determine 100% interference signal level. HAMA activity was then measured in the presence of blockers (added to sample diluent buffer) to measure the suppression of signal. Greater suppression of signal (reduced bar height) indicates a more effective HAMA blocker.

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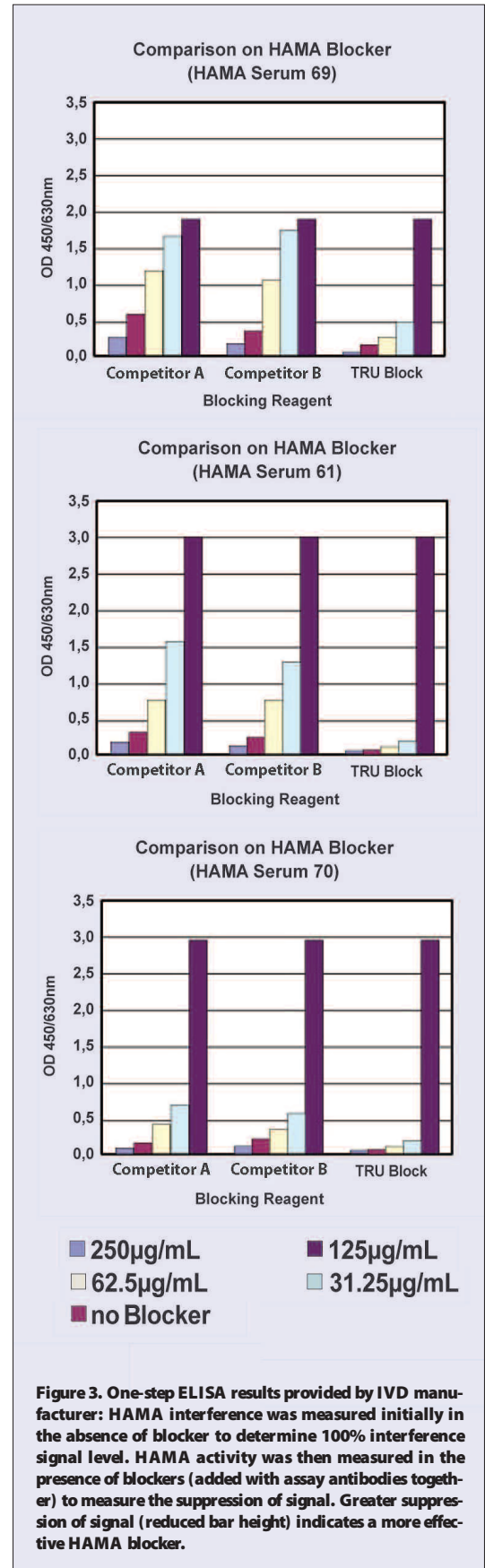
Although the frequency of these interferences is low, the false positive results have a significant negative impact on the quality and competitiveness of diagnostic assays as well as on the lives of those individuals who have been falsely diagnosed.

IVD manufacturers often use mouse serum or mouse Immunoglobulin G (IgG) to block HAMA interference in their assay. This traditional method has several known limitations including:

- Mouse IgG can block HAMA interference but not other types of HA interference.
- Mouse IgG does not block RF interference.
- Mouse IgG can only block HAMA interference passively,

by being competitively bound to a HAMA molecule at the same affinity as the assay antibodies. As a result, much higher concentrations of mouse IgG (often on the order of 10 times or higher than the concentration of the assay antibodies) are required to show sufficient blocking. This can present a challenge in miniaturized immunoassays where reduction in the amount of assay components is desired.

- Mouse IgG alone also presents a significant limitation due to the need for broader coverage against various HA/RF interferences in IVD immunoassays.



Meridian Life Science has developed an active HAMA blocker called TRU Block™. TRU Block employs an active blocking technology that results in a higher blocking efficiency than conventional HAMA blockers such as mouse IgG. TRU Block also provides broad coverage against other HA and RF interferences.

The blocking effectiveness of TRU Block was recently evaluated against mouse IgG and a well-known HA blocker. The results indicate that TRU Block can outperform both mouse IgG and the other HA blocker in immunoassays.

Some examples of recent HAMA and RF blocking studies are shown in Figure 1 and Figure 2, respectively. These studies were done using a two-step mAb-mAb ELISA with the blockers added into a sample diluent buffer. IVD manu-

facturer's data on TRU Block vs. other interference blockers in a one-step ELISA format is shown in Figure 3.

TRU Block has also been successfully used in a lateral flow rapid test assay format where it was dried down as a stripe between the sample pad and assay antibody location.

Summary

TRU Block has shown a performance advantage over various interference blockers currently on the market. TRU Block has been successfully applied in IVD assays using both ELISA and lateral flow formats. The enhanced effectiveness of TRU Block allows IVD manufacturers to add this active blocker at reduced concentrations to achieve HAMA blocker cost reductions as well as broader coverage against HA/RF. ■

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