Reducing False Results in Immunoassay Testing

Immunoassays used for human in vitro diagnostics (IVD) typically use animal-derived antibodies to recognize specific disease markers. Some patients have antibodies in their blood that can react with animal antibodies in the immunoassay and cause a false result. Although the frequency of these interferences is low, false-positive results have a significant negative impact on the quality and competitiveness of a diagnostic assay as well as on the lives of those individuals who have been falsely diagnosed.

The most well-known antibody interference is HAMA (human anti-mouse antibodies), which is due to the wide use of mouse monoclonal antibodies in diagnostic applications. However, HAMA only represents one type of heterophilic antibody (HA) interference - others include HA to animals such as goat (HAGA), sheep (HASA), and rabbit (HARA) which can cause false results when antibodies originating from these animals are used in immunoassays. In addition to HA there is another class of interference called Rheumatoid factor (RF), which is an autoantibody that reacts with the patient's own immunoglobulin (Ig) and can cross-react with animal Ig, similar to HA/HAMA interference.

In order to reduce HA, HAMA and RF assay interference, IVD manufacturers often use animal serums or purified animal IgG which can bind to low cross-reactive antibodies species against the same animal of origin.

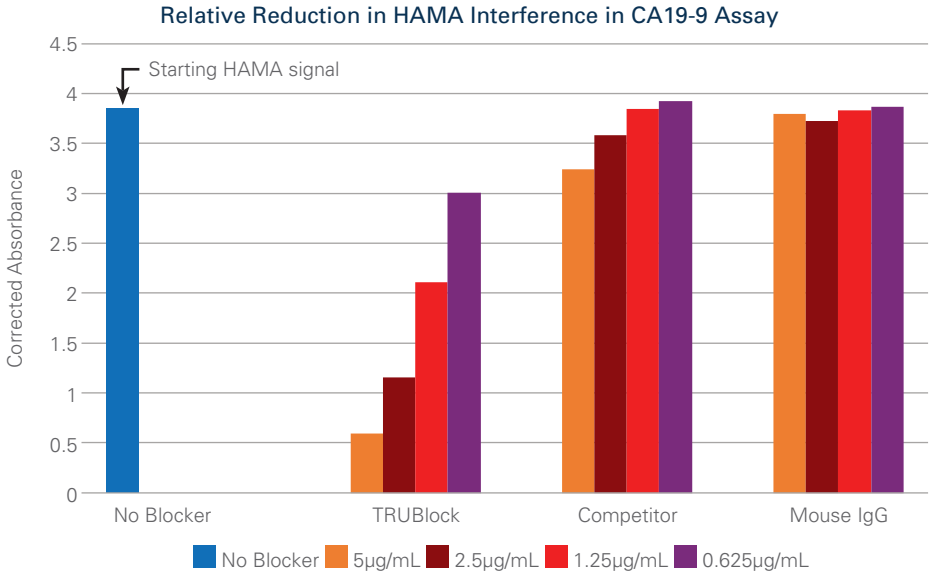

Figure 1: A double mouse monoclonal sandwich CA19-9 assay was used to measure the effectiveness of TRU Block, Mouse IgG and a competitor HA blocker in blocking HAMA interference from a commercially sourced patient sample (SD386-15). HAMA activity was measured in the absence (no blocker) and in the presence of blockers (TRU Block, Competitor, Mouse IgG) at various concentrations (0.625 µg/ml, 1.25 µg/ml, 2.5 µg/ml and 5 µg/ml). Greater suppression of the HAMA signal with no blocker (blue) indicates greater HAMA blocking effectiveness.

One example is mouse serum, or Mouse IgG, which is used to block HAMA interference. Although Mouse IgG can be effective in blocking HAMA, it also has several limitations including:

- Narrow coverage, only blocks HAMA, not other types of HA interference
- Does not block RF interference
- Requires high concentrations to sufficiently block interference
- Mechanism of action relies on passive blocking techniques

Mouse IgG and other IgG's passively block HA interference by competitively binding to an HA molecule at the same affinity as the assay antibodies and they must be used in high concentrations. Often their concentration must be more than ten times higher than the concentration of the assay antibodies and this can have a negative impact on the assay signal. It can also present a challenge in miniaturized immunoassays where a reduction in the amount of assay components is desired.

An immunoassay blocker, called TRU Block™, has been developed to remove HA, HAMA and RF interference using a completely new approach. TRU Block contains a specialized binder that is directed against antibody interference and is able to bind to HA, HAMA and RF with high affinity. Once TRU Block is bound to the assay interference, it prevents the interference from binding to other antibodies in the assay through steric

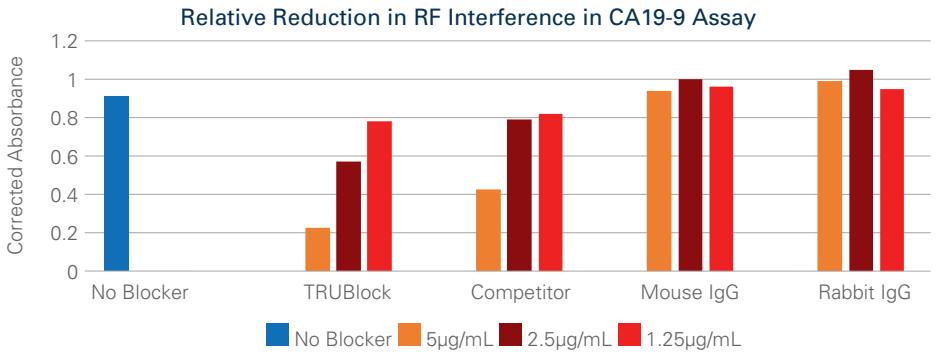

Figure 2: A double mouse monoclonal sandwich CA19-9 assay was used to measure the effectiveness of TRU Block, Mouse IgG and a competitor HA blocker in blocking RF interference from a commercially sourced patient sample (A12916H). RF activity was measured in the absence (no blocker) and in the presence of blockers (TRU Block, Competitor, Mouse IgG and Rabbit IgG) at various concentrations (1.25 mg/ml, 2.5 mg/ml and 5 mg/ml). Greater suppression of the RF signal with no blocker (**blue**) indicates greater RF blocking effectiveness.

hindrance mechanism. Performance advantages of TRU Block include broader coverage against all types of HA inference and RF, the ability to be used at a low concentration, and better blocking efficacy compared to Mouse IgG.

The blocking effectiveness of TRU Block has been evaluated against Mouse IgG and a well-known HA blocker using both two-step (Figure 1) and one-step (Figure 2) double mouse monoclonal ELISAs with blockers added to the sample diluent buffer. The results indicate that TRU Block can outperform both Mouse IgG and the other blocker in ELISA-based immunoassays. TRU Block has also been successfully used in chemiluminescent assays and lateral flow rapid tests where TRU Block was dried down as a stripe between the sample pad and assay antibody location.

SUMMARY

Heterophilic antibody interference blockers are an essential part of clinical diagnostic assays, and their importance has been documented by dozens of case studies where approved assays resulted in misdiagnosis (Bolstad, N. et al, 2013). In selecting a blocker, it is important to consider the source of the antibodies used in the assay and the types of heterophilic interference that could impact the assay. TRU Block™ is a unique HA, HAMA and RF interference blocker that can be used in ELISA, LF and chemiluminescent assay formats. It has several performance advantages over other heterophilic antibody interference blockers on the market and when used in an immunoassay, can prevent false positive and false negative results.

Bolstad, N., Warren, D. J. & Nustad, K. Heterophilic antibody interference in immunometric assays. *Best Pract Res Clin Endocrinol Metab* 27, 647–661 (2013).

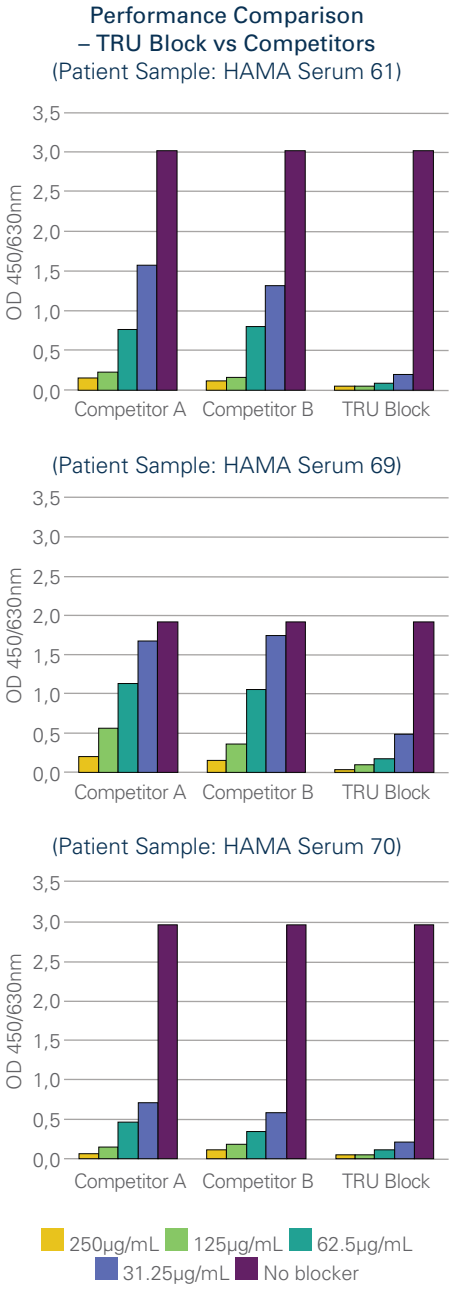

Figure 3: Customer (IVD manufacturer) One-step ELISA results: HAMA interference for three different samples (HAMA Serum 61, Hama Serum 69 and HAMA Serum 70) was measured initially in the absence of blockers to determine 100% interference signal level. HAMA activity was then measured in the presence of blockers (added with assay antibodies together) to measure the suppression of signal. Greater suppression of signal (reduced bar height) indicates a more effective HAMA blocker.

Product	Cat Number	Protein Concentration	Application
TRU Block Ready	8001	Single-step dilution with recommended dilution of 1:1000 to 1:10	ELISA & LF
TRU Block ULTRA	8000	Range: 24 - 26 mg/mL	ELISA, CLIA & LF
TRU Block	A66800H	Range: 24 - 26 mg/mL	ELISA
TRU Block 2	A66802H	Range: 24 - 26 mg/mL	ELISA, CLIA & LF
TRU Block 3	A66803H	24.3 mg/mL	ELISA, CLIA

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