

# TRU BLOCK: A Novel HAMA Blocker for Reduction of Immunoassay Interference



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## ABSTRACT

Mouse monoclonal antibodies (MAbs) are widely used in immunoassays and because of this such assays are susceptible to interference factors that interact with mouse immunoglobulins found in the test sample. One of the most common interference factors in immunoassays is human anti-mouse antibodies (HAMA). Mouse IgG is often used as a HAMA blocker but it acts as a passive blocker and requires large quantities that may be as much as 10 times the antibody reagent concentrations employed in the assay.

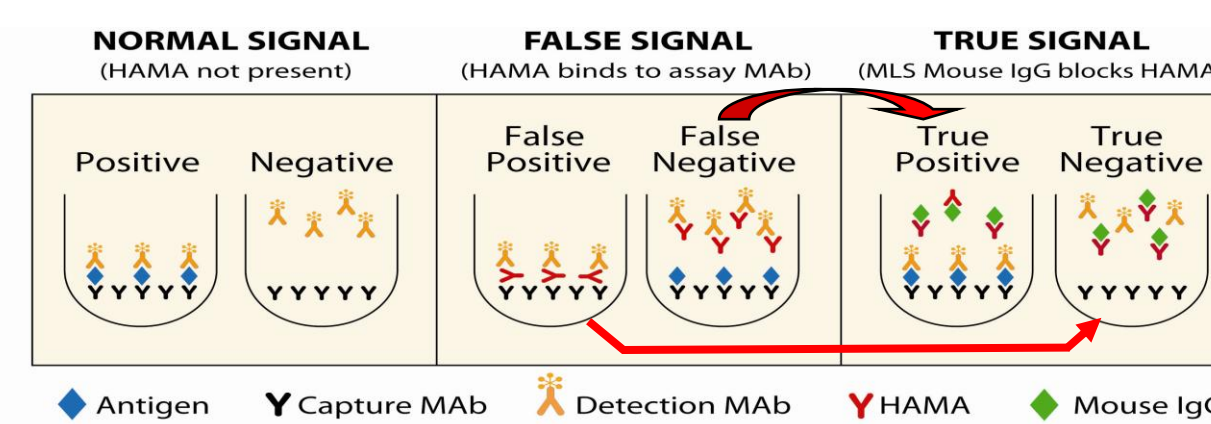
Meridian Life Science, Inc. has developed a novel HAMA blocker called TRU Block. TRU Block employs an active blocking mechanism that results in a much higher blocking efficiency as compared to conventional HAMA blockers such as mouse IgG. We studied the HAMA blocking effectiveness of TRU Block vs. mouse IgG and a well known active HAMA blocker on the market. Two commercial immunoassays measuring CA 19-9 (a tumor marker) and follicle stimulating hormone (FSH) were chosen for the blocker evaluations. The mouse IgG normally included in the original assay was removed to allow observation of the HAMA signal when performing the assay using several commercially available HAMA samples.

The results indicate that TRU Block performed more effectively as a HAMA blocker at a much lower concentration than mouse IgG. TRU Block was also equivalent to or better than the commercially available active blocker on all HAMA samples tested. In addition, TRU Block did not interfere with specific assay signal. The enhanced effectiveness of TRU Block allows this HAMA blocker to be added to immunoassays at greatly reduced concentrations as contrasted to conventional blockers making it especially useful in miniaturized immunoassays where reduction of assay components is desired.

## Introduction: HA Interference

- Heterophilic Antibody (HA), some times called Heterophile Antibody, is a group of Human Antibodies against animal IgG.
- HA interference is a well known interference factor in various Human diagnostic assays, often causing false positive or false negative results.
- Due to the wide use of Mouse Monoclonal Antibodies, the most well known HA interference is HAMA (Human Anti-Mouse Antibodies).
- The frequency of such interference is low, but the false results have significant negative impact on the quality and competitiveness of the diagnostic assays as well as on the lives of those individuals who have been falsely diagnosed.
- Similar to HAMA, HA to other animals such as Goat (HAGA), Sheep (HASA), Rabbit (HARA), etc. may cause false results when antibodies originating from such animals are used in immunoassays.
- It is difficult to clearly differentiate HAMA vs. other types of HA interference, especially due to known cross reactivity. A collection of HAMA samples may have other types of HA interference.

## Example of HAMA Interference



- The blocking mechanism of typical HAMA Blocker mouse IgG is shown above.
- The blocking mechanism of TRU Block is very different and is proprietary.

## Commercial HAMA Samples

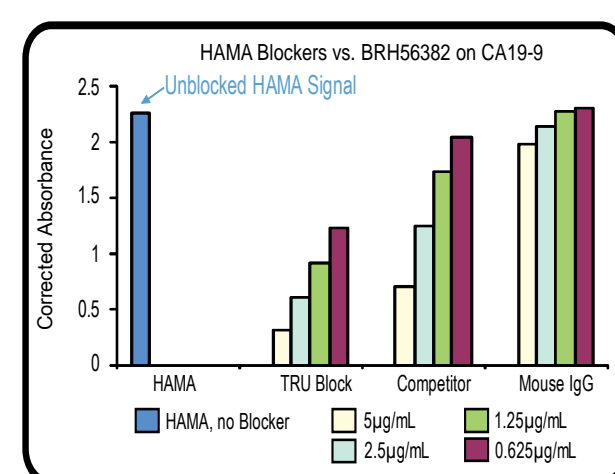
- Scantibodies, Inc.**  
Various HAMA Samples tested by MLS  
Samples are individual Natural Human HAMA
  - BioReclamation, Inc.**  
Various HAMA samples tested by MLS  
Samples are individual Natural Human HAMA
  - Roche**  
Information obtained by conversation with Roche technical support.  
This information is not stated on Roche HAMA sample spec sheet.
- Type I.** Normal Human Serum Pool spiked-in with Roche proprietary HAMA mimic from undisclosed species.
- Type II.** Normal Human Serum Pool spiked-in with proprietary HAMA mimic material of animal origin.
- Roche Blocker MAK-33 is known to work well on blocking Roche HAMA Type I & II. However, our observations are such that the HAMA mimic materials do not correlate well with HAMA serum of natural origin. Drawing conclusions on blocker performance using Roche HAMA Type I and II may not represent real life situation. HAMA samples from natural human origin may exhibit much different properties (affinity, specificity, etc.) than the HAMA mimic material.
- MLS encourages evaluation using your own collection of multiple real patient's HA and HAMA samples and avoid drawing conclusions from a single sample or from a few samples. When not available, purchase of HAMA samples from Scantibodies, Inc. and BioReclamation is recommended.

## TRU Block Performance Data

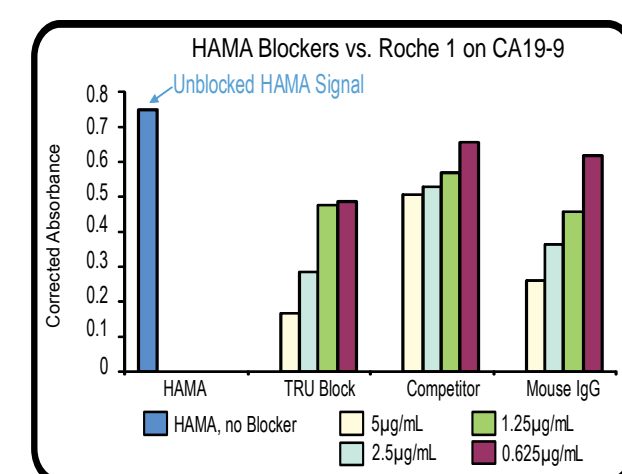
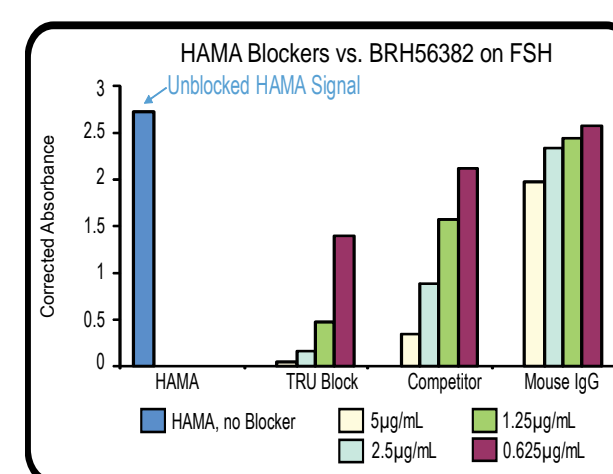
### Functional Assay Principle:

- Two commercial assays: CA 19-9 and FSH (with the assays modified as noted below)**
- Buffer lacking mouse Ig was used in place of kit buffer which contains mouse IgG as blocker.
  - HAMA blockers were mixed with sample diluent buffer.
  - Six different HAMA-reactive sera were tested.
  - Three different HAMA blockers were used separately at 4 different concentrations.
  - HAMA activity was measured initially in the absence of blocker to determine 100% signal.
  - HAMA activity was then tested in the presence of blocker(s) to measure suppression of signal.
  - Interpretation: The greater the suppression of HAMA signal the more potent the blocker.

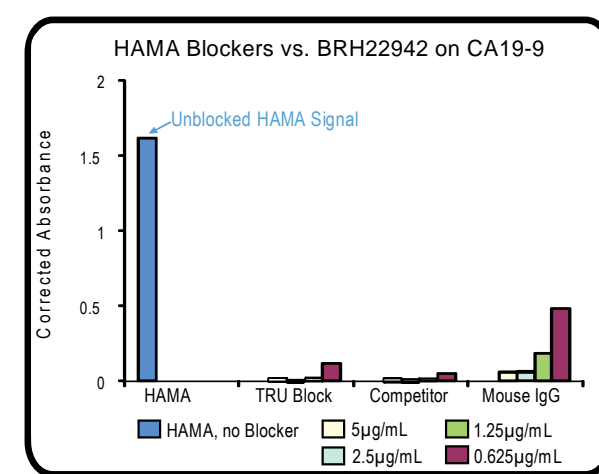
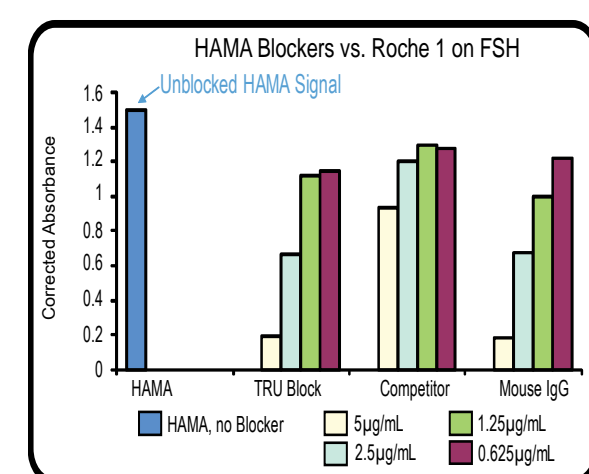
HAMA sera sources & dilution factor used		Blockers tested	
<b>Roche:</b>	Roche HAMA Type 1	1:10 dilution	
<b>Scantibodies:</b>	SD386-1	1:10	<ul style="list-style-type: none"> <li>Competitor's Active Blocker (Named as Competitor in data)</li> <li>TRU Block, Active Blocker (Named as TRU Block in data)</li> <li>Mouse IgG, passive Blocker (Named as Mouse IgG in data)</li> </ul>
	SD386-15	1:10	
<b>BioReclamation:</b>	BRH22942	1:10	
	BRH56382	1:10	
	HMSRM-DNR	1:10	



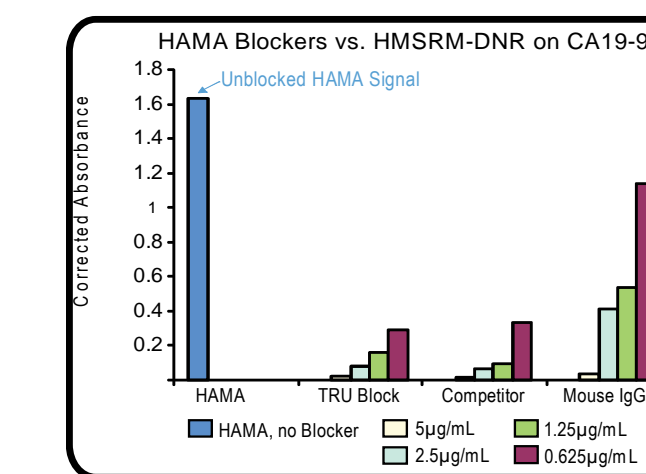
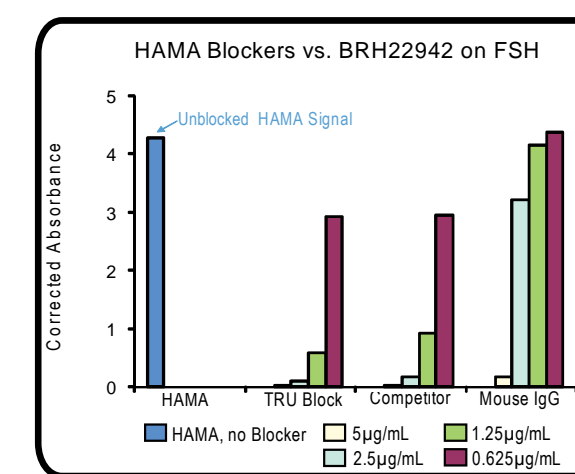
**Conclusion:** Based on CA19-9 and FSH Assays, TRU Block outperforms competitor's active blocker on BRH56382 HAMA sample.



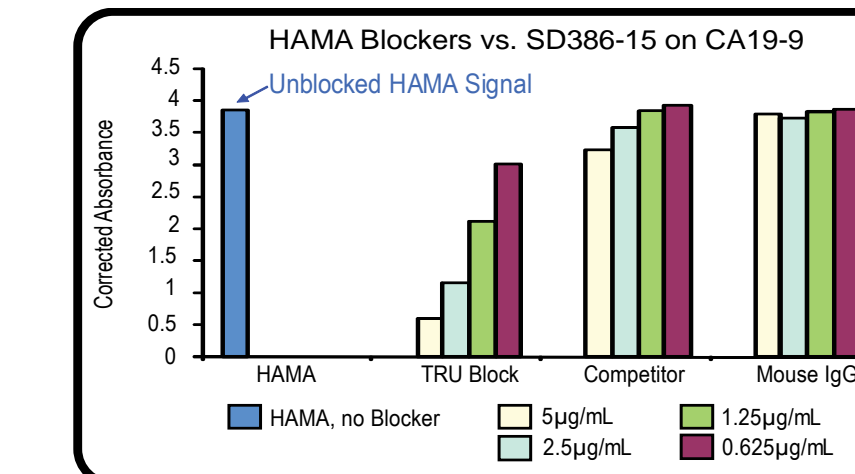
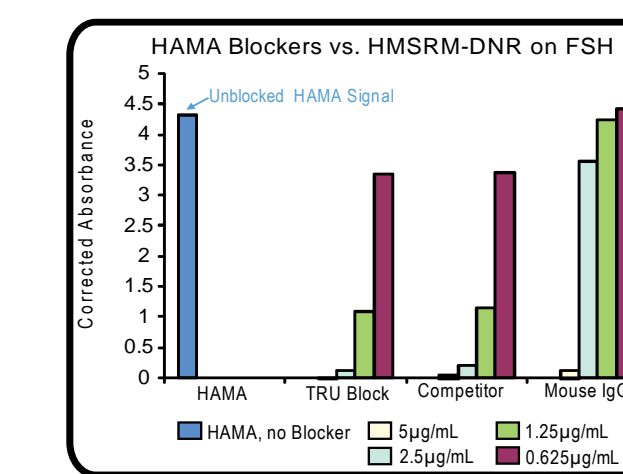
**Conclusion:** Based on CA19-9 and FSH Assays, TRU Block outperforms competitor's active blocker on Roche Type I HAMA sample.



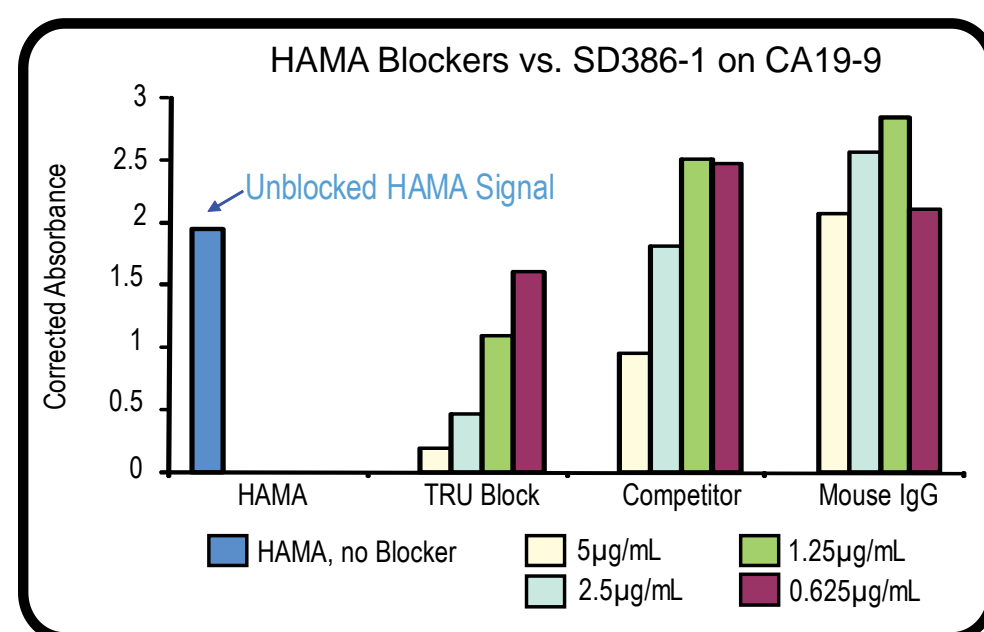
**Conclusion:** Based on CA19-9 and FSH Assays, TRU Block performs equal to competitor's active blocker on BRH22942 HAMA sample.



**Conclusion:** Based on CA19-9 and FSH Assays, TRU Block performs equal to competitor's active blocker on HMSRM-DNR HAMA sample.



**Conclusion:** Based on CA19-9 Assay, TRU Block outperforms competitor's active blocker on SD386-15 HAMA sample.



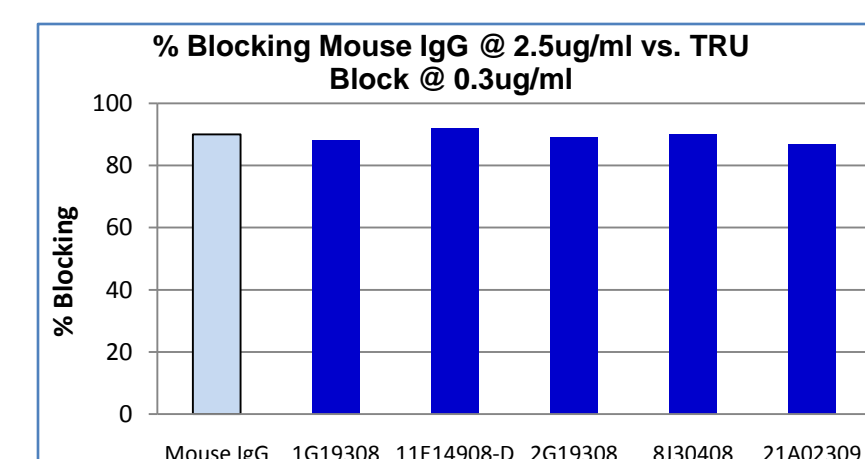
**Conclusion:** Based on CA19-9 Assay, TRU Block outperforms competitor's active blocker on SD386-1 HAMA sample.

## TRU Block QC Assay

- TRU Block QC release includes function testing on HAMA blocking performance.
- The R&D HAMA assay using a modified CA 19-9 Kit (as described earlier) was adopted for TRU Block QC.
- The assay method has been adjusted to reduce variability (% CV) in the assay and to ensure data within the ELISA linear range:
  - The unblocked HAMA activity is limited in the QC assay to OD's < 1.700 by adjusting the HAMA Sample dilutions.
  - All blockers are added at the Sample Diluent step.
  - TRU Block is tested at lower working concentrations (2.5ug/ml to 0.15ug/ml) to examine blocking performance.

## Active Blocking (TRU Block) vs Passive Blocking (Mouse IgG)

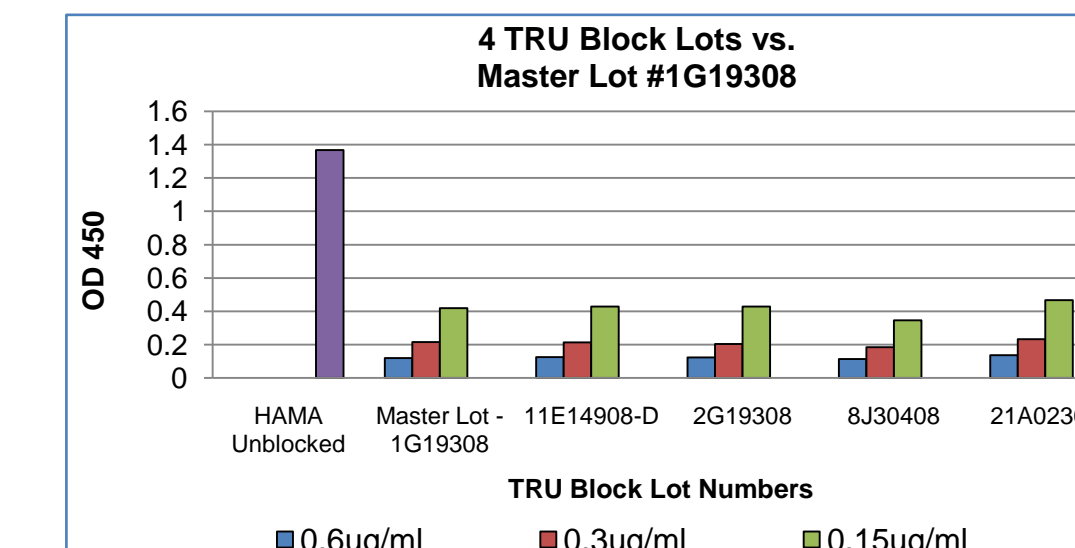
Using HAMA Sample BRH22942, TRU Block must demonstrate equal performance at more than 5x lower concentration compared to Mouse IgG.



- HAMA Sample BRH22942 was diluted 1:20 to bring the HAMA activity within OD range 1.000-1.700.
- Blockers (Mouse IgG or 5 lots of TRU Block) were added to Sample Diluents.
- Data shown are the mean of 3 replicates.
- Calculation % Blocking: [(Unblocked HAMA OD value - Blocked HAMA OD value) / Unblocked HAMA OD value] x 100
- Result: Equivalent Blocking when Mouse IgG is used at 8x the working concentration compared to TRU Block (Mouse IgG at 2.5ug/ml vs TRU Block at 0.3ug/ml).

## QC vs. Master Lot

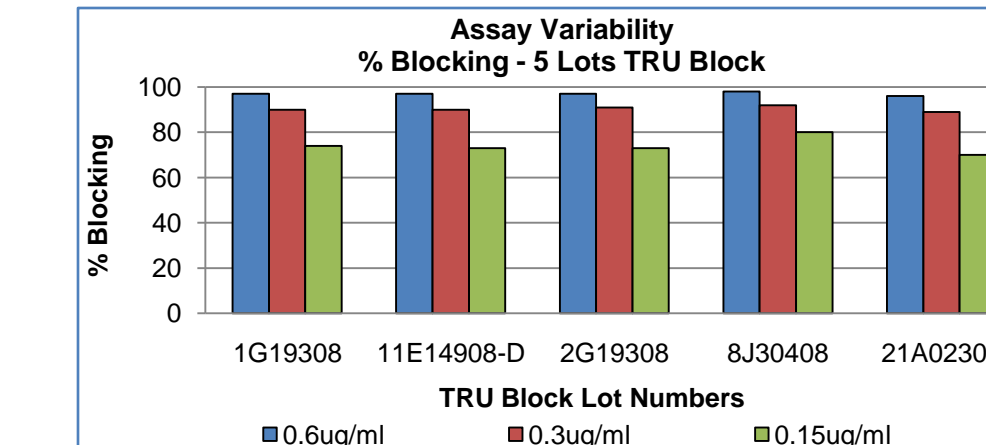
Using HAMA Sample BRH22942 and TRU Block added at 0.6ug/ml, each lot of TRU Block must meet release spec of >90% master lot performance.



- HAMA Sample BRH22942 was diluted 1:20 to bring the unblocked HAMA activity within OD range 1.000-1.700.
- 5 lots of TRU Block were added to Sample Diluents at 0.6ug/ml, 0.3ug/ml and 0.15ug/ml.
- Data shown are the mean of 9 replicates (3 wells per plate; 3 plates run on 3 different days).
- Result: 4 lots of TRU Block are within +/- 4% of TRU Block Master Lot.

## Variability of QC Release Assay

The QC Assay has an Interassay CV ≤ 10%. HAMA Sample BRH22942, Multiple TRU Block lots, multiple days.



- HAMA Sample BRH22942 was diluted 1:20 to bring the HAMA activity within OD range 1.000-1.700.
- 5 lots of TRU Block were added to Sample Diluents at 0.6ug/ml, 0.3ug/ml and 0.15ug/ml.
- Data shown are the mean of 9 replicates (3 wells per plate; 3 plates run on 3 different days).
- Calculation % Blocking: [(Unblocked HAMA OD value - Blocked HAMA OD value) / Unblocked HAMA OD value] x 100.
- Result: Assay Variability < 10% CV.

## SUMMARY

- Six different HAMA samples at varying concentrations were tested on two commercial immunoassays.
- TRU Block performs equal to or better than Competitor's Active Blocker on all HAMA samples tested.
- TRU Block production is very consistent with very little lot to lot variations.

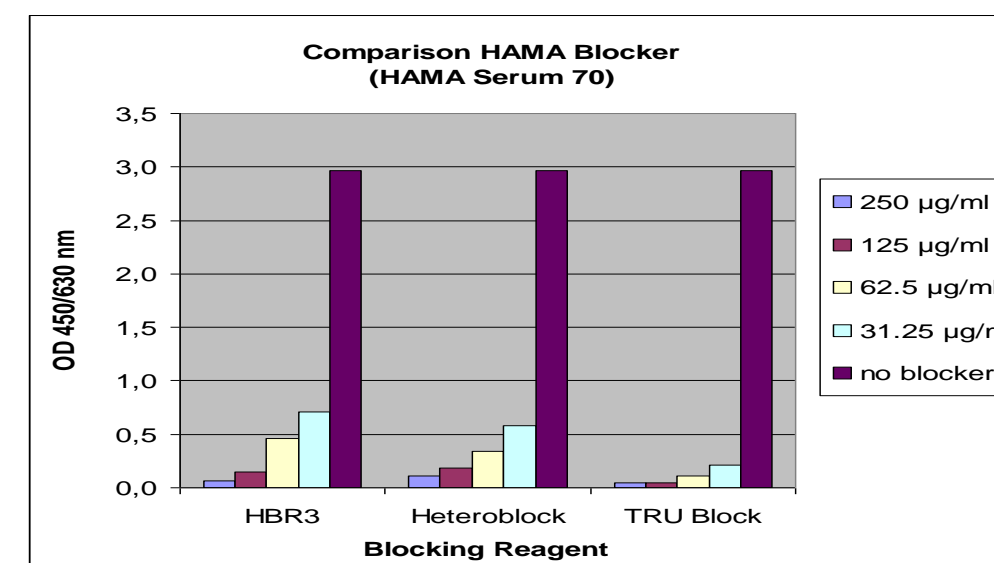
## Customer Testing Results

- Many positive results were reported by customers. A top diagnostic manufacturer reported 30% performance advantage over an existing bench-mark active blocker on the market. (Assay results cannot be presented due to confidentiality agreement)
- One diagnostic manufacturer kindly allowed Meridian Life Science to present their data (without disclosing their name) obtained under the following assay set-up:

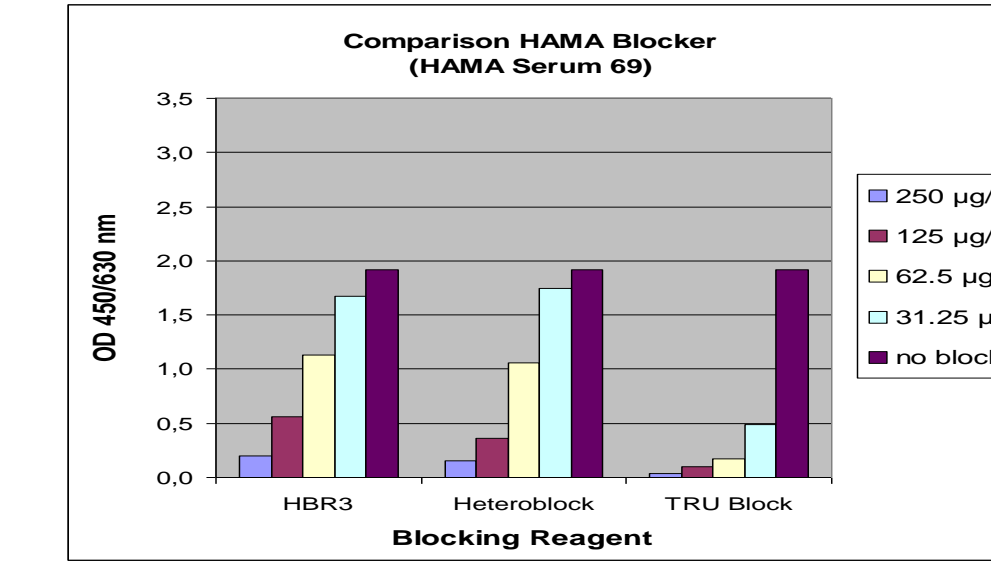
### Blockers were tested in One-step MAb/Mab Sandwich ELISA.

- Assay buffers with or without blockers were dispensed directly into the wells.
- Samples (HAMA positive) and a Biotinylated detection antibody were added to the wells.
- The mix was then incubated at room temperature for 2 hours.
- After washing, Streptavidin-HRP was added and incubated for 30 minutes.

## Customer Results #1



## Customer Results #2



## Customer Results #3

