Elimination of False Positive ELISA Signals in RF-Positive Patient Specimens

A-274

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Presented at AACC 2017 San Diego, CA

BACKGROUND

Heterophilic antibody interference remains a prevalent and persistent source of potential error in immunoassays. Spuriously high signals may lead to further diagnostic tests or procedures that are unnecessary, costly, and potentially detrimental to the patient. Rheumatoid factor (RF) is a well known interfering substance present in the majority of rheumatoid arthritis (RA) patients. This study was designed to identify false positive signals in commercial ELISA test kits observed with RF-positive serum and plasma specimens. Two biomarkers, Human Cardiac Troponin I (cTnI) and Human Mucin 16 (CA125), were selected for their clinical importance. Human Cardiac Troponin I is a critical biomarker for assessing myocardial disease, and Human Mucin 16 is the most widely used biomarker for detection of ovarian cancer.

METHODS

Ten RF-positive serum specimens (age, gender, and titer data is shown in Table 1) and nine plasma specimens obtained from patients with a diagnosis of rheumatoid arthritis (RA) (age, gender, and titer data is shown in Table 2) were tested in commercial ELISA kits, Human Cardiac Troponin I and Human Mucin 16, per the manufacturer's protocol. Kit assay diluent was also prepared according to the manufacturer's instructions. HeteroBlock®, a commercially available blocking reagent, was added directly to the assay diluent without any additional steps such as filtering or heating. Patient specimens were diluted per the manufacturer's recommendations (2- to 2.5-fold) just prior to testing with and without HeteroBlock® present in the assay diluent.

Table 1: RF-Positive Serum Specimens

Specimen ID	Gender	Age	RF Titer per Sure-Vue Latex Agglutination
S1	Female	63	160 IU
S2	Female	75	80 IU
S3	Male	71	40 IU
S4	Female	29	40 IU
S5	Male	64	20 IU
S6	Male	88	20 IU
S7	Female	66	20 IU
S8	Male	35	20 IU
S9	Female	70	20 IU
S10	Female	29	20 IU

Table 2: Plasma Specimens from Patients with a Diagnosis of RA

Specimen ID	Gender	Age	RF Titer per Beckman Nephelometer
P1	Female	61	40 IU
P2	Female	71	585 IU
P3	Female	76	353 IU
P4	Female	76	201 IU
P5	Female	57	900 IU
P6	Female	53	621 IU
P7	Female	51	884 IU
P8	Female	70	792 IU
P9	Female	43	262 IU

RESULTS

The Troponin I ELISA kit was susceptible to heterophilic interference. Elevated signals were observed for seven of the ten RF-positive serum specimens; the seven elevated signals were eliminated in the specimens prepared with assay diluent containing 150 µg/mL of HeteroBlock®, shown in Figure 1. Nine of the nine plasma specimens from RA patients produced elevated signals; the nine elevated signals were eliminated in the specimens prepared with assay diluent containing 150 µg/mL of HeteroBlock®, shown in Figure 2. The nine plasma specimens were also prepared with assay diluent containing 15 µg/mL of HeteroBlock®. At this concentration, HeteroBlock® reduced the signal for seven of the nine RA plasma specimens by 50% or more, shown in Figure 3. Assay diluent with 150 µg/mL of HeteroBlock® was spiked with known concentrations of recombinant TNNI3 (the standard provided with the test kit). The reported concentrations were as expected indicating that HeteroBlock® does not interfere with true positives, shown in Figure 4.

For the Human Mucin 16 ELISA test kit, elevated signals were observed for six of the ten RF-positive serum specimens; specifically, four of the six specimens produced a result greater than the clinically significant level of 35 U/mL. None of the ten serum specimens prepared with assay diluent containing 150 µg/mL of HeteroBlock® generated a signal above the limit of detection, shown in Figure 5. All nine of the plasma specimens from RA patients gave elevated CA125 results; seven of those nine results were greater than the clinically significant level of 35 U/mL. The elevated signals were reduced below the clinically significant level of 35 U/mL when the plasma specimens were prepared with assay diluent containing 150 µg/mL of HeteroBlock®, shown in Figure 6. The nine plasma specimens were also prepared with assay diluent containing 15 µg/mL of HeteroBlock®; at this concentration, HeteroBlock® reduced the signal for seven of the nine RA plasma specimens by 50% or more, shown in Figure 7. Assay diluent containing 150 µg/mL of HeteroBlock® was spiked with known concentrations of recombinant MUC16 (the standard provided with the test kit). The reported concentrations were as expected indicating that HeteroBlock® does not interfere with true positives, shown in Figure 8.

CONCLUSIONS

This study reinforces the need for vigilance regarding the potential for false positive results caused by heterophilic antibody interference. In this study, the addition of HeteroBlock® to the assay diluents for Human Cardiac Troponin I and Human Mucin 16 commercial ELISA kits demonstrated a simple and effective means of blocking heterophilic antibody interference.

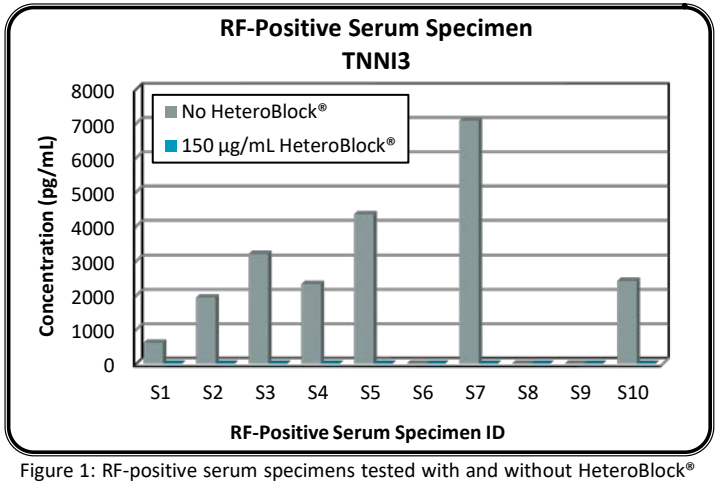


Figure 1: RF-positive serum specimens tested with and without HeteroBlock® in Human Cardiac Troponin I ELISA test kit.

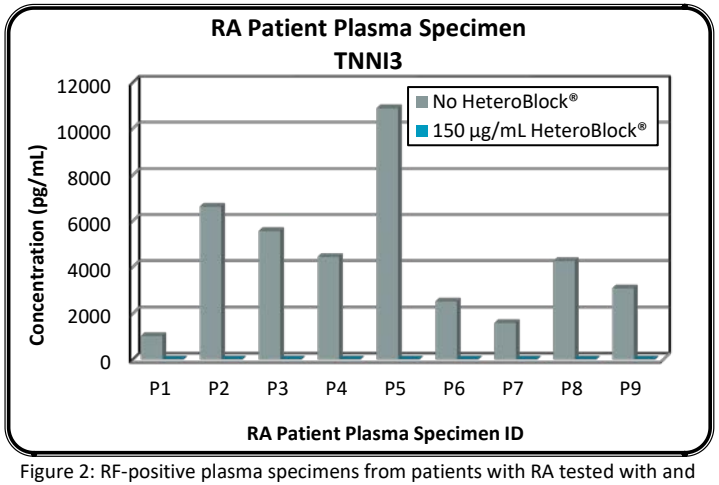


Figure 2: RF-positive plasma specimens from patients with RA tested with and without HeteroBlock® in Human Cardiac Troponin I ELISA test kit.

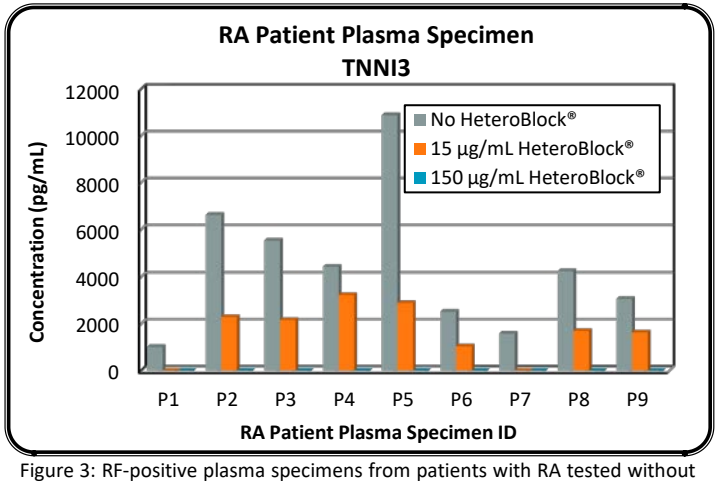


Figure 3: RF-positive plasma specimens from patients with RA tested without HeteroBlock® or with different doses of HeteroBlock® in Human Cardiac Troponin I ELISA test kit.

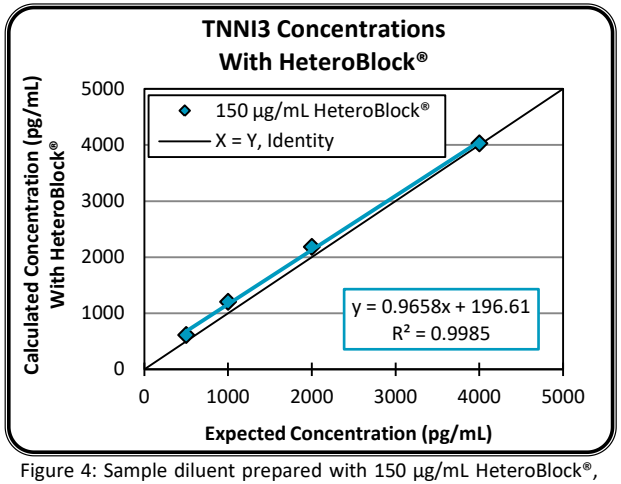
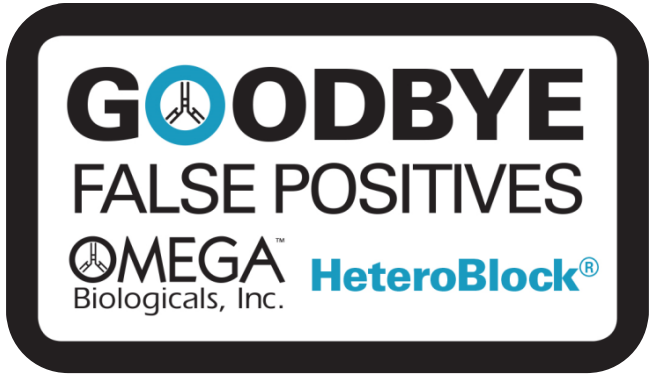


Figure 4: Sample diluent prepared with 150 µg/mL HeteroBlock®, spiked with recombinant TNNI3, and serially diluted. Observed concentration of TNNI3 was as expected in Human Cardiac Troponin I ELISA test kit.



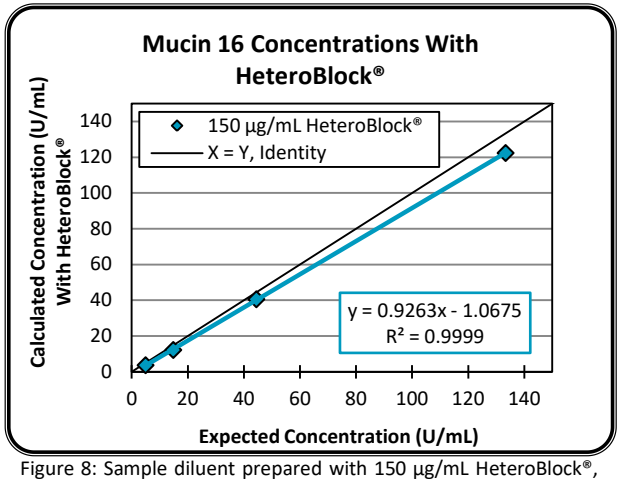


Figure 8: Sample diluent prepared with 150 µg/mL HeteroBlock®, spiked with recombinant Mucin 16, and serially diluted. Observed concentration of Mucin 16 was as expected in Human Mucin 16 ELISA test kit.

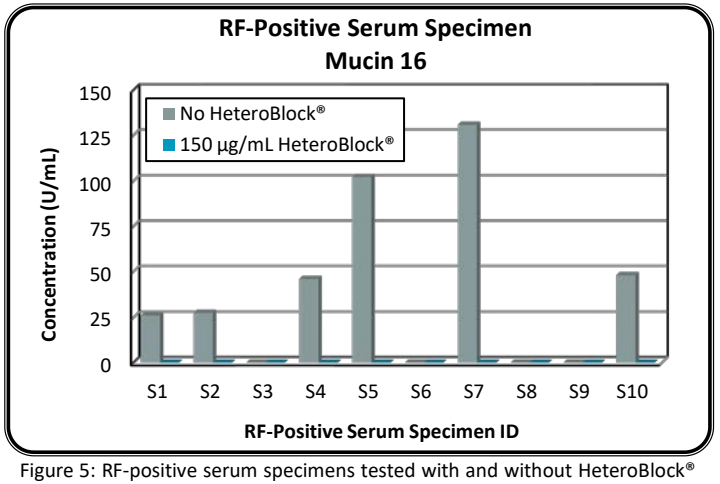


Figure 5: RF-positive serum specimens tested with and without HeteroBlock® in Human Mucin 16 ELISA test kit.

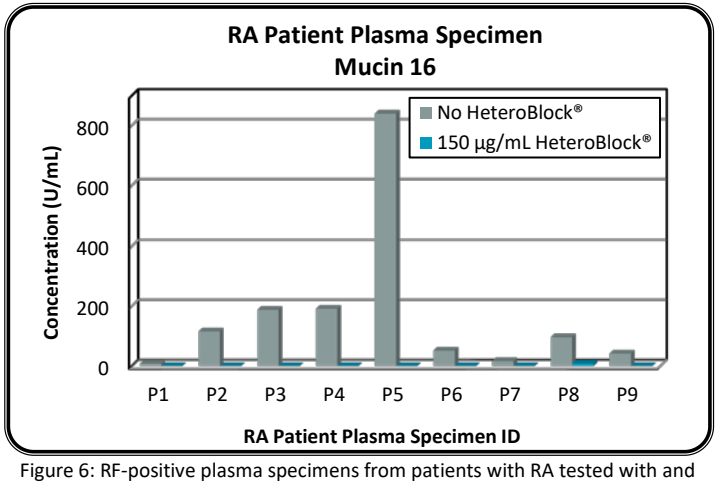


Figure 6: RF-positive plasma specimens from patients with RA tested with and without HeteroBlock® in Human Mucin 16 ELISA test kit.

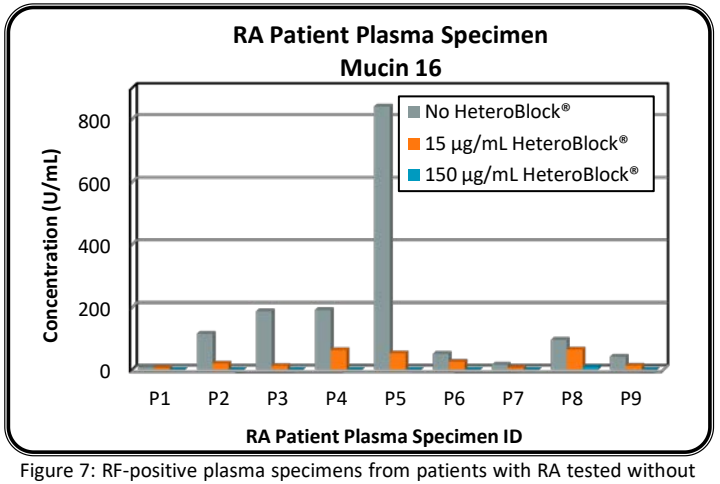


Figure 7: RF-positive plasma specimens from patients with RA tested without HeteroBlock® or with different doses of HeteroBlock® in Human Mucin 16 ELISA test kit.