Reduction of RF Interference in ELISA by Active and Passive Blocking Agents

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BACKGROUND

Heterophilic antibodies may cause problematic interference in immunoassays. Rheumatoid factor (RF) is a common heterophilic antibody found in rheumatoid arthritis (RA) patients. RF can bind non-specifically to capture and detection antibodies resulting in falsely elevated or falsely decreased signals. Addition of a blocking agent to sample diluent prior to running the assay may ameliorate false results. This study examines the effectiveness of active and passive blocking agents in reducing RF interference in patient specimens. The specimens were tested across two biomarkers, Human Cardiac Troponin I (TNNI3) and Human Mucin 16 (CA125). Both biomarkers are known to be vulnerable to RF interference.

METHODS

Ten plasma specimens from patients with a RA diagnosis (10 female, age 46-70, RF titer 107->600 IU/mL) were tested in commercial TNNI3 and CA125 ELISA kits per the manufacturers' protocol. Prior to specimen dilution, a blocking agent was added directly to the assay diluent. Three passive blocking agents (purified polyclonal mouse IgG, human IgG, and goat IgG) were tested alongside HeteroBlock®, a commercially available active blocking agent. Passive blocking agents were added to the assay diluent for a final concentration of 200 µg/mL in the diluted specimens. The active blocking agent was added to the assay diluent for a final concentration of 20 µg/mL. Patient specimens were diluted 2-fold immediately prior to testing with and without a blocking agent present in the assay diluent. Four of the ten plasma specimens were also tested with 600 µg/mL final concentration of passive blocking agents and 60 µg/mL final concentration of active blocking agent for both biomarkers.

Plasma Specimens from Patients with a Diagnosis of RA							
Specimen ID	Gender	Age	RF Titer per Beckman Coulter (AU Analyzer)	Specimen ID	Gender	Age	RF Titer per Beckman Coulter (AU Analyzer)
P22	Female	64	144 IU/mL	P27	Female	64	181 IU/mL
P23	Female	69	223 IU/mL	P28	Female	69	277 IU/mL
P24	Female	70	107 IU/mL	P29	Female	50	>600 IU/mL
P25	Female	51	>600 IU/mL	P30	Female	46	312 IU/mL
P26	Female	64	563 IU/mL	P31	Female	64	>600 IU/mL

RESULTS

Elevated signals were observed for all ten RF-positive plasma specimens prepared without a blocking agent in the assay diluents of both the TNNI3 and CA125 ELISA kits. For the CA125 tests, all plasma signals were greater than the clinically significant level of 35 U/mL. Results are summarized in the table below. Interference remained even with passive blocking agent concentrations as high as 600 µg/mL. HeteroBlock® at 60 µg/mL completely eliminated the interference for the four specimens in the TNNI3 test and reduced the interference below 35 U/mL for the four specimens in the CA125 test.

	CA125 ELISA			
	# of Specimens Tested	# Reduced more than 20%	% Reduction Average	% Reduction Range
Human IgG at 200 µg/mL	10	3 of 10	12%	0-24%
Human IgG at 600 µg/mL	4	0 of 4	7%	0-14%
Goat IgG at 200 µg/mL	10	4 of 10	26%	0-93%
Goat IgG at 600 µg/mL	4	0 of 4	6%	1-11%
Mouse IgG at 200 µg/mL	10	6 of 10	26%	0-48%
Mouse IgG at 600 µg/mL	4	3 of 4	40%	14-58%
HeteroBlock® at 20 µg/mL	10	10 of 10	73%	33-91%
HeteroBlock® at 60 µg/mL	4	4 of 4	98%	94-100%

	TNNI3 ELISA			
	# of Specimens Tested	# Reduced more than 20%	% Reduction Average	% Reduction Range
Human IgG at 200 µg/mL	10	2 of 10	11%	0-24%
Human IgG at 600 µg/mL	4	0 of 4	3%	0-6%
Goat IgG at 200 µg/mL	10	5 of 10	33%	11-89%
Goat IgG at 600 µg/mL	4	1 of 4	4%	0-5%
Mouse IgG at 200 µg/mL	10	10 of 10	42%	22-63%
Mouse IgG at 600 µg/mL	4	4 of 4	42%	23-62%
HeteroBlock® at 20 µg/mL	10	10 of 10	76%	37-100%
HeteroBlock® at 60 µg/mL	4	4 of 4	100%	100%

CONCLUSIONS

The addition of a blocking agent to assay diluent prior to specimen preparation may reduce the interference from heterophilic antibodies like RF. Passive blocking agents may be only partially effective and will likely require much higher concentrations than active blocking agents. In this study, HeteroBlock® demonstrated superior performance over the passive blocking agents, at 10% of the passive blocking agent concentration. This study also demonstrates that the active blocking agent, HeteroBlock®, has a more sensitive dose response than the passive blocking agents tested.

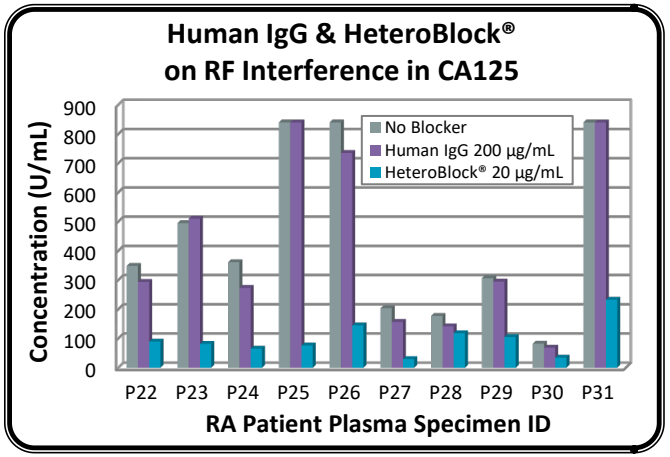


Figure 1: RF-positive plasma specimens from patients with RA tested with Human IgG and HeteroBlock® in Human Mucin 16 ELISA test kit.

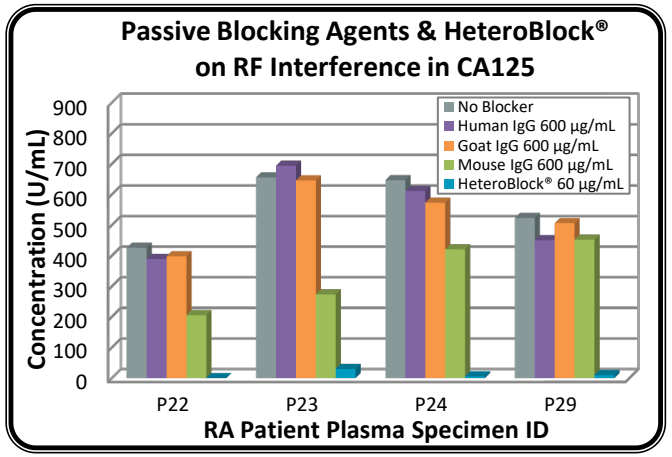


Figure 8: RF-positive plasma specimens from patients with RA tested with Passive Blocking Agents and HeteroBlock® in Human Mucin 16 ELISA test kit.

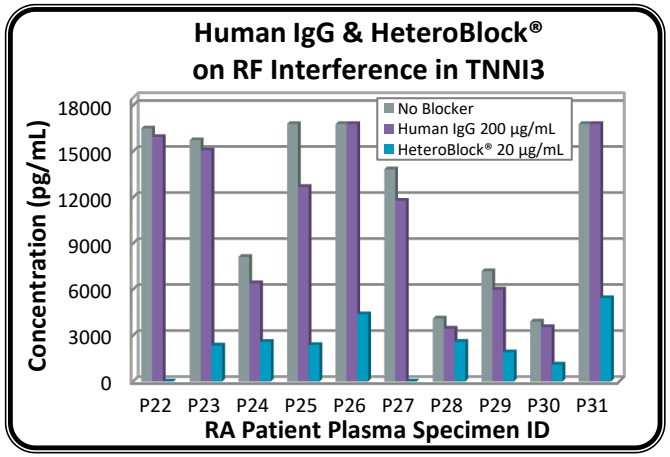


Figure 4: RF-positive plasma specimens from patients with RA tested with Human IgG and HeteroBlock® in Human Cardiac Troponin I ELISA test kit.

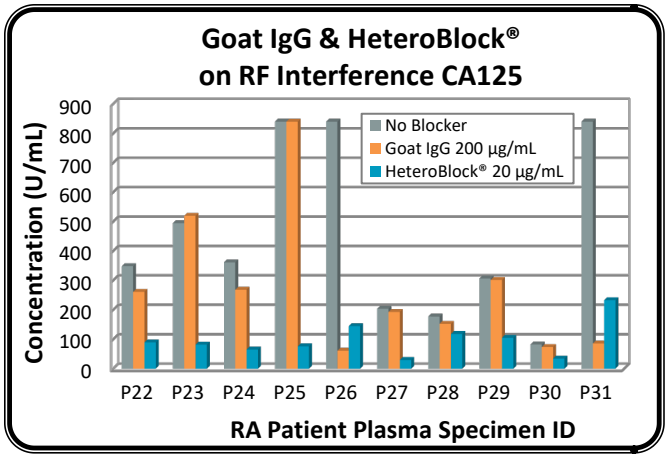
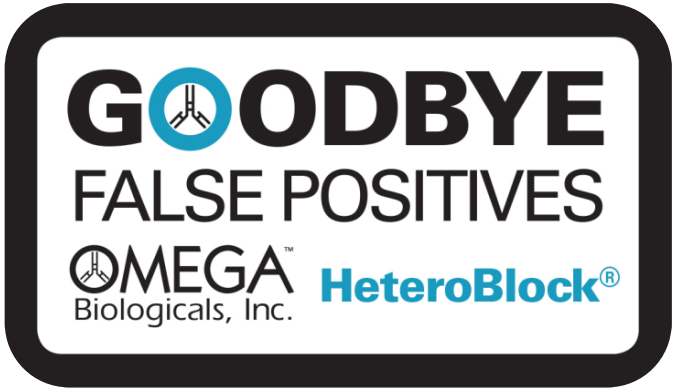


Figure 2: RF-positive plasma specimens from patients with RA tested with Goat IgG and HeteroBlock® in Human Mucin 16 ELISA test kit.



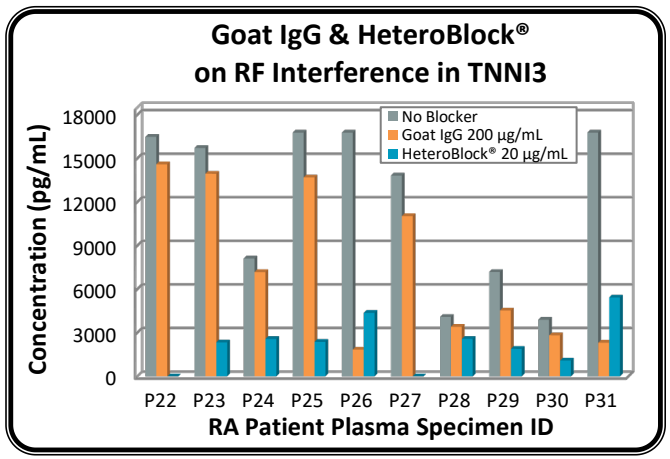


Figure 5: RF-positive plasma specimens from patients with RA tested with Goat IgG and HeteroBlock® in Human Cardiac Troponin I ELISA test kit.

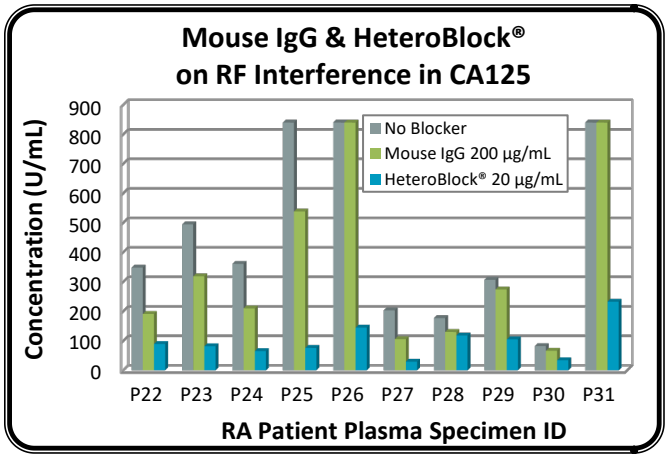


Figure 3: RF-positive plasma specimens from patients with RA tested with Mouse IgG and HeteroBlock® in Human Mucin 16 ELISA test kit.

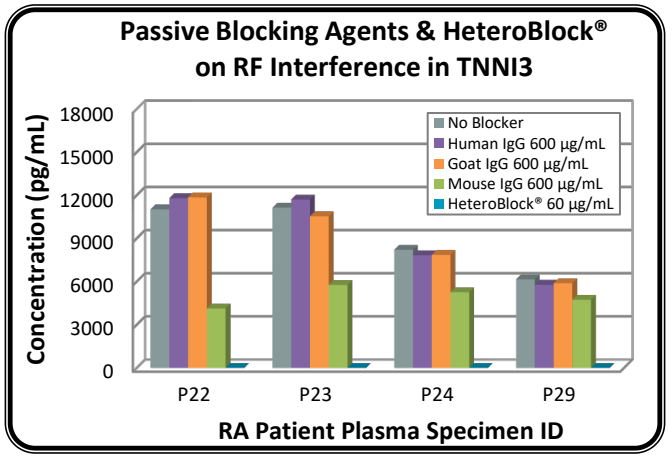


Figure 7: RF-positive plasma specimens from patients with RA tested with Passive Blocking Agents and HeteroBlock® in Human Cardiac Troponin I ELISA test kit.

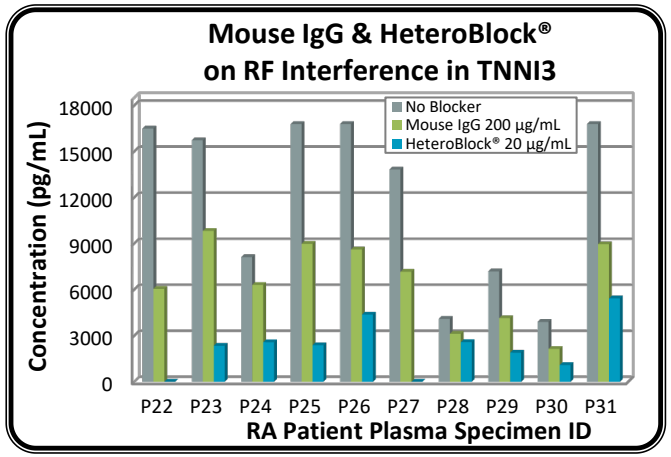


Figure 6: RF-positive plasma specimens from patients with RA tested with Mouse IgG and HeteroBlock® in Human Cardiac Troponin I ELISA test kit.