OMEGA Biologicals, Inc.™

RF Interference in Lateral Flow Assays

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BACKGROUND

A lateral flow immunoassay is often used as a quick and affordable means to aid diagnosis and decision making in patient care. Accurate results are crucial as a positive result may prompt further testing. Heterophilic antibodies may be present in patient specimens and can cause interference through non-specific binding in immunoassays. Rheumatoid Factor (RF) is one example; it can be present in the majority of Rheumatoid Arthritis (RA) patients. This study examines the performance of a lateral flow assay in the presence of RF. The assay was chosen for its clinical relevance; it is designed to identify elevated cardiac markers in Acute Myocardial Infarction (AMI) patients.

METHODS

Acquired an FDA registered, CE marked lateral flow immunoassay for determination of Cardiac Troponin I (cTnI), Creatine Kinase MB (CK-MB), and myoglobin (Myo) with different cutoff concentrations. RF positive serum specimens (n=26) were obtained, nine male, seventeen female, ages 32-99, with RF titers ranging from 80-9375 IU/mL. Eight plasma specimens from donors with a RA diagnosis were obtained, all female, ages 46-70, with RF titers ranging from 107->600 IU/mL. Patient specimens were tested according to manufacturer's recommendations; 80 µL of serum or plasma were added to the sample well; results were read after 15 minutes. Specimens suspected of false positive results were retested with HeteroBlock®, a commercially available blocking reagent. HeteroBlock® was prepared at 2 mg/mL concentration, 10 µL of the HeteroBlock® solution were added to 80 µL of serum or plasma, mixed well and allowed to stand for five minutes at room temperature before testing. Positive and negative control samples were acquired to verify kit performance. Control samples were prepared with and without HeteroBlock® and run side-by-side.

RESULTS

Of the 26 RF positive serum specimens, seven specimens tested positive for one or more cardiac biomarkers. One specimen tested positive for all three biomarkers; when retested with HeteroBlock® the signal was reduced in intensity. Four specimens tested positive for CK-MB; when retested with HeteroBlock® the signals were eliminated. Two specimens tested positive for myoglobin; when retested with HeteroBlock® one signal was reduced in intensity, one signal was unchanged. Three of the eight RA patient plasma specimens tested positive for cTnI and CK-MB. The three positive plasma specimens were retested with HeteroBlock®. The addition of HeteroBlock® eliminated the positives in all three plasma specimens. A negative and positive control were tested with and without HeteroBlock®. The addition of HeteroBlock® did not change the results of the positive or negative controls supporting the expectation that HeteroBlock® does not affect true positive or true negative results.

RF Positive Serum Specimens

Specimen ID	Gender	Age	RF Titer Per Sure-Vue Latex Agglutination	Lateral Flow Immunoassay Results	+ 20 µg of HeteroBlock®
S20	Female	45	640	Negative	N/A
S25	Female	48	640	Negative	N/A
S27	Male	40	640	Negative	N/A
S28	Male	71	1280	Myo (+), CK-MB (+), cTnI (+)	All three (+), Less intense
S29	Male	69	1280	CK-MB (+)	Negative
S30	Female	52	2560	Negative	N/A
S31	Female	76	5120	Negative	N/A
S32	Male	68	320	Negative	N/A
S33	Male	55	640	Negative	N/A
S36	Female	74	160	Negative	N/A
S37	Female	56	160	Negative	N/A
S38	Female	79	80	Negative	N/A
S39	Male	76	320	Negative	N/A
S40	Female	82	160	Negative	N/A
S41	Female	56	160	Negative	N/A
S42	Female	99	320	Myo (+)	Myo (+), Less intense
S43	Female	58	80	CK-MB (+)	Negative
S44	Male	83	320	Myo (+)	Myo (+), No change
S45	Female	56	160	Negative	N/A
S46	Male	32	80	Negative	N/A
S47	Female	70	640	Negative	N/A
S48	Female	56	160	Negative	N/A
S49	Female	55	5120	Negative	N/A

RF Positive Serum Specimens

Specimen ID	Gender	Age	RF Titer	Lateral Flow Immunoassay Results	+ 20 µg of HeteroBlock®
SK1	Male	67	3440	CK-MB (+)	Negative
SK2	Female	60	1690	Negative	N/A
SK3	Female	51	9375	CK-MB (+)	Negative

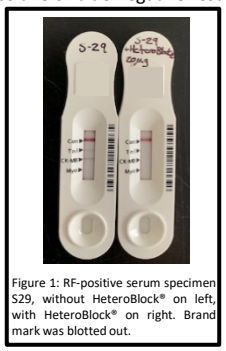

Figure 1: RF-positive serum specimen S29, without HeteroBlock® on left, with HeteroBlock® on right. Brand mark was blotted out.

Figure 2: RF-positive serum specimen SK3, without HeteroBlock® on left, with HeteroBlock® on right. Brand mark was blotted out.

Figure 3: RF-positive plasma specimen P26, without HeteroBlock® on left, with HeteroBlock® on right. Brand mark was blotted out.

Figure 4: Positive Control specimen without HeteroBlock® on left, with HeteroBlock® on right. Brand mark was blotted out.

Plasma Specimens from Patients with a Diagnosis of RA

Specimen ID	Gender	Age	RF Titer Per Beckman Coulter (AU Analyzer)	Lateral Flow Immunoassay Results	+ 20 µg of HeteroBlock®
P23	Female	69	223	Negative	N/A
P24	Female	70	107	Negative	N/A
P25	Female	51	>600	Negative	N/A
P26	Female	64	563	CK-MB (+), cTnI (+)	Negative
P27	Female	65	181	Negative	N/A
P28	Female	69	277	CK-MB (+), cTnI (+)	Negative
P30	Female	46	312	Negative	N/A
P31	Female	64	>600	CK-MB (+), cTnI (+)	Negative

CONCLUSIONS

Lateral flow immunoassays may be susceptible to interference by RF leading to false positive results. The assay surveyed used three unique biomarkers with different cutoff levels; all three biomarkers revealed some risk for interference. Specimens that caused interference for one biomarker did not necessarily interfere with other biomarkers. This study reinforces the need for vigilance regarding the potential for false positive results caused by heterophilic antibody interference.