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An Evaluation of the Effectiveness of Three Immunoglobulin G (IgG) Removal Procedures for Routine IgM Serological Testing

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Three procedures for the removal of immunoglobulin G (IgG) from human serum were evaluated for their effectiveness in eliminating false-positive results caused by rheumatoid factor and in removing IgG from serum to reduce competing-IgG interference in IgM enzyme-linked immunosorbent assay (ELISA) testing. The procedures investigated employed two anti-human IgG diluents and a recombinant protein G-filled tube. The anti-human IgG was more effective than the protein G method in eliminating false-positive results caused by rheumatoid factor and removed 5.4% more IgG from serum samples in the normal range (<1,700 mg/dl) and up to 16.4% more of the IgG from samples with elevated levels (>1,700 mg/dl). The recombinant protein G removed less IgM than the anti-human IgG diluents; however, this difference did not affect the results of the ELISA. For these reasons, the in-house-developed anti-human IgG diluent proved to be the most effective and economical for IgM serological testing.

The discovery in the early 1960s of the transient nature of the immunoglobulin M (IgM) serologic response led to the development of IgM-specific tests for infectious disease diagnosis. In theory, the new IgM tests were expected to provide physicians with reliable and timely diagnostic information and eliminate the need for testing of paired (acute- and convalescent-phase) sera (11). In the clinical setting, however, IgM testing initially suffered from two major problems: interference from rheumatoid factor (RF) and the presence of IgG in the samples which competed with IgM for epitopes on the antigen.

RFs present one of the most serious problems in IgM testing (9). RFs are autoimmune antibodies, usually of the IgM class, which recognize human IgG. In antibody testing, specific IgG antibodies present in the serum bind to the antigen, presenting a site for the anti-IgG IgM RF to bind. The IgM-class RF will then be recognized by the enzyme-labeled anti-IgM conjugate, giving rise to a false-positive IgM reaction (Fig. 1).

Another problem with IgM testing results from the effects of competing IgG in the sample. This can cause a false-negative result if there is an excess of IgG antibody, which is usually of a higher affinity and competes for the limited amount of immobilized antigen. This reduces the availability of antigen for the binding of the larger IgM antibody (Fig. 1). The main efforts to eliminate these problems so far have centered around the removal of IgG from the serum.

Several techniques for the separation of IgG from IgM to improve testing performance have been reported. One technique involves the use of protein A, a *Staphylococcus aureus* cell wall protein which has a high affinity for the Fc portion of the IgG molecule and is effective in clearing much of the IgG from serum (4). Another method for removing IgG prior to IgM testing utilizes anion-exchange chromatography (7). Both of these methods are labor-intensive, have been reported to remove much of the IgM, and are not specific for all of the IgG subclasses (6, 8). These methods are not suitable for routine, high-volume serology testing and therefore were not evaluated in this study. The methods investigated in this study include the use of a goat anti-human IgG serum and the use of a recombinant protein G coupled to Sepharose. A panel of human serum samples with total IgG ranging from 753 to 7,220 mg/dl was treated by these IgG removal methods and analyzed by rate nephelometry to quantitate the amount of IgG removed. These treated sera were then assayed by a sensitive human IgM enzyme-linked immunosorbent assay (ELISA) to quantitate the amount of IgM being removed by the treatment methods. Epstein-Barr virus (EBV) viral capsid antigen (VCA) IgM-positive and RF-positive samples were also treated to remove IgG and tested by ELISA to determine the effectiveness of eliminating RF and competing-IgG interference by these treatment methods.

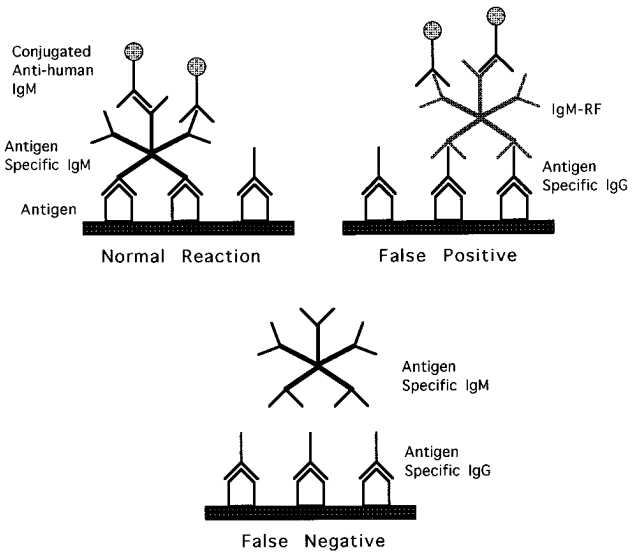

FIG. 1. False positive caused by RF interference and false negative caused by competing IgG.

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TABLE 1. Amount of IgG remaining[a] and percent removed by the three procedures

Sample	IgG (mg/dl)	IgG result with:					
		Goat anti-human diluent		GullSorb		Isocolumn	
		Amt remaining	% Removed	Amt remaining	% Removed	Amt remaining	% Removed
1	753	0	100.0	0	100.0	50	93.4
2	756	0	100.0	0	100.0	16	97.9
3	779	0	100.0	0	100.0	7	99.1
4	856	0	100.0	0	100.0	7	99.2
5	874	0	100.0	0	100.0	30	96.6
6	900	1.6	99.8	0	100.0	10	98.9
7	960	0	100.0	0	100.0	11	98.9
8	985	0	100.0	0	100.0	76	92.3
9	1,040	0	100.0	0	100.0	29	97.2
10	1,080	0	100.0	0	100.0	9	99.2
11	1,156	0	100.0	0	100.0	33	97.1
12	1,160	0	100.0	0	100.0	80	93.1
13	1,210	0	100.0	0	100.0	85	93.0
14	1,400	0	100.0	0	100.0	132	90.6
15	1,420	0	100.0	0	100.0	178	87.5
16	1,520	0	100.0	1	99.9	240	84.2
17	1,560	0	100.0	0	100.0	158	89.9
18	1,910	0	100.0	0	100.0	142	92.6
19	1,930	0	100.0	0	100.0	190	90.2
20	2,050	0	100.0	0	100.0	154	92.5
21	2,650	0	100.0	0	100.0	522	80.3
22	3,000	0	100.0	0	100.0	740	75.3
23	3,790	0	100.0	0	100.0	1,024	73.0
24	4,100	0	100.0	0	100.0	1,104	73.1
25	7,220	1,332	81.6	854	88.2	3,296	54.3

[a] Expressed in milligrams per deciliter.

MATERIALS AND METHODS

Clinical samples. Twenty-five serum samples were selected to cover a wide range of low, normal, and elevated IgG and IgM antibody levels. An additional nine samples with at least a 2-ml volume were selected for reproducibility studies. A balanced selection of 55 positive and negative EBV samples with various levels of RF were also selected for testing. Since our laboratory is a reference laboratory, testing samples submitted through other hospital and regional laboratories, the clinical information for the patients was not available to us and was unobtainable even after repeated attempts.

Goat anti-human IgG diluents. Two different brands of goat anti-human IgG diluents were used in this study, GullSorb (Gull Laboratories, Salt Lake City, Utah) and an in-house-developed diluent.

The in-house-developed diluent was made by making a 1:2 (1 part to 1 part) dilution of goat anti-human IgG Fc-specific serum (International Immunology Corporation, Murrieta, Calif.) in 0.15 M phosphate-buffered saline (PBS) (pH 7.5) with 0.1% sodium azide. The 1:2 dilution was determined by diluting different sera in titrated dilutions of the anti-human IgG goat serum. A 1:2 dilution was found to remove at least 4,100 mg of IgG per dl (see Table 1), while at dilutions of 1:4 or greater, there was IgG remaining in the serum samples. Therefore, we found a 1:2 dilution to be optimal for this lot of goat anti-human IgG serum. This dilution should be reoptimized for each new lot number. For total IgG and EBV VCA IgM ELISA testing, all samples were diluted 1:20 in the goat anti-human IgG diluent and allowed to react for 10 min. For IgG and RF testing on the nephelometer, samples were centrifuged at $\approx 4{,}000 \times g$ for 3 min in a Microfuge E (Beckman Instruments, Inc., Fullerton, Calif.). The complexes formed by goat serum antibodies binding to the human IgG caused excessive light scatter on the nephelometer and interfered with the readings if the samples were not centrifuged. These complexes did not interfere with the ELISA, as nine IgM-positive and nine IgM-negative EBV samples gave the same result when centrifuged and not centrifuged. The average coefficient of variation (CV) for the positive samples was 4.1%, with an average CV of 10.1% for the negative samples.

GullSorb was purchased in a ready-to-use dilution, and samples were treated in the same way as described for the goat anti-human IgG diluent.

Protein G affinity tubes. Quik-Sep IgM recombinant G affinity tubes were purchased from Isolab Inc. (Akron, Ohio). Each prefilled tube contains an affinity resin of recombinant protein G coupled to Sepharose. The recombinant protein G is a modified form of protein G, a cell wall protein of group G streptococci. The recombinant protein G strongly binds the Fc fragment of human IgG1 to IgG4. It does not bind human IgM, IgD, or IgE, and the protein G sequence for albumin binding has also been deleted (1, 2, 5). Fifty microliters

TABLE 2. Reproducibility studies showing percent IgG removed after treatment by the three procedures

Sample[a]	Amt not absorbed (mg/dl)	Mean % IgG removed	SD	% CV
1S	330	100.0	0.0	0.0
1G		100.0	0.0	0.0
1I		100.0	0.0	0.0
2S	688	100.0	0.0	0.0
2G		99.6	0.8	0.8
2I		100.0	0.0	0.0
3S	834	100.0	0.0	0.0
3G		100.0	0.0	0.0
3I		98.6	0.3	0.3
4S	930	100.0	0.0	0.0
4G		100.0	0.0	0.0
4I		98.8	0.0	0.0
5S	1,110	100.0	0.0	0.0
5G		100.0	0.0	0.0
5I		96.4	0.4	0.4
6S	1,488	100.0	0.0	0.0
6G		100.0	0.0	0.0
6I		97.6	0.1	0.1
7S	1,694	100.0	0.0	0.0
7G		100.0	0.0	0.0
7I		95.6	0.5	0.5
8S	3,740	100.0	0.0	0.0
8G		100.0	0.0	0.0
8I		71.1	5.2	7.3
9S	4,300	99.2	0.4	0.4
9G		99.8	0.1	0.1
9I		62.7	2.9	4.6

[a] S, goat anti-human IgG diluent; G, GullSorb; I, Isocolumn. $n = 3$ for each sample.

TABLE 3. Amount and percent of IgM remaining after treatment by the three procedures

Sample	Nonabsorbed IgM (mg/dl)	IgM result with:					
		Goat anti-human IgG diluent		GullSorb		Isocolumn	
		mg/dl	% Remaining	mg/dl	% Remaining	mg/dl	% Remaining
1	43.68	33.70	77.2	35.68	81.7	40.38	92.5
2	34.42	28.52	82.9	27.92	81.1	33.52	97.4
3	28.86	23.92	82.9	24.62	85.3	28.56	99.0
4	42.82	37.84	88.4	36.66	85.6	41.04	95.8
5	37.64	29.30	77.8	29.74	79.0	35.76	95.0
6	36.64	31.50	86.0	30.30	82.7	36.38	99.3
7	21.38	18.24	85.3	16.92	79.1	20.16	94.3
8	57.62	48.48	84.1	48.56	84.3	53.72	93.2
9	46.84	36.86	78.7	38.18	81.5	45.56	97.3
10	48.85	39.52	80.9	40.08	82.0	48.52	99.3
11	39.02	34.70	88.9	35.38	90.7	38.78	99.4
12	61.62	46.90	76.1	47.36	76.9	57.48	93.3
13	61.60	47.76	77.5	49.42	80.2	60.26	97.8
14	43.70	32.62	74.7	31.44	71.9	40.96	93.7
15	52.04	45.70	87.8	45.22	86.9	47.66	91.6
16	7.60	6.28	82.6	5.84	76.8	6.94	91.3
17	47.82	42.56	89.0	41.84	87.5	46.18	96.6
18	47.18	36.32	77.0	34.96	74.1	42.64	90.4
19	41.34	32.36	78.3	32.34	78.2	40.34	97.6
20	39.08	34.44	88.1	35.24	90.2	38.26	97.9
21	6.80	5.10	75.0	4.94	72.7	6.68	98.2
22	38.18	31.08	81.4	30.64	80.3	36.98	96.9
23	23.96	20.56	85.8	20.10	83.9	23.82	99.4
24	30.98	25.56	82.5	24.32	78.5	29.76	96.1
25	41.74	38.96	93.3	38.98	93.4	41.62	99.7
Average			82.5		81.8		96.1

of human serum was added to each prefilled tube, and the mixtures were vortexed for 5 s and allowed to react for 10 min on a rocking platform. A disc was then inserted and pushed down through the solution, compressing the resin bed, leaving over 200 μl of the IgM-containing supernatant ready for use at a 1:8 dilution. These treated samples were further diluted to 1:16 for total IgG testing, 1:20 for EBV VCA IgM ELISA testing, and 1:200 for IgM quantitation by ELISA.

The treated samples were compared with samples diluted in the regular diluent supplied with the EBV IgM ELISA kits, which did not contain an IgG absorbent.

RF quantitation. RF testing was performed on an Array 360 (Beckman Instruments, Inc.) by rate nephelometry, using aggregated human IgG as the antigen. An increase in light scatter resulting from the interaction of RF and aggregated IgG is converted to a peak rate signal which is a function of the RF concentration (10). Following calibration, the peak rate signal for the assay is automatically converted to concentration units by the analyzer. Values are reported as international units per milliliter and are calibrated to the World Health Organization reference preparation for RF (3).

IgG quantitation. The Beckman Array 360 was also used for IgG testing. The method is identical to that for RF described above, with the exception that antibody to human IgG is employed to detect human IgG in the sample. Results are reported as milligrams per deciliter.

IgM quantitation. For IgM testing, a sensitive ELISA was developed. Polystyrene Immulon 4 wells (Dynatech Laboratories, Inc., Chantilly, Va.) were coated with 100 μl of 1.5-μg/ml goat anti-human IgM monoclonal antibody (Sigma, St. Louis, Mo.) per well. The plates were coated for 20 h at 4°C and then washed for three cycles on an automated washer (WellWash 4; Denley Instruments, Durham, N.C.) with a PBS-Tween 20 buffer. The plates were then blocked for 1.5 h with 100 μl of StabilCoat (Bio-Metric Systems, Inc., Eden Prairie, Minn.) per well and stored at 4°C. Serum samples were diluted 1:20 in the goat anti-human IgG diluent and GullSorb and 1:8 in the Isocolumn. All samples were further diluted to 1:200. A standard curve was generated by making serial dilutions of purified human IgM (Sigma). One hundred microliters each of the serum dilutions and standard dilutions was then added to each microplate well, and the dilutions were tested in duplicate. The plate was incubated for 30 min at 37°C and washed for five cycles. One hundred microliters of a 1:4,000 dilution of alkaline phosphatase-goat anti-human IgM (Fc5u-specific) conjugate (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) was then added to each well, and the plate was incubated and washed as before. Finally, 100 μl of 1-mg/ml *p*-nitrophenol phosphate in diethanolamine (Sigma) was added to each well, incubation was carried out for 30 min, and the reaction was stopped with 1 N NaOH. The plates were read on a spectrophotometer (405 to 650 nm), results for duplicate wells were averaged, and values were obtained in milligrams per deciliter on the basis of the standard curve.

EBV ELISA testing. EBV VCA IgM testing was performed according to the instructions provided by the manufacturer (Ortho Diagnostic Systems, Inc., Raritan, N.J.) with the exception that the patient samples, supplied controls, and calibrator were also treated by the IgG removal procedures.

The absorbance was read at a wavelength of 490 nm, and samples were reported as positive if their absorbances were greater than the mean cutoff control and negative if the absorbances were less than the mean cutoff control.

RESULTS

IgG removal. Twenty-five human serum samples with IgG levels ranging from 753 to 7,220 mg/dl were tested by the three IgG removal procedures. (The normal range of IgG in serum is 800 to 1,700 mg/dl in human adults.) The samples were treated by the three different procedures, and then the amounts of IgG remaining in the samples were quantitated by rate nephelometry. The percentage of IgG removed was calculated by comparing the amount of IgG remaining with the amount of IgG in the untreated sample. Of the 25 samples tested, only 2 had any detectable IgG after treatment with the goat anti-human IgG diluent and GullSorb (Table 1). The goat anti-human IgG diluent removed an average of 100.0% of the IgG in samples in the normal range (<1,700 mg/dl; the detection limit of the nephelometer is 0.93 mg/dl). In samples with elevated IgG levels (>1,700 mg/dl), the goat anti-human IgG diluent removed all of the IgG up to 4,100 mg/dl and 81.6% of the IgG from a sample with an extremely elevated level of IgG, 7,220 mg/dl (Table 1, sample 25). These results were very similar to those for the GullSorb treatment, in which an average of 100.0% of the IgG was removed from sera with normal IgG levels, nearly all of the IgG up to 4,100 mg/dl was removed, and 88.2% of the IgG from the sample with the

TABLE 4. Reproducibility studies showing percent IgM remaining after treatment by the three procedures

Sample[a]	IgM not absorbed (mg/dl)	*n*	Mean % IgM remaining	SD	% CV
1S	77.7	6	85.9	2.5	3.0
1G		6	87.3	1.8	2.1
1I		6	94.1	4.1	4.3
2S	86.0	6	86.4	3.1	3.6
2G		6	89.9	4.1	4.6
2I		6	97.1	1.8	1.9
3S	68.4	6	93.2	3.3	3.6
3G		6	92.6	3.9	4.2
3I		6	98.2	2.2	2.2
4S	29.0	6	87.7	2.6	3.0
4G		6	85.7	2.7	3.2
4I		6	94.7	1.9	2.0
5S	19.2	6	91.4	2.2	2.5
5G		6	88.4	6.3	7.1
5I		6	95.3	3.1	3.2
6S	6.5	5	76.6	3.5	4.5
6G		5	74.7	5.0	6.7
6I		5	103.4	1.8	1.7
7S	21.3	6	85.1	2.2	2.6
7G		6	83.2	3.5	4.2
7I		6	96.2	4.9	5.1
8S	27.7	6	78.3	3.1	4.0
8G		6	76.3	2.3	3.0
8I		6	88.0	3.8	4.4
9S	66.3	4	91.4	0.7	0.8
9G		4	93.1	3.5	3.7
9I		4	102.4	1.9	1.9

[a] S, goat anti-human IgG diluent; G, GullSorb; I, Isocolumn.

extremely elevated level, sample 25, was removed. The Isocolumn was not as effective at removing IgG. An average of 94.6% was removed from samples in the normal range (<1,700 mg/dl). The Isocolumn showed a significant drop in IgG removal at levels above 2,050 mg/dl and removed only 54.3% of the IgG from sample 25. The reproducibility of the IgG removal procedures was investigated by testing an additional nine samples with various levels of IgG. Three separate extractions were performed on each sample by all three procedures. The standard deviation and percent CV were then calculated for the amount of IgG remaining in each sample (Table 2).

As described above, high titers of antigen-specific IgG can cause a false-negative IgM reaction (Fig. 1). To test the effectiveness of the IgG removal procedures in eliminating false-negative results, high-titer EBV IgG VCA-positive samples (1:5,260) were spiked with IgM-positive sera. When tested with the regular diluent, five of these samples yielded negative results. When treated with the goat anti-human IgG diluents, all five of these samples had positive results. Isocolumn treatment yielded four positive results and one equivocal result, which remained equivocal after retesting.

IgM quantitation. The same 25 human serum samples were also tested for total IgM, with levels ranging from 6.8 to 61.6 mg/dl. The samples were treated by the three procedures, and then the amounts of IgM remaining in the samples were quantitated by the IgM ELISA. The percentage of IgM recovered was calculated by comparing the amounts of IgM remaining in the treated samples with the amount of IgM in the untreated sample. For the 25 samples tested, an average of 82.5% of the IgM remained after treatment with the goat anti-human IgG diluent, while 81.8% of the IgM remained after treatment with GullSorb. The Isocolumn results were significantly better, with an average serum IgM recovery of 96.1% (Table 3). The reproducibility of the three IgG removal procedures for the recovery of IgM was investigated by treating an additional nine samples a minimum of four times each. The standard deviation and percent CV were then calculated for the amount of IgM remaining in each sample (Table 4).

RF interference. As described above, RF of the IgM class can cause false-positive serologic reactions by binding to IgG (Fig. 1). Of the 55 additional serum samples selected and tested by EBV IgM ELISA, 33 had RF concentrations higher than the normal range (<40 IU/ml). Of these 33 RF samples, 13 (samples 5, 9, 10, 13, 14, 25, 39, 40, 44, 50, 51, 53, and 54) had false-positive IgM reactions, while another 5 (samples 2, 8, 11, 15, and 24) were actually positive for IgM antibodies against EBV VCA (Table 5). Not all samples with elevated RF levels caused false-positive ELISA results. This may be due to the fact that the nephelometer measures all classes of RFs, including IgG and IgM. Samples which do not have IgM RF should not interfere. All 13 samples which had false-positive ELISA results caused by RF tested negative when treated with the goat anti-human IgG diluent and GullSorb. Two of these samples (Table 5, samples 10 and 54) remained positive after treatment with the Isocolumn. These samples had very elevated RF levels (2,840 and 3,660 IU/ml, respectively) and IgG levels of 2,070 and 1,840 mg/dl, respectively. After Isocolumn treatment, 136.8 mg of IgG per dl remained in sample 10 and 16 mg/dl remained in sample 54. Apparently, there was still enough IgG present after treatment to provide binding sites for the IgM RF to cause false-positive results. Since the diluents remove more IgM than the Isocolumn, another explanation for these samples may be that enough IgM was removed by the diluents to cause the IgM antibody levels to fall below the positive cutoff. This explanation does not seem as likely, since other samples, such as sample 22, had optical densities (ODs) closer to the positive cutoff than those of samples 10 and 54 but nevertheless remained positive when treated with the diluents. In addition, the ELISA positive-cutoff control was also treated by the diluents to adjust for any loss of IgM from the serum samples. Another five samples with RF levels higher than normal remained positive after treatment by all three procedures and were therefore assumed to be true positives, as no clinical information was available for these patients. Sample 18 (Table 5) was positive before treatment and remained positive with the GullSorb and Isocolumn but was negative as determined with goat anti-human IgG diluent. After this sample was retested and the same results were obtained, the goat anti-human IgG-extracted sample was centrifuged to eliminate the possibility of the goat-human IgG complexes binding to the antigen and thus causing a false-negative reaction. Upon retesting of the centrifuged sample, the results did not change, and therefore the antibody complexes do not seem to interfere with the ELISA. One other discrepancy was also noted for sample 12 (Table 5). This sample was positive when treated with the Isocolumn but was negative with the IgG-absorbent diluents and the regular diluent. The possibility of protein G contamination in the supernatant due to improper filtering was investigated by adding recombinant protein G and recombi-

TABLE 5. Comparison of positive and discrepant ELISA IgM results after treatment with the three IgG removal procedures[a]

Sample	RF (IU/ml)[b]	Regular diluent		Goat anti-human diluent		GullSorb		Isocolumn	
		Result	OD	Result	OD	Result	OD	Result	OD
2	179	POS	3.500	POS	3.439	POS	3.071	POS	4.000
5	421	POS	2.079	NEG	0.072	NEG	0.080	NEG	0.221
8	65	POS	3.500	POS	3.500	POS	2.862	POS	3.887
9	93	POS	3.448	NEG	0.056	NEG	0.060	NEG	0.490
10	2,840	POS	1.175	NEG	0.196	NEG	0.201	POS	1.445
11	107	POS	2.806	POS	0.486	POS	0.544	POS	1.012
12	183	NEG	0.559	NEG	0.234	NEG	0.248	POS	1.307
13	57	POS	1.178	NEG	0.128	NEG	0.200	NEG	0.662
14	42	POS	2.311	NEG	0.041	NEG	0.045	NEG	0.429
15	115	POS	3.491	POS	0.491	POS	0.583	POS	0.767
16	<20	POS	1.033	POS	0.609	POS	0.530	POS	1.703
18	31	POS	1.115	NEG	0.109	POS	0.671	POS	0.803
19	<20	POS	1.688	POS	1.558	POS	1.555	POS	2.338
22	<20	POS	0.833	POS	0.519	POS	0.531	POS	0.931
24	293	POS	1.845	POS	1.526	POS	1.473	POS	0.891
25	85	POS	1.762	NEG	0.123	NEG	0.176	NEG	0.434
28	<20	POS	0.901	NEG	0.042	NEG	0.048	NEG	0.167
35	<20	POS	2.275	POS	1.646	POS	1.702	POS	2.523
39	365	POS	0.795	NEG	0.078	NEG	0.050	NEG	0.235
40	548	POS	3.229	NEG	0.174	NEG	0.133	NEG	0.452
44	43	POS	2.500	NEG	0.127	NEG	0.146	NEG	0.588
50	415	POS	2.485	NEG	0.272	NEG	0.340	NEG	0.722
51	2,980	POS	2.997	NEG	0.067	NEG	0.069	NEG	0.345
53	1,980	POS	3.336	NEG	0.245	NEG	0.210	NEG	0.540
54	3,660	POS	1.496	NEG	0.340	NEG	0.258	POS	1.105
ELISA controls									
Negative			0.056		0.013		0.001		0.141
Positive			3.186		2.973		3.196		3.775
Cutoff			0.779		0.458		0.454		0.799

[a] POS, positive; NEG, negative.
[b] Normal range, <40 IU/ml.

nant protein G bound to specific IgG to the ELISA wells, but this was not found to interfere or cause elevated ODs.

DISCUSSION

The goat anti-human IgG diluent and the GullSorb diluent removed 5.4% more IgG from serum samples in the normal range (<1,700 mg/dl) and up to 16.4% more IgG from samples with >1,700 mg of IgG per dl than the Isocolumn method. This was an advantage of the goat anti-human IgG diluents, as seen for samples 10 and 54 in Table 5. Because all of the IgG was not removed by the Isocolumns, it is likely that these samples with high RF levels still caused false-positive reactions in the ELISA. Therefore, the goat anti-human IgG diluents were more effective than the Isocolumn in eliminating false-positive IgM ELISA results, but the three procedures performed equally well by removing enough IgG to eliminate false-negative reactions caused by antigen-specific competing IgG. The results of all three procedures proved to be reproducible, especially when samples within the normal concentration range of IgG were treated. Samples with IgG concentrations above the normal range showed greater deviation, especially with the Isocolumn, with which less IgG was removed (Table 2).

When the amount of IgM remaining after treatment was calculated, all three IgG removal procedures proved to be very reproducible, having an overall average standard deviation of <4 and an average CV of <4.5% (Table 4). The Isocolumn had the advantage of removing only 3.9% of serum IgM, while the goat anti-human IgG diluent and GullSorb removed 17.5 and 18.2%, respectively (Table 3). The extra IgM removed by the goat anti-human IgG diluents did not seem to affect the sensitivity of the ELISA testing, as in all cases except one (sample 18 with goat anti-human IgG diluent in Table 5), the diluents correctly identified the positive samples. When serum samples were treated with the anti-human IgG diluents, lower OD readings were noticed for EBV IgM-positive ELISA samples than in the Isocolumn treatment. For this reason, it is necessary to also treat the ELISA controls with the anti-human IgG diluents or to adjust the cutoff value.

The diluents are also much easier to use, require the same amount of serum sample, and add no extra steps to the ELISA procedure. The dilutions do not require centrifugation and can still be done in a 96-well dilution tray and transferred to an ELISA plate, thus making this method easily adaptable to automation. The Isocolumn is more labor and time intensive, as it requires an extra dilution, vortexing, incubation on a rotator, and physical manipulation to filter out the protein G from the purified IgM. This procedure is not adaptable to automation.

On the basis of additional cost per patient for the ELISA, the goat anti-human IgG diluent was the most economical, at $0.41 per patient. This cost includes all materials, reagents, and labor, as well as the cost of performing quality control testing to determine the optimal dilution for each new lot of goat serum. For this particular lot, a 1:2 dilution of the goat anti-human IgG serum was found to remove all measurable IgG through the normal range and up to 4,100 mg/dl from samples with elevated IgG levels. This dilution should not vary

greatly from lot to lot, as the manufacturer also has quality control requirements to maintain consistent goat anti-human IgG antibody levels. This diluent proved to be effective in both ELISA and indirect fluorescent-antibody assay IgM testing. In other studies, the diluent did not cause any background fluorescence in indirect fluorescent-antibody assay, and no centrifugation of the treated serum samples was required. In comparison, the commercially available GullSorb was as effective as the in-house-developed diluent but was more expensive, costing $1.92 per patient. The cost for the anti-human IgG diluents was based on the amount of diluent required for a 1:20 dilution of the patient serum. The total volume of the dilution was 240 μl (228 μl of diluent and 12 μl of serum), of which 200 μl was added to the microtiter plate for EBV IgM testing. The Isocolumn was the most expensive of the three procedures, costing $2.09 per serum treatment, on the basis of the cost of $209.00 for 100 columns. An advantage of GullSorb and the Isocolumn is that they have Food and Drug Administration clearance for in vitro diagnostic use. When the effectiveness of IgG removal, the elimination of false-positive results caused by RF, and the ease of use and lower cost were considered, the in-house-developed goat anti-human IgG diluent was the best method for IgM serological testing.

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