Use of a Simple Separation Column in Detection of Immunoglobulin M Antibody to Epstein-Barr Virus

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The use of a new commercial separation column on whole test serum improved the method for detecting immunoglobulin M antibody to Epstein-Barr virus. The efficiency of this product in absorbing interfering immunoglobulin G was similar to that of another absorption product, staphylococcal protein A, but it has the advantage of being stable without refrigeration.

The demonstration of immunoglobulin M (IgM) serum antibody to Epstein-Barr virus (EBV) capsid antigen is a rapid diagnostic test for acute infection with this virus. Immunofluorescence methods for detecting this antibody response in whole serum have been beset by problems, probably due to the presence of high levels of IgG (1, 6) and occasionally IgM rheumatoid factor (RF) to human IgG (4). A simple chromatographic column (Quik-Sep IgM Isolation System; Isolab, Inc., Akron, Ohio) has now been devised which separates serum IgM and IgG and is adaptable for routine use in a diagnostic laboratory. In this study, we compared the usefulness of the separation column on whole serum with that of another absorption method (6) utilizing staphylococcal protein A (SPA) in the detection of IgM antibody to EBV capsid antigen.

Sera from 22 patients with acute EBV infectious mononucleosis, from 6 patients with rheumatoid arthritis, and from 10 healthy controls were examined. The diagnosis of EBV infectious mononucleosis was established by the presence of typical clinical manifestations, peripheral blood lymphocytosis with atypical lymphocyte formation, heterophil antibody response, a consistent pattern of other (non-IgM) EBV-specific antibodies, and the detection of oropharyngeal EBV (3). Sera from three pediatric patients with the above characteristics, except for a heterophil antibody response, also were included. Sera from patients with rheumatoid arthritis were selected because of their high level of IgM RF. Control sera were obtained from five persons lacking antibodies to EBV and five others with old prior EBV infections (3). Test sera were divided into three portions before being tested for the virus specific IgM antibody. One portion was absorbed with SPA (SPA-absorbed serum), one was passed through the separation column (column-separated serum), and the third received no manipulation (unabsorbed serum).

The SPA absorption procedure has been described in detail previously (6). The polypropylene separation column utilized quaternary aminoethyl-Sephadex A-50 as the ion-exchange resin to provide serum IgM essentially free of IgG. The total column volume was 6.5 ml, and the cross-sectional area was 0.44 cm. For the procedure, the wash buffer (ethylenediamine and glacial acetic acid [pH 7.0]) was added to the

column, drained, and discarded. A 200- μ l portion of serum was then diluted with 5 ml of the wash solution and added to the column. This eluate was also discarded. After the column was washed in buffer, the IgM elution buffer (glacial acetic acid, sodium acetate water, and sodium chloride [pH 4.2]) was added. The eluate (2 ml of a 1:10 dilution of original serum), brought to neutral pH, was used for testing. The reduction in levels of total immunoglobulin produced by the absorption steps was determined by a rate nephelometer (Analyzer 11; Beckman Instruments, Inc., Anaheim, Calif.).

The two-coat immunofluorescence technique described by Nikoskelainen et al. (4) was used to detect IgM antibodies to EBV capsid antigen. Column separation or SPA absorption of test serum was a step added in the present study. Briefly, acetone-fixed smears of HR1-K cells treated with a tumor promoter agent, 12-*O*-tetradecanoyl-phorbol-13-acetate (2), were incubated with serially diluted serum and thereafter with fluorescein-conjugated goat antibody to human IgM (Tago, Burlingame, Calif.). The starting dilution for testing the unabsorbed sera and the column-separated or SPA-absorbed samples of these sera was 1:10. Specific fluorescence reactions were characterized by vivid yellow-green fluorescence in a fine granular pattern confined to 50 to 60% of the cells per visual field. Portions of sera were coded and tested concurrently. Fluorescence reactions were evaluated by two observers, with parallel results in 92% of cases and results within a twofold titer difference in 8% of cases. In the latter situation, the results from one observer (C.V.S.) were reported.

A decrease in reaction time between antigen and column-separated or SPA-absorbed samples from 3 to 1 h did not alter titers (titer changes of more than twofold were considered significant). However, this decrease did significantly lower the titers in six instances in unabsorbed sera. Therefore, the analysis of antibody testing was thereafter based on a 1-h reaction between antigen and column-separated or SPA-absorbed sera and a 3-h reaction between antigen and unabsorbed sera. Almost all column-separated samples of sera from patients with infectious mononucleosis has titers similar to those of SPA-absorbed or unabsorbed samples of these same sera (Table 1). The titers of these serum samples, based on the unabsorbed sample, ranged from 1:10 to 1:640 (geometric mean, 1:74).

When tested at the starting dilution of 1:10, the column-separated and SPA-absorbed samples produced less nonspe-

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TABLE 1. Comparison of titers of IgM antibody to EBV capsid antigen in unabsorbed sera versus SPA-absorbed and column-separated samples of the same sera^a

Sera	Titer in relation to unabsorbed sera	No.	
		SPA-absorbed sera	Column-separated sera
Infectious mononucleosis (n = 22)	Same	11	14
	Within twofold range ^b	10	7
	>Twofold lower	0	0
	>Twofold higher	1	1
Control (n = 10)	Same (<1:10)	10	10

^a The reaction time between antigen and SPA-absorbed and column-separated serum samples was 1 h; unabsorbed serum was reacted for 3 h with antigen. The actual starting dilution for testing all serum samples was 1:10.

^b Greater-than-twofold differences in titers were considered significant.

cific fluorescence than the unabsorbed serum (Table 2). Differences in nonspecific fluorescence between unabsorbed and column-separated or SPA-absorbed serum samples were not apparent when serum dilutions of 1:40 or greater were tested.

False-positive EBV-IgM antibody reactions were eliminated by the separation column in all six sera with elevated concentrations of RF (Table 3) (an equivocal reaction was considered negative). A large reduction in serum IgG antibody to EBV capsid antigen and only a minimal reduction of RF were produced by the separation column. The single column-separated serum sample which gave an equivocal fluorescence reaction at a dilution of 1:10 had the highest combined levels of RF, IgG antibody to EBV capsid antigen, and IgM fluorescence reaction in the unabsorbed sample of the same serum. Similar findings were obtained with the SPA-absorbed sera.

In randomly selected sera from five patients, the separation column reduced the total IgG concentration by an average ($\pm$ standard deviation) of 99.7% ($\pm 0.08\%$) and the

TABLE 2. Comparison of nonspecific background fluorescence in the EBV-IgM antibody test^a

Sera	No. with the following nonspecific background fluorescence:			
	Tr	+	++	+++
Unmodified	11	10	5	6
SPA absorbed	26	6	0	0
Column separated	27	5	0	0

^a Nonspecific background fluorescence had a diffuse or filmy appearance, was present over most of the cell smear, included extracellular localization, and was relatively weaker in intensity than specific fluorescence. Twenty-two sera from patients with infectious mononucleosis and 10 sera from controls were divided into three portions and determined unabsorbed, after SPA absorption, or after column separation. The results are based on nonspecific background fluorescence graded from trace to +++ produced by a 1:10 dilution of the test serum samples.

TABLE 3. Levels of IgM (RF)^a and IgM and IgG antibodies to EBV capsid antigen before and after SPA absorption or column separation of sera containing RF from patients with rheumatoid arthritis

Serum	Unmodified			SPA absorbed			Column separated		
	RF	IgG	IgM	RF	IgG	IgM	RF	IgG	IgM
1	143.9	1:2,560	1:80	102.5	1:10	<1:10	139.9	1:20	<1:10
2	31.5	1:2,560	1:20	21.0	<1:10	<1:10	19.3	1:10	<1:10
3	154.6	1:2,560	1:80	103.3	<1:10	<1:10	147.0	<1:10	<1:10 ^b
4	172.1	1:320	1:20	127.6	<1:10	<1:10	157.8	<1:10	<1:10
5	85.8	1:160	1:10	44.9	<1:10	<1:10	50.5	<1:10	<1:10
6	116.7	1:10	1:10	103.7	<1:10	<1:10	71.9	<1:10	<1:10

^a Quantitated by radioimmunoassay as described by Pope and McDuffy (5). The concentration was measured in micrograms per milliliter.

^b Equivocal fluorescent reaction.

total IgM concentration by an average of 48.5% ($\pm 7.5\%$). SPA absorption reduced the total IgG concentration by 99.1% ($\pm 0.8\%$) and the total IgM concentration by 38.0% ($\pm 6.1\%$).

The use of the commercially available separation column offered improvements for EBV-IgM antibody assays similar to those obtained with SPA absorption, namely, a reduction of nonspecific background fluorescence at lower dilutions of test serum, a shortened reaction time between test serum and antigen, and the removal of false-positive reactions caused by IgM RF. The column separation system is simple and permits large-scale IgM testing at low cost. Moreover, and in contrast to the SPA system, the separation column does not require refrigeration for storage or transportation.

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