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Production of Polyclonal and Monoclonal Antibodies against Group A, B, and C Capsular Polysaccharides of *Neisseria meningitidis* and Preparation of Latex Reagents

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Polyclonal and monoclonal antibodies against capsular polysaccharides of *Neisseria meningitidis* serogroups A, B, and C were produced in order to develop immunological reagents allowing both the detection of soluble antigens during meningococcal meningitis and antigenic serogrouping of *N. meningitidis* cultures. The performance characteristics of monoclonal and polyclonal antibody latex reagents were compared. For the detection of soluble polysaccharide antigen, polyclonal antibody latex reagent was selected for *N. meningitidis* A and C. The latex reagent prepared with polyclonal antibodies against *N. meningitidis* B could not detect capsular polysaccharide even at 1 mg/ml. The monoclonal antibody B latex reagent which detected 100 ng of polysaccharide per ml was therefore chosen. For the serogroup identification of *N. meningitidis*, the use of a confirmatory test results in an overall specificity of 100% with polyclonal or monoclonal antibody latex reagents.

Meningococcal meningitis is a major health problem in many countries. In France there are 600 to 800 cases per year (19), 8 to 10% of which result in death. Rapidity and reliability of serogroup identification are essential in diagnosis of meningococcal infection both for individual therapy and for prophylaxis. In France, prophylaxis with vaccines A and C for cases of contact requires an accurate and rapid diagnosis of serogroups to maximize the specific activity of preventive-therapy vaccines.

Before Kohler and Milstein (14) found that cellular hybridation allowed the production of monoclonal antibodies (MAb) of predetermined specificity, serogroup identification tests for meningococcal strains used specific polyclonal antibodies (PAb) raised in rabbits. MAb against capsular polysaccharides of meningococci of groups A, B, and C have been produced by various authors to replace PAb for routine bacteriological laboratory use, particularly against group B meningococci because of the extreme difficulty of producing antisera with group B specificity (17, 31). Moreover, MAb were considered good reagents because of their specificity and reproducibility. They also allow standardization of diagnostic tests (26).

This paper describes the production of a range of MAb with appropriate specificities to each of the group A, C, and particularly B polysaccharides of *Neisseria meningitidis*. A latex particle agglutination test was developed for their use in serogroup identification and in the detection of capsular polysaccharide antigens. These reagents were compared to those obtained with PAb. This comparison allows the preparation of the most satisfactory reagents by using MAb or PAb.

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MATERIALS AND METHODS

Bacterial strains. Meningococcal reference strains for serogroups used for MAb and PAb preparation and titration are listed in Table 1. Eighteen type strains of the genus *Neisseria*, 13 type strains of genera other than *Neisseria*, and strains of species other than members of the family *Neisseriaceae* used in this study are listed in Table 2.

One hundred twelve strains from clinical isolates collected at the Centre National de Référence des Méningocoques (Institut Pasteur, Paris, France) were used for testing the efficiency of latex reagents in serogroup identification. Their clinical origins are shown in Table 3. Strains isolated in different regions of France over a period of 3 years were included in the study. Only one isolate from each patient was used.

All strains were stored by lyophilization or frozen at −70°C (1). The strains were first isolated on selective and nonselective media (21). Bacteriological identification was similar to diagnosis of *N. meningitidis* (21): bacteria appeared as cocci in pairs or in tetrads without pigment. They were oxidase and catalase positive. The bacteria caused acidification in cystine-trypticase agar (Difco) containing glucose or maltose but not that containing fructose or sucrose. γ-Glutamyl-transferase activity (20) was observed. There was no synthesis of polysaccharides (iodine test) and no hydrolysis of tributyrin (22). Agglutination was performed as previously described (28).

Preparation of polysaccharide. Reference strains of serogroups A, B, and C used for preparation of polysaccharide are listed in Table 1. Bacteria were cultivated in Frantz media (9) supplemented with 2 g of yeast extract dialysate per liter. The culture was grown overnight at 37°C in a Psycrotherm incubator shaker (New Brunswick) at 120 rpm under 8 to 10% CO_2 to the late-logarithmic growth phase. Hexadecitrimethyl ammonium bromide was then added to the culture to a final concentration of 0.1%. The bacterial

TABLE 1. Meningococcal serogroup reference strains

Serogroup	Origin of strains	Reference	LNP[a] no.
A	M 1027 (1872)	28	1912
B	M 2092 (2779)	28	1913
C	SD 3 CM 1628 (2711)	28	1914
X	18/68 (456)	28	1915
Y	19/68 (1830)	28	1916
Z	20/68 (498)	28	1917
29E	M 521	28	636
W135	M 603	28	638
H	1890	6	3970
I	1486	6	3969
K	1811	6	3971
L	78189	2	3972

[a] LNP, Laboratoire des Neisseria, Institut Pasteur, Paris, France.

cells and the precipitated capsular polysaccharide were harvested by centrifugation. The pelleted paste was suspended in 1 M $CaCl_2$ and stirred at 4°C for 1 h. The suspension was centrifuged at 25,000 × *g* for 20 min, and the pellet was discarded. Ethanol was added to the supernatant to give a final concentration of 25% (vol/vol) ethanol, and the supernatant was stirred for 1 h and centrifuged at 25,000 × *g* for 20 min. Ethanol was added to the supernatant in order to obtain a final concentration of 80% (vol/vol) ethanol, and the samples were stored overnight at 4°C. The precipitate was collected by centrifugation at 25,000 × *g* for 20 min and washed with absolute ethanol. It was then dissolved in, and dialyzed against, distilled water. The capsular polysaccharide was treated with cold phenol, and the lipopolysaccharide was removed by centrifugation at 100,000 × *g* for 3 h. The polysaccharide was dialyzed against distilled water and freeze-dried. The final product contained less than 2% protein and nucleic acid and less than 0.01% lipopolysaccharide as measured by the *Limulus* amebocyte lysate test.

TABLE 2. Bacterial type strains used for the evaluation of sensitized latex reagents

Species	Type strain[a]	Collection no.[a]
Family *Neisseriaceae*		
N. meningitidis	ATCC 13077^T	LNP 404
N. gonorrhoeae	ATCC 19424^T	LNP 403
N. mucosa	ATCC 19696^T	LNP 405
N. subflava	NRL 30017^T	LNP 3260
N. flava	NRL 30008^T	LNP 3264
N. perflava	ATCC 10555^T	LNP 407
N. sicca	NRL 30016^T	LNP 3265
N. flavescens	ATCC 13120^T	LNP 414
N. lactamica	ATCC 23970^T	LNP 411
N. denitrificans	ATCC 14686^T	LNP 412
N. cinerea	ATCC 14685^T	LNP 415
N. polysaccharea	LNP 462^T	NCTC 11858^T
N. animalis	ATCC 19573^T	LNP 413
N. elongata	ATCC 25295^T	LNP 416
N. canis	ATCC 14687^T	LNP 419
N. ovis	ATCC 33078^T	LNP 427
N. cuniculi	ATCC 14688^T	LNP 420
N. caviae	ATCC 14659^T	LNP 418
B. catarrhalis	ATCC 25238^T	LNP 417
K. kingae	ATCC 23330^T	LNP 4136
K. indologenes	ATCC 25869^T	LNP 4103
K. denitrificans	ATCC 10995^T	LNP 4138
M. bovis	ATCC 10900^T	
M. phenylpyruvica	ATCC 23333^T	
M. osloensis	ATCC 19976^T	
A. calcoaceticus	ATCC 23055^T	LNP 6617
A. lwoffii	ATCC 15309^T	LNP 6624
A. baumannii	ATCC 19606^T	LNP 6621
A. haemolyticus	ATCC 17906^T	LNP 6625
A. junii	ATCC 17908^T	LNP 6619
A. johnsonii	ATCC 17909^T	LNP 6620
Species other than *Neisseriaceae*		
H. influenzae serotype b		CIP 52152, CIP 5494 (NCTC 8468)
E. coli K-1		CIP 8558
E. coli O1:K-1:H7	ATCC 11775^T	CIP 54.8

[a] ATCC, American Type Culture Collection (Rockville, Md.); NRL, *Neisseria* Reference Laboratory (Seattle, Wash.); NCTC, National Collection of Type Culture (London, England); CIP, Collection de l'Institut Pasteur (Paris, France); LNP, Laboratorie des *Neisseria*, Institut Pasteur (Paris, France).

TABLE 3. Clinical isolates (109 strains) used for the evaluation of sensitized latex reagents

Species and serogroup	No. of isolates of clinical origin					
	CSF	Blood	Pharynx	Sputum	Other	Total
N. meningitidis						
A	3	2	9		6	20
B	22	2	6	1	2	33
C	6	5	3		1	15
X			3	2		5
Y	3	2	1		1	7
Z			1	2	2	5
29E			3	2		5
W135	1	1	1	1		4
NA[a]				1		1
PA[b]				2		2
N. lactamica			7			7
B. catarrhalis		1	2	2		5

[a] NA, nonagglutinable.
[b] PA, polyagglutinable.

The immunological reactivity of these polysaccharides was estimated by immunodiffusion. Purified capsular polysaccharides of *N. meningitidis* group A (lot MA 1120), group B (lot 30-2), and group C (lot MC 5128), kindly provided by R. Triau (Institut Mérieux, Marcy l'Etoile, France), and *Escherichia coli* K-1, kindly provided by W. F. Vann (Office of Biologics, Food and Drug Administration, Bethesda, Md.), were used as reference antigens.

Rabbit antisera against group A and C polysaccharides prepared in the Centre National de Référence des Méningocoques and equine group B meningococcal antisera (H46) kindly provided by J. B. Robbins (National Institutes of Health, Bethesda, Md.) were used as reference antisera. Antigenic identity was found between our polysaccharide preparations and the corresponding reference antigens.

Rabbit antibody (PAb) production. Rabbit immune sera to *N. meningitidis* groups A, B, and C were made by immunization of bouscat rabbits with living bacterial suspensions as previously described (28). Antibodies were purified by salt fractionation and anion-exchange chromatography (DE52-Whatman) by the method of Levy and Sober (15).

Production of MAb. Five injections of purified polysaccharide were given intraperitoneally to BALB/c mice. Mice were injected with 10 μg of antigen on day 1 (D1) and D6 and with 5 μg on D12, followed by two final simultaneous intraperitoneal and intravenous injections (2.5 μg each) on both D13 and D14.

This procedure gave specific hybridomas against group A or C polysaccharide. For group B polysaccharide which was

known to be weakly immunogenic, Biozzi, NZB, and CBA mice were stimulated with live meningococci over a period of 30 days. Bacteria were injected intraperitoneally on D1 (5×10^7 bacteria), D9 (1×10^8), and D19 (2×10^8). Four intravenous injections with 5×10^7, 7.5×10^7, 1×10^8, and 2×10^8 bacteria were given on D20, D23, D28, and D30, respectively. Animals were partially bled 1 week after the last injection.

The sera were tested by enzyme-linked immunosorbent assay (ELISA). Only one Biozzi mouse (genetically selected for its high immune response) gave a strong specific immune response. Three days after the last injection, this Biozzi mouse was bled and the spleen was removed. Spleen cells were fused with X63-Ag8.653 myeloma cells (12) as described by Kohler and Milstein (14). Hybridoma cells were tested for production of antibody against polysaccharide by ELISA. Cells from positive wells were cloned by the limiting dilution technique in microtiter plates, and supernatants were screened by ELISA. Positive clones were expanded and injected intraperitoneally into 2,6,10,14-tetramethylpentadecane (pristane)-primed mice. MAb of the immunoglobulin M type were purified on a S200 Sephacryl column by a modified gel filtration technique (3).

ELISA. Antibodies in supernatants or in ascites were assayed by a modification of the method of Voller et al. (29). Microtiter plates (Nunc) were incubated with 1 or 5 μg of polysaccharide per ml diluted in phosphate-buffered saline (PBS) for 2 h at 37°C. Plates were washed three times with PBS containing 0.1% Tween 20 (buffer A) and saturated with buffer A containing 0.5% gelatin for 30 min. Hybridoma culture supernatants were then added. Plates were incubated for 2 h at 37°C and washed four times prior to the addition of 100 μl of peroxidase-congugated goat anti-mouse immunoglobulins (Diagnostics Pasteur, Marnes-La-Coquette, France). After 1 h of incubation at 37°C and four washes with buffer A, freshly prepared 0.2% orthophenylenediamine (Dakopatts A/S, Glostrup, Denmark) containing 0.03% H_2O_2 in 0.1 M citrate buffer, pH 5.2, was added to each well (100 μl). The peroxidase reaction was stopped by the addition of 3 N HCl (50 μl per well), and the optical density was measured at 490 nm.

The classes and subclasses of MAb were determined by ELISA using peroxidase-conjugated anti-mouse immunoglobulins specific for immunoglobulin M or different subclasses of immunoglobulin G (Miles Laboratories).

Competitive enzyme immunoassay. The following competitive procedure was used to define the antibody specificity. The concentration of MAb giving 50% maximum binding to the antigen was determined by enzyme immunoassay. Then, the antibody at this concentration was incubated overnight at room temperature with a series of concentrations of polysaccharide antigen (50 to 0.010 μg). The mixture was then incubated with the antigen immobilized on polystyrene plates for 1 h at 4°C. After the plates were washed, peroxidase-conjugated anti-mouse antibodies were added. Subsequent steps were performed as described above.

Latex reagents. The latex reagents were produced by passive sensitization of colored polystyrene particles (23) (Estapor Rhône Poulenc) with purified mouse MAb (against groups A, B, and C) or rabbit PAb (against groups A and C).

Test procedures for antigen detection. To assess the detection limits of the latex reagent, 50 μl of various concentrations of purified polysaccharide diluted in 9 g of NaCl per liter were mixed with 25 μl of latex on a test card. Agglutination was observed after 3 min.

For cerebrospinal fluid (CSF) and urine specimens, the following procedures were used. Samples of CSF (0.2 to 0.3 ml) were heated in a boiling water bath for 3 min and then centrifuged for 5 min at $3,000 \times g$. Each sample supernatant (50 μl) was mixed with 25 μl of latex reagent on a test card. Agglutination was observed after 3 min.

For serum, the samples were diluted with 100 mM EDTA (EDTA/serum, 3/1 [vol/vol]) and treated and tested as for CSF.

The sensitivity of these latex reagents was established by adding purified polysaccharides to these different biological fluids (CSF, serum, and urine). A mini-test slide, produced by C.B.S. (Worcester, Mass.), was used in order to increase the reading sensitivity of the agglutination test in the case of *N. meningitidis* group B latex reagents. The following procedure was used. Treated samples and latex reagent (50 μl each) were mixed in the well of the slide. The mixture was then diffused by capillarity into a narrow channel. The slide was read after 3 min.

Test procedure for culture serogroup identification. Several colonies isolated on agar plates (Mueller-Hinton) were mixed with one drop of sensitized latex reagent. Agglutination was followed for 1 min. Autoagglutinable and polyagglutinable strains, as defined by Vedros (28), were checked by a confirmatory test (25) as follows. Several colonies were emulsified in 0.5 ml of PBS, pH 7.4, containing 100 ng of EDTA in order to obtain turbidity superior to a no. 4 McFarland standard. Each sample was centrifuged at $3,000 \times g$ for 5 min. The agglutination test was then performed. One drop of supernatant and one drop of sensitized latex reagent were mixed, and agglutination was assessed after 1 min.

RESULTS

Characterization of MAb. The heavy chains of 22 MAb were shown to be μ and those of two antibodies were shown to be γ_3 (anti-group C). Their light chains were determined as κ.

The specificities of the MAb raised against each of the group A, B, and C polysaccharides were checked by immunodiffusion (18), agglutination test (28), and ELISA (29). In double-diffusion analysis, the specificity of each MAb was demonstrated by the single precipitin line observed with each homologous capsular polysaccharide. The MAb specific for group B capsular polysaccharide recognized also *E. coli* K-1 polysaccharide antigen. Bacterial cells of meningococcus groups A, B, and C were directly agglutinated by the corresponding ascitic fluid. Agglutination of bacteria from different *N. meningitidis* groups was observed only with homologous antibodies. In ELISA, MAb against groups A, B, and C recognized homologous antigens. The heterologous reactions all scored negative.

Competitive enzyme immunoassay, which was performed to ascertain the specificity of the antibodies, resulted in 50% inhibition of MAb against group B (clone A24-6) by 100 ng of capsular polysaccharide per ml; 50% inhibition of MAb against group A (clone H13-28) and group C (clone B4-17) was obtained with 200 ng of capsular polysaccharide per ml. No inhibition was observed with heterologous polysaccharides even with 500 μg of antigen per ml.

The three MAb chosen for the preparation of latex reagents were H13-28 for group A, A24-6 for group B, and B4-17 for group C.

Comparative studies of latex reagents coated with PAb or MAb for serogroup identification. Latex reagents coated with PAb against group A or C and latex reagents coated with

TABLE 4. Detection limits for capsular polysaccharide in various fluids

N. meningitidis latex reagent	Detection limit for dilution of polysaccharide in[a]:			
	NaCl	CSF	Serum	Urine
Group A				
PAb	2.5 ng/ml	2.5 ng/ml	25 ng/ml	10 ng/ml
MAb	25 ng/ml	ND	ND	ND
Group B				
PAb	Negative at 1 mg/ml	ND	ND	ND
MAb	100 ng/ml	100 ng/ml	5 μg/ml	Negative at 50 μg/ml
Group C				
PAb	5 ng/ml	5 ng/ml	50 ng/ml	10 ng/ml
MAb	25 ng/ml	ND	ND	ND

[a] Purified polysaccharide was diluted in 9 g of NaCl, CSF, serum, or urine per ml. Then, 25 μl of latex reagent was added to 50 μl of each polysaccharide solution. The agglutination was observed after 3 min. ND, Not determined.

MAb against group A, B, or C were prepared. The latex reagent coated with polyclonal antibodies against group B was not prepared because of the low agglutinating titer of the serum. These reagents were evaluated for serogrouping of *N. meningitidis* strains as groups A, B, and C. Their specificity was checked in agglutination tests. Twenty-two group A strains, 36 group B strains, and 16 group C strains were tested with these latex reagents; all the strains were serogrouped without ambiguity.

All the monoclonal and polyclonal latex reagents were further evaluated with *N. meningitidis* group X, Y, Z, 29E, W135, H, I, K, and L reference strains (Table 1). No agglutination was observed with any of these reference strains.

Monoclonal latex reagents were tested with 18 type strains of the genus *Neisseria* (Table 2). The type strain of *N. meningitidis* was agglutinated with group A latex. This is consistent with the fact that the type strain of *N. meningitidis* is a group A strain. Of 17 type strains of species other than *N. meningitidis*, 9 gave negative reactions with group A, B, and C latex reagents. The remaining 8 of the 17 type strains gave positive reactions. These included *Neisseria sicca* and *Neisseria cuniculi*, which are known to be autoagglutinable. In the confirmatory test, these eight strains all scored negative. No cross-reaction was observed with type strains of the genus *Moraxella* (Table 2), except for the *Moraxella osloensis* type strain, which was autoagglutinable.

Among *Kingella* species, the *Kingella kingae* type strain was agglutinated with both group A and C monoclonal latex. *Kingella indologenes* type strain was agglutinated with group A latex. Two of the six *Acinetobacter* type strains tested, *Acinetobacter junii* and *Acinetobacter haemolyticus*, gave positive reactions with both group A and group C monoclonal latex. All of the species exhibiting autoagglutination and false-positive reactions observed in this study scored negative in the confirmatory test.

The two *E. coli* K-1 capsular strains tested (Table 2) were agglutinated only with group B latex reagent.

Among different strains from clinical isolates (Table 3) tested, only one strain from each of groups X and 29E presented cross-reactivity with specific group A MAb latex. These cross agglutinations did not occur in the confirmatory test. Three of seven strains of *N. lactamica* (clinical isolates, Table 3) which gave positive reactions with group B rabbit polyclonal sera were not agglutinated with group A, B, or C monoclonal latex. The specificity study was extended to other strains. In the genus *Branhamella*, four *Branhamella catarrhalis* strains out of five strains tested were not agglutinated with group A, B, or C monoclonal latex. The fifth strain which was autoagglutinable scored negative in the confirmatory test.

Comparative sensitivities of latex particles coated with PAb or MAb in soluble antigen detection tests. The sensitivities of the latex reagents were tested with various quantities of purified polysaccharide. Table 4 shows the minimum amount of polysaccharide diluted in NaCl (9 g/liter) or biological fluids (CSF, serum, urine) that gave a positive reaction with MAb or PAb latex reagents.

The PAb latex against group A was 10 times more sensitive than the group A MAb (2.5 ng/ml compared to 25 ng/ml). In contrast, the MAb latex against group B was more sensitive (100 ng/ml) than the PAb latex. The PAb latex against group C was five times more sensitive than the group C MAb latex (5 ng/ml compared to 25 ng/ml).

In view of this data, MAb against group B and PAb against groups A and C seem to be the reagents of choice for the detection of soluble antigens. It should be noted that dilution of the samples in CSF did not alter the test sensitivity. However, dilution in serum or urine resulted in reduced sensitivity, especially for the group B reagent.

DISCUSSION

This paper describes the production of MAb and PAb against group A, B, and C *N. meningitidis* and the evaluation of latex reagents for use in serogroup identification and in the detection of polysaccharide antigens.

Our first application of these reagents was the replacement of immune sera used for *N. meningitidis* serogroup identification.

We compared latex sensitized with either PAb or MAb on available *Neisseria* strains. Agglutination was observed only with the homologous strain in all cases. Other members of the *Neisseriaceae* were not agglutinated with group A, B, or C monoclonal latex reagents as expected. A positive reaction was also obtained with *E. coli* type strain K-1, which has been reported to have the same surface polysaccharide as group B meningococci (11). It should, however, be noted that only a few strains other than the type strains of the genus *Neisseria* were used in this study.

About 15% of the results obtained in the primary test with the specific *N. meningitidis* B latex reagent gave weakly false-positive or doubtful reactions. Krambovitis (13) tested 85 bacterial cultures (21 group B and 7 *E. coli*) which were correctly identified except for 3 *E. coli* strains for which the

results were not interpretable because of the slimy nature of the colonies. Various treatments of samples for the reduction of nonspecific reactions in biological fluids (sera, urine, CSF), but not for serogroup identification, have been published (4, 7, 8, 25). In our study, biological samples were treated with EDTA to facilitate serogroup identification.

The use of this confirmatory test allows unambiguous and accurate classification of the autoagglutinating and polyagglutinating strains. All of these latex reagents were 100% specific and 100% sensitive.

The latex reagents prepared with MAb against group A, B, and C *N. meningitidis* were compared to that of similar reagents obtained with PAb in the detection of soluble antigen.

Several techniques, including counterimmunoelectrophoresis, coagglutination, and latex agglutination (10), are used for detecting antigens in biological fluids. The essential criterion is the rapidity of diagnosis compared with culture methods. Counterimmunoelectrophoresis has been used in many laboratories for several years (5), but it has been shown (24, 30) that latex agglutination is more effective than counterimmunoelectrophoresis.

Commercial latex reagents prepared with polyclonal antibodies for the diagnosis of *Streptococcus pneumoniae*, *Haemophilus. influenzae*, and group A, C, Y, and W135 *N. meningitidis* are available. Tilton et al. (27) compared some of these reagents by using coagglutination and latex agglutination and reported that the detection of group B polysaccharide was the most difficult. Only one kit used monoclonal group B latex, which has 80% sensitivity for the detection of soluble antigen in CSF samples. Other kits using group B polyclonal reagents are less sensitive. Directigen group B latex reagent was capable of detecting 30 ng of antigen per ml of spiked PBS (16). The group B latex reagent with monoclonal antibodies developed by Krambovitis (13) gives 100% specificity and sensitivity in fresh samples. The minimum amount of antigen detected in this case varied with the saccharide chain length of the sample. Our polyclonal A and C reagents detected 2.5 to 5.0 ng of soluble antigen per ml as read with the naked eye. Group B reagent could similarly detect 100 ng of antigen per ml.

In reconstituted biological fluids, the sensitivity varied according to the diluent used. The sensitivity was not modified when the polysaccharide was dissolved in NaCl or in CSF, but it decreased in serum or urine. These differences in sensitivity could be due to effects of the pH and ionic strength of the medium. If the antibodies recognize a conformational epitope and this conformation is affected by pH and ionic strength, a reduced sensitivity in urine would be expected. It should be noted that the pH and ionic strength of urine samples vary. Moreover, the protein concentration in the serum was higher than that in CSF. Polysaccharide antigens could be adsorbed onto the proteins and thus be less accessible to MAbs. It is, however, difficult to compare reagents from different origins. For antigen detection in body fluids, no clinical specimens were used in our study. The sensitivity and specificity reported are for model samples.

For detection of antigens A and C, polyclonal latex reagents gave better results than monoclonal reagents. In contrast, the best group B latex reagent is that prepared with MAb. Concerning the serogrouping, polyclonal or monoclonal latex reagents against group A and group C gave identical results. For these reasons, PAb against groups A and C and MAb against group B were selected. However, no general statement can be extrapolated from these results because PAb and MAb characteristics depend on the immunization route, type of immunogen, and strain of mouse used.

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