Staining Characteristics of Six Commercially Available Monoclonal Immunofluorescence Reagents for Direct Diagnosis of *Chlamydia trachomatis* Infections

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Using purified elementary bodies of 14 *Chlamydia trachomatis* serovars in an in vitro assay, we compared the staining characteristics of six commercially available monoclonal antibody reagents used for direct immunofluorescent staining of patient smears. Considerable variation in the degree of brightness, consistency of staining, and specificity of the six reagents was found. Monoclonal antibodies against the major outer membrane proteins of *C. trachomatis* produced brighter fluorescence, more consistent elementary body morphology, and less nonspecific staining than did monoclonal antibodies directed against chlamydial lipopolysaccharide.

As a result of new technologies for antigen detection, the laboratory diagnosis of *Chlamydia trachomatis* infection has moved from reference centers to hospital and commercial laboratories (5a). Direct staining of elementary bodies in urethral and cervical smears with fluorescein-conjugated monoclonal antibodies is widely used by many of these laboratories (1-3, 6, 9-11). There are now many commercially available reagents for this procedure, some utilizing species-specific monoclonal antibodies that bind to the major outer membrane protein (MOMP) of the organism (6, 7) and some using genus-specific antibodies that bind to a lipopolysaccharide (LPS) component (1).

In this study, we have compared the staining characteristics of six commercially available reagents used for direct detection of *C. trachomatis* elementary bodies in patient smears.

MATERIALS AND METHODS

Six commercially available, fluorescein isothiocyanate-conjugated, monoclonal antibody stains for *C. trachomatis* were tested for intensity of fluorescence and staining quality against elementary bodies of each of the 14 major serovars of *C. trachomatis*. The following stains were tested: Anti-chlamydiae mouse monoclonal antibodies (Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash.); Chlamydia direct fluorescent-antibody reagent (Difco Laboratories, Detroit, Mich.); Chlamydia direct specimen test (California Integrated Diagnostics, Inc., Berkeley, Calif.); Imagen chlamydia test (Boots Celltech Diagnostics, Inc.; Analytab Products, Plainview, N.Y.); Microtrak *Chlamydia trachomatis* direct specimen test (Syva Co., Palo Alto, Calif.); and Pathfinder *Chlamydia trachomatis* direct antigen detection system (Kallestad Diagnostics, Austin, Tex.).

Chlamydial antigens grown in HeLa 229 cells were Formalin killed, purified through Renografin (E. R. Squibb & Sons, Princeton, N.J.) density gradients, and then stored at 4°C (12). Each antigen was suspended in (i) phosphate-buffered saline (PBS), pH 7.4, and (ii) 5% fetal bovine serum (FBS) in PBS, pH 7.4. We studied the effect of exposure of elementary bodies to 5% FBS because culture specimen

swabs which are transported in a 2 M sucrose phosphate buffer supplemented with 5% FBS are a possible source of specimens for direct staining. Antigen dilutions were calculated to achieve two dilutions simulating moderate and high antigen concentrations, i.e., 5 to 20 and 50 to 200 elementary bodies per an $\times 630$ microscopic field. Actual counts sometimes varied slightly from these estimates. Antigens were held in suspension at 4°C for 5 to 6 days prior to slide preparation and staining.

Antigens were applied to microscope slides with 10 6-mm-diameter wells per slide by using a 10- μ l pipette. Each of the two concentrations of antigen was placed on five wells per slide. Two slides were used to test each serovar against each stain. The first slide contained elementary bodies exposed only to PBS, and the second contained elementary bodies exposed to 5% FBS in PBS. Slides were air dried and fixed by the method recommended by the manufacturer (by acetone or methanol or air dried). Some stains were tested by using more than one fixation method.

Each stain was tested at the concentration recommended by the manufacturer and at 1:4, 1:16, and 1:64 dilutions by using PBS, pH 7.6, containing Evans blue, bovine serum albumin, and glycerol as a diluent. Stain (10 μ l) was applied to each well and incubated by the method recommended by the manufacturer. Incubation time was either 15 or 30 min, and incubation temperature was either 35°C or room temperature. Slides were rinsed with distilled water for 10 s and mounted in a glycerol-based fluorescent mounting fluid (Syva).

Each stain was also tested against bacteria known to cross-react with chlamydial monoclonal antibodies, including *Peptostreptococcus productus* (two isolates), *Staphylococcus aureus* Cowan, and *Neisseria gonorrhoeae* IA32779 and JSK53.

Slides were read on an epifluorescence Zeiss microscope with a halogen light source (100 W) and with a Zeiss Planapo $\times 63/1.4$ oil immersion objective and Zeiss Kpl $\times \times 10/18$ eyepieces. Fluorescence was graded from negative to 3+ (-, no visible organisms; +/-, organisms just visible; 1+, light-green staining; 2+, moderate fluorescent-green staining; 3+, intense fluorescent staining). In addition, the size

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TABLE 1. Staining of *C. trachomatis* elementary bodies by six commercially available products

Intensity of fluorescence and elementary body morphology by method using ^a :																
<i>C. trachomatis</i> serovar	Anti-MOMP antibodies								Anti-LPS antibodies							
	Syva			Difco		Kallestad			Bartels		Boots Celltech		California Integrated Diagnostics			
	Acetone		MeOH	Acetone		MeOH		Acetone		Acetone		MeOH		Air Dry		
	PBS	FBS	FBS	PBS	FBS	PBS	FBS	PBS	FBS	PBS	FBS	PBS	FBS	PBS	FBS	
A	3C	3C	3C	2C	2C	2C	±	1-3V	2-3V	±-1V	1-3V	1-2V	1-3V	1-3V	1-3V	
B	3C	3C	3C	2C	2C	2C	2C	2-3V	1-3V	±-1V	1-2V	1-2V	1-2V	2-3V	2-3V	
D	3C	3C	3C	2C	2C	3C	3C	1-2V	±-2V	±-1V	±-2V	±-2V	±-2V	±-2V	1-2V	
E	3C	3C	3C	2C	2C	3C	3C	1-2V	±-2V	±-1V	±-2V	±-2V	±-2V	1-2V	1-2V	
F	3C	3C	3C	2C	2C	2C	2C	1-2V	1-2V	±-1V	±-2V	±-2V	1-2V	1-2V	1-2V	
G	3C	3C	3C	2C	2C	2C	1C	2-3V	2-3V	±-1V	1-2V	1-3V	2-3V	1-3V	1-2V	
C	3C	2V	3C	2C	2V	1C	–	1-3V	1-3V	±-1V	1-3V	±-1V	1-3V	1-3V	2-3V	
J	3C	1V	3C	2C	2V	1C	±	1-3V	±-3V	±-2V	±-2V	±-2V	±-3V	±-3V	1-3V	
H	3C	3C	3C	2C	2C	1C	±	1-3V	1-3V	±-2V	±-2V	±-3V	±-2V	1-3V	1-3V	
I	3C	2V	3C	2C	2C	1C	±	2-3V	1-3V	±-2V	±-2V	±-2V	±-3V	±-3V	1-3V	
K	3C	3C	3C	2C	2C	±	±	±-2V	1-3V	±-2V	±-2V	1-2V	1-2V	±-2V	±-2V	
L3	3C	3C	3C	2C	2C	1C	±	1-3V	1-3V	±-1V	1-2V	1-2V	1-2V	1-3V	1-3V	
L1	3C	3C	3C	2C	2C	3C	2C	1-3V	1-3V	±-1V	±-2V	1-2V	1-3V	2-3V	1-3V	
L2	3C	3C	3C	2C	2C	2C	2C	±-2V	1-3V	±-1V	±-2V	1-2V	1-2V	1-2V	1-2V	

^a Antigens were suspended in either PBS or FBS, and slides were fixed by acetone or methanol or air dried. The intensity of fluorescence is shown as ±, 1+, 2+, or 3+ as defined in Materials and Methods. Elementary body morphology is indicated as consistent (C) or variable (V).

and shape of the fluorescence-stained elementary bodies were graded as consistent or variable.

RESULTS

The intensity of observed fluorescence of elementary bodies ranged from very dim (±) to bright-green (3+) (Table 1). In general, brighter and more consistent fluorescence was observed with those products using anti-MOMP monoclonal antibodies (Syva, Kallestad, and Difco) than with those using anti-LPS antibodies (Boots Celltech, Bartels, and California Integrated Diagnostics). When elementary bodies were diluted in PBS, the intensity of staining with the Syva reagent was consistently 3+ with all 14 serovars. Under the same conditions, the Difco and Kallestad stains were slightly less bright, with Kallestad being least intense against the K-L3 and CJHI serovars. When elementary bodies were diluted in FBS and refrigerated for 5 days before being spotted on slides, all three anti-MOMP stains were reduced in brightness and the elementary bodies of some serovars lost their characteristic morphology.

Staining with reagents utilizing anti-LPS antibodies resulted in generally lesser and much more variable intensities.

The intensity of staining did not appear to be affected by incubation with FBS (Table 1). We also found that the characteristic morphology of the stained elementary bodies varied with the reagent used. We classified morphology as either consistent (C) or variable (V) (Table 1). When the anti-MOMP stains were used, elementary bodies consistently appeared as well-defined, round disks of a uniform size. The anti-LPS reagents (Bartels, Boots Celltech, and California Integrated Diagnostics) resulted in stained elementary bodies of more varied shapes and sizes. These products also stained extraneous fragments of chlamydial LPS which appeared as fluorescent artifacts.

To evaluate the specificity of the stains, we used each product to stain slides spotted with several species of bacteria, including *N. gonorrhoeae*, *S. aureus*, and *P. productus* (Table 2). Although monoclonal antibodies are theoretically specific, in reality they may cross-react with other microorganisms. Only the Syva and Kallestad stains failed to react with any of the other bacteria tested. The Difco, California Integrated Diagnostics, and Bartels stains reacted strongly with *P. productus*, while the Boots Celltech stain reacted weakly with *P. productus* and the Bartels stain reacted weakly with *S. aureus*.

TABLE 2. Staining of bacteria by six commercially available products

Strain	Intensity of fluorescence ^a with product from:					
	Syva	Difco	Kallestad	Bartels	Boots Celltech	California Integrated Diagnostics
<i>P. productus</i> (isolate 1)	—	2+	—	1+	±	2+
<i>P. productus</i> (isolate 2)	—	2+	—	2+	1+	2+
<i>N. gonorrhoeae</i> IA32779	—	—	—	—	—	—
<i>N. gonorrhoeae</i> JSK53	—	—	—	—	—	—
<i>S. aureus</i> Cowan	—	—	—	±	—	—

^a The grading of staining intensity is described in Materials and Methods.

DISCUSSION

While there have been many studies comparing various direct stains against cultures for *C. trachomatis* (1-3, 6, 9-11), there have been no clinical studies comparing the sensitivities and specificities of the various stains used for direct immunofluorescence staining of chlamydial elementary bodies. In reality, comprehensive clinical comparisons of stains would be expensive and difficult to undertake, since large numbers of patients would have to be studied to discern what probably would be small differences in sensitivity and specificity. An alternative way to compare these reagents is the type of laboratory study that we undertook. However, not all relevant variables can be tested in such an in vitro study. Factors such as the presence or absence of local antibody or cervical mucus in a specimen could influence one stain to a greater extent than others and would not be evident by the methods we used. Despite these shortcomings, the in vitro testing method we used is a useful alternative to an expensive clinical trial and can be used to evaluate new products as they are introduced or to reevaluate older ones as modifications are made.

It should be noted that various factors, such as type of microscopy equipment, microscope illumination, mounting fluid, and skill of the microscopist, may influence the results of a study such as ours. Thus, intensification of staining with both the Kallestad stain and the Syva stain was noted when a mercury light source was used instead of a halogen source (other stains were not compared). Although the mercury system intensifies fluorescence, we prefer halogen because of its greater convenience and lesser cost.

Our results demonstrate that the LPS-based stains were characterized by variable intensity of elementary body fluorescence. Schachter has previously described similar findings (5). In addition, LPS-based stains cross-reacted with *P. productus*. Previous studies have also demonstrated cross-reactions between anti-chlamydial LPS antibodies and *Acinetobacter calcoaceticus* (4). Staining of bacteria other than chlamydia presents two problems to the microscopist: distinguishing the elementary body from other organisms and recognizing an elementary body against a background of other fluorescent particles (8). Both of these problems are compounded when the elementary bodies themselves stain with variability in size and shape as they do when stained with anti-LPS antibodies are used.

In conclusion, our studies demonstrate considerable variation in the degree of brightness, consistency of staining, and specificity of the six monoclonal immunofluorescent reagents tested. In general, the anti-MOMP stains were brighter and more specific and provided more consistent staining than did the anti-LPS stains. While all of the products tested doubtlessly can provide satisfactory results, microscopists must be thoroughly trained to interpret the stain being used. If possible, slide results should be compared with culture results during the training period. Until more is published substantiating the sensitivities and specificities of genus-specific antibodies which stain LPS, we

recommend particular caution when using this group of stains.

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