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Performance of Three Commercially Available Monoclonal Reagents for Confirmation of *Chlamydia pneumoniae* in Cell Culture

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We evaluated the performance of three commercially available monoclonal antibodies for confirmation of the presence of *Chlamydia pneumoniae* in cell culture by examining their abilities to stain inclusions of eight strains of *C. pneumoniae*. The antibodies tested were two unconjugated *C. pneumoniae*-specific monoclonal reagents and one conjugated genus-specific reagent. All three produced similar intensities of staining of *C. pneumoniae*, with some strain-to-strain variation. Methanol appeared to be a better choice of fixative than acetone, which greatly reduced the intensity of fluorescence with one of the species-specific antibodies.

Chlamydia pneumoniae, the newly recognized third chlamydial species, is emerging as a common human respiratory pathogen accounting for as many as 10 to 20% of cases of community-acquired lower respiratory tract infections (2, 5, 6). Isolation of the organism in tissue culture, although initially found to be difficult, has been greatly facilitated by the use of more sensitive cell lines (3, 8). However, the performance of available reagents for culture confirmation has not been systematically evaluated. The purpose of this study was to compare the performances of two commercially available *C. pneumoniae*-specific antibodies and one genus-specific monoclonal antibody for detection of *C. pneumoniae* in tissue culture, by using the reference strain TW-183 and recent clinical isolates of *C. pneumoniae*.

The three reagents tested were *C. pneumoniae*-specific monoclonal antibody, unconjugated (Cellabs Chlamydia Cel Pn IF test; Cellabs Pty. Ltd., Brookvale, Australia), TWAR-specific monoclonal antibody, unconjugated (Washington Research Foundation, Seattle, Wash.), and the Pathfinder Chlamydia Culture Confirmation System (Kallestad Diagnostics, Chaska, Minn.), a conjugated antilipopolysaccharide monoclonal antibody.

The *C. pneumoniae* organisms used were as follows: TW-183 (Washington Research Foundation); CWL-050; CWL-029 (kindly provided by C. Black, Centers for Disease Control, Atlanta, Ga.); W6805, a clinical isolate from Wisconsin; T2023 (ATCC VR-1356); T2043 (ATCC VR-1355); and BAL 16 and BAL 37, clinical isolates from Brooklyn. All clinical isolates chosen were selected between passage numbers 3 and 4 to best reflect the number of inclusions needed for the experiment and to avoid using well established or laboratory adapted strains when possible. The *C. trachomatis* organisms used were L2/434/Bu (ATCC VR-902B), N18 (a cervical isolate that had the growth characteristics of a trachoma biovar strain), and UW-31, serotype K (ATCC VR-887).

HEp-2 cells were seeded onto 96-well microtiter plates 48 h prior to inoculation (8). All cell monolayers were examined for confluence on the day of inoculation. For each experiment, 100 μl of 10^3 inclusion-forming units of each strain per ml was inoculated into four wells on duplicate plates. All plates were centrifuged at 1,700 × *g* for 1 h and then incubated for 1 h at 35°C. Fresh overlay medium (Iscove's modified Dulbecco's medium [Gibco] containing 1 μg of cycloheximide [Sigma] per ml and 10% fetal calf serum) was added, and plates were incubated for 72 h at 35°C. At 72 h, one plate of each duplicate pair was fixed with methanol (95%) and one was fixed with acetone (50%) for 10 min. Twenty-five microliters of each *C. pneumoniae*-specific antibody was added to specific wells in the duplicate plates and plates were incubated for 30 min at 37°C in a moist chamber. After incubation, the wells were rinsed with phosphate-buffered saline (PBS), and 25 μg of the anti-mouse immunoglobulin-fluorescein isothiocyanate reagent provided by the manufacturer was added to each well. The plates were incubated again for 30 min at 37°C, after which the wells were rinsed with PBS and a drop of mounting medium was added to each. A 5-mm coverslip was added to each well, and the plate was inverted for microscopy. Staining with the conjugated genus-specific monoclonal antibody was carried out according to the manufacturer's instructions.

In order to assess the staining properties of the three monoclonal antibodies, fluorescence was evaluated blindly by three observers with a Nikon epifluorescence microscope with a halogen light source, a 20× long-focal-length objective, and 10× eyepieces. Fluorescence was graded from negative to 3+ (−, no visible organisms; ±, organisms just visible; 1+, light-green staining; 2+, moderate fluorescent-green staining; and 3+, intense fluorescent-green staining).

The results are summarized in Table 1. The two *C. pneumoniae*-specific antibodies stained all eight *C. pneumoniae* strains and did not react with any of the *C. trachomatis* strains tested. The genus-specific reagent reacted with both species. The intensity of the fluorescence varied from strain to strain, ranging from very dim (±) to bright green (3+). The lowest intensity of fluorescence was observed with *C. pneumoniae* CWL-050: 1+ with both species-specific monoclonal antibodies. Marginally brighter fluorescence (2+) was observed when CWL-050 was fixed with methanol and stained with the genus-specific antibody. Overall, methanol appeared to perform better than acetone as a fixative for staining *C. pneumoniae*. This was especially striking with the Cellabs antibody; when the cell layers were fixed with acetone, the intensity of fluorescence produced with the Cellabs reagent was poor (±) for all the *C. pneumoniae* isolates tested. Otherwise, the

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TABLE 1. Staining of *C. pneumoniae* inclusions by three commercially available monoclonal antibodies

Strain	Intensity of fluorescence[a]					
	Cellabs		Washington		Kallestad	
	Acetone	MeOH[b]	Acetone	MeOH	Acetone	MeOH
TW-183	±	3	2	3	2	3
CWL-050	±	1	1	1	1	2
CWL-029	±	3	2	3	2	2
2043	±	2	1	2	1	2
2023	±	2	3	2	3	3
BAL 37	±	3	2	2	2	2
W6805	±	3	3	3	2	2
BAL 16	±	3	3	3	3	2

[a] Shown as ±, 1+, 2+, and 3+ as defined in Materials and Methods.
[b] MeOH, methanol.

intensities of fluorescence observed with all three reagents appeared to be equivalent for staining *C. pneumoniae* inclusions.

The use of fluorescence staining for confirmation of the presence of *C. pneumoniae* in tissue culture has not been assessed systematically, in part because of the limited availability of the reagents. As the number of laboratories performing *C. pneumoniae* culture and the demand for this service increase, evaluation of these reagents becomes imperative. The results of this study suggest that the *C. pneumoniae*-specific monoclonal antibodies produced by the Washington Research Foundation and Cellabs appear to be equivalent as culture confirmation reagents for detecting *C. pneumoniae* inclusions in cell culture.

The Washington Research Foundation monoclonal antibody was produced from AR-39 (7). The Cellabs reagent was produced from IOL 207, which appears to be identical to TW-183 on the basis of microimmunofluorescence typing and sequencing of the MOMP gene (1, 4). The two antibodies produced similar intensities of staining of isolates of *C. pneumoniae* from various geographical locations.

If these reagents are not readily available, the conjugated genus-specific monoclonal antibody produced by Kallestad appears to be a reasonable alternative. One should be aware that this reagent will detect *Chlamydia psittaci* as well as *C. trachomatis*, and thus respiratory isolates obtained from young infants should be confirmed by staining with a *C. trachomatis*-specific antibody or with iodine. If there are epidemiologic data to suggest psittacosis, one could exclude the other species by differential staining.

Our data also suggest that methanol is preferable to acetone for fixation. Wang and Grayston (9) reported that fixation with methanol destroyed the antigenic reactivity of *C. pneumoniae* elementary bodies, as would be seen in a direct smear. However, this effect was not seen with *C. pneumoniae* inclusions in tissue culture, and our results confirm this observation. None of the reagents tested in our study have been evaluated for direct detection of *C. pneumoniae* in smears of patient specimens from any source, including pharyngeal, nasopharyngeal, and lower respiratory tract specimens. Grayston and colleagues have found direct fluorescent-antibody staining of throat specimens to be less sensitive than cell culture for identifying *C. pneumoniae* infection (6). We have had a similar experience and also have concern about specificity due to numerous artifacts and nonspecific staining which can occur with respiratory specimens that contain mucus. The package insert for the Cellabs reagent suggests that it may be used as a direct fluorescent-antibody reagent and provides instructions for staining smears, but the reagent has not been evaluated for this use.

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