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Efficient Culture of *Chlamydia pneumoniae* with Cell Lines Derived from the Human Respiratory Tract

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Two established cell lines, H 292 and HEp-2, originating from the human respiratory tract, were found to be significantly more efficient and practical than the currently used HeLa 229 cells for growth of *Chlamydia pneumoniae*. Six strains of *C. pneumoniae* recently isolated from patients with respiratory ailments were used as test cultures. The H 292 and HEp-2 cells yielded much higher inclusion counts for all the test strains than did HeLa 229 cells. When they were compared with each other, H 292 cells yielded more inclusions than did HEp-2 cells, and the differences were statistically significant in 10 of 18 test sets. A simple system with these two cell lines appeared to be very efficient for culturing *C. pneumoniae*. It does not require treatment of tissue cells with DEAE-dextran before infection, and it may eliminate the need for serial subpassages of specimens to increase culture sensitivity. Monolayers of these cells remained intact and viable in the *Chlamydia* growth medium so that reinfection could take place, resulting in greatly increased inclusion counts for specimens containing few infectious units. This system may make it more practical for laboratories to culture for *C. pneumoniae* for treatment of infections and outbreak intervention and will facilitate studies on this recently recognized pathogen.

Chlamydia pneumoniae, a new species of the obligate intracellular chlamydiae, has been recognized as a significant pathogen that causes infections of the human respiratory tract and possibly other organs (5, 6, 12, 13, 16). Diagnosis of infections with *C. pneumoniae* is problematic, because the syndrome usually presents few distinguishing features and culture of the organism is difficult. Hence, most clinical and epidemiological studies have relied on restrospective serologic tests for specific antibody responses. Limitations of these laboratory diagnostic techniques (5, 7) have hindered investigation of the organism and disease control.

Conditions that would promote the growth of *C. pneumoniae* in tissue cells appear to be different from some of those for *C. trachomatis*, because cell lines and techniques which enhanced the growth of *C. trachomatis* were found to be ineffective for *C. pneumoniae* (7, 9). The cell line HeLa 229 pretreated with DEAE-dextran has been used most often for culturing *C. pneumoniae* (7). Recently, the HL cell line has been found to be more efficient than HeLa 229 cells for culturing the type strain TW 183 (3) and some other isolates (8). In the search for a more efficient and practical cell culture system, we studied the growth of six recent clinical isolates of *C. pneumoniae* in selected cell lines derived from the human respiratory tract and found that two established cell lines, H 292 and HEp-2, were significantly more efficient than HeLa 229 cells for culturing all of the test strains. Our findings and a relatively simple and more efficient culture system for *C. pneumoniae* are presented in this report.

MATERIALS AND METHODS

Bacteria. *C. pneumoniae* CWL 029 (ATCC VR-1310) (2) and CM-1 were isolated from patients with pneumonia in laboratories at the Centers for Disease Control, Atlanta, Ga. Strains FML 7, FML 10, FML 12, FML 16, and FML 19 were isolated from patients with respiratory ailments in a military training camp in Norway during an adenovirus outbreak (1) and were kindly provided by Bjorn P. Berdal, University of Tromso, Tromso, Norway. All strains were isolated in HeLa 229 cells. Strain CWL 029 was used as antigen for production of monoclonal antibodies for identification of *C. pneumoniae* in tissue cells. The other six isolates in the third to fifth subpassages in HeLa 229 cells were used as inocula to test the selected cell lines.

Serotype strains of *C. trachomatis*, originally from S. P. Wang of the University of Washington, Seattle, and *C. psittaci* PI-1 and D44 were from our stock cultures.

Cell lines. All cell lines were obtained from the Cell Culture Section at the Centers for Disease Control and were passaged by standard tissue culture techniques. The following five cell lines derived from human respiratory tissues were tested in reference to HeLa 229 from cervix carcinoma: H 292 (ATCC H-292), lung carcinoma; RU 1, fetal lung fibroblast; HEp-2, larnyx carcinoma; HR 6, embryonic lung; and WI 38, embryonic lung.

Comparison of culture efficiencies of cell lines. Monolayers of tissue cells were cultured at 36°C in 5% CO_2 in the following cell growth medium: Eagle's minimal essential medium supplemented with fetal calf serum (10%), L-glutamine (2 mM), MEM nonessential amino acids (10 ml/liter; 100×; GIBCO, Grand Island, N.Y.), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (10 mM), gentamicin (10 mg/liter), and vancomycin (25 mg/liter).

The strains of *C. pneumoniae* to be used for testing the cell lines were grown in HeLa 229 cell monolayers in six-well culture plates (Costar, Cambridge, Mass.). Each monolayer was inoculated with 2 ml of the seed culture in HSC buffer (pH 7.3; 10 ml of 1.0 M HEPES, 0.2 g of $MgCl_2 \cdot 6H_2O$, 5.26 g of NaCl, 0.23 g of $CaCl_2 \cdot H_2O$, and 68.4 g of sucrose in 1 liter of deionized H_2O). The plates were centrifuged at 700 × *g* for 30 min at room temperature, and the supernatant was replaced with 3 ml of the *Chlamydia* growth medium per

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well; the *Chlamydia* growth medium consisted of the cell growth medium as the base and contained cycloheximide (1 μg/ml) (Sigma, St. Louis, Mo.), glucose (1 mg/ml), and amphotericin B (3 μg/ml). When inclusions were seen with phase-contrast microscope in at least 50% of the tissue cells, the monolayers were harvested and sonicated for 20 s. Cellular debris was removed by centrifugation (500 × *g*, 20 min, 4°C), and the elementary bodies were pelleted at 30,000 × *g* for 30 min.

Homogeneous suspensions of elementary bodies were prepared in HSC buffer by use of short sonic pulses. The infectivity units or inclusion counts of the suspensions were titrated in HeLa 229 cell monolayers on round coverslips (diameter, 12 mm) in 1-dram (3.697 ml) shell vials. The suspensions were stored at −75°C until they were used to test the cell lines.

Monolayers of the cell lines for comparison were tested simultaneously with the *C. pneumoniae* isolates by the shell vial system. Each isolate was used as an inoculum at three different concentrations. A 0.2-ml aliquot of high-concentration inoculum yielded approximately 100 to 500 inclusions per coverslip of HeLa 229 cells after 48 h of incubation, whereas the yield of a medium-concentration inoculum was about 10 to 60 inclusions, and that of a low-concentration inoculum ranged from 1 to 7 inclusions.

Each monolayer of the cell lines in the test was inoculated with 0.2 ml of the *C. pneumoniae* suspension in HSC buffer; this was followed by centrifugation at 1,700 × *g* for 30 min at room temperature. The supernatant was replaced with 1 ml of the *Chlamydia* growth medium. Unless otherwise stated, incubation was for 48 h at 36°C in 5% CO_2. The infected monolayers were fixed with methanol and stained with a mouse monoclonal antibody specific to *C. pneumoniae* and a fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin G (IgG). Inclusions were counted at ×100 magnification with a fluorescence microscope.

Effect of DEAE-dextran on growth of *C. pneumoniae*. Monolayers of test cell lines were pretreated with 10, 20, or 30 μg of DEAE-dextran (molecular weight, 500,000; Sigma) per ml in HSC buffer for 20 min at room temperature before they were infected with the *C. pneumoniae* isolates. For comparison, control groups treated with only HSC buffer were tested simultaneously.

Clinical specimens. Specimens that were previously determined to be positive or negative for *C. pneumoniae* by culturing them in HeLa 229 cells were used to test selected cell lines. Two consecutive sputum specimens taken in 3 days from a patient with pneumonia and a positive serology were used as positive specimens, and five throat swab specimens from patients with respiratory ailments but no microbiological or serological evidence of infection with *C. pneumoniae* were used as negative specimens. The test cell lines, with HeLa 229 cells used for comparison, were inoculated simultaneously with three dilutions of the specimens in HSC buffer.

Production of monoclonal antibodies. The species-specific monoclonal antibody used for the identification of *C. pneumoniae* inclusions in tissue cells was produced by the procedures of Galfre and Milstein (4), with modifications of the fusion techniques as described by Lane (10). *C. pneumoniae* CWL 029 was used as the immunogen and was partially purified by centrifugation (30,000 × *g*, 1 h, 4°C) in 35% Renografin 76 (Squibb, New Brunswick, N.J.) in HSC buffer. Six-week-old BALB/c mice were immunized at 10-day intervals with two doses of 0.5 ml of the antigen suspension in HSC buffer containing approximately 10^8 infectious units by the intraperitoneal route, which was followed by one dose of 0.2 ml by the intravenous route. Spleens were collected for fusion 3 days after the last injection.

The specificities of the monoclonal antibodies were determined by an indirect immunofluorescence test by using strains of *C. pneumoniae*, *C. psittaci*, and *C. trachomatis* as antigens and fluorescein isothiocyanate conjugates of goat anti-mouse IgG and IgM (Sigma) as secondary antibodies. A pool of ascitic fluids produced in young adult BALB/c mice with the selected monoclone was used for staining *C. pneumoniae*.

Statistical methods. Statistical analyses for comparing the efficiencies of the cell lines for culturing *C. pneumoniae* with regard to strains and inoculum concentrations, including the techniques of analysis of variance (14) and Scheffe's simultaneous confidence intervals (11), were performed for each of the 18 test sets. It was observed that the standard deviations of inclusion counts were proportional to the means. Inclusion counts on a logarithmic scale were used in the analyses. Analysis of variance for randomized complete block design was adopted to test the null hypothesis that the inclusion counts of three cell lines were the same. Each inoculum was considered as a block in this design. The test statistic for testing the null hypothesis was calculated. The test statistic would have a $F(u,v)$ distribution with the degrees of freedom $u = 2$ and $v = 10$ if the null hypothesis was true. The P value associated with the test statistic was then determined. When the hypothesis of equal means was rejected, Scheffe's 95% simultaneous confidence intervals for mean difference between cell lines were constructed, and the significances of differences among the comparisons were determined.

RESULTS

Monoclonal antibodies. Six monoclones excreting IgG antibodies to *C. pneumoniae* CLW 029 were identified. Clone 2D11 was genus specific, reacting with *C. trachomatis*, *C. psittaci*, as well as *C. pneumoniae*; and the other five clones—3F12, 3G9.1, 5E1.1, 7D10, and 8A6—were species specific, with no cross-reaction with *C. trachomatis* or *C. psittaci*. Clone 7D10 was selected for production of antibody in BALB/c mice for identifying and counting *C. pneumoniae* inclusions in tissue cultures, because this clone was well tolerated by the host and produced high titers of antibody. The fusion procedure as modified by Lane (10) appeared to be very efficient in producing monoclones to antigens of *C. pneumoniae*.

Screening of cell lines for culture efficiencies. The five cell lines were screened for their efficiencies in culturing the *C. pneumoniae* isolates relative to those of HeLa 229 cells. Monolayers of the H 292 and HEp-2 cells consistently yielded higher inclusion counts than HeLa 229 cells did, whereas inclusion counts in RU 1, HR 6, and WI 38 cells were less. The results in Table 1 for strain CM-1 are representative of those for the other strains tested. Cell lines H 292 and HEp-2 were selected for further studies in reference to HeLa 229 cells.

Effect of DEAE-dextran on growth of *C. pneumoniae*. Treatment of HeLa 229 cells with an appropriate dose of DEAE-dextran before infection increased the inclusion counts of some strains of *C. pneumoniae*. However, the enhancing effect was not observed in H 292 or HEp-2 cells. Representative results are presented in Table 2. Pretreatment with DEAE-dextran at 20 μg/ml slightly enhanced the

TABLE 1. Representative results of screening of cell lines for relative efficiencies for culturing *C. pneumoniae*

Inoculim concn[a]	Inclusion counts in the following cell lines[b]:					
	HeLa 229	RU 1	H 292	HR 6	HEp-2	WI 38
High	119 (1)	79 (0.66)	202 (1.7)	77 (0.64)	195 (1.6)	93 (0.78)
Medium	9 (1)	9 (1)	18 (2)	8 (0.8)	29 (3.2)	8 (0.8)
Low	0.5 (1)	0.5 (1)	1 (2)	0 (0)	3 (6)	0.5 (1)

[a] *C. pneumoniae* CM-1 was used as the inoculum. Relative efficiencies for the other strains varied, but H 292 and HEp-2 cells invariably yielded higher inclusion counts. The inoculum concentration was the yield of inclusions of a 0.2-ml inoculum in a monolayer of HeLa 229 cells (diameter, 12 mm) after incubation for 48 h at 36°C. Ranges of approximate inclusion yields of inocula for all *C. pneumoniae* strains were as follows: 100 to 500, for the high inoculum concentration, 10 to 60, for the medium inoculum concentration, and 1 to 7, for the low inoculum concentration.

[b] Counts are averages of two separate experiments. Culture efficiencies relative to those of HeLa 229 cells are given in parentheses.

inclusion counts of strain CM-1 in HeLa 229 cells, but no enhancing effect was observed in HeLa 229 cells for strain FML 7 at all concentrations of DEAE-dextran tested. In H 292 cells, DEAE-dextran actually depressed the inclusion counts of both strains CM-1 and FML 7.

Relative culture efficiencies of H 292, HEp-2, and HeLa 229 cells. Six independent tests were performed for each of the six *C. pneumoniae* isolates at three inoculum concentrations. Table 3 summarizes the means of inclusions (log), the mean square errors from the analysis of variance, and the *P* values associated with the test statistics as well as the significances of comparisons between the cell lines with respect to the strains by Scheffe's method (11).

Cell line H 292 was most efficient for culturing all *C. pneumoniae* strains. The HEp-2 cells were significantly more efficient than HeLa 229 cells in all tests with the exception of strain FML 7, which, when tested at the lowest inoculum concentration, yielded higher inclusion counts in HEp-2 cells than it did in HeLa 229 cells, but the difference was not significant. H 292 cells were superior to HEp-2 cells in 10 of the 18 comparisons, and the generally higher inclusion counts in H 292 cells in the other eight comparisons were not statistically different from those in HEp-2 cells, as indicated in Table 3.

TABLE 2. Effect of DEAE-dextran on growth of *C. pneumoniae* in tissue cells

Cell line and *C. pneumoniae* strain[a]	Inclusion counts after treatment with DEAE-dextran at concn (μg/ml) of[b]:		
	10	20	30
HeLa 229 cells			
CM-1	94 (99)	160 (124)	40 (40)
	5 (6)	20 (12)	3 (2)
	0 (0)	1 (2)	1 (0)
FML-7	42 (40)	366 (422)	25 (63)
	3 (2)	24 (31)	3 (4)
	0 (0)	2 (2)	0 (1)
H 292 cells			
CM-1	82 (156)	230 (418)	32 (54)
	7 (13)	10 (44)	9 (13)
	0 (0)	0 (5)	0 (1)
FML-7	61 (88)	388 (869)	51 (110)
	4 (14)	12 (78)	3 (15)
	0 (0)	0 (10)	1 (1)

[a] Each strain of *C. pneumoniae* was used at three concentrations to inoculate the tissue cells.

[b] Inclusion counts in tissue cells of matched untreated controls are given in parentheses.

Effect of prolonged incubation on growth of *C. pneumoniae*. Monolayers of H 292, HEp-2, and HeLa 229 cells remained intact and viable in the *Chlamydia* growth medium after infection with *C. pneumoniae* for at least 7 to 10 days at 36°C. In all three cell lines, inclusion counts increased with prolonged incubation beyond 48 h. Representative results are summarized in Tables 4 and 5. Consistent with results from other experiments, inclusion counts were highest in H 292 cells and were lowest in HeLa 229 cells, with counts in HEp-2 cells being between those for H 292 and HeLa 229 cells. Dramatic increases in inclusions by 12- to 59-fold were observed between 3 and 7 days after infection when an inoculum of moderate or low concentration was used (Table 4). The ratios of inclusion counts between cell lines as an indication of relative culture efficiencies after prolonged incubation are given in Table 5.

Culture of clinical material in H 292, HEp-2, and HeLa 229 cells. Specimens that were cultured previously in the HeLa 229 cell system were tested in H 292 and HEp-2 cells, with HeLa 229 cells used as a reference. The results obtained with the two positive sputum specimens from a patient with pneumonia are given in Table 6, whereas the five negative throat swab specimens from patients with respiratory ailments remained negative in all three cell lines. Because of the viscosity of the sputum specimens, the number of infectious particles in each dose of the inoculum conceivably might vary. However, in all the tests the inclusion counts in H 292 and HEp-2 cells were consistently higher than those in HeLa 229 cells. With the first specimen, at a 10^{-2} dilution as an inoculum, one of the three monolayers of HeLa 229 cells was negative, whereas all the monolayers of H 292 and HEp-2 cells were positive. By using a 10^{-3} dilution of the same specimen as an inoculum, only one monolayer of HEp-2 cells was positive. With the second specimen, one of the three HeLa 229 monolayers was negative at the 10^{-3} inoculum dilution, whereas all monolayers of H 292 and HEp-2 cells were positive, yielding 4 to 10 inclusions per coverslip.

A culture system for *C. pneumoniae*. A system for culturing *C. pneumoniae* emerged from the findings of this study and is outlined in Fig. 1. Briefly, monolayers of H 292 or HEp-2 cells are inoculated in triplicate and centrifuged at room temperature for 30 min at about 1,700 × *g*. The supernatant is replaced with the *Chlamydia* growth medium. After incubation for 48 h at 36°C, one monolayer is stained with murine monoclonal antibody specific to *C. pneumoniae* and fluorescein-conjugated anti-mouse antibody and then examined microscopically. If the specimen is negative, incubation

TABLE 3. Comparison of efficiencies of H 292, HEp-2, and HeLa 229 cell lines for culturing *C. pneumonia*

C. pneumoniae strain and concn[a]	Mean inclusion counts (log)[b]			Mean square error	*P* value	Results of Scheffe's 95% simultaneous confidence intervals[c]
	H 292	HEp-2	HeLa 229			
FML 7						
High	2.8130	2.7199	2.5095	0.0018	<0.001	***
Medium	1.8153	1.6954	1.4706	0.0024	<0.001	***
Low	0.8671	0.6464	0.4014	0.0482	0.014	*
FML 10						
High	3.0976	2.9300	2.3625	0.0102	<0.001	***
Medium	2.1302	1.9354	1.3721	0.0090	<0.001	***
Low	1.1405	0.9229	0.3709	0.0412	<0.001	**
FML 12						
High	3.1897	2.9692	2.3702	0.0078	<0.001	***
Medium	2.2142	1.9667	1.3350	0.0132	<0.001	***
Low	1.2992	0.9402	0.3172	0.0447	<0.001	***
FML 16						
High	3.0944	2.9313	2.3744	0.0046	<0.001	***
Medium	2.0599	1.8661	1.4266	0.0069	<0.001	***
Low	1.0075	0.8209	0.3304	0.0676	0.003	**
FML 19						
High	2.9723	2.7672	2.2765	0.0107	<0.001	***
Medium	1.9464	1.9200	1.2659	0.0873	0.004	**
Low	1.0363	0.7537	0.1003	0.0466	<0.001	**
CM-1						
High	2.5367	2.3947	1.9670	0.0182	<0.001	**
Medium	1.6755	1.5880	1.2040	0.0236	<0.001	**
Low	0.6464	0.4924	0.1297	0.0481	0.006	**

[a] Each strain of *C. pneumoniae* was used at three concentrations to inoculate the cell lines in simultaneous tests.
[b] Inclusion counts in logarithmic scale were used to calculate the means of six tests.
[c] ***, significant differences of mean inclusion counts for all pairwise comparisons among the three cell lines; **, significant differences between H 292 and HeLa 229 cells and between HEp-2 and HeLa 229 cells; *, significant difference between H 292 and HeLa 229 cells only.

continues for the other two slides, which should be examined after incubation for 4 to 7 days.

DISCUSSION

This study identified some features of the H 292 and HEp-2 cell lines that would make them more suitable than HeLa 229 cells for culturing *C. pneumoniae*. Several points are noteworthy. First, the much higher efficiencies of H 292 and HEp-2 cells in culturing *C. pneumoniae* were very consistent and statistically significant in all 18 comparative test sets. When tested with clinical isolates, the H 292 cell line was significantly more efficient than the HEp-2 cell line in 10 of the 18 test sets, and the actual inclusion counts for all

TABLE 4. Effect of prolonged incubation on inclusion counts of *C. pneumoniae* in three cell lines: increase in inclusion counts between days 3 and 7 of incubation

Cell lines	Concn	3 Days				7 Days			
		Inclusion counts per monolayer[a]			Mean ± SE	Inclusion counts per monolayer[a]			Mean ± SE
		1	2	3		1	2	3	
HeLa 229	High	433	442	424	433.0 ± 5.20	TNTC[b]	TNTC	TNTC	
	Medium	41	45	38	41.3 ± 2.03	580	494	399	491.0 ± 52.27
	Low	2	1	1	1.3 ± 0.33	43	107	27	50.0 ± 24.44
H 292	High	938	914	924	925.3 ± 6.96	TNTC	TNTC	TNTC	
	Medium	94	86	89	89.7 ± 2.33	1,380	1,220	1,416	1,338.7 ± 60.24
	Low	9	7	8	8.0 ± 0.58	253	183	272	236.0 ± 27.06
HEp-2	High	652	645	631	642.7 ± 6.17	TNTC	TNTC	TNTC	
	Medium	69	66	58	64.3 ± 3.28	860	823	717	800.0 ± 42.85
	Low	4	5	5	4.7 ± 0.33	106	108	130	114.7 ± 7.69

[a] *C. pneumoniae* CM-1 was used at three concentrations to inoculate monolayers of the cell lines.
[b] TNTC, Too numerous to count.

TABLE 5. Ratios of inclusion counts between cell lines as an indication of relative culture efficiencies after prolonged incubation

Cell line	Concn	Ratio ± SE[a]: 3 Days	7 Days
H 292/HeLa 229	High	2.14 ± 0.02	
	Medium	2.17 ± 0.07	2.73 ± 0.18
	Low	6.15 ± 0.94	4.00 ± 0.99[b]
H 292/HEp-2	High	1.44 ± 0.01	
	Medium	1.40 ± 0.05	1.67 ± 0.07
	Low	1.70 ± 0.10	2.06 ± 0.16
HEp-2/HeLa 229	High	1.48 ± 0.01	
	Medium	1.56 ± 0.06	1.63 ± 0.11
	Low	3.62 ± 0.55	1.94 ± 0.47[b]

[a] Denote ratio by y/z, estimated variances of y and z by var(y) and var(z), respectively, where y and z are the means of inclusion counts of the cell lines. Standard error (SE) = ratio × $[\text{var}(y)/y^2 + \text{var}(z)/z^2]^{1/2}$.

[b] All ratios were significantly different from 1 at a level of significance of 0.001, with the exceptions of the ratios H 292/HeLa 229 and HEp-2/HeLa 229 at the lowest concentration and at 7 days, which were significant only at the levels of 0.01 and 0.05, respectively.

test strains were invariably higher in H 292 cells than they were in HEp-2 cells. The H 292 cell line was the most efficient for growing *C. pneumoniae* cultures.

Second, the H 292 and HEp-2 cell lines were stable upon serial passages with respect to their sensitivities to infection with *C. pneumoniae*. During the course of this study, which lasted for more than a year, clones of H 292 and HEp-2 cells were routinely passaged by standard tissue culture techniques with no indication of changes in susceptibility to infection with all the test strains of *C. pneumoniae*. Both are established, commercially available cell lines. No special nutritional or handling requirements, other than standard tissue culture media and procedures commonly used in virology laboratories, are necessary for maintaining these cell lines.

Third, unlike HeLa 229 cells, the susceptibilities of H 292 and HEp-2 cells to infection with *C. pneumoniae* were not enhanced by treatment with DEAE-dextran. Because the effect of DEAE-dextran may vary with different preparations and different *C. pneumoniae* strains, elimination of DEAE-dextran treatment of tissue cells before infection is a significant step toward simplification of the culture procedure.

Fourth, the results of this study indicate that a culture system with H 292 or HEp-2 cells may increase culture sensitivity and simultaneously eliminate the need for serial subpassages of specimens which yield negative results on first isolation attempts. The practice of serial subpassages of negative specimens to increase culture sensitivity drastically increases laboratory work load. Monolayers of H 292 and HEp-2 cells sustained cellular integrity and viability well for at least 7 to 10 days in the *Chlamydia* growth medium after infection with *C. pneumoniae*. Dramatic increases in inclusion counts, which were probably a result of cycles of reinfection, were observed between days 3 and 7 after infection with a low dose of inoculum. Unless the original specimen is toxic to the tissue cells, serial subpassages of the

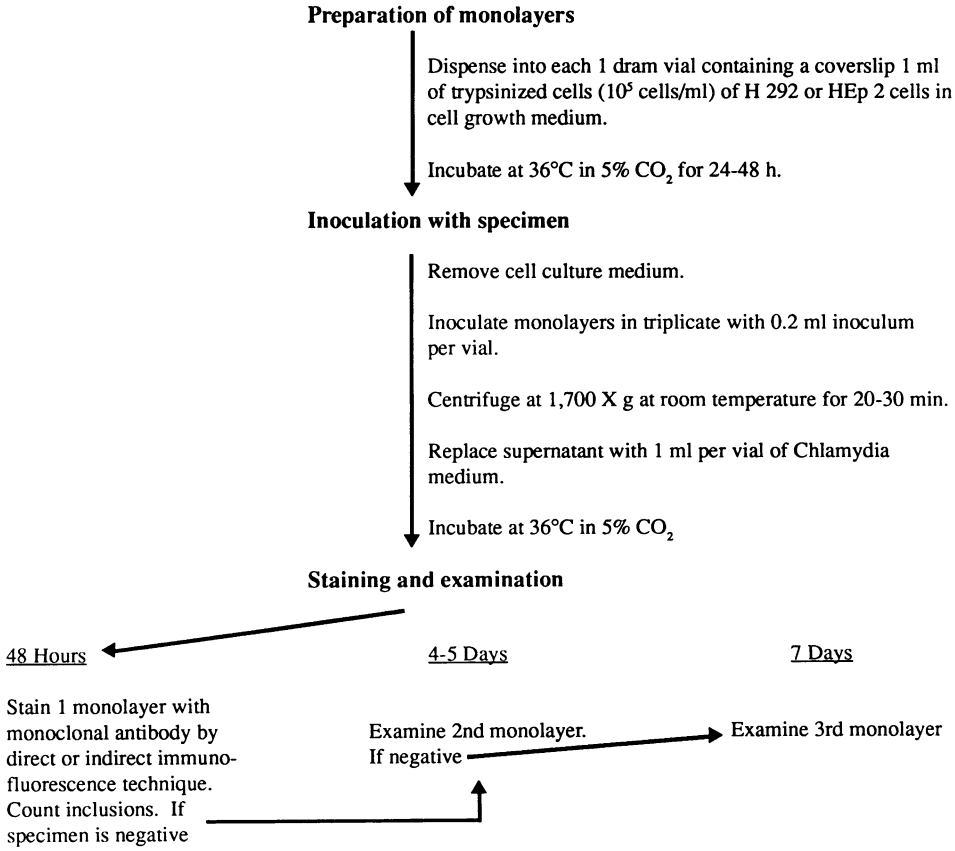

FIG. 1. Scheme of a simple, efficient procedure for culturing *C. pneumoniae*.

TABLE 6. Test of cell lines with clinical specimens[a]

Cell line	Specimen dilution[b]	Inclusion counts per coverslip					
		Sputum sample 1			Sputum sample 2		
		1	2	3	1	2	3
HeLa 229	10^{-1}	8	7	9	60	68	49
	10^{-2}	1	1	0	18	14	19
	10^{-3}	0	0	0	2	0	3
H 292	10^{-1}	14	17	19	87	94	91
	10^{-2}	1	3	1	111	74	94
	10^{-3}	0	0	0	4	9	7
HEp-2	10^{-1}	22	21	18	210	225	194
	10^{-2}	3	3	1	48	64	85
	10^{-3}	1	0	0	9	10	8

[a] Selection of specimens was made according to previous culture results for *C. pneumoniae* in HeLa 229 cells. Two positive sputum specimens taken consecutively in 3 days from a patient with pneumonia and five negative throat swabs from patients with respiratory ailments but no evidence of *C. pneumoniae* infection were used. The five throat swabs remained negative in all three cell lines.

[b] Sputum specimens were diluted in HSC buffer (pH 7.3), and three monolayers of each cell line in 1-dram vials were inoculated with each dilution. All the tests for each specimen were performed simultaneously. Inclusions were counted after incubation of tests for 48 h.

specimen to increase culture sensitivity may not be necessary for the H 292 or HEp-2 cell lines, because prolonged incubation of the originally inoculated monolayers would enhance inclusion counts.

Fifth, for higher yields of elementary bodies for studies on the organism, the H 292 cell line is superior to the HEp-2 and HeLa 229 cell lines. It appears that both H 292 and HEp-2 cell lines are well-suited for testing clinical specimens and are superior to HeLa 229 cells. However, other factors that may affect the relative performance of the cell lines for clinical specimens need to be evaluated. These factors include the types of specimens (e.g., sputum versus swabs) and the relative susceptibilities of the tissue cells to certain "toxic" factors as well as other microorganisms frequently encountered in clinical specimens.

Recently, the HL cell line has been reported to be more efficient than the HeLa 229 cell line for growing *C. pneumoniae* (3, 8). Because of the differences in experimental design and test strains, the results obtained with the HL cell line cannot be directly compared with those of the present study. However, as more cell lines are identified as being more efficient in various aspects than HeLa 229 cells for culturing *C. pneumoniae* and the culture procedure becomes technically less demanding, advances in our understanding of this pathogen and control of the diseases it causes will be greatly facilitated.

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ADDENDUM

When this manuscript was being cleared for publication, an abstract entitled "Use of HEp2 cells for improved isolation and passage of *Chlamydia pneumoniae*" was presented (15). The authors reported that HEp-2 cells were more sensitive than HL cells for the isolation and propagation of *C. pneumoniae*.

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