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Comparison of Different Methods and Cell Lines for Isolating Measles Virus

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Infectivity titers were determined for seven strains of measles virus by using various methods and cell lines. The use of B95-8 cells in a shell vial assay resulted in the highest infectivity titers. Our data suggest that B95-8 is the cell line of choice for the isolation of measles virus.

Recent epidemics of measles in the United States have generated renewed interest in isolating measles virus from clinical material (1). While the diagnosis of measles can often be made when the rash is present, many physicians remain unfamiliar with the disease. Furthermore, with an increasing population of immunosuppressed patients, typical features of measles may be absent and a diagnostic antibody response may not be demonstrable (5, 7). In these cases, viral isolation may be helpful in establishing the diagnosis.

Isolation of measles virus is thought to be best achieved in primary human or monkey kidney cells (4). The use of monolayers of these cell lines in tube cultures, however, is tedious and time-consuming, often requiring further passage before cytopathic effect or detectable antigen is evident (4). The spin amplification vial (shell vial) method, using A549 cells and monoclonal antibody, appears to be a sensitive means of isolating measles virus (9). This method is quicker and more convenient than standard tube isolation procedures (9). Two methods that use blood mononuclear cell lines have recently been reported to be sensitive for isolating measles virus from clinical specimens (2, 8).

In view of the availability of newer methods, we compared the following techniques and cells to determine the most sensitive means of measles virus isolation: (i) tube cultures of primary African green monkey kidney (AGMK), primary rhesus monkey kidney (RMK), Vero, LLC-MK2, MRC-5, or A549 cell monolayers; (ii) a shell vial method using the same six cell lines; (iii) phytohemagglutinin (PHA)-stimulated cord blood mononuclear cells (CBMC) in 48-well microtiter plates and in shell vials; and (iv) a shell vial method using B95-8 cells (Epstein-Barr virus-transformed marmoset lymphocytes).

Seven strains of measles virus isolated from acutely infected patients were used. All strains were isolated from patients' peripheral blood mononuclear cells by cocultivation with PHA-stimulated CBMC (2), passed an additional time in PHA-stimulated CBMC, and stored at −70°C prior to use. Virus dilutions were made in RPMI medium supplemented with 10% fetal bovine serum, 250 U of penicillin per ml, and 150 μg of streptomycin per ml (supplemented RPMI medium).

Tube monolayer cultures and coverslip-containing shell vials with monolayers of AGMK, RMK, Vero, LLC-MK2, MRC-5, and A549 cells were purchased from Viromed Laboratories, Inc. (Minnetonka, Minn.) and maintained in Eagle's minimum essential medium (EMEM) supplemented with 2% fetal bovine serum, 50 μg of gentamicin per ml, and 250 μg of amphotericin B per ml (supplemented EMEM). After aspiration of medium, duplicate shell vials containing each cell line were inoculated with 0.2 ml of each virus dilution. Vials were centrifuged at 1,000 × *g* for 45 min, fed 1 ml of supplemented EMEM, incubated at 37°C for 72 h, fixed in acetone, and stained with measles monoclonal antibody (Chemicon, Temecula, Calif.) in an indirect immunofluorescent-antibody (IFA) assay (8). Tube cultures were inoculated in duplicate with 0.2 ml of each virus dilution, incubated at 37°C for 1 h, fed 2 ml of supplemented EMEM as described above, and observed daily for cytopathic effect. Following a 14-day incubation, cell monolayers were scraped; duplicate samples were pooled, and a portion of the cells were placed on eight-well glass slides, fixed with acetone, and stained for the presence of measles virus antigen in an IFA assay as described above. In addition, 0.2 ml of the cell suspension was passed to shell vials containing the same cell line as the original tube cultures. These shell vials were centrifuged, incubated, fixed, and stained in the manner described above (9).

CBMC were obtained from fresh specimens of heparinized blood by centrifugation on a Ficoll-Hypaque gradient. Cells were resuspended in supplemented RPMI medium to which PHA (final concentration, 5 μg/ml) had been added. Either 48-well microtiter plates or shell vials were used. For microtiter plates, 0.2 ml of each viral dilution was inoculated, in duplicate, into wells containing 10^5 CBMC in suspension. Plates were incubated at 37°C in 5% CO_2 and observed daily for syncytium formation. Following a 6-day incubation, cells from all wells which did not have syncytia and cells from wells with the highest dilution of virus that did have syncytia were fixed with methanol and stained in an IFA assay with measles monoclonal antibody (2). In the experiment using shell vials, 0.2 ml of each viral dilution and 10^6 CBMC, in 0.2 ml of supplemented RPMI medium (without PHA), were added to duplicate vials. Vials were spun at room temperature for 45 min at 1,000 × *g*, fed 1 ml of supplemented RPMI medium containing PHA (final dilution, 5 μg/ml) and incubated at 37°C in 5% CO_2. Vials were examined daily for syncytia. Following a 6-day incubation, all vials with no syncytia, as well as the vials containing the highest viral dilution with syncytia, were centrifuged at 900 × *g* for 10 min. After supernatants were aspirated, cells on the cover

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TABLE 1. Titration of measles virus on PHA-stimulated CBMC and on four cell lines

Measles virus strain[a]	Titer obtained with[b]:								
	AGMK		RMK		MRC-5		LLC-MK2		CBMC on MT
	TC	SV	TC	SV	TC	SV	TC	SV	
A	5.5	4.5	3.5	4.5	<2	4.0	<2	3.5	5.0
B	5.5	5.5	4.5	4.5	<2	3.5	2.5	2.5	5.5
C	4.5	5.0	3.5	4.5	<2	3.5	<2	3.5	4.5
D	4.5	5.5	3.5	4.5	<2	3.5	<2	4.5	4.5
E	4.5	4.5	3.5	4.5	<2	4.0	3.5	4.0	4.5
F	4.5	5.0	4.5	4.0	<2	3.5	<2	3.0	5.0
G	4.5	5.5	4.5	5.5	<2	3.0	<2	3.0	4.5

[a] Virus strains were passaged prior to titration (see text).
[b] Expressed as $\log_{10}$ of the number of 50% tissue culture infective doses per 0.2 ml of single samples in tubes (TC) and duplicate samples in shell vials (SV) and 48-well microtiter plates (MT).

slips were fixed with methanol and stained with IFA as described above.

B95-8 cells were acquired from the American Type Culture Collection (Rockville, Md.) and maintained in supplemented RPMI medium. Shell vials containing 10^6 B95-8 cells in suspension were inoculated with virus and handled in the same manner as shell vials containing PHA-stimulated CBMC, with the exception that PHA was not added to the medium.

Tenfold dilutions of virus were used for all experiments. Endpoint dilutions were taken as the last dilution resulting in measles antigen as detected by IFA staining. If a passage of an original dilution resulted in specific fluorescence, the endpoint was taken as the dilution from which the passage was made. The 50% tissue culture infective dose was calculated by the Kärber method (6). Infectivity titers are expressed as the $\log_{10}$ of the number of 50% tissue culture infective doses per 0.2 ml of duplicate samples, unless stated otherwise.

Initially, two strains of virus (A and B) were titrated on AGMK, RMK, Vero, MRC-5, LLC-MK2, and A549 cells in shell vials. Infectivity titers for strains A and B, respectively, were 3.5 and 3.0 for Vero, 3.5 and 2.5 for A549, 3.5 and 4.5 for LLC-MK2, 4.0 and 4.0 for MRC-5, 5.0 and 4.5 for RMK, and 5.0 and 5.0 for AGMK cells.

All seven strains of virus were then titrated with tube cultures and shell vials containing the cell lines which demonstrated the highest infectivity titers (AGMK, RMK, MRC-5, and LLC-MK2 cells). The virus was also titrated in PHA-stimulated CBMC in 48-well microtiter plates. Of the four monolayer-forming cell lines, AGMK cells had the highest infectivity titers, followed by RMK cells (Table 1). With two exceptions, the shell vial method resulted in titers equal to or higher than those obtained with tube cultures that used the same cell line. Passage to shell vials after the 14-day incubation resulted in the same endpoint dilution as the original tube culture specimen in every instance but four: passage of strains A on AGMK cells and F and B on RMK cells resulted in an endpoint 1 dilution higher than that of the original tube, and passage of strain G on RMK cells resulted in an endpoint 1 dilution lower than that of the original tube. Cytopathic effect in tube cultures was difficult to distinguish from nonspecific degeneration of the monolayer. PHA-stimulated CBMC in microtiter plates resulted in slightly lower titers than AGMK cells in shell vials (Table 1). Syncytium formation in PHA-stimulated CBMC was easily observed and usually evident within 72 h. In addition, there was perfect correlation between the presence of syncytia and IFA staining (data not shown).

The most sensitive cell line (AGMK cells) and technique (shell vial) were then compared with PHA-stimulated CBMC and B95-8 cells in shell vials. The use of B95-8 cells in shell vials resulted in the highest infectivity titers (Table 2). The addition of a spin amplification step increased the titers of PHA-stimulated CBMC (Tables 1 and 2). Again, for both B95-8 and CBMC, IFA staining correlated well with the presence of syncytia: in one vial of PHA-stimulated CBMC, the IFA assay was positive when syncytia were not present, and in two vials of B95-8 cells, the IFA assay was negative when rare syncytia were present. Fluorescent staining of B95-8 cells was often weak.

Of the methods and cell lines tested, the use of B95-8 cells in shell vials resulted in the highest infectivity titers. The use of shell vials containing PHA-stimulated CBMC resulted both in slightly lower titers than the use of B95-8 cells and, in general, in higher titers than the use of cells grown as monolayers. Of the monolayer cell lines tested, AGMK cells resulted in the highest titers, and the shell vial method was generally superior to tube cultures. The use of Vero and A549 cells, which has been recommended for measles isolation, resulted in the lowest infectivity titers of all cell lines (4, 9).

These results corroborate those of Kobune et al. (8), who found B95-8 cells selected for adherence (and without a spin amplification step) to be much more sensitive than Vero, cynomolgus monkey kidney, KB, and FL cells. Although we

TABLE 2. Titration of six strains of measles virus on different cells in shell vials

Measles virus strain[a]	Titer on[b]:		
	AGMK	CBMC[c]	B95-8
A	6.0	5.5	6.5
B	3.5	6.0	6.5
C	5.0	5.5	6.0
D	6.0	6.5	6.5
E	4.5	5.0	6.5
F	5.5	5.5	6.0
G	5.5	6.0	6.0

[a] Virus was passaged prior to titration (see text).
[b] Expressed as $\log_{10}$ of the number of 50% tissue culture infective doses per 0.2 ml of duplicate samples.
[c] PHA-stimulated CBMC.

did not compare titers on B95-8 cells with and without spin amplification, such a step did result in an increase in titers on PHA-stimulated CBMC.

The use of A549 cells in shell vials was recently reported to be an efficient means of isolating measles (9). In that study, direct antigen detection from nasopharyngeal and throat swabs was about as sensitive as isolation in shell vials containing A549 cells. In view of the poor susceptibility of A549 cells in our study, the use of this cell line for comparison would call into question the sensitivity of direct antigen detection in the diagnosis of measles.

The use of MRC-5 cells in shell vials, which are available in many laboratories, resulted in intermediate titers. Discernment of measles virus antigen in IFA-stained MRC-5 cells was quite easy. This cell line may therefore offer some practical advantages. However, low titers of virus in clinical specimens are likely to go undetected if MRC-5 cells alone are used.

We used two nonadherent blood mononuclear cell types in a modified spin amplification method using shell vials. We felt that shell vials offered an advantage over microtiter plates when cells are being fixed and stained. Some cells were lost during aspiration of the supernatant prior to fixing. Furthermore, it has been our experience that nonadherent cells fix better with methanol than with acetone. On the other hand, acetone fixation of B95-8 cells results in stronger fluorescence (3). In any case, the use of adherent derivatives of B95-8 cells, which have been shown to be very sensitive measles virus hosts, might facilitate the application of this cell line to the shell vial method (8).

While our results suggest the superiority of blood mononuclear cell lines as a host for measles virus, other factors may have resulted in their improved susceptibility. In the spin amplification assays with monolayer cell types, incubation periods of 72 h prior to fixing and staining were used. While most dilutions in the assays with blood mononuclear cells developed syncytia within 72 h, the additional incubation period (total, 6 days) resulted in increased infectivity titers. In addition, all strains of virus were originally isolated from the peripheral blood mononuclear cells of adults infected with measles and passed once in CBMC. While our strains were very early passages, it is possible that they had adapted (either in vivo or in vitro) to grow preferentially in blood mononuclear cell lines. Nonetheless, the sensitivity of B95-8 cells in specimens obtained directly from patients, including throat and nasal swabs, has been demonstrated (8).

Clinical and serological data remain the keystones of measles diagnosis. Viral isolation, however, may be required to establish the diagnosis in some patients. Our data suggest that B95-8 is the cell line of choice and offers a sensitive, convenient, and rapid means of measles virus isolation.

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