Detection of mumps virus in clinical specimens by rapid centrifugation culture and conventional tube cell culture

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Abstract

Conventional tube cell culture was compared with a 2 day and 5 day spin-amplified shell vial indirect immunofluorescence assay for the detection of mumps virus in swabs from the area of Stensen’s duct. The sensitivity and specificity of the shell vial assay were 95.9 and 100%, respectively. The shell vial detected 66.3% of the positive cultures within 2 days of inoculation while the first positive results were available by conventional tube cell culture after 3 days (1.6%) reaching 72.4% of all culture positive specimens after 7 days. These data suggest that a centrifugation shell vial indirect immunofluorescence assay may be useful for rapid detection of mumps virus in clinical specimens. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Laboratory diagnosis of mumps virus infection is based mainly on the detection of specific IgM antibodies in serum. However, mumps virus infection may occur without the induction of IgM antibodies, particularly in subjects vaccinated previously (Briss et al., 1994). Virus isolation by conventional tube cell culture (TC) has been used for confirmation of mumps virus infection in such patients (Germann et al., 1996). This approach may delay the diagnosis for several days. In the last 10 years, TC has been modified by the use of shell vial (SV) culture after low-speed centrifugation to shorten detection time and enhance viral recovery (Gleaves et al., 1985). This technique combined with the staining of cells infected by
virus using a labelled monoclonal antibody allows the detection of several viruses within 1–5 days (Olsen et al., 1993; Klespies et al., 1996). The purpose of this study was to develop and evaluate a spin-amplified shell vial assay for rapid detection of mumps virus thus avoiding blood samples in children with suspected mumps virus infection.

2. Materials and methods

2.1. Patients

In 1994, 349 children presenting with parotitis during a period of high mumps virus activity in western Switzerland were included. Vaccination history was verified by reviewing the vaccination certificates.

2.2. Specimens

A total of 349 swabs from the area of Stensen’s duct were taken. All swabs were placed immediately into virus transport medium (2 M sucrose-phosphate containing 0.1 mg ml\(^{-1}\) of gentamicin and 2.5 mg ml\(^{-1}\) of amphotericin B) and sent to the laboratory at ambient temperature.

2.3. Conventional TC

Vero cell monolayers were inoculated with 0.2 ml of the specimen, incubated at 36°C for 10 days and read daily for cytopathic effect (CPE). One blind passage was carried out on all TC’s lacking CPE after 10 days with incubation at 36°C for another 10 days. Cells were scraped, resuspended in phosphate-buffered saline (PBS), mounted on a slide, fixed with acetone–methanol (1:1) and stained for mumps virus by indirect immunofluorescence assay (IFA) after primary incubation and after blind passage. The presence of mumps virus in cultures showing typical CPE was confirmed by IFA.

2.4. SV

Two vero cell shell vials were inoculated with 0.2 ml of the specimen, centrifuged at 700 × g for 40 min and incubated in a CO\(_2\) incubator at 36°C with two uninoculated vero cell SV as the negative control. The positive control consisted of mumps virus strain YLB92 which has been isolated earlier in our laboratory (Ströhle et al., 1996). After 2 and 5 days the cell monolayers were washed twice with PBS, fixed with acetone and stained by indirect IFA.

2.5. Indirect IFA

150 μl of monoclonal antibody to mumps virus (Bio-Science Products, Emmenbrücke, Switzerland) were added to the slides and were incubated for 30 min at 36°C in a humid chamber and washed twice with PBS. 150 μl fluorescein isothiocyanate-labelled rat anti-mouse antibody (Bio-Science Products) with 0.01% of Evans blue were added to the slides and they were incubated for another 30 min at 36°C in a humid chamber, washed twice with PBS, rinsed with distilled water, and air dried. The slides were mounted in buffered glycerol, pH 8.6 and examined with a fluorescent microscope. Slides containing cells (mainly syncytial cells) exhibiting granular cytoplasmic fluorescence were considered positive for mumps virus.

3. Results

3.1. Specimens excluded from the analysis

Eighteen specimens were excluded from the analysis for the following reasons: toxicity for inoculated cells, 13 (TC 4, SV 9); and growth of a different virus, 5 (3 herpes simplex viruses type 1, one poliovirus type 1 and one enterovirus).

3.2. Patients

A total of 331 swabs from 331 patients were examined. The patients consisted of 145 females and 186 males with a mean (median) age of 7.1 (6.25) years (range 3 months–63 years). Previous vaccination against mumps virus was documented for 235 (71%) of the patients. Large scale failure of one particular mumps vaccine was demon-
strated in Switzerland and published elsewhere (Germann et al., 1996).

3.3. Comparison of SV and conventional TC

The results are summarized in Table 1. Mumps virus was detected in 243 of 331 specimens (73.4%), 228 were positive by both tests, 10 by TC only (confirmed by IFA) and 5 by SV only. When TC was defined as the ‘gold standard’ for isolation of mumps virus, the SV assay had a sensitivity of 95.8% and a specificity of 94.6%. Assuming that the five specimens positive by SV and negative by TC represented true positives (clinically typical mumps cases with exposure to subjects with laboratory-documented mumps virus infection) the sensitivity of the SV assay remained unchanged at 95.9%, but the specificity reached 100%.

The SV assay detected 66.3% (161/243) of the positive samples within a 2-day incubation period and the remaining 29.6% (72/243) within 5 days, while TC detected only 21.4% (52/243) within this 5 day period. Fig. 1 shows the cumulative rate of mumps virus detection by SV and TC from day 2 to day 16.

The median time to detection by TC of specimens positive by SV assay on day 2 or day 5 was 6 days (IQR 2, range 3–11) and 7 days (IQR 3, range 4–16), respectively, whereas samples negative by SV assay took a median of 9.5 days (IQR 4, range 6–15) to be detected by TC (Fig. 2).

Eighteen of 238 (7.6%) TC positive samples were detected after blind passage only. Thirteen of those 18 specimens were positive by the SV assay (4 at 2 days and 9 at 5 days).

4. Discussion

The SV assay has been used to detect cytomegalovirus (Gleaves et al., 1985), respiratory viruses (Olsen et al., 1993) and enteroviruses (Klespies et al., 1996) within 1–5 days of incubation with a high sensitivity (> 90%). Confronted with a mumps epidemic in Switzerland (Germann et al., 1996), we attempted to reduce the time to diagnosis by using the SV assay for the detection of mumps virus. In this study, the majority (66%) of mumps viruses from specimens of the upper respiratory tract were detected after an incubation of 2 days using a commercially available monoclonal antibody which has been used previously for the identification of mumps virus grown in conventional TC in our laboratory.

Vaccine coverage of > 80% in most established market economies has reduced markedly the incidence of mumps or virtually eliminated the infection (Peltola et al., 1994). However, several outbreaks in subjects vaccinated previously have been described. Primary as well as secondary vaccine failure has been demonstrated in such individuals by the presence or absence of IgM antibodies to mumps virus. With decreasing numbers of mumps virus infections, rapid laboratory confirmation is required, particularly in single cases or in an incipient outbreak. The detection of mumps virus in swabs from the area of Stensen’s duct or from saliva does not require blood samples and is thus preferred by the parents of children. It also permits the correct diagnosis even in patients with secondary vaccine failure lacking IgM antibodies to mumps virus. In addition, virus detection is possible very early during primary infection (or after primary vaccine failure) when antibodies may not yet be present in the serum.

The sensitivity of the SV assay seems to be related to the viral load in the clinical specimen.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of shell vial assay and conventional tube cell culture for the detection of mumps virus from clinical specimens</th>
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<tr>
<td>Shell vial assay</td>
<td>Conventional tube cell culture</td>
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<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>228</td>
</tr>
<tr>
<td>Negative</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Sensitivity, 95.9%; Specificity, 100%.

<sup>b</sup> These specimens were considered true positives for clinical and epidemiological reasons (exposure to laboratory-documented mumps cases).

<sup>c</sup> Presence of mumps virus in tube cell culture was confirmed by monoclonal antibody.
Fig. 1. Time to detection of mumps virus by shell vial assay (bars) and conventional tube cell culture (squares). The bars and the squares represent the cumulative percentage of positive samples (total = 243).

(Fig. 2). The application of the SV technique may thus be particularly useful if the swab is taken early during the clinical course. The addition of TC may increase the sensitivity of virus detection from specimens taken late in the course of the illness.

The SV technique is easy to perform, available in most diagnostic virology laboratories and is superior to TC in terms of the time interval until the virus is detected. Even for the remaining 30% which are detected within 5 days of incubation, the delay is minimal compared to TC remaining below 22% after this interval. The loss of sensitivity of the SV assay of about 4% compared to TC seems negligible with respect to the gain of time. The SV assay has replaced TC in our clinical laboratory after the described mumps epidemic. Its performance for the confirmation of mumps cases has been very satisfactory. The very small difference between the sensitivities of SV and TC results in almost identical positive and negative predictive values, particularly with lower pre-test probabilities as expected in non-epidemic situations.
If the virus strains are to be kept for further sequence analysis, TC can be carried out in addition to the SV assay. However, the preservation of the primary specimen for this type of analysis may be preferable and can be done based on the SV result. This allows the sequencing of mumps virus genes prior to passage of the virus in cell culture, which may rapidly induce mutations and selection of certain variants (Boriskin et al., 1992; Cusi et al., 1996).

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References


