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Utility of neutralization test for laboratory diagnosis of suspected mumps

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ABSTRACT

Mumps is an infectious disease caused by mumps virus (MuV), which belongs to the family *Paramyxoviridae and* genus *Rubulavirus*. Typical symptoms of mumps include fever and swelling of the parotid glands; however, mumps can be asymptomatic. Mumps is diagnosed by molecular and serological methods (i.e., PCR and Enzyme Immunoassay [EIA]); however, both methods have pros and cons. This study was performed to compare the diagnostic utility of a focus reduction neutralization test (FRNT) to that of MuV-specific commercial IgM and IgG antibody EIA in patients suspected of having mumps. One hundred-eighty six samples collected during mumps outbreak in 2012–16 were studied. Samples (n = 80) were tested by all the three serological assays and showed 70.4%, 83% and 92.5% positivity by IgM EIA, IgG and FRNT, respectively. In all, 58.8% samples (n = 47) tested positive in all three assays. Concordance between mumps RT-PCR and IgM EIA was highest during the first 2–5 days and decreased with increasing time post-onset. Mumps FRNT results agreed with those of RT-PCR/IgM EIA from the second week onwards, whereas the results of mumps IgG EIA agreed with those of RT-PCR/IgM EIA from post-onset days 3–10. These findings suggest the utility of a FRNT for laboratory diagnosis of mumps in countries whose populations are not immunized against this infection.

List of Abbreviations

EIA
enzyme immuno assay
FRNT
focus reduction neutralization test
MuV
mumps virus

Mumps, a childhood disease caused by MuV, is characterized by fever and swelling of the parotid glands. MuV also infects other organs, such as the genital organs, pancreas, thyroid, salivary glands, urinary tract and central nervous system. MuV is a member of the *Paramyxoviridae* family and *Rubulavirus* genus and has a single-stranded, negative sense RNA genome with 15,384 nucleotides that encodes seven different proteins 1. It has been shown by standard small hydrophobic gene sequencing that MuV has 12 genotypes 2.

Mumps-containing vaccines have not been included in India's universal immunization program. The Indian Academy of Pediatrics has suggested including a first dose of mumps vaccine at 9 months and second dose at 16–24 months [3]. Although mumps is a public health problem in India, it remains ignored because there is no surveillance system. To date, circulation of mumps genotypes C and G viruses in India has been reported [4].

The clinical diagnosis of mumps requires laboratory confirmation by mumps-specific IgM antibodies in serum or oral fluid specimens or detection of MuV RNA by RT-PCR [2]. However, the utility of mumps IgM EIAs to confirm mumps infection in an immunized population has limitations. The time of specimen collection is crucial for MuV RNA detection. Hence, diagnosis of mumps by RT-PCR is valuable in both unimmunized and immunized patients [5-7]. Mumps IgM detection is highly specific but the sensitivity is lower in immunized individuals. Hence, an additional test such as mumps IgG EIA (or IgG avidity) would confer an added advantage [8].

Few studies on the laboratory diagnosis of mumps in an unimmunized population by using serological and molecular tools are available. Hence, we undertook a study to determine correlations between three serological assays for mumps diagnosis. Interestingly, these serological assays are based on different principles, measuring MuV-specific IgM, IgG and neutralizing antibodies.

MATERIALS AND METHODS

Altogether, 186 clinical specimens were referred for the diagnosis of mumps from four States and two Union Territories of India. Informed consent for collection of the specimens was obtained from all participants and their anonymity was preserved. Epidemiological and clinical information about the individuals with suspected mumps was collected using standard case investigation forms available from the National Institute of Virology Pune, India. The samples were obtained during the years 2012–16 and came from 93 male and 93 female participants. No participant had been vaccinated for mumps; 175 were aged ≤15 years and 11 >15 years.

Serum samples were subjected to MuV specific IgM and IgG antibody detection using commercial kits (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The assays were carried out and the results interpreted according to the manufacturer's instructions.

For FRNT, serum samples ($n = 80$) were heat inactivated in a 56°C water bath for 30 min, after which serum dilutions (1:2 to 1:256) were prepared. Virus–serum neutralization reactions were performed by adding equal volumes of diluted samples at 37°C in a 5% CO_2 incubator for 2 hr. For the challenge experiments, MuV genotypes C or G or N dilutions giving 30–45 viral foci per 10 µL inoculum per well were used in the FRNT. Non-neutralized MuV was detected by immunocolorimetric staining [9]. Briefly, one-day-old Vero cell monolayers were prepared in 96-well tissue culture plates. Cell concentrations were adjusted to 2×10^4 cells/mL and 100 µL cell suspension added to each well. Virus–serum reactions (20 µL) was inoculated into each well and, after 3–4 hr adsorption at 37°C in a 5% CO_2 incubator, the cells were overlaid with 0.8% carboxymethyl cellulose (BDH GPR, Poole, UK) and the plates incubated. Two days of post-infection, the plates were developed. The volumes of primary antibody, secondary antibody and substrate were reduced (50 µL) in the FRNT protocol and the plates washed manually. After development, blue stained viral foci were counted by eye and 50% neutralization titers were deduced using the Kärber formula [10]. A

mumps neutralizing antibody titer >1:4 is considered positive, as described earlier 10.

Throat swabs (*n* = 70), urine specimens (*n* = 2) and acute serum samples (*n* = 71) collected from 143 of 186 individuals clinically suspected of having mumps were tested by mumps RT-PCR as previously described 11, 12 and their MuV genotypes determined.

A descriptive statistical analysis was performed using PASW version 18.

RESULTS

Mumps specific IgM EIA was positive in 70.4% (131/186) of the samples and IgG EIA in 76.4% (139/182) of them. Ten samples showed IgM antibodies but not IgG antibodies, whereas 33 were negative for IgM antibodies but had IgG antibodies. Altogether, 118 samples had both IgM and IgG antibodies and 21 were negative for both.

Comparison of IgM EIA, IgG EIA and FRNT assays

A sufficiently large subset of samples (*n* = 80) was subjected to FRNT testing. Results of FRNT were compared with those of IgM EIA and IgG EIA. Overall positivity by FRNT was 92.5% (74/80) followed by 85% (68/80) for IgG EIA and 77.5% (62/80) for IgM EIA. Altogether, 58.8% samples (47) tested positive in all three assays. FRNT and IgM EIA had 72.5% (58/80) agreement. Among the 18 IgM negative samples, 17 had neutralizing antibodies whereas IgM antibodies were detected in five FRNT negative samples. Altogether, 57 cases showed the presence of both IgM and neutralizing antibodies; a single sample was negative for both IgM and neutralizing antibodies. There was 85% overall agreement between FRNT and IgG EIA. Both IgG and neutralizing antibodies were present in 65 of 80 samples but negative in three. Three samples negative for neutralizing antibodies had IgG while nine samples negative by IgG EIA were positive by FRNT. Mumps specific IgG EIA and IgM EIA had a concordance rate of 80%.

There was 69.9%, 78.7%, and 94.6% positivity in samples from female participants and 71%, 87.1%, and 90.7% positivity in those from male participants by IgM EIA, IgG EIA and FRNT, respectively. Seropositivity according to age group is depicted in Figure 1. Overall, seropositivity was higher by mumps FRNT, followed by mumps IgG EIA and IgM EIA.

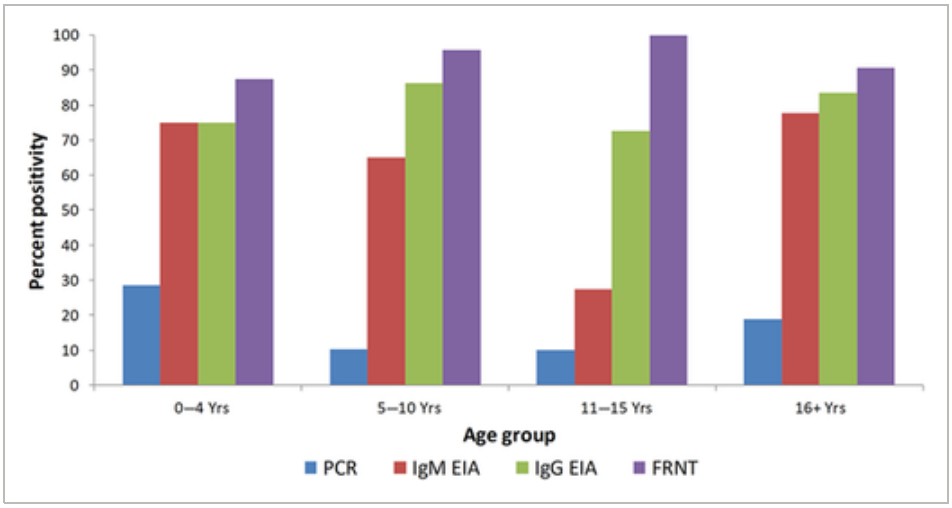

Figure 1

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Mumps positivity by age group. [Color figure can beviewed at wiley onlinelibrary.com]

Day of onset of disease and positivity for the assessed assays, including RT-PCR

Mumps small hydrophobic gene RT-PCR positivity was noted in 24 of 143 clinical specimens. Mumps IgM antibodies were detected in 22 of 24 specimens and mumps IgG antibodies in 15 of 24. Sequencing revealed circulation of genotype C in 19 and genotype G in five specimens.

Correlations between post-onset days of mumps and MuV RT-PCR, IgM EIA, IgG EIA and FRNT were studied (Table 1) and it was found that highest RT-PCR positivity occurred up to 5 days of onset and thereafter decreased, as expected. However, mumps IgM positivity remained almost constant for at least up to two weeks. As expected, mumps IgG positivity increased with time post-onset, whereas mumps FRNT was positive in all samples from Day five onwards (Fig. 2). Concordance between mumps RT-PCR and IgM EIA was highest during the first 2–5 days and decreased thereafter with time. Mumps FRNT agreed with RT-PCR/IgM EIA from the second week (8 days post-onset) onwards, whereas mumps IgG EIA agreed with RT-PCR/IgM EIA from post-onset days 3–10 (approximately).

Table 1. Mumps onset, percent positivity of assessed tests and their concordance

Post-onset days (mean)		Samples tested by PCR & IgM EIA	MuV PCR positivity (%)	MuV IgM EIA positivity (%)	†Concordance MuV PCR and IgM EIA (%)	Samples tested by MuV IgG EIA	MuV IgG EIA positivity (%)	†Concordance MuV PCR/ IgM EIA and IgG EIA (%)	Samples tested by MuV FRNT	p
1–2 (1.5)		22	22.7	63.6	59.1	22	59.1	59.1	16	
3–4 (3.5)		30	43.3	63.3	73.3	30	70.0	76.7	14	
5–6 (5.5)		37	8.1	67.6	40.5	34	82.4	76.5	16	
7–9 (8)		24	12.5	66.7	37.5	23	91.3	78.3	8	
10+(14)‡		30	0.0	60.0	40.0	30	96.7	63.3	11	
Total		143				139			65	

†Concordance = (both positive and negative)/tested × 100; ‡For 10 + group, the median (rather than the mean) is supplied.

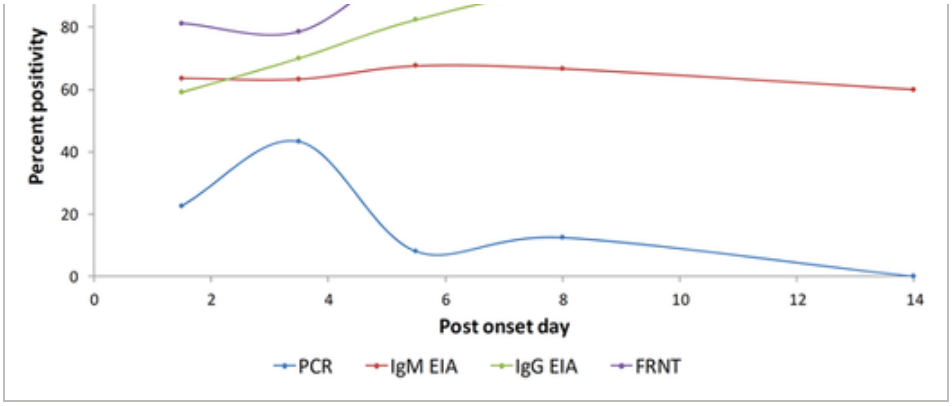

Figure 2

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Mumps positivity by post-onset day. [Color figure can be viewed at wiley onlinelibrary.com]

DISCUSSION

This study documents the utility of three serological assays for the diagnosis of mumps in an unimmunized population. It was found that specimen collection time and type of specimen are crucial for selecting serological tests. MuV specific IgM-class antibody appears at presentation of illness 10–12 days after infection 13 and may last for 2–6 months 14. In the present study, mumps virus-specific IgM antibodies were detected in 131 of 186 specimens (70.4%) and IgG antibodies in 151 of 182 (83%). A study from the Czech Republic documented that a combination of mumps-specific IgM and IgA EIAs showed increased seropositivity from 52% to 72% 15. Similar findings were noted in the present study when the results of all the three assays were combined. It is interesting to note that about 55.2% of healthy individuals are positive by FRNT (National Institute of Virology, 2014). Because of the unavailability of a mumps IgG avidity testing facility, recent or past mumps infection could not be determined in these individuals with suspected mumps, as described in previous studies 7, 16.

Altogether, 26.34% of specimens from individuals suspected clinically of having mumps showed neither MuV RNA nor MuV IgM antibodies; those individuals may therefore not have had true mumps. A recent study from Italy showed 19.8% Epstein–Barr virus positivity in persons with symptomatic mumps 17. Further studies are required to determine the contribution of different viruses to mumps-like illness reported in unimmunized individuals. Amongst the samples (n = 53) that were negative by both mumps IgM and RT-PCR, 39 showed presence of either mumps IgG or neutralizing antibodies, indicating previous mumps infection. However, mumps RT-PCRs and real time PCRs are preferred methods for rapid diagnosis in the acute phase of this disease. In the present study, most samples were serum samples collected at different time points from disease onset, so enzyme immunoassays or neutralization tests were the methods of choice for laboratory diagnosis.

Previously, mumps FRNT was established, and its sensitivity and specificity documented with the gold standard plaque reduction neutralization test and mumps specific IgG antibody EIA 9, 10. In addition, published cross-neutralization studies with MuV genotype C, G and N indicate minor differences in FRNT titers without any qualitative differences in FRNT results 18. Therefore, the present findings on three

different challenge viruses (MuV genotype C, G, and N) may not influence FRNT results in individuals suspected of having mumps.

In conclusion, concordance between mumps RT-PCR and IgM EIA is greatest during the first 2–5 days and decreases thereafter with time. Mumps FRNT results agree with those of RT-PCR/IgM EIA from the second week onwards, whereas the results of mumps IgG EIA agree with those of RT-PCR/IgM EIA from post-onset days 3–10. Thus, the utility of a FRNT for laboratory diagnosis of mumps is feasible in the countries whose populations are largely unimmunized. The present study had a few limitations. (i) Not all samples were tested by all three serological assays; thus, overall seropositivity may differ. (ii) Mumps IgM EIA and RT-PCR were not performed on all specimens; thus, assay correlations may not be accurate by IgG EIA and FRNT.

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DISCLOSURE

The authors declare that they do not have a commercial interest or financial arrangements with any company or other association that might pose a conflict of interest regarding this work.

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