Isolation of Measles Virus from Clinical Specimens Using B95a and Vero/hSLAM Cell-lines

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SUMMARY

The clinical presentation of acute measles is normally quite typical, especially in the presence of Koplik's spots, that laboratory test is seldom required to confirm the diagnosis. However, with wide measles vaccination coverage and the extensive use of immunosuppressive chemotherapy, the diagnosis of atypical manifestations of acute measles may require laboratory confirmation. When compared with B95a cell-line, this study shows that the Vero/hSLAM cell-line is sensitive and is recommended for use in the primary isolation of wild-type measles virus from clinical specimens. Throat swab and urine specimens are the clinical specimens of choice and both are recommended for optimal isolation of measles virus from patients suspected of acute measles virus infection.

KEY WORDS:
Measles virus, B95a cell-line, Vero/hSLAM cell-line

INTRODUCTION

Measles is a highly contagious disease characterized by a prodrome of fever, coryza, cough, and conjunctivitis for three to four days followed by the appearance of Koplik's spots and a generalized maculopapular rash. Measles virus (MV) infection induces lifelong immunity, but this acute infection also induces immunesuppression resulting in increased risk of complications to other opportunistic infections, and in rare instances, persistent infection of the nervous system such as subacute sclerosing panencephaltis[1,2]. Despite the availability and global use of a successful live attenuated vaccine, measles remains a significant of childhood morbidity and mortality in developing countries. Outbreaks continue to occur in developed nations[3-5].

The measles virus (MV) is an enveloped, single stranded negative-sense RNA virus which belongs to the genus *Morbillivirus* under the family *Paramyxoviridae*. Measles virus naturally infects only humans but can be transmitted to subhuman primates[1,2]. Due to the induction of lifelong immunity and the absence of an animal reservoir, maintenance of MV in the human population requires large numbers of susceptible individuals for it to sustain continuous person-to-person transmission. As in the case of smallpox, subclinical and asymptomatic measles virus infection is uncommon in immunocompetent individuals. Thus, together with the availability of the highly effective live attenuated measles vaccine, World Health Organization (WHO) has earmarked to eliminate measles in the WHO Western Pacific Region by 2012[6]. The Ministry of Health Malaysia in February 2003 decided to initiate measles elimination in Malaysia through mass measles vaccination starting in 2004 and the National Public Health Laboratory was designated as the WHO national measles laboratory.

Currently, the symptoms of acute measles are quite typical, especially in the presence of Koplik's spots, that a virus laboratory is seldom called upon to make a diagnosis. However, as the mass vaccination programme takes effect, physicians may become less familiar with the clinical presentation of the disease, and vaccination itself may lead to the emergence of atypical measles. In addition, as more patients undergo immunosuppressive therapy, the typical presentation of acute measles may be altered. Thus, diagnosis of these atypical manifestations may require laboratory support[7]. In-lined with its designation as the WHO national measles laboratory, the National Public Health Laboratory has developed all the required diagnostic tests, including serology, molecular diagnostic and virus isolation, for the laboratory confirmation of acute measles since 2004. This study evaluated the type of clinical specimens and cell-line types that were found to be most suitable for measles virus isolation and identification during the recent measles outbreak in Peninsular Malaysia at the end of 2004 and in Sarawak, East Malaysia in the beginning of 2005, where measles virus genotype D9 was isolated in both outbreaks.

MATERIALS AND METHODS

Clinical specimens, consisting of respiratory secretions (nasal swab, throat swab) in viral transport medium and urine of at least 10 ml in plain sterile universal bottle, were collected from patients with clinical diagnosis of acute measles. The clinical specimens, together with patients' serum samples derived from respective 5 ml of venous blood, kept cool by ice-pack were transported to the National Public Health Laboratory within 24 hours of collection.

The swab specimen in viral transport medium was lightly vortexed and treated with antibiotics (crystalline penicillin 100,000 I.U./ml and streptomycin 100 μg/ml). After an hour of treatment, the specimen was inoculated in duplicates (100 μl and 200 μl respectively) onto freshly seeded monolayer of Vero/hSLAM and B95a cells cultured in JM tubes. The inoculated culture tubes were incubated at 37^{0}C in an ambience of 5% CO_2 and examined daily for a period of at

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least 10 days for the appearance of cytopathic effect (CPE). Those that developed CPE were harvested, washed with sterile phosphate buffered saline and evidence of measles virus was confirmed by indirect immunofluorescence (IF) using a commercial typing anti-measles monoclonal antibody (Cat. No: 5030, Chemicon International, Temecula, USA). At the end of 10 days, an aliquot of cells was also harvested from the culture tubes without obvious evidence of CPE and similarly tested by indirect IF for the presence of measles virus.

Ten ml of urine was carefully transferred into a sterile 15-ml Falcon tube and the cell suspension was pelleted by centrifugation at 150g for 10 minutes. After centrifugation, the excess urine was decanted into a beaker containing disinfectant for liquid waste. The cell pellet with about 200 to 300 µl of remaining urine was resuspended in 2 ml of viral transport medium and similarly treated with the same concentration of antibiotics as the swab specimen. After an hour of antibiotic treatment, the urine specimens were inoculated into both culture cell-lines, incubated and processed in the same manner as the swab specimens.

All serum samples were tested for the presence of anti-measles specific IgM using a commercial measles IgM-capture ELISA kit (Cat. No: OWLI 15, Dade Behring, Marburg, Germany) in accordance to the manufacturer's instructions.

Data analysis was performed using Epi Info6 computer free software programme from the Center for Disease Control and Prevention, Atlanta, USA. A probability (p) value of 0.05 or less was taken as the level of significant association for each ordinal variable with the relevant adjusting variables.

RESULTS

In the two outbreaks, 160 patients were clinically suspected of having acute measles and 393 clinical specimens consisting of 152 serum samples, 120 urine samples, 110 throat swabs and 11 nasal swabs were collected for laboratory confirmation of the illness. Anti-measles IgM antibody was detected in the sera of 73 (48%) patients and measles virus was isolated from clinical specimens of 40 (25%) patients. Of the 40 patients with positive measles virus isolation, anti-measles specific IgM was also detected in the serum samples of 27 patients (67.5%).

Measles virus was isolated from 84 specimens (45 from throat swabs, 37 from urine specimens, and two from nasal swabs) using both type of cell-lines. The type of clinical specimens that were measles virus culture positive and the type of cell-lines in which the virus were isolated are shown in Table I. There is a slight increase in the number of measles virus isolated from throat swabs as compared to the urine specimens (45 verses 37) but there is no significant difference in the virus isolation rate based on sample type ($\chi^2 = 1.54$, p = 0.0.2140). More measles virus were isolated in Vero/hSLAM cell-line than that of B95a cells but there is also no significant difference in the virus isolation rate between the two cell-lines used in this study ($\chi^2 = 1.03$, p = 0.3112).

Only 30 patients had both throat swabs and urine collected simultaneously for comparison of the efficacy of measles virus isolation rate with respect to sample type and cell-line as shown in Table II and Table III. Using both B95a and Vero/hSLAM cells, 33 (33/60, 55.0%) measles virus isolates were obtained from urine and 34 (34/60, 56.7%) from throat swabs (Table II). There is no significant difference in the isolation rate based on type of specimen ($\chi^2 = 0.00$, p = 1.0000). Twenty nine specimens had measles virus isolated in B95a cell-line and 38 in Vero/hSLAM cell-line (Table II). All the 29 measles virus isolates that showed CPE in B95a cell-line also gave syncytial CPE in Vero/hSLAM cell-line whereas 9 virus isolates that showed CPE in Vero/hSLAM cell-line but did not show evidence of CPE in B95a cell-line. Vero/hSLAM cell-line was more sensitive than B95a cell-line (38 verse 29) for primary isolation of measles virus from clinical samples but statistical analysis shows no significant difference ($\chi^2 = 2.16$, p = 0.1413).

Of the 30 patients with positive MV isolation, only eight (8/30, 26.7%) patients had measles virus isolated from both type of specimens. Measles virus was isolated from the urine but not the throat swabs of 10 (10/30, 33.3%) patients and only from the throat swabs of 12 (12/30, 40%) patients (Table III). Though more MV was isolated in Vero/hSLAM cell-line than B95a cell-line, evidence of virus replication as shown by the presence of CPE was noted earlier in B95a cell-line (range 2 to 9 days, mean: 4.8 days) than in Vero/hSLAM cell-line (range: 3 to 11 days, mean: 6.3 days) (Table III).

Table I: The number and type of clinical specimens and cell-lines that yield positive measles virus isolates.

Type of specimen	Cell-line used					
	B95a		Vero/hSLAM		Total	
	Number tested	Culture +ve (%)	Number tested	Culture +ve (%)	Number tested	Culture +ve (%)
Nasal swab	11	1 (9.1)	11	1 (9.1)	22	2 (9.1)
Throat swab	110	19 (17.3)	110	26 (23.6)	220	45 (20.5)
Urine	120	18 (15.0)	112	19 (17.0)	232	37 (15.9)
Total	241	38 (15.8)	233	46 (19.7)	474	84 (17.7)

Table II: The number of measles virus isolated from the 30 patients with paired urine and throat swab specimens based on the sample type and cell-line used for virus isolation.

Cell-line	Urine	Throat swab	Total
B95a	15	14	29
Vero/hSLAM	18	20	38
Total	33	34	67

Table III: Patients with both throat swabs and urine collected simultaneously for comparison of the efficacy of measles virus isolation rate with respect to sample type and cell-line used for virus isolation.

			Post-inoculation cytopathic effect (day)	
Patient	**Lab. No.**	**Type of Specimen**	**B95a cell**	**Vero/hSLAM cell**
1	MI/05/033	Throat swab	-	-
		Urine	-	9
2	MI/05/035	Throat swab	-	9
		Urine	-	-
3	MI/05/036	Throat swab	-	-
		Urine	3	4
4	MI/05/041	Throat swab	3	4
		Urine	3	4
5	MI/05/048	Throat swab	6	10
		Urine	-	-
6	MI/05/049	Throat swab	8	10
		Urine	-	-
7	MI/05/060	Throat swab	8	10
		Urine	-	-
8	MI/05/074	Throat swab	-	-
		Urine	9	9
9	MI/05/085	Throat swab	-	-
		Urine	-	9
10	MI/05/089	Throat swab	-	9
		Urine	6	4
11	MI/05/095	Throat swab	-	-
		Urine	4	6
12	MI/05/096	Throat swab	9	9
		Urine	9	9
13	MI/05/097	Throat swab	6	6
		Urine	5	9
14	MI/05/098	Throat swab	-	-
		Urine	3	3
15	MI/05/100	Throat swab	-	5
		Urine	-	-
16	MI/05/101	Throat swab	3	4
		Urine	3	3
17	MI/05/102	Throat swab	-	4
		Urine	-	-
18	MI/05/112	Throat swab	-	-
		Urine	4	5
19	MI/05/118	Throat swab	-	-
		Urine	4	3
20	MI/05/121	Throat swab	-	-
		Urine	6	5
21	MI/05/126	Throat swab	4	3
		Urine	-	-
22	MI/05/131	Throat swab	3	3
		Urine	-	-
23	MI/05/133	Throat swab	3	3
		Urine	-	-
24	MI/05/134	Throat swab	2	2
		Urine	2	2
25	MI/05/138	Throat swab	-	11
		Urine	-	-
26	MI/05/142	Throat swab	-	11
		Urine	-	11
27	MI/05/143	Throat swab	3	4
		Urine	-	-
28	MI/05/146	Throat swab	4	8
		Urine	-	-
29	MI/05/160	Throat swab	6	9
		Urine	5	5
30	MI/05/161	Throat swab	-	-
		Urine	5	5
Total number of measles virus isolates			**(29)**	**(38)**

DISCUSSION

Measles virus (MV) has most successfully been isolated from peripheral blood mononuclear cells or respiratory secretions inoculated onto primary human or monkey kidney cells[1,2]. Once the virus has been isolated in culture, it can be adapted to replicate in a variety of primary cell cultures and cell-lines. Due to problems with the use of animal to obtain the primary cells, long term maintenance of primary cell culture, and possibility of contamination of primary cells with simian viruses, continuous monkey kidney cell lines were subsequently and commonly used for primary MV isolation[8-10]. However, several blind passages are often required before propagation of wild-type MV from clinical specimens that lead to production of syncytial cytopathic effect can be detected in tissue culture[8-10].

Recently, reproducible primary isolation of MV has been reported using an Epstein-Barr virus (human herpesvirus 4, HHV4) transformed marmoset monkey B lymphocyte line (B95a) and human cord blood leucocytes[11,12]. Subsequently, a commonly used continuous monkey kidney cell-line (Vero) derived from the African Green monkey was genetically modified to express the human SLAM (signaling lymphocytic activation molecule) receptor (Vero/hSLAM) and was found to be just as sensitive for primary isolation of wild-type MV[13]. This small study shows that whenever wild-type MV from clinical specimen was isolated in B95a cell-line, it can also be isolated in Vero/hSLAM cell-line but not the reverse. This finding indicated that Vero/hSLAM was more receptive than B95a cells for isolation of wild-type MV though statistical analysis did not show any significant difference. This may have been attributed to the small sample size of this study. In view of the fact that B95a cell-line also contains HHV4, it is recommended that Vero/hSLAM cell-line alone should be sufficient for use in the national measles diagnostic laboratory to carry out isolation of MV. The suitability of using Vero/hSLAM cells alone for primary isolation of MV from patients has been supported by other previous studies and the World Health Organization[13,14].

Processing peripheral blood mononuclear cells for isolation of MV requires higher demand of technical laboratory support. This may not be readily available in most diagnostic laboratories especially in developing countries. Respiratory secretions and/or urine specimens are the acceptable alternatives. This study shows that though there was no significant difference, both specimen types are thus recommended for isolation of MV from patients suspected of acute measles. This is supported by the finding that out of the 30 patients, eight (26.7%) patients had MV isolated from both urine and throat swabs. MV was isolated from urine specimen but not the throat swabs of 10 (33.3%) patients and only from throat swabs of 12 (40%) patients.

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