A Study on the Clinical Application of a Rapid Diagnostic Reagent for Measles

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ABSTRACT

Measles is an acute febrile rashes and infections caused by the measles virus belonging to the paramyxovirus. Each year more than twenty million peoples are affected to measles all over the world and 242,000 patients are leading to death. Traditionally the diagnosis of measles had been based on clinical symptoms in daily clinical practice. In recent years, many clinical cases do not show typical clinical manifestations and laboratory confirmation of the disease is definitely required. Lateral flow-based immuno-chromatography reagents are widely used for rapid point-of-care testing in Japanese general clinics, because it does not require special skills and facilities. We have developed a rapid diagnostic reagent for measles that employs the lateral flow-based immuno-chromatography. The correlation of the results between obtained by the diagnostic reagent and RT-PCR assay, the diagnostic reagent and detection of serum IgM antibodies, and laboratory diagnosis and clinical findings or symptoms was investigated. The correlation of the results between obtained by the diagnostic reagent and RT-PCR was the positive results that match rate of 45.5%, 100% negative match, correlation of the diagnostic reagent and detection of serum IgM antibodies positive matches to 55.6%, 100% negative match. In what had been diagnosed with measles in clinical findings so far identified with differential diagnosis of measles cases obtained by laboratory confirmation. It was concluded that the lateral flow-based rapid diagnostic reagent for measles was useful in general clinics.

Key words: *Measles, rapid diagnosis, RT-PCR, immuno-chromatography*

Kızamık için Hızlı Tanısal Ayıraçın Klinik olarak Kullanımı

ÖZET

Kızamık paramiksovirüs ailesine bağlı kızamık virüsünün yolaçtığı ateşli döküntülü bir akut infeksiyondur. Her yıl dünya üzerinde yirmi milyondan fazla kişi hastalıktan etkilenmekte ve 242.000 hasta ise ölmektedir. Geleneksel olaral kızamık tanısı günlük pratikte klinik semptomlar ile konulmaktadır. Yakın zamanda, birçok hasta tipik klinik özellikler göstermemektedir ve hastalığın laboratuvar doğrulaması kesin gerekli olmaktadır. Yan akım tabanlı immün kramotografi ayıraçları özel eğitim ve beceri gerektirmediğinden Japon genel kliniklerinde hızlı bakım testinde geniş olarak kullanılmaktdır. Yan akım tabanlı immün kramotografi ile uygulanan bir hızlı tanısal ayıraç geliştirdik. Tanısal ayıraç ve RT-PCR, tanısal ayıraç ve serum IgM antikorları, laboratruvar tanı ve klinik bulgular veya semptomlar arasındaki korelasyon sonuçları araştırıldı. Tanısal ayıraç ve RT-PCR arasındaki korelasyon sonuçları pozitif %45.5, negatif %100 oranında iken tanısal ayıraç ve serum IgM antikorları için pozitiflik %45.5, negatiflik %100 bulundu. Klinik bulgular ile tanı konulan kızamık olguları laboratuvar doğrulaması ile ayrıcı tanı yapılarak ortaya konulmuştur. Sonuç olarak, kızamık için bulunan yan akım tabanlı hızlı tanısal ayıraç genel klinikler için yararlı bulunmuştur.

Anahtar kelimeler: *Kızamık, hızlı tanı, RT-PCR, immünokramotografi*

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INTRODUCTION

Measles is a viral infectious disease caused by the measles virus, an enveloped negative-strand RNA virus of the Morbillivirus genus in the Paramyxoviridae. There is no specific treatment for measles and most patients recover within 2–3 weeks. However, particularly in malnourished children and people with lowered immunity, measles can cause serious complications, including blindness, encephalitis, severe diarrhea, ear infection and pneumonia. There is no clinically-useful rapid diagnosis for measles over the world. Measles was estimated to infect 20 million people per year worldwide, and to result in 242,000 deaths (1,2). Although so-called Koplik's spots are known as typical symptoms of measles, a typical clinical case often shows no symptoms and that makes early diagnosis quite difficult (3,4). Since atypical cases of measles are not uncommon, diagnosis based on laboratory testing is required (5,6). Laboratory diagnosis by IgM antibody-detection, RT-PCR assay, and viral isolation are now established for the confirmation of the diagnosis of measles infection (7,8). Because of high sensitivity and specificity, these methods are the most reliable for laboratory diagnosis of Measles. However, although they are effective for epidemiological investigation, they are not suitable for use in daily clinical practice (9). Not from the importance of rapid diagnosis, the experimental laboratory diagnosis of measles has been reemphasized to establish the daily application in general clinics (10).

The immuno-chromatographic method, including lateral flow-based rapid diagnosis reagents, is widely used for daily point of-care testing in almost all Japanese clinics. These reagents have been developed and marketed for the diagnosis of respiratory infectious disease such as influenza (11-13). To detect the measles virus in clinical samples, a reagent has to react with each of wild 23 genotypes (14-16). In a previous study, more than 100TCID50 of measles virus was detected from nasopharyngeal swabs (17). Our country is sought against yellow fever in exceptional crisis situations as the developed countries domestic, but also western pacific countries conduct technical assistance and fulfill international obligations towards the Elimination of the 2012 goals (1). We already reported about the fundamental results of rapid diagnostic reagent for measles, this time we will report about the diagnostic significance (18). The aim of this study is to investigate the possibility of diagnosing measles with a rapid diagnostic reagent based on a lateral flow platform.

MATERIALS AND METHODS

The lateral flow-based rapid diagnostic reagent

In order to select the target protein for the lateral flow-based rapid diagnostic kit, 25 reagents were prepared with previously-developed antibodies for measles H, F, M and N proteins, and the detection limits of them were examined using cultured virus of Edmonston strain (19-22). B4 antibody to N protein exhibited 10 times higher sensitivity than the other antibodies (18). Rated measurement sensitivity using cultured measles virus, about reagent system already includes a wild stock.

Evaluation of the diagnostic reagent using clinical samples

During the time of March 2008 to July 2010 clinical samples were obtained from the 46 patients (0 to 38 years of age; average 8.1 years, 25 males, 21 female) with suspected measles infection at 14 medical facilities in the area of Tokyo, Chiba, Osaka and Hokkaido of Japan. In the collaboration facilities, nasopharyngeal specimens of patients were collected and diagnosed with the test kit. Patients suspected measles clinical findings more than throat swab taken, evaluation of rapid tests kits. The residual samples for the diagnostic reagent were analyzed with RT-PCR in the laboratory. We examined the correlation between the diagnostic reagent and RT-PCR, and the clinical symptoms in non-measles patients (23,24). Detection sensitivity compared with real-time PCR method using the clinical material collected from this time at 14 facilities.

Serum samples were also collected to detect measure-specific IgM antibodies. Commercially available EIA kits were used to detect serum antibodies against measles (25-27). Using serum and extract liquid residual by using real-time RT-PCR and antibody measurement conducted. Specimen collection was done by the physicians' responsibilities under the agreement based on ethical guidelines on clinical research (issued by the Ministry of Education and Science and the Ministry of Health, Welfare and Labor of Japan) and the Helsinki Declaration (World Medical Association).

Quantitative real-time RT-PCR method

RT-PCR analysis was used as the reference assay (22,23). For the Taq Man PCR method, we used the N3 primer and probe set described in a previous paper (22). One-step real-time RT-PCR reactions were performed in duplicate

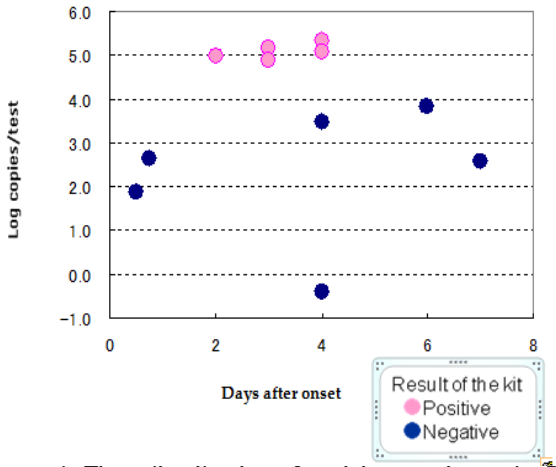

Figure 1. *Time distribution of positive specimens by RT-PCR assy.*

in an eight well optical micro tube (Applied Biosystems, Carlsbad, CA). A One Step Prime Script RT-PCR Kit (Takara-Bio, Otsu, Japan) was used for the PCR reagent. A 5 mL RNA sample or control RNA sample was analyzed in 25 mL of reaction mixture. Thermo cycling parameters included a reverse transcription step at 42.8 C for 5 min, followed by DNA polymerase activation at 95.8 C for 10 s and 40 cycles of 95.8 C for 5 s and 608C for 35 s. Real-time assays were performed with the ABI PRISM 7500 sequence detection system (Applied Biosystems). Threshold cycle (Ct) values were calculated for each reaction. Samples were considered positive only if Ct values were less than 40 for both replicates. Results are expressed as the equivalent of copies mL, according to titration of control RNA.

RESULTS

Sensitivity and specificity of the diagnostic reagent system

In 46 clinical specimens 5 were positive by the diagnostic reagent and 11 were positive by RT-PCR assay. These 5 reagent-positive specimens were also positive by RT-PCR assy. Minimum detection sensitivity of the reagent system in the clinical material was the 1 x 104copies/test. Specimens were positive in onset later in 2-4 days, 7 samples in the RT-PCR assay. Negative in the entire RT-PCR negative example of 35 samples, was a negative match rate is 100%. Correlation of the results between the reagent system and RT-PCR was shown in Table 1.

Table 1.*Correlations of the results between the diagnostic reagent and RT-PCR assay.*

		RT - PCR		
		+	-	Total
Regaent	+	5	0	5
	-	6	35	41
	Total	11	35	46

Positive predictive value : 45.5%
Negative predictive value : 100%

Analysis of clinical and laboratory findings of the patients with measles (Table 2)

We examined the correlation between the diagnostic reagent and RT-PCR assay, and the symptoms in 11 patients with positive results by RT-PCR. Serum IgM antibodies against measles were detected in 9 patients, Koplik's spots were pointed out in 9 and 2 showed no rashes. Measles D5 strain was identified from 9 specimens. Two patients without rash had not appeared in the relationship between clinical symptoms and positive matches, obtained a positive decision.

Clinical symptoms in non-measles patients

We also examined the correlation between the diagnostic reagent and PCR assay, and the symptoms in non-measles patients. Clinical findings of the 35 patients with RT-PCR negative results were summarized in Table 3. In what had been diagnosed with measles in clinical findings so far Koplik's spots, judged measles diagnosis differential diagnosis difficult clinical symptoms alone was RT-PCR negative results. Although non-measles patients showed measles-like symptoms, the diagnostic reagent did not exhibit false-positive. We confirmed that the diagnostic reagent could detect measles in clinical samples.

Time distribution of positive specimens by RT-PCR

The diagnostic reagent detected 71% (5/7) of measles cases at 2-4 day after onset of fever (Figure 1).

DISCUSSION

Measles is still globally prevalent focusing on developing countries every year and 452,000 person death cases (lethality 3~5%) are estimated to have occurred (2). WHO is formulated global measles elimination measures strategic planning for mortality reduction and

Table 2. *Clinical and laboratory findings of patients with measles.*

	Clinical symptoms											Laboratory result							
	Age	Sex	Temp.	Fever	Rash	Koplik spot	Cough	Nasal discharge	Conjunctival hyperemia	Eye discharge	Vaccin.	Days after onset of fever	Gene type	Log copies / test	kit-A				
															10 min	20 min	IgM	IgG	
1	17.1	F	ND	+	-	+	-	-	-	-	1	0.5	D5	1.9	-	-	-	24.8	64
2	7.6	F	38.6	+	+	-	-	-	-	-	ND	0.75	D5	2.6	-	-	ND	ND	ND
3	15.8	M	ND	+	-	+	+	+	-	-	1	2	D5	5.0	+	+	5.7	<2	4
4	16	F	ND	+	+	+	+	-	-	-	0	3	D5	4.9	+	+	3.1	96.6	128
5	17.3	M	ND	+	+/-	+/-	+	+	-	-	1	3	D5	5.2	+	+	5.7	2.1	<4
6	15.1	F	ND	+	+	+	+	+	-	-	0	4	D5	5.1	+	+	9.6	3.1	4
7	0.9	F	ND	+	+	+	+	+	+	-	0	4	D5	5.3	+	+	23.1	<2	<4
8	20	M	39.7	+	+	+	-	-	+	-	0	4	ND	-0.4	-	-	1.6	14.8	16
9	0.9	F	ND	+	+	-	+	+	+	+	1	4	ND	3.5	-	-	9.7	<2	<4
10	11.9	F	39.1	+	+	+	+	+	+	-	1	6	D5	3.8	-	-	24.5	5.1	16
11	9.4	M	39.4	+	+	+	+	+	+	+	0	7	D5	2.6	-	-	25.0	12.3	64
11	7.6	M	38.6	+	4	-	-	-	-	-	ND	0.75	D5	2.6	-	-	ND	ND	ND

Table 3. *Clinical symptoms in non-measles patients.*

	n
Clinical Symptoms	
Fever	35
Rash	25
Cough	6
Koplik Spot	21
Conjunctival Hyperemia	12
Nasal Discharge	12
Reagent	
Positive	0
Negative	35

regional elimination. While many countries conducted a thorough vaccination and surveillance, almost close to the eradication of achieving in the part already to Americas, Europe, South Africa and Middle East countries. Realization of the measles eradication, including Japan WHO Western Pacific (WPRO) is the region most of the measures was delayed and even adopted the draft had not set conditions, currently targeting 2012 prima facie achievement (1).

Although the number of measles patients in Japan has declined once more still 1 year ~ 500 people are estimated. In respect of the measures protecting overall public health from high severity in pediatric, adult onset even in question, measles is the key (10,11). The measles elimination important in strengthening surveillance and vaccination rates of improvement. Also enhancing epidemiological surveillance and scientific monitoring system based on the characteristics of virus and gene is essential for the eradication of measles (28,29).

Molecular epidemiological characterization of wild measles virus strains is an important component of measles surveillance because to identify the source and route of the transmission and it can be a valuable tool to evaluate the effectiveness of measles control and elimination programs (6). WHO recommends that viral surveillance is conducted during all phases of measles control and that virological surveillance activities be expanded to provide an accurate description of the global distribution of measles genotypes (7,8).

Establishment of effective measures against the spread of the epidemic infection is required along with prevention by vaccination in measles. Atypical cases especially after the measles vaccine inoculation of secondary immunodeficiency, pointed out experiments laboratory diagnostic importance (3-5). Establishment of rapid reagent of measles is important not only from the standpoint of the prevention of infection, but also confirms the definitive diagnosis. Currently, traditional clinical diagnosis depending on symptomatic findings often leads to misdiagnosis of measles. Detection of measles virus can be done with a diagnostic reagents is in the study using clinical specimens in a short time (30). Also, how easy is extremely secure and high practicality as well as possible were. Suggests may can be applied as a simple and rapid diagnosis of implementation possible future, even general clinical results of more than is.

Rapid diagnosis of measles introduced as a way to compensate those challenges expected (18). Diagnostic system used in the clinical evaluation of a rapid diagnostic reagent by immuno-chromatography has helped real clinical rapid diagnosis in infections associated with such as influenza virus, RS virus, adenovirus, and Streptococcus from the superior speed and simplicity (11-13). Considering the time, difference in specificity and sensitivity comparisons with RT-PCR assay for detection of virus measles genome, immuno-chromatography was usually utilized from the beginning of the disease in general medical clinics (31).

Further study overlapping on clinical specimen types, collection methods, collecting time and times of collection with diagnostic kits currently available for adenovirus, Streptococcus and influenza virus will improve diagnostic value (11-13). Also rise of serum IgM antibody is a biological reaction due to infections (25-27), the sensitivity and specificity of difference of immunochromatography and had been estimated in this study.

As compared to influenza virus low titer of measles viruses exist in the nasal pharynx depending on time of specimen collection, the diagnostic reagent lower sensitivity than that of the RT-PCR assay. High viral load time 2 to 4 days after onset in specimens been achieved with regard to detection. A further sensitivity is required including the purification of monoclonal antibody to the practical level.

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