A point-of-care test for measles diagnosis: detection of measles-specific IgM antibodies and viral nucleic acid

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Objective To evaluate the performance of a newly developed point-of-care test (POCT) for the detection of measles-specific IgM antibodies in serum and oral fluid specimens and to assess if measles virus nucleic acid could be recovered from used POCT strips.
Methods The POCT was used to test 170 serum specimens collected through measles surveillance or vaccination programmes in Ethiopia, Malaysia and the Russian Federation: 69 were positive for measles immunoglobulin M (IgM) antibodies, 74 were positive for rubella IgM antibodies and 7 were positive for both. Also tested were 282 oral fluid specimens from the measles, mumps and rubella (MMR) surveillance programme of the United Kingdom of Great Britain and Northern Ireland. The Microimmune measles IgM capture enzyme immunoassay was the gold standard for comparison. A panel of 24 oral fluids was used to investigate if measles virus haemagglutinin (H) and nucleocapsid (N) genes could be amplified by polymerase chain reaction directly from used POCT strips.
Findings With serum POCT showed a sensitivity and specificity of 90.8% (69/76) and 93.6% (88/94), respectively; with oral fluids, sensitivity and specificity were 90.0% (63/70) and 96.2% (200/208), respectively. Both H and N genes were reliably detected in POCT strips and the N genes could be sequenced for genotyping. Measles virus genes could be recovered from POCT strips after storage for 5 weeks at 20–25 °C.
Conclusion The POCT has the sensitivity and specificity required of a field-based test for measles diagnosis. However, its role in global measles control programmes requires further evaluation.

Abstracts in عربي, 中文, Français, Русский and Español at the end of each article.

Introduction

Measles is a severe, vaccine-preventable disease that causes extensive morbidity and mortality in large parts of the world. Despite the widespread use of measles vaccine, either as a single antigen vaccine or as a component of the triple vaccine against measles, mumps and rubella (MMR), 278 358 reported cases of measles and an estimated 164 000 deaths from measles occurred worldwide in 2008.[1] Vaccine coverage is highly variable between World Health Organization (WHO) global regions. Measles has been eliminated in the Americas but continues to be endemic in the African and South-East Asia regions, where vaccine coverage is less than 80%. These regions account for approximately 94% of all global measles deaths.[1] Outbreaks continue to occur in other global regions, primarily as a result of measles virus importation into areas where vaccine coverage has fallen to a suboptimal level and a susceptible cohort has accumulated.[2–4] Most regions have elimination goals and elimination strategies based on the maintenance of high vaccination coverage, for which political commitment is required. A key component of elimination plans is surveillance to monitor impact.

Laboratory confirmation of cases of measles is a vital aspect of surveillance at all stages of control programmes because clinical diagnosis is unreliable.[5] The mainstay of laboratory confirmation is the detection of measles-specific immunoglobulin M (IgM) antibodies in serum samples.[6,7] More recently, alternative samples such as dried blood spots and oral fluids have been used for diagnosis by antibody detection. Oral fluids can also be used to detect viral ribonucleic acid (RNA) and their use is becoming increasingly common because samples can be obtained safely and non-invasively, without the risks associated with blood collection, and it improves patient compliance with specimen collection, as the procedure is simple and painless.[7–10]

Point-of-care tests (POCTs) are increasingly used for the rapid diagnosis of infections. They can be performed in a single incubation step at ambient temperature without complex electrical equipment and their results can be read visually. By increasing diagnostic capacity and facilitating rapid diagnosis in resource-poor countries, they have the potential to improve measles surveillance and the response of health authorities to possible outbreaks.

We have developed a POCT capable of detecting measles-specific IgM antibodies in both serum and oral fluid specimens. In this paper we describe the diagnostic performance of this POCT for each specimen type. We also investigated whether viral RNA could be amplified from the used test strips, as that would enhance their use in measles surveillance.

Methods

Test strips

The POCT strips for the detection of measles-specific IgM were constructed essentially as described for mumps-specific IgM POCT strips,[11] except that affinity purified $F(ab')_2$ fragment goat anti-human IgM, 1.2mg/ml (Jackson Immunoresearch Labora-

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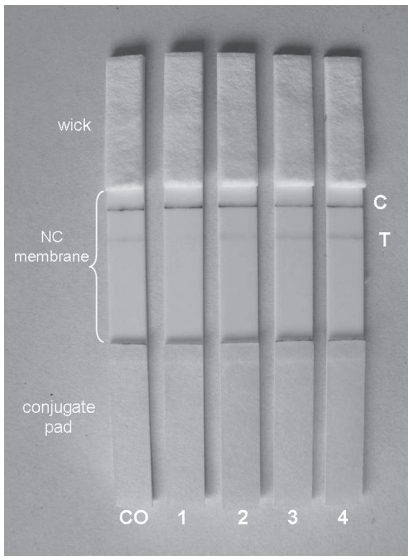
Fig. 1. **Components of the point-of-care test (POCT) and examples of signal intensities obtained during POCT testing of sera from four patients and of the cut-off control serum**



Note: Each POCT strip measures 60 × 5 mm. The photograph shows: an accuflow G glass fibre conjugate pad, on which MAb-gold conjugate is localized before use; the nitrocellulose membrane with an immobilized anti-human IgM antibody test line (T) and an anti-mouse IgG antibody control line (C) positioned approximately 14 mm and 18 mm from the conjugate pad, respectively (not visible before testing); and a cotton linter paper wick. The five POCT strips demonstrate from left to right the results of testing the cut-off control serum (CO), one negative (strip 1, enzyme immunoassay (EIA) T/CO of 0.34) and three positive specimens of varying intensity (strips 2–4, EIA T/COs of 5.4, 1.7 and 5.5, respectively).

tories, Inc., West Grove, United States of America) was used as the test line capture antibody, and monoclonal antibody to recombinant measles virus nucleoprotein[12] was conjugated to 40 nm gold colloid (BBInternational Ltd, Cardiff, England) to produce the measles-specific gold conjugate (MAb-gold conjugate). Assembled POCT strips are illustrated in Fig. 1.

Serum protocol

Recombinant measles virus nucleoprotein (rNP), expressed in yeast *Pichia pastoris* and purified by caesium chloride ultracentrifugation,[13] was diluted to 3.5 μg/ml in oral fluid extraction diluent, prepared as described previously.[14] A cut-off serum giving a weakly reactive anti-human IgM antibody test line signal in the POCT was prepared. This was done by diluting one part of a serum that tested positive for measles IgM on capture enzyme immunoassay (EIA) in 20 parts of a measles-IgM-negative human serum. The cut-off serum was tested in parallel with patient sera.

The cut-off serum and patient sera were diluted 1 part in 100 in oral fluid extraction diluent and 100 μl of the resulting dilution were mixed with 5 μl of the 3.5 μg/ml rNP in a plastic tube. POCT strips were inserted in the serum– rNP mixture to initiate the flow of reagents and were incubated at room temperature for 10 minutes. Test results were interpreted by comparing the intensity of the pink to red signal generated at the test line with patient sera, with that of the signal generated with the cut-off serum run in parallel. If the test line signal was more intense in colour than the signal obtained with the cut-off serum, the test was considered positive; if the test line signal was less intense in colour than the signal obtained with the cut-off serum or if both signals were equally intense in colour, the test was considered negative (Fig. 1).

Oral fluid protocol

The POCT protocol used for oral fluid testing was essentially the same as the one used to test sera, except that oral fluid specimens were tested undiluted, rNP was used at 35 μg/ml and the test strips were incubated for 20 minutes at room temperature. A cut-off control serum was not required for interpreting the results since background signals were not observed when testing oral fluid specimens. If the signal at the anti-human IgM test line ranged from pink to red in colour, the test was interpreted as positive for measles-specific IgM; if no signal was visible at the test line, the test was interpreted as negative for measles-specific IgM. For both serum and oral fluid testing, a signal had to be visible at the control line for the test to be considered valid.

Test performance evaluation

The performance of the POCT for the detection of measles-specific IgM in both serum and oral fluid specimens was evaluated by comparing the results with those obtained with the measles IgM capture EIA (Microimmune Ltd, Hounslow, England). Two individuals interpreted the POCT results independently at the specified incubation times. The results were then classified as positive or negative for measles-specific IgM by consensus. When only one reader observed a weak signal, the result was interpreted as negative. The EIA was performed and the results, expressed as a numerical value (quotient) calculated by dividing the optical density obtained for each test specimen (450/620 nm) by the cut-off value (T/CO), were interpreted in accordance with the manufacturer's instructions.

Serum specimens

A total of 170 serum specimens were evaluated; 100 of them had been collected from suspected cases of measles in Malaysia in 2004 (age range: 6 months to 42 years; mean: 12.4), when outbreaks were reported. The specimens originated from four federal states within Malaysia: 83 from Selangor, 11 from Melaka, 5 from Perak and 1 from Pahang.

Sixty-two serum specimens had been collected from children (age range: 6 months to 17 years; mean: 5.8) who presented with a rash during an outbreak of rubella in Ethiopia in 2003–2004. Of these children, 42 had been vaccinated against measles. Specimens were collected a mean of 7 days (range: 1–23) after the onset of the rash.

Eight serum specimens from acute or suspected cases of rubella in the Russian Federation were kindly donated by the World Health Organization's Regional Office for Europe, in Denmark. All sera were also tested by rubella IgM capture EIA (Microimmune Ltd).

Oral fluid specimens

All oral fluids from suspected measles and rubella cases received by the Virus Reference Department of the Health Protection Agency of the United Kingdom of Great Britain and Northern Ireland over a 10-day period in July 2008 were used to evaluate the POCT. The specimens had been collected as part of the national MMR surveillance programme using Oracol swabs (Malvern Medical Developments Ltd, Worcester, England) and extracted as described previously.[14] Specimens included 232 oral fluids for measles investigation, 11 for both measles and rubella testing and 39 for rubella investigation only (age range of all oral fluids: 1 month to 59 years; mean: 5.9). The 50 oral fluids submitted for rubella investigation were tested by EIA for the detection of measles IgM as well as by Microimmune rubella IgM capture EIA.

Virus nucleic acid amplification

An additional 24 oral fluid specimens received for measles surveillance testing in September 2010 were used to evaluate the extraction and amplification of viral

Table 1. **Comparison of the results obtained for 170 serum specimens with point-of-care test (POCT) for the detection of measles-specific IgM and with measles- and rubella-specific IgM capture enzyme immunoassays (EIAs)**

EIA result		POCT result		Total
Measles	**Rubella**	**Positive**	**Negative**	
Positive	Positive	5	2	
	Equivocal	0	1	76
	Negative	64	4	
Negative	Positive	5	69	
	Equivocal	0	3	94
	Negative	1	16	
Total	–	–	–	**170**

genome from POCT strips after IgM detection. Following routine surveillance testing, the specimens were stored for up to 6 weeks at 2–8 °C before this evaluation.

To investigate the stability of measles virus nucleic acid on POCT strips, the oral fluids were tested in duplicate by POCT as described earlier, then dried and stored at 20–25 °C. Nucleic acid was extracted and complementary DNA (cDNA) was prepared as described previously,[11] from an aliquot of each oral fluid on the day of point-of-care testing, from one set of dried POCT strips after overnight storage and from a second set of dried strips after 5 weeks of storage. Measles virus cDNA amplicons were generated by real-time polymerase chain reaction (PCR) for the haemagglutinin (H) gene[15] and by nested PCR targeting the nucleocapsid (N) gene.[16] Amplified N-gene products were sequenced in forward and reverse directions by dideoxynucleotide sequencing in a Genetic Analyser (Applied Biosystems, Foster City, USA), analysed with Bionumerics software version 6.1 (Applied Maths NV, Sint-Martens-Latem, Belgium) and genotyped by comparison with reference strain sequences according to WHO guidelines.[17–19]

Results

Serum

Concordant results were obtained for 157 (92.4%) of the 170 sera tested by measles-specific IgM capture EIA and POCT (Table 1). Of 76 sera that tested positive for measles-specific IgM in EIA, 69 also tested positive by POCT. This included 5 sera that also tested positive for rubella-specific IgM. Of the 94 sera that tested negative for measles-specific IgM by EIA, 88 were also negative by POCT, including 69 that had tested positive for rubella-specific IgM. Two of the 7 sera that tested positive for measles-specific IgM by capture EIA and negative by POCT had low T/COs (1.5 and 1.9) in the EIA. They had been collected 1 and 5 days, respectively, after the onset of fever. Of 74 sera that tested positive for rubella-specific IgM only, 69 tested negative by the POCT. Thus, with this set of samples the POCT had a specificity of 93.2%.

The overall performance of the POCT with all serum samples was as follows: sensitivity: 90.8% (95% confidence interval, CI: 81.94–96.22); specificity: 93.6% (95% CI: 86.62–97.62); positive predictive value: 92.0% (95% CI: 83.4–97.01); negative predictive value: 92.6% (95% CI: 85.41–96.99).

Oral fluid

The performance of the POCT on oral fluids was evaluated using a total of 282 specimens received for measles and rubella investigations (Table 2). Of the 50 oral fluids submitted for rubella investigation, 48 tested negative for both measles- and rubella-specific IgM by EIA. One oral fluid tested positive for rubella-specific IgM and one other tested positive for measles-specific IgM by EIA.

Concordant POCT and EIA results were obtained for 263 of the 278 (94.6%) oral fluids after excluding the four specimens that gave equivocal results on the measles-specific IgM capture EIA. The overall performance of the POCT with all oral fluids was as follows: sensitivity: 90.0% (95% CI: 80.5–95.9); specificity, 96.2% (95% CI: 92.6 – 98.3%); positive predictive value: 88.7% (95% CI: 79.0 – 95.0%); negative predictive value: 96.6% (95% CI: 93.2 – 98.6%).

The POCT data were further analysed as a function of T/CO on EIA and of the timing of specimen collection following the onset of symptoms (Table 3). Approximately 20% of the specimens were collected within 7 days of the onset of symptoms; 25% were collected between 7 and 14 days after onset and 31% were collected after the 14th day. The timing of specimen collection in relation to the onset of symptoms was not known for 24% of oral fluids. Seven out of the 70 oral fluids that tested positive by EIA gave discrepant POCT results. All such specimens had T/COs on EIA just above the positive cut-off value of 1.0 (range: 1.05–1.94). Collection dates were known for six of these specimens. Five of them had been collected more than 14 days after the onset of symptoms (range: 19–38). The sixth oral fluid had been collected three days after the onset of symptoms. However, this patient had also received a single dose of measles-virus containing vaccine 32 days before the specimen was collected.

Table 3 also suggests that the detection of IgM by POCT may depend on the quantity of specific IgM antibody present. All oral fluids in this panel with a T/CO of 5 or higher on IgM capture EIA were identified by POCT, regardless of the timing of specimen collection.

Table 2. **Comparison of the results obtained for 282 oral fluid specimens with point-of-care test (POCT) for the detection of measles-specific IgM and with Microimmune measles-specific IgM capture enzyme immunoassay (EIA)**

EIA result	POCT result		Total
	Positive	**Negative**	
Positive	63	7	70
Equivocal	0	4	4
Negative	8	200	208
Total	**71**	**211**	**282**

Table 3. **Results obtained for oral fluid specimens with point-of-care test (POCT) for the detection of measles-specific IgM, analysed with respect to the result quotient (T/CO) obtained in Microimmune measles-specific IgM capture enzyme immunoassay (EIA) and timing of specimen collection**

EIA		No. positive by POCT out of total tested				
		Days between onset of symptoms and specimen collection				Total
Result	T/CO[a]	0–7	8–14	>14	Unknown	
Negative	<0.8	2/42	2/51	1/64	3/51	8/208
Equivocal	0.8 to <1.0	0/0	0/1	0/1	0/2	0/4
Positive	1– 5	6/7	4/4	14/19	9/10	33/40
	5–10	1/1	7/7	3/3	2/2	13/13
	10–15	0	2/2	1/1	2/2	5/5
	15–20	1/1	1/1	0	0	2/2
	>20	4/4	3/3	1/1	2/2	10/10
No. positive by POCT out of no. positive by EIA	–	**12/13**	**17/17**	**19/24**	**15/16**	**63/70**

[a] This represents the quotient calculated by dividing the optical density obtained for each test specimen (450/620 nm) by the cut-off value.

Virus nucleic acid amplification

When measles H gene real-time PCR was performed on oral fluid aliquots and their respective POCT strips, concordant results were obtained in 23 of 24 cases (Table 4). A comparison of the threshold cycle (Ct) values obtained in the H-gene real-time PCR for oral fluids with those obtained for their matched POCT strips showed little difference between the two in the recovery of viral nucleic acid.

The H gene was detected in 16 oral fluids and in 15 of the 16 corresponding POCT strips. The measles virus N gene was amplified by nested PCR from 14 of these oral fluids and the corresponding POCT strips. The N gene was also amplified from the oral fluid aliquot and POCT strip of specimen 20, although the H gene had not been detected in either. Nucleic acid sequence analysis of the nested N-gene PCR amplicons identified viral genotype D4 in 14 of these oral fluids and viral genotype D9 in one. The genotypes and individual strains identified from the corresponding POCT strips were identical to those identified from the oral fluids (Table 4).

The remaining nine oral fluid aliquots were negative for the N gene by nested PCR. However, when nested PCR was performed on the corresponding POCT strips, N-gene amplicons were generated on five occasions. These amplicons were not derived from sequences in the specimens, but from the rNP that had been added during IgM detection, which contained trace levels of residual N-gene sequence that amplified sporadically in the absence of specimen-derived N-gene target. The fact that the contaminating N-gene sequence was not detected after one round of N-gene PCR confirms that it was present in minute quantities. The generated amplicons were confirmed to have the same Schwarz vaccine strain sequence that was used to synthesize the rNP antigen.

The measles virus N gene was amplified from oral fluid 23 and identified as a Schwarz vaccine strain during the routine surveillance testing performed before this study, but it was not detected in the oral fluid during this evaluation. Although the same vaccine strain was identified from the corresponding POCT strip, it was not possible to determine whether this originated from the oral fluid specimen or from the added rNP.

POCT results for this small panel of oral fluids were highly concordant with the results of measles-specific IgM EIA, as were the results obtained with the larger panel of oral fluids. Measles-specific IgM was not detected by POCT in only one EIA-positive oral fluid, specimen 19 (Table 4). In this case, measles was confirmed by PCR.

Stability of measles nucleic acid on POCT strips

The measles virus H gene was amplified from 16 POCT strips stored at 20–25 °C for 5 weeks. This included all 15 specimens in which the H gene was amplified from the first POCT strip as well as specimen 20, in which the H gene was not detected initially. The measles virus N gene was amplified from 12 of these 16 POCT strips after 5 weeks of storage. The nucleotide sequences obtained from 11 of these stored strips were identical to those identified from the original POCT strips. Measles viral genotype A, consistent with the nucleic acid sequence present in the rNP antigen, was identified from the stored POCT strip of specimen 20; viral genotype D4 had been previously identified in this specimen.

Discussion

Sensitive and specific tests for measles diagnosis that can be used in low-resource settings can greatly enhance surveillance. Field-based tests would facilitate the diagnosis of measles in patients with a rash illness accompanied by fever, as well as the rapid implementation of control measures. With these objectives in mind we developed a rapid POCT for the detection of measles-specific IgM based on the principle of immunochromatographic flow. In this study, the POCT showed good sensitivity, specificity and positive and negative predictive values (> 88%) and is therefore suitable for field use. However, the potential role of POCT in measles surveillance will remain unknown until wider field-based evaluations are conducted in a range of settings, including those where control activities are in the elimination phase.

An added advantage of the POCT, apart from its ability to detect measles-specific IgM, is that the test strip is an excellent matrix for capturing viral nucleic acid from oral fluid samples and preserving it for up to 5 weeks at ambient temperature. This not only makes it possible to diagnose measles cases immediately, but also permits subsequent molecular characterization of the virus at national laboratories to track transmission pathways and confirm the diagnostic accuracy of the POCT in the field.

Table 4. **Results of polymerase chain reaction (PCR) and IgM antibody testing obtained for 24 oral fluid (OF) specimens used to compare viral nucleic acid recovery from OF aliquots and from used point-of-care test (POCT) strips**

SN	Real-time H-gene PCR result and viral strain						IgM antibody		
	OF aliquot		POCT strip		Surveillance		POCT	OF EIA	
	H (Ct)	Strain	H (Ct)	Strain	H (Ct)	Strain		Result	T/CO[a]
1	36.43	D4-a	35.88	D4-a	34.58	D4-a	Pos	Pos	19.57
2	30.72	D4-b	29.48	D4-b	30.37	D4-b	Pos	Pos	30.09
3	36.64	–	ND	A	ND	NT	Neg	Neg	0.41
4	ND	–	ND	A	ND	NT	Neg	Neg	0.57
5	25.16	D4-c	27.62	D4-c	21.07	D4-c	Pos	Pos	5.54
6	30.69	D9	32.24	D9	30.08	D9	Pos	Pos	17.52
7	34.89	D4-c	35.01	D4-c	31.57	D4-c	Pos	Pos	25.40
8	34.23	D4-c	35.12	D4-c	30.29	D4-c	Pos	Pos	23.63
9	ND	–	ND	A	ND	NT	Pos	Pos	1.22
10	33.20	D4-c	33.28	D4-c	30.34	D4-c	Pos	Pos	8.32
11	32.40	D4-d	33.53	D4-d	30.29	D4-d	Pos	Pos	19.57
12	26.85	D4-d	27.91	D4-d	23.21	D4-d	Pos	Pos	1.09
13	ND	–	ND	–	ND	NT	Pos	Eqv	0.99
14	ND	–	ND	–	ND	NT	Neg	Neg	0.58
15	ND	–	ND	–	ND	NT	Neg	Neg	0.76
16	30.91	D4-c	28.26	D4-c	23.09	D4-c	Pos	Pos	9.74
17	31.88	D4-c	31.76	D4-c	30.02	D4-c	Pos	Pos	10.78
18	ND	–	ND	A	ND	NT	Neg	Neg	0.72
19	32.51	D4-b	33.32	D4-b	30.82	D4-b	Neg	Pos	27.74
20	ND	D4-c	ND	D4-c	37.50	D4-c	Pos	Pos	19.04
21	31.23	D4-c	31.86	D4-c	32.34	D4-c	Pos	Pos	15.67
22	34.07	D4-e	33.99	D4-e	33.16	D4-e	Pos	Pos	17.14
23	37.32	–	34.34	A	37.41	Vaccine	Pos	Pos	23.25
24	ND	–	ND	–	ND	NT	Pos	Pos	1.11

Ct, threshold cycle number; Eqv, equivocal; H, haemagglutinin; ND, not detected; Neg, negative; NT, not tested; Pos, positive; SN, specimen number.

[a] This represents the quotient calculated by dividing the optical density obtained for each test specimen (450/620 nm) by the cut-off value.

Note: The Ct obtained for the OF aliquots submitted for this evaluation, from POCT strips used in IgM antibody detection and from each oral fluid during routine surveillance testing, are listed. During routine surveillance testing, nested N-gene PCR and nucleic acid sequence analysis were only performed on oral fluids in which the H gene had been detected by real-time PCR. For this evaluation, real-time H-gene PCR, nested nucleocapsid-gene (N-gene) PCR and sequence analysis were performed on all 24 OF aliquots and their corresponding POCT strips. The measles virus strain designations listed are based on sequence analysis of nested PCR N-gene amplicons. Five different D4 measles virus strains were identified and each was assigned a different letter in the table: a, b, c, d, e. Strains b, c and d were obtained from more than one subject. Nested N-gene PCR negative results are indicated as –-.

The results obtained with the larger panel of oral fluids suggest that IgM detection by POCT improves as the concentration of IgM antibody increases, since all specimens with an EIA T/CO greater than 5 were identified (Table 3). However, one oral fluid specimen, number 19 in Table 4, was highly reactive in the measles-specific IgM capture EIA but was negative by POCT. That oral fluid was also found to have a high concentration of measles-specific IgG by EIA – a T/CO of 62 – but remained IgM-negative by POCT after IgG was absorbed from the sample using protein A sepharose (data not shown). On dilution of the sample one part in three and one part in six, a positive IgM result was obtained in the POCT. The total measles-specific antibody concentration in this sample may have been so high that it bound the entire rNP added, leaving no sites available for the MAb-gold conjugate to bind and form a complex that could be captured at the anti-human IgM antibody line in the POCT. Other oral fluids with high IgM and IgG EIA T/CO values were positive by POCT. This suggests that the failure to detect IgM in specimen 19 may not be simply due to a high concentration of antibody. Alternatively, some oral fluids may contain inhibitors that require dilution to enable complex formation with both specific antibody and the MAb-gold conjugate to occur. The antibody-negative result did not preclude the diagnosis of measles, since H and N genes were amplified from the POCT strips used for oral fluid testing.

The presence of trace levels of measles N-gene sequences from the rNP used in the POCT could have interfered with the amplification of the N-gene target in the oral fluids tested by POCT, but in most cases it did not. We surmise that the viral nucleic acid sequences in oral fluids from acute measles cases were present in greater quantity and were amplified preferentially from the POCT strips. This was confirmed by the identification of the same genotype that was found in the oral fluid aliquots. The possibility of removing the contaminating nucleic acid in the rNP by deoxyribonuclease and ribonuclease treatment is currently being investigated. However, the amplification of the Schwarz vaccine strain N-gene sequence from the rNP is not likely to seriously impair the identification of current wild-type measles strains, as genotype A viruses have not circulated widely in the past 20 years and the few sporadic cases of infection with these viruses have been frequently associated with recent vaccination.[20]

In its present configuration the POCT relies on some basic laboratory equipment. A robust field-based test would require further development of methods for extracting oral fluids from swabs without centrifugation, the use of disposable, volumetric pipettes or loops to deliver accurate volumes, and a plastic cassette to enclose the test strip and reduce the handling of the POCT's analytical membrane. The estimated cost of manufacturing one POCT is approximately one United States dollar. ■

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ملخص

اختبار نقطة الرعاية لتشخيص الحصبة: اكتشاف الأضداد M الخاصة بالحصبة والحمض النووي الفيروسي

الغرض تقييم قدرة اختبار نقطة الرعاية، الذي جرى تطويره حديثاً، على اكتشاف الأضداد M الخاصة بالحصبة في عينات المصل والسائل الفموي، وقياس إمكانية اكتشاف الحمض النووي الفيروسي من الشرائط المستخدمة في اختبار نقطة الرعاية.

الطريقة استخدم اختبار نقطة الرعاية لاختبار 170 عينة من المصل تم جمعها من خلال ترصد الحصبة أو برامج التحصين في أثيوبيا، وماليزيا، والاتحاد الروسي: وكان هناك 69 عينة إيجابية للغلوبولين المناعي الخاص بالحصبة، و74 عينة إيجابية للأضداد M الخاصة بالحصبة الألمانية، و7 عينات إيجابية لكل منهما. كما جرى اختبار 282 عينة من السائل الفموي مأخوذة من برنامج ترصد الحصبة، والنكاف، والحصبة الألمانية في المملكة المتحدة لبريطانيا العظمة وشمال أيرلندا. واعتبرت المقايسة المناعية لإنزيم التقاط الغلوبولين المناعي M هي المعيار الذهبي للمقارنة. وجرى تقصي 24 عينة للسائل الفموي للتعرف على إمكانية تضخيم التفاعل السلسلي للبوليميراز مباشرة من الشرائط المستخدمة في اختبار نقطة الرعاية.

النتائج أظهر الاختبار المصلي المجرى عند نقطة الرعاية حساسية قدرها 90.8% (76/69) ونوعية قدرها 93.6% (94/88)؛ ومع السوائل الفموية كانت الحساسية 90.0% (70/63) والنوعية 96.2% (208/200). وقد اكتشف كل من جين H وجين N على نحو يوثق في مصدوقيته في شرائط اختبار نقطة الرعاية، وكان بالإمكان تصنيف الجينات ومتابعتها. وأمكن اكتشاف جينات فيروس الحصبة في الشرائط المستخدمة في اختبار نقطة الرعاية بعد حفظها لمدة 5 أسابيع في درجة حرارة 20-25 درجة مئوية.

الاستنتاج يوجد لدى اختبار نقطة الرعاية الحساسية والنوعية المطلوبين لاعتباره اختباراً ميدانياً لتشخيص الحصبة. إلا أن دوره في البرامج العالمية لمكافحة الحصبة يحتاج إلى المزيد من التقييم.

摘要

麻疹诊断的床旁检测：麻疹特异性免疫球蛋白M抗体和病毒核酸检测

目的 旨在评价新开发的用于血清和唾液标本中麻疹特异性免疫球蛋白M抗体检测的床旁检测（POCT）的绩效，并评估麻疹病毒核酸是否可从使用过的床旁检测测试条中回收。

方法 用床旁检测来测试通过埃塞俄比亚、马来西亚和俄罗斯联邦的麻疹监测或疫苗接种计划收集的170个血清标本：其中69个标本的麻疹免疫球蛋白M(IgM)抗体呈阳性，74个标本的风疹免疫球蛋白M抗体呈阳性，7个标本的两种免疫球蛋白M抗体均呈阳性。另外还测试了从英分析麻疹病毒血凝素（H）和核蛋白（N）基因是否可以直接检出。**结果** 对于血清，床旁检测分别显示出90.8%（69/76）和93.6%（88/94）的敏感性和特异性；而对于唾液，敏感性和特异性则分别为90.0%（63/70）和96.2%（200/208）。

从床旁检测测试条中能可靠检测到H和N基因，并且对N基因可进行基因测序然后进行基因型分析。麻疹病毒基因在20-25°C条件下储存5周之后可从床旁检测测试条中检出。