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Development of a measles specific IgM ELISA for use with serum and oral fluid samples using recombinant measles nucleoprotein produced in *Saccharomyces cerevisiae*

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Abstract

In order to develop sensitive assays for detecting measles antibodies in oral fluid specimens, we have produced recombinant measles virus nucleoprotein (rMVN) in a yeast expression system and prepared monoclonal antibodies to the protein. Measles nucleoprotein gene from the Schwarz vaccine strain was cloned into a yeast expression vector, pFX7 under the control of the hybrid GAL10-PYK1 promoter. High levels of rMVN (20 mg/litre of yeast culture) were generated. Electron microscopy showed that the purified rMVN assembled into typical herring-bone structures. Monoclonal antibodies produced to the rMVN also reacted with native measles virus N in immunofluorescence tests. The purified rMVN and a monoclonal antibody to the rMVN conjugated to horseradish peroxidase were used to develop a measles specific IgM capture EIA (MACEIA) in both serum and oral fluid specimens. Evaluations of the MACEIA were performed by testing a) serum samples ($n = 80$) and b) paired oral fluid/serum samples from measles cases ($n = 50$, representing 16 cases) and oral fluids from controls with non-measles rash ($n = 59$, representing 48 cases). The samples were also tested for measles IgM, using a reference radioimmunoassay (MACRIA). The sensitivity and specificity of the MACEIA compared with MACRIA for a) the serum samples were 100 and 96.6% respectively and b) for paired serum/oral fluids samples 100 and 100%, respectively.

Keywords: Measles; ELISA; rMVN; Oral fluid

1. Introduction

Measles is a severe vaccine preventable disease causing extensive morbidity and mortality in large

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parts of the world. Measles virus (MV) is transmitted from person to person by respiratory droplets and there is no known animal reservoir. Measles transmission can be interrupted by immunisation. Despite the widespread use of measles vaccine, however, either as a single antigen vaccine or as a component of the triple vaccine against measles, mumps and rubella (MMR), there are still an estimated 30–40 million reported measles cases and 777 000 deaths per year globally (World Health Organisation Report, 2002). In 1997 there was an estimated 151 000 measles cases and 6500 deaths in the European Region (World Health Organisation, 1998). The World Health Organisation (WHO) has targeted the elimination of measles in Europe by the year 2007 as part of a programme for the global eradication of the disease. The strategy to achieve this aim is based on political commitment to achieve and maintain high vaccination coverage. A key component of the plan is surveillance to monitor progress.

Surveillance based on the clinical diagnosis of measles is unreliable in countries where measles incidence is low. For example, in the UK it has been shown that only a small proportion of clinically diagnosed cases can be confirmed by laboratory testing (Ramsay et al., 1997). Thus laboratory testing to confirm infection is essential for measles surveillance once the control phase of measles elimination is established.

Effective surveillance requires high compliance amongst subjects being tested. Experience in the UK has shown that the use of a non-invasive specimen, such as oral fluid, to confirm infection results in high compliance rates. Oral fluid has a number of advantages over serum/plasma for diagnosis. Oral fluid is easy to collect, store and transport. Collection requires very little skill and can be performed by older children or their parents. Oral fluid can be collected at home and in rural settings, and shipped by post for laboratory analyses. Oral fluid collection is cheap, non-invasive and free from risks of exposure to HIV and hepatitis associated with use of needles for collection of blood samples. Compliance amongst patients of all age groups is high since collection of oral fluid samples is painless.

A disadvantage of using oral fluid for measles testing is that commercial tests optimised for this specimen are not available. Tests using oral fluid require high sensitivity since antibodies in this specimen are at much lower concentrations than in serum (Parry et al., 1987). Measles IgM capture radioimmunoassay (MACRIA) has been demonstrated to detect IgM in oral fluid specimens from clinically diagnosed and seropositive confirmed cases (Perry et al., 1993). This test, however, is not easily transferred to other laboratories. In this report we describe the development of a simple measles IgM capture enzyme immunoassay (MACEIA) which employs a recombinant measles virus nucleoprotein (rMVN) and monoclonal anti-measles N antibody-enzyme conjugate. Our initial evaluations suggest that the MACEIA test will be a suitable replacement for measles MACRIA and can be transferred easily to other laboratories.

2. Materials and methods

2.1. Cloning of measles N gene

Apart from the primers used, the cloning strategy was essentially identical to that used for cloning mumps virus N gene (Samuel et al., 2002). The MVN gene was amplified by PCR from cDNA prepared after extraction and reverse transcription of reconstituted Priorix vaccine containing the measles Schwarz strain (SmithKline Beecham Pharmaceuticals, UK). The primers used in the amplification of the MVN gene included a *SPe*I site for subcloning into the yeast vector pFX7, a single ATG codon in the forward primer and a stop TAA codon in the reverse primer. The primer sequences including the *Spe*I sites (in bold) and the start and stop codons (underlined) are shown below.

Reverse (5′→3′) TT **ACT AGT** TTA GTC TAG AAG ATT TCT GTC ATT GTA CAC

Forward (5′→3′) TT **ACT AGT** ACA ATG GCC ACA CTT TTA AGG AGC TT

PCR conditions were as described previously (Jin et al., 1996) and amplified products were separated on a 2% Agarose (Seakem) gel. DNA fragments corresponding to the N gene were

excised, gel-purified and cloned into pCR® 2.1 TOPO® as described in the manufacturer's instructions (Invitrogen, NL). PCR and DNA sequencing confirmed the insertion of the MVN gene sequence in plasmids. Cloning of the amplified MVN gene into the yeast *Saccharomyces cerevisiae* vector was performed as described for cloning of mumps N (Samuel et al., 2002). The resulting plasmid pFX7–MVN was used for transformation of yeast *S. cerevisiae* strain AH22 (*leu2 his4*), the MVN gene expressed and the resulting measles nucleocapsid-like particles purified as described for recombinant mumps virus nucleocapsid-like particles (Samuel et al., 2002)

2.2. SDS-PAGE and Western blotting

Samples were analysed on Novex 10% PAGE gels (Invitrogen, NL) under reducing conditions in SDS–Tris–glycine buffer (Laemmli, 1970). The proteins were transferred to nitrocellulose electrophoretically (Towbin et al., 1979) and stained with human convalescent, measles IgG positive sera followed by an anti-human IgG HRP conjugate (Dako, UK). A monoclonal antibody to MV nucleoprotein conjugated to HRP (Chemicon, UK), derived from clone 83VIIKK2 (Bellini et al., 1986; Erdman et al., 1991), was also used. Immunostained proteins were identified using TMB blotting substrate (MBI Fermentas, Lithuania).

2.3. Electron microscopy

Suspensions of rMVN purified by ultracentrifugation were placed on 400-mesh carbon coated palladium grids. The samples were stained with 2% aqueous uranyl acetate solution and examined with a JEM-100S electron microscope.

2.4. Monoclonal antibodies

BALB/c mice were immunised with 50 ug of CsCl purified rMVN in Freund's complete adjuvant and boosted twice on day 30 and 60 after primary immunisation. Spleen cells from immunised mice were fused with mouse myeloma cells NS0/1 cells and hybridomas generated by standard techniques (Kohler and Milstein, 1975). Hybridoma cells secreting antibodies to the rMVN were identified by ELISA using rMVN coated microwells and cloned by limiting dilution. The monoclonal antibodies (MABs) were isotyped using the Monoclonal Antibody Isotyping kit, ISO-2 from Sigma.

2.5. Anti-Measles NP–HRP conjugate

IgG antibody from one clone, 12B6 was purified on a protein A column and coupled to horseradish peroxidase (HRP) by the method of Wilson and Nakane (1978).

2.6. Immunofluorescence

Confluent cultures of Vero cells were grown on cover slips in 24-well tissue culture plates and inoculated with three wild-type measles virus strains including the Loss strain (Sinitsyna et al., 1990) genotype A and strains 95/43708 (genotype D4) and 95/43709 (genotype D6) isolated at the Central Public Health Laboratory, UK. Three to 4 days post inoculation, the cells were fixed with cold acetone/PBS (80:20). Indirect immunofluorescence was carried out using MABs and goat anti-mouse IgG-FITC conjugate (Chemicon). The stained cover slips were mounted onto glass slides in mounting medium (Chemicon) and the fluorescence observed using an epifluorescence microscope (Zeiss, Germany) with filter sets for the detection of FITC fluorescence.

2.7. Measles specific IgM capture EIA (MACEIA)

Microtitre plates were coated with 100 μl per well of a 0.5 ug/ml solution of goat anti-human IgM (Jackson ImmunoResearch Laboratories Inc., US) in 0.05 M carbonate buffer, pH 9.6 and incubated for 18 h at 2–8 °C. The plates were transferred to 37 °C for 2 h and then brought to RT for 1 h. The plates were washed with PBS containing 0.05% (v/v) Tween-20 (PBS/Tw), dried and blocked with 300 μl per well of 5% Solupro (Dynagel, US) in water for 2 h at 37 °C. The solution was then aspirated and the plates dried

overnight at 37 °C and stored pouched with desiccant at 2–8 °C.

Test and control serum or plasma samples were diluted 1/200 in serum diluent (1% dried milk, Marvel, Cadbury, UK in PBS/Tw). Oral Fluid samples were used undiluted. The diluted serum/plasma or undiluted oral fluids (100 μl per well) was added to assigned wells of the anti-human IgM coated plates and incubated at 37 °C for 30 min. The wells were aspirated and washed four times with PBS/Tw using an automatic plate washer. rMVN was diluted to 0.75 μG/ml in 3% (w/v) BSA in PBS/Tw containing 1 M NaCl and 0.05% (w/v) Bronidox-L (Henkel, Germany) and 100 μl per well was added to the wells and incubated at 37 °C for 30 min. The wells were washed as above and 100 μl per well of diluted anti-measles N-HRP conjugate was added to the wells and incubated for a further 30 min at 37 °C. The wells were again washed and 100 μl per well of TMB substrate (Microimmune, UK) was added for 10 min at room temperature. The reaction was stopped by adding 100 μl per well of 0.5 M HCl. The optical densities at 450 nm and at a correction wavelength of 620 nm were read simultaneously within 5 min of stopping the reaction.

2.8. Clinical specimens

The MACEIA was evaluated using the following panels of specimens.

2.8.1. Serum Samples

Eighty serum samples previously tested by IgM capture radioimmunoassay, MACRIA and IgG capture radioimmunoassay, GACRIA (Perry et al., 1993) were investigated. This panel included 10 sera from the measles AccuPanel (Quest Laboratories, UK) and a panel of 40 sera distributed by WHO measles reference laboratories (VDRL, Melbourne, Australia and Laboratoire Nationale de Sante, Luxembourg) and 30 sera received at Central Public Health Laboratory, London, UK for routine measles testing. In addition 37 parvovirus B19 IgM positive specimens were tested to help assess assay specificity

2.8.2. Serum/oral fluid specimens

Serum and oral fluid specimens from 16 patients with measles, enrolled in a previously described clinic-based study of measles in Niteroi, Brazil (Oliveira et al., 1998) were investigated. Multiple samples ($n = 50$), comprising 23 sera and 27 oral fluids, were collected at varying times after onset of rash symptoms from this patient cohort. Specimens from control subjects, comprising 59 oral fluids collected from 48 patients with non-measles were investigated. The corresponding serum samples from these subjects were negative for measles, rubella, parvovirus B19 and dengue IgM (Oliveira et al., 1998). Sera from 27 of the 48 subjects were also tested for HHV6 and five had evidence of recent HHV6 infection (Oliveira et al., 2001). Oral fluid specimens were collected using the OraSure device (Epitope, US) according to the manufacturer's instructions.

3. Results

3.1. Expression of measles nucleocapsid like particles

Coomassie stained SDS-PAGE of *S. cerevisiae* cells harbouring pFX7–MVN, after induction with galactose revealed a major protein migrating with a molecular mass of approximately 60 kDa (Fig. 1, lanes A3, A4), consistent with the molecular weight of measles nucleoprotein reported in the literature (Spehner et al., 1991). Western blots using a measles IgG positive human convalescent serum immunostained proteins consistent with measles nucleoprotein in the galactose-induced pFX7–MVN preparation and in the CsCl purified fraction (Fig. 1, lanes B3, B4). Immunostaining was not observed with induced yeast cells harbouring the pFX7 plasmid without the MVN insert (Fig. 1, lane A1), or in the pFX7–MVN in glucose media (i.e. not induced, Fig. 1, lane A2). Monoclonal antibody, 12B6 produced in this study immunostained the 60 kDa protein consistent with nucleoprotein (Fig. 1, lanes B3, B4 and C3, C4). A MAB to the native measles virus nucleoprotein, 83VIIKK2 (Bellini et al., 1986; Erdman et al., 1991) failed to react with the yeast rMVN in

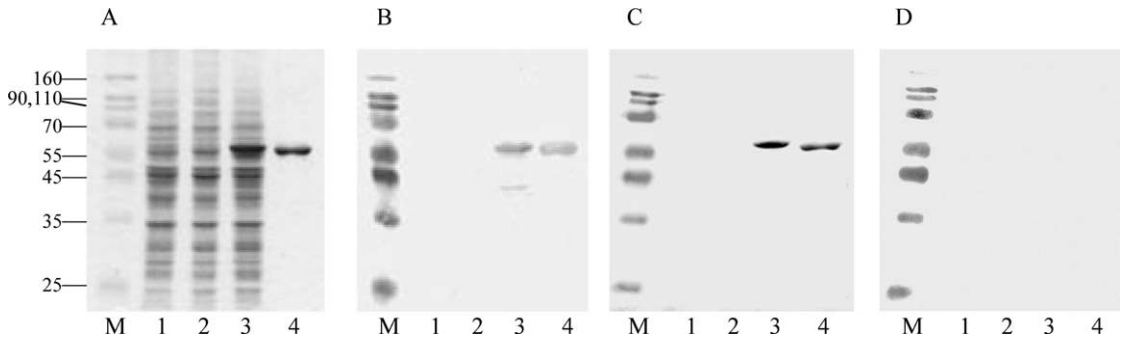

Fig. 1. SDS-PAGE of yeast lysates and CsCl centrifugation purified rMVN on a 12% acrylamide gel. (A) Coomasssie blue stained gel. (B) Immunoblot using human serum. (C) Immunoblot using Mab 12B6. (D) Immunoblot using Mab 83VIIKK2. Preparations loaded onto gels were as follows: Lane 1, *S. cerevisiae* [pFX7] in YEPG (induction) medium; Lane 2, *S. cerevisiae* [pFX7-MVN] in YEPD [growth] medium; Lane 3, *S. cerevisiae* [pFX7-MVN] in YEPG [induction] medium; Lane 4, CsCl purified measles N protein from *S. cerevisiae* [pFX7-MVN] and Lane M, pre-stained protein ladder (Fermentas AB, Vilnius) 160, 110, 90, 70, 55, 45, 35, 25, 15 and 10 kDa.

western blots (Fig. 1D), dot blots or in an indirect ELISA (data not shown).

3.2. Electron microscopy

Electron microscopy of the purified rMVN revealed structures with typical 'herring-bone' morphology: rods of $\sim$20 nm diameter with repeated seration along the edges and a central core of $\sim$5 nm of lengths varying from 20 to 1000 nm (not shown).

3.3. Yield and stability of particles

In several preparative procedures, the yield of the CsCl purified rMVN was between 18 and 22 mg/l of induced yeast cells. The rMVN was stable in CsCl at 4 °C. Treatment with 10 mM EDTA, 10 mM EGTA or 10 mM DTT did not cause dissociation of rMVN particles indicating the assembled structure does not require divalent ions or disulphide bonds for stabilisation of the structure. The particles were stable on freezing and lyophilisation.

3.4. Characterisation of monoclonal antibodies

Fifteen hybridoma cell lines producing MAbs to the yeast rMVN were generated. Eleven of these were of isotype IgG1 (including MAB secreted by clone 12B6), three were of isotype IgG2b and one was of isotype IgG2a. Thirteen of the 15 MABs immunostained rMVN in Western blots and all 15 MABs reacted specifically with rMVN and not recombinant mumps virus nucleoprotein in indirect ELISA (data not shown).

Two of the MABs tested (from clone 12B6 and 7C11) reacted with three wild type measles virus strains in immunofluorescence. The staining was localised mainly in the cytoplasm (not shown).

3.5. Evaluation of the MACEIA on serum and oral fluid specimens

3.5.1. Determination of cut-off values

3.5.1.1. For serum samples. Optical density (OD) values for 29 measles MACRIA negative serum samples, which included four rubella IgM and five mumps IgM positive serum samples, were used to determine the cut-off values for positive, negative and equivocal results in the MACEIA. The mean OD and standard deviation (S.D.) for this panel of measles IgM negative serum samples were 0.074 and 0.054, respectively. A negative control serum sample (NC), tested in triplicate in each run, was used to determine the cut-off values. A specimen giving an OD value $\geq 1.1 \times$ (mean NC + 0.15), i.e. mean + 3S.D., was defined as measles IgM positive, a specimen giving an OD $\leq 0.9 \times$ (mean NC + 0.15) was defined as measles IgM negative

and specimens giving an OD between these values were considered measles IgM equivocal.

3.5.1.2. For oral fluid samples. The cut-off values for the MACEIA on oral fluids was determined after testing 39 MACRIA negative oral fluids from subjects with non-measles rash and fever. The mean OD and S.D. for these specimens in the MACEIA were 0.027 and 0.011, respectively. A negative control (NC) serum sample was assayed in triplicate in each run. An oral fluid giving an OD $\geq$ mean NC + 0.02 in the MACEIA was defined as measles IgM positive.

3.6. Serum panel

Of the 80 serum specimens tested, 27 were positive, 51 negative and two equivocal by MACRIA.

Twenty-three of 27 MACRIA positive serum samples were positive in the MACEIA (Table 1). Two of the MACRIA positive/MACEIA equivocal samples were from the AccuPanel, one a rubella IgM positive serum specimen and the other a rheumatoid factor positive specimen. Both these specimens were negative in two commercial indirect EIA for measles IgM (Behring, Germany and Gull Laboratories, US). Two other MACRIA positive and MACEIA negative samples were from the WHO panels, one was equivocal in the Behring test and negative in the Gull test and the other negative in both commercial measles IgM tests and are therefore likely to represent MACRIA false positive results.

Forty-nine of the 51 MACRIA negative specimens were negative by the MACEIA. The two specimens giving discordant results were equivocal in the MACEIA and negative in the both commercial measles IgM tests. Thirty-six out of 37 parvovirus B19 serum specimens were negative in the IgM capture EIA. The one sample giving a positive result in the MACEIA was tested in the Behring measles IgM EIA. The sample tested negative, but gave a high OD reading in the control antigen well. This sample was re-tested in the MACEIA with and without the addition of rMVN antigen. The minus antigen test gave a high OD reading indicating a false positive reaction in the MACEIA.

Excluding the four cases where the MACRIA may represent a false positive, the sensitivity of the MACEIA compared with MACRIA was 100% (23/23; 95% CI 85.18–100%). The specificity of the MACEIA compared with MACRIA was 96.1% (49/51) or 96.6% (85/88; 95% CI 90.67–99.31%) including the parvovirus B19 specimens.

Table 1
Detection of measles specific IgM in serum samples by Maceia and Macria

Macria	Maceia			
	Pos	Neg	Eqv	Total
Pos	23	2	2	27
Neg	0	49	2	51
Eqv	0	2	0	2
Total	23	53	4	80

3.7. Serum/oral fluid panel

Of the 16 subjects from a clinic-based study in Niterói, Brazil from whom both serum and oral fluid specimens were available, 15 patients had detectable IgM in serum by both MACRIA and the MACEIA (Fig. 2). In all the IgM seropositive subjects, IgM was detected in the oral fluids by both assays. In three subjects serum was taken on the first day of rash and was IgM positive. For two of these three subjects an oral fluid specimen was also available on the first day of rash and IgM was detected by both MACRIA and MACEIA. IgM was not detected by either MACRIA or MACEIA in serum or oral fluid specimens collected 2 days after onset of rash from one subject (patient 1 in Fig. 2). The oral fluid from this subject was positive by PCR and the paired serum was also negative by a commercial measles IgM EIA (Dade Behring, UK). In the limited number of samples tested, the MACEIA for oral fluid was 100% sensitive compared with serum IgM results.

IgM was not detected in the MACEIA and MACRIA in 59 oral fluids from the 48 patients with non-measles rash and fever. The samples were taken between one and 28 days post onset of symptoms from patients ranging in age from 1

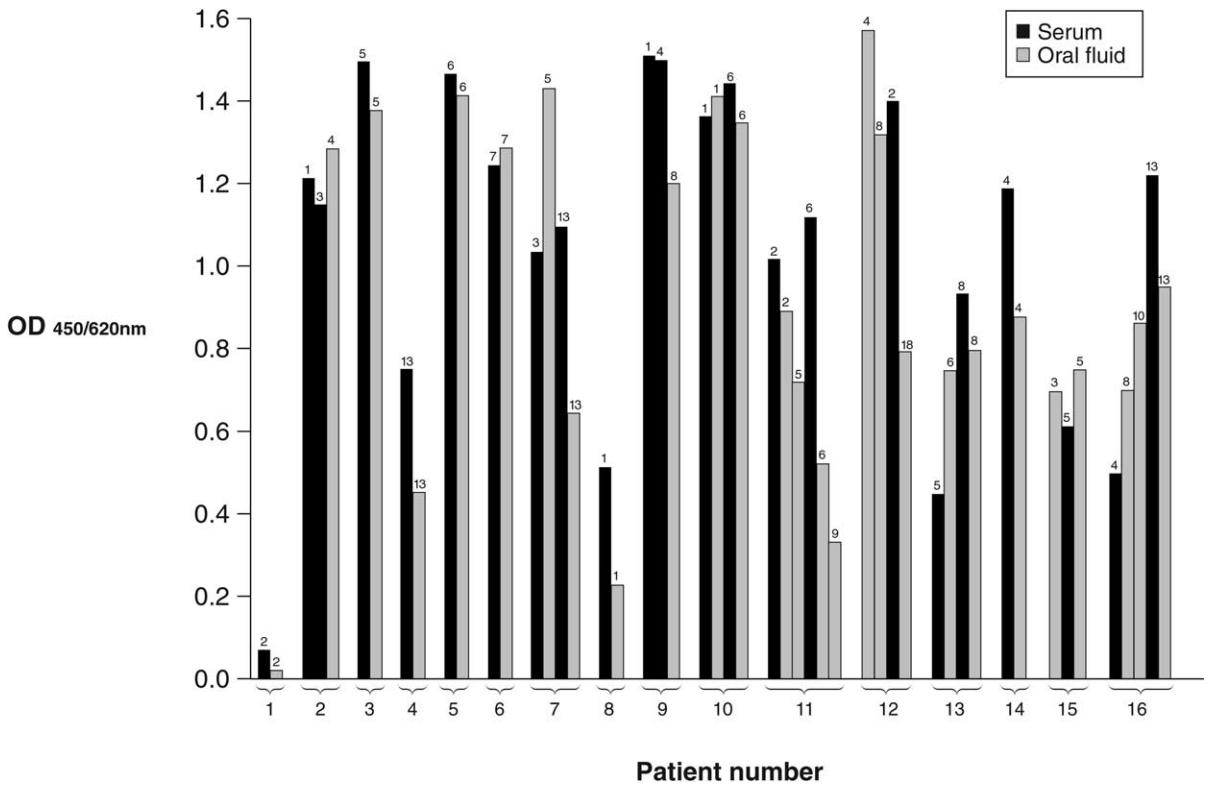

Fig. 2. Optical densities obtained in the MACEIA for the serum/oral fluid pairs from a measles study in Niteroi, Brazil (Oliveira et al., 1998). For each patient, the days post onset of rash symptoms is indicated above the histogram bar. A minimum of one oral fluid and one serum sample was available from each patient. Multiple samples were available for some patients.

month to 43 years. Thus in this limited set of samples, the specificity of the MACEIA with respect to serum results was 100% (95% CI 93.84–100%).

4. Discussion

The assembly of yeast derived rMVN into nucleocapsid-like particles was analysed by electron microscopy and density-gradient centrifugation. The morphological appearance in electron microscopy of rMVN expressed in yeast was similar to the nucleocapsid in native measles virus infected cells (Waters et al., 1972) and to rMVN expressed in other expression systems such as insect cells (Hummel et al. 1992; Fooks et al., 1993), mammalian cells (Spehner et al., 1991; Fooks et al., 1995; Warnes et al., 1994) and bacteria (Warnes et al., 1995). To the best of our knowledge this is the first report of expression of measles nucleoprotein as nucleocapsid-like particles in a yeast expression system. As observed in other expression systems, the measles nucleocapsids assembled without the assistance of other measles gene products and if cellular proteins are involved in the assembly, they appear to be conserved and found in both bacteria and eukaryotic cells. Yeast derived nucleocapsid-like particles had a buoyant density in cesium chloride of ~ 1.31 g/ml, a value also reported for native nucleocapsids from measles virus particles (Thorne and Dermott, 1976; Robbins et al., 1980) and for nucleocapsids from vaccinia virus recombinants containing the measles virus nucleoprotein gene grown in mammalian cells (Spehner et al., 1991).

Antigenic similarity of rMVN and native MVN was demonstrated since MABs to the rMVN also

reacted with N protein from wild-type measles virus strains in immunofluorescence assays. The genotypes of two of these virus strains (D2 and D6) were different from the Schwarz vaccine strain (genotype A) used for recombinant NP construction. Although the greatest diversity in measles strains is in the nucleocapsid gene sequence (Parks et al., 2001), it is noteworthy that the expressed rMVN contains sufficient conserved epitopes that are immunogenic and recognised by both immune human sera and MABs. This property has enabled the development of a measles MACEIA.

The measles MACEIA developed here was evaluated against the MACRIA routinely used for measles surveillance by oral fluid testing in the UK (Ramsay et al., 1997). The two assays were also evaluated with serum samples where discordant results could be further investigated using other commercially available tests. This demonstrated that for the serum panel, the MACEIA had high sensitivity and specificity (100 and 96.6%). In the limited evaluation with a small set of serum/oral fluid paired specimens from clinically diagnosed measles cases and controls, the MACEIA for oral fluids was 100% sensitive and specific compared with serum IgM results. Although the preliminary data presented in this report suggests that MACEIA on oral fluids is a suitable method for investigating clinically notified measles cases, evaluation with a larger number of paired serum/oral specimens from measles and control cases is needed to establish the true performance of the test.

MACEIA has obvious advantages over MACRIA for testing of oral fluids. It requires a smaller specimen volume than MACRIA, 100 compared with 200 ul, which is important for oral fluids which are tested without dilution. It is faster, taking approximately 2 h to complete the test compared with approximately 24 h for MACRIA. It avoids the use of radioisotopes making the test easy to automate and transfer to other laboratories.

In summary, the use of highly purified rMVN has allowed us to develop a simple MACEIA for measles IgM in serum and oral fluid. Our preliminary work indicates the sensitivity and specificity of the test is sufficiently high for it to be used in the surveillance of measles using oral fluid specimens. The availability of an easily transferable test for oral fluid specimens rather than serum will improve surveillance of measles and contribute to the global eradication programme.

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References

Bellini W, Englund G, Rammohan K, McFarlin DE. Evaluation of the structural proteins of the hamster neurotropic strain of measles virus with monoclonal antibodies. J Neuroimmunol 1986;11:149–63.

Erdman DD, Anderson LJ, Adams DR, Stewart JA, Markowitz LE, Bellini WJ. Evaluation of monoclonal antibody-based capture enzyme immunoassay for detection of specific antibodies to measles. J Clin Microbiol 1991;29:1466–71.

Fooks AR, Stephenson JR, Warnes A, Dowsett AB, Rima BK, Wilkinson GWG. Measles virus nucleocapsid protein expressed in insect cells assemble into nucleocapsid like structures. J Gen Virol 1993;74:1439–44.

Fooks AR, Schadeck E, Liebert UG, Dowsett AB, Rima BK, Steward M, Stephenson JR, Wilkinson GWG. High level expression of measles virus nucleocapsid protein by using replication-deficient adenovirus vector: Induction of an MHC-1-restricted CTL response and protection in a murine model. Virology 1995;210:456–65.

Hummel KB, Erdman DD, Heath J, Bellini WJ. Baculovirus expression of the nucleoprotein gene of measles virus and utility of the recombinant protein in diagnostic enzyme immunoassay. J Clin Microbiol 1992;30:2874–80.

Jin L, Richards A, Brown DWG. Development of a dual target-PCR for detection and characterization of measles virus in clinical specimens. Mol Cell Probes 1996;10:191–200.

Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–7.

Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 1970;227:680–5.

Oliveira SA, Siqueira MM, Brown DWG, Camacho LAB, Faillace T, Cohen BJ. Salivary diagnosis of measles for surveillance: a clinic-based study in Niterói, State of Rio de Janeiro, Brazil. Trans R Soc Med Hyg 1998;92:636–8.

Oliveira SA, Siqueira MM, Camacho LAB, Nogueira RM, Spinetti CCJ, Cubel Garcia RCN, Brown DWG. The

aetiology of maculopapular rash disease in Niterói, State of Rio de Janeiro, Brazil: implications for measles surveillance. Epidemiol Infect 2001;127:509–16.

Parks CL, Lerch RA, Walpita P, Wang H-P, Sidhu MS, Udem SA. Comparison of predicted amino acid sequences of measles virus strains in Edmonston vaccine lineage. J Virol 2001;75:910–20.

Parry JV, Perry KR, Mortimer PP. Sensitive assays for viral antibodies in saliva: an alternative to tests on serum. Lancet 1987;2:72–5.

Perry KR, Brown WG, Parry JV, Panday S, Pipkin C, Richards A. Detection of measles, mumps and rubella antibodies in saliva using antibody capture radioimmunoassay. J Med Virol 1993;40:235–40.

Ramsay M, Brugha R, Brown D. Surveillance of measles in England and Wales: implications of a saliva testing programme. Bull WHO 1997;75:515–21.

Robbins SJ, Bussel RH, Rapp F. Isolation and partial characterisation of two forms of cytoplasmic nucleocapsids from measles virus infected cells. J Gen Virol 1980;4:301–10.

Samuel D, Sasnauskas K, Jin L, Beard S, Zvirbliene A, Gedvilaite A, Cohen B. High level expression of recombinant mumps nucleoprotein in *Saccharomyces cerevisiae* and its evaluation in mumps IgM serology. J Med Virol 2002;66:123–30.

Sinitsyna OA, Khudaverdyan OE, Steinberg LL, Nagieva FG, Lotte VD, Dorofeeva LV, Rozina EE, Boriskin YS. Further-attenuated measles vaccine: virus passages affect viral surface protein expression, immunogenicity and histopathology pattern in vivo. Res Virol 1990;141:517–31.

Spehner D, Kirn A, Drillien R. Assembly of nucleocapsid-like structures in animal cells infected with vaccinia virus recombinant encoding the measles virus protein. J Virol 1991;65:6296–300.

Thorne HV, Dermott E. Circular and elongated forms of measles virus nucleocapsid. Nature (London) 1976;264:473–4.

Towbin H, Staehelin T, Gordan J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4.

Warnes A, Fooks AR, Stephenson JR. Production of measles nuceoprotein in different expression systems and its use as a diagnostic reagent. J Virol Methods 1994;49:257–68.

Warnes A, Fooks AR, Dowsett AB, Wilkinson GW, Stephenson JR. Expression of the measles virus nucleoprotein gene in Escherichia *coli* and assembly of nucleocapsid-like structures. Gene 1995;160:173–8.

Waters DJ, Hersh RT, Bussel RH. Isolation and characterization of measles nucleocapsid from infected cells. Virology 1972;48:278–81.

Wilson MB, Nakane PK. Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies. In: Knaapp W, Holubar K, Wick G, editors. Immunofluorescence and Related Techniques. Amsterdam: Elsevier, 1978:P215–44.

World Health Organisation, 1998. Progress towards global measles control and regional elimination, MMWR, 47, 1049–1054.

World Health Organisation Report, 2002. Global measles mortality reduction and regional elimination, 2000-2001. WER., 77, 50–55.