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REVIEW

Role of oral-fluid based measles diagnostic methods for measles global elimination

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Abstract

Measles eradication is biologically feasible. There is an availability of a safe, effective and inexpensive vaccine; a proven elimination strategy; high Local demand; and an effective global partnership and initiative to support vaccination. Measles eradication is a cost-effective scenario and a good investment to avoid expensive epidemics and save those children die due to measles. Laboratory investigations are indispensable to monitor the progress of measles elimination. This role will require the development of more sensitive diagnostic methods suitable for diagnosis and surveillance, genetic analysis of measles strains and a technology which is transferable worldwide. Measles diagnosis relies increasingly on serological tests. The practical utility of oral-fluid methods (antibody and genetic) in evaluating and refining measles immunization programs would, additionally, provide support for a global surveillance initiative. The utility of in a population survey, in a vaccine sero-conversion study and application in molecular epidemiological use is demonstrated in this review. It is to be hoped that this review will assist in the wider uptake and acceptance of methodology in both developed and developing country situation. More research needed for further evaluation of a recently developed point-of-care test for measles diagnosis: detection of measles-specific IgM antibodies and viral nucleic acid for wider use oral-fluid methodology. There is a strong case and imperative for the promotion of methods by World Health Organization in its global program of control/eradication of measles over the coming decade.

Key words: Measles elimination; Oral-fluid test methods; Laboratory diagnosis

Core tip: Laboratory investigations play a critical role in monitoring the success of measles elimination strategies. The role requires the development of more sensitive diagnostic which is transferable worldwide. Measles diagnosis relies increasingly on serological tests. Promotion of the use of oral fluid as viral diagnostic alternative to serum may be of advantage in communities where reliable age-specific notification and vaccination data are unavailable or in groups that are "hard to reach". This review will assist in the wider uptake and acceptance of oral-fluid methodology in both developed and developing country situation for global measles elimination.

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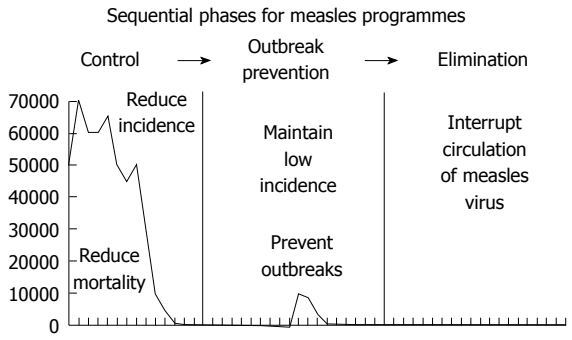

Figure 1 Phases for measles control/eradication programmes.

PHASES OF MEASLES CONTROL AND ELIMINATION

Measles is a highly contagious disease caused by a virus. It is one of the leading causes of death among young children. In 1980, before widespread vaccination, measles caused an estimated 2.6 million deaths each year. It remains one of the leading causes of death globally, despite the availability of a safe and effective vaccine. It is estimated that approximately 158000 people died from measles in 2011, mostly children under the age of five[1].

Based on implementation of a combination of vaccination and surveillance strategies, countries are considered to be in 1 of 3 stages: control, outbreak prevention, or elimination[2,3] (Figure 1).

MEASLES CONTROL

Control is defined as the reduction of disease incidence and/or prevalence to an acceptable level as a result of deliberate efforts, requiring continued interruption measures. In the control stage, the objective is to achieve high routine coverage with 1 dose of measles vaccine among infants to reduce measles morbidity and mortality. To accelerate measles control in large urban and other high-risk areas with a substantial proportion of unvaccinated children and measles associated deaths, mass vaccination campaigns targeting children aged 9 mo to 3-14 years have been recommended[4,5]. Countries in all regions have committed to the mortality reduction goal. Global measles deaths are decreased by 78% between 2000 and 2008, averting an estimated 4.3 million deaths[6,7]. The Southeast Asia region is already exceeding 90% measles mortality reduction[8].

MEASLES OUTBREAK PREVENTION

Measles outbreak prevention aims to maintain low incidence and prevent outbreaks by the administration of supplemental doses of measles vaccine through mass vaccination campaigns. As programmes plan for elimination of measles, a high coverage of single dose vaccine with supplementary immunization is assumed be sufficient to interrupt transmission[9]. A second dose is required to eliminate susceptibles from the population and interrupt measles transmission[10]. The Africa region is adopting to raise routine vaccine coverage to at least 80% and using supplemental campaigns in all non-polio-reservoir countries by 2003[11]. The Western Pacific Region from 1996 to 2009, 235 million persons received measles vaccine during 94 immunization campaigns in 30 countries and areas[12]. In the same region during 2009, 32 countries and areas provided 2 routine doses of measles vaccine[12]. The steady increase in routine measles coverage is shown from 71% to 82% globally between 2000 and 2009[8]. Between 2000 and 2008, administration of more than 600 million doses of measles vaccine in mass vaccination campaigns were made globally[7,13].

MEASLES ELIMINATION

Elimination is defined as the reduction of endemic incidence of a disease to zero as a result of deliberate efforts, requiring continued control measures. An alternative approach to documenting measles elimination are molecular evidence to confirm the lack of a circulating endemic genotype for at least one year and maintenance of 95% coverage of one dose of measles-containing vaccine, with an opportunity for a second dose[14]. It is well understood that laboratory testing and confirmation of suspected measles infection is crucial in countries that are in elimination phase of measles[15-17]. Generally, all 6 World Health Organization (WHO) regions have committed to measles elimination, and 5 (except Southeast Asia) set target date to move from regional measles mortality reduction to the regional elimination of indigenous transmission[6].

GLOBAL PROGRESSES TOWARDS ELIMINATION

Although there has been tremendous success in the reduction of measles endemic incidence in many countries with measles elimination, the total interruption of measles transmission remains a major challenge due to importation of measles cases to America and Europe regions[18-20]. For example, the ongoing transmission of endemic measles was declared eliminated in the United States in 2000[21]. However, within five months period starting from January to May 2011, 118 cases were reported in the United States in which 46% of the cases were imported[18]. The elimination of measles deaths in Southern Africa in 2000 joins the region of the Americas to be free from measles deaths[22,23]. However, from July 2003 to November 2005, 1676 laboratory-confirmed measles cases were reported in South Africa[24] and silent causalities' the disease was also reported[25].

The WHO Europe strategic plan for measles 2010-15 sets targets of 90% measles vaccination coverage, and reductions in the number of cases to fewer than five per million and in mortality by 95% compared with 2000 levels[26,27]. The 37 countries and areas of the WHO Western Pacific Region have targeted measles for elimination by 2012[12].

Between 1997 and 2011, the goal of interrupting measles transmission was adopted toward the elimination of measles in the Eastern Mediterranean Region (EMR). For the 22 EMR member countries, routine coverage with the first dose of a measles-containing vaccine increased from 70% in 1997 to 82% in 2009. Reported measles cases decreased by 86% during 1998-2008, and estimated measles mortality decreased by 93% during 2000-2008, accounting for 17% of global measles mortality reduction during that period. Despite these successes, EMR was not being able to achieve measles elimination by the end of 2010[28].

Many Progresses have been achieved toward measles elimination in the People's Republic of China between 2000-2009 and in the Russian Federation between 2003-2009[29,30]. Globally, the number of measles deaths worldwide fell by 78% between 2000 and 2008, from an estimated 733000-164000[31]. Despite the efforts measles elimination, measles remains a disease still endemic in many parts of Europe[32]. For instance, between 2009 and 2011, Austria, France, Germany, Ireland, Italy, Greece, the Netherlands, Spain, Bulgaria, Norway and United Kingdom have all seen outbreaks[32-41].

Estimates indicate that almost a quarter of all lives saved annually towards achieving Millennium Development Goal 4 are the result of progress towards achieving a 90% reduction in measles deaths [7,10,13].

STRATEGIES FOR MEASLES CONTROL AND ELIMINATION

Key strategies for the local elimination/total eradication of measles as a disease are as follows. The spread of measles infection through a population requires that a chain of invectives should be maintained. Protection against this spread of infection can be taken at two points. First, the route from susceptible to recovered (return to immunized state after vaccine uptake) can be short-circuited by the establishment of immunization[42-45]. Second is to interrupt the mixing of invectives (carrier of the infections) and susceptible with protective barriers (*e.g.*, isolation)[46]. Incidence rises as susceptible individuals enter the population. Acquisition of immunity through exposure to the wild virus or vaccination decreases the number of susceptible individual in the population and measles incidence falls [47]. The greatest potential is with vaccination.

Acquired immunity after measles illness is permanent. Live attenuated measles virus, when administered at recommended ages, produces about 85% immunity after one dose and greater than 90% immunity after two doses[5,48,49]. Vaccine-induced immunity is long lasting and protective to all the diverse geographic origin strains. Widespread vaccination has resulted in interruption of measles virus transmission in a number of countries. For instance, the Gambia in 1968-1969, the English speaking Caribbean islands, Cuba, Chile, United States over short periods in 1993, 1995, and 1996[50,51]. Similar achievements were obtained in England and Wales through 1995-2000[20]. Estimate indicates increase in routine measles coverage from 71% to 82% globally between 2000 and 2009, and from 56% to 73% in the 47 countries with the greatest burden of measles deaths[7,8].

The success of recent mass vaccination campaigns in these countries has suggested that global eradication of measles is possible biologically, technically, and operationally[19,52]. Reaching this goal will require continued commitment to increase vaccination coverage levels with a co-coordinated global effort.

Vaccine investments rose from donors in United Kingdom, Japan, United States, *etc.,* to provide additional funding to the Global Alliance for Vaccines and Immunization (GAVI) for its childhood immunization program save many children's lives. Vaccination is one of the most cost-effective health interventions[53]. Studies show that measles eradication by 2020 was found to be the most cost-effective scenario globally[54].

Programmatic and technological innovation will be needed to sustain recent successes in reduction of the global burden of measles. Delivery of the measles vaccine through the respiratory tract could help this effort[55]. It has many advantages compared to the injectable vaccines in which the major one can be stated as follows[55-58]. Respiratory delivery generates robust local and systemic immune responses which resulted in superior and longer lasting protection and boosting better responses in seropositive people than are injectable vaccines[57,58]. This route is less likely to be blocked by maternal antibodies in infants than is a subcutaneous measles vaccine. Aerosol administration of vaccines needs fewer skills than injectable vaccines. Use of non-injectable vaccines reduces the likelihood of unsafe disposal and reuse of syringes in immunization program.

An important component of the measles control and elimination strategy is information obtained from laboratory. Currently the WHO Global Measles and Rubella Laboratory Network (LabNet) include 690 laboratories serving 183 countries[59].

MEASLES VACCINATION

Different factors affect the response to immunization, such as, age and maternal antibody level[60]. Wesley *et al*[61] reported that the response to measles immunization was delayed among malnourished children. The optimal age at delivery of measles vaccine depends upon the relationship between the average age at infection and the rate of loss (average duration) of maternal antibodies specific to measles[62,63]. Maternal antibodies typically provide protection during the first 6 mo of life, but often longer[63,64]. Interference with the replication of vaccine virus is frequently still seen at the age of 12 mo[65]. As a consequence vaccination in the first year of life gives inadequate immunity to measles, meaning the earlier at the age of vaccination the lower the sero conversion rate[63,64]. The requirement for delay until maternally derived antibodies vanish is an impediment for early vaccination.

The duration of maternally derived immunity in a child depends on the mother's antibody titer, the efficiency of transfer across the placenta and the rate of catabolism in the child[66,67]. A child exposed to many infections makes a large variety of immunoglobulin G (IgG); in order to keep the total blood IgG level in the normal range, catabolism is accelerated and passively acquired antibodies are swept out at an accelerated pace. In this way, early susceptibility to measles is strongly correlated with low economic status[67]. To meet this challenge age cross-sectional sero-epidemiological surveys and sero conversion studies are important for recommending the proper age for vaccination. An evaluation of the routine immunization program in Ethiopian children, reported here[68], gives support for the WHO recommended age for measles vaccination at 9 mo[69]. The ability of a measles vaccine to induce an immune response, particularly in the presence of maternal antibody, varies according to the strain and the dose of vaccine[63].

Acquired immunity after measles illness is permanent. Live attenuated measles virus, when administered at recommended ages, produces about 85% immunity after one dose and greater than 90% immunity after two doses[5,48,49]. Vaccine-induced immunity is long lasting and protective to all the diverse geographic origin strains.

Developed and developing countries of the world have different measles vaccination policy. In developed countries children are immunized at the age between 12-18 mo (depending up on the policy of different countries), as part of a three-part mumps and rubella (MMR)-vaccine. The vaccination is not given earlier than this because children younger than 12 mo usually retain anti-measles immunoglobulin's transmitted from the mother during pregnancy. A second dose is usually given to children between the ages of four and five. In developing countries where measles is highly endemic, it is recommend that two doses of vaccine be given at six months and at nine months of age. Serological studies in developing countries have shown sero-conversion rates following immunization at age 9 mo of 80%-90%[70,71]. Generally, the two-dose schedule is beneficial when there is a need to increase net vaccine efficacy, after coverage has been maximized with a one-dose schedule[64,72,73].

Role of laboratory for measles control and elimination

Laboratory investigation will play a critical role in monitoring the success of measles control strategies[15,16,59,74]. This role will require the development of more sensitive diagnostic methods suitable for diagnosis and surveillance, genetic analysis of measles strains and a technology which is transferable worldwide. Measles diagnosis relies increasingly on serological tests[75]. Serum based diagnosis can be made by virus isolation, by demonstration of a significant increase in specific IgG titers, or by the detection of anti-measles virus (MV) IgM antibodies by using radio-immunoassays (RIA), enzyme-linked immune sorbent assays (ELISAs) and direct or indirect fluorescence-antibody techniques[10,76,77]. Genetic characterization of wild-type measles viruses from different types of specimen sources provides a means to study the transmission pathways of the virus and is an essential component of laboratory-based surveillance[78-82].

The effectiveness of an immunization program can be evaluated though serological survey methods. Using dried blood spot (DBS) sample-drops of whole blood collected on filter paper from a simple finger prick-provides a minimally invasive method for collecting blood samples in nonclinical settings for serological and genetic analysis of measles[83-87]. The measles laboratory network required the use of alternative sampling techniques for surveillance[88]. The need for techniques that obviate the requirement for blood sampling promotes the application of oral-fluid based methods to the evaluation of the immunization programs. Oral-fluid based testing has an advantage of convenience, avoidance of inadvertent transmission of blood-borne pathogens, ease of use in pediatric and geriatric populations; as well as the potential for blood-free home and work place collection of patient samples.

Of the "ten elements of surveillance" summarized by WHO[89] at least in six of them visa a VIS, morbidity reporting, epidemic reporting, laboratory investigations, individual case investigations, epidemic field investigations and surveys, the laboratory has a role in providing serological results for measles surveillance. This indicates that the general advantages of measles surveillance data depend in large part on laboratory results[59,74]. The requirement of blood specimens for laboratory results can limit the yield of data for measles surveillance. In this respect we need another source of human biological material for measles surveillance, which is inexpensive and simple to collect, acceptable to donor and collector and provides accurate representation of serological status. Oral fluid has been explored as a source of human biological material for surveillance of viral diseases[90,91]. It has clear advantages over venipuncture in surveillance and epidemiology of viral diseases. In the United Kingdom, oral-fluid sampling and screening has been used for the surveillance of measles, MMR since 1994[20]. This has permitted the impact of MMR vaccination program to be monitored and evaluated in a way which may not have been possible through blood collection alone. Measles serological surveys could play a role in the evaluation of immunization programs[92,93]. Immuno-serological cross-sectional measles surveys have particular importance to determine immunization program strategy in relation to age groups, geographic areas, socio-economic groups and risk population groups. Follow-up serological measurements in measles immunized persons has importance to determine the proportion developing immune responses, quality and extent of response, duration of response and level of protection against measles infection. Periodic measles serological surveys have advantage to identify groups who are not receiving measles vaccines or who have inadequate responses. The importance of sero epidemiology for such purposes is paramount although the necessity for vein puncture reduces the ease

Table 1 Samples for laboratory diagnosis of measles virus infections

Virus disease	Samples for virus isolation for detection of antigen	Samples for serology	Remarks
Acute measles	Blood (leukocytes), throat secretions (saliva/oral-fluid), conjunctival secretions, urine; skin biopsies	Acute and convalescent serum	Period of infectivity; prodromal stage until 1-2 d after rash; antibody rises occur at appearance of rash; in tropical measles, possibly prolonged virus excretion also in stools
Measles pneumonia	Blood (leukocytes), throat secretions, conjunctival secretions, urine	Acute and convalescent serum	Frequently no rash; prolonged period of infectivity
Acute measles encephalitis	Brain specimen (biopsy or autopsy specimen), cells in CSF	Serum and CSF	In most cases, no infectious virus is detectable; occasional local production of antibodies in the CNS
SSPE	Brain specimen (biopsy or autopsy specimen), cells in CSF, lymph node biopsy (?)	Serum and CSF	Virus antigen detected in CSF cell; virus isolation requires propagation of explants cultures and cocultivation with susceptible cells; hyper-immune antibody response; local production of antibodies in the CNS

Modified from Norrby *et al*[137]. CSF: Central spinal fluid; CNS: Central nervous system.

and acceptability of this method. New methods that obviate the requirement for blood sampling could further encourage the application of measles serological surveys for the evaluation of measles immunization program. To achieve the aforementioned roles at better performance work was undertaken for measles vaccination program evaluation and surveillance based on oral-fluid collection and screening methods[94]. The purpose of this review is, therefore, to explore the development and evaluation of oral fluid as a diagnostic specimen for measles virus with particular reference to the developing country setting. The technologies developed[68,77,81] have increased the level of sensitivity and specificity where salivary examination for measles IgG and IgM is practical and convenient. Using polymerase chain reaction (PCR) technology we found oral-fluid from measles cases to be useful in the molecular characterization of measles virus. Success of the measles vaccination program can be assessed using oral fluid specimens as markers of sero-conversion.

ORAL-FLUID AS CLINICAL SPECIMENS

Laboratory investigation will play a critical role in monitoring the success of measles elimination strategies. As we shall see in this review the role will require the development of more sensitive diagnostic methods suitable for diagnosis and surveillance, genetic analysis of measles strains and a technology which is transferable worldwide.

Measles virus can be detected from various clinical samples by using serological methods, cell cultures techniques or molecular techniques. Samples that can be collected at different stages of the measles infection for virus isolation and serological tests are outlined in Table 1.

The concentration of antibody in saliva was found at much lower levels compared to plasma[95]. This has limited its use as diagnostic specimen for viral immunological assays. However, research demonstrated that salivary antibody has two sources, the parotid and crevicular crevice, with different concentration levels of immunoglobulin's[95]. The transudate that comes from the gingival crevice, whilst being lower in concentration, closely reflects the immunoglobulin class and specificities of antibody found in plasma[91,96]. The major reason for this is that the majority of the antibody present in the transudate comes from the small capillary bed beneath the margin that separates the teeth and gum. These properties of crevicular fluid lead investigators for measurements of virological markers of immune activation as an alternative to serum.

The other problem associated to the use of saliva as a viral diagnostic fluid is the need of immunological assays that have higher sensitivity. The development of antibody capture assays, ^{125}I labeled (RIA) or ELISA, that are able to generate higher signals by capturing a higher proportion of the total immunoglobulin (present in the oral fluid) specific for the antigen under test, enabled saliva to be used for successful immunological assays[97,98]. Presently the production of purified nucleoprotein through Baculovirus expression[99] increases the utility of saliva in diagnostic enzyme immunoassays.

The value of oral-fluid in screening for human immunodeficiency virus infection is now well established with the use of IgG captures radioimmunoassay[100,101]. The methodology has been applied to oral-fluid diagnosis of measles, mumps, rubella, Epstein-Barr virus and hepatitis A and B infection[77,100-104]. Veterinarians found it useful for detecting feline immunodeficiency virus[105], and feline leukemia virus[106]. Hepatitis C virus antibodies can be able detected from oral fluid[107]. Its potential application in bacteria was demonstrated with the measurement of specific IgA antibody to *Bordetella pertussis* antigens in saliva for diagnosis of whooping cough[108]. Other possibilities were seen in the diagnosis of cysticercoids by measuring specific salivary antibody to *Taenia solium* larvae[47]. Measuring of specific IgA antibodies to gliadin is used as a screening marker for coeliac disease[109,110]. Methods that can detect microbial antibodies in oral fluid such as *Helicobacter pylori* antibodies have been developed[111]. The potential to use oral fluid as Porcine Reproductive and Respiratory Syndrome virus in swine, cardiac diagnostics, oral cancer, systemic diseases, water-borne diseases, alcohol and drug testing specimen has been the subject of considerable scientific interest[112-118]. Generally oral-fluid as diagnostic fluid has the following advantages: (1) humanitarian-the patients are spared the discomfort of

repeated venipunctures; (2) clinical- with less stress, non-risk of anemia, infection or thrombosis; (3) for children-saliva sampling is the technique of choice; (4) economic-patients can collect themselves, thereby saving technicians' time, samples may also be mailed, eliminating travel time; and (5) eliminates the issue of protection of privacy and adulteration during sample collection; the ease and low cost of collection are major benefits in large-scale studies.

STUDIES PERFORMED ON MEASLES ORAL-FLUID BASED TEST METHODS

The works so far done can be specifically summarized as follows: (1) The development of a GACELISA for the detection of measles specific IgG in oral-fluid, with performance (sensitivity and specificity) that makes it suitable for replacement of serum assays, particularly for estimating population immunity[77]. By comparison with the serum measles IgG assay, the oral fluid GACELISA had a sensitivity of 97.4% (95%CI: 95.9-98.2) and a specificity of 90.0% (95%CI: 81.9-94.3), with no significant differences observed by age group. It is concluded that the overall performance of the GACELISA was satisfactory, showing close agreement to the serum ELISA, and has potential to serve as an easily transferable tool for large scale epidemiological studies as required for the World Health Organization's program for the global control of measles; (2) The development of a MACELISA for the detection of measles specific IgM in oral-fluid, suitable in performance to replace serum assays[68]; Screening of sera was undertaken using commercial indirect ELISA kits, and of oral fluids using an in-house IgM-capture ELISA. Pre-vaccination serology showed 1.4% IgM positive, 2.0% IgG positive, and 97.0% sero negative; Post-vaccination seroprevalence of IgM and IgG was 91.3% and 85.0%, respectively, and 92.9% overall. The seroconversion rate was 92.6% (95%CI 88.2-95.7); Based on oral fluid results, 87.3% (95%CI: 82.0-91.4) of children showed specific IgM antibody conversion. These results are in support of the recommended age for measles vaccination in Addis Ababa, and show the merit of oral-fluid IgM screening as a non-invasive alternative to blood for assessing vaccine immunogenicity; (3) Demonstration of the use of these assays in the estimation of measles antibody (immunity) prevalence in the vaccine-targeted population and in monitoring the outcome of a measles vaccination program (routine and campaign) in a developing country setting[68,96-98]; (4) Demonstrate the utility of oral fluid to study the molecular epidemiology of measles virus in both developed and developing country situations in a period of accelerated measles control[81,82,109]; (5) Oral fluid for the serological and molecular diagnosis of measles in a developed country setting[109,119]. These studies demonstrate the use of oral fluid samples for the detection of measles virus in the United Kingdom and the Belgian measles surveillance system and other studies in the framework of the WHO elimination program; (6) Technical refinements of sample collection and laboratory screening of oral fluid, and, importantly, comparisons with existing methods based on serum prior to wider adoption of non-invasive methods. This work includes the evaluation oral-fluid relative to serum and DBS for the detection of measles specific IgM in suspected measles cases in relation to assay type and sample timing post onset of rash. Works done to assess the performance (sensitivity and specificity) of a commercial IgG antibody capture method for oral fluid in relation to currently used assays for serum/blood spots is in preparation for publication (Dr. Nigatu W personal communication); (7) The studies emphasize the potential and suitability of oral-fluid to substitute serum in estimating and monitoring measles IgG antibodies, during community surveys[118-120]; (8) Applicability of oral fluid collected onto filter paper for detection and genetic characterization of measles virus strains[119,121]. The former study showed molecular nested RT-PCR using oral fluid was validated against the standard assay on nasopharyngeal secretions and gave a sensitivity of 100% and specificity of 100%. The latter study demonstrate that oral fluid dried onto filter paper can be used for the detection and characterization of MV strains. Using this approach, an MV-positive sample by reverse transcriptase PCR could be obtained from 67% of serologically confirmed acute measles cases; (9) Determination of measles immunization status using oral-fluid samples [122]. The presence of antibodies in oral fluid specimens correlated with that in serum with sensitivity and specificity: measles, 97% and 100%, respectively. This study assessed protective antibodies to measles by means of an oral fluid sample with good reliability; and (10) Evaluation of 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[123]. With oral fluids POCT showed sensitivity and specificity of 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 wk at 20-25 ℃. The POCT has the sensitivity and specificity required of a field-based test for measles diagnosis. However, its role in global measles control programs requires further evaluation.

PRESENT AND FUTURE APPLICATIONS OF MEASLES ORAL-FLUID METHODS

Present applications

Community surveys of measles specific IgG/IgM are useful to guide the design of measles control programs. For example these help in (1) defining levels of immunity to measles pre- and post-vaccination efforts, *i.e.,* assessing the effectiveness of the vaccination program; (2) identifying age groups in which a significant susceptible proportion remain; and (3) assessing sero-conversion rates following vaccination. Analysis of the genetic characteristics

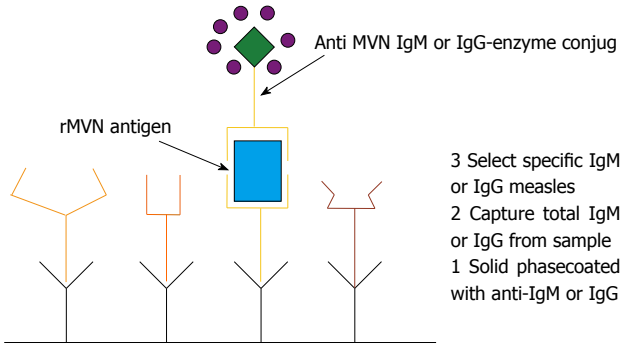

Figure 2 Principle of the"Microimmune" measles IgM or IgG Capture Enzyme Immuno-assay methodology. IgG: Immunoglobulin G; IgM: Immunoglobulin M; MVN: Medial vestibular nucleus.

of wild-type measles helps to elucidate the origin and transmission pathways of measles virus [124]. The genetic data when analyzed with other epidemiological data provides a means to assess the efficacy of measles control programs. For such molecular studies measles RNA can be detected by RT-PCR from isolates, oral fluid, blood, throat-swabs, urine collected from acute cases. There have been no systematic studies made to evaluate the relative sensitivity of these different samples. The primary role of this review is in the demonstration of the use of oral fluid as a clinical specimen for detecting IgG/IgM antibody for evaluating measles control strategies and the virus genome for molecular epidemiological studies.

Future technical development

The low concentration of IgG/IgM antibodies in oral fluid relative to other diagnostic specimens such as plasma[95] demanded the development of an enhanced immuno assays and of diagnostic techniques based on nucleic acid amplification.

Promotion of the use of oral fluid as viral diagnostic fluid requires that immunological assays have higher sensitivity. The development of antibody capture assays, either ^{125}I labeled (RIA) or ELISA, that are able to generate higher signals by capturing the proportion of specific to the total immunoglobulin (present in the oral fluid), enabled oral fluid to be used for successful immunological assays[97-99]. The RIA have associated problems in disposing of radioactive waste from aspect of health may decrease its acceptability. Capture ELISA is better for wide-scale use in many laboratories. A study also showed that the capture ELISA with saliva was more sensitive than the radioimmunoassay for specific rubella IgG[102]. Hence sensitivity enhancement is required to make best advantage of ELISA. This review shows that FITC/anti-FITC enhanced capture ELISA that can be used for population and vaccine surveys [68,77].

The production of measles antigen for measles diagnosis, such as the one we used for GAC- and MAC-ELISA, benefited from tissue culture. However, production of purified measles antigens in tissue culture can be difficult. The capture format has been revolutionized by the raising of purified antigen and monoclonal antibodies for use in oral-fluid measles diagnostics. Cloning and expression of measles genes provides a relatively straightforward alternative approach[99,125], simplifying purification and enabling large-scale production for improvement in measles oral-fluid diagnostic assays.

Kits based on the use of recombinant antigens such as the Light Diagnostic kit (Chemicon Temecula, CA, United States) benefited from the cloning and expression approach. More recently IgG and IgM kits specific to measles have been developed based on such an alternative approach by Microimmune Ltd (Brentford, Middlesex, United Kingdom) for both oral-fluid and serum samples. However, there are problems associated with the use of recombinant antigens associated with the production of "incorrectly" processed antigens by most expression systems[99,125] and the problem of using a single cloned antigen to detect a measles antigen that may vary between isolates. This may be resolved by cloning and expressing the most conserved region of the measles gene identified from sequence data of different isolates. Notwithstanding this problem measles antibody assays that are increasingly based on the use of cloned proteins will continue to play a prominent role in oral-fluid diagnostic development. Such immuno-assays may be useful in the future when they become better suited to use with automated systems that are capable of handling all stages of testing from specimen preparation to issuing of diagnostic results.

Microimmune assays are observed to be easy to use, but have not yet been evaluated under a wide range of conditions such as in highly vaccinated populations. The procedure and principle of the oral-fluid Microimmune EIA methodology is described in Figure 2.

Studies of rubella revealed problems of sensitivity in enhanced GACELISA in older age groups. This appears to be due to decay in the level of specific antibody in serum and in oral fluid[98,102,126]. Age-related variation in sensitivity was not seen as a big problem in measles assays[77,98]. However, low-level measles antibodies resulting from vaccine-induced immunity is a feature of many communities, particularly those with high-level routine immunizations coverage. Future work is required to evaluate the performance of newly developed kit assays in such settings.

Assays of measles nucleic acid are fundamentally different from those of measles antibodies, since they detect a component of the measles virus itself, rather than serological evidence of its past presence. Among the several techniques used to detect viral nucleic acids the PCR is the one widely used for detection of measles nucleic acid[127-129]. In contrast to direct hybridisation, whose application is restricted to where high concentration of the virus is present, PCR amplifies the probe signal by means of a sequential series of secondary, tertiary, *etc.* stages. The signal amplification thus increases the sensitivity of detection to a range where it can detect viruses at low concentration in various specimens[129]. PCR is suitable

for the detection of the low concentration of measles virus present in oral fluid. Actually oral fluid is better for nucleic acid extraction than serum or blood because of the absence of PCR inhibitors, such as haem or porphyrin, in the oral fluid[129]. In addition, oral fluid specimens do not need pre-treatment for nucleic acid extraction. In future developments of measles oral-fluid diagnosis based on the nucleic acid amplification systems are likely to play an increasing part. The new tool developed by Roche Molecular Biochemicals, MagNA Pure LC DNA isolation kit, for the isolation of nucleic acid from various types of specimen including oral fluid, is a breakthrough that has shortened the tedious manual RNA extraction process in measles nucleic acid detection. This is now practiced in many laboratories of industrialized countries but may be restricted to laboratories that have specialised requirements and too costly for most developing countries. Recently Health Protection Agency has 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[123]. Further evaluation of this test method under different scenario will refine the technique for wider future application.

IgG can be measured in terms of its functional binding avidity. The binding strength between the IgG and the virus antigen is supposed to be low in primary infection and changes to high in past infection. This avidity can be measured by disrupting the interaction using protein denaturants such as urea or diethylamine[130]. Diagnosis of primary infection by IgG avidity assay using serum samples has got relevance for the diagnosis of viral infection such as rubella[131-132]. The detection of antibody with low or high avidity enables a more accurate diagnosis in differentiating primary infection from past infection.

IgG avidity is useful when the IgM assay result is indeterminate. It may also help in distinguishing primary and secondary ("boosting") response to measles vaccine. Future development of IgG avidity in oral-fluid measured by GACELISA may allow specific, sensitive and accurate diagnosis of primary infection. Our study[68] show the problem of MACELISA in detecting IgM in oral-fluid samples collected at early onset of measles rash. The future development of IgG avidity that can determine IgM in early-collected oral fluid samples makes MACELISA better use.

Another interesting area to look at in the future is the differentiation between antibodies resulting from vaccine strain and wild type measles virus. This assists in defining vaccine uptake and estimating continued measles transmission. It may be difficult to explain the technical development at this stage. However, it is an area for future research.

Evaluation of a new diagnostic test has the potential sources of bias introduced by the study design. The test's discriminatory ability, sensitivity, and specificity depend upon the composition of the study population. The study design we used for evaluation of the present measles oral- fluid diagnostic assays[68,77] is an area that can be followed in the future for other viral diagnostic test evaluation.

Future wider applications of oral-fluid methods

The application of oral-fluid methods to population surveys, vaccine surveys, diagnosis of clinical cases, and case surveillance for different vaccine uptake settings of a country/district are illustrated in Table 2. The following is a description of some of the applications of oral-fluid methods.

Population surveys: Measles antibody population surveys can used to define the proportions of susceptible and immune in the population. Population immunity may result from natural measles infection or/and measles routine and campaign vaccination. Current methods cannot distinguish between the two whether the immunity is induced by wild or vaccine virus. Population immunity surveys can identify in which age groups large pockets of susceptibles remain in unvaccinated populations, in a population with routine immunization, and before and after a vaccine campaign. This would provide valuable information on the age groups to target for vaccination and effectiveness of the routine or campaign vaccination, and clues to where future outbreaks might arise. Similarly, through such surveys hard-to-reach groups in rural/urban under different geographical settings can be reached.

Population surveys may be appropriate at all stages of vaccination programmes, in country/settings of vaccine uptake for low to high, with or without campaigns/accelerated measures. Predominantly, such surveys could assess specific antibody status. However, post-campaigns there might be a role for IgM testing in community survey to establish what proportion of the population actually responded to vaccine. Based on surveys cluster sampling techniques, as for EPI vaccine cluster sampling, and using of the-shelf EIA kits, the surveys would be rapid and simple to effect.

Vaccine surveys: Vaccine surveys assess the level of population immunity attending vaccine clinic to a measles routine vaccination. It can identify the responses to routine vaccine in pre- and post-vaccinated children. The widespread use of serological determinants of vaccine responsiveness is limited by the need to carry out follow up of vaccinees at 2 (IgM) and < 4 (IgG) wk after vaccination. Oral-fluid sampling will not improve greatly up on this situation, except that compliance for second samples is likely to be greater than if blood samples are required. However, the future development of oral-fluid IgG avidity measurement cannot be ruled out that may improve this situation.

Diagnosis: Measurement of measles antibody present in oral-fluid samples provides information on the status of current and past infection by use of tests for IgG and IgM antibody. Laboratory diagnosis of suspected measles clinical cases can assist in (1) confirmation of the occurrence of measles clinical illness (2) capability of physicians to diagnose illness and (3) reporting of the infection to health department. The usefulness of

Table 2 Application of oral-fluid methods under different countries settings

Applications of oral-fluid methods	Setting for country/district		
	Low/Med uptake routine	High uptake routine	Campaign
Population survey	Methods: community surveys of IgG across wide age range. Including hard-to-reach groups, informal settlements. Purpose: immunity profiles. Identifies susceptibility gaps and age range for campaigns. Implications: increase in coverage, need for and age range for campaigns	As previous	As previous plus. Methods: Post-campaign surveys of IgG and perhaps IgM. Purpose: IgG-Identify immunity levels post-campaign. Susceptibility in target age group and outside target group. IgM-indicator of impact, *i.e.*, proportion responding to vaccine. Implications: Age-range for future campaigns; locate problems of vaccine efficacy
Vaccine surveys	Methods: Vaccine clinic samples pre- and post-vaccination. IgM and/or IgG testing. Purpose: Assess efficacy of routine vaccination. Implications: Identify cause of low efficacy.	As previous	As previous plus. Methods: IgG survey of individuals attending vaccine clinics. Purpose: Identify proportion able to respond to vaccine. Implications: Assess potential effectiveness, and suggest alternative method for delivery eg hard-to-reach groups.
Diagnosis	Not indicated while measles incidence remains high	Method: IgM testing on demand. Purpose: Confirmation of clinical diagnosis	As previous
Case surveillance: serological and genetic	Not indicated while measles transmission remains high	Method: System of reporting and oral fluid sampling from sporadic cases and outbreaks. IgM and Genotyping Purpose: Verify cases, and monitor distribution of virus and endemicity Implications: Need for additional control measures	As previous

IgG: Immunoglobulin G; IgM: Immunoglobulin M.

oral fluid in this capacity is at present hindered by the relatively low sensitivity of IgM assays in samples taken early after onset of rash. A study showed the oral fluid measles IgM detection rate increased from 63%-67% at 2 d and 3%-100% at days 6 and 7[82]. Delay in collecting a sample may be impractical. Improved sensitivity of assays remains a need.

Case surveillance: The recognition and identification of measles outbreaks and sporadic cases using a system of reporting and oral-fluid sampling is established in the United Kingdom[20,76,133]. For measles epidemic investigation in Ethiopia, where infrastructure is poor and locations of the remote, oral-fluid sampling was found to be appropriate. Especially in the situations where community beliefs or attitudes like "measles sick should not get injection" are present, in which communities declined to give blood specimens, oral-fluid specimens are preferable. Provided reasonable storage conditions while in transit or awaiting transit to the laboratory are made, oral-fluid is a robust sample for IgG testing, IgM testing and viral genome detection (United Kingdom surveillance and in these studies in Ethiopia)[77,134]. However, further stability studies of oral-fluid at different temperature in field conditions are required in the future.

Another area of increasing importance is the application of sequence data obtained from oral-fluid nucleic acid amplification techniques. Genetic information is valuable, in combination with other traditional epidemiological data, to enhance the ability to determine measles transmission pathways and to assess the success of measles control strategies[79,124,135,136].

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