Report on a Workshop on the Laboratory Diagnosis of Measles

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Abstract

The Western Pacific Region of the World Health Organization (WHO) has declared a goal of regional measles elimination with a target date of 2012. To facilitate this goal, and in order to increase the familiarity of staff from some Western Pacific national laboratories with the technique of enzyme immunoassay (EIA) for the detection of anti-measles IgM, a WHO sponsored workshop was held at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in May 2005. The workshop included participants from national laboratories in Cambodia and Lao People's Democratic Republic, and from five Pacific Island countries, Fiji, French Polynesia, Guam, New Caledonia and Papua New Guinea. An observer from Guam also participated.

In addition to increasing the workshop participants' familiarity with the Dade Behring Enzygnost® Anti- Measles Virus/IgM assay by hands-on involvement, the participants learnt to use dried venous blood spots for measles diagnosis. All participants successfully completed the practical component of the workshop. The workshop also included informal seminars on trouble-shooting problems in EIA, good laboratory practice, data management in the laboratory and transporting infectious and diagnostic material.

The EIA measles IgM calculation worksheets and the seminar on good laboratory practice were considered to be particularly useful by the majority of participants. The workshop was considered a success in terms of equipping participants with the knowledge and capacity to perform accurate measles IgM testing for both serum and dried venous blood spots. It also provided an introduction to proficiency testing. Pages 159 - 163

Introduction

In the World Health Organization (WHO) region of the Americas, a measles control and elimination program has been conducted for a decade, resulting in elimination of indigenous measles in 2002.[1] The Western Pacific Region (WPR) of the WHO has declared a goal of regional measles elimination with a target date of 2012 recommended by the WPR Measles Task Force in July 2004 and the WPR Technical Advisory Group meeting in June 2005. The recommendations for this target date will be considered again by the Regional Committee Meeting in September 2005. The Pacific Island countries and territories have designated measles as one of a number of priority diseases.

After the measles immunization campaign in many Pacific Island countries in1998 indigenous transmission of measles appeared to have been eliminated. Since then a small number of imported cases have been reported and two outbreaks have occurred. In 2002 a measles outbreak in Guam was found to be due to the falling coverage of vaccination in pre-school children. A vaccine campaign targeting children 12-59 months was implemented.[2] In 2003 a measles outbreak in the Republic of the Marshall Islands was considered to be due to low vaccine coverage and a mass vaccine campaign was organised.[3] Hospital-based active surveillance for patients with acute fever and rash, as a means of detecting both measles and rubella, is currently conducted in 58 hospitals in 20 Pacific Island countries.[4]

In countries, such as the Pacific Island countries where measles is well controlled, and infection is rare, it is important to confirm all clinical diagnoses by a reliable laboratory test. The WHO recommends that this test should be an enzyme-linked immunoassay (EIA) detecting measles immunoglobulin class M (IgM). It is also important to establish a reliable laboratory network with accredited national laboratories capable of performing accurate testing and with ready access

to reference laboratories for support and confirmatory testing. In order to improve laboratories capacity for diagnosis of measles in the Pacific Region a laboratory network – LabNet, a service of the Pacific Public Health Surveillance Network (PPHSN) – has been set up with Level 1 laboratories performing local testing. Level 2 laboratories (4 national WHO accredited measles laboratories) perform local testing and referred testing from L1 laboratories and Level 3 (reference laboratories) perform confirmatory testing, virus isolation and genotyping and provide training.

The use of an EIA to confirm infection requires the collection of blood by venipuncture. The blood should be transported to a laboratory within 24 hours and kept at 4°C prior to testing. This may be difficult in countries where laboratory facilities and infrastructure are limited, or when parents do not give permission for their infant or child to have venous blood taken. A blood spot on filter paper, obtained from venous blood, or a heel or finger prick, requires less blood and is easier to transport than whole blood or serum. Previous work from the Victorian Infectious Diseases Reference Laboratory (VIDRL) has shown that dried blood spots for the detection of anti-measles virus IgM can be transported at ambient temperature and stored at 4°C for up to 6 months prior to testing, with results consistent with those obtained from serum.[5]

In order to be confident that laboratories around the world are able to detect measles IgM reliably, quality assurance panels (QAP) have been distributed annually by VIDRL on behalf of the WHO. In 2001 the first panel was distributed to 46 laboratories, while the most recent distribution in 2003/2004 involved 99 laboratories in four of the WHO regions: the European, South East Asian, African and Eastern Mediterranean regions. The region of the Americas uses a QAP for measles and rubella distributed by the Centre for Diseases Control in the United States and China has developed national QAPs for its provincial and prefecture measles laboratories. However some laboratories in the Western Pacific region had not been included in QAP distributions prior to June 2005.

A WHO sponsored workshop was held at VIDRL between May 24-27, 2005 in order to increase the familiarity of staff from some Western Pacific national laboratories with the technique of EIA for the detection of anti-measles IgM and to introduce the use of dried venous blood (DVB) to workshop participants.

The workshop

The workshop was sponsored by the Western Pacific Regional Office of the WHO and included participants from national laboratories from two SE Asian countries, Cambodia and Lao People's Democratic Republic, and five Pacific Island countries, Fiji, French Polynesia, Guam, New Caledonia and Papua New Guinea. An observer from Guam (PIHOA Regional Laboratory Coordinator) also participated in the workshop. Workshop participants and VIDRL staff are pictured in Figure 1.

In addition to increasing the workshop participants' familiarity with the Dade Behring Enzygnost® Anti-Measles Virus/IgM assay by hands-on involvement, alternative methods for measles diagnosis were also demonstrated. These included antigen detection by immunofluorescence, viral culture and the detection of measles specific RNA by polymerase chain reaction (PCR). There were also informal seminars on trouble shooting problems in enzyme immunoassays, good laboratory practice, data management in the laboratory and transporting infectious and diagnostic material.

Figure 1. Workshop participants and VIDRL staff at the Measles 'hands-on' EIA Workshop held at the Victorian Infectious Diseases Reference Laboratory, May 24-27, 2005.

The hands-on part of the workshop was conducted in the serology laboratory at VIDRL and included three components. The first was a theoretical and practical

introduction to the EIA technique, specifically using the Dade-Behring assay. The second involved processing DVB spots that had been collected and stored at -20°C as part of enhanced measles and rubella screening in Victoria. The third exercise involved processing 12 serum samples, consisting of 6 measles IgM positive and 6 measles IgM negative specimens, all of which had previously been included in the WHO global measles QAP. The DVB panel included specimens that were positive for rubella and human parvovirus B19 specific IgM, both of which are known to cross-react with measles IgM. Participants worked in pairs for the first two components of the workshop and independently for the third session (Figure 2).

Figure 2. Workshop participants with 'hands-on'.

Results of the hands-on workshop

All participants successfully completed the introductory assay, which consisted of the kit positive and negative controls, an in-house positive quality control (QC) sample and positive and negative measles IgM samples, tested in duplicate. All results passed validation criteria and were within acceptable ranges, as specified by the manufacturer.

The DVB panel included four measles IgM confirmed cases and two other positive samples: one from a recently vaccinated child and the other a convalescent sample from a patient with measles who had seroconverted to IgG but whose IgM was no longer detectable in serum. All other samples, including four samples that were rubella IgM positive and one that was human parvovirus B19 positive, were measles IgM negative.

Table 1: Results for DVB testing for the diagnosis of measles by the detection of measles specific immunoglobulin class M (IgM)

Group	Sample	Expected result	Group result
1	1	Negative Rubella IgM positive	Negative
	2	Negative	Negative
	3	Positive Confirmed measles	Positive
	4	Negative Human parvovirus B19 IgM positive	Negative
2	5	Negative Rubella IgM positive	Negative
	6	Positive Convalescent sample, measles IgM no longer detectable in serum	Positive
	7	Positive Confirmed measles	Positive
	8	Negative	Negative
3	9	Negative Rubella IgM positive	Negative
	10	Positive Confirmed measles	Positive
	11	Negative	Negative
	12	Negative	Negative
4	13	Negative Rubella IgM positive	Negative
	14	Positive Confirmed measles	Positive
	15	Positive Recent vaccine recipient	Positive
	16	Negative	Negative

Each group achieved satisfactory results for the panel, including detecting a low level measles specific IgM in the convalescent DVB sample of the case who had seroconverted to measles (suggesting that in this case the DVB spot may have been more concentrated than the serum sample) and the DVB collected from the recent vaccine recipient. Four different DVB spots were processed by each group as outlined in Table 1.

Each participant tested an identical panel of 12 serum samples plus an in-house positive QC sample and kit controls. All tests were valid and controls were within the acceptable range according to the manufacturer's instructions. The in-house QC was included in every test performed throughout the workshop and the mean optical density (OD), as determined over the period of the workshop for the in-house QC was 0.63 with a standard deviation (SD) of 0.10. The coefficient of variation (SD/mean) for the QC was 15.9%, which is similar to the coefficient of variation for repeat in-house QC testing at VIDRL. All but one of the OD values obtained for the in-house QC sample was within 2SD of the mean of the QC sample.

Negative results were obtained for all negative panel samples and all but one result for the positive panel samples were > 0.2 (the assay cut-off for positive results). The one positive sample value with an OD less than 0.2 had an OD = 0.180 which would have been reported as equivocal and was therefore not considered aberrant. The OD results for the six positive serum specimens were all within 2SD of the individual mean for each sample. The OD values obtained by each of the eight participants are given in Figure 3.

Discussion

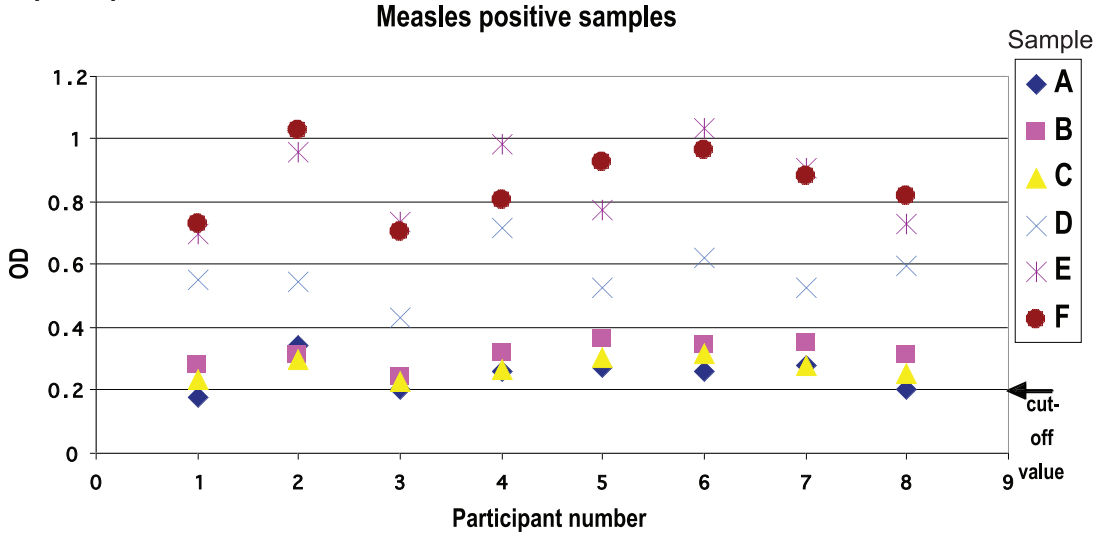
The workshop used the Dade-Behring assay as it has been extensively evaluated and is used by approximately 70% of national measles laboratories in the four regions participating in the measles quality assurance program (unpublished data at VIDRL). About half of the participants indicated that they regularly performed EIA for various diseases and no one had difficulty with the Dade-Behring assay. This was reflected in the successful completion of both the DVB and serum panels. At the completion of the workshop, participants, with the necessary import permits from their own countries, carried home the QAP that had been distributed to other laboratories around the world and a Dade Behring Measles IgM kit. The first result from this QAP was returned to VIDRL in the week following the workshop and was completely correct.

In the formal evaluation of the workshop, two aspects of the workshop were considered to be useful by the majority of participants: the EIA measles IgM calculation worksheets and the seminar on good laboratory practice.

The worksheet was designed to take participants, step by step, through the calculations required for the assay as outlined in the Dade Behring kit insert. It also included a checklist to ensure that the test was valid and that result interpretation was made correctly.

The seminar on good laboratory practice described the main factors that contribute to the safe handling of specimens in the laboratory. This included the principles of biosafety, the appropriate wearing of protective clothing, quality control of both tests and equipment, and good laboratory techniques. Good record keeping of test details, including test controls, maintenance and calibration of equipment, and staff training were

Figure 3. Optical density (OD) values for six positive samples tested by eight workshop participants.



also discussed. Participants were also provided with examples of record keeping sheets.

Another important component of the workshop was the seminar that focused on EIA trouble shooting. Participants were given a wide range of examples of when the validation criteria of an EIA may not be met, why this might have happened, and what would be the appropriate response. Informal discussion in all sessions encouraged participants to share ideas and develop networks.

Some countries that use a combined measles-rubella vaccine have extended measles control to include rubella control.[6] In the WHO region of the Americas and the European region, targets have been set for the elimination of rubella by 2010.[7,8] Enhanced rubella control may also be possible in countries within the Western Pacific region that use a combined measles-rubella vaccine. Recent work at VIDRL has confirmed that the technique using DVB for the detection of anti-measles IgM is also useful for the detection of anti-rubella IgM.[9] Moreover the International Air Transport Association has recently exempted dried blood spots from the Dangerous Goods Regulations, making their use even more practical as an alternative to whole blood (http://iata.org/whatwedo/dangerous_goods1).

The seminar on good laboratory practice described the main factors that contribute to the safe handling of specimens in the laboratory

Conclusion

The WHO sponsored measles 'hands-on' workshop was considered a success in terms of equipping participants from the Western Pacific region with the knowledge and capacity to perform accurate measles IgM testing using the Dade-Behring EIA for both serum and DVB. It was also an introduction to proficiency testing for the region. The workshop should result in an improvement in the diagnosis of measles and the EIA techniques are applicable to the diagnosis of other diseases. This success may encourage the more widespread use of DVB and will facilitate the regional initiative of measles elimination by 2012.

Acknowledgements

We are grateful to Dr Kazunobu Kojima from the WHO Western Pacific Regional Office. In addition to inviting VIDRL to host the workshop, Dr Kojima contributed to workshop presentations and reviewed this report. We would like to thank all the workshop participants for their enthusiastic involvement. We would also like to thank David Featherstone from WHO for providing valuable comments on this report.

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