VIDRL & WHO

Measles IgM Proficiency Panel 01002

Final Report

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Table of Contents

Tables and Figures

Abbreviations

AFRO	African Regional Office
ANOVA	Analysis of variance
CDC	Centers for Disease Control and Prevention, Atlanta, USA
EIA	Enzyme immunoassay
EMRO	Eastern Mediterranean Regional Office
EURO	European Regional Office
N	Negative
OD	Optical density
P	Positive
PCR	Polymerase chain reaction
QA	Quality assurance
S/CO	Sample/cut-off ratio
SEAR	South East Asian Regional Office
VIDRL	Victorian Infectious Diseases Reference Laboratory
WHO	World Health Organization

Measles IgM proficiency panel 2002

Panel number: 01002

Introduction

As the world moves towards control of measles, confirmation of clinically diagnosed measles by IgM serology will become increasingly important. Proficiency testing is an important part of measles laboratory programs as both false positive and false negative results can occur with some of the commonly used measles IgM enzyme immunoassays (EIA).

Aims:

1. To assess the proficiency of laboratories within the WHO global measles laboratory network when testing for measles IgM.
2. To identify problems with any assays routinely used in these laboratories.
3. To check the accuracy of data reporting.

Methods

Panel composition

All samples were undiluted serum samples, comprising

10 Measles IgM positive (sourced from 1999/2001 measles outbreaks in Victoria, Australia)

5 Measles IgM negative (VIDRL staff volunteers)

3 Parvovirus IgM positive (Diagnostic sera)

2 Rubella IgM positive (Diagnostic sera)

All samples were negative for HIV, Hepatitis BsAg & Hepatitis C.

WHO Panel Results

PANEL 01002

Sample	Measles IgM	Rubella IgM	Diagnosis
01002001	Positive	Negative	Measles
01002002	Positive	Negative	Measles
01002003	Positive	Negative	Measles
01002004	Negative	Negative	Healthy volunteer
01002005	Positive	Negative	Measles
01002006	Positive	Negative	Measles
01002007	Negative	Positive	Rubella
01002008	Positive	Negative	Measles
01002009	Negative	Negative	Parvovirus
01002010	Negative	Negative	Healthy volunteer
01002011	Negative	Negative	Parvovirus
01002012	Negative	Negative	Healthy volunteer
01002013	Negative	Positive*	Healthy volunteer
01002014	Positive	Negative	Measles
01002015	Positive	Negative	Measles
01002016	Negative	Negative	Parvovirus
01002017	Positive	Negative	Measles
01002018	Negative	Negative	Healthy volunteer
01002019	Positive	Negative	Measles
01002020	Negative	Positive	Rubella

*False positive result

Table 1: Panel composition detailing measles and rubella IgM status of panel number

Validation of panel

The panel was tested for Measles IgM at VIDRL using two methods:

Dade Behring Enzygnost® anti-measles virus IgM

Chemicon Light Diagnostics Measles IgM Capture Enzyme Immunoassay

Rubella IgM was tested by two methods

Beckman Access Chemiluminescent Rubella IgM

DiaSorin ETI-RUBEK-M reverse PLUS capture assay

Distribution of panel

Results were returned from 65 laboratories.

WHO regions included:

EMRO

AFRO

SEAR

EURO

Each laboratory was assigned a unique number as results were received at VIDRL. This number is known only by VIDRL and that laboratory. Figure 1 shows the approximate site of the 65 laboratories that submitted results on the QA panel.

Laboratories participating in the measles proficiency panel-01002
NB: indicators are an approximate guide

Figure 1: The global distribution of laboratories that have submitted results.

Measles serology

Statistical Analysis

Analysis was performed by laboratory and panel number. The majority of participating laboratories (77%) used the Dade Behring Enzygnost® assay. Four laboratories used an in-house assay, one laboratory did not state the method used. The remaining 10 laboratories used a range of commercial kits (7 different methods). The proportion of correct results, based on the positive/negative interpretation reported by the laboratory, was calculated for each laboratory and according to the assay used. Results interpreted as equivocal were scored as incorrect since all sera included in the panel were clearly positive or negative, however this was not considered as serious as a false negative or false positive result.

The Dade Behring group

The laboratory assigned optical density (OD) values and interpretation (positive/negative) were recorded for each of the panel numbers. The positive/negative cut off was assumed to be 0.2 unless stated otherwise. OD values for all positive samples were combined for all laboratories and inspected for normality. This inspection was repeated separately for all negative samples and individually for each of the twenty panel numbers. Data were analysed using STATA 7.0 software.

As the OD values were normally distributed, the mean and standard deviation of the combined positive and combined negative OD values was calculated for each laboratory. The individual laboratory mean positive and mean negative OD value and corresponding standard deviation values were compared with the mean positive and mean negative OD value and corresponding standard deviation values calculated from combined positive and combined negative OD values for all laboratories using the Dade Behring assay method. Separate analysis of the positive and negative panel numbers, based on the laboratory designation was performed. Laboratory number 92 was excluded from this analysis since OD values were not recorded.

Laboratories were compared directly with each other and analysis of variance (ANOVA) was used to compare the mean results from each laboratory with those of other laboratories.

Other assay groups

Four laboratories used an in-house assay, two used the Microimmune assay and two the Seiken assay. The remaining four laboratories used a variety of assay methods. Comparisons between laboratories using assays other than Dade Behring Enzygnost® were not attempted since there were an insufficient number of laboratories using the same kit for meaningful analysis. The OD values obtained by laboratories using commercial EIA kits were plotted but no further comparisons were made.

Results

Reporting of kit details

Lot numbers

6 laboratories did not supply any lot or reagent details (One was an in-house assay).

1 laboratory did not state the method used or any kit details

1 laboratory reported the catalogue number instead of the lot number.

Expiry dates

One laboratory used a kit after the recorded expiry date.

6 laboratories did not record any expiry dates (4 of these were reported as in-house assays).

Results analysed by kit

Kit Details

One laboratory tested the panel using two separate assay methods resulting in 66 separate sets of results.

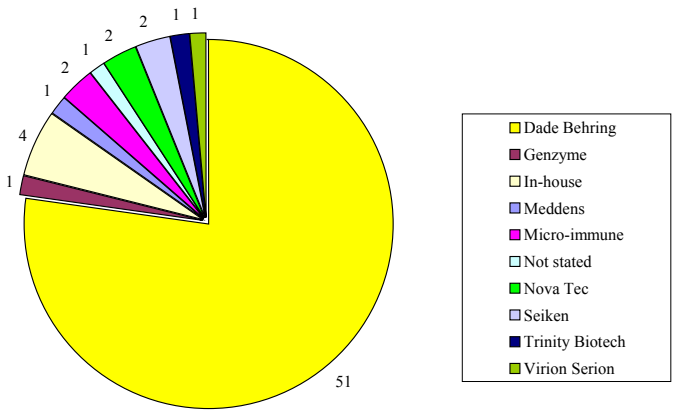

Figure 2: Distribution of kit type used for measles IgM testing of panel 01002.

Assay	WHO region			
	AFRO	EMRO	EURO	SEARO
Dade Behring	17	1	19	14
In-house assays			4	
Other commercial assays			10	
Not stated	1			
Total	18	1	33	14

Table 2 Type of assays used by WHO region.

Assay	Number of laboratories using assay	Number of samples correctly identified				
		20	19	18	17	15
Dade Behring	51	39	9	1	1	1*
In house assays	4	3	1			
Other/ not stated	11	3	5	2	1	

*1 laboratory only tested 18 samples

Table 3: Number of correct results by assay type.

Assay	Laboratories (n)	Proportion of laboratories with all positives correct	Proportion of laboratories with all negatives correct
Dade Behring	51	82%	90%
Other commercial kits/ not stated	11	64%	64%
In-house assays	4	100%	75%

Table 4: Proportion of laboratories correctly identifying all positive and negatives by assay type.

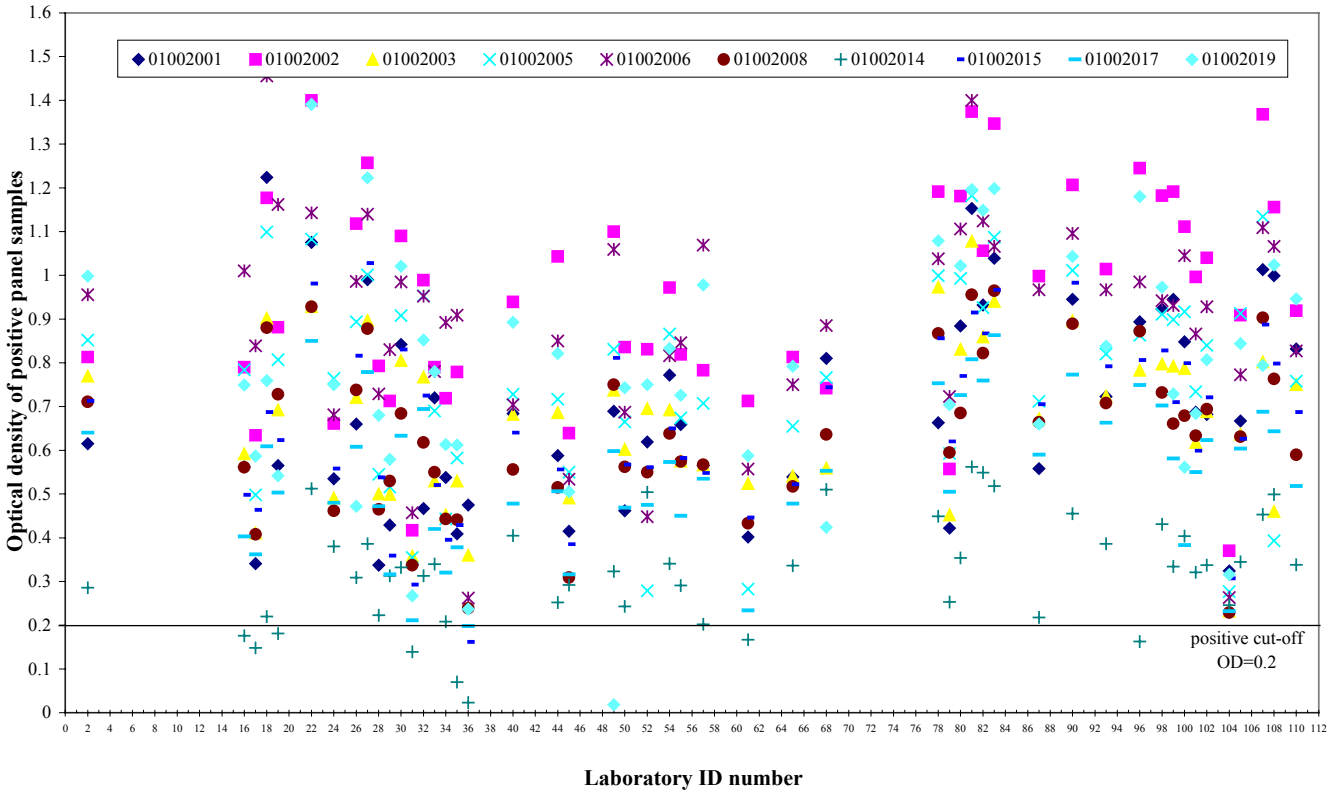

Figure 3: OD values for each positive sample by laboratory for laboratories using the Dade Behring assay.

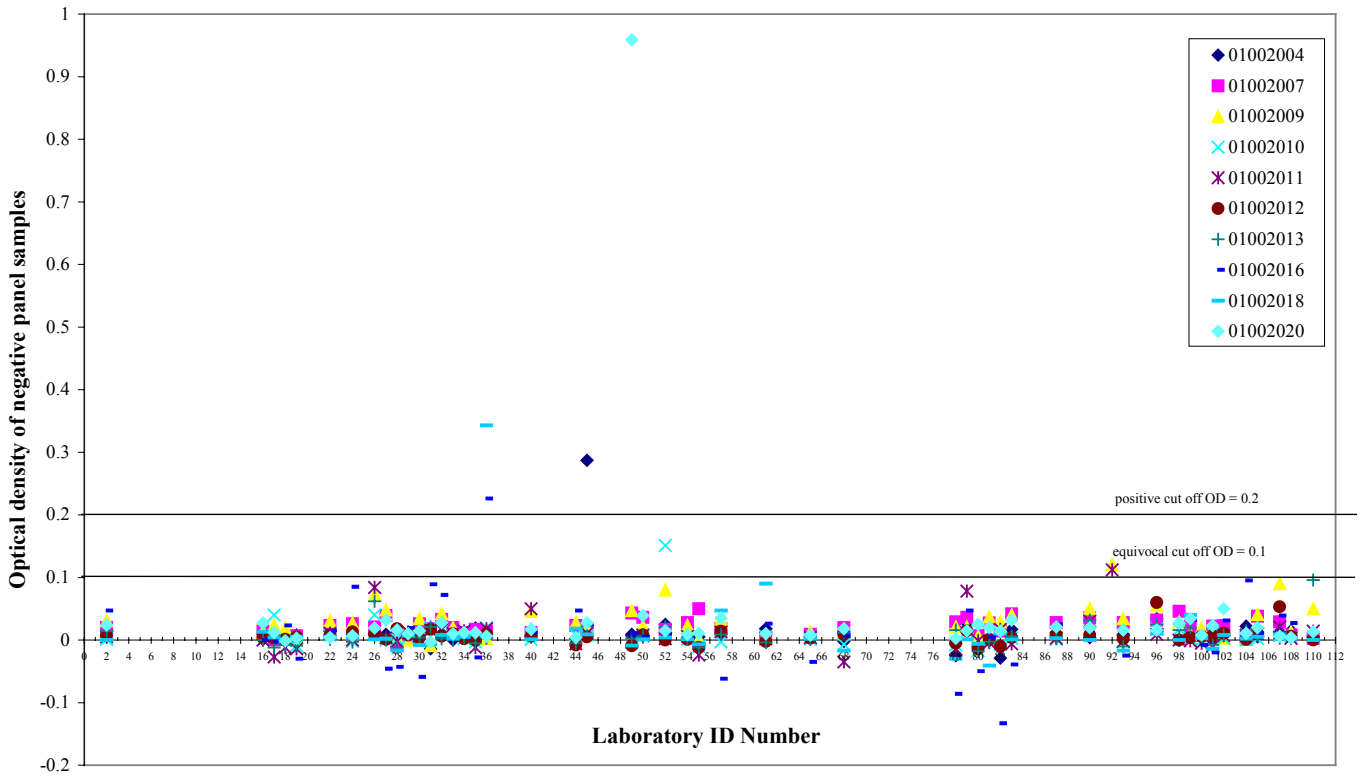

Figure 4: OD values for each negative sample by laboratory for laboratories using the Dade Behring assay.

Results analysed by panel number

Forty-five (45) laboratories achieved a perfect score (20/20).

One laboratory tested for measles IgM by 2 methods.

Score	Number of labs (%)
20/20	45 (68%)
19/20	15 (23%)
18/20	3 (4.5%)
17/20	2 (3%)
15/20	1 (1.5%)
TOTAL	66

Table 5: The proportion of participating laboratories achieving total scores based on positive/negative interpretation submitted to VIDRL.

Results by panel number

Panel no.	001	002	003	004	005	006	007	008	009	010
Measles IgM status	**P**	**P**	**P**	**N**	**P**	**P**	**N**	**P**	**N**	**N**
% correct	98.5	98.5	100	94	97	100	100	98.5	98.5	98.5

Panel no.	011	012	013	014	015	016	017	018	019	020
Measles IgM status	**N**	**N**	**N**	**P**	**P**	**N**	**P**	**N**	**P**	**N**
% correct	98.5	100	100	85	98.5	97	98.5	95.5	97	95.5

Table 6: Proportion of laboratories reporting correct result for each individual panel number.

Analysis of discrepant results

Panel no.	001	002	004	005	008	009	010
Measles IgM status	**P**	**P**	**N**	**P**	**P**	**N**	**N**
Positive			3				
Negative							
Equivocal	1		1	1		1	1
Not tested		1		1	1		
Total	***1***	***1***	***4***	***2***	***1***	***1***	***1***

Panel no.	011	014	015	016	017	018	019	020
Measles IgM status	**N**	**P**	**P**	**N**	**P**	**N**	**P**	**N**
Positive				2		3		3
Negative		4					2	
Equivocal	1	6	1		1			
Not tested								
Total	***1***	***10***	***1***	***2***	***1***	***3***	***2****	***3****

***** One laboratory tested the panel by 2 methods and reversed samples 19 and 20.

Table 7: Result classification for panel samples not correctly identified by reporting laboratories for measles IgM.

Rubella serology

Twenty laboratories tested the whole panel for Rubella IgM, and twenty-eight laboratories tested between 8 and 11 samples.

Kit Details

Two laboratories used kits after the recorded expiry date.

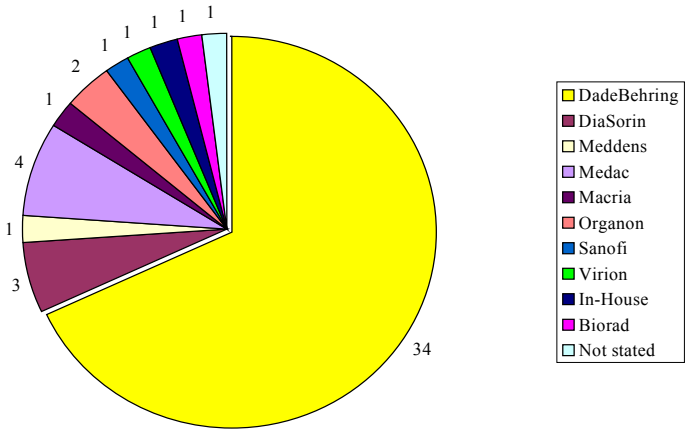

Figure 5: Distribution of kit type used for rubella IgM testing of panel 01002.

Analysis of discrepant results

Panel No.	005	006	009	010	011	016	019
Diagnosis	Measles	Measles	Parvovirus	Healthy adult	Parvovirus	Parvovirus	Measles
Rubella IgM POS	1	2			1		1*
Rubella IgM EQUIV			1	1	4	1	1

* Sample 019 & 020 were reversed by the testing laboratory

Table 8: Details of result classification for those panel samples which were not correctly identified by reporting laboratories for rubella IgM.

Discussion

Measles

The panel was distributed to National and Regional Measles reference laboratories within the WHO global measles laboratory network and 65 laboratories returned results for analysis. The results overall were very encouraging. A score of 100% was achieved by 68% of laboratories. Ninety-six percent of laboratories achieved a score of 90% or greater.

The majority of participants (77 %) used the Dade Behring Enzygnost® anti-measles-virus IgM assay for measles diagnosis, facilitating analysis of the variation of reactivity of samples for these laboratories. The number of users of other kits was too few for any meaningful statistical analysis.

Overall there were thirty-four aberrant results, 14 were reported as equivocal. Ideally all equivocal results should be repeated however the limited volume of sample provided to each laboratory may have prevented full investigation of these samples.

Of the remaining twenty aberrant results three samples were not tested, two samples were incorrectly reported twice since the reporting laboratory incorrectly labelled samples numbers 019 and 020 and used two testing methods.

Thus thirteen incorrect results were submitted on 5 panel numbers (004, 014, 016, 018, 020). The true status of sample 014 was measles IgM positive, the remaining four samples were measles IgM negative. Sample 014 which 4 laboratories reported measles IgM negative, was collected 5 days post onset of rash. A nose and throat swab collected at the same time was positive for measles virus RNA by RT- PCR.

Sample 004, collected from a healthy volunteer with no clinical illness was reported by three laboratories as IgM positive. Two of these laboratories used the Seiken assay and one laboratory used the Dade Behring assay.

Three laboratories reported sample 018, also from a normal healthy adult, as positive. Two laboratories used the Novatec assay and one laboratory used the Dade Behring assay. Two other samples were falsely reported as containing measles IgM, one of which was parvovirus IgM positive (sample 016), the other rubella IgM positive (sample 020). These results demonstrate the importance of confirmatory testing when determining measles diagnosis.

Good data reporting is just as important as obtaining the correct result. One laboratory did not submit OD values for the samples tested and 8 laboratories did not submit the cut-off values.

Rubella

While this QAP panel was not composed with rubella proficiency testing in mind, it has provided an opportunity for some comparative rubella serological testing on well characterised specimens across the global laboratory network. The number of panel specimens tested for rubella IgM varied as some laboratories tested all specimens, and others only those specimens negative on measles IgM testing.

Sample 013 has been determined to be an unsatisfactory sample for rubella IgM QAP testing since it has been found to be IgM positive by a number of commercial kits but is not from a patient with a clinical illness. This sample was excluded from further analysis. Seventy-two percent of laboratories reported all tested samples correctly, and the remaining laboratories reported only one incorrect result (apart from one laboratory that tested for IgG instead of IgM).

Most of the aberrant results (8) were in the equivocal range. Ideally all equivocal results should be repeated however the limited volume of sample provided to each laboratory may have prevented full investigation of these samples.

Five results from four panel samples (005, 006, 011 & 019) were reported as positive (one laboratory reversed samples 019 & 020). The four other samples were measles IgM positive (3) and parvovirus IgM positive (1).