VIDRL & WHO

Measles IgM Proficiency Panel 00801

Final Report

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Abbreviations

AFRO	African Regional Office
ANOVA	Analysis of variance
CDC	Centers for Disease Control and Prevention
EMRO	Eastern Mediterranean Regional Office
EURO	European Regional Office
N	Negative
OD	Optical density
P	Positive
QA	Quality assurance
S/CO	Sample/cut-off ratio
SEAR	South East Asian Regional Office
VIDRL	Victorian Infectious Diseases Reference Laboratory
WHO	World Health Organisation
WPRO	Western Pacific Regional Office

Measles IgM proficiency panel 2001

Panel number: 00801

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).

Aim

The aims of this panel are to assess the proficiency of laboratories in the WHO laboratory network testing for measles IgM and to identify problems with any assays routinely used in these laboratories and also check the accuracy of data reporting.

Methods

Panel composition

All samples were undiluted serum samples, comprising

10 Measles IgM positive (sourced from 1999 measles outbreak in Victoria, Australia)

5 Measles IgM negative (VIDRL staff volunteers)

2 Parvovirus IgM positive (Diagnostic sera)

2 Rubella IgM positive (Diagnostic sera)

1 Dengue IgM positive (supplied by the WHO Arbovirus Reference Lab, Qld. Australia)

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

Table 1 details the composition of the panel by sample number and validated result of testing.

WHO Panel Results

PANEL 00801

Sample	Measles IgM	Rubella IgM	Status
00801001	Positive	Negative	MEASLES IgM POSITIVE
00801002	Positive	Negative	"
00801003	Positive	Negative	"
00801004	Positive	Negative	"
00801005	Positive	Negative	"
00801006	Positive	Negative	"
00801007	Positive	Negative	"
00801008	Positive	Negative	"
00801009	Positive	Negative	"
00801010	Positive	Negative	"
00801011	Negative	Positive	MEASLES IgM NEGATIVE
00801012	Negative	Negative	"
00801013	Negative	Negative	"
00801014	Negative	Negative	"
00801015	Negative	Negative	"
00801016	Negative	Negative	PARVO IgM POSITIVE
00801017	Negative	Negative	"
00801018	Negative	Positive	RUBELLA IgM POSITIVE
00801019	Negative	Positive	"
00801020	Negative	Negative	DENGUE IgM POSITIVE

Table 1: Panel composition detailing IgM status of panel number

Validation of panel

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

Dade Behring Enzygnost anti-measles virus IgM

Chemicon Light Diagnostics Measles IgM Capture Enzyme Immunoassay

Before general distribution the panel was tested by CDC, Atlanta USA and the Central Public Health Laboratory, Colindale UK. The results obtained by the other two laboratories confirmed those obtained by VIDRL.

Distribution of panel

Results were returned from 46 laboratories (including CDC& PHLS)

WHO regions included:

EMRO

WPRO

AFRO

SEAR

EURO

Each laboratory was assigned a dedicated 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 46 laboratories (and VIDRL) who submitted results on the QA panel.

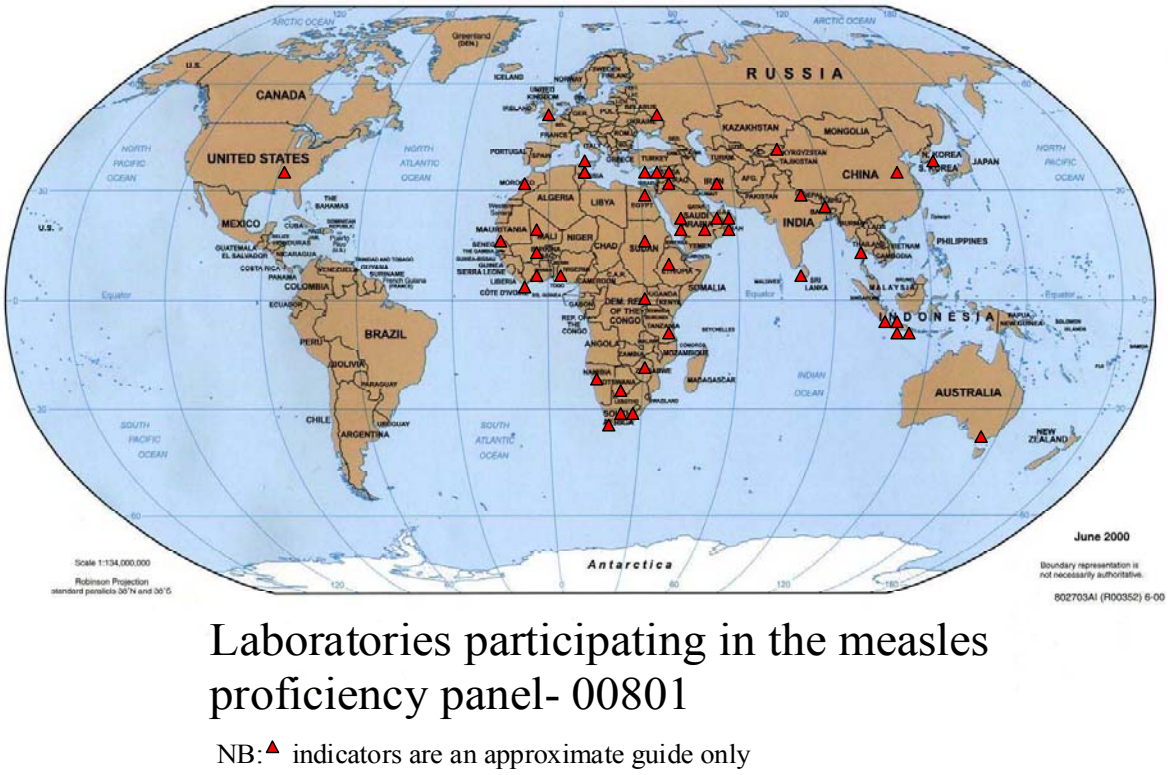

Figure 1: The global distribution of the laboratories that have submitted results.

Statistical Analysis

Analysis was performed by laboratory and panel number. A range of kits was used in participating laboratories and detailed analysis was performed by grouping kits. These groups were laboratories using the Dade Behring kit (n=34), laboratories using Chemicon/Light Diagnostics (n= 4), in-house kits derived from CDC (n=5) and laboratories using other commercial or unspecified kits (n=7). One laboratory used an RIA kit and these results were not analysed further. The proportion of correct results, based on the positive/negative interpretation reported by the laboratory, was calculated by laboratory and according to kit used.

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 OD values was calculated for each laboratory. Individual laboratory mean OD and standard deviation values were compared with the mean and standard deviation calculated from combined data for all panel numbers and all laboratories. Separate analysis of the positive and negative panel numbers, based on the laboratory designation was performed. Laboratories with extreme results on inspection of the data were removed from the calculation of the mean positive and negative OD values and panel number 00801006 was removed as it was incorrectly identified by 20% of the laboratories.

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 kit groups

Those laboratories using the Chemicon/ Light Diagnostic assay method and the CDC in-house method used different cut off values. A sample to cut-off (S/CO) ratio was therefore calculated for these laboratories and the mean of this ratio was compared for laboratories using these assay methods.

The remaining eight laboratories used a variety of assay methods. The OD values obtained by laboratories using commercial EIA kits was plotted but no further comparisons were made.

Results

Reporting of kit details

Lot numbers

13 laboratories did not supply any lot or reagent details (8 were reported as in-house assays).

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

Expiry dates

Two laboratories used kits that were past the expiry date.

13 laboratories did not supply any expiry dates (8 were reported as in-house assays).

Results analysed by kit

Kit Details

Figure 2 shows the number and type of kits used by the 46 participating laboratories. Five laboratories used 2 assays resulting in 51 separate sets of results.

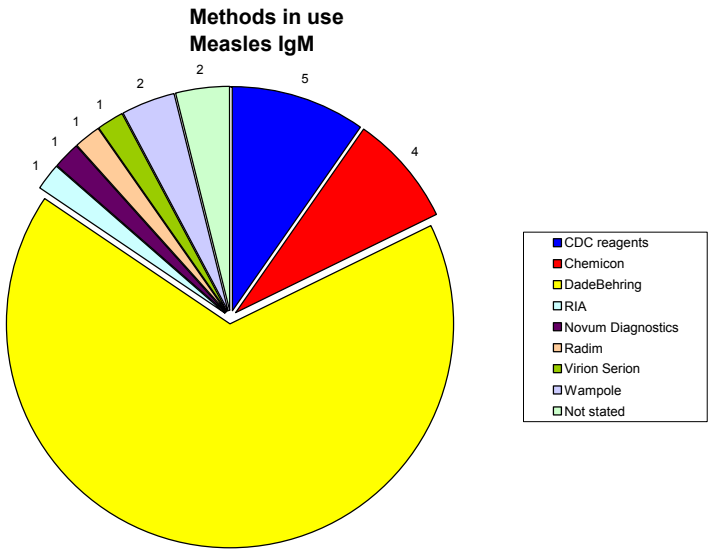

Figure 2: The breakdown of kits used in testing the panel.

Table 2 demonstrates the number of correct results by kit.

KIT	NUMBER OF LABS USING KIT	NUMBER OF SAMPLES CORRECTLY IDENTIFIED				
		20	19	18	17	15
CDC Reagents (In-house assay)	5*	4				
Chemicon	4	3		1		
Dade Behring	34	23	10		1	
In house RIA	1		1			
Other/ not stated	7	3	2	1		1

- *1 laboratory only tested 16 samples
- The ten laboratories that scored 19 using the Dade Behring kit all reported panel number 00801006 as negative or equivocal.

Table 2: The kits used versus the number of correct results that each laboratory achieved.

Kit Method Used	Number of laboratories using kit (n)	Proportion of laboratories with all positives correct	Proportion of laboratories with all negatives correct
Dade Behring	34	76%	91%
Other commercial kits	9	89%	56%
In-house EIA kits	5	100%	80%
Not stated/ RIA	3	100%	33%

Table 3: The proportion of Laboratories correctly identifying all positive and negatives by kit method used.

73% of aberrant positive results were reported as equivocal.

47% of aberrant negative results were reported as equivocal.

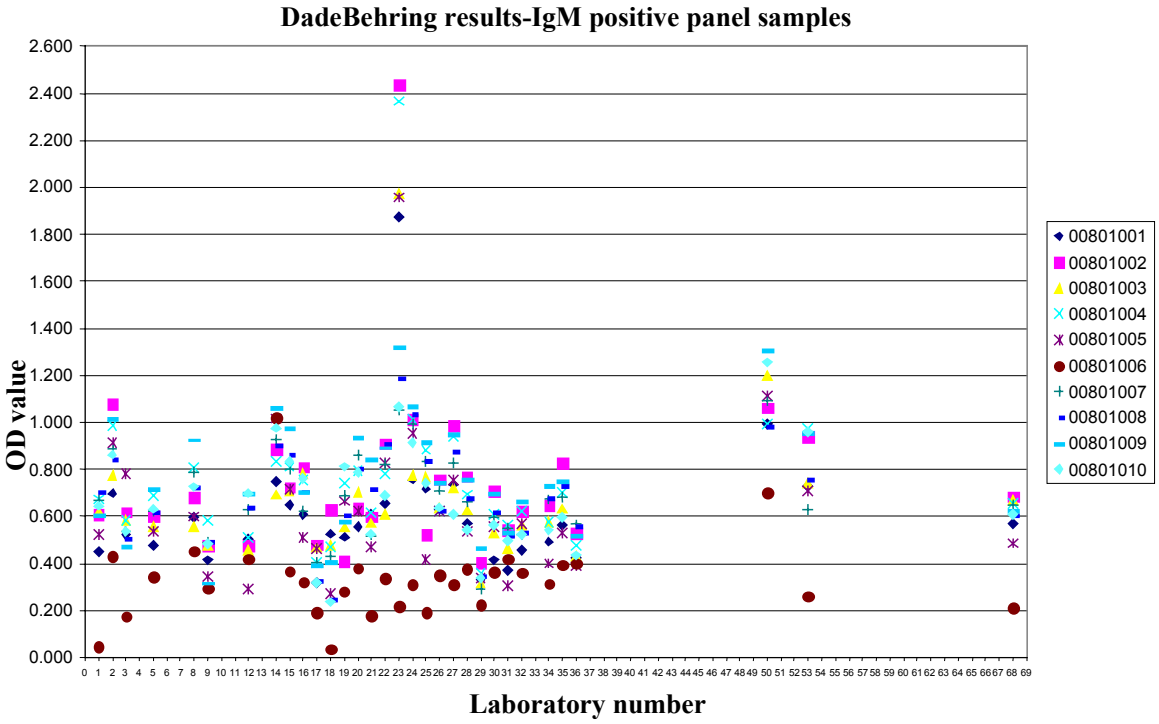

Figure 3: OD values for each positive panel sample by laboratory for laboratories using the Dade Behring kit. The cut-off is 0.2 OD units.

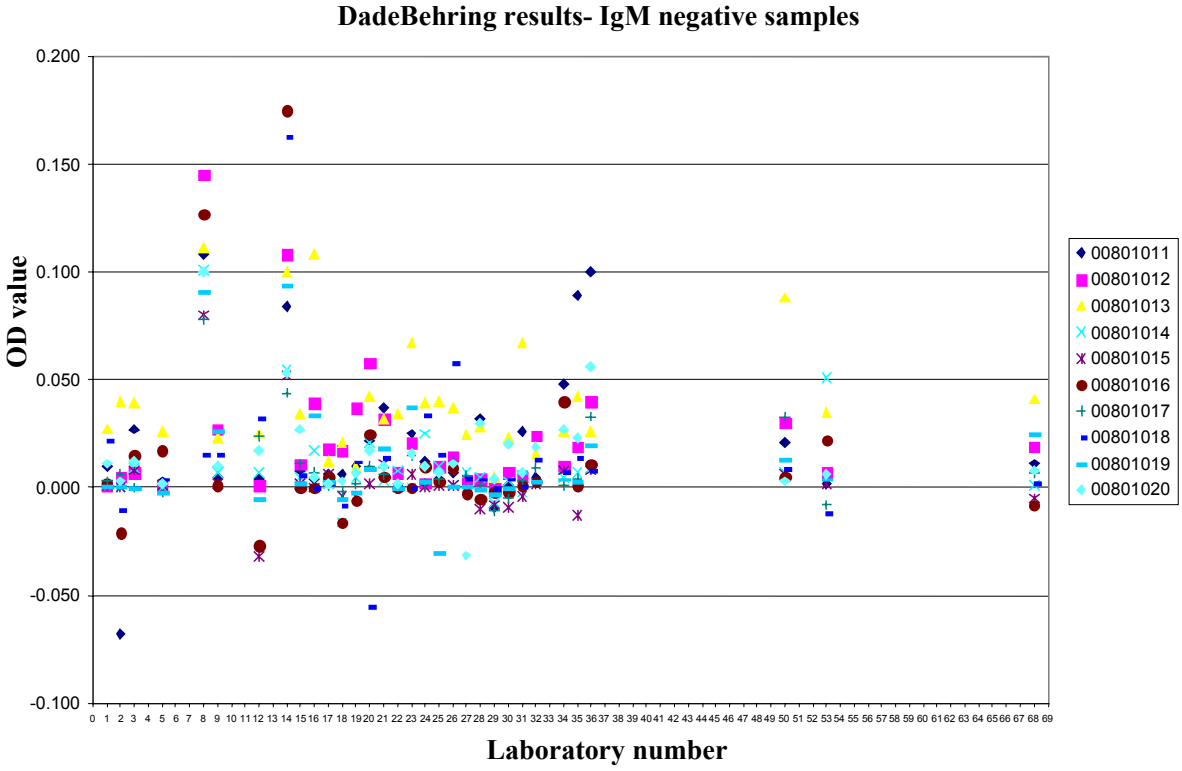

Figure 4: OD values for each negative panel sample by laboratory for laboratories using the Dade Behring assay. The cut-off is 0.2 OD units, a true negative is <0.1OD units and equivocal range 0.1- 0.2 OD units.

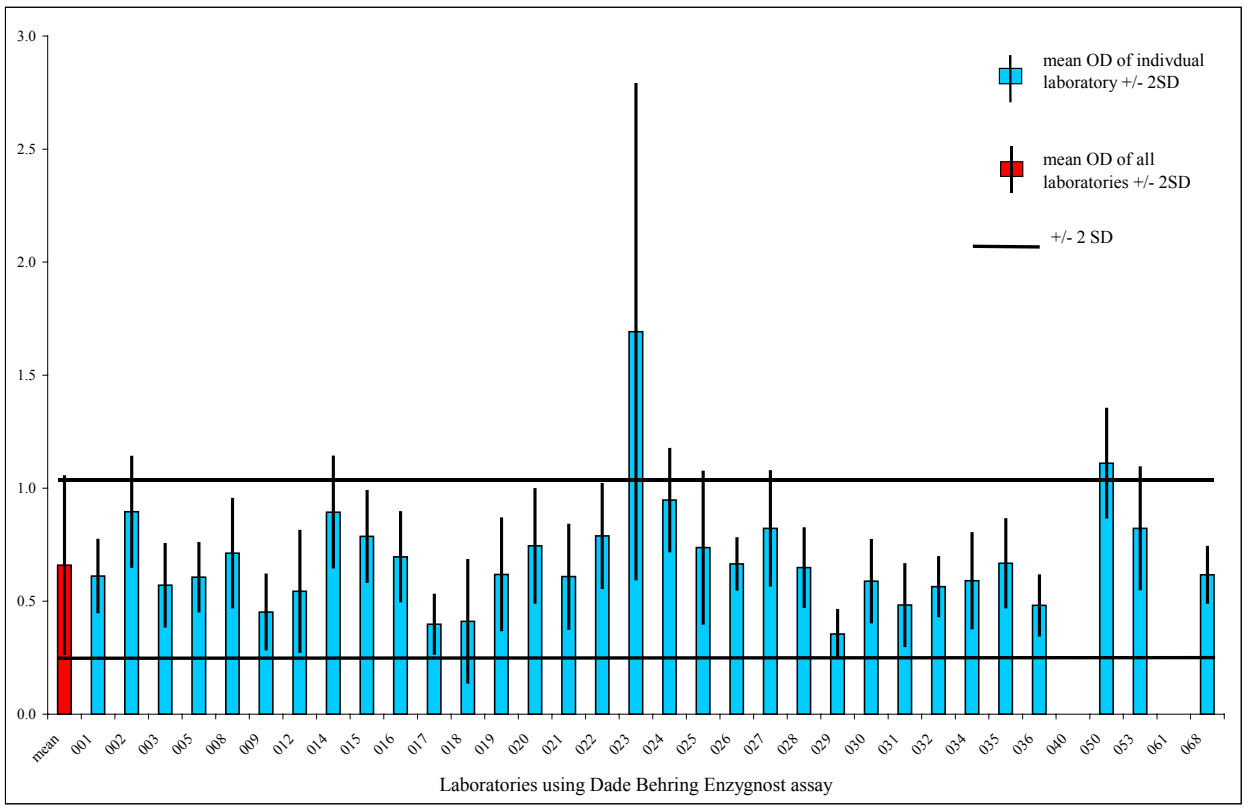

Figure 5: Mean OD value +/- 2SD for all positive panel samples for all laboratories combined and for each individual laboratory using the Dade Behring assay.

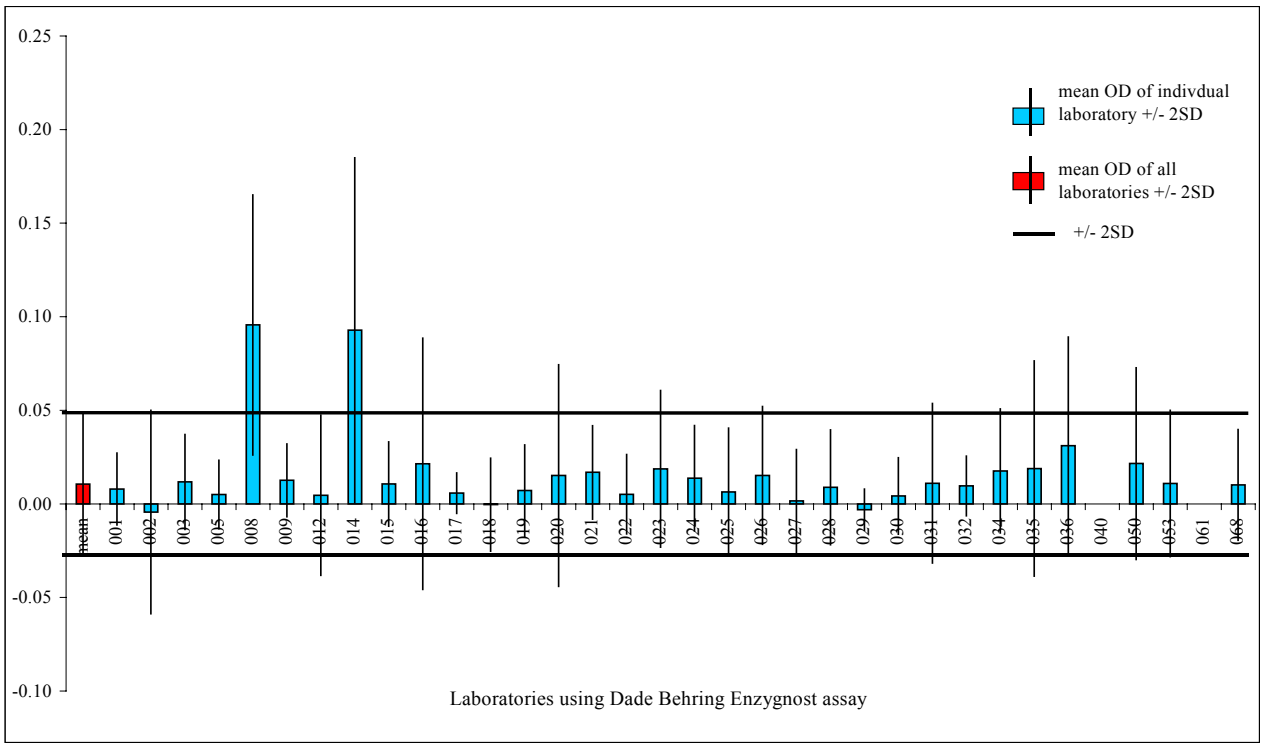

Figure 6: Mean OD value +/- 2SD for all negative panel samples for all laboratories combined and for each individual laboratory using the Dade Behring assay.

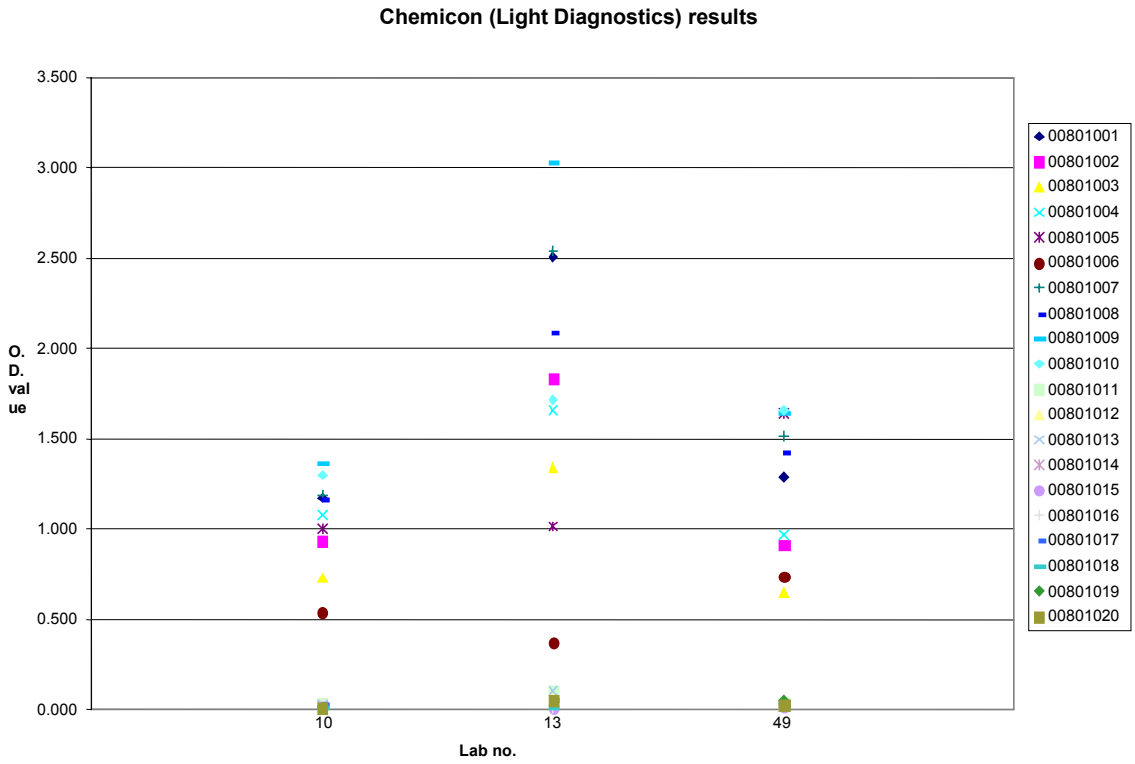

Figure 7: OD values of all panel samples for laboratories using the Chemicon / Light Diagnostics assay.

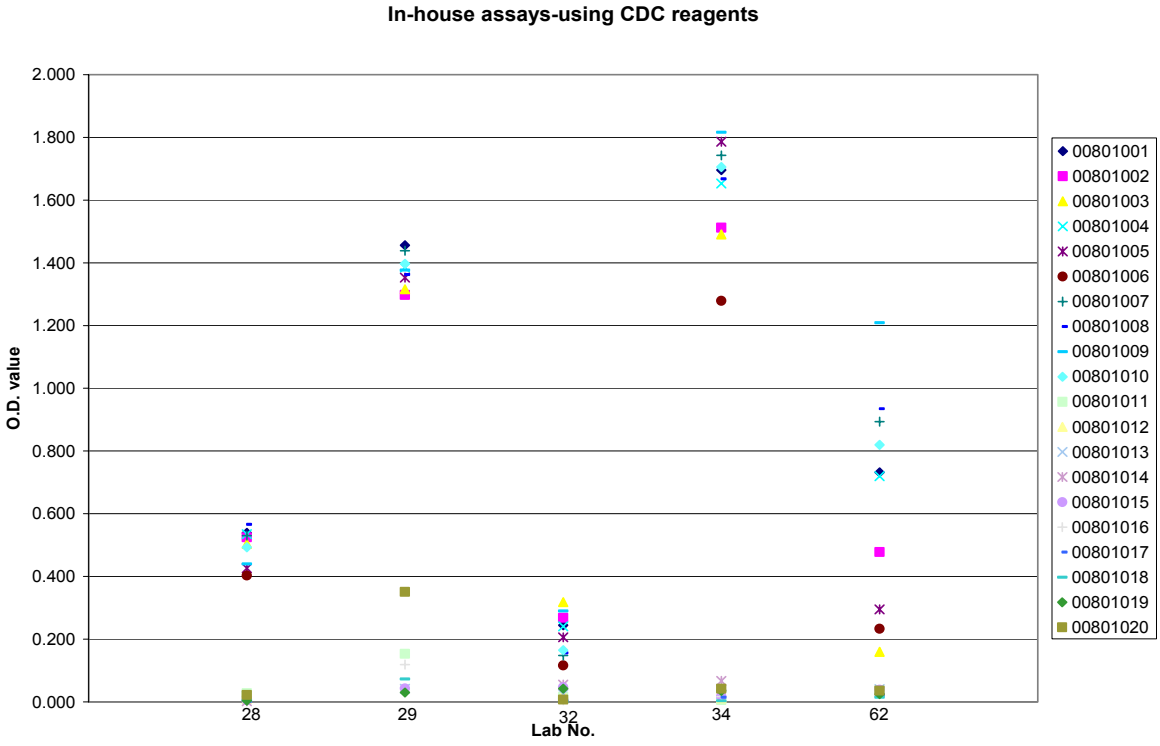

Figure 8: OD value of all panel samples for laboratories using CDC reagents in an in-house assay.

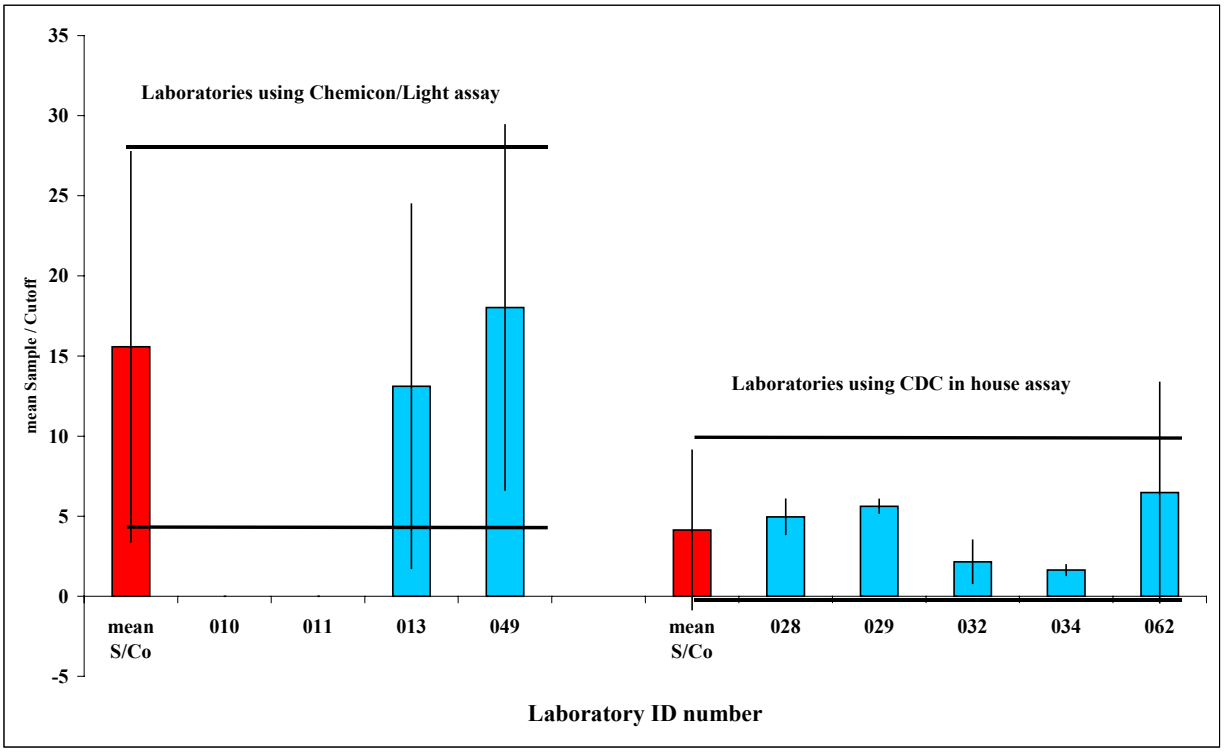

Figure 9. Mean sample/cutoff ratio +/- 2SD for combined positive samples for all laboratories using either Chemicon/Light Diagnostics or CDC in-house assay method and mean sample/cutoff ratio for each individual laboratory.

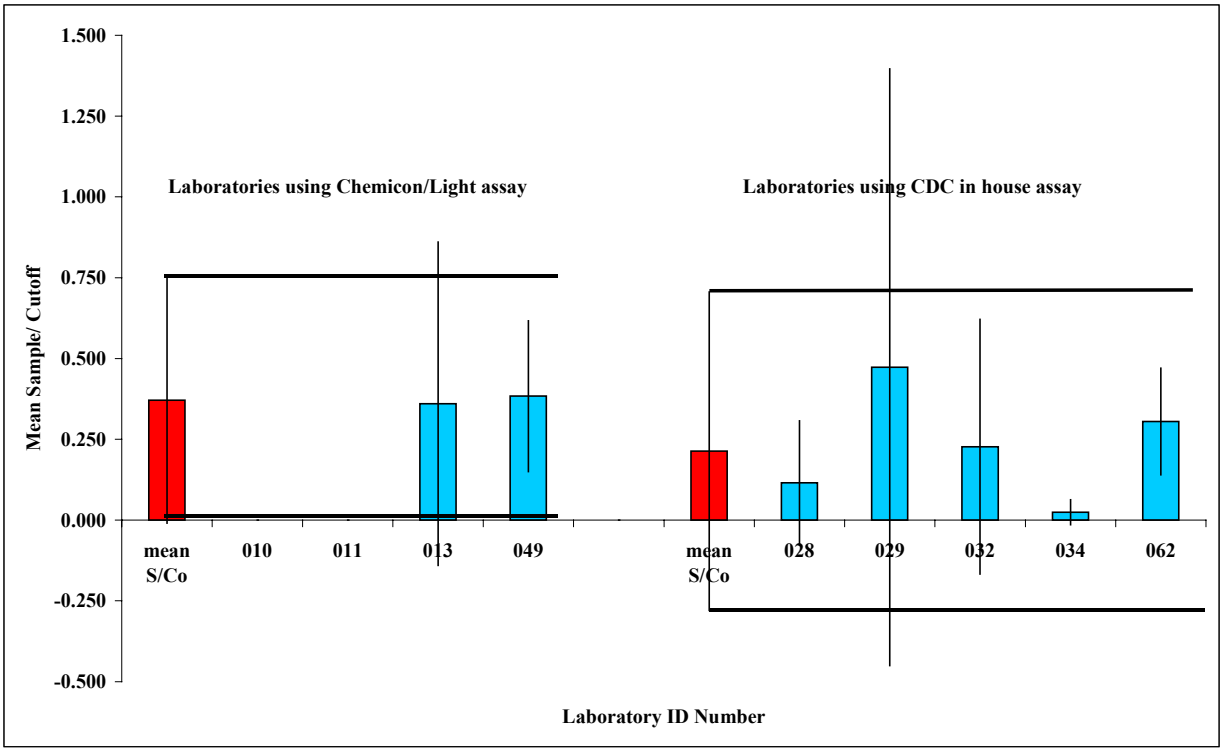

Figure 10. Mean sample/cutoff ratio +/- 2SD for combined negative samples for all laboratories using either the Chemicon/Light Diagnostics or CDC in-house assay method and mean sample/cutoff ratio for each individual laboratory.

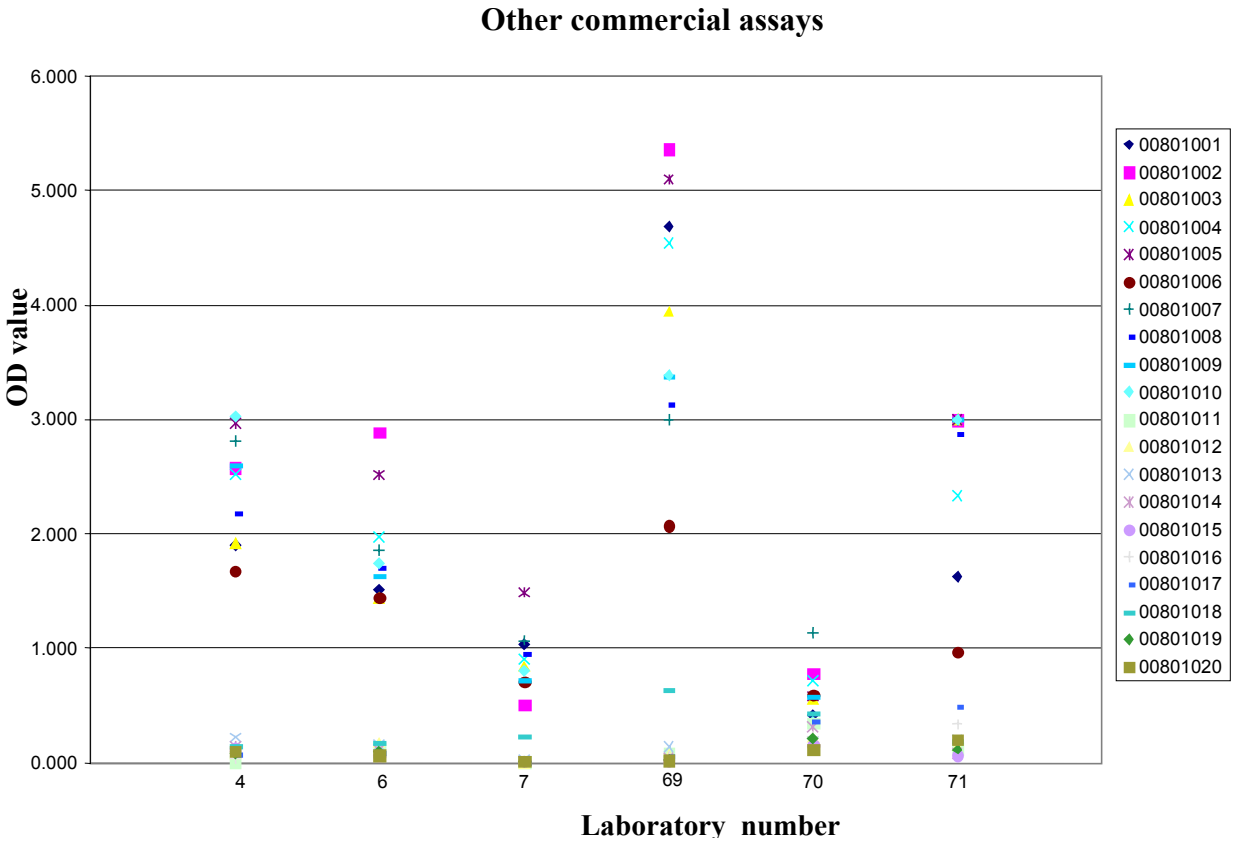

Figure 11: OD value of all panel samples for remaining laboratories using a selection of commercial assays other than Dade Behring or Chemicon.

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Results analysed by panel number

Thirty (30) laboratories achieved a perfect score (20/20).

Five laboratories tested for measles IgM by 2 methods.

SCORE	NUMBER OF LABS
20/20	30
19/20	12
18/20	2
17/20	1
15/20	1
TOTAL	46*

Table 4: Scores achieved by participating laboratories assessed by the P/N interpretations as returned to VIDRL. Of the 5 laboratories reporting two methods only two had discrepancies between the method. *When two methods were used the highest score was used.

Results by panel number

Panel no.	001	002	003	004	005	006	007	008	009	010
Number correct	51 100%	51 100%	51 100%	51 100%	50 98%	41 80%	51 100%	50 98%	50 98%	51 100%
Measles IgM status	P	P	P	P	P	P	P	P	P	P

Panel no.	011	012	013	014	015	016	017	018	019	020
Number correct	46 90%	50 98%	48 94%	49 96%	51 100%	51 100%	48 94%	49 96%	51 100%	49 96%
Measles IgM status	N	N	N	N	N	N	N	N	N	N

Table 5: Number (percentage) of laboratories reporting correct result for each individual panel number.

Analysis of discrepant results

Panel no.	005	006	008	009	011	012	013	014	017	018	020
Measles IgM status	P	P	P	P	N	N	N	N	N	N	N
Positive					2			1	2	2	1
Negative		3									
Equivocal	1	6		1	3		3				1
Not tested		1	1			1		1	1		
Total	***1***	***10***	***1***	***1***	***5***	***1***	***3***	***2***	***3***	***2***	***2***

Table 6: Details of result classification for those panel samples which were not correctly identified by reporting laboratories.

Panel numbers **006** and **011** were most frequently assigned incorrectly. The history of these samples is outlined below:

Patient data panel number **006**

Young adult – laboratory confirmed measles

	Measles IgG	Measles IgM	Measles PCR
15/03/99	0.02 negative	Negative	
18/03/99	0.11 equivocal	0.68 positive	
23/03/99*	***0.23 positive***	***0.62 positive***	***positive***

*Sample 006 in panel

Table 7: The serological profile of panels number 00801006. This sample gave the most varied results in the measles IgM positive group.

Patient data panel no**. 011**

Healthy adult, no clinical illness

Measles IgG positive and IgM negative

Discussion

The panel was distributed globally to National and Regional Measles reference laboratories within the WHO measles network and 46 laboratories returned results for analysis.

The results overall were very encouraging. A score of 100%was achieved by 65% of laboratories. Ninety-six percent of laboratories achieved a score of 90% or greater. The majority of participants (67 %) used the Dade Behring Enzygnost IgM measles kit for investigating measles so we were able to analyse the variation of reactivity of samples for these laboratories. The number of users of other kits was too small for any meaningful statistical analysis.

For the laboratories using Dade Behring kits for measles IgM, one false positive IgM result was reported for the negative samples by laboratory 61. This laboratory did not submit any sample data, only an interpretation of positive or negative, so we were unable to confirm if it was a transcription error or a true false positive. There were also two results reported in the equivocal range.

For the measles positive samples all negative and equivocal results reported were specimen no. 00801006, except for laboratory 9 who reported three equivocal results quoting a cut-off value not consistent with the manufacturer's recommendation. Sample 00801006 was a young adult with laboratory confirmed measles. The sample used in the panel was collected eight days after onset of illness. Sixty percent of aberrant results were actually reported as equivocal. Ideally all equivocal results should be repeated but unfortunately due to the number of participants in the program we were unable to supply volumes large enough to allow repeat testing.

Four labs reporting equivocal results reported OD values very close to the cut-off. One lab reported an equivocal result but their value stated is actually over the cut-off value for positive results. Another laboratory using a Dade Behring kit reported an OD of 0.295 usually considered positive with a stated cut-off of 0.20, but their cut-off was 0.357. Perhaps a review into the reason for adjusting the cut-off is required. Overall the

aberrant results were in a 10% range of the cut-off or due to variation from the manufacturer's recommended interpretation of the values obtained.

The users of alternative assays to Dade Behring performed well. Equivocal results were the main reason that a perfect score was not obtained. The exception was laboratory number 70 which reported four false positive results. A review of the kit in use and/or laboratory technique would be advisable.

Four laboratories out of seventeen in this group reported a total of 5 samples as Measles IgM false positive. These samples were:

2 Parvovirus IgM positive samples
2 Rubella IgM positive samples
1 Dengue IgM positive sample

This highlights the need for careful interpretation of laboratory results in a routine diagnostic situation and also the necessity of using a kit or in-house assay, which has a high level sensitivity and specificity in the hands of that laboratory.
Good data reporting is just as important as obtaining the correct result. Five laboratories did not supply any values for the samples tested and 8 laboratories did not supply the cut-off values.