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Standardization of measles, mumps and rubella assays to enable comparisons of seroprevalence data across 21 European countries and Australia

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SUMMARY

The aim of the European Sero-Epidemiology Network is to establish comparability of the serological surveillance of vaccine-preventable diseases in Europe. The designated reference laboratory (RL) for measles, mumps, rubella (MMR) prepared and tested a panel of 151 sera by the reference enzyme immunoassay (rEIA). Laboratories in 21 countries tested the panel for antibodies against MMR using their usual assay (a total of 16 different EIAs) and the results were plotted against the reference results in order to obtain equations for the standardization of national serum surveys. The RL also tested the panel by the plaque neutralization test (PNT). Large differences in qualitative results were found compared to the RL. Well-fitting standardization equations with $R^2 \geqslant 0{\cdot}8$ were obtained for almost all laboratories through regression of the quantitative results against those of the RL. When compared to PNT, the rEIA had a sensitivity of 95·3%, 92·8% and 100% and a specificity of 100%, 87·1% and 92·8% for measles, mumps and rubella, respectively. The need for standardization was highlighted by substantial inter-country differences. Standardization was successful and the selected standardization equations allowed the conversion of local serological results into common units and enabled direct comparison of seroprevalence data of the participating countries.

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INTRODUCTION

Surveillance of population immunity is an important component in the evaluation of intervention programmes as it allows an assessment of the past and the current risk of infectious diseases in a population. This is particularly relevant for vaccine-preventable diseases for which alternative vaccination strategies may be employed. When vaccination coverage is suboptimal, susceptible individuals accumulate, which can lead to future outbreaks. Serological surveys allow the early identification of susceptible cohorts, allowing targeted interventions, e.g. catch-up campaigns to be undertaken [1–4]. Serological surveys can also be used to evaluate the impact of campaigns and ascertain elimination status [5–7]. The aim of the European Sero-Epidemiology Network (ESEN2) project was to coordinate and harmonize the serological surveillance of immunity to a variety of vaccine-preventable infections in Europe in order to improve vaccination policies and strategies. ESEN2 was started in 2001 and is the continuation of the successful original project ESEN [8] by extending the coordination of serological surveillance to more diseases (as well as diphtheria, pertussis, measles, mumps, and rubella also included are hepatitis A and B, and varicella zoster) and to more countries (22 instead of the original eight).

For epidemiological purposes the enzyme immunoassay (EIA) is the widely used method to detect antibodies to measles, mumps, and rubella in serum. However variations in both the sensitivity and specificity of several commercial EIAs have been reported as well as inter-laboratory variability when the same assay is used [9–11]. Standardization of assays is a pre-condition to ensure direct comparability of any seroepidemiology results obtained during the project. In this paper the results of standardizing the measles, mumps and rubella (MMR) assays are presented.

MATERIALS AND METHODS

Standardization procedure

The method used for standardization is given in detail by Kafatos *et al*. [12] and was similar to that used for the first ESEN project [11]. In brief the aim was to construct a reference panel containing enough sera (~150) to represent the antibody values with high, low, equivocal and negative levels for MMR. The panels were distributed to and tested by a laboratory in each of the participating countries using their established antibody detection method. The same method had to be used for testing the national serum bank which was collected by each country according to the project guidelines [8].

After the first round of testing of the reference panel the quantitative results were plotted against those of the reference laboratory (RL). For the purpose of the analysis, results above the upper detection limit were doubled and those below the lower detection limit were halved [11]. Outliers were identified and a linear, quadratic or sigmoid regression line fitted and the proportion of the variance explained by different regression lines was calculated (R^2).

For each country agreement of the non-standardized qualitative results with those of the RL was calculated. Agreement within negatives is defined as the percentage of the RL negative samples (e.g. $n=28$ for measles antibodies) each laboratory found negative, agreement within positives is the percentage of the RL positive or equivocal samples (e.g. $n=123$ for measles antibodies) found positive or equivocal in each laboratory, and overall agreement is the percentage of the 151 samples where each laboratory gave the same qualitative result as the RL.

The reference panel was tested for a second time during testing of the national serum bank. The results from this second round were compared with the results of the first test to identify potential assay drift [12]. The choice of the standardization equation was based on the results that were obtained during testing the national serum bank [12]. To successfully obtain standardized results the chosen regression line should fit the data well around the equivocal range and should ideally explain at least 80% of the variance ($R^2 > 0{\cdot}8$). The coordinating centre to whom all data were sent for calculation and evaluation was the Health Protection Agency Centre for Infections, UK.

Countries that tested their national serum bank more than one year prior to the start of the project required a different approach for standardization called back-standardization [12]. A panel of about 150 sera was selected randomly from the national serum bank of those countries using back-standardization after stratifying the samples by result (positive, equivocal, negative). This national panel was tested by the RL as well as by the laboratory in the country concerned. The results were processed as described above.

Overall 22 countries took part in the standardization of the measles, mumps, and rubella results

(Table A1, available in the online Appendix). Four countries (Finland, Israel, Spain, Sweden) had already completed the testing of their national serum bank more than 1 year prior to back-standardization being performed (Finland only for mumps). Luxembourg and the Czech Republic had also already tested their national serum banks, however, as this was $\leqslant 1$ year ago and they were still using the same assays back-standardization was not required. Nevertheless these countries retested a panel of the national serum bank to confirm that no major changes of the results had occurred. These countries only tested the reference panel once as did Greece and The Netherlands who participated only for the purpose of method comparison of all three antigens or of selected antigens (Australia for mumps and rubella, Finland for measles and rubella, Spain for rubella).

Preparation of the reference panel

The preparation and distribution of the reference panel was undertaken by the reference laboratory, the National Reference Centre on Measles, Mumps, Rubella (NRC MMR) at the Robert Koch Institute, Berlin, Germany. The panel consisted of 151 samples. The majority of samples (111) were collected in 2001 by the PHLS in Preston from students in the North West of England. Between 4·2 ml and 9·5 ml of sera were obtained from each student, most of whom had immunity against measles, mumps and rubella acquired either through natural infection or by vaccination. As an inadequate number of negative sera were collected, 16 samples were created from 42 children (pooling 2–3 sera) whose sera were negative for measles, mumps and/or rubella antibodies. The remaining 24 samples of the panel were prepared using the 2nd International Standard for anti-measles serum, human (66/202), the 2nd British Standard for anti-rubella serum, human (67/182) and the 2nd Working Standard for anti-mumps serum (3/2000) and seven dilutions of these standards. The latter standard was prepared in the RL using a blood donation of a patient with acute mumps and it provided 600 U after calibration against the 1st mumps Working Standard [11].

The sera of the reference panel were tested by the RL four times in 2001 using commercial EIAs (Enzygnost, Dade Behring, Germany), which provide quantitative antibody values for measles and rubella in IU/ml and for mumps in titres. The mean antibody values were calculated and the samples classified as negative, equivocal, and positive (Table A2, available online). Aliquots of 0·2 ml of all 151 sera and standard dilutions were prepared, stored at $-25\,^{\circ}$C until shipment to the laboratories in the participating countries.

Serological methods

The RL used commercial EIAs to detect virus-specific IgG against measles, mumps and rubella (Enzygnost) in the samples of the reference panel. The tests were performed according to the manufacturer's instructions, allowing quantification by measuring the optical density (OD) of a single serum dilution (1:231) of the antigen and control antigen also. The difference of these ODs (ΔOD) corrected by an internal control were used to evaluate the qualitative result according to the following cut-off values: OD $<0{\cdot}100=$ negative, OD 0·100–0·200 = equivocal, OD $>0{\cdot}200=$ positive. Quantitative specific antibody values (SAV) were calculated with the equation: $\log_{10}\ \mathrm{SAV} = \alpha * \Delta\mathrm{OD}^{\beta}$, where α and β were constants specific for the reagent's batch [13]. The SAV according to the defined cut-off were considered as shown in Table A2 (online).

The laboratories of the 22 participating countries used different commercial as well as in-house EIAs providing quantitative values which started at different detection limits (Table A1, online). Nearly half of the participants used the same kit as the RL (ten for measles and mumps antibodies, nine for rubella).

The reference panel was also tested using the plaque neutralization test (PNT) to check the sensitivity and specificity of the reference EIAs (Enzygnost). The PNT to detect measles and rubella antibodies was performed at the RL, using a standard procedure [14]. Serum samples were diluted beginning at 1:2, incubated with virus dilutions of 20–30 plaque-forming units (p.f.u.) (measles virus strain L16, rubella virus strain RA27/3) before the inoculation of cell monolayers. The neutralizing antibody titre was the reciprocal of the highest serum dilution which caused $\geqslant 50\,\%$ reduction of plaque number. Titres of $\geqslant 1{:}2$ were positive and corresponded after calibration against the above-mentioned International Standards to $\geqslant 0{\cdot}04 \pm 0{\cdot}02$ IU/ml measles antibodies and $\geqslant 4 \pm 2$ IU/ml rubella antibodies respectively. The PNT to detect mumps antibodies was performed at the laboratory of Berna Biotech Ltd, Berne, Switzerland. Dilutions of sera were incubated with mumps virus (wild virus strain '9337-Altstetten', 15–50 p.f.u.).

Monolayer of Vero cells, grown on Lab TekII culture plates were incubated with the serum-virus mixture for 5 days after adding an overlay of carboxymethylcellulose. Plaques were detected by immunofluorescence technique. Neutralizing antibody titre was defined as the reciprocal of the highest serum dilution which neutralized at least 50% of viral plaques in comparison to the control and titres of $\geqslant 1:2$ were positive. The anti-mumps PNT was developed and validated using in-house positive and negative sera according to international guidelines [15, 16].

The qualitative results of testing the reference panel by EIA (Dade Behring) were compared with those obtained by PNT. For calculating the sensitivity and specificity of the EIA the equivocal EIA results were included as positives.

RESULTS

First and second round of panel testing

After the first testing of the MMR reference panel two participants (Cyprus, Malta) had to change the EIA kit for detection of measles and mumps antibodies as the chosen assay did not work adequately (R^2 values were not acceptable). It transpired after the first testing and plotting of the results of all participants against those of the RL that an extension of the detection limits could improve the regression lines and the R^2 values. Therefore values below the recommended negative cut-off were calculated using the measured OD values [12] (Table A1, online).

Most of the participants tested the MMR panel twice. The assay drift between the first and second test was mostly minor and the correlation between the results of the two test runs was sufficiently good ($R^2 > 0{\cdot}8$, detailed results available at the ESEN2 website [17]). Primarily, the results of the second test of the reference panel were chosen because this was performed during the testing of the national serum bank. The identification of outliers performed as previously described [12] revealed overall a small number of $\leqslant 5$ (3·5%) per participant among the measles, mumps and rubella results. No sample number was found to be a systematic outlier.

Results of standardization

The agreements for overall positive (including equivocal) and negative results obtained before standardization by all participants in relation to the reference results are given in Tables 1–3 for measles, mumps and rubella, respectively. The chosen optimal standardization equation with the corresponding R^2 value and effect of standardization illustrated by the equivocal ranges in local units before and after standardization is shown for the measles, mumps, and rubella results in Tables 1–3, respectively. Plots of all the quantitative results against the RL along with the fitted regression lines can be found at the ESEN2 website [17].

Measles results

Comparison of qualitative results. A high overall agreement (>95%) was shown by most of the laboratories (8/10) using the same kit as the RL (Dade Behring) as well as the laboratories (7/11) that used different EIAs than the RL. An especially low overall agreement was observed in those laboratories using the Hycor kit [Belgium (56%), Bulgaria (84%)] which showed a strong underestimation of positive results (47–81%, Table 1).

Quantitative comparisons. Mostly quadratic regression equations (19/21) were selected. The R^2 values were generally well above 0·90 and a R^2 of $\geqslant 0{\cdot}95$ was more often obtained (6/10) in laboratories using the same EIA as the RL than in laboratories using other EIAs (4/11). The R^2 value was $<0{\cdot}90$ in only two laboratories (Israel 0·79, Sweden 0·89). Both countries performed back-standardization (Table 1). Examples for plotting the local results of three participants (Belgium, Romania and Sweden) against those of the RL are given in Figure A1 (top row) (see online Appendix).

Mumps results

Comparison of qualitative results. A high overall agreement (>95%) was noted for five out of 11 laboratories using the same kit as the RL and one showed only 78% agreement (Israel, which performed back-standardization). For five out of 10 laboratories using other EIAs than the RL an overall agreement of >95% was also noted, but it was below 90% in three laboratories (Cyprus, Bulgaria, Malta). The low overall agreement was mostly caused by a considerable underestimation of negatives (45–76%), but low agreement of positive results was also observed (Cyprus 87%, Malta 79%) (Table 2).

Quantitative comparisons. In total an R^2 value of $\geqslant 0{\cdot}95$ was obtained in only three out of 21

Table 1. *Comparison of qualitative measles results of all participants with those of the reference laboratory testing the MMR reference panel by different EIAs, the chosen optimal regression equation and the equivocal ranges before and after standardization for each participant*

		Agreement of qualitative results in %			Quantitative results			
					Optimal standardization equation		Equivocal ranges in local units	
Used kit DB	Country Germany (RL)	Negatives ($n=28$)	Positives‡ ($n=123$)	Overall ($n=151$)	Equation of standardization line§	R^2	Before std. 0·15–0·35 IU/ml	After std. 0·15–0·35 IU/ml
DB	Australia	71	100	95	$y=0{\cdot}93x+0{\cdot}15$	0·91	0·15–0·30	0·24–0·53
DB	England & Wales	100	98	99	$y=0{\cdot}11x^2+1{\cdot}06x-0{\cdot}31$	0·97	0·15–0·35	0·08–0·17
DB	Finland*	100	98	99	$y=0{\cdot}09x^2+1{\cdot}02x-0{\cdot}16$	0·99	0·15–0·34	0·12–0·25
DB	Greece*	100	97	97	$y=0{\cdot}13x^2+1{\cdot}14x-0{\cdot}12$	0·94	0·15–0·35	0·11–0·24
DB	Israel†	77	98	95	$y=0{\cdot}07x^2+0{\cdot}87-0{\cdot}10$	0·79	0·15–0·35	0·17–0·33
DB	Lithuania	93	97	96	$y=0{\cdot}12x^2+1{\cdot}05x-0{\cdot}29$	0·96	0·15–0·35	0·08–0·18
DB	Luxembourg	92	99	98	$y=0{\cdot}06x^2+1{\cdot}00x-0{\cdot}06$	0·95	0·15–0·34	0·14–0·31
DB	Romania	96	100	99	$y=0{\cdot}10x^2+1{\cdot}01x-0{\cdot}18$	0·97	0·15–0·30	0·11–0·24
DB	Slovenia	96	99	99	$y=0{\cdot}04x^2+1{\cdot}08x-0{\cdot}02$	0·98	0·17–0·31	0·13–0·31
DB	Spain†	100	100	100	$y=-0{\cdot}06x^2+1{\cdot}01x+0{\cdot}20$	0·94	0·15–0·35	0·21–0·53
Biotech	Eire	100	97	98	$y=-0{\cdot}11x^2+0{\cdot}39x+0{\cdot}27$	0·98	0·90–1·10	0·75–1·17
ER	Cyprus	100	90	92	$y=0{\cdot}15x^2+1{\cdot}00x-0{\cdot}20$	0·93	0·25	0·12–0·24
HC	Belgium	100	47	56	$y=0{\cdot}06x^2+0{\cdot}74+1{\cdot}15$	0·94	27–40	3·81–6·68
HC	Bulgaria	100	81	84	$y=0{\cdot}03x^2+0{\cdot}63x+1{\cdot}50$	0·94	27–40	10·03–16·56
HU	Latvia	100	95	96	$y=0{\cdot}07x^2+0{\cdot}66x-0{\cdot}06$	0·93	0·40–0·60	0·28–0·45
In-house	The Netherlands*	100	97	97	$y=0{\cdot}18x^2+1{\cdot}08x-0{\cdot}29$	0·94	0·20	0·09–0·18
In-house	Sweden†	89	93	93	$y=0{\cdot}32x^2+1{\cdot}02x-0{\cdot}41$	0·89	0·15–0·35	0·09–0·16
SK	Czech Republic	100	100	100	$y=-0{\cdot}25x^2+0{\cdot}97x+3{\cdot}10$	0·96	200–400	135–4·03
VR	Hungary	90	100	98	$y=0{\cdot}15x^2+0{\cdot}93x-0{\cdot}01$	0·95	0·15–0·20	0·21–0·40
VR	Malta	93	99	98	$y=0{\cdot}10x^2+0{\cdot}86x-0{\cdot}20$	0·92	0·15–0·20	0·14–0·27
VT	Slovakia	97	98	98	$y=0{\cdot}51x+1{\cdot}42$	0·97	9·0–11·0	10–15

DB, Dade Behring; ER, Euroimmun; HC, Hycor; HU, Human; SK, Seiken; VR, VirionSerion; VT, Virotech; *n*, samples of the reference panel; std., standardization; RL, reference laboratory.

* Countries took part for quality assurance only.

† Countries performed back standardization.

‡ Positive including equivocal sera.

§ y is the $\log_{10}$ local result; x is the $\log_{10}$ of reference result (Germany).

Table 2. *Comparison of qualitative mumps results of all participants with those of the reference laboratory testing the MMR reference panel by different EIAs, the chosen optimal regression equation and the equivocal ranges before and after standardization for each participant*

					Quantitative results			
		Agreement of qualitative results in %			Optimal standardization equation		Equivocal ranges in local units	
Used kit DB	Country Germany (RL)	Negatives ($n=42$)	Positives‡ ($n=109$)	Overall ($n=151$)	Equation of standardization line§	R^2	Before std. 230–500	After std. 230–500
DB	Australia*	100	99	99	$y=0{\cdot}94x+0{\cdot}09$	0·96	230–500	204–424
DB	England & Wales	89	99	97	$y=0{\cdot}93x+0{\cdot}21$	0·94	230–500	255–525
DB	Finland†	98	93	95	$y=0{\cdot}30x^2-0{\cdot}70x+2{\cdot}39$	0·91	230–500	257–485
DB	Greece*	98	91	93	$y=0{\cdot}08x^2+0{\cdot}38x+0{\cdot}93$	0·82	230–500	188–345
DB	Israel†	45	99	78	$y=0{\cdot}14x^2+0{\cdot}14x+1{\cdot}48$	0·83	231–543	390–755
DB	Lithuania	95	100	99	$y=0{\cdot}04x^2+0{\cdot}70x+0{\cdot}39$	0·98	150	185–372
DB	Luxembourg	83	100	96	$y=-0{\cdot}06x^2+1{\cdot}12x+0{\cdot}16$	0·92	231–525	295–557
DB	Romania	83	100	96	$y=0{\cdot}06x^2+0{\cdot}68x+0{\cdot}46$	0·94	150–300	252–540
DB	Slovenia	83	100	95	$y=1{\cdot}04x+0{\cdot}03$	0·94	190–370	306–687
DB	Spain†	76	100	95	$y=0{\cdot}12x^2+0{\cdot}30x+1{\cdot}36$	0·85	230–500	547–1106
DB	Sweden†	65	100	89	$y=0{\cdot}25x^2-0{\cdot}38x+2{\cdot}06$	0·89	230–500	361–717
Captia	Eire	97	97	97	$y=-0{\cdot}09x^2+1{\cdot}05x-2{\cdot}01$	0·94	0·9–1·1	0·9–1·5
ER	Cyprus	92	87	89	$y=0{\cdot}86x-1{\cdot}04$	0·87	20	10–19
HC	Belgium	90	97	95	$y=0{\cdot}33x^2-0{\cdot}42x+0{\cdot}63$	0·89	27–40	30–79
HC	Bulgaria	54	100	88	$y=-0{\cdot}05x^2+0{\cdot}94x+0{\cdot}12$	0·91	27–40	115–196
HU	Latvia	89	100	97	$y=0{\cdot}93x-0{\cdot}67$	0·95	39–46	34–69
In-house	The Netherlands*	92	99	97	$y=-0{\cdot}06x^2+1{\cdot}04x-0{\cdot}46$	0·92	40	46–81
SK	Czech Republic	74	98	92	$y=-0{\cdot}16x^2+1{\cdot}66x-0{\cdot}50$	0·82	200–400	337–653
VR	Hungary	95	99	98	$y=0{\cdot}74x+0{\cdot}19$	0·94	70–100	87–154
VR	Malta	98	79	84	$y=0{\cdot}18x^2-0{\cdot}17x+1{\cdot}30$	0·9	150–200	80–142
VT	Slovakia	93	97	96	$y=0{\cdot}58x-0{\cdot}44$	0·91	9–11	9–13

DB, Dade Behring; ER, Euroimmun; HC, Hycor; HU, Human; SK, Seiken; VR, VirionSerion; VT, Virotech; *n*, samples of the reference panel, std., standardization; RL, reference laboratory.

* Countries took part for quality assurance only.

† Countries performed back standardization.

‡ Positive including equivocal sera.

§ y is the $\log_{10}$ local result; x is the $\log_{10}$ of reference result (Germany).

laboratories and the R^2 was $<0{\cdot}90$ in seven out of 21 laboratories; among these were three of the four participants that had performed back-standardization. No difference was observed between laboratories using the same EIA or using different EIAs than the RL (Table 2). The regression equations are linear (7/21) as well as quadratic (14/21). Figure A1 (middle row) (see online) shows three examples for plotting the local results of participants (Bulgaria, Hungary and Spain) against those of the RL.

Rubella results

Comparison of qualitative results. The overall agreement was very good (mostly 99%) in laboratories using the same kit as the RL (Dade Behring) with one exception (Israel, which had performed back-standardization). Laboratories using the EIA kit from DiaSorin showed a lower overall agreement of 93–95% with a systematic underestimation of positive results. In two of the eight remaining laboratories a low overall agreement of 90% and 88% was noted, mainly caused by underestimation of negative results (Table 3).

Quantitative comparisons. The regression equations were quadratic (13/21), linear (5/21) as well as sigmoid (2). All laboratories using the EIA kit of Dade Behring or DiaSorin showed R^2 values of $\geqslant 0{\cdot}90$, most of them (9/13) had a $R^2 \geqslant 0{\cdot}95$. In laboratories using other EIA kits or in-house EIA the R^2 ranged from 0·81 to 0·98 and only few laboratories (3/8) showed R^2 values of $\geqslant 0{\cdot}95$. Figure A1 (bottom row) (see online) shows three examples for plotting the local results of participants (Lithuania, The Netherlands, Slovakia) against those of the RL.

Dilutions of the 2nd British Standard for anti-rubella serum included in the reference panel were blindly tested by the participating laboratories. The levels of anti-rubella virus-IgG in IU/ml detected in the dilutions differed widely from the expected values. In the four dilutions containing 40, 20, 15 and 10 IU/ml the following minimal and maximal values were reported: 22–70; 13–39; 5–28; and 5–20 IU/ml, respectively. The measured SAV in the dilutions of the standards for anti-measles and anti-mumps serum differed in a similar wide range (data not shown).

Evaluation of the MMR reference panel by PNT

For measles antibodies the EIA showed, in comparison to PNT, a sensitivity of 95·3% (122/128 positives) and a specificity of 100% (23/23 negatives). The EIA for mumps antibodies had in comparison to the PNT a lower sensitivity of 92·8% (103/111), and the specificity was only 87·1% (34/39) because three equivocal and two positive sera were negative by PNT. The sensitivity of the EIA for rubella antibodies was 100% (105/105), but the specificity was only 93·7% (30/32) since two sera with equivocal EIA results were negative by PNT.

DISCUSSION

The aim of the ESEN2 project was to provide comparable seroprevalence data for vaccine-preventable diseases, and in particular measles, mumps and rubella, across the participating countries. The EIA is the suitable method for investigating large numbers of sera since kits are commercially available and the assay can be automatically performed.

However, as we and other investigators have demonstrated, there are differences in the reported results [9–11]. In our study, we noticed a wide range of overall agreement (56–100%) of the qualitative results of the RL compared with those of the laboratories using other kits than Dade Behring, either due to a strong underestimation of positive results (47%, measles antibodies by the Hycor kit; Hycor Biomedical GmbH, Kassel, Germany) or of negative results (58%, rubella antibodies by the Platelia kit; Bio-Rad S.A., Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). Even if laboratories used the same kit as the RL cases of underestimation were found among positive results; but more often among the negative results (mainly mumps results).

All the rubella kits and most of the measles kits employed by the different laboratories provided the antibody values in IU. The high variation of the antibody values for the different standard dilutions in the panel blindly tested by the participants indicate that the results are not always reliable despite the fact that they are expressed in IU/ml. The wide variety of the EIA results obtained by the different kits in the different laboratories underlines the necessity of a standardization of the results of antibody testing to achieve an inter-country comparability of the seroprevalence data.

Results of standardization

For all participants and all antigens satisfactory standardization equations were obtained that explained

Table 3. *Comparison of qualitative rubella results of all participants with those of the reference laboratory testing the MMR reference panel by different EIAs, the chosen optimal regression equation and the equivocal ranges before and after standardization for each participant*

					Quantitative results			
		Agreement of qualitative results in %			Optimal standardization equation		Equivocal ranges in local units	
Used kit DB	Country Germany (RL)	Negatives ($n=35$)	Positives‡ ($n=116$)	Overall ($n=151$)	Equation of standardization line§	R^2	Before std. 4–7 IU/ml	After std. 4–7 IU/ml
DB	Australia*	94	99	98	$y=1{\cdot}15x-0{\cdot}19$	0·97	4–7	3–6
DB	Finland*	97	100	99	$y=0{\cdot}14x^2+0{\cdot}55x+0{\cdot}27$	0·99	4–6·5	4–7
DB	Greece*	100	96	97	$y=0{\cdot}10x^2+0{\cdot}73x+0{\cdot}13$	0·92	5–10	4–7
DB	Israel†	80	100	94	$y=0{\cdot}87x+0{\cdot}19$	0·91	4–7	5–8
DB	Lithuania	97	100	99	$y=0{\cdot}09x^2+0{\cdot}89x-0{\cdot}09$	0·98	4–7	3–5
DB	Luxembourg	97	100	99	$y=0{\cdot}05x^2+0{\cdot}75x+0{\cdot}23$	0·97	4–6	5–8
DB	Romania	97	100	99	$y=0{\cdot}10x^2+0{\cdot}74x+0{\cdot}19$	0·95	4–7	5–8
DB	Slovenia	94	100	99	$y=0{\cdot}05x^2+0{\cdot}93x+0{\cdot}10$	0·98	4–7	5–8
DB	Spain*	91	100	98	$y=0{\cdot}98x+0{\cdot}13$	0·97	4–7	5–9
DO	Belgium	100	91	93	$y=-0{\cdot}09x^2+1{\cdot}31x-0{\cdot}34$	0·96	9–11	3–5
DO	Czech Republic	100	93	95	$y=0{\cdot}09x^2+0{\cdot}74x+0{\cdot}07$	0·90	9–11	4–6
DO	Hungary	100	92	94	$y=-0{\cdot}06+(2{\cdot}58/(1+\exp(2{\cdot}96-2{\cdot}40x)))$	0·95	10–15	3–5
DO	Slovakia	100	94	95	$y=0{\cdot}68+(1{\cdot}68/(1+\exp(4{\cdot}80-3{\cdot}61x)))$	0·97	9–11	6–8
Abbott	Malta	65	96	90	$y=0{\cdot}25x^2+0{\cdot}25x+0{\cdot}53$	0·86	5–10	6–8
Biokit	Eire	100	99	99	$y=0{\cdot}87x+0{\cdot}31$	0·98	10–15	7–11
In-house	The Netherlands*	91	98	97	$y=0{\cdot}81x+0{\cdot}57$	0·93	10	11–18
In-house	Sweden†	100	97	97	$y=0{\cdot}11x^2+0{\cdot}56x+0{\cdot}42$	0·91	7·4–10	6–9
Microgen	England & Wales	100	99	99	$y=0{\cdot}12x2+0{\cdot}83x+0{\cdot}14$	0·95	4–7	5–8
PL	Bulgaria	87	97	95	$y=0{\cdot}81x+0{\cdot}63$	0·88	10–15	13–21
PL	Latvia	58	96	88	$y=0{\cdot}20x^2+0{\cdot}40x+0{\cdot}71$	0·81	10–15	11–16
Radim	Cyprus	100	91	93	$y=-0{\cdot}46x2+2{\cdot}67x$-1·41	0·98	8–10	1–3

DB, Dade Behring; DO, DiaSorin; PL, Platelia; *n*, samples of the reference panel; std., standardization; RL, reference laboratory.

* Countries took part for quality assurance only.

† Countries performed back standardization.

‡ Positive including equivocal sera.

§ y is the $\log_{10}$ local result; x is the $\log_{10}$ of reference result (Germany).

most of the variability ($R^2 = 0{\cdot}80$) with only one exception (Tables 1–3). R^2 values of $< 0{\cdot}90$ were reported more often with standardization of mumps results (7) than either measles (3) or rubella (3). This reflects a higher variability of the mumps results as already noted on the comparison of qualitative results and indicates that the mumps antigen used in EIA kits is very different. A further cause may be the relatively high percentage of equivocal and low positive mumps antibody values in the sera of the panel, another cause might be the genetic variability of the different mumps virus strains used in the EIAs [18, 19]. Participants that used back-standardization obtained mostly lower R^2 values and also the lowest R^2 value (0·79, measles results of Israel) was achieved by a participant who performed back-standardization.

The comparison of equivocal ranges before and after standardization demonstrates the changes entailed after using the chosen regression equation. In case of underestimation of positive results by any kit the standardization resulted in lowering the original equivocal range (e.g. the measles results of Belgium, Bulgaria, Sweden) or in case of overestimation of positive results standardization resulted in raising the original equivocal range (e.g. mumps results of Israel, Bulgaria). When the standardization equation is applied on the results of the national serum bank the seroprevalence data of the respective country will change as illustrated by the following example. A slight underestimation of positive measles results (90%) was obtained by the Euroimmun EIA kit (Euroimmun AG, Lübeck, Germany) in the Cyprus laboratory (Table 1). The equivocal range decreased by standardization. Therefore, the proportion of seropositive samples increased in the corresponding seroprofile (Fig. A2, online). Applying this procedure to the seroprevalence data will ensure direct comparability with those of other countries participating in the inter-country comparison.

Evaluation of EIA results by PNT

The sera of the MMR reference panel were also measured by the PNT for measles, mumps and rubella antibodies to evaluate the sensitivity and specificity of the EIA (Dade Behring) used by the RL. The PNT is a very sensitive and functional method whereby the antibodies neutralize the capacity of the virus to infect cells [20]. Neutralizing antibodies are commonly assumed to correlate with protection.

Measles antibodies

Other authors reported a slightly lower sensitivity (92%, 88%) for the EIA from Dade Behring in comparison to PNT because they did not consider the equivocal sera [9, 10]. The equivocal values of the reference EIA range from 0·15 to 0·35 IU/ml; all equivocal sera of the panel were positive by PNT with 0·17–1·2 IU/ml. Therefore, it seems justified to add the proportion of equivocal to the positive sera in the scope of the ESEN2 project. The antibody level which gives protection against infection or illness is still under debate. Individuals with a measles antibody level of $> 0{\cdot}20$ IU/ml measured by PNT were protected against typical measles [9, 21]. On the other hand persons with PNT titres $< 0{\cdot}05$ IU/ml developed only an asymptomatic infection [22].

Mumps antibodies

Several studies compared different types of EIAs and neutralization tests besides other methods for mumps antibody detection and found a good agreement [23, 24] as well as discrepancies [25, 26]. The antibody values measured by PNT and an in-house EIA did not correlate well as was also found in our comparison. In vaccine trials a higher sensitivity for EIA was found [27, 28], but there is evidence that antibodies to mumps cross-react with other paramyxoviruses, such as human parainfluenza, particularly in EIA [29, 30]. Further, it was shown that the mumps virus strain used in the neutralization test had a strong effect on the measured titres [26]. The assessment of immunity to mumps virus remains problematical due to the lack of an International Standard for human mumps serum and the discrepancies in detecting mumps antibodies.

Rubella antibodies

The equivocal values of the reference EIA range from 4 to 7 IU/ml and sera with > 7 IU/ml are considered to be positive. Since the specificity of the EIA compared to PNT was not 100% it is proposed that the proportion of sera with equivocal values should not be added to positive sera in the scope of ESEN2. However, the protective titre is assumed to be 10 IU/ml (for the United States [31]) or 15 IU/ml [32, 33]. These values are of special interest for the evaluation of the percentage of women of childbearing age with a sufficient rubella antibody level in the range of seroprevalence studies.

The results of this paper demonstrate the importance of the standardization procedure to control inter-assay and laboratory differences. By applying the selected standardization equations we will be able to undertake international comparisons of measles, mumps and rubella seroprevalence data in Europe and provide a sound knowledge base with which to evaluate MMR vaccine policies in Europe. These results will be presented in further papers published within the scope of ESEN2. The presented method and results are also valuable and useful for countries which did not participate in the project.

APPENDIX. Members of ESEN2

Belgium: S. Broodhaers, P. van Damme, L. D. Cock, H. Theeten, R. Vranckx. **Bulgaria:** N. Gatcheva, Z. Mihneva, V. Voynova. **Cyprus:** C. Hadjianastassiou, M. Zarvou. **Czech Republic:** B. Kriz, V. Nemecek. **England & Wales:** N. Andrews, N. Gay, W. J. Edmunds, L. Hesketh, G. Kafatos, E. Miller, P. Morgan-Capner, A. Nardone, R. Pebody. **Finland:** I. Davidkin, R. M. Ölander, M. Valle. **Germany:** W. Hellenbrand, W. Thierfelder, A. Tischer. **Greece:** C. Anastassopoulou, A. Hatzakis, A. Tsakris. **Hungary:** K. Bartha, A. Csohan, I. Karacs, I. Lontai, M. Melles, Zs. Molnar, Zs. Pauliny. **Ireland:** M. Carton, L. Jones, D. O'Flanagan. **Israel:** Y. Aboudy, D. Cohen, M. Green, E. Marva, Z. Smetana. **Italy:** G. Gabutti, A. Giammanco, G. Icardi, C. Rota, S. Salmaso, C. von Hunolstein. **Latvia:** J. Bebris, A. Duks, J. Perevoscikovs, I. Rezebergs, I. Selga, I. Velicko. **Lithuania:** V. Baksenas, A. Griskevicius, J. Suracienne. **Luxembourg:** J. Mossong, F. Schneider. **Malta:** A. Amato-Gauci, C. Barbara. **The Netherlands:** G. Berbers, H. de Melker, **Romania:** V. Alexandrescu, D. Butur, E. Lupulescu, A. Pistol, R. Viorica. **Slovakia:** B. Černáková, J. Lančová, E. Máderová, I. Rovný, M. Slačiková. **Slovenia:** A. Kraigher, K. Prosenc. **Spain:** F. de Ory, J. M. Echevarria, M. V. Martínez de Aragón. **Sweden:** R. M. Carlsson, K. Johansen, E. Reizenstein.

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DECLARATION OF INTEREST

None.

NOTE

Supplementary information accompanies this paper on the Journal's website (http://journals.cambridge.org).

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