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Comparison of Commercial Enzyme Immunoassay Kits with Plaque Reduction Neutralization Test for Detection of Measles Virus Antibody

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Four commercially available enzyme immunoassay (EIA) kits were evaluated in comparison with the plaque reduction neutralization (PRN) test for detection of measles virus antibody. The EIA kits, Enzygnost (Behring), Diamedix, Vidas (bioMerieux Vitek), and Measlestat (Biowhittaker), were assessed with two PRN cutoff titers: a PRN titer of 8, the lowest detectable antibody level by the PRN test under the test conditions, and a titer of 120, which has been shown to be the minimum protective antibody titer. At a PRN cutoff titer of 8, the sensitivity was 88.2, 91.1, 74.6, and 69.8% for Behring, Diamedix, Vidas, and Biowhittaker EIA tests, respectively, with negative predictive values ranging from 22.7 to 45.5%. The specificity was 93.8% for Diamedix and 100% for the rest. At a PRN cutoff titer of 120, the sensitivity and specificity, respectively, were 100 and 90.7% (Behring), 98.2 and 58.8% (Diamedix), 90.6 and 94.5% (Vidas), and 85.7 and 96.4% (Biowhittaker). At this PRN cutoff titer, the negative predictive values of all EIA tests improved considerably, ranging from 70.7 to 100%. The EIA results showed an excellent association with PRN results when the PRN titers of the test samples were either <8 or >1,052. Discrepancies occurred especially when testing samples having PRN titers in the range of 8 to 120, indicating lack of sensitivity of the EIA tests in detecting measles virus antibody at low levels. Maternally derived measles virus antibody at this level has been shown to interfere with measles vaccine response in children and hence has implications from the standpoint of measles immunization. The ready availability, ease of operation, and rapid turnaround time are strong plus points of the EIA kits, and they could be useful in a clinical laboratory setting for routine application, but they may have limited use in vaccine-related studies and seroepidemiological surveys.

Measles remains one of the leading causes of childhood morbidity and mortality in developing countries and is still a major public health concern in developed countries. Continued outbreaks of measles in highly immunized populations have led to reassessment of immunization strategies (3, 5, 10, 11). Measles control has a high priority in many countries, and it is important that questions surrounding possible vaccine failures and declining immunity be addressed so that the strategy to eliminate measles may be evaluated and strengthened. In this context, it is important that highly sensitive and specific laboratory tests are used to accurately determine the antibody level resulting from vaccination and the level of antibody that persists in those who were previously vaccinated as immunity to measles is interpreted on the basis of measles virus-specific serum antibody levels. Clinical laboratories mostly use enzyme immunoassay (EIA) to test for measles virus antibody. EIA has been reported to be more sensitive and specific than hemagglutination inhibition and complement fixation tests (4, 12, 15). However, an important consideration is the validity of commercial measles EIA kits produced by a variety of manufacturers.

The plaque reduction neutralization (PRN) test is an enhanced version of the standard neutralization test for detection of measles virus antibody. The PRN test measures the serum dilution capable of preventing 50% of plaque formation by measles virus in cell cultures (2). This test has been reported to be 10-fold more sensitive than the standard neutralization test, 60-fold more sensitive than the hemagglutination inhibition test, 200-fold more sensitive than the complement fixation test, and more sensitive and specific than the EIA method to detect measles virus antibody (2). Therefore, the PRN test is now considered to be the most reliable technique for detection of measles virus antibody. On the basis of an efficacy study during an outbreak of measles, Chen et al. have reported that PRN titers of <120 were not protective against measles, titers of >120 but <1,052 may protect against classic measles but not against mild clinical infections, and those of >1,052 indicate full protection (6). Although this interpretation was based on a small study, it has provided some indication of minimum protective PRN antibody titer (6). Nevertheless, the PRN test is not widely used because it is slow, highly labor intensive, technically demanding, and not suitable for routine application in the clinical laboratory setting.

We had an opportunity to evaluate commercial measles EIA kits in comparison with the PRN test for detection of measles virus antibody while carrying out measles vaccine studies. We had on hand over 1,500 pre- and post-measles-mumps-rubella (MMR) immunization serum samples collected from children, and these were utilized in the evaluation study. We determined the ability of commercial EIA kits to detect measles virus antibodies in test samples with a PRN cutoff titer of 8, the lowest antibody level detectable by the PRN assay under our test conditions. In addition, we also assessed the ability of the test kits to delineate protective antibody levels based on a PRN

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cutoff titer of 120. This report summarizes our findings and the performance characteristics of four commercial EIA tests.

MATERIALS AND METHODS

EIA test. Four commercially available measles EIA kits were included in this evaluation study. These were Enzygnost anti-measles virus–immunoglobulin G (IgG) (Behring, Marburg, Germany), Diamedix measles IgG microassay (Diamedix Corporation, Miami, Fla.), Vidas measles IgG (bioMerieux Vitek Inc., Hazelwood, Mo.), and Measlestat (Biowhittaker Inc., Walkersville, Md.). The Behring EIA test uses microtiter plates with one set of wells coated with the measles virus antigen derived from simian kidney cells and a second set of wells coated with a control antigen derived from uninfected simian kidney cells. The antigen-antibody complex is detected by peroxidase-conjugated anti-human IgG with TMB-hydrogen peroxidase as the enzyme substrate. The optical density (OD) is read spectrophotometerically, the difference in absorbance between the test and control wells is determined, and the final test values are generated. These are qualitatively interpreted as negative, equivocal, or positive for antibodies to measles virus. This system also offers an option for automatic quantitative determination of measles virus antibody titer for samples yielding equivocal or positive results. The Diamedix EIA test also uses microtiter plates but coated with only the measles virus antigen. The antigen-antibody complex is detected by alkaline phosphatase-conjugated anti-human IgG with *p*-nitrophenyl phosphate as the enzyme substrate. The results are read spectrophotometerically, and the OD readings are converted to EIA units and interpreted as negative, equivocal, or positive for antibodies to measles virus. The Vidas EIA uses a fully automated approach with all assay steps and assay temperatures controlled by an instrument. It utilizes a special receptacle, which serves as the solid phase coated with the measles virus antigen, as well as a pipettor for the assay. The antigen-antibody complex is detected by alkaline phosphatase-conjugated anti-human IgG with 4-methylumbelliferyl phosphate as the enzyme substrate. The intensity of the fluorescent product 4-methylumbelliferone is measured by an optical scanner, the results are analyzed automatically, and reports are generated with quantitative test values. The test values are interpreted as negative, equivocal, or positive. The Biowhittaker EIA test is similar to that of the Diamedix system except that it utilizes phenolphthalein monophosphate as the enzyme substrate and the OD readings are converted into predicted index values. The predicted index values are interpreted semiquantitatively as negative or equivocal or as low, mid-, or high positives. All EIA tests were carried out according to the manufacturers' instructions by one experienced technologist.

PRN test. The PRN test was carried out as previously described by Albrecht et al. (2). Our technologists received their initial training in Paul Albrecht's laboratory at the National Institutes of Health, Bethesda, Md. Subsequently, we also carried out parallel testing of over 200 samples blindly to ensure reproducibility and quality assurance. A low-passage Edmonston B strain (HEK7V1 6.22-81) obtained from Albrecht was used as the challenge virus in the PRN test. This viral strain had been propagated once in Vero cells at the National Institutes of Health laboratory and was passaged twice in Vero cell culture (Biowhittaker) in our laboratory. The stock viral culture was harvested in equal volumes of Hanks' minimal essential medium (MEM) and Earle's MEM, plaque titrated, and stored at −70°C in 2-ml aliquots. This stock yielded an average of 30 PFU/ml at a dilution of 10^5. This was diluted in Spinner medium to yield 25 to 35 PFU in the final serum-virus inoculum mixture of 100 μl per cell culture well of the PRN assay.

The PRN test controls included the World Health Organization (WHO) measles virus antibody standard (8) (5 IU, lot no. 66/202; WHO International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, England) and two in-house standards, one having a low titer and the other with a high titer of measles virus antibody. The WHO standard was rehydrated with 2 ml of distilled H_2O to which 23 ml of Spinner medium containing 3% fetal bovine serum (Gibco BRL, Grand Island, N.Y.) was added to obtain a working stock of 200 mIU/ml. All standard sera were aliquoted, stored at −70°C, and diluted and used in each PRN assay identical to the test samples. Vero cells (Biowhittaker) at passage levels 140 through 180 were used for the PRN test throughout the study. The PRN test was carried out by an experienced technologist who was blinded to the results of the EIAs. Briefly, 1 ml of suspension of Vero cells in Eagle's MEM containing 5% fetal bovine serum (Gibco) was seeded into 24-well (16-mm diameter) cell culture plates at a concentration of 200,000 cells per well, and the monolayers were grown to near confluence. With the exception of the WHO serum standard, all serum samples were inactivated at 56°C for 30 min. Six serial dilutions of each of the test serum samples and standard sera were prepared in Spinner medium in either twofold dilutions (1:4 to 1:128) or fourfold dilutions (1:4 to 1:4,096) depending on the expected titer range. One hundred twenty microliters of each one of these dilutions was mixed with an equal volume of virus suspension diluted to contain approximately 50 to 70 PFU/100 μl and incubated for 105 min. The growth medium was removed from the cell culture wells, 100 μl of each of the serum-virus mixtures (starting serum dilution, 1:8) was transferred into two cell culture wells, and the plates were incubated for 75 min. The inoculum was then removed, the monolayers were covered with an overlay (1 ml per well) containing carboxymethyl cellulose in Leibovitz's L-15 medium (Gibco), and the plates were incubated for 4 days. The monolayers were stained in situ by adding neutral red in MEM to the overlay medium, and the plates were incubated for an additional day. Throughout the PRN assay, all incubations were carried out at 35°C under 5% CO_2. Following the final incubation step, the overlay medium was removed, and the monolayers were fixed with 10% formalin and air dried. The plaques were counted, and the average count of two wells was taken for each dilution of test samples assayed. The 50% end point was determined by the Karber formula. In order to account for any variability in the test procedure, each sample was tested in duplicate and the average counts of PFU were taken in determining the titer. As the PRN assay was carried out in cell culture wells of 16 mm in diameter, the challenge virus suspension was adjusted to yield 25 to 35 PFU per well. Since the lowest antibody titer detectable under the test conditions was 8 PRN units, samples having no detectable antibody were considered to have a PRN titer of <8.

Test serum panels. Two panels of test sera were used in the evaluation. The main test panel comprised 229 serum samples. This panel had overall PRN titers ranging from <8 to 10,000, with 17 samples having a PRN titer of <8, 41 samples with titers of between 8 and 120, 110 samples with titers of between 120 and 1,052, and 61 samples with titers of >1,052. These were from a collection of serum samples obtained from children of 1 to 16 years of age who were previously immunized with MMR vaccine. The main test panel was used for simultaneous testing of all four EIA kits in comparison with the PRN test. A second test panel comprised 1,287 pre- and postimmunization serum samples obtained from 12- to 15-month-old children. This test panel was used for further evaluation of the Behring EIA kit.

RESULTS

The WHO standard was used at a concentration of 200 mIU/ml in the PRN test, and this yielded a mean PRN titer of 94, and the low- and high-level in-house controls gave a mean PRN titer of 30 and 224, respectively. Only those PRN assays in which the WHO standard titer varied by <20% were considered valid.

The performance of the four EIA test kits was evaluated with two cutoff levels of PRN titers, a PRN titer of 8 (17 mIU/ml), the lowest antibody level detectable by the PRN test

TABLE 1. Relative performance of commercial EIA tests in detecting measles virus antibody on the basis of a PRN titer of 8 as cutoff[a]

EIA brand name/ manufacturer	Result for test	PRN cutoff titer		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Agreement[b] (%)	No. of samples with indeterminate results (%)
		≥8	<8						
Enzygnost/Behring	+	164	0	88.2	100.0	100.0	43.6	181/203 (89.2)	26 (11.4)
	−	22	17						
Diamedix	+	185	1	91.1	93.8	99.5	45.5	200/219 (91.3)	10 (4.4)
	−	18	15						
Vidas/bioMerieux	+	147	0	74.6	100.0	100.0	25.4	164/214 (76.6)	15 (6.6)
	−	50	17						
Measlestat/Biowhittaker	+	134	0	69.8	100.0	100.0	22.7	151/209 (72.2)	20 (8.7)
	−	58	17						

[a] A total of 229 serum samples with PRN measles virus antibody titers ranging from <8 to 10,000 were tested for measles virus antibody with EIA kits.
[b] Number of samples showing agreement out of total number of samples yielding reactive or nonreactive results.

TABLE 2. Relative performance of commercial EIA tests to delineate protective measles virus antibody titer on the basis of a PRN titer of 120 as cutoff[a]

EIA brand name/ manufacturer	Result for test	PRN cutoff titer: ≥120	PRN cutoff titer: <120	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Agreement[b] (%)	No. of samples with indeterminate results (%)
Enzygnost/Behring	+	160	4	100.0	90.7	97.6	100.0	199/203 (98.0)	26 (11.4)
	−	0	39						
Diamedix	+	165	21	98.2	58.8	88.7	90.9	195/219 (89.0)	10 (4.4)
	−	3	30						
Vidas/bioMerieux	+	144	3	90.6	94.5	98.0	77.6	196/214 (91.6)	15 (6.6)
	−	15	52						
Measlestat/Biowhittaker	+	132	2	85.7	96.4	98.5	70.7	185/209 (88.5)	20 (8.7)
	−	22	53						

[a] A total of 229 serum samples with PRN measles virus antibody titers ranging from <8 to 10,000 were tested for measles virus antibody with EIA kits.
[b] Number of samples showing agreement out of total number of samples yielding reactive or nonreactive results.

under our assay conditions, and a PRN titer of 120 (255 mIU/ml), which considered as the minimum protective antibody titer. When the EIA results obtained with the main test panel of 229 serum samples were analyzed with a PRN titer of 8 as the cutoff, the sensitivity of the EIA test kits was found to range from a low of 69.8% for Biowhittaker to a high of 91.1% for Diamedix (Table 1). While the positive predictive value was >99% for all four kits, the negative predictive value ranged from 22.7 to 45.5%. The percent agreement was highest for Diamedix at 91.3 whereas the percent indeterminate results were highest for Behring EIA at 11.4. When the EIA results were analyzed with a PRN titer of 120 as the cutoff, the level of sensitivity was found to increase for all four kits with an attendant decrease in specificity (Table 2). The decrease in specificity was particularly striking for the Diamedix EIA, which dropped to a level of 58.8%. While the negative predictive value was higher for all four EIA systems at the PRN cutoff titer of 120, among the test kits, it was considerably lower for both Vidas EIA (77.6%) and Biowhittaker EIA (70.7%) compared with Diamedix and Behring EIA. Overall, the Behring EIA system performed best at a PRN cutoff titer of 120, albeit yielding the highest percent indeterminate results (Table 2). The various formats of quantitative-semiquantitative readings generated by the commercial EIA kits were compared with four ranges of PRN titers as reported by Chen et al. (6). This showed a wide variation and overlap of test values within each EIA system (Table 3). The EIA systems yielded discrepant results almost exclusively with samples that had PRN titers of between 8 and 1,052 (Table 4).

As the Behring EIA kit was found to perform better in our initial evaluation for measuring protective antibody levels based on a PRN titer of 120, this test was chosen for further assessment with the second panel of 1,287 serum samples. In this series, at a PRN cutoff titer of 8, a considerable improvement in the negative predictive value was observed compared with that obtained with the main test panel, i.e., 84.3 versus 43.6%. Also, there was a considerable decrease in the percent indeterminate results, i.e., 1.6 versus 11.4% (Tables 1 and 5). At a PRN titer of 120 as the cutoff, the overall performance was found to be about the same as that observed with the main test panel (Tables 2 and 5).

DISCUSSION

The PRN test is now considered to be the "gold standard" for detection of measles virus antibody because of its greatly increased sensitivity with no loss in specificity (2, 5, 6, 14). However, currently the PRN test is carried out in maybe only a few laboratories in the world because of technical and practical difficulties. We used the WHO international anti-measles virus serum standard as a control in PRN assays as part of our quality assurance, and this also allows for the conversion of our PRN antibody titers into international units for comparative analysis.

While the relevance of serum antibody to susceptibility or immunity is an unsettled issue, and there is no agreed standard serum correlate of protective immunity, the reported protective PRN antibody titer of ≥120 is based on a serological study of an outbreak of measles (6). In addition, the use of the PRN test has provided evidence that maternally derived measles virus antibody present at the time of MMR immunization (at levels not detectable by other serological tests) interferes with the attenuated live measles virus vaccine, resulting in poor response in young children (1, 13). For these reasons, we consider the PRN titers to be the most predictive of the currently available serological markers for measles virus antibody status and used this assay to evaluate commercial EIA kits for detection of measles virus antibody.

TABLE 3. Correlation between PRN measles virus antibody titer and EIA ODs of commercial test kits

PRN titer	No. of samples	Mean (range of EIA ODs) obtained with test: Enzygnost (Behring)[a]	Diamedix[b]	Vidas (bioMerieux)[c]	Measlestat (Biowhittaker)[d]
<8	17	0.0072 (0–0.024)	11.02 (3.3–31.4)	0.0400 (0.01–0.14)	0.0412 (0–0.17)
8–120	41	0.1205 (0–0.942)	22.08 (0–70.0)	0.2932 (0.01–1.63)	0.4027 (0–1.97)
120–1,052	110	0.5743 (0.118–1.607)	56.33 (0.4–171.8)	1.1659 (0–2.86)	1.2761 (0.25–2.41)
>1,052	61	1.4648 (0.435–2.549)	109.86 (42.1–230.7)	2.4208 (0.82–3.89)	2.3321 (0.56–3.97)

[a] OD: <0.1, negative; 0.1 to 0.2, equivocal; >0.2, reactive.
[b] EIA units: <15, negative; 15 to 20, equivocal; >20, reactive.
[c] Test value threshold: <0.5, negative; ≥0.5 to <0.7, equivocal; ≥0.7, reactive.
[d] Predicted index value: ≤0.79, negative; 0.80 to 0.99, equivocal; ≥1.0, positive (1.00 to 2.49, low positive; 2.50 to 4.29, mid-positive; ≥4.30, high positive).

TABLE 4. Distribution of test samples in terms of PRN titer ranges and relative performance of EIA tests

PRN titer range	No. of serum samples	No. of samples with result for test:											
		Enzygnost (Behring)			Diamedix			Vidas (bioMerieux)			Measlestat (Biowhittaker)		
		+	−	I[a]	+	−	I	+	−	I	+	−	I
<8	17	0	17	0	1	15	1	0	17	0	0	17	0
8–120	41	4	22	15	20	15	6	3	35	3	2	36	3
120–1,052	110	99	0	11	104	3	3	83	15	12	73	21	16
>1,052	61	61	0	0	61	0	0	61	0	0	59	1	1

[a] I, indeterminate.

The main test panel was put together specifically to assess the ability of EIA tests to detect measles virus antibody titers in the range of <8 to 500 PRN units as well as to discriminate protective antibody titers. Our data comparing the performance of EIA tests based on a PRN cutoff titer of 8 showed a wide range of sensitivities and negative predictive values (Table 1). Although PRN titers in the range of 8 to 120 may be difficult to interpret in terms of protective immunity, the presence of maternally derived measles virus antibody in this range has implications in response to measles vaccination in young children as previously reported (1, 13). On the basis of our initial evaluation, it is reasonable to assume that the Diamedix test would be more sensitive than the other EIA tests in detecting low levels of maternal antibody. We, however, could not evaluate this test further in our second series of testing because of limited volume of preimmunization sera obtained from 1-year-old children. Regardless, it is clear from our data that the EIA tests that we evaluated are generally insensitive in detecting measles virus antibody at low levels and therefore unlikely to provide reliable information in measles vaccine-related studies and seroepidemiological surveys. The EIA tests, with the exception of Behring EIA, were also found to be less sensitive than the PRN test in determining protective antibody levels based on a PRN titer of 120, and in addition, the negative predictive values of these EIA systems ranged from 70.7 to 90.9% (Table 2). Therefore, the use of such EIA tests could result in underestimation of the prevalence of protective antibody levels in population-based studies. While the negative predictive value of the Diamedix EIA test, based on a PRN titer of 120, was considerably higher than those of the Vidas and Biowhittaker tests, i.e., 90.9 versus 77.6 and 70.7%, respectively, the Diamedix test suffered from a false-positive rate of over 40% (Table 2). Thus, the use of such a test in this setting could lead to overestimation of the prevailing level of protective antibody titers.

In our initial evaluation, the Behring EIA system was found to have the overall best performance. Hence, this system was chosen for further evaluation with 1,287 pre- and postimmunization serum samples. In this series of evaluation, there was a significant improvement in the negative predictive value of this kit at a PRN cutoff titer of 8. Also, there was a considerable reduction in indeterminate results compared with those obtained with the main test panel (Tables 1, 2, and 5). This was because the test panel used in this series comprised mostly samples that either had no antibody at all (preimmunization samples) or had antibody at PRN titers of >350 (postimmunization samples). This observation confirms our initial findings with the four EIA systems with the main test panel, i.e., that the reliability of EIA results is dependent on the titers of measles virus antibody in test samples. Almost all EIA discrepant results were found with samples having PRN titers in the range of 8 to 1,052 (Table 4). In addition, the quantitative values generated by individual EIA systems showed considerable overlap between the various ranges of PRN titers (Table 3). This may, therefore, pose difficulty at times in interpreting EIA results of single test samples, particularly in terms of protective immunity. Of course, the concern on the other hand might be the validity of the protective antibody titer as reported by Chen et al. (6). Although this was based on a study carried out in an outbreak setting, it was a small study. The application of a PRN titer of ≥120 as a definitive basis of protective immunity may require additional data, and accordingly, our findings should be interpreted with caution. Regardless, the quantitative titers generated by EIA systems may be useful when testing paired sera for diagnostic purposes.

The discrepancies observed between the EIA and PRN tests may be largely due to the differences in the antigen used in EIA tests. EIA tests generally utilize whole-virus antigen for this purpose. The proteins of measles virus include the large protein, phosphoprotein, nucleocapsid protein, membrane-matrix protein, hemagglutinin, and fusion protein. Therefore, EIA tests may detect antibodies directed against all viral antigens while the PRN test detects only the antibodies directed against specific proteins, i.e., the envelope proteins, hemagglu-

TABLE 5. Performance of Enzygnost (Behring) EIA test in detecting measles virus antibody on the basis of PRN titers of 8 and 120 as cutoffs[a]

PRN cutoff titer	No. of samples with result:		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Agreement[b] (%)	No. of samples with indeterminate result (%)
	+	−						
≥8	623	100	86.2	98.9	99.0	84.3	1,161/1,267 (91.6)	20 (1.6)
<8	6	538						
≥120	588	7	98.8	93.9	93.5	98.9	1,219/1,267 (96.2)	20 (1.6)
<120	41	631						

[a] Based on 1,287 pre- and post-MMR II immunization serum samples from 12- to 15-month-old children.
[b] Number of samples showing agreement out of total number of samples yielding reactive or nonreactive results.

tinin, and fusion protein. Also, EIA tests measure only IgG antibodies whereas the PRN test would theoretically measure all classes of measles virus-specific immunoglobulins. Regardless, some in-house developed EIA tests have been reported to be comparable to the PRN test (7, 9, 14).

Besides the performance characteristics of the EIA tests, we also assessed the ease of their operation in a routine clinical laboratory setting. Of the four EIA systems evaluated, the Vidas EIA procedure was found to have the best overall acceptability in this setting because it requires no predilution of test samples and is a fully automated hands-off operation and the results are available in about 40 min. We also observed a very high degree of intra- and interassay reproducibility with this system during the initial trial runs. The Diamedix procedure requires no predilution of test samples either, takes about 3.5 h to complete a batch of tests, and is mostly automated, whereas the Behring EIA system requires predilution of test samples, takes about 5 h to complete a batch of tests, and is less automated than the Diamedix procedure. The Biowhittaker procedure is the least automated of the four EIA procedures and consequently requires a considerable amount of hands-on time. It also requires predilutions of test samples and up to six controls and standards but takes about 1.5 h to complete a batch of tests.

Commercial measles EIA test kits offer a standardized procedure, a common source of supply, and ease of operation. Nevertheless, there is a need to determine the validity of individual EIA products in comparison with a highly sensitive and specific test such as the PRN test, particularly if these products are to be used in studies dealing with vaccine response and immune status assessment. In our vaccine study dealing with about 650 children, 9% were found to have low levels of maternal antibody at preimmunization at 12 months of age as determined by the PRN test, and these children did not respond to measles vaccine as well as those who had no detectable maternal antibody at the time of immunization (13a). Our data and those of others have shown that even low levels of maternal antibody present at the time of immunization are quite effective in blocking the infectivity of attenuated measles virus vaccines (1, 13a). In this context, the ability of EIA tests to detect low levels of maternal antibody becomes an important criterion, and PRN titers in the range of 8 to 120 could be used as a basis for evaluating EIA tests in this setting. Maternal antibody at low levels, however, may not be protective against wild measles virus. As already stated, there is some evidence to suggest that a PRN titer of $\geq$120 may be protective when exposed to the wild virus (6). Therefore, for the purpose of immune status assessment, it appears reasonable to evaluate EIA kits based on a PRN titer of 120 pending additional data on protective antibody titers in measles. Continuing outbreaks of measles have generated renewed worldwide interest in the prevention and control of measles, and this has resulted in an increased demand for laboratory support. It is important that clinical and research laboratories consider quality assurance carefully when using commercial products in the above contexts since there is a need for reliable information so that the strategy to eliminate measles may be evaluated and strengthened.

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