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Comparison of measles plaque reduction neutralization test (PRNT) and measles virus-specific IgG ELISA for assessment of immunogenicity of measles-mumps-rubella vaccination at 5–7 months of age and maternal measles antibodies

Dorthe Maria Vittrup [a,b,*], Andreas Jensen [b], Michelle Malon [b], Anne Cathrine Zimakoff [b], Jesper Kiehn Sørensen [b], Brickley Littell [c], Eric A.F. Simões [c], Jannet Svensson [d,e], Lone Graff Stensballe [b,e,a,1]

[a] Blegdamsvej 9, 2100 Copenhagen E, Denmark
[b] The National Danish University Hospital, Rigshospitalet, The Juliane Marie Center, Pediatric Department, Denmark
[c] University of Colorado, Anschutz Medical Campus, Aurora, CO, USA
[d] Herlev University Hospital, Department of Pediatrics, Denmark
[e] Institute for Clinical Medicine, University of Copenhagen, Denmark

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ABSTRACT

Background: Assessing the risk of measles outbreaks and identifying the susceptible parts of the population is essential to timely intervention. Infants between 6–12 months are increasingly susceptible to measles but evaluating the performance of high throughput enzyme immunoassays (ELISAs) in infants < 9 months of age is lacking.

Methods: A commercially available ELISA kit (Creative Diagnostics, DEIA359) for estimating measles seroprotection was evaluated in infants 5–7 months of age. In an immunogenicity substudy in the Danish MMR trial conducted between 2019–2021, infants (and mothers at baseline) were sampled before and one month after measles-mumps-rubella vaccination (MMR) or placebo as well as one month after routine MMR at 15 months. Measles IgG ELISA was compared to the gold standard but labor-intensive measles plaque reduction neutralization test (PRNT) by Pearson and Spearman correlations and by estimating sensitivity, specificity, and positive and negative predictive values (PPV and NPV).

Findings: Measles IgG levels compared to PRNT antibodies had a Pearson's correlation coefficient between 0.10–0.24. Seroprotection rates measured by ELISA in young infants were 10–14% lower than measured by PRNT. The sensitivity of the ELISA to detect serological protection compared to PRNT in the infant population differed markedly across sampling time points and was 14%, 40%, and 92% at baseline, post-intervention, and post-routine MMR, whereas the specificity was 99%, 93%, and 43%, respectively. The PPV and NPV were 68% and 87% in infants at baseline.

Interpretation: The correlation between measles IgG and PRNT antibodies was low. Seroprotection was underestimated using ELISA. High-accuracy tests are needed to avoid misclassifications and practices that lead to primary or secondary vaccine failure or retention of vaccination in outbreak settings. Baseline PPV and NPV suggested some applicability of ELISA in predicting serological protection in this age group. However, PRNT may be the only accurate estimator of serological protection in young infants.

* Corresponding author at: Blegdamsvej 9, 2100 Copenhagen E, Denmark.
E-mail addresses: Dorthe.maria.vittrup.01@regionh.dk (D.M. Vittrup), Lone.graff.stensballe@regionh.dk (L. Graff Stensballe).
[1] Trial Sponsor.

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Introduction

Measles is a potentially severe and very contagious disease, which implies a high degree of population immunity to achieve herd immunity. [1,2] Measles serosurveillance, i.e., the measurement of seroprevalence of measles antibodies in a population, is an important tool to monitor population immunity and, by proxy, vaccination coverage and long-term vaccine-induced immunity. Accordingly, measles serosurveillance can identify immunity gaps and thereby assist in faster management of measles outbreaks with focused efforts on vaccinating individuals likely to be susceptible.[3] High throughput, effective, and precise methods are needed to evaluate herd immunity and facilitate prompt actions to contain outbreaks.

Infants are of particular interest as they are at the highest risk of suffering severe consequences from measles infection, including death and severe sequelae.[4] In the post-vaccine era, infants are susceptible to measles from a younger age due to lower vaccine-induced levels of antibodies in their mothers, which are transferred transplacentally to the infant, resulting in an important immunity gap until receiving their first measles-containing vaccine (MCV). Current recommendations include the first MCV from 12 months of age in elimination settings, at 9 months of age in endemic settings, and from 6 months of age in high-risk settings.[5].

An assessment of accuracy and comparability of the different measures of humoral immunity in young infants is currently needed since they may differ from those of older individuals. A central aspect is the effective inhibition of infant vaccine responses by maternal antibodies, which may arise when administering MCV in early infancy [6], and another critical aspect is that infant immune responses are not entirely comparable to older individuals. Lower levels of antibodies are generated upon immunization and are also likely to display lower avidity, further reducing protection.[6] Thus, precise estimates of immunity in infants are crucial for guiding feasible and effective national or even regional vaccination schedules with optimal timing of vaccination: early enough to provide protection prior to exposure to measles and late enough for the maternal antibodies to wane adequately to avoid negative impact on the immunogenicity of MCVs.[5,7] Applying the right tool to measure immunity is essential to obtain the correct information for guiding the optimal vaccination strategies.

Furthermore, immunogenicity studies of vaccines need highly accurate laboratory tests to estimate vaccine responses. The gold standard method, "plaque reduction neutralization assay" (PRNT), is a good proxy of clinical protection against measles,[8] and the protective threshold (≥120 mIU/mL) [9] has been widely accepted and applied for decades. However, the certainty of this cutoff has been questioned based on the scarcity of data.[10] Moreover, the method is labor-intensive, does not provide a rapid test result, and is not easily transferred between laboratories.[11].

The comparison of a cost-effective, high-throughput method like enzyme-linked immunosorbent assays (here indirect ELISA) and the more precise PRNT has been performed several times. [3] Comparisons show that ELISAs display lower sensitivity and underestimate seroprotection rates compared to PRNT, especially in individuals with low levels of antibodies around the protective threshold [8,11]; however, the analyses have shown moderate to good correlation otherwise [8] and with varying levels of sensitivity and specificity.[3] Importantly, this has only sparsely been evaluated in 6-month-old infants.[3] Maturation of the immune system occurs at great speed within the first year of life. Thus, antibodies may be measured differently in assays developed to measure antibodies from mature immune systems. As the infant immune response is generally lower, the risk of misclassification as being seronegative is higher when the test sensitivity is lowered, and antibody levels are around the cutoff value.[11].

The results reported in the present paper are findings from the Danish MMR trial evaluating the intervention MMR versus placebo (solvent: sterile water) in 5–7-month-old infants.[12] Samples were collected at baseline in the infants and their mothers and in the infants one month after intervention and after routine MMR at 15 months of age.[13] The main scope of the paper is a comparison of responses measured by the two methods: measles PRNT and measles IgG by indirect ELISA.

Methods

Ethics

The trial protocol was approved by the Capital Region Biomedical Research Ethics Committee (H-16041195), the Danish Medicines Agency, and the Danish Data Protection Agency (J.no. 2015–41-4508). All legal guardians signed informed consent forms prior to participation. The trial was performed in accordance with the principles of the Declaration of Helsinki.

Study design and participants

The samples were collected as part of the Danish double-blind, placebo-controlled RCT evaluating immunogenicity of MMR in 5–7-month-old infants.[12,13] Samples were collected in > 10 % of the participants (immunogenicity population N=749, correlation population N=718). [12] Selection of infants participating in the immunogenicity study was based on parental interest in this part of the trial and was not selected by staff. Infants were randomized in a 1:1-ratio to receive either M−M−RVaxPro or placebo (solvent: sterile water). Sampling was performed as cubital venipuncture preceded by local anesthetic band-aids (25 mg lidocaine/25 mg prilocaine) at baseline right before receiving the allocated intervention, three to five weeks after intervention, and routine MMR at 15 months of age. Mothers were sampled at baseline. All families were recommended to let the child adhere to the Danish national immunization program with MMR at 15 months and 4 years of age. During the study period, 19 cases of measles were verified in all of Denmark (during 2019 and 2020, none in 2021).[14] Hence, it is reasonable to assume that antibodies present in the infants at baseline were passively acquired from their mothers transplacentally during pregnancy, and that the responses mounted by infants over time were induced by MMR vaccination and not by measles infection.

Samples and laboratory methods

The samples used for analysis were collected in serum clot activator tubes. Serum was separated from cells by centrifugation at 2000 G for 10 min, transferred to cryotubes and stored at −80° Celsius until analysis. Measles IgG ELISA and measles PRNT utilized samples which underwent one and three freeze–thaw cycles, respectively.

Samples were analyzed using two laboratory methods: Commercial Measles IgG ELISA kits [15] and a modified version of a published PRNT protocol.[11] Modifications included but were not limited to using 0.4 % carboxymethylcellulose for the overlay medium and a fixation and staining protocol that included methanol and crystal violet (see supplement for the applied protocol). We used serial, 6-step, 4-fold dilutions, and samples were run in duplicate. The measles virus strain was a laboratory-adapted Edmonston strain. All samples from a mother-infant dyad were run in the same PRNT run and on the same ELISA plate to ensure that differences between samples arose from changes in immunity and not from variations in the laboratory methods. The WHO anti-measles antibody third International Standard (WHO third IS) with a concentration of neutralizing antibodies at 3000 mIU/mL was included in every PRNT run.[16] Plaques were manually counted on a lightbox, and the average count between two corresponding wells was used in the Kärber formula to calculate the concentration of neutralizing antibodies. All laboratory processes were performed manually.

Calculations

For the PRNT, the Kärber formula was used to calculate the ND50 titer: the dilution at which 50 % of measles plaque formation was inhibited by the sample used. This titer could then be converted to a standardized concentration in mIU/mL by applying the WHO-conversion factor based on the WHO-control performance in that specific run to make all runs comparable. The WHO-conversion factor was obtained by calculating the ND50 titer for the WHO third IS for that specific run and multiplying it with the known concentration of the WHO third IS. A level of neutralizing antibodies above 120 mIU/mL is perceived as protective against clinical disease.[9,10].

For ELISA, four-parameter logistics based on the calibrators' optical densities of the run was used to convert the samples' ODs measured in the lab to titers of antibodies. This conversion was performed in R using the function drm from the drc package. Measles calibrators had concentrations expressed in NovaTec Units (NTUs). The concentrations of NTUs in the calibrators A to D are seen in Table 1 below:

A measles IgG level above 200 mIU/mL is considered an indicator of seroprotection against measles infection.[17] The qualitative interpretation of the antibody level in each sample as to whether it is providing clinical protection is shown in Table 2 and based on calibration with the WHO third IS. The measles IgG kit has been validated against the WHO third IS by the manufacturer, and thus, it is possible to convert the titer result into the more readily interpretable mIU/mL and the qualitative assessment of the level being either negative, equivocal, or positive (see Table 2).

Statistical analysis

The main population was defined by mother-infant dyads with at least one infant sample available for both PRNT and IgG ELISA. To include the maximum number of observations, all samples with both a measles PRNT and a measles IgG ELISA result were included in the present study. The correlation was evaluated using both Pearson and Spearman correlations. The analyses were performed in Stata v.18.0.

Results

Quantitative results

The main population was N=718 mother-infant dyads. Infants were 6.4 months old (average) at baseline sampling when the intervention (MMR/placebo) was administered (Table 3). They were born by primarily immune mothers (either vaccinated, WT-measles infected or both, Table 3). Follow-up sampling was performed approx. four weeks after intervention and routine MMR (27 days, Table 3). Measles antibody levels were low in infants at baseline (MMR group 25 mIU/mL (95 % CI; 20–29) and placebo group (29 mIU/mL (95 % CI; 25–34)) and post-intervention (MMR group 120 mIU/mL (95 % CI; 102–141), and placebo group (25 mIU/mL (95 % CI; 22–29), but high in post-routine MMR (MMR group 1815 mIU/mL (95 % CI; 1552–2123), and placebo group 1181 mIU/mL (95 % CI; 1038–1344)) in the PRNT as well as in the ELISA (Table 4). Mothers had high antibody levels in both assays as well (Table 4).

In the comparison of measles IgG ELISA and measles PRNT, we found Pearson correlation coefficients between 0.10 and 0.24 across sampling types (mother, infant at baseline, post-intervention, and post-routine MMR), and Spearman correlations being between 0.22 and 0.52 (Fig. 1). The strongest correlation in the Pearson correlation analysis was found in infants at baseline; however, for the Spearman correlation, the strongest correlations were found in mothers and infants at post-intervention (randomly assigned MMR/placebo, Fig. 1).

Table 1
Calibrators, concentrations, and interpretations for the measles IgG ELISA kit. Antibody concentration in calibrators is reported in NovaTec Units (NTUs).

Calibrator	Interpretation	Concentration in calibrator
A	Negative	1
B	Cut-off	10
C	Weak positive	40
D	Positive	250

Table 2
Translation of measles IgG ELISA results from NTU to mIU/mL in a qualitative manner.

Interpretation	Level NTU	Level mIU/mL
Positive	>11 NTU	>220 mIU/mL
Equivocal	9–11 NTU	120–220 mIU/mL
Negative	< 9 NTU	< 120 mIU/mL

Table 3
Demographics based on randomization group presented for the population with at least one child sample included in the correlation analysis (N=718).

	Participants in the immunological correlation study		
	Total N	**MMR N (%)**	**Placebo N (%)**
Baseline characteristics	718	326 (45.4)	392 (54.6)
Sex boys	718	175 (53.7)	203 (51.8)
Mean infant age in months [a]	718	6.4 (6.4–6.5)	6.4 (6.4–6.5)
Age at randomization < 6 months	718	45 (13.8)	36 (9.2)
Mean sampling interval in days at intervention [b]	708	27 (18–41)	27 (20–42)
Mean sampling interval in days at routine MMR [b]	660	26 (3–78)	27 (20–90)
Premature (GA<37 weeks)	703	27 (8.4)	14 (3.7)
Mean maternal age in years [a]	711	33 (33–34)	33 (32–33)
Household income per year (USD)	707	9 (2.8)	11 (2.8)
Less than 27,000		37 (11.6)	58 (15.0)
Between 27000–54000		274 (85.6)	318 (82.2)
More than 54,000			
Maternal measles immunization status [c]	656	15 (5.1)	11 (3.1)
Previously infected		273(92.2)	329 (91.4)
Vaccinated		8 (2.7)	18 (5.0)
Both previously infected and vaccinated		0 (0.0)	2 (0.6)
Not immunized			
Maternal year of birth (a proxy for measles exposure)	718	128 (39.3)	138 (35.2)
Before 1986		42 (12.9)	60 (15.3)
1986–1987		156 (47.9)	194 (49.5)
After 1987			

N (%) within the population for non-missing data. GA: gestational age. USD: US Dollars. [a] Ages were reported as means with 95% confidence intervals in parenthesis. [b] Sampling interval defined as time from intervention until follow-up blood sampling reported as days in mean (range). [c] Self-reported maternal immunization status.
The number of observations reported here differs from the numbers presented in the original publication of immunogenicity in the trial, as the criterion that the infant needed to have a PRNT result at post-intervention to be included was changed to include infants with a result in both measles IgG ELISA and PRNT at any given timepoint.

Qualitative results

Seroprotection levels were between 4–17 percentage points lower in the ELISA compared to the PRNT analysis, depending on the sampling time point. The best agreement was in the samples collected post-routine MMR at 15 months of age (disagreement was 4 % in the MMR group and 7 % in the placebo group). Sensitivity and specificity of the measles IgG ELISA compared to the measles PRNT varied greatly across sampling time points. Sensitivity was 14 %, 40 %, and 92 % at baseline, post-

Table 4
Descriptive statistics on antibody measurements by measles PRNT and measles IgG ELISA. Absolute levels are reported as arithmetic mean concentration (AMC (range)) and geometric mean concentration (GMC (95 %CI)), and seroprotection rates (SPR). SPR is the ratio of participants with an antibody level above the protective threshold. For PRNT>120 mIU/mL, for ELISA IgG 11 and 9 NTU, respectively, depending on whether the equivocal results are regarded protective or not.

	MMR				Placebo			
	Mother	Baseline	Post int.	Post routine	Mother	Baseline	Post int.	Post routine
Measles PRNT	N=333	N=285	N=290	N=266	N=399	N=349	N=356	N=337
AMC	2743 (3–119513)	80 (1–1829)	455 (2–37295)	3595 (4–87948)	1641 (5–51366)	74 (2–1731)	67 (2–1429)	1937 (3–13407)
GMC	667 (561–793)	25 (20–29)	120 (102–141)	1815 (1552–2123)	712 (625–813)	29 (25–34)	25 (22–29)	1181 (1038–1344)
SPR (%)	87 %	16 %	47 %	97 %	91 %	14 %	13 %	96 %
Measles IgG ELISA	N=333	N=285	N=290	N=266	N=399	N=349	N=356	N=337
Titer AMC	37 (1–292)	2 (0–32)	12 (0–82)	53 (1–198)	37 (0–255)	3 (0–63)	2 (0–44)	37 (1–281)
SPR (%), 11 NTU	76 %	2 %	34 %	93 %	74 %	4 %	2 %	89 %
SPR (%), 9 NTU	79 %	4 %	40 %	94 %	77 %	4 %	3 %	91 %

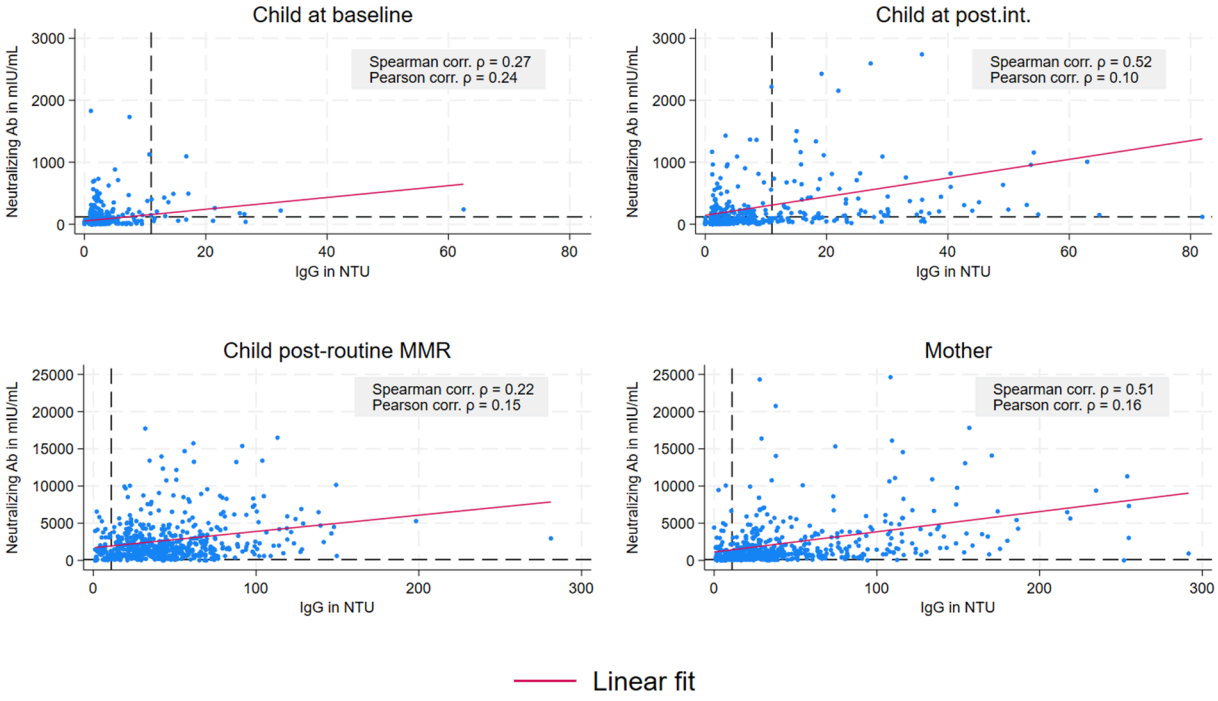

Fig. 1. Measurement of measles neutralizing antibodies by PRNT plotted against measurements of measles IgG by ELISA. Correlation estimates are provided for all sample types separately and are based on all observations with the given sample type. However, the plots are showing a selected range of observations, but all values are included in the linear fit and correlation estimates. N.B., the y-axis varies: it is shared between baseline and post-intervention, and between children at 15 months and mothers. Protective cutoffs at 120 mIU/mL and 11 NTU in PRNT and ELISA, respectively, are presented as dotted lines. Censored values per graph: Baseline (N=0), post-intervention (PRNT>4,000 mIU/mL; N=4), post-routine MMR (PRNT>25,000 mIU/mL; N=2), and mother (PRNT>25,000 mIU/mL; N=4).

intervention, and post-routine MMR, respectively, and specificity was 99 %, 93 %, and 43 %, accordingly. The ELISA performed best in correctly identifying samples as protective or non-protective in the baseline samples with PPV 68 % and NPV 87 %. In samples with expected high levels of antibodies (mothers and post-routine MMR), NPV was low as the ELISA failed to detect relatively low, yet likely still protective levels of antibodies.

Discussion

In this sub-study on immunogenicity within a trial of MMR or placebo at 5–7 months of age, the main finding, the correlation between measles neutralizing antibodies measured by PRNT and measles IgG measured by commercial ELISA, was low to moderate in both Pearson and Spearman correlations independent of sampling time point. This finding was in accordance with other studies of this correlation, albeit lower [3]; however, this correlation has not been thoroughly investigated for infants, which may partly explain the disagreement.[3] Immunity gaps in infants are emerging due to earlier waning of maternal antibodies, and correspondingly, measles cases in infants are rising.[7] This observation warrants a deeper understanding of which methods to apply for accurately estimating measles seroprotection in infants, as it is essential in estimating the susceptibility in this specific population characterized by less mature immune systems diverging from the more mature immune systems including passive antibody presence and reduced immunogenicity. [18].

Measurement of measles IgG by a commercial ELISA kit did not precisely predict serological protection, i.e., antibody levels exceeding the cutoff levels (200 mIU/mL for measles IgG and 120 mIU/mL for measles neutralizing antibodies measured in PRNT), compared to the gold standard method, the PRNT. Other commercial ELISA kits have shown better precision in other studies evaluating other age groups. [3]

In a study of 9-month-old infants [8], post-intervention levels of antibodies exhibited a stronger correlation coefficient (0.59) for measles IgG measured through manually performed ELISA compared to neutralizing antibodies measured by measles PRNT. The tests had the most significant disagreement within the lowest values, a pattern similar to our study.[8] Further, they found the ELISA sensitivity and specificity to be 72 % and 97 %, and the corresponding PPV and NPV were 99.7 % and 22 %, respectively.[8] The findings by Cohen et al.[8] and the present study confirm that ELISAs have low sensitivity for low levels of antibodies,[8] which is the predominant state in infants both pre- and post-vaccination due to reduced immunogenicity of early measles immunization (Table 5). We did not confirm a strong correlation when analyzing the samples from the mothers in opposition to other studies in adults with correlations above 0.7 between measles PRNT and measles IgG ELISA.[19] Likely, the mothers in the present trial were last exposed to measles (infection or vaccination) during childhood.[20] In contrast, the other study evaluated primarily recently challenged (WT-exposure or vaccination) individuals with resulting high levels of circulating antibodies.[19] Thus, the conditions for the performance of the ELISA in the other study were better, which resulted in a higher correlation.

For disagreements within individuals in absolute levels of antibodies, high levels measured in the PRNT with low levels measured in the ELISA were problematic in children after routine MMR at 15 months of age (Fig. 1). The very high levels measured in the measles PRNT are reliably measured until the upper limit of quantification (ULQ) at the highest dilution applied in the assay (8192) multiplied with the WHO conversion factor for each specific run. However, a sensitivity analysis of the correlation estimates is provided in the supplement, in which values below the lower limit of quantification (LLQ) and above ULQ in either the PRNT or the ELISA were omitted. The sensitivity analysis did not change the correlation estimates (supplementary Table 2).

The two methods differ in mechanical aspects that may affect the correlation. The PRNT measures neutralizing antibodies, which can be any isotype of antibodies, and especially when measuring short-term responses app. one month after immunization (MMR group post-intervention and placebo group post-routine-MMR in the current study) [21], IgM is expected to be present and must be expected to provide some neutralizing activity in a serum sample. IgG ELISA quantifies measles-specific antibodies of the IgG isotype, but not all are neutralizing. The primary antigens recognized by neutralizing antibodies are the fusion (F-) and hemagglutinin (H-) proteins, but other viral proteins can be recognized and are targets for non-neutralizing antibodies, e.g., nucleocapsid. [8,22] Further, a primary humoral immune response is characterized by producing lower levels of antibodies with lower avidity compared to a secondary humoral immune response elicited by re-exposure to the antigen.[21,23] Low avidity-antibodies may be washed away by rigorous washing procedures in the ELISA, which are not part of the PRNT procedures, lowering the sensitivity of the ELISA for low-avidity antibodies. Likewise, the secondary humoral response is characterized by higher levels of specific antibodies with increased affinity. A factor that would be expected to increase the correlation.

In a recent review, sensitivity and specificity were shown to vary greatly between commercial ELISA kits (sensitivity range 0 %-98.9 %, specificity range 58.8 %-100.0 %), but with median (IQR) sensitivity at 90.6 [86.6–95.2] and median (IQR) specificity = 100.0 [88.7–100.0].[3] The pooled sensitivity to detect seroprotection in the ELISA was around 80 % in the present study (results from all samples pooled together, not shown in the table) is a well-described phenomenon when compared to the measles PRNT with a protection cutoff at 120 mIU/mL [24] and not specific for young infants. However, the performance of the ELISA is especially challenged when the antibody levels are close to the protective cutoff (baseline and post-intervention samples, Table 5), which is the case in many infants both pre- and post-immunization due to low levels of maternal antibodies months after birth and lower immunogenicity of early administration of MCVs. The ELISA generally

Table 5
Analyses of agreement between measles IgG ELISA and measles PRNT. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) areis presented both when treating equivocal results as protective (>9 NTU regarded protective) and non-protective (only > 11 NTU regarded protective). The numbers are reported pooled within sampling time point and for randomization groups separately.

	Sensitivity	Specificity	PPV	NPV
Treating equivocal results as non-protective (>11 NTU is considered protective)				
Baseline samples	13/95 (14 %)	533/539 (99 %)	13/19 (68 %)	533/615 (87 %)
MMR	5/45 (11 %)	239/240 (100 %)	5/6 (83 %)	239/279 (86 %)
Placebo	8/50 (16 %)	294/299 (98 %)	8/13 (62 %)	294/336 (88 %)
Post-intervention samples	73/182 (40 %)	431/464 (93 %)	73/106 (69 %)	431/540 (80 %)
MMR	72/136 (53 %)	126/154 (82 %)	72/100 (72 %)	126/190 (66 %)
Placebo	1/46 (2 %)	305/310 (98 %)	1/6 (17 %)	305/350 (87 %)
Post-routine MMR samples	535/580 (92 %)	10/23 (43 %)	535/548 (98 %)	10/55 (18 %)
MMR	240/258 (93 %)	1/8 (13 %)	240/247 (97 %)	1/19 (5 %)
Placebo	295/322 (92 %)	9/15 (60 %)	295/301 (98 %)	9/36 (25 %)
Mother samples	510/654 (78 %)	41/78 (53 %)	510/547 (93 %)	41/185 (22 %)
MMR	233/291 (80 %)	22/42 (52 %)	233/253 (92 %)	22/80 (28 %)
Placebo	277/363 (76 %)	19/36 (53 %)	277/294 (94 %)	19/105 (18 %)
Treating equivocal results as protective (>9 NTU is considered protective)				
Baseline samples	18/95 (19 %)	531/539 (99 %)	18/26 (69 %)	531/608 (87 %)
MMR	9/45 (20 %)	238/240 (99 %)	9/11 (81 %)	238/274 (87 %)
Placebo	9/50 (18 %)	293/299 (98 %)	9/15 (60 %)	293/334 (88 %)
Post-intervention samples	79/182 (43 %)	418/464 (90 %)	79/125 (63 %)	418/521 (80 %)
MMR	78/136 (57 %)	116/154 (75 %)	78/116 (67 %)	116/174 (67 %)

(continued on next page)

Table 5 (*continued*)

	Sensitivity	Specificity	PPV	NPV
Placebo	1/46 (2 %)	302/310 (97 %)	1/9 (11 %)	302/347 (87 %)
Post-routine MMR samples	545/580 (94 %)	10/23 (43 %)	545/558 (98 %)	10/45 (22 %)
MMR	243/258 (94 %)	1/8 (13 %)	243/250 (97 %)	1/16 (6 %)
Placebo	302/322 (94 %)	9/15 (60 %)	302/308 (98 %)	9/29 (31 %)
Mother samples	534/654 (82 %)	40/78 (51 %)	534/572 (93 %)	40/160 (25 %)
MMR	242/291 (83 %)	21/42 (50 %)	242/263 (92 %)	21/70 (30 %)
Placebo	292/363 (80 %)	19/36 (53 %)	292/309 (94 %)	19/90 (21 %)

has a negative result output, which results in falsely high specificity in the samples known to have low to no antibodies (baseline and post-intervention) and very low sensitivity due to misclassifying the protective samples as also being negative. This could be explained by low levels of antibodies in infants at baseline and in the post-intervention sample and high levels in the mothers and the samples collected after routine MMR at 15 months of age. This is an important challenge for making vaccine schedule recommendations, as the presence of even low levels of maternal antibodies greatly inhibits responses in the infant.[13] Because of an overall underestimation of protective levels of antibodies in the ELISA assay, sensitivity was especially low for the early sampling time points.

Changing the interpretation status of the equivocal results from non-protective by using the >11 NTU criterion to protective by using the >9 NTU criterion, only slightly increased the sensitivity of the ELISA. Several studies have concluded that equivocal results in ELISAs should preferably be considered protective levels of antibodies [3]. However, it only marginally changed the results and did not change the conclusions in the current study.

Although not representing a correlation to clinical protection, measurement of measles-specific T cells has a place in immunogenicity studies of MCVs in providing evidence of cellular immunogenicity as shown previously.[25] Measles-specific T cells are deterministic in the elimination of measles infections and for the generation of a long-lasting and effective humoral vaccine response [26], however, including T cell analyses in serosurveillance is currently not relevant due to the lack of an established cutoff for clinical protection.

The measles IgG ELISA was only run in unicate, but an average of a triplicate run would have been ideal. However, no variations among the assay plates were observed when comparing the control readings (supplement Table 1).The difference in number of freeze–thaw cycles between samples utilized for measles IgG ELISA and PRNT (one and three, respectively) is a limiting factor in comparability between samples, however, measles antibodies have been shown to be robust to freeze–thaw cycles, [27] and therefore, the low to moderate correlation between the two assays was not explained by this factor.

Several staff members were involved in performing the laboratory analyses, and the PRNT can be especially sensitive to variation in procedures due to several factors with biologically active properties (live measles virus and Vero cells).[11] The WHO third IS partly mitigated this variation, as it was included in every run of the PRNT, enabling a comparison between runs and functioned as a quality control of each run. On the other hand, the study describes measles antibodies in a unique and rather large population in a unique setting: the young infants right before and after early MMR in a measles-elimination setting.[28] This provides a novel understanding of the fitness of ELISA for serosurveillance and immunogenicity outcomes in vaccine studies in infants.

The baseline results showed that ELISA underestimates the seroprotection rate by more than 10 %. Underestimating the seroprotection rate in infants could lead to concerns regarding the duration of passive immunity and the development of immunization policies regarding early MCV that would not serve the purpose of successfully immunizing susceptible infants but could adversely lead to primary or secondary vaccine failure instead. Especially antibody levels around the protective threshold tend to be misclassified as negative.[19] If levels in the equivocal range and lower in the ELISA are actually protective, which seems to be the case in head-to-head comparisons between ELISAs and PRNT [8], it would change the picture regarding young infant susceptibility to measles, i.e., proportion without persisting antibodies at 6 months of age and also change the ratio of infants obtaining protection following immunization. Immunogenicity of MCVs is found to be lower in young infants [6], why ELISAs may not be a fit for estimating seroprotection following early MCV either. The clinical consequences of obtaining low levels, yet above the protective threshold, of antibodies as a primary vaccine response is not entirely understood. [6,29].

Emerging immunity gaps due to vaccine-induced maternal immunity with earlier waning in the 6–12-month-old infants call for action since they are at a higher risk of suffering from measles morbidity and mortality than measles cases in older age groups.[4] Overestimation of protection based on measles IgG ELISA is also an issue since unpredicted outbreaks could occur and lead to delays in outbreak responses and a wider spread of measles, counteracting the global efforts to obtain measles elimination.[7] In outbreak situations, a rapid result on whether a measles-exposed person is protected or should be offered post-exposure prophylaxis (PEP) can greatly enhance the chance of containing the spread of measles, decrease the individual risk of developing measles, and reduce the number of unnecessary vaccines administered to already protected individuals. This result cannot be provided rapidly by PRNT, and in such situations, EIAs are useful tools.

Findings in measles serosurveillance should be cautiously interpreted for individuals with low, yet likely still protective, levels of antibodies. The accuracy of the test needs to be high to avoid misclassifications and practices that lead to primary or secondary vaccine failure or retention of post-exposure prophylaxis in outbreak settings. An approach already in use in Canada [30] to supplement EIAs with PRNT analysis in case of a negative or equivocal result increases the sensitivity by several percentage points. However, this strategy relies on a high NPV of the EIA, which does not seem to be the case for ELISA in young infants. Instead, we suggest that samples from young infants are analyzed using a measles PRNT.

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CRediT authorship contribution statement

Dorthe Maria Vittrup: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Data curation. **Andreas Jensen:** Writing – review & editing, Visualization, Validation, Supervision, Software, Formal analysis, Data curation. **Michelle Malon:** Writing – review & editing, Validation, Project administration, Investigation. **Anne Cathrine Zimakoff:** Writing – review & editing, Validation, Project administration, Investigation. **Jesper Kiehn Sørensen:** Writing – review & editing, Validation, Project administration, Investigation. **Brickley Littell:** Writing – review & editing, Methodology,

Investigation. **Eric A.F. Simões:** Writing – review & editing, Supervision, Project administration, Methodology. **Jannet Svensson:** Writing – review & editing, Supervision, Project administration. **Lone Graff Stensballe:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Lone Graff Stensballe reports financial support was provided by Innovation Fund Denmark. Dorthe Maria Vittrup reports a relationship with MSD Denmark that includes: speaking and lecture fees. Dorthe Maria Vittrup reports a relationship with Sanofi-Aventis France SA that includes: consulting or advisory. MM received grants from Helsefonden, The Beckett Fund, and the Rosalie Petersen's Fund.

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Data availability

Data will be made available on request.

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Appendix A. Supplementary material

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