SUPPLEMENTAL MATERIAL

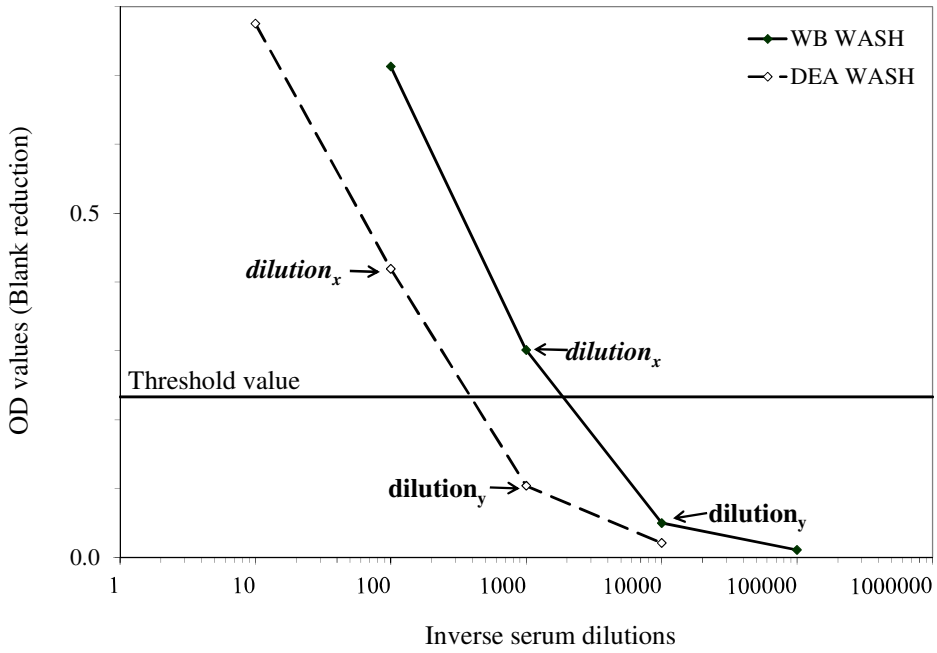

FIG S1 Modified formula to calculate end-titer avidity index percentages (etAI%) (22). Dilution series washed either with wash buffer or diethylamine depicted for reference. OD is optical density value.

etAI% = (*end-titer* diethylamine curve / *end-titer* wash buffer curve) X 100,

where

end-titer= $\text{dilution}_x^{-1} \text{x} 10^a$

where

$a = \log 10 \text{ X } (OD_x - OD \text{ threshold value})/OD_x - OD_y)$

where

10 is the dilution factor

OD_x is the optical density value at dilution_x

OD threshold value is 0.9 X calibrator value as described in kit's insert

OD_y is the optical density value at dilution_y

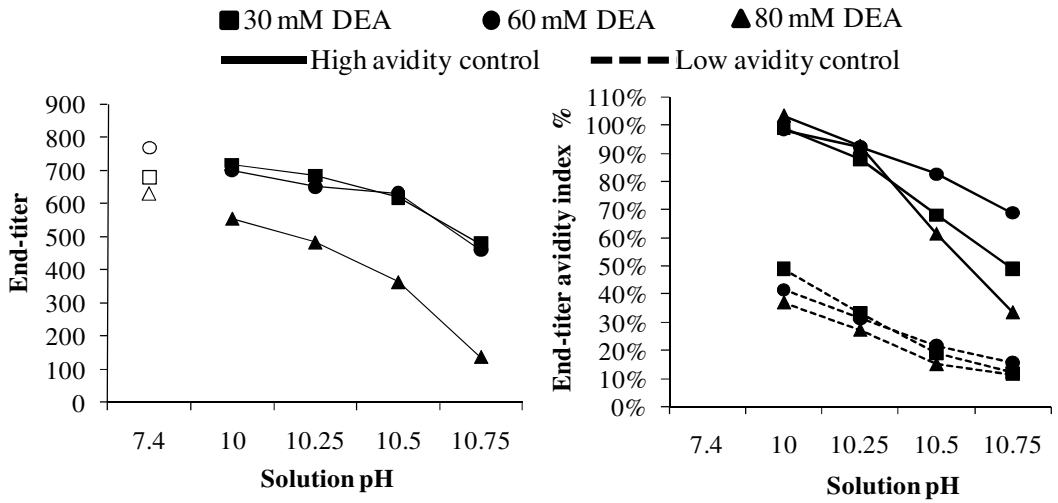

FIG S2 Effect of diethylamine (DEA) molarity and pH on binding of coating measles virus antigen (left panel) and on binding of measles-specific antibodies (right panel). Open symbols are treatment with wash buffer. Solid symbols are treatments with DEA solutions at specified molarities. Original pH for these solutions was >11. The pH of the DEA solution increases with molarity. In preliminary experiments, the effect of higher pH values and higher molarities was already observed. A solution of 8.75mM DEA (pH 9.3) was not effective in eluting antibodies expected to be of low avidity, while a solution of 17.5mM DEA (pH 10.9) consistently washed away high avidity antibodies. A solution of 60 mM DEA adjusted to a pH of 10.25 (±0.1) was selected. In our hands, 6M, 7M, and 8M urea destabilized the antigen and resulted in loss of optical density signal (data not shown).

Avidity results based on a single dilution

Materials and methods: Comparison of single serum dilution and serial serum dilutions was performed using all samples in groups A and B (Table1). Single serum dilution points at 1:100 (n=154) and 1:10 (n=53) were taken out of the serum dilutions curves used to calculate etAI%. Avidity results were calculated as avidity index (AI%):

AI%= (*OD* after diethylamine wash / *OD* after wash buffer wash) X 100,

where *OD* is optical density at the selected dilution (either 1:100 or 1:10)

The AUCs were compared using MedCalc for Windows, version 8.1.1.0 (MedCalc Software, Belgium. Microsoft® Office EXCEL 2003 was used to calculate coefficients of determination (R^2). *Results:* The AUCs were not significantly different (P=0.763). The AUC at dilution 1:100 was 0.959 (95%CI: 0.914 to 0.984). At dilution 1:100, R^2 was 0.889 and at dilution 1:10 R^2 was 0.918. At 1:100, a threshold greater than 80% lead to an assay specificity of 95.08% (95%CI: 86.3-98.9) and sensitivity of 94.62% (95%CI: 87.9-98.2). *Conclusion:* In those situations where time or resources are limited, this avidity assay could be performed using a single dilution. An AI% threshold should be established for the serum dilution (1:20) that is used in the Captia™ assay protocol.

Interpretation of intermediate avidity results

Intermediate avidity results are generally uninterpretable, but can be used in two situations. First, except for underlying conditions that may result in potentially slower IgG avidity maturation (as in HIV-infections, Down syndrome, or organ transplant recipients) intermediate avidity results in samples collected 4 to 8 weeks after rash onset from unvaccinated individuals suggest recent infection with measles virus (S1, S2, 27). To confirm this possibility, high avidity antibodies should be detected in a second sample collected a few weeks later. Second, when investigating SVF with elevated PRN titers, intermediate avidity results can be interpreted as suggestive of secondary immune responses, since low avidity results were not observed in samples collected from persons with long-standing immunity. Noteworthy, intermediate avidity results have been obtained in a healthy individual born during the pre-vaccine era, in recently vaccinated persons with rash and fever illness, and in young infants with classic measles with possible interference of maternal antibody.

REFERENCES

S1. **Kusters, M., C. M. Jol-van der Zijde, M. van Tol, W. E. Bolz, L. A. Bok, M. Visser, and E. de Vries.** 2011. Impaired avidity maturation after tetanus toxoid booster in children with Down syndrome. The Pediatric infectious disease journal **30:**357-359.

S2. **Lutz, E., K. N. Ward, and J. J. Gray.** 1994. Maturation of antibody avidity after primary human cytomegalovirus infection is delayed in immunosuppressed solid organ transplant patients. Journal Of Medical Virology **44:**317-322.