A New Method with General Diagnostic Utility for the Calculation of Immunoglobulin G Avidity

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Received 22 February 1999/Returned for modification 13 April 1999/Accepted 16 June 1999

The reference method for immunoglobulin G (IgG) avidity determination includes reagent-consuming serum titration. Aiming at better IgG avidity diagnostics, we applied a logistic model for the reproduction of antibody titration curves. This method was tested with well-characterized serum panels for cytomegalovirus, Epstein-Barr virus, rubella virus, parvovirus B19, and Toxoplasma gondii. This approach for IgG avidity calculation is generally applicable and attains the diagnostic performance of the reference method while being less laborious and twice as cost-effective.

The diagnosis of acute viral and some other microbial infec-
tions often relies on the serological detection of immunoglobu-
lumin M (IgM) antibodies, but the available techniques have serious pitfalls that may lead to erroneous interpretations (3, 4, 9, 11). This problem is of particular importance for infections during the first trimester of pregnancy, which should be diagnosed as exactly as possible (8, 10, 18). The differential assay of high-avidity and low-avidity IgG antibodies can be used as an alternative or a complement to the IgM antibody assay and is gaining popularity as a diagnostic method for the assessment of the time of infection. In protein-denaturing avidity enzyme immunoassays (EIAs), the patient’s IgG (e.g., in serum) is allowed to bind to its antigen, followed by elution with or without a protein denaturant, such as urea. From the proportion of IgG remaining antigen bound, the time of primary infection can be deduced.

The most straightforward procedure for the calculation of avidity is a comparison of EIA absorbances in single (fixed) dilutions of serum. This procedure is quite sensitive and specific for the diagnosis of several different microbes (3, 6, 7, 13, 17, 18) but is affected to some extent by the concentration of specific IgG (7). The two-step avidity assay developed by Lecoeur and Pucheu is based on the selection of working dilutions according to the level of specific IgG in each specimen (12). Another means of improved avidity calculation is based on the α method, in which the antibody titer is derived from a single dilution of serum by use of the formula log_{10} titer = α(OD^β), in which OD is optical density and α and β are constants specified by the assay manufacturer for each batch of kit reagents (5, 19). The avidity technique based on end-point titration of IgG (2, 8, 10, 11, 16, 17) is not influenced by IgG concentration but is relatively laborious and reagent consuming. Due to its excellent sensitivity and specificity, we consider this approach the reference method for several microbes (9). Aiming at low cost combined with high performance, we have applied a logistic procedure (14) for avidity calculation; here, we evaluate its diagnostic value.

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FIG. 1. End-point titration curves for CMV IgG avidity determinations. Views are as seen on the computer screen. In each curve pair, the upper one was obtained without urea and the lower one was obtained with urea. Calculations were done with the reference method and 4 + 4 data points (dilutions of serum) (A), with the logistic model and 4 + 4 data points (B) and 2 + 2 data points (C), and with the log-log model and 2 + 2 data points. This CMV IgG is of low avidity. PBST, phosphate-buffered saline containing 0.05% Tween 20.
titration curve with no dilution. Thus, only two parameters had to be fitted for each curve.

**Log-log model.** For comparison with the logistic model, a piece of software which functions by linear interpolation of log(absorbance) versus log(dilution) values for native and denatured samples was developed. The end-point titer of both samples was calculated, and the ratio of end-point titers was taken to be the output avidity result. In fact, this process amounted to using the function \[ \log(f(x)) = ax + b \] for the curve fitting instead of the logistic function. As above, \( x \) was the logarithm of the dilution ratio.

**RESULTS**

In the reference method, EIA absorbances were plotted against serum dilutions on a semilogarithmic scale, and the individual data points (4+4 dilutions per sample) were united by straight lines (Fig. 1A). Under the same conditions with 4+4 serum dilutions, the logistic model produced curvilinear, or “smooth,” IgG titration curves, which often bypassed individual data points (Fig. 1B). With 2+2 serum dilutions per sample, the same logistic model produced IgG titration curves that resembled those obtained with 4+4 serum dilutions (Fig. 1C) but, at the curve ends, met their data points precisely. The log-log model displayed linear IgG titration curves when both axes were linear (Fig. 1D).

The logistic model operating with 2+2 dilutions per sample was tested with all the serum panels, and the results were compared with those obtained with the reference method (operating with 4+4 data points). Overall, the two methods showed excellent correlation; the correlation coefficients for all four viruses and the one protozoan were \( r = 0.94 \) (Fig. 2). Also illustrated are the domains (bordered by broken lines) in which the avidity values obtained could be allowed to move without...
a change in diagnosis. For the >1,000 samples studied, only once, in the parvovirus serum panel, was there disagreement between the two methods; the reference method produced a pathological value of low avidity (12%), whereas the 2+2 logistic method produced a nonpathological value of high avidity (26%). This single crossover was due to a deviant Elia data point caused by an apparent pipetting error; however, this error was well tolerated by the 4+4 logistic method, which produced a borderline-avidity result (17%).

The diagnostic value of the simple log-log model was determined with the large toxoplasma serum panel. As depicted in Fig. 2E, this model also corresponded fairly well to the reference method, yielding only two false high-avidity results. However, the logistic model (with 2+2 data points) was even more accurate \( (r, 0.96) \) producing no false avidity results and fewer crossovers to or from the borderline avidity zone (Fig. 2F).

**DISCUSSION**

We applied and evaluated curve-fitting methods for IgG avidity calculations. The diagnosis of four viruses (a nonenveloped single-stranded DNA virus, an enveloped single-stranded RNA virus, and two enveloped double-stranded DNA viruses) and one protozoan could be accomplished reliably with the logistic model and only \( 2+2 \) dilutions per sample. Success with *T. gondii*, an immunologically and structurally complex pathogen, is particularly noteworthy because simple indices obtained from single dilutions of serum are insufficient for its avidity determination (7, 8). Given previously published work (15), we were somewhat surprised to observe that the diagnostic performance of the log-log model lagged behind that of the more elaborate logistic model only slightly. The explanation may arise from the fact that in avidity determinations, two parallel (with and without a protein denaturant) titration curves are generated by the same model, the inherent errors of which are abolished when the two titration end-point values are divided.

In conclusion, the logistic approach for IgG avidity calculations is generally applicable, attains the diagnostic performance of the reference method, and is twice as cost-effective.

**ACKNOWLEDGMENTS**

We thank Lea Hedman for expert technical assistance. This work was supported by the Helsinki University Central Hospital Research and Education Fund, the Finnish Technology Advancement Fund, the Center for International Mobility, and the Sohlberg Foundation.

**REFERENCES**