Figure S2

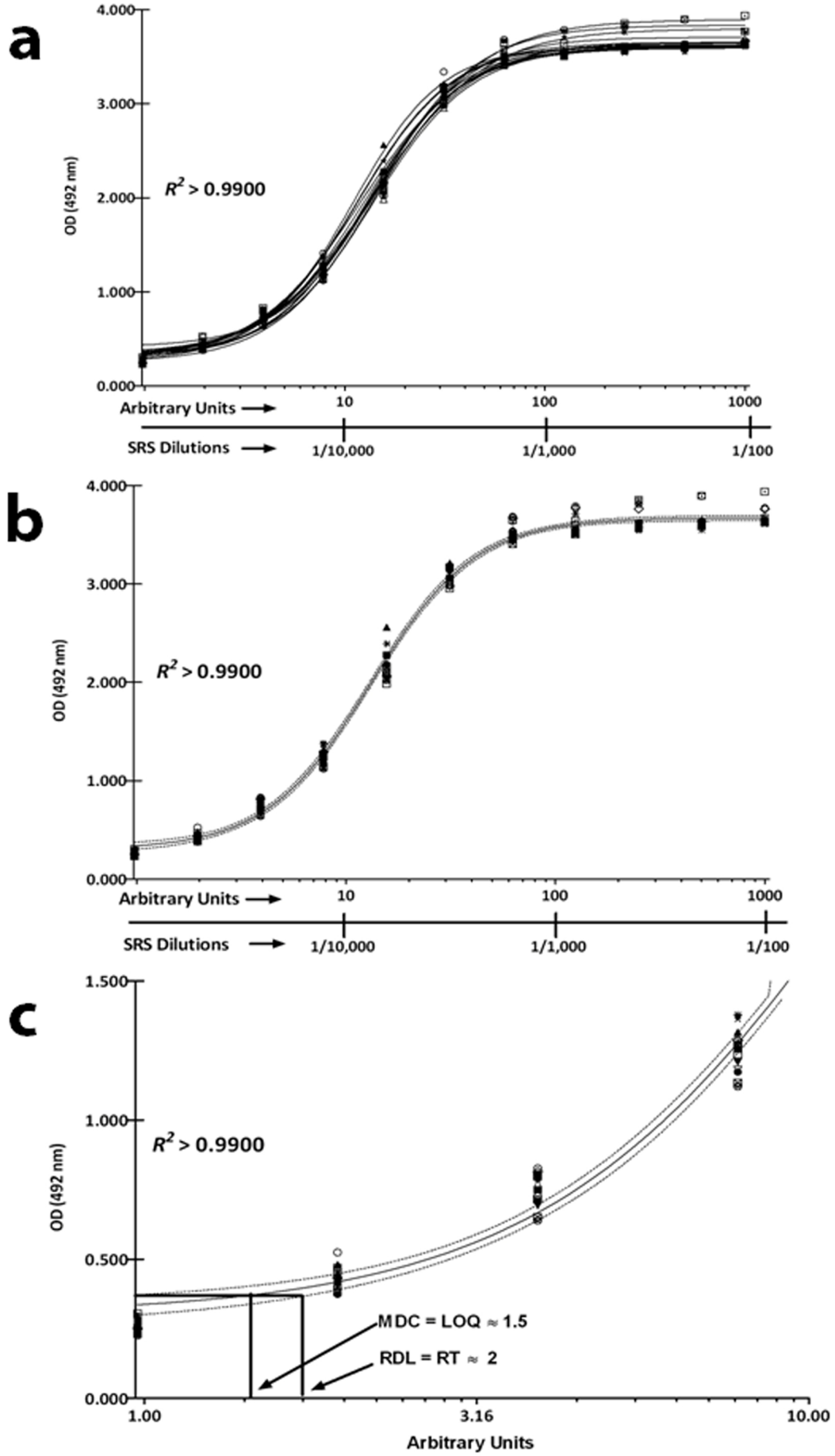
a
4.000
3.000
2.000
1.000
0.000
OD (492 nm)
R^2 > 0.9900
Arbitrary Units
10
100
1000
SRS Dilutions
1/10,000
1/1,000
1/100
b
4.000
3.000
2.000
1.000
0.000
OD (492 nm)
R^2 > 0.9900
Arbitrary Units
10
100
1000
SRS Dilutions
1/10,000
1/1,000
1/100
c
1.500
1.000
0.500
0.000
OD (492 nm)
R^2 > 0.9900
MDC = LOQ ≈ 1.5
RDL = RT ≈ 2
1.00
3.16
10.00
Arbitrary Units

Figure S2. An illustration of the relationship of Reliable Detection Limit when used as a Reactivity Threshold to the Standard Calibration Curve by 4-parameter logistic-log modeling. The Determination of the Reliable Detection Limit as Reactivity Threshold for the assays by using 4-parameter logistic-log modeling of 25 runs of a Standard Reference Serum to estimate a "Standard Calibration Curve". The RDL is interpolated from the lower 95% Confidence Interval of the lower asymptote of the 4-PL modeled logistic log curve. (a) The Standard Reference Serum was developed by pooling the sera from 10 female BALB/c mice (Taconic) 6-8 weeks old that were administered i.p 50 µg of AnAPN1/ Alhydrogel® in a prime (day 0) and boost (day 28) regimen and bled on days -1, 14, 28 (boost), with a terminal bleed on day 42 (see also Table 1). Sera were pooled from the terminal bleed and then tested for the presence of antibodies anti-AnAPN1 IgG. The Standard Calibration Curve is derived by serially diluting this SRS starting at 1:100 across the ELISA plates 12 times. The resulting curve is sigmoidal, with extended upper and lower asymptotes, which makes it amendable to by 4 parameter logistic-log modeling: $F(x) = ((A-D)/(1+((x/C)^B))) + D$. Also on this figure is the assignment of Arbitrary Units of Antibody. The lowest dilution of antibody used to make the SCC (1:100) is assigned 1000 Arbitrary units of Antibody, which is the highest number given that the lowest dilution would have the highest OD 492nm as shown in Panel B, where is has an $OD492_{nm}$ of 3.982. The subsequent dilution points are assigned units accordingly (e.g., a dilution of 1:1000 receives 100 units of antibody or a tenth of the units assigned to the 1:100 dilution, with the assumption there are fewer antibodies). Here the horizontal axis represents Arbitrary Units of Antibody on a log scale and the vertical axis represents the OD at 492_{nm}. (b) Figure showing all 25 SRS runs combined into a single curve referred to as the Standard Calibration Curve which the mean of the 25 curve (solid line) and its 95% Confidence Intervals (dashed lines). (c) Figure shows a magnified view of lower asymptote SCC shown in Panel B indicating the Minimal Detection Concentration (MDC) and Reliable Detection Limit of mouse IgG against AnAPN1 [1-2]. The lowest concentration of analyte that can be detected with a specific degree of probability in a diluted serum sample is defined as the minimum detectable concentration (MDC). The lowest concentration of analyte that has a high probability of producing a response significantly greater than the response at zero concentration of analyte is defined as the reliable detection limit (RDL). More specifically, the MDC is the concentration of anti-AnAPN1 IgG corresponding to the interpolated intersection of the lower asymptote of the upper 95% confidence interval (95% CI) with the 4-PL fit of the SCC (panel B). The RDL is the concentration of anti-AnAPN1 IgG corresponding to the interpolated intersection of the upper 95% CI asymptote with the lower-95% CI of the SCC. Accordingly to Quinn et al [2], the MDC and RDL are distinct and statistically robust measurements of the lower limits of detection for an indirect ELISA assay, with the RDL being the more conservative of the two. The Reactivity Threshold is a term that refers to the application of the RDL to current assays where it is used to categorize a serum of an animal is determined to be "reactive" or "non-reactive". Here, the OD value is converted to an anti-AnAPN1 IgG concentration as expressed in Arbitrary Units as explained above.

References

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