

# Development of a Rapid Gold Nanoparticles-Based Lateral Flow Immunoassay for the Identification of Dengue Virus

Manuscript ID: biosensors-1728240

## Supplementary Material

**Supplementary Information 1.** Equations and calculations to determine the TCID<sub>50</sub> and PFU.

Table S1 shows data for titration of virus stocks.

**Table S1.**

Log of virus dilution	Positive units	Total units	Ratio percent (A/B)*100
-1	6	6	100
-2	5	6	83.33
-3	2	6	33.33
-4	2	6	33.33
-5	1	6	16.66

With these results, the log viral titer was calculated by the Reed-Muench method [1], first calculating the proportionate distance;

$$\text{Proportionate distance (PD)} = \frac{\% \text{ mortality greater than 50\%} - 50\%}{\% \text{ mortality greater than 50\%} - \% \text{ mortality below 50\%}}$$

$$PD = \frac{83.33 - 50}{83.33 - 33.33} = \frac{33.33}{50} = 0.66$$

To calculate the viral titer, it was determined as follows:

*Negative logarithm of LD50 endpoint titer = PD \* negative Logarithm of factor dilution*

$$= 0.66 * -1 = -0.66$$

**Log LD50 titer** = *Negative logarithm of the lower dilution (next above 50% mortality)*  
 – *Negative logarithm of LD50 endpoint titer*

$$\text{LD50 titer} = (-2) + (-0.66) = -2.66$$

$$\text{Log LD50 titer} = 10^{-2.66}$$

From this value, the TCID<sub>50</sub>/mL was determined with the following equation:

$$\text{Infectious doses per unit volume} = \frac{\frac{1}{\log \text{LD50 titer}}}{\text{viral inoculum volume}}$$

$$= \frac{\frac{1}{10^{-2.66}}}{0.1 \text{ mL}}$$

$$\text{TCID}_{50}/\text{mL} = 4.58 \times 10^3$$

The value TCID<sub>50</sub>/mL was converted to plaque forming units per mL (PFU/mL) using the Poisson distribution [2-5]. This conversion is an estimate based on the rationale that the limiting dilution, which would infect 50% of the cell layers challenged, would be expected to produce a single plaque in a cell monolayer.

To estimate PFU/mL from TCID<sub>50</sub>/mL, the Poisson distribution can be applied; P(o) is the proportion of negative tubes and 'm' is the mean number of infectious units per volume (PFU/mL),

$$P(o) = e(-m)$$

For any titer expressed as TCID<sub>50</sub>, P(o)=0.5; m= -ln 0.5; therefore, the value of e is 0.693 ≈ 7 [2]. Then;

$$\text{PFU/mL} = 4.58 \times 10^3 * (0.7) = 3.2 \times 10^3$$

To estimate total PFU value, the PFU/mL was multiplied by the volume in mL used in the strip (since the undiluted stock was used);

$$\text{Total PFU} = 3.2 \times 10^3 * 0.160 = 5.12 \times 10^2$$

**Supplementary Table S2.** Determination of the hydrodynamic diameter (relative size), ζ-potential and distribution (polydispersity index) of the AuNP conjugates.

Sample	Hydrodynamic diameter	ζ -Potential	Polydispersity index
AuNP CIT	67.69	-34.9	0.2

AuNP-BSA	103.5	-24.17	0.136
AuNP- 4G2 [1 µg]	107.87	-23.43	0.120
AuNP- 4G2 [3 µg]	117.20	-24.83	0.167
AuNP- 4G2 [6 µg]	106.63	-22.30	0.139

## References

1. Horstmann, D. M. DIAGNOSTIC PROCEDURES FOR VIRAL AND RICKETTSIAL INFECTIONS (4th Ed.). Am. J. Public Heal. Nations Heal. 1970, 60 (5), 950–951.
2. The American Type Culture Collection (ATCC). Virology Guide. Available in <https://www.atcc.org/-/media/resources/culture-guides/virology-guide.pdf?rev=a3816afe46cb499c90265fcf6456d2e3>.
3. Logan, M., Manalil, J., Notte, C., Kearse, C., George, S., Zeiser, A., Farrell, P., & Aucoin, M. G. (2019). A flow cytometric granularity assay for the quantification of infectious virus. *Vaccine*, 37(47), 7090–7099. <https://doi.org/10.1016/j.vaccine.2019.02.059>
4. Manenti, A., Molesti, E., Maggetti, M., Torelli, A., Lapini, G., & Montomoli, E. (2021). The theory and practice of the viral dose in neutralization assay: Insights on SARS-CoV-2 "doublethink" effect. *Journal of virological methods*, 297, 114261. <https://doi.org/10.1016/j.jviromet.2021.114261>
5. Stanley, S., Hamel, D. J., Wolf, I. D., Riedel, S., Dutta, S., Contreras, E., Callahan, C. J., Cheng, A., Arnaout, R., Kirby, J. E., & Kanki, P. J. (2022). Limit of Detection for Rapid Antigen Testing of the SARS-CoV-2 Omicron and Delta Variants of Concern Using Live-Virus Culture. *Journal of clinical microbiology*, 60(5), e0014022. <https://doi.org/10.1128/jcm.00140-22>