Annex 1: procedure for ELISA technique using ELISA kit (DIALAB® Austria) and *Emax* ELISA plate reader, E11865 model (*Molecular Devices*® USA)

1. All reagents were allowed to reach room temperature for 15 minutes before use.
2. A 1:40 dilution of Wash Buffer Concentrate with distilled water was prepared.
3. Sample diluent (100 ul, 92 drops) was added into the appropriate wells except the blank well, negative well (one well each for blank and positive control well and 2 wells for negative control)
4. 10ul specimen was added to the well, beating by pipettor repeatedly until liquid turn blue, 50ul of negative and positive control were dispensed into the negative and positive well separately. No liquid was added to the blank control well.
5. Microtiter wells were flicked for 30 seconds and allowed to mix well. The plate was affixed to sealing template and incubated at 37°C for 20 minutes.
6. The plate was then taken out wash Buffer added to each well and absorbed after 20 seconds. It was repeated 5 times until each well was dried.
7. 1 drop (50ul) of HRP conjugate was dispensed to each well except the blank well. Gently vibration mixture, mixed well, affixed to sealing template. Then incubated at 37°C for 20 minutes
8. Wash Buffer was added to each well and absorbed after 20 seconds. It was repeated 5 times until each well was dried.
9. 1 drop (50ul) of Substrate A and 1 drop (30ul) of Substrate B were dispensed into every well except blank well. Incubated at 37°C for 10 minutes. Gently vibration mixture, mixed well and incubated at 37°C for 10 Minutes.
10. Then, the plate was taken out, 1 drop (50ul) of Stop solution added except the blank well, mixed well, and then read with a micro well reader for the result.