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**Title: Standard Operating Procedure (SOP) for Quantification of IgG antibodies against *Plasmodium falciparum* GLURP<sub>25-514</sub> (RO)**

**Introduction:**

The IgG level is determined by an indirect ELISA method using a single serum / plasma dilution obtained by checker-board titration experiments. The optical density (OD) of the test sample is converted into arbitrary units (A.U.) by means of a standard-curve from known concentrations of purified human polyclonal IgG.

Wells of microtiter plates are coated with either GLURP<sub>25-514</sub> antigen or serial dilutions of the purified human polyclonal IgG. Diluted test-samples and controls (primary antibody) are added to the wells containing the GLURP<sub>25-514</sub> antigen and specific antibodies against GLURP are revealed by Peroxidase conjugated rabbit anti-human IgG (secondary antibody). Bound secondary antibody is quantified by colouring with ready to use TMB (3,3', 5,5'-Tetramethylbenzidine) substrate. Optical density (OD) is read at 450 nm with a reference at 620nm in a plate reader, and the OD value of the test-sample is converted into A.U. by means of a standard curve on each plate.

**(a) Preparation of Standard curve:**

For antibody quantification, each assay includes a calibration (standard) curve obtained with a polyclonal reference IgG (1.0 mg/ml). For the standard curve, serial dilutions (2-fold) of standard IgG proteins are coated directly to duplicate wells in columns 1 and 2 using 100 µl / well. Concentrations of standard IgG are 100; 50; 25; 12.5; 6.25; 3.13; 1.56; 0.78 ng / ml. In order to prepare the standard reference curve for 12 plates, first make a working stock of 500 ng/ml by transferring 5 µl of the 1.0 mg / ml standard into 9.995 ml of coating buffer (1:2000).

Then prepare 8 tubes labelled 1 to 8 for a 2-fold serial dilution of the standard

1. To tube 1, add 8 ml of coating buffer.
2. Add 3 ml of coating buffer each to tubes 2 to 8.
3. Transfer 2 ml from the working stock (500 ng/ml) to tube 1, and mix well.
4. Transfer 3 ml from tube 1 into tube 2, and mix well.
5. Transfer 3 ml from tube 2 into tube 3, and mix well.
6. Transfer 3 ml from tube 3 into tube 4, and mix well.
7. Transfer 3 ml from tube 4 into tube 5, and mix well.
8. Transfer 3 ml from tube 5 into tube 6, and mix well.
9. Transfer 3 ml from tube 6 into tube 7, and mix well.
10. Transfer 3 ml from tube 7 into tube 8, and mix well.

## b. Working procedure:

- Coat the microtiter plate with 1) 100 µl per well of serial dilutions of the standard reference IgG solution in PBS coating buffer in columns 1 and 2 on each plate and 2) 100 µl per well of rGLURP-R0 in PBS coating buffer at 1.0 µg/ml in columns 3 to 12. Cover the plates with a plastic sealer and label each plate with antigen name, Isotype or IgG subclass, series number, date and personnel initial. The plates can be stored for 21 days in the fridge at 2 to 8 °C.
- Dilute serum / plasma samples 1:200 in serum dilution buffer. The dilutions can be stored for 21 days in the fridge at 2 to 8 °C.
- Remove the plates from the fridge and wash them four times in washing buffer. For each washing step leave the plate filled with washing buffer for 1 minute before it is emptied. Pad the plates dry and add 150 µl of blocking buffer and keep at room temperature for 1 hour.
- Empty the plates, pad them dry, and wash the plates 4 times in washing buffer. Add 100 µl of dilution buffer to wells in columns 1 and 2. Add 100 µl of the diluted 4 positive and the 2 negative control samples and a PBS blank to duplicate wells in columns 3 and 4.
- Add 100 µl of the diluted test sample to duplicate wells in columns 5 to 12. Incubate for 2 hours with shaking on a rocker platform at room temperature.
- Wash the plates 4 times in washing buffer. For each washing step leave the plate filled with washing buffer for 1 minute before it is emptied.
- Add to each well 100 µl of Peroxidase-conjugated goat anti-human IgG diluted 1:3000 in dilution buffer. Incubate for 1 hour with shaking on a rocker platform at room temperature.
- Wash the plates 4 times in washing buffer. For each washing step, leave the plate filled with washing buffer for 1 minute before it is emptied.
- Add to each well 100 µl of colour solution. Incubate at room temperature for 30 min in the dark.
- Add to each well 100 µl 0.2 M H<sub>2</sub>SO<sub>4</sub> and read the absorbance at 450 nm, ref. 620 nm on a plate reader and save the results in a text file
- The conversion of absorbance values into A.U. is performed by using Microsoft Excel-based curve-fitting program.
- For each test fill out a work protocol

## References:

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