	DEPARTMENT of PARASITOLOGY BPRC Rijswijk	SOP: 001 Version: 001
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	Standard Operating Procedure for **Enzyme Linked ImmunoSorbent Assay (ELISA)** **Written by: Sumera Y. Younis, MSc** **Reviewed by: Ed J. Remarque, PhD**	Date: 19/10/12

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A.1. Aim

The detection and quantification of antibodies (Ab) by an Enzyme Linked ImmunoSorbent Assay. This assay can measure Ab titers from 0.78 ng/mL to 100 ng/mL IgG from serum, supernatant or plasma, depending on which coating is used.

B.1. Equipment and instruments

- ELISA plates: Greiner Bio-one # 655092
- 50 mL tubes: Greiner Bio-one # 22761
- Pipettes: 2 mL: ALP # PN2E1
  5 mL: ALP # PN5E25
  10 mL: ALP # PN10E25
  25 mL: ALP # PN25E1
- Deep well 96-well plates 2.2 mL: VWR # 82006-448
- Plate washer: Bio-Tek instruments, ELx405
- Plate reader: BIORAD microplate reader, model iMark

B.2. Materials

- Coating antigen: 1 µg/mL AMA-1, HBsAg or Ag85A.
- Coating Buffer: PBS (Gibco # 10010-031)
- Blocking Buffer: PBS + 0.05% v/v Tween-20 + 3% w/v BSA
  Tween-20: Sigma # P1379
  BSA: Sigma # A9647
- Wash Buffer: PBS + 0.05% v/v Tween-20
- Dilution Buffer: PBS + 0.05% v/v Tween-20 + 0.5% w/v BSA
- Standard e.g. pool of final bleed (day 70) of animals immunised with AMA-1, HBsAg or Ag85A
  Available through PHARVAT - WHO
- Goat Anti-Mo total IgG, IgG1, IgG2b or IgG2c conjugated to horseradish peroxidase (HRP)
  Total IgG: Invitrogen # G21040
  IgG1: Invitrogen # A10551
  IgG2b: Invitrogen # M32407
  IgG2c: Southern Biotech # 1079-05
- Substrate Buffer: ready-to-use TMB plus 2 substrate # 4395A Kem-en-Tec, Denmark
- Stop solution: 2mM H_2SO_4
  To prepare ± 1 L: add 110 mL H_2SO_4 (Merck # 1.00731.1000) to 880 mL distilled water

C.1. Procedure

1. Coat ELISA plates with the antigens at the optimal concentration in Coating Buffer (see scheme below), add 100 µL per well.
2. Incubate overnight (o/n) at 4°C covered with a plastic seal or lid.
3. Remove coating solution from plates by inverting the plates with a vigorous wrist action.
4. Block with 200 µL/well of Blocking Buffer.
5. Incubate 2 hour at Room Temperature (RT).
6. Wash plates using a plate washer with the following sequence: aspirate wells, fill with 200 µL Wash Buffer and aspirate wells again, repeating this cycle five times.
7. Prepare samples and standards:

Dilute pre immune samples 100x and 500x in Dilution Buffer and dilute day 42 and day 70 bleed samples 20 000 – 640 000 times - depending on IgG subclasses (see table below).
Prepare the standard in 2-fold serial dilution in deep well plates, starting dilutions according to table below for the four subclasses.
* Note: pre-immune samples only tested against total IgG

8. Add 100 µL of Dilution Buffer to (empty) wells prior to titration of the samples.
9. Add 150 µL of each pre diluted sample to the first wells and make a 3-fold serial dilution over 5 wells using 50 µL of first well. The standard IgG sample is serial diluted in 2-fold over 7 wells. Skip the eighth well; fill final well with Dilution Buffer only, as a true negative control. Check templates below for position samples and standard.
10. Incubate 1 hour at RT.
11. Wash plates using a plate washer with the following sequence: aspirate wells, fill with 200 µL Wash Buffer and aspirate wells again, repeating this cycle five times.
12. Add 100 µL/well HRP-conjugate in the right dilution (see table below).
13. Incubate 1 hour at RT.
14. Wash plates using a plate washer with the following sequence: aspirate wells, fill with 200 µL Wash Buffer and aspirate wells again, repeating this cycle five times.
15. Add 100 µL/well ready-to-use TMB plus 2 substrate.
16. Incubate 20-30 minutes at RT while checking for optimal colour development visually along incubation time.
    * Note: IgG2b takes more time to develop
17. Stop reaction by adding 50 µL/well stop solution.
18. Read OD at 450 nm on a Microplate Reader.

C.2. Scheme

Action	Material	Supplier	Concentration	Dilute in	Incubate
Coating	AMA-1 HBsAg Ag85A	BPRC Lionex GmbH	1 µg/mL 1 µg/mL 1 µg/mL	Coating buffer	o/n 4°C
Blocking	PBS-T-BSA	Sigma # P1379 Sigma A-9647	0.05% Tween 3% BSA		2 hr RT
IgG Standard	See table standard			Dilution buffer	1 hr RT
Samples	Pre immune sera Immune sera – day 42-70		Start 100 – 500x See table samples	Dilution buffer	1 hr RT
Conjugate	Go anti Mo total IgG-HRP Go anti Mo IgG1-HRP Go anti Mo IgG2b-HRP Go anti Mo IgG2c-HRP	Invitrogen # G21040 Invitrogen # A10551 Invitrogen # M32407 Southern Biotech # 1079-05	1:1000 1:1000 1:500 1:1000	Dilution buffer	1 hr RT
Substrate	Ready-to-use TMB	Kem-en-Tec # 4395A	100 µL/well		20-30 min.
Stop Solution	2mM H_2SO_4	Merck # 1.00731.1000	50 µL/well		

D.1. Table standards

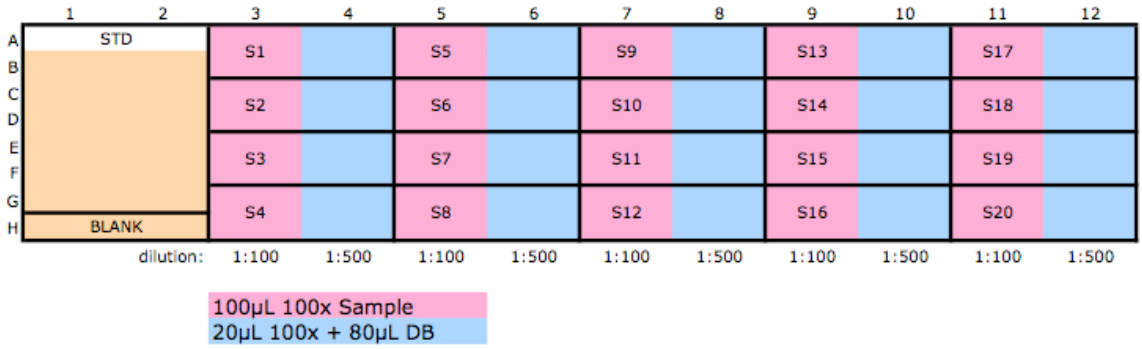
	AMA-1	HBsAg	Ag85A
Total IgG	1:160 000	1:160 000	1:80 000
IgG1	1:160 000	1:160 000	1:80 000
IgG2b	1:20 000	1:40 000	1:5 000
IgG2c	1:40 000	1:40 000	1:10 000

D.2. Table day 42 – 70 sample dilutions

	AMA-1	HBsAg	Ag85A
Total IgG	1:640 000	1:200 000	1:160 000
IgG1	1:640 000	1:200 000	1:200 000
IgG2b	1:40 000	1:150 000	1:20 000
IgG2c	1:120 000	1:75 000	1:20 000

E.1. Template

Day 0



Day 42-70