Competition ELISA

Created by: Kwadwo Kusi Date: August 2007

Aim

To demonstrate the antigen specificities of malarial antibodies in immune serum or plasma by direct competition with different allelic forms of the homologous antigen.

Materials

- ELISA plates (Greiner microlon cat# 655092)
- Recombinant antigens (AMA-1, MSP-1, DiCos 1,2,3 etc) – for coating and competition. Use at 1 µg/ml in Coating Buffer.
- Coating Buffer : PBS, pH 7.2 – 7.4 (Gibco)
- Blocking Buffer : PBS + 0.05% v/v Tween-20 + 3% w/v BSA
- 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 of animals immunized with AMA-1, MSP-1, DiCos 1,2,3 or total Hu, Rb, Rt or Mo IgG.
- Negative control: serum or IgG from a non-immunized Rb, Mo, Ra or unexposed Hu
- Anti Hu, Rb, Ra or Mo IgG or IgM conjugated to Alkaline Phosphatase. Use 1:1250 in Dilution Buffer
- DEA buffer : 0.15% w/v $MgCl_2*6H_2O$ (e.g. 0.75 g) in 500 ml MQ + 492 µl Diethanolamine pH 9.8
- Substrate: p-Nitro Phenyl Phosphate hexahydrate (pNPP) (Fluka cat# 71768). Use at 1 mg/ml in DEA Buffer
- Plate washer
- Plate reader
- Curve-fitting program (ELISA 023)

7.15.1 Determination of Serum Dilution

An initial titration is carried out to determine the optimal dilution of serum to use in the subsequent competition assay.

1. Coat plates O/N at 4°C with 100 µl/well recombinant Ag in PBS.
2. Block for at least 1.5 hours with 200 µl/well Blocking Buffer.
3. Wash with program 09 on the plate washer (See protocol ***2.6** ELISA washer*)
4. Titrate standard (and negative control) IgG or serum 2-fold in dilution buffer, starting from an appropriate dilution.
5. Titrate antisera in dilution buffer, 2-fold from 1:10,000 or more concentrated if Ab titres are expected to be low (e.g. in infants). Final volume is 100 µl/well.

6. Incubate for 2 hr at RT.
7. Wash as before.
8. Add 100 µl/well AP-conjugated Goat antibody (anti-Hu, Rb, Ra, Mo). Incubate for 1 hr at RT.
9. Wash as before.
10. Add 100 µl/well pNPP substrate and read OD at 405 nm after 30 minutes.

After transforming ODs to arbitrary units (AUs) and correcting for variation (ELISA 023 curve-fitting program), choose a dilution of the serum (corresponding to an AU of 1 or greater) that falls within the linear region of the standard curve. For the subsequent competition assay, antisera should be diluted to 2X the prefered dilution determined above since half the original titration volume (50 µl) will be used.

7.15.2 Competition ELISA

1. Coat plates O/N at 4°C with 100 µl/well recombinant Ag in PBS.
2. Block for at least 1.5 hours with 200 µl/well Blocking Buffer.
3. Wash with program 09 (See protocol ***2.6*** *ELISA washer*).
4. Titrate standard and negative control IgG or serum 2-fold in Dilution Buffer, starting from an appropriate dilution (see below for possible plate layout).
5. Add 50 µl Dilution Buffer to all Ag titration wells except Column 1 wells. Add 75 µl of soluble/competitor Ag (60 µg/ml in Dilution Buffer) to wells in Column 1 and do a 3-fold titration (using 25 µl from Column 1 wells) out to Column 9, leaving Column 10 wells with dilution buffer but no Ag.
6. Add 50 µl/well of serum, diluted to 2x the desired dilution (determined in *7.15.1* above), to all Ag titration wells (columns 1 to 10).
7. Incubate for 2 hr at RT.
8. Wash as before.
9. Add 100 µl/well AP-conjugated goat antibody (anti-Hu, Rb, Ra, Mo).
10. Incubate for 1 hr at RT.
11. Wash as before.
12. Add 100 µl/well pNPP substrate and read OD at 405 nm after 30 minutes.

Plate layout for competition assay

	1	2	3	4	5	6	7	8	9	10	11	12
Soluble antigen 1, µg/ml	30	10	3.3	1.1	0.37	0.12	0.04	0.014	0.005	No Ag	Neg01	Neg01
	30	10	3.3	1.1	0.37	0.12	0.04	0.014	0.005	No Ag	Neg02	Neg02
Soluble antigen 2, µg/ml	30	10	3.3	1.1	0.37	0.12	0.04	0.014	0.005	No Ag	Neg03	Neg03
	30	10	3.3	1.1	0.37	0.12	0.04	0.014	0.005	No Ag	Neg04	Neg04
Soluble antigen 3, µg/ml	30	10	3.3	1.1	0.37	0.12	0.04	0.014	0.005	No Ag	Neg05	Neg05
	30	10	3.3	1.1	0.37	0.12	0.04	0.014	0.005	No Ag	Neg06	Neg06
	Std01	Std02	Std03	Std04	Std05	Std06	Std07	Std08	Std09	Std10	blank	blank
	Std01	Std02	Std03	Std04	Std05	Std06	Std07	Std08	Std09	Std10	blank	blank