	DEPARTMENT of PARASITOLOGY BPRC Rijswijk	SOP: 001 Version: 001
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	Standard Operating Procedure for AMA1, HBsAg and Ag85A formulations in Alhydrogel®, Squalene-in-water and QS21-Liposomes **Written by: Sumera Y. Younis, MSc Reviewed by: Ed J. Remarque, PhD**	Date: 19/10/12

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A.1. Aim

The formulation of AMA1, HBsAg and Ag85A in Alhydrogel®, Squalene-in-water and QS21-Liposomes

A.2. Brief description

The formulations described here yield 20 µg for AMA1, 100 µg for HBsAg and 200 µg for Ag85A at an injection volume of 1 mL. Per immunisation 50 µL injections were prepared.

B.1. Equipment and instruments

- Laminar Flow Cabinet: Clean Air
- Terumo Insulin syringes 0.5 mL: Multimedi # 14.BS=05M2713
- 15 mL tubes: Greiner Bio-one # 188271

B.2. Materials

- AMA1 vials (62.5 µg per vial; 3 mL vials): BPRC
- Ag85A vial (228 µL; 2192 µg/mL per vial): Lionex GmbH # LRP-0004.4
- HBsAg vial (6.56 mg/mL per vial; 100 µL): VFL, Lausanne
- NaCl 0.9% (Saline for injection): 9501C12
  Use unopened bottle, discard after use.
- Alhydrogel® 2% (AH 1173 µg; 0.345 mL): Brentag Biosektor
- SWE (SWE LE 009; 0.345 mL): VFL, Lausanne
- Cholesterol-DOPC Liposomes (LIPO-003; 0.138 mL): VFL, Lausanne
- LS saponin (LS-001; 0.138 mL): VFL, Lausanne

C.1. Preparation adjuvants

QS21-liposomes preparation

1. Take 135 µL cholesterol-DOPC liposomes
2. Add 135 µL of LS saponin
3. Add 67.5 µL saline and mix gently

AlOH and SWE are ready-to-use

C.2. Preparation antigens

AMA1 dilution @ 40 µg/mL

1. Reconstitute one vial containing 62.5 µg in 1562.5 µL saline.
2. Final concentration: 40 µg/mL

HBsAg dilution @ 200 µg/mL

1. Take 25.9 µL HBsAg @ 6.56 mg/mL
2. Add 824.1 µL saline and mix gently
3. Final concentration: 200 µg/mL

Ag85A dilution @ 400 µg/mL

1. Take 1 vial of Ag85A and take out 200 µL and transfer to 1.25 mL vial
2. Add 896 µL saline and mix gently

3. Final concentration: 400 µg/mL

D.1. Formulations for control vaccines

AlOH control

1. Take 40 µL AlOH.
2. Add 40 µL saline and mix gently.
3. Incubate for 1 hour at RT.
4. Take up 50 µL of the mixture into a labeled insulin syringe for one mouse.

SWE control

1. Take 40 µL SWE.
2. Add 40 µL saline and mix gently.
3. Take up 50 µL of the mixture into a labeled insulin syringe for one mouse.

QS21-Liposomes control

1. Take 40 µL QS21-liposomes.
2. Add 40 µL saline and mix gently.
3. Take up 50 µL of the mixture into a labeled insulin syringe for one mouse.

D.2. Formulations for mouse experiments

AMA1

Adjuvant-Formulations AMA1 - AlOH

1. Take 270 µL AMA1 at 40 µg/mL.
2. Add 270 µL AlOH and mix gently.
3. Incubate for 1 hour at RT.
4. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

Adjuvant-Formulations AMA1 - SWE

1. Take 270 µL AMA1 at 40 µg/mL.
2. Add 270 µL SWE and mix gently.
3. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

Adjuvant-Formulations AMA1 - QS21-Liposomes

1. Take 270 µL AMA1 at 40 µg/mL.
2. Add 270 µL QS21-liposomes and mix gently.
3. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

HBsAg

Adjuvant-Formulations HBsAg - AlOH

1. Take 270 µL HBsAg at 200 µg/mL.
2. Add 270 µL AlOH and mix gently.
3. Incubate for 1 hour at RT.
4. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

Adjuvant-Formulations HBsAg - SWE

1. Take 270 µL HBsAg at 200 µg/mL.
2. Add 270 µL SWE and mix gently.
3. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

Adjuvant-Formulations HBsAg - QS21-Liposomes

1. Take 270 µL HBsAg at 200 µg/mL.
2. Add 270 µL QS21-liposomes and mix gently.
3. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

Ag85A

Adjuvant-Formulations Ag85A - AlOH

1. Take 270 µL Ag85A at 400 µg/mL.
2. Add 270 µL AlOH and mix gently.
3. Incubate for 1 hour at RT.
4. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

Adjuvant-Formulations Ag85A - SWE

1. Take 270 µL Ag85A at 400 µg/mL.
2. Add 270 µL SWE and mix gently.
3. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.

Adjuvant-Formulations Ag85A - QS21-Liposomes

1. Take 270 µL Ag85A at 400 µg/mL.
2. Add 270 µL QS21-liposomes and mix gently.
3. Fill labeled insulin syringes with 50 µL of the mixture for each mouse.