ANNEXURE 1

OPTIMISED METHOD FOR MYCOLIC ACID-LIPOSOME IMMOBILISATION

1.1 Liposome preparation
1. Aliquot MA in 100μg quantities
2. Make fresh PC and cholesterol stock solutions of 10mg/ml in cold chloroform
3. Add necessary quantities of PC and cholesterol stock solutions to one vial of MA (100μg)
4. Dry the mixture in a heat block at 85°C under a steady stream of nitrogen gas.
5. Add 2ml PBS containing 0.025% (w/v) sodium azide and 1mM EDTA (PBS/AE) to the dried lipids
6. Melt the lipids at 85°C in a heat block for ten minutes or until no residual flakes are visible
7. Vortex the solution at 100% for one minute
8. Sonify with a micro probe sonicator set at pulsed, 20 duty cycles, output level two for one minute
9. Vortex the solution at 100% for one minute

1.2 Liposome immobilisation
1. Achieve a stable baseline with PBS/AE for at least five minutes
2. Aspirate cells, add 50μl of 20μg/ml CPC in PBS/AE and incubate for ten minutes
3. Wash the cells five times each with 60μl PBS/AE and add 25μl of PBS/AE
4. Incubate for at least five minutes and until a stable baseline has been achieved.
5. Add 25μl of the liposome solution and incubate for ten minutes
6. Wash cells five times with 60μl PBS/AE, and immediately wash with five times 60μl 1mg/ml saponin in PBS/AE. Note: Saponin concentration should be titrated first to optimal blocking capacity concentration
7. Incubate for ten minutes and until a stable baseline has been achieved
8. Wash five times with 60μl PBS/AE and add a final 25μl PBS/AE
1.3 Interaction analysis and regeneration

1. Enter a baseline event as soon as a stable baseline has been achieved after the PBS/AE wash. Baseline should be monitored for at least five minutes.

2. Add 25µl of the desired test sample at an appropriate dilution to the cells and enter an association event.

3. Incubate for five to ten minutes depending on association velocity.

4. Wash five times with 60µl PBS/AE to initiate dissociation and enter a dissociation event.

5. Continue dissociation for five minutes before regenerating the cells as follow:

6. Wash three times with 50µl 95% ethanol.

7. Follow immediately with three times 80µl PBS/AE and five times 60µl PBS/AE over one minute.

8. Wash five times with 60µl 12.5M KOH and incubate for two minutes.

9. Follow with three times 80µl PBS/AE and five times 60µl PBS/AE over one minute.
ANNEXURE 2

OPTIMISED METHOD FOR GANGLIOSIDE-LIPOSOLE IMMobilISATION

2.1 Liposome preparation
1. Aliquot Gm1 in 100μg quantities
2. Make fresh PC stock solution of 1mg/ml in cold chloroform
3. Add necessary quantity of PC stock solution to one vial of Gm1 (100μg)
4. Dry the mixture in a heat block at 85°C under a steady stream of nitrogen gas.
5. Add Tris based buffer (50mM Tris, 200mM NaCl, 3mM NaN3, 1mM Na2EDTA) to the dried lipids to a concentration of 0.5mg/ml
6. Vortex the solution at 100% for one minute
7. Sonify with a micro probe sonicator set at pulsed, 20 duty cycles, output level two for one minute
8. Vortex the solution at 100% for one minute

2.2 Liposome immobilisation
Ganglioside-liposomes are immobilised with residual HCl remaining in the cells after regeneration. Therefore the regeneration protocol should be followed first and then immobilisation can be continued.
1. Aspirate the HCl solution remaining after regeneration and add 50μl of the liposome solution
2. Incubate for ten minutes before washing cells four times with 60μl Tris buffer, four times with 60μl 10mM NaOH and another five times with 60μl Tris buffer finishing with a 25μl Tris buffer addition
3. Incubate for five minutes and until a stable baseline has been achieved

2.3 Interaction analysis and regeneration
1. Enter a baseline event as soon as a stable baseline has been achieved after the Tris buffer wash. Baseline should be monitored for at least five minutes
2. Add 25μl of the desired test sample at an appropriate dilution to the cells and enter an association event.
3. Incubate for five to ten minutes depending on association velocity
4. Wash five times with 60μl Tris buffer to initiate dissociation and enter a dissociation event
5. Continue dissociation for five minutes before regenerating the cells as follow:
6. Wash four times with 60μl absolute ethanol
7. Follow immediately with seven times 100μl water, five times 60μl KOH and incubate for two minutes
8. Wash seven times with 100μl water and follow with three times 2M HCl and four times 20mM HCl
9. Achieve a stable baseline for at least five minutes before aspirating for the next round of immobilisation