**Supplementary Methods 2. Dilution to achieve desired dose of BCG**

BCG: Bacillus Calmette–Guérin

**Group A. BCG SSI 2 - 8 x 105 pfu**

* *Supplied as: 1 vial SSI reconstituted in 1mL contains 10 doses of 2 - 8 x 105 pfu (\*taken as 5x105 pfu for this calculation)*
* For the specified volume of 0.15mL to contains the specified dose of 2 - 8 x 105 pfu the solution needs to be diluted to a concentration of 2 - 8 x 105 pfu/1.5mL (0.15mL x 10 =1.5mL)

i.e 1.5mL - 1mL = 0.5mL

* Therefore, if 0.5mL of saline is added to the reconstituted BCG SSI:

150 microlitres contains 2 - 8 x 105 pfu

**Group B. BCG TICE 2 - 8 x 10*5* pfu**

* *Supplied as: 1 vial TICE in 1mL saline contains 2 - 8x108 pfu (\*taken as 5.0x108 for this calculation)*
* To attain the specified dose of 2 - 8 x 10*5* pfu (\*taken as 5 x 10*5* pfu for this calculation) in the specified volume of 0.15mL, the solution needs to be diluted to the following concentration:

[required concentration = desired dose/ desired volume]

[3.3x106 pfu/mL = 5 x 10*5* pfu/0.15mL]

* To attain the required concentration from the supplied concentration the dilution factor needs to be calculated:

[DF = current concentration/desired concentration]

[5.0x108 pfu/mL ÷ 3.3x106 pfu/mL = dilution factor of 150]

* To achieve a final dilution factor of 1/150, serial dilutions have to be carried out according to the following formula:

[D(final) = DA x DB x etc]

Staring with 1/10 dilutions (ie. If DA = 1/10 and DB = 1/10):

DC = D(final) / (DA x DB)

DC = 1/150 / (1/10 x 1/10)

DC = 6.7/10

Therefore, if we dilute (1/10 x 1/10 x 6.7/10) i.e. 1mL in 9mL, then 1mL of this in 9mL, then 6.7ml of this in 3.3mL we have a 1/150 dilution.

150 microlitres contains 2 - 8 x 105 pfu

**Group C. BCG SSI 6 - 24 x 105 pfu**

* *Supplied as: 1 vial SSI reconstituted in 1mL contains 10 doses of 2 - 8 x 105 pfu (\*taken as 5x105 pfu for this calculation)*

For the specified volume of 0.15mL to contain the specified dose of 6 - 24 x 105 pfu the solution needs to be diluted to a concentration of 6 - 24 x 105 pfu/0.5mL

Therefore, if the SSI is reconstituted in 0.5mL diluent (1mL x 0.5 = 0.5mL)

150 microlitres contains 6 - 24 x 105 pfu

**Group D. BCG TICE 6 - 24 x 105 pfu**

*Supplied as: 1 vial TICE in 1mL saline contains 2 - 8x108 pfu (\*taken as 5.0x108 for this calculation)*

* To attain the specified dose of 6 - 24 x 105 pfu (\*taken as 15 x 105 pfu for this calculation) in the specified volume of 0.15mL, the solution needs to be diluted to the following concentration:

[required concentration = desired dose/ desired volume]

[1x107pfu/mL = 15 x 105 pfu/0.15mL]

* To attain the required concentration from the supplied concentration the dilution factor needs to be calculated:

[DF = current concentration/desired concentration]

[5.0x108 pfu/mL ÷ 1x107 pfu/mL = dilution factor of 50]

* To achieve a final dilution factor of 1/50, serial dilutions have to be carried out according to the following formula:

[D(final) = DA x DB x etc]

Staring with a 1/10 dilutions (ie. If DA = 1/10):

DB = D(final) / DA

DB = 1/50 ÷ 1/10

DB = 2.0/10

Therefore, if we dilute (1/10 x 2.0/10) i.e. 1mL in 9mL, then 2.0mL of this in 8.0mL, we have a 1/50 dilution

150 microlitres contains 6 - 24 x 105 pfu