Investigator's Brochure Appendix VII

Investigational product dose preparation sheets can be found below:

Dose Preparation Overview

pGM169/GL67A or 0.9% (w/v) NaCl placebo will be administered to the nose and/or the lungs of trial subjects. Randomisation of trial subjects to pGM169/GL67A or 0.9% (w/v) NaCl placebo is co-ordinated by the Imperial College Trials Unit.

For nasal administration, the nominal dose administered will be 2mL pGM169/GL67A or placebo. For lung administration, the nominal dose administered will be 5mL of pGM169/GL67A or placebo.

Sterile 0.9% (w/v) NaCl saline solution for intravenous infusion is supplied by pharmacy and used to prepare nasal and lung placebo doses as appropriate.

pGM169/GL67A is supplied by the sponsor in single-use packages sufficient to prepare a 10mL aliquot of pGM169/GL67A.

One 10mL aliquot of pGM169/GL67A is used to fill a nasal spray device sufficient to deliver the 2mL necessary for nasal administration.

One 10mL aliquot of pGM169/GL67A is used to prepare up to two (2) 5mL lung doses. Thus up to two lung doses may be prepared from one single-use package.

Preparation of 10mL pGM169/GL67A Worksheet

Final Material For Clinical trial use only

Date:	Patient ID:
Pharmacy Batch Number:	10ml Aliquot Number:

Data entry by:	Data checked by:

Time box removed from freezer: _____hrs	
Time recorded by:	Time checked by:

Raw Material	**Manufacturer**	**Form**	**Batch number**	**Retest or expiry date**	**Quantity**	**Assembled by**	**Check by**
pGM169/GL67A	NHSBT/CBC	Pouch					
pGM169/GL67A	NHSBT/CBC	Box					
pGM169 (Blue crimp)	VGX	vial					
GL67A (Red crimp)	Octoplus	vial					
Water For Injection		Ampoule					
Container	**Manufacturer**	**Form**	**Batch number**	**Expiry date**	**Quantity**	**Assembled by**	**Check by**
5ml Syringe	BD	Syringe					
1ml Syringe	BD	Syringe					
Interim container Double lumen syringe		Barrel					
		Plunger					
		Mixer					
Final container		Universal tube (Sterilin)					

Cleaning log checked by:	Temperature log checked by:
Approval to proceed: Pharmacist/Senior Technician	

Isolator is clean and free of items not required for this procedure.

Signed:_____

Method Step 1- Prepare Pneumatic mixer

1) Follow SOP CFGT/SOP/601/SD LMD2 Pneumatic mixer: Open cylinder valve with gas spanner to pressurise regulator. Ensure adequate cylinder pressure (>70 psi) before commencing studies.

Pressure adequate Yes _____check
No _____check

Method Step 2- Hydration of GL67A

2a) Allow vials to warm to room temperature for 10-15 minutes.
Time vials at room temperature: Time removed from freezer (from page 1): _____ hrs
Time ready to start: _____ hrs

Timings recorded by:	Timings checked by:

If time ready to start <10minutes then wait until 10 minutes has elapsed.

2b) Add 5.25 ml of sterile water for injection to the GL67A vial through the septum using a sterile green 21G butterfly needle and a 5 ml and 1 ml syringe.
Volume measured by _____ Volume checked by_____

2c) As per SOP CFGT/SOP/603/SD GL67A Hydration, place the GL67A vial firmly in rack of the vortexer. Set the speed to 2500 and the time to 40 minutes and then switch on the vortexer.

2d) Vortex the vial of GL67A on a setting of 2500 for forty (40) minutes.

2e) During hydration of GL67A (red crimp vial) in the vortexer, occasionally gently shake the vial of pGM169 (blue crimp vial) to encourage thawing.

2f) Remove vial of GL67A (red crimp vial) from vortexer and visually inspect to confirm that the material is completely dissolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).
Note that in some cases it may take longer than 40 min for the lipid to dissolve completely. If lipid is not completely dissolved, use the vortexer as described for additional 10 minute cycles until the material is completely dissolved. If total vortexing material exceeds 100 minutes, discard GL67A

Additional Vortex start time: _____ am / pm
Final Vortex stop time: _____ am / pm
Total vortexing time: _____ minutes

Timings recorded by:	Timings checked by:

Method Step 3- Formation of pGM169/GL67A

3a) Visually inspect appearance of solution in pGM169 (blue crimp) vial to confirm that the material is completely thawed (no visible lumps of material in suspension).

Note that in some cases the pGM169 may not be completely thawed. If pGM169 is not completely thawed, gently shake vial to encourage thawing until there are no visible lumps of material in suspension.

GL67A and pGM169 solution homogeneous by visual inspection:

Assessed by:	Checked by:

3b) Check calibration of the pneumatic mixer is within acceptable limits (SOP CFGT/SOP/601/SD LMD2 Pneumatic mixer).

Adjustment required: _____ Yes
_____ No

Assessed by:	Checked by:

3c) Fill double lumen syringe as described in SOP CFGT/SOP/602/SD Preparation of pGM169/GL67A complexes with five (5) ml of solution from GL67A (red crimp) vial through the septum using a sterile 16 gauge needle and sterile 5 ml syringe and transfer to lumen two (2) of double lumen syringe.

Volume measured by:_____ Volume checked by:_____

3d) Fill double lumen syringe as described in SOP CFGT/SOP/602/SD with five (5) ml of solution from pGM169 (blue crimp) vial through the septum using a sterile 16 gauge needle and sterile 5 ml syringe and transfer to lumen one (1) of double lumen syringe.

Volume measured by:_____ Volume checked by:_____

3e) Insert double lumen syringe into pneumatic mixer as described in SOP CFGT/SOP/602/SD, place 20 ml steralin immediately below the protruding static mixer element to collect mixed formulation and operate as described in SOP CFGT/SOP/602/SD.

Activate static mixer: _____ Check

3e) Record time preparation was completed: _____ hrs

Time recorded by:	Time checked by::

Method Step 4- Labelling of pGM169/GL67A investigational product

4a) Calculate and record dosage expiry time.

Dosage expiry time = time preparation was completed (recorded at 3e) plus 4 hours.

Record dosage expiry time: _____ hrs

Time calculated by:	Time checked by:

4b) Label Sterilin Universal tube containing final dosage form with label adhering to following format:

Master Label:	Sample Label:
pGM169/GL67A Batch: 10mL Patient ID: Expiry time: Date: 10ml Aliquot number: Room temperature storage Clinical trial use only	

4c) Confirm final dosage form labelling and release:

Labelling

Number of labels issued		*Number of units expected*	
Labels issued by		Units prepared by	
Labels checked by		Units checked by	
Number of labels used		Number of units prepared	
Number of samples used		Number of units issued	
Discrepancy		Discrepancy	
Explanation for discrepancy		Explanation for discrepancy	
Label reconciliation Completed by		Product reconciliation Completed by	

Product Release

Number of units for inspection	
Number of units rejected	
Discrepancy	
Comment	

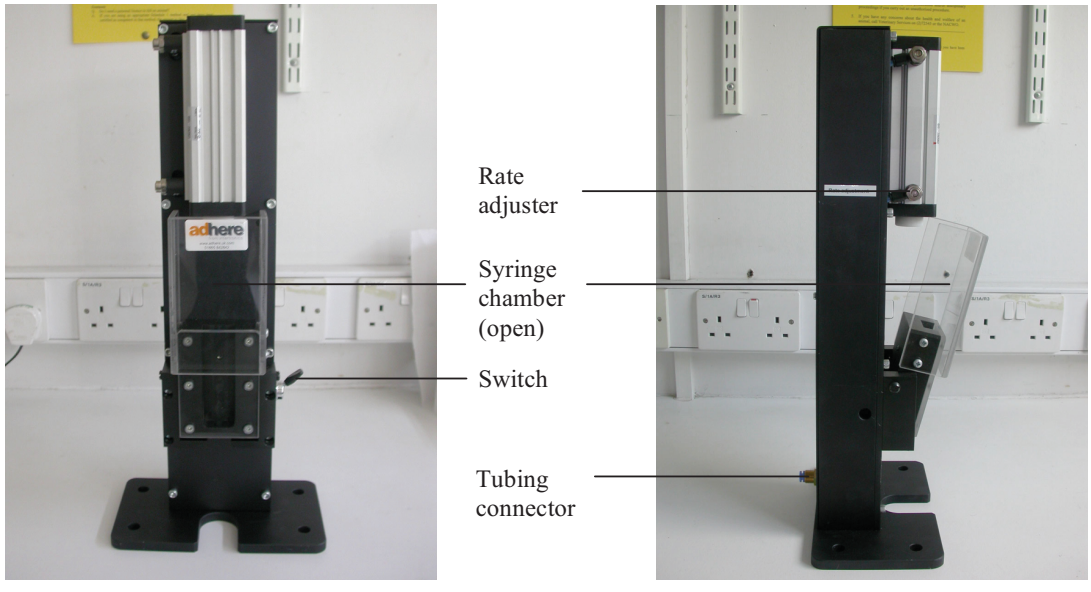
Pharmacists inspection completed: Name	Date
Releasing Pharmacists sign off: Name	Date

SOP: CFGT/SOP/601/MD

LMD-2 Pneumatic mixer

Introduction

The LMD-2 pneumatic mixer device is a specialised piece of equipment designed specifically for use within the UK Cystic Fibrosis Gene Therapy Consortium to enable rapid and reproducible mixing of lipid and DNA components for clinical applications. The device consists of a fully adjustable pneumatic piston that can be used in conjunction with a dual lumen syringe and static mixer to achieve controlled and reproducible mixing of plasmid DNA and GL67A components of gene transfer formulations.



Procedure

1. LMD-2 Setup

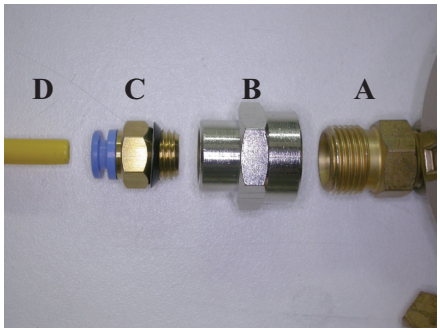
1.1 Required apparatus

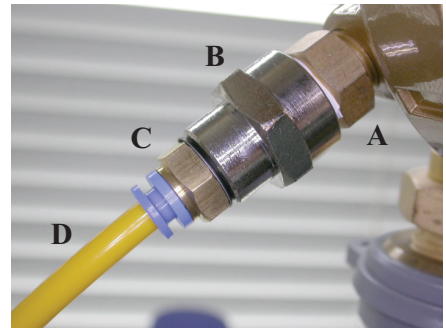
LMD-2 pneumatic mixing device (Intertronics Ltd)
Compressed air/nitrogen cylinder capable of 50 psi output pressure
Suitable gas regulator with G3/8 male output connector
G3/8 to G1/4 steel sleeve connector (RS Components Cat 367-5590)
G1/4 to 6 mm push-fit straight connector (RS Components Cat 726-718)
6 mm nylon pressure tubing (RS Components Cat 726-718)
Gas joint sealing tape (RS Components Cat 231-964)

Setup

Attach gas regulator to suitable compressed air/nitrogen cylinder and open cylinder valve with gas spanner to pressurise regulator. Ensure adequate cylinder pressure (>200 psi) on the right hand dial before commencing studies. If pressure is too low then replace cylinder.

Connect 6mm pressure tubing to regulator via G1/4 to 6 mm push-fit connector and G3/8 to G1/4 steel sleeve connector.





Wrap one layer of gas joint sealing tape around G3/8 male thread on regulator outlet connector (A)

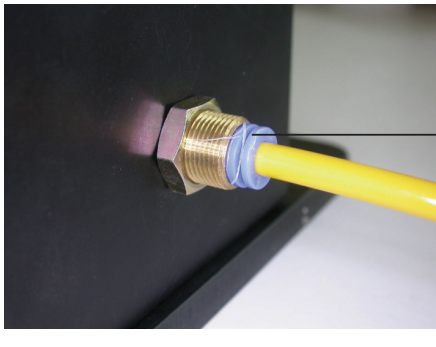
Thread G3/8 to G1/4 steel sleeve connector (B) onto regulator outlet (A) and tighten into place with 24 mm spanner

Thread G1/4 to 6 mm push-fit connector (C) into G3/8 to G1/4 steel sleeve connector (B) and tighten into place with 16 mm spanner

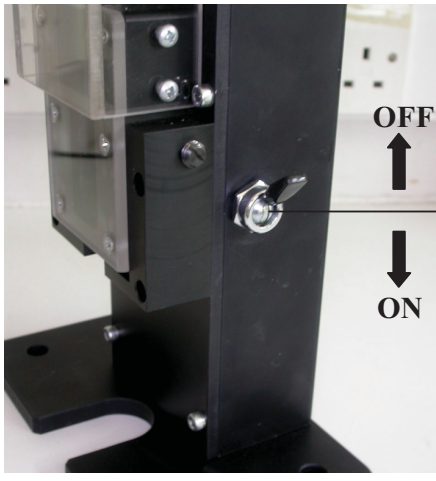
Insert length of 6mm pressure tubing (D) into G1/4 to 6 mm push-fit connector (C). Push firmly into place and check that it is secure by gently trying to pull the tubing free

If the tubing needs to be removed, press firmly down on the blue securing ring at the connector outlet whilst pulling the tube free. This does not require a great deal of force and if there is considerable resistance ensure that the securing ring is depressed sufficiently. Do not try to force the tubing out as this will result in damage to the connector.

Insert the other end of the 6mm pressure tubing into the 6 mm push fit connector located at the base of the rear of the LMD-2 (see below). Ensure secure connection as above.



Close syringe chamber door on front of LMD-2 by pushing it flat against the body of the device and ensure pneumatic switch is in the OFF (switch upwards) position (see below).



pressurise LMD-2 by opening gas regulator tap (turn clockwise) and turning until output pressure on regulator reads 50 ± 5 psi (left hand dial).

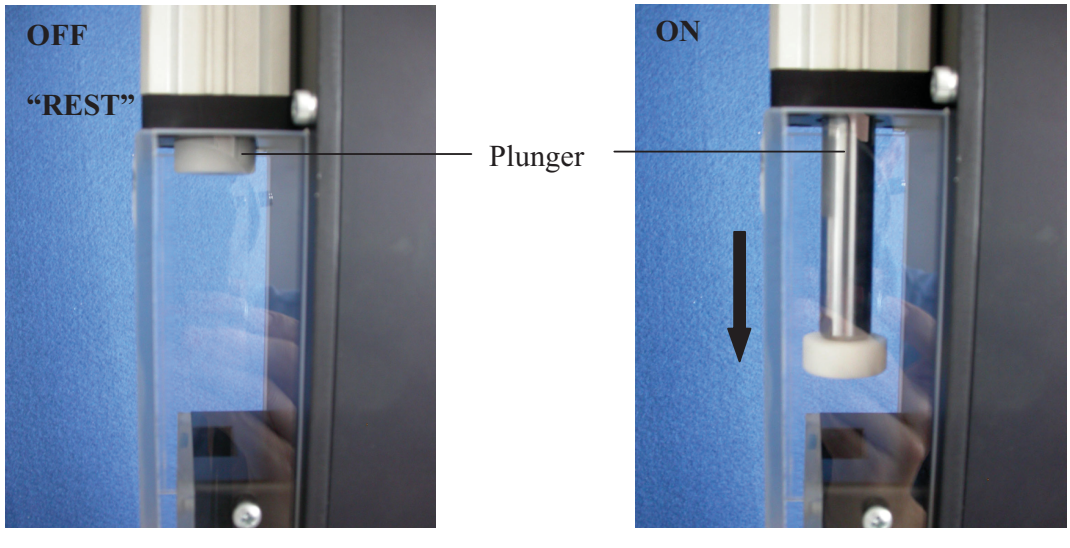
Check for any leaks evident as "hissing" or obvious escape of gas from connections and tubing. If leaks are present, turn off the regulator (turn handle anti-clockwise), allow any gas within the system to escape and then tighten affected connector or apply sealing tape where necessary. Re-pressurise system.

LMD-2 is now charged and ready to use

2. Operation and calibration of LMD-2

2.1 The LMD-2 device utilises gas pressure from the compressed air cylinder to drive a plunger that in turn depresses the plunger on the dual syringe mixing device.

2.2 With the switch in the OFF position the pneumatic plunger remains in its "REST" position at the top of the syringe chamber (see below).



2.3 To activate the device, ensure that the syringe door is closed and depress the switch to the ON position (down). The plunger will descend through the syringe chamber until it reaches the bottom.

2.4 IMPORTANT – the plunger will not automatically return to the top of the chamber. Once the plunger reaches the bottom of the chamber you MUST immediately deactivate the device by lifting the switch to the OFF position (up) to prevent damage to the device. This will also return the plunger to the "REST'' position.

2.5 A flow regulator valve positioned on the left hand side of the mixer unit determines plunger speed. Regulator valve is labelled "Rate adjustment >" (see below) and is adjusted using a flat screwdriver.

2.6 To adjust the plunger speed insert screwdriver into slot on regulator valve and turn clockwise for SLOWER and anti-clockwise for FASTER plunger movement.

2.7 To determine the plunger velocity use a stopwatch accurate to $1/100^{th}$ second to time a full depression from the top of the chamber to the bottom with no mixer syringe inserted into device.

2.8 With plunger in the "REST" position and switch turned OFF. Activate device and start stopwatch. When plunger reaches the bottom of the chamber stop stopwatch and record time. Turn switch OFF to return plunger to "REST" position.

2.9 Adjust regulator valve until desired plunger velocity is achieved. A turn to clockwise will slow the velocity.

2.10 All mixer studies should be carried out with plunger taking 4 ± 0.5 s to travel from the top of the chamber to the bottom. This range is equivalent to mixing speeds of 2.1 to 2.7 ml/s/syringe.

2.11 Plunger velocity should be confirmed and adjusted, if necessary, prior to each use.

3. Disassembly of the LMD-2

3.1 The LMD-2 can be left pressurised between mixing runs. However, it is recommended that it should be depressurised if it is to be left unused for periods greater than 2 hr.

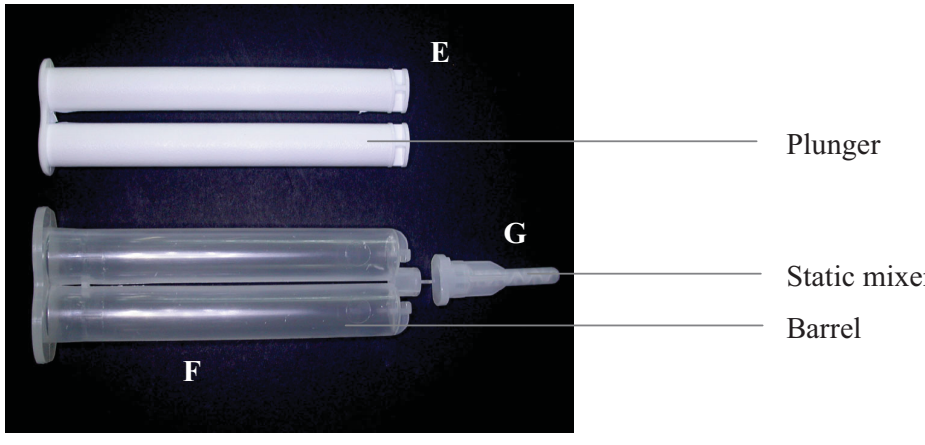
3.2 To depressurise the LMD-2 turn off the gas regulator on the cylinder by turning the handle anti-clockwise.
3.3 NOTE – output pressure on regulator will still read approximately 50 psi as the tubing between the regulator and the mixing device REMAINS PRESSURISED.
3.4 To release pressure in tubing repeatedly activate/deactivate the LMD-2 several times.
3.5 Upon each activation the measured pressure on the regulator output dial will fall as gas within the system is purged.
3.6 Repeat until regulator output dial reads 0 psi.
3.7 It is now safe to disconnect tubing if required or simply re-pressurise system for next mixing experiments.

SOP: CFGT/SOP/602/MD

Preparation of pGM169/GL67A complexes with the LMD-2

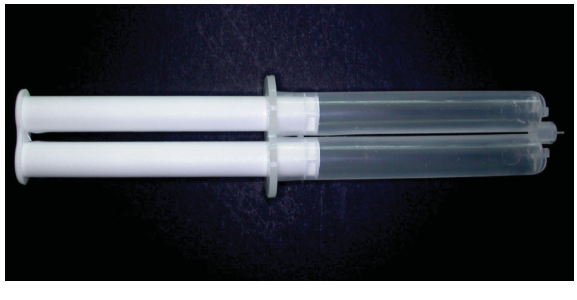
Required apparatus

LMD-2 pneumatic mixing device setup and calibrated as described above
Dual lumen syringe barrels (Plas-Pak Industries Cat 14B35(S505A)
Dual lumen syringe plungers (Plas-Pak Industries Cat 14C35(S446)
Eight element static mixers (Plas-Pak Industries Cat 003M08B005-3(S732)
16G disposable needles with Luer fitting hub (Sigma Cat Z118036)
5 ml sterile disposable syringes with Luer fitting hub
Thawed vial of pGM169 plasmid
Thawed and re-suspended vial of GL67A



Method

1 For preparation of 10 ml aliquot of pGM169/GL67A complexes.
2 Fully insert a single dual lumen syringe plunger (E) into a single dual lumen syringe barrel (F).
3 Withdraw plunger until end of plunger approaches the end of the syringe barrels. At this point there will be resistance to any further withdrawal of the plungers and the syringe barrels will be filled with air (see below).



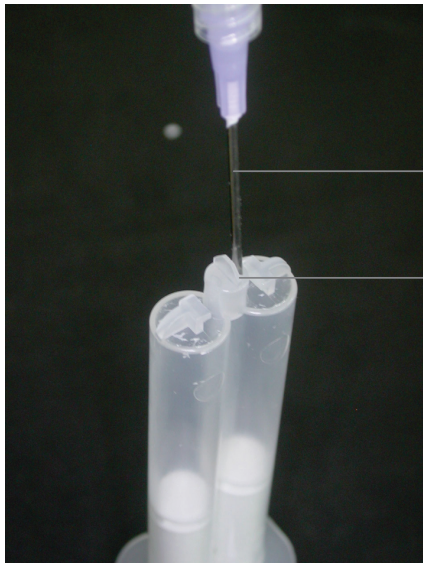
4 Hold dual lumen syringe assembly with the syringe barrels uppermost and the plunger pointing downwards. Support in this inverted position.

5 Attach a 16G needle to a disposable 10ml syringe and take up 5ml of room air into the syringe barrel.

6 Holding a vial of pGM169 upright, insert the needle through the top of the rubber stopper on the vial and inject the 5 ml of air into the vial. Hold the needle firmly in place to resist the increased pressure in the vial trying to push it out.

7 With the syringe now emptied, carefully invert the vial with the needle and syringe still *in situ*. Keeping the tip of the needle below the surface of the DNA solution, withdraw 5 ml of pGM169 into the syringe.

8 Remove the needle and syringe from the DNA vial and insert into one barrel of the dual lumen syringe assembly via the small hole at the tip of the barrel (see below).



9 Carefully inject 5 ml of pGM169 into the syringe barrel.

10 Remove and discard 5 ml syringe and 16G needle.

11 Repeat the procedure using a fresh 16G needle and 5 ml syringe to transfer 5 ml of re-suspended GL67A from the vial to the other barrel on the dual lumen syringe.

12 Whilst keeping the dual lumen syringe inverted, attach the 8 element static mixer (G) to the end of the dual lumen syringe by pushing into place and then rotating the mixer 90˚ clockwise to lock into position beneath plastic retainers.

13 It is now safe to hold the dual lumen syringe assembly containing 5ml of pGM169 and 5ml of GL67A vertically with the static mixer pointing downwards. Some air (1-2ml) will remain within the syringe barrels. This is normal and important to allow complete emptying of the syringes upon activation of the mixer.

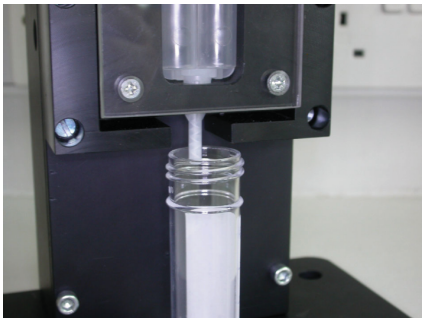
14 Check calibration of LMD-2 is within acceptable limits (see setup and calibration section of SOP: CFGT/SOP/601/MD).

15 Open the syringe chamber door of the pressurised and calibrated (see SOP: CFGT/SOP/601/MD) LMD-2 by pulling the top of the anterior clear perspex panel gently forwards – you will hear a small escape of gas as you open the door. This is normal and prevents activation of the device whilst the door is open.

16 Insert the dual syringe assembly into the syringe chamber such that the tip of the static mixer protrudes through the hole at the bottom of the chamber (see below).

17 Close the syringe door by pushing it gently backwards until it clicks into place. The device is now primed and ready for operation.

18 Place a sterile 20ml Sterilin tube immediately below the protruding static mixer element to collect mixed formulation (see below).



19 Activate pneumatic mixer by turning the switch on the right hand side of the device to the ON position (down). The pneumatic plunger will descend and push the dual plunger of the dual lumen syringe assembly. Mixed pGM169/GL67A complexes will be ejected from the static mixer into the 20 ml Sterilin collection tube.

20 When the dual syringe has been fully depressed, quickly return the switch to the OFF position (up) to return the pneumatic plunger to the “REST” position.

21 Screw cap into place on 20 ml Sterilin tube and store at room temperature for 20 min before use.

22 Open syringe chamber door and remove and discard empty dual lumen syringe. Removal is aided by pushing the static mixer vertically upwards from beneath.

23 Mixer is now ready for next sample.

SOP: CFGT/SOP/603/MD

GL67A Hydration

Required apparatus

VWR-DVX2500 vortexer
Foam rack

Method

1. Place the vial containing GL67A and water (red crimp vial) into the foam rack (Figure 1)

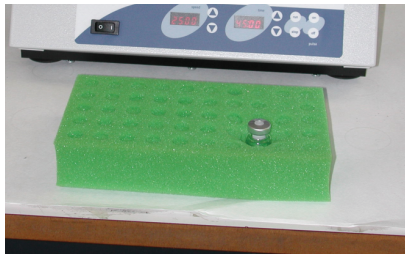


Figure 1

2. Place the foam rack into the VWR-DVX2500 vortexer (Figure 2)

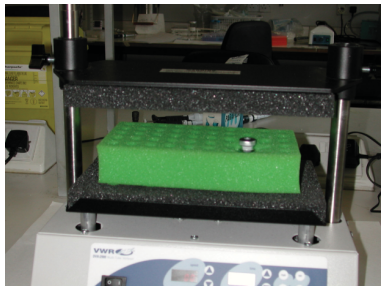


Figure 2

3. Lower the top plate onto the foam rack and tighten screws on both sides (Figure 3)

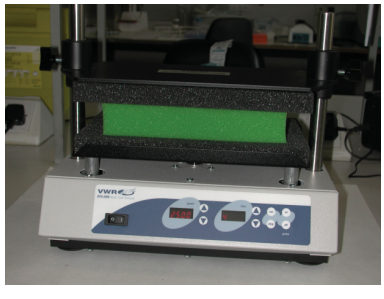


Figure 3

4. Set to maximum speed (2500 rpm) for 40 min and press run button

5. After required time unscrew top plate and lift up, remove vial and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

6. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button

7. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

8. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

9. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

10. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

11. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

12. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

13. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

14. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

15. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

16. If the lipid is not dissolved repeat steps 1-3. Set to maximum speed (2500 rpm) for 10 min and press run button.

17. Repeat step 5 and check that lipid has completely resolved (no white residues on the bottom of the vial, no visible lumps of material in suspension).

18. Discard GL67A, if the total vortexing time exceeds 100 min.