Safety Documentation

Please select the forms you require by selecting the check boxes below.
You can select more than one.

☑ **Risk Assessment** ☑ **Method Statement** ☑ **Chemicals COSHH**

Once you have made your selections, scroll down and complete the forms.

Buttons: [+] will add a row to a list [X] will delete a row from a list

You may save this file to a local drive at any time.
When you have finished, save the file to a local drive and email it to your supervisor for authorisation.

Supervisors - There is a sign-off section at the end of the document set that must be completed.

Staff may "self authorise", (as a supervisor), but the forms must still be submitted to the DSO for approval.

IMPORTANT:

YOU ***MUST NOT*** START ANY PRACTICAL WORK UNTIL THESE FORMS HAVE BEEN RETURNED TO YOU WITH **BOTH** YOUR SUPERVISOR's AND DSO's APPROVAL SIGNATURES ATTACHED.

Please complete these fields

School or Service	Wolfson School of Mechanical, Electrical and Manufacturing Engineering
Department	Centre for Biological Engineering
Originator name	Sotiria Toumpaniari
email address	s.toumpaniari@lboro.ac.uk
Location	H27, H34
Project / Activity / Task	Decellularization of porcine tissues
Supervisor Name	Sotiris Korossis

Version : 2.19

Loughborough University
Centre for Biological Engineering

Risk Assessment

Reference: SAF/MEME/6889

Location: H27, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Decellularization of porcine tissues

Is this process risk assessment for a : (✓) Laboratory / Workshop ( ) General use

Category 1: Machinery & work equipment:				
Design and Construction	Mechanical hazards	Electrical hazards	Radiation hazards	+
N/A	N/A	Electrical test lables current	N/A	x
		Short circuit/Overload		x
Category 2: Workplace				+
Slips/Trips/Falls on the level				x
Category 3: Hazardous and/or Harmful substances				+
Flammable substances				x
Corrosive substances				x
Irritant substances				x
Exposure to Covid				x
Category 4: Work activity				+
Lone working out of hours				x
Category 5: Work organisation				+
N/A				x

Explain the risks associated with these hazards

People / Groups at risk: Operator and people in proximity — x

Enter risk details here:-	Impact	Probability	Risk Score
Slips/Trips/Falls on the level	Slightly Harmful	Unlikely	Low

What are the control measures?	Lowers Impact	Lowers Probability	+
Organise room to have nothing on the floor that can be a trip hazard. Reduce movement between labs if possible. Any spillages must be cleaned up immediately according to relevant CBE SOP	Significantly	Moderately	x

Residual Risk: Low

Process Risk Assessment Form (Continued)

People / Groups at risk	Operator and people in proximity		x
Enter risk details here:-	Impact	Probability	Risk Score
Aerosols/splashes from irritant substances & sensitiser	Harmful	Likely	High
What are the control measures?	Lowers Impact	Lowers Probability	+
Work in fume hood	Significantly	Significantly	x
Only trained users can use chemicals	Significantly	Significantly	x
Wear appropriate PPE appropriate to and according to COSHH	Significantly	Significantly	x
		Residual Risk	Low

People / Groups at risk	Operator and people in proximity		x
Enter risk details here:-	Impact	Probability	Risk Score
Hydrochloric acid can cause exothermic reaction	Harmful	Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Do not mix with amines, aldehydes, permanganates, for example potassium permanganate. Operator must be competent in the handling and use of hazardous materials	Significantly	Significantly	x
		Residual Risk	Low

People / Groups at risk	Everyone in the room		x
Enter risk details here:-	Impact	Probability	Risk Score
Ignition or formation of inflammable gases/vapours	Harmful	Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Do not mix hydrochloric acid with aluminium, carbides, fluorine, metals, bases, sulphides.	Significantly	Significantly	x
		Residual Risk	Low

People / Groups at risk	Operator and people in proximity		x
Enter risk details here:-	Impact	Probability	Risk Score
Hydrogen release	Harmful	Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Avoid contact of hydrochloric acid with metals.	Significantly	Significantly	x
		Residual Risk	Low

People / Groups at risk	Operator only		x
Enter risk details here:-	Impact	Probability	Risk Score
Corrosion	Harmful	Likely	High
What are the control measures?	Lowers Impact	Lowers Probability	+

Process Risk Assessment Form (Continued)

Avoid contact of hydrochloric acid with metals.	Significantly	Significantly	x

Residual Risk
Low

People / Groups at risk	Everyone in the room	x

Enter risk details here:-	Impact	Probability	Risk Score
Toxic substances	Harmful	Unlikely	Medium

What are the control measures?	Lowers Impact	Lowers Probability	+
Work in fume hood and wear appropriate PPE.	Significantly	Significantly	x
Only trained users can use chemicals.	Significantly	Significantly	x

Residual Risk
Low

People / Groups at risk	Operator only	x

Enter risk details here:-	Impact	Probability	Risk Score
Splashes from hydrochloric acid	Very Harmful	Likely	Unacceptable

What are the control measures?	Lowers Impact	Lowers Probability	+
Use butyl-rubber gloves with minimum layer thickness: 0.7 mm, break through time: > 480 min. Preferably, KCL 898 Butoject®	Significantly	None	x

Residual Risk
Medium

People / Groups at risk	Everyone in the room	x

Enter risk details here:-	Impact	Probability	Risk Score
Flammable substances	Harmful	Likely	High

What are the control measures?	Lowers Impact	Lowers Probability	+
Do not use chemicals at high temperatures	Significantly	Significantly	x
Remove sources of ignition	Significantly	Significantly	x

Residual Risk
Low

People / Groups at risk	Operator only	x

Enter risk details here:-	Impact	Probability	Risk Score
Electrocution	Very Harmful	Highly Unlikely	Medium

What are the control measures?	Lowers Impact	Lowers Probability	+
Bi-annual PAT testing, visual inspection of cables and connectors prior to start	Significantly	Significantly	x
Keep liquids away from mains	Significantly	Significantly	x

Residual Risk
Low

Process Risk Assessment Form (Continued)

People / Groups at risk	Everyone in the room		x
Enter risk details here:-	Impact	Probability	Risk Score
Fire due to electrical causes	Harmful	Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Bi-annual PAT testing, ensure equipment within current inspection date, visual inspection of cables and connectors prior to start	Significantly	Significantly	x
Carbon dioxide fire extinguisher	Significantly	Significantly	x
		Residual Risk	Low

People / Groups at risk	Operator only		x
Enter risk details here:-	Impact	Probability	Risk Score
Lone working	Harmful	Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Should out of hours working be required, permission to work out of hours must be obtained prior to work commencing. Sign in using the lone working Power App (https://www.lboro.ac.uk/services/health-safety/loneworking/). It is advised also to inform security so that they are aware of your location on campus for the duration of your lone working/out of hours . Security staff are trained First Aiders - mobile no. freefone 0800 526966 Inform academic supervisor and a colleague of intention to lone work and state duration of stay. Lone working duty officer will be appointed. If duration out of hours is longer than 2 hours arrange to be accompanied, as these are high category labs. Ensure you have mobile phone on person at all times. ALWAYS remember to log out of lone working app when leaving building at completion of the work	Moderately	Moderately	x
		Residual Risk	Low

+ Add another Risk

Who may be at risk as a result of this activity?

Personnel Group	Maximum (Task setup/ Re-configuration)	High (Performing the task)	Medium (Observing the task)	Low (Present, but not involved)	Lone Working (Out of hours)	No Exposure Permitted	Total
Academic Staff	0	1	0	0	0	0	1
Technical Staff	0	1	0	0	0	0	1
Research Staff (PDRA)	1	1	0	0	0	0	2
Research Students (PhD)	0	1	0	0	0	0	1
Students (Undergraduate / MSc)	0	1	0	0	0	0	1

Process Risk Assessment Form (Continued)

Personnel Group	Maximum (Task setup/ Re-configuration)	High (Performing the task)	Medium (Observing the task)	Low (Present, but not involved)	Lone Working (Out of hours)	No Exposure Permitted	Total
Visitors	0	0	0	0	0	0	0
Others - Over-type as needed	0	0	0	0	0	0	0
Total	1	5	0	0	0	0	6

With these controls in place, the risk is:

This activity is MEDIUM RISK. Extra controls are required to make the activity adequately controlled.

Loughborough University
Centre for Biological Engineering
Safety Method Statement

Reference: SAF/MEME/6889

Location: H27, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Decellularization of porcine tissues

What equipment will be used in this activity?	+
Orbital shaker	x
Minisart Syringe Filter	x
Sterilin pots	x
Autoclave	x
Biological safety cabinet	x
Fume hood	x
Scales	x
Pipette	x
Pipette tips	x
Duran bottles	x
Volumetric cylinder	x
pH meter	x
Aspirator	x
Fume Hood	x

What training must be completed to do this activity?	+
CBE code of practice, SOP003, SOP004, SOP037, SOP038, SOP048	x

What chemicals are being used? (These must be included in the COSHH Form)	+
Polymixin B sulphate salt powder	x
Vancomycin hydrochloride hydrate	x
Gentamycin sulphate	x
DPBS without calcium, magnesium x10	x
Ethylenediaminetetraacetic acid disodium salt dihydrate	x
Sodium Dodecyl Sulfate (SDS)	x
Tris	x
Triton X-100	x
Sodium hydroxide	x
Hydrochloric acid (6N)	x
CASO bouillon	x

Spill and accident procedures.	+
Spillages can be cleaned up with an absorbent cloth/tissue using 1:20 Chemgene. Specific disposal procedures must be followed depending on the chemicals that have been described below in COSHH.	x

Safety Method Statement (Continued)

Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)	+
Leave a note with details of the user and name of the chemical asking not to move anything from the area.	x

References.	+
CBE code of practice, SOP003, SOP004, SOP037, SOP038, SOP048	x

Detailed sequential description of the process

Process step	Precautionary measures and comments	+
Prepare Duran bottles where the solutions are going to be made and kept.	Be cautious not to drop glassware and break.	x
Measure the powder using using scales.	Always measure powders under fume hood in H25 or H34 to avoid breathing dust.	x
Pour powders in bottles under fume hood	Always measure powders under fume hood in H25 or H34 to avoid breathing dust.	x
Add liquids in the bottles to make solutions	Handle liquids carefully and have absorbent tissue nearby.	x
When required to modify the pH, add as required sodium hydroxide or hydrochloric acid dropwise and check pH.	Be careful not to pour liquid on the pH meter.	x
Filter sterilise solutions.	Make sure that the receiving container can fit all teh liquid.	x
Add appropriate solutions in dissected samples in BSC.	Be careful not spilling solution and treat waste according to COSSH forms.	x
Change solutions in BSC using aseptic technique.	Be careful not to contaminate your samples.	x

Loughborough University
Centre for Biological Engineering

COSHH Form

Reference: SAF/MEME/ 690 - 692, 69

Location: H27, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Decellularization of porcine tissues

CHEMICAL NAME

Vancomycin hydrochloride hydrate

CAS No. 1404-93-9

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Low

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.25	g	1	Semi Closed	Non-Volatile Liquid	☐ Eyes ☑ Skin ☑ Inhaled ☐ Ingested	Low

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description
H317 May cause an allergic skin reaction.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
H334 May cause allergy or asthma symptoms or breathing difficulties i	P280 Wear protective gloves/protective clothing/eye protection/face protection.
	P284 Wear respiratory protection.
	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com
	P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/p

Justify the use of this chemical: Vancomycin hydrochloride hydrate is used to inhibit growth of gram-positive bacteria, including penicillin-resistant staphylococci in the samples.

How will the precautions listed above be implemented?

Use personal protective equipment (gloves and lab coat). Use chemical in BSC or fume hood. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.

Special Storage and Containment Measures	Disposal Method
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Store in freezer at -20 °C.	Collect in labelled bottle with other antibiotics and when bottle is full, dispose in Gas Pod 1.

How will spillages be dealt with?

Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.
Click here to see spill procedures

Absorbent cloth / tissue

CHEMICAL NAME

Gentamicin sulfate

CAS No. 1405-41-0

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Low

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.25	g	1	Semi Closed	Non-Volatile Liquid	☐ Eyes ☑ Skin ☐ Inhaled ☐ Ingested	Low

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description
H317 May cause an allergic skin reaction.	P280 Wear protective gloves/protective clothing/eye protection/face protection.

	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	x
Justify the use of this chemical:	Gentamicin sulfate is used to inhibit growth of primarily gram-negative bacteria in the samples.	

How will the precautions listed above be implemented?

Use personal protective equipment (gloves and lab coat). Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Use the powder form of the chemical in BSC or fume hood. Do not let product enter drains.

Special Storage and Containment Measures	Disposal Method	
Store in the fridge at 2 - 8 °C. Keep container tightly closed in a dry and well-ventilated place.	Collect in labelled bottle with other antibiotics and when bottle is full, dispose in Gas Pod 1.	x
How will spillages be dealt with?	*Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* *Click here to see spill procedures*	
Absorbent cloth / tissue		

CHEMICAL NAME

Ethylenediaminetetraacetic acid disodium salt

CAS No. 6381-92-6

W.E.L. (Itel / stel)

Hazard Rating: High

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
5	g	48	Semi Closed	Dusty Solid	☐ Eyes ☐ Skin ☑ Inhaled ☐ Ingested	Low

x

OVERALL RISK: Low

Hazard Statement and Description	Precaution Statement and Description	
H332 Harmful if inhaled.	P260 Do not breathe dust/fume/gas/mist/vapours/spray.	x
H373 Causes damage to organs through prolonged or repeated expos	P271 Use only outdoors or in a well-ventilated area.	x
H412 Harmful to aquatic life with long lasting effects.	P273 Avoid release to the environment.	x
	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com	x
	P312 Call a POISON CENTER or doctor/physician if you feel unwell.	x
	P314 Get medical advice/attention if you feel unwell.	x
	P501 Dispose of contents/container to an approved waste disposal plant	x

How will the precautions listed above be implemented?

Use personal protective equipment (lab coat and goggles). Avoid contact with skin and eyes. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Avoid breathing dust. Do not let product enter drains.

Special Storage and Containment Measures	Disposal Method	
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.	Dilute prepared solution to 1-10mM and pour down the drain.	x
How will spillages be dealt with?	*Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* *Click here to see spill procedures*	
Absorbent cloth / tissue		

CHEMICAL NAME

Sodium dodecyl sulfate

CAS No. 151-21-3

W.E.L. (Itel / stel)

Hazard Rating: High

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
500	ml	48	Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☑ Inhaled ☐ Ingested	Low

x

OVERALL RISK: Medium

COSHH Form (Continued)

Hazard Statement and Description	Precaution Statement and Description	+
H315 Causes skin irritation.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray.	x
H318 Causes serious eye damage.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	x
H335 May cause respiratory irritation.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	x
	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com	x
	P312 Call a POISON CENTER or doctor/physician if you feel unwell.	x
	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov	x
	P310 Immediately call a POISON CENTER or doctor/physician.	x

How will the precautions listed above be implemented?
Use personal protective equipment (lab coat and goggles).

Special Storage and Containment Measures	Disposal Method	+
Keep container tightly closed in a dry and well-ventilated place. Containers which arePOur it opened must be carefully resealed and kept upright to prevent leakage. Store in cool place.	Pour it in drain and add copious amounts of water.	x
How will spillages be dealt with?	*Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* *Click here to see spill procedures*	
Absorbent cloth / tissue		

CHEMICAL NAME

Triton X-100

CAS No. 9036-19-5

W.E.L. (Itel / stel)



Hazard Rating: High

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
5	ml	48	Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☐ Inhaled ☑ Ingested	Low

x **OVERALL RISK:** Medium

Hazard Statement and Description	Precaution Statement and Description	+
H302 Harmful if swallowed.	P273 Avoid release to the environment.	x
H315 Causes skin irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	x
H318 Causes serious eye damage.	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.	x
H410 Very toxic to aquatic life with long lasting effects.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov	x
	P313 Get medical advice/attention.	x

How will the precautions listed above be implemented?
Use personal protective equipment (lab coat and goggles). Do not breathe aerosols. Avoid substance contact with skin and eyes. If product enter drains, dilute it with plenty of water.

Special Storage and Containment Measures	Disposal Method	+
Keep solution in a tightly-closed container. Store in a dry, cool and well-ventilated place.	Pour it in drain and add copious amounts of water.	x
How will spillages be dealt with?	*Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* *Click here to see spill procedures*	
Absorbent cloth / tissue		

COSHH Form (Continued)

CHEMICAL NAME

Sodium hydroxide

CAS No. 1310-73-2

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
1	ml	1	Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☐ Inhaled ☐ Ingested	Low

Hazard Statement and Description	Precaution Statement and Description
H290 May be corrosive to metals.	P260 Do not breathe dust/fume/gas/mist/vapours/spray.
H314 Causes severe skin burns and eye damage.	P280 Wear protective gloves/protective clothing/eye protection/face protection.
	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminate
	P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for
	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov

How will the precautions listed above be implemented?

Wear PPE- nitrile gloves, lab coat, closed shoes, goggles.

Special Storage and Containment Measures	Disposal Method
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.	Solution can be neutralised with hydrochloric acid and then, be discarded in drain. Pellets can be discarded in yellow bag.

How will spillages be dealt with?

Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.
Click here to see spill procedures

Absorbent cloth / tissue

CHEMICAL NAME

Hydrochloric acid

CAS No. 258148

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.5	ml		Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☑ Inhaled ☐ Ingested	Low

Hazard Statement and Description	Precaution Statement and Description
H290 May be corrosive to metals.	P280 Wear protective gloves/protective clothing/eye protection/face protection.
H314 Causes severe skin burns and eye damage.	P301 + P330 + P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
H318 Causes serious eye damage.	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminate
H335 May cause respiratory irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov
	P310 Immediately call a POISON CENTER or doctor/physician.

How will the precautions listed above be implemented?

COSHH Form (Continued)

Wear PPE- nitrile gloves, lab coat, closed shoes, goggles. It can be used on the bench, but it is preferable to be used in fume hood in H34.
Avoid contact with the following:
Exothermic reaction with amines, aldehydes, permanganates, for example potassium permanganate.
Risk of ignition or formation of inflammable gases or vapours with: aluminium, carbides, fluorine, metals, bases, sulphides.
Risk of explosion with: alkali metals, sulphuric acid.
Gives off hydrogen by reaction with metals.
Corrosive to metals.

Special Storage and Containment Measures	Disposal Method	+
Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.	Solution can be neutralised with sodium hydroxide and then, be discarded in drain.	x
How will spillages be dealt with?	*Please note: any material used to clean up a spill of hazardous material must also be disposed of as hazardous material.* *Click here to see spill procedures*	
Spill kit		

+ Add another chemical

Statement of work (Process to be undertaken)

Show image

Preparation of solutions:
• PBS (11L total): Prepare 11L that are required for the preparation of disinfection solution, 12 washes, and 1 sterility check.
• Disinfection solution: Disinfection solution is made up by supplementing 500 ml of PBS with 0.1-1 mg·ml-1 of gentamicin, 0.1-1 mg·ml-1 of polymyxin B and 0.01-0.1 mg·ml-1 of vancomycin hydrochloride. The pH is adjusted to 7.2 - 7.4, and the solution is sterilised by filtration using a Stericup filter unit with 0.22 µm pore size filter.
• Hypotonic buffer plus EDTA (2.7 mM, 10mM Tris): Prepare a stock solution of EDTA of 0.5M. Then, take 5.4ml from this solution and dissolve it in 900ml of distilled water, then add Tris (1.21g) to make the hypotonic buffer. The pH is adjusted to 8.0 – 8.2 and the volume made up to 1000ml. The solution is autoclaved at 121°C for 20 min and stored at room temperature for long term storage.
• SDS (0.1-1% w/v) in distilled water: Prepare 0.5% w/v solution, filter sterilise and store it for long term storage.
• Triton X-100 (0.1-1% w/v) in distilled water: Dissolve 5ml in 995ml of distilled water. Stir until dissolved. Filter sterilise it and then store it for up to 2 months roughly.
• CASO bouillon for sterility assessment: Prepare 5 x CASO bouillon stock solutions by adding 75g of CASO bouillon to 500ml of distilled water. The solution is autoclaved at 121°C for 20 min and stored at 4°C.

Decellularisation procedure:
1st day:
1. Add samples in containers.
2. Add disinfection solutions (37°C; 185 ± 5 rpm; 1h)
3. Remove disinfection solution.
4. Add hypotonic buffer (RT;185 ± 5 rpm; 0-12h). Change the solution after 6 hours minimum.
5. Remove hypotonic buffer.
6. Hypotonic buffer (RT;185 ± 5 rpm; 12-24h) (NOTE: leave overnight, so to treat the sample in the solution for a total of at least 24h).
The following steps should take place at RT; 185 ± 5 rpm)
7. Remove hypotonic solution.
8. Add Triton X-100 (x2, change every 12h)
9. Remove solution.
10. SDS (x2, change every 12h)
11. Remove previous solution
12. Add PBS 1x (2x 12h washes)
13. Store tubes with samples in sterile PBS with 1% v/v P/S at 4° C for up to 3 months
14. Cut a small piece of decellularized tissue and put it in soy casein medium.
15. Assess sterility, in terms of medium turbidity, after 14 days incubation.

Personal protection requirements not covered in the precaution statements above.

Closed shoes, over shoes (required for work in CBE)

COSHH Form (Continued)

Sources of information and references

Vancomycin- https://www.sigmaaldrich.com/GB/en/product/sigma/sbr00001?context=product

Gentamicin- https://www.sigmaaldrich.com/GB/en/product/sial/g1914?context=product

EDTA- https://www.sigmaaldrich.com/GB/en/product/sigma/e5134# https://ehs.ucsf.edu/chemicals-approved-drain-disposal#E

SDS- https://www.sigmaaldrich.com/GB/en/product/sigma/05030?context=product#

Triton X-100- https://www.merckmillipore.com/GB/en/product/Triton-X-100,MDA_CHEM-108603

Sodium hydroxide- https://www.sigmaaldrich.com/GB/en/product/sigald/795429?context=product

Hydrochloric acid- https://www.sigmaaldrich.com/GB/en/product/sigald/258148?context=product

Reference to **existing approved** Risk Assessment

SAF/MEME 6511

With the current controls, the risk of using these chemicals is: Medium

Supervisor to check that the process involving the safe use of these chemicals has been satisfactorily evaluated

Supervisor and Departmental Safety Office (DSO) Sign-off.

Supervisors

Please check the documents above and if you want to approve them:

1) Electronically sign this document
2) Save it to a local drive (You will be prompted to do this)
3) eMail the signed document to the DSO.

DSO

Please review the documents above and if you want to approve them:

1) Enter the reference numbers as appropriate
2) Electronically sign this document
3) Save it to a local drive (You will be prompted to do this)
3) eMail the signed document to the originator

IF YOU DO NOT WANT TO AUTHORISE THE FORMS,

Please do not sign the form, but click the "Not Approved" check-box and return it to the originator by email stating why and what you expect them to do to put it right in the comments box below.

Not Approved ☐

Supervisors Signature: Professor Sotiris Korossis — Digitally signed by Professor Sotiris Korossis Date: 2021.08.14 13:30:15 +01'00'

Form Reference Numbers

Risk Assessment	Method Statement	COSHH Assessment
SAF/MEME/6889	SAF/MEME/6889	SAF/MEME/ 690 - 692, 6

DSO Signature: Keven G Smith — Digitally signed by Keven G Smith Date: 2021.09.02 17:41:12 +01'00'

This document set must be reviewed and re-approved at the following times:

1) After the first occurrence of the activity described above (Review only)
2) After any change to the procedure or reagents used
3) After any incident resulting from this activity
4) At least annually from the date of approval

Next Review: 2 Sep 2022

Review comments