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	Decellularisation protocol 2
Supervisor Name	Prof Sotiris Korossis

Version : 2.19

Loughborough University
Centre for Biological Engineering

Risk Assessment

Reference: SAF/MEME/6846

Location: H27, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Decellularisation protocol 2

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	Electrical test lables current	N/A	x
Category 2: Workplace				+
Slips/Trips/Falls on the level				x
Category 3: Hazardous and/or Harmful substances				+
Corrosive substances				x
Irritant substances				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 only — x

Enter risk details here:-	Impact	Probability	Risk Score
Electrical shock from using fume hood	Harmful	Unlikely	Medium
What are the control measures?	**Lowers Impact**	**Lowers Probability**	+
Equipment has bi-annual PAT testing and visual checking of cables and connectors prior to use	Significantly	Significantly	x

Residual Risk: Low

People / Groups at risk: Operator only — x

Enter risk details here:-	Impact	Probability	Risk Score
Aerosols from hazardous/ harmful substances	Harmful	Unlikely	Medium
What are the control measures?	**Lowers Impact**	**Lowers Probability**	+
Wear appropriate PPE suited to task of chemical use	Significantly	Significantly	x

Process Risk Assessment Form (Continued)

Work in fume hood and follow the COSSH forms and CBE SOP 9see below)	Significantly	Significantly	x
		Residual Risk	Low

People / Groups at risk	Operator only		x
Enter risk details here:-	Impact	Probability	Risk Score
Corrosive substance	Harmful	Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Use chemical resistant tray in fume hood when preparing solutions. Follow msds and lab rules on chemical use Substances should be safely stored in correct hazard cupboards when not in use	Significantly	Significantly	x
		Residual Risk	Low

People / Groups at risk	Operator and people in proximity		x
Enter risk details here:-	Impact	Probability	Risk Score
Slips trips and falls	Harmful	Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Ensure that the work area is kept clear of chemicals not in use, and ensure good housekeeping is maintained and floors are kept clean and clear of obstructions Clean any spillages immediately to relevant CBE SOP (see below)	Slightly	Slightly	x
		Residual Risk	Medium

People / Groups at risk	Everyone in the room		x
Enter risk details here:-	Impact	Probability	Risk Score
Exposure to Covid-19	Very Harmful	Highly Unlikely	Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Follow all national, local Covid-19 guidelines, and respect local Lab rules. Wear face covering when in the buildings / workshop / lab, regularly sanitise hands, cleanse surfaces, social distance Frequent washing (min 20 seconds)/ sanitizing of hands, gloves may be worn. Touch points and surfaces to be cleansed / wiped down prior to/after use. Distancing should be 2 metre: 1M+ is allowed where all concerned are wearing face coverings. Check local Covid tier rating.	None	Moderately	x
		Residual Risk	Low

+ Add another Risk

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

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	1	0	0	0	2
Research Staff (PDRA)	1	1	1	1	0	0	4
Research Students (PhD)	0	2	2	2	0	0	6
Students (Undergraduate / MSc)	0	0	0	0	0	0	0
Visitors	0	0	0	0	0	0	0
Others - Over-type as needed	0	0	0	0	0	0	0
Total	1	5	4	3	0	0	13

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/6846

Location: H27, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Decellularisation protocol 2

What equipment will be used in this activity?	+
Orbital shaker	x
Stericup filter unit	x
Duran bottles	x
Autoclave	x
200mL sterile containers	x
Stripettes	x
Pipette gun	x

What training must be completed to do this activity?	+
Chemical use	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
EDTA	x
SDS	x
Tris	x
Triton X-100	x
Sodium hydroxide	x
Hydrochloric acid (6N)	x
CASO bouillon	x

Spill and accident procedures.	+
Take up with liquid-absorbent paper and leave it in the fume hood until it solidifies. Dispose it in the yellow bins.	x
Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with substance.	x

Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)	+
In case of an emergency, ensure that you close the lid of the Kaiser's glycerol gelatine. Leave a note with your name, date and chemical asking not to be touched.	x

References. +

Safety Method Statement (Continued)

CBE code of practice	x
SOP004	x
SOP037	x
SOP038	x
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 and liquid using volumetric cylinders for large quantities or strippetes and pipette gun for small quantities.	Always measure chemicals under fume hood in H27 or H34 to avoid breathing dust.	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
Sterilise filter all liquids using stericup filter unit in BSC.	Handle liquids carefully and have absorbent tissue nearby.	x
Add appropriate solutions in samples.	Be careful not spilling solution and treat waste according to COSSH forms.	x
Place samples on orbital shaker.	Ensure samples are stable before starting the orbital shaker.	x

Loughborough University
Centre for Biological Engineering

COSHH Form

Reference: SAF/MEME/1113 - 1116

Location: H27, H34

Originator: Sotiria Toumpaniari

Project / Activity / Task: Decellularisation protocol 2

CHEMICAL NAME

Vancomycin hydrochloride hydrate

CAS No. 1404-93-9

W.E.L. (Itel / stel)

Hazard Rating: High

OVERALL RISK: Medium

Amount used		Period of use (hrs)	The process is:	Physical State		Exposure Potential
0.25	ml	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.
H334 May cause allergy or asthma symptoms or breathing difficulties i	P302 + P352 IF ON SKIN: Wash with plenty of soap and water.
Justify the use of this chemical:	Vancomycin is a glycopeptide antibiotic used to treat severe but susceptible bacterial infections such as MRSA (methicillin-resistant Staphylococcus aureus) infections. A variety of antibiotics are going to be used in this process to protect the development of potential growth of bacteria.

How will the precautions listed above be implemented?

Use personal protective equipment (gloves and lab coat). Use chemical in BSC or fume hood in H27. 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.	Aqueous waste - Collect in bottle and when full, place it 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	Lyophilised Solid	☐ 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.
Justify the use of this chemical:	Gentamicin is an aminoglycoside antibiotic used in the treatment of several gram-negative infections. In this case, it is used to prevent growth of gram negative bacteria.

COSHH Form (Continued)

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 chemical in BSC or fume hood in H27. 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.	Aqueous waste - Collect in bottle and when almost full place it 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*

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
5	ml	24	Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☑ Inhaled ☐ Ingested	Low

OVERALL RISK: **Medium**

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

How will the precautions listed above be implemented?	
Use personal protective equipment (lab coat and gloves). Avoid contact with skin and eyes. Avoid breathing spray formation by using chemical in fume hood in H27or H34 lab.	
Special Storage and Containment Measures	**Disposal Method**
Keep the SDS solution in a tightly-closed container. Store in a dry, cool and well-ventilated place. Do not refrigerate, as the SDS will precipitate out of solution.	Diluted SDS can be poured in drain and add copious amounts of water.
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	24	Semi Closed	Non-Volatile Liquid	☑ Eyes ☑ Skin ☐ Inhaled ☑ Ingested	Low

OVERALL RISK: **Medium**

Hazard Statement and Description	Precaution Statement and Description
H302 Harmful if swallowed.	P273 Avoid release to the environment.
H315 Causes skin irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.

COSHH Form (Continued)

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 gloves 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.	Aqueous waste - Collect in bottle and when almost full place it in gas pod 1. If diluted, can be discarded in drain and then, 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		

+ Add another chemical

Statement of work (Process to be undertaken)

Show image

Preparation of solutions:
• Prepare PBS that is required for the preparation of disinfection solution and 12 washes.
• Disinfection solution: Disinfection solution is made up by supplementing 500 ml of PBS with 0.5 mg·ml-1 of gentamicin, 0.2 mg·ml-1 of polymyxin B and 0.05 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.
• Distilled water for 4 changes
• 0.5% (v/v) Triton X-100 in distilled water for 2 changes
• 0.5% (v/v) SDS in distilled water for 2 changes

Decellularisation procedure:

1. Add samples in containers.
2. Add disinfection solutions (37°C; 185 ± 5 rpm; 1h).
3. Remove disinfection solution.
4. Add distilled water (RT;185 ± 5 rpm; 0-6h).
5. Remove water.
6. Add 0.5 % Triton X-100 (RT;185 ± 5 rpm; 0-24h; x2, change every 12h).
7. Remove solution.
8. Add 0.5 % SDS (RT;185 ± 5 rpm; 0-24h; x2, change every 12h).
9. Remove solution.
10. Add distilled water (RT;185 ± 5 rpm; 0-24h; x2 change every 15 min and then x1 for 24 h).
11. Remove previous solution.
12. Add PBS 1x (12x 12h washes).
13. Store tubes with samples in sterile PBS for up to 3 months.

Personal protection requirements not covered in the precaution statements above.

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

Sources of information and references

https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=P4932&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fp4932%3Flang%3Den
https://ehs.ucsf.edu/chemicals-approved-drain-disposal#E

https://www.sigmaaldrich.com/catalog/product/sigma/05030?lang=en&region=GB

Reference to **existing approved** Risk Assessment

http://www.ncbe.reading.ac.uk/SAFETY/SDS/SodiumDodecylSulphate.pdf

https://www.merckmillipore.com/GB/en/product/msds/MDA_CHEM-108603?Origin=PDP

https://www.sigmaaldrich.com/GB/en/product/sigma/sbr00001#

https://www.fishersci.co.uk/shop/products/corning-cellgro-gentamicin-sulfate-3/15313761

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: 2021.03.26 10:54:36 Z

Form Reference Numbers

Risk Assessment	Method Statement	COSHH Assessment
SAF/MEME/6846	SAF/MEME/6846	SAF/MEME/1113 - 1116

DSO Signature: Keven G Smith — Digitally signed by Keven G Smith Date: 2021.07.01 14:38:45 +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: 26/03/2022

Review comments