

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	School of Aeronautical, Automotive, Chemical and Materials Engineering
Department	Department of Chemical Engineering
Originator name	Nishant Joglekar
email address	n.joglekar@lboro.ac.uk
Location	Centre for Biological Engineering
Project / Activity / Task	Use of DMSO as a cryoprotectant for the cryopreservation of cells
Supervisor Name	Karen Coopman and Elizabeth Ratcliffe

Risk Assessment

Reference

Location

Originator

Project / Activity / Task

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 cables current	N/A	+
Category 2: Workplace				
Risk of asphyxiation (Oxygen depletion)				+
Slips/Trips/Falls on the level				+
Category 3: Hazardous and/or Harmful substances				
Liquid Nitrogen / Cryogenics				+
exposure to Covid-19				+
Category 4: Work activity				
Other Work related hazard (Freezing involves dealing with liquid nitrogen which can result in asphyxiation if precautions are not taken)				+
Other Work related hazard (Handling liquid nitrogen can result in cold burns if precautions are not taken)				+
Category 5: Work organisation				
N/A				+

Explain the risks associated with these hazards				
People / Groups at risk	<input type="text" value="Everyone in the room"/>			+
Enter risk details here:-	Impact	Probability	Risk Score	
<input type="text" value="Asphyxiation risk"/>	<input type="text" value="Very Harmful"/>	<input type="text" value="Highly Unlikely"/>	Medium	
What are the control measures?	Lowers Impact	Lowers Probability	+	
<input type="text" value="Door propped open when handling cryobanks"/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	+	
<input type="text" value="Room to be well ventilated"/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	+	
			Residual Risk	
			<input type="text" value="Low"/>	
People / Groups at risk	<input type="text" value="Operator only"/>			+

Process Risk Assessment Form (Continued)

Enter risk details here:- Cold burns	Impact Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Appropriate PPE including heavy duty gloves worn Must be competent in handling of cryo materials	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- Frostbite to eyes	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Appropriate PPE including safety glasses and a face visor to be worn	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Operator and people in proximity		x
Enter risk details here:- Slips trips and falls	Impact Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Any spillages cleaned up immediately as per COSHH below and to CBE SOP (below). Keep area clear and tidy and clear any obstructions from the floor	None	Moderately	x
			Residual Risk Low
People / Groups at risk	Everyone in the room		x
Enter risk details here:- Exposure to Covid 19	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Follow all national, local and University Covid-19 guidelines, and respect local Lab rules. Frequent washing / sanitizing of hands / gloves to be carried out. Common touch points and surfaces to be cleaned / wiped down before and after use. Social distancing should be 2 metre. Check local Covid tier rating	None	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
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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
Academic Staff	0	0	0	0	0	0	0
Technical Staff	0	1	0	0	0	0	1
Research Staff (PDRA)	0	1	0	0	0	0	1
Research Students (PhD)	0	1	0	0	0	0	1
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	0	3	0	0	0	0	3

With these controls in place, the risk is:

The activity is LOW RISK - and is effectively controlled

Safety Method Statement

Reference SAF/MEME/6693

Location Centre for Biological Engineering Originator Nishant Joglekar

Project / Activity / Task Use of DMSO as a cryoprotectant for the cryopreservation of cells

What equipment will be used in this activity?	+
Pipettes and Pipette Tips	X
Cryovials	X
-80°C freezer	X
Passive cooling device CoolCell®/ Mr Frosty	X
Liquid nitrogen dewar	X
BSC	X

What training must be completed to do this activity?	+
Cell culture	X
Aseptic techniques	X
Cryopreservation	X
Liquid nitrogen	X

What chemicals are being used? (These must be included in the COSHH Form)	+
Dimethylsulfoxide (DMSO)	X
Fetal Bovine Serum (FBS)	X
Liquid Nitrogen	X

Spill and accident procedures.	+
<p>For DMSO & FBS</p> <p>For a small spill (less than 10ml), alert people in immediate area of spill. In case of a leaking container, turn the container leak-side up to prevent the escape of liquid. Cover the spill area with paper towels soaked with 1% Virkon solution and leave for 10 minutes. Place the soaked paper towels (and other virkon soaked items) into a yellow biohazard disposal bag. Wipe the spill and adjacent area with further paper towels soaked in 1% Virkon solution and place the used towels and gloves in the yellow biohazard bag/container. Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Place all reusable contaminated PPE (eg labcoat, goggles etc) in an autoclave bag/ container for decontamination (for reusable items not contaminated with virkon). Place non reusable items (eg gloves, shoe covers) in a yellow biohazard disposal bag. Wash hands and other potentially contaminated areas again with soap and water. Inform lab staff when clean-up is complete and fill in the spill record in the logbook.</p> <p>For a large spill (greater than 10ml), alert other laboratory staff and leave the laboratory immediately. Leave the BSC switched on and any cultures inside cabinet. Close the lab doors and post warning signs to prevent others entering the laboratory. Report the incident to the Laboratory Manager. For significantly large spills (i.e. >100 ml) contact the local DSO for advice before proceeding. If authorised, assemble a clean-up team consisting of three people: one to observe and direct the clean-up procedure, and the other two, who must be properly trained, to carry out the procedure. Complete and fill in the spill record in the logbook.</p> <p>For Liquid Nitrogen</p> <p>If correct procedures have been followed, then the spill will be in a well-ventilated area and will be allowed to evaporate.</p> <ol style="list-style-type: none"> Move any other personnel away from the spill area. Prop open doors to the laboratory if additional ventilation is required If oxygen monitors are alarming (<18 % O₂) then immediately evacuate the area and contact the Laboratory Manager and Departmental Safety Officer. 	X

Safety Method Statement (Continued)

<p>For Liquid Nitrogen</p> <p>A small spill consists of < 100ml, If correct procedures have been followed, then the spill will be in a well-ventilated area and will be allowed to evaporate.</p> <p>a. Move any other personnel away from the spill area.</p> <p>b. Prop open doors to the laboratory if additional ventilation is required</p> <p>c. If oxygen monitors are alarming (<18 % O₂) then immediately evacuate the area and contact the Laboratory Manager and Departmental Safety Officer.</p> <p>If the volume of liquid nitrogen spilled is ≥ 100 ml (Major External Spill) and external to the CBE laboratory then:</p> <p>a. Immediately evacuate the area.</p> <p>b. Cordon off the spill area and prevent any individual (whether staff member, student or general public) from accessing the spill area.</p> <p>c. Allow the liquid nitrogen to evaporate into the atmosphere.</p> <p>d. Contact the Laboratory Manager and Departmental Safety Officer.</p> <p>If the volume of liquid nitrogen spilled is ≥ 100 ml (Major Internal Spill) within the CBE laboratory then:</p> <p>a. All personnel must immediately evacuate the surrounding area or risk death from asphyxiation.</p> <p>b. Immediately contact the Laboratory Manager and Departmental Safety Officer.</p> <p>c. If the spill is very large (> 10 L) and/or in an enclosed space, a complete evacuation of the building may be necessary.</p> <p>d. The Fire Service should also be alerted to the situation if there is serious risk of combustion.</p>	X
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Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)	+
Remove contaminated PPE or clothing. Alert other laboratory staff and leave the laboratory immediately while leaving the BSC switched on and leaving any cultures inside the cabinet. Wash hands and other potentially contaminated areas with soap and water.	X
Make sure that the DMSO container is firmly closed and is left in a well-ventilated area.	X
Make safe the liquid nitrogen if possible before evacuating. If it is not possible to make the liquid nitrogen safe, then evacuate the building and immediately inform the Fire Brigade to the risks posed by liquid nitrogen (oxygen depletion and combustion in particular).	X

References.	+
SOP003;	X
SOP013; Safe use and Maintenance of Liquid Nitrogen Stores	X
SOP031; Cryopreservation and storage of Mammalian Cells	X
SOP038; Biological Spill Response	X

Detailed sequential description of the process

Process step	Precautionary measures and comments	
Make up freezing media by adding 5ml of DMSO to 45ml of FBS to make a total of 50ml. This will be a concentration 10% DMSO in FBS. For immediate use, keep at room temperature/in a 37°C water bath. For short-term storage, keep in a fridge or for long-term storage, store in a -20°C freezer. Freezing media must be warmed in a 37°C water bath before use to prevent cold shock.	Wear nitrile gloves and a lab coat. Work in the BSC.	X
Label 1.8ml cryovials and perform cell count.	Wear nitrile gloves and a lab coat.	X
Following centrifugation, aspirate the supernatant, leaving a cell pellet. Immediately resuspend the cell pellet in freezing medium with gentle pipetting to obtain target freezing cell density. The will be frozen at 1 million cells per ml.	Wear nitrile gloves and a lab coat. Work in the BSC.	X

Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
Aliquot freezing medium/cell mix in the pre-labelled cryovials (1ml/ vial). this step along with resuspension should be done swiftly to prevent large amount of DMSO uptake into the cells as this can affect cell viability on thawing.	Wear nitrile gloves and a lab coat. Work in the BSC.	X
Confirm each loaded cryovial is properly closed by hand tightening with an extra twist to ensure closure without damaging O-rings or the vial threads.	Wear nitrile gloves and a lab coat. Work in the BSC.	X
Transfer into Mr Frosty or similar and put in -80°C freezer until frozen (4-16hrs). Then transfer to cryobanks and update cryoslips and the Pro Curo software.	Wear nitrile gloves and a lab coat. For transferring to cryobanks, wear heavy duty gloves (no nitrile gloves) and face visor. Use metal tray on the floor, prop doors open to make sure well ventilated. Check Oxygen monitor before opening Cryobank.	X
For rapid thawing, transfer the cryovials from the cryobanks to 37°C water bath and hold the vials in the water bath to thaw them until a slither of ice remains.	Wear nitrile gloves and a lab coat. When taking out the cryovials from the cryobanks, wear heavy duty gloves (no nitrile gloves) and face visor. Use metal tray on the floor, prop doors open to make sure well ventilated. Check Oxygen monitor before opening Cryobank.	X
Wipe and put into BSC	Wear nitrile gloves and a lab coat.	X
To remove the DMSO from the cell suspension, transfer the suspension to a falcon tube and centrifuge at 200g for 5mins. Then aspirate the supernatant and resuspend the cells in 1ml fresh media.	Wear nitrile gloves and a lab coat.	X

COSHH Form

Reference SAF/MEME/938- 939

Location Centre for Biological Engineering

Originator Nishant Joglekar

Project / Activity / Task Use of DMSO as a cryoprotectant for the cryopreservation of cells

CHEMICAL NAME				Hazard Rating		OVERALL RISK: Low
Dimethylsulfoxide (DMSO)				Low		
CAS No.	67-68-5	Amount used	Period of use (hrs)	The process is:	Physical State	Exposure Potential Low
W.E.L. (Itel / stel)		5 ml	0.1	Semi Closed	Non-Volatile Liquid	
				<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested		

Hazard Statement and Description	Precaution Statement and Description	+
No Hazard Statements applicable	No Precaution statements applicable	x

How will the precautions listed above be implemented?

N/A

Special Storage and Containment Measures	Disposal Method	+
Must be stored in a cool, well ventilated area with the lid being tightly closed. DMSO is combustable and must hence not be stored near sources of ignition.	Before being diluted with FBS, the DMSO must be disposed of via the cytotoxic waste route. Once diluted with FBS, the freezing mix can be disposed of via the biological liquid waste route.	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	

For a small spill (less than 10ml), alert people in immediate area of spill. In case of a leaking container, turn the container leak-side up to prevent the escape of liquid. Cover the spill area with paper towels soaked with 1% Virkon solution and leave for 10 minutes. Place the soaked paper towels (and other virkon soaked items) into a yellow biohazard disposal bag. Wipe the spill and adjacent area with further paper towels soaked in 1% Virkon solution and place the used towels and gloves in the yellow biohazard bag/container. Remove all PPE immediately upon leaving the work area and as soon as possible if overtly contaminated. Place all reusable contaminated PPE (eg labcoat, goggles etc) in an autoclave bag/container for decontamination (for reusable items not overtly contaminated with virkon). Place non reusable items (eg gloves, shoe covers) in a yellow biohazard disposal bag. Wash hands and other potentially contaminated areas again with soap and water. Inform lab staff when clean-up is complete and fill in the Spill Record in the logbook.


For a large spill (greater than 10ml), alert other laboratory staff and leave the laboratory immediately. Leave the BSC switched on and any cultures inside cabinet. Close the lab doors and post warning signs to prevent others entering the laboratory. Report the incident to the Laboratory Manager. For significantly large spills (i.e. >100 ml) contact the local DSO for advice before proceeding. If authorised, assemble a clean-up team consisting of three people: one to observe and direct the clean-up procedure, and the other two, who must be properly trained, to carry out the procedure.

CHEMICAL NAME				Hazard Rating		OVERALL RISK: Low
Fetal Bovine Serum				Low		
CAS No.	N/A	Amount used	Period of use (hrs)	The process is:	Physical State	Exposure Potential Low
W.E.L. (Itel / stel)		45 ml	0.1	Open	Non-Volatile Liquid	
				<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested		

Hazard Statement and Description	Precaution Statement and Description	+
No Hazard Statements applicable	No Precaution statements applicable	x

COSHH Form (Continued)

How will the precautions listed above be implemented?		
N/A		
Special Storage and Containment Measures	Disposal Method	+
Stored in -20 freezer for long term storage, short term storage fridge.	Biological waste - aspirate and treat with virkon before disposal down the sink with copious amounts of water.	x
How will spillages be dealt with?	<i>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</i>	
Absorbent cloth / tissue		

CHEMICAL NAME				Hazard Rating High		OVERALL RISK:	x
Liquid nitrogen							Low
CAS No.	N/A	Amount used	Period of use (hrs)	The process is:	Physical State	<input type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	
W.E.L. (l/te / stel)		5	1	0.1	Open	Non-Volatile Liquid	

Hazard Statement and Description	Precaution Statement and Description	+
H281 Contains refrigerated gas; may cause cryogenic burns or injury.	P282 Wear cold insulating gloves/face shield/eye protection.	x

How will the precautions listed above be implemented?

Wear PPE, including lab coat, insulating gloves (no nitrile gloves underneath) and face protection.

Special Storage and Containment Measures	Disposal Method	+
Make sure area is well ventilated. Keep liquid nitrogen containers clean and free of oil, grease or other materials which may become hazardous in contact with cryogenic fluids or condensed oxygen. Containers stored in a location away from fire risk, sources of heat and ignition. Container caps in place.	Allow liquid nitrogen to evaporate into the atmosphere.	x
How will spillages be dealt with?	<i>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</i>	

If the volume of liquid nitrogen spilled is < 100 ml (Minor Spill):

- Move any other personnel away from the spill area and allow evaporation.
- Prop open doors to the laboratory if additional ventilation is required (determined by oxygen monitor alarms)
- If oxygen monitors are alarming (<18 % O2) then immediately evacuate the area and contact the Laboratory Manager and Departmental Safety Officer.

If the volume of liquid nitrogen spilled is ≥ 100 ml (Major External Spill) and external to the CBE laboratory:

- Immediately evacuate the area.
- Cordon off the spill area and prevent any individual (whether staff member, student or general public) from accessing the spill area.
- Allow the liquid nitrogen to evaporate into the atmosphere.
- Contact the Laboratory Manager and Departmental Safety Officer.

If the volume of liquid nitrogen spilled is ≥ 100 ml (Major Internal Spill) within the CBE laboratory:

- All personnel must immediately evacuate the surrounding area or risk death from asphyxiation.
- Immediately contact the Laboratory Manager and Departmental Safety Officer.
- If the spill is very large (> 10 L) and/or in an enclosed space, a complete evacuation of the building may be necessary.
- The Fire Service should also be alerted to the situation if there is serious risk of combustion.

+ Add another chemical

COSHH Form (Continued)

Statement of work (Process to be undertaken)

Cryopreservation of cells

Show
Image

Personal protection requirements not covered in the precaution statements above.

Shoe covers, Heavy duty gloves, face visor

Sources of information and references

<https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=GB&language=en&productNumber=276855&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F276855%3Flang%3Den>;
<https://www.chemadvisor.com/matheson/database/msds/00202589000800003.pdf>;
SOP003; SOP038; SOP013

Reference to **existing approved** Risk Assessment

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

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

Form Reference Numbers

Risk Assessment

SAF/MEME/6693

Method Statement

SAF/MEME/6693

COSHH Assessment

SAF/MEME/938- 939

DSO Signature

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:

16 Feb 2022

Review comments