

## 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	Dania Ebrahim
email address	d.ebrahim-21@lboro.ac.uk
Location	CBE;H23
Project / Activity / Task	Investigation of the effect of cryopreservation on the differentiation ability of MSCs
Supervisor Name	Dr Karen Coopman

# 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				
Slips/Trips/Falls on the level				+
Slips/Trips/Falls on the level				X
Category 3: Hazardous and/or Harmful substances				
Biological substances (Infection) - Cell work				+
Biological substances (Infection) - Cell work				X
Flammable substances - Refer to COSHH forms below for formaldehyde, Oil Red O solution and Alican Blue				X
Corrosive substances - refer to COSHH forms below for formaldehyde and Alican Blue				X
Handling of skin/eye irritation causing substances - refer to COSHH forms below for formaldehyde and Oil Red O solution				X
Cancer causing substances - refer to COSHH form below for formaldehyde				X
Category 4: Work activity				
Highly repetitive actions- standard cell culture can be repetitive				+
Highly repetitive actions- standard cell culture can be repetitive				X
Category 5: Work organisation				
N/A				+
N/A				X

Explain the risks associated with these hazards				
People / Groups at risk	<input type="text" value="Operator only"/>			X
Enter risk details here:-	Impact	Probability	Risk Score	
<input type="text" value="Biological Hazard - MSCs used"/>	<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="Refer to BRA ( all waste will be disposed appropriately as per SOP003)"/>	<input type="text" value="Significantly"/>	<input type="text" value="Significantly"/>	X	
			Residual Risk	
			<input type="text" value="Low"/>	
People / Groups at risk	<input type="text" value="Operator and people in proximity"/>			X

## Process Risk Assessment Form (Continued)

Enter risk details here:- Slips trips and falls	Impact Slightly Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Work area to be kept clean and tidy - any floor based obstacles or hazards should be cleared away	Slightly	Moderately	x
			Residual Risk Low
People / Groups at risk	Operator and people in proximity		x
Enter risk details here:- Handling of flammable substances	Impact Slightly Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
Refer to COSSH forms below for formaldehyde, Oil Red O solution and Alican Blue	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- Handling of corrosive substances	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
refer to COSSH forms below for formaldehyde and Alican Blue	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- Handling of skin/eye irritation causing substances	Impact Slightly Harmful	Probability Highly Unlikely	Risk Score Low
What are the control measures?	Lowers Impact	Lowers Probability	+
refer to COSSH forms below for formaldehyde and Oil Red O solution	Significantly	Significantly	x
			Residual Risk Low
People / Groups at risk	Operator only		x
Enter risk details here:- Handling of cancer causing substances	Impact Very Harmful	Probability Highly Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
refer to COSSH form below for formaldehyde	Moderately	Moderately	x
			Residual Risk Low
People / Groups at risk	Operator only		x

## Process Risk Assessment Form (Continued)

Enter risk details here:- use Electrical equipment	Impact Harmful	Probability Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Be within current PAT inspection date - visual check of cables and connectors prior to use LEVs within current inspection date	None	Moderately	x
			Residual Risk Low
People / Groups at risk	Everyone in the room		x
Enter risk details here:- Possible exposure to Covid	Impact Harmful	Probability Unlikely	Risk Score Medium
What are the control measures?	Lowers Impact	Lowers Probability	+
Follow all current national, local and University Covid guidelines, and respect local Lab rules. Frequent washing / sanitizing of hands to be carried out. Ventilate area Respect social distance	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
Academic Staff	0	0	0	0	0	0	0
Technical Staff	0	0	0	1	0	0	1
Research Staff (PDRA)	0	0	0	1	0	0	1
Research Students (PhD)	0	1	0	0	0	0	1
Students (Undergraduate / MSc)	0	1	0	1	0	0	2
Visitors	0	0	0	0	0	0	0
Others - Over-type as needed	0	0	0	0	0	0	0
<b>Total</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>5</b>

With these controls in place, the risk is:

Process Risk Assessment Form (Continued)

**The activity is LOW RISK - and is effectively controlled**

## Safety Method Statement

Reference SAF/MEME/7141

Location CBE;H23

Originator Dania Ebrahim

Project / Activity / Task Investigation of the effect of cryopreservation on the differentiation ability of MSCs

### What equipment will be used in this activity?

	+
Biological Safety Cabinet (BSC)	X
Water Bath	X
Centrifuge	X

### What training must be completed to do this activity?

	+
Aseptic technique	X
Standard cell training	X
Standard CBE training	X

### What chemicals are being used? (These must be included in the COSHH Form)

	+
Formaldehyde solution	X
Oil Red O solution	X
Alcian Blue	X
Alizarin Red	X

### Spill and accident procedures.

	+
For small spillages of any substances, use an absorbent cloth / tissue with 1:20 chemgene to clear up the spillage and dispose of the cloth / tissue in the yellow stream waste.	X

### Procedure in the event of an emergency. (How to leave the process in a safe condition in such an event)

	+
Make sure all chemical containers are tightly closed and upright. Leave BSC on, and exit the laboratory. Remove all contaminated PPE and wash hands with soap and water.	X
Close laboratory doors and post warning signs to prevent others entering the laboratory and report the incident to the Laboratory Manager.	X

### References.

	+
CBE-BRA-183	X
SAF/MEME/766/769	X
SDS for formaldehyde- <a href="https://www.sigmaaldrich.com/GB/en/sds/sial/252549">https://www.sigmaaldrich.com/GB/en/sds/sial/252549</a>	X
SOP003, SOP038, SOP039	X
SDS for Oil Red O Solution- <a href="https://www.sigmaaldrich.com/GB/en/sds/sigma/o1391">https://www.sigmaaldrich.com/GB/en/sds/sigma/o1391</a>	X
SDS for Alican Blue- <a href="https://www.sigmaaldrich.com/GB/en/sds/mm/tms-010">https://www.sigmaaldrich.com/GB/en/sds/mm/tms-010</a>	X
SDS for Alizarin Red - <a href="https://www.sigmaaldrich.com/GB/en/sds/sial/a5533">https://www.sigmaaldrich.com/GB/en/sds/sial/a5533</a>	X

### Detailed sequential description of the process

Process step	Precautionary measures and comments	+
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## Safety Method Statement (Continued)

Process step	Precautionary measures and comments	+
<p>1) Thaw and culture MSCs from working cell bank as per standard protocol or use cells that are already in culture and seed in appropriate culture vessel and culture to 80-90% confluency.</p> <p>2) Seed MSCs into culture vessels at <math>5 \times 10^3</math> cells/cm<sup>2</sup>. For classical stain differentiation assay, seed into a 12-well plate.</p> <p>3) Incubate the cells in MSC Growth Medium at 36°C to 38°C in a humidified atmosphere of 4–6% CO<sub>2</sub> for a minimum of 2 hours up to 4 days.</p> <p>For osteogenic differentiation:</p> <p>4) Replace media with pre-warmed Complete Osteogenesis Differentiation Medium and continue incubation. Refeed cultures every 3–4 days.</p> <p>5) After 21 days or longer under differentiating condition, remove media from 12-well plate and rinse once with DPBS. Fix cells with 4% formaldehyde solution for 30 minutes.</p> <p>6) After fixation, rinse wells twice with distilled water and stain cells with Alizarin Red S prepared in water for 2–3 minutes.</p> <p>7) Rinse wells three times with distilled water, visualize under light microscope and capture images for qualitative or quantitative analysis.</p> <p>For adipogenic differentiation:</p> <p>4) Replace media with pre-warmed Complete Adipogenesis Differentiation Medium and continue incubation. Refeed cultures every 3–4 days.</p> <p>5) After 7 days or longer under differentiating conditions, remove the media from the 12 -well tissue culture plate and rinse once with DPBS. Fix cells with 4% formaldehyde solution for 30 minutes.</p> <p>6) 2. After fixation, rinse wells twice with DPBS, apply 1:100 dilution Oil Red O and incubate for 15–30 minutes.</p> <p>7) Rinse twice with DPBS, visualize under fluorescent microscope and capture images for qualitative or quantitative analysis.</p> <p>For chondrogenic differentiation:</p> <p>4) Generate micromass cultures by seeding 5-µL droplets of cell solution in the center of 12-well plate.</p> <p>5) After cultivating micromass cultures for 2 hours under high humidity conditions, add warmed chondrogenesis media to culture vessels and incubate in 37°C incubator with 5% CO<sub>2</sub>.</p> <p>6) Refeed cultures every 2–3 days</p> <p>7) After 14 days or longer under differentiating conditions, remove media from culture vessel, rinse once with DPBS, and fix cells with 4% formaldehyde solution for 30 minutes.</p> <p>8) After fixation, rinse wells with DPBS and stain cells with 1% Alcian Blue solution for 30 minutes.</p> <p>9) Rinse wells three times with 0.1 N HCl, add distilled water to neutralize the acidity, visualize under light microscope, and capture images for analysis. Blue staining indicates synthesis of proteoglycans by chondrocytes.</p>	<p>Wear gloves, lab coat and shoe covers at all times.</p> <p>Wear safety glasses and keep chemical containers tightly closed.</p>	<p>+</p> <p>X</p>



# COSHH Form

Reference SAF/MEME/985, 1487 - 14

Location CBE;H23

Originator Dania Ebrahim

Project / Activity / Task Investigation of the effect of cryopreservation on the differentiation ability of MSCs

<b>CHEMICAL NAME</b>	 	Hazard Rating <span style="border: 1px solid black; padding: 2px; color: red;">High</span>	<span style="border: 1px solid black; padding: 2px; color: red; font-weight: bold;">OVERALL RISK:</span> <span style="border: 1px solid black; padding: 2px; color: green; font-weight: bold; font-size: 1.2em;">Medium</span>
Formaldehyde solution  CAS No. <span style="border: 1px solid black; padding: 2px;">50-00-0</span> W.E.L. (Itel / stel) <span style="border: 1px solid black; padding: 2px;"> </span>	Amount used: <span style="border: 1px solid black; padding: 2px;">5</span> ml Period of use (hrs): <span style="border: 1px solid black; padding: 2px;">1</span>	The process is: <span style="border: 1px solid black; padding: 2px;">Semi Closed</span> Physical State: <span style="border: 1px solid black; padding: 2px;">Non-Volatile Liquid</span>	Exposure Potential <span style="border: 1px solid black; padding: 2px; color: green;">Low</span>  <input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input checked="" type="checkbox"/> Inhaled <input checked="" type="checkbox"/> Ingested

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description	+
H226 Flammable liquid and vapour.	P201 Obtain special instructions before use.	X
H301 + H307 Toxic if swallowed or in contact with skin.	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.	X
H314 Causes severe skin burns and eye damage.	P280 Wear protective gloves/protective clothing/eye protection/face protection.	X
H317 May cause an allergic skin reaction.	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated	X
H330 Fatal if inhaled.	P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position com	X
H335 May cause respiratory irritation.	P310 Immediately call a POISON CENTER or doctor/physician.	X
H341 Suspected of causing genetic defects.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remov	X
H350 May cause cancer.		X
H370 Causes damage to organs.		X

Justify the use of this chemical: Required for fixing of cells for differentiation analysis

How will the precautions listed above be implemented?

Training will be provided before handling of the chemical.  
 Gloves, safety glasses and lab coat will be worn.  
 Will be kept away from any heat source/flame/hot surfaces.  
 In case of contact with skin will take off all contaminated clothing, skin will be washed with soap and water.  
 In case of contact with eye, eyes will be washed with water + use eye wash station  
 if inhaled, go out to fresh air and call physician  
 if swallowed: fresh air. Call a doctor immediately (mention methanol ingestion).

Special Storage and Containment Measures	Disposal Method	+
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Keep container tightly closed in a dry and well-ventilated place. Keep away from heat and sources of ignition. Keep locked up or in an area accessible only to qualified or authorized persons. The recommended storage temperature 15 - 25 °C	Any tissues/strippetes with traces of formaldehyde should be disposed as cytotoxic sold waste using the yellow stream waste route. Any pipette tips with traces of formaldehyde should be disposed in purple cytotoxic sharps containers.	X
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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.</i> <a href="#" style="color: blue; font-size: 0.8em;">Click here to see spill procedures</a>	
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Most likely a small spill (1-3ml). Cover and wipe spill with paper towel. Clean spill, cabinet walls, work area and other equipment with 1:20 chemgene and 70% IMS.



COSHH Form (Continued)

<b>CHEMICAL NAME</b> <b>Oil Red O solution</b>			Hazard Rating <b>High</b>	<b>X</b>
CAS No. <b>1320-06-5</b>	Amount used <b>5</b> ml	Period of use (hrs) <b>1</b>	The process is: <b>Semi Closed</b>	Physical State <b>Non-Volatile Liquid</b>
W.E.L. (Itel / stel)			<input checked="" type="checkbox"/> Eyes <input type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential <b>Low</b>
				<b>OVERALL RISK:</b> <b>Medium</b>



Hazard Statement and Description	Precaution Statement and Description	
H225 Highly flammable liquid and vapour.	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.	<b>X</b>
H319 Causes serious eye irritation.	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	<b>X</b>
H336 May cause drowsiness or dizziness.		<b>X</b>
		<b>X</b>

How will the precautions listed above be implemented?

Will be kept away from any heat source/flame/hot surfaces.  
In case of contact with skin will take off all contaminated clothing, skin will be washed with soap and water.  
In case of contact with eye, eyes will be washed with water + use eye wash station

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 cool place.	Any tissues/strippetes with traces of Oil Red O should be disposed as cytotoxic solid waste using the yellow stream waste route. Any pipette tips with traces of Oil Red O should be disposed in purple cytotoxic sharps containers.	<b>X</b>
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. <a href="#">Click here to see spill procedures</a></i>	

Most likely a small spill ( between 1-3ml) within the BSC. Cover and wipe spill with paper towel. Then clean spill, cabinet walls, work area and other equipment with 1:20 chemgene and 70% IMS.

<b>CHEMICAL NAME</b> <b>Alican Blue</b>			Hazard Rating <b>High</b>	<b>X</b>
CAS No. <b>64-19-7</b>	Amount used <b>5</b> ml	Period of use (hrs) <b>1</b>	The process is: <b>Semi Closed</b>	Physical State <b>Non-Volatile Liquid</b>
W.E.L. (Itel / stel)			<input checked="" type="checkbox"/> Eyes <input checked="" type="checkbox"/> Skin <input type="checkbox"/> Inhaled <input type="checkbox"/> Ingested	Exposure Potential <b>Low</b>
				<b>OVERALL RISK:</b> <b>Low</b>

Hazard Statement and Description	Precaution Statement and Description	
H226 Flammable liquid and vapour.	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.	<b>X</b>
H314 Causes severe skin burns and eye damage.	P233 Keep container tightly closed.	<b>X</b>
	P240 Ground/bond container and receiving equipment.	<b>X</b>
	P280 Wear protective gloves/protective clothing/eye protection/face protection.	<b>X</b>
	P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminate	<b>X</b>
	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove	<b>X</b>

How will the precautions listed above be implemented?

# COSHH Form (Continued)

Gloves, safety glasses and lab coat will be worn. Will be kept away from any heat source/flame/hot surfaces. In case of contact with skin will take off all contaminated clothing, skin will be washed with soap and water. In case of contact with eye, eyes will be washed with water + use eye wash station if inhaled, go out to fresh air and call physician if swallowed: Drink water (two glasses at most). Avoid vomiting. Call physician.		+
Special Storage and Containment Measures	Disposal Method	+
Keep container tightly closed in a dry and well-ventilated place. Keep away from heat and sources of ignition.	Any tissues/strippetes with traces of Alien Blue should be disposed as cytotoxic solid waste using the yellow stream waste route. Any pipette tips with traces of Alican Blue should be disposed in purple cytotoxic sharps containers.	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.  <a href="#">Click here to see spill procedures</a></i>	
Most likely a small spill (1-3ml). Cover and wipe spill with paper towel. Clean spill, cabinet walls, work area and other equipment with 1:20 chemgene and 70% IMS.		

CHEMICAL NAME	Hazard Rating		OVERALL RISK:
<b>Alizarin Red S</b>	Low	x	Low
CAS No. <input type="text" value="130-22-3"/> <input type="text" value="130-22-3130"/> W.E.L. (Itel / stel) <input type="text"/>	Amount used: <input type="text" value="5"/> <input type="text" value="ml"/> Period of use (hrs): <input type="text" value="1"/>	The process is: <input type="text" value="Semi Closed"/> Physical State: <input type="text" value="Dusty Solid"/>	Exposure Potential: <input type="text" value="Low"/>
<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?		
No precautions as chemical considered not a hazardous substance		
Special Storage and Containment Measures	Disposal Method	+
Store in cool place. Keep container tightly closed in a dry and well-ventilated place.	Disposed using yellow stream waste	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.  <a href="#">Click here to see spill procedures</a></i>	
Most likely a small spill (1-3ml). Cover and wipe spill with paper towel. Clean spill, cabinet walls, work area and other equipment with 1:20 chemgene and 70% IMS.		

+ Add another chemical

### Statement of work (Process to be undertaken)

Determining the ability of MSCs to differentiate post-thaw	Show Image
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Personal protection requirements not covered in the precaution statements above.

Shoe covers
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### Sources of information and references

SDS Formaldehyde solution- <a href="https://www.sigmaaldrich.com/GB/en/sds/sial/252549">https://www.sigmaaldrich.com/GB/en/sds/sial/252549</a> SDS Oil Red O Solution- <a href="https://www.sigmaaldrich.com/GB/en/sds/sigma/o1391">https://www.sigmaaldrich.com/GB/en/sds/sigma/o1391</a> SDS Alican Blue- <a href="https://www.sigmaaldrich.com/GB/en/sds/mm/tms-010">https://www.sigmaaldrich.com/GB/en/sds/mm/tms-010</a> SDS Alizarin Red S- <a href="https://www.sigmaaldrich.com/GB/en/sds/sial/a5533">https://www.sigmaaldrich.com/GB/en/sds/sial/a5533</a> SOP003, SOP038, SOP039	Reference to <b>existing approved</b> Risk Assessment <input style="width: 100%;" type="text" value="SAF/MEME 6749"/>
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## COSHH Form (Continued)

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

### Form Reference Numbers

Risk Assessment

SAF/MEME/7141

Method Statement

SAF/MEME/7141

COSHH Assessment

SAF/MEME/985, 1487 - 1

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:

31 Mar 2023

Review comments