Safety Documentation

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

☐ **Process 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	PRAVEENKUMAR KAVERI
email address	P.Kaveri@lboro.ac.uk
Location	Loughborough University, Wolfson School T 2.08 B
Project / Activity / Task	Biofilm staining
Supervisor Name	Dr. Sourav Ghosh

Version : 2.15

Loughborough University
CENTRE FOR BIOLOGICAL ENGINEERING

COSHH Form

Reference: SAF/MEME/933

Location: Loughborough University, Wolfson School T 2.08 B

Originator: PRAVEENKUMAR KAVERI

Project / Activity / Task: Biofilm staining

CHEMICAL NAME

Crystal Violet

CAS No. 548-62-9

W.E.L. (Itel / stel) Less than 0.5m

Amount used		Period of use (hrs)	The process is:	Physical State
0.25	g	2	Semi Closed	Volatile Liquid

- [x] Eyes
- [x] Skin
- [x] Inhaled
- [x] Ingested

Hazard Rating: High

Exposure Potential: Low

OVERALL RISK: Medium

X

This chemical has a high health risk associated with it.

Hazard Statement and Description	Precaution Statement and Description
H350 May cause cancer.	P201 Obtain special instructions before use.
H412 Harmful to aquatic life with long lasting effects.	P202 Do not handle until all safety precautions have been read and understood.
H225 Highly flammable liquid and vapour.	P273 Avoid release to the environment.
H319 Causes serious eye irritation.	P280 Wear protective gloves/protective clothing/eye protection/face protection.
H302 Harmful if swallowed.	P308 IF exposed or concerned:
H318 Causes serious eye damage.	P313 Get medical advice/attention.
H400 Very toxic to aquatic life.	P501 Dispose of contents/container to ...
H410 Very toxic to aquatic life with long lasting effects.	P405 Store locked up.
H301 Toxic if swallowed.	No Precaution statements applicable
H311 Toxic in contact with skin	No Precaution statements applicable
H331 Toxic if inhaled.	No Precaution statements applicable
H370 Causes damage to organs.	No Precaution statements applicable
Justify the use of this chemical:	Chemical is supplied as a powder and will be prepared using water in a 1%(v/v) solution. This is a very commonly used chemical to stain bacteria and is needed for standard methodology

How will the precautions listed above be implemented?

Use personal protective equipment as required. Ensure adequate ventilation. Avoid dust formation. Keep people away from and upwind of spill/leak. Evacuate personnel to safe areas.

Special Storage and Containment Measures	Disposal Method
Keep containers tightly closed in a dry, cool and well-ventilated place. Protect from direct sunlight	As the chemical is carcinogenic, the CBE cytotoxic waste procedure fo
	Should not be released into the environment. Waste is classified as h
	Dispose of this container to hazardous or special waste collection po
	Do not flush to sewer. Waste codes should be assigned by the user b

How will spillages be dealt with?

COSHH Form (Continued)

Spill kit

+ Add another chemical

Statement of work (Process to be undertaken)

Show image

Crystal violet is a general bacterial staining dye which is preferred than safranin due to high optical density.
Crystal violet will be disposed as cytotoxic waste.

Ob1. To grow biofilm in microtitre plates

1. Streak frozen bacterial stock on Luria broth (LB) agar plate
2. Grow overnight
3. Dilute the overnight culture 1:100 into fresh medium for biofilm assays.
4. Add100 µL of the dilution per well in a 96 well dish
5. For quantitative assays, use 4-8 replicate wells for each treatment.
6. Incubate the microtiter plate for 4 -24 hrs. at 37°C

Ob2. Evaluate or quantify the biofilm using colorimetric dye (visual observation)

1. After incubation, dump out cells by turning the plate over and shaking out the liquid.
2. Gently submerge the plate in a small tub of water; Shake out water.
3. Repeat this process
4. Add125 µL of a 0.1% solution of crystal violet in water to each well of the microtiter plate.
5. Incubate the microtiter plate at room temperature for 10-15 min.
6. Rinse the plate 3-4 times with water by submerging in a tub of water.
7. Shake out and blot vigorously on a stack of paper towels to rid the plate of all excess cells and dye.
8. Turn the microtiter plate upside down and dry for a few hours or overnight.
9. Photograph the wells when dry for qualitative assays.
10. Add125µL of 30% acetic acid in water to each well of the microtiter plate to solubilize the CV.
11. Incubate the microtiter plate at room temperature for 10-15 min.
12. Transfer 125µL of the solubilized CV to a new flat-bottomed microtiter dish.
13. Quantify color intensity of the micro wells visually.

Decontamination procedure
1. After each experiment the culture flask, buffers and the microplates will be treated with 1% virkon solution for 24 hours, and disposed c
2. The biofilm formed plates will be disposed through cytotoxic waste method.

Reference SOP
CBE SOP003 - Disposal of biological waste

Personal protection requirements not covered in the precaution statements above.

Wear protective clothing as described in Section 8 of this safety data sheet. Provide adequate ventilation. Keep unnecessary and unprotected personnel away from the spillage. Treat the spilled material according to the instructions in the clean-up section.

Environmental precautions
Avoid discharge into drains or watercourses or onto the ground.

COSHH Form (Continued)

Sources of information and references	Reference to **existing approved** Risk Assessment
SAFETY DATA SHEET Crystal Violet According to Regulation (EC) No 1907/2006, Annex II, as amended	SAF/MEME/6688 & Biological risk assessment (PK)

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: Sourav Ghosh — Digitally signed by Sourav Ghosh Date: 2020.12.10 11:14:37 Z

Form Reference Numbers

Risk Assessment	Method Statement	COSHH Assessment
		SAF/MEME/933

DSO Signature: Keven G Smith — Digitally signed by Keven G Smith Date: 2021.02.05 16:17:21 Z

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: 5 Feb 2022

Review comments