Document Number:	**SASoM/METHOD/109.v2**
Title:	**'COMET' Assay**
Version:	**v2**
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Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
v1	01/11/18	Original
V2	11/11/2020	Update

Controlled

1.0 Purpose –
This SOP describes the current procedure for performing a COMET assay in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM performing the COMET assay.

3.0 Responsibilities –
All staff involved in the performing a COMET assay are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –
This protocol is for the detection of DNA double-strand break although it is also possible to look at single strand break by doing an Alkaline comet assay. A suitable reference for the assay "The comet assay: a method to measure DNA damage in individual cells" can be found in Nature Protocols (Olive et al., 2006).

Materials:

1% agarose: 250 mg Low Melting Point Agar (LMPA) in 25 mL PBS (adjust volumes for your experiment.)

Neutral Lysing Solution: 2% sarkosyl, 0.5 M Na2EDTA, 0.5 mg/ mL proteinase K (pH 8.0). 1g Sarkosyl and 9.3g Na2EDTA in 40mL distilled water, adjust pH to 8.0 with 10M NaOH. Add 1.25mL of Proteinase K (20 mg/mL) to get 0.5mg/mL and make up to a final volume of 50 mL, keep at 4°C.

Electrophoresis Buffer: 90 mM Tris buffer, 90 mM boric acid, 2 mM Na2EDTA (pH 8.0). 32.707g Tris base, 16,694g boric acid and 2.233g Na2EDTA in 2.5L distilled water. Adjust pH to 8.0 and make up to 3 L with distilled water.

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Methods:

Preparation of sample slides (conducted under low light conditions):

Prepare 1% low melting point agarose (250 mg/ 25 mL PBS) in a microwave until fully dissolved and then keep in a water bath at 40°C. Dip slides into molten 1% agarose and wipe one side clean. Allow agarose to air-dry to a thin film – slides can be prepared ahead of time and stored with desiccant.

Sample Preparation:

Remember to include (i) an untreated control as well as (ii) a positive control treated with 10µM of H_2O_2 10min prior to trypsin. Trypsinize cells and keep them in ice-cold PBS - adjust the cell density to $2x10^5$ cells/mL in PBS. Samples can be kept at -80°C if experiment cannot be done immediately.

Label glass slides (using a pencil). Mix 100µL of cells with 900uL of 1% Low Melting Point Agar at 40°C. Mix and rapidly pipet 100µL of cell suspension onto the pre-coated slide – avoid air bubbles. Use a coverslip to flatten the sample and make it even. Once the gel has set, carefully remove the coverslip. Submerge slides in a dish

containing neutral lysis solution, put the dish at 37°C for 1h in the dark (it can be left for up to 20h, Olive et al., 2006).

Electrophoresis Conditions:

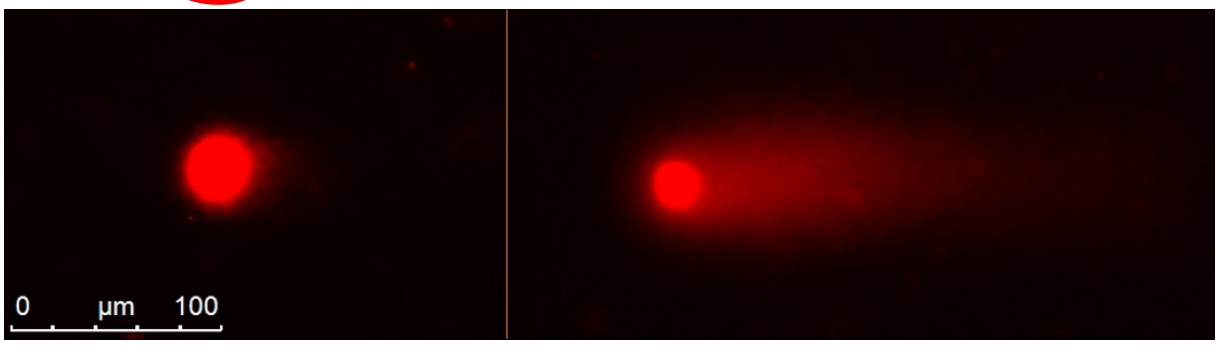
Pour off the lysing solution and submerge the slides in electrophoresis buffer for 30min at RT. Repeat 2 more times. Place the slides close together in an electrophoresis chamber and then carefully fill it with freshly prepared buffer (pH 8.0) until slides are covered. Run the electrophoresis for 25min at 20V in the cold room. Remove the slides, rinse and neutralize in 400mL distilled water in a coplin jar.

Slide Staining:

Pipet 100µL of a 10µg/mL stock solution of propidium iodide directly onto the slide and incubate for 20min – in the dark. Rinse slides with 400mL distilled water to remove excess stain. Slides can be stored in a humidified, dark chamber in the cold room (at 4°C) up to 3 days. If longer, then dip them in 100% ethanol and dry them; they can be kept like this for several months but need to be rehydrated and stained before analysis.

Analysis:

Use an epifluorescence microscope to observe the slides. Images can be analysed with 'OpenComet' - a free 'Image J' plugin. The image below shows two typical comets which represent an untreated control (left) and a positive control H_2O_2 (right).



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5.0 Personal protection –
A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

6.0 Spillages –
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –
All staff should under go training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/011 and RA/BIOL/004

8.2 SOP - SASoM/METHOD/

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9.0 Approval and sign off –

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