Document Number:	**SASoM/METHOD/049.v5**
Title:	**Transwell Boyden Chamber Migration Assay**
Version:	**v5**
Author:	**Peter Mullen**

Effective from:	09/09/2021
Valid to:	09/09/2023

SOP History		
Number	Date	Reason for Change
v1	29/08/2013	Original
v2	10/08/2015	Update
v3	10/08/2017	Update
V4	10/08/2019	Update
V5	09/09/2021	Update

1.0 Purpose –
This SOP describes the current procedure for Transwell Boyden Chamber Migration Assay for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in Transwell Boyden Chamber Migration Assay work.

3.0 Responsibilities –
All staff involved in cell culture 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 –

For each experimental condition, set up three Boyden chambers, i.e. a triplicate. Cells migrate to a higher serum environment (chemotaxis).
Supplier of Boyden chambers is VWR, UK (cat no. 734-0038).

Day 1:

1. Add 0.5 mL aliquot of serum-free cell suspension (5e5 cells,1e6 cells per mL) to the top chamber of 24-well chambers with 8.0 µm pores (BD Biosciences) and add media supplemented with 20% serum to the lower chamber.
2. Incubate cells for 16-24h at 37^0C in a humidified atmosphere of 5% (v/v) CO_2.

Day 2:

3. Use a cotton swab to scrub the top of the insert membrane free of cells and perform three PBS washes, use a cotton swab to scrub each time. Stain cells on the lower surface of the Boyden chamber using 0.3% crystal violet (Sigma-Aldrich) in 80% PBS/ 20% ethanol solution previously filtered through a sterile 0.22 µm syringe driven filter (Elkay). Place staining solution in wells of 24-well chambers and add Boyden chambers to this. Stain for 2-24h at RT.

4. Count the number of cells on the lower surface of each chamber using a Zeiss Axiovert 40CFL microscope and take representative digital images of migration Boyden chambers as a visual record.

5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

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 100% ethanol or water.

7.0 Training -

All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessments –
CHARM RA22141 Preparation of cell culture media and additives
CHARM RA20158 In Vitro cell proliferation assays

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 09/09/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 09/09/2021

QA release by:

Name: John O' Connor

Position: QA Manager

Signature:  Date: 09/09/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE