Document Number:	**SASoM/METHOD/097.v3**
Title:	**Preparation of Chicken Red Blood Cells (CRBCs) for use as a ploidy calibration standard in DNA Flow Cytometry studies.**
Version:	**v3**
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Effective from:	11/11/2020
Valid to:	11/11/2022

SOP History		
Number	Date	Reason for Change
v1	18/08/2016	Original
V2	18/08/2018	Update
V3	11/11/2020	Update

1.0 Purpose –

This SOP describes the current procedure for preparing whole blood cells from freshly drawn chicken blood.

2.0 Scope –

The scope of this document is to describe how blood is taken from chickens and subsequently prepared for use as a calibration standard in flow cytometry.

3.0 Responsibilities –

All staff involved in looking in preparing chicken red blood cells 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 –

1. Blood can be taken from either male or female chickens but since blood from a female chicken contains slightly less DNA per cell, its use as a calibration standard is dependent upon the sex of the bird. Whilst this is not an issue, it is nevertheless critical to know whether the blood was taken from (i) a male or (ii) a female chicken.
2. Blood has in the past been successfully taken from the wing vein.
3. Up to three (3) pink-capped BD Vacutainer® K2E (EDTA) 10mL collection tubes (BD catalogue #367525) were used to collect blood from a live chicken and then inverted / mixed thoroughly to ensure that coagulation did not occur. Tubes were then transferred to the lab as quickly as possible. Tubes can be transported at room temperature and do not need to be kept on ice. In an ideal world we would like to collect 5-10mL of uncoagulated whole blood in total.
4. *Prepare 'Vindelov's Citrate Storage Buffer' by dissolving 85.5g Sucrose (S9378, Sigma) and 11.76g of Trisodium Citrate (301287F, BDH) in 800ml distilled water. Add 50ml DMSO (140214P, BDH) and adjust to pH7.6 (with 2-3 drops of concentrated HCl). Make up to 1000ml with distilled water and store at 4ºC.*
5. An aliquot of the blood (2mL) was removed from the EDTA tube and then added to Vindelov Citrate Storage Buffer (11mL) to give a total volume of 13mL.
6. Prepare a 1:100 dilution (10μL in 100mL) of this sample and then perform a cell count using a haemocytometer. On a previous occasion (29/07/2009) this cell count was found to be 5.52 x 10^6 cells / mL, suggesting that 2mL of chicken blood will contain between 50 - 75 x 10^6 cells.
7. Since Vindelov recommends aliquoting the CRBCs at a concentration of 1.45 x 10^6 cells / mL, cells were further diluted in Vindelov Citrate Storage Buffer as required. Aliquots (50 μL) were then made and frozen at -20º for long term storage.

5.0 Personal protection -

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

6.0 Spillages -

Always clean up and disinfect any spills immediately after use, only you know what you have spilt and are aware of its hazard.

7.0 Training -

All staff should undergo training in this technique before performing procedure.

8.0 Related documents –

SOPS - SASoM-METHOD-018-Flow Cytometric DNA Analysis.

9.0 Approval and sign off –

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