A Protocol for the Stabilisation and Flow Cytometric Analysis of Whole Blood Samples from Chicken and Duck

1. Collect the whole blood sample from either the brachial (wing) vein, the jugular vein or medial metatarsal vein of the subject as appropriate.

2. Insert a needle of appropriate gauge for the size of the subject into the vein of choice then blood can either be aspirated into a syringe or allowed to drip freely from the needle into an appropriate collection tube. The samples should be collected into EDTA as the anticoagulant of choice for use with TransFix.

3. Stabilise the samples by the addition of **1 part TransFix to 5 parts whole blood (e.g. 0.2ml TransFix to 1ml whole blood)[1].** Blood samples should be treated with TransFix immediately after collection, but failing this blood must be less than 4 hours old* when it is treated with TransFix. Do not refrigerate the sample before treatment with TransFix.

4. Mix the sample gently by inversion at least 10 times and store at 2-25°C for up to 72 hours* prior to analysis. **Do not vortex.**

5. If refrigerated, allow the stabilised blood sample to return to room temperature (18-25°C) before preparing it for cellular analysis.

6. Examine the sample using routine flow cytometry evaluation.

   N.B. The dilution factor must be accounted for when calculating absolute cell counts i.e. multiply the cell count by 1.2.

   N.B. Cross linking of proteins may occur when using TransFix. We therefore recommend that all antibody conjugates are validated in association with TransFix prior to routine use.

***Studies have demonstrated that this ratio of TransFix to whole blood gives comparable absolute counts versus unfixed samples analysed within 4 hours of collection up to 72 hours after collection. However, these finding have not been validated at Cytomark so we recommend titrating the volume of TransFix to whole blood to find the optimal ratio for your application.**

[1]C. Seliger et al., Veterinary Immunology and Immunopathology *145 (2012) 86– 99* & Personal correspondence with Dr S Härtle.

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