Preparing Blood Controls for Immunophenotyping – Towards Meeting the ISO15189:2012 Standard in the Clinical Flow Laboratory

The ISO standard entitled 'ISO 15189: Medical laboratories - Particular requirements for quality and competence', refers to the '*use of quality control materials that react to the examining system in a manner as close as possible to patient samples*' (section 5.6.2.2), and that '*the use of independent third party control materials should be considered, either instead of, or in addition to, any control materials supplied by the reagent or instrument manufacturer* '(section 5.6.2.2, Note 2).

It is common practice in immunophenotyping and immune monitoring laboratories to use a patient sample as a daily or even weekly control for flow cytometry. In addition, when a rare immunophenotype is detected there is an opportunity to preserve it for teaching or comparison purposes.

All samples left untreated are subject to apoptosis of cells and proteolytic degradation leading to immediate changes in the phenotypic profile. This poses a challenge, not only with analysing the sample in a short time scale but when preserving samples of interest for re-testing.

Simple addition of TransFix to a blood sample enables stabilisation of the immunophenotype for up to 14 days. Subject to validation at a clinical flow facility, this could provide a solution when such reference material is not available and would address the clauses referenced above.

Therefore, using TransFix, it is possible to stabilise blood samples (diseased and healthy) for use as a reference tool during flow cytometry analysis.

Protocol – Preparing a Blood Control

1. Collect blood by venepuncture into an EDTA vacuum tube according to CLSI document H3-A62.

2. Carefully remove the blood collection tube cap and determine the volume of anti-coagulated whole blood within the vacuum tube.

3. Pipette into the blood collection tube the appropriate volume of TransFix at the ratio of 0.2ml TransFix per 1ml of blood. For TransFix Sample Storage Tubes, add 1ml blood to the tube. Note: Blood samples should be treated with TransFix immediately after collection, but failing this, blood must be less than 6 hours old when it is treated with TransFix. Do not refrigerate the sample before treatment with TransFix.

4. Replace the cap on the blood collection tube, ensuring that there is no leakage and mix gently by inversion at least 10 times. Inadequate or delayed mixing may result in inaccurate test results. Do not vortex.

5. Once TransFix treated, the stabilised sample can be transferred to smaller test tubes to prevent repetitive cooling and warming thereby preserving the phenotypic profile of the sample.

6. Store/transport the TransFix treated blood for up to 14 days at 2 - 8°C or for up to 4 days at 18 - 25°C.

7. If refrigerated, incubate the treated blood sample at room temperature (18 - 25°C) for 15 minutes prior to use. Then mix the treated blood by rolling the tube between the hands 10 times and by

inverting. Take care when opening blood collection tubes and briefly spin TransFix Sample Storage Tubes to reduce the build-up of blood in the caps.

8. Perform analysis by flow cytometry in accordance with the manufacturer's instructions. A 'stain, lyse no wash' sample preparation method is recommended. Blood stabilised by TransFix should be analysed within 6 hours before being returned to 2 - 8°C storage for future use, if necessary.

Note a; Light scatter positions of cells stabilised by TransFix may differ slightly from those of untreated cells.

Note b; The dilution factor must be accounted for when calculating absolute cell counts. This can be done by multiplying the value given by the manufacturer to the absolute counting beads by 1.2 so that absolute cell counts are automatically corrected for TransFix treated blood samples.

Note c; Studies have shown that moderately high levels of haemolysis, icterus and lipemia do not affect the results. Grossly haemolysed samples should be rejected.

Products

TransFix Sample Storage Tubes
TransFix