Flow Cytometry - method

A brief explanation of What we do and Why: Flow Cytometry has long been established as the methodology of choice for the typing of haematological malignancies. Our expert team of Biomedical Scientists headed by Mr Peter Richardson (Section Head) can provide this service using blood, bone marrow, serous effusions, Cerebro-spinal fluid or biopsy material.

Open all sections

Sample Processing

Blood, marrow and heavily-bloodstained fluids are subjected to an Ammonium chloride lysis protocol. This is a low ionic strength buffer, which compromises red cell integrity, causing lysis, whilst leaving the Leukocytic fraction relatively unscathed. An unintended issue of this protocol is that, as well as destroying mature erythrocytes, it will also remove some of the later elements of erythroid differentiation, leaving an incomplete record. Hence, in cases of myelodysplasia (MDS), this may lead to slightly higher blast cells counts (i.e.: CD34+CD117+ cells) than would be discernable with morphological appraisal of a fresh, particulate smear. In any case, BSH and WHO guidelines still recommend the latter as the 'gold-standard' in such cases. In cases other than MDS, we have no evidence of any substantial disturbance to the population distribution in a sample. It is worth pointing out, however, that we get the best results with a fresh sample (one or two days old)- older samples, those in excess of 3 days, may show changes in population distribution that may prejudice the interpretation of cytometry results. Our experience is that bone marrow samples tend to store better than blood samples, possibly because of the relatively nourishing mix of active compounds to be found in the former. CSF samples can pose a problem for the Cytometrist. Due to the low volumes drawn and the relative paucity of cells, it is, unfortunately, the norm to be unable to collect sufficient events to be able to obtain statistically satisfactory results (NB: in the world of Cytometry, we acquire 'events' not cells!). In an ideal world we would prefer a sample volume of at least 5ml, although we are aware that this can be an issue for the Clinician and the Patient. Our experience with Fine Needle Aspirate (FNA) biopsy material has not been encouraging. In the main, we find they do not usually provide the numbers of cells that we would prefer to work with. As with CSF, Flow Cytometry is an unforgiving technology and low cell numbers are prejudicial to satisfactory analysis. In the event of biopsy material arriving, we do prefer larger fragments in a suitable medium (this can be saline, PBS or a tissue culture fluid, but, please, no azide, no aldehydics, and no preservative additives at all).

Staining

When we have obtained a satisfactory preparation of cells, we have to choose which panel of antibodies is appropriate. This decision is based on the information supplied to us on the request form by the Clinician. It is an unfortunate truth that the less information we receive, the more difficult that decision is. However, we do our best!We currently operate a Becton-Dickinson FACS Canto 2 digital Flow Cytometer, with three lasers- 'red', 'blue', and 'violet'. This combination of light sources enables us to stain cells with 7 different antibodies, each conjugated to a different fluorescent dye ('fluorochrome'). However, it is possible, with careful selection, to increase that number to at least 10 antibodies. In daily practice 7 is our current limit. With the addition of each new fluorochrome we can obtain more information on each individual cell; however everything in life comes with a price tag and with each new fluorochrome the technical issues and difficulties increase. To explain this phenomenon is not easy, but, briefly, the closer two target antigens are to each other on the cell surface, and the more structurally-similar are two fluorochromes to each other, there is an increased risk of 'interference' in the dynamics of the fluorescence process.Having decided on our panel of suitable antibodies, we aliquot a small volume (50ul) of the sample into tubes, and add a tiny volume (typically 2ul) of an appropriate antibody-fluorochrome preparation. These we obtain from commercial sources. In the past, as we (the CIS) are part of a larger academic department in a University, we had access to high-quality monoclonal and polyclonal antisera produced by various research groups. The problem with using this grade of product in a diagnostic laboratory is that, in order to use these products as diagnostic reagents, it is necessary to have detailed documentation for compliance reasons. Hence, it is preferable to use commercial products, as the 'paperwork' is their responsibility.Having mixed cells and antisera, these are incubated, fixed and washed, ready for Cytometry. The stained material is read by the Cytometer, and the results are displayed using appropriate software, in our case 'FacsDiva' by Becton-Dickinson. The results are analysed and printed. In addition, we also make digitalised records which are stored on the University of Birmingham Server.

Urgent Requests

We do accept that some samples have a greater Clinical urgency than others. We are happy to provide a 'same-day' service for patients presenting with Acute Leukaemias and High Grade lymphomas. A small aliquot of bone marrow or blood (as appropriate) is stained with a small panel of antibodies to determine lineage and differentiation stage. Our aim is to have a rapid screen that will give information sufficient for the Clinician to advise the patient and/or initiate treatment on the same day as sample biopsy. The results of this assay are usually telephoned directly to the requesting Clinician (or proxy), so we would request that a telephone number be provided (preferably a mobile phone) in order to expedite the process in as efficient a manner as possible. We would also be very grateful if a telephone call warning us of the arrival of such a specimen could be made.Sadly, we do not operate an out-of hours service, and Urgent samples arriving near to our closing time may be processed early the following morning. This will be at the discretion of the Section Head or Deputy. The effective operating hours of the Cytometry service are 07:00 to 17:00, Monday to Friday inclusive.

Reporting

The final written report is issued, authorised, and released for printing and posting. We also offer a secure e-mail service to requesting Clinicians and other interested parties. This may be arranged if application is made to the Laboratory Manager, Mr Tim Plant|. Our aim is to provide a three-day turn-around time. If a sample is received on Day 1 (i.e. working days), we will process on Day 2 and have the results on the Pathologist's desk by the middle of the afternoon of that day. By Day 3, the completed report will be issued, printed, posted and e-mailed.

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