Automated PBMC isolation for biobanking

Since its inception in 2008, IBBL has been a biobanking pioneer, developing a high quality repository of samples to support biomedical research into a variety of diseases. Using a Freedom EVO® workstation, the center has developed an automated workflow for the processing and cryopreservation of a wide range of biological materials, and has recently added viable peripheral blood mononuclear cells to its standard whole blood extraction portfolio.

IBBL (Integrated BioBank of Luxembourg) is at the forefront of disease-oriented biobanking, using cutting-edge automation technologies to build up a large collection of disease-specific samples for research into new approaches for the diagnosis and treatment of a wide range of conditions. Working closely with the neighboring Centre Hospitalier de Luxembourg, IBBL catalogs and cryogenically preserves biological materials – including tumor samples, serum, blood, plasma, urine and feces – from individuals with specific diseases, for national and international projects.

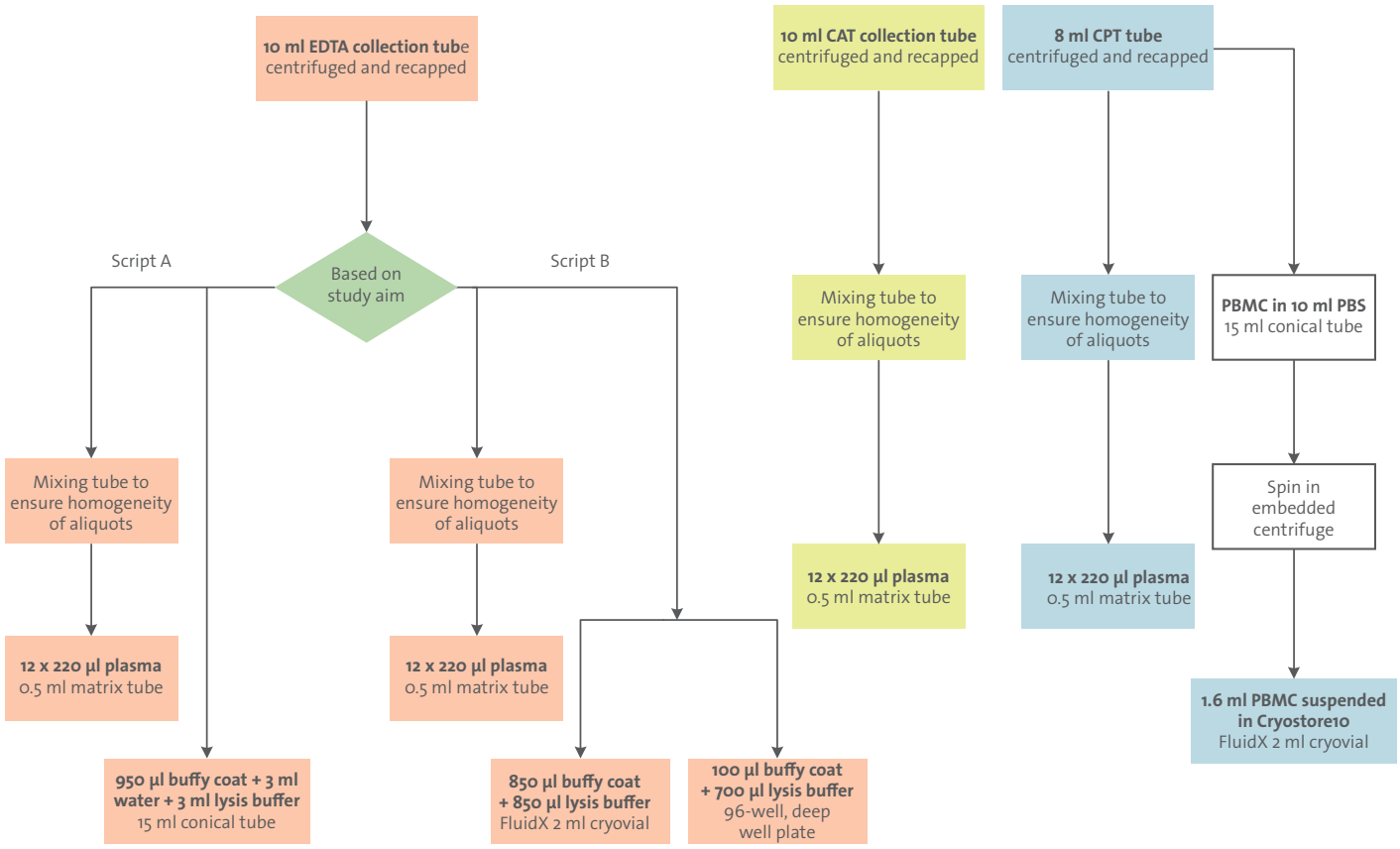
IBBL has a strong history of working in partnership with Tecan, and has already developed a number of innovative automated sample preparation protocols using a Freedom EVO 200 platform (Tecan Journal, Issue 3, 2012). The center's latest project – to automate extraction of viable peripheral blood mononuclear cells (PBMCs) from whole blood – further demonstrates the advantages of IBBL's automated approach. Dr Fay Betsou, Chief of Biospecimen Science, explained: "PBMCs – primarily lymphocytes and monocytes – are of increasing interest for vaccine development and validation, and we have seen a steady increase in requests from our collaborators within the pharmaceutical industry for PBMC-based functional assays. These blood cells are traditionally extracted manually using a Ficoll-Paque method. This gradient-based technique is both laborious and time consuming, taking a technician approximately four hours to isolate PBMCs from 10 samples."

Left to right: Gaël Hamot, Conny Mathay and Fay Betsou with the Freedom EVO workstation

IBBL has developed a straightforward automated protocol for PBMC extraction based on the use of standardized citrate anticoagulant tubes (BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate). By ensuring the same fill volume in each tube, the team has been able to identify the optimum pipetting height following centrifugation, allowing efficient isolation of PBMCs while minimizing the number of granulocytes present in the final preparation. Fay continued: "Unlike the buffy coat extraction process, which uses the Freedom EVO's Tube Inspection Unit to identify the optimal pipetting height, this protocol is based on the mean hematocrit of the general population. Despite this, the recovery rates are comparable to the manual process – 61 % recovery with the automated process compared to 69 % when performed manually. This is very satisfactory for our needs and, more importantly, cell viability is virtually unaffected – 74 % compared to 79 % for manual processing."

Centrifuged, decapped CPT tubes are placed on the Freedom EVO workstation, and the plasma-suspended PBMCs are transferred into intermediate tubes, which are respun using

Schematic input/output of the blood workflow



IBBL's blood sample processing workflow

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the platform's integrated centrifuge. The supernatant is subsequently discarded and the PBMC pellet is resuspended in cryopreservation medium in cryotubes. The processed samples are placed into cryostorage, first in a progressive rate freezer to avoid damaging the cells, and then into liquid nitrogen for long-term storage. "Best practice for cryopreservation is to normalize the cell concentration in each vial, however this is not possible using our standard workflow as cryopreservation medium is toxic at room temperature, and so samples need to be frozen quickly to maintain cell viability. This is not generally an issue as the automated process is very reliable and reproducible, however the Freedom EVO gives us the flexibility to resuspend cells in wash buffer instead of cryopreservation medium if precise cell concentrations are required for specific projects."

The PBMC protocol has now been fully validated[1] and implemented as part of IBBL's standard sample collection workflow, allowing it to be run alongside a number of other scripts, such as the extraction of the buffy coat from blood. "Having implemented this protocol as part of a composite script, we can now automatically process different specimen types in parallel. Although high throughput is not our primary focus, the Freedom EVO offers walkaway processing of 24 patient samples in under three hours, allowing the technician to perform other activities while it is running," Fay concluded.

1 Hamot *et al*. Method Validation for Automated Isolation of Viable Peripheral Blood Mononuclear Cells. *Biopres Biobank*, 2015, in press.

To find out more about Tecan's biobanking solutions, visit **www.tecan.com/biobanking**

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The PBMCs are extracted from centrifuged whole blood