Policy for the Laboratory Performance Quality Assurance Program of IVRN Tier One Laboratories

Overall aim: To provide certification to laboratories participating in cryopreservation of serum, plasma and peripheral blood mononuclear cells (PBMC), and to facilitate quality assurance for involvement of IVRN Tier One laboratories in handling of specimens from clinical trials or cohort studies.

Structure: Under direction of the IVRN Steering Committee, a Quality Assurance Program (QAP) for separation and cryopreservation of PBMC is conducted twice-yearly. The QAP is coordinated by Dr Wayne Dyer at UNSW in Sydney, and involves: shipment of QA specimens to the participating laboratories, as well as local collection of healthy donor comparison samples; shipment of cryopreserved PBMC specimens to UNSW for assessment of viability, recovery and functional activity; preparation of de-identified performance reports; and follow-up with Tier One laboratory staff when performance is below standard. In addition, the QAP Coordinator may visit laboratories to provide remedial training when discussions fail to resolve a technical issue identified during a QA round. Blood collection from HIV+ and healthy donors for the QAP is coordinated by the IVRN. The regularly updated Laboratory Manual for the PBMC processing protocol is available to all laboratories (www.ach4.org.au/quality-assurance-program).

Scope: Reliable separation and cryopreservation of PBMC requires both specialised laboratory equipment and significant technical skills. PBMC cryopreserved from subjects participating in clinical trials and cohort studies must be of the highest quality to ensure the feasibility of subsequent studies of immune function and virological parameters. These premises underpin the activities of the IVRN Tier One laboratory network. Accordingly, participation in the IVRN QAP and ongoing accreditation based upon agreed performance standards is essential.

Overview of the assessment process: A standard blood donation (~600ml) is drawn from one HIV+ and one healthy donor, into CPDA1 anticoagulant blood collection packs, and 2 x 15ml aliquots from each donor are shipped at ambient temperature by overnight courier to each of the participating Tier One laboratories. The ambient temperature is monitored and recorded during shipment by a Tiny Tag™ device. The blood samples are protected from extreme ambient temperature fluctuations during air freight by packing between gel packs pre-warmed to approximately 25°C. The blood is then processed simultaneously the following morning by each of the Tier One laboratories, according to the IVRN protocol, along with a freshly collected blood sample from a locally sourced HIV- donor. Total available PBMC in the whole blood samples is determined using a Coulter Act Diff cell differential counter, as the sum of lymphocytes and monocytes. Frozen PBMC from all three donors are shipped back from each Tier One laboratory to the IVRN testing laboratory in Sydney. All specimens are thawed and tested on the same day by the same scientist. Viability is determined by manual counting of Trypan Blue-stained cells, and absolute counts for yield determined on the Coulter Act Diff cell differential counter. PBMC function is determined by interferon (IFN)-γ ELISPOT, in response to a pool of 23 HLA class I-restricted T cell epitopes from human cytomegalovirus, Epstein-Barr virus and influenza virus (CEF) to assess CD8+ T cell function, and PMA/ionomycin to determine total IFN-γ release.

Assessment performance standards: Fractionation and cryopreservation of PBMC from one of the single donor blood specimens is deemed satisfactory if:

- Fractionation yield of a minimum 30% of the total PBMC, as determined by automated counts of the whole blood specimen;
- Post thaw PBMC viability is >80%;

- Post thaw recovery of viable PBMC is between 75% and 125% of the stated vial contents;
- ELISPOT response to CEF antigen and background, mean – SD and mean + SD, respectively (applicable to the IVRN supplied specimens only), and total response to PMA + ionomycin >5000 spots/10^6 PBMC (all PBMC specimens).

Performance required for ongoing certification as a Tier 1 Laboratory: The performance standards (above) must be attained from at least one PBMC specimen (IVRN donor or local donor), from at least 2 out of the past 3 QAP rounds. Non-participation in a QAP round is designated as a failed result. A certificate of satisfactory performance will be issued to each successful laboratory after each QAP round.

All results for performance within the QA are fully confidential and are not discussed with other participating laboratories or sponsors that may fund IVRN for specimen collection. However, only certified laboratories will be recommended to participate in clinical trial sample collection. Only the QAP project director and IVRN Steering Committee will be aware of individual laboratory results. If there is a change in performance status, it is the responsibility of the participating laboratory to communicate their performance level, certified or otherwise, with any relevant sponsor.

Remedial action if a laboratory fails to maintain certification:

- Upon losing fully "Certified" status, a laboratory will be issued with an "Certified - Under Review" report, which recommends that the laboratory continue participation in current clinical trials and cohort studies, but involvement in new studies be deferred. Laboratory staff will be contacted by the QAP coordinator with the aim of identifying potential causes for the below standard performance, and interventions put in place to achieve the quality standard.
- After two consecutive failed attempts at satisfactory performance, the laboratory will be classified as "Unsatisfactory". In due regard for confidentiality of the status of each laboratory, it is the responsibility of the laboratory that is downgraded to "Unsatisfactory" status to notify the relevant clinical trial sponsor of this change of status. The IVRN will not distribute any details of laboratory performance to a third party. The consequence of this change in status is for negotiation between the laboratory and the clinical trial coordinator/sponsor.
- The IVRN Steering Committee will negotiate a remedial plan with the head of a laboratory that becomes "Unsatisfactory" to assist in improving performance. If the response is deemed acceptable, "Certified Under Review" status will be reinstated upon attainment of a satisfactory result in the subsequent QAP round. If the negotiation is unsuccessful, termination of Tier One laboratory status will be recommended to the IVRN Steering Committee.

Contact details for enquiries on the QAP process:

QAP Coordinator
Dr Wayne Dyer
P: 0408 039 329
E: ivnrqap@sharkdentist.com.au

IVRN Steering Committee
Professor Andrew Lloyd
P: 02 9385 2534
E: a.lloyd@unsw.edu.au