

Investigation of Patients with Suspected HLH – RCH Immunology Laboratory **Instructions for Other External Laboratories**

The tests below must be BOOKED AHEAD with the RCH Immunology Laboratory. To arrange testing, ring the laboratory (03-93455725), or page or email the Immunopathology Consultant (03-93455522 pager 6744 or sharon.choo@rch.org.au).

Recommended immunology tests

1. Perforin expression
2. NK cell degranulation (CD107a expression)
3. SAP and XIAP expression
4. Activated T cells
5. Soluble CD25 (IL-2 receptor alpha)
6. [NK cell cytotoxicity – second line test - performed if perforin or NK degranulation abnormal]

Blood samples required for testing

- o Lithium heparin (no gel) – 7 ml (if lymphocyte count $>1.0 \times 10^9/L$)
- o EDTA – 0.5 ml
- o If requesting cellular tests – 0.5ml serum/gel for soluble CD25 can be sent with the above blood samples

If requesting sCD25 ONLY

Collect 0.5ml serum/gel, separate and freeze at $-20^{\circ}C$ **within 24 hours of collection** and send frozen to RCH Immunology Lab

Healthy Control Information

In addition, the following blood samples should be obtained from a **healthy adult control**, collected at a similar time as the patient:

- o Lithium heparin (no gel) – 7 ml
- o EDTA – 0.5ml

The **healthy control** should meet the following requirements:

- o Age ≥ 18 years
- o Healthy on the day (no intercurrent illness)
- o Not taking any systemic immunosuppressant medication
- o Not pregnant
- o Not a relative of the patient

The RCH Immunology Laboratory recommends that the **healthy control** reads the attached 'Control Information Statement' and signs the 'Control Consent' form prior to his/her blood being collected. The signed consent form, the attached 'Clinical Details' form and a pathology request form should accompany the samples to RCH. The Control's blood tubes should be labelled 'Travel Control' as the name and the DOB of the Control as the DOB.

Sample Transport

It is essential that the blood samples arrive at RCH as soon as possible because cell function tests (heparin/EDTA samples) must be assayed within 24h of collection. To avoid unnecessary delays, refer to the instructions below:

- o Collect the samples late morning or early afternoon. We do not recommend collecting the blood in theatre as this usually results in specimen handling errors and/or delays.
- o Send the samples by **overnight courier** - to arrive at RCH Laboratory Services **by 0900** the next day. Specifically request that RCH is the FIRST drop off in the morning (RCH is close to the airport).
- o Do NOT centrifuge the patient/control samples (our lab will centrifuge/separate the serum for sCD25 testing).
- o Keep the patient and control samples at **room temperature**.
- o Label the package 'Biological Sample - URGENT'.

Address for courier delivery

Attention: Dr Sharon Choo
Laboratory Services (Immunology Lab)
4th Floor East Building **via green lifts**
Royal Children's Hospital 50 Flemington Road
PARKVILLE VIC 3052 Ph: (03) 9345 5725

After the package has been collected by the courier, please contact the RCH Immunology Lab (03-93455725) with the following information: (a) name of the courier company and (b) consignment number of the package.