We’re Back!

After a brief hiatus, the DIL Newsletter is back! We look forward to describing interesting cases and our new assays. A lot has been going on and we are excited to update everyone.

We would like to hear from our Clients. We invite you to share your questions and comments with us. This can be regarding existing assays, new assays that you might be interested in, the way we report results, other services that we can provide, etc. Feel free to send/fax/email your comments to us: Fax 513-636-3861; Email: immunodeficiencies@cchmc.org

Severe combined immunodeficiency (SCID) includes a group of inherited immunodeficiencies characterized by a profound reduction or absence of T cells with or without a reduction or absence of NK and/or B cells. SCID is a syndrome caused by mutations in genes responsible for the development and function of lymphocytes [1]. In some cases, the molecular defect prevents only T cell function, while B cell development is less impaired. However, as B cells require T cell signaling to function appropriately, a severe T cell abnormality precludes effective humoral immunity.
The following chart shows the possible diagnostic assays and aberrations in cell counts in the several SCID categories. The screening tests performed in the Cincinnati Children's Diagnostic Immunology Lab are also related to the genetic abnormality causing each SCID category.

The patient suspected of having SCID requires clinical evaluation and studies including the measurement of immunoglobulin levels, absolute numbers and percentages of lymphocyte subsets (T, B, and NK), and assessment of T cell function (the Mitogen Stimulation Assay) to determine their response to mitogens, such as phytohemagglutinin (PHA) and concanavalin A (ConA).

SCID patients typically have lymphopenia and reduced mitogen proliferative responses. The incidence of SCID is estimated to be 1:50,000 to 1:500,000 live births. Approximately half of SCID cases are X-linked. The incidence of autosomal recessive SCID will be higher where consanguineous marriage is common [2].

### T-B+NK+ SCID
- **IL-7a deficiency, alpha chain (CD127)**
  - DIL Test: CD127/132, Lymph Subset, Mitogens
  - Gene: IL7RA
- **ZAP-70**
  - DIL Test: Mitogens, ZAP-70
  - Gene: ZAP70
- **CD3 chain components**
  - **CD3 delta**
    - DIL Test: Mitogens
    - Gene: CD3D
  - **CD3 epsilon**
    - DIL Test: Mitogens
    - Gene: CD3E
  - **CD3 zeta**
    - DIL Test: Mitogens
    - Gene: CD3Z
- **CD45**
  - DIL Test: CD45 RARO, Lymph Subset, Mitogens
  - Gene: PTPRC

### T-B+NK- SCID
- **X-linked SCID, gamma chain (CD132)**
  - DIL Test: CD127/132, Mitogens, pSTAT5
  - Gene: IL2RG
- **Jak-3 (Janus kinase 3 deficiency)**
  - DIL Test: Lymph Subset, Mitogens, pSTAT5
  - Gene: JAK3

### T-B-NK+ SCID
- **RAG deficiency (may cause Ommen Syndrome)**
  - DIL Test: CD45 RARO, Lymph Subset, Mitogens
  - Gene: RAG1, RAG2
- **ARTEMIS (radiation-sensitive)**
  - DIL Test: CD45 RARO, Lymph Subset, Mitogens
  - Gene: DCLRE1C

### T-B-NK- SCID
- **Adenosine deaminase (purine metabolism deficiency)**
  - DIL Test: CBC, Lymph subset, Mitogens
  - Gene: ADA
- **Nucleoside phosphorylase deficiency**
  - DIL Test: CBC, Lymph subset, Mitogens
  - Gene: PNP

### MHC Class deficiencies
- **TAP deficiency (MHC Class I)**
  - DIL Test: MHC Class I by flow, Mitogens
  - Gene: TAP 1, TAP 2
- **MHC Class II deficiency**
  - DIL Test: MHC Class II by flow, Mitogens
  - Gene: CIITA, RFXANK, RFXS, RFXAP

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**Additional Reading and References:**
Patient Vignette

A 5-month old male presented with chronic diarrhea, poor oral intake and dehydration. Blood and stool were positive for salmonella and the stool also was positive for rotavirus. A bronchoalveolar lavage revealed PCP pneumonia. These infections were treated and immunologic evaluation was performed, which indicated essentially no T-cells present in the peripheral blood and near normal absolute number of B-cells but hypogammaglobulinemia in the serum and decreased natural killer cell function. An initial diagnosis of Severe Combined Immunodeficiency (SCID) was made after noting the marked T-cell deficiency with hypogammaglobulinemia.

HIV, Diphtheria, and Tetanus antibodies were negative but not suggestive of non-exposure given the working diagnosis of SCID. Abnormal staining was observed in the surface CD132 as seen in the figures below. The blue line represents the patient’s CD132 staining pre-transplant (on the left) and post-transplant (on the right). The red line represents a healthy, adult peripheral blood sample run concurrently with the patient’s. Observe the decreased CD132 in the pre-BMT sample.

After the initial immune work-up, a IL2RG sequencing was ordered from the CCHMC Molecular Genetics Laboratory for confirmatory testing. This assay is PCR-based sequencing of the entire coding region of the interleukin 2 receptor gamma chain (IL2RG) gene and its intron/exon boundaries. It detects about 99% of mutations in the IL2RG gene in males. In this case, the IL2RG Gene Mutation Analysis showed: Allele 1: 373_374 ins A (E121fsX167).

The patient was placed on the BMT unit during his pre-BMT evaluation until a suitable donor was found. His donor was a male with an 8/8 match. Six-month post-BMT immune studies showed essentially normal Mitogen Proliferation studies and most prophylactic antibiotics were discontinued. There was mixed donor chimerism with 92% donor T cell engraftment after BMT.

### Reference Ranges

<table>
<thead>
<tr>
<th></th>
<th>pre-BMT</th>
<th>1yr. post-BMT</th>
<th>Reference Ranges</th>
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<tbody>
<tr>
<td>CD3 %</td>
<td>4</td>
<td>75</td>
<td>39-73</td>
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<tr>
<td>CD3 ABS</td>
<td>40</td>
<td>2839</td>
<td>1400-8000</td>
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<tr>
<td>CD4 %</td>
<td>3</td>
<td>40</td>
<td>25-50</td>
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<tr>
<td>CD4 ABS</td>
<td>31</td>
<td>1532</td>
<td>900-5500</td>
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<td>CD8 %</td>
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<td>21</td>
<td>11-32</td>
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<tr>
<td>CD8 ABS</td>
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<td>CD19 %</td>
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<tr>
<td>CD16/56 ABS</td>
<td>12</td>
<td>379</td>
<td>100-1400</td>
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**DIAGNOSIS** – The diagnosis of SCID should be suspected in children with any of the following:

- Unexplained lymphopenia
- Recurrent fevers
- Failure to thrive
- Chronic diarrhea
- Recurrence of severe episodes of thrush, mouth ulcers, RSV, HSV, VZV, measles, influenza, parainfluenza 3, or other serious infections
- Adverse reactions (infections) caused by live vaccines, such as BCG, rotavirus vaccine, or varicella vaccine
- A family history of SCID
The Diagnostic Immunology Laboratories, consisting of the Clinical Immunology Laboratory and the Research Immunology Laboratory, are committed to providing the highest quality, comprehensive clinical testing available to aid in the detection, diagnosis and treatment of pediatric immunologic, as well as oncologic and hematologic, disorders. We’re committed to applying scientific advances to promote efficiency, enhance patient care and improve clinical utility.

The clinical diagnostic laboratories are in compliance with all major regulatory agencies including CLIA (Clinical Laboratory Improvement Amendments), CAP (College of American Pathologists), HCFA (Health Care Financing Administration), HIPAA (Health Insurance Portability and Accountability Act) and JCAHO (Joint Commission on Accreditation of Healthcare Organizations).

The current menu of immunologic assays and information regarding shipping instructions is published on the last page of this Newsletter. The accompanying Test Requisition Form can be obtained through our website. Previous editions of the Newsletter can also be found at this website: www.cchmc.org/DIL

New Tests Now Available:
- Apoptosis, Fas-mediated
- CD45 RARO – revised panel
- CD52 Surface Expression
- MHC Class I & II and ZAP-70
- Neopterin, peripheral blood and CSF
- pSTAT5
- WASP Transplant Monitor Panel

New Tests Down the Pipeline:
- Campath – Plasma Levels
- Extended Mitogen Panel (PHA, PMA Calcium Ionophore at three concentrations, IL-2, CD3/CD28
- Restimulation Induced Cell Death (RICD) Assay (complements the Apoptosis assay)

New Employees (Hired 2010-2011):
- Vijaya Chaturvedi
- Lori Davis
- Lisa Durbin
- David Ingala
- Jan Martin
- Kannan Meganathan
- Lisa Neumeier
- Erika Owsley
- Sabina Sylvest

Please visit our website or call us with any inquiries:
Ph: 513-636-4685
Fax: 513-636-3861
www.cchmc.org/DIL

Cincinnati Children’s Hospital – Bird’s Eye View
Current Menu of Available Tests:

### Diagnostic Immunology Laboratory

**Ph:** 513-606-4666  
**Fax:** 513-606-3981  
**www.chmc.org/DIL**

Test Requisition Form - 101011

**Please call with the courier and tracking number of the package.**

### PATIENT & SAMPLE INFORMATION

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<tr>
<th>Patient Name</th>
<th>Patient Identification Number</th>
<th>Date of Sample</th>
<th>Time of Sample</th>
<th>Has the patient undergone SMT?</th>
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<td>No □ Yes – date of SMT</td>
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**Diagnosis or reason for testing**

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<th>Race: □ African-American □ American Indian □ Asian □ Caucasian □ Hispanic □ Other (specify)</th>
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<tr>
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**Notes:**
1. Volume requested assume a normal absolute lymphocyte count (ALC). If the ALC is abnormal, please call the lab for adjusted volume requirements when ordering any of the following tests: Antigen Stimulation, Mitogen Stimulation, CTL Function, NK Function, or CD107a Mobilization.
2. It is strongly recommended that a Lymphocyte Subset also be ordered when ordering a B Cell Panel, CD52, or NK Function.
3. Results of a same day CBC/Diff must accompany the sample where indicated.
4. Neopterin CSF samples should be shipped at 2-8°C. Neopterin EDTA samples should be shipped at room temperature.

### CURRENT IN THE UPTICKING ISSUE:

- Updated RARO Panel
- A look at the new WASP Transplant Monitor Panel