From the Diagnostic Immunology Laboratories

IN THIS ISSUE:

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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

Good to see you!

THIS ISSUE'S FOCUS - SCID

Severe combined immunodeficiency (SCID) includes a group of inherited immunodeficienes 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.

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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].

Additional Reading and References:

1. Geha RS, Notarangelo LD, Casanova JL, et al. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol 2007; 120:776.
2. Suliaman, F, Al-Ghonaium, A, Harfi, H. High incidence of severe combined immune deficiency in the Eastern Province of Saudi Arabia. Pediatr Asthma Allergy Immunol 2006; 19:14.

T-B+NK+ SCID	**DIL Test**	**Gene**
IL-7a deficiency, alpha chain (CD127)	CD127/132, Lymph Subset, Mitogens	IL7RA
ZAP-70	Mitogens, ZAP-70	ZAP70
CD3 chain components		
CD3 delta	Mitogens	CD3D
CD3 epsilon	Mitogens	CD3E
CD3 zeta	Mitogens	CD3Z
CD45	CD45 RARO, Lymph Subset, Mitogens	PTPRC
T-B+NK- SCID		
X-linked SCID, gamma chain (CD132)	CD127/132, Mitogens, pSTAT5	**IL2RG**
Jak-3 (Janus kinase 3 deficiency)	Lymph Subset, Mitogens, pSTAT5	JAK3
T-B-NK+ SCID		
RAG deficiency (may cause Omenn Syndrome)	CD45 RARO, Lymph Subset, Mitogens	RAG1, RAG2
ARTEMIS (radiation-sensitive)	CD45 RARO, Lymph Subset, Mitogens	DCLRE1C
T-B-NK- SCID		
Adenosine deaminase (purine metabolism deficiency)	CBC, Lymph subset, Mitogens	ADA
Nucleoside phosphorylase deficiency	CBC, Lymph subset, Mitogens	PNP
MHC Class deficiencies		
TAP deficiency (MHC Class I)	MHC Class I by flow, Mitogens	TAP 1, TAP 2
MHC Class II deficiency	MHC Class II by flow, Mitogens	CIITA, RFXANK, RFX5, RFXAP

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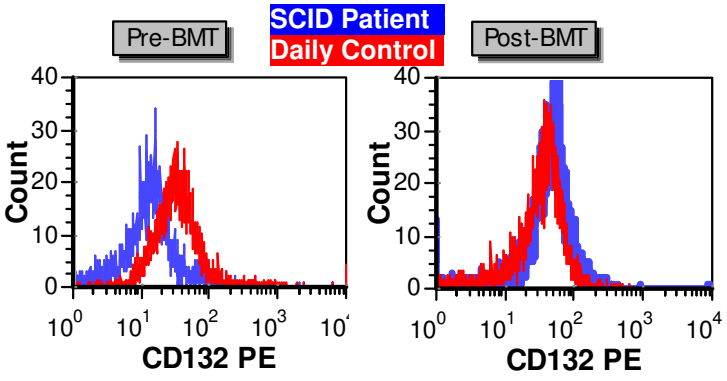
Patient Vignette

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.

	pre-BMT	1yr. post-BMT	Reference Ranges
CD3 %	4	75	39-73
CD3 ABS	40	2839	1400-8000
CD4 %	3	40	25-50
CD4 ABS	31	1532	900-5500
CD8 %	2	21	11-32
CD8 ABS	15	787	400-2300
CD19 %	92	15	17-41
CD19 ABS	904	569	600-3100
CD16/56 %	1	10	3-16
CD16/56 ABS	12	379	100-1400

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.

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

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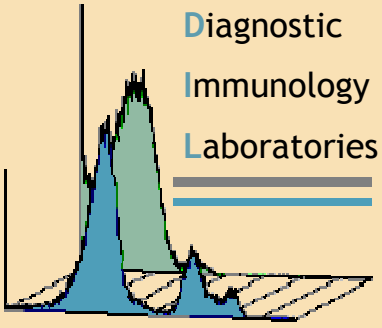
The Diagnostic Immunology Laboratories, consisting of the Clinical Immunology Laboratory and the Research Immunology Laboratory, are committed to providing the highest quality, comprehensive clinical testing available to aid in the detection, diagnosis and treatment of pediatric immunologic, as well 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

CONTACT US

Please visit our website or call us with any inquiries:
Ph: 513-636-4685
Fax: 513-636-3861
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

Cincinnati Children's Hospital – Bird's Eye View

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Current Menu of Available Tests:

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change the outcome

Diagnostic Immunology Laboratory
Ph: 513-636-4685 Fax: 513-636-3861
www.cchmc.org/DIL

Test Requisition Form - 101011

Send to: **Julie Beach-Hematology/Oncology R2328**
Cincinnati Children's Hospital Medical Center
3333 Burnet Avenue
Cincinnati, OH 45229-3039
MUST be received within 24 hrs of being drawn.
Maintain all samples, unspun, at room temperature.
Use Diagnostic Specimen packs and **FIRST OVERNIGHT PRIORITY SHIPPING** to ensure timely delivery.
The lab operates M-F only. Closed Saturday and Sunday.

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

PATIENT & SAMPLE INFORMATION

Patient Name	Patient Identification Number	Date of Sample	Time of Sample
Date of Birth	Gender: ☐ Male ☐ Female	Has the patient undergone BMT? ☐ No ☐ Yes – date of BMT _____	
Diagnosis or reason for testing			Diagnosis Code
Race: ☐ African-American ☐ American Indian ☐ Asian ☐ Caucasian ☐ Hispanic ☐ Other (specify) _____			
Medications:			

TESTS REQUESTED

☐ ALPS Panel	3ml (1ml) EDTA	**CBC/Diff**[3]
☐ Antigen Stimulation	10ml (5ml) Sodium Heparin[1]	
☐ Apoptosis (Fas-mediated)	20ml (10ml) ACD-A	
Note: Sample must be <24 hrs old and received Thursday AM		
☐ B Cell Panel [2]	3ml (1ml) EDTA	**CBC/Diff**[3]
☐ BAFF, Plasma Levels	3ml (1ml) EDTA	
☐ CD40L (CD154)	5ml (3ml) Sodium Heparin	
☐ CD45RA/RO*NEW REVISED PANEL*	3ml (1ml) EDTA	
☐ CD52 Expression[2]	3ml (1ml) EDTA	
☐ CD64 (Leuko64)	1ml (0.5ml) EDTA	
☐ CD107a Mobilization	10ml (5ml) Sodium Heparin[1]	
Note: CD107a is a 2 day test and cannot be accepted on Fridays		
☐ CD127 / CD132	3ml (1ml) EDTA	**CBC/Diff**[3]
☐ CTL Function	10ml (5ml) Sodium Heparin[1]	
☐ Cytokines, Intracellular	3ml (2ml) Sodium Heparin	
☐ Cytokines, Plasma	5ml (3ml) EDTA	
☐ EBV Immortalized Cell Line	3ml Sodium Heparin	
☐ Check here if this is a research sample; signed consent required		
☐ EDN (Eosinophil-Derived Neurotoxin)	3ml (1ml) EDTA	
☐ Eotaxin-3	3ml (1ml) EDTA	
☐ Foxp3	3ml (1ml) EDTA	**CBC/Diff**[3]
☐ iNKT	3ml (1ml) EDTA	

☐ Lymphocyte Activation Markers	3ml (2ml) Sodium Heparin
☐ Lymphocyte Subsets	3ml (1ml) EDTA
☐ MHC Class I & II	3ml (1ml) EDTA
☐ Mitogen Stimulation	10ml (5ml) Sodium Heparin[1]
☐ Neopterin	3ml (1ml) EDTA, or CSF[4]
☐ Neutrophil Adhesion Markers(CD18/CD11b)	3ml (1ml) EDTA
☐ Neutrophil Oxidative Burst	3ml (1ml) EDTA
☐ NK Function[2]	10ml (5ml) Sodium Heparin[1]
☐ Perforin/Granzyme B	3ml (1ml) EDTA
☐ PNH Screen (CD59)	3ml (1ml) EDTA
☐ pSTAT5	3ml (1ml) EDTA
☐ SAP (XLP1)	3ml (1ml) Sodium Heparin
☐ Soluble CD163	2ml (1ml) EDTA
☐ Soluble IL-2R	3ml (1ml) EDTA
☐ Sorted Engraftment	call to schedule
☐ TCR α/β / TCR γ/δ	3ml (1ml) EDTA
☐ TCR V beta Repertoire	3ml (2ml) EDTA
☐ WASP	3ml (1ml) Sodium Heparin
☐ WASP Transplant Monitor	3ml (1ml) Sodium Heparin
☐ XIAP (XLP2)	3ml (1ml) EDTA
☐ ZAP-70 (NOT for CLL)	3ml (1ml) EDTA
☐ Other _____	

Notes:
1. Volumes 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.

IN THE UPCOMING ISSUE:

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

FOCiS Federation of Clinical Immunology Societies www.focisnet.org

jmcn Jeffrey Modell Centers Network

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