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Laboratory User Manual
for
Paediatric Allergy & Immunology Laboratory

Version: 06

Date of Issue: 01 Oct 2023

Approved by Lee Bee Wah

Accredited Laboratory SAC-SINGLAS LA-2022-0810-H

Table of Contents

Laboratory Information

- **Laboratory Address:**
  MD1 Tahir Foundation Building Level 15
  Department of Paediatrics
  Yong Loo Lin School of Medicine, National University of Singapore
  12 Science Drive 2 (via Science Drive 4)
  Singapore 117549

- **Contact number:** +65 66013306

- **E-mail:** NUHPaedsimm@nuhs.edu.sg

- **Opening hours of the Laboratory:**
  9:00 – 17:30, Monday to Friday (Closed on Saturday/Sunday/Public Holiday)

Service Agreement

1. PROCESSING OF REQUESTS AND ENQUIRIES FOR TESTING OF SAMPLES

1.1 Requests and enquiries for the testing of samples (each a "**Request**") to the Laboratory shall be processed via phone call or email. The person making a Request is herein referred to as a "**Requestor**".

1.2 For internal Requests, the Requestor shall submit such Requests by ordering via the EPIC System. The Requestor shall print out the order form, which must be submitted together with the sample to the Laboratory unless agreed otherwise by the Laboratory.

1.3 For external Requests:

(a) the Laboratory shall provide the Requestor with the request form to be completed by him/her; or

(b) the Requestor is permitted to use his/her own organization's request form so long as said request form indicates the tests required.

1.4 Where a Requestor makes a verbal request, the Requestor shall send confirmation email stating the nature of the Request within 24 hours from the time the verbal Request was made. The Laboratory reserves the right to withhold testing of the sample until the Laboratory receives the confirmation email for the Request.

1.5 All Laboratory Services requests shall be ordered by a physician.

2. EVALUATION AND REJECTION OF SAMPLES

2.1 Prior to carrying out any testing of samples in a Request, the Laboratory will evaluate the sample(s) provided by the Requestor to determine if the sample(s) pass the sample acceptance criteria for the requested test(s).

2.2 In the event that the sample acceptance criteria are not met, the Laboratory will notify the Requestor of such non-conformity:

(a) Where both the Laboratory and the Requestor agree to continue to use the sample for the requested test(s), notwithstanding the non-conformity, the test report for the sample will indicate the nature of the non-conformity (where applicable) and that the Requestor should take caution when interpreting the test results; or

(b) Where the Laboratory is unable to proceed with the requested test(s) or the Requestor agrees not to proceed with the requested test(s), the Laboratory will provide a sample rejection form stating the reasons for rejection to the Requestor.

3. COMPETENCIES AND RESOURCES OF THE LABORATORY

3.1 The Laboratory shall ensure that its Laboratory personnel conducting the requested test(s) has the necessary competencies to carry out said test(s).

3.2 The Laboratory shall ensure that it has sufficient resources to perform the requested test(s). In the event that the Laboratory is unable to perform the requested test(s), the Laboratory shall notify the Requestor.

4. ADVICE ORDERING TESTS AND INTERPRETATING TEST RESULTS ON

Should the Requestor require clinical advice in respect of the ordering of test(s) and/or the interpretation of test results, the Requestor may contact the Laboratory regarding such requirements. The Laboratory will consult its clinicians and thereafter provide the Requestor with the clinical advice.

5. HANDLING OF COMPLAINTS

The Laboratory will record complaints made to the Laboratory. Depending on the clinical implication(s) of the complaint, the Laboratory will assign a duly authorized Laboratory personnel to handle the conduct of the complaint.

Where the complaint involves major clinical implication(s), the authorized Laboratory personnel shall conduct an investigation into the complaint.

6. PERSONAL DATA PROTECTION ACT 2012 (“PDPA”)

Where the Request(s) and/or sample(s) contain information comprising personal data as defined in PDPA, the Laboratory shall adopt appropriate and reasonable measures to ensure that it complies with the personal data provisions set out in PDPA (where applicable) in respect of the personal data.

List of Laboratory Services

1. Lymphocyte Subsets Enumeration by Flow Cytometry
2. Neutrophils Oxidative Burst Test by Dihydrorhodamine (DHR)
3. Lymphocytes Proliferation Assay
4. Flow Cytometric Cross Match
5. MSMD assay for IL-12/IFN-γ production
6. CD40 Ligand Assay
7. CD62L Shedding Assay
8. STAT-3 Phosphorylation Assay
9. Anti-IFN-γ Antibody Assay
10. IL-10 Receptor Functional Assay
11. TB T-SPOT Test

See detailed information for each assay in Appendix I

Instructions for Completion of the Request Form

- Request from NUH
  - Request via EPIC System on tests request
- Request from Referral Institutions
  - Write tests request on their own ordering form or use our Laboratory Request Form
- Request from overseas
  - Send request via our email

Instructions for Sample Collection and Handling Instructions

1. Check the specimen requirement for respective assay as listed in the table in next page prior collecting samples. Ensure samples will be collected with correct tube type, preservative and amount. Please take note that some assays are performed on specific days only.
2. Samples are collected through venipuncture.
    a. Identify the patient. Ask patient to state their name and IC number. The information must match the requisition.
    b. Select the appropriate vein for venipuncture.
    c. Put tourniquet on the patient about 3-4’ above the venipuncture site
    d. Clean the puncture site by making a smooth circular pass over the site with the 70% alcohol pad, moving in an outward spiral from the zone of penetration. Do not touch the puncture site after cleaning.
    e. Perform the venipuncture.
    f. Gently invert blood tube 10 time to mix anticoagulant. Do not shake or mix vigorously.
3. Ensure sample collection tubes are labeled with correct patient information (Name, IC number, DOB).
4. Check the specimens handling requirements. All specimen should be transported in a leak-proof biohazard bag/container and arrive to our Laboratory before the cut-off time.

Summary of Assay & Specimen Information

Assay	Specimen requirements	Analytic Time (days)	Maximum Laboratory Time	Remarks
Lymphocyte Subsets Enumeration by Flow Cytometry	• 3ml EDTA blood for all CD markers ordered on the same day (min 2ml) • 1ml for infants (<15months) • Specimens are to be transported at room temperature no later than 4pm on date of blood drawn. Specimen taken on weekend must arrive within 48 hours from time of blood drawn.	1-2	3-4 working days	
Neutrophils Oxidative Burst Test by Dihydrorhodamine (DHR)	• 3ml lithium heparin blood from patient (minimum 2ml) and a non-related healthy control preferred. (PLEASE INFORM LABORATORY should a related healthy control blood is sent) • 1 ml for infants (<15months) • Reach the laboratory latest by 3pm	1-2	3-4 working days	
Lymphocytes Proliferation Assay	<u>PHA & Con A assay</u> • 10ml sodium heparin blood from patient • 3-5ml sodium heparin blood for patient age <2 years • 10ml sodium heparin blood from a healthy control <u>Anti-CD3 & anti-CD3/CD28 assay</u> • 10ml sodium heparin blood from patient • 3-5ml sodium heparin blood for patient age <2 years • 10ml sodium heparin blood from a healthy control • Please send 20ml or more for each assay should patient be lymphopenia (less than 1000 lymphocytes per ul)	7-8	8-10 working days	**Mondays and Fridays only** For Monday sample, no Public Holidays on either Thursday or Friday of that week. For Friday sample, no Public Holidays on either Monday or Tuesday of the following week.

	• Specimens are to be transported at room temperature and reach the laboratory by 12 noon. Overnight specimens will not be processed.			
Flow Cytometric Crossmatch	• 20ml sodium heparin blood from donor and 5ml plain tube from patient • Specimens are to be transported at room temperature and reach the laboratory by 12 noon on day of blood drawn. • Overseas sample collection to contact laboratory for transportation instructions.	1-2	3-4	
MSMD assay for IL-12/IFN-γ production	• Collect 15ml of blood from patient and control in sodium heparin tube • 5ml sodium heparin blood for patient age <2 years • Specimens are to be transported at room temperature and must reach the laboratory before 12 noon. Overnight specimens will not be processed.	5-7	7-10	Mondays to Wednesdays only. No Public Holidays on following 2 days after sample receipt.
CD40 Ligand Assay	• 3ml sodium heparin blood from patient (minimum 2ml) and a non-related healthy control preferred. (PLEASE INFORM LABORATORY should a related healthy control blood is sent) • 1 ml for infants (<15months) • Specimens are to be transported at room temperature and reach the laboratory before or by 12 noon. Overnight specimens will not be processed.	1-2	3-4	
CD62L Shedding Assay	• 3ml sodium heparin blood from patient (minimum 2ml) and a non-related healthy control preferred. (PLEASE INFORM LABORATORY should a related healthy control blood is sent) • 1 ml for infants (<15months) • Specimens are to be transported at room temperature and reach the laboratory latest by 12 noon. Overnight specimens will not be processed.	1-2	3-4	

Loss of function STAT-3 Phosphorylation Assay	• 10ml sodium heparin blood from patient (minimum 6ml) and a non-related healthy control preferred. (PLEASE INFORM LABORATORY should a related healthy control blood is sent) • 3 ml for infants (<15months) • Specimens are to be transported at room temperature and reach the laboratory latest by 12 noon. Overnight specimens will not be processed.	2-3	3-4	
Anti-IFN-γ Antibody Assay	• 3ml EDTA blood from patient • If samples cannot be shipped on the same day. Plasma are to be collected and kept cold (frozen preferred) and dispatch with **ice** on the next working day. • Specimens taken on the same day are to be transported at room temperature and reach the laboratory latest by 5pm.	3	10	
IL-10 Receptor Functional Assay	• 10ml sodium heparin whole blood from both patient and a normal healthy control • 3 ml for infants (<15months) • Samples to be transported in room temperature and reach laboratory by 12 noon.	2-3	3-4	
TB T-SPOT Test	• 15 ml lithium heparin from patient with (WBC count <2.0 x 10^6) • 10 ml lithium heparin for patient age >10 years • 4ml lithium heparin age 2-9 years • 2ml lithium heparin for patient age <2 years • Samples to be transported in room temperature and reach laboratory by 3 pm.	2-3	3-4	Mondays to Thursday only except public holidays and eve of public holidays.

Appendix I: Detailed Information of Each Assay

Lymphocyte Subsets Enumeration by Flow Cytometry

Useful for

Diagnosis of immunodeficiency disorders involving lymphocyte quantities

- Adjusting management of immunodeficiency disorders that involve lymphocyte numbers
- Monitoring lymphocyte reconstitution after transplantation

Principle

Quantitation of lymphocyte markers is useful for diagnosis and management of certain immunodeficiency disorders. Each disorder has a clinical profile that, when combined with the lymphocyte numbers and matched to the IUIS Classification, aids in diagnostic accuracy. An example of a primary immunodeficiency disorder is Severe Combined Immunodeficiency Disorder (SCID). An example of a secondary immunodeficiency disorder is HIV infection.

Method

Flow Cytometry. Fresh whole blood is stained using specific fluorescence-tagged monoclonal antibodies, which can be detected through flow cytometry. This assay aims to measure percentages and absolute counts of various CD markers on lymphocytes from fresh whole blood and evaluate immunological status.

Specific Instruction for Order

Any add on test markers if needed, please contact the laboratory within 48 hours from time of blood drawn

Specimen Requirement

- 3ml EDTA blood from patient (minimum 2ml)
- 1 ml for infants (<15months)

Shipping Instructions

- Specimens are to be transported at room temperature and reach the laboratory no later than 4pm on date of blood drawn.
- Specimen taken on weekend must arrive within 48 hours from time of blood drawn, and no later than 4pm.

Reject due to

- Mislabeled or unlabeled specimens
- Use of incorrect blood tubes
- Clotted blood specimen
- Insufficient specimen volume
- Extremely Low lymphocyte count (less than 100 lymphocytes per ul)
- Incorrect conditions in transportation/storage of blood tubes, or exceeding the 48 hours from time of blood drawn

Specimen Stability Information

Specimen must be kept at room temperature and arrived at laboratory no longer than 48 hours from time of blood drawn

Days and Times Test Performed

Monday through Friday except Public Holidays

Analytic Time

1-2 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

48 hours from time of blood drawn

Reference value

Refer to tables below

Limitations

There are no major limitations to the procedure

Reference Value

Reference Range for Lymphocyte Subsets (Percentage and Absolute Count)

Normal reference values are corrected for age and gender in our local population based on

Lee BW et al. Age- and Sex-Related Changes in Lymphocyte Subpopulations of Healthy Asian Subjects: From Birth to Adulthood

Cytometry (Communications in Clinical Cytometry) 26: 8-15 (1996)

		At Birth		2days - 11mths		1 - 6yrs		7 - 18yrs		19 - 40yrs	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
CD2	%	60 – 74	70 - 78	59 - 67	60 - 72	61 - 70	65 - 74	63 - 75	66.5 - 75.5	70 - 81	70 - 80
	Absolute Count	1448 – 2804	1086 - 2166	1851 - 3347	2726 - 3878	1642 - 3271	1946 - 3169	1122 - 2154	1290 - 2241	821 - 1127	914 - 1409
CD3 (T Cells)	%	53 – 74	65 - 76	56 - 67	60 - 71	56 - 68	62 - 70	65 - 72	64 - 72.5	61 - 73	67 - 76
	Absolute Count	1180 – 1983	853 - 2110	1767 - 3292	2765 - 3516	1488 - 2948	1934 - 2948	1062 - 2208	1302 - 2190	732 - 1002	939 - 1338
CD4	%	36 – 48	44 - 53	33 - 39	37 - 48	29 - 40	30 - 40	27 - 34	29.5 - 35.5	27 - 39	30 - 43
	Absolute Count	835 – 1944	1035 - 1870	892 - 2289	1687 - 2424	954 - 2059	1037 - 1784	579 - 1573	616 - 1080	360 - 515	416 - 742
CD8	%	13.5 - 19.5	18.5 - 24.5	16 - 24	16 - 21	19 - 25	20 - 27	23 - 30	24 - 33.5	24 - 32	26 - 35
	Absolute Count	367 – 778	352 - 929	726 - 1490	751 - 1137	698 - 1170	657 - 1179	524 - 1151	507 - 961	298 - 415	355 - 841
CD4/CD8	%	1.9 - 2.5	1.7 - 3.0	1.6 - 2.2	1.9 - 2.9	1.1 - 2.0	1.2 - 2.0	1.0 - 1.4	0.9 - 1.4	0.9 - 1.5	1.0 - 1.6
CD20 (B Cells)	%	12 - 22	12 - 20	23 - 30	21 - 33	18.5 - 28	19 - 29	15 - 20	14 - 21	9 - 14	10 - 15
	Absolute Count	297 - 569	196 - 446	685 - 197	989 - 1696	485 - 1200	570 - 1391	249 - 615	259 - 547	93 - 175	130 - 243
CD56+CD16 (NK Cells)	%	16 - 27	9 - 16	8 - 16	7 - 14	9 - 19	7 - 16	11 - 24	11 - 23	17 - 28	10 - 19
	Absolute Count	389 - 1093	157 - 387	267 - 752	302 - 846	313 - 563	275 - 567	251 - 645	275 - 456	233 - 362	149 - 288
Activated T Cells	% Tac on CD3	4.1 - 12.5	4.6 - 8.1	4.5 - 8.6	4.0 - 7.6	3.5 - 8.0	3.1 - 7.0	2.8 - 8.0	4.4 - 8.7	1.7 - 14.5	2.8 - 8.1
	% DR on CD3	1.5 - 4.6	1.3 - 1.9	4.4 - 13.0	3.8 - 8.1	7.5 - 17.7	7.4 - 17.6	5.9 - 16.9	7.8 - 19.5	6.1 - 16.9	7.4 - 14.1

Reference Range for Naïve & Memory B Cells (Percentage)

Normal ranges for Naïve & Memory B Cells are not established in local population
Reference ranges for B cell is taken from Piątosa et al. Cytometry Part B (Clinical Cytometry) 78B:372-381 (2010)

	0-7days	8days - 2mths	2 - 5mths	5 - 9mths	9 - 15mths	15 - 24mths	2 - 5yrs	5 - 10yrs	10 - 16yrs	>16yrs
$IgD^{+}CD27^{+}$ on B cells	2.6-12.0	1.7-6.5	2.5-8.7	2.8-7.4	3.0-10.7	4.1-13.9	2.7-19.8	5.2-20.4	4.6-18.2	7.0-23.8
$IgD^{-}CD27^{+}$ on B cells	1.0-7.2	1.5-7.1	0.3-9.0	1.6-7.0	1.4-11.9	3.9-13.6	4.7-21.2	10.9-30.4	8.7-25.6	8.3-27.8
$IgD^{+}CD27^{-}$ on B cells	55.0-91.3	85.7-94.8	82.1-95.2	85.5-93.4	76.5-94.7	68.1-89.3	54.0-88.4	47.3-77.0	51.3-82.5	48.4-79.7
$IgD^{-}CD27^{-}$ on B cells	2.1-39.3	1.1-7.9	0.7-5.0	0.8-4.4	0.6-3.0	1.1-10.1	1.5-12.4	2.3-11.0	2.7-13.4	2.2-10.0
$IgM^{high}CD38^{high}$ on B cells	1.2-41.9	4.1-43.9	11.4-38.4	7.2-19.7	3.6-12.7	3.3-16.5	3.1-12.3	4.6-8.3	1.4-13.0	0.9-5.7
$IgM^{-}CD38^{high}$on B cells	0.2-3.2	0.2-2.7	0.4-3.3	0.2-4.0	0.4-5.5	0.5-3.0	0.6-4.0	0.6-5.3	0.6-6.5	0.4-2.4
$CD21^{low}$ on B cells	3.6-26.2	6.7-23.1	7.1-31.1	6.2-20.3	4.3-23.1	5.1-21.3	4.1-24.4	5.9-25.8	2.9-13.2	3.2-19.6

Neutrophils Oxidative Burst Test by Dihydrorhodamine (DHR) Flow Cytometric Test

Useful for

Diagnosis of chronic granulomatous disease or its carrier.

Principle

Dihydrorhodamine 123 (DHR) is a substrate of hydrogen peroxide that was metabolized by NADPH oxidase from superoxide ions yield in Neutrophil Oxidative Burst. DHR is oxidized to a fluorescent rhodamine, which can be detected, and measured using flow cytometry. This assay aims to measure and determine the degree of neutrophil oxidative burst function. Complete myeloperoxidase (MPO) deficiency can yield a false-positive result.

Method

Flow Cytometry

Specific Instruction for Order

Nil

Specimen Requirement

- 3ml lithium heparin blood from patient (minimum 2ml) and a non-related healthy control preferred. **(PLEASE INFORM LABORATORY should a related healthy control blood is sent)**
- 1 ml for infants (<15months)

Shipping Instructions

- Specimens are to be transported at room temperature and reach the laboratory latest by 3pm. Sample must arrive laboratory within same day from time of blood drawn.

Reject due to

- Mislabeled or unlabeled specimens
- Use of incorrect blood tubes
- Clotted blood specimen
- Insufficient specimen volume

Specimen Stability Information

Same day from time of blood drawn

Days and Times Test Performed

Monday through Friday except Public Holidays

Analytic Time

1-2 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

Specimen will not be kept

Reference Value

- Stimulation Index (SI) ≥ 66.1
- Coefficient of variation (CV) ≤ 53.8

Limitations

Complete myeloperoxidase (MPO) deficiency can yield a false-positive result

Lymphocyte Proliferation Assay

Useful for

Diagnosis of immunodeficiency disorders where lymphocyte proliferation is suppressed.

Principle

Lymphocyte proliferation assays are "qualitative" tests that assess the ability of lymphocytes to multiply in response to a trigger (such as a pathogen). This test aids in the diagnosis of immunodeficiency disorders where cell-mediated immunity is clinically impaired; it is particularly useful when the lymphocyte subsets (the quantitative tests) return as apparently-normal despite the clinical context being suspicious. An example of an immunodeficiency disorder with impaired lymphocyte proliferation, is HIV.

Method

This assay describes the degree of lymphocyte proliferation upon stimulation with mitogens, such as Phytohaemagglutinin (PHA) and Concanavalin A (ConA); as well as anti- CD3 and anti-CD28 costimulation.

Specific Instruction for Order

Please contact laboratory to confirm the availability or request of any stimulus to the assay

Specimen Requirement

- Overnight specimens will not be processed. Please send 20ml or more for each assay should patient be lymphopenia (less than 1000 lymphocytes per ul). See below for details.

<u>PHA & Con A assay</u>

- 10ml sodium heparin blood from patient
- 3-5ml sodium heparin blood for patient age <2 years
- 10ml sodium heparin blood from a healthy control

<u>Anti-CD3 & anti-CD3/CD28 assay</u>

- 10ml sodium heparin blood from patient
- 3-5ml sodium heparin blood for patient age <2 years
- 10ml sodium heparin blood from a healthy control

Shipping Instructions

- Specimens are to be transported at room temperature and reach the laboratory by 12 noon

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Low lymphocytes count (less than 1000 lymphocytes per ul)

- Specimen arrived laboratory past the cut-off time

Specimen Stability Information

Same day from time of blood drawn

Days and Times Test Performed

- Mondays and Fridays only
- For Monday sample, no Public Holidays on either Thursday or Friday of that week
- For Friday sample, no Public Holidays on either Monday or Tuesday of the following week

Analytic Time

7-8 working days

Maximum Laboratory Time

8-10 working days

Specimen Retention Time

Specimen will not be kept

Reference Value

(ug/ml)	PHA (0.1)	PHA (0.5)	Con A (7.5)	CD3 (0.01)	CD3 (0.01)/ CD28 (2)
CPM	>2,937	>41,226	>33,408	>20,357	>40,224

Limitations

Lack of B cells or monocytes in blood samples will cause false negative results

Flow Cytometric Crossmatch

Useful for

The detection of the presence of circulating HLA antibodies against donors in potential allograft recipients.

Principle

The flow cytometric crossmatch is performed by incubating donor peripheral blood mononuclear cells with the serum of a potential recipient. If donor-specific HLA antibodies are present, they will bind to the HLA molecules on the surface of donor's cells. Assessment of alloantibody binding is performed by the addition of a fluorochrome labeled anti-human immunoglobulin reagent.

Method

Flow cytometry

Specific Instruction for Order

For recipient, pls state if previously treated with rituximab
For donor, pls state if any treatment done such as GM-CSF etc.
For overseas specimen, please contact the laboratory before sample collection.

Specimen Requirement

- 20ml sodium heparin blood from donor and 5ml plain tube from patient

Shipping Instructions

- Specimens are to be transported at room temperature and reach the laboratory by 12 noon on day of blood drawn
- Overseas sample collection to contact laboratory for transportation instructions

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Specimen arrived laboratory past the cut-off time

Specimen Stability Information

3 days

Days and Times Test Performed

Monday through Friday except Public Holidays

Analytic Time

1-2 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

Specimen will not be kept unless requested

Reference Value

- T cell: Positive > 54 Median channel shift
- B cell: Positive > 69 Median channel shift

Limitations

The results of flow crossmatch need to be evaluated along with the patient's current antibody screening results and reviewed for concordance

Mendelian Susceptibility to Mycobacterial Disease (MSMD) - IL-12/IFN-γ production

Useful for

Preliminary test for the in-born defect in the IL-12/IFN-γ production pathway associated with non-tuberculous mycobacterial infections, fungal and viral infections.

Principle

Inborn defect in the loop of IL-12/IFN-γ signaling pathway resulted in the lack of production of IL-12/IFN-γ cytokine. In the assay, patient's cells are stimulated with BCG and IFN-γ/IL-12 in vitro. The ability of patient's cells to produce IL-12 and IFN-γ are analyzed in the culture supernatant.

Method

In vitro cell culture and ELISA

Specific Instruction for Order

Nil

Specimen Requirement

- Collect 20ml of blood from patient and healthy control in sodium heparin tube
- 5ml sodium heparin blood for patient age <2 years

Shipping Instructions

- Specimens are to be transported at room temperature and must reach the laboratory before 12 noon. Overnight specimens will not be processed.

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Specimen arrived laboratory past the cut-off time

Specimen Stability Information

Same day from time of blood drawn

Days and Times Test Performed

Mondays to Wednesdays only and providing the following two days of sample receiving day have no public holiday

Analytic Time

5-7 days

Maximum Laboratory Time

7-10 working days

Specimen Retention Time

Specimen will not be kept

Reference Value

This assay is not meant for quantifying the level of IL-12 and IFN-γ. It is for qualitative purpose.

Limitations

- This assay examines the responses of patient cells to IL-12/IFN-γ stimulation and hence mainly detects two discrete groups of defects:
  - IL-12/IL-12 receptor
  - IFN-γ receptor/STAT-1
- A normal result does not exclude conditions with increased susceptibility to Mycobacterial infections that is beyond the IL-12/IFN-γ cytokine loop. Please correlate clinically and refer to a specialist in immunodeficiency if needed.
- ELISA kits used in this assay are for research purposes only, however, it will not affect the interpretation of the qualitative results.

CD40 ligand (CD40L) Assay

Useful for

Screening for X-linked hyper-IgM syndrome in patients with CD40 deficiency or carrier status in females.

Principle

Patients with this disorder usually present with unusual, severe or recurrent infections of the lower respiratory tract by 2-3 years of life and may have other infections that point to a significant defect in humoral immunity. CD40 Ligand (CD40L) is a co-stimulatory molecule expressed primarily on $CD4^+$ T lymphocytes. Interaction of CD40L with CD40, expressed on antigen-presenting B lymphocytes, is critical for an intact defensive response. Marked reduction in CD40L expression is observed in immune deficiencies such as Hyper-IgM. This test aims to detect the expression of CD40L on $CD4^+$ T cells upon stimulation with PMA and Ionomycin.

Method

Flow cytometry

Specific Instruction for Order

Nil

Specimen Requirement

- 3ml sodium heparin blood from patient (minimum 2ml) and a non-related healthy control preferred. **(PLEASE INFORM LABORATORY should a related healthy control blood is sent)**
- 1 ml for infants (<15months)
- Overnight specimens will not be processed

Shipping Instructions

- Specimens are to be transported at room temperature and reach the laboratory before or by 12 noon

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Specimen arrived laboratory past the cut-off time

Specimen Stability Information

Same day from time of blood drawn

Days and Times Test Performed

Monday through Friday except Saturday/Sunday/Public Holidays

Analytic Time

1-2 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

Specimen will not be kept

Reference Value

- Normal: Present with single peak
- Abnormal:
  - Completely absent: CD40L deficiency
  - Two peaks on CD40L histogram: Carrier status

Limitations

Approximately 20% of XL-HIGM patients have activated CD4 T cells with normal surface expression of CD40L, but aberrant function (Seyama K, Nonoyama S, Gangsaas I, et al: Mutations of the CD40 ligand gene and its effect on CD40 ligand expression in patients with X-linked hyper IgM syndrome. Blood 1998; 92:2421-2434). This assay did not detect binding function of CD40L to CD40.

CD62L shedding assay

Useful for

Identification of functional defects in Interleukin-1 receptor associated kinases (IRAK)-4 and MyD88 in the Toll-like Receptor (TLR) pathways**.**

Principle

IRAK-4 or MyD88 deficiency impairs neutrophil activation through ablation of TLR mediated microbial sensing, therefore patients are susceptible to recurrent serious bacterial and fungal infections. This also detects patients with a defect in a protein in another TLR pathway, UNC93b. Patients with a deficiency in this protein are predisposed to Herpes simplex virus (HSV) encephalitis.

Interpretation: Granulocytes of patients with IRAK-4 or MyD88 deficiency will still express CD62L after treatment with lipopolysaccharide (LPS, TLR4 agonist) and the compound R848 (TLR7/8 agonist) but lose CD62L expression as a result of treatment with PMA. Granulocytes of UNC-93B deficient patients would still express CD62L with R848 but lose CD62L with both LPS and PMA.

Method

Flow Cytometry

Specific Instruction for Order

Nil

Specimen Requirement

- 3ml sodium heparin blood from patient (minimum 2ml) and a non-related healthy control preferred. **(PLEASE INFORM LABORATORY should a related healthy control blood is sent)**
- 1 ml for infants (<15months)
- Overnight specimens will not be processed

Shipping Instructions

- Specimens are to be transported at room temperature and reach the laboratory latest by 12pm

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Specimen arrived laboratory past the cut-off time

Specimen Stability Information

1 day

Days and Times Test Performed

Monday through Friday except Public Holidays

Analytic Time

1-2 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

Specimen will not be kept

Reference Value

This is a qualitative assay

Normal: CD62L can be downregulated upon LPS stimulated

Limitations

There are no major limitations to the procedure

Loss of function STAT-3 phosphorylation (pSTAT-3) - autosomal dominant hyper IgE Syndromes

Useful for

Diagnosis autosomal-dominant (AD) hyper IgE Syndromes.

Principle

The hyper IgE syndrome (HIES) is a rare primary immunodeficiency characterized by elevated serum IgE, rash and recurrent bacterial infections of the skin and lung. There are two forms of HIES, an autosomal dominant (AD) and an autosomal recessive (AR) form. STAT3 mutations are responsible for most cases of autosomal-dominant (AD) HIES. The mutation and deletions in the DOCK8 gene were found to underline the majority of cases with AR-HIES. This assay examines the function of STAT-3 phosphorylation by means of flow cytometry.

Method

Flow Cytometry

Specific Instruction for Order

Nil

Specimen Requirement

- 10ml sodium heparin blood from patient (minimum 6ml) and a non-related healthy control preferred. **(PLEASE INFORM LABORATORY should a related healthy control blood is sent)**
- 3 ml for infants (<15months)
- Overnight specimens will not be processed

Shipping Instructions

- Specimens are to be transported at room temperature and reach the laboratory latest by 12 noon

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Specimen arrived laboratory past the cut-off time

Specimen Stability Information

1 day

Days and Times Test Performed

Monday through Friday except Public Holidays

Analytic Time

2-3 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

Specimen will not be kept

Reference Value

This is a qualitative assay

Normal: phosphorylated STAT-3 can be detected

Limitations

There are no major limitations to the procedure

Anti-Interferon Gamma (α-IFN-γ) Antibody Assay

Useful for

Adult-onset immunodeficiency (AOID) with anti-interferon-γ (IFN-γ) autoantibodies (autoAbs) is an emerging immunodeficiency syndrome in Asian countries. The presence of neutralizing anti-IFN-γ autoAbs are significantly associated with severe disseminated opportunistic infections.

Principle

Autoantibodies against interferon-γ are associated with severe disseminated nontuberculous mycobacterial infections. This test examines the presence of anti-IFN-γ autoAbs by detecting the inhibitory effects of patient's serum/plasma on IFN-γ induced STAT-1 phosphorylation as well as on the detection of input IFN-γ by enzyme linked immunoassay (ELISA).

Method

Flow cytometry and ELISA

Specific Instruction for Order

Nil

Specimen Requirement

- 3ml EDTA blood from patient.
- If samples cannot be shipped on the same day. Plasma are to be collected and kept cold (frozen preferred) and dispatch with **ice** on the next working day.

Shipping Instructions

- Specimens taken on the same day are to be transported at room temperature and reach the laboratory latest by 5pm.
- Overnight samples (plasma) to be transported on ice packs.

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes

Specimen Stability Information

3 days at 4^{o}C, 1 year in -80^{o}C

Days and Times Test Performed

Every Monday

Analytic Time

3 working days

Maximum Laboratory Time

10 working days

Specimen Retention Time

Positive samples will be retained for subsequent comparison with future sample

Reference Value

This is a qualitative assay

Normal: Absence of anti-IFN-γ antibody

Limitations

There are no major limitations to the procedures

IL-10 Receptor Functional Assay by flow cytometry

Useful for

Examination for the deficiency of IL-10 receptor

Principle

IL-10 is an immune regulatory cytokine with anti-inflammatory property. IL-10/IL-10 receptor deficiency is associated with severe early onset enterocolitis. This test examines the deficiency of IL-10 receptor through a functional assay. Patient's cells are stimulated with IL-10 and the phosphorylation of signal transducer and activator of transcription 3 (STAT3) which is an adaptor protein involved in the IL-10/IL-10R signaling pathway is examined. This assay does not examine for the deficiency of IL-10.

Method

Flow cytometry

Specific Instruction for Order

Nil

Specimen Requirement

- 10ml sodium heparin whole blood from both patient and a normal healthy control

Shipping Instructions

- Samples to be transported in room temperature and reach laboratory by 12 noon

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Specimen arrived laboratory past the cut-off time

Specimen Stability Information

1 day

Days and Times Test Performed

Mondays to Thursday only. Testing is not available on public holidays and the eve of public holidays.

Analytic Time

2-3 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

Specimen will not be kept

Reference Value

This is a qualitative assay

Normal: phosphorylated STAT-3 can be detected

Limitations

There are no major limitations to the procedure

TB T-SPOT Test

Useful for

Detection of effector T cells that have been specifically activated by *Mycobacterium tuberculosis* (MTB) antigens as an aid in the diagnosis of MTB infection.

Principle

The immune response to infection with *Mycobacterium tuberculosis* (MTB) is mediated predominantly through T cell activation. As part of this response, T cells are sensitized to MTB antigens and the activated effector T cells, both CD4+ and CD8+, produce the cytokine interferon-gamma when stimulated by these antigens. The TB T-SPOT test uses the enzyme-linked immunospot (ELISPOT) methodology to enumerate *M. tuberculosis*-sensitized T cells by capturing interferon-gamma in the vicinity of T cells from which it was secreted

Method

Enzyme-linked immunospot (ELISPOT)

Specific Instruction for Order

Nil

Specimen Requirement

Collect lithium heparin whole blood from patient.

- WBC count <2.0 x 10^6 15 ml
- Age ≥10 years 10 ml
- Age 2-9 years old 4 ml
- Age < 2years 2 ml

Shipping Instructions

- Samples to be transported in room temperature and to reach the laboratory by 3 pm on the same day of blood collection.

Reject due to

- Mislabeled or unlabeled specimens
- Blood taken in incorrect vacutainer tubes
- Clotted blood specimen
- Insufficient blood volume

Specimen Stability Information

32 hours from time of blood collection

Days and Times Test Performed

Monday through Thursday except public holidays and eve of public holidays.

Analytic Time

2-3 working days

Maximum Laboratory Time

3-4 working days

Specimen Retention Time

Unprocessed excess blood sample may be kept till the next day of sample receiving

Reference Value

This is a qualitative assay
Normal: Negative

Limitations

- The test should be used and interpreted only in the context of the overall clinical picture.
- A negative test result does not exclude the possibility of exposure to or infection with *M. tuberculosis*.
- The test detects TB infection but does not differentiate between active and latent TB infection