Overview

Useful For
Assessing T-cell function in patients on immunosuppressive therapy, including solid-organ transplant patients
Evaluating patients suspected of having impairment in cellular immunity
Evaluation of T-cell function in patients with primary immunodeficiencies, either cellular (DiGeorge syndrome, T-negative severe combined immunodeficiency: SCID, etc) or combined T- and B-cell immunodeficiencies (T- and B-negative SCID, Wiskott Aldrich syndrome, ataxia telangiectasia, common variable immunodeficiency, among others) where T-cell function may be impaired
Evaluation of T-cell function in patients with secondary immunodeficiency, either disease related or iatrogenic
Evaluation of recovery of T-cell function and competence following bone marrow transplantation or hematopoietic stem cell transplantation

Reflex Tests

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGSTM</td>
<td>Additional Flow Stimulant, LPMGF</td>
<td>No, (Bill Only)</td>
<td>No</td>
</tr>
</tbody>
</table>

Testing Algorithm
If insufficient peripheral blood mononuclear cells (PBMCs) are isolated from the patient's sample due to low WBC counts or specimen volume received, selected dilutions or stimulants may not be tested at the discretion of the laboratory to ensure the most reliable results. Testing with 1 stimulant will always be performed. When adequate specimen is available for both stimulants to be tested, an additional test ID will be reflexed and billed separately.

Method Name
FlowCytometry

NY State Available
Yes

Specimen

Specimen Type
WB Sodium Heparin

Shipping Instructions
Specimens are required to be received in the laboratory weekdays and by 4 p.m. on Friday. Draw and package specimen as close to shipping time as possible. Ship specimen overnight in an Ambient Shipping Box-Critical Specimens Only (T668 following the instructions in the box).

It is recommended that specimens arrive within 24 hours of draw.
Specimens arriving on the weekend may be canceled.

**Necessary Information**

1. Date and time of draw and ordering physician name and phone number are required.

2. Specify "Mitogen" to differentiate from "Antigen" testing. This information is required.

**Specimen Required**

For serial monitoring, we recommend that specimen draws be performed at the same time of day.

**Supplies:** Ambient Shipping Box-Critical Specimens Only (T668)

**Container/Tube:** Green top (sodium heparin)

**Specimen Volume:**

- <3 months: 1 mL
- 3 months-5 years: 2 mL
- 6-18 years: 3 mL
- >18 years: 10 mL

**Collection Instructions:** Send specimen is original tube. **Do not aliquot.**

**Blood Volume Recommendations Based on Absolute Lymphocyte Count (ALC)**

<table>
<thead>
<tr>
<th>Mitogen Only</th>
<th>Blood Volume for Minimum PHA Only</th>
<th>Blood Volume for Minimum PHA and PWM</th>
<th>Blood Volume for Full Assay</th>
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</thead>
<tbody>
<tr>
<td>ALC</td>
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<td></td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>&gt;6.5 cc</td>
<td>&gt;8.5 cc</td>
<td>&gt;22 cc</td>
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<tr>
<td>0.5-1.0</td>
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<td>8.5 cc</td>
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<td>1.1-1.5</td>
<td>3.0 cc</td>
<td>4.0 cc</td>
<td>10 cc</td>
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<tr>
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<td>1.0 cc</td>
<td>3 cc</td>
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<tr>
<td>&gt;5.0</td>
<td>0.5 cc</td>
<td>0.8 cc</td>
<td>2 cc</td>
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<table>
<thead>
<tr>
<th>Mitogen and Antigen</th>
<th>Blood Volume for Minimum of Each Assay</th>
<th>Blood Volume for Full Assay</th>
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</table>
Test Definition: LPMGF
Lymphocyte Proliferation, Mitogens

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<tr>
<th>&lt;0.5</th>
<th>&gt;28 cc</th>
<th>&gt;60 cc</th>
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<tbody>
<tr>
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<td>60 cc</td>
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<td>1.1-1.5</td>
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<td>30 cc</td>
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<td>3.1-4.0</td>
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<td>10 cc</td>
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<td>4.1-5.0</td>
<td>3.5 cc</td>
<td>8 cc</td>
</tr>
<tr>
<td>&gt;5.0</td>
<td>2.5 cc</td>
<td>6 cc</td>
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Specimen Minimum Volume
1 mL

Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
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<tbody>
<tr>
<td>Gross lipemia</td>
<td>Reject</td>
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</table>

Specimen Stability Information

<table>
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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tbody>
<tr>
<td>WB Sodium Heparin</td>
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Clinical and Interpretive

Clinical Information

The method of determining impaired T-cell function by culturing human peripheral blood mononuclear cells (PBMC) in vitro with mitogenic plant lectins (mitogens) such as phytohemagglutinin (PHA) and pokeweed mitogen (PWM) has been part of the diagnostic immunology repertoire for many years.(1,2) The widely used method for assessing lymphocyte proliferation has hitherto been the measurement of 3H-thymidine incorporated into the DNA of proliferating cells. The disadvantages with the 3H-thymidine method of lymphocyte proliferation are:

1. The technique is cumbersome due to the use of radioactivity
2. It does not allow discrimination of responding cell populations in response to stimulation
3. It does not provide any information on contribution of activation-induced cell death to the interpretation of the final result

Further, decreased lymphocyte proliferation could be due to several factors, including overall diminution of T-cell proliferation or decrease in proliferation of only a subset of T cells, or an apparent decrease in total lymphocyte proliferation due to T-cell lymphopenia and under-representation of T cells in the PBMC pool. None of these can be discriminated by the thymidine uptake assay, but can be assessed by flow cytometry, which uses antibodies to identify specific responder cell populations. Cell viability can also be measured within the same assay without requiring additional cell manipulation or specimen.
Mitogens are very potent stimulators of T-cell activation and proliferation independent of their antigenic specificity.(3) It has been suggested that mitogens can induce T-cell proliferative responses even if they are incapable of responding adequately to antigenic (physiologic) stimuli. Therefore, abnormal T-cell responses to mitogens are considered a diagnostically less sensitive but more specific test of aberrant T-cell function. Lectin mitogens have been shown to bind the T-cell receptor, which is glycosylated through its carbohydrate moiety, thereby activating quiescent T cells. Mitogenic stimulation has been shown to increase intracellular calcium (Ca++) in T cells, which is absolutely essential for T-cell proliferation. While PHA is a strong T-cell mitogen, PWM is a weak T-cell mitogen, but it also induces B-cell activation and proliferation as well.

For this assay, we use a method that directly measures the S-phase proliferation of lymphocytes through the use of Click chemistry. In the Invitrogen Click-it-EdU assay, the Click chemistry has been adapted to measure cell proliferation through direct detection of nucleotide incorporation. In the assay, an alkyne-modified nucleoside is supplied in cell-growth media for a defined time period and is incorporated within cells. The cells are subsequently fixed, permeabilized, and reacted with a dye-labeled azide, catalyzed by copper. A covalent bond is formed between the dye and the incorporated nucleotide, and the fluorescent signal is then measured by flow cytometry.(4) Specific proliferating cell populations can be visualized by the addition of cell-specific antibodies. Cell viability, apoptosis, and death can also be measured by flow cytometry using 7-AAD and Annexin V.

The Click-it-EdU assay has already been shown to be an acceptable alternative to the 3H-thymidine assay for measuring lymphocyte/T-cell proliferation.(5)

The absolute counts of lymphocyte subsets are known to be influenced by a variety of biological factors, including hormones, the environment, and temperature. The studies on diurnal (circadian) variation in lymphocyte counts have demonstrated progressive increase in CD4 T-cell count throughout the day, while CD8 T cells and CD19+ B cells increase between 8:30 am and noon, with no change between noon and afternoon. Natural killer (NK)-cell counts, on the other hand, are constant throughout the day. Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration. In fact, cortisol and catecholamine concentrations control distribution and, therefore, numbers of naive versus effector CD4 and CD8 T cells. It is generally accepted that lower CD4 T-cell counts are seen in the morning compared with the evening, and during summer compared to winter. These data, therefore, indicate that timing and consistency in timing, of blood collection is critical when serially monitoring patients for lymphocyte subsets.

**Reference Values**

Viability of lymphocytes at day 0: > or = 75.0%

Maximum proliferation of phytohemagglutinin as % CD45: > or = 49.9%

Maximum proliferation of phytohemagglutinin as % CD3: > or = 58.5%

Maximum proliferation of pokeweed mitogen as % CD45: > or = 4.5%

Maximum proliferation of pokeweed mitogen as % CD3: > or = 3.5%

Maximum proliferation of pokeweed mitogen as % CD19: > or = 3.9%

**Interpretation**

Abnormal test results to mitogen stimulation are indicative of impaired T-cell function if T-cell counts are normal or only modestly decreased. If there is profound T-cell lymphopenia, it must be kept in mind that there could be a "dilution" effect with under-representation of T cells within the peripheral blood mononuclear cell (PBMC) population that could result in lower T-cell proliferative responses. However, this is not a significant concern in the flow cytometry assay, since acquisition of additional cellular events during analysis can compensate for artificial reduction in proliferation due to lower T-cell counts.
There is no absolute correlation between T-cell proliferation in vitro and a clinically significant immunodeficiency, whether primary or secondary, since T-cell proliferation in response to activation is necessary, but not sufficient, for an effective immune response. Therefore, the proliferative response to mitogens can be regarded as a more specific but less sensitive test for the diagnosis of infection susceptibility.

It should also be kept in mind that there is no single laboratory test that can identify or define impaired cellular immunity, with the exception of an opportunistic infection.

Controls in this laboratory and most clinical laboratories are healthy adults. Since this test is used for screening and evaluating cellular immune dysfunction in infants and children, it is reasonable to question the comparability of proliferative responses between healthy infants, children, and adults. One study has reported that the highest mitogen responses are seen in newborn infants with subsequent decline to 6 months of age, and a continuing decline through adolescence to half the neonatal response.(6) In our evaluation of 43 pediatric specimens (of all ages) with adult normal controls, only 21% and 14% were below the tenth percentile of the adult reference range for pokeweed (PWM) and phytohemagglutinin (PHA), respectively. A comment will be provided in the report documenting the comparison of pediatric results with an adult reference range and correlation with clinical context for appropriate interpretation.

It should be noted that without obtaining formal pediatric reference values it remains a possibility that the response in infants and children can be underestimated. However, the practical challenges of generating a pediatric range for this assay necessitate comparison of pediatric data with adult reference values or controls.

Lymphocyte proliferation responses to mitogens and antigens are significantly affected by time elapsed since blood collection. Results have been shown to be variable for specimens assessed more than 24- and less than 48-hours postblood collection; therefore, lymphocyte proliferation results must be interpreted with due caution and results should be correlated with clinical context.

Cautions

When interpreting results it should be kept in mind that the range of lymphocyte proliferative responses observed in healthy, immunologically competent individuals is large. The reference ranges provided will be helpful in ascertaining the magnitude of the normal response.

Lymphocyte proliferation to mitogens is known to be affected by concomitant use of steroids, immunosuppressive agents, including cyclosporine, tacrolimus (FK506), Cellcept (mycophenolate mofetil), immunomodulatory agents, alcohol, and physiological and social stress.

Specimens older than 24 hours may give spurious results.

Diminished results may be obtained in cultures that contain excess neutrophils or nonviable cells.(7)

Timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets. See data under Clinical Information.

Clinical Reference


Performance

Method Description

Peripheral blood mononuclear cells in RPMI 1640 medium supplemented with L-glutamine and 5% human AB serum are incubated unstimulated or stimulated with varying concentrations of pokeweed (PWM)- and phytohemagglutinin. A daily experimental normal control is included with each batch of patient samples to serve as an internal control.

Following incubation, the cells are assessed for proliferation and stained with the following markers: CD45+ lymphocytes, CD3+ T cells, CD69+ activated cells, and CD19+ B cells (PWM only). Results are reported for the percent viable cells on day 0, as well as the percentage of proliferating cells of total lymphocytes, T cells, and B cells (PWM only). (Package insert: 4, Invitrogen Click-iT-EdU; Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday

Do not send specimen after Thursday.

Analytic Time

8 days

Maximum Laboratory Time

11 days

Specimen Retention Time

Entire specimen is used in preparation of the assay

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.
Test Definition: LPMGF
Lymphocyte Proliferation, Mitogens

Test Classification
This test was developed using an analyte specific reagent. Its performance characteristics were determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
86353
86353 (if appropriate)

LOINC® Information

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<th>Test ID</th>
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<th>Order LOINC Value</th>
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<td>LPMGF</td>
<td>Lymphocyte Proliferation, Mitogens</td>
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<tbody>
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<td>Interpretation</td>
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