

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

Test Id	Reporting Name	Available Separately	Always Performed
MGSTM	Additional Flow Stimulant, LPMGF	No, (Bill Only)	No

Testing Algorithm

To ensure the most reliable results, if insufficient peripheral blood mononuclear cells are isolated from the patient's sample due to low white blood cell counts or specimen volume received, selected dilutions or stimulants may not be tested at the discretion of the laboratory.

Testing with one stimulant will always be performed. When adequate specimen is available for both stimulants to be tested, the second stimulant will be evaluated at an additional charge.

Method Name

Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

WB Sodium Heparin

Shipping Instructions

Testing is performed Monday through Friday. The test may be canceled if specimens are not received in the laboratory by Friday at 4 pm (CST). Samples arriving on weekends and observed holidays may be canceled.

Collect 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 collection.

Necessary Information

1. Date and time of collection are required.
2. The ordering healthcare professional's name and phone number are required.

Specimen Required

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

Container/Tube: Green top (sodium heparin)

Specimen Volume: 20 mL

See tables for information on recommended volume based on absolute lymphocyte count

Pediatric Volume:

- <3 months: 1 mL
- 3-24 months: 3 mL
- 25 months-18 years: 5 mL

Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**

Additional Information: For serial monitoring, it is recommended that specimen collection be performed at the same time of day.

Table. Blood Volume Recommendations Based on Absolute Lymphocyte Count (ALC)

Mitogen only			
ALC x 10(9)/L	Blood volume for minimum phytohemagglutinin (PHA) only	Blood volume for minimum PHA and pokeweed mitogen (PWM)	Blood volume for full assay
<0.5	>6.5 mL	>8.5 mL	>22 mL
0.5-1.0	6.5 mL	8.5 mL	22 mL
1.1-1.5	3.0 mL	4.0 mL	10 mL
1.6-2.0	2.0 mL	2.5 mL	7 mL
2.1-3.0	1.5 mL	2.0 mL	6 mL
>3.1	1.0 mL	1.5 mL	4 mL
4.1-5.0	1.0 mL	1.0 mL	3 mL
>5.0	1.0 mL	1.0 mL	2 mL

Mitogen and antigen		
ALC x 10(9)/L	Blood volume for minimum of each assay	Blood volume for full assay
<0.5	>28 mL	>60 mL

0.5-1.0	28 mL	60 mL
1.1-1.5	12 mL	30 mL
1.6-2.0	8.5 mL	20 mL
2.1-3.0	6.5 mL	15 mL
3.1-4.0	4.5 mL	10 mL
4.1-5.0	3.5 mL	8 mL
>5.0	2.5 mL	6 mL

Specimen Minimum Volume

See Specimen Required

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
WB Sodium Heparin	Ambient	48 hours	GREEN TOP/HEP

Clinical & 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) A widely used method for assessing lymphocyte proliferation has hitherto been the measurement of (3)H-thymidine incorporated into the DNA of proliferating cells. The disadvantages with the (3)H-thymidine method of lymphocyte proliferation are as follows:

1. The technique is cumbersome due to the use of radioactivity.
2. It does not distinguish between different cell populations responding 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 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 distinguished 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^{2+}) in T cells, which is essential for T-cell proliferation. While PHA is a strong T-cell mitogen, PWM is a weak T-cell mitogen but induces B-cell activation and proliferation as well.

This assay uses 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 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-aminoactinomycin D (7-AAD) and annexin V.

The Click-iT-EdU assay has shown to be an acceptable alternative to the $(3)\text{H}$ -thymidine assay for measuring lymphocyte/T-cell proliferation.(5,6)

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 a.m. and noon, with no change between noon and afternoon. Natural killer-cell counts, on the other hand, are constant throughout the day. Circadian variations in circulating T-cell counts negatively correlate 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(7) and during summer compared to winter.(8) 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: $\geq 75.0\%$

Maximum proliferation of phytohemagglutinin as % CD45: $\geq 49.9\%$

Maximum proliferation of phytohemagglutinin as % CD3: $\geq 58.5\%$

Maximum proliferation of pokeweed mitogen as % CD45: $\geq 4.5\%$

Maximum proliferation of pokeweed mitogen as % CD3: $\geq 3.5\%$

Maximum proliferation of pokeweed mitogen as % CD19: $\geq 3.9\%$

Interpretation

Abnormal mitogen stimulation test results are indicative of impaired T-cell function if T-cell counts are normal or only modestly decreased. If there is profound T-cell lymphopenia, there could be a dilution effect with under-representation of T cells within the peripheral blood mononuclear cell 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.

No single laboratory test can identify or define impaired cellular immunity on its own.

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.⁽⁹⁾ In an in-house 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 and phytohemagglutinin, 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.

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 between 24- and 48-hours post blood collection; therefore, lymphocyte proliferation results must be interpreted with due caution and results should be correlated with clinical context.

Cautions

When interpreting results, note that the range of lymphocyte proliferative responses observed in healthy, immunologically competent individuals is large. The reference values (based on healthy donors) provided will be helpful in ascertaining the magnitude of the patient 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 yield spurious results.

Diminished results may be obtained in specimens that contain excess neutrophils or nonviable cells.⁽¹⁰⁾

Timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

Clinical Reference

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6. Clarke ST, Calderon V, Bradford JA. Click chemistry for analysis of cell proliferation in flow cytometry. Curr Protoc Cytom. 2017;82:7.49.1-7.49.30. doi:10.1002/cpcy.24

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8. Paglieroni TG, Holland PV. Circannual variation in lymphocyte subsets, revisited. Transfusion. 1994;34(6):512-516

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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). (Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

8 to 11 days

Specimen Retention Time

Not retained. Entire specimen is used in preparation of the assay

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

Fees & 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 account representative. For assistance, contact [Customer Service](#).

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 US Food and Drug Administration.

CPT Code Information

86353
86353 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
LPMGF	Lymphocyte Proliferation, Mitogens	69018-0

Result ID	Test Result Name	Result LOINC® Value
32318	Viab of Lymphs at Day 0	33193-4
32320	Max Prolif of PHA as % CD3	57741-1
32319	Max Prolif of PHA as % CD45	69038-8
32323	Max Prolif of PWM as % CD19	69037-0
32322	Max Prolif of PWM as % CD3	69020-6
32321	Max Prolif of PWM as % CD45	69019-8
32317	Interpretation	69052-9
32324	Mitogen Comment	48767-8