MAYO CLINIC LABORATORIES

Test Definition: LPAGF

Lymphocyte Proliferation to Antigens, Blood

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

This test is **not intended** for assessment of maternal engraftment.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
AGSTM	Additional Flow Stimulant, LPAGF	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

Ordering Guidance

This test **should not be ordered** for patients younger than 3 months unless there is a clinical history of candidiasis. For more information see Cautions.

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)

Antigen only		
ALC x 10(9)/L	**Blood volume for minimum *Candida albicans* (CA) and tetanus toxoid (TT) Only**	**Blood volume for full assay**
<0.5	>18.5 mL	>40 mL
0.5-1.0	18.5 mL	40 mL
1.1-1.5	8.5 mL	20 mL
1.6-2.0	6.0 mL	12 mL
2.1-3.0	4.5 mL	10 mL
3.1-4.0	3.0 mL	6 mL
4.1-5.0	2.5 mL	5 mL

>5.0	2.0 mL	4 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

Determining impaired T-cell function by culturing human peripheral blood mononuclear cells (PBMC) in vitro with recall antigens, including *Candida albicans* (CA) and tetanus toxoid (TT), has been part of the diagnostic immunology repertoire for many years.(1,2) A widely used method for assessing lymphocyte proliferation to antigens 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:

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.

Antigens, like CA and TT, have been widely used to measure antigen-specific recall (anamnestic) T-cell responses when assessing cellular immunity. In fact, it may be more revealing about cellular immune compromise than assessing the response of lymphocytes to mitogens because the latter can induce T-cell proliferative responses even if those T cells are incapable of responding adequately to antigenic (physiologic) stimuli. Therefore, abnormal T-cell responses to antigens are considered a diagnostically more sensitive, but less specific, test of aberrant T-cell function.(2)

Antigens used in recall assays measure the ability of T cells bearing specific T-cell receptors to respond to such antigens when processed and presented by antigen-presenting cells. The antigens used for assessment of the cellular immune response are selected to represent antigens, seen by a majority of the population, either through natural exposure (CA) or as a result of vaccination (TT).

This assay uses a method that directly measures the S-phase proliferation of lymphocytes through the use of Click chemistry. 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)H-thymidine assay for measuring lymphocyte/T-cell proliferation.(3,4)

The degree of impairment of antigen-specific T-cell responses can vary depending on the nature of the cellular immune compromise. For example, some, but not all, patients with partial DiGeorge syndrome, a primary cellular immunodeficiency, have been reported to have either decreased or absent T-cell responses to CA and TT.(5) Similarly, relative immune compromise, especially to TT, has been reported in children with vitamin A deficiency, but the measurements have been largely of the humoral immune response. Since this requires participation of the cellular immune compartment, it can be postulated that there could be a potential impairment of antigen-specific T-cell responses as well.(6)

Reference Values

Viability of lymphocytes at day 0: > or =75.0%
Maximum proliferation of *Candida albicans* as % CD45: > or =5.7%
Maximum proliferation of *Candida albicans* as % CD3: > or =3.0%
Maximum proliferation of tetanus toxoid as % CD45: > or =5.2%
Maximum proliferation of tetanus toxoid as % CD3: > or =3.3%

Interpretation

Abnormal antigen 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 underrepresentation 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.

In the case of antigen-specific T-cell responses to tetanus toxoid (TT), there can be absent responses due to natural waning of cellular immunity, if the interval between vaccinations has exceeded the recommended period, especially in adults. In such circumstances, it would be appropriate to measure TT-specific T-cell responses 4 to 6 weeks after a booster vaccination.

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 antigens can be regarded as a more sensitive, but less specific, 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. It is reasonable to expect robust T-cell-specific responses to TT in children without cellular immune compromise, as a result of repeated childhood vaccinations. The response to *Candida albicans* can be more variable depending on the extent of exposure and age of exposure. 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.

Cautions

There is no clinical utility to assessing antigen responses in infants younger than 3 months due to limited antigen exposure and vaccination. The only exception would be infants who develop candidiasis prior to 3 months of age.

When interpreting results, note 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.

Lymphocyte proliferation responses to antigens (and mitogens) 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. Specimens more than 24-hours old may yield 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' lymphocyte subsets (specifically T cells in this context) and their diurnal variation can potentially affect the magnitude of the proliferative response, especially in patients who already have severe T-cell lymphopenia. 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(8) and during summer compared to winter.(9)

Clinical Reference

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Performance

Method Description

Peripheral blood mononuclear cells in RPMI 1640 medium supplemented with L-glutamine and 20% human AB serum are added to wells that contain either medium plus 20% AB serum alone (unstimulated) or varying concentrations of *Candida albicans* and tetanus toxoid antigens. Cells are analyzed by flow cytometry for day 0 viability as outlined below. After 6 days of incubation, EdU (thymidine analog) is added to all wells, where it becomes incorporated into the synthesizing DNA during a final 18- to 24-hour incubation period. A daily experimental normal control is included with each batch of patient samples to serve as an internal control.

On day 7 following the second incubation, the cells are stained for proliferation via a copper-catalyzed click chemistry

reaction where the EdU, an alkyne, is covalently bonded to a fluorescent azide. Cells are also stained for the following markers: CD45+ lymphocytes, CD3+ T cells, and CD69+ activated T cells. Results are reported for the percent viable cells on day 0, as well as percent proliferating cells within each group of lymphocytes and T cells.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
11 to 14 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
LPAGF	Lymphocyte Proliferation, Antigens	69042-0

Result ID	Test Result Name	Result LOINC® Value
32328	Max Prolif of CA as % CD3	69015-6
32327	Max Prolif of CA as % CD45	69014-9
32330	Max Prolif of TT as % CD3	69029-7

32329	Max Prolif of TT as % CD45	69016-4
32325	Interpretation	69052-9
32331	Antigen Comment	48767-8
32326	Viab of Lymphs at Day 0	33193-4