MAYO CLINIC LABORATORIES

Test Definition: LPA3P

Lymphocyte Proliferation to Anti-CD3/Anti-CD28 and Anti-CD3/Interleukin-2 (IL-2), Flow Cytometry, Blood

Overview

Useful For

A second-level test after lymphocyte proliferation to mitogens (specifically phytohemagglutinin) has been assessed

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

Evaluation of T-cell function in patients receiving immunosuppressive or immunomodulatory therapy

Evaluation of T-cell function in the context of identifying neutralizing antibodies in patients receiving therapeutic anti-CD3 antibody immunosuppression for solid organ transplantation or autoimmune diseases, such as type 1 diabetes

This panel is **not useful as** a first-level test for assessing lymphocyte (T-cell) function.

Reflex Tests

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

Testing Algorithm

To ensure the most reliable results, if insufficient peripheral blood mononuclear cells are isolated from the patient's specimen 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 performed with at least one stimulant will be reported. When adequate specimen is available, the second and third stimulants will be evaluated, each 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 specimen 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 table 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)

ALC	Blood volume for minimum aCD28 only	Blood volume for minimum of aCD3, aCD28, and IL-2	Blood volume for full assay
<0.5	>15 mL	>28 mL	>50 mL

0.5-1.0	15 mL	28 mL	50 mL
1.1-1.5	6.5 mL	12 mL	24 mL
1.6-2.0	4.5 mL	8.5 mL	16 mL
2.1-3.0	3.5 mL	6.5 mL	12 mL
3.1-4.0	2.5 mL	4.5 mL	8 mL
4.1-5.0	1.8 mL	3.5 mL	6 mL
>5.0	1.5 mL	2.5 mL	5 mL

Specimen Minimum Volume

See Specimen Required

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

T-cell stimulation in vitro is used extensively in the diagnostic immunology arena for facilitating T-cell proliferation and evaluation of T-cell function in a variety of clinical contexts.(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 of 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 the contribution of apoptosis or 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 peripheral blood mononuclear cell 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.

While mitogens such as phytohemagglutinin activate T cells by binding to cell membrane glycoproteins, including the T-cell receptor (TCR)-CD3 complex, there are mitogenic or co-mitogenic antibodies, including those directed against the CD3 coreceptor that can stimulate T-cell proliferation. Typically, anti-CD3 antibodies provide an initial activation signal but do not induce significant proliferation, and the addition of a costimulatory antibody (anti-CD28) provides the stimulus for robust proliferation.(3) An exogenous T-cell growth factor, such as interleukin-2 (IL-2), may also be used as an alternate to anti-CD28 costimulation, and in patients with suspected IL-2 receptor-associated signaling defects, it may be more helpful than the use of anti-CD28. IL-2, an autocrine cytokine, has been demonstrated to be critical in T-cell proliferation.(4,5) The interaction of IL-2 with the IL-2 receptor (IL-2R) plays a central role in regulation of T-cell proliferation.(4) Triggering of the TCR leads to synthesis of IL-2 in certain T-cell subsets and induction of high-affinity IL-2Rs in antigen- or mitogen-activated T cells, and the binding of IL-2 to IL-2R ultimately leads to T-cell proliferation. The use of exogenous IL-2 in association with anti-CD3 allows discrimination of whether T cells, which cannot proliferate to other mitogenic signals, can respond to a potent growth factor such as IL-2. Stimulation of T cells with soluble antibodies to anti-CD3 (and the associated TCR complex) causes mobilization of cytoplasmic calcium and translocation of protein kinase C from the cytoplasm to the cell membrane. This stimulation also causes induction of phosphatidylinositol metabolism and subsequent IL-2 production for proliferation.(6) T-cell activation induced by anti-CD3 antibody requires prolonged stimulation of protein kinase C, which apparently can be achieved by the concomitant use of the anti-CD28 antibody for costimulation without addition of other mitogenic stimuli, such as phorbol myristate acetate.(7)

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. The Click-iT-EdU assay has been shown to be an acceptable alternative to the (3)H-thymidine assay for measuring lymphocyte/T-cell proliferation.(8,9)

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.(10) 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 and annexin V.

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, is 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, the 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(11) and during summer compared to winter.(12) 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 anti-CD3 as % CD45: > or =19.4%
Maximum proliferation of anti-CD3 as % CD3: > or =20.3%
Maximum proliferation of anti-CD3 + anti-CD28 as % CD45: > or =37.5%
Maximum proliferation of anti-CD3 + anti-CD28 as % CD3: > or =44.6%
Maximum proliferation of anti-CD3 + IL-2 as % CD45: > or =41.7%
Maximum proliferation of anti-CD3 + IL-2 as % CD3: > or =46.2%

Interpretation

Abnormal anti-CD3/anti-CD28/interleukin-2 (IL-2) 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, it must be kept in mind that there could be a "dilution" effect with underrepresentation of T cells within the peripheral blood mononuclear cells 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. The evaluation of T-cell proliferation to anti-CD3/IL-2 is likely to be helpful in assessing T-cell function in patients with refractory responses to other mitogenic and antigenic stimuli, specifically in the context of IL-2-receptor signaling defects, enabling greater mechanistic insight into the origins of T-cell dysfunction.

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 any mitogenic stimulus, including anti-CD3/anti-CD28, 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.(13) In our evaluation of 43 pediatric samples (of all ages) with adult normal controls, only 21% and 14% were below the tenth percentile of the adult reference range for the mitogens, pokeweed mitogen and phytohemagglutinin, respectively. Comparisons between pediatric and adult data have not been performed for anti-CD3/anti-CD28 due to unavailability of prospective blood samples from healthy or patient pediatric donors for purposes of analytical validation.

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 (including anti-CD3 stimulation) 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 the range of lymphocyte proliferative responses observed in healthy, immunologically competent individuals is large. The reference values provided (based on healthy donors) 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 (CsA), tacrolimus (FK506), CellCept (mycophenolate mofetil), immunomodulatory agents, alcohol, and physiological and social stress. Calcineurin inhibitors, such as CsA and tacrolimus would specifically inhibit interleukin-2 (IL-2)-stimulated proliferation since they specifically block production of IL-2 after T-cell activation. Also, IL-2-receptor-targeting monoclonal antibodies, such as daclizumab and basiliximab will also interfere with IL-2-stimulated proliferation.

Anti-CD3 proliferation should not be used as a first-line test when assessing lymphocyte proliferative responses. Ideally, mitogen proliferation (especially to phytohemagglutinin) should be assessed first, followed by antigens. If there are abnormal results to either, especially the former, it would be worthwhile to pursue additional testing for lymphocyte proliferation to anti-CD3 panel.

Specimens older than 24 hours may yield spurious results.

Diminished results may be obtained in specimens that contain excess neutrophils or nonviable cells.(14)

Clinical Reference

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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 anti-CD3, anti-CD3+anti-CD28, or anti-CD3+IL-2. 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, and CD69+ activated cells. Results are reported for the percent viable cells on day 0, as well as the percentage of proliferating cells of total lymphocytes and T cells.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

5 to 8 days

Specimen Retention Time

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

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

Test Definition: LPA3P

Lymphocyte Proliferation to Anti-CD3/Anti-CD28 and Anti-CD3/Interleukin-2 (IL-2), Flow Cytometry, Blood

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 x 2
86353 (as appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
LPA3P	Lymphocyte Proliferation, aCD3	59063-8

Result ID	Test Result Name	Result LOINC® Value
35203	Viab of Lymphs at Day 0	33193-4
35171	Max Prolif, soluble aCD3 as % CD45	81760-1
35172	Max Prolif, soluble aCD3 as % CD3	81756-9
35173	Max Prolif, soluble aCD28 as % CD45	81759-3
35174	Max Prolif, soluble aCD28 as % CD3	81758-5
35176	Max Prolif, soluble IL2 as % CD45	81755-1
35177	Max Prolif, soluble IL2 as % CD3	81757-7
35204	aCD3 Comment	48767-8
35205	Interpretation	69965-2