Lab Dept: Chemistry

Test Name: LYMPHOCYTE PROLIFERATION, ANTIGENS

General Information

Lab Order Codes: LBAB

Synonyms: Blastogenesis Antigens; Immune Competence; Lymphocyte Phytohemagglutinin; Lymphocyte Transformation

CPT Codes: 86353 – Lymphocyte transformation, mitogen or antigen induced blasotgenesis

Test Includes: Peripheral blood monoculear cells (2x10[6]) cells/mL) in RPMI 1640 medium supplemented with L-glutamine and 20% human AB serum) are added in duplicate in 16 wells of a sterile saline, flat-bottom, 48-well culture plate that contains either medium plus 20% AB serum alone (unstimulated) or varying concentrations of antigens: CA (1, 10 and 20 micrograms/mL) and TT (0.5, 1 and 2.5 micrograms/mL). Cells are analyzed by flow cytometry for Day 0) viability as outlined below. After 6 days on incubation, EdU (thymidine analog) is added to all wells, where it becomes incorporated into the synthesizing DNA during a final 18-24 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 duplicate wells are prepared separately, the first set for viability with viability stain 7-ADD, apoptosis stain Annexin V, and lymphocyte marker CD45. The second set is 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 percent viable cells on Day 0, as well as percent proliferating cells within each group of lymphocytes and T-cells.

Logistics

Test Indications:	Assessing T-cell function in patients on immunosuppressive therapy, including solid-organ transplant patients. Evaluation of patients suspected of having diminished cellular immune function. Evaluation of T-cell function in patients with primary immunodeficiencies, either cellular (DiGeorge syndrome, T-negative CSID, 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 (BMT) or hematopoietic stem cell transplantation (HSCT).
Lab Testing Sections:	Chemistry - Sendouts
Referred to:	Mayo Medical Laboratories (MML Test: LPAGF)
Phone Numbers:	MIN Lab: 612-813-6280 STP Lab: 651-220-6550
Test Availability:	Monday – Thursday at 3 PM. The cut off draw time of 3 PM on Thursday allows for specimen transport to Mayo prior to the last set up time of the week on Friday at 4 PM. Specimens submitted outside of this timeframe will be rejected.
Turnaround Time:	11 to 14 days, test set up Monday - Friday
Special Instructions:	Specimens must reach the reference lab within 24 hours of collection. See Collection for important information. For serial monitoring, it is recommended that the sample be collected at the same time of day per collection.

Specimen

Specimen Type:	Whole blood
Container:	Green top (Sodium heparin) tube
Draw Volume:	Draw volume varies by age. **Only draw Minimum Volume if absolutely necessary.** Reference table below:

Patient Age	**Requested Volume**	**Minimum Volume**

<3 months	1 mL	1 mL
3 – 24 months	3 mL	1 mL
25 months – 18 years	5 mL	2 mL
>18 years	20 mL	6 mL

Processed Volume: Same as Draw Volume

Collection: Routine venipuncture. **Note:** Specimens must be filled by needle through the stopper to maintain a closed system. **DO NOT** fill tube by removing the stopper. Invert gently to mix.

Special Processing: Lab Staff: **Do Not** Centrifuge. Specimen should remain in original collection container. Store and ship at ambient temperatures. Specify Antigen vs. Mitogen on tube. Forward promptly.

Patient Preparation: None

Sample Rejection: Specimens other than whole blood; anticoagulants other than sodium heparin; frozen specimens; gross hemolysis; gross lipemia; mislabeled or unlabeled specimens

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Interpretive

Reference Range:

Reference name:	**Result:**
Viability of Lymphs at Day 0	>=75.0%
Max Proliferation of CA as %CD45	>=5.7%
Max Proliferation of CA as %CD3	>=3.0%
Max Proliferation of TT as %CD45	>=5.2%
Max Proliferation of TT as %CD3	>=3.3%

Critical Values: N/A

Limitations:

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.

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 >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. Test specimens >24 hours old may give spurious results.

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

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 sever 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 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 naïve 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 the summer compared to winter.

Methodology:

Flow cytometry

References:

Mayo Medical Laboratories Web Page December 2017

Updates:

1/4/2006: MML changed units from DPM to S.I. The S.I. is a measure of proliferation of the patient's cells compared to cells from a normal control tested simultaneously. MML has always tested a normal control along with patient specimens, but this is not apparent from the way results were being reported prior to 1/4/2006.
1/18/2011: Method change, reference range change, draw volume update.
7/22/2015: Ref range update.