Lab Dept:	**Chemistry**
Test Name:	**LYMPHOCYTE PROLIFERATION, MITOGENS**

General Information

Lab Order Codes:	MPRB
Synonyms:	Mitogen Profile; Mitogen Studies; Blastogenesis Mitogens; Mitogen Stimulation
CPT Codes:	86353 – Lymphocyte transformation, mitogen or antigen induced blastogenesis
Test Includes:	Peripheral blood mononuclear cells (500,000 cells/mL) in RPMI 1640 medium supplemented with L-glutamine and 5% human AB serum are added in duplicate to 16 wells of a sterile, flat-bottom, 48-well culture plate that contains either medium plus 5% AB serum alone (unstimulated) or varying concentrations of mitogens PWM (0.5, 5, and 10 micrograms/mL) and PHA (0.5, 2.5, and 5 micrograms/mL). Cells are incubated for 72 hours, after which EdU (thymidine analog) is added to all wells where it becomes incorporated into the synthesizing DNA during a second incubation of 18 to 24 hours. A daily experimental normal control is included with each batch of patient samples to serve as an internal control. Following the second incubation, the duplicate wells are prepared separately, the first set for viability with viability stain 7-AAD, 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, CD69+ activated cells, and CD19+ B cells (PWM only). Results are reported for the percent viable, dead, and apoptotic lymphocytes, as well as percent proliferating cells with in each group of lymphocytes, T cells, and B cells (PWM only).

Logistics

Test Indications:

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 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 (BMT) or hematopoietic stem cell transplantation (HSCT).

Lab Testing Sections: Chemistry - Sendouts

Referred to: Mayo Medical Laboratories (MML Test: 60591/LPMGF)

Phone Numbers: MIN Lab: 612-813-6280

STP Lab: 651-220-6550

Test Availability: Daily, 24 hours

Turnaround Time: 8-11 days, test set up Monday - Friday

Special Instructions: Specimen must arrive within 24 hours of draw. Send specimen Monday - Thursday only. 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 (Na Heparin) tube

Draw Volume: Draw volume varies by age. Reference table below:

Patient Age	Requested Volume	Minimum Volume
<3 months	1 mL	1 mL
3 months – 5 years	2 mL	1 mL

6 – 18 years	3 mL	1 mL
>18 years	10 mL	1 mL

Processed Volume: Same as Draw Volume

Collection: Routine venipuncture, send specimen to the laboratory immediately after collection. **Note:** Specimens must be filled by needle through the stopper to maintain a closed system. **DO NOT** fill tube by removing the stopper.

Special Processing: Lab Staff: **Do Not** centrifuge. Send in original collection tube. Keep at room temperature. Specify “mitogen” to differentiate from “antigen” testing. 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

---

Interpretive

Reference Range:

Reference name:	**Result:**
Viability of Lymphs at Day 0	>= 75.0%
Max Proliferation of PHA as %CD45	>=49.9%
Max Proliferation of PHA as %CD3	>=58.5%
Max Proliferation of PWM as %CD45	>=4.5%
Max Proliferation of PWM as %CD3	>=3.5%
Max Proliferation of PWM as %CD19	>=3.9%

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 at 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. 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 for lymphocyte subsets.
Methodology:	Flow cytometry
References:	Mayo Medical Laboratories Web Page January 2011
Updates:	1/4/2006: MML changed units from DPM to %NC. 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. 9/2/2008: Removed %NC from reporting units. Extended turnaround time, previously listed as 6-9 days. 1/18/2011: Method change, reference range change, draw volume update. 1/19/2011: Viability of Lymphs at Day 0 reference value change.