Test Definition: GLICP

CD8 T-Cell Immune Competence Panel, Global, Whole Blood

Overview

Useful For

Determining over immunosuppression within the CD8 T-cell compartment, when used on transplant recipients and patients with autoimmune disorders receiving therapy with immunosuppressant agents

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
TBBS	QN Lymphocyte Subsets: T, B, and NK	Yes	Yes
GLIC	CD8 Immune Competence, B	Yes	Yes

Testing Algorithm

When multiple specimen types are required to perform a panel of tests, the laboratory will perform the tests for which the appropriate specimen type was received, and the laboratory will cancel those for which the appropriate specimen was not received.

Method Name

Flow Cytometry

NY State Available

No

Specimen

Specimen Type

Whole Blood EDTA
WB Sodium Heparin

Ordering Guidance

This test requires multiple whole blood specimens to perform all testing. If only one whole blood specimen type is received, only the testing associated with that specimen type will be performed. Be advised that this may change the degree of interpretation received with the report.

Shipping Instructions

Testing is performed Monday through Friday. Specimens not received by 4 p.m. (CST) on Friday may be canceled.

Collect and package specimens as close to shipping time as possible. Ship specimens overnight in an Ambient Shipping

Box-Critical Specimens Only (T668) following the instructions in the mailer. It is recommended that specimens arrive within 24 hours of collection.

Samples arriving on the weekend and observed holidays may be canceled.

Necessary Information

Date and time of collection and ordering healthcare professional name and phone number are required.

Specimen Required

Two separate whole blood specimens, EDTA and (possibly 2 tubes) sodium heparin, are required.
For serial monitoring, it is recommended that specimens are collected at the same time of day.

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

Specimen Type: EDTA whole blood
Container/Tube: Lavender top (EDTA)
Specimen Volume: 3 mL
Collection Instructions:
1. Send whole blood specimen in original tube. **Do not aliquot.**
2. Label specimen as TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK) Cells, Blood
Additional Information: For serial monitoring, it is recommended that specimen collection be performed at the same time of day.

Specimen Type: Sodium heparin whole blood
Container/Tube: Green top (sodium heparin)
Specimen Volume: 15 mL
Collection Instructions:
1. Send whole blood specimen in original tube. **Do not aliquot.**
2. Label specimen as GLIC / CD8 T-Cell Immune Competence, Global, Blood

Specimen Minimum Volume

Sodium heparin whole blood: 10 mL
EDTA whole blood: 1 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Ambient	52 hours	PURPLE OR PINK TOP/EDTA
WB Sodium Heparin	Ambient	48 hours	GREEN TOP/HEP

Clinical & Interpretive

Clinical Information

CD8 T cells play an important role in the immune response to viral or intracellular infectious agents, as well as antitumor immunity and immune surveillance.

Upon activation, CD8 T cells mediate a variety of effector functions, including cytokine secretion and cytotoxicity. Interferon-gamma (IFN-gamma) is one of the early cytokines produced by CD8 T cells; it is released within a few hours of activation.(1) The cytotoxic function is mediated by the contents of the cytolytic granules.(1) Cell-surface mobilization of the cytolytic granule components, CD107a and CD107b, also known as lysosome-associated membrane proteins LAMP-1 and LAMP-2, occurs when CD8 T cells mediate their cytolytic function and degranulate.(2)

CD8 T-cell activation occurs either through the T-cell receptor peptide major histocompatibility complex or by use of a mitogen (eg, phorbol myristate acetate and the calcium ionophore ionomycin). Mitogen-mediated activation is antigen nonspecific.

Impairment of global CD8 T-cell activation (due to inherent cellular immunodeficiency or as a consequence of over-immunosuppression by therapeutic agents) results in reduced production of IFN-gamma and other cytokines, reduced cytotoxic function, and increased risk for developing infectious complications. Agents associated with over-immunosuppression include the calcineurin inhibitors (eg, cyclosporine A, FK506 [Prograf/tacrolimus], and rapamycin [sirolimus]), antimetabolites (eg, mycophenolate mofetil), and thymoglobulin.

Immunosuppression is most commonly used for allograft maintenance in solid-organ transplant recipients, to prevent graft-versus-host disease in allogeneic hematopoietic stem cell transplant patients, and to treat patients with autoimmune diseases. In these settings, reducing the risk for developing infectious complications as a result of over-immunosuppression is a clinical challenge.

Therapeutic drug monitoring is routinely used in the transplant practice to avoid overtreatment and to determine patient compliance. However, the levels of drugs measured in blood specimens do not directly correlate with the administered dose due to individual pharmacokinetic differences.(3) Furthermore, drug levels may not necessarily correlate with biological activity of the drug. Consequently, it may be beneficial to consider modification of the immunosuppression regimen based on the patient's level of functional immune competence.

This assay provides a means to evaluate over-immunosuppression within the CD8 T-cell compartment (global CD8 T-cell function). Intracellular IFN-gamma expression is a marker for CD8 T-cell activation. Surface CD107a and CD107b are markers for cytotoxic function. This test may be most useful when ordered at the end of induction immunosuppression and 2 to 3 months after maintenance immunosuppression to ensure that global CD8 T-cell function is not compromised. The test may also provide value when immunosuppression is increased to halt or prevent graft rejection, to provide information on a balance between over-immunosuppression with subsequent infectious comorbidities and under-immunosuppression with resultant graft rejection.

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.(4) Circadian variations in circulating T-cell counts have been shown to be negatively correlated with plasma cortisol concentration.(5-7) In fact, cortisol and catecholamine concentrations control distribution and therefore, numbers of naive versus effector CD4 and CD8 T cells.(5) It is generally accepted that lower CD4 T-cell counts are seen in the morning compared to the evening(8) and during summer compared to winter.(9) These data, therefore, indicate that timing and consistency in timing of blood collection is critical when serially monitoring patients for lymphocyte subsets.

Reference Values

The appropriate age-related reference values will be provided on the report.

Interpretation

Interferon-gamma (IFN-gamma) and CD107a and CD107b expression below the defined reference range are consistent with a global impairment in CD8 T-cell function, most likely due to over immunosuppression.

IFN-gamma, CD107a, and CD107b levels greater than the defined reference range are unlikely to have any clinical significance.

Cautions

This assay is specific only for CD8 T cells; it does not provide information for overall T-cell competence.

Further studies are needed to determine if, within the reference range, certain levels of interferon-gamma, CD107a, and CD107b expression confer greater or lesser degrees of risk for infectious disease.

Timing and consistency in timing of blood collection are critical when serially monitoring patients for lymphocyte subsets. See Clinical Information

Clinical Reference

1. Betts MR, Casaza JP, Patterson BA, et al. Putative immunodominant human immunodeficiency virus-specific CD8(+) T-cell responses cannot be predicted by MHC class I haplotype. J Virol. 2000;74(19):9144-9151. doi:10.1128/jvi.74.19.9144-9151.2000
2. Peters PJ, Borst J, Oorschot V, et al. Cytotoxic T-lymphocyte granules are secretory lysosomes, containing both perforin and granzymes. J Exp Med. 1991;173(5):1099-1109. doi:10.1084/jem.173.5.1099
3. Venkataramanan R, Shaw LM, Sarkozi L, et al. Clinical utility of monitoring tacrolimus blood concentrations in liver transplant patients. J Clin Pharmacol. 2001;41(5):542-551. doi:10.1177/00912700122010429
4. Carmichael KF, Abayomi A. Analysis of diurnal variation of lymphocyte subsets in healthy subjects and its implication in HIV monitoring and treatment. 15th Intl Conference on AIDS, Bangkok, Thailand, 2004, Abstract # B11052
5. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. Blood. 2009;113(21):5134-5143. doi:10.1182/blood-2008-11-190769
6. Dimitrov S, Lange T, Nohroudi K, Born J. Number and function of circulating human antigen presenting cells regulated by sleep. Sleep. 2007;30(4):401-411. doi:10.1093/sleep/30.4.401
7. Kronfol Z, Nair M, Zhang Q, Hill EE, Brown MB. Circadian immune measures in healthy volunteers: Relationship to hypothalamic-pituitary-adrenal axis hormones and sympathetic neurotransmitters. Pyschosom Med. 1997;59(1):42-50. doi:10.1097/00006842-199701000-00006

8. Malone JL, Simms TE, Gray GC, Wagner KF, Burge JR, Burke DS. Sources of variability in repeated T-helper lymphocyte counts from HIV 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. J Acquir Immune Defic Syndr (1988). 1990;3(2):144-151
9. Paglieroni TG, Holland PV. Circannual variation in lymphocyte subsets, revisited. Transfusion. 1994;34(6):512-516
10. Cabral-Marques O, Schimke LF, de Oliveira EB Jr, et al. Flow cytometry contributions for the diagnosis and immunopathological characterization of primary immunodeficiency diseases with immune dysregulation. Front Immunol. 2019;10:2742. doi:10.3389/fimmu.2019.02742
11. Meesing A, Abraham RS, Razonable RR. Clinical correlation of cytomegalovirus infection with CMV-specific CD8+ T-cell immune competence score and lymphocyte subsets in solid organ transplant recipients. Transplantation. 2019;103(4):832-838. doi:10.1097/TP.0000000000002396

Performance

Method Description

CD8 T-Cell Immune Competence, Global:
Peripheral blood mononuclear cells (PBMC), which contain CD8 T cells, are stimulated with a mixture of phorbol myristate acetate and ionomycin, and with stimulatory signals derived using antibodies against the costimulatory molecules CD28/CD49d. The cells are simultaneously treated with a mixture of brefeldin A and monensin, which blocks extracellular secretion of interferon-gamma (IFN-gamma), enabling intracellular retention and detection of the protein. PBMC that have not been stimulated are used as a control to determine the background levels of IFN-gamma and CD107a and CD107b. The cells are analyzed on the Becton Dickinson FACS Canto flow cytometer, and analysis involves gating (defining) of the CD8 T cells using an antihuman CD8 antibody. Specific IFN-gamma, CD107a, and CD107b signals are determined within the "gated" CD8 T-cell population. Global CD8 T-cell immune competence is measured by the amount of IFN-gamma produced (CD8 T-cell functional activity) and surface expression of CD107a and CD107b (cytotoxicity assessment) relative to the unstimulated control and is interpreted on the basis of the reference range determined from healthy adult donors.(Unpublished Mayo method)

Quantitative Lymphocyte Subsets: T, B, and Natural Killer:
The T, B, and natural killer-cell surface marker assay uses monoclonal antibodies to identify the various membrane antigens, and flow cytometry to enumerate the number of cells expressing these differentiation antigens. CD14 is used to exclude monocytes, thereby improving accuracy and enhancing the purity of the lymphocyte population. The results are reported as the percent of lymphocytes that are total T cells (CD3+), CD3+CD4+ T cells, CD3+CD8+ T cells, natural killer (CD16+56+, CD3-), and B-lymphocytes (CD19+), and the absolute number of each cell type per mL of blood. The assay is a 7-color no-wash procedure and the absolute counts are calculated from internal bead standards. In addition, the total lymphocyte count and the CD4:CD8 ratio are reported.(Hoffman RA, Kung PC, Hansen WP, Goedstien G. Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA. 1980:77(8):4914-4917; Mandy FF, Nicholson JK, McDougal JS; CDC. Guidelines for performing single-platform absolute CD4+ T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. Centers for Disease Control and Prevention. MMWR Recomm Rep. 2003;52(RR-2):1-13)

PDF Report

No

Day(s) Performed
Monday through Friday

Report Available
3 to 6 days

Specimen Retention Time
EDTA whole blood: 4 days; Peripheral blood mononuclear cells: 7 days

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
86355-B cells, total count
86357-Natural killer (NK) cells, total count
86359-T cells, total count
86360-Absolute CD4/CD8 count with ratio
86356 x 2

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
GLICP	CD8 Immune Competence Panel	80721-4

Result ID	Test Result Name	Result LOINC® Value
30644	CD107a/b	95203-6
30643	IFN-g	95204-4
30645	Interpretation	69052-9
4054	% CD16+CD56 (NK cells)	8112-5
4055	CD16+CD56 (NK cells)	20402-4

3322	CD3 (T Cells)	8122-4
3319	% CD4 (T Cells)	8123-2
3325	CD4 (T Cells)	24467-3
3326	CD8 (T Cells)	14135-8
3327	4/8 Ratio	54218-3
3321	CD45 Total Lymph Count	27071-0
3318	% CD19 (B Cells)	8117-4
3316	% CD3 (T Cells)	8124-0
3320	% CD8 (T Cells)	8101-8
6657	Comment	80722-2
624173	CD19 (B Cells) (TBBS)	8116-6
624009	%_Sample Viability	33193-4
624010	%_Lymphocyte Viability	33193-4