Test Definition: GLIC

CD8 T-Cell Immune Competence, Global, Blood

Overview

Useful For

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

Method Name

Flow Cytometry

NY State Available

No

Specimen

Specimen Type

WB Sodium Heparin

Shipping Instructions

Testing performed Monday through Friday. Specimens not received by 4 p.m. Central time on Friday may be canceled.

Samples arriving on the weekend 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 mailer.

It is recommended that specimens arrive within 24 hours of collection.

Necessary Information

Ordering healthcare professional name and phone number are required.

Specimen Required

Supplies: Ambient Shipping Box-Critical Specimens Only (T668)
Container/Tube: Green top (sodium heparin)
Specimen Volume: 15 mL
Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**

Specimen Minimum Volume

10 mL

Reject Due To

Gross	Reject

hemolysis	
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

The 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 overimmunosuppression 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 overimmunosuppression 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 overimmunosuppression is a clinical challenge.

Therapeutic drug monitoring is routinely used in the transplant practice to avoid overtreatment and to determine patient compliance. But, the levels of drugs measured in blood 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 overimmunosuppression 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 overimmunosuppression with subsequent infectious comorbidities and underimmunosuppression 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

Interferon-gamma (IFN-gamma) expression (as % CD8 T cells): 10.3-56.0%

CD107a/b expression (as % CD8 T cells): 8.5-49.1%

Reference values have not been established for patients who are <19 years of age.

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 overimmunosuppression.

The IFN-gamma and 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 and CD107a and b expression confer greater or lesser degrees of risk for infectious disease.

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

Supportive Data

The 95% confidence interval reference values were determined from 102 healthy adult donors.

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
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
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
4. Carmichael KF, Abayomi A. Analysis of diurnal variation of lymphocyte subsets in healthy subjects in the Caribbean, and its implication in HIV monitoring and treatment. Afr J Med Med Sci. 2006;35(1):53-57
5. Dimitrov S, Benedict C, Heutling D, et al. Cortisol and epinephrine control opposing circadian rhythms in T-cell subsets. Blood. 2009;113(21):5134-5143
6. Dimitrov S, Lange T, Nohroudi K, Born J. Number and function of circulating antigen presenting cells regulated by sleep. Sleep. 2007;30(4):401-411
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
8. Malone JL, Simms TE, Gray GC, et al. Sources of variability in repeated T-helper lymphocyte counts from HIV 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important. J AIDS. 1990;3(2):144-151
9. Paglieroni TG, Holland PV. Circannual variation in lymphocyte subsets, revisited. Transfusion 1994;34:512-516
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 26;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

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 BD FACSCanto flow cytometer and analysis involves gating (defining) of the CD8 T cells using an antihuman CD8 antibody. Specific IFN-gamma and 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)

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

3 to 6 days

Specimen Retention Time

PBMC: 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

86356 x 2

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
GLIC	CD8 Immune Competence, B	80222-3

Result ID	Test Result Name	Result LOINC® Value
30644	CD107a/b	95203-6
30643	IFN-g	95204-4
30645	Interpretation	69052-9