Overview

Useful For
Quantitation of natural killer (NK)/natural killer T (NKT) cell subsets as well as quantitation of specific cell-surface and intracellular proteins required for NK cell function

Method Name
Flow Cytometry

NY State Available
Yes

Specimen

Specimen Type
WB Sodium Heparin

Advisory Information
Mayo Lab Director/Consultant approval is required prior to ordering test, call 800-533-1710.

A minimum number of CD16+CD56+ NK cell events (as measured by flow cytometry in the laboratory) are required to perform the NKSP test. Please be advised that any sample that does not meet this minimum requirement will have the NKSP test converted to QNKS / Natural Killer (NK)/Natural Killer T (NKT) Cell Subsets, Quantitative. Further, a minimum CD45 lymph count (as measured by flow cytometry in the laboratory) is required to report the NKSP test. If that requirement is not met (eg, patients with severe lymphopenia), the test will be canceled and an alternate test will be suggested (TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK)).

Shipping Instructions
Send specimen Monday through Thursday only. Specimen must arrive within 24 hours of draw and by 10 a.m. on Friday. Draw and package specimen as close to shipping time as possible. Ship specimen overnight.

Necessary Information
Ordering physician's name and phone number are required.

Specimen Required
For serial monitoring, we recommend that specimen draws be performed at the same time of day.

Container/Tube: Sodium heparin

Specimen Volume: 5 mL

Collection Instructions: Send specimen in original tube. Do not aliquot.

Forms
If not ordering electronically, complete, print, and send a Benign Hematology Test Request Form (T755) with the specimen.

Specimen Minimum Volume
Clinical and Interpretive

Clinical Information
Natural killer (NK) cells are derived from pluripotent hematopoietic stem cell precursors, but develop independently of the thymus. They comprise a key lymphocyte subset (approximately 10%-15% of peripheral blood mononuclear cells) and are a constituent of the innate immune system, since these cells do not rearrange their germline DNA to obtain specificity. NK cells serve an important role in host defense against viral infections, as well as tumor surveillance. They are also a component of the adaptive immune response through cytokine production. NK cell functions are governed by a balance between activating receptors and inhibitory receptors.

NK cells are identified by expression of different cell-surface receptors and they are not a homogeneous population.(1) In general, the most common combination of surface markers used to identify the majority of NK cells is the absence of CD3 (CD3-), along with expression of CD56 (neural cell adhesion molecule) and CD16 (low-affinity IgG Fc receptor-FcgammaRIII). However, not all NK cells express the CD56 and CD16 markers uniformly and, therefore, can be divided into subsets based on expression of these 2 molecules.(2) The CD16+ CD56+/− (dim or negative) that are CD3- are referred to as cytotoxic NK cells, while the CD56+ (bright) CD16- NK cells are called regulatory or cytokine secreting NK cells.(3) These are not only phenotypically and functionally distinct subsets but also developmentally separate. The majority of human NK cells (approximately 90%) have dim expression of CD56 and moderate to high levels of CD16, as well as perforin and granzymes (2 proteins mediating cytolytic activity), and are therefore high in cytotoxic capability. The remaining minority (approximately 10%) of NK cells are the CD56(bright) cytokine-producing NK cells. Therefore, cytotoxicity and cytokine production are the major functions of NK cells. Cytotoxicity can be subdivided into (1) natural cytotoxicity directed largely toward virally infected cells or tumor cells, in the absence of prior stimulation or immunization, and (2) antibody-dependent cellular cytotoxicity (ADCC) directed against antibody-coated target cells.(4) Circulating NK cells are enriched for the CD56(dim) phenotype, while within the lymph nodes, NK cells are largely CD56(bright). This differential localization is related to the pattern of homing receptors expressed on NK cells: CD56(dim) NK cells express homing markers for inflamed peripheral sites, while CD56(bright) NK cells express receptors for secondary lymphoid organs. The majority of circulating human NK cells, which have cytokytic function and phenotype (CD56(dim)), are CD27−, while the CD56(bright) cells are CD27+. Therefore, the absence of CD27 expression identifies cytotoxic effector cells within the mature NK cell subsets.(5) Other markers are:

-NKp46 (CD335) is a marker expressed on the majority of human NK cells and is an activating receptor involved in non-major histocompatibility complex (MHC)-restricted natural NK cytotoxicity. It is expressed in all resting and activated NK cells, including the minor CD56(bright) cytokine-producing population. NKp46 is considered to be involved in tumor cell eradication in vivo.
-NKG2D is an activating receptor expressed on all NK cells, as well as on natural killer T (NKT) cells. NKG2D has been described as being relevant in tumor surveillance and organ transplantation.(6)

-CD69 is a marker for NK-cell activation and triggers NK-mediated cytolytic activity and sustains NK-cell activation.(7)

-CD95 (Fas or APO-1) is a marker expressed on a variety of immune cells, including lymphocytes and NK cells. CD95 is involved in mediating programmed cell death or apoptosis and has been shown to associate with NK cell regulatory function in multiple sclerosis.(8)

-CD107a and CD107b (lysosomal-associated membrane proteins 1 and 2: LAMP-1 and LAMP-2) expression are markers of NK cell functional activity and correlate with both cytokine production and NK-cell-mediated lysis of target cells.(9)

-Perforin, granzyme A, and granzyme B are components of the cytolytic granules in NK cells and associated with NK cell cytotoxic function, while interferon gamma (IFN-gamma) is produced by NK cells on activation.(10)

-NK cells also secrete other cytokines including tumor necrosis factor alpha (TNF-alpha), interleukin-1 (IL-1), IL-3, and granulocyte monocyte-colony stimulating factor (GM-CSF).

NKT cells represent a specialized T-cell population that is distinct from conventional T cells. They express an invariant T-cell receptor (TCR) that recognizes self and bacterial glycosphingolipid antigens presented by the MHC class I-like molecule, CD1d.(11) The development of NKT cells is also unique from regular T cells, as NKT cell precursors are positively selected by CD4+CD8+ cortical thymocytes and the signaling pathways differ from the conventional T cells. Activated NKT cells rapidly produce large amounts of Th1 and Th2 cytokines that transactivate other immune components and, therefore, NKT cells are involved in both innate and adaptive immune responses.(11)

NK cell deficiencies can be present as part of a larger immunological syndrome or as an isolated deficiency. Some of the primary (monogenic) immunodeficiencies that affect NK cell function or numbers include autoimmune lymphoproliferative syndrome (ALPS) related to CASP8 (caspase 8 mutations); familial hemophagocytic lymphohistiocytosis (FHL) types 2, 3, and 4 due to mutations in the PFP1 (encoding perforin), UNC13D (encoding the Munc13-4 protein), and STX-11 (encoding syntaxin -11), respectively; Hermansky-Pudlak syndrome (AP3B1); Papillon-Lefèvre syndrome (CTSC, cathepsin C); nuclear factor kappa-beta essential modulator deficiency (NEMO) due to mutations in the IKBKG gene; severe combined immunodeficiencies due to mutations in the IL-2RG, JAK3, ADA, PNP, ADK2 genes; bare lymphocyte syndrome (TAP2 gene); X-linked inhibitor of apoptosis deficiency (XIAP); X-linked lymphoproliferative disease (XLP); XLP-1 (due to mutations in the SAP gene); Griscelli syndrome (RAB27A gene); Chediak-Higashi syndrome (LYST gene); and Wiskott-Aldrich syndrome (WAS gene).(12) Patients with X-linked inhibitor of apoptosis protein (XIAP) deficiency have been variably reported as having either normal numbers of NKT cells (13) or low numbers of NKT cells.(14) The apparent discrepancy in the numbers of NKT cells is likely related to the difference in size of the sample control groups and disease stage of patients between the 2 reports. At the present time, the role of XIAP in development of NKT cells has not been clearly delineated.

The isolated NK cell deficiencies include the absolute NK cell deficiency (ANKD), the classic NK cell deficiency (CNKD), and the functional NK cell deficiency (FNKD). NK cell function is absent in ANKD and CNKD and deficient in FNKD, while NK cells are present in the latter, but absent in the former 2 conditions. NK cells are absent only in ANKD and present in both CNKD and FNKD.(12) NK cell dysfunction has also been reported in systemic juvenile rheumatoid arthritis and macrophage activation syndrome.(15) There is also more data emerging on the pathogenic role of NK cells in atopic and autoimmune diseases.(4)

HIV-1 patients show a gradual loss of NK cells that correlates with disease progression. There is a selective loss of CD56(dim) NK cells, while the numbers of CD56(bright) NK cells remain the same. There appears to be a defect in
NK cells also play an important role in regulating viral infections, and their deficiency predisposes to susceptibility with herpes virus infections. NKG2D expression has been reported to decrease during human CMV infection. NK cells that express inhibitory receptors to self-MHC class I molecules are called "licensed," which means they are functionally more responsive to stimulation, while "unlicensed" NK cells lack receptors for self-MHC class I and are hyporesponsive. Contrary to the hypothesis that "licensed" NK cells are key for viral immunity, the depletion of "unlicensed" NK cells impairs control of viremia, suggesting that these cells are critical for protection against viral infection.

NK-cell lymphocytosis is seen in NK-neoplasias, extranodal NK/T-cell lymphoma, aggressive NK-cell leukemia, and blastic NK-cell lymphoma. Chronic NK-cell lymphocytosis (CNKL) is an indolent disorder characterized by proliferation of CD3-CD56+CD16- NK cells. Epstein-Barr virus (EBV) can infect nonneoplastic NK cells, and there is an expansion of CD16+CD56(dim) NK cells. Chronic active EBV infection involving NK cells can present with severe inflammatory and necrotic skin reactions typically associated with EBV+ NK-cell lymphoproliferative disease.

This assay provides both absolute and relative quantitation of various NK-cell subsets relative to total NK cells (NK cell subsets) or total lymphocytes (NKT cells) and can be used for assessment in the following clinical contexts: HIV, primary immune deficiencies with NK cell defects, NK-cell lymphocytosis, solid-organ transplantation, immune reconstitution following bone marrow or hematopoietic cell transplantation, evaluation of NK cells in neoplasias, only for quantitation (not for diagnosis or classification of NK cell malignancies).

Reference Values
The appropriate age-related reference values will be provided on the report. Pediatric reference values are not available and therefore, interpretation will be based on adult ranges with appropriate cautionary statements in the interpretation.

Interpretation
Interpretive comments will be provided, where applicable, along with reference range values for adult samples. Since a separate pediatric reference range could not be established at this time, interpretation of pediatric samples will be made using the adult reference range as an approximate guideline. For the surface marker and intracellular protein expression on natural killer (NK) subsets, relevant values that are abnormal will be provided in a table format within the interpretation along with textual interpretive comments. If results for surface and/or intracellular subsets are quantitatively normal, then only interpretive comments will be provided without actual numeric data. Clients may request numerical data for specific subsets that are not included within the report through the Laboratory Director.

Cautions
Natural killer (NK)/natural killer T (NKT) cell subset panel (NKSP) is a complex, expensive, and highly specific immunophenotyping test intended for evaluating patients with primary immunodeficiencies affecting the NK cell compartment, patients who have received allogeneic hematopoietic cell transplant because of an underlying primary immunodeficiency, and certain subsets of HIV patients. It is not intended for analysis of NK cell function, nor is it meant for patients with malignancies and/or those who have received chemotherapy or immunotherapy for these conditions (except for NK and NKT cell lymphomas and leukemias). The test is not useful in patients with chronic fatigue syndrome, chronic Lyme disease, chronic insomnia, or other related conditions either.

For total NK cell quantitation, it would be appropriate to order the standard lymphocyte subset quantitation test (TBBS / Quantitative Lymphocyte Subsets: T, B, and Natural Killer (NK)).
This test should ideally not be ordered in patients over the age of 40 years without prior discussion with Lab Directors on clinical utility and interpretation in specific clinical contexts.

**Clinical Reference**


7. Borrego F, Robertson MJ, Ritz J, et al: CD69 is a stimulatory receptor for NK cell and its cytotoxic effect is blocked by CD94 inhibitory receptor. Immunology 1999;97:159-165


2005;102(8):2886-2891


Performance

Method Description

This assay is done in 3 components: absolute counts, surface markers, and intracellular markers, by flow cytometry in whole blood.

The absolute quantitation of natural killer (NK) subsets is performed as a 2-tube assay with BD Trucount tubes. Tube 1 provides the isotype control for CD56 and CD16 markers. A combination of antibodies directed against the following surface markers are used to identify the 4 major NK subsets as well as natural killer T (NKT) cells. Whole blood is stained with the surface marker antibodies in the dark for 20 minutes, followed by RBC lysis for 10 minutes in BD FACS Lysing solution. Samples are analyzed on a BD FACS Canto II flow cytometer and data analysis is performed in FACS DIVA 6.1 software. (Unpublished Mayo method)

Surface staining of various markers on these 5 NK subsets is performed as a 5-tube assay. Tube 1 serves as isotype control for CD56 and CD16 in tubes 2 through 5, while tube 2 serves as the isotype control for markers in tubes 3 through 5. A panel of antibodies conjugated to various fluorochromes is used in this component of the assay and includes: CD3, CD45, CD16, CD56, CD27, CD95, CD107a/b, CD69, NKp46, and NKG2D. Whole blood is incubated with the various antibody combinations in tubes 1 through 5 in the dark for 20 minutes. RBC lysis is performed as mentioned above. Samples are centrifuged and resuspended in flow staining buffer and the samples are run and analyzed as previously described. (Unpublished Mayo method)

Intracellular staining of NK subsets is performed as a 4-tube assay, with tubes 1 and 2 serving as isotype controls in a manner similar to the surface staining assay. The following surface markers are used for NK subset identification: CD3, CD45, CD16, and CD56. Intracellular proteins, such as perforin, Granzyme A and B, and IFN-gamma are assessed in each of the 5 NK subsets, using specific antibodies labeled with various fluorochromes. The initial staining procedure is as previously described. Following RBC lysis, samples are fixed and permeabilized using the BD CytoFix/CytoPerm kit and then stained with above-mentioned intracellular markers. Analyses of samples are performed as described above. (Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday

Do not send specimen after Thursday. Specimen must be received by 10 a.m. on Friday.
Test Definition: NKSP

NK/NKT Subset Panel

Analytic Time
3 days

Maximum Laboratory Time
4 days

Specimen Retention Time
4 days

Performing Laboratory Location
Rochester

Fees and 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 Regional Manager. 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 U.S. Food and Drug Administration.

CPT Code Information

86357

86359

86356 x3

LOINC® Information

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