MAYO CLINIC LABORATORIES

Test Definition: QNKS

Natural Killer (NK)/Natural Killer T-Cell Subsets, Quantitative, Blood

Overview

Useful For

Quantitation of the major natural killer (NK)-cell subsets relative to total NK cells (NK cell subsets) or total lymphocytes (NK T cells)

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

This test is **not useful for** diagnosis or classification of NK cell malignancies.

This test **should not be used** for assessing NK cell cytotoxic function.

Method Name

Flow Cytometry

NY State Available

Yes

Specimen

Specimen Type

WB Sodium Heparin

Ordering Guidance

This assay does not measure cell-surface or intracellular proteins on natural killer or natural killer T-cell subsets.

A minimum CD45 lymph count (as measured by flow cytometry in the laboratory) is required to report this 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] Cells, Blood).

Shipping Instructions

Send specimen Monday through Thursday only. Specimen must arrive within 24 hours of collection and by 10 a.m. Central time on Friday.

Collect and package specimen as close to shipping time as possible. Ship specimen overnight.

Necessary Information

The ordering healthcare professional's name and phone number are required.

Specimen Required

Container/Tube: Green top (sodium heparin)
Specimen Volume: 3 mL
Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot.**
Additional Information: For serial monitoring, it is recommended that specimens are collected at the same time of day.

Specimen Minimum Volume

0.2 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
WB Sodium Heparin	Ambient	30 hours	GREEN TOP/HEP

Clinical & 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-Fc gamma RIII). 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 natural cytotoxicity directed largely toward virally infected cells or tumor cells, in the absence of prior stimulation or

immunization, and 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 cytotoxic 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)

Natural killer T (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 variants); familial hemophagocytic lymphohistiocytosis (FHL) types 2, 3, and 4 due to variants in the *PFP1* (encoding perforin), *UNC13D* (encoding the Munc13-4 protein) and *STX-11* (encoding syntaxin -11), respectively; Hermansky-Pudlak syndrome (*AP3B1*); Papillon-Lefevre syndrome (*CTSC*: cathepsin C); nuclear factor kappa-beta essential modulator deficiency (NEMO) due to variants 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 protein deficiency (*XIAP* gene); X-linked lymphoproliferative disease (XLP): XLP-1 (due to variants 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. NKT 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)

Patients with HIV-1 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 differentiation from immature CD56- NK cells to mature CD56(dim) NK cells (16), with an expansion of the former (CD56-CD16+) NK cells in HIV viremic patients.(17) Differential mobilization of NK-cell subsets has also been reported related to acute exercise, with CD56(bright) NK cells being less responsive than CD56(dim) NK cells and the ratio of CD56(bright):CD56(dim) favors the former at least up to 1-hour post-exercise.(18)

NK cells also play an important role in regulating viral infections, and their deficiency predisposes individuals to susceptibility with herpes virus infections. NKG2D expression has been reported to decrease during human cytomegalovirus infection.(19) 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 is an indolent disorder characterized by proliferation of CD3-CD56+CD16- NK cells. Epstein-Barr virus (EBV) can infect nonneoplastic NK cells(20), 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.(21)

Reference Values

The appropriate age-related reference values will be provided on the report. Pediatric reference values are not available for patients younger than 7 years and therefore, interpretation will be based on the 7- to 17-year old ranges with appropriate cautionary statements in the interpretation.

Interpretation

Interpretive comments will be provided, where applicable, along with reference range values for adult patients and pediatric patients from age 7 to 17 years. Since a separate pediatric reference range could not be established for patients younger than 7 years at this time, interpretation of these samples will be made using the 7- to 17-year old reference range as an approximate guideline.

Cautions

Results should be interpreted in conjunction with the relevant clinical context and other appropriate immunological analyses.

Clinical Reference

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Performance

Method Description

Test Definition: QNKS

Natural Killer (NK)/Natural Killer T-Cell Subsets, Quantitative, 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 cells. Whole blood is stained with the surface marker antibodies in the dark for 20 minutes, followed by red blood cells 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 8.0 software.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
3 to 4 days

Specimen Retention Time
4 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 x3
86359
86357

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
QNKS	Quantitative NK/NKT Subsets	98073-0

Test Definition: QNKS

Natural Killer (NK)/Natural Killer T-Cell Subsets, Quantitative, Blood

Result ID	Test Result Name	Result LOINC® Value
NKA45	Total Lymphs (CD45+)	27071-0
NKP3N	% Total CD45+CD3- cells	89311-5
NKA3N	Total CD45+CD3- cells	89313-1
NKP	% Total NK Cells	8112-5
NKA	Total NK Cells	9728-7
NKPCY	% Cytotoxic NK (CD16++CD56+)	In Process
NKACY	Cytotoxic NK (CD16++CD56+)	42188-3
NK56P	% Cytokine-Producing NK (CD56++)	8133-1
NK56A	Cytokine-Producing NK (CD56++)	14113-5
NKPL3	% Total CD45+CD3+ Cells	In Process
NKAL3	Total CD45+CD3+ Cells	89312-3
NKTP	% NKT cells (CD3+CD56+)	17135-5
NKTA	NKT cells (CD3+CD56+)	26858-1
QNKSI	Quantitative NK/NKT Interpretation	69052-9