Neutrophil Antibody Detection and Identification

Versiti offers several flow cytometric tests for the detection and identification of neutrophil antibodies.

Neutropenia results from either under production or increased destruction. Neutropenia due to increased destruction is often caused by antibodies that bind to the neutrophils and hasten their clearance from the circulation.[1] Antibodies can form against neutrophil-specific alloantigens and neutrophil antigens shared with other cells (Table 1).[2] Neutrophil antibodies are important in clinical conditions including neonatal alloimmune neutropenia,[3] autoimmune neutropenia,[4,5] transfusion-related acute lung injury (TRALI)[6,7], and drug-induced neutropenia.[8,9]

Table 1: Neutrophil Antigens Identified by Human Alloantibodies

Antigen	Common Name	Antigen Frequency (%)	
Neutrophil-specific:		Caucasian	African American
HNA-1a	NA1	54	47
HNA-1b	NA2	88	84
HNA-1c	SH	5	22
HNA-2a	NB1	97	unknown
Shared antigens:			
HNA-3a	5b	97	unknown
HNA-4a	MART[a]	92	unknown
HNA-5a	OND[a]	99	unknown

Drug-Dependent Neutrophil Antibodies:

Serum is tested against normal donor neutrophils in the presence and absence of the suspected drug(s). Reactions in the presence of drug but not in its absence indicate the presence of drug-dependent antibodies.

Neutrophil Antibody Detection:

Level 1 – Neutrophil Antibody Screen: Patient serum is screened against donor neutrophils for antibodies against HNA-1a, -1b, -1c, -2a, -3a, and HLA Class I. Neutrophil alloantibodies and autoantibodies are detected.

Level 2 – Neutrophil Antibody Screen and HLA Antibody Screen: Samples demonstrating positive reactions in the Level 1 Screen are candidates for additional testing against HLA antigens. If HLA antibodies are detected, the serum is adsorbed with platelets and retested against donor neutrophils. Antibody reactivity detected with platelet-adsorbed serum is considered neutrophil-specific.

Level 3 – Neutrophil Antibody Identification: Level 1 and Level 2 are performed first. If a neutrophil specific antibody is found, serum is screened against an expanded panel of donor neutrophils to determine which neutrophil antigen (HNA-1a, -1b, -1c, -2a, -5a) the antibody recognizes.

TRALI – Order TRALI Testing for each patient or donor sample submitted.

Indications for testing:

Autoimmune Neutropenia (AIN): Primary AIN occurs in both adults and children as an isolated hematologic disorder not associated with other disease factors. Patients frequently present with neutrophil counts less than 500/mm3 and recurrent infections of mild to moderate severity. Neutrophil-reactive antibodies can be detected in the sera of patients with this disease, especially in children. Antibodies often show specificity for the HNA-1a antigen.

Neutrophil antibodies and AIN also occur as a secondary phenomenon in other autoimmune diseases including systemic lupus erythematosus, Felty's syndrome, rheumatoid arthritis, and myasthenia gravis.

Drug-induced Neutropenia: Flow cytometry has been shown to be effective for detection of neutrophil drug-dependent antibodies.[8] Many drugs have been implicated as causes of immune-neutropenia. For instance, our laboratory has described quinine-dependent neutrophil antibodies.[9]

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Neonatal Alloimmune Neutropenia (NAN): In NAN, the mother is immunized by fetal neutrophil antigens inherited from the father. Maternal IgG antibodies cross the placenta and destroy fetal neutrophils.[3] The most common neutrophil alloantigen incompatibilities are HNA-1a, -1b, -1c and NB1. Unlike its erythrocyte counterpart, hemolytic disease of the newborn, NAN can occur during the first pregnancy and has been estimated to occur once in every 500 live births. Antibodies can be detected in the maternal serum by testing with a panel of normal donor neutrophils. Testing with the father's neutrophils is necessary to detect antibodies to low frequency antigens. Neutrophil genotyping of both parents can be useful for confirming maternal antibody specificity and in providing counseling regarding future pregnancies.

Transfusion-Related Acute Lung Injury (TRALI): TRALI is a serious non-hemolytic transfusion reaction.[6,7] Reactions can occur within minutes of onset of transfusion, and can result in death. TRALI reactions are believed to occur when leukocyte antibodies in the transfused blood react with antigens on the recipient's white blood cells. Testing the blood donor's plasma for antibodies may be informative. Antibodies to the HNA-1a, -2a, -3a, and HLA Class I and Class II antigens have all been implicated in cases of TRALI.[14,15]

Test method:

Flow cytometry: Flow cytometry is a highly sensitive method for detection of neutrophil antibodies.[10,11,12] Patient serum is incubated with isolated donor neutrophils typed for HNA-1a, -1b, -1c, -2a, -3a, -4a. Binding of serum antibodies is detected using fluorescent-labeled polyclonal antibodies specific for human IgG and IgM. In order to distinguish HLA antibodies from neutrophil-specific antibodies, positive samples are adsorbed with normal platelets to remove HLA Class I antibodies, and testing is repeated. Level 2 & Level 3 testing include detection of HLA Class I & Class II antibodies using a sensitive flow cytometry method.

Assay sensitivity and limitations:

Some strong HLA Class I antibodies might be difficult to distinguish from neutrophil-specific antibodies. Antibodies against some low frequency neutrophil antigens might not be detected.

Reporting of results:

Positive – Neutrophil-reactive antibodies detected

Negative – No neutrophil-reactive antibodies detected

Specimen requirements:

Suspected disorder	Recommended test	Sample requirements
Autoimmune Neutropenia (AIN)	Neutrophil Antibody Screen and HLA Antibody Screen	5ml of serum, refrigerated
Drug-induced Neutropenia	Drug-dependent Neutrophil	5ml of serum, refrigerated
Neonatal Alloimmune Neutropenia (NAN)	Neutrophil Antibody Identification	5ml of serum, refrigerated
Transfusion/ Related Acute Lung Injury (TRALI)	TRALI	5ml serum and EDTA whole blood from both patient and donor, refrigerated

SHIP

Shipping requirements:

Place specimen and requisition into plastic bags, seal and insert into a Styrofoam container. Surround with ice packs, seal the Styrofoam container and place in a sturdy cardboard box, then tape securely. Ship the package in compliance with your overnight carrier guidelines. Send to:

Versiti Client Services
Platelet and Neutrophil Immunology Laboratory
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

ORDER

Required forms:

Please complete all pages of the requisition form.

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CPT Codes/Billing/Turnaround time:

Test code:

Neutrophil Antibody Screen (5102)

Neutrophil Antibody Screen with REFLEX to HLA Antibody Screen (5110)

Neutrophil Antibody Screen with REFLEX to 5113 (5119)

Neutrophil Antibody Screen and HLA Antibody Screen (5112)

Neutrophil Antibody Identification and HLA Antibody Screen (5113)

CPT codes: For recommended CPT codes, visit the versiti.org/test-catalog

Turnaround time: 7-10 days

References:

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9. Wu GG, Curtis BR, Shao YL, Aster RH. Analysis of quinine-dependent neutrophil-reactive antibodies in patients with quinine-induced hemolytic uremic syndrome (HUS) and quinine-induced thrombocytopenia by flow cytometry and immunoprecipitation. Transfusion 1993;33 (Suppl.).
10. Curtis BR, Reno CK, Aster RH. Neonatal alloimmune neutropenia due to maternal IgG antibodies against the neutrophil alloantigen HNA-1c (SH): a report of five cases. Transfusion 2005;45:1308- 1313.
11. Davoren A, Saving K, McFarland MG, Aster RH, Curtis BR. Neonatal neutropenia and bacterial sepsis associated with placental transfer of maternal neutrophil-specific autoantibodies. Transfusion 2004;44:1041-1046.
12. Pei R, Wang C, Tarsitani S, et al. Simultaneous HLA class I and class II antibody screening with flow cytometry. Human Immunology 1998;59:313-2.
13. Lucas G, Rogers S, de Haas M, Porcelijn L, Bux J. Report on the fourth international granulocyte immunology workshop: progress toward quality assessment. Transfusion 2002;42:462-468.
14. Davoren A, Curtis BR, Shulman AF, et al. TRALI due to granulocyte-agglutinating human neutrophil antigen-3a (5b) alloantibodies in donor plasma: a report of 2 fatalities. Transfusion 2003;43:641-45.
15. Kopko PM, Popovsky MA, Mackenzie MR, Paglieroni TG, Muto KN, Holland PV. HLA class II antibodies in transfusion-related acute lung injury. Transfusion 2001;41:1244-48.

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