Neutrophil Antibody Detection And Identification

**REFERENCES:**

**METHOD:** Flow cytometry. Flow cytometry is a highly sensitive method for detection of neutrophil antibodies. 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 polycional antibodies specific for human IgG and IgM. In order to distinguish HLA antibodies from neutrophil-specific antibodies, positive samples are absorbed 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.

**CREDENTIALS:** The Platelet & Neutrophil Immunology Lab (PNIL) is a CLIA licensed laboratory with an established Quality Program that includes participation in the International Granulocyte Immunology Workshop. The lab was founded in 1972 to provide support for clinical evaluation of immune platelet disorders. Flow cytometric testing for neutrophil antibodies was developed and implemented in 1995. Only a few laboratories in the world can adequately perform this technically complex testing.

**Table 1. Neutrophil Antigens Identified by Human Alloantibodies.**

<table>
<thead>
<tr>
<th>ANTIGEN</th>
<th>COMMON NAME</th>
<th>ANTIGEN FREQUENCY (%)</th>
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<tbody>
<tr>
<td>HNA-1a</td>
<td>NA1</td>
<td>Caucasian 54</td>
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<td>HNA-1b</td>
<td>NA2</td>
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</tr>
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<td>HNA-5a</td>
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**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:

Client Services/Platelet & Neutrophil Immunology Laboratory
BloodCenter of Wisconsin
638 N. 18 St.
Milwaukee, WI 53233
800-245-3117, ext. 6250

**TURNAROUND TIME:** 7-10 days

**REFERENCES:**

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**Recommended Test**

**Drug-dependent Neutrophil Sample Requirements**

- **5 ml of serum and EDTA whole blood** from both patient and donor, refrigerated.

**REFERENCE INTERVAL:**

- Positive: Neutrophil-reactive antibodies detected
- Negative: No neutrophil-reactive antibodies detected

**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.

**Nomenclature:**

- Granulocytes are a category of white blood cells, characterized by the presence of cytoplasmic granules under light microscopy. They are also referred to as polymorphonuclear leukocytes (PMN). The granulocytes include neutrophils, eosinophils and basophils. The most abundant granulocyte is the neutrophil, which has neutrally staining granules. Often the terms “granulocyte” and “neutrophil” are used interchangeably.

**Reasons for Referral - Suspected Disorders:**

**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. Many drugs have been implicated as causes of immune-neutropenia. For instance, our laboratory has described quinine-dependent neutrophil antibodies.

**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. 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 determine which neutrophil antigen (HNA-1a, -1b, -1c, -2a, -3a, and HLA Class I and Class II) the antibody recognizes. Antibodies against HNA-1a, -1b, -1c, -2a, -3a, and HLA Class I and Class II antigens have all been implicated in cases of NAN.

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. The most common neutrophil alloantigen incompatibilities are HNA-1a, -1b, -1c, -2a, -3a, 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 determine 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

**Reference Interval:**

- Positive: Neutrophil-reactive antibodies detected
- Negative: No neutrophil-reactive antibodies detected

**Testing Suggested:**

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

**CPT Codes:**

- TRALI: 86021, 86828

**Testing Offered:**

**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. CPT Codes: 86021

**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. CPT Codes: 86021, 86828

**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. CPT Codes: 86021, 86828

**Specimen Requirements:**

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<tr>
<th>Suspected Disorder</th>
<th>Recommended Test</th>
<th>Sample Requirements</th>
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<td>Neutrophil Antibody Screen and HLA Antibody Screen</td>
<td>5 ml of serum, refrigerated.</td>
</tr>
<tr>
<td>Drug-Induced Neutropenia</td>
<td>Drug-dependent Neutrophil Antibodies</td>
<td>5 ml of serum, refrigerated.</td>
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**CPT Codes:**

- 86021, each additional drug: 86021

**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.

**CPT Codes:**

- 86021, each additional drug: 86021

**Testing Schedule:** Assays are set up once each week. Results are available within 7-10 days. Please contact the laboratory at (800)-245-3117, ext. 6255, for the current test schedule or to discuss your particular situations.