Antibody Testing and Gene Therapy

This fact sheet will provide valuable information on **antibody testing**, which is one important factor in determining eligibility for gene therapies currently being studied in clinical trials.[1] In a clinical trial, other eligibility factors may include age, type of genetic mutation, baseline mobility and function, and prior exposure to an investigational or commercially available therapy.[2]

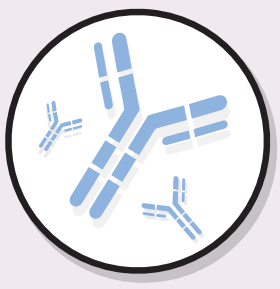
An introduction to Antibodies

Antibodies are a protein used by the immune system to identify and neutralize foreign objects, like bacteria or viruses. Antibodies are also known as immunoglobulins, a term that is often written as Ig. The human body makes different antibodies, or immunoglobulins, to fight different things.

There are 5 different types of antibodies: IgG, IgM, IgA, IgE, and IgD.[13]

The two most relevant to systemic gene therapy are IgG and IgM as they are both found in blood and other body fluids that are distributed throughout the entire body. IgA, IgE, and IgD are usually found in smaller amounts or are more localized to specific areas like mucus membranes.

- **Immunoglobulin G (IgG)** – This is the most common antibody. It's in blood and other body fluids and protects against bacterial and viral infections. IgG can take time to form after an infection or immunization.
- **Immunoglobulin M (IgM)** – This is the first antibody the body makes when it fights a new infection. It is found mainly in blood and lymph fluid.
- **Immunoglobulin A (IgA)** – This antibody is found mostly in mucous membranes.
- **Immunoglobulin E (IgE)** – These antibodies trigger allergies and protect against parasites. Small amounts are found in skin, lungs, and mucous membranes.
- **Immunoglobulin D (IgD)** – We are still learning about these antibodies. They are found in very small amounts and bind to B-cells, a component of the immune system.

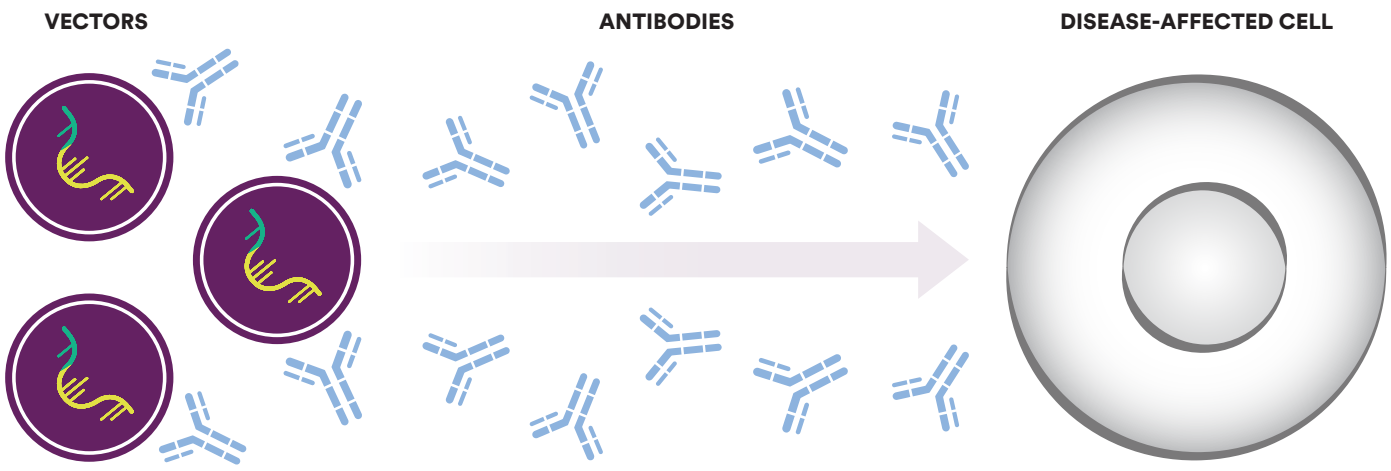


Further Antibody Classifications

- **Neutralizing (NAbs)[14]** – After an infection, it can take some time for the body to produce highly effective neutralizing antibodies. Neutralizing antibodies persist to protect against future encounters with a virus or bacteria. Only a small subset of the many antibodies that bind to a virus are capable of neutralization. Neutralizing antibodies not only bind to a virus, but they bind in a manner that blocks infection. They do this in a variety of ways – by blocking the virus' ability to bind to a receptor in the body, or binding to the virus to prohibit it from uncoating and effectively infecting the body. When virologists refer to serotypes and seroprevalence in research studies, the reference is typically for neutralizing antibodies.
- **Non-neutralizing antibodies (Binding antibodies)[15]** – These antibodies bind to the virus, but they do not interfere with their ability to infect the body. They can help flag the viral particles, serving as a signal that the virus or bacteria in question needs to be targeted by immune cells. Non-neutralizing antibodies are also known to help slow the spread of infection in other ways by recognizing invaders and signaling to other parts of the immune system that there is a problem.[16,17]

Why does antibody testing play a role in determining eligibility for a gene therapy?

Prior to treatment, individuals must be tested for **preexisting antibodies*** because they may prevent the therapy from working as intended.[1,3]



Gene therapies are delivered via a **vector,** which aims to deliver functioning genes to disease-affected cells in the body.[4] The vector is a delivery vehicle that aims to bring the functioning gene into target cells.[5] The vector is selected based on its ability to get into these target cells. One of the most common vectors used in gene therapy is the adeno-associated virus (AAV) vector. Because vectors are not naturally found in the body, the immune system may respond to them as if they are foreign invaders and work to eliminate them with the help of antibodies.[4,6] For this reason, antibody testing plays an important role in determining eligibility for therapy.[1,3]

*Preexisting antibodies are those already present in the body that may recognize a gene therapy's vector.[6]

How do preexisting antibodies develop against gene therapy vectors?

Individuals may develop preexisting antibodies that recognize a gene therapy vector **even if they've never received a gene therapy before**. Some viruses naturally present in the environment are similar to vectors used in gene therapy, and if exposed, an individual's immune system can make antibodies against the virus that also recognize the vector.[6] Currently, there is no way to prevent the development of these naturally occurring antibodies or to know if an individual has been exposed to one of these viruses without an antibody test.[7]

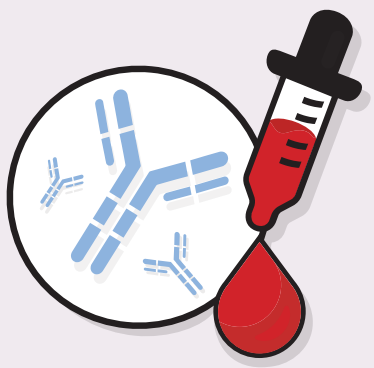
Importantly, approved Duchenne therapies like steroids and exon skipping **do not create antibodies.**[8,9]

How are antibody tests used to help determine eligibility for a gene therapy?

Antibody testing is the **only way** to determine if an individual has preexisting antibodies that may prevent a gene therapy from working as intended.[1,6]

Helpful definitions

- **Antibody titer:** a measurement of the amount of antibodies in a blood sample that can recognize a vector[10]
- **Seropositive (elevated):** preexisting antibodies are detected in the body at levels **above** a predefined threshold. Individuals who are seropositive are **ineligible** for gene therapy or clinical trials.[1]
- **Seronegative (not elevated):** no preexisting antibodies are detected in the body, or they are present at levels **below** a predefined threshold[3]



Individuals will need to take the antibody test **specific to the gene therapy they are considering**. The type of antibody test needed may differ depending on the vector used and the therapy's manufacturer. Currently, **no universal antibody test exists**.[11]

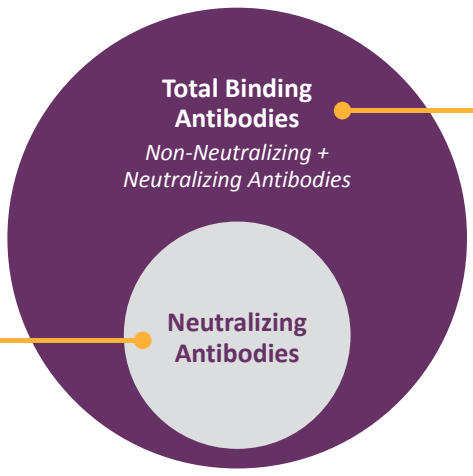
Additionally, the concentration of antibodies in the body can change over time, and an individual who was initially seronegative can become seropositive. That's why antibody testing **must be performed at the time** that an individual is being considered for participation in a gene therapy clinical trial, regardless of whether they have received an antibody test before.[1,3]

Two different types of antibody tests may be used in clinical trials[12]:

Neutralizing Antibody Test

This test screens for neutralizing antibodies, a subset of total binding antibodies.

Neutralizing antibodies recognize specific vector types and prevent them from delivering functioning genes to disease-affected cells, potentially preventing a gene therapy from working as intended.



Total Binding Antibody Test

This test screens for both neutralizing antibodies and non-neutralizing antibodies, which may provide a more comprehensive assessment of the potential for immune responses.

Non-neutralizing antibodies recognize specific vector types and may cause immune-related side effects.

When considering a clinical trial for gene therapy, ask your doctor what type of antibody test will be used.

References: **1.** Colella P, Ronzitti G, Mingozzi F. Emerging issues in AAV-mediated *in vivo* gene therapy. *Mol Ther Methods Clin Dev.* 2017;8:87-104. **2.** U.S. National Library of Medicine. A multicenter, randomized, double-blind, placebo-controlled trial for Duchenne muscular dystrophy using SRP-9001. Identifier NCT03769116. Accessed March 19, 2021. https://clinicaltrials.gov/ct2/show/NCT03769116 **3.** Shirley JL, de Jong YP, Terhorst C, Herzog RW. Immune responses to viral gene therapy vectors. *Mol Ther.* 2020;28(3):709-722. **4.** Asher DR, Thapa K, Dharia SD, et al. Clinical development on the frontier: gene therapy for Duchenne muscular dystrophy. *Expert Opin Biol Ther.* 2020;20(3):263-274. **5.** Chira S, Jackson CS, Oprea I, et al. Progresses towards safe and efficient gene therapy vectors. *Oncotarget*. 2015;6(31):30675-30703. **6.** Meliani A, Leborgne C, Triffault S, Jeanson-Leh L, Veron P, Mingozzi F. Determination of anti-adeno-associated virus vector neutralizing antibody titer with an *in vitro* reporter system. *Hum Gene Ther Methods.* 2015;26(2):45-53. **7.** Barnes C, Scheideler O, Schaffer D. Engineering the AAV capsid to evade immune responses. *Curr Opin Biotechnol.* 2019;60:99-103. **8.** Hodgens A, Sharman T. Corticosteroids. *StatPearls.* 2021. Accessed March 19, 2021. https://www.ncbi.nlm.nih.gov/books/NBK554612 **9.** Jean-Baptiste R, Kozaczynska J, van Deutekom S, et al. Immunogenicity assay for detection of anti-dystrophin antibodies in serum of Duchenne muscular dystrophy patients following therapeutic antisense-induced exon skipping. *Abstracts, Neuromuscular Disorders* 2011;21:712. **10.** U.S. National Library of Medicine. Antibody titer blood test. Accessed March 19, 2021. https://medlineplus.gov/ency/article/003333.html **11.** Camino E. Gene therapy and immune response: understanding challenges and strategies. Accessed on March 19, 2021. https://www.parentprojectmd.org/understanding-gene-therapy-immunity **12.** Fitzpatrick Z, Leborgne C, Barbon E, et al. Influence of pre-existing anti-capsid neutralizing and binding antibodies on AAV vector transduction. *Mol Ther Methods Clin Dev.* 2018;9:119-129. **13.** Corley, R., 2004. Antibodies. In: G. Pier, J. Lyczak and L. Wetzler, ed., Immunology, Infection, and Immunity. ASM Press. **14.** Payne, S. ed., 2017. Chapter 6 - Immunity and Resistance to Viruses. In: Viruses. Academic Press. **15.** Cheedarla, N. and Hanna, L., 2018. Chapter 7 - Functional and Protective Role of Neutralizing Antibodies (NAbs) Against Viral Infections. In: V. Buddolla, ed., Recent Developments in Applied Microbiology and Biochemistry. [online] Academic Press. **16.** Tirado SM, Yoon KJ. Antibody-dependent enhancement of virus infection and disease. Viral Immunol. 2003;16(1):69-86. doi:10.1089/088282403763635465. **17.** Kulkarni R. Antibody-Dependent Enhancement of Viral Infections. Dynamics of Immune Activation in Viral Diseases. 2019;9-41. Published 2019 Nov 5. doi:10.1007/978-981-15-1045-8_2.

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