

# HLA Disease Association (DA) Panels

## Introduction:

The Disease Association Panel supplemental information document is to be used by the health providers as a reference when selecting the panel of interest as well as a guide to interpretation of the test results. The Disease Association Panel test allows the requestor to order HLA typing from a battery of pre-selected HLA specificities known to be associated with certain diseases or drug-induced adverse reactions based on available evidence. The confirmation of presence or absence of HLA factors associated with each disease or drug-induced adverse reactions can make a significant impact on the diagnosis and/or treatment of patients. Clinicians should refer to the supplemental information document for information on clinical indication, methodology, limitations, clinical background and references.

## Panels (click on the disease of interest to be directed to the appropriate page)

Panel #	Disease or Hypersensitivity	Page #	Associated HLA Allele	Locus	Typing resolution	Reported resolution
1	<a href="#">Abacavir Hypersensitivity</a>	3	HLA B*57:01	HLA-B	Intermediate	Intermediate
2	<a href="#">Allopurinol Hypersensitivity</a>	4	HLA B*58:01	HLA-B	Intermediate	Intermediate
3	<a href="#">Spondyloarthropathies</a> (Ankylosing Spondylitis, Reactive Arthritis, Juvenile Arthritis)	7	HLA-B27	HLA-B	Intermediate	Serology
4	<a href="#">Eye Disease</a> HLA Association (Acute Anterior Uveitis HLA-B27, Behçet's Disease HLA-B51, Birdshot chorioretinopathy HLA-A29)	11	HLA-B27 HLA-B51, HLA-A29	HLA-A, HLA-B	Intermediate	Serology
5	<a href="#">Carbamazepine Hypersensitivity</a>	16	HLA-B*15:02, HLA-A*31:01	HLA-A, HLA-B	Intermediate	Intermediate
6	<a href="#">Celiac Disease (Celiac Genetics)</a>	18	HLA-DQA1 and HLA-DQB1 associated alleles and DQ2/DQ8 heterodimers	HLA-DQA1, HLA-DQB1	Intermediate	Intermediate
7	<a href="#">Narcolepsy</a>	24	HLA-DQB1*06:02, DQA1*01:02	HLA-DQA1, HLA-DQB1	Intermediate	Intermediate
8	<a href="#">Pediatric Acute-onset Neuropsychiatric Syndrome (PANS)</a>	27	HLA-B38, HLA-B5, HLA-B51, HLA-B52, HLA B27, HLA-Bw4	HLA-B	Intermediate	Serology
9	<a href="#">Psoriasis</a>	29	HLA-B27, HLA-Cw6	HLA-B, HLA-C	Intermediate	Serology
10	<a href="#">IL-1 and IL-6 inhibitors Hypersensitivity</a>	32	HLA-DRB1*15:01, HLA-DRB5*01:01 HLA-DQB1*06:02, HLA-DQA1*01:02,	HLA-DRB1, HLA-DRB345, HLA-DQB1, HLA-DQA1,	Intermediate	Intermediate
11	<a href="#">HLA typing Custom Panel (Intermediate resolution)</a>	35	Select any or all	Class I: HLA-A, HLA-B, HLA-C Class II: HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DRB345, HLA-DPA1, HLA-DPB1	Intermediate	Intermediate
12	<a href="#">HLA NGS typing (Full High Resolution)</a>	37	High Res includes Class I & Class II (cannot be ordered separately)	HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DRB345, HLA-DPA1, HLA-DPB1	High for all loci	High

## 1. Abacavir Hypersensitivity HLA-B\*57:01 Association

### a. Clinical Indication:

As HLA-B\*57:01 is associated with significantly increased risk of hypersensitivity reactions when Abacavir is administered, screening for this allele is recommended for patients prior to initiation of Abacavir therapy.

### b. Methodology:

Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

### c. Limitations:

The absence of HLA-B\*57:01 allele does not preclude the possibility of developing hypersensitivity reaction to abacavir, although the risk is lower. The incidence of suspected abacavir hypersensitivity reaction in some clinical trials was reported to be 1% in subjects not carrying HLA-B\*57:01. [1] The PCR-SSO methodology used for typing of HLA-B locus may not be able to rule out some rare alleles other than B\*57:01. The presence of these rare alleles instead of B\*57:01 is unlikely. Risk of hypersensitivity to abacavir may also be affected by other genetic or non-genetic factors not covered by this test. Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA).

### d. Clinical Background:

Abacavir is used as part of the antiretroviral therapy in the treatment of human immunodeficiency virus (HIV) infection. Abacavir hypersensitivity has been found to be associated with the HLA-B\*57:01 allele across different ethnic groups (Caucasians, Hispanics, and some Asian and African populations) [2][3]. According to the 2017 Statement from the US Food and Drug Administration (FDA), due to the potential for severe and possibly fatal hypersensitivity reactions with abacavir, all patients should be screened for the HLA-B\*57:01 allele prior to initiating therapy with abacavir

### e. References:

[1] Dean, L., 2018. Abacavir Therapy and HLA-B\* 57: 01 Genotype. In Medical Genetics Summaries [Internet]. National Center for Biotechnology Information (US)

[2] Sousa-Pinto B., Pinto-Ramos J., Correia C., Goncalves-Costa G., et al. Pharmacogenetics of abacavir hypersensitivity: A systematic review and meta-analysis of the association with HLA-B\*57:01. J Allergy Clin Immunol. 2015;136(4):1092–4.e3

[3] Martin M.A., Klein T.E., Dong B.J., Pirmohamed M., et al. Clinical pharmacogenetics implementation consortium guidelines for HLA-B genotype and abacavir dosing. Clinical pharmacology and therapeutics. 2012;91(4):734–8

## **2. Allopurinol Hypersensitivity HLA-B\*58:01 Association**

### **a. Clinical Indication:**

The presence of the HLA-B\*58:01 allele is strongly associated with increased risk for developing severe cutaneous adverse reactions (SCAR), including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug rash with eosinophilia and systemic symptoms (DRESS syndrome) after treatment with allopurinol. Risk levels and allele frequencies are considered as part of the regimen for allopurinol therapy. Thus, in patients who have received allopurinol, the HLA-B\*58:01 allele can be used as a predictive marker to identify patients with increased risk of developing allopurinol-induced SCAR.

### **b. Methodology:**

Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

### **c. Limitations:**

The absence of HLA-B\*58:01 indicates low or reduced risk of SCARs but does not preclude the development of an adverse reaction to allopurinol, and does not replace the need for therapeutic drug monitoring or other clinical testing. Other genetic and nongenetic factors that may influence allopurinol-related adverse reactions are not evaluated in this assay.

This test is designed to specifically test the HLA-B\*58:01 allele and other HLA loci will not be evaluated. Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles. Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA).

### **d. Clinical Background:**

Allopurinol is a commonly prescribed medication used to inhibit xanthine oxidase in patients with gout, hyperuricemia, and in cancer patients undergoing chemotherapy [1]. However, allopurinol is a frequent cause of adverse drug reactions, including SCARs, which are a group of life-threatening conditions that include SJS, TEN, and DRESS syndrome [2]. Symptoms include rash, combined with eosinophilia, leukocytosis, fever, hepatitis and progressive kidney failure. Allopurinol-induced SCAR typically develops within weeks or a few months after initiation of treatment and the incidence is estimated to be 0.1-0.4%. [1] Some patients can develop serious adverse events with mortality rates reaching up to 25% [3].

The presence of the major histocompatibility complex class I allele HLA-B\* 58:01 has been shown to be strongly associated with the development of SCARs in patients treated with allopurinol. The mechanism has not been fully elucidated. Some theory suggests a direct pharmacological reaction of the drug and immune receptor of drug-specific T cells to induce T cell activation and strengthened by interaction with HLA-B\*58:01. There is also evidence supporting MHC-restricted presentation of drug or drug metabolites for T cell activation. The HLA-B\*58:01 and high doses of oxypurinol can act synergistically to result in a higher risk of T-cell sensitization. Due to this increased risk of SCAR, the Clinical Pharmacogenomics Implementation Consortium (CPIC) recommend HLA-B\*58:01 genotyping when considering allopurinol as treatment, and that allopurinol should not be prescribed to patients who test positive for the allele [4,5].

The frequency of the HLA-B\*58:01 allele varies with ethnicity, for example, the allele frequency is 5.8% in Asian populations, 3.5% in African American populations, 1.5% in Hispanic populations, and 0.5% in Caucasian populations [6]. Allopurinol-induced SCARs are especially associated with HLA-B\* 58:01 in those of Han Chinese, Korean and Thai descent [1]. The negative predictive value of HLA-B\* 58:01 in East Asian patients is very high, but the positive predictive value is low due to the high frequency of the HLA-B\* 58:01 allele (8-15% in Southeast Asians and Asian-Indians) and the rarity of these severe reactions [7]. Due to the severity of adverse reactions, the American College of Rheumatology recommends testing for HLA-B\* 58:01 allele prior to initiation of allopurinol therapy in select patient populations that are at higher risk for severe allopurinol hypersensitivity reactions, including all Han Chinese and Thai patients irrespective of renal function, as well as Koreans with stage 3 or higher chronic kidney disease [5,8].

#### e. References:

[1] Dean, L., 2016. Allopurinol Therapy and HLA-B\* 58: 01 Genotype. In Medical Genetics Summaries [Internet]. National Center for Biotechnology Information (US).

[2] Hung SI, Chung WH, Liou LB, Chu CC, Lin M, Huang HP, Lin YL, Lan JL, Yang LC, Hong HS, Chen MJ, Lai PC, Wu MS, Chu CY, Wang KH, Chen CH, Fann CS, Wu JY, Chen YT. HLA-B\*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc Natl Acad Sci U S A*. 2005 Mar 15;102(11):4134-9.

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- [6] Maiers, M., Gragert, L. and Klitz, W. High-resolution HLA alleles and haplotypes in the United States population. *Human Immunology*, (2007), 68(9), pp.779-788.
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### **3. Spondyloarthropathies HLA-B27 Association (Ankylosing Spondylitis/Reactive Arthritis/Psoriatic Arthritis/Juvenile Arthritis)**

- a. Clinical Indication:  
This test can be used as an ancillary testing to assist with diagnosis of HLA-B\*27-associated spondyloarthropathies
- b. Methodology:  
Low – Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)
- c. Limitations:  
HLA antigens other than HLA-B are not evaluated, at first, in this testing. Rare sequence variants not covered by the assay probes may lead to inaccurate test result or interpretation. A negative test results of HLA-B27 does not preclude the possibility of developing spondyloarthropathies. There is the option of testing additional HLA loci such as HLA-C, since secondary susceptibility signals, in addition to those associated with HLA-B locus, have been previously reported in the literature.

An occasional sample may not be satisfactory for testing. In those cases, an additional collection of this specimen may be required for testing.

For human genetic inheritable conditions and mutations. This test was developed and its performance characteristics determined by Stanford Blood Center. The U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

- d. Clinical Background:

#### Ankylosing Spondylitis (AS):

There is a strong association between ankylosing spondylitis and the HLA-B27 antigen. Upwards of 90% of patients with AS carry B27, whereas its frequency in general population is less than 8%, making B27 antigen an important marker for disease diagnosis. In addition, B27 positivity may affect the AS phenotype severity and susceptibility. Other features of AS, such as age of onset, sex, and family history also seem to be influenced by HLA-B27. [1,2] However, an individual's B27 status cannot be used to predict ankylosing spondylitis; out of all B27-positive individuals, only about 1-2% will develop the disease [3]. The most prevalent HLA-B27 subtypes in AS are HLA-B\*27:05 (Caucasian populations), HLA-B\*27:04



(Chinese populations), and HLA-B\*27:02 (Mediterranean populations). On the other hand, two subtypes, HLA-B\*27:06 and HLA-B\*27:09, seem unrelated to AS. [4-6]

## Reactive Arthritis (ReA):

Reactive arthritis is an infection-induced systemic illness characterized by a sterile synovitis developing secondary to a bacterial infection localized in a distant organ/system, usually in the genitourinary or gastrointestinal tract. ReA is considered as part of the spectrum of the spondyloarthritis. [7,8] it has been established that microbial degradation products are present in the inflamed synovia. [9] A classification into HLA-B27-associated and nonassociated forms has also been proposed. The HLA-B27 nonassociated arthritides are presently reviewed as distinct entities under the general heading of reactive arthritides. [10]

## Juvenile Idiopathic Arthritis (JIA):

Juvenile idiopathic arthritis, previously referred to as Juvenile Rheumatoid Arthritis (JRA) or Juvenile Chronic Arthritis (JCA), is the most common chronic inflammatory arthropathy of childhood. It is a heterogeneous group of diseases characterized by arthritis of unknown origin with onset before age of 16 years and persisting for more than 6 weeks. [11,12] It is influenced by both genetic and environmental factors. There are both HLA and non-HLA genetic factors associated with disease susceptibility. The association between HLA-B27 and the enthesitis-related arthritis (ERA) subtype category of JIA is well described; HLA B27 has been reported in 60-90% of patients with ERA [13]. HLA-B27 was also reported to be negatively associated with long term remission status and more likely to have a chronic course with the eventual development of axial disease. [14] Furthermore, the association between HLA-B27 and other JIA subtype categories has been suggested in some studies, but not as well established as ERA. [15-17]

## Psoriatic Arthritis (PsA):

Psoriatic arthritis (PsA) is a heterogeneous disease with diverse clinical and radiographic manifestations [18]. Thus, patients often differ considerably from one another in the type of their clinical manifestations, including whether or not they exhibit dactylitis, enthesitis, asymmetric or symmetric sacroiliitis, or joint deformity, but also in the presence and type of joint damage on plain radiography [19]. Several features in PsA suggest that an autoimmune mechanism may drive the disease. Using improved psoriatic arthritis (PsA) case ascertainment based on CLASSification criteria for Psoriatic ARthritis (CASPAR) classification criteria and more precise methods of allele determination, susceptibility to PsA has recently been strongly associated with inheritance of several class I major histocompatibility complex (MHC) alleles of both human leukocyte antigen (HLA)-B and HLA-C loci and their haplotypes [19]. Thus, it has been described that HLA-B\*27 is more common among PsA patients with axial disease, whereas B\*38 and B\*39 are increased among those with peripheral polyarthritis [20].

e. References:

Ankylosing Spondylitis:

- [1] Akassou, A and Bakri, Y. 2018. Does HLA-B27 status influence ankylosing spondylitis phenotype?. Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders, 11, p.1179544117751627.
- [2] Reveille, JD & Weisman, MH. The epidemiology of back pain, axial spondyloarthritis and HLA-B27 in the United States. Am. J. Med. Sci. 345, 431–436 (2013).
- [3] Zhu W, He X, Cheng K, et al. Ankylosing spondylitis: etiology, pathogenesis, and treatments. Bone Res. 2019;7:22.
- [4] Taurog, J. D. The mystery of HLA-B27: if it isn't one thing, it's another. Arthritis Rheum. 56, 2478–2481 (2007).
- [5] D'Amato, M. et al. Relevance of residue 116 of HLA-B27 in determining susceptibility to ankylosing spondylitis. Eur. J. Immunol. 25, 3199–3201 (1995).
- [6] Koh, W. H. & Boey, M. L. Ankylosing spondylitis in Singapore: a study of 150 patients and a local update. Ann. Acad. Med. Singap. 27, 3–6 (1998).

Reactive Arthritis:

- [7] Colmegna, I., Cuchacovich, R. and Espinoza, L.R. (2004). HLA-B27-associated reactive arthritis: pathogenetic and clinical considerations. Clinical Microbiology Reviews, 17(2), pp.348-369.
- [8] García-Kutzbach A, Chacón-Súchite J, García-Ferrer H, Iraheta I. Reactive arthritis: update 2018. Clin Rheumatol. 2018;37(4):869-874.
- [9] Toivanen, P. (2001). From reactive arthritis to rheumatoid arthritis. J. Autoimmun. 16:369-371.
- [10] Kuipers JG, Köhler L, Zeidler H. Reactive or infectious arthritis. Ann Rheum Dis. 1999;58(11):661-4.

Juvenile Idiopathic Arthritis:

- [11] Hersh AO, Prahalad S. Immunogenetics of juvenile idiopathic arthritis: A comprehensive review. J Autoimmun. 2015;64:113-24.



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[13] Colbert RA. Classification of juvenile spondyloarthritis: Enthesitis-related arthritis and beyond. Nat Rev Rheumatol. 2010;6(8):477-85

[14] Berntson L, Damgård M, Andersson-Gäre B, et al. HLA-B27 predicts a more extended disease with increasing age at onset in boys with juvenile idiopathic arthritis. J Rheumatol. 2008;35:2055–2061.

[15] Thomson W, Barrett RD, Donn R, et al. Juvenile idiopathic arthritis classified by the ILAR criteria: HLA associations in UK patients. Rheumatology. 2002;41:1183–1189.

[16] Savolainen HA, Lehtimäki M, Kautiainen H, et al. HLA B27; a prognostic factor in juvenile chronic arthritis. Clin Rheumatol. 1998;17:121–124.

[17] Żuber Z, Kania U, Król-Zdechlikiewicz A, et al. Analysis of clinical symptoms and laboratory profiles in children with juvenile idiopathic arthritis in Malopolska region (Poland) in the Years 2007-2010. Mac J Med Scien. 2014;7:56–61.88

## Psoriatic Arthritis:

[18] Taylor, W., Gladman, D., Helliwell, P., Marchesoni, A., Mease, P. and Mielants, H.. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, (2006), 54(8), pp.2665-2673.

[19] FitzGerald O, Haroon M, Giles JT, Winchester R. Concepts of pathogenesis in psoriatic arthritis: genotype determines clinical phenotype. Arthritis research & therapy. 2015 Dec 1;17(1):115.

[20] Queiro R, Morante I, Cabezas I, Acasuso B. HLA-B27 and psoriatic disease: a modern view of an old relationship. Rheumatology. 2016 Feb 1;55(2):221-9.

## 4. Eye Disease HLA Association

### A. Acute Anterior Uveitis HLA-B27 Association

a. Clinical Indication:

This test can be used as an ancillary testing to assist with diagnosis of acute anterior uveitis.

b. Methodology:

Low – Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

c. Limitations:

HLA antigens other than HLA-B are not evaluated in this testing. Rare sequence variants not covered by the assay probes may lead to inaccurate test result or interpretation. A negative test results of HLA-B27 does not preclude the possibility of developing acute anterior uveitis.

An occasional sample may not be satisfactory for testing. In those cases, an additional collection of this specimen may be required for testing. This test was developed and its performance characteristics determined by Stanford Blood Center. The U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

d. Clinical Background:

Uveitis is the most common form of inflammatory eye disease and an important cause of visual impairment. [1-2] Anterior uveitis is the predominant form of uveitis in most populations. [3] Acute anterior uveitis (AAU) is strongly associated with HLA-B27. [4] AAU associated with HLA-B27 accounts for about 18–32% of all cases of AAU in western countries. [1,3] HLA-B27 antigen increases the relative risk of AAU by 26 times. [4] AAU is the most common extra-articular manifestation of spondyloarthropathies and occurs in 20%–30% of patients with ankylosing spondylitis. [5] Approximately 50% of all patients with HLA-B27 AAU develop an associated seronegative arthritis, and about 25% of the patients initially diagnosed with HLA-B27 seronegative arthritis develop AAU. [3] Compared to the HLA-B27 negative AAU, the HLA-B27 positive AAU affects males 1.5-2.5 times more often than females, generally has earlier disease onset, high tendency for recurrences and significant association with other spondyloarthropathies. [1]

e. References:

- [1] Chang JH, McCluskey PJ, Wakefield D. Acute anterior uveitis and HLA-B27. *Surv Ophthalmol.* 2005;50(4):364-88.
- [2] Rothova A, Suttorp-van Schulten MS, Frits Treffers W, et al. Causes and frequency of blindness in patients with intraocular inflammatory disease. *Br J Ophthalmol.* 1996 Apr;80(4):332-6.
- [3] Wakefield D, Chang JH, Amjadi S, et al. What is new HLA-B27 acute anterior uveitis? *Ocul Immunol Inflamm.* 2011;19(2):139-44.
- [4] Brewerton DA, Caffrey M, Nicholls A, et al. Acute anterior uveitis and HL-A 27. *Lancet.* 1973;302(7836):994-6.
- [5] Gouveia EB, Elmann D, Morales MS. Ankylosing spondylitis and uveitis: overview. *Rev Bras Reumatol.* 2012;52(5):742-56.

## **B. Behçet's Disease HLA-B51 Association**

a. Clinical Indication:

The HLA-B51 (a serologic split of the HLA-B5 broad antigen specificity) is strongly and consistently associated with increased risk for Behçet's Disease in different populations of various ethnicities. Testing for this HLA allele group can be used to identify patients with increased risk for developing Behçet's Disease.

b. Methodology:

Low – Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

c. Limitations:

HLA antigens other than HLA-B are not evaluated in this testing. Rare sequence variants not covered by the assay probes may lead to inaccurate test result or interpretation. A negative test results of HLA-B51 does not preclude the possibility of developing Behçet's Disease.

An occasional sample may not be satisfactory for testing. In those cases, an additional collection of this specimen may be required for testing.

This test was developed and its performance characteristics determined by Stanford Blood Center. The U. S. Food and Drug Administration has not approved or cleared this

test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

d. Clinical Background:

Behçet disease (BD) is a multisystem autoinflammatory disease with a chronic, relapsing-remitting course, characterized predominantly by mucocutaneous lesions and ocular involvement. [1,2] BD has a geographical predominance in countries located between 30°N and 45°N latitude around the Middle East, the Mediterranean, China, Japan, and along the Silk Route. [3] HLA-B51, one of the split antigens of HLA-B5, is the most established genetic risk factor for BD to date. [2][4-6] The odds ratio for individuals carrying HLA-B51(B5) allele to develop BD compared with no-carriers was found to be 5.78 [6]. Its contribution to the overall genetic BD susceptibility was estimated to be about 20% [7,8].

e. References:

- [1] Leccese P, Alpsoy E. Behçet's Disease: An Overview of Etiopathogenesis. *Front Immunol.* 2019;10:1067.
- [2] Wallace, GR. HLA-B\* 51 the primary risk in Behçet disease. *Proc Natl Acad Sci U S A.* 2014;111(24):8706-7.
- [3] Salmaninejad A, Zamani MR, Shabgah AG, et al. Behçet's disease: An immunogenetic perspective. *J Cell Physiol.* 2019;234(6):8055-8074.
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- [5] Mizuki N, Ota M, Yabuki K, et al. Localization of the pathogenic gene of Behçet's disease by microsatellite analysis of three different populations. *Invest Ophthalmol Vis Sci* 2000;41(12):3702-8.
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## **C. Birdshot chorioretinopathy HLA-A29 Association**

a. Clinical Indication:

The HLA-A29 is strongly associated with Birdshot chorioretinopathy, and can be used as an ancillary testing to assist with diagnosis of this disorder.

b. Methodology:

Low – Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

c. Limitations:

HLA antigens other than HLA-A are not evaluated in this testing. Rare sequence variants not covered by the assay probes may lead to inaccurate test result or interpretation. A negative test results of HLA-A29 does not preclude the possibility of developing acute anterior uveitis.

An occasional sample may not be satisfactory for testing. In those cases, an additional collection of this specimen may be required for testing.

This test was developed and its performance characteristics determined by Stanford Blood Center. The U.S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

d. Clinical Background:

Birdshot chorioretinopathy (BCR) is a bilateral, autoimmune posterior uveitis with a distinct clinical phenotype. [1] BCR is a relatively rare form of uveitis and the prevalence is estimated to fall between 38/100,000 and 115/100,000 for European and US populations, and between 0.2-1.7/100,000 for all populations. BCR is most prevalent in Caucasian populations and predominantly seen in middle-aged individuals. [1] In the US, BCR accounts for approximately 8% of posterior uveitis. About 85-95% of BCR patients express HLA-A29, as compared to 7% of general population. [2,3] Despite the strong association of HLA-A29 with BCR, its exact role in the pathogenesis of BCR remains largely unknown. [5,6] Because there were reports of rare HLA-A29 negative BCR cases, the presence of HLA-A29 was included as a supportive, and not absolute diagnostic criterion in the research diagnostic criteria for BCR defined in the UCLA International Workshop. [1,6].

e. References:

- [1] Minos E, Barry RJ, Southworth S, et al. Birdshot chorioretinopathy: current knowledge and new concepts in pathophysiology, diagnosis, monitoring and treatment. *Orphanet J Rare Dis.* 2016;11(1):61.
  
- [2] Zucchiatti, I, Miserocchi, E., Sacconi, R., Bandello, F. and Modorati, G. HLA-A29positive uveitis: birdshot chorioretinopathy, what else. *Case reports in ophthalmology.* 2013;4(3):287-293.
  
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## 5. Carbamazepine Hypersensitivity HLA-B\*15:02 and HLA-A\*31:01 Association

### a. Clinical Indication:

HLA-B\*15:02 is a genetic marker strongly associated with increased risk for developing hypersensitivity reactions affecting the skin, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) after treatment with carbamazepine, particularly in patients with Southeast Asian ancestry. The HLA-A\*31:01 allele is associated with risk of a wider range of hypersensitivity reactions, including maculopapular exanthema (MPE), drug reaction with eosinophilia and systemic symptoms (DRESS) in different populations, and has a much weaker association with SJS/TEN. FDA recommends that testing for HLA-B\*15:02 should be done for patients prior to initiating Carbamazepine (or its keto-analog Oxcarbazepine) therapy.

### b. Methodology:

Low – Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

### c. Limitations:

The absence of HLA-B\*15:02 and HLA-A\*31:01 indicates normal risk of carbamazepine-induced hypersensitivity reactions but does not preclude the development of an adverse reaction to carbamazepine. A negative result does not indicate protection from reactions to carbamazepine and does not replace the need for therapeutic drug monitoring or other clinical testing. Other genetic and non-genetic factors that influence carbamazepine-related adverse reactions are not evaluated in this assay.

This test is designed to specifically test the HLA-B\*15:02 and HLA-A\*31:01 alleles, and other HLA loci will not be evaluated. Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles. Treatment with carbamazepine should be discontinued in all patients if severe hypersensitivity symptoms develop, regardless of HLAB\*15:02 or HLA-A\*31:01 status.

If a sample is not sufficient or satisfactory to perform the assay, an additional specimen should be collected for repeat testing. Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA). FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

## d. Clinical Background:

Carbamazepine is an anticonvulsant drug that is often used as a first-line agent to treat seizure disorders, and also used to treat bipolar disorder and pain in trigeminal neuralgia. In about 10% of cases, carbamazepine can induce hypersensitivity reactions that typically affect the skin. [1] Some reactions are mild, causing maculopapular exanthema (MPE); however, severe conditions, including SJS, TEN, and Dress syndrome, can be life-threatening. [1,2]

The presence of one or two copies of the major histocompatibility complex class I allele HLAB\*15:02 or HLA-A\*31:01 has been shown to be strongly associated with the development of carbamazepine-induced hypersensitivity reactions [3]. The HLA-B\*15:02 allele is strongly associated with carbamazepine-induced SJS/TEN, while the HLA-A\*31:01 allele is more strongly associated with DRESS and MPE. The frequency of HLA-B\*15:02 allele varies with ethnicity, with the highest prevalence in East Asian (6.9%), Oceanian (5.4%), and South/Central Asian (4.6%) populations; much lower in Japanese (<1%) and Korean (<2.5%) populations; and largely absent in Caucasians, African-Americans, Middle Easterners, and Hispanics/South Americans (<1%) [1, 2]. The HLA-A\*31:01 allele, on the other hand, is found in most populations.

Due to this increased risk of potentially serious or fatal adverse reactions, the FDA issued a health alert in 2007 and recommended HLA-B\*15:02 testing before initiating carbamazepine in patients with ancestry in at-risk populations [3]. The Clinical Pharmacogenomics Implementation Consortium (CPIC) recommended that carbamazepine should not be prescribed to patients who test positive for the HLA-B\*15:02 or HLA-A\*31:01 allele [2]. Carbamazepine-induced hypersensitivity reactions usually develops within the first 4-28 days of therapy; therefore, patients who have been continuously taking carbamazepine for greater than 3 months without developing cutaneous reactions are at low risk (but not zero) of developing adverse events in the future, regardless of their HLA-B\*15:02 status [2].

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## 6. Celiac Disease HLA-DQ Association (Celiac Genetics)

### a. Clinical Indication:

This test aids in the diagnosis of celiac disease (CD) by providing the HLA-DQA1 and HLA-DQB1 genotype at the two-field allele detection level. This test will report the presence or absence of HLA-DQ2 (DQA1\*05:01 or 05:05 and DQB1\*02:01 or 02:02) and HLA-DQ8 (DQA1\*03 and DQB1\*03:02), as well as an estimated risk for CD based on the literature. This test is not indicated as an initial diagnostic screen for CD, and it is mainly considered for its high negative predictive value. It may be indicated in scenarios where tissue biopsy is not possible, infants never exposed to gluten, young children who might not make antibodies, patients with indeterminate serology or biopsies, relatives of biopsy-diagnosed individuals, patients with IgA deficiency, and patients on a self-imposed gluten-free diet (GFD) unwilling to undergo gluten challenge.

### b. Methodology:

PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

### c. Limitations:

This test is designed to specifically test the HLA-DQA1 and HLA-DQB1 loci, and other HLA loci, genetic and non-genetic factors potentially contributing to CD predisposition will not be evaluated by this assay.

Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles. An occasional sample may not be satisfactory for testing. In those cases, an additional collection of this specimen may be required for testing.

Development and performance characteristics of this HLA test is determined by Stanford Blood Center (SBC). It has not been cleared or approved by the US Food and Drug Administration (FDA). FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

### d. Clinical Background:

Celiac disease (CD) is an autoimmune condition triggered by gluten ingestion in genetically predisposed individuals. Partly undigested gluten peptides cross the intestinal barrier and

are modified by tissue transglutaminase 2 (TG2), which generates epitopes that are then presented to autoreactive gluten-specific T cells. This triggers an immune response that attacks the villi of the small intestine, leading to malabsorption of nutrients and secondary symptoms such as fatigue, joint pain, and gastrointestinal discomfort. The prevalence of CD is estimated to be approximately 1% in Europe and North American populations, but the disease often goes undiagnosed because many patients have silent or atypical forms [1]. Early diagnosis and treatment are important to prevent long-term complications like intestinal cancers, infertility and additional autoimmune conditions [1,2].

CD has a strong hereditary genetic component, with the HLA region alone being the major CD-predisposing factor, accounting for approximately 40% of the disease heritability [3-5]. Specific variant alleles of the HLA class II genes HLA-DQA1 and HLA-DQB1 are known to contribute to the risk of developing CD. HLA-DQA1 and DQB1 loci encode the alpha and beta subunits of the HLA-DQ molecule, which are heterodimers located on the cell surface of antigen-presenting cells (APCs) and present peptides to CD4+ T cells. The genetic variants with highest association with CD are HLA-DQA1\*05:01, -DQA1\*05:03 or -DQA1\*05:05 and HLA-DQB1\*02:01 or -DQB1\*02:02 (being the most common in the general population), which can combine in cis (on the same chromosome) or trans (on two homologous chromosomes) to form the so-called HLA-DQ2.5 heterodimer molecule. At a lower scale in comparison to DQ2.5, increased risk to CD is also associated with heterodimer DQ2.2, which is made up of HLA-DQA1\*02 (\*02:01 being the only most common) and -DQB1\*02 (\*02:01 or 02:02 being the most common in the general population) subunits. Thirdly, the HLA-DQB1\*03:02 and -DQA1\*03 (\*03:01/03:02/03:03 being the most common in the general population) variants combine to form the HLA-DQ8 heterodimer molecule, which also predisposes patients to CD. Each of these variants and each given variant combination carries a unique risk of CD. The combination of HLA-DQA1\*05 and DQB1\*02 (either in cis or trans) encodes the DQ2.5 heterodimer molecule, which appears to have the strongest association with CD, and depending on the study set, up to 97% of CD patients are DQ2.5 positive (compared with 35% of the general population) [6,7]. More than 90% of patients with celiac disease will have a full DQ2 (i.e. one or more copies of the predisposing HLA-DQA1 or -DQB1 variants), but some may only have DQ8 or one half of the DQ2 heterodimer molecule [1,2]. The various genetic combinations of these alleles have been well-studied to provide a CD risk gradient associated with each particular HLA-DQ status (DQA1 and DQB1 subunits and their combinations) and DQ2/DQ8 homozygosity/heterozygosity [6,8]. An abbreviated version of the risk gradient is shown in the Table 1 [9-13].

HLA testing is mainly valued in clinical management for its negative predictive value since CD is highly unlikely when DQ predisposing alleles are absent, while a positive result only implies a genetic predisposition. HLA may be used to screen first-degree relatives due to the higher prevalence of CD among relatives of celiac patients [8,12], since 20% of sibs and 6%

of parents, positive for the HLA-DQA1/-DQB1 predisposing alleles, are affected [4,10]. The test may be used amongst CD patient families to determine their DQA1/DQB1 risk gradient, thereby allowing better counseling and appropriate follow-up visits [7,12]. In the meantime, a negative gene test result may have psychological impact to make individuals feel reassured of their low CD risk.

Table 1: Estimated Risk of Celiac Disease based on HLA-DQ Genotypes

HLA-DQ genotype	Disease Risk
<b>1- Homozygous DQA1*05/DQB1*02</b>  (DQA1*05:01, DQA1*05:01 + DQB1*02:01, DQB1*02:01, almost always in subjects homozygous for DRB1*03:01 haplotype)  *In bibliography this genotype may be cited as DQ2.5 in double dose	Very High Risk
<b>2- DQA1*05, DQA1*03 + DQB1*02, DQB1*03:02</b>  (DQA1*03:01, DQA1*05:01 + DQB1*02:01, DQB1*03:02; almost always in subjects carrying DRB1*03:01 and DRB1*04-DQB1*03:02)  *In bibliography this genotype may be cited as DQ2.5, DQ8	Very High Risk
<b>3- DQA1*05, ≠ DQA1*05+ DQB1*02, DQB1*02</b>  (DQA1*05:01, DQA1*02:01/03:03 + DQB1*02:01, DQB1*02:02; almost always in subjects carrying DRB1*03:01 and DRB1*07:01/DRB1*04/DRB1*09, DQB1*02:02)  *In bibliography this genotype may be cited as DQ2.5/DQ2.2	High Risk
<b>4- Heterozygous DQA1*05/DQB1*02</b>  (DQA1*05, ≠DQA1*02 or DQA1*05, ≠DQB1*02) + DQB1*02  *In bibliography this genotype may be cited as DQ2.5, with a single dose of DQB1*02	High Risk
<b>5- DQA1*05, DQA1*05 + DQB1*02, ≠DQB1*02</b>  *In bibliography this genotype may be cited as DQ2.5, with a single dose of DQB1*02	High Risk

<p>6- Homozygous DQB1*03:02</p> <p>(DQA1*03, DQA1*03 + DQB1*03:02, DQB1*03:02; almost always in subjects with DRB1*04, DRB1*04 and DQB1*03:02,DQB1*03:02)</p> <p>*In bibliography this genotype may be cited as DQ8 homozygous</p>	Moderately increased risk
<p>7- DQA1*05 Negative + DQB1*03:02, DQB1*02</p> <p>(almost always in subjects with DRB1*04, DRB1*04/DRB1*07/DRB1*09 and DQB1*03:02, DQB1*02:02)</p> <p>*In bibliography this genotype may be cited as DQ8/DQ2.2</p>	Moderately increased risk
<p>8- Negative for DQA1*05+ DQB1*02, DQB1*02</p> <p>( No DQA1*05 with a double dose of DQB1*02:02; almost always in subjects homozygous for DRB1*07:01 and DQB1*02:02)</p> <p>*In bibliography this genotype may be cited as DQ2.2 homozygous</p>	Moderately increased risk
<p>9- DQB1*03:02, ≠DQB1*03:02/02:01/02:02</p> <p>*In bibliography this genotype may be cited as DQ8 heterozygous or DQ8 positive, DQ2 negative</p>	Low Risk
<p>10- ≠ DQA1*05, ≠DQA1*05 + DQB1*02, ≠DQB1*02</p> <p>(No DQA1*05 + One allele of DQB1*02; almost always DRB1*07:01, heterozygous and Negative for haplotypes bearing DQA1*05)</p> <p>In bibliography this genotype may be cited as DQ2.2 heterozygous</p>	Low Risk
<p>11- Patient is negative for both DQB1*02 and DQB1*03:02 (DQ2 and DQ8 negative)</p>	Genotypes associated with susceptibility to celiac disease are absent.

Notes: HLA-DQ2.5 heterodimers are encoded by DQA1\*05 and DQB1\*02 alleles; HLA-DQ2.2, by DQA1\*02 and DQB1\*02 alleles; and HLA-DQ8, by DQB1\*03:02 allele in combination with DQA1\*03.



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## 7. Narcolepsy HLA-DQB1\*06:02 Association

### a. Clinical Indication

The HLA-DQB1\*06:02 allele is strongly associated with narcolepsy with cataplexy and present in 85-95% of Type 1 narcolepsy patients. HLA genotyping, along with an assessment of cataplexy and sleep testing, helps diagnose or rule out type 1 narcolepsy. DQB1\*06:02 is present in only 40-50% patients with Type 2 narcolepsy, which is not much higher than the rate of the allele in the general population (12-38%). Testing in these cases are not generally recommended.

In addition, prior studies have shown that almost all patients with narcolepsy carry the DQA1\*01:02-DQB1\*06:02 heterodimer versus approximately 25% of controls. Both alleles were suggested to be involved in predisposition to narcolepsy.

### b. Methodology: Low – Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

### c. Limitations

The absence of HLA-DQB1\*06:02 or HLA-DQA1\*01:02 does not preclude the development of narcolepsy. A negative result does not replace the need for close clinical monitoring or other clinical testing. Alleles other than HLA-DQB1\*06:02 and HLA-DQA1\*01:02 are not evaluated. Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles. Other genetic and nongenetic factors that influence narcolepsy are not evaluated in this assay.

If a sample is not sufficient or satisfactory to perform the assay, an additional specimen should be collected for repeat testing.

Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA). FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

### d. Clinical Background

Narcolepsy is a chronic sleep disorder characterized by unusual sleep-wake cycles.

Narcolepsy patients suffer from excessive daytime sleepiness, and they will often fall asleep involuntarily even while performing activities. Patients can also experience sleep paralysis and hallucinations while falling asleep or waking up. Cataplexy, a sudden loss of muscle control often triggered by strong emotions, is a prominent symptom seen in patients with type 1 narcolepsy (previously termed narcolepsy with cataplexy) and not in patients with type 2 narcolepsy (previously termed narcolepsy without cataplexy) [1-3]. Narcolepsy is likely caused by environmental exposures in genetically susceptible individuals. It is postulated that the cells in the hypothalamus secreting hypocretin, a neurotransmitter that regulates sleep and arousal, are selectively destroyed by immune cells [3].

The prevalence of narcolepsy has been reported to be between 25 and 50 per 100,000 people. In one study, the average incidence for narcolepsy with cataplexy was 0.74 per 100,000 person-years, and the average incidence for narcolepsy was 1.37 per 100,000 person-years. The actual number of individuals with narcolepsy may be higher because many cases are likely undiagnosed or misdiagnosed [1, 3].

Narcolepsy with cataplexy is strongly associated with HLA-DQB1\*06:02, which is present in 85-95% of type 1 narcolepsy patients, and not ethnicity-specific. Individuals carrying heterozygous HLA-DQB1\*06:02 has 7-25 fold higher risk to develop narcolepsy, and homozygosity for HLA-DQB1\*06:02 increases risk additional 2-4 fold. In individuals with heterozygous DQB1\*06:02, the relative risk for narcolepsy increases with presence of DQB1\*03:01 and decreases with DQB1\*05:01 and DQB\*06:01. [4-5] HLA-DQB1\*06:02 is present in only 40-60% of patients with type 2 narcolepsy and 75% of familial cases. [5-6] This DQB1\*06:02 allele is common in the general population and seen in 12% of Asians, 25% of Caucasians, and 38% of African Americans, whereas only a small percentage of these individuals develop narcolepsy. Therefore, this HLA allele, despite being the only established genetic risk factor, is neither necessary nor sufficient to trigger narcolepsy. [2] HLADQB1\*06:02 is not highly specific for type 2 narcolepsy and testing is not generally recommended [7].

Interestingly, complex genetic associations between multiple risk alleles were shown to contribute to the genetic predisposition to human narcolepsy. [8]. Moreover, a concept of the allele competition effect was proposed, which may explain the protective effects of various HLA combinations in narcolepsy. Almost all patients with narcolepsy carry the DQA1\*01:02-DQB1\*06:02 heterodimer versus approximately 25% of controls [9]. Both DQA1\*01:02 and DQB1\*06:02 were predicted to be important for peptide binding. Since DQA1\*01:02 is also commonly found in other DQB1 haplotypes not associated with narcolepsy without DQB1\*06:02, DQA1\*01:02 alone does not predispose to narcolepsy. Because DQA\*01:02 is always present together with DQB1\*06:02, it is impossible to test whether DQB1\*06:02

alone predisposes to narcolepsy using existing data, although the allele competition model suggested so [9].

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## **8. Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) HLA-B38, HLA-B5, HLA-B51, HLA-B52, HLA-B27, and Bw4 association**

### **a. Clinical Indication:**

The presence of HLA-B38, HLA-B5, HLA-B51, HLA-B52, HLA B27 and Bw4 have been found to be associated with Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) in a study performed at Stanford using data from the Stanford PANS clinic. There was no association found with the HLA Class II genes. A second PANS cohort will be analyzed to determine if these results are reproducible.

### **b. Methodology:**

Low – Intermediate = PCR-SSO (HLA Typing with PCR and Sequence-Specific Oligonucleotide Hybridization-based method)

### **c. Limitations:**

This test is designed to specifically type the HLA-B locus and other HLA loci will not be evaluated. Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles. This assay is based on a study performed at Stanford on a single cohort from the Stanford PANS clinic. To date, no other studies have been performed to evaluate HLA associations in patients with PANS.

If a sample is not sufficient or satisfactory to perform the assay, an additional specimen should be collected for repeat testing.

Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA).

### **d. Clinical Background:**

PANS is a clinical diagnosis given to children who have a sudden onset of obsessivecompulsive symptoms and/or severe eating restrictions and at least two concomitant cognitive, behavioral, or neurological symptoms, typically following infections such as *Streptococcus pyogenes*, varicella, and *Mycoplasma pneumoniae*. The cause of PANS has not been elucidated, but in most cases is thought to be triggered by a variety of etiologies. [1, 2] Patients with PANS have also been observed to have co-existing arthritis and autoimmune diseases, and/or first-degree family members with autoimmune diseases.

A study performed at Stanford found that PANS was significantly associated with HLA-B antigens, including HLA-B38, HLA-B5, HLA-B51, HLA-B52, and HLA-B27. Further investigation into HLA-B27 reveals that the B\*27:02 allele is strongly associated. As these



associated alleles share the HLA-Bw4 epitope, amino acid level analysis was performed and reveals significant amino acid level associations at positions 80-83 (the Bw4 epitope). There is no association found with the HLA Class II genes. [3]

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## 9. Psoriasis HLA-Cw6 and HLA-B27 Association (Psoriasis and Psoriatic arthritis)

### a. Clinical Indication:

HLA-C\*06 is a strong genetic component for the predisposition to the autoimmune reactions that result in Psoriasis (isolated cutaneous-only known as PsO). The presence of the HLA-Cw6 antigen is associated with early onset psoriasis vulgaris (type 1) and guttate psoriasis.

The presence of HLA-B\*27 is associated many inflammatory disorders and spondyloarthropathic conditions. Testing for HLA-B\*27 can be used as ancillary testing to support the diagnosis and determine a therapeutic regimen in patients with HLA-B\*27-associated joint disease with psoriatic arthritis (PsA).

### b. Methodology:

Low – Intermediate = PCR-SSO (HLA Typing with Sequence-Specific Oligonucleotide Primed PCR)

### c. Limitations:

The absence of the HLA-Cw6 or HLA-B27 antigen does not preclude the development of psoriasis. A negative result does not replace the need for close clinical monitoring or other clinical testing. Antigens other than HLA-Cw6 and HLA-B27 are not evaluated. Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles. Other genetic and non-genetic factors that influence psoriasis or psoriatic arthritis are not evaluated by this assay.

If a sample is not sufficient or satisfactory to perform the assay, an additional specimen should be collected for repeat testing.

Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA). FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

### d. Clinical Background:

Psoriasis is a chronic inflammatory and hyperproliferative skin disease with a prevalence ranging from 0.1-11.4% [1]. There are complex interactions between genetic components, environmental factors, and immune-mediated pathways that can influence the clinical

presentation, areas of involvement, and response to treatment in different patients with psoriasis.

Psoriasis vulgaris (plaque-type) is the most common clinical form and accounts for 80-90% of all cases of psoriasis. Symptoms include dry, raised, red skin lesions covered with silvery scales. These plaques might be itchy or painful and can occur anywhere on the body. Psoriasis can be classified based on genetic and demographic factors. Type 1 has a positive family history, starts before the age of 40, and is associated with HLA-Cw6. Conversely, type 2 does not show a family history, presents after age 40, and is not associated with HLA-Cw6. Type 1 accounts for about 75% of persons with psoriasis. Psoriatic arthritis is a form of chronic and inflammatory arthritis that can occur in small joints of hands in association with skin psoriasis. The prevalence of psoriatic arthritis differs with ethnicity, ranging from 64-72%. [1].

HLA-Cw6 is one of the most strongly associated psoriasis susceptibility alleles shown to play an important role in the pathogenesis of the disease. It has been hypothesized that HLA-Cw6 may affect both the innate system causing abnormal lymphocyte function as well as the adaptive immune system via antigen presentation to T-cells [1]. The HLA-Cw6 allele frequency varies in different populations and can range from 14-59%. The percentage of patients with psoriasis carrying HLA-Cw6 ranges from 11-77%. HLA-Cw6 positive rate in patients with psoriasis is generally higher in Caucasians than in Asians. [1] It is thought that HLA-Cw6 is a biomarker of skin involvement in psoriatic disease and has little or no influence in the development of joint disease.

The association of HLA-B\*27 with spondyloarthropathies is well documented and has been shown to be a stronger genetic marker for psoriatic arthritis than for psoriasis in population-based analyses. [2-3] The mechanism is hypothesized to be immune-mediated in that the molecules encoded by the HLA-B\*27 alleles recognize self-peptides derived from proteins found in enthesal and synovial sites. T-cell clones specific for these self-peptides are inappropriately activated, which is perpetuated by the continual exposure to self peptides [4].

Overall, about 10-20% of psoriasis patients (5-10% HLA-C\*06 positive patients and 30-60% of HLA-B\*27 positive patients) develop psoriatic arthritis. HLA-C\*06 is present in 60% of patients with psoriasis patients and ~30% of patients with psoriatic arthritis. HLA-B\*27 is present in ~20% of patients with psoriatic arthritis and 5% of patients with psoriasis. Studies have suggested that there could be two genetic pathways to psoriatic arthritis since several MHC alleles operate independently to confer varying disease phenotypes. One is through the function of HLA-B\*27, and another is through the function of haplotypes containing the HLA-C\*06 allele. These two forms of psoriatic arthritis that share the psoriasis phenotype are subtly different. It has been shown that the HLA-C\*06 alleles confer a phenotype with more severe skin disease and, on average, a long interval ( $\geq 10$  years)

between the appearance of psoriasis and the development of joint disease as seen in psoriatic arthritis [4]. In patients with HLA-B\*27, the joint disease component appears more synchronously with the cutaneous component, and psoriatic arthritis is more likely than in the presence of HLA-C\*06.

Based on these findings, testing for HLA-Cw6 and HLA-B27 aids in the diagnosis of psoriasis and/or psoriatic arthritis, if other clinical signs and symptoms are present.

e. References:

[1] Chen L, Tsai TF. HLA-Cw6 and psoriasis. Br J Dermatol. 2018 Apr;178(4):854-862.

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## 10. IL-1 and IL-6 Inhibitors Hypersensitivity

### a. Clinical Indication:

Reactions classified as drug reaction with eosinophilia and systemic symptoms (DRESS) occur in some patients with systemic-onset juvenile idiopathic arthritis (sJIA) or adult-onset Still's disease (AOSD) treated with IL-1 or IL-6 inhibitors. Notably, these delayed hypersensitivity reactions (DHR) are strongly associated with common HLA-DRB1\*15 class II haplotypes.

### b. Methodology: Low – Intermediate = PCR-SSO (HLA Typing with Sequence-Specific Oligonucleotide Primed PCR)

### c. Limitations:

This test is designed to specifically type the HLA-DRB1, -DRB3/4/5 -DQB1 and DQA1 genes, and other HLA loci will not be evaluated. Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles.

This assay is based on a case/control study [1] comprising a multicenter series of patients with Still's disease with features of inhibitor-related DRESS (n=66) and drug-tolerant Still's controls (n=65). Clinical data were retrospectively analyzed from all Still's subjects and 94/131 were typed for HLA. Therefore, data from this study is still insufficient as a basis for specific recommendations on when or if it is safe to use these inhibitors in patients with Still's disease with the reaction-associated HLA haplotypes. Further research is needed to determine underlying mechanisms and additional risk factors for DRESS reactions associated with inhibitors of IL-1 and IL-6. In other inflammatory conditions, DRESS reactions have been reported during treatment with these drugs; however, data is sparse and relevance beyond Still's disease is currently unknown.

If a sample is not sufficient or satisfactory to perform the assay, an additional specimen should be collected for repeat testing. Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration(FDA).

### d. Clinical Background:

Systemic juvenile idiopathic arthritis (sJIA) is a chronic, inflammatory disease of childhood, observed worldwide, with an incidence of 0.4–0.9/100,000 in North America and Europe [2]. A similar disease occurs in adults (adult-onset Still's disease (AOSD); incidence: 0.2–0.4/100,000) [3]. sJIA and AOSD are collectively known as Still's disease. Still's disease is characterized by a combination of arthritis and systemic inflammation, manifest as daily fever spikes, evanescent macular rash and serositis. A known complication risk in Still's is secondary hemophagocytic lymphohistiocytosis, also called macrophage activation syndrome in the setting of rheumatic disease.

The usual pulmonary complications of Still's are pleuritis and pleural effusion [2,4], but parenchymal lung disease (LD) is very rare, with sporadic reports and one initial multicentre series of 25 cases (Kimura et al, 2013) in the literature [5]. However, in the last decade, increasing numbers of cases of high fatality, parenchymal LD are being detected worldwide. A majority of these are characterized by unusual clinical and radiological features, in association with a variant of pulmonary alveolar proteinosis/endogenous lipid pneumonia often with pulmonary vascular involvement [6-8].

The emergence of LD has coincided with increasing therapeutic use of the inhibitors of interleukin-1 (IL-1; anakinra, canakinumab, and rilonacept) or IL-6 (tocilizumab). These drugs were introduced into the management of Still's about 15 years ago. This approach is rapidly effective in >65% of patients, implicating these inflammatory mediators as key drivers of sJIA [9-11]. Most of the Still's patients with LD have a history of exposure to one or more of these agents [6-8].

Saper et al. first observed reactions classified as drug reaction with eosinophilia and systemic symptoms (DRESS) among a small group of sJIA patients who developed the LD described above during treatment with the above IL-1 or IL-6 inhibitors [7]. Based on this, in subsequent work [1,12], Saper et al. conducted a multicentre study including 66 patients with sJIA or AOSD who had a drug-related reaction to these IL-1 and/or IL-6 inhibitors. An additional 65 apparently drug-tolerant patients were enrolled in the study. In a retrospective analysis of clinical data, 65 of 66 of the drug-reactive cases were verified to classify as DRESS according to RegiSCAR criteria. HLA data was available for 94 of the 131 patients. HLA-DRB1\*15 haplotypes were strikingly enriched in those with DRESS as compared with self-identified ancestry-matched drug-tolerant study participants ( $P = 6.3 \times 10^{-10}$ ) and compared with ancestry-matched individuals in the International Childhood Arthritis Genetics Consortium (INCHARGE) sJIA cohort ( $P = 7.5 \times 10^{-13}$ ) [1]. The HLA genetic risk factor was found to be shared across the delayed reactions associated with these inhibitors, analyzed individually or as a group. A very strong association of the HLA-DRB1\*15:01 allele and the haplotype-linked HLA-DRB5\*01:01 was observed in white Still's subjects. Although this study was limited by the relative scarcity of non-white subjects, results suggested that, in addition to HLA-DRB1\*15:01, other alleles of the HLA-DRB1\*15 family (15:01, \*15:03, \*15:06) are linked to risk of inhibitor-triggered reaction in these populations. The distribution of subjects with HLA-DRB1\*15:XX argued the risk applies across ancestry groups, as found in some other HLA/DHR associations [13]. Carriers of DRB1\*15:01, \*15:03, \*15:06 alleles are common (27% (White), 15% (Hispanic), 27% (Black) and 16% (Asians), respectively) in US populations [14]. This study cohort did not allow analysis of HLA-DRB1\*15:02, a high frequency DRB1\*15 allele in Asian populations [1]. Approximately 20% of the subjects with the DRESS reaction/LD do not carry the risk alleles [1]. Therefore, it will be important to determine in future studies if other genetic factors confer risk, both in those with and those without the DRB1\*15 risk alleles.



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## 11. HLA Typing Custom Panel (Intermediate resolution)

### a. Clinical Indication:

Different DNA-based molecular techniques are used for HLA typing to define HLA alleles and allele groups depending on the clinical application. HLA typing plays a critical role in both solid organ and hematopoietic stem cell transplantation (HSCT), and is also used in drug interactions, platelet refractoriness, autoimmune diseases, cancer, allergy, infectious diseases, and vaccine development.

HLA typing and determining whether alloimmunization has occurred may inform diagnoses, treatment options, and transfusion support plans. Numerous methods for HLA typing exist that include a single, multiple, or all clinically relevant HLA loci. In addition, these different methods may generate different degrees of detail regarding the HLA type depending on the specific treatment needed. For example, solid organ transplantation typically requires a low-to intermediate-resolution typing to determine HLA antigens whereas hematopoietic stem cell transplantation commonly requires high-resolution typing to determine the HLA alleles.

PCR-based HLA typing includes both MHC Class I and Class II regions: HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DRB3/4/5, DPA1, DPB1 loci.

### b. Methodology: Low – Intermediate = PCR-SSO (HLA Typing with Sequence-Specific Oligonucleotide Primed PCR)

### c. Limitations:

If a sample is not sufficient or satisfactory to perform the assay, an additional specimen should be collected for repeat testing. Diagnostic errors can occur due to rare sequence variations, or the presence of rare and undocumented alleles.

Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA).

### d. Clinical Background:

The HLA system is comprised of an important set of genes that influence the immune system and is characterized by a high degree of polymorphism in the human genome. HLA alleles are known to be associated with increased risk of certain disease processes, and hypersensitivity to certain medications. Thus, HLA typing information plays a major role in both solid organ and hematopoietic stem cell transplantation (HSCT), drug testing, platelet refractoriness, autoimmune diseases, cancer, allergy, infectious diseases, and vaccine development [1].

Low-resolution typing is composed of the first field in the DNA-based nomenclature and generally corresponds to the serological typing result. Intermediate-resolution typing results include a subset of alleles sharing the digits in the first field of their allele name and for which some alleles sharing those digits are excluded.

e. References:

[1] Fung MK, Benson K. Using HLA typing to support patients with cancer. Cancer Control. 2015 Jan;22(1):79-86.

## 12. Full HLA Typing (High Resolution)

### a. Clinical Indication:

Different DNA-based molecular techniques are used for HLA typing to define HLA alleles and allele groups depending on the clinical application. HLA typing plays a critical role in both solid organ and hematopoietic stem cell transplantation (HSCT), and is also used in drug interactions, platelet refractoriness, autoimmune diseases, cancer, allergy, infectious diseases, and vaccine development.

HLA typing and determining whether alloimmunization has occurred may inform diagnoses, treatment options, and transfusion support plans. Numerous methods for HLA typing exist that include a single, multiple, or all clinically relevant HLA loci. In addition, these different methods may generate different degrees of detail regarding the HLA type depending on the specific treatment needed. For example, solid organ transplantation typically requires a low-to intermediate-resolution typing to determine HLA antigens whereas hematopoietic stem cell transplantation commonly requires a high-resolution typing to determine the HLA alleles.

HLA typing by next-generation sequencing includes both MHC Class I and Class II regions: HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DRB345, DPA1, DPB1 loci.

### b. Methodology:

High resolution = NGS (HLA Typing by Next Generation Sequencing method)

### c. Limitations:

Rare diagnostic errors can occur due to primer site mutations.

If a sample is not sufficient or satisfactory to perform the assay, an additional specimen should be collected for repeat testing.

Development and performance characteristics of this HLA test is determined by Stanford Blood Center. It has not been cleared or approved by the US Food and Drug Administration (FDA).

### d. Clinical Background:

The HLA system is comprised of an important set of genes that influence the immune system and is characterized by a high degree of polymorphism in the human genome. HLA alleles are known to be associated with increased risk of certain disease processes, and hypersensitivity to certain medications. Thus, HLA typing information plays a major role in both solid organ

and hematopoietic stem cell transplantation (HSCT), drug testing, platelet refractoriness, autoimmune diseases, cancer, allergy, infectious diseases, and vaccine development [1].

High-resolution typing is defined as a DNA-based typing result that includes a set of alleles specifying and encoding the same protein sequence of an HLA molecule.

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[1] Fung MK, Benson K. Using HLA typing to support patients with cancer. *Cancer Control*. 2015 Jan;22(1):79-86.