Recommendations for performance and interpretation of the HLA test for Coeliac Disease in Italy (a document approved by CSN-AIC, SIGU and AIBT)

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**CSN-AIC:**
National Scientific Committee of the Italian Coeliac Association (Clinical Practice)

**SIGU:**
Italian Society of Human Genetics (Genetics)

**AIBT:**
Italian Association of Immunogenetics and Transplantation Biology (HLA typing)
Recommendations for HLA testing

**Aims:**

- to promote the *appropriate use* of the test
- to define the requirements for *laboratories* that can perform the test
- to provide details on *how* the testing is to be performed
- to propose a standard form for *test reports*
- to recommend a *correct interpretation* of results
Introduction

CD is multifactorial disease in which HLA-DQ represent the main susceptibility genes.

HLA typing in CD has a limited diagnostic value, and only assesses the risk of a person to develop the disease.

Analysis of HLA susceptibility genes has mostly negative predictive value, since the absence of risk alleles makes highly unlikely the development of the disease (but does not exclude it!). In the literature the frequency of DQ2 and DQ8 negative patients varies between 0 and 3%.
When to offer testing (avoiding useless analysis!)

HLA typing is recommended for:
- patients with an uncertain diagnosis of CD or
- asymptomatic individuals with an increased risk for CD (relatives, associated diseases) to decide whether to continue the follow-up in case of negative serology.[1]

[1] New ESPGHAN Guidelines - Husby et al. JPGN 2012:
  - children with strong clinical suspicion of CD and high specific antibodies (TG2 > 10x normal levels or anti-DGP, in children younger than 2 years). In presence of DQ2/DQ8 and EMA positivity the diagnosis could be done without biopsy.

For groups at risk for CD, start the screening with DQ2 and DQ8 typing.
Recommendations for HLA testing

Which laboratories can perform testing

Labs providing the genetic tests must document experience in molecular biology and participate in quality control programmes.

Tests should be undertaken in laboratories with an accreditation by the Italian Society of Human Genetics (SIGU) or by the European Federation for Immunogenetics (EFI)

EFI site http://www.efiweb.eu/ under EFI affairs reports: 1. standards, 2. accreditation 3. accredited Laboratories
DQ2/DQ8 association

CD patients

\[ DQA1*05, DQB1*02 \] >80%
\[ DQA1*03, DQB1*03:02 \] ≈10%
\[ DQB1*02 (DQA1*05 neg) \] ≈5%

Rare cases do not have any risk allele
**HLA-DR associations**

*DRB1* alleles do not modify the risks of CD that only depend on *DQ* genes. Their determination may be helpful as a validation of the results, because of the strong *linkage disequilibrium* between DR and DQ loci.
Alleles that have to be tested

The **molecular** typing **must** include:

- $DQA1^*03$, $DQA1^*05$, $DQB1^*02$ and $DQB1^*03:02$
- negative and positive controls

For completeness it **could** be added:

- $DRB1^*03$, *04, *07, *11 and *12

It’s **recommended** to check the $DQB1^*02$

homozygous status
# Recommendations for HLA testing

## Form suggested for Genetic Test Results

<table>
<thead>
<tr>
<th>IDENTIFICATION DATA OF THE LABORATORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surname and Name</td>
</tr>
<tr>
<td>Date of birth</td>
</tr>
<tr>
<td>Identification Number</td>
</tr>
<tr>
<td>Referring Physician Name</td>
</tr>
<tr>
<td>Kind of sample (peripheral blood, saliva..)</td>
</tr>
<tr>
<td>Date of sample</td>
</tr>
<tr>
<td>Reason for submission (uncertain diagnosis, associated disease, affected family member..)</td>
</tr>
<tr>
<td>Assay performed</td>
</tr>
<tr>
<td>Method used</td>
</tr>
<tr>
<td>Alleles tested</td>
</tr>
</tbody>
</table>
The presence of any of these conditions is indicative of susceptibility to coeliac disease and does not imply the development of the disease that must be verified by serological tests and intestinal biopsy.

- Presence of the DQ2 heterodimer (\textit{DQA1*05, DQB1*02})
  Status \textit{DQB1*02} homozygous: present, absent, not determined

- Presence of only the beta chain of the DQ2 dimer (\textit{DQB1*02})

- Presence of the DQ8 heterodimer (\textit{DQA1*03, DQB1*03:02})

The presence of any of these conditions is indicative of susceptibility to coeliac disease and does not imply the development of the disease that must be verified by serological tests and intestinal biopsy.

- Absence of HLA at risk of coeliac disease

This condition makes the occurrence of coeliac disease highly unlikely.

For any clarification on the interpretation of results please contact the laboratory that performed the test or a medical expert in the genetics of coeliac disease.

Date ............./............./.............

Operator Director
Recommendations for HLA testing

Summary

HLA typing for Coeliac Disease:

- gives information only about the risk of disease
- must be performed just when indicated
- should be conducted in laboratories participating in quality controls
- must consider all the DQ associated alleles
- should be reported in a standard form so as to make the interpretation easier
Co-Authors

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Antonio Amoroso
*Italian Society of Human Genetics (SIGU)*

and

Ettore Cardi

THANKS!
<table>
<thead>
<tr>
<th>Risk allele</th>
<th>DQ2 and DQ8</th>
<th>1:7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homozygous DQ2</td>
<td>1:10</td>
</tr>
<tr>
<td></td>
<td>DQ8 and B1*02</td>
<td>1:24</td>
</tr>
<tr>
<td></td>
<td>Homozygous B1*02</td>
<td>1:26</td>
</tr>
<tr>
<td></td>
<td>DQ2</td>
<td>1:35</td>
</tr>
<tr>
<td></td>
<td>DQ8</td>
<td>1:89</td>
</tr>
<tr>
<td></td>
<td>Only B1*02</td>
<td>1:210</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Only A1*05</th>
<th>1:1842</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other alleles</td>
<td>1:2518</td>
</tr>
</tbody>
</table>

Megiorni et al. Human Immunology 2009;70:55-59

Risk\(_{(j)}\) = 1:N\(_{(j)}\)
N\(_{(j)}\) = ctrs\(_{(j)}\)/pts\(_{(j)}\) × 100