Tests for molecular diagnostics of HLA associated diseases

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RNDr. David Elsnic

Tests for molecular diagnostics of HLA associated diseases

BAG Health Care GmbH develops and distribute worldwide wide range of HLA tests. Recently we focus on molecular tests and produce both SSP and SSO tests. Due our big experiences in HLA field we produce high quality tests for HLA associated diseases. We keep out tests up to date with latest knowledge about associations to provide for our customers most accurate diagnostics.

- HISTO TYPE Celiac Dissease   BAG HISTO SPOT DQB1 / DQA1

- HISTO TYPE Narcolepsy

- HISTO TYPE B57

- HISTO TYPE B27

BAG HEALTH CARE

Celiac disease

Celiac disease is an autoimmune reaction triggered by gluten which is an ingredient of different cereals. If not diagnosed early this leads to chronic inflammation and destruction of the small intestine. Celiac disease is strongly associated with the DQA1*05:01- DQB1*02:01 and DQA1*03-DQB1*03:02 haplotype. Additionally, DR3, DR7 and DR11 alleles can be used as genetic markers.

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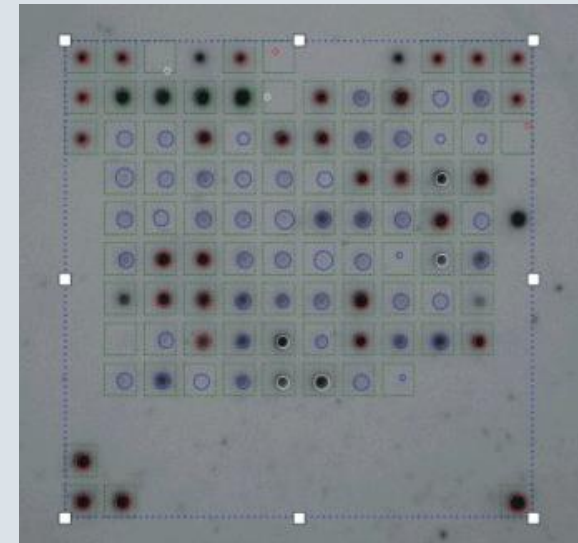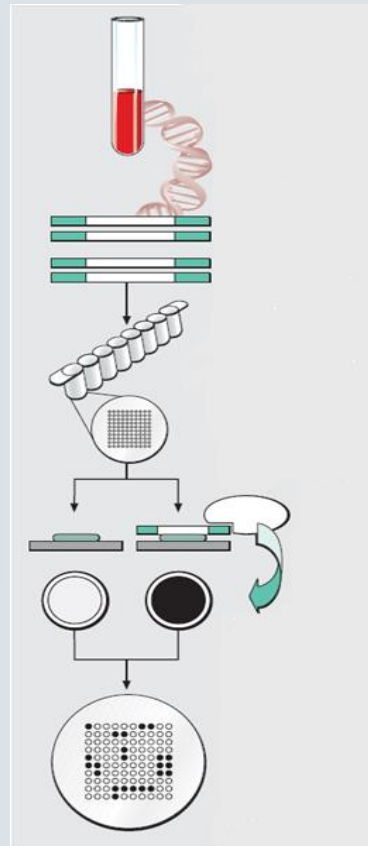
BAG HISTO TYPE COELIAC DISEASE HAPPY PACK

- Simple SSP method with products identification on gel.
- Interpretation either manually with diagram or with our dedicated software Histo Match..
- 23 PCR mixes designed for precise typing of all relevant alleles (HLA-DQ2 a HLA-DQ8) which are asassociated with CD.
- It is not full HLA typization, so therefore: HLA specialist not needed for interpretation.
- Ideal in combination with serological EIA tests.
  - HLA negative result is important (exclusion of CD)
  - Positive serological result is important (confirmation of CD)
- Non invasive method
- Fast and for reasonable price
- Results valid for whole life (EIA and biopsy has to be repeated).
- DRB1 region included for better results interpretation.
- Standard method in whole Europe.
- **Patients suspicious for CD.**
- **Relatives of CD patients.**
- **Conformed CD patients for severances prediction** (2xDQ2>DQ8+DQ2 > DQ2 > DQ8)

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BAG HISTO SPOT DQB1 / DQA1

- SSO PCR test for coeliac disease diagnose for users with high throughput of samples. With this test you can easily interpret up to 200 samples per day. Assay is performed automatically on MR. SPOT processor and interpreted also automatically with BAG software – HISTO MATCH.

Auswertediagramm / *Evaluation diagram*

HISTO TYPE Celiac Disease

HLA DRB1 – DQA1 – DQB1 associations: | Reaction pattern: | Susceptibility to Celiac Disease:
1. DRB1*03 - **DQA1*05:01** - **DQB1*02:01**　　1, **5, 6, 19**　　　high →
2. DRB1*04 - **DQA1*03:01** - **DQB1*03:02**　　2, **12, 13, 14, 15, 18**　　high → very high
3. DRB1*07 - DQA1*02:01 - **DQB1*02:02**
　　DRB1*11 - **DQA1*05:05** - DQB1*03:01 　　3, 4, **6, 7**, 9, 10, 11, (15), 17, **23**　high
4. DRB1*07 - **DQA1*02:01** - DQB1*02:02　　3, **6, 7, 17**　　low
In case of a HLA-DQB1*02 homozygous sample, the risk of CD development is 5 times higher than by a DQB1* 02 heterozygous sample

Ser.Type	Spezifitäten / Specificities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	Positive Reactions																								
	Lenght of Internal control in bp	1070	1070	1070	1070	**429**	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
DR3	DRB1*03:01:01:01-01:03,01:05-41,43-67N,68N-75,77-80,82,83 / *11:07,53,103,105,107,125 / *14:104,111w / DRB3*01:14	1																							
DR4	DRB1*04:01:01-05:11w,05:12-62,64-81N,82-94N,95-109,111-119N,120N-124 / *03:49 / *08:38 / *11:102:01,102:02		2																						
DR7	DRB1*07:01:01:01,01:01:02,01:02w-01:04,03-09,10N-21			3																					
DR11	DRB1*11:01:01-21,23-29,31w,32,33w,34,35w-37:02,39-52w,53-65:02w,66,68-70,72-96w,97-129 / *03:08,65				4																				
DQ2	DQB1*02:01:01-01:05,04,07?					5 !	6																		
DQ2	DQB1*02:02,06w						6	7																	
DQ2	DQB1*02:03							7	8																
-	DQB1*02:05					?	6	?																	
DQ7(3) / -	DQB1*03:01:01:01-01:01:03,01:03-01:06,16,19,21,22,24,27-29,35,36,42									9	10	11				15									
DQ7(3)	DQB1*03:01:02									9	10	11													
DQ8(3) / -	DQB1*03:02:01,02:02,02:04,05:03,05:04,11,18,32,37												12	13	14	15									
DQ8(3)/DQ3	DQB1*03:02:03,05:01,05:02												12		14										
DQ9(3) / -	DQB1*03:03:02:01-03:02:03,03:04,15,17,26,30,31,33,34,38,39,41,43														14	15									
DQ7(3)	DQB1*03:04									9	10	11	12	13		15	16								
DQ3 / DQ4	DQB1*03:03:03,06,20,25 / DQB1*04:01:01-07														14										
-	DQB1*03:02:05,07												12	13		15									
-	DQB1*03:08												12	13	14										
-	DQB1*03:09,44									9	10					15									
DQ8(3)	DQB1*03:10										10	11			14	15									
-	DQB1*03:12										10				14	15									
-	DQB1*03:13															15									
-	DQB1*03:14										10		12	13		15	16								
DQ4	DQB1*04:02:02								8						14										
-	DQB1*04:08											11			14										
-	DQA1*02:01																	17							
-	DQA1*03:01:01																		18						
-	DQA1*05:01:01:01,01:01:02, 01:02?																			19					
-	DQA1*05:02																				20				
-	DQA1*05:03, 06, 07																			19		21*			
-	DQA1*05:04																			?			22		
-	DQA1*05:05:01:01-01:01:03,08-11																							23	
-	Kontaminationskontrolle/*Contamination Control*																								24
	Länge in bp / *length in bp*	220	200	140	177/171	800/171 !	150	140	150	105	200	185	120	95	145	135	135	105	485	235	90	200	205	235	

w = schwache Reaktion / *weak reaction*　　/ ! Die Größe der internen Kontrolle in Mix 5 ist 429bp / *The size of the internal control in mix 5 is 429bp* !
*　　Die spezifische Bande in Mix 21 kann schwächer sein als die anderen spezifischen Reaktionen. / *The specific band in Mix 21 may be weaker than the other specific bands.*

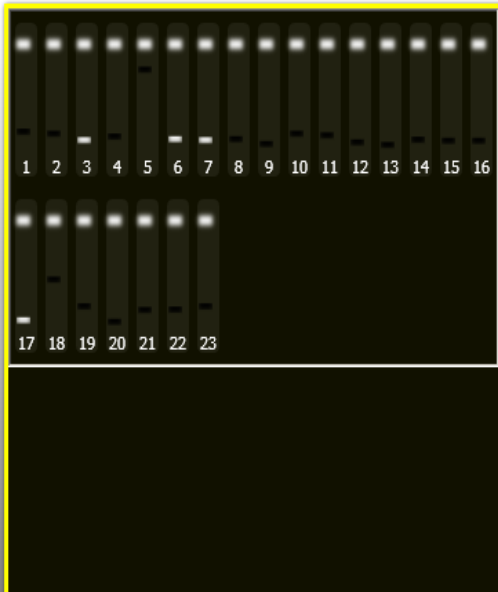# Interpretation with Histo Match software

- Dedicated software HISTO MATCH for our SSP and SSO test if for our customers free of charge.
- Suitable for:
  - easy and fast interpretations
  - Creating and printing reports
  - Protected results database

- HM for BAG HistoType Celiac disease kit:

- Easy interpretation with virtual gel picture
- Missing controls tests interpretation
- Clean typization reports
- Precise model for CD predisposition
- Possible rare alleles included in report
- Results database

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Patient:
Sample ID **720417** Status Initial

Celiac Disease DQB1_ 316S1 (IMGT v. 3.13.1)

All 4D CWD User

Coeliac

Summary **DQ2.x/DQ2 [Coeliac Risk: High]**

DRB1*07:01:01:01/ 07:01:01:02/ 07:01:01:03/ 07:01:04/ 07:03/ 07:04/ 07:05/ 07:06/ 07:07/ 07:08/ 07:09/ 07:10N/ 07:11/ 07:12/ 07:13/ 07:1
DQB1*02:02:01/ 02:02:02/ 02:10/ 02:11
DQA1*02:01

HLA-DRB1
Suggested Ambiguous Result
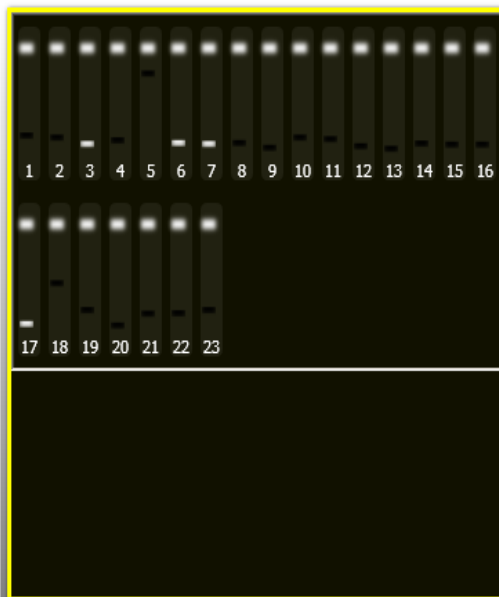
DRB1*07,-; DRB1*07 ,
DRB1*07

HLA-DQB1
Suggested Ambiguous Result

DQB1*02,-; DQB1*02 ,
DQB1*02; DQB1*02 ,
DQB1*03

HLA-DQA1
Suggested Ambiguous Result

DQA1*02,-; DQA1*02 ,
DQA1*05

Results History Comments

Hit Table **DQB1_ 316S1**   *IMGT* **3.13.1**   *Version* **18.7.2013**

				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
				220	200	140	175	800	150	140	150	105	200	185	120	95	145	135	135	105	485	235	90	200	205	235
	Group/Allele	MM	CWD																							
❯	DQB1*02,-(2)	0	[2]																							
❯	DRB1*07 , DRB1*(	0	[1]																							
❯	DQB1*02 , DQB1*:	0	[1]																							

Home Patients Samples Worklists Kits Users Processors Setup Log Off

BAG HEALTH CARE

Patient:
Sample ID 720417 Status Initial

Celiac Disease DQB1_ 316S1   (IMGT v. 3.13.1)

All 4D CWD User

1

Coeliac

Summary DQ2.x/DQ2 [Coeliac Risk: High]

Coeliac Risk: High

DQ2.x		DQ2	
ß-chain	a-chain	ß-chain	a-chain
DQB1*02	DQA1*x	DQB1*02	DQA1*x

HLA-DRB1
Suggested Ambiguous Result

DRB1*07,-; DRB1*07 ,
DRB1*07

HLA-DQB1
Suggested Ambiguous Result

DQB1*02,-; DQB1*02 ,
DQB1*02; DQB1*02 ,
DQB1*03

HLA-DQA1
Suggested Ambiguous Result

DQA1*02,-; DQA1*02 ,
DQA1*05

Results | History | Comments

Hit Table **DQB1_ 316S1**   IMGT **3.13.1**   Version **18.7.2013**

Group/Allele	MM	CWD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
			220	200	140	175	800	150	140	150	105	200	185	120	95	145	135	135	105	485	235	90	200	205	235
DQB1*02,-(2)	0	[2]																							
DRB1*07 , DRB1*(	0	[1]																							
DQB1*02 , DQB1*	0	[1]																							

Home | Patients | Samples | Worklists | Kits | Users | Processors | Setup | Log Off

Patient: 720417   **28.1.2014**

Name: **David elsnic**

Gender: **Male**

Blood Group: **A   +**   CMV: **–**

Comments:

Sample ID: **720417** Collected: **28.1.2014**

Sample Received: 28.1.2014 16:33:37

Extraction Method:

Tests

Tests

Worklist

Extraction
Date Performed
Analyzed By   david
Date Analyzed   28.1.2014
Approved By
Date Approved
Authorized By
Date Authorized

Kit
Batch: DQB1_ 316S1
Expiry: 30.9.2015
Hit Table: Hit Table: DQB1_ 316S1, IMGT db: 3.13.1, ver:18.7.2013
Method: SSP

Suggested Ambiguous Result

Summary: DQ2.x/DQ2 [Coeliac Risk: High]

Filter: Display All Alleles

Serology: DR7/DRNull/DR-;DQ2/DQ-;DQA1?

Assigned NMDP: DRB1*07:JKZW, DRB1*07:JKZW;DQB1*02:BPPD, DQB1*XX:XX;DQA1*02:01,
                    DQA1*XX:XX
Possible NMDP: DRB1*07:JKZW, DRB1*07:JKZW;DQB1*02:BPPD,
                    DQB1*XX:XX;DQA1*02:01, DQA1*XX:XX

Group Result:
DRB1*07,-   [2]
DRB1*07 , DRB1*07   [1]
DQB1*02,-   [2]
DQB1*02 , DQB1*02   [1]
DQB1*02 , DQB1*03   [1]
DQA1*02,-   [2]

Patient: 720417   **28.1.2014**

DQA1*02 , DQA1*05   [1]

Allelic Result:

DRB1*07:01:01:01/ 07:01:01:02/ 07:01:03/ 07:01:04/ 07:03/ 07:04/ 07:05/ 07:06/ 07:07/ 07:08/
07:09/ 07:10N/ 07:11/ 07:12/ 07:13/ 07:14/ 07:15/ 07:16/ 07:17/ 07:18/ 07:19/ 07:20/ 07:21 ,
DRB1*07:01:01:01/ 07:01:01:02/ 07:01:03/ 07:01:04/ 07:03/ 07:04/ 07:05/ 07:06/ 07:07/ 07:08/
07:09/ 07:10N/ 07:11/ 07:12/ 07:13/ 07:14/ 07:15/ 07:16/ 07:17/ 07:18/ 07:19/ 07:20/ 07:21
DRB1*07:01:01:01/ 07:01:01:02/ 07:01:03/ 07:01:04/ 07:03/ 07:04/ 07:05/ 07:06/ 07:07/ 07:08/
07:09/ 07:10N/ 07:11/ 07:12/ 07:13/ 07:14/ 07:15/ 07:16/ 07:17/ 07:18/ 07:19/ 07:20/ 07:21 ,
DRB1*07:01:02

DQB1*02:05 , DQB1*02:05

DQB1*02:02:01/ 02:02:02/ 02:10/ 02:11 , DQB1*02:02:01/ 02:02:02/ 02:10/ 02:11

DQB1*02:05 , DQB1*02:07/ 02:08/ 02:09

DQB1*02:05 , DQB1*02:02:01/ 02:02:02/ 02:10/ 02:11

DQB1*02:05 , DQB1*02:06

DQB1*02:07/ 02:08/ 02:09 , DQB1*02:02:01/ 02:02:02/ 02:10/ 02:11

DQB1*02:02:01/ 02:02:02/ 02:10/ 02:11 , DQB1*02:06

DQB1*02:05 , DQB1*03:23

DQB1*02:02:01/ 02:02:02/ 02:10/ 02:11 , DQB1*03:23

DQA1*02:01 , DQA1*02:01

DQA1*02:01 , DQA1*05:01:02

Could Not Exclude

Positive Reactions

3, 6, 7, 17

Ignored Reactions

Comments

NARCOLEPSY

- Narcolepsy belongs to a groups of sleeping disorders (Dyssomnias) with symptoms like daytime somnolence, sleep paralysis and hallucinations. 98 % of the Caucasian patients with narcolepsy have the DQB1*061:02 haplotype. Therefore, HLA typing is helpful to confirm or exclude a diagnosis.

- Prevalence is 0,02–0,18% of whole population, (depending on ethnicity).

- Association between narcolepsy and HLA is long known.

- 85-95% narcolepsy patients carry HLA haplotype DRB*15:01-DQA1*01:02-**DQB1*06:02,** in common population is this haplotype present just in 30%

- Strongest association is towards subtype **DQB1*06:02**, which therefore constitutes best marker for narcolepsy.

- *Test great for exclusion (when results negative) of narcolepsy, positive results has weak prediction value.*

BAG HISTO TYPE Narcolepsy

- Simple SSP method with amplicons interpretation on gel
- Interpretation manually with diagram
- Just 8 PCR mixes for detailed examination of all HLA-DR, DQA and DQB allels known to be associated with narcolepsy.
- It is not full HLA typization, just selected alleles, therefore HLA specialist not required for interpretation
- Non invasive method
- Fast and for reasonable price
- Results valid for whole life
- *All patients suspected for narcolepsy.*

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Narcolepsy intepretation diagram

Abacavir

- Treatment with antiretroviral drugs containing the active substance Abacavir (mainly in HIV therapy) is only allowed when the existence of HLA-B*5701 is excluded by molecular genetic methods, since otherwise it is possible that a hypersensitivity reaction against Abacavir may occur.

HISTO TYPE B57 Happy Pack

- SSP PCR kit for exclusion of HLA-B*57:01. Contains 7 mixes + contamination control.

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Morbus Bechterew et al.

- There is a strong association between HLA-B27 and a group of seronegative arthritic diseases like Morbus Bechterew or Morbus Reiter. Determination of HLA-B27 is an important tool in the diagnosis of these diseases.

HISTO TYPE B27 Happy Pack

- SSP PCR kit for detection of HLA-B*27. Contaisns 1 mix.

HISTO TRAY B27

- Srological test for B*27 detections.