Validation of a novel single-drop rapid HLA-DQ2/-DQ8 method to identify people susceptible to celiac disease

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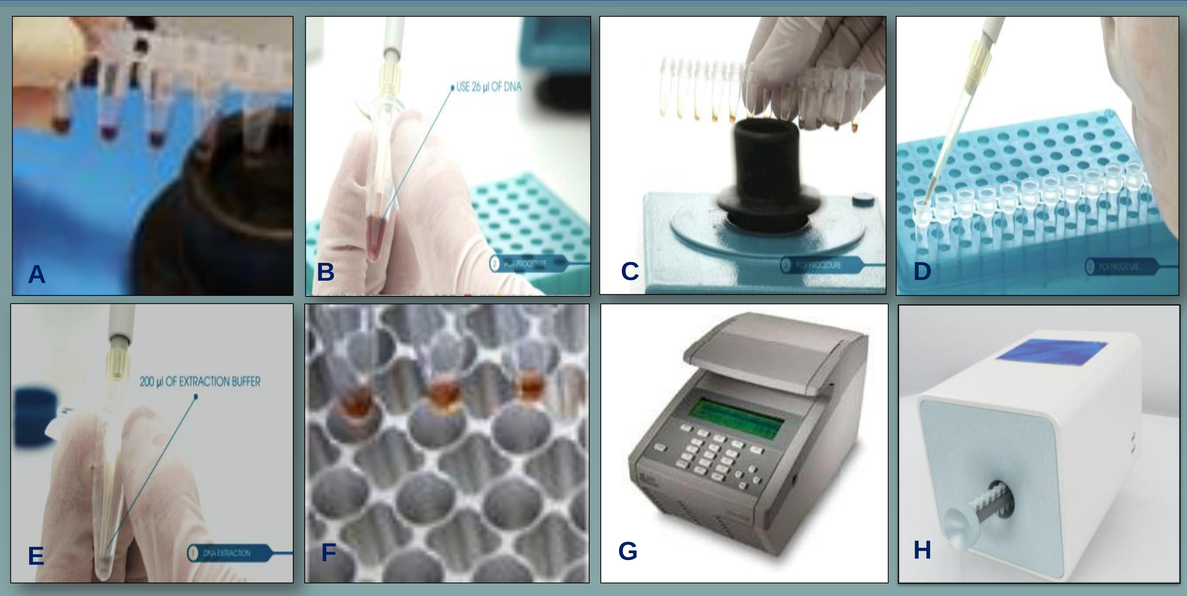
Introduction

- Human Leucocyte Antigen (HLA) HLA-DQ2 &/or -DQ8 is a major genetic predisposing factor for Celiac disease (CD) [1]
- About 90% of CD patients carry HLA DQ2 allele (DQA1*05 DQB1*02) & rest 5 % carry HLA DQ8 allele (DQA1*03 DQB1*0302) [2]
- HLA -DQ2/-DQ8 typing is considered as in additional diagnostic test for CD specifically for uncertain cases [3]
- Determination of HLA -DQ2/-DQ8 alleles is not the final diagnostic but absence of these alleles excludes an individual for CD risk [4]
- We have assessed efficacy of a new-fangled sequence specific primer based rapid single PCR reaction HLA DQ typing method to detect HLA DQ2/DQ8 alleles & compared with the conventional HLA typing method i.e. PCR-sequence specific oligonucleotide probes (PCR-SSOP)



Methodology: A-B: Blood collection & DNA isolation; C- F: PCR preparation; G-H: Detection & interpretation of result

Methodology

- A new rapid HLA class II allele typing method, "Celiac Gene Screen Kit" developed by BioDiagene S.R.L (Palermo, Italy) is introduced for the determination of HLA -DQ2/-DQ8 alleles associated with CD
- During June 2017, the rapid HLA typing was performed on the stored EDTA blood samples collected from the bio-repository of the Celiac clinic, department of gastroenterology & Human Nutrition, All India Institute of Medical Sciences, New Delhi, India
- CD diagnosis was made on the basis of ESPGHAN criteria
- This unique DQ-CD typing kit uses single blood drop (fresh/EDTA stored) & permits a rapid, specific & unambiguous detection of specific HLA class II alleles & covers the most specific alleles for CD shown in Table-1

DQA1*0201	DQB1*02	DRB1*03/*04
DQA1*03	DQB1*03:01/03:0	DRB1*07
DQA1*05	DQB1*03:02/03:05	DRB1*11/*12

Table 1: HLA – DQA1, -DQB1 & -DRB1 alleles detect by rapid HLA method

Test Procedures:

Whole methodology has been divided into two major steps

Step 1: Determination of diagnostic performance of Celiac Gene Screen HLA DQ typing kit (Assessment for concordance)

- A total of n=100 known status of HLA-DQ2/-DQ8 samples, previously characterized by the standard SSO-PCR testing were typed by Celiac Gene Screen HLA test, results of both the HLA typing methods were compared

Step 2: Determination of HLA DQ status through Celiac Gene Screeng HLA DQ Typing Method

- To validate the rapid HLA method , 300 samples (CD n=141; First degree relatives of CD patients n= 56 & Controls n=103) were additionally determined their HLA-DQ2/-DQ8 status with rapid HLA method
- HLA status was never determined in these 300 samples
- Celiac Gene Screen involves following major steps: a preliminary lysis of EDTA blood sample, DNA amplification (1 reaction/test) & fluorescence detection using BioRun Reader.
- Whole HLA DQ typing process requires almost 90 mins. During the procedure manufactures guidelines were strictly followed

Major Steps	Medium	Duration
DNA isolation	BioDiagene Extraction buffer	~1 min.
DNA amplification	Conventional thermocycler (PCR)	90 min. (1 reaction/test)
Gene detection	Fluoroscence detection (BioRun reader)	~20 Sec.

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Results

Step 1: Assessment of concordance

- Of 100 known HLA results, 79 were already characterized as positive for HLA-DQ2/-DQ8 & 21 were characterized as HLA-DQ2/-DQ8 negative by standard HLA typing test (PCR-SSOP)
- All 79 reported positive & 21 reported negative for HLA-DQ2/-DQ8 by Celiac Gene Screen rapid HLA DQ typing test as well
- This shows 100% concordance rate between both HLA typing method

Step 2: Determination of HLA DQ status

Sample category	No. of samples	HLA-DQ +ve (%; 95%CI)	HLA-DQ –ve (%; 95%CI)
CD	141	118 (84%; 78-90)	23 (16%; 10-22)
FDRs of CD	56	48 (86%; 85-87)	8 (14%; 5-23)
Controls	103	52 (50%; 40-60)	51 (50%; 46-60)

Discussion

Conventional HLA typing	Celiac Gene Screen HLA DQ typing Kit
Time consuming	Rapid : less performance time
Complex instrumentation	Easy instrumentation
Tough result interpretation	Very easy
Requires an expert laboratory personal	No such requiremennt
Can not established in normal laboratory	Suitable for any basic laboratory
Costly	Reasonably cheaper

Conclusion

- Celiac Gene Screen HLA DQ typing method showed an excellent concordance with standard test
- Celiac Gene Screen HLA DQ typing method could be a cost reducing & an effective tool for CD gene screening
- Further valiadation is needed in a large number of samples

References

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The authors declare no conflict of interest