High rates of variation in HLA-DQ2/DQ8 diagnostic testing:

Results from an RCPAQAP coeliac disease pilot program

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Coeliac disease (CD) is a chronic inflammatory enteropathy caused by an autoimmune response to dietary gluten in genetically predisposed individuals[1]. The diagnosis of CD relies on demonstrating the presence of coeliac-specific antibodies and small intestinal villous atrophy with improvement upon exclusion of dietary gluten[2].

A key feature of CD is its strong dependence on the presence of susceptibility genes encoding for HLA DQ2.5, DQ2.2 or DQ8[3]. These specific HLA types are seen in more than 98% of Europeans and Australians with CD[4,5]. HLA typing therefore achieves near-perfect sensitivity and negative predictive value for CD in the general population, making it exceptionally useful as a test to exclude CD when the susceptibility genotypes are absent[3]. However, as approximately half of the Australian population express HLA DQ2.5, DQ8, and/or DQ2.2 the presence of these HLA types has poor positive predictive value and low specificity for CD[5].

CD diagnostic HLA genotyping is on the increase and is associated with community awareness of CD in relation to a gluten-free diet. This has rendered the traditional gluten testing with serology and histology uninformative[3]. As HLA testing is often used to exclude a diagnosis of CD, it is imperative that the results are both accurate and clearly reported to general practitioners. Australasian guidelines were therefore developed to optimise for testing and reporting of HLA results[3]. However, their uptake appears limited to date[6].

Our findings from this study highlight concerns with the detection of HLA-DQ2/DQ8 and the reporting of the results and underscore an urgent need to ensure pathology providers offering HLA testing are involved in an external quality assurance (EQA) program, and that a set-of-guidelines to standardise testing and reporting are adhered to.

Methods

DNA was extracted from five patients and sent to ten coeliac disease testing laboratories. Each laboratory was monitored for proficiency testing using techniques specific to each participating laboratory. Coeliac disease reports were submitted to the RCPAQAP for data analysis.

Results

(i) Assessment of genotyping

90% of laboratories did not determine the full HLA DNA sequence and did not use the current recommended HLA reporting nomenclature (Table 1).

Only one laboratory scored maximum points for genotyping and for using the current HLA reporting nomenclature. One laboratory provided a false DQ2 positive result for Case 1.

(ii) Assessment of interpretation

40% of laboratories did not provide any clinical interpretation and/or did not comment on the limitation of using HLA typing in isolation. 30% of laboratories did not report on the relative CD risk in relation to the specific genotype detected. 90% of laboratories did not provide any supporting references.

(iii) Assessment of methodology

80% of laboratories did not report on the sensitivity or limitations on their specific CD testing assay and 20% failed to report on the methodology used.

(iv) Assessment of overall performance

Only laboratory scored above 80%. Two laboratories scored between 65%–68%. The remainder scored below 61% (Figure 1).

Discussion

Best practise guidelines for CD diagnostics would recommend that each clinical report contain information on the three key testing categories of genotyping, interpretation and methodology[3]. This study identified best-practice shortcomings for analysis in each of these categories (Table 1). For genotyping, the largest discrepancies were a failure to determine the full HLA DNA allelic sequence and in not using the recommended HLA reporting nomenclature (Table 1). For clinical interpretation, 40% of laboratories did not provide any clinical interpretation for all cases, or did not make any comment regarding the usefulness or limitation of using HLA typing in isolation. Only one laboratory provided supporting references to support their clinical interpretations for the genotypes identified in each case. For methodology, the largest discrepancy was a failure to report on the levels of sensitivity or limitations on the specific testing assay.

This level of reporting may lead to ambiguities for correct clinical classification that could impact on patient treatment.

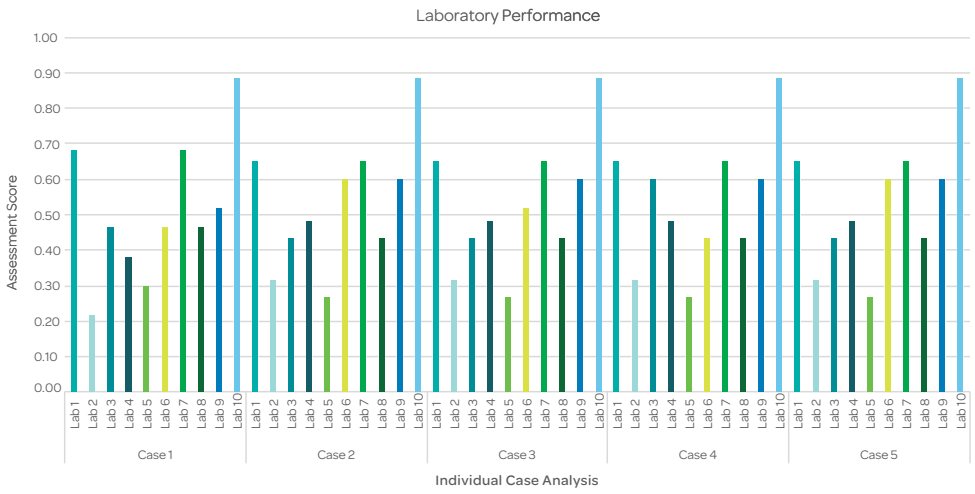
The combined average assessment score from the three testing categories can be used to provide an overall indication of laboratory performance. In this study, performance assessment indicated that one laboratory scored 88% and two laboratories scored between 65%–68% of the maximum possible score for all cases tested. The remaining seven laboratories all scored less than 61% (Figure 1). These data indicate that only one laboratory is performing at levels that would be deemed acceptable for best practise (i.e. scoring >71%), two laboratories may be borderline for underperforming (i.e., scoring between 61% and 70%), and seven of the ten laboratories are possibly underperforming (i.e. scoring <61%) for using CD diagnostic best practise guidelines.

The issues identified from this study highlight the important role of EQA providers in helping to identify problems and to raise standards for laboratory diagnostic analyses of genetic disorders.

Table 1: Assessment criteria and deductive scoring used for the quality monitoring of coeliac disease reporting. The Observed Frequency column represents the percentage of laboratories failing to adhere to the recommended guidelines for data reporting.

Category	Criterion	Deduction	Observed Frequency
Genotyping	No deduction	0	-
	Critical genotyping error	2	10%
	Complete HLA allele sequence not determined	1	90%
	Not reporting zygosity	0.2	80%
	Not correctly using HLA nomenclature	0.2	90%
Interpretation	No deduction	0	-
	Critical interpretation error	2	-
	No clinical interpretation provided	1.5	40%
	No comment on negative result to exclude CD	1	10%
	Comment on HLA typing in isolation not provided	0.5	40%
	No statement on observed genotype and CD	0.5	30%
	No references to support of clinical interpretation	0.2	90%
Methodology	No deduction	0	-
	No statement about the assay used	1.5	20%
	Limitations/sensitivity of assay used not provided	0.5	80%

Figure 1. Laboratory performance for the combined categories of genotyping, interpretation and methodology. Clinical reports from each testing laboratory were individually assessed for each of the five suspected CD cases.



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