WHY IS IMMUNOASSAY STANDARDISATION SO HARD?

Joanna Sheldon

Protein Reference Unit, South West London Pathology, St George's Hospital, London, UK

Good immunoassays depend on the optimisation of antibody, antigen, pH, detection system, temperature and time. The complexity of these methods is hidden behind the 'kit-based' and 'black box' technologies that we now require to cope with the demands of workload, turnaround times and accreditation. The starting point of immunoassay will be the antigen that will need to be purified from its normal matrix. Antigens are typically proteins and the purification steps may degrade or denature them; nevertheless, the purified antigen can be used to inoculate animals to make antibodies. The antibody characteristics can vary between species and even between individual animals so anti-serum is often blended to improve the specificity and sensitivity and to try to maintain the characteristics. The measurand may be in such low concentration that the antibody needs to be bound to another matrix to increase the sensitivity and this step can change the how the antigen and antibody bind. We can pick the 'best' antibody but it must be compatible with other methodological elements, e.g., assay speed, detection system, working temperature, pH, etc., and all these components need optimisation for a robust and appropriate immunoassay – standardisation is never going to be easy!

RECOMMENDATIONS FOR MEMORY B CELL REPORTING

Louise Wienholt

The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), St Leonards, Sydney, Australia

The enumeration of B cell subsets by flow cytometry is increasingly being performed as a routine diagnostic test in laboratories, both in Australasia and internationally. Results of pilot External Quality Assurance (EQA) programs performed by The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) have shown that at least 16 Australasian pathology laboratories are routinely reporting B cell subset panels. These pilot studies have also revealed a marked discrepancy in the panels (number and type of fluorochromes used), as well as the reporting algorithm (if cell populations are reported as % of total lymphocytes or % of B cells). This has led to significant variation in the values of B cell subsets values reported.

Three seminal papers have proposed phenotypic and prognostic classification criteria for common variable immunodeficiency disorder (CVID) patients based on B cell subpopulations. These are known as the 'Freiburg', 'Paris' and 'Euroclass' classifications. Each classification has methodological variations, which make the interpretation of results in routine clinical practice difficult. To improve the standardisation of results reported by Australasian laboratories, we undertook a review of the classifications to establish minimum recommendations for diagnostic pathology laboratories who report B cell subset results used in the clinical characterisation of CVID. These recommendations include sample type to be used, minimum populations and units

reported and the requirement for reference ranges and interpretive commentary.

HIGH ACCURACY OF HLA-B*27 GENOTYPING BY ALLELE-SPECIFIC REAL-TIME POLYMERASE CHAIN REACTION IN A HETEROGENEOUS POPULATION

Helena S. Jang¹, Anné Proos², Lisa Koe², Janet Anderson¹, Richard Fulton¹, Suran L. Fernando¹

¹*Department of Immunology, NSWHP, Royal North Shore Hospital, Sydney, Australia; and* ²*Department of Molecular Genetics, NSWHP, Royal North Shore Hospital, Sydney, Australia*

Objective: HLA-B*27 is a risk marker for ankylosing spondylitis. We compared three methods of HLA-B*27 detection in a New South Wales (NSW) population – flow cytometry, rs4349859 single nucleotide polymorphism (SNP) assay and allele-specific real-time polymerase chain reaction (RT-PCR) analysis of exons 2 and 3.

Methods: Over a five-month period, 549 samples underwent flow cytometric testing and RT-PCR-high-resolution melt analysis of rs4349859 SNP and of exon 2 (5' fragment) and exon 3. In the third method, positive samples underwent confirmation with fluorescent resonance energy transfer (FRET) RT-PCR of exon 2 fragments, 2a and 2b. HLA-B*27 and other genotypes were confirmed by Sanger sequencing of a 600-bp fragment of exons 2 and 3.

Results: In our cohort, rs4349859 SNP method had 77.5% sensitivity and 97.2% specificity, with false-negative results for non-Caucasians. Screening with exon 2 (5' fragment) and exon 3 RT-PCR produced 100% sensitivity. Confirmation with exon 2 FRET RT-PCR provided 100% specificity. HLA-B*27 molecular testing was able to subgroup type all samples. Genotyping was able to resolve seven indeterminate flow cytometry results.

Conclusion: HLA-B*27 testing by genotyping was more sensitive and specific than rs4349859 SNP and flow cytometric assays. Our HLA-B*27 genotyping assay has replaced the current flow cytometric HLA-B*27 assay for the heterogeneous NSW population.

DIAGNOSING COMMON VARIABLE IMMUNODEFICIENCY DISORDERS IN THE ERA OF GENOME SEQUENCING

Rohan Ameratunga, See-Tarn Woon

Auckland Hospital, Grafton, New Zealand

Common variable immunodeficiency disorders (CVID) are the most frequent symptomatic primary immune deficiency in adults. There is no single clinical feature or laboratory test that can confirm the diagnosis with certainty, since by definition, there is no known cause for these conditions. Over the last 17 years, an increasing number of genetic defects have been identified in patients with a CVID phenotype. If a causative mutation is identified, patients are removed from the umbrella diagnosis of CVID and are deemed to have a CVID-like disorder caused by a specific primary immunodeficiency/inborn error of immunity (PID/IEI). Currently, causative mutations are identified in about 25% of patients with a CVID phenotype in non-consanguineous populations. In the remaining patients, the explanation for these disorders remains unclear.