To establish a test for MRP8/14 protein in childhood arthritis

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INTRODUCTION

Juvenile idiopathic arthritis (JIA) is an autoimmune condition that affects about 1 in 1000 children under the age of 16, causing joint inflammation. Early treatment of inflammation is essential for minimising joint destruction, but prolonged medication is not desirable as the drugs used have significant side-effects. Unfortunately, the presence of symptoms of joint inflammation means that the inflammatory process is already substantial. Ideally, a laboratory biomarker would identify disease before symptoms appear, meaning anti-inflammatory medications can started earlier.

Research has found that myeloid related proteins (MRP) subtypes 8 & 14 function as markers of localised inflammation. In JIA, a number of studies have found that MRP8/14 is a useful and accurate marker of predicting not only whether there are signs of inflammation that mean that treatment should be initiated, but the risk of flare of disease for patients that are currently on medication, to determine whether treatment can be safely stopped.

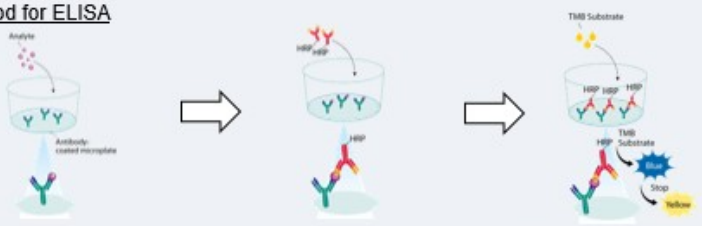
The research group of Professor Lucy Wedderburn, had shown that it was possible to identify a subset of JIA patients who would respond well to treatment with the cytotoxic drug methotrexate as they had high concentrations of MRP8/14 in their serum prior to treatment (Moncrieffe et al. Rheumatology 2013). Working with Professor Wedderburn's group, the Clinical Immunology lab at Great Ormond Street Hospital verified the Buhlmann MRP 8/14 ELISA kit for use in the clinical diagnostic lab.

AIM

The aim of this project was to establish the MRP 8/14 ELISA for use in a clinical diagnostic laboratory. This involved validating the manufacturer's data for performance characteristics such as intra- and inter-assay variability, and linearity, as well as establishing independent quality control material.

METHOD

Method for ELISA



A monoclonal capture antibody (mAb) highly specific to the MRP8/14 heterodimeric and polymeric complexes is coated onto the microtiter plate.

Serum samples are diluted and incubated in the wells, and if MRP8/14 is present, this will bind to the specific antibody.

A second monoclonal detection antibody (Ab) conjugated to horseradish peroxidase (HRP) detects the MRP8/14 molecules bound to the monoclonal antibody coated onto the plate after a washing step.

After incubation and a further washing step, tetramethylbenzidine (TMB) will be added (blue color formation) followed by a stopping reaction (change to yellow color). The absorption is measured at 450 nm.

Figure 1 Schematic of ELISA method

Method for validation

- To establish intra-assay variability: two QC samples were run five times on the same assay
- To establish inter-assay variability: three QC samples were run ten times on different assays
- To establish linearity: a QC sample was diluted out over five dilutions, MRP concentration measured for each and the percentage recovery calculated.

Figure 2 Buhlmann ELISA Kit

RESULTS

- Average intra-assay variability was 5% which compared favourably with the manufacturer's data of 4%.
- Average Inter-assay variability was 12%, compared to 6% suggested by the manufacturer. Although this was higher than expected, this is in line with the performance of other ELISAs carried out in the laboratory, and was deemed to be acceptable.
- Linearity performance was also good. The percentage range was 97% to 110%; this is better than the manufacturer's data of 85% to 110%.

	Kit Mid QC (ng/ml)	Kit High QC(ng/ml)
Results	5076	10892
	4947	9928
	5401	9827
	4927	9508
	5035	10182
Mean (ng/ml)	5098	9903
Standard Deviation (ng/ml)	191	634
%CV	4	6

Figure 3 Intra-assay variability

	In-house Low QC (ng/ml)	In-house Medium QC(ng/ml)	In-house High QC (ng/ml)
Results	1376	7300	11400
	1586	5553	10000
	1351	5122	10193
	1666	6447	13539
	1711	6123	12333
	1516	5811	10744
	1648	5926	10644
	1416	5857	9504
	1097	5413	10173
	1143	5076	10802
Mean (ng/ml)	1451	5843	10252
Standard Deviation (ng/ml)	203	631	1169
%CV	14	11	11

Figure 4 Inter-assay variability

	In-house High QC (ng/ml)		
Dilution	Observed	Expected	O/E (%)
100	1102	1002	110
200	518	501	103
300	351	332	105
400	245	250	98
500	194	200	97
Mean			107
Range			97%-110%

Figure 4 Linearity of assay

CONCLUSIONS & FOLLOW-UP

- The Buhlmann MRP 8/14 ELISA kit is suitable for use in a clinical diagnostic laboratory, showing good performance characteristics
- The test is now available to request by clinicians on a routine basis, with a turnaround time of 8 weeks to allow for batching of samples.
- A clinical audit of the period March to December 2015 showed that 105 patient samples were analysed for MRP 8/14. In almost 60% of requests, the result of the MRP test directly influenced the clinical decision making process, demonstrating the value that this test has brought to the management of these patients.