FROM ELISA TO TURBIDIMETRY

MRS DELPHINE LE BRIS & DR ISABELLE BERNARD – LABORATORY CEF-BOULARD

ESTABLISHING AN ASSAY FOR FECAL CALPROTECTIN

The fecal calprotectin assay was set up at BIOMNIS (our Lyon site) in 2010. The test requests were low at that time and the biologists decided to use the BÜHLMANN Quantum Blue® for measuring fecal calprotectin. This technique provides fast results. As the demands were increasing each day, the BIOMNIS laboratory has had to make the decision to change the technique where larger test numbers can be efficiently measured and has switched to the BÜHLMANN fCAL® ELISA in 2012. When the BIOMNIS Laboratory decided to outsource all the coprology examinations to our CEF-Front de Seine laboratory, we started to perform calprotectin using the BÜHLMANN ELISA method on a DYNEX DS2 microplate reader provided by BÜHLMANN.

Transition to turbidimetry

Several criteria encouraged us to switch to the BÜHLMANN fCAL® turbo: Adaptability on our AU5800 BECKMAN COULTER analyzer without any additional manipulation of the CALEX®, the gain in terms of time to reporting results (as well as gain in hands-on time), the widening of the measuring range which better meets the needs of gastroenterologists, and the good comparison of both methods, ELISA and turbidimetry, respectively (see graph 1) were decisive.

The change from one technique to another may have seemed labourious, but this is not the case: We proceeded by risk analysis to establish the project plans for the method change. The risk analysis was carried out on 24/04/2017 and we switched to the new method on 07/06/2017.

In practice, risk analysis involves bringing together the different members of the project team around a framework that allows us to scan all of our business processes and thus determine the actions needed to change the method.

> Widening of the measuring range which better meets the needs of gastroenterologists, and the good comparison of the both methods, ELISA and turbidimetry, respectively

The protocol was adapted to the AU5800 by the BÜHLMANN team in a few hours and validated by the same team using samples previously prepared and predefined by us.

Then, the verification of the method was done according to our internal procedures.

The verification studies took some time but were very conclusive. The method shows nice repeatability, even better than the ELISA. Comparisons have shown that the results are well comparable between both assays.

Furthermore, BÜHLMANN provided us with a lot of documentation and evaluation results from other laboratories that allowed us to get a good understanding of the method's performance and to be confident to safely implement a very new method.

Expectations of the change of methods and the benefits

The main expectation was a gain on reporting deadlines. There are still few organizational points that need to be refined but overall, today we are returning our calprotectin results at "D0 – same day". (A technical delay of 5:00h on average from reception of the

samples to the technical validation of the result is given by internal logistics.)

In practice: The samples are received at the CEF-Front de Seine twice a day: 70% at 7:30 am and the rest at 11 am.

Extractions with CALEX® or Smart-Prep tubes are carried out at the CEF-Front de Seine by experienced staff right after receiving the samples.

Once the extractions are completed, the tubes are then brought inside transport boxes equivalent to those of the blood/urine samples to the CEF-BOULARD site where our AU5800 is located. We only had to adapt the racks.

CALEX® tubes can be placed directly onto BECKMAN racks.

Result can be obtained within 10 minutes after loading the extracts.

The wide measuring range up to 2000 µg/g without the need for additional dilution provides a real advantage over the ELISA.

MORE DETAILS ON PRE-ANALYTICS

CALEX® Cap extraction tubes

The extraction of the raw stool is a crucial step to obtain good results. We have been working with all the tubes proposed by BÜHLMANN since we started measuring fecal calprotectin. At first, we weighed the stool. It took a lot of time (almost 30 minutes per sample). Then we switched to Schebo® Quick Prep ™ tubes to avoid weighing each sample. Finally, since its development in 2015, we have tested and adopted the CALEX® Cap tubes.

These tubes allow a much more standardized extraction since the factors of variation are minimized due to less manipulation for the technicians, therefore less risks and saving a lot of time.

HOW DID YOU PROCEED FOR ACCREDITATION?

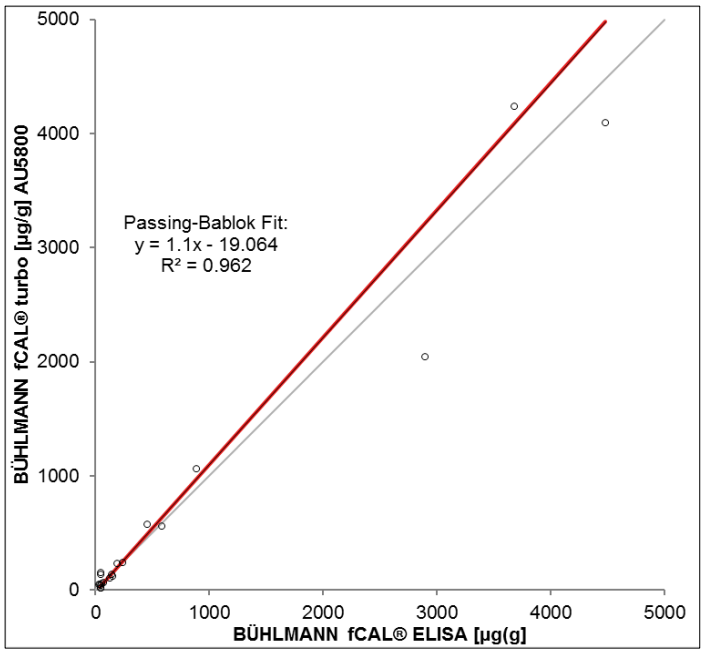
We have been accredited by COFRAC (French Committee for Accreditation) for the BÜHLMANN fCAL® ELISA assay in 2015 (N° 8-3182). As soon as the turbidimetric assay was validated, we also accredited this method. For this, it was sufficient to proceed according to our flexible range management since the calprotectin remains in scope of the same BIOCHBM family (sub group of COFRAC: general and special biochemistry). We created a method file in which we estimated repeatability, reproducibility, accuracy, uncertainty, comparison between the old and the new method, inter-sample carry-over (see Table 1 & Graph 1). To this, we added the additional publications on the subject, the establishment of external quality controls (we work with INSTAND), and the risk analysis on the AU5800. In addition, it was necessary to inform COFRAC about the change of the method via our detailed list of the examinations we already accredited.

HOW WAS THE COLLABORATION WITH BÜHLMANN AND THE SUPPORT FOR CHANGE OF METHODS?

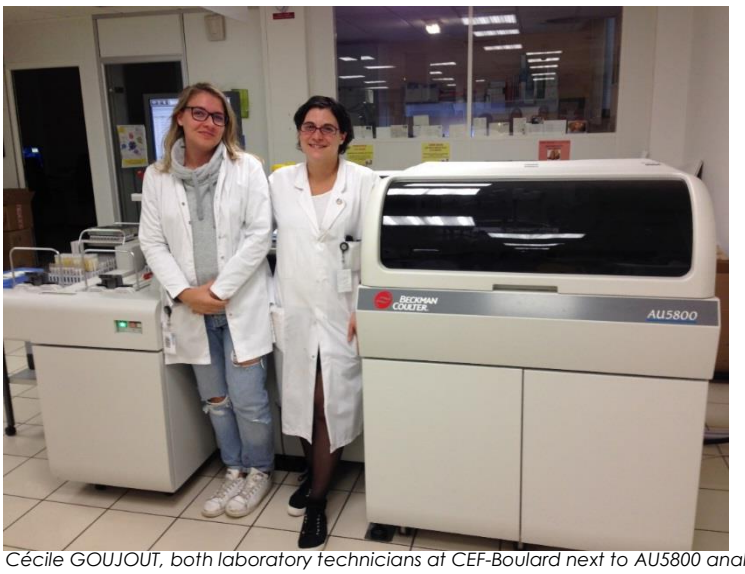
Very good. BÜHLMANN is a long-standing partner of BIOMNIS on the LYON site and we have been working with them since 2014. Our BÜHLMANN contacts are always attentive, responsive and available for all our requests. The exchanges are constructive and transparent; a real collaboration has taken place. We know we can count on them.

Repeatability							
Samples	**Number of values (N)**	**Mean**	**SD**	**CV (%)**	**CV (%) by manufacturer**	**CV (%) acceptance criteria of laboratory**	**Conclusion**
Level low	20	43.78	2.672	5.1	8.94	15	Confirmed
Level high	20	1140.566	12.252	1.07	1.54	15	Confirmed
Inter-run precision							
Samples	**Number of values (N)**	**Mean**	**SD**	**CV (%)**	**CV (%) by manufacturer**	**CV (%) acceptance criteria of laboratory**	**Conclusion**
Level low	21	73.891	5.5	7.44	1.17	15	Confirmed
Level high	21	242.293	8.134	3.36	0.83	15	Confirmed

Table 1: repeatability and inter-run precision



Gaph 1: Method comparison BÜHLMANN fCAL® ELISA and BÜHLMANN fCAL® turbo with 20 samples.



Gwenaëlle LEPRETRE (left) and Cécile GOUJOUT, both laboratory technicians at CEF-Boulard next to AU5800 analyzer from BECKMAN

BÜHLMANN thanks the laboratory team for the time to set up the turbidimetric assay and for this interview.