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Fecal Calprotectin Laboratory Testing at Fraser Health

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Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal system, including Crohn's disease and ulcerative colitis as the two major classes. Fecal calprotectin (FC) is a protein released by the neutrophils during intestinal inflammation; and it is one of the most studied and widely used biomarker for IBD management, although not disease specific. For patients without known IBD, FC can aid in the screening of IBD or distinguishing IBD from other functional bowel disorders such as irritable bowel syndrome. On the other hand, FC test can also help monitoring disease activity, response to treatment and prediction of disease relapse for patients with known IBD. Due to the relatively high sensitivity and moderate specificity of FC testing in IBD management, its use reduces the number of patients requiring specialist referral for invasive endoscopy and biopsy testing.

We implemented FC test at Fraser Health in January 2021 using commercial assay reagent and stool extraction device. Currently, all FC test requires a stool extraction step prior to analysis. This sample preparation has resulted in great measurement variability between laboratories. Although it is still the "gold standard", the manual stool weighing method is time and labor consuming, prone to errors and limits assay throughput. In our laboratory, we evaluated a commercial stool extraction device - BÜHLMANN Calex® Cap for preparing different stool samples for FC measurement. The device contains a pin with grooves able to carry an approximate amount of stool, the sample is then diluted in corresponding buffer volume. We assessed the reproducibility of stool extraction among various stool types and operators across multiple consecutive days' stool extraction. Total analytical imprecision of our FC test was calculated from within and between run CV,

ranged from 0.99% to 4.25%. Using the BÜHLMANN Calex® Cap extraction device, the overall CV of FC measurement was determined to be 15.04% across all stool types, operators and different days of extraction. We observed a larger CV when extracting semi-solid and liquid stools compared to the solid stools. This is most likely due to the non-homogenous nature of the stool sample, the sampling location and also the accuracy of pipetting liquid stool for analysis. In addition, there is a minor CV variation between different operators and days of extraction.

Beside the lack of stool preparation standardization among laboratories, there is also a large variation in terms of the current commercially available FC assays. Differences raised from assay techniques, antibody used, calibrators as well as the analytical measuring range; some assays have narrowing ranges may not be optimal for disease monitoring purposes (1). Previous study has looked at the inter-assay variability between different commercial FC assays; the correlations were found to be acceptable but quantitative agreement were poor (2). At our laboratory, we have compared our FC measurements with two reference laboratories who are running using different assay platforms, the same phenomenon was observed. FC reference ranges are often assay depended and thus results from different laboratories may not be interchangeable; monitoring patient with one assay platform is highly recommended.

Currently, we are accepting stool samples with all appearance including liquid and bloody stools. Other factors could cause elevated FC beside inflammation are: infectious, neoplastic, allergic, age (<4 and >70 years) and medication (3); for patient taking non-steroidal anti-inflammatory drugs and proton pump inhibitors, the current recommendation is to suspend for two weeks prior to testing.

Stool is a very complex sample matrix; FC concentration can vary by the interval between bowel movement, stool relative water content and many more. Some researchers have found a large intra-individual variability of FC measurement to be CV around 50% (4, 5). And by combining the pre-analytical variation, analytical variation and biological variation altogether, the reference change value (the difference between measurements that

exceed the difference expected due to total test variation) has determined to be at around 100% (6); therefore, FC measurement change greater than 100% from the same patient are more likely to be clinically relevant and biologically significant.

From January to the beginning of October 2021, we have analyzed close to 600 FC stool samples in our laboratory. MSP-paid is 77%, and 23% is self-paid; current BC MSP only payable to patients with an existing diagnosis of IBD. Since we are accepting patients from all age groups, around 17% of our samples analyzed are from pediatrics.

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