Evaluation of a faecal calprotectin method using the OC-SENSOR PLEDIA.
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Background
- Faecal calprotectin (f-cal) is often used to aid the diagnosis of inflammatory bowel disease (IBD) over irritable bowel syndrome (IBS) and to monitor ongoing prognosis.
- Currently patients send faecal samples in ‘poo-pots’ to laboratories for analysis where an aliquot of the sample is removed by the lab staff and transferred to an extraction device.
- This is an unpleasant job and consumes a large amount of time within the laboratory.
- The extraction fluid is then either decanted from the device or in some instances the devices are loaded directly onto analysers for analysis.
- The faecal immunochemical test (FIT) for haemoglobin is used for bowel cancer screening and also for triaging patients with symptoms suggestive of bowel cancer.
- The use of this test requires samples to be transferred directly into a collection device by patients.
- Eiken Chemical Co., Ltd. (Japan) have developed a calprotectin method (OC-FCa) using the same faecal immunochemical test (FIT) collection device and analyser (OC-SENSOR PLEDIA) used for faecal haemoglobin (f-Hb), including for bowel cancer screening programmes.
- Using this method, a calprotectin result can be obtained simultaneously with a f-Hb result from the same device.

Aim
- This study aimed to perform an analytical evaluation of the Eiken OC-FCa using the OC-SENSOR PLEDIA.

Method
Using calprotectin solutions provided by Eiken we determined:
- Limit of blank (LOB), limit of detection (LOD) and limit of quantitation (LOQ)
- Within-run imprecision - 2 concentrations, n=20, between-run imprecision - 3 concentrations over 20 days, n=80.
- Linearity - 10 dilutions (1.0-0.1) over the analytical range 20-2720 µg calprotectin/g faeces for 2 separate starting points of 2758 and 335 µg/g.
- Prozone - 6 dilutions of 1 sample, expected concentrations 1563-50,016 µg/g.
- Recovery - 2 series, volume replacement of low concentration sample (70 µg/g) with high concentration sample (1013 µg/g) or buffer, n=24.

Using patient samples we assessed:
- Carryover - Two pools were created, one high (H) and one low (L), using patient samples measuring 2689 µg/g and 93 µg/g respectively. Three aliquots of each solution were measured, one set following the other. The carryover factor (k) was calculated from the equation: k = (L1 – L3)/(H3 – L3).
- A method comparison with the BÜHLMANN fCAL® turbo (BÜHLMANN Laboratories AG, Switzerland), n=39.

Results
Table 1. Results for the analytical evaluation of the Eiken OC-FCa using the OC-SENSOR PLEDIA

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>LOD</th>
<th>LOQ</th>
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<tbody>
<tr>
<td>Detectability</td>
<td>8 µg/g</td>
<td>20 µg/g</td>
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<tr>
<td>Imprecision</td>
<td>Within-run 247 µg/g, 516 µg/g, 1.7%</td>
<td>Between-run 49 µg/g, 98 µg/g, 992 µg/g, 4.9%</td>
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<tr>
<td>Linearity</td>
<td>R² values &gt;0.99 for both assessments</td>
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<tr>
<td>Prozone</td>
<td>Samples at threshold concentration of 37512 and 50016 µg/g gave ‘PR’ (Prozone error code). Samples between 3126 and 25008 µg/g gave ‘OR’ (Over-range) error code.</td>
<td></td>
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<tr>
<td>Recovery</td>
<td>99.6%</td>
<td></td>
</tr>
<tr>
<td>Carryover</td>
<td>k = -0.06%</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1](image.png) Bland-Altman plot of the sample comparison between the Eiken OC-FCa and BÜHLMANN fCAL® turbo methods.

Conclusion
- The OC-FCa method performed well in all aspects of the evaluation – matching or exceeding the manufacturers claims where comparable.
- The method comparison showed a clear positive bias when compared to the BÜHLMANN fCAL® turbo.
- With the lack of standardisation for faecal calprotectin a clinical study is required to evaluate the impact of the positive bias and establish suitable cut-off levels.
- The OC-FCa offers the potential to screen for both Hb and calprotectin from a single sample to aid primary care with the distinction between bowel cancer, IBD and IBS which often present with overlapping symptoms.

Acknowledgements
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