

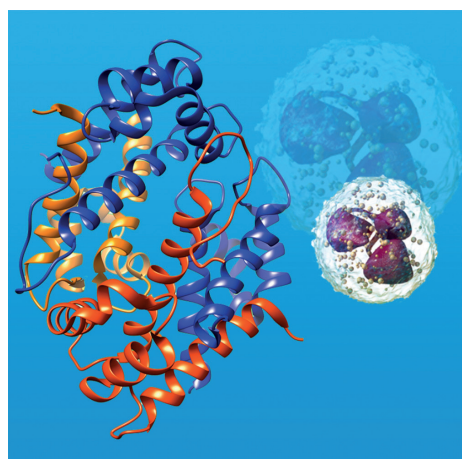
# Everything Is Under Control!

Preparation of a Simple Internal Quality Control for Faecal Calprotectin.

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As Programme Director of the UK NEQAS for Faecal Markers, I am often asked if I can recommend a suitable internal quality control (IQC) material for Faecal Calprotectin. Until quite recently, there has been no commercially-produced IQC available but all is not lost – with a bit of know-how, it is perfectly possible to make your own bespoke IQC, tailored to your exact requirements.



**Calprotectin (MRP8/14 or S100A8/A9) is a protein found extensively in the cytoplasm of neutrophils. It has been shown to be extremely useful in the diagnosis and monitoring of inflammatory diseases in the gastrointestinal tract.**



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## Now for the science bit.....

Due to the large number of pre-analytical steps involved in Faecal Calprotectin analysis, it is necessary to create two different types of IQC: the first will examine the consistency of the pre-analytical aspects of the process (sampling, preparation and handling of the extract) and the second, the consistency of your assay.

### Extraction Control:

To determine the consistency of your sampling and extraction process, you will simply need some homogenised stool containing a decent, measurable level of Calprotectin.

You don't want it to be too high – aim for a concentration of 200-400 µg/g. Ideally, the stool will have been obtained from a single patient donor, however, it's often much easier to pool together remnant material from a number of different samples with similar concentrations.

The leftover samples would ideally, have been frozen immediately after analysis to preserve the Calprotectin and prevent any degradation.

- Thaw out the remnant samples at 4°C overnight.
- Pool them together in a suitable vessel and mix well. You can use a variety of sanitised methods for this (vortex, stomacher etc.) but personally, I have found that you cannot beat a good ol' mixing bowl and spatula!

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- And, just like cake mixture, stir well whilst continually scraping the stool from the sides of the bowl into the centre.
- If there is fibrous material present, it may be necessary to push the stool through a coarse sieve to remove the lumps! This leaves what's known in the trade as the '[insert trade name of famous chocolate-hazelnut-spread here] portion'.
- Keep stirring and scraping for at least 10 minutes and voila – homogenised stool!
- Now all that remains is for you to split the pool into single-use, grape-sized aliquots. Use the smallest containers that you can find to minimise the amount of air to which the sample is exposed.
- Freeze the aliquots at -80°C\* for a minimum of 24 hours.

## Setting the Target Value

- Thaw one of the aliquots for a few hours at 4°C, mix well and prepare multiple extracts from it.
- Analyse each of the extracts and take a mean of the results obtained.
- Once they have been analysed, discard the extracts.
- Set your acceptance criteria for the IQC around this mean.
- Each time you run your assay, simply remove an aliquot of stool from the freezer, thaw, mix-well, extract and analyse in the usual way.



### Internal Assay Control:

The preparation of an IQC material suitable for monitoring the consistency of your assay, is a slightly more involved process during which you will need to make a bulk extract:

For this type of IQC, I would recommend that you prepare three different extracts each containing a different level of Calprotectin: one at a measurable level below the cut-off e.g.  $\sim 50 \mu\text{g/g}$ , one at or slightly above the cut-off ( $\sim 80\text{--}120 \mu\text{g/g}$ ) and one somewhere between  $200\text{--}600 \mu\text{g/g}$ .

- For each IQC level, prepare homogenised stool using the method outlined above.
- Next, decide on the total volume of extract you wish to prepare. This will depend upon how often you intend to run the IQC, the volume required for each analysis and the manufacturer's quoted stability for the extract when frozen. If it is not possible to purchase the buffer directly from the manufacturer, you may need to harvest it from the extraction devices.
- The kit insert for the extraction buffer/device will quote the required ratio of stool to buffer. For example, the CALEX® Cap requires a stool:buffer ratio of 1:500 so if you wish to make up 100 mL of extract, weigh out  $200 \mu\text{g}$  stool and add 100 mL buffer.
- Using an accurate balance, weigh out the correct mass of stool for the volume of extract being prepared (see ratio quoted in kit insert).
- Weigh the stool into the container in which the extract will be made. For liquid stool samples, use a pipette to measure out the recommended volume of stool required.
- Based on the mass of stool weighed into the container, calculate the exact volume of buffer required to give the correct ratio of stool:buffer quoted in the kit insert.
- Using the most accurate tool to hand (volumetric pipette, measuring cylinder etc.), measure out the total volume of buffer needed and add it into the vessel containing the stool.
- Vortex the extract for 30 minutes. Centrifuge the extract at  $3000g$  for 10 minutes. Decant off the supernatant, portion into single-use aliquots and freeze at  $-80^\circ\text{C}^*$  for a minimum of 24 hours.<sup>1</sup>



- Set your acceptance criteria for the IQC around this mean.
- Each time you run your assay, remove an aliquot of each different level of IQC from the freezer, thaw, mix-well and analyse in the usual way.

And there you have it, a simple IQC preparation that is tailored for use with your assay.

\*If you do not have access to a  $-80^\circ\text{C}$  freezer, store at the lowest temperature available and follow the manufacturer's guidance regarding stability at that temperature. Do not analyse stool or extract that has been stored for longer than the manufacturer recommends.

### Reference

1. Whitehead, S, French, J, Brookes, M et al. Between-assay variability of faecal calprotectin enzyme-linked immunosorbent assay kits. *Ann Clin Biochem* 2013; 50: 53–61.

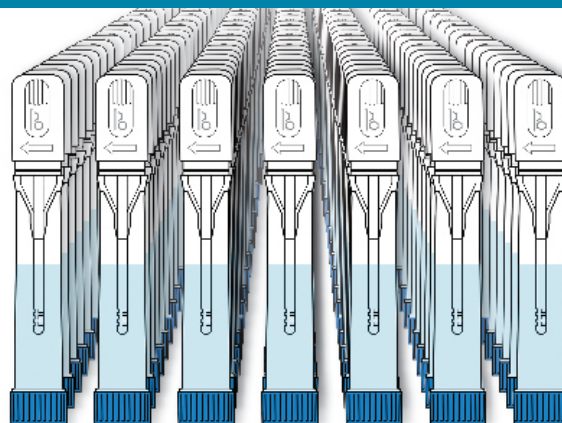
## CALEX® Cap - Faecal Calprotectin Extraction Made Easy

Faecal calprotectin sample preparation for the BÜHLMANN assays is quick, clean and consistent with the CALEX® Cap extraction device.

It offers a simplified workflow solution for faecal calprotectin extraction without the need to weigh, decant or further dilute samples.

The tube is pre-filled with buffer to give optimal dilution for maximum extraction efficiency.

CALEX tubes can be directly loaded onto automated ELISA processors or laboratory track systems.



Find out more at [www.calprotectin.co.uk/calex](http://www.calprotectin.co.uk/calex)