

## Are human fecal samples an appropriate matrix to measure mucus layer thickness?

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The intestinal mucus layer is important for the maintenance of gut barrier function, as it separates the intestinal epithelium from gut microbes. Most work on mucus layers has been conducted in animal studies, due to ethical restrictions in accessing human intestinal tissue. However, Kamphuis and colleagues have shown recently that the mucus layer forms around luminal contents and is excreted with the feces<sup>1</sup>. Thus, we investigated whether human feces samples are appropriate sample material to study intestinal mucus.

For two weeks four volunteers collected feces samples and recorded feces consistency using the Bristol Stool Index. Samples were taken in duplicate and immediately fixated with Carnoy's fixative. After fixation samples were paraffin embedded, sectioned and stained with alcian blue. Mucus layers were analyzed using an Axiovert S100 microscope.

The average mucus layer thickness for the individuals ranged from 15 µm to 30 µm. Maximum differences between the duplicates (taken from the same fecal sample) were huge, ranging from 21 µm to 28 µm. Further, a variety of problems occurred, such as empty sample tubes or not-sectionable samples, due to consistency problems. Thus, only 54 % of samples could be analyzed.

The results of this pilot study show that even though it is possible to analyze the mucus layer on fecal samples, this material might not be suitable to analyze mucus layer thickness systematically in larger study populations due to the huge intra-sample variability.

### Reference:

Kamphuis et al 2017: "Mucus organisation is shaped by colonic content; a new view" in Scientific Reports