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### Establishing techniques for processing and analyzing anal fistulas in Crohn's disease patients via flow cytometry

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**Background:** Patients with Crohn's disease are at an increased risk of developing anal fistulas. Anal fistulas in Crohn's disease patients are heavily infiltrated by CD45R positive T cells as well as CD20+ B cells with minor infiltration of CD68 positive macrophages and CD4/CD161 positive T cells. The aim of this study is to conduct a pilot study exploring if flow cytometry is a viable method to describe anal fistulas of Crohn's disease patients.

**Methods:** Tissues were processed mechanically and then separated from undesired cells and debris using either Ficoll-Paque gradient or BD FACS lysing solution. Cells in suspension were stained with fluorophore-conjugated CD4, CD20, CD45R, and CD68 antibodies and run through BD FACSCanto II. Results:

As predicted, tissues were able to be processed into single cell suspension and all four markers (CD4, CD20, CD45R and CD68) were detected in the sample fistulas. Discussion: The first problem encountered was the concentration of cells that the Ficoll-Paque gradient yielded, which was later corrected with the use of BD FACS Lysing Solution. Problems with staining intensity were also detected. Increasing the incubation period to one hour in room temperature and the utilization of BD FACS Lysing Solution enhanced staining of CD20-APC and CD45R-PE. Conclusions: Ultimately, with decreased autofluorescence and stronger staining detection, flow cytometry can be easily applied to the study of cell populations and present antigens in anal fistulas. Conducive to individual patient analysis using flow cytometry, fluorochrome panels, such as more antigens and different fluorophores, will be altered to best fit the study. Biography Jacie has completed her Master of Science at the age of 22 years from the University of Seville and is currently working at the University of Chicago in a IBS/microbiology lab. She is looking to apply to PhD programs and has spent the last 3 years researching IBS and Crohn's disease. She is passionate about understanding the cellular mechanisms of gastrointestinal diseases and is interested in improving the lives of those affected.

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