Profiling 115 proteins in colon and duodenal organoid supernatants by multiplex immunoassay

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Introduction

The MILLIPLEX® PLEXpedition Screening Panel is a configurable multiplex immunoassay kit that can simultaneously measure up to 115 targets including cytokines, chemokines, growth factors, matrix metalloproteinases, and biomarkers of bone health, metabolism, and cardiovascular disease. These 115 proteins were initially profiled in a diverse collection of human biofluids, including serum, plasma, cerebrospinal fluid, urine, milk, synovial fluid, and bronchoalveolar lavage (BAL). To evaluate the utility of this kit in *ex vivo* research models, we assessed 3dGRO® Gastrointestinal Organoids cultured in supplemented L-WRN Conditioned Medium. Supernatants were collected after 48 and 72 hours from matched colon and duodenal organoid cultures from a 21-year-old donor. The vast majority of the 115 analytes were detectable in at least one of the supernatant samples and were increased in the organoid supernatants relative to the conditioned media control. However, proteins exogenously added to the organoid culture media (Insulin, EGF) were above the upper limit of quantification. Most analytes were increased at the 72-hour timepoint relative to the 48-hour timepoint. A subset of analytes exhibited differential expression based on the tissue of origin, displaying relative increases in either the colon or duodenal organoid supernatants. In summary, this study demonstrates how a multiplex immunoassay can be applied to organoid research and used to measure >100 proteins in organoid supernatants.

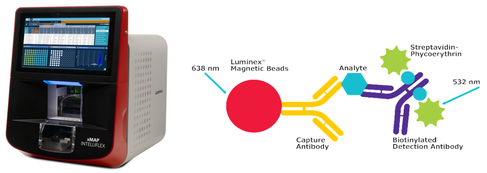


Figure 1: MILLIPLEX® immunoassay format.
MILLIPLEX® assays use magnetic microspheres (beads) conjugated to capture antibodies. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set, allowing researchers to simultaneously measure the analytes targeted by the capture antibodies. Native protein is analyzed by means of a "sandwich" immunoassay, pairing the capture beads with a biotinylated detection antibody.

Methods

Cell Culture and Sample Collection: DMEM/F12 PLUS (SCM162) was used as the basal organoid culture media. To prepare 1X complete media, the basal media was combined with L-WRN Conditioned Media (SCM105) and its recommended supplements. The 3dGRO® gastrointestinal organoids used in this study were obtained from a healthy 21-year-old male donor. Matched colon (SCC321) and duodenal (SCC322) patient-derived organoids were cultured in complete media and the cell culture supernatants were collected after either 48 hours or 72 hours of culture.

Immunoassays and Data Analysis: The MILLIPLEX® PLEXpedition Screening Panel was used to evaluate all samples according to the kit protocol on the xMAP® INTELLIFLEX® instrument (pictured above). Data analysis was performed using the Belysa® Immunoassay Curve Fitting Software (Cat. No. 40-122).

Results

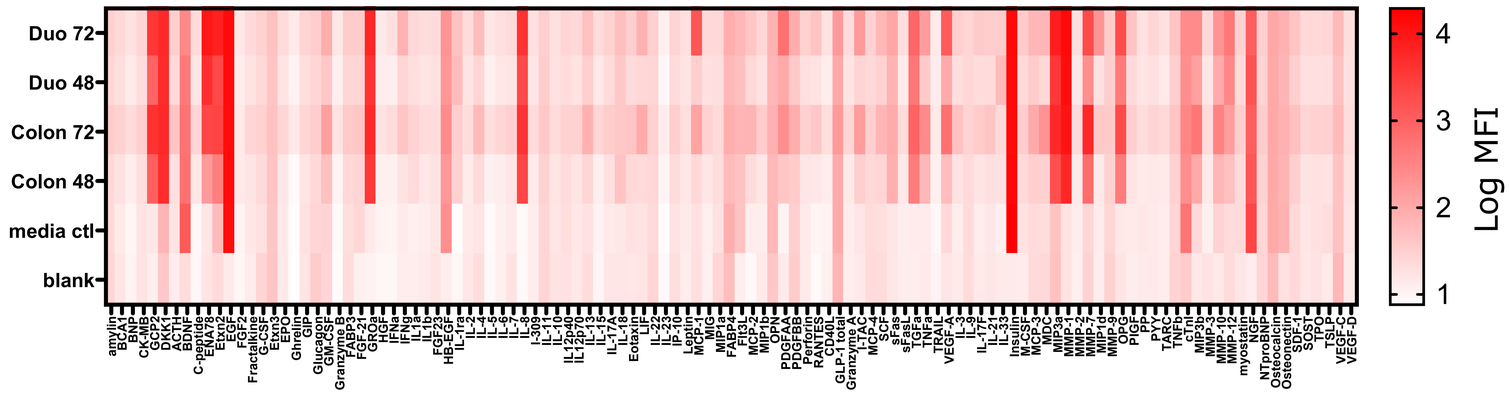
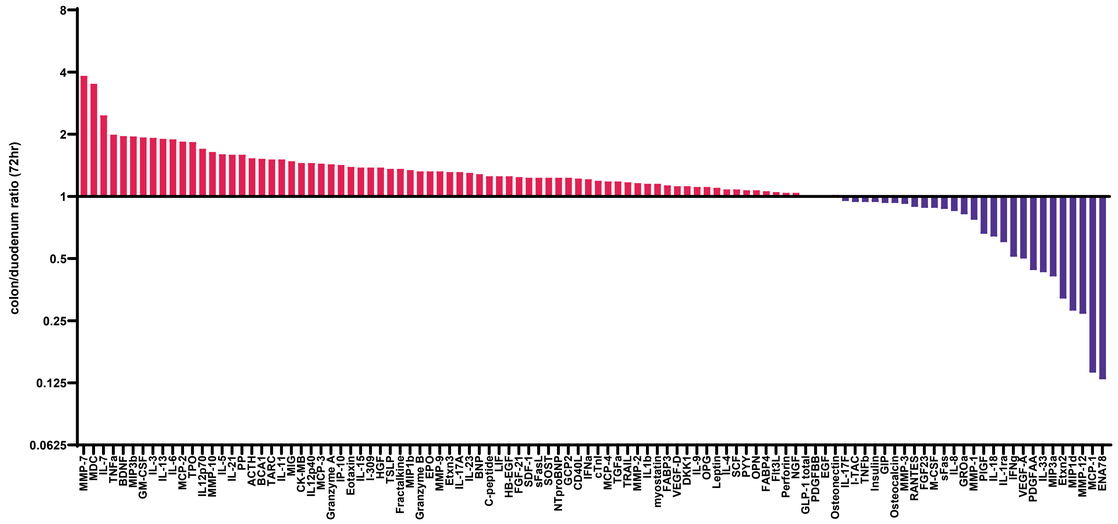


Figure 2. Measurement of 115 analytes in organoid cell culture supernatant with MILLIPLEX® PLEXpedition Screening Panel. 3dGRO® gastrointestinal organoids were cultured in supplemented L-WRN Conditioned Medium. Supernatants were collected at the 48 hour and 72-hour timepoints. Matched colon and duodenal (Duo) organoid supernatants and the media-only control (media ctl) were profiled using MILLIPLEX® PLEXpedition Screening Panel and the median fluorescence intensity was compared between conditions. Proteins that were exogenously added to the culture media (Insulin, EGF) were present ubiquitously across all samples. Most analytes showed accumulation in the organoid culture supernatant between the 48-hour and 72-hour timepoints.

A



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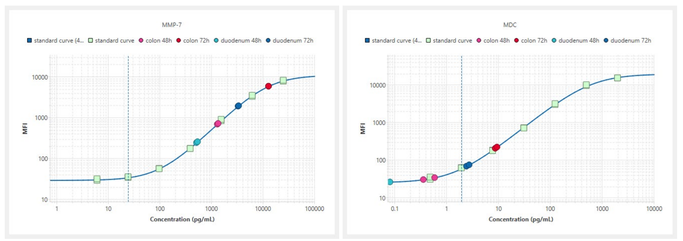

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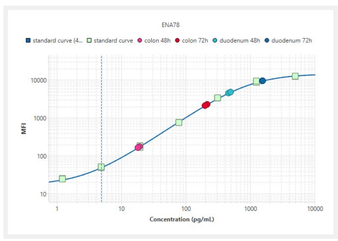

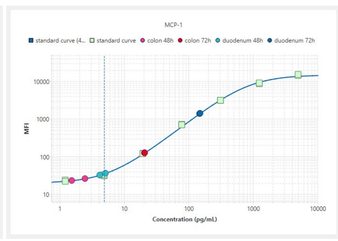

Figure 3. Relative enrichment of proteins in colon or duodenal organoid supernatant.
(**A**) Proteins were measured in organoid supernatant as described above and then ranked based on enrichment in colon or duodenal organoid supernatant at the 72-hour timepoint. Standard curve overlays generated in Belysa® Immunoassay Curve Fitting Software are shown below for (**B**) the two proteins most enriched in the colon organoid supernatant relative to the duodenal organoid supernatant and (**C**) the two proteins most enriched in the duodenal organoid supernatant relative to the colon organoid supernatant.

Summary

MILLIPLEX® PLEXpedition Screening Panel (Cat. No. HPLX1-115SP) is a configurable multiplex immunoassay kit to facilitate measurement of up to 115 protein biomarkers. Here we measured the relative abundance of these 115 proteins in colon and duodenal organoid culture supernatant along with the culture media control. Proteins varied in abundance, with known media additives insulin and EGF ubiquitously present. The concentrations of most proteins in the supernatant were increased at 72 hours relative to the 48-hour timepoint. Relative enrichment in the supernatant of either the colon or the duodenal organoids was observed for several proteins including MMP-7, MDC, ENA-78, and MCP-1. These results highlight the utility of MILLIPLEX® immunoassays for the measurement of protein biomarkers in organoid culture systems.

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