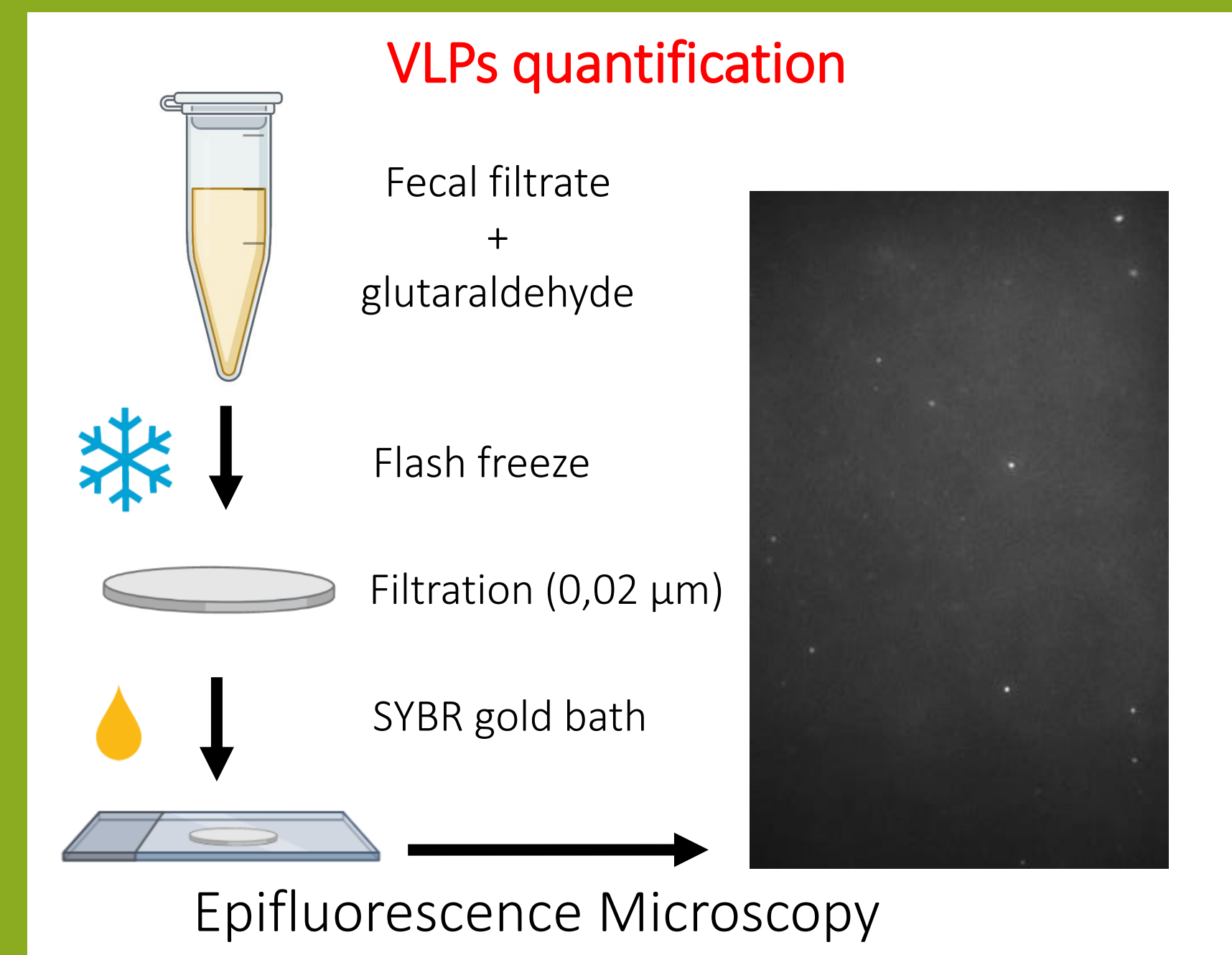
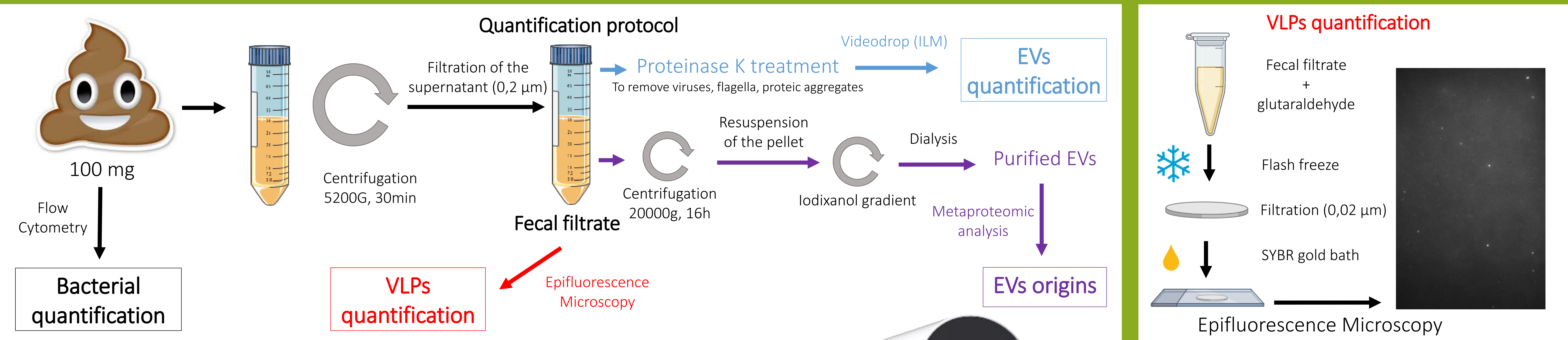
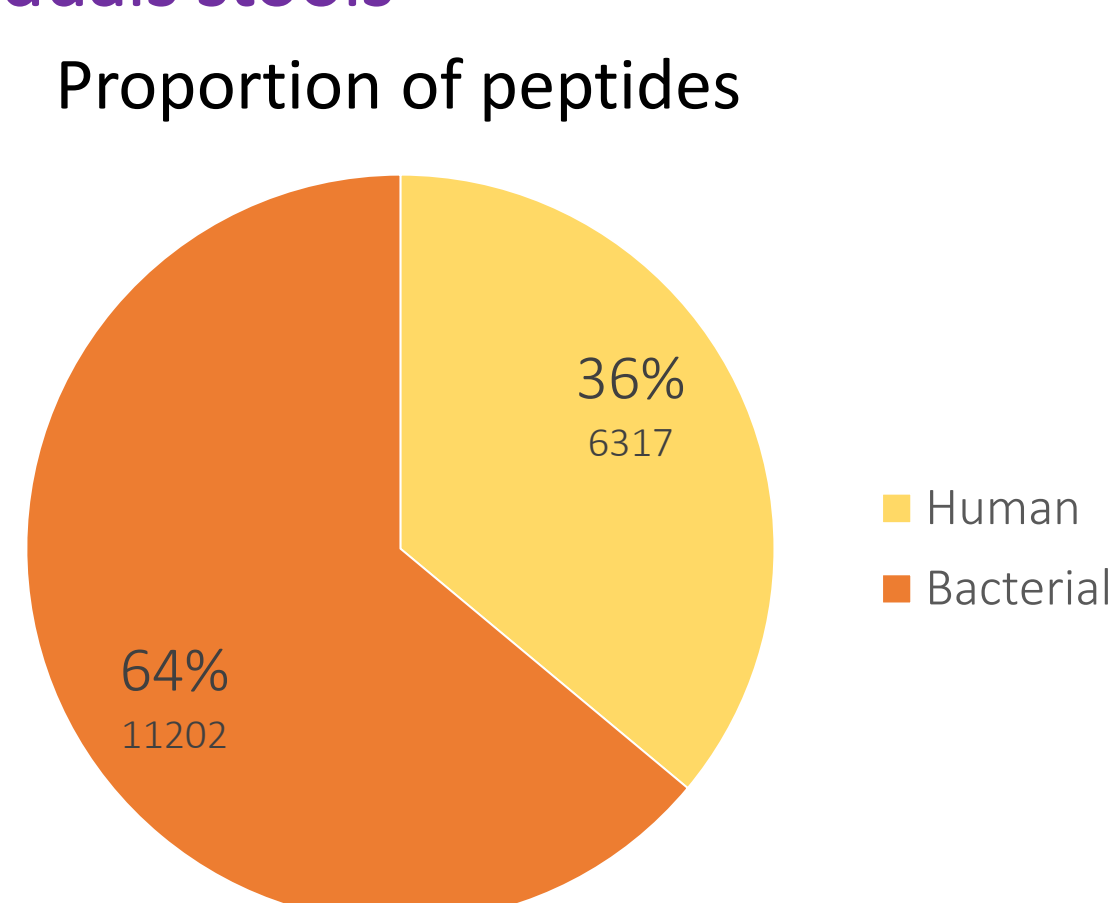
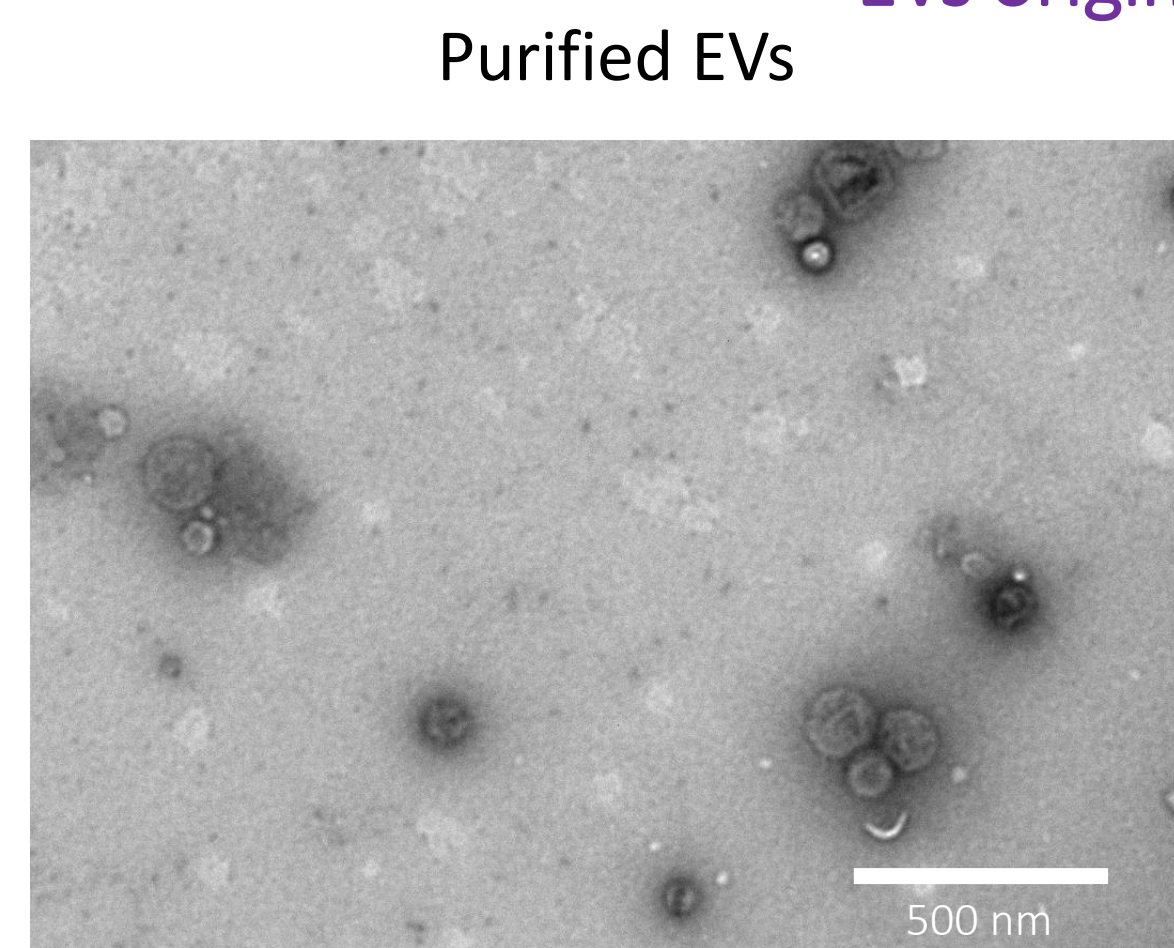


Introduction

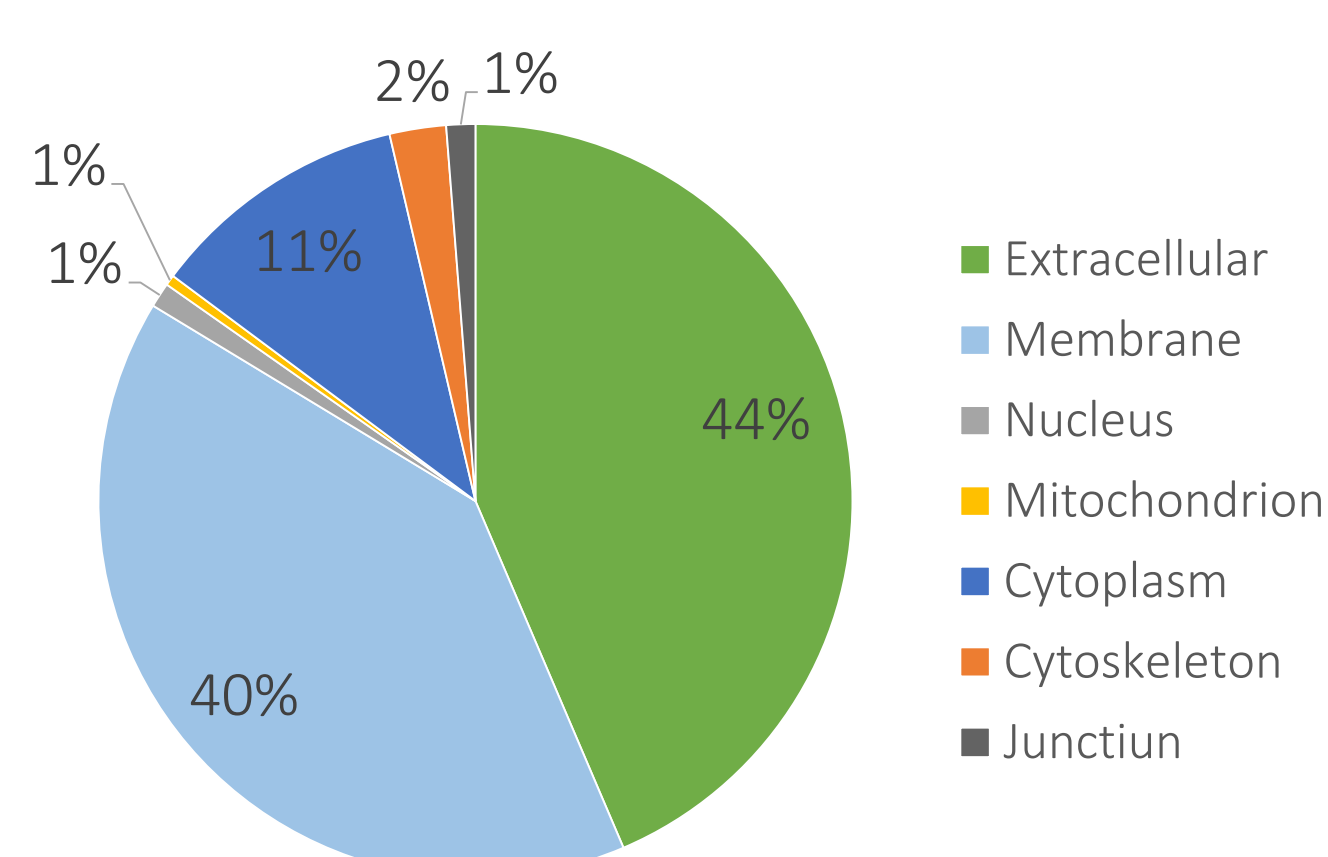
It is now largely accepted that the intestinal microbiota plays a key role in Intestinal Bowel Diseases (IBD). There are indications that phages, the viruses infecting bacteria, but also extracellular vesicles (EVs), might play a part in this family of diseases. For a better understanding of their roles, we have developed a protocol to quantify these different nanoparticles. The stools of ten healthy individuals were studied: EVs, Virus-Like Particles (VLPs) and bacteria were quantified using respectively the Videodrop, a recent device based on Interferometric Light Microscopy (ILM); epifluorescence microscopy; and Flow Cytometry (FCM). In addition, metaproteomic analysis were performed on purified EVs by high resolution mass spectrometry (LC-MS/MS) to determine the origin and the diversity of stools EVs.



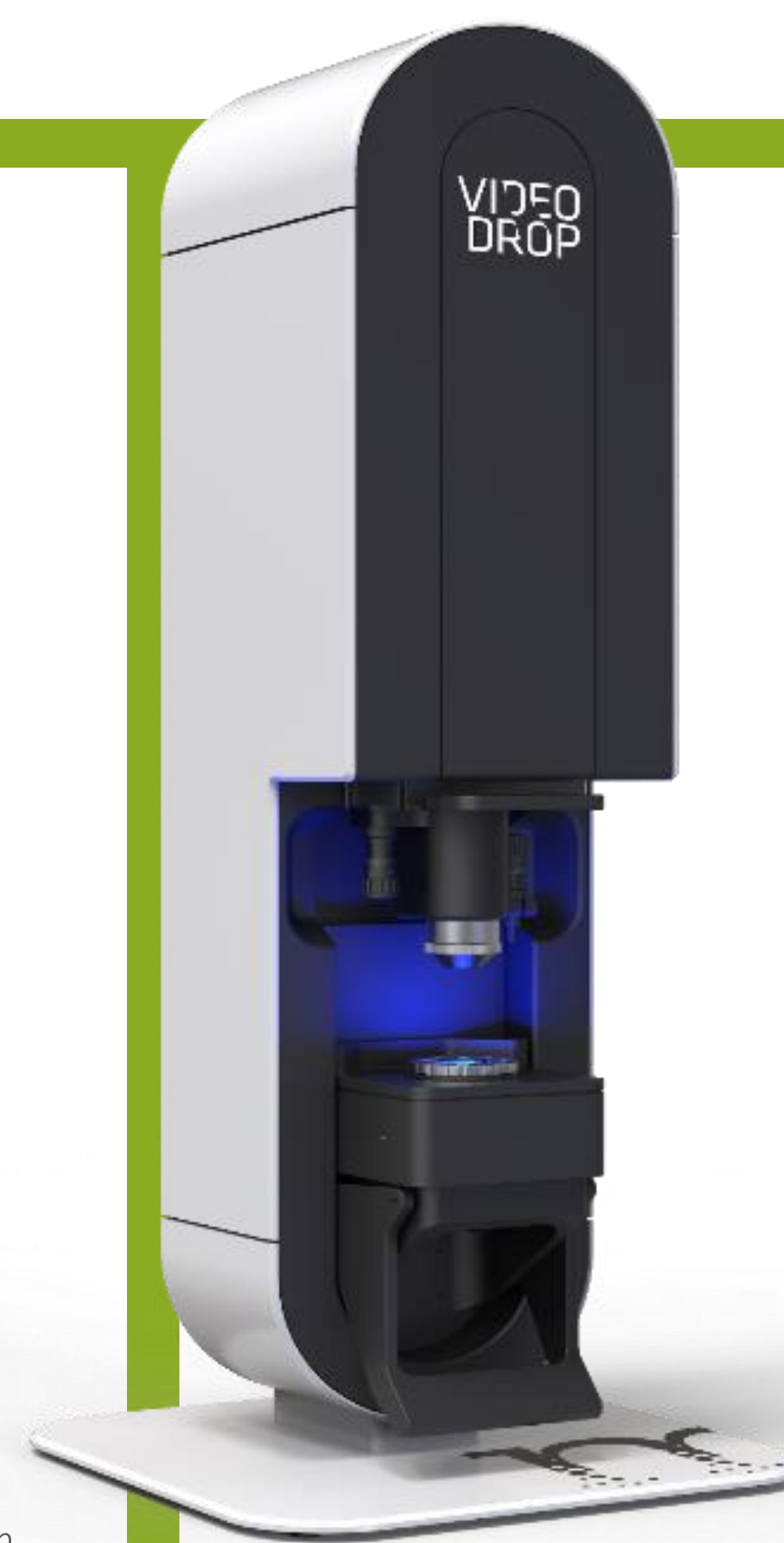
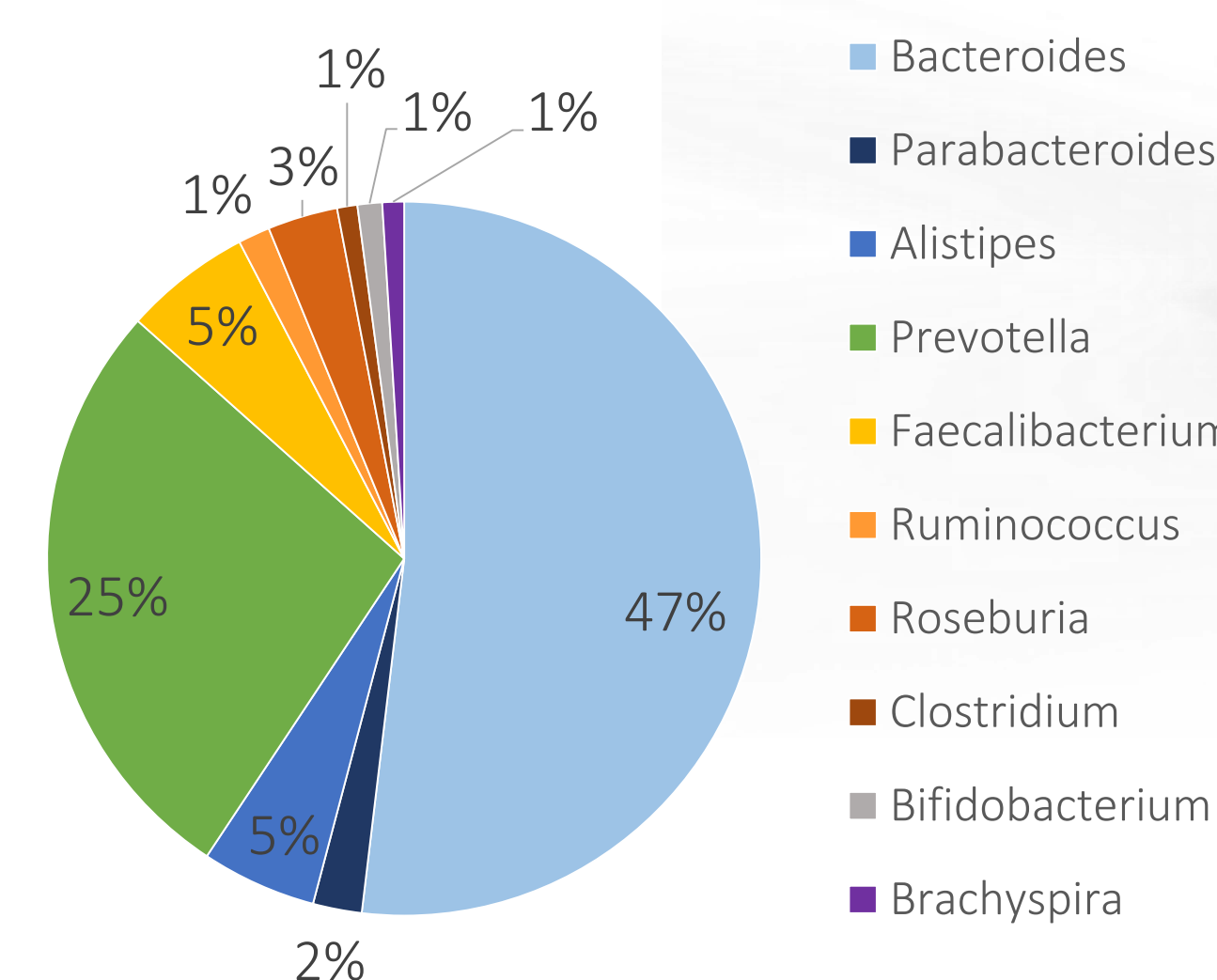
EVs origins in healthy individuals stools



Human peptide counts per localization



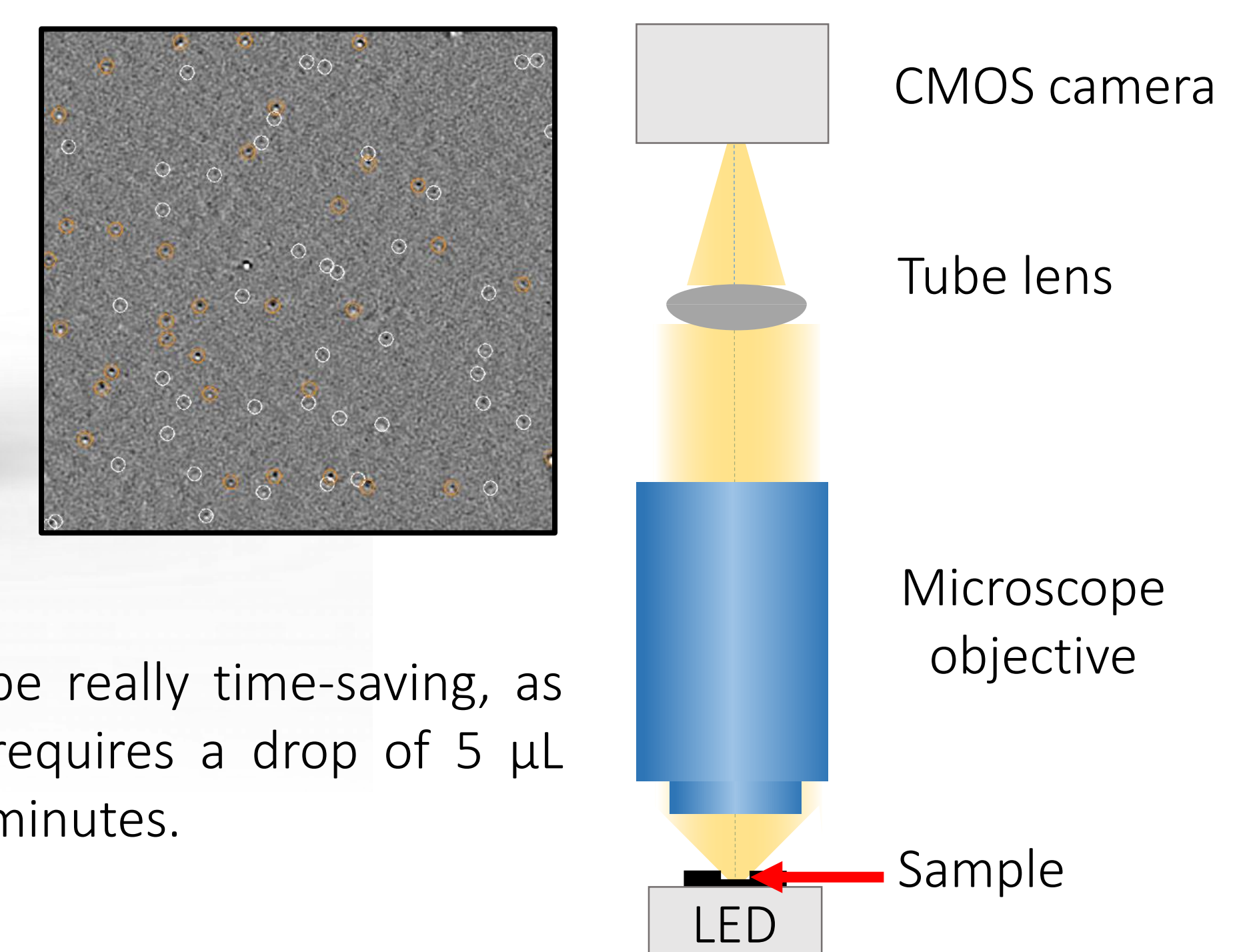
Bacterial peptide counts per taxonomic level



The Videodrop can be really time-saving, as an acquisition only requires a drop of 5 µL and lasts less than 2 minutes.

EVs quantification

ILM uses a transmission brightfield microscope used as a homodyne interferometer to detect, count, and track nanoparticles. Counting particles allows to measure the concentration, while tracking their Brownian motion allows to measure individually their hydrodynamical diameter and compute the sample size distribution.



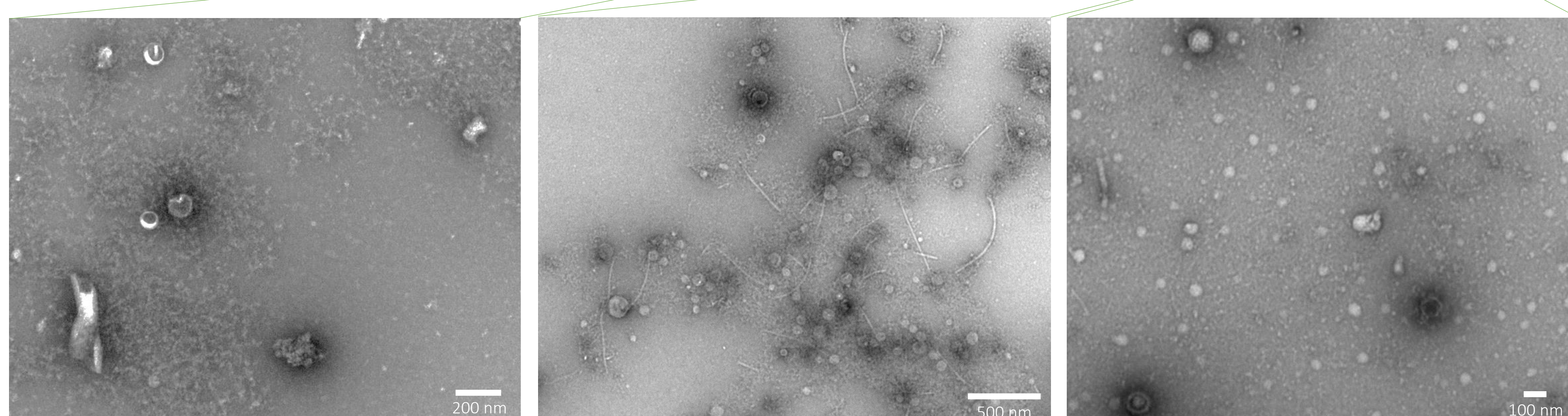
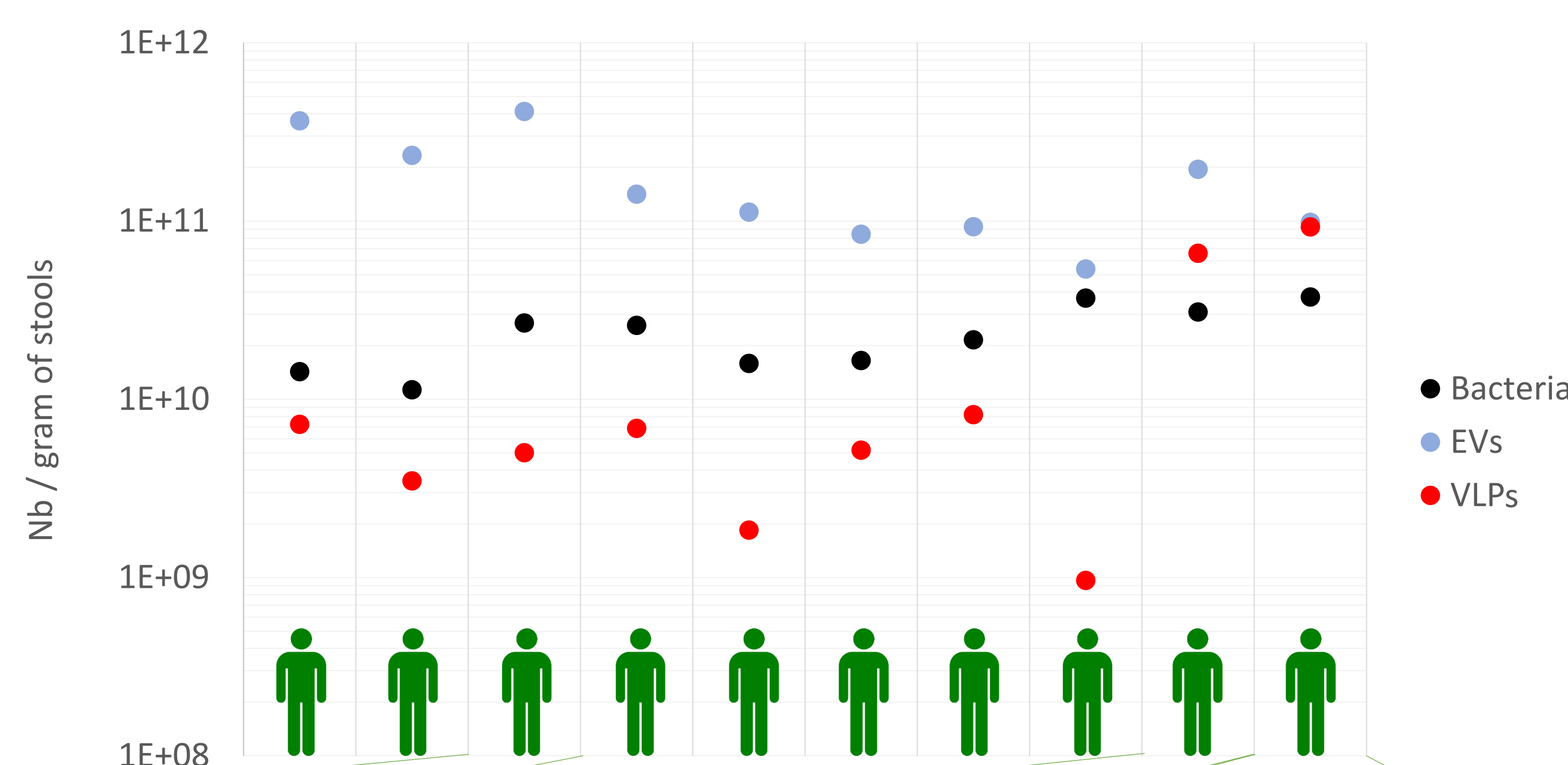
Bacteria, EVs, and VLPs quantification in healthy individuals stools

EVs and VLPs quantities are variable in the stools of our healthy donors.

EVs are very abundant, ranging from 5×10^{11} to 10^{12} per gram of stools.

The VLPs concentrations range from 10^9 to 10^{11} per gram of stools. VLPs were found to be 10-fold more concentrated in the stools of two healthy individuals.

The concentration of bacteria is less variable from an individual to another.



Conclusions

- We have developed a protocol to quantify bacteria, EVs, and VLPs in human stools. The Videodrop enabled a quick quantification of EVs.
 - Our first results in healthy individuals show variable inter-individual quantities of bacteria, EVs, and especially VLPs.
 - In two healthy individuals, we detected higher virus to bacteria ratios by epifluorescence microscopy. The presence of virions was confirmed by TEM.
 - Metaproteomic analysis of EVs revealed the diversity of their origins. 36% of the peptides found are human, and their subcellular localization highly suggest that they originate from EVs.
 - Bacteroides are the major producers of EVs.
- Quantifications are underway in IBD samples.

