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EUROPEAN SOCIETY OF HOSPITAL PHARMACEUTICAL TECHNOLOGIES

COM24-18490

Feasibility study of implementing flow cytometry in a fecal microbiota transplant preparation unit

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Introduction

Native stool

Controls of transplants (out of clinical trials):

- Screening for pathogens in stool
- Quantity (minimum 30g for 25g and 55g for 50g of stool)
- Macroscopic examination (consistency, presence of foreign elements, blood, etc.)

Fecal suspension (0.9% NaCl + Glycerol)

No characterization of:

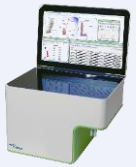
- Type of microorganisms (MO) present?
- Number of MO in a transplant?
- Percentage of bacterial viability?
- Proportion of other components of the fecal microbiota?

Flow cytometry would allow:

- Enumeration of MO
- Proportion of viable MO: fluorescent staining with differing permeability based on membrane integrity

Materials et methods

Tests on 2 cytometers



CyFlow® Cube 6 (Sysmex®)

Staining: CyStain™ Green/Red

Triplicate, 23 stools

Blue laser

CytoFLEX® S (Beckman Coulter®)

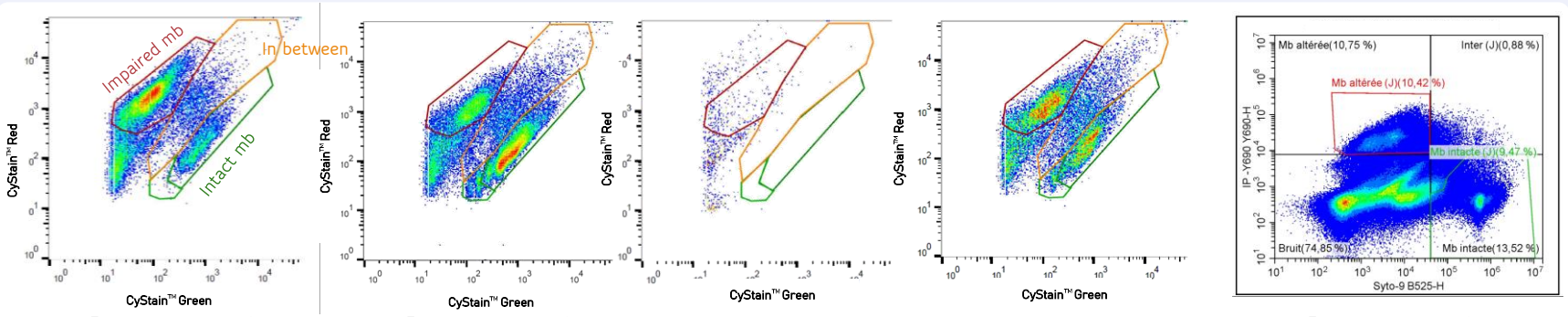
Staining: SYTO™9/ Iodure de propidium (IP)

Simplicate, 4 stools

Blue and yellow lasers

Mb : Membrane, MO : Microorganisms, CV : coefficient of variation

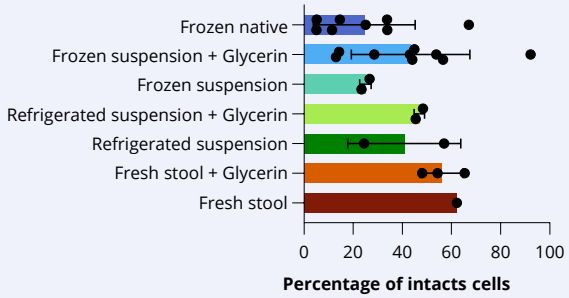
Results

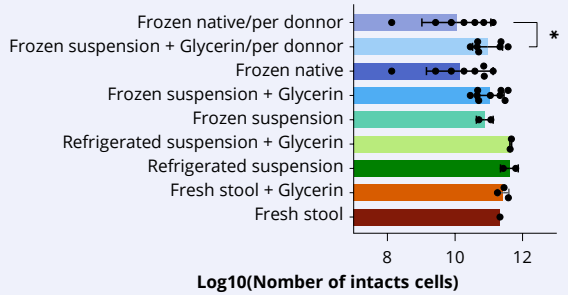


Frozen suspension 18 days | Frozen suspension + Glycerin | Frozen suspension + Glycerin unmarqued | Frozen native stool | Frozen suspension + Glycerin

	Cyflow®	CytoFLEX®
Price	Equivalent ~ 50 k€	
Training	Complete by the supplier	
Nomber of manipulation	+	- (automated homogenization and rinsing)
Upgrade	Change device	Laser addition possible without changing the device
Ergonomics	Integrated computer	Integrated sheath fluid and waste bottles

Criteria	Results
CV permeability of membrane	<13%
CV enumeration	<2% (in log MO/g of stool)
Enumeration	10^9 and 10^{12} MO/g of stool, consistent with the literature
Stool preparation	▪ No prior centrifugation or filtration ▪ Dilution to 10^{-6}: to achieve 10^{10}–10^{11} MO/g of stool (if outside this range, analyze the lower or higher dilution)





Wilcoxon tests

Conclusion

- ⇨ Rapid, repeatable results
- ⇨ Method does not provide information on: type of MO or other components of the fecal microbiota
- ⇨ Collaboration with other teams to enhance expertise
- ⇨ Supports innovation towards new pharmaceutical forms