

WP5: Microbiome & Metabolome	Security: PU	1/4
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DISCOVERIE

Project No. 848228

DISCOvERIE

**Development, diagnostic and prevention of gender-related
Somatic and mental COMorbitiEs in iRRitable bowel syndrome In
Europe**

Workpackage 5 Deliverable D5.1

**[Report on fecal sampling and transport of IBS cohorts in the project
and the quantitative microbiome profiling]**

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Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	X

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Consortium

Participant N°	Participant Legal Name	Country	Partner's Acronym
1	Fundacio Hospital Universitari Vall d'Hebron-Institut de recerca	ES	VHIR
2	Goeteborgs Universitet	SE	UGOT
3	Stichting Katholieke Universiteit	NL	SKU
4	University College Cork – National University of Ireland, Cork	IE	UCC
5	Johann Wolfgang Goethe – Universitatfrankfurt Am Main	DE	GUF
6	Katholieke Univeriteit Leuven	BE	KUL
7	Universitätsklinikum Heidelberg	DE	UKL-HD
8	Alma Mater Studiorum – Università Di Bologna	IT	UNIBO
9	VIB VZW	BE	VIB
10	Universiteit Maastricht	NL	UM
11	Semmelweis Eguetem	HU	SU
12	Universitatea de Medicina Si Farmacie Iuliu	ROU	UMF
13	Anaxomics Biotech, S.L.	ES	AX
14	Hycult Biotechnology BV	NL	HB
15	Emergentec Biodevelopment GMBH	AU	EMTEC
16	Nanostring Technologies Inc	USA	NSTG
17	Cliclab Technologies Inc	ES	Cliclab
18	Followhealth SL	ES	hITc
19	BYTE Factory Systems SL	ES	BF

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General hints for writing a deliverable:

List of abbreviations:

BL: Baseline

FU1: First follow-up (after one year)

Executive summary

Deliverable D5.1 describes the current status of baseline faecal sample collection and shipment. Additionally, it provides information on the collection procedures implemented, sampling handling protocols, storage conditions and transportation. The document includes the overall success of the samples collected during the project.

Introduction

Irritable Bowel Syndrome (IBS) is a persistent gastrointestinal condition characterized by abdominal discomfort and irregular bowel movements, including constipation and/or diarrhoea. Despite being a common disorder, the underlying mechanisms of IBS remain poorly understood and are thought to involve various factors, including the gut microbiota. However, mainly using 16S rRNA amplicon sequencing, where although it is possible to approximate the taxonomic composition of the bacterial communities, a detailed characterization of the functional potential of the gut microbiome is still needed. To fill such a gap, the WP5 will perform shotgun sequencing and metabolomics over the faecal samples for the participants of the DISCOVERIE cohort collected from the nine different collection centres.

Report

The deliverable 5.1 focuses on collecting the faecal samples for the IBS cohorts for further metagenomic and metabolomic profiling. In total, 1,538 faecal sample collection kits have been shipped to partner sites according to the distribution in [Table 1](#) as of 23/02/2024. The sample kits include a mat where the faecal sample can be divided into droplets for further use. The samples have been frozen from the day of collection to the day the samples arrived at the facilities of the VIB Raes' lab. All the samples are kept at -80°C to avoid any bacterial growth that could contaminate the sample.

As of 23/06/2023, 841 faecal samples have been returned, of which 754 BL samples (out of 800 total subjects) and 87 FU1 samples (out of 300 total subjects (maximum of 2 years of follow-up)) ([Table 1](#)). Originally, these samples were expected to reach the laboratory of Prof. Jeroen Raes, Wp5 co-leader, by June 2022. However, delays resulting from the COVID-19 pandemic and transportation challenges postponed the shipment, and the samples were not received until May 2023. During the nine months from May 2023 to February 2024, samples were

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preprocessed and catalogued for storage in Prof Raes's laboratory facilities for further manipulation and data generation.

The data generated includes ([Details in D5.2](#)):

1. The total bacterial cell count quantification using flow cytometry of the 841 samples.
2. The faecal moisture estimation for the 841 samples.
3. The DNA extraction and the sequencing library preparations for 841 samples.
4. The Illumina Novaseq sequencing of 841 samples, of which 83% succesful.
5. The metabolite extraction and preprocessing of faecal metabolites for 840 samples.
6. The high-resolution, non-targeted metabolomics profiling of 840 samples.

Table 1: Status of the kits sent and samples received

	UGOT	KUL	VHIR	UNIBO	UM	UMF	SKU/RUMC	GUF	SU
Country	Sweden	Belgium	Spain	Italy	Netherlands	Romania	Netherlands	Germany	Hungary
WP leader	Magnus Simren	Lukas Van Oudenhove	Javier Santos	Giovanni Barbara	Daisy Jonkers	Dan Lucien Dumitrascu	Alejandro Arias Vasquez	Andreas Reif	Istvan Bitter
BASELINE									
IBS patients	102	102	102	102	102	102			
disease controls			39				39	39	39
healthy controls	17	17	17						
total 1 per site	119	119	158	102	102	102	39	39	39
FOLLOW-UP									
IBS patients	51	51	51	51	51	51			
disease controls			39				39	39	39
healthy controls	17	17	17						
total 2 per site	68	68	107	51	51	51	39	39	39
total 3 per site (BASELINE + FOLLOW-UP)	187	187	265	153	153	153	78	78	78
Kits shipped – update 16feb2024	202	287	334	130	240	110	66	84	85
Samples received – update 16jun2023	112 (BL)	98 (BL), 22 (FU1)	167 (BL), 10 (FU1)	90 (BL), 6 (FU1)	102 (BL), 37 (FU1)	75 (BL)	35 (BL)	34 (BL), 11 (FU1)	41 (BL), 1 (FU1)

It is important to remark that we sent all samples of baseline for sequencing and metabolic profiling in the same batch, to minimize any possible batch effect issue.

It is important to remark that due to technical problems in the VIB Metabolomics core related to the installation of their equipment ([Details in D5.2](#)), we were pushed to subcontract a company, increasing the delivery time in the metabolome profiling. Following the financial rules for subcontracting activities, we engaged with four companies ([Detailed in D5.2](#)) and conducted thorough negotiations to secure the most cost-effective service possible.

Conclusion

In conclusion, the shipment of the samples is now complete by covering all available samples at baseline and 87 of the follow up.