STUDY FRAMEWORK

The alchemy project is a collaboration between Universidade Católica Portuguesa and the American company Amyris, currently carried out in the Centro de Biotecnologia e Química Fina (CBQF) facilities, located on the 3rd floor of the Biotechnology building, Universidade Católica Portuguesa, campus Foz do Douro and scientifically coordinated by Prof. Manuela Pintado (CBQF director). Within the Alchemy project framework, the **GIOTA platform** was designed to establish standardized gastrointestinal *in vitro* models to support the development of new functional ingredients for human nutrition. This is not a clinical trial[1], but a study comprising a number of sequential stages, 1, 2 and 3, in which the ingredients fermentability, digestion and absorption and prebiotic properties, respectively, are assessed. Stage 1 and 3 requires the use of human fecal material as inoculum for the fermentability assessment and gut microbiota impact studies. Therefore, dependent of the donation of human stool from healthy adult volunteers, which will be further processed by the GIOTA platform and used exclusively within this study framework, according to the guidelines established in the national law (n.º 21/2014 from 16 of April) for clinical studies.

All volunteers are previously informed of the study framework and objectives. Their rights and personal data protection and confidentiality are assured, in accordance to the National Committee of Data Protection (CNPD) guidelines, and reserved for research purposes only. Before donation, the volunteers are asked to carefully read this study factsheet and guidelines, collection protocol (SOP GT7) and sign the consent form below if willing to freely collaborate. In addition, the volunteers are asked to fill in the questionnaire, attached to this document, regarding their nutritional and life style habits to complement the study. All donors are aware that their participation in this study is voluntary and free of monetary remuneration.

Hence, as a potential donor, the GIOTA platform kindly asks you to carefully read this document, fill in the attached questionnaire and sign the informed study consent, if willing to participate in this study.

We are grateful for your participation!

The GIOTA platform

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[1] This study is out of the scope of the Regulation (EU) No 536/2014 as thus type of study are typically considered to have the lowest risk. See additional information (1)

Informed study consent[2]

1. Study factsheet

The GIOTA platform was designed to establish standardized human *in vitro* models for gut microbiota studies. The platform is responsible for designing, optimizing and implementing a number of gastrointestinal and colonic fermentation *in vitro* models able to mimic the gastrointestinal tract, including the digestion and absorption phases, and the fermentation stage in the colon. The results will enable to study the digestion, (oral, gastric and intestinal), absorption (small intestine) and fermentation (colon) processes and assess the different ingredients bioacessibility, bioavailability and impact on gut microbiota population. Using a number of molecular, microbiological and biochemical methods the platform will provide valuable information on the identification and quantification of the colonic bacteria population and their metabolites (e.g., SCFA) profile produced during the fermentation of the target ingredients being developed within the Alchemy project.

2. Purpose

Stage 1 and 3 of this study requires the use of human fecal material as inoculum for the fermentability assessment and gut microbiota impact studies, in accordance with the study framework described above. Therefore, the platform is recruiting healthy adult volunteers to collaborate in this study by providing a stool sample in several occasions throughout the study. To be an eligible donor, the volunteer will have to fulfill the requirements (section 3) described below.

3. To be eligible, the donor must:

- Have a normal omnivorous diet;
- Have not ingested any antibiotics or other medicines known to affect the gut microbiota, for at least 6 months previously to the donation;
- Age between 18 and 50 years old;
- Have not been submitted to extreme stress or intensive exercise for the last 12 h previous to the donation;

[2] According to the guidelines described in article 18º of the national law 12/2005 of 26 of January – see additional information (2)

- Have not consumed any probiotics-enriched (e.g., probiotic yogurt) or fiber-rich products (see examples in figure 5)[3] at least for 3 days previous to the donation;
- Absent of any gastrointestinal disorders (see examples in figure 6)[4];
- Absent of diarrhea;
- Have not ingested alcoholic drinks the day before to the donation;
- Be willing to provide a stool sample in several occasions throughout the study;
- Fill in the attached questionnaire and sign in a consent form.

4. Stool samples donation and data collection information

a) The decision of donating their stool samples and answering the questionnaire attached is voluntary, whereby, the donor is entitled to refuse without any further consequence;

b) Personal data protection and study results disclosure will be in accordance with the National Committee of Data Protection (CNPD)[5] and protected by current ethical and clinical studies national and international legislation[5];

c) Research will be under the supervision of the UCP Ethics committee, which will ensure that all the regulations are applied and complied with;

d) All biological samples collected and derived products, as well as all the information given by the questionnaires will be included in a database and coded to keep the donor's anonymity.

e) The donor's consent can be revoked at any time with no need of further justification, either by the donor itself or by their family, in case of death or incapacity. In this case, all samples and samples derived products and data will be destroyed. However, the same does not apply to the data already processed and results already published or part of other studies.

f) The samples can be shared with other research groups however it will obey to the same rules and will not be used for commercial end;

g) No monetary compensation will be given to the donors and all donors will agree to freely volunteer.

h) A collection and delivery protocol (SOP GT7) will be provided and explained to each volunteer. Fecal samples will be collected by the volunteers themselves at the Alchemy project facilities or at any other place of their choice, according to the given procedure. The sample

[3] SOP GT7
[4] SOP GT7
[5] See additional information attached (2)

should be delivered to the GIOTA platform within 2h of collection. The volunteer must strictly follow steps described in SOP GT7

i) Samples will be exclusively used within the project framework and processed within the Alchemy project facilities, located on the third floor of the Biotechnology Building of Universidade Católica Portuguesa, Rua Diogo Botelho, 1327, 4169-005 Porto, Portugal.

5. Communication and data disclosure

The data resulting from this study or studies will be published anonymously and grouped according to the publication target and never individually or by name.

6. Predicted physical risks

No health or physical risks are associated to this study. At the most, the donor can experience some type of discomfort, driven by the fact that the biological samples are stool samples.

Declaration of informed consent form

Donor's full name: _____
Attributed code: _____

I, _____, with the **ID number**: _____, **declare** to have acknowledged the terms and conditions of this research study and voluntarily accepted to participate by donating my biological sample (stool) and filling in a nutritional and life style questionnaire. I consent the use of my biological sample(s), exclusively for research purposes and have understood that I can withdraw my participation at any time and ask for the destruction of my samples, with no further consequences or discrimination.

1) The aims of this study were explained correctly and clearly, and I was given the opportunity to ask any question(s) regarding the study and/or my participation.
□ Yes
□ No

2) I was informed that my biological samples will be characterized at a microbiological and metabolic level.
□ Yes
□ No

3) I accept to participate voluntarily in this study by providing my stool sample and authorizing its preservation and use for future research studies
□ Yes
□ No

4) I am aware that my participation is voluntary and free, and I am willing to answer honestly to the nutritional and lifestyle questionnaire bellow
□ Yes
□ No

_____ _____
(Date) (Participant's signature)

I have discussed this research study with the volunteer, using an appropriate and clear language. I have informed the volunteer about this study's nature and its potential benefits and risks and I believe the volunteer has understood correctly my explanations.

_____ _____
(Date) (Researcher's signature)

This document was signed in duplicate. One signed copy will be provided to the volunteer

Nutritional and lifestyle questionnaire

Research Project Title - Establishment of a gastrointestinal platform using *in vitro* models for development of new functional ingredients for human nutrition.

GIOTA team (by alphabetic order) – Célia Costa, Diana Oliveira, Nelson Mota de Carvalho and Ana Raquel Madureira

Please read the questions carefully and answer honestly. Fill in the boxes with an X.

1. Did you read the factsheet for this study?

☐ Yes ☐ No

2. If you asked questions, did you receive appropriate answers?

☐ Yes ☐ No ☐ Not applicable

3. Do you understand that you are free to leave this study at any time without giving any justification?

☐ Yes ☐ No

4. Do you agree to participate in this study?

☐ Yes ☐ No

5. How old are you?

_____

6. What is your height (meters) and weight (kg)?

_____

7. Which ethnic group do you belong to (e.g. European, African, Asian, southern American, etc…)?

_____

8. What is your gender?

☐ Male ☐ Female ☐ Rather not answer

9. How would you define your diet (choose all the options that applies to you)?

☐ Omnivore ☐ Vegetarian ☐ Vegan ☐ Other _____

☐ High meat consumption ☐ High vegetables consumption

☐ High fiber consumption ☐ Low sugar consumption

☐ High sugar consumption ☐ High protein consumption

☐ Low protein consumption ☐ Dairy consumption

10. If you consume dairy products, how often? (e.g., milk, cheese, yogurts, etc…)?

☐ Every day ☐ Almost every day ☐ 2 to 3 times a week

☐ Rarely ☐ Never

11. Do you exercise (e.g. running, gym, sports, hiking, etc. ...)?

☐ Every day ☐ Almost every day ☐ 2 to 3 times a week

☐ Rarely ☐ Never

12. Do you smoke?

☐ Yes How many cigarettes per day? _____ ☐ No

13. Do you take chronic medication (e.g. diabetes, hypertension, autoimmune diseases, etc.)?

☐ Yes _____ ☐ No

14. Do you suffer from any intestinal discomfort (e.g. irritable bowel syndrome, Crohn's disease, *Helicobacter pylori*, etc…)?

☐ Yes _____ ☐ No

15. Do you take prebiotics, probiotics or other dietary supplements that benefits the intestinal microbiota (e.g. Inulin, UL – 250 ®, Actimel®, Activia®, etc…)?

☐ Yes ☐ No ☐ Do not known

16. In case you answered yes to the previous question, how often do you take such products?

☐ Every day ☐ Almost every day ☐ 2 to 3 times a week

☐ Rarely

17. When was the last time you took antibiotics?

☐ Last week ☐ Last month ☐ Last 3 months

☐ Last 6 months ☐ Last 12 months ☐ More than 12 months ago

18. When was the last time you were admitted to the hospital?

☐ Last week ☐ Last month ☐ Last 3 month

☐ Last 6 month ☐ Last 12 month ☐ More than 12 months ago

The GIOTA platform appreciates your collaboration!

Any questions please contact the GIOTA Platform team on the following e-mails (in alphabetical order):

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ADDITIONAL INFORMATION (1)

Annex II: Decision tree to establish a whether a trial is a "clinical trial"

Note: this Annex and in particular the definition for a low-interventional trial are still under discussion in the expert group on clinical trials**.** This algorithm and its endnotes[6] will help you answer that question. Please start in column A and follow the instructions. Additional information is provided in the notes at the end of the table. If you have doubts about the answer to any of the questions, contact the clinical trials unit of your competent authority.

	A	B	C	D	E	F
A CLINICAL TRIAL OF A MEDICINAL PRODUCT?					**A NON-INTERVENTIONAL STUDY?**	**A LOW-INTERVENTION CLINICAL TRIAL?**
Is a medicinal product administered before or during the start of the clinical trial **NO**	Is it a medicinal product (MP)?[i] **NO**	Is it not a medicinal product?	What effects of the medicine are you looking for?	Why are you looking for those effects?	How are you looking for those effects?	Is the product authorised in any EU Member State?
If a medicinal product is administered before the start of the clinical trial, and it falls under current practice, please go to column E. If a medicinal product is administered before the start of the clinical trial and it falls not under current practice, column E is excluded. If a medicinal product is administered after the start of the clinical trial, please go to column A.	If you answer no to all the questions in column A, the activity is not a clinical trial on a MP. If you answer yes to any of the questions below go to column B.	If you answer yes to the question below in column B the activity is not a clinical trial on a MP. If you answer no to this question below go to column C.	If you answer no to all the questions in column C the activity is not a clinical trial under the scope of Regulation EU No 536/2014. If you answer yes to any of the questions below go to column D.	If you answer no to all the questions in column D the activity is not a clinical trial under the scope of Regulation EU No 536/2014. If you answer yes to any of the questions below go to column E.	[redacted]	[redacted]
	A.1. Is it a substance[ii] or combination of substances presented as having properties for treating or preventing disease in human beings? A.2. Does the substance function as a medicine? i.e. can it be administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action or to making a medical diagnosis or is otherwise administered for a medicinal purpose? A.3.Is it an active substance in a pharmaceutical form?	B.1. Are you only administering any of the following substances? • Human whole blood[iii]; • Human blood cells; • Human plasma; • A food product[iv] (including dietary supplements) not presented as a medicine; • A cosmetic product[v] • A medical device	C.1. To discover or verify/compare its clinical effects? C.2. To discover or verify/compare its pharmacological effects, e.g. pharmacodynamics? C.3. To identify or verify/compare its adverse reactions? C.4. To study or verify/compare its pharmacokinetics, e.g., absorption, distribution, metabolism or excretion?	D.1. To ascertain or verify/compare the efficacy[vi] of the medicine? D.2. To ascertain or verify/compare the safety of the medicine?		

[6] Cf. Article 1(2) of Directive 2001/83/EC, as amended

ii Substance is any matter irrespective of origin e.g. human, animal, vegetable or chemical that is being administered to a human being.

iii This does not include derivatives of human whole blood, human blood cells and human plasma that involve a manufacturing process.

iv Any ingested product which is not a medicine is regarded as a food. A food is unlikely to be classified as a medicine unless it contains one or more ingredients generally regarded as medicinal and indicative of a medicinal purpose.

v The Cosmetic Directive 76/768/EC, as amended harmonises the requirements for cosmetics in the European Community. A "cosmetic product "means any substance or preparation intended for placing in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and mucous membranes of the oral cavity with the view exclusively or principally to cleaning them, perfuming them or protecting them in order to keep them in good condition, change their appearance or correct body odours.

vi Efficacy is the concept of demonstrating scientifically whether and to what extent a medicine is capable of diagnosing, preventing or treating a disease and derives from EU pharmaceutical legislation.

ADDITIONAL INFORMATION (2)

Applicable and informative legislation

1) National laws for personal data protection - Comissão Nacional de Proteção de Dados (CNPD)

- **Lei n.º 67/98 de 26 de Outubro -** Lei da Protecção de Dados Pessoais relativa à protecção das pessoas singulares no que diz respeito ao tratamento dos dados pessoais e à livre circulação desses dados) https://dre.pt/web/guest/pesquisa/-/search/239857/details/maximized
- **Aditamento à Lei n.º 67/98, de 26 de Outubro** - É aditado à Lei n.º 67/98, de 26 de outubro, o artigo 45.º -A, (Inserção de dados falsos) da Lei n.º 103/2015 de 24 de agosto https://www.cnpd.pt/home/legis/nacional/Lei103_2015.pdf
- **Regulamento n.º 1/2018 de 16 de Outubro** relativo à lista de tratamentos de dados pessoais sujeitos a Avaliação de Impacto sobre a Proteção de Dados https://www.cnpd.pt/home/decisoes/regulamentos/regulamento_1_2018.pdf
- **Lei n.º 58/2019 de 8 de Agosto** - Assegura a execução, na ordem jurídica nacional, do Regulamento (UE) 2016/679 do Parlamento e do Conselho, de 27 de abril de 2016, relativo à proteção das pessoas singulares no que diz respeito ao tratamento de dados pessoais e à livre circulação desses dados http://www.pgdlisboa.pt/leis/lei_mostra_articulado.php?nid=3118&tabela=leis&ficha=1&pagina=1&so_miolo=

2) International legislation for personal data protection

- **Directiva 95/46/CE** do Parlamento Europeu e do Conselho, de 24 de Outubro de 1995, relativa à protecção das pessoas singulares no que diz respeito ao tratamento de dados pessoais e à livre circulação desses dados https://eur-lex.europa.eu/legal-content/PT/TXT/?uri=CELEX:31995L0046
- **Regulation (EU) 2016/679** of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02016R0679-20160504

3) Other applicable legislation

3.1) National

- **Lei n.º 12/2005 de 26 de Janeiro** - Informação genética pessoal e informação de saúde https://dre.pt/pesquisa/-/search/624463/details/maximized
- **Lei n.º 21/2014, de 16 de Abril,** Aprova a lei da investigação clínica https://www.infarmed.pt/documents/15786/1068535/036-B1_Lei_21_2014_1alt.pdf
- **Declaração de Helsínquia** da Associação Médica Mundial Princípios Éticos para a Investigação Médica em Seres Humanos (versão de Outubro 2013) (Adapted Portuguese version) https://ispup.up.pt/docs/declaracao-de-helsinquia.pdf

3.2) International

- International Declaration on Human Genetic Data, 16 October 2003 http://portal.unesco.org/en/ev.php-URL_ID=17720&URL_DO=DO_TOPIC&URL_SECTION=201.html