ONCOSCREEN

D5.1 PHASE A CLINICAL STUDY - INITIATION PACKAGE

Date 31/08/2023

Funded by the European Union

Submission date: 31/08/2023

Due date: 31/08/2023

DOCUMENT SUMMARY INFORMATION

Grant Agreement No	101097036	**Acronym**	ONCOSCREEN
Full Title	A European "shield" against colorectal cancer based on a novel, more precise and affordable risk-based screening methods and viable policy pathways		
Start Date	1 January 2023	**Duration**	48 months
Project URL	https://oncoscreen.health/		
Deliverable	D5.1: Phase A Clinical Study - Initiation package		
Work Package	WP5- ONCOSCREEN Advancements and Novelties on CRC Diagnostics		
Type	Clinical Study	**Dissemination Level**	CO
Lead Beneficiary	FIRALIS		
Contributions	MAINZ, BEIA, TIMELEX, ROSENBAUM		
Authors	Céline Meyer (FIRALIS)		
Co-authors	Stéphanie Boutillier (FIRALIS)		
Reviewers	Gabrielle Ostreicher (UMC-MAINZ) Ivan Polak (ROSENBAUM)		

DISCLAIMER

COPYRIGHT MESSAGE

DOCUMENT HISTORY

Version	Date	Changes	Contributor(s)	Comments
V0.1	07.08.2023	The table of contents was prepared.	FIRALIS	Discussed the table with involved partners
V0.2	15.08.2023	The version was shared with partners.	ROSENBAUM MAINZ FIRALIS TIMELEX	Comments and input received
V0.3	28.08.2023	A new version of the deliverable was prepared and shared with the coordinator	FIRALIS	Feedback received and incorporated
V0.4	29.08.2023	Initial version of the Initiation Package was integrated and send for review to the coordinator	FIRALIS	Inputs were formatted and integrated
V0.5	30.08.2023	Pre-final version sent to the reviewers; peer review comments addressed	ROSENBAUM MAINZ FIRALIS	Incorporated comments from peer reviewers
V1.0	31.08.23	Final version after some minor edits. Submitted to EC	EXUS FIRALIS	Quality assurance and submission to the EU portal

PROJECT PARTNERS

Partner	Acronym
EXUS SOFTWARE MONOPROSOPI ETAIRIA PERIORISMENIS EVTHINIS	EXUS
UNIVERSITAETSMEDIZIN DER JOHANNES GUTENBERG-UNIVERSITAET MAINZ	UMC-Mainz
INSTITUTE OF COMMUNICATION AND COMPUTER SYSTEMS	ICCS
FIRALIS	Firalis
UNIVERSITATSKLINIKUM SCHLESWIG-HOLSTEIN	UKSH
UNIVERSITAET zu LUEBECK	UzL
LIETUVOS SVEIKATOS MOKSLU UNIVERSITETAS	LSMU
MEDIZINISCHE UNIVERSITAT GRAZ	MUG
INSTITUTO PORTUGUES DE ONCOLOGIA DO PORTO FRANCISCO GENTIL, EPE	IPO
INSTITUTUL ONCOLOGIC PROF. DR. ALEXANDRU TRESTIOREANU BUCURESTI	IOB
TECHNION - ISRAEL INSTITUTE OF TECHNOLOGY	TECHNION
UNIVERSIDADE DO MINHO	UMINHO
UNIVERSITEIT VAN TILBURG	TLBG
VLAAMSE INSTELLING VOOR TECHNOLOGISCH ONDERZOEK N.V	VITO
ETHNIKO KENTRO EREVNAS KAI TECHNOLOGIKIS ANAPTYXIS	CERTH
INNOVATION SPRINT	iSPRINT
SCIENTIFIC ACADEMY FOR SERVICE TECHNOLOGY EV	SERVTECH
AINIGMA TECHNOLOGIES	AINIGMA
CATALINK LIMITED	CATALINK
KONNEKT ABLE TECHNOLOGIES LIMITED	KT
BEIA CONSULT INTERNATIONAL SRL	BEIA
UNIVERSIDAD DE LA RIOJA	URIOJA
TIME.LEX	time.lex
CARR COMMUNICATIONS LIMITED	CARR
MINISTRY OF HEALTH	MoHGR
PAGALBOS ONKOLOGINIAMS LIGONIAMS ASOCIACIJA	POLA LT
EUROPACOLON PORTUGAL- ASSOCIACAO DE LUTA CONTRA O CANCRO DO INTESTINO	ECPT
ELLINIKI ETAIREIA OGKOLOGIAS PEPTIKOU	HSGO
EUROPEAN SOCIETY OF DIGESTIVE ONCOLOGY	ESDO
FUNDATIA YOUTH CANCER EUROPE	YCE
MEDIZINISCHE UNIVERSITAT INNSBRUCK	MUI
LIETUVOS RESPUBLIKOS SVEIKATOS APSAUGOS MINISTERIJA	MoH-LT
EY ADVISORY SPA	EY
AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	CSIC
UNIVERSITE DE FRANCHE-COMTE	UFC
ROZENBAUM KONSULTING	ROSENBAUM
GIE AXA	GIE AXA
ASSOCIATION GERCOR	GERCOR
CC ASSURED	CC ASSURED

LIST OF ABBREVIATIONS

Abbreviation	Description
ANSM	Agence Nationale de Sécurité du Médicament et des produits de santé
BMI	Body Mass Index
BRC	Biological Registration and Collection
CA	Consortium Agreement
CRA	Clinical Research Associate
CRO	Contract Research Organization
CRC	Colorectal Cancer
CRF	Case Report Form
CS	Clinical Study
CT	Clinical Trial
D	Deliverable
EDC	Electronic Data Capture
FIT	Fecal Immunochemical Test
GA	Grant Agreement
GDPR	General Data Protection Regulation
IC	Informed Consent
IPR	Intellectual Property Rights
KPI	Key Performance Indicator
PI	Principal Investigator
SOP	Standard Operating Procedure
WP	Work Package

Executive Summary

The Phase A Clinical Study Package-Initiation Package constitutes the first part of the Clinical Study Package for the Clinical Studies (CS) of the ONCOSCREEN project, which is performed within the Task 5.1. The second part will be specified later in the deliverable D5.2, which will cover the Phase B of the Clinical Study.

The main objective of this deliverable is to design in detail the Clinical Trial (CT) phases and provide the documentation to the hospital sites' Ethics Committees in view of its approval. Indeed, preparation and submission of the necessary documentation to the ethical committees is performed in this deliverable. Once the ethical committee approves the package, the documentation prepared will allow the clinical partners to implement the Clinical Study and to start the subject recruitments. These documents are written with main contributions by the relevant partners, allowing the ONCO-VOC, ONCO-CTC, ONCO-NMR, and ONCO-CRISPR tools to be tested.

Ten countries all around the Europe are part of the CT in the ONCOSCREEN project and will reflect the diversity of the European population, socio-economic factors or inequalities within EU countries and regions.

The Clinical Study is a comprehensive plan of action. Outcomes of the CT are the expected results of Phase A.

The clinical study initiation package includes among others:

- The **clinical study protocol**, that contains the details about objectives, design and methodology of the clinical study.
- The **Biological Registration and Collection registration number**.
- The **Investigator Brochure**, which targets the Principal Investigators (PIs), who are responsible of the CT in their respective clinical sites.
- The **Standard Operating Procedures** (SOPs), which are available for each tool under the scope of the CS.
- The **Participant Information Sheet** and the **Informed Consent** (IC), which include the process in which a health care provider educates a patient about the risks, benefits, and alternatives of a given procedure or intervention, and accordingly collect the patient's consent.
- The trial **Key Performance Indicators** (KPIs), which are crucial to ensure the quality of the Clinical Trial and will be setup according to the European laws and regulations.

TABLE OF CONTENTS

LIST OF TABLES

1. Introduction

During the first phase of the clinical study, we will identify the different expression patterns of the four ONCOSCREEN diagnostic solutions (ONCO-VOC, ONCO-CRISP, ONCO-NMR, and ONCO-CTC) in patients with colorectal cancers (CRC) and healthy controls with high risk for CRC, and also initially estimate their sensitivity and specificity. The Phase A Clinical Study-Initiation Package consists of different parts. The main part focus on the Phase A Clinical Study, which will be conducted in 10 different countries involving the recruitment of 700 subjects in total. Six clinical sites are project partners (UMC-MAINZ, UzL, LSMNU, IOB, IPO, UFC), one is the French GERCOR Cluster that may involve from 2 to 30 different clinical sites, and finally ROSENBAUM partner (CRO company), who will be responsible for 9 different clinical sites from Greece, Hungary, Slovakia, Czech Republic, and Bulgaria. The other parts described in this package are especially dedicated to the Case Report Form and the Standard Operating Procedures.

The overall management and monitoring are made by FIRALIS and ROSENBAUM (CRO companies), and UMC-MAINZ is the Clinical Study Sponsor. The ONCOSCREEN-CS will be secured through the prolonged experience of project partners in clinical trials, the 2 CROs and the UMC-MAINZ, which is also the Scientific Coordinator. During both Phases (A and B), an equal distribution of males and females will be performed.

This deliverable will be complemented by the D5.2 Phase B Clinical Study - Initiation package, and then by the D5.3 and D5.4 Midterm recruitment reports for the Phase I and Phase II Clinical Study, respectively.

1.1. The need of Clinical Study Package in the project

The deliverable D5.1 presents a detailed overview of the Initiation Package related to the ONCOSCREEN Phase A Clinical Study, which is a multicentric study. This Initial Package is important for the proper implementation of the Clinical Study in the various clinical sites involved. Moreover, part of the documents included in this Initiation Package that describe the processes and methods, will allow the partners to use in the same manner the four ONCOSCREEN diagnostic solutions, leading to identify their different expression patterns, and to also estimate their sensitivity and specificity initially.

1.2. Deliverable objectives

Table 1: Description of Action: Task 5.1

ONCOSCREEN DoA requirements	Deliverable addressing the requirements	Brief description
Task 5.1 Clinical Trial design and preparation, study protocol and KPI definition	D5.1 Phase A Clinical Study – Initiation Package	This deliverable includes all the documents that will be necessary for the Phase A Clinical Study implementation and its approval by the various Ethic Committees.

1.3. Relationship with other deliverables and tasks

This deliverable is closely linked to other projects tasks and the deliverables listed in the Table 2.

Table 2: Linkages between D5.1 and other ONCOSCREEN tasks and deliverables

Deliverable	Description of the deliverable	Link to D5.1
T5.2	End users training and upskilling	Clinical protocol is needed to prepare the training and support to all clinical study actors
T5.4	Clinical Trials Implementation and validation	Clinical protocol is needed by ethical committee to authorize the implementation of a clinical trial
T5.5	Health Technology Assessment, Effectiveness and Quality Assurance Trial evaluation	This task is based on the results of the task 5.4 which needs information from clinical protocol
D5.2	Phase B Clinical Study - Initiation package	This deliverable will adapt the D5.1 initial package documents to the clinical trial Phase B
D5.3	Phase A Clinical Study-Midterm recruitment report	D5.3 is dependent from the D5.1, which manages the clinical trial Phase A starting

1.4. Deliverable structure

This deliverable is composed of different sections. The Section 2 reminds the objectives. The Section 3 describes the Clinical Study, which details its objectives, design and methodology. Section 4 is dedicated to the Investigator Brochure that targets the Principal Investigators (PIs), who are responsible of the CT in their respective clinical sites. In addition to that, the Section 5 presents the Standard Operating Procedures (SOPs), which are available for each tool under the scope of the CS. The Section 6 presents the Participant Information Sheet, which is the process in which a health care provider educates a patient about the risks, benefits, and alternatives of a given procedure or intervention. In addition, this section also describes the Informed Consent (IC) and the Consent Withdrawal Forms that are used for the collection of the patient's consent. The Section 7 illustrates the Case Report Form (CRF), which is a document specifically designed to record all protocol-required information on each recruited subject in the clinical study. The Section 8 covers the Trial Key Performance Indicators (KPIs). Such KPIs are crucial for ensuring the quality of the Clinical Trial and will be setup according to the European laws and regulations. The Section 9 describes the outcomes of the initiation package. Finally, the section 10 concludes the deliverable with the next steps.

2. Objectives

This deliverable is important to start and implement the Clinical trial. Indeed, the documents, which constitute the Clinical Study Initiation Package are fundamental because they shape the whole Phase A of the clinical trial.

The main objective of this deliverable is to create a full set of documents needed for the Clinical Study implementation. This includes the gathering of information from different partners of the consortium such as technical partners who contributed to the SOPs, and from clinical partners who contributed to design the clinical protocol. This will allow all the partners to work according to the same procedures, in order to generate homogenous and high-quality results that will be comparable between all the clinical sites involved in this study.

All the documents included in the Initiation Package must be submitted to the ethic committees to get their approvals before starting the Clinical Study. Each country or clinical site has its own ethic committee and the Initiation Package will be submitted to each committee separately for its evaluation and approval.

3. Clinical Study

A research study involving human subjects is designed to answer specific questions about the safety and efficacy of a biomedical intervention (devices), or new ways of using a known device. The CS is a comprehensive plan of action that contains information concerning the goals of the study, details of the subject recruitments, details of the safety monitoring, and all aspects of the design, methodology, and analysis. FIRALIS coordinates the clinical partners and has implemented regular meetings since the beginning of the project. Every two weeks, the content of documents belonging to the clinical initiation package have been discussed. This allowed to the consortium to well progress on the clinical protocol writing, which is nearly finalized. Different ONCOSCREEN partners contributed to the protocol writing. Thus, UMC-MAINZ and BEIA defined the inclusion/exclusion criteria. UMC-MAINZ also contributed to the sample data analysis and statistical methods section. ROSENBAUM contributed to the Quality Insurance and Quality Control section. TIMELEX worked on the Ethics section.

ONCOSCREEN-CS-Phase A: This is going to be a multi-center cross-sectional case-control study for the collection of data that will develop and finalize the screening and diagnostic solutions and firstly estimate their respective and potential complementary diagnostic performance (initial assessment of sensitivity and specificity, and other KPIs).

The Phase A-CS plans to recruit a total of 700 subjects. The control group is going to be enrolled using FIT as a pre-screening test. Thus, subjects with 40-50 years that are of high risk for CRC defined by the presence of CRC heredity, obesity (BMI>30), history of long smoking, alcohol consumption and/or hyperlipidemia are going to be enrolled to the healthy control group when having a negative FIT screening. The patient group is going to be enrolled with the consecutive recruitment of colonoscopy-examined and biopsy proven newly diagnosed CRC patients, excluding those who underwent surgical resection or received chemotherapy and/or other oncological treatment

3.1 Inclusion Criteria

For the Patient Group

1. Signature of the informed consent indicating that the subject accepts to participate in the study and to comply with the requirements and restrictions inherent in this study.
2. Male or female subjects aged ≥18 years
3. Subject has undergone a FIT screening test
4. Subject is diagnosed with CRC by colonoscopy and tissue biopsy
5. CRC is at stage considered well resectable
6. Subject is able to comply with all study procedures
7. Covered by a Health Insurance System

For the healthy control group

1. Signature of the informed consent indicating that the subject accepts to participate in the study and to comply with the requirements and restrictions inherent in this study
2. Male or female subjects aged ≥18 years
3. Subject has undergone a FIT screening test
4. Otherwise healthy individuals with recognized risk factors for CRC development defined by either heredity and/or obesity and/or smoking and/or excess alcohol consumption and/or hyperlipidemia.

3.2 Exclusion Criteria

For the Patient Group

1. Legal incapacity or limited legal capacity
2. Subject did not sign the Informed Consent form
3. Subject has previous history of CRC surgery
4. Subject has history of other cancer types
5. Subject received treatment by chemotherapy or other targeted oncological treatments
6. Subject who, according to the investigator's assessment, presents with an unstable medical condition contraindicating the performance of the planned blood test, stool test or breath test
7. Pregnancy and/or breastfeeding

For the healthy control group

1. Legal incapacity or limited legal capacity
2. Subjects who did not sign the Informed Consent form
3. Subject is diagnosed with CRC by colonoscopy and tissue biopsy
4. Previous history of cancer (any type)
5. Presence of any relevant organic, systemic or metabolic disease
6. Gastrointestinal disorders or other serious acute or chronic diseases
7. Participation in another interventional study
8. Pregnancy and/or breastfeeding
9. Known drug and/or alcohol abuse
10. Using any form of nicotine or tobacco

The CS is subject to a declaration of registration making it possible to obtain a registration number, called Biological Registration and Collection (BRC) number. This has been done with the ANSM French agency ("Agence Nationale de Sécurité du Médicament et des produits de santé"). The Phase A Clinical Study is registered under the BRC number: **2023-A01793-42** (see Annex A).

4. Investigator Brochure

The Investigator Brochure (IB) is a compilation of the current clinical and non-clinical information on the investigational medical devices relevant to the clinical investigation. The different sections of the brochure, which is presented in the Annex B explain the studies already done and/or data available for the four different devices ONCO-CRISPR, ONCO-CTC, ONCO-NMR, and ONCO-VOC. The technical features of the devices are described, as well as the analytical validation parameters, including specificity, sensibility, and their performance. A dedicated section relative to the ongoing clinical study is also part of the IB. This section compiles relevant scientific literature available relating to the safety, clinical benefits to subjects, design characteristics and intended purpose of the device. The IB is part of the clinical trial package that has to be submitted to the competent authorities and ethics committees and must be approved before the clinical trial can start. The IB is based on information provided by both technical and clinical partners. They give input to shape the document in order that each clinician will understand the way the tools are working and the way the results generated by the tools will help a better care of patients suffering from colorectal cancer.

5. SOPs

A Standard Operating Procedure (SOP) is a specific procedure that describes step by step the activities necessary to complete the tasks in accordance with standards pre-established by the developer of the device. It is a set of written instructions that documents a routine or repetitive activity followed by an organization. The SOPs, which have been prepared based on the close collaboration with relevant partners are describing the technical features of the following devices: ONCO-VOC, ONCO-CTC, ONCO-CRISPR, and ONCO-NMR. All the technical partners, who have developed involved an ONCOSCREEN tool, written the related SOP.

The SOP structure is divided in different sections. It specifies the appropriate matrix needed for each device, the protocol to perform the collection of the matrix, and its potential processing before to use the tool. It also describes and explains how to use it in an optimal way and finally how to store the samples. The ONCO-CTC, ONCO-CRISPR, and ONCO-NMR are each described by a single SOP. The ONCO-VOC is combining different documents to explain the device, the conditions of utilisation, the structure of the device and the recommendations to the patient. As all the four tools are new and innovative, SOPs are really helpful to give to clinicians the maximum of information for using them. Thanks to the development of these SOPs, the variations that can be observed due to differences in the tool utilization will be minimized and will guarantee the quality of values obtained at the end of the measurements. The prepared SOPs are presented in detail in Annex C of this deliverable.

6. Participant Information Sheet/Informed Consent Form/Consent Withdrawal Form

The Participant Information Sheet document (presented in Annex D) is written with the goal of informing the participants of the study about the CT. It is the process in which a health care provider educates a patient about the risks, benefits, and alternatives of a given procedure or intervention. Indeed, it is important that the patient must be competent to make a voluntary decision about whether to undergo the procedure. The IC is both an ethical and legal obligation of medical practitioners and originates from the patient's right to direct what happens to their body. Implicit in providing informed consent is an assessment of the patient's understanding, rendering an actual recommendation, and documentation of the process.

The following are the required elements for documentation of the informed consent discussion: (1) The nature of the procedure, (2) The risks and benefits and the procedure, (3) Reasonable alternatives, (4) Risks and benefits of alternatives, and (5) Assessment of the patient's understanding of elements 1 through 4. The Informed Consent (IC) is part of mandatory documentation for all clinical trials involving human beings. The consent process must respect the patient's ability to make decisions and adhere the individual hospital rules for CS (Shah, 2023).

The Consent Withdrawal Form is a document allowing participants study to withdraw their consent and to not be part of the study anymore. All data collected from them will not be used.

Specific sections about the General Data Protection Regulation (GDPR) explain to the study participants how the data generated during the study will be used, protected, and stored. This document was designed according to the Data Management Plan, that includes inputs from all the consortium partners.

Any updates discussed and agreed by the consortium will be communicated to the patients in advance and will be reported in the next D5.2. In case that significant updates are made it will be requested from patient to sign an amended section or a new consent form (see Annex D).

7. Case Report Form

The Case report form (CRF) is a printed, optical, or electronic document designed to record all protocol-required information on each subject in a clinical research study. The CRF facilitates the completed and standardized data collection that promotes efficient processing, analysis, and reporting of information, as well as exchange of data across sites and to the Sponsor/Principal Investigator/Data Coordinating Center. This document is filled for every subject by Clinical Research Associate (CRA)/Nurse based on information from the record file of the subject enrolled in the study and will be upload on an Electronic Data Capture (EDC) platform. The CRF is designed in order to reflect the protocol as it has been developed in this project. Among the data collected, there are measurable variables and medical condition of the subject. Then data will be exported from the EDC platform and be processed (see Annex E).

The platform provider of the EDC is carefully chosen by FIRALIS team. There was a need for a platform easy to use for end users such as data managers, principal investigators, and clinical research associates and providing an optimal way to conduct the clinical study monitoring. FIRALIS contacted 8 providers to find the best fit for the Phase A study and needs (see Annex E).

8. Trial KPIs

The following table depicts the description of KPIs that will be used for evaluating the CT. It is noted that the exact target will be decided and documented in the next iteration of phase A deliverable (D5.3).

Table 3: Description Key performance indicators that will be used for evaluating the success of the clinical trial.

KPI	KPI Metric	Means of verification
Appointment of Staff to Key Roles	Number of subjects/patients recruited per clinical center	Each site should have as a minimum a PI, a Clinical Project Manager, and a CRA/Nurse
Follow-up of clinical sites activation	Number of sites activated after 3 months	On site visit
Recruitment rate/clinical site	Number of patients enrolled	Monitoring through EDC platform
Highlighting sites underperforming recruitment	Set a threshold of patients to be enrolled/per month	Monitoring through EDC platform
Quality of the data relative to the patients in the eCRF	Low number queries	Monitoring through EDC platform
Modification of data justification	Audit Trail	Monitoring through EDC platform
Quantification of protocol deviation	Number of protocol deviation per site	Monitoring through EDC platform
Quality control of clinical sites	Quality markers	On site visit
Patient engagement	% withdrawing from intervention	Monitoring through EDC platform

9. Outcomes

This initiation package contains all the documents that are essential to inform the ethical committees about the ONCOSCREEN-CS, in order to obtain their approvals. As described in previous sections the CS involves various steps that need to be followed that we briefly describe next to better understand the current and future steps.

The work on the clinical study design and protocol started just after the ONCOSCREEN project was launched. This part was discussed iteratively during regular meetings with clinicians and other partners. From Month 2 (See Table 4), FIRALIS started to contact various EDC platform providers for requesting quotes and demos, narrowing the selection of the final provider. Then, the partners worked on the conception of the Patient Information Sheet and the Informed Consent forms (TIMELEX and UMC-MAINZ), the SOPs and the investigator brochure (technical partners, ICCS, UMC-MAINZ, and FIRALIS) starting from months 3.

With all these different steps, the expected outcome for this part is the submission of the CS initiation package to the local ethical committee of each clinical sites.

Following this step, as soon as the ethical committee approvals will be obtained, the implementation of the Phase A-CS in the clinical sites will be performed. This will allow the PIs to start recruitments of patients and healthy subjects.

Table 4: Gantt Chart for T5.1

WP 5 Tasks-Months	Leader	M1	M2	M3	M4	M5	M6	M7	M8
T5.1 Clinical Trials Design Implementation and Validation	**FIRALIS**								
Clinical Study design and protocol	FIRALIS								
EDC platform	FIRALIS								
Investigator Brochure	FIRALIS								
Participant Information Sheet/Informed Consent Form/Consent Withdrawal Form	FIRALIS								
SOPs	FIRALIS								

10. Next steps

Thanks to the work already done by the ONCOSCREEN partners, the Initiation Package is well progressed, reaching to an adequate maturity level, as it can be evidenced by the documents provided in the Annexes of this deliverable. However, there are some details which will be finalized in the forthcoming period in order to cover all aspects before the submission to the ethic committees. Currently, the consortium is actively working on finalising the last details of the various documents (*e.g.*, the section "Data analysis and statistical method" in the Clinical Study protocol), in order to obtain a high-quality Initiation Package and avoid follow up questioning and delays by the ethic committees. The consortium expects that the package will be completed by Month 9. The final package will be included in the next deliverable D5.3.

The next deliverables related to the WP5 are:

- The D5.3 (due on month 18) is the Midterm recruitment report related to the Phase A-CS, that will explain the progress of the recruitments performed among the clinical sites involved.

- The D5.2 (due on month 23) corresponds to the initial package related to Phase B-CS. As for the D5.1, the Initiation Package will include the Clinical Study Protocol, the CRF, the Informed Consent Form/Participant Information Sheet/Consent Withdrawal Form, and the Investigator Brochure.

Conclusion

This deliverable focuses on providing information about the implementation of the Clinical Trial Phase A. Thanks to a collective work, regular meetings with technical and clinical partners, as well as feedbacks and inputs from ONCOSCREEN partners, FIRALIS generated this initiation package. All documentation provided in this deliverable will be crucial for the beginning of the ONCOSCREEN-CS. Indeed, the clinical package a) Clinical Study, b) Investigator Brochure, c) SOPs, d) Patient Information Sheet and Informed Consent/Withdrawal Forms, e) CRF, needs to be approved following its submission to the various ethical committees. Once the CS is approved, the CT can be initiated in the clinical sites. The SOPs will help clinical team to use the ONCO-tools on an optimal way and the Investigator Brochure will provide all information needed by the health professionals to perform the CT under the best conditions.

Annex A: Clinical Study (BRC & Protocol)

This annex provides details about the objectives, design, and methodology of the clinical study protocol. It also includes the clinical study registration number or Biological Research and Collection (BRC) number, obtained with French ANSM Agency.

ONCOSCREEN Clinical Study

Multicentre observational study for the data collection, development and evaluation of the performance of novel CRC screening and diagnostic methods- PHASE A

Sponsor Study ID:

IDRCB no:

Version:

Version date:

INVESTIGATOR SIGNATURE PAGE

Protocol Title: Multicenter observational study for the data collection, development and evaluation of the performance of novel CRC screening and diagnostic methods- PHASE A

Protocol Version and date:

PROTOCOL SYNOPSIS

Study Title	Multicentre observational study for the data collection, development and evaluation of the performance of novel CRC screening and diagnostic methods- PHASE A (ONCOSCREEN-CS-Phase A)
Clinical Investigation Centers/Departments	To be defined.
Principal Investigator(s)	To be defined.
Objectives	**Primary Objective** To identify the different expression patterns of Oncoscreen diagnostic tools (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) in CRC patients and healthy controls. **Secondary objectives** 1. To estimate the sensitivity and specificity of the Oncoscreen diagnostic tools (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) when comparing CRC patients to Healthy Controls 2. To evaluate the performance of the Oncoscreen tools for CRC screening in individuals at high risk for development of colorectal cancer 3. To gather Colonoscopy and Tissue Images data from 50 CRC recognized patients that will allow the training of junior endoscopists to validate the Algorithms retrospectively and not during the coloscopy procedures
Study Design	The ONCOSCREEN Phase A clinical study is a multi-center case-control prospective study for the collection of data that will develop and finalize the ONCOSCREEN screening and diagnostic solutions.

Target Population	The ONCOSCREEN Phase A clinical study aims to enroll male and female adults (at least 18 years old) that are of average/high risk of colon cancer as defined by the study eligibility criteria
Study Endpoint(s)	**Primary Endpoint** The primary endpoint of this study is the differential expression patterns of Oncoscreen tools in CRC patients and healthy controls using colonoscopy as the reference method. **Secondary Endpoint(s)** • The specificity of the Oncoscreen tools when screening for CRC in high-risk population using colonoscopy and FIT as the reference methods. • The sensitivity of the Oncoscreen tools when screening for CRC in high-risk population using colonoscopy and FIT as the reference methods.
Eligibility criteria	To be defined.
Number of participants	To be defined.
Study duration	To be defined.

BACKGROUND & RATIONALE

Colorectal Cancer (CRC) is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women[1]. CRC can often be prevented through regular screening. Although colonoscopy-based screening programs result in a significant decrease in CRC incidence, the compliance rate of the people to perform the required screening is still too low and not the desired one[2]. Fecal Immunohistochemical Tests (FITs) is another widely used screening modality for CRC but has certain constrains to use, such as relatively low sensitivity and the need of multiple sampling (three) to reach the full screening potential[3]. The development of novel, more practical screening methods can effectively increase the screening rates for CRC through non-invasive, repeatable, cost-effective, easy-to-use, and patient-friendly procedures. This is of particular importance in different European societies and population subgroups, since an increase in the incidence of Early-Onset CRC is currently noticed which is significantly associated with risk factors such as heredity, obesity, smoking, alcohol abuse and hyperlipidemia [4,5]. Within this framework, ONCOSCREEN will develop a multi-tier diagnostic solution towards an improved CRC screening. Furthermore, ONCOSCREEN will consider specific socio-economic determinants which increase the regional or national CRC risks, thus exploiting new solutions, particularly in younger high-risk individuals in our European communities. For the validation of the developed solution, a clinical validation study (titled "ONCOSCREEN-CS") will be conducted, to assess its effectiveness, sensitivity and specificity in detecting CRC at an early stage. During the first phase of the study (**ONCOSCREEN-CS-Phase A)**, we will identify the different expression patterns of the four diagnostic solutions (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) in CRC patients and healthy controls with high risk for CRC, and also initially estimate their sensitivity and specificity. During the second phase (**ONCOSCREEN-CS-Phase B)** the ONCOSCREEN solution will be clinically validated.

Within the **ONCOSCREEN-CS-Phase A**, a sub-study will be conducted aiming to train Non-Experts/Junior Colonoscopists and Junior Histopathologists. The rationale for the sub-study is provided below.

Rationale of the sub-study (please also check 1.3.1.5 and 6): In a large study[6] that examined more than 15,000 tandem colonoscopies and 10,000 adenomas, the miss rate for polyps was 28% and adenomas 26%. According to the European guidelines for colonoscopy in general (not only screening) have defined an overall aim of 40% Polyp Detection Rate (PDR) and 25% Adenoma Detection Rate ADR[7]. in a comparison study between Junior and Senior colonoscopists for a Pre-intervention group of patients, the detection rate of adenomas for the junior colonoscopists was 11.57% compared to 16.2% of the senior colonoscopists having a statistically significant difference[8]. While the impact of the missed lesions during the colonoscopy are responsible for 50%–60% of interval cancers[9]. demand for screening colonoscopy has additionally continued to rise over the past two decades. As a result, the current workforce of gastroenterologists is unable to meet the needs for CRC screening. Therefore, alternative solutions are needed to improve

this disparity in the long term, with non-physician endoscopists (like Nurse Practitioners) being a potential option. There were two studies in which trained nurse practitioners conducted colonoscopies, with mean ADR 35.6% in 1012 subjects and 26.7 % in 528 subjects respectively.

Within ONCOSCREEN with the use of explainable AI powered by open source Deep Neural Networks and automated medical image segmentation models, will automatically suggest potential areas of interest "explaining" the confidence level, the classification type and other meta-data information (algorithm, training dataset). At the end of the session, the ONCOSCREEN Real Time AI-Assisted Colonoscopy module will automatically calculate the correctly classified information and will show an individual risk score. The selected areas of pathological interest will be stored and asynchronously expert colonoscopists can annotate as correctly classified or not.

A biopsy consists out of several hundred glands. During the diagnostic process, a pathologist has to identify the dysplastic glands (one dysplastic gland can change the complete diagnoses. These images are also playing a major role in the pathological diagnosis process. The analysis of Whole slide images (WSI) provides pathologists with a thorough insight into the data content and enables accurate diagnosis of tumours and cancer sub-types. However, the evaluation of WSIs for tissue classification is easily affected by many subjective factors. Such as:

a) training b) experience c) evaluation condition or d) time pressure for each pathologist that could result to different diagnosis judgement. Deep learning applied to WSIs has the potential to generate new clinical tools that are more accurate, reproducible, and objective than current clinical techniques while also delivering fresh insights into various pathologies. WSIs, on the other hand, are multi-gigabyte images with typical resolutions of 100, 000 x 100, 000 pixels, substantial morphological diversity, and a variety of artifacts. Practitioners are confronted with two significant difficulties. On the one hand, the visual understanding of the images, which is hampered by morphological variance, artifacts, and typically small data sets, and on the other hand, the current state of the hardware's inability to facilitate learning from images with such high resolution, necessitating the use of artificial intelligence and Deep Neural Networks[10,11,12]. ONCOSCREEN will provide the AI-Assisted Tissue Image Analysis for supporting pathologists in accurate and timely classification of early stage CRC histopathological images.

OBJECTIVES

Primary Objective

To identify the different expression patterns of Oncoscreen diagnostic tools (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) in CRC patients and healthy controls.

Secondary objectives:

1. To estimate the sensitivity and specificity of the Oncoscreen diagnostic tools (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) when comparing CRC patients to Healthy Controls
2. To evaluate the performance of the Oncoscreen tools for CRC screening in individuals at high risk for development of colorectal cancer
3. To gather Colonoscopy and Tissue Images data from 50 CRC recognized patients that will allow the training of junior endoscopists to validate the Algorithms retrospectively and not during the coloscopy procedures

ENDPOINTS

Primary Endpoint

The primary endpoint of this study is the differential expression patterns of Oncoscreen tools in CRC patients and healthy controls using colonoscopy as the reference method.

Secondary Endpoint(s)

- The specificity of the Oncoscreen tools when screening for CRC in high-risk population using colonoscopy and FIT as the reference methods.

- The sensitivity of the Oncoscreen tools when screening for CRC in high-risk population using colonoscopy and FIT as the reference methods.

STUDY DESIGN AND PROCEDURES

The ONCOSCREEN Phase A clinical study is a multi-center case-control prospective study for the collection of data that will develop and finalize the ONCOSCREEN screening and diagnostic solutions.

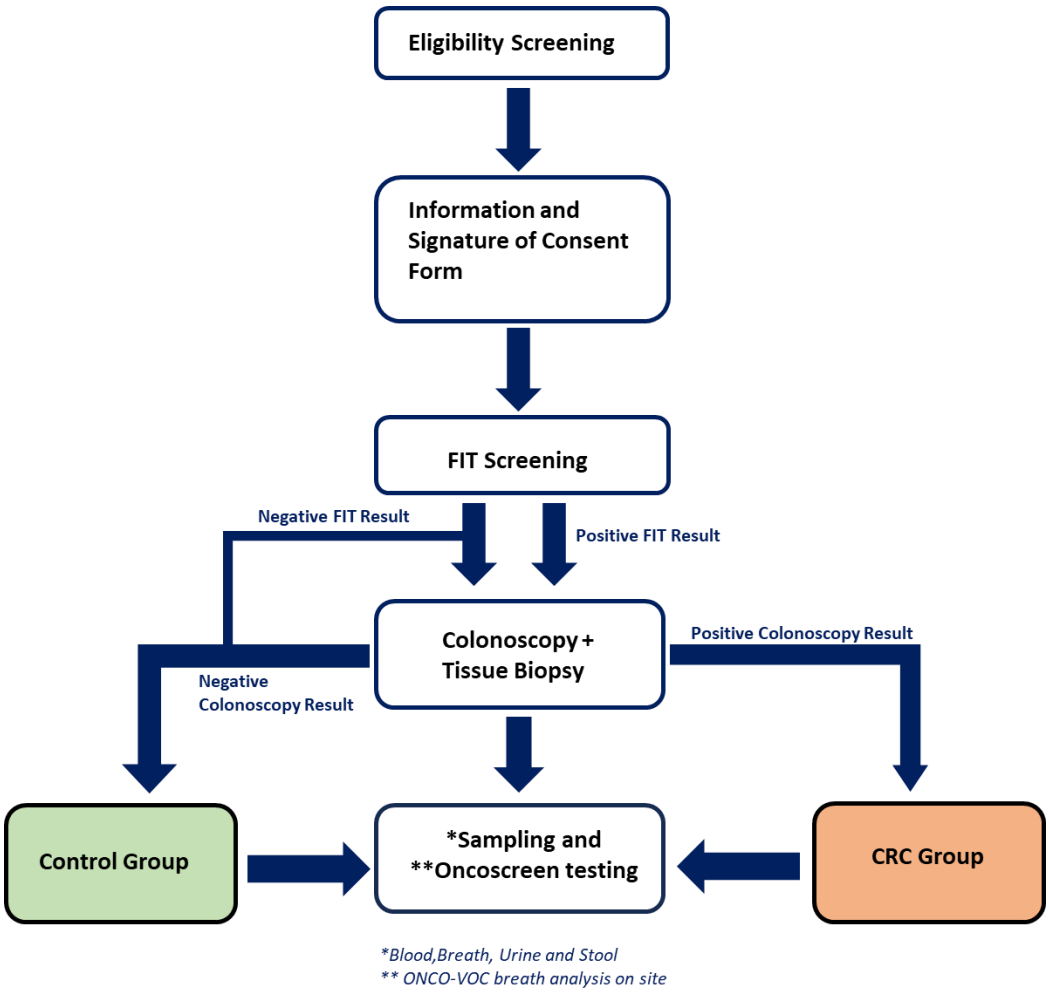


Figure 1 ONCOSCREEN PHASE A Study Flow chart

Participants will be screened for study eligibility and informed about the study by the investigator. Subjects who consent to participate will be enrolled into the study and screened using a FIT test. Participants who return a positive FIT test will be invited for a mandatory screening colonoscopy. Participants with a negative FIT test will also be invited to a facultative screening colonoscopy. Participants with a positive colonoscopy result will be assigned into the cases group (CRC Patient), while those with a negative colonoscopy result will be assigned into the control group (see Subject enrollment manual annex). Participants who returned a FIT negative test and declined to undergo a

colonoscopy will be assigned to the control group. All subjects enrolled into the study will be required to provide biological specimen samples (stool, blood, urine and breath as detailed in table 1) for testing using the Oncoscreen tools (see sampling and testing manual annex (to be defined)x). Additionally, samples will be archived for use in further developing the Oncoscreen tools and for additional CRC related research. Corresponding clinical data will be collected for analysis using a case report form.

Type of tube	Sample	Qte	Volume
PAXgene® DNA tubes	Blood	1	8.5ml
PAXgene® RNA tubes	Blood	1	2.5ml
cell-free dna bct	Blood	2	20.0 ml
tube S-Monovette K3 EDTA	Blood	1	9.0 ml
tube S-Monovette Serum	Blood	1	9.0 ml
total blood volume	**49.0 ml**		
Tenax tubes	breath	1	250.0ml
total breath volume	**250.0 ml**		
Faeces tubes 107x25mm	Faeces	1	
total Faecal volume			

Table 5 Oncoscreen Phase A Biological samples

The results of the ONCOSCREEN tools will be compared with the results of the colonoscopy and of the FIT test to evaluate diagnostic and screening performances (initial assessment of sensitivity and specificity and other KPIs). Testing of subject's specimens will be blinded to all subject clinical information including the colonoscopy and FIT results. Investigators, technicians and subjects participating in the study will be blinded to ONCOSCREEN tools test results, and the results of the tests will not be used for clinical management of study participants.

These data from the colonoscopy and the tissue biopsies will be used to validate the AI algorithms and test it on junior experts in endoscopy and histopathology, RETROSPECTIVELY and not during colonoscopy session. This study will provide impactful data on the diagnostic potential of the ONCOSCREEN proposed solutions with the aim to improve the CRC screening in our societies, especially for high-risk population who are younger and not yet included in the current screening guidelines.

Biological specimens collected within the study will be sent to the Oncoscreen partner laboratories for testing and use in further developing other CRC related diagnostic and screening tools.

The duration of the study participation for each enrolled participant will be approximately (to be defined) days from enrollment into the study.

The total duration of the study from the first participant first visit to last participant last visit is estimated to be (to be defined) months.

Study procedure

Study participants, both patients with CRC and healthy volunteers will undergo screening assessments which will be scheduled and conducted according to local site standards of care

procedures. The assessments to be performed during the screening visit (Visit 0) and the testing visit (visit 1) are summarized in table 2. Visit 1 should be completed within 28 days of completing the FIT screening test.

	Visit 0 Screening	Visit 1 Testing (D1-D28)
Informed Consent Form	X	
Demographic data	X	
Risk factors	X	
Preexisting conditions / Medical history / Relevant surgical history	X	
Inclusion/ Exclusion Criteria	X	
Concomitant medication	X	
Physical evaluation		
Height	X	
Weight	X	
Vital signs (Pulse, BP, T)	X	
Physical exam	X	
Non-invasive tests		
Breath Test		X
Urine and stool sample		X
Blood sample		X
FIT screening test	X	
Invasive tests		
Colonoscopy (Endoscopy / SES- CD with biopsies)		X
Questionnaires		
Health Professional questionnaire		X
Study Participants questionnaire		X

Table 6 Oncoscreen Phase A summary of assessments

STUDY DEVICES

ONCO-VOC

The ONCO-VOC is a screening test based on Volatile Organic Compounds (VOC). VOC analysis devices available today in the market, are mainly based on various derivatives of mass spectrometry, are fairly large, only semi-portable, inaccurate, and affected by humidity and other confounding factors. Low- cost miniaturized devices are either based on the detection a single biomarker in breath or, they do not show sufficient sensitivity and specificity for a wide population CRC screening program. The innovative breath analyzer, developed by TECHNION is based on sensor array of nanomaterials in conjugation with AI.

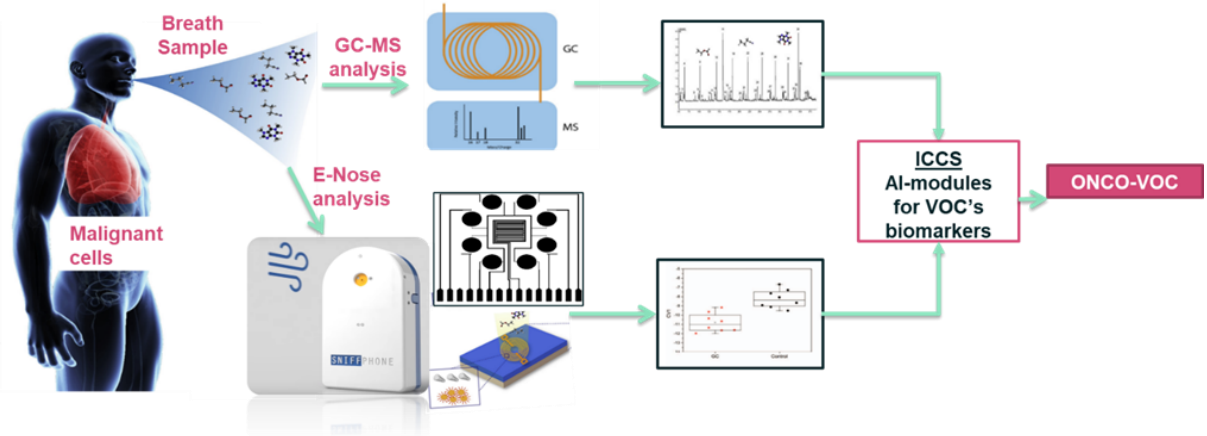


Figure 2 ONCO-VOC testing workflow

To perform the test (see the ONCO-VOC testing instructions annex (to be defined)), a short-exhaled breath sample (2-3sec.) will be evaluated in less than 2 minutes by the ONCO-VOC breath analyzer that includes 8 nano-sensors, in an array format. These sensors are cross-reactive towards the VOCs adsorbed on each of the sensors in the array and change their electrical properties. Analysis of the electro-chemical signal responses with pattern recognition algorithms, will give early indication for distinguishing between CRC-linked and healthy categories.

CRC investigation in 160 samples have shown 85% sensitivity, 94% specificity.

ONCO-CRISPR

The ONCO-CRISP is a screening test based on CRISPR-Cas9 biomarkers. The ONCO-CRISPR tool and human CRISPR microRNAs will enable the detection of clinically relevant biomarkers in colorectal adenomas, colorectal carcinomas in tissue and non- or minimal invasive obtained patient samples.

The test consists of a detection dipstick, a reporter, a Cas protein (nuclease, DNA/RNA cutting enzyme) and a guiding RNA, primers, isothermal amplification enzymes and input material that could be RNA or DNA that contains the biomarker of interest. The test will show a negative or a positive result on a used test strip. Testing will be done according to the testing instructions (annex (to be defined)).

Figure 3 Example of the ONCO-CRISPR tool on the left and a hCRISPR biomarker on the right (red is positive)

The tool addresses CRC testing needs by increasing accuracy (sensitivity >0.90 specificity >0.90), affordable/user-friendly tools, quick results in less than two hours, detection of early-stage CRC, equipment free and deliverable to the end-users. Patients will benefit from the ONCO-CRISPR tool which uses a minimally invasive procedure for obtaining a correct diagnosis.

ONCO-NMR

NMR is a highly robust method with excellent reproducibility and minimal variation between measurements. It has been used to study cancer samples in many studies and has been shown to provide good signatures for cancer and cancer progression. The method has been utilized extensively in other cancer settings, in particular for breast cancer, and recently also for colorectal cancer *Vignoli, A. et al. Exploring Serum NMR-Based Metabolomic Fingerprint of Colorectal Cancer Patients: Effects of Surgery and Possible Associations with Cancer Relapse. Applied Sciences 11, 11120 (2021).* UzL has developed a standardized NMR test for the analysis of blood serum and plasma samples. NMR metabolomics provides approx 40 metabolic parameters, ca. 100 detailed lipoprotein parameters. The test is a highly robust with excellent reproducibility and minimal variation between measurements. Sample testing will be performed according to the instructions..

ONCO-CTCs

CTCs as epithelial cancer cells are able to move, migrate and invade blood vessels after epithelial-mesenchymal transition (EMT) and have been characterized as the main causal factor of tumor metastasis mediation. Compared to other cancer biomarkers, CTCs contain molecular and biological information about the tumor as a whole, supporting single cell analysis and ongoing changes in tumors at all stages. At the same time, phenotypic and molecular characteristics of CTCs, can reveal the mechanism of

pathogenesis and metastasis of CRC and identify specific mutations in target genes. In contrary with the conventional theory that the metastatic dissemination of cancer cells represents the final stage of a deteriorating process, it has been found that CTCs often disseminate at the early stages during the process of tumorigenesis, invading distant organs and eventually developing into overt metastatic lesions. Therefore, the detection of CTCs in the circulation may be proved a feasible way to improve the early diagnosis and treatment of patients with CRC prior to metastasis.

CTCs can be found in blood as independent cells, and clusters of both CTCs alone and cells aggregates comprising of neutrophils, platelets, and CTCs. Thus, blood-based analysis of CTCs could therefore function as a "liquid biopsy," allowing repeated sampling.

ONCO-CTC is a biofluidgnostics platform for early-diagnose CRC and treatment personalization being developed by UMINHO. UMINHO will use a well-established photolithography technology and will explore the different designs for microfluidics fabrication, for focusing on the detection of isolated CTCs that are usually formed at twice the rate of CTC clusters.

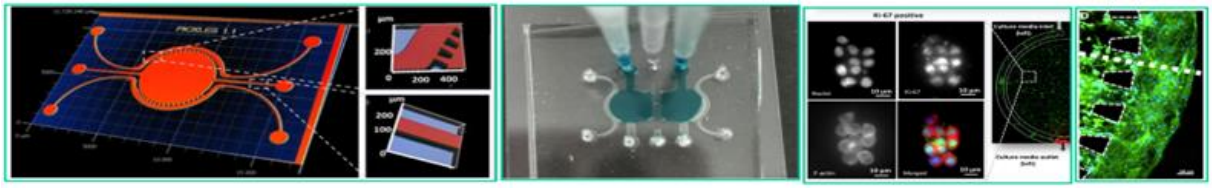


Figure 4 CRC Tumour-on-a-chip published model of UMINHO.

CRC cancer CTCs have a diameter less than 10 μm and a cross-sectional area of 40–65 μm2, similar to white blood cells. The proposed ONCO-CTC chip platform is based on size exclusion isolation and detection of CTCs. The ONCO-CTC tool will consist of costly-effective CRC diagnostic chips/test kit. It will provide qualitative and quantitative information across the cancer continuum (initiation, progression, metastasis and relapse), not only providing indication/detection insights, but also classification capabilities within a period of 15-60 minutes.

STUDY POPULATION AND SAMPLE SIZE

Study Population

The ONCOSCREEN Phase A clinical study aims to enroll male and female adults (at least 18 years old) that are of average/high risk of colon cancer as defined by the study inclusion and exclusion criteria.

Inclusion Criteria

For the Patient Group

1. Signature of the informed consent indicating that the subject accepts to participate in the study and to comply with the requirements and restrictions inherent in this study.
2. Male or female subjects aged ≥18 years
3. Subject has undergone a FIT screening test
4. Subject is diagnosed with CRC by colonoscopy and tissue biopsy
5. CRC is at stage considered well resectable
6. Subject is able to comply with all study procedures
7. Covered by a Health Insurance System

For the healthy control group

1. Signature of the informed consent indicating that the subject accepts to participate in the study and to comply with the requirements and restrictions inherent in this study
2. Male or female subjects aged ≥18 years
3. Subject has undergone a FIT screening test
4. Otherwise healthy individuals with recognized risk factors for CRC development defined by either heredity and/or obesity and/or smoking and/or excess alcohol consumption and/or hyperlipidemia.

Exclusion Criteria

For the Patient Group

1. Legal incapacity or limited legal capacity
2. Subject did not sign the Informed Consent form
3. Subject has previous history of CRC surgery
4. Subject has history of other cancer types
5. Subject received treatment by chemotherapy or other targeted oncological treatments
6. Subject who, according to the investigator's assessment, presents with an unstable medical condition contraindicating the performance of the planned blood test, stool test or breath test
7. Pregnancy and/or breastfeeding

For the healthy control group

1. Legal incapacity or limited legal capacity
2. Subjects who did not sign the Informed Consent form
3. Subject is diagnosed with CRC by colonoscopy and tissue biopsy
4. Previous history of cancer (any type)
5. Presence of any relevant organic, systemic or metabolic disease
6. Gastrointestinal disorders or other serious acute or chronic diseases
7. Participation in another interventional study
8. Pregnancy and/or breastfeeding

9. Known drug and/or alcohol abuse
10. Using any form of nicotine or tobacco

Sample size

For the power calculation a medium incidence rate between the countries was chosen. Whereby, the country differences in incidences as well as on the sensitivity or specificity can be still be analyzed based on the current consortium. Sample size calculation was performed on the basis that sensitivity and specificity for FIT and colonoscopy were assessed in a 95% CI +/- .05 around the true value, i.e., if the sensitivity would be .90, it would lie between .85 and .95. For the FIT N = 370 patients and N = 365 healthy controls and for the colorectal test a minimal number of N = 148 cancer patients and N = 869 healthy controls would need to be screened (Buderer, 1996). Based on the higher incidences due to red meat or living in Hungary an incidence of 50% was chosen which led to a necessary power for the FIT N = 592 patients and N = 146 healthy controls with high risk.

The power calculations are based on: Buderer, N, M. F. (1996). Statistical methodology: I. Incorporating the Prevalence of Disease into the Sample Size Calculation for Sensitivity and Specificity. Academic Emergency medicine, 3, 9, p 895-900. For this approach of the power calculation the 95% CI +/- .05 around the true values, the proposed sensitivity between .85 and .95 and the incidences was included. Hereby the well-known risk factors of heritability, smoking, obesity as well as live style of the different countries were chosen for the power estimation.

DATA COLLECTION

The study participants who are interested in participating in the study will sign an informed consent. Once the consent is signed data such as demographics and medical history will be obtained and recorded into the CRF . An electronic version of the CRF will be used for harmonized data collection and validation across all clinical sites. The informed consent forms signed by the participants are retained by the respective clinical center, and the date of obtaining it will be recorded in the CRF. Anonymized subject data will be collected on CRFs filled in by the investigator team at each clinical site and sent to the sponsor. The clinical site will send a copy of available colonoscopy reports and applicable histopathology reports and/or other diagnostic information to the Sponsor. All the reports that are sent to the sponsor will be redacted to mask the personal information of the participants prior to being sent to the sponsor. All the data managed on this project will be anonymous.

END OF STUDY

Study end will be determined as defined in the protocol when the last participant completes the last visit and examinations. Subject will be considered completed from the

study when they have fulfilled all the visits and examinations required by the protocol or till the point of their withdrawal.

SAMPLE RETENTION

Samples collected for the study will be retained to develop and evaluate the performance of biomarker assays to detect cancers as per the protocol. Retained samples will be anonymized of all protected health information, and clinical study data will only be associated by a subject identification number. Samples will be stored for no more than 20 years in the on-site biorepository and will be used for developing and evaluating the performance of biomarker assays for cancer detection. Specimens from the biorepository will be destroyed under standard biohazardous material protocol and record of the removal and destruction will be maintained for 7 years by the Sponsor. Retained subject samples will not be used for any other purposes.

ADVERSE EVENT REPORTING

An adverse event (AE) is any unfavorable or unintended sign, symptom, or disease temporally associated with the use of an investigational product or protocol-imposed intervention, regardless of attribution. Adverse events commonly associated with colonoscopy will not be collected. Such events include: Abdominal discomfort, bowel irregularity, bleeding, intestinal perforation, and adverse reaction to the sedation.
Surgical intervention, such as, but not limited to, colectomy and sub-mucosal resection are part of routine patient care for subjects with CRC or AA; therefore, adverse events commonly associated with surgeries will not be collected. Such events include: Bleeding, blood clots in the legs (deep vein thrombosis) and lungs (pulmonary embolism); infection; injury to organs near the colon, such as the bladder and small intestines; and tears in the sutures that reconnect the remaining parts of the digestive system.

QUALITY CONTROL AND QUALITY ASSURANCE

CRO and relevant laboratory partners, will implement and maintain quality assurance and quality control systems with written standard operating procedures (SOPs), to ensure that all laboratory and clinical data are generated and recorded in accordance with the protocol.

The activities associated with the data management of this study will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Study investigators/sites will enter data directly into an electronic data capture system by completing the eCRF via a secure internet connection. Data entered into the eCRF must be verifiable against source documents at the study site. Any changes to the data entered into the electronic data capture system will be recorded in the audit trail.

Site initiation visits, site interim monitoring visits and site close-out visits will be performed in-line with the ICH GCP principles directly on site or remotely. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Authorized CRAs will verify that the clinical trial is conducted, and data are generated, documented and reported in compliance with the protocol, GCP and the applicable ethics and regulatory requirements.

To ensure compliance with Declaration of Helsinki, Good Clinical practice, applicable Regulatory and Ethics requirements, sponsor may conduct a quality assurance audit. Additionally, an authorized representative of the Independent Ethics Committees, and Regulatory Authority eventually may visit the site to perform audits or inspections. The purpose of an audit or inspection is to independently examine all study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed and accurately reported according to the protocol, ICH GCP and any applicable ethics and regulatory requirements. The Investigator should contact the CRO immediately if contacted by Ethics Committee or Regulatory Authority about an audit or inspection.

DATA HANDLING AND RECORD KEEPING

Data confidentiality

The participants will be given a study identification that ensures anonymization of the participant and thus no information to identify the participants will be included. Only coded data will be transferred to third parties and other countries, which in no case will contain information that can directly identify the participant (such as name and surname, initials, address, social security number, etc.). If this transfer occurred, it would be only for the study and guaranteeing confidentiality. Study promoter will comply with the EU Regulation 2016/679 to process and protect the data and will keep a record of all the treatment activities that are carried out.

Any participant can limit the processing of incorrect data, request a copy or that the data provided for the study can be transferred to a third party (portability). To exercise these rights, participant should contact Principal Investigator of the study or the Data Protection Officer of the clinical site. Moreover, participant has the right to contact the Data Protection Agency if he/she is not satisfied.

All the participants reserve the right to withdraw from the participation at any time. In such cases data already collected is not removed but no new data will be collected.

Record keeping

The data collected for the study will be kept for 10 years after the study completion. Subsequently, personal information will only be kept by the clinical center for participant health care, and by the promoter for other scientific research purposes if the participant has given its consent to do so and if this is allowed by the law and applicable ethical requirements.

Protocol deviations

The site should make all efforts not to deviate from any of the study procedures or requirements as described in this protocol, except where necessary to eliminate immediate risks to the subject(s). Any deviations from this protocol must be reported to the Sponsor and documented properly.

Source documents

Investigator sites must maintain proper record of all study documents, medical records, examination reports and other medical reports. The clinical sites must permit trial-related monitoring, audits, EC reviews, and regulatory inspection(s) by providing direct access to source data/documents. Only authorized and trained personnel will have access to the CRF and study related documents.

ETHICS

1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended 64th WMA General Assembly, Fortaleza, Brazil, October 2013).

2. ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice E6 (R2), and other relevant local legislation.

3. Independent Ethical Committee (IEC)

The protocol, informed consent and any accompanying material provided to the patient will be submitted by the investigator to an Independent Ethical Committee for review. Approval from the committee must be obtained before starting the study. Any modifications made to the protocol, informed consent or material provided to the patient after receipt of the Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements. The IEC approval report must contain details of the trial (title, protocol number and version), documents evaluated (protocol, informed consent material) and the date of the approval.

4. Medical/Clinical Safety standards; Incidental findings

An incidental and unexpected findings policy will be prepared.

Additionally, should any such findings occur, they will be evaluated by the clinical team on a case-by-case basis and reported if necessary.

The reporting of the incidental findings will be done based on the nature of these findings. Those individual incidental findings that are not regarded as adverse events or are mild adverse events will be solved according to usual clinical practice standards; they will be addressed within the clinical setting and reported to monitoring responsible personnel. Serious incidental findings or those considered as severe adverse events will be reported to the trial committee, according to the regulatory rules.

Incidental findings will be addressed according to the standards governing Good Clinical Practice Regulation.

5. Informed Consent

It is the responsibility of the Investigator to obtain written informed consent from each subject prior to entering the trial or, where relevant, prior to evaluating the subject's suitability for the study. The informed consent document used by the Investigator for obtaining the subject's informed consent must be reviewed and approved by the IEC. All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he will be exposed, and the mechanism of treatment allocation. In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki. Information will be given in lay terms and under no circumstances will pressure of any kind be exerted either on the individual participant or her/his family. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered for the study. This must be done in accordance with the national and local regulatory requirements. The written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative; if the person concerned is unable to write, oral consent in the presence of at least one witness may be given in exceptional cases, as provided for under national legislation. The clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study. A copy of the signed informed Consent Form must be provided to the patient or the patient's legally authorized representative. The original copy of the patient's signed written consent will be kept by the centre in the proper section of the Investigator Site File and must be available for verification by study monitors at any time. Consent will be a continuing process, researchers will foster a continuous dialogue with study participants and inform them of anything new related to the trial.

The European Commission - Research Directorate-General provides guidance on informed consent in the context of clinical trials and this guidance will be respected http://ec.europa.eu/research/participants/data/ref/fp7/89807/informed-consent_en.pdf.

6. Patient data protection and procedures regarding patient information and obtaining consent

The consent to the participation in a research study should be clearly distinguished from the consent to the processing of personal data (within the meaning of the GDPR), the latter being a separate legal requirement. Obtaining one does not waive the obligation to collect the other. Therefore, the Informed Consent Form will incorporate wording that complies with the data protection and privacy legislation (GDPR). In agreement with this wording, patients will authorize the collection, processing (use) and disclosure of their study data by the Investigator and by those who need that information for the purposes of the study (consent of the study participants will constitute the required legal basis for the

processing of their personal). Moreover, the informed consent form (or a document attached thereto) will provide the study participants with the following information as required under the GDPR:

- identity and contact detail of the data controller/joint controllers (and – if applicable - their representatives; DPOs – if appointed);
- the source from which the data originated (if collected not from the data subject himself) and if applicable, whether it came from publicly accessible sources;
- the purpose of processing;
- the legal basis for processing (such as e.g. consent);
- the categories of recipients of data (if any; e.g. external vendors/processors/OncoScreen partners);
- in cases where the controller/joint controllers intend to transfer personal data outside the EEA, justification for such transfer along with the reference to appropriate safeguards and the means to obtain access to or a copy of those;
- data storage duration, or criteria used to determine such storage duration;
- data subjects' rights and how to exercise them;
- the right to withdraw consent (where consent was the basis for processing) and that such withdrawal will not affect the lawfulness of processing based on consent that took place before its withdrawal;
- the right to lodge a complaint with a competent supervisory authority;
- the existence of profiling including meaningful information about the logic involved, as well as the significance and the envisaged consequences of such processing for the data subject;
- the possibility that the data will be further processed for scientific research purposes.

As already explained above, the above information must – in compliance with the transparency principle – be concise, transparent, intelligible and easily accessible in order to avoid information fatigue for the data subjects, who must consider many elements, sometimes fairly technical.

The Informed Consent Form will explain that how the study data will be stored, maintaining confidentiality in accordance with the European and national data legislation.

The Informed Consent Form will also explain that for data verification purposes, authorized representatives of Sponsor/Promoter, a regulatory authority, an Ethics Committee may require direct access to parts of the hospital or practice records relevant to the study, including patients' medical history.

REFERENCES

- Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89–103.
- Kwack WG, Lim YJ. Current Status and Research into Overcoming Limitations of Capsule Endoscopy. Clin Endosc. 2016 Jan;49(1):8–15.
- Lee JK, Liles EG, Bent S, Levin TR, Corley DA. Accuracy of fecal immunochemical tests for colorectal cancer: systematic review and meta-analysis. *Ann Intern Med*. 2014;160(3):171. doi:10.7326/M13-1484
- O'Sullivan DE, Sutherland RL, Town S, Chow K, Fan J, Forbes N, Heitman SJ, Hilsden RJ, Brenner DR. Risk Factors for Early-Onset Colorectal Cancer: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2021 Jan 29:S1542-3565(21)00087-2.
- Patel SG, Karlitz JJ, Yen T, Lieu CH, Boland CR. The rising tide of early-onset colorectal cancer: a comprehensive review of epidemiology, clinical features, biology, risk factors, prevention, and early detection. Lancet Gastroenterol Hepatol. 2022 Mar;7(3):262-274.
- Zhao S, Wang S, Pan P, Xia T, Chang X, Yang X, Guo L, Meng Q, Yang F, Qian W, Xu Z, Wang Y, Wang Z, Gu L, Wang R, Jia F, Yao J, Li Z, Bai Y. Magnitude, Risk Factors, and Factors Associated With Adenoma Miss Rate of Tandem Colonoscopy: A Systematic Review and Meta-analysis. Gastroenterology. 2019 May;156(6):1661-1674.e11. doi: 10.1053/j.gastro.2019.01.260. Epub 2019 Feb 6. PMID: 30738046.
- Kaminski M F, et al.Performance measures for lower gastrointestinal endoscopy: a European Society of Gastrointestinal Endoscopy (ESGE) Quality Improvement Initiative. Endoscopy. 2017;49:378–397
- Deng, B., et. Al., (2016). The Impact of Colonoscopy Quality Control Table on Adenoma Detection Rates. Gastroenterology research and practice, 2016, 2548109. https://doi.org/10.1155/2016/2548109
- Robertson D.J., et Al., Colorectal cancers soon after colonoscopy: a pooled multicohort analysis. Gut. 2014; 63: 949-956
- Dimitriou N, Arandjelovi´c O and Caie PD (2019) Deep Learning for Whole Slide Image Analysis: An Overview. Front. Med. 6:264. doi: 10.3389/fmed.2019.00264
- BenTaieb A, Ghassan Hamarneh G. Predicting cancer with a recurrent visual attention model for histopathology images. In: Frangi AF, Schnabel JA, Davatzikos C, Alberola-López C, Fichtinger G, editiors. Medical Image Computing and ComputerAssisted Intervention. Vol. 11071. Springer International Publishing (2018). p. 129–37. Available online at: https://link. springer.com/book/10.1007/978-3-030-00934-2
- Tellez D, Litjens G, van der Laak J, Ciompi F. Neural image compression for gigapixel histopathology image analysis. arXiv. (2018). doi: 10.1109/TPAMI.2019.2936841

Agence nationale
de sécurité du médicament
et des produits de santé

REGISTER FORM ON
BIOLOGICAL RESEARCH AND COLLECTIONS (BRC)

Date : 10/08/2023

1. INFORMATION ON THE APPLICANT

Applicant : Firalis
(Person or entity)

Category : Commercial

Address : Firalis-35 rue du Fort

City : Huningue

zip code : 68330

Country : France

Siret : 50494436400018

Contact name : Mrs Céline Meyer

EMail : celine.meyer@firalis.com

Telephone : 0389911320

Fax : 0389911320

2. INFORMATION ON THE FILE

Title of biological research

ONCOSCREEN- A European "shield" against colorectal cancer based on novel, more precise and affordable risk-based screening methods and viable policy pathways

BRC Identification number : 2023-A01793-42

Type of BRC : Medical device or Medical device In vitro diagnostic

Type of file : Initial application - Other cases

Annex B: Investigator Brochure

The Investigator Brochure (IB) is a compilation of the current clinical and non-clinical information on the investigational medical devices (ONCO-Tools), relevant for the clinical investigation.

Investigator Brochure

ID RCB Number: 2023-A01793-42

STUDY Number:
Study Title:

Multicentre observational study for the data collection, development and evaluation of the performance of novel CRC screening and diagnostic methods.

Document type	Investigator Brochure
Author	Dr.Céline Meyer PhD
Total number of pages	22
Project	ONCOSCREEN

Version Number	1.0
Date	August 30 2023

Signature page for the Sponsor UMC-MAINZ:

SPONSOR Representative

Address:
Johannes Gutenberg-University Clinic, Mainz, Germany
1.Dept. Medicine, Prof. Peter R. Galle
Langenbeckstraße 1, 55131 Mainz

Tel: +49 (0) 6131-176076 **Fax:** +49 (0) 6131-173438

E-mail: markus.moehler@unimedizin-mainz.de

Name: Prof .Markus Mohler MD, PhD

Date: Signature:

CONFIDENTIALITY

This investigator brochure (IB) document is the property of the sponsor UMC Mainz. It shall not be divulged, published or otherwise disclosed without the written consent of ONCOSCREEN consortium.

It describes confidential results and information compiled on the clinical data on the ONCOSCREEN consortium technical partners investigational IVD product(s) that are relevant to the study of these product(s) in human subjects. Its purpose is to provide the investigators involved in the clinical studies on such IVD products with the information to facilitate their understanding of the rationale for, and their compliance with, many key features of the protocol and related procedures. The IB also provides insight to support the clinical management of the study subjects during the course of the clinical study. The investigator/recipients shall treat the IB as a confidential document for the sole information and compliance with the study protocol and related procedures.

The IB document is also shared with regulatory authorities and ethics committees, whenever appropriate.

The IB will be revised in compliance with a sponsor's written procedures at a time appropriate depending on the stage of development and the generation of relevant new information.

BRIEF SUMMARY

With Colorectal Cancer (CRC) being accountable for 12.4% of all deaths due to cancer, and with only 14% of EU citizens participating in screening programmes, there is an urgent need for accurate, non-invasive, cost-effective screening tests based on novel technologies and an increased awareness on the disease and its detection. Furthermore, personalized approaches for screening are needed, to consider genetic and other socioeconomic variables and environmental stressors that lead to different onsets of the disease. ONCOSCREEN responds to these challenges by developing a risk-based, population-level stratification methodology for CRC, to account for genetic prevalence, socio-economic status, and other factors. It complements this methodology by a) developing a set of novel, practical, and low-cost screening technologies with high sensitivity and specificity, b) leveraging AI to improve existing

methodologies for CRC screening, allowing for the early detection of polyps and provision of a personalized risk status stratification, and c) providing a mobile app for self-monitoring and CRC awareness raising. Furthermore, ONCOSCREEN develops an Intelligent Analytics dashboard for policy makers facilitating effective policy making at regional and national levels. Through a multi-level campaign, the above-mentioned solutions are tested and validated. For the clinical solutions specifically, a clinical validation study has been planned with the participation of 4100 enrolled patients/citizens. To ensure the adoption of the developed solutions by the healthcare systems, their cost-effectiveness and financial viability will be assessed. The 48-months duration project will be implemented by a multidisciplinary consortium comprising of 38 partners, including technical solutions providers, hospitals, Ministries of Health as policy makers, legal and ethics experts, Insurance companies, involving actively end-users/citizens in all phases of implementation through targeted workshops.

INTRODUCTION

It is estimated that, in EU-27 countries in 2020, Colorectal Cancer (CRC) accounted for 12.7% of all new cancer diagnoses and 12.4% of all deaths due to cancer. It is the second cause of cancer death in men (after lung cancer) and the third one in women (after breast and lung cancers). The methods that are recommended for CRC screening include stool-based and direct visualization tests. However, in order to achieve the benefits of screening, re-evaluate abnormal results from stool-based and visualisation tests, subjects should be followed up with colonoscopy. This method encompasses risks for the subjects which include belly pain and discomfort, bleeding, bad reaction to anaesthesia, infection and the colonoscopy preparation risks. Human error in diagnosis of CRC cancer or a diagnosis at a late stage due to unwillingness of human subjects to conduct the screening, leads to multiparametric undesirable consequences ranging from reduced life expectancy of diagnosed patients and changes in their Quality of Life (QoL) and higher costs for the patients and the overall healthcare system. Additionally, the time for the delivery of CRC results is a very important factor1. Despite recommendations from EU, it is estimated that only 14% of European citizens participate in CRC screening programs. Fear and other socio-economic factors of patients are not studied in depth and may contribute negatively to prevention and early diagnosis. Furthermore, several CRC screening solutions have been published in scientific articles, however very few have been validated in large scale clinical trials and certainly not at an EU-wide level with the involvement of an adequate number of countries. The management of screen-detected pre-cancerous lesions and early disease is intended to reduce CRC mortality. When diagnosed at stage I, the overall 5-year survival rate is around 90%, whereas it is only around 10% in the metastatic stage IV. It is estimated that, only around 13% of patients are diagnosed at stage IV. The overall CRC trends present large disparities among EU countries, especially in the survival rates which are highest in Western Europe and lowest in some countries of Eastern Europe, which is distant from a health-equal Europe in regard to access. Additional factors beyond age, such as sex and gender, race and ethnicity and lifestyle could improve the prevention strategies and update the recommendations from policy makers2. From the patient's perspective, other barriers include fear, socio-demographic, psychosocial, economic/geographic factors, as well as awareness, understanding, or lifestyle. For clinicians and healthcare providers, low screening recommendations, poor coordination and communication between patients and providers, or lack of follow-up actions hinders the success of such programs. At a higher scale, health system policy makers, may be hesitant to adopt radical measures due to costrelated constraints and the capacity to move patients from screening to colonoscopy to effective treatment. A more detailed patient-level data risk-based screening program is needed to control for comorbidities, oncological therapies, stage-related variations at time of diagnosis, and other factors in the screened and non-screened populations3.

BACKGROUND AND RATIONALE ON ONCO-TOOLS

ONCO-VOC

The ONCO-VOC is a screening test based on Volatile Organic Compounds (VOC). VOC analysis devices available today in the market, are mainly based on various derivatives of mass spectrometry, are fairly large, only semi-portable, inaccurate, and affected by humidity and other confounding factors. Low- cost miniaturized devices are either based on the detection a single biomarker in breath or, they do not show sufficient sensitivity and specificity for a wide population CRC screening program. The innovative breath analyzer, developed by TECHNION is based on sensor array of nanomaterials in conjugation with AI.

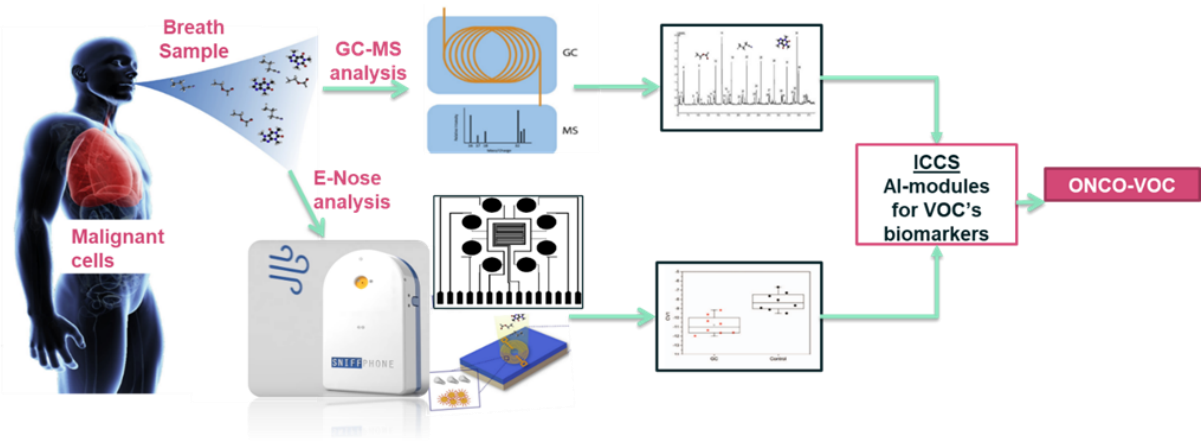


Figure 5 ONCO-VOC testing workflow

To perform the test, a short-exhaled breath sample (2-3sec.) will be evaluated in less than 2 minutes by the ONCO-VOC breath analyzer that includes 8 nano-sensors, in an array format. These sensors are cross-reactive towards the VOCs adsorbed on each of the sensors in the array and change their electrical properties. Analysis of the electro-chemical signal responses with pattern recognition algorithms, will give early indication for distinguishing between CRC-linked and healthy categories.

CRC investigation in 160 samples have shown 85% sensitivity, 94% specificity.

ONCO-CRISPR

The ONCO-CRISP is a screening test based on CRISPR-Cas9 biomarkers. The ONCO-CRISPR tool and human CRISPR microRNAs will enable the detection of clinically relevant biomarkers in colorectal adenomas, colorectal carcinomas in tissue and non- or minimal invasive obtained patient samples.

The test consists of a detection dipstick, a reporter, a Cas protein (nuclease, DNA/RNA cutting enzyme) and a guiding RNA, primers, isothermal amplification enzymes and input material that could be RNA or DNA that contains the biomarker of interest. The test will show a negative or a positive result on a used test strip. Testing will be done according to the testing instructions.

Figure 6 Example of the ONCO-CRISPR tool on the left and a hCRISPR biomarker on the right (red is positive)

The tool addresses CRC testing needs by increasing accuracy (sensitivity >0.90 specificity >0.90), affordable/user-friendly tools, quick results in less than two hours, detection of early-stage CRC, equipment free and deliverable to the end-users. Patients will benefit from the ONCO-CRISPR tool which uses a minimally invasive procedure for obtaining a correct diagnosis.

ONCO-NMR

NMR is a highly robust method with excellent reproducibility and minimal variation between measurements. It has been used to study cancer samples in many studies and has been shown to provide good signatures for cancer and cancer progression. The method has been utilized extensively in other cancer settings, in particular for breast cancer, and recently also for colorectal cancer *Vignoli, A. et al. Exploring Serum NMR-Based Metabolomic Fingerprint of Colorectal Cancer Patients: Effects of Surgery and Possible Associations with Cancer Relapse. Applied Sciences 11, 11120 (2021).* UzL has developed a standardized NMR test for the analysis of blood serum and plasma samples. NMR metabolomics provides approx 40 metabolic parameters, ca. 100 detailed lipoprotein parameters. The test is a highly robust with excellent reproducibility and minimal variation between measurements. Sample testing will be performed according to the instructions in annex.

ONCO-CTCs

CTCs as epithelial cancer cells are able to move, migrate and invade blood vessels after epithelial-mesenchymal transition (EMT) and have been characterized as the main causal

factor of tumor metastasis mediation. Compared to other cancer biomarkers, CTCs contain molecular and biological information about the tumor as a whole, supporting single cell analysis and ongoing changes in tumors at all stages. At the same time, phenotypic and molecular characteristics of CTCs, can reveal the mechanism of pathogenesis and metastasis of CRC and identify specific mutations in target genes. In contrary with the conventional theory that the metastatic dissemination of cancer cells represents the final stage of a deteriorating process, it has been found that CTCs often disseminate at the early stages during the process of tumorigenesis, invading distant organs and eventually developing into overt metastatic lesions. Therefore, the detection of CTCs in the circulation may be proved a feasible way to improve the early diagnosis and treatment of patients with CRC prior to metastasis.

CTCs can be found in blood as independent cells, and clusters of both CTCs alone and cells aggregates comprising of neutrophils, platelets, and CTCs. Thus, blood-based analysis of CTCs could therefore function as a "liquid biopsy," allowing repeated sampling.

ONCO-CTC is a biofluidgnostics platform for early-diagnose CRC and treatment personalization being developed by UMINHO. UMINHO will use a well-established photolithography technology and will explore the different designs for microfluidics fabrication (Figure 4), for focusing on the detection of isolated CTCs that are usually formed at twice the rate of CTC clusters.

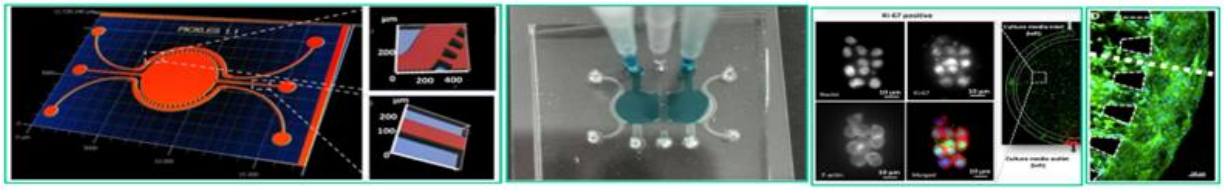


Figure 7 CRC Tumour-on-a-chip published model of UMINHO.

CRC cancer CTCs have a diameter less than 10 μm and a cross-sectional area of 40–65 μm2, similar to white blood cells. The proposed ONCO-CTC chip platform is based on size exclusion isolation and detection of CTCs. The ONCO-CTC tool will consist of costly-effective CRC diagnostic chips/test kit. It will provide qualitative and quantitative information across the cancer continuum (initiation, progression, metastasis and relapse), not only providing indication/detection insights, but also classification capabilities within a period of 15-60 minutes.

PRESENT CLINICAL STUDY

Scientific rationale

Colorectal Cancer (CRC) is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women1. CRC can often be prevented through regular screening. Although colonoscopy-based screening programs result in a significant decrease in CRC incidence, the compliance rate of the people to perform the required screening is still too low and not the desired one2. Fecal Immunohistochemical Tests (FITs) is another widely used screening modality for CRC but has certain constrains to use, such as relatively low sensitivity and

the need of multiple sampling (three) to reach the full screening potential3. The development of novel, more practical screening methods can effectively increase the screening rates for CRC through non-invasive, repeatable, cost-effective, easy-to-use, and patient-friendly procedures. This is of particular importance in different European societies and population subgroups, since an increase in the incidence of Early-Onset CRC is currently noticed which is significantly associated with risk factors such as heredity, obesity, smoking, alcohol abuse and hyperlipidemia 4,5. Within this framework, ONCOSCREEN will develop a multi-tier diagnostic solution towards an improved CRC screening. Furthermore, ONCOSCREEN will consider specific socio-economic determinants which increase the regional or national CRC risks, thus exploiting new solutions, particularly in younger high-risk individuals in our European communities. For the validation of the developed solution, a clinical validation study (titled "ONCOSCREEN-CS") will be conducted, to assess its effectiveness, sensitivity and specificity in detecting CRC at an early stage. During the first phase of the study (ONCOSCREEN-CS-Phase A), we will identify the different expression patterns of the four diagnostic solutions (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) in CRC patients and healthy controls with high risk for CRC, and also initially estimate their sensitivity and specificity. During the second phase (ONCOSCREEN-CS-Phase B) the ONCOSCREEN solution will be clinically validated.

Within the ONCOSCREEN-CS-Phase A, a sub-study will be conducted aiming to train Non-Experts/Junior Colonoscopists and Junior Histopathologists. The rationale for the sub-study is provided below.

Rationale of the sub-study (please also check 1.3.1.5 and 6): In a large study6 that examined more than 15,000 tandem colonoscopies and 10,000 adenomas, the miss rate for polyps was 28% and adenomas 26%. According to the European guidelines for colonoscopy in general (not only screening) have defined an overall aim of 40% Polyp Detection Rate (PDR) and 25% Adenoma Detection Rate ADR7. in a comparison study between Junior and Senior colonoscopists for a Pre-intervention group of patients, the detection rate of adenomas for the junior colonoscopists was 11.57% compared to 16.2% of the senior colonoscopists having a statistically significant difference8. While the impact

of the missed lesions during the colonoscopy are responsible for 50%–60% of interval cancers9. demand for screening colonoscopy has additionally continued to rise over the past two decades. As a result, the current workforce of gastroenterologists is unable to meet the needs for CRC screening. Therefore, alternative solutions are needed to improve this disparity in the long term, with non-physician endoscopists (like Nurse Practitioners) being a potential option. There were two studies in which trained nurse practitioners conducted colonoscopies, with mean ADR 35.6% in 1012 subjects and 26.7 % in 528 subjects respectively.

Within ONCOSCREEN with the use of explainable AI powered by open source Deep Neural Networks and automated medical image segmentation models, will automatically suggest potential areas of interest “explaining” the confidence level, the classification type and other meta-data information (algorithm, training dataset). At the end of the session, the ONCOSCREEN Real Time AI-Assisted Colonoscopy module will automatically calculate the correctly classified information and will show an individual risk score. The selected areas of pathological interest will be stored and asynchronously expert colonoscopists can annotate as correctly classified or not.

A biopsy consists out of several hundred glands. During the diagnostic process, a pathologist has to identify the dysplastic glands (one dysplastic gland can change the complete diagnoses. These images are also playing a major role in the pathological diagnosis process. The analysis of Whole slide images (WSI) provides pathologists with a thorough insight into the data content and enables accurate diagnosis of tumours and cancer sub-types. However, the evaluation of WSIs for tissue classification is easily affected by many subjective factors. Such as: a) training,b) experience c) evaluation condition or d) time pressure for each pathologist that could result to different diagnosis judgement. Deep learning applied to WSIs has the potential to generate new clinical tools that are more accurate, reproducible, and objective than current clinical techniques while also delivering fresh insights into various pathologies. WSIs, on the other hand, are multi-gigabyte images with typical resolutions of 100, 000 x 100, 000 pixels, substantial morphological diversity, and a variety of artifacts. Practitioners are confronted with two significant difficulties. On the one hand, the visual understanding of the images, which is hampered by morphological variance, artifacts, and typically small data sets, and on the other hand, the current state of the hardware's inability to facilitate learning from images with such high resolution, necessitating the use of artificial intelligence and Deep Neural Networks10,11,12. ONCOSCREEN will provide the AI-Assisted Tissue Image Analysis for supporting pathologists in accurate and timely classification of early stage CRC histopathological images.

Study objectives, design and population

Primary Objective

To identify the different expression patterns of Oncoscreen diagnostic tools (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) in CRC patients and healthy controls.

Secondary objectives:

1. To estimate the sensitivity and specificity of the Oncoscreen diagnostic tools (ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) when comparing CRC patients to Healthy Controls
2. To evaluate the performance of the Oncoscreen tools for CRC screening in individuals at high risk for development of colorectal cancer
3. To gather Colonoscopy and Tissue Images data from 50 CRC recognized patients that will allow the training of junior endoscopists to validate the Algorithms retrospectively and not during the coloscopy procedures

Study design

The ONCOSCREEN Phase A clinical study is a multi-center case-control prospective study for the collection of data that will develop and finalize the ONCOSCREEN screening and diagnostic solutions.

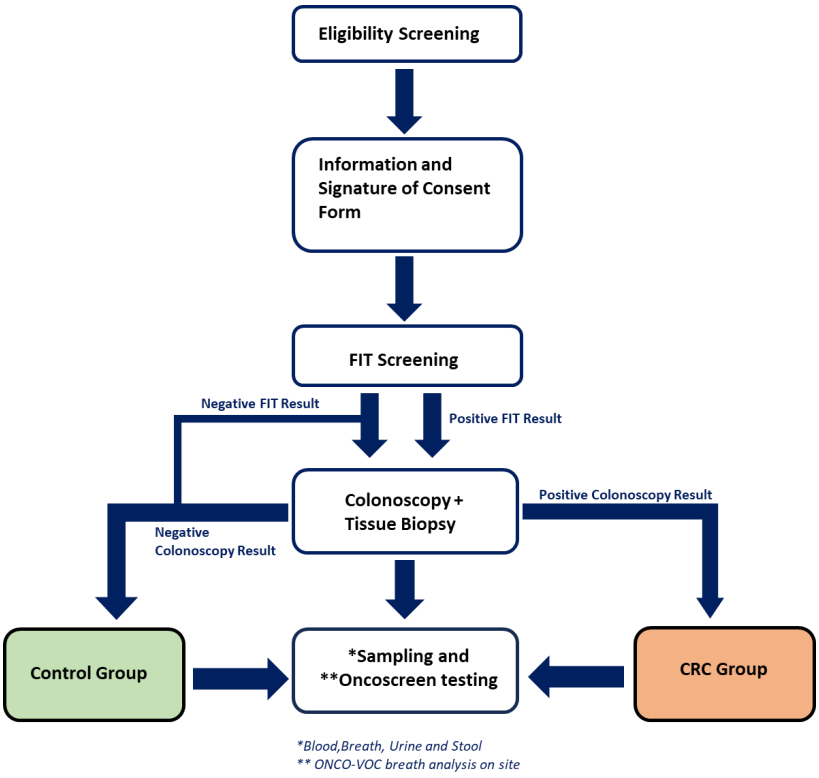


Figure 8 ONCOSCREEN PHASE A Study Flow chart

Participants will be screened for study eligibility and informed about the study by the investigator. Subjects who consent to participate will be enrolled into the study and

screened using a FIT test (ref iFOBT). Participants who return a positive FIT test will be invited for a mandatory screening colonoscopy. Participants with a negative FIT test will also be invited to a facultative screening colonoscopy. Participants with a positive colonoscopy result will be assigned into the cases group (CRC Patient), while those with a negative colonoscopy result will be assigned into the control group. Participants who returned a FIT negative test and declined to undergo a colonoscopy will be assigned to the control group. All subjects enrolled into the study will be required to provide biological specimen samples (stool, blood, and breath as detailed in table 1) for testing using the Oncoscreen. Additionally, samples will be archived for use in further developing the Oncoscreen tools and for additional CRC related research. Corresponding clinical data will be collected for analysis using a case report form.

Study population

The ONCOSCREEN Phase A clinical study aims to enroll male and female adults (at least 18 years old) that are of average/high risk of colon cancer as defined by the study inclusion and exclusion criteria.

Inclusion & exclusion criteria

Inclusion Criteria

For the Patient Group

1. Signature of the informed consent indicating that the subject accepts to participate in the study and to comply with the requirements and restrictions inherent in this study.
2. Male or female subjects aged ≥18 years
3. Subject has undergone a FIT screening test
4. Subject is diagnosed with CRC by colonoscopy and tissue biopsy
5. CRC is at stage considered well resectable
6. Subject is able to comply with all study procedures
7. Covered by a Health Insurance System

For the healthy control group

1. Signature of the informed consent indicating that the subject accepts to participate in the study and to comply with the requirements and restrictions inherent in this study
2. Male or female subjects aged ≥18 years
3. Subject has undergone a FIT screening test
4. Otherwise healthy individuals with recognized risk factors for CRC development defined by either heredity and/or obesity and/or smoking and/or excess alcohol consumption and/or hyperlipidemia.

Exclusion Criteria

For the Patient Group

1. Legal incapacity or limited legal capacity
2. Subject did not sign the Informed Consent form
3. Subject has previous history of CRC surgery
4. Subject has history of other cancer types
5. Subject received treatment by chemotherapy or other targeted oncological treatments
6. Subject who, according to the investigator's assessment, presents with an unstable medical condition contraindicating the performance of the planned blood test, stool test or breath test
7. Pregnancy and/or breastfeeding

For the healthy control group

1. Legal incapacity or limited legal capacity
2. Subjects who did not sign the Informed Consent form
3. Subject is diagnosed with CRC by colonoscopy and tissue biopsy
4. Previous history of cancer (any type)
5. Presence of any relevant organic, systemic or metabolic disease
6. Gastrointestinal disorders or other serious acute or chronic diseases
7. Participation in another interventional study
8. Pregnancy and/or breastfeeding
9. Known drug and/or alcohol abuse
10. Using any form of nicotine or tobacco

Primary and secondary endpoints

Primary Endpoint

The primary endpoint of this study is the differential expression patterns of Oncoscreen tools in CRC patients and healthy controls using colonoscopy as the reference method.

Secondary Endpoint(s)

- The specificity of the Oncoscreen tools when screening for CRC in high-risk population using colonoscopy and FIT as the reference methods.

- The sensitivity of the Oncoscreen tools when screening for CRC in high-risk population using colonoscopy and FIT as the reference methods.

End of the study

Study end will be determined as defined in the protocol when the last participant completes the last visit and examinations. Subject will be considered completed from the study when they have fulfilled all the visits and examinations required by the protocol or till the point of their withdrawal.

Anonymization of samples and data

Each sample collection tube should bear an adhesive sticker with the information according to the specific matrix type. Patient details should be written in a legible hand written form on the sample tubes with indelible pen BEFORE collection of samples from the subject.

Details must include the following:

- Matrix type **(1)**
- Unique Patient Number (UPN) **(2)**
- Date (DD/MM/YYYY) **(3)**
- Time of collection (use the 24h clock format) **(4)**

Sticker labels, for each matrix type, will be in the following format:

Serum (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

RNA (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

Unique Patient Number:

Subjects will be assigned a Unique Patient Number according to the chronological order of inclusion that will be a specific and unique code created for each subject of 12 digits. The UPN codes will be always used to refer the country and the correspondent subject information. The UPN number should include information about:

a- Study or protocol small name (4 characters: ONCO)

b- Country code of sample collection (2 characters according to the ISO country code)

c- Centre number: (2 digits: 01)

d- Patient number (4 digits: from 0001 to 9999)

Example UPN: ONCO FR 01 0021

Unique Sample Number:

Unique Sample Number (USN) will include the subject code ending in 2 characters that represent the sample type (serum / plasma subtype / urine / tissue type / DNA) and a digital addendum for counting of longitudinal samples. ***Codes for Sample Type***

Fresh whole blood (Lithium-Heparin tube)	WB
Serum	SE
Plasma EDTA	ED
Plasma Li-Heparin	LH
PBMC	PB
Spot Urine	US
PAXgene RNA tube	PX
PAXgene DNA tube	PD

The USN number should include information about:

• Subject number or UPN (12 digits)

• Sample type (2 digits)

• Time points (2 digits)

Example USN: for "first serum" extraction

USN = ONCO FR 01 0021 **SE01**

Biological sample volume and number

Type of tube	Sample	Qte	Volume
PAXgene® DNA tubes	Blood	1	8.5ml
PAXgene® RNA tubes	Blood	1	2.5ml
cell-free dna bct	Blood	2	20.0 ml
tube S-Monovette K3 EDTA	Blood	1	9.0 ml
tube S-Monovette Serum	Blood	1	9.0 ml
total blood volume	**49.0 ml**		
Tenax tubes	breath	1	250.0ml
total breath volume	**250.0 ml**		
Faeces tubes 107x25mm	Faeces	1	
total Faecal volume			

Table 7 Oncoscreen Phase A Biological samples

Adverse event reporting

An adverse event (AE) is any unfavorable or unintended sign, symptom, or disease temporally associated with the use of an investigational product or protocol-imposed intervention, regardless of attribution. Adverse events commonly associated with

colonoscopy will not be collected. Such events include: Abdominal discomfort, bowel irregularity, bleeding, intestinal perforation, and adverse reaction to the sedation.

Surgical intervention, such as, but not limited to, colectomy and sub-mucosal resection are part of routine patient care for subjects with CRC or AA; therefore, adverse events commonly associated with surgeries will not be collected. Such events include: Bleeding, blood clots in the legs (deep vein thrombosis) and lungs (pulmonary embolism); infection; injury to organs near the colon, such as the bladder and small intestines; and tears in the sutures that reconnect the remaining parts of the digestive system.

Data handling and record reporting

Data confidentiality

The participants will be given a study identification that ensures anonymization of the participant and thus no information to identify the participants will be included. Only coded data will be transferred to third parties and other countries, which in no case will contain information that can directly identify the participant (such as name and surname, initials, address, social security number, etc.). If this transfer occurred, it would be only for the study and guaranteeing confidentiality. Study promoter will comply with the EU Regulation 2016/679 to process and protect the data and will keep a record of all the treatment activities that are carried out.

Any participant can limit the processing of incorrect data, request a copy or that the data provided for the study can be transferred to a third party (portability). To exercise these rights, participant should contact Principal Investigator of the study or the Data Protection Officer of the clinical site. Moreover, participant has the right to contact the Data Protection Agency if he/she is not satisfied.

All the participants reserve the right to withdraw from the participation at any time. In such cases data already collected is not removed but no new data will be collected.

Record keeping

The data collected for the study will be kept for 10 years after the study completion. Subsequently, personal information will only be kept by the clinical center for participant health care, and by the promoter for other scientific research purposes if the participant has given its consent to do so and if this is allowed by the law and applicable ethical requirements.

Protocol deviations

The site should make all efforts not to deviate from any of the study procedures or requirements as described in this protocol, except where necessary to eliminate

immediate risks to the subject(s). Any deviations from this protocol must be reported to the Sponsor and documented properly.

Source documents

Investigator sites must maintain proper record of all study documents, medical records, examination reports and other medical reports. The clinical sites must permit trial-related monitoring, audits, EC reviews, and regulatory inspection(s) by providing direct access to source data/documents. Only authorized and trained personnel will have access to the CRF and study related documents.

Study participants, both patients with CRC and healthy volunteers will undergo screening assessments which will be scheduled and conducted according to local site standards of care procedures. The assessments to be performed during the screening visit (Visit 0) and the testing visit (visit 1) are summarized in table 2. Visit 1 should be completed within 28 days of completing the FIT screening test.

Study Procedures

	Visit 0 Screening	**Visit 1 Testing (D1-D28)**
Informed Consent Form	X	
Demographic data	X	
Risk factors	X	
Preexisting conditions / Medical history / Relevant surgical history	X	
Inclusion/ Exclusion Criteria	X	
Concomitant medication	X	
Physical evaluation		
Height	X	
Weight	X	
Vital signs (Pulse, BP, T)	X	
Physical exam	X	
Non-invasive tests		
Breath Test		X
Urine and stool sample		X
Blood sample		X
FIT screening test	X	
Invasive tests		

Colonoscopy (Endoscopy / SES- CD with biopsies)		X
Questionnaires		
Health Professional questionnaire		X
Study Participants questionnaire		X

Table 8 Oncoscreen Phase A summary of assessments

Ethics

1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended 64th WMA General Assembly, Fortaleza, Brazil, October 2013).

2. ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice E6 (R2), and other relevant local legislation.

3. Independent Ethical Committee (IEC)

The protocol, informed consent and any accompanying material provided to the patient will be submitted by the investigator to an Independent Ethical Committee for review. Approval from the committee must be obtained before starting the study. Any modifications made to the protocol, informed consent or material provided to the patient after receipt of the Ethics Committee approval must also be submitted by the investigator to the Committee in accordance with local procedures and regulatory requirements. The IEC approval report must contain details of the trial (title, protocol number and version), documents evaluated (protocol, informed consent material) and the date of the approval.

4. Medical/Clinical Safety standards; Incidental findings

An incidental and unexpected findings policy will be prepared.

Additionally, should any such findings occur, they will be evaluated by the clinical team on a case-by-case basis and reported if necessary.

The reporting of the incidental findings will be done based on the nature of these findings. Those individual incidental findings that are not regarded as adverse events or are mild

adverse events will be solved according to usual clinical practice standards; they will be addressed within the clinical setting and reported to monitoring responsible personnel. Serious incidental findings or those considered as severe adverse events will be reported to the trial committee, according to the regulatory rules.

Incidental findings will be addressed according to the standards governing Good Clinical Practice Regulation.

5. Informed Consent

It is the responsibility of the Investigator to obtain written informed consent from each subject prior to entering the trial or, where relevant, prior to evaluating the subject's suitability for the study. The informed consent document used by the Investigator for obtaining the subject's informed consent must be reviewed and approved by the IEC. All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he will be exposed, and the mechanism of treatment allocation. In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki. Information will be given in lay terms and under no circumstances will pressure of any kind be exerted either on the individual participant or her/his family. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered for the study. This must be done in accordance with the national and local regulatory requirements. The written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative; if the person concerned is unable to write, oral consent in the presence of at least one witness may be given in exceptional cases, as provided for under national legislation. The clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study. A copy of the signed informed Consent Form must be provided to the patient or the patient's legally authorized representative. The original copy of the patient's signed written consent will be kept by the centre in the proper section of the Investigator Site File and must be available for verification by study monitors at any time. Consent will be a continuing process, researchers will foster a continuous dialogue with study participants and inform them of anything new related to the trial.

The European Commission - Research Directorate-General provides guidance on informed consent in the context of clinical trials and this guidance will be respected http://ec.europa.eu/research/participants/data/ref/fp7/89807/informed-consent_en.pdf.

6. Patient data protection and procedures regarding patient information and obtaining consent

The consent to the participation in a research study should be clearly distinguished from the consent to the processing of personal data (within the meaning of the GDPR), the latter being a separate legal requirement. Obtaining one does not waive the obligation to collect the other. Therefore, the Informed Consent Form will incorporate wording that complies with the data protection and privacy legislation (GDPR). In agreement with this wording, patients will authorize the collection, processing (use) and disclosure of their study data by the Investigator and by those who need that information for the purposes of the study (consent of the study participants will constitute the required legal basis for the processing of their personal). Moreover, the informed consent form (or a document attached thereto) will provide the study participants with the following information as required under the GDPR:

- identity and contact detail of the data controller/joint controllers (and – if applicable - their representatives; DPOs – if appointed);
- the source from which the data originated (if collected not from the data subject himself) and if applicable, whether it came from publicly accessible sources;
- the purpose of processing;
- the legal basis for processing (such as e.g. consent);
- the categories of recipients of data (if any; e.g. external vendors/processors/OncoScreen partners);
- in cases where the controller/joint controllers intend to transfer personal data outside the EEA, justification for such transfer along with the reference to appropriate safeguards and the means to obtain access to or a copy of those;
- data storage duration, or criteria used to determine such storage duration;
- data subjects' rights and how to exercise them;
- the right to withdraw consent (where consent was the basis for processing) and that such withdrawal will not affect the lawfulness of processing based on consent that took place before its withdrawal;
- the right to lodge a complaint with a competent supervisory authority;
- the existence of profiling including meaningful information about the logic involved, as well as the significance and the envisaged consequences of such processing for the data subject;
- the possibility that the data will be further processed for scientific research purposes.

As already explained above, the above information must – in compliance with the transparency principle – be concise, transparent, intelligible and easily accessible in order to avoid information fatigue for the data subjects, who must consider many elements, sometimes fairly technical.

The Informed Consent Form will explain that how the study data will be stored, maintaining confidentiality in accordance with the European and national data legislation.

The Informed Consent Form will also explain that for data verification purposes, authorized representatives of Sponsor/Promoter, a regulatory authority, an Ethics Committee may require direct access to parts of the hospital or practice records relevant to the study, including patients' medical history.

Administratives rules

Curriculum vitae

A current copy of the curriculum vitae describing the experience, qualification and training of each Investigator will be signed, dated and provided to the Sponsor prior to the beginning of the clinical study.

Record retention in study site

The Investigators must maintain confidential all study documentation, and take measures to prevent accidental or premature destruction of these documents. The Investigators should retain the study documents at least fifteen (15) years after the completion or discontinuation of the clinical study. However, applicable regulatory requirements should be taken into account in the event that a longer period is required. The Investigators must notify the Sponsor prior to destroying any study essential documents following the clinical study completion or discontinuation. If the Investigators' personal situation is such that archiving can no longer be ensured by them, the Investigators shall inform the Sponsor and the relevant records shall be transferred to a mutually agreed upon designee.

CONFIDENTIALITY

All information disclosed or provided by the Sponsor (or any company/institution acting on their behalf), or produced during the clinical study, including, but not limited to, the clinical study protocol, the CRF, and the results obtained during the course of the clinical study, is confidential, prior to the patent and/or the publication of results. The Principal Investigators, the Co-Investigator and any person under their authority agree to keep confidential and not to disclose the information to any third party without the prior written approval of the Sponsor. However, the submission of this clinical study protocol and other necessary documentation to the Ethics Committee (IEC/CPP) is expressly permitted, the IEC/CPP members having the same obligation of confidentiality.

The Principal Investigator and the Co-Investigator shall use the information solely for the purposes of the clinical study, to the exclusion of any use for their own or for a third party's account. Furthermore, the Co-Investigators and the Sponsor agree to adhere to the principles of personal data confidentiality in relation to the subjects, Co-Investigators and its collaborators involved in the study.

REFERENCES

- Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89–103.
- Kwack WG, Lim YJ. Current Status and Research into Overcoming Limitations of Capsule Endoscopy. Clin Endosc. 2016 Jan;49(1):8–15.
- Lee JK, Liles EG, Bent S, Levin TR, Corley DA. Accuracy of fecal immunochemical tests for colorectal cancer: systematic review and meta-analysis. Ann Intern Med. 2014;160(3):171. doi:10.7326/M13-1484
- O'Sullivan DE, Sutherland RL, Town S, Chow K, Fan J, Forbes N, Heitman SJ, Hilsden RJ, Brenner DR. Risk Factors for Early-Onset Colorectal Cancer: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2021 Jan 29:S1542-3565(21)00087-2.
- Patel SG, Karlitz JJ, Yen T, Lieu CH, Boland CR. The rising tide of early-onset colorectal cancer: a comprehensive review of epidemiology, clinical features, biology, risk factors, prevention, and early detection. Lancet Gastroenterol Hepatol. 2022 Mar;7(3):262-274.
- Zhao S, Wang S, Pan P, Xia T, Chang X, Yang X, Guo L, Meng Q, Yang F, Qian W, Xu Z, Wang Y, Wang Z, Gu L, Wang R, Jia F, Yao J, Li Z, Bai Y. Magnitude, Risk Factors, and Factors Associated With Adenoma Miss Rate of Tandem Colonoscopy: A Systematic Review and Meta-analysis. Gastroenterology. 2019 May;156(6):1661-1674.e11. doi: 10.1053/j.gastro.2019.01.260. Epub 2019 Feb 6. PMID: 30738046.
- Kaminski M F, et al.Performance measures for lower gastrointestinal endoscopy: a European Society of Gastrointestinal Endoscopy (ESGE) Quality Improvement Initiative. Endoscopy. 2017;49:378–397
- Deng, B., et. Al., (2016). The Impact of Colonoscopy Quality Control Table on Adenoma Detection Rates. Gastroenterology research and practice, 2016, 2548109. https://doi.org/10.1155/2016/2548109
- Robertson D.J., et Al., Colorectal cancers soon after colonoscopy: a pooled multicohort analysis. Gut. 2014; 63: 949-956
- Dimitriou N, Arandjelovi´c O and Caie PD (2019) Deep Learning for Whole Slide Image Analysis: An Overview. Front. Med. 6:264. doi: 10.3389/fmed.2019.00264
- BenTaieb A, Ghassan Hamarneh G. Predicting cancer with a recurrent visual attention model for histopathology images. In: Frangi AF, Schnabel JA, Davatzikos C, Alberola-López C, Fichtinger G, editiors. Medical Image Computing and ComputerAssisted Intervention. Vol. 11071. Springer International Publishing (2018). p. 129–37. Available online at: https://link.springer.com/book/10.1007/978-3-030-00934-2
- Tellez D, Litjens G, van der Laak J, Ciompi F. Neural image compression for gigapixel histopathology image analysis. arXiv. (2018). doi: 10.1109/TPAMI.2019.2936841

Annex C: SOPs

A Standard Operating Procedure (SOP) is a specific procedure that describes step by step the activities necessary to complete the tasks in accordance with standards pre-established by the developer of the device. Here are the SOPs related to each ONCOSCREEN-Tools.

SOP

CRC CRISPR POCT

Abbreviations

Abbreviation	Definition
CRC	Colorectal cancer
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR-Cas	Clustered Regularly Interspaced Short Palindromic Repeats - CRISPR-associated protein
DETECTR	DNA Endonuclease Targeted CRISPR trans Reporter
DNA	Desoxyribo Nucleic Acid
FW	Forward
gRNA	Guide RNA
mL	Milliliter
POCT	Point-of-care test
PEG	Polyethylene glycol
RNA	Ribo Nucleic Acid
(RT)-RPA	(Reverse-transcriptase)-Recombinase Polymerase Amplification
RV	Reverse
SOP	Standard operating procedure
µL	Microliter
µM	Micromolar

Objective

CRISPR-Cas based point-of-care test to detect clinical CRC biomarkers in stool or blood samples.

Equipment, consumables, and reagents

Name	Supplier	Cat. Number	Storage
DEPC treated water	Life Technologies Europe BV / ThermoFisher	AM9906	Room temp.
10 x 2 mL IDTE pH 8.0 (1X TE Solution)	IDT	N/A	-20°C
TwistAmp® Basic	Twist Dx Limited	TABAS03KIT	-20°C
(RT)-RPA Amplification primer pair	Biolegio	Custom	-20°C
10X NEBuffer 2.1	New England Biolabs	B7202S	-20°C
1X NEBuffer 2.1 containing 2% Polyethylene glycol 6000 (PEG)	N/A	N/A	Room temp.
EnGen® Lba Cas12a (Cpf1) protein	Bioké / Cell signaling technology	NEB M0653S / NEB M0653T	-20°C
M-MLV Reverse Transcriptase (200 U/µL)	Life Technologies Europe BV / ThermoFisher	28025013	-20°C
RiboLock RNase Inhibitor	Life Technologies Europe BV / ThermoFisher	EO0381	-20°C
LbaCas12a gRNA	Biolegio	Custom	-20°C
LbaCas12a Reporter RNA for lateral flow read out	IDT	Custom	-20°C
Positive and negative control samples	Biolegio	Custom	-20°C
Samples to test, DNA and/or RNA extracted from fecal samples	N/A	N/A	-20°C
Milenia GenLine HybriDetect	Milenia Biotec GmbH	MGHD 1	2-8°C

Pipette tips	N/A	N/A	N/A
Pipettes	N/A	N/A	N/A
Sterile 1.5 mL tubes	N/A	N/A	N/A
37°C Heat block	N/A	N/A	N/A
42°C Heat block	N/A	N/A	N/A
Microcentrifuge	N/A	N/A	N/A
Timer	N/A	N/A	N/A

Safety procedures

No hazardous substances or mixtures according to Regulation (EC) No 1272/2008.

These substances/mixtures contain no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Body Protection

Flame retardant antistatic protective clothing (laboratory coat).

In case of skin contact: Rinse skin with water.

In case of eye contact: Rinse out with plenty of water. Remove contact lenses.

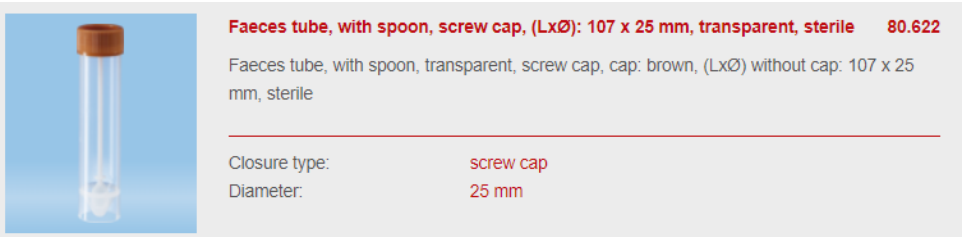
Extinguishing media: Water Foam Carbon dioxide (CO2) Dry powder, but no limitations of extinguishing agents are given.

Operating instructions

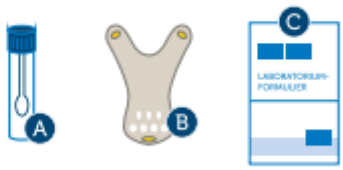
Sample collection procedure

Stool samples: Samples will be collected by the Oncoscreen consortium. Written informed consent will be obtained from each study participant. Early or late forms of CRC or a patient being healthy needs to be confirmed by biopsy and colonoscopy for the Oncoscreen phase A study, for the phase B study this confirmation is not needed, since in

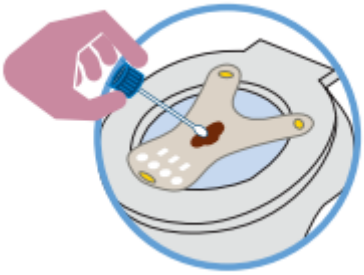
this phase it is anticipated that although the patient has specific symptoms the patient is healthy and without CRC until proven otherwise. All stool samples will be collected pre-operatively. The stool samples will be collected by subjects in their homes on the day before colonoscopy or biopsy procedures by using a "hat" or specimen collection container that fit under a toilet seat (see below). Then stool aliquots need to be collected using a faeces tube, with spoon and screw cap (see example). After collection the faces tube needs to be stored at 4 °C, labelled with the provided patient sticker and put into a sealed plastic bag and transferred within 24 hours to the clinical location. The collected sample needs to be transported preferably cooled to the clinical location where the colonoscopy or biopsy will take place. Here the sample administration needs to be organized so that the faeces sample will be included in the Oncoscreen study (see Sample Labelling).



Faeces collection tube



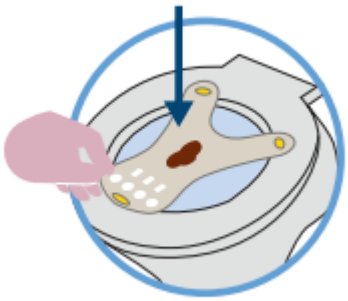
A) Faeces tube, B) Faeces collector, Oncoscreen form



B) Faeces collector, which can also be replaced by a fecotainer, see https://www.fecesvanger.nl/en_GB/c-3804543/the-fecotainer/

C) fill the faeces tube to 1/3.

D) Lock the faeces tube by screwing the cap and label the faeces tube with the provided oncoscreen patient information.



E) If the paper faeces collector is used this can be put in the toilet and flushed away when wetted. If the fecotainer (right picture) https://www.fecesvanger.nl/en_GB/c-3804543/the-fecotainer/ is used it can be wasted as a normal disposable.

Blood samples

For blood collection to isolate genomic DNA the SOP of Firalis can be used, see SOP-Blood Collection using PAXgene DNA Tube (Firalis). For the PAXgene RNA Tube the same procedure is applicable. In the Oncoscreen phase A study it is important to know whether the patient has CRC (early or late) or is healthy and needs to be confirmed by biopsy and colonoscopy. For the phase B study this confirmation is not needed, since in this phase it is anticipated that although the patient has specific symptoms the patient is healthy and without CRC until proven otherwise, which needs to be detected with the CRISPR-Cas prototype validated in the phase A study of Oncoscreen.

Sample labelling

Anonymization of samples and data

Each sample collection tube should bear an adhesive sticker with the information according to the specific matrix type. Patient details should be written in a legible hand written form on the sample tubes with indelible pen BEFORE collection of samples from the subject.

Details must include the following:

- Matrix type **(1)**
- Unique Patient Number (UPN) **(2)**
- Date (DD/MM/YYYY) **(3)**
- Time of collection (use the 24h clock format) **(4)**

Sticker labels, for each matrix type, will be in the following format:

Serum (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

RNA (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

Unique Patient Number:

Subjects will be assigned a Unique Patient Number according to the chronological order of inclusion that will be a specific and unique code created for each subject of 12 digits. The UPN codes will be always used to refer the country and the correspondent subject information. The UPN number should include information about:

a- Study or protocol small name (4 characters: ONCO)

b- Country code of sample collection (2 characters according to the ISO country code)

c- Centre number: (2 digits: 01)

d- Patient number (4 digits: from 0001 to 9999)

Example UPN: ONCO FR 01 0021

Unique Sample Number:

Unique Sample Number (USN) will include the subject code ending in 2 characters that represent the sample type (serum / plasma subtype / urine / tissue type / DNA) and a digital addendum for counting of longitudinal samples. ***Codes for Sample Type***

Fresh whole blood (Lithium-Heparin tube)	WB
Serum	SE
Plasma EDTA	ED
Plasma Li-Heparin	LH
PBMC	PB
Spot Urine	US
PAXgene RNA tube	PX
PAXgene DNA tube	PD

The USN number should include information about:

- Subject number or UPN (12 digits)
- Sample type (2 digits)
- Time points (2 digits)

Example USN: for “first serum” extraction

USN = ONCO FR 01 0021 **SE01**

(Instructions for sample labelling will be provided by Firalis, according to clinical centre requests, for global harmonization)

Sample processing

Stool sample processing

DNA Preparation and Bacterial Quantification and identification by PCR and CRISPR-Cas

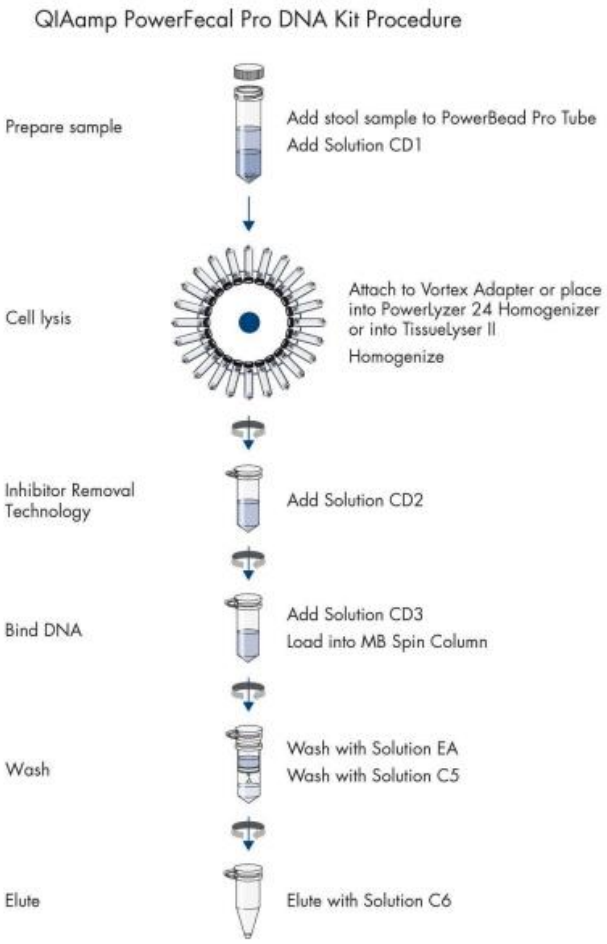
Stools samples (20-100mg) were digested overnight in 0.7mL molecular grade lysis buffer (100mM TrisHCl pH 8.5, 5mM EDTA pH 8.0, 0.2% SDS, 200mM NaCl, 1 mg/ml proteinase K) at 55°C with rotation. The samples need to be centrifuged at 20,000xg for 5 minutes, whereafter the liquid portion will be moved to equal volume isopropanol. The precipitated DNA needs to be recovered and resuspended in 0.4mL TE buffer. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) needs to be added, mixed by inversion, centrifuged at 20,000xg for 5min, then the aqueous phase needs to be transferred to a fresh tube. If aqueous phase is milky, the phenol:chloroform:isoamyl alcohol step needs to be repeated. 0.1 volume of 3M sodium acetate pH 5.2 and 2 volumes of 100% ethanol needs to be added, incubated at -20C for one hour, and centrifuged at 20,000xg for 15min. The pellet needs to be washed two times by adding 0.5mL of 70% ethanol, centrifuging at 20,000xg for seven minutes, and discarding supernatant. The DNA pellet needs to be resuspended in molecular grade water. For stool samples, alternatives are available that would allow working without dangerous chemicals. This is the old-fashioned method, alternatively these kits can be used, so that the researcher is not exposed to toxic chemical such as phenol and chloroform.

QIAamp PowerFecal Pro DNA Kits For the isolation of microbial DNA from stool and gut samples

https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-powerfecal-pro-dna-kit

QIAamp Fast DNA Stool Mini Kit For isolation of gDNA from stool samples

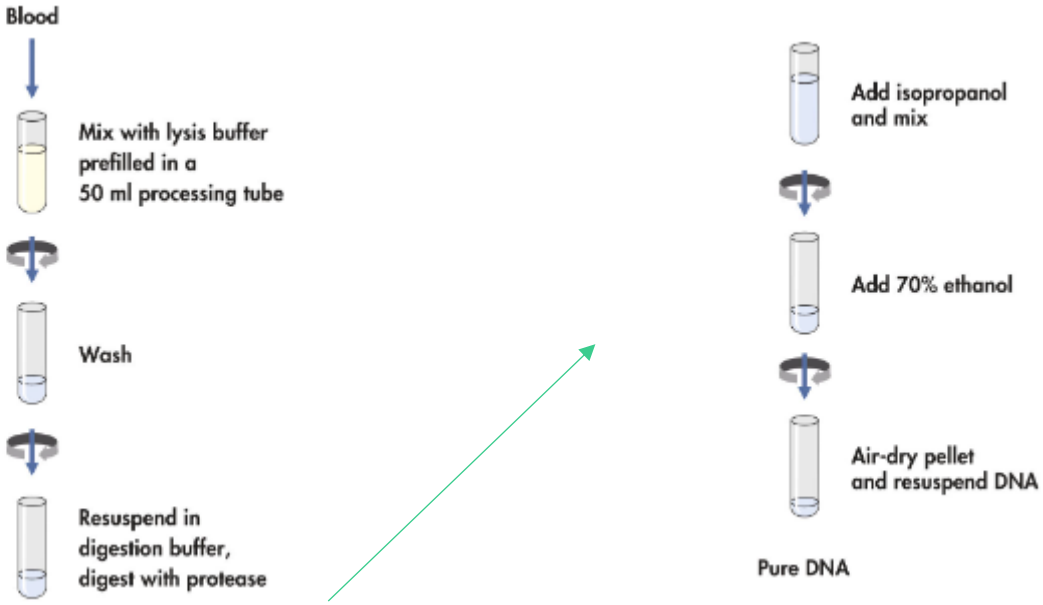
https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-fast-dna-stool-mini-kit?catno=51604



Blood sample processing

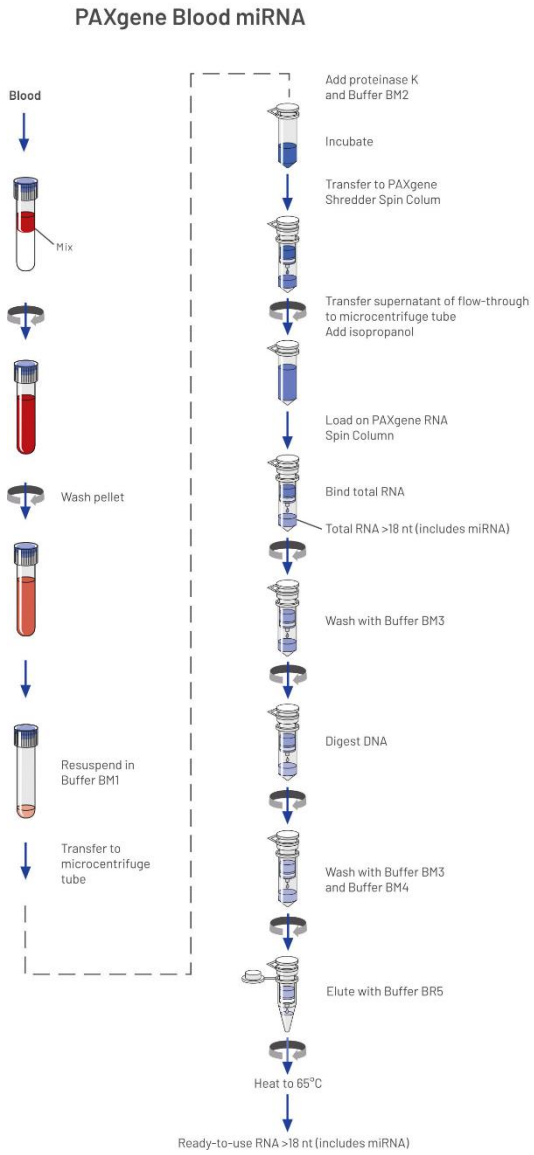
DNA isolation

For DNA isolation from the PAXgene Blood DNA tubes, the blood is transferred to processing tubes (supplied already filled with cell lysis buffer), and the solution is mixed to lyse red and white blood cells. Cell nuclei and mitochondria are pelleted by centrifugation, washed, and resuspended in digestion buffer. Protein contaminants are removed by incubation with a protease. DNA is precipitated in isopropanol, washed in 70% ethanol, dried, and resuspended in resuspension buffer.



RNA isolation

For RNA isolation from the PAXgene blood RNA tubes, the PAXgene blood miRNA kit Cat. No. /ID:763134 needs to be used which will enable the isolation of miRNAs and total RNA. Cell lysis and stabilization of intracellular RNA takes place upon collection of whole blood in PAXgene Blood RNA Tubes, effectively preventing changes to gene expression profiles by ex vivo gene regulation or RNA degradation. After removal of cellular debris, kit buffers provide optimal conditions for RNA molecules to bind to the silica membrane. Contaminants are washed away and trace DNA is removed by treatment with DNase I. Pure RNA, including miRNA, is then eluted in a low-salt elution buffer and denatured by heating, see procedure below.



Sample aliquoting

Faeces DNA 400 – 500ng/ul per 100ul elution buffer obtained from 200mg faeces

PAXgene RNA 3–18 µg/80ul obtained from 2.5 ml blood sample

PAXgene DNA 150–500 µg/1ml obtained from 8.5 ml blood sample

Sample storage and shipment

Collected faeces samples can be stored for long-term after labelling and aliquating at –80 °C until further processing (DNA extraction). Genomic DNA isolated from faeces material can be stored at 4 °C and for long-term the –80 °C can be used.

The PaxGene Blood DNA or RNA tube can be stored for long-term at –80 °C.

ONCO-CRISPR procedure

A. Preparings

Dilute gRNA

- For gRNA (5nmol), add 20µL of nuclease-free 1X TE buffer (Tris-EDTA, pH8.0) for a final concentration of 250µM (250pmol/µL). Total of 20µL of 250µM.
- Then dilute gRNA to working stock (3 months stable, -20 °C):
    - To make a 10µM working stock, add 2-5µL of 250µM sgRNA oligo to 120µL of the provided DEPC water to make a total of 125µL of 10µM (10 pmol/µL) sgRNA. This will be your working stock.

Prepare 1X NEBuffer 2.1

- To get a 1000 µL 1X NEBuffer 2.1 solution, mix 100 µL of 10X stock NEBuffer 2.1 with 900 µL of DEPC water.

Prepare 1X NEBuffer 2.1 with PEG (2%)

- 2% à 20 mg PEG 6000 in 1 mL 1X NEBuffer 2.1

Prepare Reporter working stock (10 µM)

- To get a 100 µL 10 µM solution, mix 10 µL of 100 µM oligo stock with 90 µL of IDTE buffer.

Turn on incubator: at 37°C and prepare Ice.

B. Isothermal Amplification, (RT)-RPA

1. Prepare a (RT)-RPA master mix in 1.5 mL Eppendorf tubes for …X… reactions/samples (include control samples and some extra volume). One (RT)-RPA reaction mix with one dissolved pellet aliquot is sufficient for four reactions/samples.
    a. Start by adding reagents number 1 to 4 together in the following order.
    b. On ice, carefully resuspend the (RT)-RPA reaction mix in 5 single pellet aliquot tubes. Then transfer the entire reconstituted reaction back to the initial 1.5 mL Eppendorf tube.
    c. When performing RT-RPA, add M-MLV Reverse Transcriptase to the mixture.

Reagent	**Volume (4 reactions/samples)**	**Volume 5x (20 reactions/samples)**
1. TwistAmp Rehydration Buffer (from the TwistAmp Basic kit)	29.5 µL	146.5 µL
2. DEPC water	11.45 µL	57.25 µL
3. DETECTR FW Primer nr (50µM)	1 µL	5 µL
4. DETECTR RV Primer nr (50µM)	1 µL	5 µL
5. Pellet aliquot tube	1 tube	5 tubes

6. M-MLV Reverse Transcriptase (200 U/µL) (only RT-RPA)	0.8 µL	4 µL

1. For each sample to be tested, add 2 µL extracted sample in a new 1.5 mL Eppendorf tube.
2. To the (RT)-RPA master mix, per 4 reactions/samples, add 2.5 µL of 280mM Magnesium Acetate (from the TwistAmp Basic kit) and vortex briefly. (Note: (RT)-RPA reactions start as soon as MgOAc is added.)
3. Add 10 µL of the reconstituted (RT)-RPA reaction mix to the 1.5 mL sample tubes (from step 3).
   Mix thoroughly and incubate RPA reactions at 37°C for 40 minutes. After incubation, place the reaction back on ice immediately. (Note: If tubes are opened after amplification there is a great risk of contamination of work surfaces with amplicon. After the run is completed, you can either immediately proceed to Cas12 DETECTR detection or store the reaction at 4 °C for 2–3 d. Alternatively, reactions can be stored at −20 °C for up to several weeks.)

C. Detection of DNA sequence using Cas12

1.
   a. LbaCas12a: dilute the 100µM LbaCas12a stock solution 50x in DEPC water to make a 2 µM (2pmol/µL) LbaCas12a working solution. (for immediately use)
   b. Or use a 1µM LbaCas12a stock solution.
2. Make a CRISPR cleavage master mix for …X… samples, in the following order. Divide the mix per sample, 18.0 µL in a 1.5 mL tube and set at room temperature:

Reagent	**Volume for nr. reactions**			
	1	**5**	**10**	**19**
1. DEPC water	12.5 µL	64.5 µL	129.0 µL	237.5 µL
2. 10X NEBuffer 2.1	2.0 µL	10.0 µL	20.0 µL	38.0 µL
3 LbCas12a				
1.0 µM	1.6 µL	2.0 µL	4.0 µL	30.4 µL
2.0 µM	0.8 µL	1.0 µL	2.0 µL	15.2 µL
4. 10 µM gRNA	1.0 µL	5.0 µL	10.0 µL	19.0 µL
5. Cas12 Reporter (10 µM)	0.9 µL	4.5 µL	9.0 µL	17.1 µL
Total	**18.0 µL**	**90.0 µL**	**180.0 µL**	**342.0 µL**

3. After incubation of (RT)-RPA reactions, add 2 µL of the (RT)-RPA reactions to the 18 µL CRISPR cleavage mix.

4. Incubate at 37°C for 40 minutes.
5. Store the (RT)-RPA samples at -20°C.
6. Add 80 ul of 1X NEBuffer 2.1 with 2% PEG to each 20ul reaction and mix thoroughly.
7. Place a HybriDetect Dipstick into each reaction tube and allow the lateral flow strip to run for 2 minutes at room temperature and make a picture at 3 minutes.

Associated Documents & Forms

INTABAS-v3.0-TwistAmp-Basic-Kit-Quick-Guide-INTABAS.pdf (twistdx.co.uk)

https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/paxgene-blood-mirna-kit

https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-powerfecal-pro-dna-kit

https://www.qiagen.com/us/products/discovery-and-translational-research/sample-collection-stabilization/dna/paxgene-blood-dna-kit

Yamagata, H., Kobayashi, A., Tsunedomi, R. *et al.* Optimized protocol for the extraction of RNA and DNA from frozen whole blood sample stored in a single EDTA tube. *Sci Rep* **11**, 17075 (2021). https://doi.org/10.1038/s41598-021-96567-2

PAXgene blood DNA tubes

Reducing Variability and Improving Workflow in Collecting, Transporting, and Processing Blood Samples for Genomic DNA Purification Using the PAXgene Blood DNA System (EN)

Groelz et al., ASHG 2004

PAXgene blood RNA tubes

Guenther et al. Performance Evaluation Study of the PAXgene Blood RNA System with Regulatory Compliance, AMP, 2005.

Guenther et al. In Situ Stability of RNA in Blood Samples Stored at –20°C and –70°C in PAXgene Blood RNA Tubes; ISBER, 2009.

Guenther et al. Automated, Low Throughput RNA Purification for Whole Blood using the PAXgene Blood RNA System, CHI Genomic Sample Prep, 2009.

Guenther et al. Development and Optimization of a Protocol for Automated RNA Purification using the PAXgene Blood RNA System, AACR, 2007.

Guenther and McCluskey. Maintaining the Stability and Integrity of RNA from Whole Blood Samples, CLI, 2008.

SOP

Blood Samples Collection and Enrichment Procedure for Isolation of CTC's

Abbreviations

CTCs: Circulating Tumor Cells
DMSO: Dimetilsulfóxido
EDTA: Ethylene Diamine Tetra-acetic Acid
FBS: Fetal Bovine Serum
GLP: Good Laboratory Practice
PBMCs: Peripheral Blood Mononuclear Cells
PBS: Phosphate Buffered Saline
RT: Room Temperature
UPN: Unique Patient Number
USN: Unique Sample Number

Objective

This SOP is tailored to the requirements of the 3B's -UMINHO QMS, in the context of the ONCOSCREEN project, and aims to provide clear guidelines for the collection of blood Samples from cancer patients to isolate CTCs. However, this SOP can be used to isolate CTCs for any other project, not just the Oncoscreen project. Specifically, the SOP covers

THE USE OF CELLSAVE (CELLSEARCH®), STRECK (CELL-FREE DNA BCT®) OR EDTA (BD VACUTAINER®) TUBES FOR BLOOD COLLECTION AND SUBSEQUENT MANIPULATIONS.

Equipment, consumables, and reagents

Name	Supplier	Cat. Number	Storage
CELLSAVE PRESERVATIVE TUBES - CELLSEARCH®	Menarini Silicon Biosystems, Inc 2023	15137	RT
CELL-FREE DNA BCT® - STRECK (100 TUBE BOX)	Menarini Silicon Biosystems, Inc 2023	230470	RT
EDTA - BD VACUTAINER® TUBES	VWR	BDAM367864	RT
HISTOPAQUE 1077	MP BIOMEDICALS, LLC	190837	2 - 8°C
DULBECCO'S PHOSPHATE BUFFERED SALINE	MERCK LIFE SCIENCE S.L.	D5652	RT
FETAL BOVINE SERUM, QUALIFIED, ONE SHOT FORMAT, EU APPROVED SOUTH AMERICA ORIGIN (10*50ML)	LIFE TECHNOLOGIES EUROPE BV	A3160801	-20°C
DIMETILSULFÓXIDO (DMSO), ULTRA PURO	VWR	N182 (AMRESCO)	RT
ROSETTESEP™ CTC ENRICHMENT COCKTAIL CONTAINING ANTI-CD36	STEMCELL TECHNOLOGIES	15127, 15167	2 - 8°C
DULBECCO'S PHOSPHATE BUFFERED SALINE WITH 2% FETAL BOVINE SERUM	STEMCELL TECHNOLOGIES	07905	2 - 8°C
CELLSAVE PRESERVATIVE TUBES - CELLSEARCH®	Menarini Silicon Biosystems, Inc 2023	15137	RT
LYMPHOPREP™	STEMCELL TECHNOLOGIES	07811, 07861, 07801, 07851	4 - 30°C
CENTRIFUGE WITH "BRAKE-OFF" FOR 50 ML FALCON	Eppendorf	Model - 5810R Rotor a-4-62	N/A

Safety procedures

ALL PROCEDURES MUST BE PERFORMED FOLLOWING GOOD LABORATORY PRACTICE (GLP) GUIDELINES BY QUALIFIED LABORATORY OR CLINICAL STAFF.

To ensure the safety of the personnel handling the blood samples and aliquoting procedures, it is mandatory to wear appropriate personal protective equipment, including double gloves, goggles, facial mask, and a laboratory coat. These measures are essential to minimize the risk of contamination and ensure the integrity of the samples.

To maintain a high level of hygiene, sterility, and prevent the potential transmission of contaminants, it is mandatory to clean the procedural benches and equipment thoroughly before and after each use. This measure will help ensure the validity and reliability of the results obtained and minimize the risk of cross-contamination between samples.

For optimal safety and protection against potential hazards, all procedures should be conducted in the biological hood, as needed. This measure will help prevent the release of biological agents into the laboratory and minimize the risk of exposure to the personnel performing the procedures.

Used needles must be discarded in a biohazard container approved for sharps disposal following the established recommended procedure. This measure will help prevent injuries and minimize the risk of infection transmission. Any unused or contaminated blood samples must be discarded in a biohazard container labeled appropriately. The container must be handled following the recommended procedure for biological waste disposal.

Operating instructions

Sample collection procedure

Specific Tubes and consumables will be required, but they will be adapted based on the available resources and time available for processing the sample.

ideally CellSave (CELLSEARCH®) or Streck (Cell-Free DNA BCT®) tubes should be used for the protocol involving sample processing within **24 hours**, while EDTA (BD Vacutainer®) tubes will be employed as a last resort.

IMPORTANT NOTE: For processing samples collected after a long period (**72-96 hours**), Streck (Cell-Free DNA BCT®) tubes are recommended to preserve the sample integrity.

All liquid preservatives and anticoagulants should be clear and colorless. If they are colored or contain precipitates, they should not be used.

EDTA spray-coated additives may have a brownish appearance, but this does not affect the performance of the EDTA additive.

Do not use an expired tube for sampling (check the expiration date on the tube, which is typically indicated as the last day of the month). Expired and unused tubes must be disposed according to the in-house hazardous waste disposal procedures.

Blood collection tubes containing additives must be entirely filled to ensure the correct blood to additive ratio and, therefore, the quality of the final sample.

Sample processing procedure

- Before collecting blood samples, ensure that the collection tubes are at RT (18°C-25°C) and properly labeled with patient identification information.

- COLLECT WHOLE BLOOD ASEPTICALLY BY VENIPUNCTURE OR FROM A VENOUS PORT INTO A COLLECTION TUBE ONLY.

- FILL THE TUBE UNTIL BLOOD FLOW STOPS TO ENSURE THE CORRECT RATIO OF SAMPLE TO ANTICOAGULANT AND PRESERVATIVE. IMMEDIATELY MIX BY GENTLY INVERTING IT EIGHT TIMES TO PREVENT CLOTTING. INADEQUATE OR DELAYED MIXING MAY RESULT IN INACCURATE TEST RESULTS.

- BLOOD SAMPLES MAY BE STORED OR TRANSPORTED IN THE PRESERVATIVE TUBES.

CAUTION: VISUALLY INSPECT EACH SAMPLE FOR CLOTTING BEFORE PROCEEDING TO THE ENRICHMENT STEP. CLOTTED SAMPLES MUST BE DISCARDED.

Sample labelling

(Instructions for sample labelling will be provided by Firalis, according to clinical centre requests, for global harmonization). An example of Labelling procedures established at UMINHO follows:

Anonymization of samples and data

Each sample collection tube should bear an adhesive sticker with the information according to the specific matrix type. Patient details should be written in a legible hand written form on the sample tubes with indelible pen BEFORE collection of samples from the subject.
Details must include the following:

- Matrix type **(1)**
- Unique Patient Number (UPN) **(2)**
- Date (DD/MM/YYYY) **(3)**
- Time of collection (use the 24h clock format) **(4)**

Sticker labels, for each matrix type, will be in the following format:

Serum (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

RNA (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

Unique Patient Number:

Subjects will be assigned a Unique Patient Number according to the chronological order of inclusion that will be a specific and unique code created for each subject of 12 digits. The UPN codes will be always used to refer the country and the correspondent subject information. The UPN number should include information about:

a- Study or protocol small name (4 characters: ONCO)
b- Country code of sample collection (2 characters according to the ISO country code)

c- Centre number: (2 digits: 01)
d- Patient number (4 digits: from 0001 to 9999)
Example UPN: ONCO FR 01 0021

Unique Sample Number:
Unique Sample Number (USN) will include the subject code ending in 2 characters that represent the sample type (serum / plasma subtype / urine / tissue type / DNA) and a digital addendum for counting of longitudinal samples. ***Codes for Sample Type***

Fresh whole blood (Lithium-Heparin tube)	WB
Serum	SE
Plasma EDTA	ED
Plasma Li-Heparin	LH
PBMC	PB
Spot Urine	US
PAXgene RNA tube	PX
PAXgene DNA tube	PD

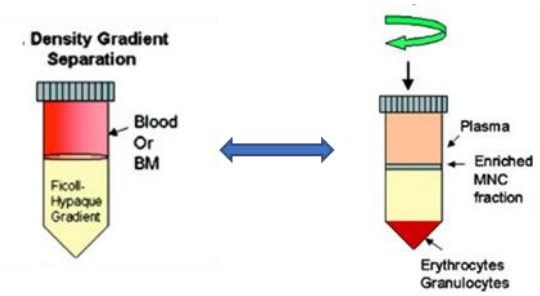
The USN number should include information about:
• Subject number or UPN (12 digits)
• Sample type (2 digits)
• Time points (2 digits)
Example USN: for "first serum" extraction
USN = ONCO FR 01 0021 **SE01**

Sample processing

ENRICHMENT OF THE MONONUCLEAR CELL (MNC) FRACTION

- MIX THE BLOOD IN THE PRESERVATIVE TUBE BY MANUALLY INVERTING IT FIVE TIMES. THEN, CAREFULLY REMOVE THE RUBBER STOPPER.

- USING A MICROPIPETTE WITH A STERILE TIP, TRANSFER 5 ML OF BLOOD FROM THE PRESERVATIVE TUBE INTO A CORRESPONDING 15 ML CENTRIFUGE TUBE PROVIDED WITH 3 ML OF HISTOPAQUE (DENSITY GRADIENT MEDIUM).



NOTE: THIS STEP SHOULD BE PERFORMED WITH CAUTION TO PREVENT THE MIXING OF THE BLOOD/BUFFY COAT WITH THE DENSITY GRADIENT MEDIUM.

- Centrifuge the sample at 400 x g for 30 minutes at RT using **A swing bucket centrifuge**. Set the centrifuge brake to "off" or if your centrifuge provides a variable braking feature, set the brake to the lowest brake setting. Following sample centrifugation, visually inspect each sample tube for separation of plasma and red blood cells.

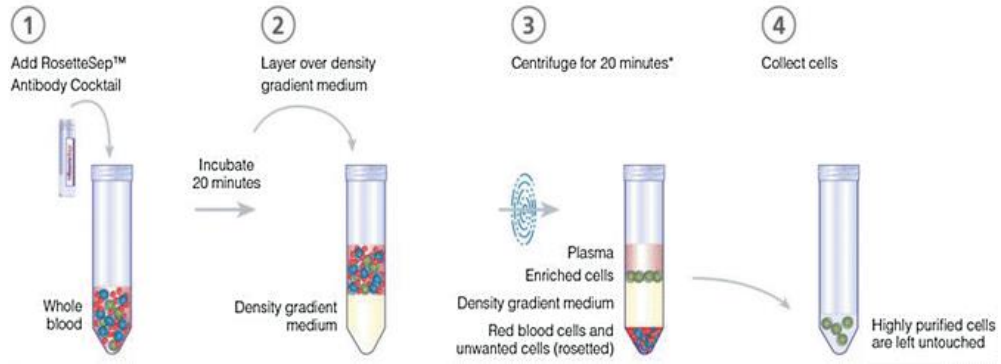
- Collect the Mononuclear cells layer (cloudy) localized between the yellow and the clear bands into a new tube.

- Add PBS to Wash and centrifuge for 10 minutes at 250 x g. Reject the supernatant and keep the pellet.

- Resuspend the PBMCs in FBS with 10% DMSO and store at -80ºC, for example in Mr. Frosty.

Enrichment step using RosseteSep CTC Enrichment Cocktail

- The RosetteSep™ antibody cocktail is a mixture that attaches to unwanted cells in human whole blood, forming immunorosettes (Blood cells and unwanted cells). These immunorosettes increases the density of the unwanted (rosetted) cells, making them pellet alongside the free RBCs when the blood sample is centrifuged over a density gradient medium. As a result, the Desired cells are left untouched (non-labelled with antibody) and can be easily collected as a highly enriched population at the interface between the plasma and the density gradient medium.



- Use whole peripheral blood collected within the last 24 hours and stored at room temperature;

- Collect sample (up to 15 mL per tube);

- Add RosetteSep™ Cocktail to the sample (50 uL/mL);

- Mix and incubate at RT for 20 min;

- Dilute the sample with the recommended medium and mix gently - Equal volume to sample;

- Add the density gradient medium to the required tube (for 5 mL whole blood, add 1.5 mL; for 14 mL whole blood, add 3 mL);

- Add the diluted sample to the tube containing the density gradient medium (carefully to minimize mixing);

- Centrifuge at 1200 x g for 20 min (brake off);

- Collect the enriched cells by harvesting the enriched cell layer with a pipette and transfer them to new tube;

- Wash the enriched cells, fill up with recommended medium (Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905);

- Centrifuge at 300 x g for 10 minutes.

- repeat the process twice.

- Resuspend the cells in recommended medium (Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum (Catalog #07905).

Sample storage and shipment

After collection, samples must be stored in upright position, shipped, and processed within 96 hours of collection to ensure optimal quality (including transportation).

Samples must be stored at temperatures of 15-30°C (59-86°F).

DO NOT REFRIGERATE SAMPLES.

SOP

NMR Metabolomics

Abbreviations

NMR = Nuclear Magnetic Resonance

EDTA = Ethylenediaminetetraacetate

x g = Minimum centrifugation rate / [g]

Objective

Generate metabolomics biomarkers for CRC.

Equipment, consumables, and reagents

Tubes for blood taking

Plasma

Name	Supplier	Cat. Number
S-Monovette K3 EDTA, 9 ml, code red, for plasma separation	Sarstedt	02.1066.001
S-Monovette K3 EDTA, 4.9 ml, code red, for plasma separation	Sarstedt	04.1931.001
Or similar from other manufacturers		

Serum

Name	Supplier	Cat. Number
S-Monovette Serum, 9 ml, code white, with clot activator	Sarstedt	02.1063.001
S-Monovette Serum, 4.9 ml, code white, with clot activator	Sarstedt	04.1934.001
Or similar from other manufacturers		

Sample storage vials

Name	Supplier	Cat. Number
Safe-Lock-Vials 1,5 ml	Eppendorf	0030120086
Safe-Lock-Vials 2 ml	Eppendorf	0030121597
Vial 1,5 ml, screw cap	Biozym	710704
Or similar from other manufacturers		

Sample storage box

Freezer storage boxes should not exceed 130 x 130 x 50 mm.

Safety procedures

Operating instructions

Notes:

Ones frozen, samples must not thaw – Handle and transport on dry ice!

The test person should be fasted for eight hours if possible, unless a specific study designs is applied.

Sample collection procedure

Collect blood in serum S-Monovette or plasma EDTA-S-Monovette. Send min 500μL for NMR metabolomics measurements.

*Centrifugation conditions for the S-Monovette according to the manufacturer's instructions

https://www.sarstedt.com/fileadmin/user_upload/99_Broschueren/NEU/780/20_780_0500_200_zentrifugationsbedingungen_s_monovette_1021.pdf

Plasma

EDTA is used as anticoagulant for plasma preparation.

1) Collect blood samples from a peripheral vein directly into EDTA-S-Monovette.

2) Turn the EDTA-S-Monovette 180° (invert, do not shake!) 2 to 3 times immediately after blood collection to ensure that the blood is completely mixed with the anticoagulant. Store the EDTA-S-Monovette upright at room temperature until plasma separation is complete.

3) Centrifugation at 2500-3000 x g, 10 min, 18-25°C.

4) Transfer the plasma supernatant into a 15 ml centrifuge tube (e.g. Falcon) without aspirating blood cells using disposable pipette tips. Close tube and turn plasma 180° (invert, do not shake!) 2 to 3 times.

5) Aliquot in at least 500 µl plasma into labeled cryotubes. Freeze as quickly as possible at -80°C. Store vials in boxes (not in bags).

Serum

1) Collect blood samples from a peripheral vein into the serum collection tube with clotting activator.

2) Turn the serum S-Monovette 180° (invert, do not shake!) 2 to 3 times immediately after blood collection.

3) Store the serum S-Monovette upright at room temperature for at least 30 minutes, until the coagulation is completely finished (min. 30 min). (Observe coagulation times according to manufacturer's instructions (min. 30 min!).

4) Centrifugation at 1800-2500 x g, 10 min, 18-25°C .

6) Transfer the serum into a pre-cooled 15 ml centrifuge tube (e.g. Falcon) without aspirating blood cells using disposable tips. Close tube and turn serum 180° (invert, do not shake!) 2 to 3 times.

7) Aliquot and freeze serum samples as quickly as possible after centrifugation.

8) Aliquot at least 500 µl serum into pre-cooled labeled cryovials. Freeze immediately at -80°C. Store vials in boxes (not in bags).

Sample labelling

Anonymization of samples and data

Each sample collection tube should bear an adhesive sticker with the information according to the specific matrix type. Patient details should be written in a legible hand written form on the sample tubes with indelible pen BEFORE collection of samples from the subject. Details must include the following:

- Matrix type **(1)**
- Unique Patient Number (UPN) **(2)**
- Date (DD/MM/YYYY) **(3)**
- Time of collection (use the 24h clock format) **(4)**

Sticker labels, for each matrix type, will be in the following format:

Serum (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

RNA (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

Unique Patient Number:

Subjects will be assigned a Unique Patient Number according to the chronological order of inclusion that will be a specific and unique code created for each subject of 12 digits.

The UPN codes will be always used to refer the country and the correspondent subject information. The UPN number should include information about:

a- Study or protocol small name (4 characters: ONCO)
b- Country code of sample collection (2 characters according to the ISO country code)
c- Centre number: (2 digits: 01)
d- Patient number (4 digits: from 0001 to 9999)
Example UPN: ONCO FR 01 0021

Unique Sample Number:
Unique Sample Number (USN) will include the subject code ending in 2 characters that represent the sample type (serum / plasma subtype / urine / tissue type / DNA) and a digital addendum for counting of longitudinal samples. ***Codes for Sample Type***

Fresh whole blood (Lithium-Heparin tube)	WB
Serum	SE
Plasma EDTA	ED
Plasma Li-Heparin	LH
PBMC	PB
Spot Urine	US
PAXgene RNA tube	PX
PAXgene DNA tube	PD

The USN number should include information about:
• Subject number or UPN (12 digits)
• Sample type (2 digits)
• Time points (2 digits)
Example USN: for "first serum" extraction
USN = ONCO FR 01 0021 **SE01**

Sample processing

As described above:

Prepare serum or EDTA plasma according to
https://www.sarstedt.com/fileadmin/user_upload/99_Broschueren/NEU/780/20_780_0500_200_zentrifugationsbedingungen_s_monovette_1021.pdf

Serum: 10min centrifugation at 1800-2500g and 18-25°C

EDTA-Plasma: 10min centrifugation at 2500-3000g and 18-25°C

Sample aliquoting

500μL

Sample storage and shipment

Ideally frozen on dry ice.

Associated and Referenced Documents & Forms

*Centrifugation conditions for the S-Monovette according to the manufacturer's instructions

https://www.sarstedt.com/fileadmin/user_upload/99_Broschueren/NEU/780/20_780_0500_200_zentrifugationsbedingungen_s_monovette_1021.pdf

There are currently no known markers for CRC using NMR metabolomics, even though there are publications which show feasibility:

- T. Buergel, J. Steinfeldt, G. Ruyoga, M. Pietzner, D. Bizzarri, D. Vojinovic, J. Upmeier Zu Belzen, L. Loock, P. Kittner, L. Christmann, N. Hollmann, H. Strangalies, J. M. Braunger, B. Wild, S. T. Chiesa, J. Spranger, F. Klostermann, E. B. Van Den Akker, S. Trompet, S. P. Mooijaart, N. Sattar, J. W. Jukema, B. Lavrijssen, M. Kavousi, M. Ghanbari, M. A. Ikram, E. Slagboom, M. Kivimaki, C. Langenberg, J. Deanfield, R. Eils, U. Landmesser, *Nat Med* **2022**, *28*, 2309–2320.
- Vignoli, E. Risi, A. McCartney, I. Migliaccio, E. Moretti, L. Malorni, C. Luchinat, L. Biganzoli, L. Tenori, *IJMS* **2021**, *22*, 4687.

SOP
Sampling room requirements for Breath

Objective

- To avoid confounding factors in breath analysis related to the breath collection room potential air contamination.
- is to ensure proper handling with the equipment for quantitative breath sample collection. The provided requirements concern exclusively the breath sampling protocol related to the WP3 of the ONCOSCREEN project.

Operating instructions

2.1 Collection room requirements

Where possible the sample collection room should be located far from storerooms of food, drugs, solvents or disinfectants. Breath samples should not be taken in freshly build, or renovated buildings to avoid high background of volatile organic compounds. The area of the collection room should be larger than 10 m^2. The room should also be free from any major sources of volatile organic compounds such as:

- drugs
- solvents and disinfectants
- kitchen waste, food, beverages
- cosmetics, perfumes

Any identified sources of VOCs (containers, bottles trashcans) should be removed minimum one week before sampling.

2.2 Room Temperature

Air temperature has an impact on the sampling protocol. Sampling should be performed at room temperature (i.e. 20-25°C). Samples taken at temperatures outside this range should be discarded.

2.3 Presence of humans

When possible, only the sampling officer and the study subject should be present in the collection room. The presence of other people during sampling should be avoided as well as the presence of any humans in the sampling room min 1 h prior the sampling.

2.4 Room for collecting samples from cancer patients and controls (non-cancer subjects)

Collection of breath samples in the same room and same timeframe for cancer patients and control subjects within the same WP is highly recommended.

If this is not done, the premises for sample collection should be coded and should be identifiable.

SOP
Instructions to the study personnel involved in breath sample collection

Objective

The aim of this document is to approach how to avoid inappropriate results of the breath test due to incorrect preparation for the breath sampling as well as due to VOCs originating from the medical staff, proper documentation of potential confounders. The provided requirements concern exclusively the breath sampling protocol related to the WP1 of the VOGAS project.

Operating instructions

1. Information to be communicated to the study subject prior to the day of breath collection

The potential study subjects should be informed by a telephone call or in writing (printed information sheets) at least 24 hours prior to the time of the planned procedure in order to ensure compliance with the requirements. This is considered that in exceptional cases (e.g. the potential study subject is admitted to the hospital for the next day surgery) this might not be possible.

The provided instructions should include the following restrictions (timeframe relates to the time period preceding the moment of planned sampling):

1) Fast for at least 12 hours;
2) Refrain from coffee, tea and soft drinks for at least 12 hours;
3) Refrain from smoking for at least 2 hours;
4) Avoid alcohol for at least 24 hours;
5) Do not clean teeth at least 2 hours before the procedure (no brushing, no mouthwash, no flossing if the floss has any aroma);
6) Avoid chewing gum and any mouth fresheners for at least 12 hours;
7) Refrain from using cosmetics/fragrances prior to the procedure on the day of test;
8) Avoid excessive physical activity (gym, jogging, cycling, intense physical work) at least 2 hours prior to the test.

Medication that are used for regular treatment according to medical indications should not be discontinued.

Use of water is allowed.

2. **What requirements the involved study personnel should follow**

The room should be free from any major sources of volatile organic compounds such as:

- drugs
- solvents and disinfectants
- kitchen waste, food, beverages
- cosmetics, perfumes.

Therefore, the staff is prohibited from using cosmetics or perfumes. Eating and using disinfectants in the test room is also prohibited.

3. **Data collection**

Before to start the breath sample collection, staff must ask the patient the clinical questionnaire: personal data, confounding factors and 24h dietary recall (see Appendix 1.,2.,3.).

4. **Instructions related to the breath sampling procedure**

These instructions have to be communicated to the study subject before breath sample collection. The study personnel should ensure that the instructions are followed.

The study subject should be advised to sit quietly for minimum 10 min. prior the breath sampling to avoid temporal changes in levels of breath VOCs related to body movement, or changing of the body posture.

The study subject should breath in a normal, relaxed way involving only the tidal volume of the lungs. The study subject should avoid breathing manoeuvres such as deep breathing, breath holding, or hyperventilation.

5. **Breath sample collection instructions**

Breath samples could be collected for one of the following purposes / analyses:

1) Samples for GC-MS analysis (to be collected in adsorbent media for transportation to Innsbruck for analysis)
2) For analysis in the breath analyser (data acquisition will take place on site)

Ideally parallel collections of samples for different analysis (No 1 & 2; No 1 & 3) would be conducted.

Procedures No.1. and No.2. are described in detail in specific SOPs.

Appendix i. Clinical questionnaire - personal data. English version.

PERSONAL DATA

Inclusion criteria:

- Adult individuals (>18 years of age)
- Having signed consent form
- Able to provide a breath sample
- Willingness to collaborate
- For the cancer group: gastric adenocarcinoma has to be documented histologically (histological diagnosis following gastric surgery is also accepted) or patients being confirmed adenocarcinoma during the course of the study
- For the non-cancer group: available biopsy data with biopsies from the corpus and antrum as the minimum within 2 months prior to the breath sampling to 2 months after breath sampling (if no therapy change (chemotherapy, antibiotics, PPIs) has occurred during this window

Exclusion criteria:

- Other (than gastric cancer) active known malignant diseases
- Ongoing neoadjuvant chemotherapy
- Stomach surgery in the past (except vagotomy and ulcer suturing)
- Inflammatory bowel disease
- End-stage renal insufficiency
- DM type I
- Bronchial asthma (active)
- Small bowel resections in the past

---

1. **Patient code:** __ __ __ __ __ __ __ __
2. **Name:**_____
3. **Surname:**_____
4. **State phone code:** + __ __ __
5. **Phone:** __ __ __ __ __ __ __ __
6. **Code of the interviewer:** __ __ __ __ __ __ __ __ __ __
7. **Research Center:**_____
8. **Sex:**
   - Female
   - Male
9. **Date of birth (dd.mm.yyyy.):** __ __.__ __.__ __ __ __.
10. **Signed VOGAS consent form:**
    - No
    - Yes
11. **Signature date VOGAS (dd.mm.gggg.):** __ __.__ __.__ __ __ __.

12. Your ethnic group (self-defined):
- Caucasian
- Asian
- Hispanic / Latino / Latin American
- Black
- Indigenous population
- Mixed

13. Place of birth:
- National
- Foreign

14. Have any of your 1st degree relatives (parents, brother, sister) had stomach cancer?
- No
- Yes
-

15. Did you have malignant disease in the past?
- No
- Yes

16. Medical history:
- Diabetes mellitus type II
- Asthma
- Celiac Disease
- Inflammatory bowel disease (Crohn's disease, Ulcerative colitis)
- Chronic kidney disease
- None

17. Have you ever had chemotherapy?
- No
- Yes

17.1. What month:_____
17.2. What year:_____

18. Have you ever had radiotherapy?
- No
- Yes

19. Have you ever had organ transplantation?
- No
- Yes

20. Height of study participants, cm:_____

21. Weight of study participants, kg:_____

22. Interview completed: ___.___._____.

Appendix ii. Clinical questionnaire - confounding factors. English version.

CONFOUNDING FACTORS

1. **Date of execution:** __ __.__ __.__ __ __ __.
2. **Time of execution:**_____
3. **Air quality of the room (evaluated by the interviewer):**
   - ☐ Regular
   - ☐ Smelly, not ventilated
   - ☐ Hot
   - ☐ The smell of disinfectants, solvents
   - ☐ The smell of food/kitchen or waste
   - ☐ Other perfumes, odors
4. **Room's air sample No.1:**__ __ __ __ __ __
5. **Breath sample No.1.:** __ __ __ __ __ __
6. **Breath sample No.2.:** __ __ __ __ __ __
7. **Room's air sample No.2 (Ulm):**__ __ __ __ __ __
8. **Breath sample No.3. (Ulm):**__ __ __ __ __ __
9. **Are you a smoker?**
   - ○ No
   - ○ Yes
10. **When was the last time you smoked?**
    - ○ last hour
    - ○ in the last 2 hours
    - ○ more than 2 hours ago
    - ○ more than 4 hours ago
    - ○ more than 8 hours ago
11. **When was the last time you drank coffee/tea?**
    - ○ last hour
    - ○ in the last 2 hours
    - ○ more than 2 hours ago
    - ○ more than 4 hours ago
    - ○ more than 8 hours ago
12. **When was the last time you ate?**
    - ○ last hour
    - ○ in the last 2 hours
    - ○ more than 2 hours ago
    - ○ more than 4 hours ago
    - ○ more than 8 hours ago
13. **Have you cleaned or used chewing gum/breath fresheners in the last 2 hours?**
    - ○ No
    - ○ Yes
14. **Have you used perfume or toilet water today?**

- No
- Yes

15. **When was the last time you drank alcohol?**
    - last hour
    - in the last 2 hours
    - more than 2 hours ago
    - more than 4 hours ago
    - more than 8 hours ago

16. **Have you done any heavy physical activity in the last hour?**
    - No
    - Yes

17. **Have you used PPIs within the last two weeks?**
    - No
    - Yes

18. **Have you used antibiotics within the last month?**
    - No
    - Yes

19. **Assessment of medical personnel:**
    - ☐ There is no smells/perfume/odor that could potentially affect the results of the test
    - ☐ Fragrance of deodorant use could be smelled
    - ☐ Cosmetics used (evident)
    - ☐ Other smells/odors could be sensed

20. **Comments on the test execution:**
    - ☐ Test performed according to requirements
    - ☐ It was difficult for the participant to exhale enough, but the test was carried out
    - ☐ The participant could not perform the test due to physical problems
    - ☐ The participant could not perform the test because he/she did not correctly understand the instructions even after repeated attempts
    - ☐ The test has not been performed due to technical problems

21. **Test was performed by**:_____

Appendix iii. Clinical questionnaires - 24-hour dietary recall. English version.

24h DIETARY RECALL

1. Do you follow a special diet?

Diet	No	Yes
Vegetarian diet	○	○
Vegan diet	○	○
Diabetic diet	○	○
Gluten free diet	○	○
Lactose free diet	○	○
Raw food diet	○	○

2. Did you eat mammalian meat in the last 24 h?

Mammalian meat	Boiled, steamed	Fried	Grilled, barbequed	Smoked	Salted, dried	Other cooked	Can't tell
Beef	□	□	□	□	□	□	□
Pork	□	□	□	□	□	□	□
Lamb	□	□	□	□	□	□	□
Goat	□	□	□	□	□	□	□
Other/unknown	□	□	□	□	□	□	□

3. Did you eat poultry in the last 24 h?

Poultry	Boiled, steamed	Fried	Grilled, barbequed	Smoked	Salted, dried	Other cooked	Can't tell
Chicken	□	□	□	□	□	□	□
Duck	□	□	□	□	□	□	□
Quail	□	□	□	□	□	□	□
Other/unknown	□	□	□	□	□	□	□

4. Did you eat any fish or seafood in the last 24 h?

Fish, seafood	Boiled, steamed	Fried	Grilled, barbequed	Smoked	Salted, dried	Other cooked	Can't tell
Fish	□	□	□	□	□	□	□
Shrimp	□	□	□	□	□	□	□
Octopus	□	□	□	□	□	□	□
Squid	□	□	□	□	□	□	□
Other/unknown	□	□	□	□	□	□	□

5. Did you eat any reptiles or amphibians in the last 24h?

Reptiles, amphibians	Boiled, steamed	Fried	Grilled, barbequed	Smoked	Salted, dried	Other cooked	Can't tell
Snakes	☐	☐	☐	☐	☐	☐	☐
Frogs	☐	☐	☐	☐	☐	☐	☐
Alligators	☐	☐	☐	☐	☐	☐	☐
Turtles	☐	☐	☐	☐	☐	☐	☐
Other/unknown	☐	☐	☐	☐	☐	☐	☐

6. **Did you eat any vegetables in the last 24 h?**

Vegetables	Fresh	Cooked
Cucurbits (pumpkins, courgettes, cucumbers etc.) - fresh	☐	☐
Okra	☐	☐
Roots vegetables (onions, carrots, turnips, ginger, beets, garlic, radishes, etc.)	☐	☐
Nightshade vegetables (tomatoes, eggplants, okra, peppers etc.)	☐	☐
Legume vegetables (beans, peas etc.)	☐	☐
Cruciferous vegetables (broccoli, kale, cabbage, brussels sprouts, collard greens etc.)	☐	☐
Perennial vegetables (asparagus, watercress, wild leeks etc.)	☐	☐
Tubers (potatoes, sweet potatoes, taro, tapioca, yam etc.)	☐	☐
Other/unknown	☐	☐

7. **Did you eat any mushrooms in the last 24h?**
   - No
   - Yes
     - ☐ Fresh
     - ☐ Cooked

8. **Did you eat any nuts or seeds in the last 24h?**
   - No
   - Yes

9. **Did you eat/drink any dairy products in the last 24 h?**

	Milk	Fermented milk	Yogurt	Kephir	Cheese and other dairy products
Cow	☐	☐	☐	☐	☐
Buffalo	☐	☐	☐	☐	☐
Goat	☐	☐	☐	☐	☐
Other/unknown	☐	☐	☐	☐	☐

10. **Did you eat any type of eggs in the last 24 h?**
    - No
    - Yes

11. **Did you use any kind of sauces in the last 24h?**
    - No
    - Yes

12. Did you eat any fruits in the last 24h?

Fruits	Fresh	Cooked
Citrus fruits (grapefruit, lime, orange, mandarin etc.)	☐	☐
Drupe, stone fruits (apricots, nectarine, coconut, etc.)	☐	☐
Soft, fleshy fruits (peaches, kiwi fruit, strawberries, etc.)	☐	☐
Apple, pear	☐	☐
Berries (currants, blackberries etc.)	☐	☐
Pineapple	☐	☐
Other/unknown	☐	☐

13. Did you eat any snacks (candies, cakes, chocolate, jelly) in the last 24 h?

	No	Yes
Candies	○	○
Cakes	○	○
Chocolate	○	○
Jelly	○	○
Other/unknown	○	○

14. Did you drink any drinks in the last 24h?

	No	Yes
Herbal tea	○	○
Black/green tea	○	○
Flavoured tea	○	○
Fermented tea (Kombucha)	○	○
Coffee	○	○
Chocolate-based drinks	○	○
Industrialized juice	○	○
Beer	○	○
Wine	○	○
Tequila, Whisky	○	○
Gin	○	○
Saquê	○	○
Vodka	○	○
Other/unknown	○	○

15. Did you use any spices in the last 24 h?

	No	Yes
Chilli pepper	○	○
Red pepper	○	○
Long pepper	○	○
Pepper	○	○
Nutmeg	○	○
Star-anise	○	○
Ginger	○	○
Sesame	○	○
Basil	○	○
Chamomile	○	○
Hickory	○	○
Tarragon	○	○
Cubeb	○	○
Bay leaf	○	○
Cinnamon	○	○
Mustard	○	○
Wasabi	○	○
Allspice	○	○
Clove	○	○
Anise	○	○
Caraway	○	○
Celery	○	○
Chervil	○	○
Coriander	○	○
Cumin	○	○
Dill	○	○
Fennel	○	○
Parsley	○	○
Saffron	○	○
Cardamom	○	○
Turmeric	○	○
Vanilla	○	○
Mint	○	○
Other/unknown spices	○	○

16. Interview completed: __ __.__ __.__ __ __ __.

Appendix iv. Clinical questionnaires – Medication documentation prior to the breath analysis. English version.

(Attributable to 1 month prior enrolment, including for the day of enrolment if not indicated otherwise)

Sedation prior surgery ☐ - started (to be detailed below in the list)

PPIs (Proton pump inhibitors), such as omeprazole, esomeprazole, pantoprazole, rabeprazole, lanzoprazole

Brand	Generic name	Dose	Last intake
		_____ mg x ____	
		_____ mg x ____	

Have PPIs been used within the last 2 weeks: ☐ - NO ☐ - YES,

Antibiotics

Brand	Generic name	Dose	Last intake

Have antibiotics been used within the last month: ☐ - NO ☐ - YES,

Probiotics, including food supplements (e.g. Actimel)

Brand	Generic name	Dose	Last intake

Have probiotics been used within the last week: ☐ - NO ☐ - YES

Chemotherapy drugs

Brand	Generic name	Dose	Last intake

Have chemotherapy been ever received: ☐ - NO ☐ - YES

NSAID (non-steroidal anti-inflammatory drugs) or aspirin

Brand	Generic name	Dose	Last intake

Drugs for cardiovascular disease

Brand	Generic name	Dose	Last intake

Drugs for respiratory disease including inhaled medication

Brand	Generic name	Dose	Last intake

Drugs for endocrine disease, including insuline, hormonal therapy

Brand	Generic name	Dose	Last intake

Other drugs

Brand	Generic name	Dose	Last intake

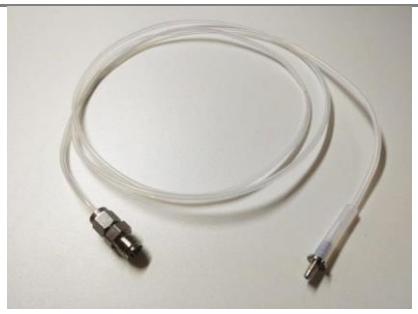
SOP
CO2 controlled breath sampling into Tenax tubes

Objective

The aim of this document is to assure proper handling with the equipment for quantitative breath sample collection. The provided requirements concern exclusively the breath sampling protocol related to the WP3 of the ONCOSCREEN project.

Equipment, consumables, and reagents

Name	Supplier	Cat. Number	Item Photo
Disposable mouthpieces 22F	Intersurgical	1931000	
Elbow 15AD/ 22AD-15ID	Intersurgical	2714000S	
Reusable PTFE ¼" nut	provided by UIBK		

CO_2 measurement IRMA airway adapter	Masimo	01-02-0879	
CO_2 IRMA mainstream analyzer	Masimo *(can be replaced by any commercial capnograph)*	200101	
Tenax inert sorbent tubes	Markes	C1-C(TO BE DEFINED)X-5003	
Teflon tube equipped with a Luerlock stainless steel connector and ¼" to 1/8" stainless steel adapter	in-house made		
Glass Syringe 250 mL equipped with a Luer-lock connector	Socorex	155.05250	
Stopcock Discofix C	Braun Melsungen AG	BRAUN-4095111	

CapLok tool	Markes	C-CPLOK	
PTFE ¼" ferrules	Markes int.	C-FP200	
¼" stainless steel nut			

Safety procedures

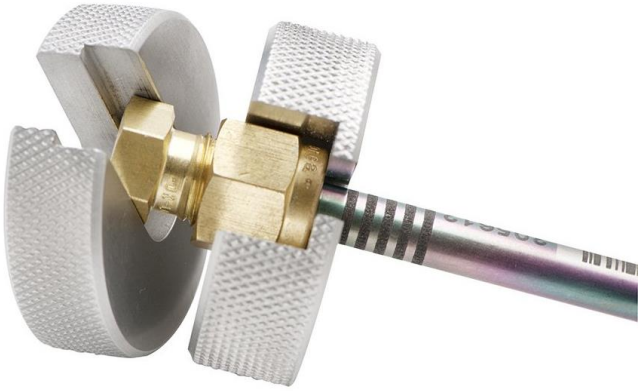
Operating instructions

Sample collection procedure - Sampler assembling for breath sampling

1.1 Connect the 250 mL syringe with the Teflon tube using the Discofix Stopcock.

1.2 Screw manually the PTFE nut on the elbow. Do not tighten it.

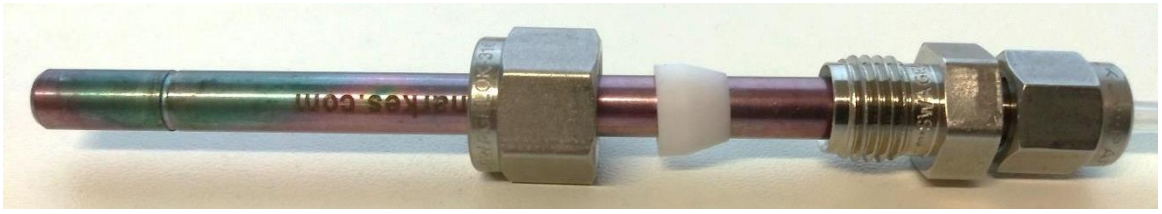
1.3 Remove the storage caps from the sorbent tube using the CapLok tool. Do not use any wrenches!



1.4 Put the ¼" steel nut on the non-grooved end of the Tenax tube.

1.5 Put the ¼" PTFE ferrule nut on the non-grooved end of the Tenax tube with the conical end toward the end of the Tenax tube.

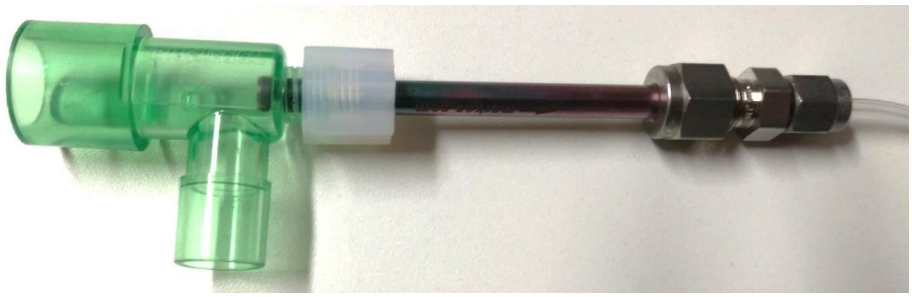
1.6 Insert the non-grooved end of the Tenax tube into the stainless steel adapter till you feel resistance.



1.7 Tighten the nut manually and next using the CapLok tool.

1.8 Inspect if the tube is firmly fixed in the adapter (it should not move out).

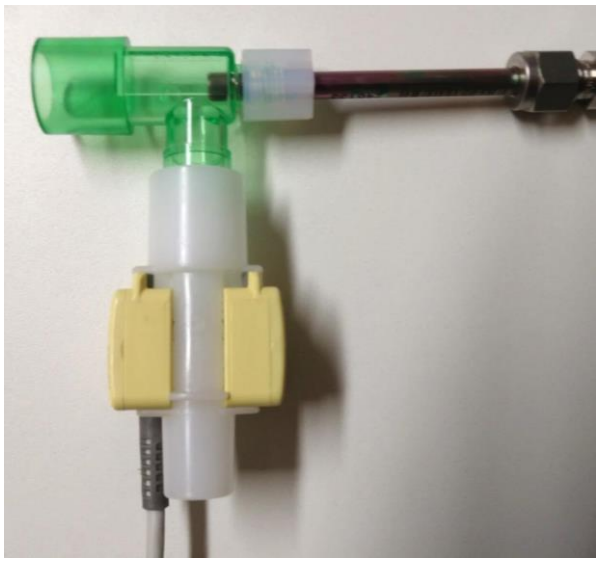
1.9 Insert the grooved end of the Tenax tube into the elbow via the PTFE nut until the PTFE ferrule drops into the groove.



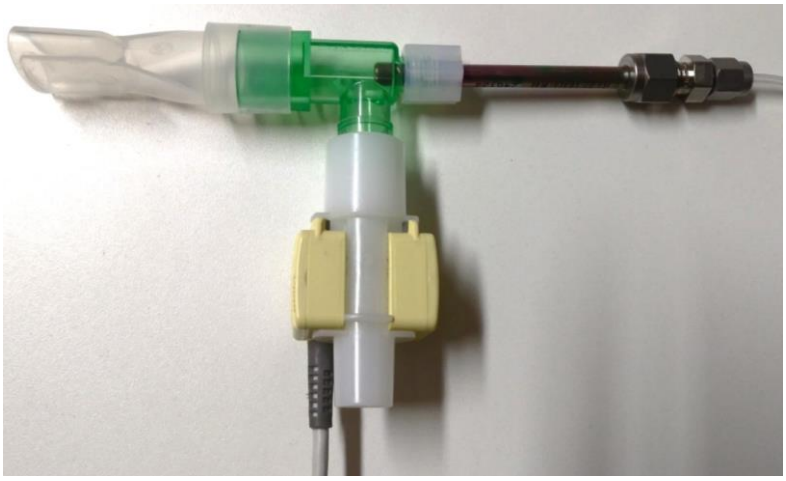
1.10 Tighten the PTFE nut manually.

1.11 Inspect if the tube is fixed in the elbow (it should not move out).

1.12 Connect the CO2 IRMA analyzer to the elbow. (or CO2 sensor/capnometer)



1.13 Connect the 22F mouthpiece to the elbow



1.14 Connect the USB connector of the CO2 IRMA analyzer to the computer (start capnometer If other CO2 sensor is used).

1.15 Start the Datalogger program for the IRMA analyzer.

2. Setting of the Datalogger program (for the IRMA CO2 analyzer)

This procedure should be performed only once during the first run of the Datalogger program on a new computer.

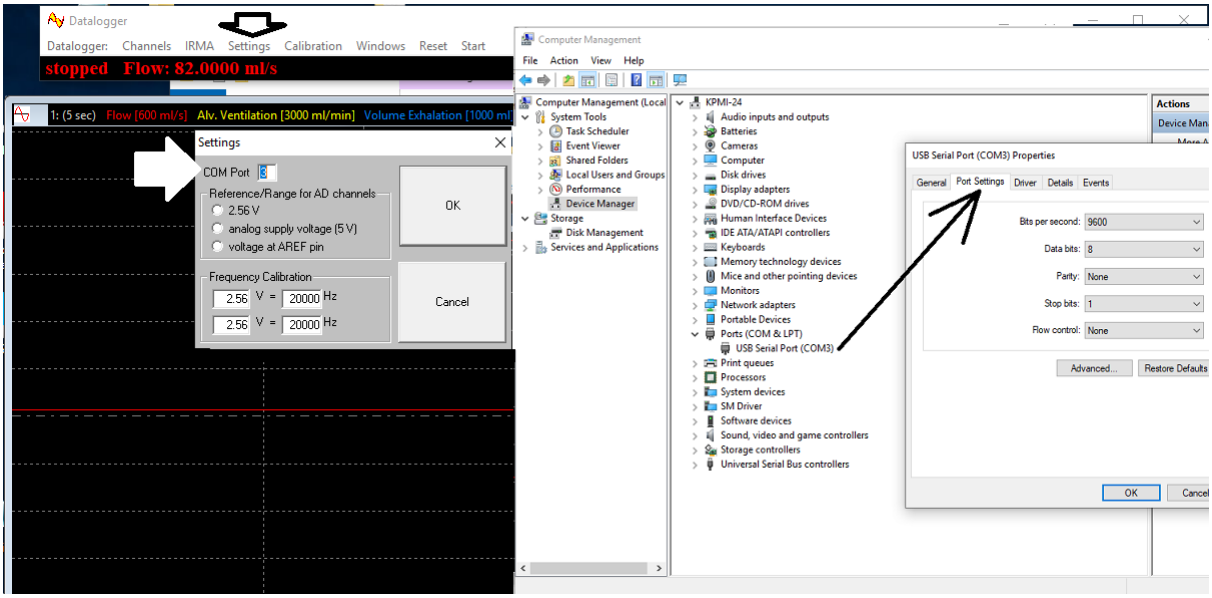
1. Connect the CO_2 IRMA analyzer to the computer
2. Identify the COM port assigned to the CO_2 IRMA analyzer to the computer.
3. Start the Datalogger program
4. Set the number of the COM port assigned to the CO_2 IRMA analyzer in the Datalogger program

   Settings -> COM Port -> OK (see figure below)

5. Check if the CO_2 IRMA analyzer has been properly identified by the Datalogger program (the red line in the visualization window should react to the CO_2 changes)

COM port settings:

- speed – 9600 bit/s
- data bit – 8
- parity – none
- stop bits – 1
- flow control – none



The system is ready for breath sampling.

Number of samples

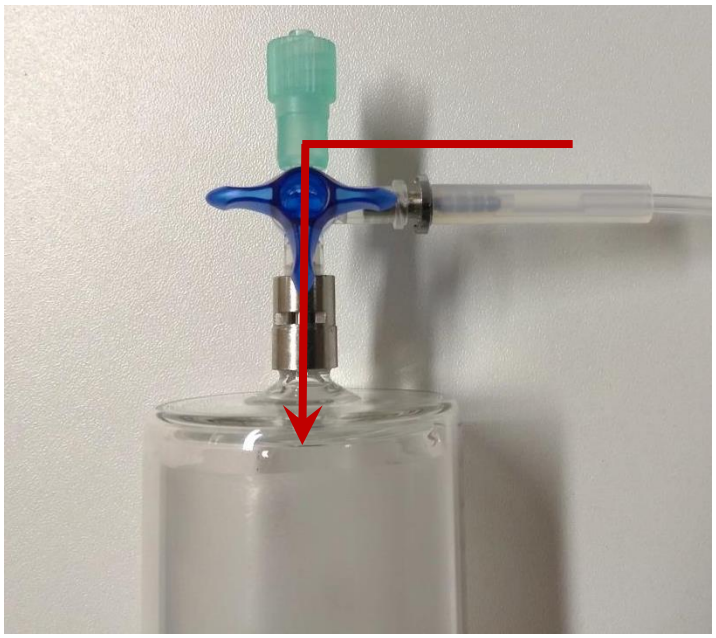
For GS-MS: 1 room air sample for each collection day, 1 breath samples.

For breath analyser: 1 room air sample, 1 breath sample.

Breath sampling into Tenax tubes

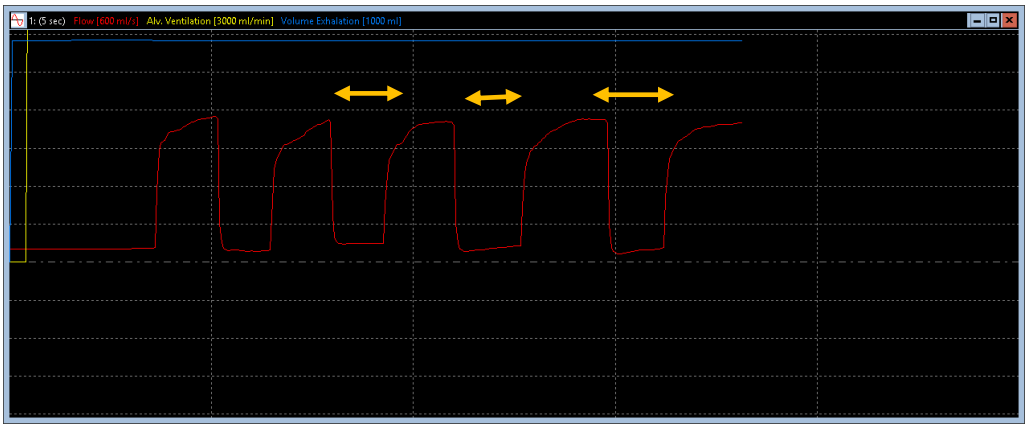
The subject **may take a rest and pause at any time during** the breath collection procedure. If the subject is tired after the first absorbent tube collection process has been accomplished and seems unable to collect the second sampling, the collector may stop the procedure.

1. Advise the subject to sit quietly for minimum of 10 min.
2. Advise the subject to breath in a relaxed manner and to avoid breathing maneuvers such as deep breathing, breath holding, or hyperventilation.
3. Turn the stopcock so as it connects the Teflon tube with the syringe.

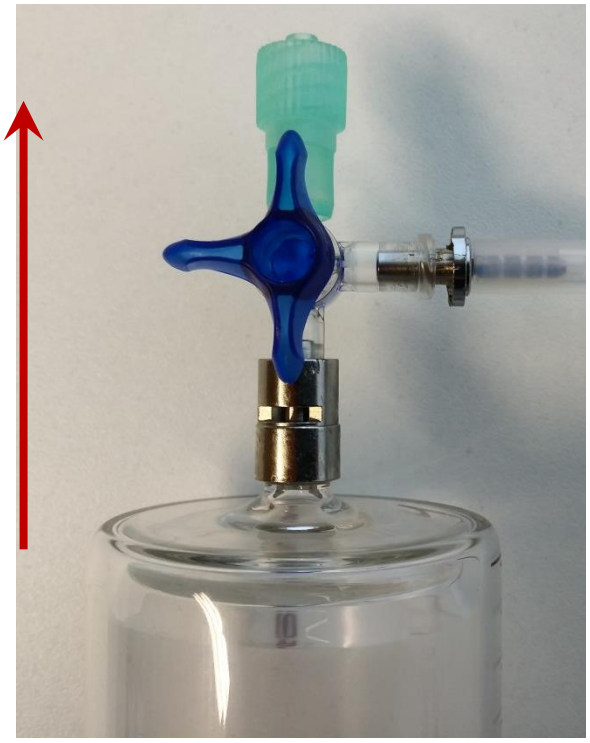


4. Advise the subject to hold the sampler, however, without touching the Tenax tube and breathe via the mouthpiece.

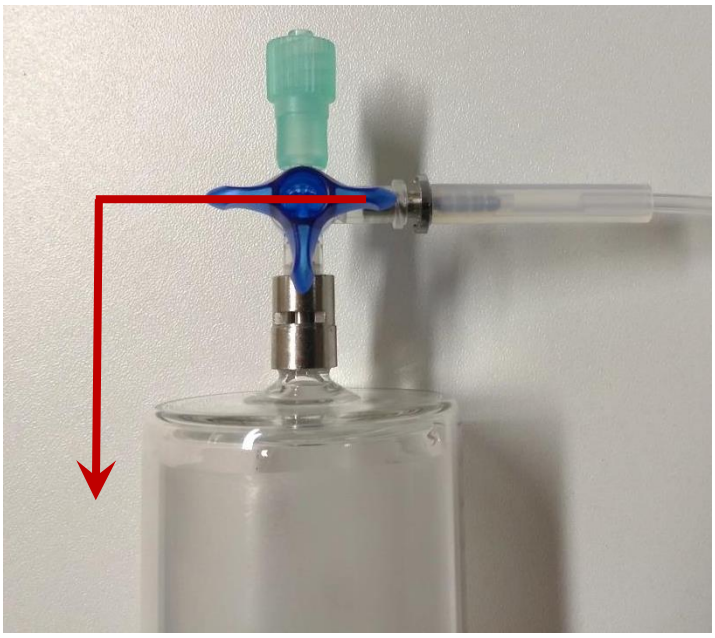
5. Inform the subject that he/she can stop breathing via mouthpiece at any time and make a break during sampling.
6. Observe the CO_2 profiles on the screen of the capnometer/computer.



7. When the CO_2 signal reaches maximum value (end-tidal phase of the exhalation) draw rapidly 10-15 ml of the breath gas.
8. Repeat (7) until 250 mL of breath gas is collected.
9. Turn the stopcock so as it connects the syringe with the vent outlet and closes the Teflon tube.



10. Remove air from the syringe.
11. Turn the stopcock so as it connects the Teflon tube with the syringe.



12. Repeat points 6-10 to collect the next 250 mL of breath gas.

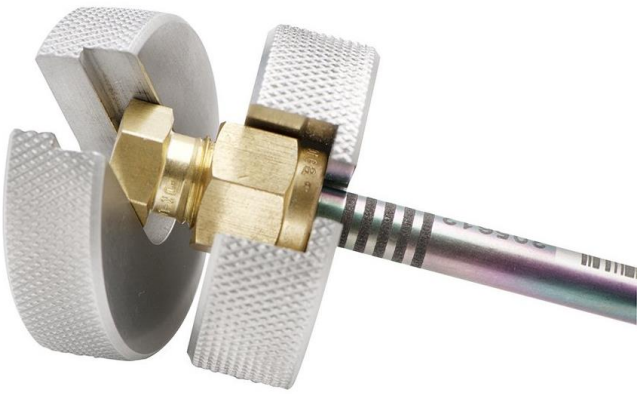
The breath sampling is accomplished.

Sampler assembling for room air sampling.

1. Connect the 250 mL syringe with the Teflon tube using the Discofix Stopcock.

2. Screw manually the Teflon nut on the elbow. Do not tighten it.

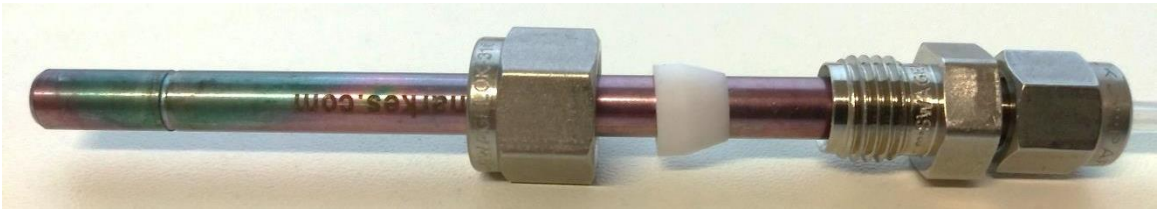
3. Remove the storage caps from the sorbent tube using the CapLok tool. Do not use any wrenches!



4. Put the ¼" steel nut on the non-grooved end of the Tenax tube.

5. Put the ¼" PTFE ferrule nut on the non-grooved end of the Tenax tube with the conical end toward the end of the Tenax tube.

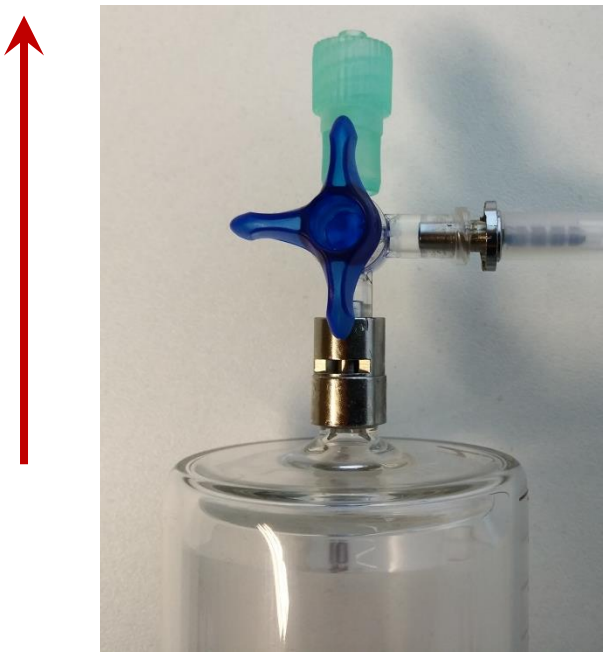
6. Insert the non-grooved end of the Tenax tube into the stainless steel adapter till you feel resistance.



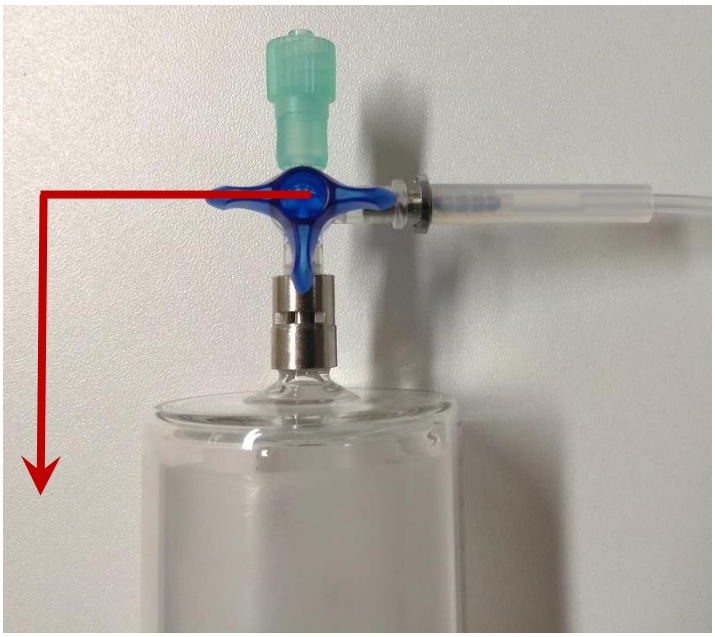
7. Tighten the nut manually and next using the CapLok tool.

Room air sampling

1. Inspect if the tube is firmly fixed in the adapter (it should not move out).
2. Draw slowly 250 ml of the room air.
3. Turn the stopcock so as it connects the syringe with the vent outlet and closes the Teflon tube.



4. Push the plunger and remove air from the syringe.
5. Turn the stopcock so as it connects the Teflon tube with the syringe.



6. Repeat points 10-12 to collect next 250 mL of the breath gas.

Disassembling of the breath sampler

1. Disconnect the CO_2 IRMA analyzer (or Capnometer) from the green elbow.

2. manually unscrew the PTFE nut on the elbow. Gently remove the tube from the elbow part. If necessary, unscrew completely the PTFE nut.

3. Remove the PTFE nut from the Tenax tube.

4. Unscrew the steel nut on the non-grooved end of the Tenax tube using the CapLok tool.

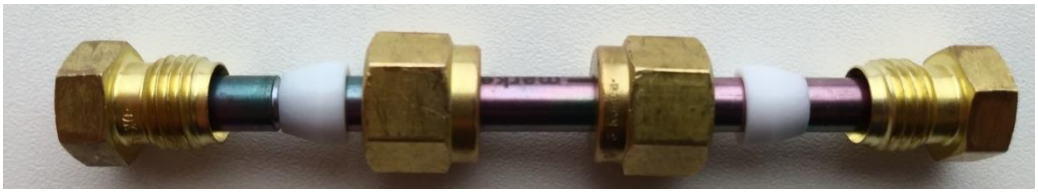
5. Remove the steel nut and the Teflon ferrule from the Tenax tube.

6. Unscrew completely both parts of the storage caps.

7. Put brass ¼" nuts on both ends of the Tenax tube.

8. Put the ¼" PTFE ferrules nut on both ends of the Tenax tube with the conical end toward the ends of the Tenax tube.

9. Insert both ends of the tube into the plugs nut till you feel resistance.



10. Screw both parts of the storage caps manually and next with the CapLok tool. Make sure that the tube is firmly fixed in the storage caps. Do not overtighten!

11. Disconnect mouthpiece from the elbow.

Device Cleaning

All device collection parts must be cleaned after each patient. The capnography connection tube and breath collector must be washed with ethanol solution (70%) and dried for 30 minutes.

Sample labelling

Anonymization of samples and data

Each sample collection tube should bear an adhesive sticker with the information according to the specific matrix type. Patient details should be written in a legible hand written form on the sample tubes with indelible pen BEFORE collection of samples from the subject.

Details must include the following:

- Matrix type **(1)**
- Unique Patient Number (UPN) **(2)**
- Date (DD/MM/YYYY) **(3)**
- Time of collection (use the 24h clock format) **(4)**

Sticker labels, for each matrix type, will be in the following format:

Serum (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

RNA (1)

- **UPN: |ONCO|_|_|_|_|_|_|_|_| (2)**
- **Date: |_|_|-|_|_|-|_|_|_|_| (3)**

Unique Patient Number:

Subjects will be assigned a Unique Patient Number according to the chronological order of inclusion that will be a specific and unique code created for each subject of 12 digits. The UPN codes will be always used to refer the country and the correspondent subject information. The UPN number should include information about:

a- Study or protocol small name (4 characters: ONCO)

b- Country code of sample collection (2 characters according to the ISO country code)

c- Centre number: (2 digits: 01)

d- Patient number (4 digits: from 0001 to 9999)

Example UPN: ONCO FR 01 0021

Unique Sample Number:

Unique Sample Number (USN) will include the subject code ending in 2 characters that represent the sample type (serum / plasma subtype / urine / tissue type / DNA) and a digital addendum for counting of longitudinal samples. ***Codes for Sample Type***

Fresh whole blood (Lithium-Heparin tube)	WB
Serum	SE
Plasma EDTA	ED
Plasma Li-Heparin	LH
PBMC	PB
Spot Urine	US
PAXgene RNA tube	PX
PAXgene DNA tube	PD

The USN number should include information about:

• Subject number or UPN (12 digits)

• Sample type (2 digits)

• Time points (2 digits)

Example USN: for “first serum” extraction

USN = ONCO FR 01 0021 **SE01**

(Instructions for sample labelling will be provided by Firalis, according to clinical centre requests, for global harmonization)

Sample storage and shipment

To ensure the proper stability of breath VOCs Tenax tubes should be stored at -80°C and transported on dry ice. Tubes should be frozen a.s.a.p. after sampling and the storage time should not exceed 6 weeks. The following protocol should be applied:

1. Manually check if the storage caps are properly installed on the tube (the tube should not move from the caps out).
2. Put the tube into the freezer (-80°C).
3. Wait 3-4 minutes.
4. Gently retighten the storage caps using the CapLok tool until the caps are firmly fixed. Do not overtighten!
5. Put the tube again into the freezer (-80°C).

Annex D: Participant Information Sheet, Informed Consent Form & Consent Withdrawal Form

The Participant Information Sheet document is written at destination of the subjects to give them information about the CT. The Informed Consent process must respect the patient's ability to make decisions and adhere the individual hospital rules for CS. The Consent Withdrawal Form is a document allowing study participants to withdraw their consent.

Participant Information sheet

[Add the clinical site's logo]

Participant Information sheet

for

Study title: *Multi-centre observational study for the data collection, development and evaluation of the performance of novel CRC screening and diagnostic methods - PHASE A*

Full project title: A European "shield" against colorectal cancer based on novel, more precise and affordable risk-based screening methods and viable policy pathways

Short project title: ONCOSCREEN

[Date: _____]

You are being invited to take part in the ONCOSCREEN project. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following participant information sheet carefully before deciding whether or not to join the study. If you consent to take part, then you will be asked to sign an **informed consent form**. If you decide not to take part, there will be no disadvantage to you and we thank you for your time in considering our project. If after reading this participant information sheet, you are still unsure or uncertain about anything, then we are happy to answer any questions you may have. You should not sign the informed consent form until your questions have been answered and you are happy to join the study.

We understand that time is valuable to you and this might represent an additional burden to you in terms of the time you will spend in reading this information. Therefore, we greatly appreciate your time taken to go through this participant information sheet.

What is ONCOSCREEN?

ONCOSCREEN is a research and innovation project funded by the European Commission under the European Union's Horizon Europe research and innovation programme (grant agreement no. 101097036). The ONCOSCREEN consortium consists of 39 partner organisations, representing 15 countries across Europe, EXUS AI Labs being the coordinator of the project. A complete list of all of the ONCOSCREEN consortium members can be found at the end of this participant information sheet (see Appendix 1 below).

ONCOSCREEN seeks to develop new methods and technologies for colorectal cancer ("**CRC"**) screening and early detection. It aims to develop a risk-based, population-level stratification methodology for CRC, to account for genetic prevalence, socio-economic status, and other factors. Within this framework, ONCOSCREEN will:

- develop a set of novel, multi-tier low-cost diagnostic technologies (called ONCO-VOC, ONCO-CRISP, ONCO-NMR, ONCO-CTC) with high sensitivity[1] and specificity[2], towards an improved CRC screening,
- leverage Artificial Intelligence (AI)[3] to improve existing methodologies for CRC screening, allowing for the early detection of polyps and provision of a personalized risk status stratification,
- provide a mobile app for self-monitoring and CRC awareness raising (so-called ONCO-CAWA),
- develop an intelligent analytics dashboard tool for policy makers to facilitate effective policy making at regional and national levels.

More information about the project can be found at https://oncoscreen.health/.

For the validation of the developed diagnostic solutions (that is to assess their effectiveness, sensitivity and specificity in detecting CRC at an early stage), a clinical validation study titled "**ONCOSCREEN-CS**" will be conducted in two phases:

During the first phase (**ONCOSCREEN-CS-Phase A**), we will identify the different expression patterns of the four diagnostic solutions (ONCO-VOC, ONCO-CRISP, ONCO-

1 Sensitivity is a measure of how good a test is in demonstrating whether the patient really has a condition or not (the true positive rate). A high sensitivity test means that it detects the presence of a condition with relatively few indicators. It is expressed in percentage (%).

2 Specificity is a measure of negativity for those patients who do not have the investigated condition (the true negative rate). A highly specific test means that it really rules out a diagnosis if a patient does not have the indicators. It is expressed in percentage (%).

3 Artificial intelligence is a technique or a system that can do a task using intelligence like human intelligence.

NMR, ONCO-CTC) in CRC patients and healthy controls with high risk for CRC, and also initially estimate their sensitivity and specificity.

During the second phase (**ONCOSCREEN-CS-Phase B**) the ONCOSCREEN solutions will be clinically validated.

What is the purpose of the study?

The **ONCOSCREEN Phase A** clinical study is a multi-centre case-control prospective study for the collection of data that will help develop the ONCOSCREEN screening and diagnostic solutions. In plain terms, the study's main aim is to get data from people either diagnosed with colorectal cancer or those with recognized risk factors for CRC development in order to develop more accurate and cheaper screening and diagnostic tools such as:

- **ONCO-VOC**: a breath biopsy screening test (breathalyser) based on Volatile Organic Compounds (VOC). The innovative tool is based on sensor array of nanomaterials in conjugation with Artificial Intelligence, helps detect CRC through breath analysis.
- **ONCO-CRISP:** is a screening test based on CRISPR-Cas9 biomarkers. The ONCO-CRISPR tool and human CRISPR microRNAs will enable the detection of clinically relevant biomarkers in colorectal adenomas, colorectal carcinomas in tissue and non- or minimal invasive patient obtained samples (blood samples).
- **ONCO-NMR**: standardized NMR test for the analysis of blood serum and plasma samples to provide signatures for cancer and cancer progression.
- **ONCO-CTC**: is a biofluidgnostics platform for early-diagnosis of CRC and personalized treatment

(jointly referred to as "**ONCOSCREEN diagnostic tools**").

Additionally, a sub-study will be conducted aiming to train non-expert and junior colonoscopists and histopathologists with the AI assisted polyp detection algorithms, to increase the adenoma detection rate and to reducing the human error and reveal new opportunities for effective cost reduction in the long term.

Why have I been invited to take part?

You have been invited to take part as you are a person who meets the study eligibility criteria (i.e. you are a male/female adult of at least 18 years old and you either

- have been diagnosed with CRC or
- are a healthy individual with recognized risk factors for CRC development defined by either heredity and/or obesity and/or smoking and/or excess alcohol consumption and/or hyperlipidaemia.

Your data will allow us to develop new screening and diagnostic tools (described above), and improve the accuracy of CRC screening and testing as well as to decrease both the invasiveness and the costs of the currently used CRC screening and diagnostic methods.

Do I have to take part?

No. This research study is done totally on a voluntary basis. If you choose to take part after reading this information sheet, we will ask you to sign a consent form. You are free to make that choice and there will be no adverse consequences if you refuse to give their consent.

Also, You are free to withdraw from this study without disadvantage and without having to provide a reason at any point in time. To withdraw your consent, please contact the research team and they will provide you with a consent withdrawal form to sign.

What will happen to me if I take part in this study?

Participants who consent to take part will be enrolled into the study and screened using a FIT test. A FIT test (*Faecal Immunochemical Test*) is a stool test designed to identify possible signs of bowel disease. It detects minute amounts of blood in faeces (faecal occult blood). Many bowel abnormalities which may develop into cancer over time, are more likely to bleed than normal tissue. So, if there is blood in the stool this can indicate the presence of abnormalities in the bowel.

Participants who return a positive FIT test will be invited to undergo screening colonoscopy.

Participants with a negative FIT test will also be invited to a facultative screening colonoscopy.

Participants with a positive colonoscopy result will be assigned into the cases group (CRC Patient), while those with a negative colonoscopy result will be assigned into the control group.

Participants who returned a FIT negative test and declined to undergo a colonoscopy will be assigned to the control group.

All participants enrolled in the study will be required to provide other biological specimen samples for testing such as stool, blood, urine and breath, using the ONCOSCREEN diagnostic tools. Corresponding clinical data will be also collected for analysis.

Additionally, samples will be archived for use in further developing the ONCOSCREEN diagnostic tools and for additional CRC related research.

The results of the ONCOSCREEN tools will be compared with the results of the colonoscopy and of the FIT test to evaluate their diagnostic and screening performance.

What are the possible benefits of taking part?

Although there is no actual and immediate medical benefit from the study for you, your data will help us achieve the ONCOSCREEN objectives. The study will provide impactful data on the diagnostic potential of the ONCOSCREEN proposed solutions with the aim to

improve the CRC screening in our societies, especially for high-risk population who are younger and not yet included in the current screening guidelines. Biological specimens collected within the study will be sent to the ONCOSCREEN partner laboratories for testing and used in further development of other CRC related diagnostic and screening tools and improving cancer care in general. Beyond the project duration, your data will help other researchers to develop similar technologies in support of cancer care. As a result, this will help early diagnosis and more efficient treatment of cancer in the future.

Please note that participation is totally voluntary, and no remuneration will be given to participants who join the study.

What are the possible disadvantages and risks of taking part?

It is not believed that there are any major risks of taking part in this study.

However, the medical procedures which you may undergo in the course of the study may be associated with certain adverse effects, some of which are mild some serious.

Adverse reactions associated with:

1. **Blood collection**
    - **a.** Commonly occurring
        - dizziness during or after blood collection
        - fatigue, light-headedness, nausea
        - sudden decrease in blood pressure resulting in loss of consciousness
        - bruising (hematoma)
        - bleeding, swelling, pain, tenderness
        - anxiety/fear
    - **b.** rarely occurring
        - allergic reaction to the antiseptic or band-aid or bandage or latex
        - hypersensitivity reaction type 1
        - anaphylactic reaction
        - infection (in immunocompromised patients)
        - phlebitis (inflammation of a vein)
        - sepsis

2. **Colonoscopy**

    - **a. Commonly occurring**
        - abdominal pain or discomfort (cramping, pain)
        - bowel irregularity
    - **b. Rarely occurring**
        - bleeding
        - adverse reaction to anaesthesia

- Postpolypectomy Electrocoagulation Syndrome (if there is a need to cut off a polyp, the doctor will use a tool with an electrical current to seal the area, which, though rarely, can go too deep, burning and inflaming the tissues)
- infection
- intestinal perforation

Can I withdraw from the study?

As already mentioned above, You are free to withdraw from the study at any point after you sign the informed consent form. If you wish to do so, please contact the research team at [*clinical site's name*] and they will provide you with the **consent withdrawal form**.

Please note that if you decide to exercise your right to discontinue your participation in the study (that is: to withdraw your consent for the participation in this study), this will be unequivocal to withdrawing your consent for the processing of your personal data as well. As a result, no new personal data will be collected by us and data already collected will be deleted (whenever feasible). However, some of your information may have to be stored for safety reasons and archiving obligations (as required by law) and to this extent, we might not be able to delete it. Also, be aware that withdrawal will not affect your medical records, which will be kept at the clinical site at which you were treated, in accordance with the applicable law on medical documentation.

Please, be informed, that the withdrawal shall not affect the lawfulness of processing which took place up until the time of such withdrawal. Any data already collected may be used to determine the results of the study, without altering them.

Will I be insured?

The project participants are insured through [_____]

What types of my data will be used for the study?

The type of data that you will be asked to share with will encompass:

- information such as name, age, medical history, relevant surgical history, preexisting conditions, concomitant medication, lifestyle (e.g. smoking history)
- physical evaluation data (height, weight, pulse, BP, T, physical exam results)
- laboratory tests/data resulting from testing of your biological samples (e.g., blood, urine, stool), i.e. breath biopsy, FIT screening test, blood tests
- colonoscopy images/videos
- histopathological images/tissue biopsy results
- other data provided by you in patient questionnaires.

What are my other rights with respect to the processing of my personal data?

Aside from the already discussed **Right of withdrawal**, You shall have a number of other rights with respect to your personal rights, such as:

- **Right of access** i.e. the right to receive confirmation as to whether or not your personal data are processed, what types of personal data are processed and in what manner; this encompasses the right to access all or part of your personal data that are collected, processed or, where applicable, disclosed to third parties (including a free copy) in the course of the study.
- **Right of rectification:** you shall have the right to have your data corrected if it is no longer accurate, is incomplete or not up to date.
- **Portability right**: i.e. the right to request that we transmit your personal data to another controller in a structured, commonly used, and machine-readable format. This right only applies in so far as the processing is based on your explicit consent and the processing is carried out by automated means (meaning electronically).
- **Right of deletion:** You have the right to request that your data be erased, whenever they are no longer needed for the purpose for which they were originally collected or whenever you withdrew your consent and we lack any other basis for the use of your data.
- **Right of restriction:** under certain conditions, You have the right to request that the processing of your data be limited i.e. the data may only be stored, but not processed (e.g. during the time your request for correction of your data is being assessed; when your personal data are no longer needed for the purposes mentioned herein but you require them for the establishment, exercise, or defence of a legal claim; when such processing was unlawful but you prefer restriction to erasure).

Please inform the clinical site's Data Protection Officer or other privacy contact of the [*clinical site's name]* listed at the end of this participant information sheet if you wish to exercise any of the above rights.

What happens when the research study ends? What will happen to my data and samples? How long is my data going to be stored?

As described above, Your personal data will be collected from You and analyzed for the purposes of this study, during the project's duration (i.e. until January 1, 2027).

The data collected for the study will be kept for 10 years after the study completion. Subsequently, personal information will only be kept by *[name of the clinical site*]

for participant health care, and by the promoter for other scientific research purposes if the participant has given its consent to do so and if this is allowed by the law and applicable ethical requirements.

After the study ends, we will anonymize your data and make it available through the ONCOSCREEN project repository (ONCO-BIOBA) for re-use by other researchers. The data will be stored there for indefinite time, as long as your *[clinical site's name*[4]*]* maintains

[4] This may be a hospital, a gastroenterology unit, a general practitioner unit, or family outpatient unit.

their contribution to the repository. If you do not consent to the re-use of your data after the project ends, it will be deleted at the end of the project. This does not affect your medical records, which will be kept at the hospital/gastroenterology unit/general practitioner unit/family outpatient unit in accordance with the applicable law on medical documentation.

Please note that the results of the study may be published in medical journals and presented at scientific meetings; in this case data will be anonymized and it will not be possible to trace any results back to you, so those publications will remain available after the above-mentioned period(s).

Samples collected for the study will be retained to develop and evaluate the performance of biomarker assays to detect cancers. Retained samples will be anonymized (i.e. all personal identifiers will be removed), and clinical study data will only be associated by a subject identification number. Samples will be stored for no more than 20 years in the on-site biorepository and will be used for developing and evaluating the performance of biomarker assays for cancer detection. Specimens from the biorepository will be destroyed under standard biohazardous material protocol and record of the removal and destruction will be maintained for 7 years by the study sponsor. Retained subject samples will not be used for any other purposes.

Will my data be kept confidential?

YES. All information we get from you will be maintained in a strictly confidential way.

During the project period (till January 1, 2027): Your data will be collected at the where you are treated in [*the clinical site's name*]. Data will be stored electronically in a secure local storage in [*clinical site to provide details*] in accordance with the clinical site's security protocols. The clinical site will de-identify (pseudonymize) your data prior to sharing with other partners of the ONCOSCREEN consortium (for full list of the partners see Appendix 1 to this participant information sheet). This means that your name or any other directly and indirectly identifying information will be removed. After this, a random code will be attached to your data to maintain confidentiality. The table that will link your code to your personal/identifiable data (mapping table) will **ONLY** be held and accessed by the research team at [*the clinical site's name*] which will be stored securely and separately from the shared data. This will ensure that other partners in the ONCOSCREEN project can access the pseudonymized data only.

Your de-identified data will be used by the joint controllers of the ONCOSCREEN project (for full list please see Appendix 1 hereto) only for the purposes of the ONCOSCREEN project (as described above).

After the end of the project (post project data availability) (after January 1, 2027): Your data will be anonymized. Such data will be stored in the dedicated infrastructure of *[the clinical site's name]* and/or a secure central location developed by the ONCOSCREEN project and located in one of the EU countries that participate in the ONCOSCREEN

project. Unidentifiable (fully anonymized) study data will be used for research purposes by other researchers (from outside the ONCOSCREEN consortium through the project's repository), in particular in the field of artificial intelligence (AI) for cancer care/research to train their technologies.

What is the legal basis for the use of my personal data?

We rely on your **freely given, specific, informed and unambiguous explicit consent** to process your (health) data, which you have provided by clear affirmative action through signing the informed consent form (Articles 6.1.a and 9.2.a of the GDPR) to:

1) use your personal data as part of this study
2) re-use your personal data for the purposes of future scientific research in the area of cancer research

We rely on **a legal obligation** (Article 6(1)(c) GDPR) whenever we process your data to comply with national or EU law.

Who is organising and acting as data controller for the study?

The data in this study is jointly controlled by all of the 39 partners within the ONCOSCREEN consortium, i.e.: **EXUS, UMC-Mainz, ICCS, Firalis, UKSH, UzL, LSMU, MUG, IPO, IOB, Technion, UMINHO, TLBG, VITO, CERTH, iSPRINT, SERVTECH, AINIGMA, CATALINK, KT, BEIA, URIOJA, TIMELEX, CARR, MoHGR, POLA, ECPT, HSGO, YCE, MUI, MoH-LT, EY, CSIC, UFC, ROSENBAUM, GIE AXA, GERCOR, CC RL, CC SV**. For full names of the ONCOSCREEN partners see Appendix 1 below.

All of the partners have signed a Joint Controllers Agreement, which sets forth their respective rights and obligations with respect to the personal data that is being processed within ONCOSCREEN. If you wish to obtain a copy of this arrangement, please contact your clinical site's Data Protection Officer or other privacy contact listed at the end of this participant information sheet.

Who will receive or have access to the personal data?

During the project, your anonymized data will be made available to some of the partners of the ONCOSCREEN consortium, in order to allow them to develop their ONCOSCREEN tools and technologies. Note that any code linking you to your personally identifiable information will not be disclosed to those partners. All biological material that will be shared with other ONCOSCREEN partners for analysis will be returned to [*clinical site's name*] or destroyed thereafter.

For the time being, we do not anticipate any transfers of data outside of the European Union (EU), to countries which do not afford the same level of protection for personal data as that afforded under the EU law, except for transfers to Israel, where one of the ONCOSCREEN partners (i.e. Technion) is located. Please note however, that data transfers to Israel can take place freely, just as it they were taking place within the EU, based on the

European Commission's adequacy decision[5] which found Israel to ensure level of protections essentially equivalent to that afforded under the EU law (GDPR).

Should transfers to any other third countries be concerned in the future, these will be subject to appropriate safeguards and you will be informed about it.

After the end of the project, your anonymized data may also be used by other researchers beyond the ONCOSCREEN consortium (through the ONCOSCREEN directory) for the purpose of training and validating technologies related to cancer research and healthcare improvement in general.

Please note, that that for data verification purposes, a regulatory authority or an Ethics Committee may require direct access to parts of the clinical site's records relevant to the study, including patients' medical history.

Who has reviewed the study?

The study has received a favourable ethical opinion from the Research Ethics Committee of the [*Name of the ethics committee at each clinical site*].

How will the results of the study be shared?

The results will be a part of several reports which will be made available to the ONCOSCREEN project partners.

After the project ends the results of the studies may be made available to other researchers (outside the ONCOSCREEN project) for the purpose of further CRC research. We will also be disseminating the findings of the study via journal articles and at relevant research conferences. It will not be possible to identify you or others from any such publications, as results will be anonymous (unidentifiable) and aggregated for the whole participants' group.

What if I have a concern about my participation in the study?

If you have any concerns about your participation in this study or if you experience any distress as a result of taking part in the study, please speak to the research team at your clinical site *[clinical site's name]* and they will do their best to answer your questions. Contact details can be found at the end of this information sheet.

You may also file a complaint about the processing your personal information to the appropriate data protection supervisory authority (DPA). The contact information for the

[5] 2011/61/EU Commission Decision of 31 January 2011 pursuant to Directive 95/46/EC of the European Parliament and of the Council on the adequate protection of personal data by the State of Israel with regard to automated processing of personal data; available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32011D0061

DPA in your country is available at: https://edpb.europa.eu/about-edpb/about-edpb/members_en.

Who should I contact if I wish to discuss anything related to this study?

If you wish to discuss anything related to this study please contact the research team. Contact details for the research team at [*clinical site's name*] in [*country name*] are provided below.

Contact details for further information.

The research team at [*clinical site's name*]

Further information may be obtained from:

Name of person: [_]
Job title: [_]
Job address: [_]
Email: [_]

Who should I contact about my personal data?

If you should have any queries regarding the processing of your personal data, or if you should wish to exercise any of your rights with respect to your personal data that we process, please contact the Data Protection Officer (DPO) *[or privacy contact]* at [*clinical site's name*].

DPO/privacy contact *[Here each clinical site shall indicate the contact details of their data protection officer (DPO) or other privacy contact]*

Name of DPO: [_]
E-mail: [_]
Phone no: [_]

Thank you for taking the time to read this information sheet. We will be thankful if you decide to take part in this study.

Appendix 1 Full details of Joint Controllers

1. **EXUS SOFTWARE MONOPROSOPI ETAIRIA PERIORISMENIS EVTHINIS (EXUS)**, PIC 916943523, established in THEANOUS 15, ATHENS 118 54, Greece,
2. **UNIVERSITAETSMEDIZIN DER JOHANNES GUTENBERG-UNIVERSITAET MAINZ (UMC-Mainz)**, PIC 994850722, established in Langenbeckstrasse 1, Mainz 55131, Germany,

3. **INSTITUTE OF COMMUNICATION AND COMPUTER SYSTEMS (ICCS),** PIC 999654356, established in Patission Str. 42, ATHINA 10682, Greece,
4. **FIRALIS (Firalis),** PIC 997607753, established in RUE DU FORT 35, HUNINGUE 68330, France,
5. **UNIVERSITATSKLINIKUM SCHLESWIG-HOLSTEIN (UKSH),** PIC 999845349, established in Ratzeburger Allee 160, Lübeck 23538, Germany,
6. **LIETUVOS SVEIKATOS MOKSLU UNIVERSITETAS (LSMU),** PIC 972782446, established in A MICKEVICIAUS GATVE 9, KAUNAS 44307, Lithuania,
7. **MEDIZINISCHE UNIVERSITAT GRAZ (MUG)**, PIC 999836231, established in AUENBRUGGERPLATZ 2, GRAZ 8036, Austria,
8. **INSTITUTO PORTUGUES DE ONCOLOGIA DO PORTO FRANCISCO GENTIL, EPE (IPO**), PIC 996835827, established in RUA ANTONIO BERNARDINO ALMEIDA, PORTO 4200-072, Portugal,
9. **INSTITUTUL ONCOLOGIC PROF. DR. ALEXANDRU TRESTIOREANU BUCURESTI (IOB),** PIC 987466015, established in SOSEAUA FUNDENI 252, BUCHAREST 022328, Romania,
10. **TECHNION - ISRAEL INSTITUTE OF TECHNOLOGY (TECHNION),** PIC 999907720, established in SENATE BUILDING TECHNION CITY, HAIFA 32000, Israel,
11. **UNIVERSIDADE DO MINHO (UMINHO),** PIC 999995505, established in LARGO DO PACO, BRAGA 4704 553, Portugal,
12. **TILBURG UNIVERSITY- UNIVERSITEIT VAN TILBURG (TLBG)**, PIC 999899475, established in WARANDELAAN 2, TILBURG 5037 AB, Netherlands,
13. **VLAAMSE INSTELLING VOOR TECHNOLOGISCH ONDERZOEK N.V. (VITO),** PIC 999645238, established in BOERETANG 200, MOL 2400, Belgium,
14. **ETHNIKO KENTRO EREVNAS KAI TECHNOLOGIKIS ANAPTYXIS (CERTH),** PIC 998802502, established in CHARILAOU THERMI ROAD 6 KM, THERMI THESSALONIKI 57001, Greece,
15. **INNOVATION SPRINT (iSPRINT),** PIC 919699584, established in RUE CLOS CHAPELLEAUX-CHAMPS 30 BTE 1.3.30, BRUXELLES 1200, Belgium, SCIENTIFIC ACADEMY FOR SERVICE TECHNOLOGY EV (SERVTECH), PIC 996374010, established in BEHLERTSTR 3A HAUS 2B, POSTDAM 14467, Germany,
16. **AINIGMA TECHNOLOGIES (AINIGMA),** PIC 892135579, established in KAPELDREEF 60, LEUVEN 3001, Belgium,

17. **CATALINK LIMITED (CATALINK),** PIC 908583578, established in CHARITINIS SAKKADA 5, NICOSIA 1040, Cyprus,
18. **KONNEKT ABLE TECHNOLOGIES LIMITED (KT),** PIC 949273914, established in FDW HOUSE BLACKTHORN BUSINESS PARK COES ROAD, DUNDALK CO LOUTH, Ireland,
19. **BEIA CONSULT INTERNATIONAL SRL (BEIA),** PIC 999685687, established in STREET POIANA NARCISELOR 12 1ST FLOOR APARTMENT 3 SECTOR 1, BUCURESTI 010158, Romania,
20. **UNIVERSIDAD DE LA RIOJA (URIOJA),** PIC 999471414, established in AVENIDA DE LA PAZ 93, LA RIOJA 26006, Spain,
21. **TIME.LEX** (time.lex), PIC 991228063, established in JOSEPH STEVENSSTRAAT 7, BRUSSEL 1000, Belgium,
22. **CARR COMMUNICATIONS LIMITED (CARRCOMMS),** PIC 998823163, established in FITZWILLIAM PLACE, DUBLIN D02 T296, Ireland,
23. **MINISTRY OF HEALTH (MoHGR),** PIC 950261665, established in ARISTOTELOUS STREET 17, ATHINA, Greece,
24. **PAGALBOS ONKOLOGINIAMS LIGONIAMS ASOCIACIJA (POLA LT),** PIC 938334351, established in A. MICKEVICIAUS G. 9, KAUNAS 44307, Lithuania,
25. **EUROPACOLON PORTUGAL- ASSOCIACAO DE LUTA CONTRA O CANCRO DO INTESTINO (ECPT)**, PIC 887002436, established in ESTRADA INTERIOR DA CIRCUNVALACAO 6657 - 1 SALA 145, PORTO 4200-172, Portugal,
26. **ELLINIKI ETAIREIA OGKOLOGIAS PEPTIKOU (HSGO),** PIC 886461758, established in IERA ODOS 354, CHAIDARI ATHINA 124 61, Greece,
27. **EUROPAISCHE VEREINIGUNG DER GASTROINTESTINALEN ONKOLOGIE(EUROPEAN SOCIETY OF DIGESTIVE ONCOLOGY - ESDO) (ESDO),** PIC 886472234, established in ALSER STRASSE 4, WIEN 1090, Austria,
28. **FUNDATIA YOUTH CANCER EUROPE (YCE),** PIC 920105529, established in CALEA MANASTUR 42B, CLUJ NAPOCA 400372, Romania,
29. **MEDIZINISCHE UNIVERSITAT INNSBRUCK (MUI),** PIC 999855437, established in CHRISTOPH PROBST PLATZ 1, INNSBRUCK 6020, Austria,
30. **LIETUVOS RESPUBLIKOS SVEIKATOS APSAUGOS MINISTERIJA (MoH-LT),** PIC 933839468, established in VILNIAUS G 33, VILNIUS LT 01506, Lithuania,
31. **EY ADVISORY SPA (EY),** PIC 996143926, established in VIA MERAVIGLI 14, MILANO 20123, Italy,

32. **AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS (CSIC),** PIC 999991722, established in CALLE SERRANO 117, MADRID 28006, Spain,
33. **UNIVERSITE DE FRANCHE-COMTE (UFC),** PIC 999863100, established in 1 RUE CLAUDE GOUDIMEL, BESANCON 25000, France,
34. **ROZENBAUM KONSULTING (ROSENBAUM GROUP),** PIC 889265155, established in UL 6TI SEPTEMVRI 13 B, SOFIA 1000, Bulgaria,
35. **GIE AXA (GIE AXA),** PIC 963794426, established in AVENUE MATIGNON 23, PARIS 75008, France,
36. **ASSOCIATION GERCOR (GERCOR),** PIC 884903162, established in 151 RUE DU FAUBOURG SAINT ANTOINE, PARIS 75011, France,
37. **LOUWEN ROGIER (CC RL),** PIC 885483610, established in WERKMANSBEEMD 13, OOSTERHOUT 4907 EW, Netherlands,
38. **SANNE VOOGD (CC SV),** PIC 885483707, established in ENCLAVEBERG 181, ROOSENDAAL 4708 EH, Netherlands

Written Informed Consent

[Add organization/hospital logo here]

Written Informed Consent

for

Study title: Multicenter observational study for the data collection, development and evaluation of the performance of novel CRC screening and diagnostic methods- PHASE A

Full project title: A European "shield" against colorectal cancer based on novel, more precise and affordable risk-based screening methods and viable policy pathways

Short project title: ONCOSCREEN

Please tick the appropriate boxes:	Yes	No

1. Taking part in the study

• I have read and understood the study information sheet, dated *[insert date*] or it has been read to me. I have been handed a copy of the information and of this informed consent form. I have been able to ask questions about the study and my questions have been answered to my satisfaction.		
• I consent voluntarily to be a participant in this study and understand that I can withdraw from the study without having to give a reason as described in the participant information sheet.		
• I understand that personal information is held about me, however that my directly identifiable information, such as my name, will not be shared beyond the study team at [*insert clinical site's name*].		
• I have had the opportunity to discuss the implications of sharing or not sharing information about me.		

2. Use of the information in the study (during the timeframe of the ONCOSCREEN project)

• I agree to my personal data, including health data, and data relating to me collected during the study, being processed by		

ONCOSCREEN consortium partners (full list and names are provided in the information sheet, "**Joint Controllers**") for research purposes as described in the participant information sheet, dated [*insert date*], i.e. to develop more accurate and cheaper CRC (colorectal cancer) screening and diagnostic tools.

3. Future use and re-use of the information by others after the end of the ONCOSCREEN project

- I give permission for Joint Controllers to anonymize my health related data (including in particular my FIT test results, colonoscopy images and reports, histopathology reports, blood, urinal and stool samples, and other medical and diagnostic information) for the purpose of depositing them in the ONCOSCREEN data repository (ONCO-BIOBA), so it can be used in the future by other researchers outside the ONCOSCREEN project, for the research and learning purposes of training and validating technologies related to cancer research and healthcare improvement in general. | | |

4. Signatures

_____	_____	_____
Name of participant [IN CAPITALS]		Date and Signature

Statement by the Researcher/ Clinician taking consent:

I have accurately read out and explained the Information Sheet to the potential participant, and to the best of my ability made sure that the participant understands it. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this informed consent form (ICF) has been provided to the participant.

_____	_____	_____
Name of Researcher/ Clinician [IN CAPITALS]	Date	Signature

Consent withdrawal

[Add organization/hospital logo here]

Consent withdrawal

for

Full project title: A European "shield" against colorectal cancer based on novel, more precise and affordable risk-based screening methods and viable policy pathways

Short project title: ONCOSCREEN

I the undersigned, hereby declare that I wish to withdraw my consent for my participation in the study entitled *[insert the study title]* which was given on *[insert date].*

- I understand that upon my withdrawal no new personal data will be collected from me and data that has been already collected will – to the broadest extent possible - be deleted, though some of my information may have to be stored for safety reasons and archiving obligations (as required by law) and to this extent, it might not be deleted
- I understand that this withdrawal does not affect the processing of my personal data which was done prior to it; any data already collected may be used to determine the results of the study, without altering them.
- I acknowledge that my withdrawal will not affect my medical records, which will be kept at the hospital in accordance with the applicable law on medical documentation.

DATE and PLACE [*insert date and place*]

_____

PARTICIPANT SIGNATURE

_____

PARTICIPANT FULL NAME

Annex E: Case Report Form

The Case report form (CRF) is a printed, optical, or electronic document designed to record all protocol-required information on each subject in a clinical research study.

ID RCB Number:	STUDY Number:

PATIENT ID: ONCOFR01 _ _ _ _
PATIENT NO

CRF Completed ☐

CRF Signed ☐

CRF Monitored ☐

Study Protocol Version 1.0 –2023
CRF Design Version 1.0 –2023

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ | _ |	
	Page name: Demography	Visit: Visit 1

INFORMED CONSENT

Please ensure that the patient has signed the informed consent in front of the investigator after receiving all the needed information.

Signed consent to participate in the study: ☐ Yes ☐ No

Date when the participant signed informed consent: |__|__|/|__|__|/|__|__|__|__|
DD MM YYYY

Identification

Visit date: |_|_|/|_|_|/|_|_|_|_|

DD MM YYYY

Initial Name : |_| Initial First name : |_|
Gender : ○ Male ○ Female
Date of Birth : |__|__| / |___|___|___|___| *Age :* /___/___/./___/

FIT test results

☐ Positive ☐ Negative
Add reference

Day of the FIT Test: |__|__|/|__|__|/|__|__|__|__|

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |	
	Page name: Inclusion criteria	Visit: Visit 1

Eligibility for Inclusion

Inclusion Criteria

*All the questions should be answered by "Yes". If only one "No" is selected, the subject **will not be included** in the study.*		
1. Signature of the informed consent to participate indicating that the subject has understood the purpose as well as the procedures required by the study and that he accepts to participate in the study and to comply with the requirements and restrictions inherent in this study	○ Yes	○ No
2. Patient positive for the FIT Test	○ Yes	○ No
3. Able to comply with all study procedures	○ Yes	○ No
4. Male or female subjects aged ≥18 years	○ Yes	○ No
5. Covered by a Health Insurance System	○ Yes	○ No

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |
	Page name: Exclusion criteria / Visit: Visit 1

Exclusion Criteria

*All questions should be answered by "NO". If only one "YES" is selected the subject **shall be***			
1.	Legal incapacity or limited legal capacity	○ Yes	○ No
2.	Subject who **did not sign the Informed Consent form**	○ Yes	○ No
3.	Subject who, in the judgment of the Investigator, is likely to be non-compliant during the study, or unable to cooperate because of a language problem or poor mental development	○ Yes	○ No
4.	Subject without health insurance	○ Yes	○ No
5.	Pregnant woman	○ Yes	○ No
6.	Subject being in the exclusion period of another study or planned by the "national volunteer file"	○ Yes	○ No
7.	Patient who, according to the investigator's assessment, presents with an unstable medical condition contraindicating the performance of the planned blood test, stool test or breath test.	○ Yes	○ No

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |
	Page name: Screening failure / Visit: Visit 1

Screening Failure

The patient is in screening failure: ○ Yes ○ No

/ If Yes */*

Reason: ○ Non respect of inclusion / exclusion criteria
○ Refuse to sign consent
○ Other reason

Precise : _____

Physical Exam and Vital Signs

Height:	| | | | | cm
Weight:	| | | | kg
Day of the first gastro-intestinal disorders:	|_|_|/|_|_|/|_|_|_|_| DD MM YYYY

Concomitant medications

Medications:

Medication name		Indication	Start date dd/mm/year	End date dd/mm/year	Ongoing
Med1.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med2.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med3.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med4.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med5.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med6.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med7			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med8.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med9.			| | /| | /| | | | |	| | /| | /| | | | |	☐
Med10.			| | /| | /| | | | |	| | /| | /| | | | |	☐

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |
	Page name: Medical history Visit: Visit 1

Family history of cancer:

Type of cancer	Family member

Personal history of cancer:

Type of cancer	Differentiation Grade
○ Adenocarcinoma ○ Colloid Carcinoma ○ Signet ring cell carcinoma ○ Carcinoma from particular histological type	○ Good ○ Moderately ○ Little

Method of diagnosis:

Type of treatment:

Type of treatment
○ Immunotherapy ○ Radiation Therapy ○ Hyperthermia ○ Surgery ○ Chemotherapy ○ Hormone Therapy ○ Photodynamic Therapy ○ Stem Cell Transplant ○ Targeted Therapy

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |	
	Page name: Surgical history	Visit: Visit 1

List of previous surgeries related or not to cancer:

Type of surgery	Day
	|__|__|/|__|__|/|__|__|__|__| DD MM YYYY

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |	
	Page name: Laboratory report	Visit: Visit 1

Laboratory report

Report : ○Done ○Not done

/ If Not done */:*
Reason: ……

/ If done */*
N° lab : |__|
/ Report in the form Laboratory Terminals the corresponding data */*

Date of sampling : |__|__| / |__|__| / |__|__|__|__|
Date of the report : |__|__| / |__|__| / |__|__|__|__|

Parameters	Results Visit 1	Units Please enter unit used	If abnormal value (*)
Haemoglobin	|__|__|.|__|		□ CNS □ CS
WBCs	|__|__|.|__|__|__|		□ CNS □ CS
Neutrophils	|__|__|.|__|__|__|		□ CNS □ CS
Lymphocytes	|__|__|.|__|__|__|		□ CNS □ CS
Monocytes	|__|__|.|__|__|__|		□ CNS □ CS
Platelets	|__|__|__|		□ CNS □ CS
LDH	|__|__|__|.|__|__|		□ CNS □ CS

Other			□ CNS	□ CS
			□ CNS	□ CS

(*) CNS: Clinically Not Significant, CS: Clinically Significant.

Colonoscopy

Colonoscopy performed □ Yes □ No

If yes, Date of Colonoscopy: |__|__|/|__|__|/|__|__|__|__|
DD MM YYYY

Results:

Colonoscopy: □ Complete □ Incomplete

Boston Preparation score: |__|/ 9

Use of Chromoendoscopy: □ Yes □ No

Prescence of Polyps: □ Yes □ No

Location:

Size

Shape

Ulcerations

Resection:

If yes:

Complete

Monobloc

If non,

Biopsy

Grade of CRC according to the European Guidelines (0, I, II, III, IV): ....

Blood sampling n°1 : □ Done □ Not done

/* If done */

Day : |__|__| / |__|__| / |__|__|__|__|

/* If not done */

Reason : □ Omission □ Patient refusal □ Other : _____

Reference number : |__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__|

Number of tubes : |__|__|

Visit 1			
Time of sampling		| | |:| | |	
Is the participant in fasting condition?		¡Yes ¡No	
		Yes / No	**Nb of tubes**
Was whole blood taken ? (tube STRECK 10 ml)	ONCO-CTC	¡Yes ¡No	
Was whole blood for Plasma taken? (tube S-Monovette K3 EDTA 9 ml)	ONCO-NMR	¡Yes ¡No	
Was whole blood for Serum taken? (tube S-Monovette Serum 9 ml)	ONCO-NMR	¡Yes ¡No	
Was whole blood for RNA taken? (tube PAXgene RNA 2.5 ml)	ONCO-CRISPR	¡Yes ¡No	
Was whole blood for DNA taken? (tube PAXgene DNA 2.5 ml)	ONCO-CRISPR	¡Yes ¡No	

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |	
	Page name: Feces sampling	Visit: Visit 1

Feces sampling

Feces sampling n°1 : □ Done □ Not done

/* If done */

Day : |__|__| / |__|__| / |__|__|__|__|

/* If not done */

Reason : □ Omission □ Patient refusal □ Other : _____

Reference number : |__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__|

Number of samples : |__|__|

Visit 1			
Time of sampling		| | |:| | |	
Is the participant in fasting condition?		¡Yes ¡No	
		Yes / No	**Nb of tubes**
Was faeces taken ? (Feaces tube 107 x 25 mm)	ONCO-CRISPR	¡Yes ¡No	

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |	
	Page name: Breath sampling	Visit: Visit 1

Breath sampling

Breath sampling n°1 : □ Done □ Not done

/* If done */

Day : |__|__| / |__|__| / |__|__|__|__|

/* If not done */

Reason : □ Omission □ Patient refusal □ Other : _____

Reference number : |__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__||__|__|__|__|

Number of samples : |__|__|

Visit 1			
Time of sampling		| | |:| | |	
Is the participant in fasting condition?		¡Yes ¡No	
		Yes / No	**Nb of tubes**
Was breath air taken ? (tube Tenax 250 ml)	ONCO-VOC	¡Yes ¡No	

Tube	Volume	Analysis
1 tube Tenax	250 ml	ONCO-VOC

ONCO-VOC Result:

ONCOSCREEN Clinical Study ONCO	Patient ID: | _ | _ | _ | _ |	
	Page name: End of study	Visit: Visit 1

End of Study

Did the patient leave the study prematurely: ○ Yes ○ No
/ If Yes */*
Reason of withdrawal :
○ No cancerous lesion during the colonoscopy
○ Consent withdrawal
○ Tumoral progression
Day of progression : |__|__|/|__|__|/20|__|__|
○ Death
Day of the death : |__|__|/|__|__|/20|__|__|
○ Refusal
Day of the refusal : |__|__|/|__|__|/20|__|__|
○ Lost to follow-up
Day of the last news : |__|__|/|__|__|/20|__|__|
○ other, precise: ___

Annexes

Lab terminals:

N° of Laboratory: |__|

Parameters	Lower limit	Higher Limit	Units Please enter unit used
Haemoglobin	|__|__|.|__|	|__|__|.|__|	
WBCs	|__|__|.|__|__|__|	|__|__|.|__|__|__|	
Neutrophils	|__|__|.|__|__|__|	|__|__|.|__|__|__|	
Lymphocytes	|__|__|.|__|__|__|	|__|__|.|__|__|__|	
Monocytes	|__|__|.|__|__|__|	|__|__|.|__|__|__|	
Platelets	|__|__|__|	|__|__|__|	
LDH	|__|__|__|.|__|	|__|__|__|.|__|	
Other			