The MISTRAL Study

SITE TRAINING

Status

- 21 sites out of 23 are now trained (23 after today)
- 21 sites are open for enrolment
- 1st patient was enrolled 1 September 2022
- 755 patients have been enrolled until now
- The last sites have opened in the last few months and we are seeing an uptick in recruitment in the new year

Agenda

- Background
- Study Design and Objectives
- Study Procedures
  - Patient identification and enrolment
  - Sample collection and questionnaire
  - MISTRAL follow-up visit
- Laboratory
- Next Steps and Site and Participant Engagement

Training aims

- Go through key study procedures
- Particular focus on highlighting MISTRAL specific procedures and sampling considerations (which are different from EuroSIDA)

Background

HIV and the microbiome

- Numerous studies have explored the interaction between HIV and the gut microbiome (1 - 3).
- Gut microbial changes (known as dysbiosis) are affected by HIV infection, but also a number of key lifestyle factors associated with HIV, including
  - Sexual practice – MSM have been shown to have higher microbial diversity (4)
  - ART
  - Diet
- Gut microbial dysbiosis is also linked with increased immune activation and various inflammatory markers – and may be causally related to the development of a number of serious non-AIDS events

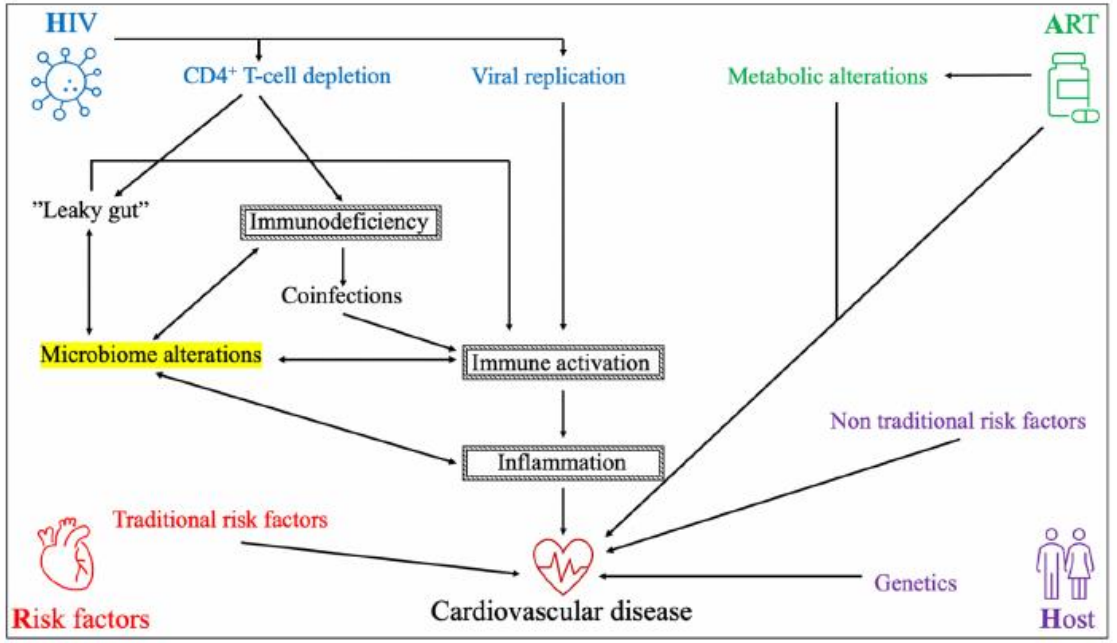


Figure 1: An illustration of the potential pathways behind the increased risk of CVD in PLWH. Virus related mechanisms such as CD4⁺ T-cell depletion and viral replication potentially cause persistent immune activation and inflammation due to immunodeficiency, leaky gut syndrome and microbial alterations. ART might contribute to the increased risk of CVD due to metabolic alterations or through a more direct pathway, although treatment with ART might have a net cardioprotective effect due to reconstitution of CD4⁺ T-cell count and decreased viral load.

Kronborg. MSc Thesis. 2022

1. Williams et al. 2016 Cellular Microbiology
2. Bandera et al. 2018. Current Opinion in HIV and AIDS
3. Dillon et al. 2016. AIDS
4. I. Vujkovic-Cvijin et al. 2020. Nat Com.

Limitations in the literature

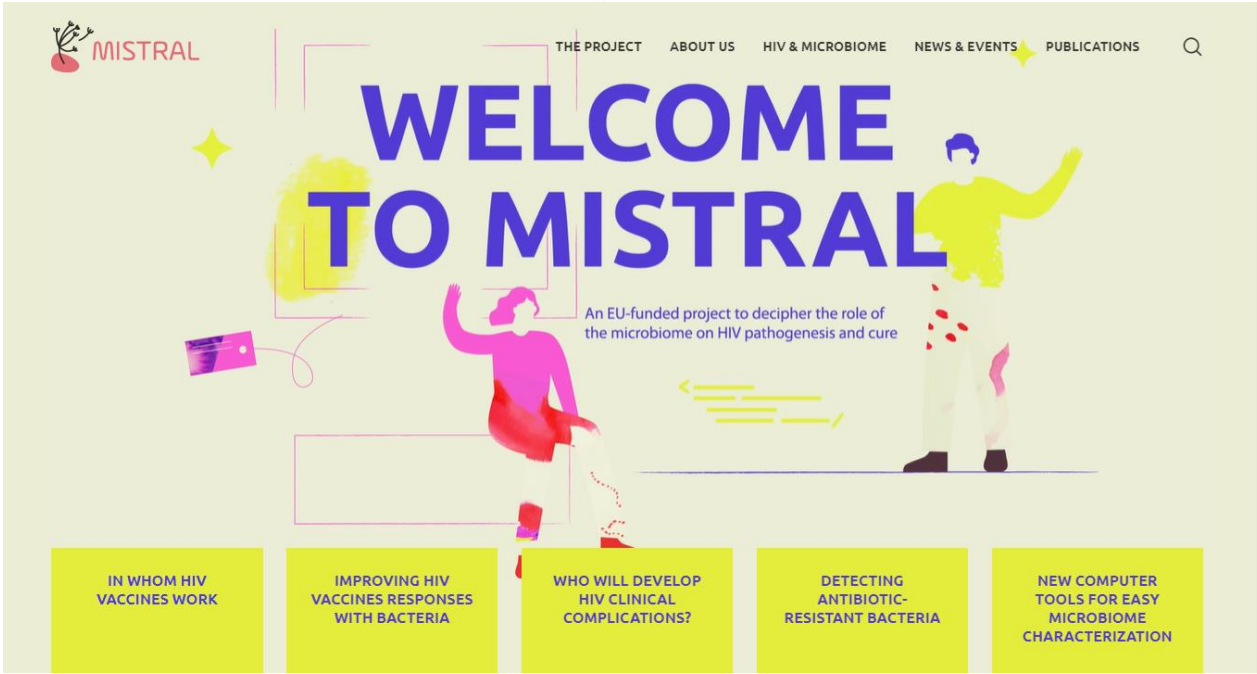
- To date studies into the gut microbiome are limited by
  - Sample size
  - A lack of control for key confounders (particularly diet and sexual practice)
  - Lack of association with hard clinical endpoints
  - Cross sectional study designs
- In order to inform future interventional strategies, larger, well characterised cohorts with adequate follow-up are needed

MISTRAL

- With this in mind, we, together with Roger Paredes and the EU funded Horizon2020 MISTRAL consortium, sought to address key questions related to the influence of the microbiome on HIV pathogenesis
- This protocol addresses Work Package 4 - Gut microbiome correlates of serious AIDS/non-AIDS events – and CHIP is leading this Work Package
- Other work packages address other key questions surrounding HIV and the microbiome as well as data analysis and sharing for these key data (see the MISTRAL website for further details https://www.mistral-hiv.eu/)

Roger Paredes

PRINCIPAL INVESTIGATOR



MISTRAL funded work with WP4

- Horizon 2020 funding has allowed for the collection and some analysis of clinical samples (including stool) and data
- Key analyses include – shotgun metagenomic sequencing of all collected stool samples
- Metabolomics and proteomics of both stool and plasma for a subset of participants
- Establishment of a biobank for additional analyses that are as of yet unfunded

Study Design & Objectives

Study objectives

Primary objective

- To strengthen and evaluate the understanding of the association between the gut microbiome composition and the risk of developing serious AIDS and non-AIDS events (SNAEs), including cardiovascular events

Secondary objectives

- To evaluate the associations between the gut microbiome composition and function and pathologic increases in inflammation and coagulation mediators in PLWH

- To develop a risk score which makes use of information in the gut microbiome as well as other risk factors separately for the different endpoints.

Study Design

- Observational study
- Aim is to recruit up to 1,000 participants from established EuroSIDA sites and follow them until end of 2025
- Participants can be existing EuroSIDA participants or new persons followed at EuroSIDA sites
- Blood and stool collection and MISTRAL questionnaire will occur at baseline and one follow-up visit
- Follow-up clinical data collection will occur during yearly EuroSIDA data collection (Oct-Dec)
- Asides from the additional sample collection and MISTRAL questionnaire, all other data collection and study procedures are the same as EuroSIDA – hopefully easing burden on sites

- EuroSIDA participants will continue to be followed as part of the EuroSIDA protocol after the MISTRAL follow-up period
- We are seeking additional funding to follow the Non-EuroSIDA participants beyond the 5 years (protocol has been designed to allow this, but funding is not guaranteed)

Eligibility

Enrollment Criteria:

- HIV-1 positive persons
- Age ≥50 years old
- Prospectively followed in a EuroSIDA site

Exclusion Criteria:

- Creatinine Clearance <50*
- Child-Pugh C end-stage liver disease
- Any ongoing severe life-threatening disease
- Experiencing any of the following events prior to inclusion:
  - myocardial infarction
  - stroke
  - an invasive cardiovascular procedure
  - AIDS-defining infections (diagnosed within 5 years of MISTRAL enrolment)
  - Prior AIDS cancer or non-AIDS cancer (excluding non-melanoma skin cancer)

* Results of the creatinine routine tests taken are inserted in this link in the eCRF to calculate creatinine clearance: https://www.mdcalc.com/creatinine-clearance-cockcroft-gault-equation

Data collection

- Data collection will occur in REDCap
- https://www.chip-crf.info/redcap/
- If anyone at your site needs access, please contact MISTRAL study staff mistral.rigshospitalet@regionh.dk

For detailed REDCap MISTRAL instructions see the Manual of operations on the CHIP website

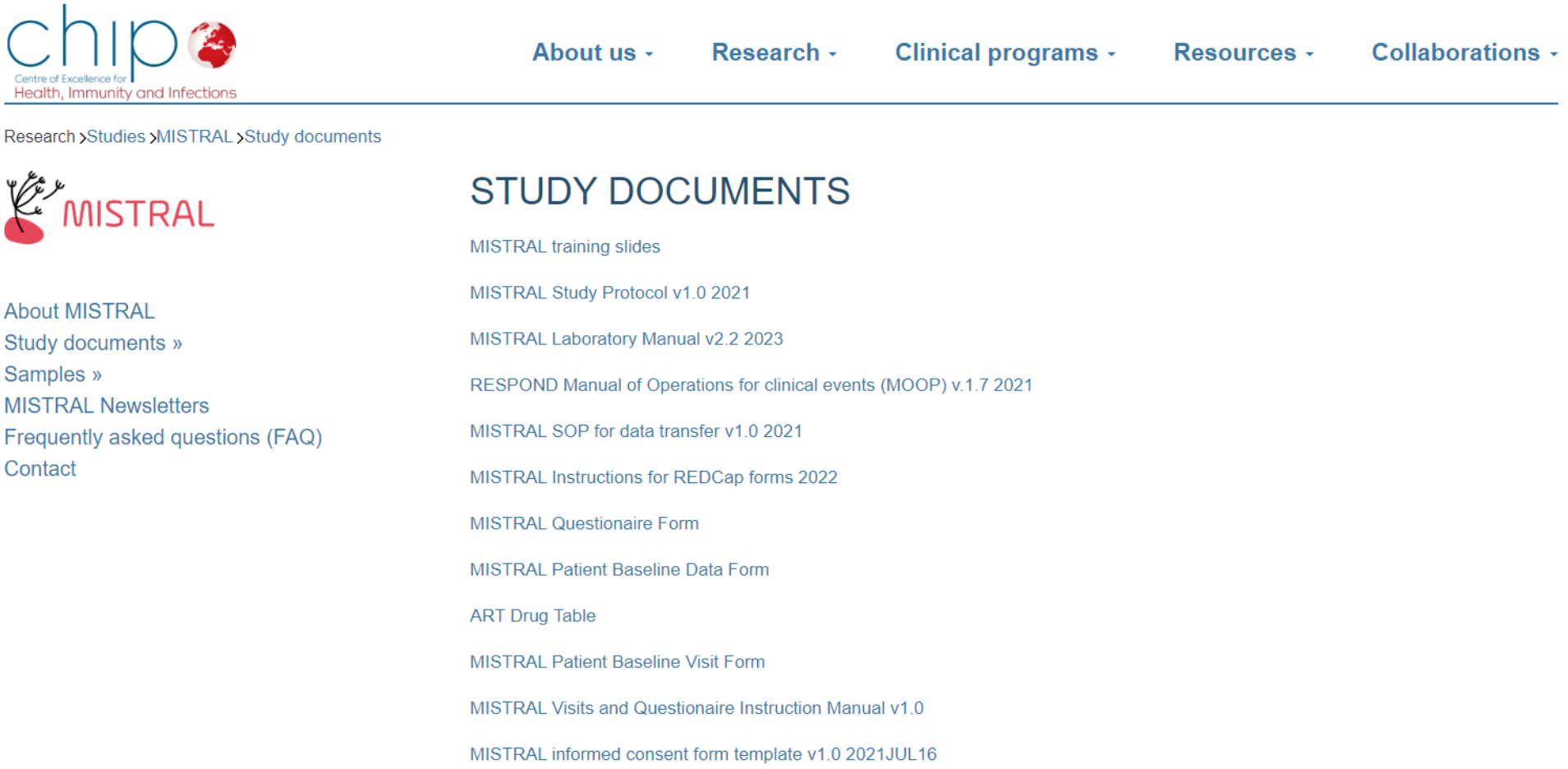
https://www.chip.dk/Portals/0/MISTRAL/REDCap%20instructions%20for%20participants%20in%20MISTRAL_Final_2022AUG19.pdf?ver=2022-08-19-140022-040&timestamp=1660910424442

Materials provided by CHIP

- Site file
- Programmed Scanner for registration of aliquoted sample vials in REDCap
- For sample collection:
  - Stool Specimen Collection Kit
  - EDTA without separator (lavender-top) collection tubes – for both whole blood and plasma collection
- For processing/storage/transport:
  - Labels for vials
  - Sterile, rigid inoculating loops for processing stool
  - 1.8 mL cryovials for storage/transport
  - Grid boxes to store vials

Study documents and additional instructions

ALL STUDY DOCUMENTS CAN BE FOUND AT THE CHIP WEBSITE:
https://chip.dk/Research/Studies/MISTRAL/Study-documents



Study Procedures

MISTRAL specific procedures

- MISTRAL specific procedures include sample collection and participant questionnaire

	Visit 1	Visit 2 (10-24 months after visit 1)
MISTRAL baseline data	X	
MISTRAL Enrolment clinical data	X[a]	
Sample collection (Plasma, Whole Blood and Stool)	X	X
MISTRAL Questionnaire	X	X

a. Only for participants not already part of EuroSIDA

See the MISTRAL Visits and Questionaire Instruction Manual v1.0 June 2022 on the website for further details.

Sample collection strategy

- Important to plan for how to collect samples (particularly stool) for these two MISTRAL visits
  - No MISTRAL study procedures can occur prior to consent being signed
  - We require that you complete the MISTRAL questionnaire with the patient and collect the blood **AFTER** the participant has returned a stool sample
- If a participant consents and is able to provide a stool sample at the same visit, then they can complete all the enrolment procedures in one day
- However, participants may not be able to provide a stool sample ‘on demand’ and they will need to take the stool sample collection kit home, before returning to complete the questionnaire and have blood taken
- The collection kit does not expire, but the stool sample should be returned to the site to be processed within 48 hours of defecation (and stored in the **fridge** until this has occurred)
- A plan for collection of follow-up sample collection is also required – e.g. if stool kits can be sent out in advance of their second MISTRAL visit
- We understand that coordinating this is an additional burden on the participants and sites, but the stool samples are the most important sample type and we want to ensure these are collected
- We encourage all sites to share successful strategies or common issues – either via email or at planned investigator meetings

REDCap data collection
MISTRAL Visits

Patient baseline data, questionnaire and samples forms must be completed for all participants at Visit 1. **At Visit 2 only** questionnaire and sample forms should be completed.

Patient ID:	Visit 1			Visit 2	
	Patient baseline data	Questionnaire	Samples	Questionnaire	Samples

Only for participants not yet part of EuroSIDA

Follow-up procedure using standard EuroSIDA forms

- For all participants, follow-up forms (standard EuroSIDA forms) will be completed in accordance with annual EuroSIDA data collection (October-December) for the duration of the study (5 years)
    - For existing EuroSIDA participants, these are the normal EuroSIDA follow-up forms that appear in your REDCap
    - For non-EuroSIDA participants, these forms will be called MISTRAL follow-up forms (this is exactly the same as the EuroSIDA form)
- As with EuroSIDA, the forms that you need to complete for each participant (regardless of whether it is a EuroSIDA or MISTRAL follow-up form) will be pre-loaded into REDCap prior to the start of the data collection period – so you just complete what is there
- If you enrol a non-EuroSIDA participant into MISTRAL after the data download for that year has been completed then they may not have a follow-up form in REDCap for their first year (non-EuroSIDA participants only)

Clinical events

- As in EuroSIDA, collect details on clinical events on the RESPOND Event Form and cause of death on the CoDe event form
- Refer to SOP from EuroSIDA for instructions on how to complete these forms

https://www.chip.dk/Portals/0/files/RESPOND/Study%20documents/RESPOND%20Manual%20of%20Operations%20MOOP__Version%201.7.pdf

Patient Identification and Enrolment

Identification of patients

- CHIP will provide a list of eligible EuroSIDA patients based on most recent data collection
- Additional patients that meet the eligibility criteria and are followed at your site who are not part of EuroSIDA are also able to enrol into MISTRAL, but note that these individuals require a more detailed enrolment form (to capture the information that is already in place for EuroSIDA participants)

Consent

- No study procedures can occur prior to consent
- There are three consent forms and a GDPR information document for the participant
  - MISTRAL study main (required)
  - Future research as part of MISTRAL (required)
  - Genomics (optional)
- As the intention of this study is to create a biobank for research purposes, the consent to future research is essential for participation in the MISTRAL study
- Participants **do not** need to consent to genomics to be part of the main MISTRAL study
- If approved by your local ethics committee, a MISTRAL participant information brochure (in English) can be found here:

https://chip.dk/Research/Studies/MISTRAL/Study-documents

Assigning PID number

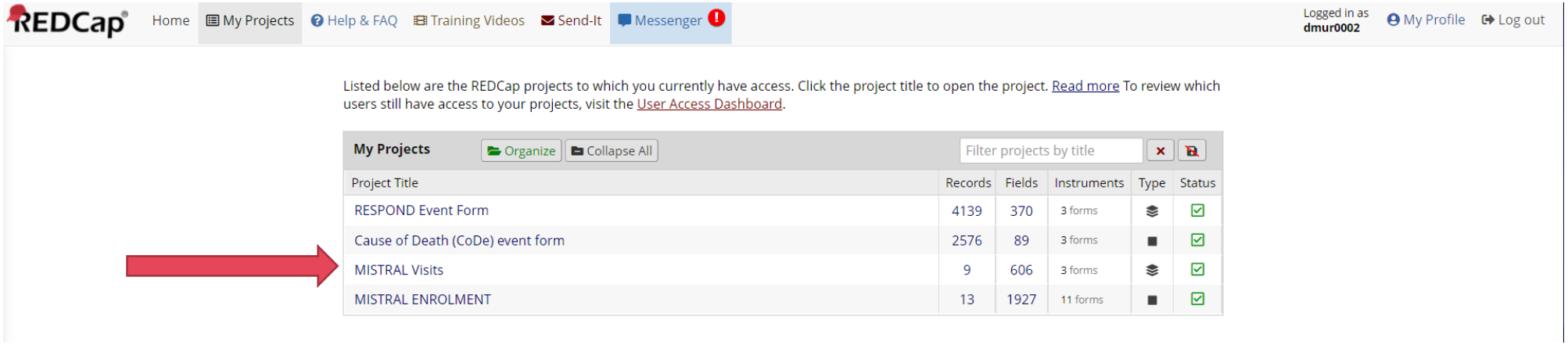
- List all patients enrolled into MISTRAL on the site specific decodification list (provided as part of the site file)
- For existing EuroSIDA patients, use their existing EuroSIDA ID
- For non-EuroSIDA participants, The PID code is a 7-digit code, consisting of a 3-digit center code followed by a 4-digit participants code. The participant code for the MISTRAL study starts with 8001. You should enrol participants as XXX-8001, XXX-8002, etc.

Patient code	Patient name and date of birth	Local identification

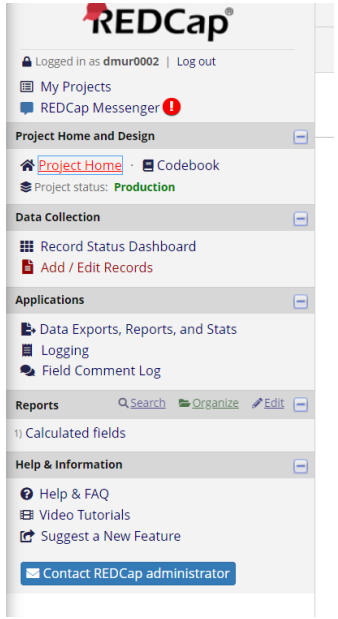
Enrol participant in REDCap

https://www.chip.dk/Research/Studies/MISTRAL/Study-documents → Instructions for enrolment

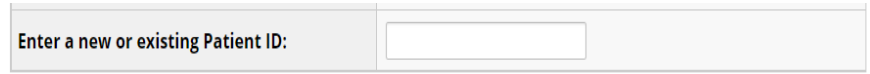
Step 1. Click on the MISTRAL Visits (not enrolment)



Step 2. Click on the Add/Edit record

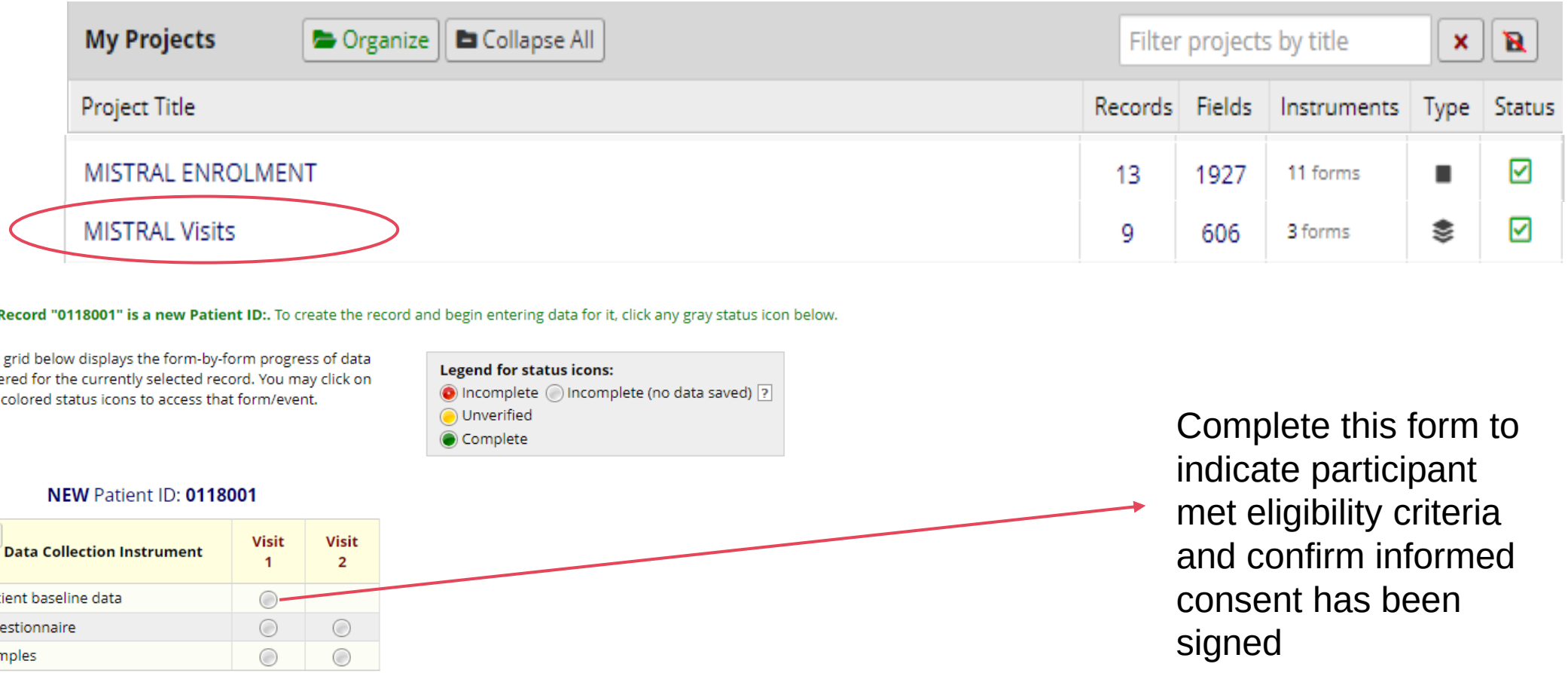


Step 3. Enter the PID (as outlined in the previous slide)



Congratulations, your participant is now enrolled!

You can now complete relevant data



Complete this form to indicate participant met eligibility criteria and confirm informed consent has been signed

Enrolment form

(new participants only)

Patient ID:	Section A1 - Demography and HIV status	Section A2 - Basic Clinical Information	Section B1 - Laboratory Values	Section B2 - CD4, CD8 and HIV-RNA	Section B3 - Hepatitis Virology and Fibrosis Screening	Section B4 - COVID-19	Section C1 - Antiretroviral Treatment	Section C2 - Medication related to Cardiovascular Disease and Diabetes	Section C3 - Treatment Against Hepatitis C	Section D - Severe Opportunistic Infections and Sexually Transmitted Infections	Status

Sample Collection and Questionnaire

Stool collection

- Provide participant with a pre-packaged Stool Specimen Collection Kit
- This kit contains:
  - Instructions for collection and storage https://youtu.be/a3uGHqWz7P8
  - Collection kit and envelope for storing (please note the envelope is for storing of the stool, not for mailing. The participant should not mail the stool back to the clinic
  - Bristol stool chart (image)
- Note, that after collection the participant should refrigerate the stool sample and return to the site as soon as possible (within 48 hours of sample collection)
- Note that the participant should not freeze the sool sample at home – only refrigeration
- You should process the stool sample and freeze as soon as possible once the participant has delivered the sample to the clinic

Blood collection

- 6mL EDTA tubes have been provided for both plasma and whole blood collection
- Do not collect blood until after the stool sample has been delivered back to the site
- Collect as per normal site procedure

MISTRAL

Data Collection Instrument	Visit 1 2021-03-09	Visit 2 2021-03-04
Patient baseline data		
Questionnaire		
Samples		

Stool sample

Did the patient have a stool sample collected?	○ Yes ○ No ○ Unknown reset
The stool sample collection date is registered in the Questionnaire	
Please scan the barcode or manually type the sample code for stool sample aliquot 1 below (if typing, include the dash (-))"	11 characters remaining xxx-xxx-xxx
Please scan the barcode or manually type the sample code for stool sample aliquot 2 below (if typing, include the dash (-))"	11 characters remaining xxx-xxx-xxx
Please scan the barcode or manually type the sample code for stool sample aliquot 3 below (if typing, include the dash (-))"	11 characters remaining xxx-xxx-xxx
Please scan the barcode or manually type the sample code for stool sample aliquot 4 below (if typing, include the dash (-))"	11 characters remaining xxx-xxx-xxx
Please scan the barcode or manually type the sample code for stool sample aliquot 5 below (if typing, include the dash (-))"	11 characters remaining xxx-xxx-xxx
Please scan the barcode or manually type the sample code for stool sample aliquot 6 below (if typing, include the dash (-))"	11 characters remaining xxx-xxx-xxx

Plasma sample

Did the patient have plasma samples collected?	○ Yes ○ No ○ Unknown reset

Whole blood sample

Did the patient have whole blood samples collected?	○ Yes ○ No ○ Unknown

EuroSIDA

MISTRAL Questionnaire

- The MISTRAL Questionnaire has been developed in order to inform MISTRAL researchers about how participants' diet, time and frequency of defecation, medical history and usage and lifestyle data may or may not impact long-term prognosis and outcomes for people living with HIV
- Before completing the Questionnaire, please restate the purpose of the Questionnaire for the participant – particularly the importance of collecting information that may be sensitive (e.g. sexual practice)
- Once the participant has returned the stool sample, go through the questionnaire with them and enter the data directly into REDCap or the downloaded questionnaire (next slide)
- This can be done over the phone or virtually, but the participant should not self-complete the questionnaire.
- Should take between 20-30 min. to complete.
- For clarity around specific questions, see the MISTRAL Visits and Questionnaire instruction manual in the Study Documents page

Download the questionnaire

https://chip.dk/Research/Studies/MISTRAL/Study-documents

STUDY DOCUMENTS

MISTRAL training slides

MISTRAL Study Protocol v1.0 2021

MISTRAL Laboratory Manual v2.2 2023

RESPOND Manual of Operations for clinical events (MOOP) v.1.7 2021

MISTRAL SOP for data transfer v1.0 2021

MISTRAL Instructions for REDCap forms 2022

MISTRAL Questionaire Form

MISTRAL Patient Baseline Data Form

ART Drug Table

Does the participant have a gluten-free diet: no consumption of products with gluten?	◯ Yes ◯ No ◯ Unknown
How many days per week do you eat red meat?	◯ 0 ◯ 1-3 ◯ 4-5 ◯ 6-7 ◯ Unknown
What is the average number of portions of fruit and/or vegetable that you consume per day (e.g., one bell pepper, a handful of peas or one apple)?	◯ 0 ◯ 1-2 ◯ 3-5 ◯ > 5 ◯ Unknown
What is the average number of portions of dairy/milk that you consume per day (e.g., one glass or cup of yogurt or milk (200 ml) or two slices (60 g) of hard cheese or 2 spoons (25 g) of soft cheese)?	◯ 0 ◯ 1-2 ◯ 3-5 ◯ > 5 ◯ Unknown
What is the average number of portions of fiber/whole	◯ 0

Follow-up MISTRAL Visit

Follow-up MISTRAL visit

- Should occur between 10-24 months after the first sample collection
  - Although **ideally ~12 months** after the first enrolment/baseline sample collection
- Again, stool sample should be delivered to site prior to completing MISTRAL questionnaire and collecting blood – and delivery of stool sample should be coordinated with the participant

Summary of study procedures

1. Identify candidate patients and check eligibility criteria
2. Obtain informed consent
3. Provide participant with Faecal Collection Kit
4. Assign or record patient ID (PID) on site decodification list
5. Register the participant in REDCap
6. Complete baseline information (Patient baseline data form (all participants) and MISTRAL enrolment form (non-EuroSIDA only))
7. When participant returns stool sample, collect blood and complete the MISTRAL Questionnaire with the participant
8. Enter the questionnaire data in the REDCap form
9. Enter details on stool and blood samples in the REDCap form
10. Plan for next visit with the participant
11. Complete EuroSIDA follow-up (including event reporting) during the yearly reporting period (Oct-Dec)
12. When the participant returns for their second MISTRAL study visit, re-do steps 3 and steps 7-9
13. Once the second set of samples has been returned, only follow-up through normal EuroSIDA procedures

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Laboratory

Overview of containers for specimen collection and storage/transport

Specimen	Collection Frequency	Collection Container Type	Collection Volume	Aliquots per Collected Specimen	Aliquot Container Type (i.e. for storage/transport)
Stool	2x (Baseline, 10M-24M)	Sterile container	1 teaspoon per collection*	6 (6x 300mg in each tube)	1.8 mL screw top cryovials
Plasma	2x (Baseline, 10M-24M)	EDTA without separator (lavender-top)**	2 x 6 mL per collection	6 (***6x 1 mL in each vial)	1.8 mL screw top cryovials
Whole Blood	2x (Baseline, 10M-24M)	EDTA without separator (lavender-top)**	1 x 6 mL per collection	4 (****4x 1 mL in each vial)	1.8 mL screw top cryovials

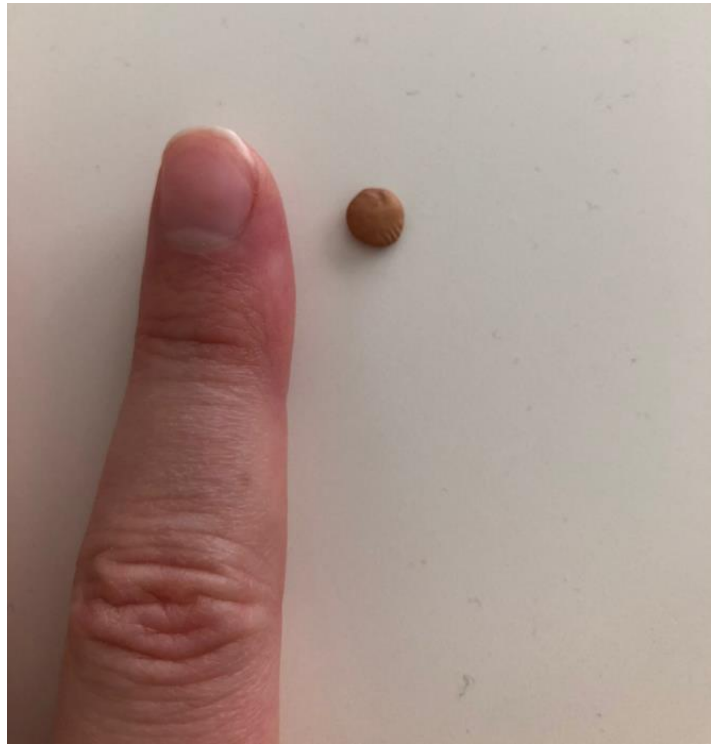
***** provided by the participant
** no PPT tubes
*** Fill as many vials as possible with 1 mL, better to have 5 vials of 1 mL than 6 of 0.8 mL
**** Fill as many vials as possible with 1 mL, better to have 3 vials of 1 mL than 4 of 0.8 mL

Stool sample processing

Processing of stool sample:
Please see section 5.1 in the Laboratory Manual
Please note: **The stool specimen should be processed within 48 hours of defecation and as soon as possible from the participant delivering the sample**.

- Using aseptic technique and the provided inoculating loop take 6 stool aliquots of 300 mg each (approximately the size of a lentil) and transfer each to a cryovial for storage and transport.

- If there is a limited amount of specimen (i.e. not enough for 6 x 300 mg), please fill as many vials as possible with 300 mg and distribute the remaining



Blood sample processing

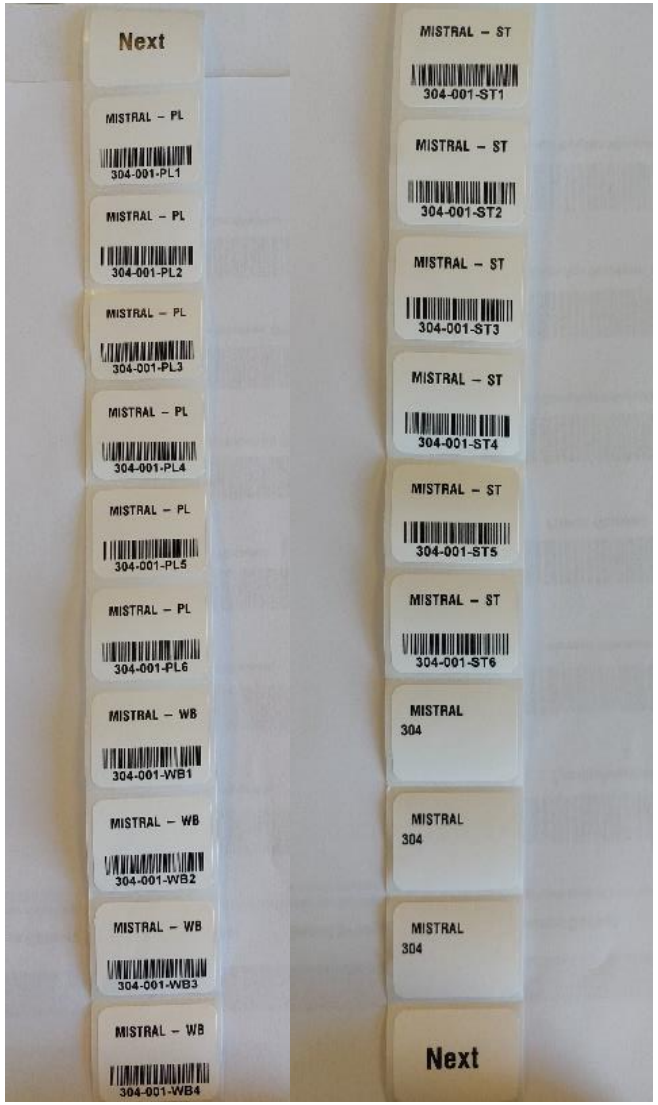
Processing of EDTA plasma samples
Please see section 5.2 in the Laboratory Manual
Please note: **Take plasma ONLY when a stool specimen has been provided**

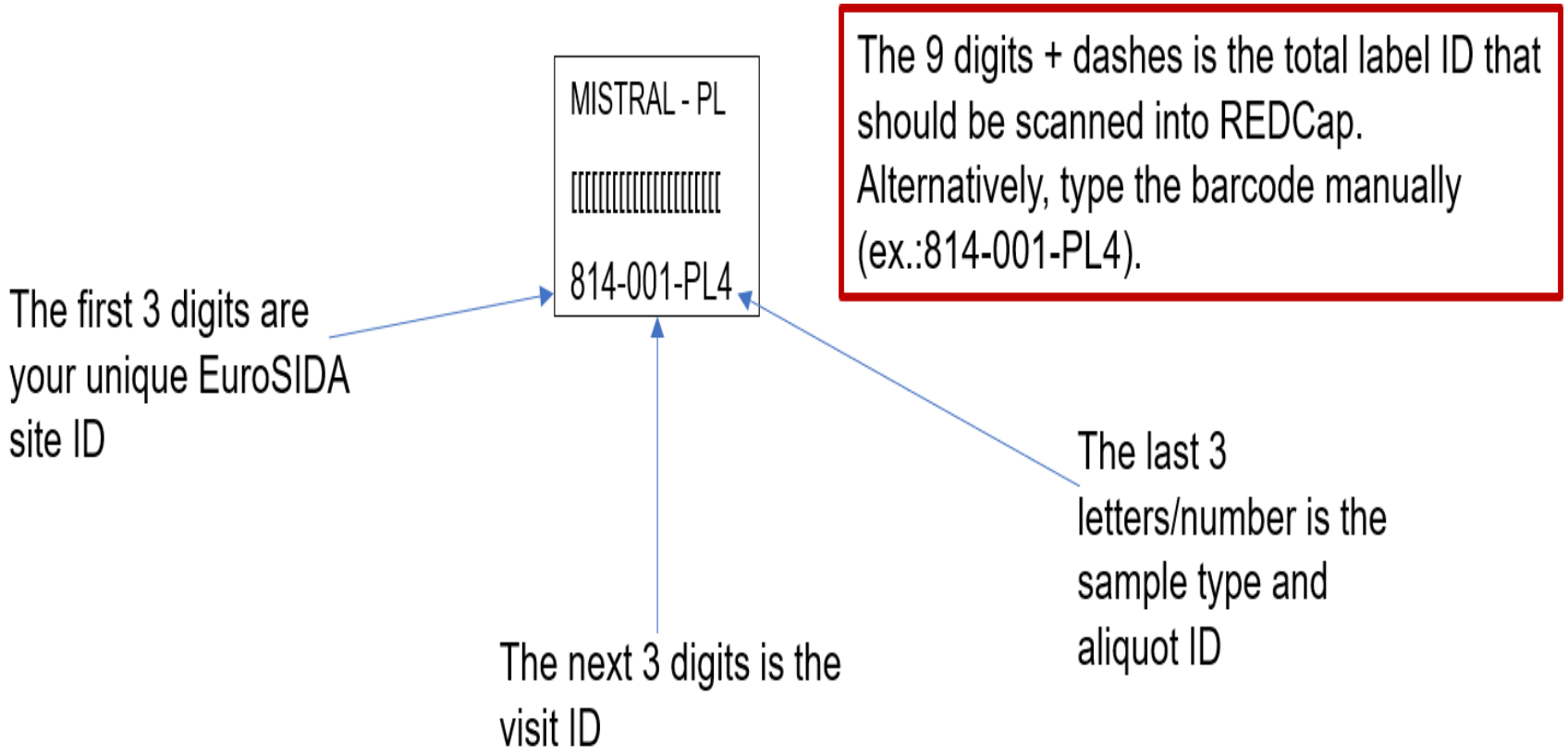
Processing of whole blood specimens
Please see section 5.3 in the Laboratory Manual
Please note: **Take whole blood ONLY when a stool specimen has been provided**

Labels for sample vials

- CHIP will provide preprinted labels for aliquoted blood and stool samples
- Each roll is divided into sets of 19 labels with a divider marked “Next” is inserted between each set
- Use one set of labels per participant visit
- Each set contains

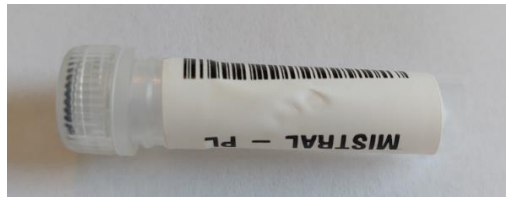
1. Labels for stool samples (marked with ST) x 6
2. Labels for plasma samples (marked with PL) x 6
3. Labels for whole blood samples (marked with WB) x 4
4. Three extra labels only preprinted with MISTRAL and site number (spares in case something goes wrong with the original)




MISTRAL - PL
814-001-PL4
The first 3 digits are your unique EuroSIDA site ID
The next 3 digits is the visit ID
The last 3 letters/number is the sample type and aliquot ID
The 9 digits + dashes is the total label ID that should be scanned into REDCap. Alternatively, type the barcode manually (ex.:814-001-PL4).

Placement of labels

Please place the label on the vial with the text aligned with the vial and so the text is read from the top of the vial and down. Ensure that it is possible to see the content of the vial on the side of the tube:

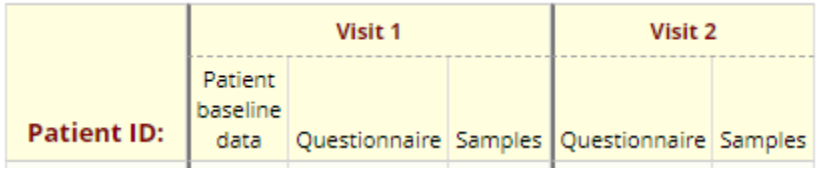


Barcode scanner

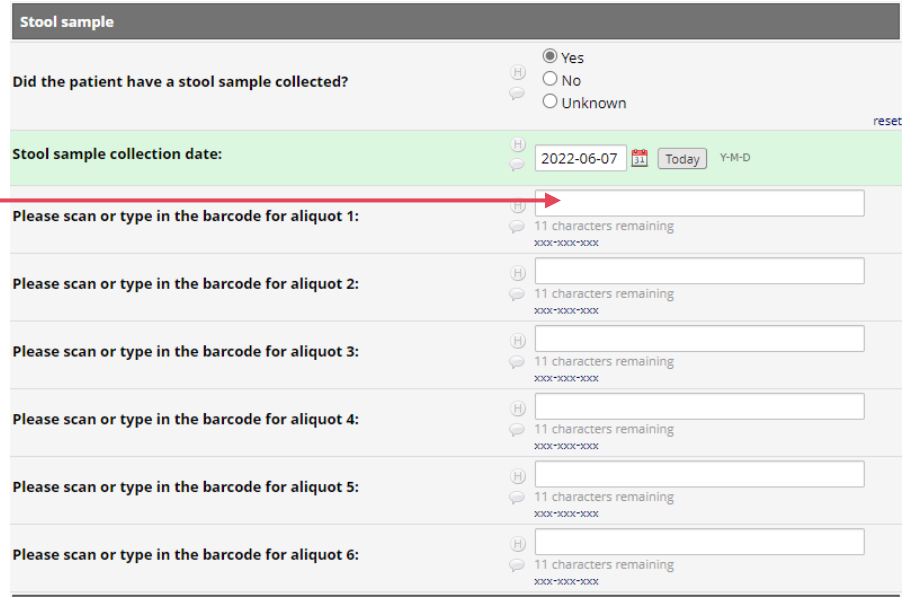
You will receive this scanner to use for scanning the sample barcodes.

It will be programmed for your country. In case it needs reprogramming, please see the Laboratory Manual section 5.4

Scan vial barcode into REDCap
Go to samples under the relevant visit in the REDCap system:



Stool samples: Place the curser in the first field and use the barcode scanner to scan the barcode on the first stool sample vial (ST1)



Repeat for all:
6 stool samples
6 plasma samples
4 whole blood samples

List of stored samples

Use one set of labels per participant per visit.

The label will be linked to the participant and visit in REDCap. In section A.1 REDCap enter the 6 digits after the site number (patient ID, sample type and aliquot ID) collected for the specific visit as well as collection date of samples .

Also be sure to record the samples in the MISTRAL list of stored samples (Appendix B in the Laboratory Manual).

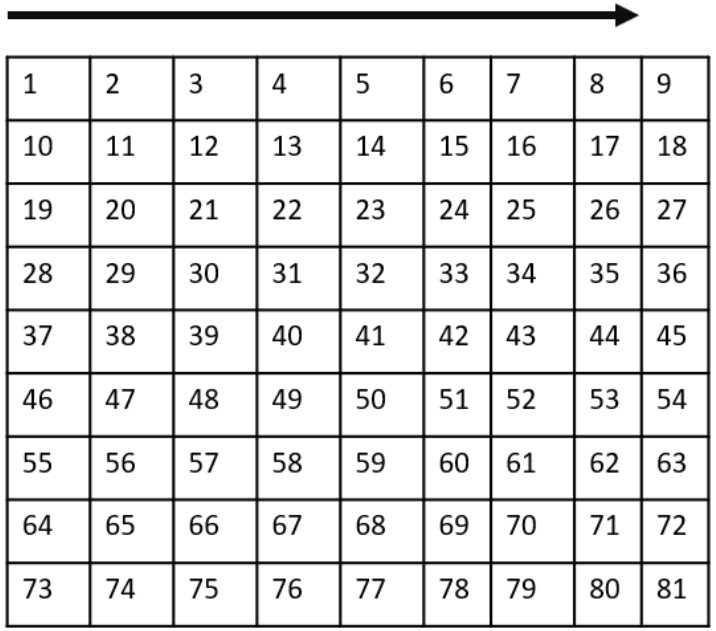
Example of list of stored samples

MISTRAL Study: List of Stored Samples				
Hospital:		Country:		
Centre Code:		Investigator:		
Participant ID xxx-xxxx	**Specimen Collection Date**	**Label ID** (excl. centre code, e.g. 001-PL4)	**Grid box number**	**Comments**

Storage

EDTA Plasma, Whole Blood and Stool samples should be stored in grid-boxes.

The figure to the right depicts the order in which the samples should be placed in the grid box.



1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26	27
28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54
55	56	57	58	59	60	61	62	63
64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	81

- All samples should be stored locally until ready to be shipped to the coordinating centre at CHIP.
- When ready to ship samples, please contact the coordinating centre Laboratory and Shipping Coordinator at CHIP.
- The coordinating centre will contact the courier to be used. Only the courier services designated by the coordinating office may be used. The courier will provide all packing and shipping materials.

Next Steps

What next

- Review all study documents
- Prepare to receive study materials
- Discuss and plan for coordination of stool sample collection

Site and patient engagement

- The success of this study relies heavily on both site staff and participant interest and engagement
- We plan regular newsletters and investigator meetings to keep everyone updated
- Scientific manuscripts completed as part of this study will be made available to all through the CHIP and MISTRAL website
- We predict there will be a number of publications resulting from the samples and data collected as part of this protocol – as is standard in EuroSIDA , each manuscript will include EuroSIDA site investigators as part of the writing group and each manuscript will acknowledge all site staff and participants for their contribution
- Investigators are also able to submit proposals to utilise these samples and data – in line with standard EuroSIDA policies – these proposals will be reviewed by both the EuroSIDA and MISTRAL scientific steering committees

- We welcome feedback on how to improve engagement with both site staff and participants
- To follow the conduct of the entire consortium, please visit https://www.mistral-hiv.eu/the-project/ and follow on social media

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