

MISTRAL Study Laboratory Manual

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1 Introduction and Contact Information

1.1 Introduction

The purpose of the MISTRAL study Laboratory Manual is to provide information on necessary laboratory requirements as well as details regarding the procedures for collecting, processing and shipping specimens for the MISTRAL study. All specimens should be sent to the coordinating site and central biobank repository at CHIP in Copenhagen, Denmark. A list of which materials are supplied by the site laboratory and which are supplied by CHIP is provided at the end of this manual.

Specimens should be collected, processed and shipped according to the directions in this manual.

1.2 CHIP Contact Information

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An electronic copy of the MISTRAL Laboratory Manual is available on <https://chip.dk/Research/Studies/MISTRAL>.

2 Laboratory Assessment Checklist

Site laboratories that process and store MISTRAL study specimens are required to have appropriate facilities. Please use the check lists A and B below to ensure your laboratory complies with the necessary requirements.

A. Required

1. Laboratories must have certification by the appropriate local authority or international organization.
2. Laboratories must be able to process all appropriate specimens within the time frame stipulated in **Section 5, Sample Processing** of this manual.
3. Laboratories must have centrifuges that can reach the speeds required (1500 g).
4. Laboratories must have availability of storage space in a -70/-80°C freezer. If the site has a -20°C freezer, there must be a procedure in place to transfer the specimens (on dry ice) to

a -70/-80°C freezer within 4 days and to track the specimens so that this 4-day window is assured for all specimens.

B. Strongly Recommended

1. A freezer alarm system that will alert a person if a freezer goes down and a plan to assure that specimens remain at -70/-80°C.
2. A standard operating procedure in place to respond to incidents that threaten the integrity of the specimens - incidents such as power failures, freezer failures, storms etc.

3 Participant Privacy Protection Warning

Please strive to ensure that personal information written on the tube by the participant is not seen outside the local processing area.

4 Specimen Collection

4.1 General Specimen Collection Information

Participants eligible for the study will review and undergo informed consent. Once consented, participants will receive a stool specimen collection kit (see **Appendix A**) to be used at home or on site **which should be returned within 48 hours of defecation**. During the site visit, the participant will submit their stool specimen and have blood drawn for both plasma and whole blood specimens (please see later sections for which tubes to use for specimen collection). Investigators from participating sites will complete the MISTRAL Enrolment form and the MISTRAL Visits form. For MISTRAL participants already enrolled in EuroSIDA, only the MISTRAL Visits form should be completed as enrolment data is already collected through EuroSIDA.

Participants will also provide a follow-up stool, plasma and whole blood specimen at either their year 1 or year 2 visit. Again, participants will receive a stool specimen collection kit to be used at home or on site **which should be returned within 48 hours of defecation**. During the site visit, the participant will again submit their stool specimen and have blood drawn for both plasma and whole blood specimens. Investigators from participating sites will complete the forms for Visit 2 in the MISTRAL Visits form.

The specimens to be collected are summarized in the table below.

	Visit 1 Enrolment	Visit 2 (10-24 months post enrolment)
Stool	x	x
Plasma	x	x
Whole Blood	x	x



5 Sample Processing and Storage

5.1 Stool Specimens

Stool specimens will, as previously mentioned, be collected by the participant either on site or at home. The participant will deliver their stool specimen in a sterile tube and should have collected approximately 1 teaspoon of stool (approximately the size of a cherry). Do not dispose of specimens with less than 1 teaspoon of stool; please process what is available.

When the site receives the stool specimen from the participant, the stool specimen should be kept at 4°C until the site is ready to process the specimen. **DO NOT** freeze the sample prior to processing. **The stool specimen should be processed within 48 hours of defecation and as soon as possible after the participant delivers the sample.**

When ready to process the stool specimen, the site should do the following:

1. 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 provided 1.8 mL cryovial for storage and transport. Each vial must contain at least 300 mg of stool: 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 <300 mg to the already filled vials, even if this means there will be less than the required number of aliquots stored. **Do NOT** add any preservative or stabilizer.
2. Clearly label the vials with the provided Stool (ST) labels according to the labelling guidance in section 5.4.
In case of using a spare label write the following information on the label with a pen, permanent pencil or securline marker:
 - a. Visit ID (e.g. 001)
 - b. Specimen type: ST (Stool) and aliquot number
3. Store the stool specimen vials immediately at -70/80°C. If -70/80°C is not available, stool specimens may be stored in a -20°C freezer for up to 4 days but must then be transferred on dry ice to a -70/80°C freezer. If stool has been stored in freezers with temperatures above -50°C, or if more than 48 hours have passed from defecation until stool has been frozen, this should be clearly indicated under the comments section on the list of stored samples when specimens are shipped to the coordinating center.
4. Keep a list of stored specimen vials including the following information:
 - a. The participant ID (the 7-digit PID number)
 - b. Specimen processing date
 - c. Label ID (e.g. 999-001-PL4)
 - d. Grid box number
 - e. Comments, if relevant

A template of the list of stored samples is available in **Appendix B**.

Please refer to **Section 5.4** for an overview of labelling procedure and **Section 5.5** for an overview of container types to be used for collection and storage/transport.

Please refer to **Section 6, Sample Shipping** if there are less/more than the proposed number of aliquots.



5.2 EDTA Plasma Specimens

EDTA plasma specimens are to be taken when the participant submits their stool specimen. **Take plasma ONLY when a stool specimen has been provided.**

1. Collect **2x 6 mL** of blood in 2x lavender-top (EDTA without separator) tubes. Do NOT use a separator tube such as PPT.
2. Immediately after collection, gently invert the tube 5 times to mix the blood and additive
3. Maintain blood at room temperature until it is centrifuged. Process and store blood within four hours of drawing it. For best results, centrifuge **within 2 hours of blood collection**.
4. Centrifuge whole blood at room temperature at 1500g for 15 minutes so the plasma and cells are separated
5. Using a graduated sterile pipette and following aseptic techniques, remove plasma, without disturbing the red cell layer, and distribute the plasma into 6 aliquots with 1 mL in each aliquot. I.e. transfer 1 mL of plasma to a storage/transport vial (1.8 mL screw top cryovials) and repeat 5 times resulting in 6 storage/transport vials with 1 mL of plasma in each. Each transport vial must contain at least 1 mL of plasma. Screw the caps on tightly. Do not tape the caps on.
6. Clearly label the vials with the provided Plasma (PL) labels according to the labelling guidance in section 5.4.
In case of using a spare label write the following information on the label with a pen, permanent pencil or securline marker:
 - a. Visit ID (e.g. 001)
 - b. Specimen type: PL (Plasma) and aliquot number
7. Store plasma samples at -70/80°C. If -70/80°C is not available, plasma specimens may be stored at a -20°C freezer for up to 4 days but then must be transferred on dry ice to a -70°/80° freezer. If plasma has been stored in freezers with temperatures above -50°C, or if more than 6 hours have passed from drawing of blood until plasma has been frozen, this should be clearly indicated in the comments section on the list of stored samples when specimens are shipped to the coordinating center.
8. Keep a list of stored specimen vials including the following information:
 - a. The participant ID (the 7-digit PID number)
 - b. Specimen processing date
 - c. Label ID (e.g. 999-001-PL4)
 - d. Grid box number

A template of the list of stored samples is available in **Appendix B**.

Please refer to **Section 5.4** for an overview of labelling procedure and **Section 5.5** for an overview of container types to be used for collection and storage/transport.

Please refer to **Section 6, Sample Shipping** if there are less/more than the proposed number of aliquots.



5.3 Whole Blood Specimens

Whole blood specimens are to be taken when the participant submits their stool specimen. **Take whole blood ONLY when a stool specimen has been provided.**

1. Collect **1x 6mL** of whole blood in a lavender-top (EDTA without separator) tube. Do NOT use a separator tube such as PPT.
2. Maintain blood at room temperature. Process and store blood within four hours of drawing it.
3. Using a graduated sterile transfer pipette and following aseptic techniques, distribute the whole blood into 4 aliquots with 1 mL in each aliquot: i.e. transfer 1 mL of whole blood to a storage/transport vial (1.8 mL screw top cryovials) and repeat 3 times resulting in 4 storage/transport vials with 1 mL of whole blood in each. Each transport vial must contain at least 1 mL of whole blood. Screw the caps on tightly. Do not tape the caps on.
4. Clearly label the vials with the provided Whole Blood (WB) labels according to the labelling guidance in section 5.4.
In case of using a spare label write the following information on the label with a pen, permanent pencil or securline marker:
 - a. Visit ID (e.g. 001)
 - b. Specimen type: WB (Whole Blood) and aliquot number (e.g. WB3)
5. Store whole blood samples at -70/80°C. If -70/80°C is not available, whole blood specimens may be stored at a -20°C freezer for up to 4 days but then must be transferred on dry ice to a -70°/80° freezer. If whole blood has been stored in freezers with temperatures above -50°C, or if more than 6 hours have passed from drawing of blood until the whole blood has been frozen, this should be clearly indicated in the comments section on the specimen list when specimens are shipped to the coordinating center.
6. Keep a list of stored specimen vials including the following:
 - a. The participant ID (the 7-digit PID number)
 - b. Specimen processing date
 - c. Label ID (e.g. 999-001-PL4)
 - d. Grid box number

A template of the list of stored samples is available in **Appendix B**.

Please refer to **Section 5.4** for an overview of labelling procedure and **Section 5.5** for an overview of container types to be used for collection and storage/transport.

Please refer to **Section 6, Sample Shipping** if there are less/more than the proposed number of aliquots.

5.4 Labelling

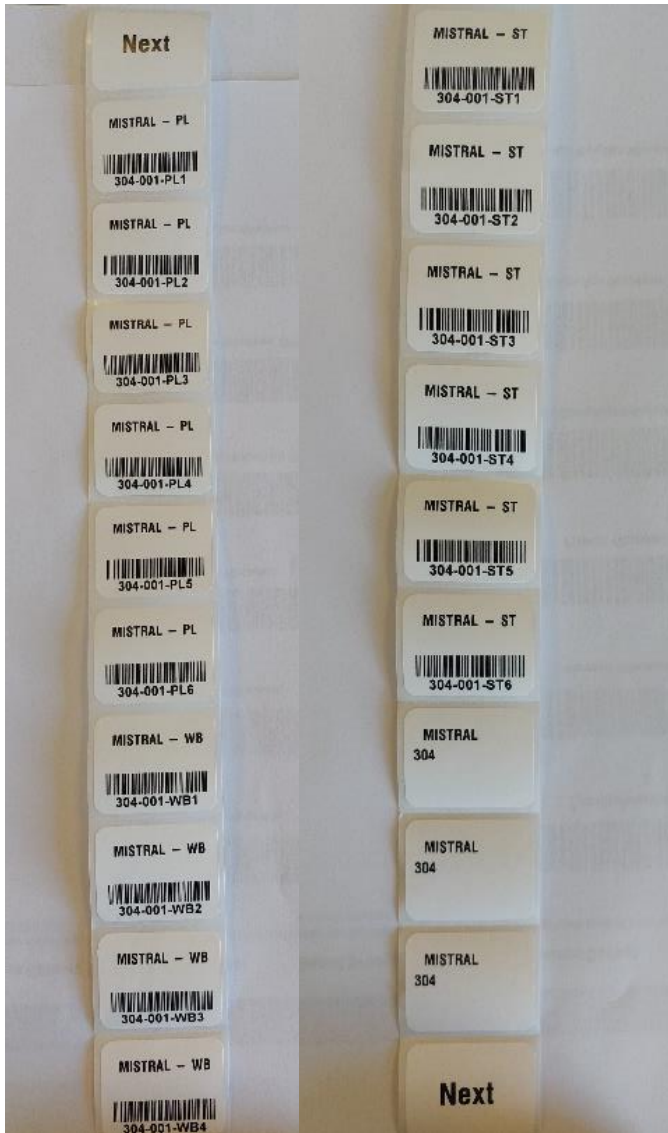
Label use instruction

CHIP will provide pre-printed labels for vials for blood and stool samples.

You have received rolls of labels that should be used for the cryovials. The roll is divided into sets of 19 labels (6 plasma, 4 whole blood, 6 stool and 3 spare labels) per participant per visit and a

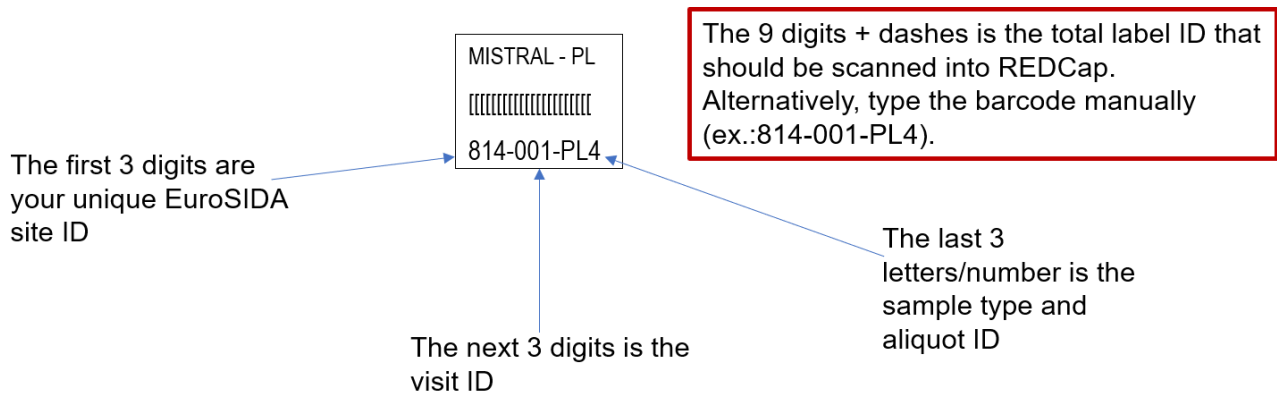
divider marked “Next” is inserted between each set. The labels are barcode-numbered for each sample*.

As mentioned, each set of labels contains labels for one participant and one visit. In each set you will find:



1. Labels for stool samples (*marked with ST*) x 6 – use these to label the 6 stool aliquots
2. Labels for plasma samples (*marked with PL*) x 6 – use these to label the 6 plasma aliquots
3. Labels for whole blood samples (*marked with WB*) x 4 – use these to label the 4 whole blood aliquots
4. Three extra labels only preprinted with MISTRAL and site number – use these as spares if needed. Please add the visit ID, sample type and aliquot ID in handwriting

*The pre-printed label contains a pre-printed sample label ID which should never be changed. The label ID has no resemblance with the Patient ID. The label ID and the Patient ID will be linked automatically when the sample label-ID is entered in REDCap.



Use one set of labels per participant per visit.

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:



The label will be linked to the participant and visit in REDCap.

In REDCap under MISTRAL Visits choose Samples under Visit 1 or Visit 2 and scan the label with the provided or your own barscanner for each sample collected for the specific visit and choose date of sample collection. Alternatively, type the barcode manually (ex. 203-001-PL1).

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

Please be sure to record the samples in the MISTRAL list of stored samples (Appendix B in the Laboratory Manual), please refer to section 5.1 (stool specimens), 5.2 (EDTA plasma specimens) and 5.3 (whole blood specimens) for details.

Barcode scanner instruction

You have also received a barcode scanner to use for scanning the vial labels into REDCap. We have programmed the scanner for use in your country. Please test the barcode scanner in the REDCap system when you receive it, before enrolling the first patient.

If the scanner needs reprogramming, please find the Quick Start Guide in the box. The scanner should be plugged into a USB port on your PC. Please scan the barcode in the guide adding a tab key:



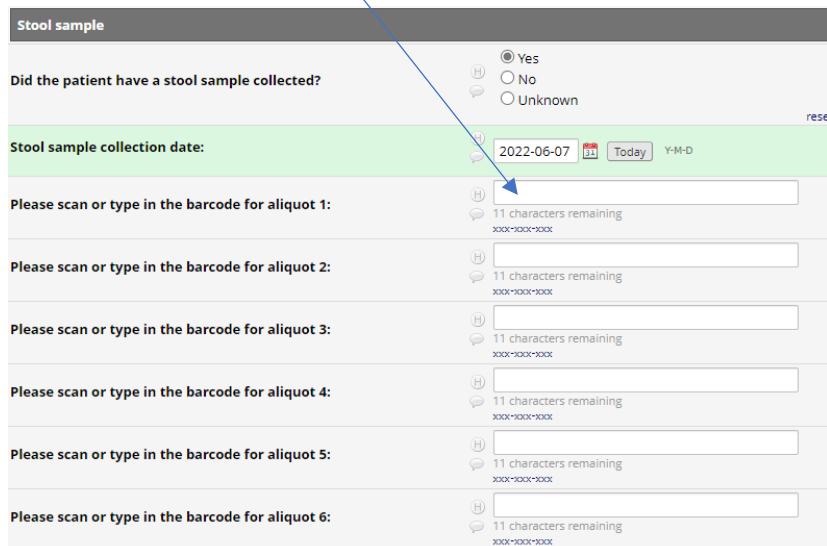
Also scan the barcode for your country, see appendix C.

Scan vial barcode into REDCap

Go to samples under the relevant visit in the REDCap system:

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

Stool samples: Place the cursor 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 and 4 whole blood samples

5.5 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 (Enrolment and 10M-24M)	Sterile container	1 teaspoon per collection*	6 (6x 300mg in each tube)	1.8 mL screw top cryovials
Plasma	2x (Enrolment and 10M-24M)	EDTA <u>without separator</u> (lavender-top)**	2 x 6 mL per collection	6 (***6x 1 mL in each vial)	1.8 mL screw top cryovials
Whole Blood	2x (Enrolment and 10M-24M)	EDTA <u>without separator</u> (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

Important: These collection tables do NOT include the blood needed for local lab tests.

6 Sample Shipping

After processing, transport vials should contain at least 1 mL of specimen and less than 1.8 mL, for whole blood and plasma, and at least 300 mg (the size of one lentil) and less than one half of the collection tubes volume, for stool samples.

6.1 Less than the Required Number of Aliquots


All transport vials must contain at least 1 mL of blood or plasma and at least 300 mg of stool (the size of one lentil). If there is not enough specimen left for these volumes in a transport vial please distribute the remaining specimen to the other vials, even if this means there will be less than the required number of aliquots stored. I.e. it is better to send fewer vials with the correct amount of specimen, than the correct number of vials with an incorrect minimum amount of specimen.



6.2 Grid Box Vial Storage and Grid Box Numbering

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

The figure below 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. Samples should be shipped every one to two years when instructed by CHIP.

When ready to ship samples, please contact the coordinating centre Laboratory and Shipping Coordinator at CHIP. You may also request a shipment from the study website at <https://chip.dk/Research/Studies/MISTRAL/Samples/Order-Shipment>.

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.

7 Materials

7.1 Materials Provided by the Site Laboratory

- **Stool specimens:**
 - For collection:
 - All materials will be provided by CHIP
 - For processing/storage/transport:
 - All materials will be provided by CHIP
- **EDTA plasma specimens:**
 - For processing/storage/transport:
 - Graduated transfer pipettes
- **Whole blood specimens:**
 - For processing/storage/transport:
 - Graduated transfer pipettes



7.2 Materials Provided by CHIP

- **Stool specimens:**
 - For collection:
 - Faecal Collection Kit including container (home-based stool collection by participants (see Appendix A))
 - For processing/storage/transport:
 - Labels for vials
 - Sterile, rigid inoculating loops for processing
 - 1.8 mL cryovials for storage/transport
 - Grid boxes to store vials
 - All packing and shipping materials via the courier at time of shipping
- **EDTA plasma specimens:**
 - For collection:
 - EDTA without separator (lavender-top)
 - For processing/storage/transport:
 - Labels for vials
 - 1.8 mL cryovials for storage/transport
 - Grid boxes to store vials
 - All packing and shipping materials via the courier at time of shipping
- **Whole blood specimens:**
 - For collection:
 - Tubes for whole blood EDTA without separator (lavender-top)
 - For processing/storage/transport:
 - Labels for vials
 - 1.8 mL cryovials for storage/transport
 - Grid boxes to store vials
 - All packing and shipping materials via the courier at time of shipping

For registration of vials in REDCap, a scanner will also be provided by CHIP.







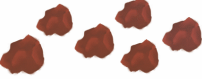


8 Appendix A: Stool Sample Collection

Participant instructions for stool sample collection will be included as part of the stool collection kit.

1. Urinate completely before defecating
2. Set up Fe-Col faeces collection paper in toilet according to printed instructions (see below)
3. Clean the perineal area (i.e. genitals, anus and area in between) with a soaped sponge
4. Cleanse the perineal area with abundant water to wash off the soap
5. Dry the perineal area with a clean, unused towel
6. Defecate on the Fe-Col faeces collection paper
7. Put on gloves provided with the kit
8. Open the sample container without touching the inner rims
9. Collect a portion of stool with the provided spatula. Do not fill the container excessively. If stool is solid, collect around the size of a cherry (or around 1 teaspoon), slightly more or less is fine. If the stool is liquid, collect approximately 20 mL directly into the stool container
10. Close the stool container, place inside the provided envelope/sealable plastic bag
11. Remove and dispose of gloves in the trash
12. Write your first name, last name, date of birth, and date and time of sample collection on the sample container label
13. Take note of stool consistency type using the Bristol Stool Form Scale (see below) and include the insert in the provided envelope/sealable plastic bag
14. Flush Fe-Col faeces collection paper (including any remaining stool) down the toilet according to instructions provided with the kit
15. Refrigerate the stool container (approximately 4-5° Celsius). If a refrigerator is not available, keep the stool in a cool dry place or defecate in the morning and immediately bring the sample to the site
16. Bring the stool container and the self-identified stool consistency type to the clinic within 48 hours (at the latest) of collecting the stool sample.

A stool collection instructional video is located here, <https://youtu.be/a3uGHqWz7P8>. This video will also be provided to participants in with the stool collection kit.

Bristol Stool Form Scale

Type 1		Separate, hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces Entirely liquid

Reference: Heaton, K W & Lewis, S J 1997, 'Stool form scale as a useful guide to intestinal transit time'. Scandinavian Journal of Gastroenterology, vol. 32, no. 9, pp.920-924.

9 Appendix B: List Template for Stored Specimens

[illegible]

10 Appendix C: Country Barcodes for Scanner

USB and Keyboard Wedge Country Keyboard Types (Country Codes)

Scan the bar code corresponding to the keyboard type. For a USB host, this setting applies only to the USB Keyboard (HID) device. If the keyboard type is not listed, see [Emulate Keypad on page 3-9](#) for the USB HID host. For a Keyboard Wedge host, see [Alternate Numeric Keypad Emulation on page 6-6](#).



NOTE When changing USB country keyboard types the scanner automatically resets. The scanner issues the standard startup beep sequences.



IMPORTANT 1. Some country keyboard bar code types are specific to certain Windows Operating Systems (i.e., XP, and Win 7, or higher). Bar codes requiring a specific Windows OS are noted so in their bar code captions.

2. Use the **French International** bar code for Belgian French keyboards.



***US English (North American)**



Albanian



Arabic (101)



Arabic (102)



Arabic (102) AZERTY

Country Codes (Continued)**Azeri (Latin)****Azeri (Cyrillic)****Belarusian****Bosnian (Latin)****Bosnian (Cyrillic)****Bulgarian (Latin)**

Country Codes (Continued)



Bulgarian Cyrillic (Typewriter)
(Bulgarian -Windows XP
Typewriter - Win 7, or higher)



Canadian French Win7



Canadian French (Legacy)



Canadian Multilingual Standard



Chinese (ASCII)

Country Codes (Continued)**Croatian****Czech****Czech (Programmer)****Czech (QWERTY)****Danish****Dutch (Netherlands)**

Country Codes (Continued)



Estonian



Faeroese



Finnish



French (France)



**French International
(Belgian French)**



French (Canada) 95/98

Country Codes (Continued)**French (Canada) 2000/XP***

*Note that there is also a country code bar code for *Canadian Multilingual Standard on page B-8*. Be sure to select the appropriate bar code for your host system.

**Galician****German****Greek Latin****Greek (220) Latin****Greek (319) Latin****Greek**

Country Codes (Continued)



Greek (220)



Greek (319)



Greek Polytonic



Hebrew Israel



Hungarian



Hungarian_101KEY



Icelandic

Country Codes (Continued)**Irish****Italian****Italian (142)****Japanese (ASCII)****Kazakh****Korean (ASCII)****Kyrgyz**

Country Codes (Continued)



Latin American



Latvian



Latvian (QWERTY)



Lithuanian



Lithuanian (IBM)



Macedonian (FYROM)



Maltese_47KEY

Country Codes (Continued)**Mongolian****Norwegian****Polish (214)****Polish (Programmer)****Portuguese (Brazil)
(Windows XP)****Portuguese (Brazilian ABNT)****Portuguese (Brazilian ABNT2)**

Country Codes (Continued)



Portuguese (Portugal)



**Romanian
(Windows XP)**



**Romanian (Legacy)
(Win 7, or higher)**



**Romanian (Standard)
(Win 7, or higher)**



**Romanian (Programmer)
(Win 7, or higher)**



Russian

Country Codes (Continued)**Russian (Typewriter)****Serbian (Latin)****Serbian (Cyrillic)****Slovak****Slovak (QWERTY)****Slovenian****Spanish**

Country Codes (Continued)



Spanish (Variation)



Swedish



Swiss French



Swiss German



Tatar



Thai (Kedmanee)



Turkish F

Country Codes (Continued)**Turkish Q****UK English****Ukrainian****US Dvorak****US Dvorak Left****US Dvorak Right**

Country Codes (Continued)



US International



Uzbek



Vietnamese