

ASPREE-X^T Microbiome Study

SAMPLE COLLECTION INSTRUCTIONS

This instruction pamphlet comprises four sections:

SECTION 1: INTRODUCTION

SECTION 2: SAMPLE COLLECTION – OVERVIEW

SECTION 3: SAMPLE COLLECTION – DETAILED INSTRUCTIONS

SECTION 4: FINAL CHECKLIST

SECTION 1: INTRODUCTION

Thank you for your participation in this study. These instructions accompany the Participant Information and Consent Form (PICF) and explain the collection and sample return process. The PICF describes the goals of the study, who is funding and running it, and who to contact for additional information. You should read through this first, and if happy to participate, complete the details on the last page (sign and date). Using one of the barcode stickers included in the kit, stick this into the space provided at the top righthand corner of the last signed page. **You can keep the explanatory part of PICF for your records, but the signature page (the last page), needs to be sent back to us with your sample collection.**

It is important to read all of the instructions prior to starting, and if you have any questions, or are unsure of a step, please call us at the ASPREE Co-ordinating Centre on the free call number – 1800 728 745. The entire process is straightforward and hygienic.

Please collect, and return your samples as soon as possible after your final collection. If you make a mistake, give us a call, we can always send you another kit.

*For all general ASPREE-X^T enquiries
please call the National Co-ordinating Centre on*

1800 728 745

What do you have to do?

This kit provides you with the equipment needed to take 3 stool samples, ideally from the one 'visit' to the bathroom and **from the same stool**, followed the next day by a light swab of the tongue as soon as you wake up. Additionally, two questionnaires are included: one about diet and bowel movements, the other relating to the tongue swab and dental health.

On the following pages we walk you through these steps:

DAY 1: Collection of stool samples and completion of the stool questionnaire.

DAY 2: Upon waking (before brushing teeth), collection of tongue swab sample, completion of the tongue swab questionnaire and finally, the return of completed sample kit.

Before starting, check that your kit contains all of the materials listed below. If you are missing any items, please let the study team know as soon as possible (1800 728 745), and we can arrange to send the missing items to you.

Kit Contents

- 3 TUBES - each to hold a small sample of stool (a scraping) from one bowel motion.

It is preferable to add to the tubes in the following order:

1. **Purple** capped Ethanol stool collection tube - packet labelled with an orange #1;
2. **Purple** capped OMNIgene-GUT stool collection tube - purple # 2 packet;
3. Shorter, anaerobic stool collection tube in a foil wrapped packet - black # 3 packet.

- 1 TONGUE (ORAL) SWAB white capped OMNIgene-ORAL tube - blue # 4 packet to collect a sample from your tongue.

- Instructions on how to collect the samples (*this pamphlet*)

- COLLECTION ACCESSORIES:

2 toilet liners

Spatulas for stool sample collection (*packet of three*)

- LABELLING AND PACKAGING ACCESSORIES:

Sheet of barcode stickers

(*one barcode label for each tube, one for each questionnaire and one for the Consent page*)

Kit Contents (continue)

- One specimen bag (with absorbent pad) for each sample tube
- One air cushion (to secure package contents prior to mailing back)

- TWO QUESTIONNAIRES

- One Stool Sample Questionnaire
- One Tongue Swab Questionnaire

- POSTAGE BOX AND PREPAID EXPRESS POST SACHEL.

Figure 1: The contents of the collection kit are shown below:

1. Postage air cushion; **2.** Kit postage box with prepaid Express Post satchel; **3.** Purple capped stool collection tube with ethanol (tube #1); **4.** Purple capped stool tube called the 'OMNIgene-GUT' tube (tube #2); **5.** The short 'anaerobic' stool tube in a foil packet (tube#3); **6.** The white capped tongue swab kit (OMNIgene-ORAL, tube#4); **7.** Labels (barcode stickers) to affix to tubes and paperwork; **8.** Specimen bags for collection tubes; **9 & 10.** Both questionnaires; **11.** Stool collection spatulas; and **12.** Toilet liners.

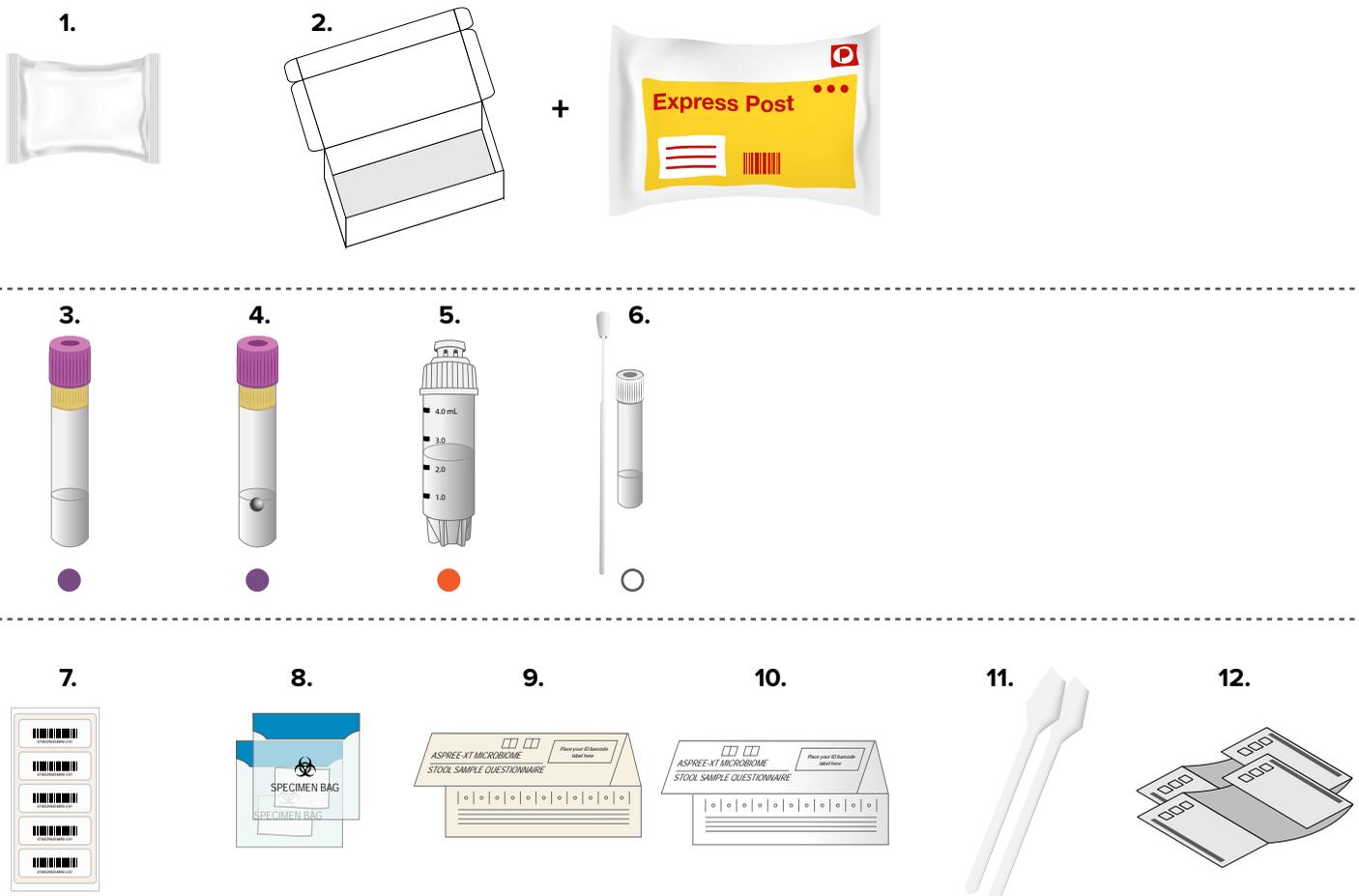


Figure 2: Packets

A The purple capped stool collection tube with ethanol **B** Purple capped OMNIgene-GUT stool collection tubes **C** The anaerobic stool tube in a foil packet **D** The oral tongue swab packet contains the brush-like swab and a white capped collection tube

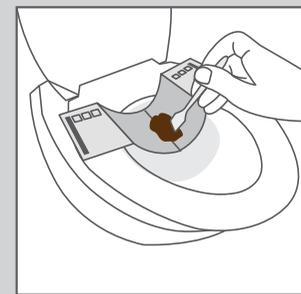
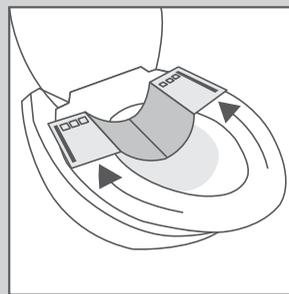
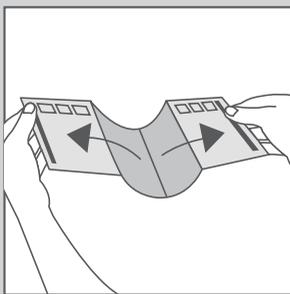


WHAT YOU WILL NEED TO HAVE ON HAND:

- A pen (to complete your questionnaires)
- A cup (in which to support your tubes upright)
- A folded wad of toilet paper (to rest your spatula on in between collections)

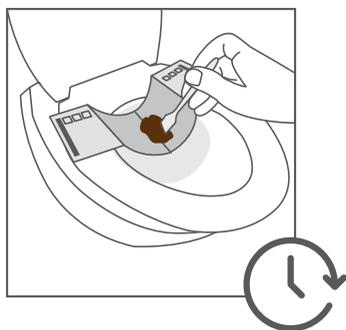
IMPORTANT NOTES:

1. If you are currently taking antibiotics or have recently taken antibiotics (in past 2 months), please DO NOT collect your samples. Wait 2 months after finishing your antibiotics before collecting your samples.



2. Follow the instructions in the toilet liner accessory and peel apart from the arrows shown on the top corners of both sides of the toilet liner:

Attach the collection paper to the toilet seat by pressing firmly down to stick to the seat.



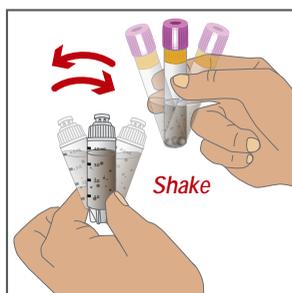
3. **Collect the samples quickly** – the toilet accessory paper will only hold for approximately 2-5 minutes after coming in contact with the stool sample.

4. If your stool sample is all liquid (if you have diarrhoea with no solid material), please wait until your next bowel movement to collect the sample (use the extra toilet liner).
5. The stool sample must be collected free of urine or toilet water. Do NOT urinate onto the toilet liner: if possible, empty your bladder first, flush the toilet, and then stick the toilet liner to the seat. If the stool becomes contaminated with urine or falls into the toilet, do NOT collect the sample. Instead, try again at your next bowel movement using the additional toilet liner.
6. Collected samples can be kept at room temperature until mailed back to us.

Keep this instruction pamphlet separate from everything else and retain until you have completed the steps for posting back the kit.

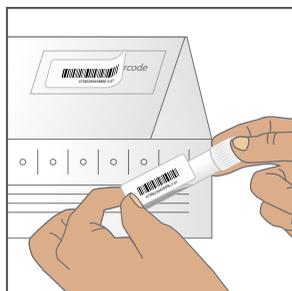
The back page is a final checklist which can be detached and used to cross check that all steps have been completed, before mailing back the packaged kit in the Express Post satchel.

SECTION 2: SAMPLE COLLECTION – OVERVIEW



DAY 1:

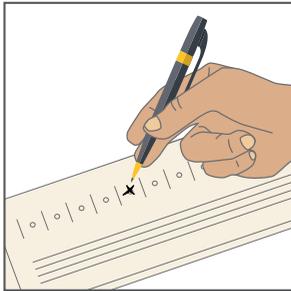
Collect stool samples, seal tubes tightly, shake well and apply the barcodes, then place in specimen bags and seal (see details in Section 3 below). Note, if you have difficulty attaching the barcode to the shorter tube, then attach one to the specimen bag instead.



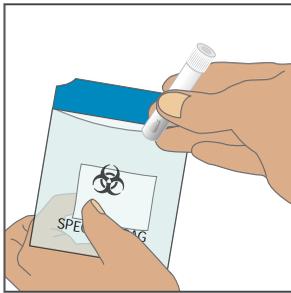
Complete stool questionnaire, and attach the barcode to the tube and questionnaire.

DAY 2

The day after collecting your stool samples, collect your tongue swab sample using the tongue swab kit (white tube), first thing before eating, drinking, smoking or brushing your teeth (see details in Section 3 below). Apply the barcode sticker and place in specimen bag and seal.



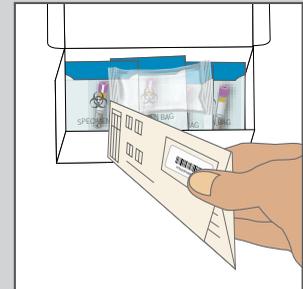
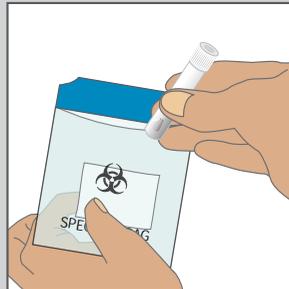
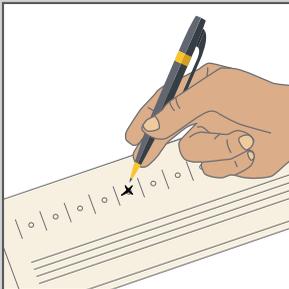
Complete the tongue swab questionnaire and stick on the barcode sticker.



Place all specimen bags and questionnaires into the box.

Sample Return:

Use the 'Final Checklist' (*Section 4, last page of this pamphlet*) to ensure all steps have been completed and all samples, questionnaires and signed consent page are present with barcode stickers attached before closing the postage box. Any leftover stickers can be returned or thrown in the bin.



Please read on for detailed instructions on how to collect and return your samples.

SECTION 3: SAMPLE COLLECTION – DETAILED INSTRUCTIONS

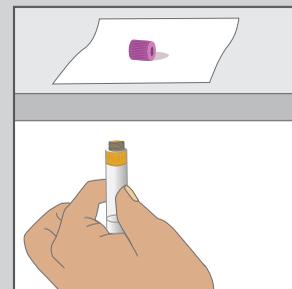
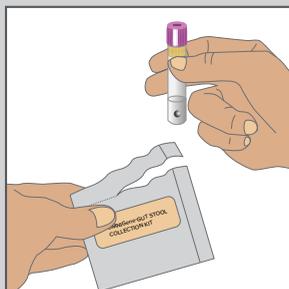
When collecting your three tubes of stool sample tubes from the same ‘visit to the toilet’ (i.e. the one bowel motion), do so from the same individual stool, if possible. Remember, it is best that your stool sample does not come into contact with urine or toilet water, so try to empty your bladder before sticking the toilet liner to the seat.

NOTE: The tubes are not designed to stand upright on their own. Placing the tubes in a cup will allow you to have both hands free to complete your collections. Please be careful not to spill the preservative that is in the tubes. If any of the liquid comes in contact with your skin or eyes, wash with water.

Wait Until AFTER All Three Stool Samples Have Been Collected to Shake the Tubes.

You can use the one spatula for all three collections: the spatula can be rested on the wad of folded toilet paper in between each collection.

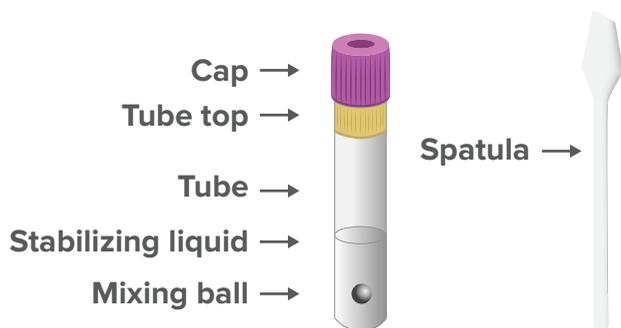
Follow these instructions for each of the three stool collection tubes – both of the purple capped tubes and the smaller tube.



1. FIRST TUBE COLLECTION (*purple capped ethanol tube, orange number 1 packet*)

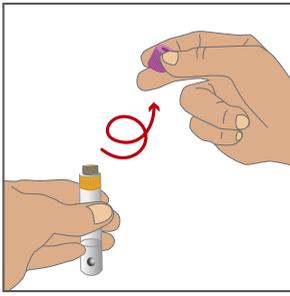
NOTE: the collection process for the second purple capped tube (OMNIgene-GUT tube) is the same process as described here for the first tube.

COLLECTION DEVICE



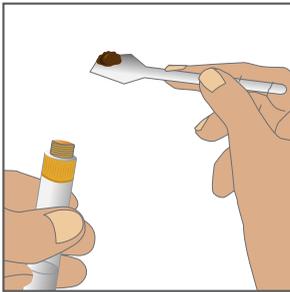
STEP 1

Find the packet labelled with an orange number 1, containing the purple capped tube, open it, and remove the tube. Discard the outer packaging.



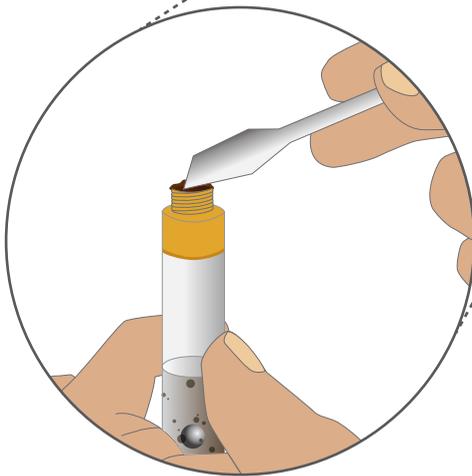
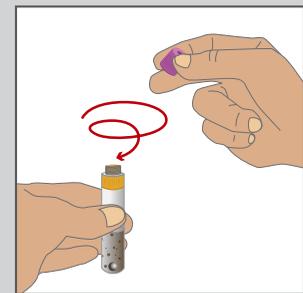
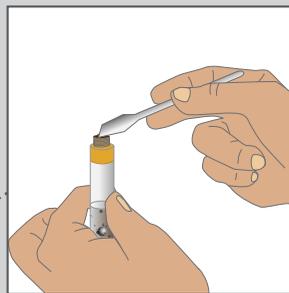
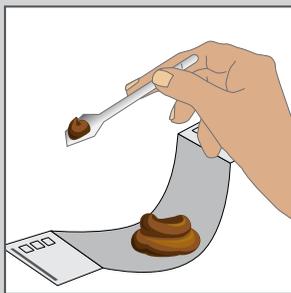
STEP 2

Hold the yellow tube top and unscrew **ONLY** the purple cap from the tube (leave the yellow tube top in place – see figure on the left hand side). Place the cap aside on a clean surface and stand your tube in the cup.



STEP 3

Use the spatula to collect a small scoop of stool sample into the yellow tube top. Repeat scoops until the sample fills the yellow tube top. Do **NOT** push the sample into the tube.



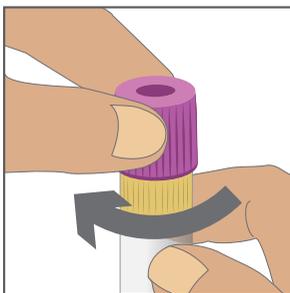
Scrape across the tube top to level the sample and remove any excess stool.

The total amount of stool in the tube should be approximately the size of a jelly bean. It is not necessary to provide any more than this.



STEP 4

Screw the purple cap back on the tube tightly. Wipe the exterior of tube with toilet paper if necessary.



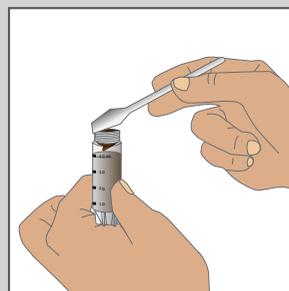
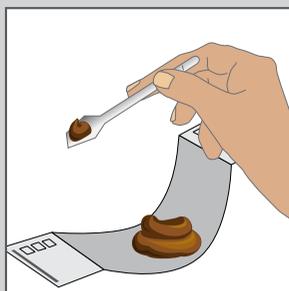
Place this tube to the side, and commence the next tube collection

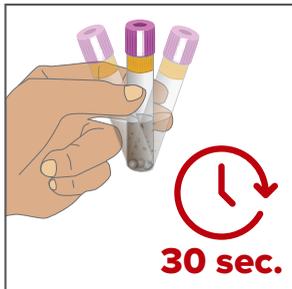
2. SECOND TUBE COLLECTION (*purple capped OMNIgene-GUT tube, packet with a purple #2*)

The collection process for the second purple capped tube (with yellow collar or top) is the same as for the first tube. Please repeat the steps listed above and move on to third tube collection.

3. THIRD TUBE COLLECTION (*foil packet with black number 3*)

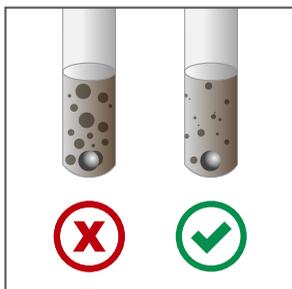
Although the third tube is smaller in size, and does not contain the yellow tube collar, the sample collection principles are the same. Scrape the stool sample across the neck of the tube to ensure stool collects within the tube and screw the cap back onto the tube.





NOW THAT YOU HAVE COMPLETED THE 3 STOOL COLLECTIONS:

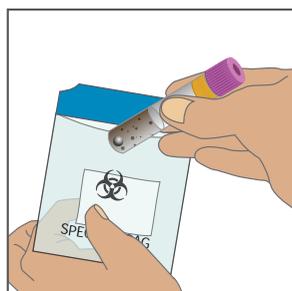
Shake each tube vigorously in a back and forth motion for at least 30 seconds each



Shake until there are no solid lumps visible.



For all three tubes, apply the barcode label running lengthways down the tube. Don't worry if it covers any of the writing on the tube, or does not fit exactly on the shorter tube (if you have trouble with the shorter tube just label the specimen bag).

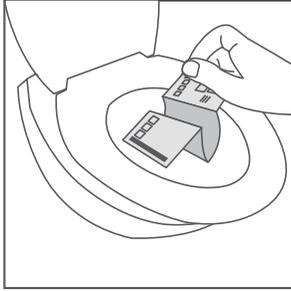


Then place each tube into its own individual specimen bag.

Do NOT remove the absorbent pad from inside the bag. Seal each individual bag by removing the plastic strip from sticky line, press out excess air, fold over and seal.



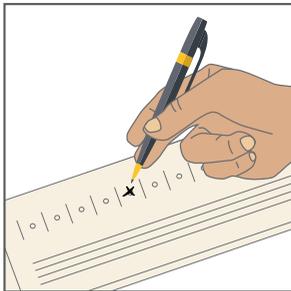
You are now ready to discard the spatula and all packaging in the bin (DO NOT FLUSH), after all collections are completed.



The toilet liner (collection paper) is designed to ‘dissolve’ in water and is safe to flush (including septic tank systems).

ALL STOOL SAMPLE COLLECTIONS HAVE NOW BEEN COMPLETED.

Wash your hands with soap and warm water.



Fill out the stool sample questionnaire completely and affix a barcode label in the space provided.

PLEASE COLLECT YOUR TONGUE SWAB SAMPLE TOMORROW.

DAY 2: ORAL TONGUE SWAB COLLECTION INSTRUCTIONS

IMPORTANT:

You will need to use the oral tongue collection kit (white capped tube) soon after waking, and ideally, on the day following your stool sample collection (or within the next 2-3 days).

DO NOT EAT, DRINK, SMOKE, BRUSH YOUR TEETH, OR USE MOUTH WASH BEFORE PROVIDING A TONGUE SWAB SAMPLE.

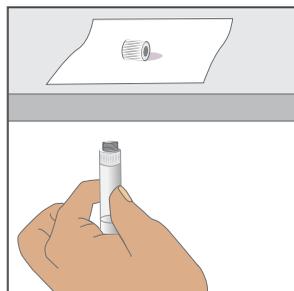
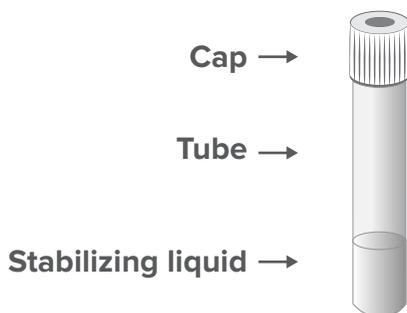
If you happened to do one or some of these things, please wait at least 30 minutes before collecting your tongue swab sample, or postpone the collection until the next day.

Read all instructions prior to collection.

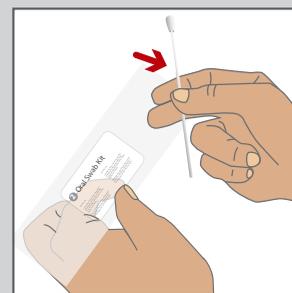
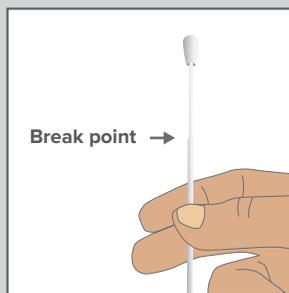
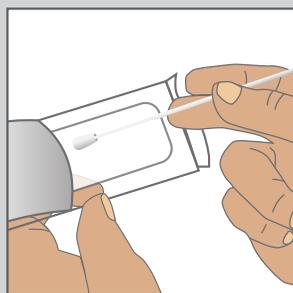
STEP 1

Open the white oral tongue swab collection kit, remove and open the tube packet first.

COLLECTION DEVICE

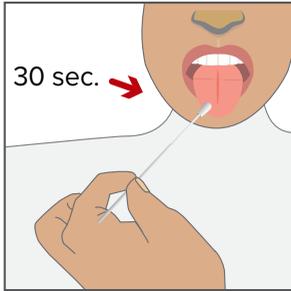


Unscrew the cap and place aside on a clean surface, and stand the open tube in the support cup, to give you two free hands. Now open the swab packet.



IMPORTANT NOTE:

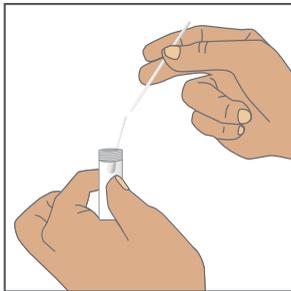
The swab should only be held by the handle, Do NOT touch the swab above the break point nor brush the top or head of the swab to any other surface.



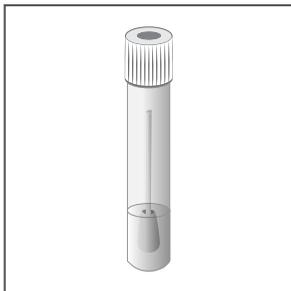
STEP 2

Gently rub the upper surface of your tongue with the swab for 30 seconds. Avoid rubbing the teeth and other parts of the mouth.

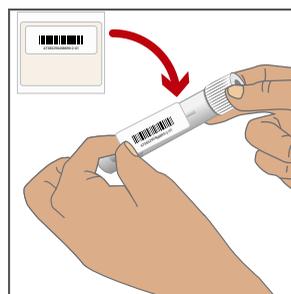
IMPORTANT: DO NOT bend the swab during collection, or touch the tip to any other surface aside from the top of your tongue.



Immediately insert the swab into the bottom of the tube and, as shown left, snap the shaft off at the break point (the swab tip remains in the tube of liquid).



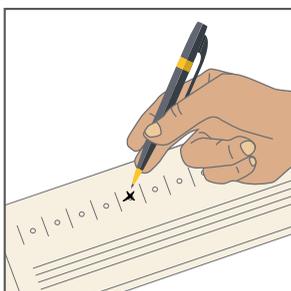
Screw the cap back on the tube tightly.



STEP 3

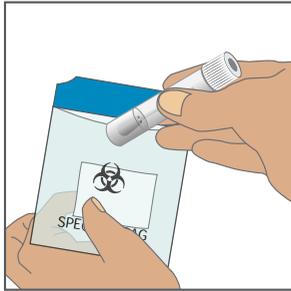
Locate the barcode label and affix one lengthways to the tube. Do not worry if you cover any other labelling or writing on the tube.

Discard the swab handle and packaging in the garbage.



STEP 4

Fill out the tongue swab sample questionnaire completely and affix a barcode label in the space provided (top righthand corner of the questionnaire).



STEP 5

Insert the collected sample into its own specimen bag. Do NOT remove the absorbent pad from inside the bag.

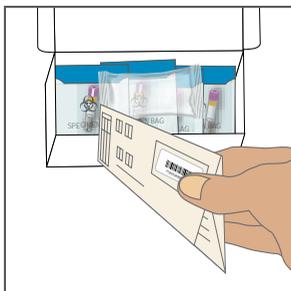
Peel off the tape covering the sticky strip at the top of the specimen bag, press out any excess air and fold over to seal tightly.

Place a barcode label on the outside of the bag.

YOUR SAMPLE COLLECTION PLAN (3 stool tubes, one tongue swab tube, two questionnaires and signed consent page) IS NOW COMPLETE.



Place the completed questionnaires and signed consent into the kit postage box. Place all sealed bags back into the kit postage box.



Please use the final checklist on the following page to cross check you have packaged all components into the kit postage box.



Once all items are inside the box, peel the plastic strip off the box lid, close the box, and then press down firmly across its entire length to seal the box. Place this box into the Prepaid Express Post satchel provided and fill in your contact details on the back of the satchel.



Please put the sealed Express Post satchel in a yellow Express Post mailbox or drop it off at your local post office (PLEASE DO NOT DROP IT OFF IN THE RED STANDARD POST BOX). Express postage has already been prepaid.

Please mail your samples as soon as possible, ensuring they do not stay in hot environments such as cars, for prolonged periods.

IF YOU HAVE QUESTIONS PLEASE CALL YOUR STUDY CO-ORDINATOR OR ASPREE-XT ON 1800 728 745.

SECTION 4: FINAL CHECKLIST

Before sealing up the kit postage box, check that all items have been completed and are included in the package:

<input type="checkbox"/>	ALL 3 STOOL COLLECTION TUBES, EACH WITHIN A SEPARATE SPECIMEN ZIP LOCK BAG WITH ABSORBENT PAD:	
	<input type="checkbox"/>	Purple capped ethanol stool collection tube, with a barcode sticker running lengthways down the tube
	<input type="checkbox"/>	Purple capped OMNIGene-GUT stool collection tube, with a barcode sticker running lengthways down the tube
	<input type="checkbox"/>	Small anaerobic stool collection tube with a barcode sticker running lengthways down the tube
<input type="checkbox"/>	THE WHITE-CAPPED TONGUE SWAB (OMNIGENE-ORAL) TUBE, WITH BARCODE STICKER AFFIXED LENGTHWAYS, AND PLACED IN A SEPARATE SPECIMEN BAG.	
<input type="checkbox"/>	TWO COMPLETED QUESTIONNAIRES, EACH WITH BARCODE STICKER AFFIXED.	
	<input type="checkbox"/>	One Stool Sample Questionnaire
	<input type="checkbox"/>	One Tongue Swab Questionnaire
<input type="checkbox"/>	SIGNED CONSENT PAGE (LAST PAGE OF PICF) WITH BARCODE STICKER AFFIXED.	
<input type="checkbox"/>	AIR CUSHION PLACED ON TOP OF THE COLLECTION BAGS AND PAPERWORK.	

Thank you for participating in the ASPREE-XT Microbiome Study!