

Diet and Health Study 2014

Protocol for 24-hour urine collection

1. Background & aims

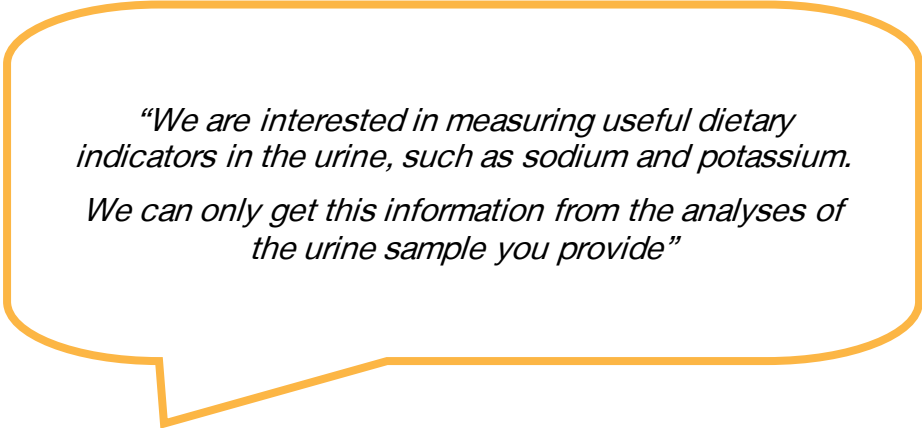
Dietary sodium (salt) consumption has been shown to contribute to high blood pressure which is a major risk factor in the development of cardiovascular disease. There is documented evidence that on average Western diets contain too much sodium (salt) and too little potassium. The Foods Standards Agency (FSA) has estimated that 75% of dietary salt intake comes from processed foods.

In May 2003 The Scientific Advisory Committee on Nutrition (SACN) published its report on Salt and Health. This recommended that salt levels should be reduced to a daily average of 6g per day for adults and children aged 11 and older.

It is not possible to obtain accurate estimates of salt intake from food intake information, mainly because the amount of salt added to food in cooking or at the table is not captured. Therefore, the best way to estimate salt intake is to analyse its presence in the urine using a complete 24-hour collection.

We are also measuring potassium (a marker of fruit and vegetable intake).

We do not want to draw too much attention to the fact that our main interest is sodium excretion, a measure of salt intake, because this may prompt participants to eat less salt than usual during the collection period. Therefore, we would like nurses to repeat the following information, provided in the leaflet for participants:



“We are interested in measuring useful dietary indicators in the urine, such as sodium and potassium. We can only get this information from the analyses of the urine sample you provide”

2. Eligibility for 24-hour urine collection

Men and women aged 19-64 years

- **Including** participants who refuse to take PABA (*para*-aminobenzoic acid) tablets but are willing to carry out the 24-hour urine collection.
- **Including** participants who CANNOT take PABA (e.g. those allergic to hair dye, sunscreen or supplements or those on sulphonamides) but are willing to carry out the 24-hour urine collection without PABA.

Before the visit

The participant will have been contacted by phone and agreed to be visited by a nurse. The participant is sent a letter with an FAQ leaflet explaining the study. The nurse follows up with a phone call to make an appointment followed by a letter confirming the appointment.

First visit

At the first nurse visit, the nurse will explain the purpose and procedures of the urine collection in more detail, encourage and answer questions, check eligibility, provide the participant with detailed leaflets about PABA and the urine collection instructions, obtain written consent and deliver the equipment to the participant.

The nurse will assign a day of the week to the participant when they are to carry out the 24-hour collection. This day will be chosen randomly by CAPI, and is done in order to ensure that sufficient samples are collected on weekdays, as our diet differs between weekdays and weekends. If a participant cannot collect on this day, another day will be chosen randomly and assigned.

At this visit the nurse will also make arrangements for the second visit to sub-sample and post the urine (the same day or the day after the 24-hour collection) to MRC HNR for analysis.

Second visit

This will take place on the day or one day after the 24-hour urine collection. The nurse will thoroughly mix and collect two aliquots of the 24-hour urine sample and dispose of the remaining urine down the participant's toilet and equipment in the household waste. The nurse will ensure that the samples are labelled correctly, and prepare packaging and despatch of the samples. The nurse will also check that the participant completed the 24-hour urine collection sheet (Annex 10) - in particular checking start and end time, any missed collections or missed PABA tablets, certain medication and dietary supplements/vitamins taken during the collection period. Finally, participants will be handed a **£15** gift card for their participation in this study. This gift card will be activated in CAPI by the nurse and be ready for the participant to use between 48 and 72 hours after the nurse's visit.

3. Participant documents

The following documents will be provided for the study:

| Participant Documents |
|--------------------------------|
| PHE/FSA Letter (optional) |
| Advance Letter |
| FAQ leaflet |
| Nurse appointment letter |
| 24-hour urine leaflet |
| PABA leaflet |
| Consent form |
| 24-hour urine collection sheet |

Consent form (carbonised)

The consent form will be signed by the participant and the nurse to indicate that the participant is willing to take part and to take PABA as part of the 24-hour urine collection. The consent form will be carbonised so that the participant can retain one copy and a second copy can be returned to the office.

Participant instructions

The 24-hour urine collection leaflet provides the participant with details about how to collect the 24-hour sample.

PABA information leaflet

The PABA information leaflet gives information about the PABA tablets. Each participant will be asked to take three tablets during the 24-hour collection period and to record details about when these were taken. PABA levels will be measured in the urine sample to assess the completeness of the 24-hour collection.

24-hour urine collection sheet

The 24-hour urine collection sheet will be completed by the participant, checked by the nurse and sent to HNR with the samples. Information from the urine collection sheet will be entered by the nurse into CAPI. The participant will be asked to choose a start time that will work for both days.

The nurse will fill in the participant details and the assigned date for the 24-hour collection, and remind the participant about when to take the PABA tablets. Ideally, the tablets should be taken at evenly spaced intervals during the collection period:

- **Start day:** get up and go to the toilet, flush it away and note the time. This will be the 24-hour urine start time. (DO NOT COLLECT THIS URINE)
- Take the first PABA tablet and note the time
- Collect all urine into the equipment provided
- Take the second PABA tablet 4-6 hours after taking the first tablet
- Continue to collect all urine into the equipment provided
- Take the third tablet 4-6 hours after the second tablet
- **Next day:** get up and go to the toilet and collect the urine sample into the equipment provided. Note the time. This will be the end time of the urine collection and should be EXACTLY 24-hours after the start time. (COLLECT THIS URINE)

As noted above, CAPI will randomly assign start dates for participants.

The participant will be asked to complete the 24-hour urine collection sheet during the collection period. Details needed are: date and time of 24-hour sample and date and time PABA tablets are taken. The date and time of any missed urine collections and the names of dietary supplements/vitamins and certain medication taken during this period will also be recorded.

4. The first nurse visit: introducing the 24-hour urine collection

CAPI Prompt

If participants are eligible, a prompt will appear in CAPI asking the nurse to explain the purpose of the 24-hour urine collection and the use of PABA, explain the instructions for collection in detail, obtain consent and provide participants with written instructions and equipment needed for the study.

The nurse will also answer any questions the participant may have. If the participant is willing to take part, they will be asked to initial the appropriate boxes on the consent form.

PABA exclusion criteria

Participants are still eligible if they do not want to take PABA but are willing to carry out the 24-hour urine collection. The following participants cannot take PABA, but they are still eligible to take part if they are willing to carry out the 24-hour urine collection:

- those who are allergic to dietary supplements/vitamins, hair dyes or sunscreen lotions
- those who are lactose intolerant
- those who take sulphonamide antibiotics during the 24-hour urine collection

Informed consent

The participant should initial the consent form to indicate that they have read the 24-hour urine leaflet and PABA leaflet and would like to take part in the study. Participants will be encouraged to take PABA if they can do so. Participants are still eligible if they do not want to

take PABA but are willing to carry out the 24-hour urine collection. The nurse will go through the instructions as per protocol except for PABA.

All participants will be instructed on the completion of the urine collection sheet.

Equipment

At the first nurse visit the following equipment will be provided:

- 5L screw cap container to collect urine.
- *Optional:* 2L capacity screw cap container for urine
- Funnel
- Jug
- 3 PABA tablets
- Carrier bags for transporting the equipment

Procedures

The nurse will assign a day of the week to the participant when they are to carry out the 24-hour collection. This day will be chosen randomly by CAPI, if a participant cannot collect on this day; another day will be chosen randomly and assigned. There is no limit to the number of times randomisation can occur.

Reassignment of dates should only be offered if the random date is impractical for the participant or if the date falls on a period day for female participants.

Sample collection

The requirement is to collect all urine which enters the bladder over 24-hours. Therefore, the 24-hour collection period should start immediately after the first urine void of day 1 is disposed down the toilet. From this moment the bladder is starting to fill with urine again so this is recorded as the START time. The 24-hour period will include the first void at the same time on the following day. This void on the second day denotes the END time. This should be EXACTLY 24 hours after the start time. For example, if the participant starts the 24-hour collection after the first void on a Tuesday at 7am then they stop collecting after their first void on Wednesday which should also be at 7am.

During the period of collection, participants will be asked to pass all urine into the plastic jug and then pour it in the 5L container using the funnel provided.

It is extremely important that all urine produced over the 24-hour period is included in the combined collection sample. During this period, participants will be asked to pass stools after passing urine, so that urine is not lost. When participants are away from home they will have the option to take a 2L collection container, the jug and funnel with them instead of the larger container. However, on return anything collected into the 2L container must be transferred to the main 5L container to mix it with the preservative.

Females will be instructed to collect urine on non-period days.

PABA (Para-aminobenzoic acid)

Each participant will have three PABA tablets which are to be taken at evenly spaced intervals throughout the collection period. The last PABA tablet should be taken at least 8 hours before the final urine collection on the second day. This time is needed for PABA clearance through

the kidneys to ensure that all PABA is excreted by the time the participant collects their final sample. The participant will be asked to retain the silver PABA blister pack and return it to the nurse on the second visit.

PABA is an intermediate in the synthesis of folic acid in bacteria. PABA is consumed in small amounts as part of our usual diet and is found, for example, in liver, kidney, brewer's yeast, molasses, whole grains, mushrooms and spinach, and can be made by intestinal bacteria. Larger amounts of PABA are found in some supplement preparations. Following ingestion, PABA is passively absorbed mainly from the small intestine. From there, it enters the portal circulation. Some metabolism of PABA occurs in the liver and PABA and its metabolites are mainly excreted in the urine. PABA may interfere with sulphonamide based antibiotics. The latter excludes participants from taking PABA. Although not directly harmful to the participant PABA may stop the anti-bacterial from working. Please note we will not ask participants to stop any of their sulphonamide medication but simply ask to not take PABA. In CAPI the nurse will have a short list of medications that exclude participants from taking PABA and on a laminated sheet.

Because some medications and dietary supplements/vitamins may interfere with the assay we use to determine PABA it is important to record them on the urine collection sheet.

The PABA tablet is very small and most people find it easy to swallow whole. It is not recommended to dissolve it in water or any other drink. The participant can crush the tablet between teeth. PABA has an acidic taste but there is no long lasting after taste.

Analysis of the PABA will allow us to check whether the sample is complete, however, it is important that participants also record any missing samples on the urine collection sheet, even if just through spillage.

24 Hour Urine Collection Instructions

Choose a START time suitable for both days (e.g. 7AM)

At **START** time day 1 go to the toilet as normal and DO NOT collect urine. Note time on form.

At START time day 1 take PABA tablet 1 and note time on form.

Pass all subsequent urine into the jug and add to the 5L container. Keep container in a cool place and note any missed collections.

At 4-6 hours after START time take PABA tablet 2 and note time on form.

Pass all subsequent urine into the jug and add to the 5L container. Keep container in a cool place and note any missed collections.

At 4-6 hours after PABA tablet 2 time take PABA tablet 3 and note time on form. Keep PABA packaging to give to nurse.

Pass all subsequent urine into the jug and add to the 5L container. Keep container in a cool place and note any missed collections.

EXACTLY 24 hours after START time (e.g. 7AM) pass urine into the jug and add to the container. The urine collection is now **FINISHED**. Write time on form.

5. The second nurse visit: Pick up samples

During the second visit the nurse will weigh the 24-hour urine collection, thoroughly mix and obtain two aliquots of the 24-hour sample. This visit should take place as soon as possible on the day or the day after the 24-hour sample collection is complete so that samples reach MRC HNR for processing without further delay.

Note: The participant must be present for the second visit so that the nurse can go through the collection sheet with the participant, prompt for missing information and hand over the gift card.

If samples are delayed for any reason, nurses should still send the samples but clearly indicate the reason for the delay on the despatch note.

24-hour urine collection equipment for the nurse

- Salter Electron scales for weighing the urine collection container **set to kg**
- 2 x 10ml Sarstedt urine monovettes with extension tubes
- Disposable gloves and work mat
- Postal container and packing material for despatching the samples
- 24-hour urine collection sheet and despatch note
- Labels for the Sarstedt urine monovettes

Urine sub-sampling procedure

The container with the 24-hour collection must be weighed twice according to protocol and the weight recorded on the despatch note. After weighing the urine, the collection container must be **inverted and rotated at least 20 times** so that the 24-hour urine sample is mixed thoroughly.

The disposable working mat should be used when performing the sub-sampling. Some urine from the 24-hour collection should be transferred to the jug. For each participant, two aliquots should be collected from the 24-hour urine sample. The Sarstedt monovettes should be filled with urine from the jug using the extension tubes and labelled using the labels provided, after they have been checked. After each monovette has been filled, the extension tube is removed, the monovette sealed with a plastic cap and the monovette plunger stalk snapped.

After collecting the samples, the nurse or participant will dispose of the rest of the 24-hour collection in the toilet (not from a height). Containers can be rinsed and disposed of with household waste. If it is not possible to dispose of equipment at the address, the nurse will pack away the equipment used and take this away for recycling at home or in public recycling containers.

The completeness of the 24-hour urine collection will be verified in two ways: through participants self-report and the use of PABA as an objective measure. During the 24-hour collection, participants are asked to record any missed urine collections on the urine collection sheet. Therefore, it is important that at the second visit that the nurse checks this sheet and ensures with participants whether the sample is complete, and if not, notes on how many occasions urine was not included in the sample. If urine has been missed, interpretation of result relies on having this information.

If participants finished their collection too early (i.e. collected for less than 20 hours) or continued for too long (i.e. longer than 28 hours) their sample will be unusable for analysis. CAPI will prompt you, where this is the case, when entering the collection period noted on the collection sheet.

The nurse may still aliquot the urine collection at their discretion and participants will still receive their £15 gift card for their effort.

Participant incentives

Finally, the nurse will thank the participant for their co-operation. The nurse will hand over a **£15** gift card and activate it by entering a unique 9 digit number as prompted by CAPI. The nurse will advise the participant that gift cards take up to 48h for activation, 72h in case of a weekend or bank holiday.

6. Labelling, packaging and despatch

The two aliquots from the 24-hour sample will be labelled. Packaging will take place during the second nurse visit and despatched as soon as possible after the visit.

The nurse will ensure that all samples are properly sealed. Each of the two samples will be placed into a recyclable rigid noax tube that contains an absorbent liner (in case of leakage) and packaged into the postal box provided. The box is then placed in the pre-labelled padded envelope and posted to HNR.

Samples must be despatched within 48 hours of the end of the 24-hour urine collection. The delay may be longer if the second visit takes place on a Saturday, but the samples should be collected by Royal Mail on Monday morning. The samples should be stored in a cool place between the second visit and postage.

Note for samples out of range:

If the urine collection period falls out of range for usable samples (i.e. collected for less than 20 hours or more than 28 hours) it is up to nurses discretion whether to aliquot the urine collection.

However, unusable samples must not be despatched to HNR and collection sheet and despatch note (if completed) should be returned to the office with the consent form,