

Appendix T The 24-hour urine sample: collecting and processing the urine and assessment of completeness of collection

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T.1 Introduction

This appendix gives further information about the 24-hour urine collection procedures, including details of the training of the nurses, procedures for obtaining a 24-hour urine collection, storage, assay auditing and representativeness of the complete urine collections included in the data analysis. Also included in this appendix is information about protocols for assessing completeness of the 24-hour urine collection.

Results for sodium excretion and estimated salt intake in Wales are presented in chapter 7 and tables 7.1-7.4. Urinary potassium, urea, creatinine and nitrogen results are presented in Appendix S. Details about urine analytical methodologies and quality control are included in appendix U.

T.2 Ethical approval

As described in chapter 2 ethical approval was granted by a Multi-Centre Research Ethics Committee (MREC) for all aspects of the survey protocol, including obtaining a 24-hour urine collection, measurement of urinary analytes and for storing urine sample residues for potential use in future analyses related to nutrition and health. Permission was also given to administer to each consenting participant a total of three 80mg tablets of *para*-aminobenzoic acid (PABA) to be taken at appropriate intervals, the quantitative excretion of which indicates a complete 24-hour urine collection.

The ethical approval allowed for a 24-hour urine collection to be obtained from fully continent participants aged four years and over.¹

T.3 Consent

Written consent was required for the following aspects of the 24-hour urine collection:

- Taking PABA tablets
- laboratory analysis of the 24-hour urine
- storing urine residues for potential future analysis of additional analytes related to nutrition and health

For children aged 4 to 15 years, written consent was sought from a parent or legal guardian, with written assent from the child where possible (see chapter 2 for more detail of procedures for obtaining informed consent).

T.4 Exclusion criteria

Participants were asked at the nurse visit (stage 2) if they were willing to make a 24-hour urine collection. All participants aged four years and over were encouraged to take part as long as they were fully continent. Those with conditions which could lead to a bad reaction to PABA (e.g. lactose intolerance; a previous allergic reaction to hair dye, sunscreen or a vitamin preparation) or who were taking sulphonamides² were excluded from taking PABA.

Participants excluded from or unwilling to take PABA were not excluded from taking part in the 24-hour urine collection.

T.5 Equipment used

To do the 24-hour collection, participants were provided with the following standard equipment:

- plastic jug and funnel
- 5 litre capacity screw cap (jerry can) container to serve as the storage container for urine. This contained a small amount (4g) of the anti-bacterial preservative boric acid (in crystal form)
- 2 litre capacity screw cap container for collections made away from the home. This was also used as an overflow container should the participant fill the 5 litre jerry can
- plastic carrier bags for transporting the equipment away from the home

- an aide-memoire safety pin for the participant to pin the under- and outer garments together during the period of the collection to remind that the specimen of urine about to be passed should be collected
- three PABA tablets, one to be taken at three specified time periods during the day to verify completeness of the 24-hour urine collection
- small coloured stickers to distinguish equipment if there were two participants in the same household
- a Urine Collection Sheet

T.6 24-hour urine collection: training, procedures and instructions

Information about the recruitment and general training of nurses is provided in chapter 2. Key elements relating to the 24-hour urine collection are summarised below.

T.6.1 Nurse training

During the face-to-face briefings, the nurses were instructed on the practical procedures related to 24-hour urine collections, given a practical demonstration of the equipment used to collect urine and measure its volume and of the dispatch procedures.

T.6.2 First Nurse Visit

During the first nurse visit to the participant's home the nurses were instructed to:

- assess the participant's eligibility for providing a 24-hour urine collection and for taking PABA and explain the procedure in detail
- provide detailed leaflets about PABA (see appendix H) and the urine collection instructions
- obtain the participant's consent to undertake the 24-hour urine collection
- instruct the participant about taking PABA, where appropriate
- deliver the necessary equipment
- agree a date with the participant(s) for when they would carry out the 24-hour urine collection

- book an appointment for the second visit (as soon as possible after completion of the collection) for the nurse to take aliquots of the 24-hour urine collection
- record the details of the visit in CAPI
- provide a reminder appointment card

T.6.3 Urine collection protocol

Participants who agreed to participate were asked to collect all urine they passed during a 24-hour period. The 24-hour period started with the first early morning urine which the participant was instructed to discard, recording this as the “start time”. All urine passed after this was collected, starting from the second morning urine passed of the 24-hour collection day, and ending with the first urine passed the following morning.

The nurse discussed and agreed with the participant on which day of the week the collection would be made. In order to maximise response participants were allowed to make the collection on a day of their choice and the majority preferred to collect their sample at the weekend (or on another non-work / school day). However, in recognition that diet often differs between weekdays and weekend days, nurses encouraged adult participants to collect their sample on a weekday where possible and children were asked to collect their sample on a non-school day. Females were instructed to collect their urine when not menstruating.

Participants were instructed not to urinate directly into the storage jerry can but instead to pass urine into the plastic jug, and then pour the sample into the 5 litre collection container using the funnel provided. Plastic bags were provided to carry the equipment (including a smaller 2 litre collection container) if participants were not at home for some of the collection period.

Participants were also asked to take one PABA tablet at three specified times, at evenly spaced intervals throughout the day of the collection, starting after discarding the first urine passed. The first tablet was therefore taken at the start of the collection period and ideally at 8am (no later than 12 noon), the second at approximately 12

noon (no later than 4pm) and the last tablet at approximately 6pm (no later than 10pm). As evidence of compliance, participants were asked to retain and return the empty blister pack. Analysis of PABA excretion provided a measure of the completeness of the 24-hour urine sample (see section T.8).

Before leaving the household, the nurse completed key information on a Urine Collection sheet, namely the participant details, the agreed start date of the 24-hour collection and whether the participant had consented to take PABA tablets (see appendix H). This sheet was then completed by the participant during the collection period to document: the time they took the PABA tablets, the start and finish times of their urine collection, any urine not collected (i.e. missed) in the container, and any medication or supplements taken during the collection period. The importance of obtaining a complete urine collection and the need to provide as much information as possible on the Urine Collection Sheet was stressed.

T.6.4 Second nurse visit

The second nurse visit took place as soon as possible after the 24-hour collection period, usually the day of completion or the following day. At this visit, the nurse mixed the urine thoroughly and aliquoted four sub-samples, disposing of the remaining urine and equipment. To do this the nurse was supplied with the following:

- spring balance for weighing the filled urine collection container
- 4 x 10ml Sarstedt Urine monovette, 4 x quills, 1 small beaker
- disposable gloves, apron, disposable work mat, postal container and packing material for dispatching the samples and dispatch note
- labels for the urine samples

The jerry can with the 24-hour collection was weighed twice by the nurse and both weights (kg) were recorded on the dispatch sheet and in CAPI. The nurse then mixed the urine thoroughly, carried out the sub-sampling procedure using the Sarstedt monovettes and discarded the remainder of the 24-hour collection. The next task was to confirm participant ID, to label the sub-sample monovettes and to check that the Urine Collection Sheet was complete, in particular the start and end time, report of any missed collections or missed PABA tablets and any medications/

supplements taken during the collection period. This information was entered into CAPI. The nurse then packaged and posted the samples, the Urine Collection Sheet, the empty PABA blister pack (to confirm how many tablets had been taken) and the dispatch paperwork to the laboratory at HNR.

Finally, each participant was given a promissory note and a High Street voucher was subsequently sent out from the survey office for their participation in the survey.

T.7 Sample reception, tracking and storage

Urine samples (monovettes) and documents were identified, logged and tracked via the use of pre-printed barcode labels.

Prior to the start of each fieldwork assignment, nurses were sent strips of unique barcode labels for every participant who had agreed to see them. These labels were used to identify all urine tubes and documents associated with each participant. A unique barcode label was affixed to each urine tube collected from the participant at the time of sub-sampling.

Upon receipt at HNR, each barcode-labelled urine monovette was scanned into a computerised sample tracking system (ItemTracker (International) Ltd, Birkenhead, UK). Received samples were cross-checked, using the barcode, against the list of expected samples to ensure that all samples had been received and were correctly labelled.

T.8 Assessment of completeness of collections

Sodium excretion in the urine approximates to 24-hour intake of sodium in the diet, only if the 24-hour urine collection is complete. The collection of a complete 24-hour urine output is a demanding task for participants and previous experience has shown that such collections may be incomplete. Participants were asked to record start and finish times and to acknowledge missed voidings, in addition to recording drugs or supplements taken during this time on their Urine Collection Sheet. However, it is accepted that relying solely on participant information may be unreliable.

In order to obtain confirmation that a collection represents all the urine passed in the 24-hour period, participants were asked to take one 80mg tablet of PABA at three specified times during the urine collection day. PABA is cleared by the kidneys within 8 hours of ingestion. Therefore, if the PABA tablets are taken in accordance with the protocol and all the urine passed during the day is collected, the excretion of PABA should be approximately the same as the dose given. A lower recovery of PABA may indicate that some urine was missed or discarded instead of being collected, or that the PABA tablets were not taken in accordance with the required protocol. The methods used for measuring PABA excretion are described in detail in appendix U.

Participants who elected not to take PABA or who were unable to do so were also eligible to take part in the 24-hour urine protocol. Completeness of 24-hour urine collections by these participants was assessed by examining the timings they recorded for start and finish time and the record that no urine had been missed during this time.

T.8.1 Standard criteria for classifying complete collections

24-hour urine collections were classified as 'complete' by either of two criteria, 'complete by PABA' or 'complete by claim'. These are jointly referred to as 'standard criteria' for completeness and have been applied to urine collections from adults and from children. Tabulated data refer to collections which have been judged to be complete. Due to limited cell sizes (less than 30) for children aged 4 to 6 years and 7 to 10 years (sex combined), descriptive statistics have not been provided for children aged 4 to 10 years for sodium and estimated salt intake (chapter 7) nor for potassium, creatinine, urea or nitrogen (appendix S).

i. Complete by PABA (where the participant has reported taking three PABA tablets and the amount of PABA recovered in the urine collection is consistent with completeness), jointly referred with criteria (ii) below as the 'standard criteria' for assessing completeness of 24-hour urine collections.

Those who consented to take PABA recorded the time at which they had taken each tablet. For those who reported taking each of the three 80mg PABA tablets as

specified during the day (approximately at mealtimes), urinary recovery over 24-hours as assessed by PABA excretion was used as an indication of completeness.

The methods used for measuring PABA excretion are described in detail in appendix U. In Year 2, all urines were initially analysed using the conventional colorimetric procedure. This assay is subject to interference and approximately 20% of urines analysed during Year 2 were therefore re-assayed by HPLC, in accordance with the published protocol. For Years 3, 4 and 5 all urines were analysed by the specific HPLC method only, in order to better standardise interpretation.

A recent study conducted at HNR (unpublished data) showed that for the colorimetric assay the appropriate lower cut-off for completeness (mean -2SD) in healthy adults is 85%, and for the HPLC method the appropriate cut-off for completeness in healthy adults is 70%; these take account of both inter-individual biological variation and analytical precision.

Urine collections containing between 70%-104% PABA when assayed by HPLC or containing 85% to 119% PABA when assayed colorimetrically were deemed to be complete. Data are included in the tables.

Urine collections with a PABA recovery greater than 119% by colorimetry were assumed to contain interfering substances and were re-assayed by HPLC.

Collections showing PABA recovery greater than 104% by HPLC were considered improbably high and therefore unreliable, and were excluded from the dataset.

Urine collections with a PABA recovery under 85% by colorimetry or below 70% by HPLC were considered incomplete and also were excluded from the dataset.

“Correction” formulae for incomplete PABA excretion were not applied in the NDNS RP.

ii. Complete by claim (where the participant has reported taking less than three PABA tablets and reported (i.e. claimed) collection of all urine passed during 23 to 25 hours), jointly referred with criteria (i) above as the ‘standard criteria’ for assessing completeness of 24-hour urine collections.

Urine data from collections made by individuals who elected not to take PABA and who recorded they had completed a full 24-hour urine collection were also included if they recorded start and finish times within one hour of a 24-hour collection period (i.e. recorded urine collected between 23 to 25 hours) and recorded no missed urine. In addition, 24-hour urine collections made by participants who consented to take PABA but recorded that they did not take all three PABA tablets were also included where they recorded that their urine collection was complete, as above. Excluding the results from these individuals did not materially alter the descriptive statistics.

T.8.2 Alternative criterion for classifying complete collections from children aged 4 to 10 years

Children aged 4 to 10 years are more likely to have difficulty swallowing tablets than older participants so compliance with the PABA protocol is likely to be poorer in this age group, particularly in the younger end of the age range. Application of the standard completeness criteria described above may have resulted in a preponderance of children towards the older end of the age group (i.e. 7 to 10 years). Therefore, data for children aged 4 to 10 years have also been provided in accordance with an alternative child criterion where collections were regarded as “complete” when they were claimed to include all urine passed for 23 to 25 hours from the start time *irrespective* of PABA excretion. Urine collections deemed complete by this alternative child (claim only) criterion are tabulated in separate columns in tables T.2-T.3 and solely in table T.1b.

Due to limited cell sizes (less than 30) for children aged 4 to 6 years and 7 to 10 years (sex combined), descriptive statistics have not been provided for children aged 4 to 10 years for sodium and estimated salt intake (chapter 7) nor for potassium, creatinine, urea or nitrogen (appendix S).

Details regarding the number and representativeness of useable collections for the different sex and age groups are presented in this appendix and in tables T.1a-T.3.

T.9 Number and representativeness of useable urine collections

The numbers of 24-hour urine collections analysed for sodium and for the supplementary analytes, and the numbers judged to be complete and included in the

descriptive statistics, are reported in chapter 7 and appendix S, respectively and in table T.1a.

The criteria for quantitative PABA excretion and participant claim as described in chapter 7, section 7.3 were applied to determine whether each urine collection was complete, i.e. represented the whole 24-hour urine output.

Urine collections were obtained from 461 participants aged four years and over.³ For the age groups reported in chapter 7 (participants aged 11 years and over) 1 24-hour urines from 374 individuals (158 males and 216 females) were received at HNR and were analysed for sodium, potassium, urea, nitrogen and creatinine. Of the received samples, 55.6% (208/374) were classified as 'complete' of which 50% (104/208) were from males and 50% were from females (104/208) and are included in the descriptive statistics presented in tables 7.1-7.4 and S.1. The remaining 44.4% of collections (166/374) were classified as 'incomplete or unreliable', 32.5% (54/166) from males, 67.5% (112/166) from females) and have been omitted from the descriptive statistics in tables 7.1-7.4 and S.1.

Table T.2 confirms that within each age group the age distribution of participants providing complete collections included in the descriptive statistics was not materially different from that of those whose collections had been rejected as "incomplete or unreliable".

(Table T.2)

T.10 Urine collection days

Table T.3 shows the distribution of complete 24-hour urine collections by day of the week broken down by age group and sex. For the age groups reported in chapter 7 and appendix S (those aged 11 years and over); 45.2% (94 / 208) of complete samples were collected from Monday to Friday, and 54.8% (114 / 208) were collected at the weekend, based on the standard criteria for all age groups. The higher proportion of weekend days is a result of participant preference for collecting at the weekend, and children being asked to collect on a non-school day; see T.6.3. The ratio of weekday to weekend days was not materially different between male and female participants but there are clear differences between age groups.

T.11 Urine analysis: laboratory procedure

At the second nurse visit, all samples were labelled and dispatched to HNR where the analysis of sodium and potassium was carried out using an ion selective electrode on the Siemens Dimension® Xpand clinical chemistry system with the QuikLYTE® module. Analysis of urea and creatinine was carried out simultaneously on the same instrument using standard clinical assays (see appendix U).

Completeness of 24-hour urine collections was assessed using the PABA recovery method; PABA was assayed at HNR using methods based on the colorimetric method of Bingham and Cummings⁴ and on the HPLC method of Jakobsen et al.⁵ Analysis of urinary nitrogen was carried out at the Institute for Biological, Environmental and Rural Sciences (IBERS), previously the Institute of Grassland and Environmental Research (IGER), University of Aberystwyth, using the LECO Nitrogen Determinator (see appendix U).

The concentration of each analyte in the urine was multiplied by the volume of the 24-hour collection, (derived from the weight of the filled container recorded by the nurse minus the weight of the empty container), to obtain the amount excreted in 24 hours.

T.12 Assay auditing

Samples were analysed in batches at intervals. Internal quality control (QC) samples were included with all analytes to monitor precision and external quality assurance schemes (e.g. NEQAS) were included where available in order to validate performance against peer laboratories. Details are provided in appendix U.

¹ Due to small cell sizes for those aged 4 to 10 years, data are only provided in chapter 7 and appendix S for participants aged 11 years and over.

² This is due to sulphonamides being similar in structure to PABA.

³ Considering participants in all age groups, urine samples from 60.6% of males (123/ 203) and 45.0% of females (116/ 258) were deemed complete and included in the statistical analysis, based on the standard criteria for all age groups.

⁴ Bingham S and Cummings J H. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24h urine collections in man. *Clin Sci (Lond)* 1983; 64(6):629-35.

⁵ Jakobsen J, Ovesen L, Fagt S, et al. Para-aminobenzoic acid used as a marker for completeness of 24-hour urine: Assessment of control limits for a specific HPLC method. *Eur J Clin Nutr* 1997; 5: 514.