



## 20 **Summary**

### 21 **Purpose**

22 Urinary spot samples are a promising method for the biomonitoring of micronutrient intake in  
23 children. Our aim was to assess whether urinary spot samples could be used to estimate the 24-  
24 h urinary excretion of potassium, phosphate, and iodine at the population level.

### 25 **Methods**

26 A cross-sectional study of 101 children between 6 and 16 years of age was conducted. Each  
27 child collected a 24-h urine collection and three urinary spot samples (evening, overnight and  
28 morning). Several equations were used to estimate 24-h excretion based on the urinary  
29 concentrations of each micronutrient in the three spot samples. Various equations and spot  
30 combinations were compared using several statistics and plots.

### 31 **Results**

32 Ninety-four children were included in the analysis (mean age: 10.5 y). The mean measured 24-  
33 h urinary excretions of potassium, phosphate, and iodine were 1.76 g, 0.61 g, and 95 µg  
34 respectively. For potassium, the best 24-h estimates were obtained with the Mage equation and  
35 morning spot (mean bias: 0.2 g, correlation: 0.27, precision: 56%, and misclassification: 10%).  
36 For phosphate, the best 24-h estimates were obtained with the Mage equation and overnight  
37 spot (mean bias: -0.03 g, correlation: 0.54, precision: 72%, and misclassification: 10%). For  
38 iodine, the best 24-h estimates were obtained with the Remer equation and overnight spot (mean  
39 bias: -8 µg, correlation: 0.58, precision: 86%, misclassification: 16%).

40 **Conclusions**

41 Urinary spot samples could be a good alternative to 24-h urine collection for the population  
42 biomonitoring of iodine and phosphate intakes in children. For potassium, spot samples were  
43 less reliable.

## 44 **Introduction**

45 Monitoring of micronutrient intake at the population level is essential to adjust food supply and  
46 policies for the optimal health of populations and to assess the effectiveness of nutrition  
47 interventions [1]. The monitoring of micronutrient intake is most commonly done with dietary  
48 questionnaires. However, this method is prone to several biases, such as reporting, recall,  
49 misclassification, and measurement biases [2]. In children, the monitoring of micronutrient  
50 intake is especially important due to its impact on growth. However, biases with dietary  
51 questionnaires are highly prevalent in children. It is even more difficult to complete dietary  
52 questionnaires in this population and therefore biases can be strong. Therefore, alternatives to  
53 dietary questionnaires, such as urinary biomonitoring [3, 4], are of interest for this population  
54 group to overcome these biases.

55 A standard biomonitoring method is the collection of 24-h urine in which biomarkers, such as  
56 sodium, are measured [5]. This method presents however major practical challenges, especially  
57 for children. To allow a better and easier biomonitoring of micronutrient intake, urinary spot  
58 samples have been proposed as an alternative. For sodium, urinary spot samples have been  
59 compared to 24-h urinary excretion in adults in several studies [5–7] and in children only in  
60 very few studies [8, 9]. For other micronutrients intake, such as potassium, phosphate or iodine,  
61 even less studies comparing urinary spot samples and 24-h urine have been conducted [10–12].

62 Taking advantage of a study in which 24 h urinary collection and several spot samples were  
63 collected, our aim was therefore to determine whether urinary spot samples can be used to  
64 estimate 24-h urinary excretion of iodine, potassium, and phosphate as quantitative biomarkers  
65 in dietary intake.

## 66 **Methods**

67 A biomonitoring study was conducted among children between 6 and 16 years of age in canton  
68 of Valais between September 2016 and February 2018. The main objective of this study was to  
69 assess whether 24-h urinary sodium excretion could be estimated with urinary spot samples.  
70 The detailed methods of this study and the results have been published elsewhere [8, 13]. We  
71 used these samples for the present study.

72 Children between 6 and 16 years of age, without any disease potentially altering the  
73 consumption and excretion of sodium and with sufficient knowledge of the local language to  
74 understand the content of the information forms, and visiting the local hospital or pediatric  
75 health centers were invited to participate in the study. Upon enrolment, the children were  
76 weighed and measured with light clothes and without shoes by a trained nurse or a research  
77 assistant.

## 78 **Ethical considerations**

79 Ethical approval was obtained by the Ethics Committee of canton of Vaud, Switzerland (CER-  
80 VD, identification number: 2015-01178). Written informed consent was obtained from the  
81 parent (or legal guardian) of the child. Children below 14 years of age gave oral consent and  
82 children above 14 years of age gave written consent.

## 83 **Urine samples**

84 Urine collection was done at home over three consecutive days (day 1 to day 3), which  
85 consisted, consecutively, of a) one evening spot (last void before going to bed) on day 1, b) one  
86 24-h urine on day 2 (starting after the last void before going to bed on day 1 and finishing with  
87 the last void before going to bed on day 2), c) one overnight spot (first void upon rising in the

88 morning) on day 3, and d) one morning spot (second void upon rising in the morning) on day  
89 3. To ensure a complete urine collection over 24 hours, written and oral instructions were given  
90 to the participants and their parents, and urine collection pots were provided. Participants were  
91 instructed to maintain their usual diet and liquid intake during urine collection.

92 During urine collection, participants and parents were instructed to keep the urine samples in  
93 closed containers in the fridge at a temperature between 4-8°C and to bring them to the  
94 laboratory no later than 48 hours after urine collection. The urine samples were stored at -20°C  
95 until analysis. Potassium, phosphorus and creatinine concentrations were measured using a  
96 Cobas® c-501 analyzer (Roche). Iodine concentrations were measured with an isotope dilution,  
97 inductively coupled plasma-mass spectrometry method [14].

## 98 **Statistical analysis**

99 The total 24-h urinary excretions of potassium, phosphate, iodine, and creatinine were  
100 calculated by multiplying the concentration in the 24-h urine sample by the total volume of the  
101 sample and by adjusting for self-reported collection times to represent an exact 24-hour  
102 duration. A 24-h creatinine excretion of less than 0.1 mmol per kilogram of body weight was  
103 considered an indication of incomplete 24-h urine collection [15] and was corrected to equal to  
104 0.1 mmol. The ratios between potassium, phosphate, iodine, one at a time in the numerator, and  
105 creatinine concentration in the denominator were calculated for the 24-h urine and the three  
106 urinary spots samples.

107 To transform the urinary concentrations of potassium, phosphate, and iodine in the urinary spots  
108 into 24-h urinary excretion estimates, the following equations were used: Remer [15] and Mage  
109 [6, 16] for potassium, phosphate, and iodine, Kawasaki [17, 18] for potassium, Robinson-Cohen  
110 [11] for phosphate, and Montenegro-Bethancourt [12] and Zimmermann [19] for iodine (see

111 detailed equations in **Appendix 1**). To compare the estimated 24-h urinary excretions from the  
112 different equations and spots with the corresponding measured 24-h urinary excretions, several  
113 statistics were used: mean bias, i.e., mean difference between the estimated and measured 24-h  
114 with the micronutrient excretion; Pearson correlation coefficient between estimated and  
115 measured excretion; precision, i.e., proportion of children with a between estimated and  
116 measured excretion difference within  $\pm 1$  SD of the 24-h mean; misclassification, i.e., the  
117 proportion of children who were incorrectly classified to  $\geq 3.5$  g/day or  $< 3.5$  g/day for potassium  
118 [20];  $\geq 1.0$  g/day or  $< 1.0$  g/day for phosphate [21]; and  $\geq 120$   $\mu\text{g/day}$  or  $< 120$   $\mu\text{g/day}$  for iodine  
119 [19]. Moreover, scatterplots and Bland-Altman diagrams [22, 23] were plotted for each spot  
120 and equation to allow visual comparisons.

121 Statistical analyses were conducted with R (version 3.3.1) and R Analytic Flow (version 3.0.6).

## 122 **Results**

### 123 **Participants' characteristics**

124 Among the 101 children recruited, 94 were able to collect a 24-h urine sample and were  
125 included in the analyses. There were 39 girls (41%). The children were on average 10.6 years  
126 of age (SD: 2.9, range: 6-16). They weighted 36.2 kg (SD: 14.2, range: 17.4-88.0) and were  
127 142 cm (SD: 17, range: 113-186) tall.

### 128 **Potassium**

129 The mean concentrations of potassium were 54 g/L (SD: 20) in the 24-h urine samples, 55 g/L  
130 (SD: 38) in the evening spots, 41 g/L (SD: 23) in the overnight spots, and 82 g/L (SD: 46) in  
131 the morning spots. The potassium-to-creatinine ratios were higher in the 24-h urine than in the  
132 spot samples: 19.5 mmol/mmol (SD: 114.1) in the 24-h urine samples; 6.8 mmol/mmol (SD:

133 4.0) in the evening spots; 4.1 mmol/mmol (SD: 2.2) in the overnight spots; and 9.7 mmol/mmol  
134 (SD: 5.1) in the morning spots. The 24-h potassium urinary excretion measured in the 24-h  
135 urine samples was 1.76 g/24-h (SD: 0.68; min: 0.37; max: 4.38). The distribution of this variable  
136 is shown in **Appendix 2A**.

137 Comparisons between the 24-h potassium excretion measured in the 24-h urine samples and  
138 estimated in the three urinary spot samples with the different equations are shown in **Table 1**.  
139 The smallest bias was with the Kawasaki equation and the overnight spot. The highest  
140 correlation was with the Mage equation and the evening spot. The highest precision was with  
141 the Kawasaki equation and the overnight spot. The lowest misclassification was with the  
142 Kawasaki equation and the overnight spot. The scatterplots are shown in **Figure 1** and the  
143 Bland-Altman plots in **Appendix 3A**. In the scatterplots, the Remer and the Mage equations  
144 with the morning spot show the best results. The Bland-Altman plots indicate that the difference  
145 between estimated and measured are the smallest with the overnight spots. Overall, the equation  
146 and spot combination that provided the best estimates was the Mage equation with the morning  
147 spot.

## 148 **Phosphate**

149 The mean concentrations of phosphate were in 24 g/L (SD: 11) in the 24-h urine samples; 31  
150 g/L (SD: 17) in the evening spots; 36 g/L (SD: 15) in the overnight spots; and 22 g/L (SD: 12)  
151 in the morning spots. The phosphate-to-creatinine ratios were higher in the 24-h urine than in  
152 the spot samples: 7.0 mmol/mmol (SD: 35.9) in the 24-h urine samples; 3.7 mmol/mmol (SD:  
153 1.3) in the evening spots; 3.5 mmol/mmol (SD: 1.1) in the overnight spots; and 2.4 mmol/mmol  
154 (SD: 1.0) in the morning spots. The total 24-h phosphate excretion in the 24-h urine samples  
155 was 0.61 g/24-h (SD: 0.27; min: 0.13; max: 1.79). The distribution is shown in **Appendix 2B**.

156 Comparisons between the 24-h phosphate excretion measured in the 24-h urine samples and  
157 estimated in the three urinary spot samples with the different equations are shown in **Table 2**,  
158 the scatterplots are shown in **Figure 2**, and the Bland-Altman plots in **Appendix 3B**. The  
159 smallest bias was with the Mage equation and the evening spot. The highest correlation was  
160 with the Mage equation and the overnight spot. The highest precision and the lowest  
161 misclassification were with the Remer equation and the overnight spot. In the scatterplots, the  
162 Remer equation with the overnight spot shows the best results. The scatterplots also show that  
163 the estimates with Robinson-Cohen 2 equation almost do not vary. Overall, the equation and  
164 spot combination that provided the best estimates was the Mage equation with the overnight  
165 spot.

## 166 **Iodine**

167 The mean concentrations of iodine were 115  $\mu\text{g/L}$  (SD: 53) in the 24-h urine samples, 155  $\mu\text{g/L}$   
168 (SD: 91) in the evening spots, 150  $\mu\text{g/L}$  (SD: 72) in the overnight spots, and 124  $\mu\text{g/L}$  (SD: 58)  
169 in the morning spots. The iodine-to-creatinine ratios varied widely between the different urine  
170 samples: 265 mmol/mmol (SD: 171) in the 24-h urine samples; 154 mmol/mmol (SD: 125) in  
171 the evening spots; 894 mmol/mmol (SD: 5) in the overnight spots; and 110 mmol/mmol (SD:  
172 5) in the morning spots. The total 24-h iodine excretion in the 24-h urine samples was 95  $\mu\text{g}/24$ -  
173 h (SD: 45; min: 19; max: 287). The distribution is shown in **Appendix 2C**.

174 The comparisons of the 24-h iodine excretion measured in the 24-h urine samples and estimated  
175 in the three urinary spot samples with the different equations are shown in **Table 3**, the  
176 scatterplots are shown in **Figure 3**, and the Bland-Altman plots in **Appendix 3C**. The smallest  
177 bias was with the Montenegro-Bethancourt equation and the overnight spot. The highest  
178 correlation was with the Remer equation and the overnight spot. The highest precision was with  
179 the Montenegro-Bethancourt equation and the overnight spot. The lowest misclassification

180 were with both the Remer and the Mage equation and the overnight spot. In the scatterplots, the  
181 Remer equation with the overnight spot shows the best results. The scatterplots appear similar  
182 between all the equations, except with the Zimmermann equation which shows an  
183 overestimation. Overall, the equation and spot combination that provided the best estimates was  
184 the Remer equation with the overnight spot.

## 185 **Discussion**

### 186 **Summary of findings**

187 Our study including 94 children between 6 and 16 years of age suggests that urinary spot  
188 samples could be an alternative to 24-h urine collections for the population biomonitoring of  
189 the intake of some micronutrients in children. For potassium, the best 24-h estimates were  
190 obtained with the Mage equation and morning spot (mean bias: 0.15 g, correlation: 0.27,  
191 precision: 56%, and misclassification: 10%). For phosphate, the best 24-h estimates were  
192 obtained with the Mage equation and overnight spot (mean bias: -0.03 g, correlation: 0.54,  
193 precision: 72%, and misclassification: 9%). For iodine, the best 24-h estimates were obtained  
194 with the Remer equation and overnight spot (mean bias: -8 µg, correlation: 0.58, precision:  
195 86%, misclassification: 22%). This suggests that urinary spot samples could be an alternative  
196 to 24-h urine collections for the biomonitoring of iodine and phosphate intakes in children in  
197 the population. For potassium, the spot samples seemed to be less reliable.

### 198 **Comparison with other studies**

199 Potassium excretion in 24-hour urine is considered as a biomarker of absolute intake and the  
200 recommended method to assessing daily potassium intake [24]. About 77% of potassium intake  
201 is excreted in urine and 18% in stool [24]. In a study including 1083 people aged 35-70 years  
202 [7], the Kawasaki formula provided the best agreement and least bias to estimate 24-hour  
203 urinary potassium excretion from a morning spot urine.

204 Phosphate is not commonly assessed in urine to measure intake, unlike potassium and iodine.  
205 However, interest in phosphate is rising as its intake, hence its excretion, is believed to have  
206 increased with the rise of use of food additives [25]. Little literature exists on the subject. One

207 study with 32 adults showed that phosphorus intake based on weighed dietary records correlates  
208 strongly with 24-h urine excretion [25].

209 Urinary excretion of iodine is considered to reflect a high portion of dietary intake, as > 90% is  
210 excreted in the urine within 24 to 48 hours by adults [16] and is relatively constant over the  
211 time of the day [26], making urinary spots a very interesting alternative to 24-h urine  
212 collections. In the DONALD study, where 180 children collected a 24-h urine sample and, a  
213 few days later, a casual urine spot sample, and from which the Montenegro-Bethancourt  
214 equation was constructed, the correlation between the measured and estimated 24-h iodine  
215 excretion was moderate ( $r = 0.41$  to  $0.47$ ) and similar to our study ( $r = 0.43$  to  $0.54$ ). In a study  
216 of 400 adults, the 24-h iodine excretion estimated with the Mage equation and different spots  
217 was compared with a 24-h urine collection [27]; this study found mean biases similar to our  
218 study ( $-9$  to  $16 \mu\text{g/d}$ ; our study:  $-18$  to  $8 \mu\text{g/d}$ ). The equation by Zimmermann seemed not to  
219 provide satisfactory estimates, whatever the spot considered.

## 220 **Strengths and limitations**

221 The strengths of this study are that: 1) the 24-h urine collection was checked for completeness  
222 and corrected in case of incompleteness; 2) three different timed urinary spots were collected;  
223 3) various equations were compared; 4) several statistics and plots were used to assess which  
224 equation and spot combination provided the best estimates.

225 The main limitation of this study was that only one 24-h urine sample was collected per child  
226 and therefore we could not measure the day-to-day variation in excretion. As a result, we could  
227 only assess whether urinary spots were useful to replace 24-h urine collections for group- or  
228 population-level estimates, not for individual-level estimates. In fact, for instance, up to ten  
229 urinary samples could be needed to accurately estimate the individual-level excretion of iodine

230 [28]. Another limitation was that only three timed urinary spots were collected and no afternoon  
231 spot was collected. Finally, the equations used and compared were found through a non-  
232 systematic search of the literature and potentially more equations could have been identified  
233 through a systematic search. Some of these equations were developed in an adult population  
234 (Kawasaki and Robinson-Cohen), another in both adults and children (Mage), and the others in  
235 children (Montenegro-Bethancourt, Remer and Zimmermann).

### 236 **Future research**

237 It would be useful to replicate this study and to compare spots and equations in another sample  
238 of children in order to confirm which equation and spot are best to estimate 24-h urinary  
239 excretion of phosphate and iodine, and eventually potassium. To improve the reliability of  
240 urinary spots for potassium, it is possible that a combination of several spots would be more  
241 informative than a single spot. In addition, collecting multiple 24-h urine collections and  
242 multiple spots would allow the assessment of the validity of spots to estimate the average 24-h  
243 urinary excretion of micronutrients at the individual level. Moreover, other micronutrients  
244 could be measured in the urine and be used as biomarkers of intake. For example, urea in the  
245 urine could be measured as this could be a biomarker for protein intake [29]. Finally, it would  
246 be useful to conduct a study where the participants change their intake in micronutrients to  
247 assess whether these changes are measurable with the urinary spots and as a result could be  
248 used to assess the effectiveness of nutrition interventions.

### 249 **Conclusions**

250 Our findings suggest that urinary spot samples could be an alternative to 24-h urine collections  
251 for the population biomonitoring of iodine and phosphate intakes in children between 6 and 16  
252 years of age if adequate timing and equations are used. In our study, the most reliable

253 estimations were obtained with the Mage and Remer equations using the overnight spot sample.  
254 For potassium, spot samples collected in the evening, overnight, and morning appeared to be  
255 less reliable.

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264 **Conflicts of interest**

265 The authors declare no conflicts of interest.

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- 352

353 **Table captions**

354 **Table 1. Comparison between 24-h potassium urinary excretion measured in 24-h**  
355 **collections and estimated with equations.** Bias: mean difference between estimated and  
356 measured 24-h potassium excretion; correlation: Pearson correlation between estimated and  
357 measured 24-h potassium excretion; precision: proportion of children with a difference  
358 between estimated and measured potassium excretion of less than 1 SD of the mean measured  
359 24-h potassium excretion; misclassification: proportion of children misclassified to  $\geq 3.5$  g or  
360  $< 3.5$  g potassium excretion per day.

361 **Table 2. Comparison between 24-h phosphate urinary excretion measured in 24-h**  
362 **collections and estimated with equations.** Bias: mean difference between estimated and  
363 measured 24-h phosphate excretion; correlation: Pearson correlation between estimated and  
364 measured 24-h phosphate excretion; precision: proportion of children with a difference  
365 between estimated and measured phosphate excretion of less than 1 SD of the mean measured  
366 24-h phosphate excretion; misclassification: proportion of children misclassified to  $\geq 1$  g or  $< 1$   
367 g phosphate excretion per day.

368 **Table 3. Comparison between 24-h iodine urinary excretion measured in 24-h collections**  
369 **and estimated with equations.** Bias: mean difference between estimated and measured 24-h  
370 iodine excretion; correlation: Pearson correlation between estimated and measured 24-h  
371 iodine excretion; precision: proportion of children with a difference between estimated and  
372 measured iodine excretion of less than 1 SD of the mean measured 24-h iodine excretion;  
373 misclassification: proportion of children misclassified to  $\geq 120$   $\mu\text{g}$  or  $< 120$   $\mu\text{g}$  iodine  
374 excretion per day.

375

376 **Figure captions**

377 **Figure 1. Scatterplot of measured 24-h potassium excretion versus estimated 24-h**  
378 **potassium excretion from urine spot samples using different equations in g/d.** Black  
379 continuous line: identity line, i.e. perfect correlation; black dashed lines: 1 SD difference  
380 between measured and estimated excretion; red dotted lines: threshold for high potassium  
381 intake; blue dashed line: linear regression.

382 **Figure 2. Scatterplot of measured 24-h phosphate excretion versus estimated 24-h**  
383 **phosphate excretion from urine spot samples using different equations in g/d.** Black  
384 continuous line: identity line, i.e. perfect correlation; black dashed lines: 1 SD difference  
385 between measured and estimated excretion; red dotted lines: threshold for high phosphate  
386 intake; blue dashed line: linear regression.

387 **Figure 3. Scatterplot of measured 24-h iodine excretion versus estimated 24-h iodine**  
388 **excretion from urine spot samples using different equations in  $\mu\text{g/d}$ .** Legend: Black  
389 continuous line: identity line, i.e. perfect correlation; black dashed lines: 1 SD difference  
390 between measured and estimated excretion; red dotted lines: threshold for adequate iodine  
391 intake; blue dashed line: linear regression.