

Example of a protocol for validation of a food picture book based on the PILOT-PANEU project¹

¹ The content of this Appendix is used with permission of the PILOT-PANEU project (Ambrus et al., 2013).

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1. Background

The methods used to collect information about dietary intake, as dietary recall, food frequency questionnaires and food diaries, all have in common that a large part of the random error is caused by estimating the portion size (Cypel et al., 1997). Weighing of the served portions is considered to be the most accurate method for measuring food intake. This method has some disadvantages as being time consuming, costly and requiring a high level of cooperation from respondents (Rutishauser, 2005). An alternative to weighing the food portion size is to use a variety of aids to help participants more accurately estimate the amounts of food consumed. These aids include household measures, abstract shapes and food photographs, among others (Cypel, Guenther et al., 1997; Venter et al., 2000; Byrd-Bredbenner and Schwartz, 2004). The advantages of food photographs are: being easily adaptable to local conditions, cheap, reproducible and transportable (Huybregts et al., 2008). A number of studies reported the benefits of using photographs to help individuals estimating portion sizes (Nelson et al., 1996; Robson and Livingstone, 2000; Frobisher and Maxwell, 2003; Lillegaard et al., 2005; Subar et al., 2010).

The applicability of photo series for quantification of food portions should be validated before they are included in the picture book. There are several types of validation studies described in the literature, but despite this there is no gold standard method for the validation of food picture books (FPB) (Faggiano et al., 1992; Cypel, Guenther et al., 1997; Nelson and Haraldsdottir, 1998; Ovaskainen et al., 2008; Subar, Crafts et al., 2010). Three elements could affect portion-size report by a subject: perception, conceptualization and memory (Nelson et al., 1994). The perception involves the ability of a subject to relate the quantity of a food that is present in reality to an amount depicted in a photo. Conceptualization concerns a subject's ability to make a mental construct of an amount of food that was previously consumed, but it is not present in reality, and to relate that to a portion shown on a photo. The memory affects the precision of the conceptualization. Some researchers focus on the validation within the context of a dietary assessment method, such as 24-hour recall or food frequency questionnaire. However, the finding obtained through this approach could reflect errors associated with the method itself, from sources other than related to food portion size estimate using pictures.

2. Aims

This protocol aims to describe the process of validation of food portion size pictures, using two approaches:

- 1) Visual perception of food portions by comparison with food pictures;
- 2) Conceptualization and memory, using the same food pictures to identify the portions self-served at least 1 hour before.

3. Subjects

The subject of the study is a stratified sample, reflecting age and gender distribution of the target population. Participants with a nutritional background should not be included.

4. Sample Recruitment

There are several possible approaches to recruit the sample for the validation study. Each research coordinator should select the recruitment strategy that best suits for the purpose.

For example participants could be invited to go to a predefined place. People can be recruited through the researchers contact nets (for example, friends, social networks, office intranet, etc.). Each invited person can come along with other people (friends or relatives), from the same or different age group, to participate in the study.

Informed consent forms must be signed by all participants. Adolescents (10-17 yrs) also need to have the informed consent form signed by themselves and their legal representatives.

This consent form may be delivered to the participant before the study via email or personally and should be returned on the day of the field work.

5. Incentives

To facilitate the recruitment and the participants' motivation incentives may be used. The participants should know if an incentive will be given in advance for their participation in the study. Incentives can be: offering a bag with some educational material published by the organization conducting the study, a piece or a basket of fruit, or others.

6. Sample size

Usually the sample size estimation assumes that the study variable has a normal distribution. Sample size is calculated through the following formula (Nelson and Haraldsdottir, 1998):

$$n = \sigma^2(z_{\alpha/2} + z_{\beta})^2 / d^2$$

Where σ^2 is the variance of portion size estimates and d is the average difference between real and estimated portion size. The variance expressed as a coefficient of variation normally varies from 25% to 80%, whereas average differences range from as low as 5% to as high as 100% (of real weigh) or more (Nelson and Haraldsdottir, 1998). The usual aim is to have statistically significant differences (α) at the 5% level (α) with 80% power (β).

The significance level of 5%, represents the probability of finding differences when they do not exist, in about 5 times in 100. On the other hand the power represents the probability of finding differences if they exist. It is directly related with the sample size. A value of 80% is usually accepted as adequate for this kind of studies.

E.g. to detect a difference of 25% with a significance of 5% and power of 80% with an assumption of 50% of coefficient of variation 32 validations per food item are needed. A sample size of 32 is only for testing the sample in total, without differentiating any subgroup (eg. age).

According to previous studies the distribution of some estimated food portions is skewed. In that case, sample size estimation should be determined with the following formula (Noether, 1987):

$$N(a) = \left[(z_{\alpha} + z_{\beta}) / \arcsin(2p - 1) \right]^2$$

Where p represents the probability of an observation is greater than the hypothetical median.

E.g. aiming a significance of 5% ($Z_{\alpha} = 1.64$), 80% power ($Z_{\beta} = 0.84$) and assuming that the probability of an observation being greater than the hypothetical median is at least twice as large as the probability of an observation being smaller than the hypothetical median ($p=2/3$) 54 validations per food item are needed.

If the distribution of the difference between the estimated and real portions were normal and it had a bias higher or equal to 0.25, it is expected, assuming a standard deviation of 1, that 2/3 of individuals had values above zero. So, a minimum sample size should be calculated to be able to have significant differences for a bias higher than 0.25.

It is advisable in any case, to increase the sample size by 20%, to account for any drop off and by that to guarantee the minimum sample size required.

7. Selection of portion to be included in validation

In case of visual perception method, the selected food items will be validated in different portion (or pictures). All portions from one photo series should be validated. Alternatively 50% of portions could be selected from each photo series. For example if a photo series has 6 portions, the portion 1, 3 and 6 should be chosen. We advise to keep the average of the portions selected constant for each food.

8. Study Design

8.1. Validation by the perception method

8.1.1. Field work

Setting – Preparation before field work

1. Calibration of the digital kitchen scales having readability of 1 g (Supplementary information 1).
2. Preparation of the portion sizes chosen from the food items selected by a kitchen staff. Each food item should be prepared and served on a plate as naturally as possible, according to local practices. The weight of each portion should be as similar as possible to the indicated in the picture book.
3. The selected food portions should be randomly distributed in a table. Ordering the portions by its weight or by food item could lead to biased estimates and it is not advisable.
4. The photo series should be placed at the right hand side of the plate. Each food portion should be served on a similar plate as the one used in picture book. For example in the case of EPIC-Soft picture series a white plate of 26 cm diameter should be used. A fork and a knife should be placed, respectively, at the left and at the right hand side of the plate (for more details see IARC's "*Preparation of photos for food portion quantification in EPIC-Soft – Reference Guidelines*") (Crispim et al., 2011).

During field work

1. It is advised to schedule the participants in small groups with difference of minimum 15 minutes between each other. After the participants' arrival, the aims and the procedures of the study should be explained to them and the signed informed consent forms must be collected. To each participant a code should be assigned and an answer form (Supplementary information 2) should be given.
2. The participants should record the estimated portions on one of the answer forms.
3. Each participant should estimate the portion of each food by comparing the plate with the corresponding photo series. The participant should never go back to see plates he/she has already seen and a researcher should be available to help the participants in case they have any doubt about the process.
4. Upon completing the perception part of the validation process the answer form should be handed over to an assistant, who will check the form for inconsistencies.
5. The memory approach should be conducted previously to the perception method to avoid the increased awareness about the portions/weights in the memory exercise induced by the perception exercise.

8.2. Validation by the memory method

The memory method including the conceptualization enables us to predict the error associated with the use of memory during the recall process, minimum 1 hour after individual serving a usually consumed amount of food.

Two different approaches could be used:

- Only serving the food;
- Serving the food, and eating some of them from different servings provided that appropriate food safety conditions are guaranteed.

The recall of the served portion sizes, in both processes, happens about at least 1 hour after serving the food.

It is advisable to think in advance how to entertain the participants during the time of the validation study. Satisfactory ideas could be inviting a chef for a demonstration of healthy cuisine or other leisure activity that does not involve quantification of food items.

The food items validated through this method are the same used in the perception method.

Setting – Field work

- The food is only served by the participants:
 - The self-service line must be arranged displaying the white plates and the food items that are used for the validation study;
 - The researcher should record the weight of the empty plate;
 - Each participant serves himself freely from the displayed food items;
 - The researcher should record the weight of the plate together with the served food items.
 - At least one hour later the participant is invited to recall the amount of served foods using the photo series from the picture book. The recall should be done in a separated room.
 - The answer form used should be the same as in perception method.
- The food is served and then some of them are eaten by the participants
 - The participants should be divided into groups, according to available meals and facilities.
 - For each group self-service lines must be arranged. A first self-service line displays the food items to be validated. A second self-service line could be available containing a few more side dishes, for a more realistic combination of the food items.
 - The researcher should record the participant's code and weigh the food portions served by the participants during the process.
 - The Participants serves themselves freely of one food item at a time, beginning at the first self-service line. After serving each food item from the first self-service line, the plate should be weighed and the weight registered. This means that the participant should take the plate to be weighed in the scale each time he/she takes a food item and then go back to get another food item. The researcher assisting the process should register, by order, the name of the food items weighed. The weight of the empty plate should be also recorded.
 - After finish taking the desired items from the first self-service line, the participants can serve themselves freely from the food items of the second self-service line. These items do not need to be weighed or registered.
 - Participants are allowed to eat the meal.
 - One hour later, counting from the time each participant finishes serving himself, the participant is invited to recall the portion sizes served using the photo series from the picture book to identify the quantity served. The recall should be done in a separated room.
 - The answer form used should be the same as used for the perception method.

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9. Handling data

For handling data the database should have a long format and not a wide format: each line should represent the estimated portion by food, plate and observer for perception approach and by food and observer for memory approach. (Please see the example below).

| Food ID | Estimated portion | Real portion | Estimated weight | Real weight | Plate ID | Observer ID |
|---------|-------------------|--------------|------------------|-------------|----------|-------------|
| 52 | 2 | 1 | 118 | 87 | 1 | 1 |
| 52 | 2 | 3 | 118 | 175 | 2 | 1 |
| 52 | 5 | 5 | 298 | 298 | 3 | 1 |
| 52 | 1 | 1 | 87 | 87 | 1 | 2 |
| 52 | 3 | 3 | 175 | 175 | 2 | 2 |
| 52 | 5 | 5 | 298 | 298 | 3 | 2 |
| 29 | 2 | 2 | 50 | 50 | 4 | 1 |
| 29 | 2 | 3 | 50 | 70 | 5 | 1 |
| 29 | 3 | 4 | 70 | 100 | 6 | 1 |
| (...) | | | | | | |

10. Statistical Analyses

Participants' characteristics should be described through frequencies (%), mean and standard deviation (SD) and range.

10.1. Perception method

1) Description by food portion (plate)

The estimated portions' (or weight, in grams) distribution by plate within each food is not likely to have quantitative distribution, considering that the range of estimated portions in each plate is small (the distribution will have only 2 or 3 portions selected from the maximum total of the possible response options). In this case a non-normal distribution is assumed and non-parametric tests are to be used.

For the description of estimated portions (or weight, in grams), median values (and respective interquartile ranges) should be used. The results should be stratified by variables that could modify the observer's estimates (e.g. sex and age).

The difference between estimated and real amount of food items by country, should be tested with one-sample sign test.

The mean and standard deviation of difference between estimated picture number and real portion number, should be calculated to evaluate if the picture is acceptable or not. The picture may be considered acceptable, for instance, if the $|\text{mean difference}| \leq 0.25$ and $\text{SD of difference} \leq 0.5$, there is an overestimation if the $|\text{mean difference}| > 0.25$ and $\text{SD of difference} \leq 0.5$ and underestimation if the $|\text{mean difference}| < -0.25$ and $\text{SD of difference} \leq 0.5$; the picture is not precise if $\text{SD of difference} > 0.5$.

2) Description by food and picture book

The estimated portions' (or weight, in grams) distribution by each food is likely to have parametric distribution. In this case it is possible to use parametric tests if data are normally distributed. Before starting this analysis it is necessary to check if data are indeed normally distributed.

For the description of estimated portions (or weight in grams), mean values (and respective standard deviations/variances) or median values (and respective interquartile ranges) should be used. The results should be stratified by variables that could modify the observer's estimates (e.g. sex and age).

A generalizability study evaluating the performance of the picture book as a whole, using both for perception and memory method could be performed. The reproducibility (= measuring consistently) and validity (= measuring what it is supposed to measure) are assessed through this test.

10.1.1. Reproducibility Study

As all observers estimate the same plates, the degree of agreement between observations should be evaluated. *Mixed effects models* are used to estimate the variance explained by each component in the estimated portions for all foods. The components of the *first model* are *plate* (the displayed portion) and *observer* (individual).

Mixed effects models are used when having repeated measures, in our case, we have repeated measures from the same *plate* and *observer*, so will have random effects for the two components.

The *mixed effects models* have the following formula (McGraw and Wong, 1996):

$$y_{ij} = \beta + b_i + b_j + \varepsilon_{ij} \quad (1)$$

y_{ij} is the estimated portion of observer j for plate i , β is the mean estimated portions across the population of plates and observers being sampled, b_i is a random variable representing the deviation from the population mean of the mean *estimated portions* for the i^{th} plate, b_j is a random variable representing the deviation from the population mean of the mean *estimated portions* for the j^{th} observer, and ε_{ij} is the residual representing the deviation in *estimated portions* for observer j on plate i from the mean *estimated portion* for plate i and observer j .

The sum of all components variance and the residual variance ($\sigma_i^2 + \sigma_j^2 + \sigma_{ij}^2$) allows to estimate the total variance and the percentage of the total variance of each component.

The percentage of the total variance from the component plate is equal to the inter-observer reliability (ICC by observer, formula 2). The standard deviation of measurement error (SDME) is calculated as the square root of the residual variance (formula 3). The ICC by observer and SDME allowed assessing the agreement by observers.

$$ICC_{observer} = \sigma_i^2 / (\sigma_i^2 + \sigma_j^2 + \sigma_{ij}^2) \quad (2)$$

$$SDME = \sigma_{ij} \quad (3)$$

A second model should be estimated by adding a third component, food (k), that could explain the variance in the estimated portions for all foods. If the real portions, x , are different by foods, a second model should be calculated adjusting the *estimated portion* for the mean of the real portion of each food, x_k , in order to assess if the *estimated portion* are systematically higher or lower when compared to the real portion of each food (b_k) and if this is different by observer (b_{kj}).

$$y_{kij} = \beta_0 + (\beta_1(x_k - \bar{x}_k)) + b_k + b_i + b_j + b_{kj} + \varepsilon_{kij} \quad (4)$$

In order to assess the agreement in estimated portions (or weight, in grams) among observers for each food, a similar model to model 1 can be used to calculate the ICC by observer and the respective SDME among observers for each food.

If real portions are different by foods a calibration should be simulated by adjusting the model for the mean of the real portion of each food, in order to assess if the estimated portions are systematically higher or lower when compared to the real portion.

Similar model can be used to estimate the ICC by observer and the respective SEM by food, in order to assess if there were some foods had lower or higher ICC and SEM by observer.

10.1.2. Validity Study

This section allows us assessing if the picture book is measuring what it is supposed to measure. If the ICC by observer is high, the mean estimated portion for each plate by all observer should be

calculated. *Mixed effects models* are used to estimate the general bias between the real portions and the estimated portions and the respective agreement using the ICC and SDME.

The mixed effects model had random effect by *plate*, and fixed effect that compare the *real* and the *estimated portion*:

$$y_{il} = \beta_0 + \beta_1 \times l + b_i + \varepsilon_{il}, l=0,1 \quad (5)$$

$$ICC_{agreement} = \sigma_i^2 / (\sigma_i^2 + \sigma_l^2 + \sigma_{il}^2) \quad (6)$$

$$SDME = \sigma_{il} \quad (7)$$

y_{io} is the real portion of plate i and y_{il} is the estimated portion of plate i , β_0 is the mean real portions across the population of plates being sampled, β_1 is the mean difference of the estimated portion from the real portions across the population of plates being sampled, b_i is a random variable representing the deviation from the population mean of the mean of the real and estimated *portions* for the i^{th} plate, and ε_{ij} is the residual representing the deviation in the real or estimated portion on plate ' i ' from the mean of the real and estimated *portions* for the i^{th} plate. The *Likelihood ratio test* comparing the results obtained with eq. 5 with a model without the term β_1 is used to assess if there were differences between the real and estimated portions.

If the analysis is stratified by food, the mean, median, variance and standard deviation for the estimated and real portion of each food should be calculated. The ICC by observer and SDME can be calculated as indicated in formula (2) and (3).

The difference between the *estimated* and *real portion* within each food can be compared using *linear mixed effects models* with random effects by *observer* as indicated in formula (8).

The β term showed if there is significant difference between estimated and the real portion.

$$difference_{ij} = \beta + b_i + b_j + \varepsilon_{ij} \quad (8)$$

10.2. Memory method

The estimated and served portions should be described (or weight, in grams) using mean (and respective standard deviation/variance) or median (and respective inter-quartile range) values. The results should be stratified by variables that could modify the observer's estimates (e.g. sex and age).

For evaluation of the differences in the amount of weighed food [g] between male and female can be evaluated by applying Mann-Whitney test or t-Student test.

To test differences between estimated and weighed amount of food consumed one hour before Wilcoxon test or t-Student test for paired samples can be used.

10.2.1. Reproducibility study

The reproducibility in the memory method cannot be studied, because the different observers do not measure the same portion at different occasions.

10.2.2. Validity study

Mixed effects models are used to estimate the general bias between the real portions and the estimated portions and the respective agreement using the ICC and SDME as indicated in formula (5), (6) and (7). Similar model is used to estimate the ICC and SDME for each food.

The *Likelihood ratio test* comparing model 5 with a model without the term β_1 is used to assess if there were differences between the real and estimated portions.

10.2.3. Criteria

For portion data the following criteria were defined: differences (absolute mean difference between the real portions and estimated portions) lower than 0.25 portion indicate *no bias*, between 0.25 and

0.5 portion indicate *low bias*, between 0.5 and 1.0 portion indicate *moderate bias*, and higher than 1.0 portion indicate *large bias*. The agreement is measured using the intra-class correlation coefficient (ICC) and standard deviation of measurement error (SDME). Guidelines for interpreting ICC statistics suggest that values between 0.81 - 1.00 indicate *almost perfect agreement*, 0.61 - 0.80 *substantial agreement*, 0.41 - 0.60 *moderate agreement*, 0.21 - 0.40 *fair agreement*, and values less than 0.21 are *poor or slight agreement* (Landis and Koch, 1977). If SDEM is lower than 0.5 portion it indicates *no error*, if it is between 0.5 and 1.0 portion it indicates *moderate error*, and if it is higher than 1.0 portion it indicates *large error*.

For weight data (in grams) the following criteria was defined: bias (absolute mean difference between the real weight and estimated portions) was lower than A indicates no bias, between A and B portion indicates *low bias*, between B and C portion indicates *moderate bias*, and higher than C portion indicates *large bias*. Regarding the, If SDME is lower than B it indicates *no error*, if between B and C portion it indicates *moderate error* and higher than C portion it indicates *large error*. A = $\frac{1}{4}$ C; B = $\frac{1}{2}$ C; C = average weight between portions (see appendix 3).

If a photo series fails to be validated, corrective actions should be taken, such as for instance: applying correction factors to the quantities expressed on the pictures (calibration). Alternatively another photo series or another method should be used to quantify that food or a new photo series should be created.

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Supplementary information

1. Calibration of scales for portion size determination.

The portion sizes shall be measured preferably with ± 0.1 g but a minimum of ± 1 g readability. Commercial kitchen scales may have a reading of 0.1 g (affecting the repeatability standard deviation) but it does not guaranty the same accuracy along the weighing range of about 10-600 g. Therefore the scales used for testing the applicability of picture series shall be calibrated before use. The calibration of scales can be carried out as follows.

1.1. Equipment:

1. Scales to be calibrated: 0.1 readability and uniquely identified (e.g. A, B, C, etc)
2. Calibrated standard weight series (usually < 1 g up to 200 g).

Notes:

- (a) The standard weights should preferably have certificate of the corresponding National Organisation accredited for calibration of scales. If such a weight series is not available use weights manufactured for analytical laboratory use.
 - (b) Never touch the reference weights with bare hand! Use forceps to move the weights
3. A-grade volumetric flasks 250 and 500 ml nominal volumes.

1.2. Procedure:

1. Place the scales on a flat levelled surface, check the cleanness of weighting platform.
2. Test '0' reading after pushing the tare bottom and record the reading
3. Repeat the procedure minimum 5 times

Note: if the reading is varying more than 1 unit (0.1 g) then the scale is instable. Wait for warming it up or replace it with another scale;

4. Place 10g reference weight on the scale and record the reading. Repeat the test 5 times;
5. Repeat step 4 with reference weights of 50g, 100g, 200g and 350g loads (use combination of reference weights). If the largest reference weight is only 100g, use the possible combinations of weights to increase the load.
6. Tare (250ml, only if 100g the largest reference weight) 500ml A-grade volumetric flask, fill it with clean water up to mark and weight and record its mass. Repeat the procedure from filling the flask to mark 5 times.

Note: at room temperature the density of clean tap water can be considered to be 1.0g/cm^3

1.3. Calculation:

Calculate the average and relative standard deviations of readings. Plot recorded average weights as a function of the nominal reference weights. Calculate the parameters of linear regression. (The calculation is shown only for one balance.)

1.4. Evaluation of results:

Balance A: linearity is good: $y=1.008-0.0432x$, $R^2=1$,

the relative deviation from the nominal reference weight is $<2\%$ over the calibrated range of 10-500 g

Reproducibility: $<1\%$

Suggested acceptability criteria:

Linearity $R^2>0.99$

Reproducibility: $CV < 1.5\%$

Calculation of the mass of food portions

Nominal weight of the food portion is 170 g

Accurate mass of food portion: $M = (170 + 0.0432) / 1.0081$

2. Validation answer forms (perception and memory).

2.1. Answer form for selection portions at or between pictures (perception and memory).

Please indicate with X the quantity you see comparing to those shown on the photographs. You can choose one of the 6 portions (1, 2, 3, 4, 5 or 6) showed in the food photograph or a quantity of the food presented in between 2 portions.

| Plate | | Quantity (choose only one) | | | | | | | | | | | |
|-----------------|-----------------|----------------------------|--------------------|---|--------------------|---|--------------------|---|--------------------|---|--------------------|---|------------------|
| Plate/ food1 | Lower than 1 | 1 | Between 1 and 2 | 2 | Between 2 and 3 | 3 | Between 3 and 4 | 4 | Between 4 and 5 | 5 | Between 5 and 6 | 6 | Higher than 6 |
| Plate 2 | Lower than 1 | 1 | Between 1 and 2 | 2 | Between 2 and 3 | 3 | Between 3 and 4 | 4 | Between 4 and 5 | 5 | Between 5 and 6 | 6 | Higher than 6 |
| Plate 3 | Lower than 1 | 1 | Between 1 and 2 | 2 | Between 2 and 3 | 3 | Between 3 and 4 | 4 | Between 4 and 5 | 5 | Between 5 and 6 | 6 | Higher than 6 |
| Plate n | Lower than 1 | 1 | Between 1 and 2 | 2 | Between 2 and 3 | 3 | Between 3 and 4 | 4 | Between 4 and 5 | 5 | Between 5 and 6 | 6 | Higher than 6 |

2.2. Example for record sheet for selection portions between pictures on decimal scale

Code:

Age: Gender: male female

Highest level of education: General school Secondary school Collage University No education

Portion size estimation: Please indicate with X on the scale the size of portions in relation to those shown on the pictures!

| | Estimated amount of food on the plate | | | | | |
|--------|---------------------------------------|---|---|---|---|---|
| I. | 1 | 2 | 3 | 4 | 5 | 6 |
| II. | 1 | 2 | 3 | 4 | 5 | 6 |
| III. | 1 | 2 | 3 | 4 | 5 | 6 |
| IV. | 1 | 2 | 3 | 4 | 5 | |
| V. | 1 | 2 | 3 | 4 | 5 | |
| VI. | 1 | 2 | 3 | 4 | 5 | |
| VII. | 1 | 2 | 3 | 4 | 5 | |
| VIII. | 1 | 2 | 3 | 4 | 5 | |
| IX. | 1 | 2 | 3 | 4 | 5 | |
| X. | 1 | 2 | 3 | 4 | 5 | 6 |
| XI. | 1 | 2 | 3 | 4 | 5 | 6 |
| XII. | 1 | 2 | 3 | 4 | 5 | 6 |
| XIII. | 1 | 2 | 3 | 4 | 5 | |
| XIV. | 1 | 2 | 3 | 4 | 5 | |
| XV. | 1 | 2 | 3 | 4 | 5 | |
| XVI. | 1 | 2 | 3 | 4 | 5 | |
| XVII. | 1 | 2 | 3 | 4 | 5 | |
| XVIII. | 1 | 2 | 3 | 4 | 5 | |
| XIX. | 1 | 2 | 3 | 4 | 5 | 6 |
| XX. | 1 | 2 | 3 | 4 | 5 | 6 |
| XXI. | 1 | 2 | 3 | 4 | 5 | 6 |

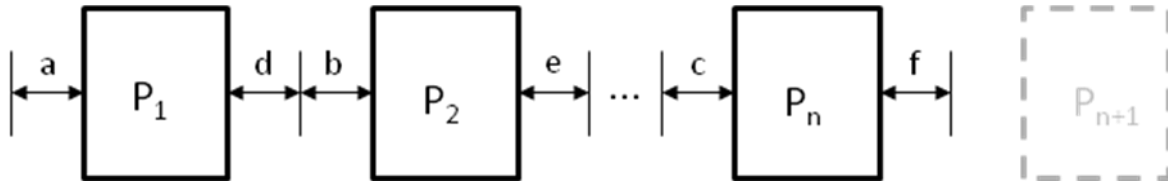
3. Average weight between portions estimate

$$A = \frac{1}{4} C$$

$$B = \frac{1}{2} C$$

C = average weight between portions

Picture series scheme



$$B = \frac{[a] + [b] + \dots + [c] + [d] + [e] + \dots + [f]}{2n}$$

$$B = \frac{\left[P_1 - \left(\frac{P_0 + P_1}{2} \right) \right] + \left[P_2 - \left(\frac{P_1 + P_2}{2} \right) \right] + \dots + \left[P_n - \left(\frac{P_{n-1} + P_n}{2} \right) \right]}{2n} +$$

$$\frac{\left[\left(\frac{P_1 + P_2}{2} \right) - P_1 \right] + \left[\left(\frac{P_2 + P_3}{2} \right) - P_2 \right] + \dots + \left[\left(\frac{P_n + P_{n+1}}{2} \right) - P_n \right]}{2n} =$$

$$B = \frac{3P_n - P_{n-1} - P_1}{4n}$$

Where,

n, total number of pictures in the picture series

P_0 (portion 0, 0 g) = 0 g ;

P_1 (portion 1 of the picture series, in g);

P_2 (portion 2 of the picture series, in g)...

P_n (last portion of the picture series, in g);

$P_{n+1} = (2P_n) - (P_{n-1})$ (estimated portion, higher than the last portion of the pictures series).