Sample collections from healthy volunteers for biological variation estimates’ update: a new project undertaken by the Working Group on Biological Variation established by the European Federation of Clinical Chemistry and Laboratory Medicine

Abstract

Background: Biological variation (BV) data have many fundamental applications in laboratory medicine. At the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) the reliability and limitations of current BV data were discussed. The EFLM Working Group on Biological Variation is working to increase the quality of BV data by developing a European project to establish a biobank of samples from healthy subjects to be used to produce high quality BV data.

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Methods: The project involved six European laboratories (Milan, Italy; Bergen, Norway; Madrid, Spain; Padua, Italy; Istanbul, Turkey; Assen, The Netherlands). Blood samples were collected from 97 volunteers (44 men, aged 20–60 years; 43 women, aged 20–50 years; 10 women, aged 55–69 years). Initial subject inclusion required that participants completed an enrolment questionnaire to verify their health status. The volunteers provided blood specimens once per week for 10 weeks. A short questionnaire was completed and some laboratory tests were performed at each sampling consisting of blood collected under controlled conditions to provide serum, K,EDTA-plasma and citrated-plasma samples.

Results: Samples from six out of the 97 enrolled subjects were discarded as a consequence of abnormal laboratory measurements. A biobank of 18,000 aliquots was established consisting of 120 aliquots of serum, 40 of EDTA-plasma, and 40 of citrated-plasma from each subject. The samples were stored at –80 °C.

Conclusions: A biobank of well-characterised samples collected under controlled conditions has been established delivering a European resource to enable production of contemporary BV data.

Keywords: biobank; biological variation; preanalytical phase.

Introduction

The 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) “Defining analytical performance specifications 15 years after the Stockholm Conference” was held in Milan in November 2014 [1]. The primary aim of the Conference was to investigate whether the hierarchy of analytical performance specifications established at the Stockholm Conference [2] was still valid or in need of change [3, 4].

The outcome of the Strategic Conference was that there should be a focus on three models to define analytical performance specifications. The first model is based on clinical outcomes, the second on biological variation (BV), and the third identified as state-of-the-art [4]. It was proposed that the first model is only useful for application to clinical measurands where there are strong and direct links between the examination result, the clinical decision and clinical outcome (e.g. glucose, total cholesterol, Hba1c), whereas the second model, based on BV data, has the advantage of applicability to a much wider range of measurands [4]. Appropriately quantified and characterised BV data have many applications. In addition to the setting of performance specifications, the data can also be used to define significance of change between measurements within a subject [5, 6], and to assess the utility of conventional population-based reference intervals [7]. To make the data accessible for these important applications, a large database of BV has been made available to laboratory medicine practitioners via the Westgard website [8]. The database is now widely used and referenced as an important source for clinical application. It was originally established, and continues to be maintained by Dr Carmen Ricos and colleagues through the offices of the Spanish Society of Clinical Biochemistry and Molecular Pathology (SEQC). The criteria used to evaluate the reliability of the data included in the database were recently published by Perich et al. [9].

As a consequence of the importance of BV data in the practice of laboratory medicine, there has been a recent focus on the quality of the data available for application. Recently published reviews and presentations at the EFLM 1st Strategic Conference raise doubts about the quality of BV data in current use and have led to concerns that their utility may be limited [10–13]. It has become clear there are many factors that may impact upon the application and transferability of BV data across populations and over time. These range from methodological factors associated with the production of the data and affecting the quality of the observations to true variability with respect to the population studied [11]. With the exception of a minority of common measurands, the currently available BV data available for use in clinical settings originate mainly from a limited number of historical scientific publications that may have employed methods of examination that in current practice would be considered obsolete, or in other studies employed methods that are subject to generational changes in analytical sensitivity and specificity. Other issues that compromise the quality and transferability of the derived BV data arise from the design of BV studies and subsequent data management [10, 14].

The importance of these complexities and limitations was recognised in a recent issue of Clinical Chemistry and Laboratory Medicine dedicated to the 1st Strategic Conference. Here it was proposed that ideally a BV database should include only results of appropriately designed and delivered studies [3]. A focus on the quality of existing data is therefore required and a drive towards delivery of high quality new studies and publications to identify and generate data to populate any future database. On behalf of the EFLM Working Group...
Materials and methods

Involving laboratories

The project is a multicentre study involving six laboratories in five different European countries:
- Servizio Medicina di Laboratorio, Ospedale San Raffaele (OSR), Milan, Italy [Coordinating centre]. Laboratory certified ISO 9001;
- Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway. Laboratory accredited ISO 15189;
- Department of Laboratory Medicine, Hospital Universitario La Paz, Madrid, Spain. Laboratory accredited ISO 15189;
- Department of Laboratory Medicine, University Hospital, Padua, Italy. Laboratory certified ISO 9001;
- Acibadem University, School of Medicine, Department of Biochemistry, Atasehir, Istanbul, Turkey. Laboratory accredited ISO 15189;
- Certe, Wilhelmina Ziekenhuis Assen, Europaweg-Zuid 1, 9401 RK Assen, The Netherlands. Laboratory accredited by CCKL (Dutch accreditation based on ISO 15189).

The protocol was first approved by the Institutional Ethical Review board of OSR in agreement with the World Medical Association Declaration of Helsinki. The documentation was then shared and approved by the ethical board of each centre, or by the regional Ethics Committee where needed.

Individual subjects were recruited to a list of volunteers via the local laboratory teams from their networks of colleagues, friends and acquaintances. The selected volunteers agreed to have blood drawn once per week for a 10-week period.

The collection started in all centres between the 13th and the 16th week of 2015. The protocol was followed strictly step by step in order to minimise pre-analytical variability. Following centrifugation serum and plasma samples separated from the drawn blood were aliquotted and frozen locally at ~80 °C and stored until the end of the study period. At this point all specimens from the entire cohort were transported from each centre on dry ice to the coordinating laboratory in Milan where they are stored in a dedicated freezer at ~80 °C.

Study enrollment and withdrawal

Inclusion criteria: In order to be eligible to participate in this study, a subject should meet all of the following criteria:
1. Agree and sign the informed consent;
2. Feel subjectively well;
3. Be older than 18 years of age;
4. Ideally he/she should not be taking any medication. Any subject taking medications or vitamin supplements had to report their active substance name, dose, and frequency to be able to identify these persons at a later stage;
5. Ideally he/she should not smoke or drink alcohol. Any subject taking tobacco or alcohol had to report type and amount.

Exclusion criteria: Subjects were excluded from participation if any of the following criteria were met:
1. Known diabetes and prescribed oral or insulin therapy, or fasting serum glucose >70 mmol/L;
2. History of chronic liver or kidney disease (γ-glutamyl transferase [γGT] >150 U/L; estimated glomerular filtration rate [eGFR] <60 mL/min per 1.73 m² using the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation) [18];
3. Dyslipidaemia (total cholesterol >6.5 mmol/L);
4. Family history of thalassemia syndrome and other haemoglobinopathies;
5. Results of examinations that clearly point to a severe chronic disease (cancer, cardiovascular or neurological) or acute disease (C-reactive protein [CRP]);
6. Known carrier state for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV);
7. History of being a hospital in-patient or otherwise seriously ill during the previous 6 weeks;
8. Blood donation in the previous 3 months;
9. Female subjects who were pregnant, breastfeeding, or within 1 year after childbirth;
10. Any other significant disease or disorder that, in the opinion of the investigator, could either put the subjects at risk because of participation in the study or could influence the results of the study.

Enrolment/baseline (visit 1, day 0): At the baseline visit informed consent was obtained from the subject after assessing the eligibility against the inclusion/exclusion criteria by filling in an enrollment questionnaire that collected data under four main headings (Table 1).
The subjects also underwent phlebotomy and the following examinations were performed: whole cell blood count (including red blood cell [RBC] count, white blood cell [WBC] count, haemoglobin [Hb], haematocrit, mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], mean corpuscular haemoglobin concentration [MCHC], red cell distribution width [RDW], platelets [PLT] and mean platelet volume [MPV]); and biochemistry investigations (including fasting glucose, creatinine, total cholesterol, triglycerides, γ GT, alanine amino transferase [ALT], creatine kinase [CK], CRP); and measurements of serum indices (haemolysis, icterus and lipaemia indices).

Post recruitment visits (visit 2–visit 10): Following the baseline visit the individual subjects were asked to attend on nine further occasions for further phlebotomy and to complete a short questionnaire (Table 2). Any adverse events that might impact on variability were noted at this time. The subjects were requested to avoid excessive physical exertion/exercise for 3 days before sampling; to avoid excessive eating and alcohol the night before the visit, to fast overnight for at least 10 h and to avoid smoking just before the blood collection. At each of these post recruitment visits samples were analysed in the local laboratories. Serum triglycerides, ALT, CK, CRP and serum indices were measured and the results assessed against local reference

Table 1: Main information asked in enrolment questionnaire.

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Life style</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of birth</td>
<td>Last food intake</td>
</tr>
<tr>
<td>Gender</td>
<td>Nutritional status (special diet)</td>
</tr>
<tr>
<td>Race</td>
<td>Drinking alcohol habits</td>
</tr>
<tr>
<td>BMI</td>
<td>Smoking history</td>
</tr>
<tr>
<td></td>
<td>Physical activity and exercise</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medical condition</th>
<th>For women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last illness time</td>
<td>Information about menstrual cycle</td>
</tr>
<tr>
<td>Hospitalisation</td>
<td>Hormone therapy or contraceptive pills</td>
</tr>
<tr>
<td>Allergic condition</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
</tr>
<tr>
<td>Current doctor’s care</td>
<td></td>
</tr>
<tr>
<td>Medication on a regular basis</td>
<td></td>
</tr>
<tr>
<td>Vitamin supplements or herbal remedies</td>
<td></td>
</tr>
<tr>
<td>Exposure to any hazardous chemicals</td>
<td></td>
</tr>
</tbody>
</table>

| Family history                |                                |
|-------------------------------|                                |
| Cancer                        |                                |
| Cardiovascular disease        |                                |
| Kidney disease                |                                |
| Diabetes                      |                                |
| Liver disease                 |                                |
| Thyroid disease               |                                |
| Thalassemia syndrome and other haemoglobinopathies | |
| Neurological disease          |                                |
| Mental disease                |                                |

The subjects were measured and the results assessed against local reference laboratories. Serum triglycerides, ALT, CK, CRP and serum indices were measured and the results assessed against local reference laboratories. Serum triglycerides, ALT, CK, CRP and serum indices were measured and the results assessed against local reference intervals and criteria to enable decisions around inclusion or exclusion of the collection from the study.

Subject withdrawal: Subjects were free to withdraw from participation in the study at any time upon request. Moreover an investigator could terminate a study subject’s participation if any clinical adverse event, laboratory abnormality, or other medical condition or situation occurred such that continued participation in the study would not be in the best interest of the subject or when the subject met an exclusion criterion (either newly developed or not previously recognised) that precluded further study participation.

Sample collection, processing and storage

Study procedures/evaluations: For each subject blood samples were drawn on a set day (Tuesday to Friday), and at the same hour between 08.00 and 10.00 at each weekly visit. It was possible to have blood samples drawn by the same phlebotomist at most visits further minimising variation.

When a subject for any reason was not available on the programmed day, the collection schedule was exceptionally changed to the day before or the day after. In a few cases (maximum two subjects/centre) it was necessary to extend the collection period by 1 week to enable the collection of the required number of samples. The blood was collected after the participant had been sitting quietly for at least 10–15 min to avoid variation due to postural influence.

The collection tubes used were provided by BD (Becton, Dickinson and Co, Italy), were identical in each laboratory.

Laboratory procedures/evaluations: At the first blood collection 42 mL were drawn into seven tubes:
- One KEDTA-plasma tube: plastic, 3 mL (13×75 mm) [BD code 368856]. It was used only during the first collection for the whole cell blood count;
- One KEDTA-plasma tube, plastic, 10 mL (16×100 mm) [BD code 367525];
- Three citrate-plasma tubes: buffered 0.109 mol/L sodium citrate tube, plastic 2.7 mL (13×75) [BD code 363048];
- Two serum tubes: clot activator, silicone coated, plastic, 10 mL (16×100 mm) [BD code 367820].

Specimen collection procedures: Specimen collection followed the procedures for collection of diagnostic blood specimens by venipuncture according to the CLSI guideline document H3-A6 [19].

Specimen preparation, handling, and storage

EDTA-plasma

The samples were stored on ice until centrifuged at 4 °C at 3000 g for 10 min in a swing-out centrifuge within 1 h after the phlebotomy. Platelet-poor plasma was removed without disturbing the sedimented cells (buffy coat) and aliquoted in Nalgene cryovials (Greiner bio-one GmbH, code 122263). Four aliquots of 1 mL were prepared, as quickly as possible, and frozen at –80 °C, or below, for long-term storage.

Citrate-plasma

The samples were centrifuged at 3000 g for 10 min at room temperature within 1 h of the blood being drawn. Platelet-poor plasma was
removed without disturbing the sedimented cells (buffy coat) and aliquoted in Nalgene cryovials. Four aliquots of 1 mL were frozen rapidly by immersion in a bowl with methanol and dry ice, and then stored at –80°C, or below, for long-term storage.

Serum
The samples were stored at room temperature for at least 30 min and maximum 2 h then centrifuged at 3000 g for 10 min at room temperature.

After separation of the serum, the specimen were promptly separated into 12 aliquots of 0.8 mL in Nalgene cryovials and stored at –80°C or below [20].

Number of samples/subject: For each subject four + four aliquots of EDTA- and citrated-plasma and 12 aliquots of serum were obtained for each sampling. This resulted in 20 aliquots of material at each time point delivering a total 200 aliquots/subject after 10 collections.

Specimen codification: In order to guarantee the privacy of enrolled subjects, all samples were identified by a unique code.

Sample shipment: At the end of the sample collection phase the samples from all of the subjects at each centre were sent as a single delivery, frozen on dry ice, to the co-ordinating central laboratory. The combined collection of samples from all centres form the biobank and are stored at –80°C in a dedicated freezer at the co-ordinating laboratory in Milan. Following analysis, all of the samples remaining at the end of the agreed study period will be destroyed.

### Results

#### Study population

Eighty three out of 105 enrolled subjects completed all 10 scheduled collections. Eleven subjects completed nine collections, three subjects eight and two subjects only seven collections.

Three subjects were not included in the final cohort after application of the inclusion/exclusion criteria at the first collection, five people withdrew during the study for personal reasons.

The final study population for sample collection consisted of 97 healthy volunteers of which there were 44 males aged 20–60 years, 43 females 20–50 years, and 10 females 50–69 years. Number, gender and age of enrolled subjects for each lab are shown in Table 3.

Further exclusions from the final cohort were based on the laboratory measurements made at each visit. This led to further exclusion of six subjects: five had several ALT and γGT values higher than their reference intervals (9–59 and 12–68 U/L, respectively, both for male) [21] suggesting liver disease:

1. A male with elevated ALT and γGT with a significant decreasing trend (ALT from 102 to 43 U/L; GGT from
Table 3: Number, gender and age of enrolled subjects by each laboratory.

<table>
<thead>
<tr>
<th></th>
<th>Men (20–60 years)</th>
<th>Women (20–50 years)</th>
<th>Men (50–70 years)</th>
<th>Women (50–70 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy – Milan (20 subjects)</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>60 (55–59)</td>
</tr>
<tr>
<td>Italy – Padua (16 subjects)</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>Norway (15 subjects)</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>63 (63–63)</td>
</tr>
<tr>
<td>The Netherlands (13 subjects)</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>60 (59–60)</td>
</tr>
<tr>
<td>Spain (16 subjects)</td>
<td>7</td>
<td>9</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Turkey (17 subjects)</td>
<td>8</td>
<td>1</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>43</td>
<td>10</td>
<td>60 (55–69)</td>
</tr>
</tbody>
</table>

Table 4: Characteristics of population studied at the baseline, after exclusion of six subjects with measurements indicative of active disease.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male</th>
<th>Female &lt;50 years</th>
<th>Female &gt;50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>91</td>
<td>38</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>Age, years</td>
<td>35 (21–69)</td>
<td>35 (22–59)</td>
<td>34 (21–49)</td>
<td>61 (55–69)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.5 (17.6–32.5)</td>
<td>24.4 (18.0–32.5)</td>
<td>21.3 (17.6–27.3)</td>
<td>22.1 (18.6–27.5)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.80 (3.55–6.99)</td>
<td>5.16 (4.11–6.99)</td>
<td>4.66 (3.55–5.40)</td>
<td>4.97 (4.09–6.16)</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>71 (52–120)</td>
<td>82 (56–120)</td>
<td>65 (52–88)</td>
<td>69 (54–83)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.70 (3.20–6.90)</td>
<td>4.84 (3.20–6.90)</td>
<td>4.50 (3.20–6.58)</td>
<td>5.95 (4.37–6.80)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.81 (0.40–2.79)</td>
<td>0.96 (0.46–2.79)</td>
<td>0.72 (0.40–1.89)</td>
<td>0.85 (0.67–1.47)</td>
</tr>
<tr>
<td>γGT, U/L</td>
<td>14 (7–74)</td>
<td>20 (9–74)</td>
<td>12 (7–30)</td>
<td>12 (9–24)</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>19 (8–51)</td>
<td>25 (13–51)</td>
<td>17 (8–47)</td>
<td>17 (14–23)</td>
</tr>
<tr>
<td>CK, U/L</td>
<td>98 (38–512)</td>
<td>135 (71–512)</td>
<td>78 (38–414)</td>
<td>102 (77–257)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.9 (0.0–8.5)</td>
<td>1.0 (0.0–8.5)</td>
<td>0.7 (0.0–5.1)</td>
<td>0.5 (0.1–3.0)</td>
</tr>
<tr>
<td>WBC, 10⁹/L</td>
<td>5.8 (2.8–11.2)</td>
<td>5.8 (3.9–8.9)</td>
<td>6.0 (2.8–11.2)</td>
<td>5.4 (3.8–7.3)</td>
</tr>
<tr>
<td>RBC, 10¹²/L</td>
<td>4.80 (3.92–5.80)</td>
<td>5.15 (4.37–5.80)</td>
<td>4.56 (3.92–5.20)</td>
<td>4.74 (4.27–5.00)</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>142 (102–172)</td>
<td>154 (136–172)</td>
<td>134 (102–162)</td>
<td>139 (128–154)</td>
</tr>
<tr>
<td>Ht, %</td>
<td>42.1 (33.4–52.0)</td>
<td>45.8 (39.4–52.0)</td>
<td>40.4 (33.4–47.7)</td>
<td>41.7 (38.9–45.4)</td>
</tr>
</tbody>
</table>

Median and range. ALT, alanine amino transferase; BMI, body mass index; CK, creatinine kinase; CRP, C-reactive protein; γGT, γ-glutamyl transferase; Hb, haemoglobin; Ht, hematocrit; PLT, platelets; RBC, red blood cell count; WBC, white blood cell count.

79 to 50 U/L at the 1st and 10th collection, respectively. This subject may have had a subclinical viral infection;

2. A male with elevated ALT (five values from 77 to 106 U/L) and CK (six values between 2300 and 5800 U/L) activities during several collections;

3. A male with elevated ALT during several collections (five values between 70 and 100 U/L), and high γGT (94 U/L) at 1st collection. This subject was also excluded for suspected subclinical viral infection;

4. A male with elevated ALT (five values between 70 and 100 U/L) during several collections (unknown liver problem);

5. A male with elevated ALT (three collections with values between 60 and 80 U/L), CK (one value higher than 450 U/L with a baseline of about 100 U/L) and CRP concentrations (three values >8 mg/L);

6. A male with raised CK (one value higher than 18,000 U/L, another >4500 U/L) and raised ALT on a number of occasions (values from 60 to 200 U/L).

However, we included a subject with body mass index (BMI) >30 kg/m² (BMI was 32.5 kg/m²) and four women with Hb between 100 and 120 g/L in whom all other clinical values were within the reference intervals.

The characteristics of the finally selected study population (91 subjects) and the clinical values at the baseline, after the exclusion of six subjects described earlier, are shown in Table 4.

The short questionnaire (Table 2) completed by each subject at each phlebotomy visit enabled single samples to be excluded from the final sample set. The information in combination with values observed in the haematology and biochemistry analyses also performed at the time of...
each visit enabled an objective explanation for single outlying results to support the decision. Variances were calculated for each measurand across the sample set for each subject. Where high variance was identified compared to the rest of the group, the data were plotted to enable a visual inspection of the distribution of the data. When a particular value was visually identified as unusual and therefore potentially an outlier the information within the short questionnaire was accessed to identify a possible cause for the discrepancy and grounds for rejection of the particular collection.

This process is exemplified by two examples involving outlying CK and CRP results as shown in Figures 1 and 2.

In total, 29 samples from 26 subjects were discarded (see Supplemental Data, Table 1).

Discussion

Several critical reviews have demonstrated that available BV data attributed to a number of measurands are poorly characterised and derived [10–14]. The authors reported that the published BV data for a single analyte and its various measurands can demonstrate a high degree of heterogeneity in the values published. The reasons for the observed differences in published BV data are often not clear while the potential causes may be manifold. Sources of uncertainty in the data may be attributable to: (1) pre-analytical issues (study design, enroled population, number of samples, frequency of collections, stability of samples); (2) analytical issues (instrument and analytical method used, number of replicates, number of runs); and (3) post-analytical data management. This list of causes is by no means exhaustive.

Users of published BV data face a challenge. That challenge is to achieve an understanding of the uncertainty around published BV data and an understanding as to whether the data are transferable to their practise. The user therefore needs to be aware of the limitations of historical data and providers of new data need to ensure that their new data are appropriately derived and characterised to be fit for use. The issue of historical data requires there is a critical appraisal process to enable assessment of the veracity and applicability of the data in the appropriate clinical context. Providers of future BV data can deliver confidence and address uncertainty by providing studies that conform to an ideal standardised approach/model. This will enable delivery of robust, well-characterised data from well-designed experiments that apply to contemporary methods and are transferable across health care systems. With this latter goal in mind, the WG-BV project reported here has been configured to follow accurately the optimal design identified by Fraser and Harris [17]. Six European clinical laboratories followed the agreed-upon protocol for the recruitment of subjects and the collection and processing of blood specimens. This approach is both rigorous, demanding and comparatively complex, but while providing challenges it also provides opportunities to the laboratory community. Every effort...
has been made in the execution of this project to deliver a large biobank of samples collected under optimal conditions and appropriately documented to be used to generate high quality BV data.

Pre-analytical phase

Removal of confounding pre-analytical variation has been a focus of the project to enable harmonisation of the pre-analytical procedures across the participating sites. This approach, in conjunction with careful gathering of data from the subject, delivers valuable documented information about the samples that will increase the quality of the BV data produced.

Healthy subjects have been selected with clearly identified inclusion and exclusion criteria, which were obtained through use of questionnaires. Combining the responses to questionnaires with the results obtained by measurements performed in each local centre has enabled determination of the healthy status of each subject at the point of every collection across the length of the whole study. This approach allowed the identification of outlier results and then exclusion of individual samples from the overall cohort or from individual sample collections.

Further potential variation has been avoided by standardisation of the phlebotomy system. The collection tubes were provided by a single manufacturer and the phlebotomists have strictly followed procedures within a clearly defined protocol to limit pre-analytical phase variation. A standardised protocol was also utilised by all centres to process and manage the collected samples prior to storage. Variability attributable to storage conditions and future integrity of the samples has been addressed by bulk transfer of specimens at the end of the study from the participating centres to the co-ordinating lab with subsequent long-term frozen storage at –80 °C.

Analytical phase

Analysis of the biobank samples to deliver the BV data will constitute the next phase of the project. The biobank provides a resource to which contemporary methods can be applied. The current biological database spans in excess of 40 years and many of the existing data sets were derived utilising methods that are obsolete today. Generational changes in methods can deliver greater accuracy as a consequence of improved specificities and lower limits of detection. Such changes will impact on the BV data obtained.

Post-analytical phase

A weakness of currently available BV data is that they are often the product of poorly designed studies (e.g. insufficient number of samples, samples measured in singleton) [14] and inadequate or inappropriate statistical analysis [10–13]. The study design (number of subjects, samples from each subject, and number of replicates) has a great impact on the reliability with which we can estimate the BV data and on the confidence intervals (CI) for the calculated CVs. There are a few published papers reporting BV data calculated from a large numbers of subjects [22–26]. Those studies involve a low number of collections from each subject (from four to six), often with measurements performed in singleton on the day of the collection.

The relative merits of alternate approaches to gathering BV data may be subject to future discussion. However, the approach that we have chosen for our study is that proposed by Fraser and Harris [17], which enables delivery of well documented and characterised data with CI around the estimates of BV. Røraas et al. [14] have reported that the number of samples taken from individual subjects is crucial in order to reduce the width of CI around the calculated within-subject biological variation (CVI). This is most important if the ratio between assay analytical standard deviation (SDa) and within-subject standard deviation (SDI) is lower than 1. Our study delivers a biobank containing 10 usable collections for the majority of 91 healthy subjects enrolled in the BV project. This provides a resource for the generation of new BV data with opportunities to update the existing literature and BV data repositories, with BV data that are appropriately characterised that have confidence limits determined around the estimates. Such an approach will enable the production of high quality data that are transferable across different populations and laboratories.

Conclusions

The BV project designed by the EFLM WG-BV will deliver BV studies based on a high number of healthy subjects. The established biobank delivers a resource upon which to commence phase 2 of the project, which is the examination phase. The numbers of collections per subject means that the resultant data will be from studies of high statistical power delivering high quality estimates of variation with tight CI. The resource will enable generation of BV data using contemporary analytical techniques for a potentially large number of measurands as it contains 200 aliquots of material from each of 91 healthy subjects. This
vast biobank, in which multiple sample types are available, provides the opportunity to determine biological variation for a wide range of target analytes including the older, common chemistry analytes as well. This updated data is critical for the derivation of analytical performance specifications for future and current practice and address the concerns raised in the EFLM 1st Strategic Conference around the quality of BV data.

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References


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