A pilot of the elfe longitudinal cohort: feasibility and preliminary evaluation

Amivi Oleko\(^a\), Fotini Betsou\(^a\), Hélène Sarter\(^a\), Claire Gerdil\(^a\), Isabelle Desbois\(^c\), Marie Aline Charles\(^d\), Henri Leridon\(^e\), Stéphanie Vandentorren\(^a\)

\(^a\)Department of Environmental Health, French Institute for Public Health Surveillance Institute, Saint Maurice, France, \(^b\)Integrated Biobank of Luxembourg IBBL, Luxembourg, \(^c\)French blood establishment, EFS, France, \(^d\)National Institute of Health and Medical Research, Inserm, France, \(^e\)Institute of demography, Paris, France

Correspondence:

Dr Stéphanie Vandentorren, Department of Environmental Health, French Institute for Public Health Surveillance, 12 rue du Val d'Osne, F-94 415 Saint Maurice, France

Tel: +33 1 41 79 68 20
Fax: +3 31 41 79 67 68
E-mail: s.vandentorren@invs.sante.fr
Abstract

Elfe will be a nationally French cohort of 20 000 children followed from birth to adulthood. Biological samples will be taken at birth to evaluate the foetal exposition to several substances. A pilot study was carried out in October 2007 to test the pre-analytical factors that affected sample quality. A variety of fractions was collected by the midwife after delivery from different blood collection tubes. Options in the collection process were two daily transports of samples; centralized and standardized processing methodology and storage of multiple aliquots in liquid nitrogen or at – 80°C. We analysed preanalytical factors that could have affected coagulation and then soluble CD40 Ligand (sCD40L) as a quality control tool of the serum quality. Cord blood was collected from 82% and urine from 84% of women who agreed to be followed up in the ELFE project. The use of the syringe was the main factor correlated with coagulation (RR 2.79 [1.47; 5.31], p<0.01). Maternity unit status was also associated with coagulation (RR 1.48 [1.03; 2.13] in a private maternity unit versus a public maternity) as well as time between collection and centrifugation (RR 1.03 [1; 1.07] when time between collection and centrifugation increases from 1 hour). There were no extremely low sCD40L values indicating extreme exposures to room temperatures. This quality control study has suggested the robustness of the pilot sample handling and storage protocol which can be used at a large scale level.
Introduction

ELFE (Etude Longitudinale Française depuis l'Enfance) is a nationally representative cohort of 20,000 children scheduled to be launched in France in 2011. ELFE will take a multidisciplinary approach, and aims to assess the impact of environmental exposures and socio-economic and familial factors on children’s development, health and behaviour. Children will be followed from birth through to adulthood over a period of 20 years.\(^1\) Inclusion in the cohort will be based on an initial enrolment interview of the mother when the child is born, so that we can obtain retrospective data on exposures during pregnancy, and then a prospective follow-up of the child. The recruitment of newborns precludes real-time collection of samples and data during pregnancy, although foetal exposures affect the future health of the child.\(^2-5\) The assessment of the prenatal environment will rely on the interview with the mother and the collection of biological samples at the child’s birth.\(^4,6\)

A pilot study was carried out in October 2007 to apply and evaluate methods for data and samples collection of cord blood, maternal urine, breast milk and maternal hair. The preanalytical conditions (the different steps from collection to storage) that are known to be responsible for more than 60% of laboratory errors ought to be considered.\(^7\) Researchers need to be aware of the various preanalytical factors in order to optimize accuracy, reproducibility and comparability of research results, and so the handling details of the samples should be documented and preanalytical conditions validated by a reliable quality control method.\(^8\) The objective of this report is to describe 1) the acceptability of the biological sample collection by both the mothers and the healthcare teams and 2) the preanalytical factors that affected sample quality for blood and serum.

The protocol for collection, processing and archiving of biological samples was developed through a consultation of the scientific community involved in the ELFE project. ELFE has to
provide resources to support a wide range of future scientific projects. The operating procedures tested in the pilot phase concerned: sample collection, temporary storage temperature and transport to a central processing facility, processing, aliquoting and long-term storage. We then analysed the effect of various preanalytical factors (namely transport, collection material used and status of maternity unit) on coagulation. We measured a storage-associated quality control biomarker in serum from cord blood. That is an indicator that the samples meet a minimum of preanalytical specifications and be suitable for a large range of downstream biological analyses.

**Materials and Methods**

**Population**

The pilot survey included all children born in hospital maternity units from 1-4 October 2007 in the Seine Saint Denis district and Rhone-Alpes region in France. For practical reasons, the survey was restricted to single or twin births. Two to three days after delivery, mothers were invited to sign a consent form explaining the general aims of the study and why we were collecting biological samples. Consent forms for biological samples and data collection were dissociated. The participants were given the right to withdraw from the study at any time. Seventy five percent of the maternity units contacted (n=30) agreed to take part in the pilot study. The acceptance rate of mothers who were invited to participate in the study was 54% (n=300). Ninety per cent of mothers who agreed to participate in the study also agreed to provide biological samples, and biological samples were collected for 80% of this population (10% could not be collected because of staff had too much to do during delivery of the child). The sample collection had received ethical approval in June 2010.

**Sample collection, processing and storage**

The ELFE biobank has been set up to establish a long term repository of biological
material, in order to support a wide range of present and future scientific questions. These cannot always be anticipated. Part of the biobank will be stored for further research. A multidisciplinary approach was taken to develop a protocol for the collection, processing and archiving of samples, rather than focusing on specific downstream analyses. A variety of fractions (serum, plasma, white and red cells, whole blood) were collected from two different blood collection tubes (K$_2$-EDTA spray dried tubes and serum tubes with clot activator coating) which allow analysis of a wide range of biomarkers. We opted for two daily transports of samples; centralized and standardized processing methodology and storage of multiple aliquots in liquid nitrogen or at – 80°C were performed. A unique barcode was attributed to each sample by the central processing facility; the barcode linked each blood collection tube with the unique participant-identifier number in order to prevent mislabeling of samples or sample mix-ups when transferred from clinical to research staff. A midwife collected cord blood (expected volume: 20 ml) and urine (expected volume: 200 ml) in the delivery room and stored them at 4°C in the maternity unit. The K$_2$-EDTA blood collection tubes were inverted ten times to mix the anticoagulant with the whole blood. Two or three days later, another midwife explained the project, obtained a consent form and collected breast milk (expected volume: 5 ml) and maternal hair from the mother in the maternity unit. Mothers were asked to collect and freeze their breast milk one month later at home (expected volume: 100 ml) and send it to the laboratory by mail in a special flask at ambient temperature. Whole blood and urine were maintained at 4°C in the maternity unit for a maximum of 24 hours prior to centrifugation (for blood) and 48 hours before storage. The blood in the K$_2$-EDTA tube was then sent to one of two central facilities located in blood transfusion centres (EFS), where it was centrifuged immediately on reception at 2500 g for ten minutes. The EFS centres coordinated transport (twice a day in temperature-controlled shipping boxes to central processing platforms), processing, aliquoting and freezing of the
samples.

The different samples from each individual were aliquoted into 0.5 ml or 1 ml aliquots for long term frozen storage. Haematological parameters (NFS) were assessed as samples arrived at the central laboratory in order to streamline processing and minimise quality control issues. Different blood fractions were separated by centrifugation at 2500 g for 10 minutes at 4°C. The aliquots were stored at –80°C in cryovials.

Analysis of various preanalytical factors

Cord blood coagulates easily (fetal hematocrit index is high at about 50%) and the way the samples were collected was probably suboptimal in the delivery context, due to the high workload of midwives. We performed an analysis of several factors that could have affected the risk of coagulation: the material for the cord blood sampling (needle extraction or collection of dripping blood), the technicity level of the maternity unit (from level 1 for lowest to level 3 for highest technical infrastructure available according to the technical level of the maternity (with level 1 for maternity with no neonatology service, level 2 for maternity with a neonatal unit and level 3 for maternity with a neonatal intensive care unit), the status of the maternity unit (private or public), the filling level of the blood collection tube; the duration of transport (in particular the time between delivery and cord blood collection (H0), the time between cord blood collection and reception at the processing centre (H1) and the time between cord blood collection and centrifugation (H2)). As the distributions of H1 and H2 were not normal, log transformations were used. The non-normal distributions could be explained by different workflows in the different maternity units. We performed a two-sample Wilcoxon test. We used a logistic or linear regression model to assess the association between these factors and the presence of a clot in the sample.
Analysis of sample quality of cord blood serum

CD40L is a protein which has shown a high sensitivity to temperature fluctuations in previous studies and can be used as a quality control tool.\(^9\) This biomarker decreases to a level distinguishable from the lowest level that is naturally occurring in response to specific variations in sample handling conditions.\(^10\) A quantitative sandwich enzyme immunoassay (Quantikine Human sCD40 Ligand Immunoassay kit, R&D Systems) was used according to the manufacturer’s instructions. This assay was based on CD40L-specific polyclonal capture with detection antibodies and colorimetric detection at 450nm. sCD40L concentrations were calculated according to the standard curve generated. Detection of sCD40L concentration below 4.3 ng/ml indicated exposure to at least 48 hours at +20°C (p<0.025).\(^10\) Using a cut-off provides the most sensitive and specific way of detecting "out of specifications" serum samples. The reported method provides a sensitivity of 97.5% for all samples whose value is higher that 6ng/ml and a specificity of 97.5% for all samples whose value is below 4.3ng/ml (values between 4.3 and 6ng/ml correspond to the grey zone).

Results

Collection of biological samples

In the pilot study, we recruited 301 women out of 571 new borns in 28 maternity units. Cord blood was collected from 82% and urine from 84% of women who agreed to participate to the ELFE project. The acceptance for cord blood donation ranges from 50% to 100% and for urine donation, from 36% to 100% according to the maternity unit (table 1).

The pilot study included two processing centres, one in each of the two districts. They received a total of 1976 specimens, and the different specimens from each mother were processed into 29 aliquots of 0.5 ml of whole blood, 11 aliquots of 10 ml of urine, 10 aliquots...
of 0.5 ml of breast milk collected in the maternity unit and 15 aliquots of 10 ml of breast milk collected at home for long term frozen storage. About 20% of the blood samples were coagulated (n=90) upon reception and less than 12% arrived in the laboratory more than 24 hours after collection in the maternity unit. The median time between collection and centrifugation was 21.2 h (interquartile range 15.9 to 25.8 hours). Whole blood was tested for a standard range of haematological parameters (red and white cell counts). The median delay between centrifugation and freezing was 4.4 hours with a minimum of 0 and a maximum of 14 hours.

**Analysis of various preanalytical factors**

We studied the association between specific collection and processing factors and the coagulation status of the samples: the status of the maternity unit, the collection materials used and the transportation delay. A great variation of coagulation according to the maternity unit was observed (coagulated samples proportion ranged from 0% to 100%). Coagulation was more frequent in samples from private maternity units than from public ones (27.8% versus 16.5%); coagulation was also more frequent when a needle was used for collection (41.7% versus 16.0%) (Table 2).

Table 3 describes the time between collection and centrifugation according to coagulation status. The average transport time of coagulated samples was higher than that of non-coagulated samples (median: 20.9 for non-coagulated samples versus 22.7 for coagulated samples).

In order to study the effects of preanalytic factors on the coagulation, a log-Poisson regression was performed, linking coagulation status to district, needle use, status of maternity, time between collection and centrifugation. Log-Poisson modelling has been chosen as an alternative to logistic modeling when odds ratio is not a good approximation of relative risks:
indeed coagulation proportions are high and depending on explaining variables, for examples 16% versus 42% for public versus private maternities.

This model showed that the use of the syringe was the main factor correlated with coagulation (RR 2.79 [1.47; 5.31], p<0.01, for syringe use versus no syringe use in Seine-Saint-Denis). Maternity unit status and time between collection and centrifugation are also associated with coagulation:

- RR 1.03 [1; 1.07] when time between collection and centrifugation increased from 1 hour.
- RR 1.48 [1.03; 2.13] when sample was collected in a private maternity unit (public maternity unit was taken as reference).

No association between coagulation and time at room temperature, spent between reception and centrifugation, was observed.

**Analysis of sample quality in cord blood serum**

sCD40L was analysed in 271 serum aliquots. Mean was 15.62 ng/ml (SD 4.52), range 3.8 to 27.3 ng/ml. No significant difference was observed between serum samples prepared from coagulated and non-coagulated blood specimens. A significant correlation was observed between sCD40L levels and delay between centrifugation and storage (R=0.15, p=0.015).

We did not observe any extremely low sCD40L values indicating extreme exposures to room temperatures. Only 6 serum samples exhibited borderline sCD40L concentrations of 3.8 - 4.9 ng/ml, suggesting a possible 48 hour exposure to +20°C. The 6 serum samples exhibiting borderline sCD40L concentrations had time between collection and centrifugation > 18 hours and/or between collection and storage > 21 hours.
Discussion

The response rate for biological collection was satisfactory for cord blood and urine (more than 80% of women) but lower for milk collection. This can be explained because the rate of breast feeding was 68% (n= 204) at delivery and 42% (n=126) accepted to collect milk for research purposes. This rate decreased to 48 % (n= 144) at one month post-delivery and 15% (n=46) accepted to collect it by then. No quality biomarker for preanalytical validation of milk or urine is available to our knowledge. Therefore, we focused our analyses on sample quality in serum and blood.

Biospecimen characterization and preanalytical validation is one of the major challenges facing modern biobanking.\textsuperscript{(11)} In this pilot survey, we performed a first evaluation of the collection, transport, processing, and storage methods of samples for the ELFE project. The average time to storage (from collection of blood and urine to storage at ultra-low temperature) was 25 hours, which is similar to other large scale studies (for example, the UK Biobank has an average time to archiving of 23 hours) even if blood samples were not centrifuged in the clinic setting before shipping to the central laboratory, as it was done in UK biobank for instance.\textsuperscript{(12)} This process was a compromise between feasibility on a large scale and the requirements of many scientific investigations. We centralized and standardized processing in a single blood transfusion centre to ensure a high quality data trail. We used two geographically separate repositories. The secure data audit trail was maintained by using quality management system ISO 9001-2008.

The use of health service infrastructure to collect biological samples at the maternity unit for epidemiological purposes is a challenge. Indeed, the conditions under which biological samples are collected and processed in the course of providing health care in maternity units could be inadequate for the measurement of less stable biochemical markers of
epidemiological interest, such as cytokines.\textsuperscript{(13)} The procedures used for sample collection, processing and storage have a major impact on the future scientific usefulness of ELFE.\textsuperscript{(14)} Thus, about 20\% of the blood samples were coagulated upon reception. That could be explained by practical reasons in the delivery room, as immediate or delayed cord clamping, or other factors within control due to different conditions in the maternities (e.g. high workload of staff); these could be also reasons for partially filled tube, as well as the lack of sufficient volume of available blood. These differing conditions between maternity units could explain the association between hospital private status and higher rate of coagulation. Private hospitals are less research oriented and have not the same academic background or practices as public maternity units. Moreover, coagulation was also more frequent when a needle was used for collection probably because physiological adaptation to delivery trauma induces hypercoagulability in the newborn's blood\textsuperscript{(15)}. Consequently, all mechanical factors such as the syringe shearing force and mechanical pressure through aspiration induce platelet and tissular factor activation and coagulation. This activation is not induced by natural dripping of blood.

A quality control study was performed to test the robustness of the pilot sample handling and storage protocol, by measuring sCD40L which is an indicator of the delay of exposure of serum to room temperature. It has previously been shown that the sCD40L levels in cord blood are lower than in peripheral blood, which further consolidates the results of this study in terms of cord blood-derived serum integrity.\textsuperscript{(16)} Finally, it should be kept in mind that although serum sample integrity was successfully assessed on the basis of sCD40L assay, novel quality control tools (such as surveillance of freeze-thaw cycles temperatures) need to be developed to assess other types of preanalytical variation and to allows a more complete characterisation of large cohort samples.
In this pilot survey, we validated the collection, transport, processing, and storage methods of samples for the ELFE project. A quality control study was performed to test the robustness of the pilot sample handling and storage protocol, by measuring sCD40L which is an indicator of the delay of exposure of serum to room temperature. Studies of serum sample integrity need to be more investigated as preanalytical validation is one of the major challenges facing modern biobanking. In the mean time, it can be highly recommended to carefully record all potentially critical preanalytical variables, with tools such as a Standard PREanalytical Code (SPREC) (16).

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Reference List


