Logistics of collection and transportation of biological samples and the organization of the central laboratory in the ELSA-Brasil

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ABSTRACT

The ELSA *(Estudo Longitudinal de Saúde do Adulto* – Brazilian Longitudinal Study for Adult Health) is a multicenter cohort study which aims at the identification of risk factors associated with type 2 diabetes and cardiovascular diseases in the Brazilian population. The paper describes the strategies for the collection, processing, transportation, and quality control of blood and urine tests in the ELSA. The study decided to centralize the tests at one single laboratory. The processing of the samples was performed at the local laboratories, reducing the weight of the material to be transported, and diminishing the costs of transportation to the central laboratory at the Universidade de São Paulo Hospital. The study included tests for the evaluation of diabetes, insulin resistance, dyslipidemia, electrolyte abnormalities, thyroid hormones, uric acid, hepatic enzyme abnormalities, inflammation, and total blood cell count. In addition, leukocyte DNA, urine, plasma and serum samples were stored. The central laboratory performed approximately 375,000 tests.

DESCRIPTORS: Clinical Laboratory Information Systems. Diagnostic Techniques and Procedures. Logistics. Hematologic Tests. Urinalysis. Multicenter Studies as Topic, methods. Cohort Studies.

INTRODUCTION

Among the exposure variables in epidemiological studies of chronic diseases, physiological, biochemical, hormonal and inflammatory data are included. The determination of these variables was legitimated in the Framingham Heart Study, initiated in 1948, when five thousand volunteers attended the project's investigation center (IC) to be submitted to a clinical assessment and tests.[12] The most important result was the identification of elevated serum cholesterol as a higher risk factor for coronary heart disease, which triggered the "cholesterol theory". Subsequently, other cohorts, like the Atherosclerosis Risk in Communities (ARIC) study[33] and the Multi-ethnic Study of Atherosclerosis (MESA),[5] used a similar methodology, with collection of biological material at the ICs. Contrasting to these results, cohorts like the Nurses' Health Study,[4] the Physicians' Health Study[19] and the Women's Health Study[8] decided to include a larger number of participants by means of correspondence. The collection of biological material was performed by the volunteer (always a health professional), using a collection kit sent by mail and picked up by a transportation company after the collection. Blood collection by the participant enables it to be performed after the baseline of studies of the type case-cohort and nested case-control, using frozen samples. A significant example of this strategy was the identification of the association of the ultrasensitive C-reactive protein with coronary heart disease in the Physicians' Health Study, with 22,071 participants, but in which only 1,086 analyses were performed: 543 participants with myocardial infarction and 543 without the disease.[27]

Cohort studies on chronic diseases enable the identification of risk markers in primary cohorts and prognostic markers in the survival cohorts of active diseases. Both the risk markers and the prognostic markers can be classified according to physiopathology as: metabolic, lipid, of renal function, of vascular function, oxidative stress, angiogenesis, and of tissue necrosis.[6] It is possible to identify, in urine samples, metabolites that are products of the interaction among several factors: diet-related, environmental, drug-related, of the intestinal microbiota and genetic factors.[21]

Independently of the strategy for biological material collection, the tests are usually performed at one or more central laboratories or by specific groups of tests, according to the technical sophistication that is required.[5,33] However, all the analyses referring to a specific test are always centralized at the same laboratory.

The *Estudo Longitudinal de Saúde do Adulto* (ELSA-Brasil - Brazilian Longitudinal Study for Adult Health) aims to identify the risk fo cardiovascular diseases and diabetes in a population that is apparently healthy, focusing on the identification of biomarkers at the initial stage of the atherosclerotic process. The ELSA-Brasil also aims to identify biomarkers involved in the process of the causal network that starts with body weight gain and is crystallized with the development of type 2 diabetes.[3] From the operational point of view, the ELSA-Brasil decided, since its beginning, that the volunteer would visit the IC to undergo all the measurements and collection of biological material, following the model of cohorts like Framinghan[12] and ARIC.[33] The ELSA-Brasil is the first multicenter study in the country with a single protocol executed uniformly at the six centers. To achieve this, a central laboratory was constituted for the performance of all the blood and urine tests of the study; in addition, it was responsible for the standardization of the DNA extraction methodology.

In a study with the characteristics of the ELSA-Brasil, the performance of tests at one single laboratory provides many advantages, like the reduction in inter-laboratory variability and the utilization of the same inputs and consumables in the performance of biochemical dosages. Nevertheless, this does not reduce the need of great caution concerning the pre-analytical aspects, described in this paper. The pre-analytical variables contribute to reduce the accuracy of a laboratory test, and are responsible for between 32% and 75% of the total variance of results attributed to errors (excluding biological variability). This paper focuses on the following pre-analytical aspects: collection place, identification of participants, preparation of participants, collection site(s), tourniquet application and time, venipuncture technique, order of tubes collection, collection volume, handling of tubes and processing of biological samples, centrifugation, freezing and transportation of biological material.

DESCRIPTION OF THE PROCEDURES

Since the beginning of the organization of the study, the researchers discussed about the need of centralizing or not the performance of the tests. Two models were possible: decentralized collection and performance of the tests or decentralized collection with transportation of the collected material to a central laboratory, common to the entire study.

Of the six centers, the São Paulo one had greater tradition in the area of clinical analyses, and it is located inside the *Hospital Universitário* (University Hospital) of the *Universidade de São Paulo* (USP). Many of the IC researchers in São Paulo had already used the laboratory in other research projects, with good results. Besides the clinical basis, the University Hospital was undergoing an excellent political moment, in which it was desired that it would be a place not only for care provision and teaching, but also research.

The Laboratory of the University Hospital has implemented a Quality Management System and received the ISO 9001/2000 certification on 04/11/2006. It was accredited by the Clinical Laboratories Accreditation Program on 12/18/2009 (2005), and participates in an Inter-laboratory Proficiency program. In addition, it has an Internal Quality Control Program with at least two levels of control to each analyte, with high-performance automated equipment and a team of qualified personnel that assure the quality of the performed tests. The study's final decision was to centralize the tests, except for the blood cell count, which would be carried out locally for technical reasons. The blood cell count in the ELSA-Brasil was performed with automated equipment, at laboratories with internal and external quality controls, which participate in laboratory proficiency programs, like the National Quality Control Program[a], the Program of Proficiency in Laboratory Tests[b] and the Laboratory Accreditation Program of the College of the American Pathologists.[c]

Fasting blood glucose and after the ingestion of a glucose solution are the key points of the study, as they represent one of the criteria that will define the presence of diabetes in a study that focuses on cardiovascular disease and on diabetes itself. Decentralizing the glucose tests would introduce an important error factor in the determination of one of the main variables of the study. Recent studies have shown that blood collection, with subsequent centrifugation and freezing of the plasma, has proved to be quite stable for future dosage of glucose.[7,11] Recently, the Study of Latinos (SOL) also decided to centralize the performance of the tests, including glucose.[32]

The procedures for the collection of biological samples were standardized to assure uniformity at all the ELSA ICs and followed the recommendations of the Brazilian Society of Clinical Pathology/Laboratory Medicine for blood collection (2005).[d] Blood collection was divided into two stages: after a 12-hour fasting and 2 hours after the ingestion of a glucose solution.[2] In the participants with diabetes, the oral glucose tolerance test was replaced by a standardized food load. Besides the tests performed two hours after the ingestion of the glucose solution or food load, the study established that biological material for storage must be collected on the second blood collection. Few studies will have the same conditions as ELSA's of evaluating biochemical and inflammatory markers in the serum after ingestion of a glucose solution.

Another highlight was the standardization of the food load in the form of a snack composed of industrialized food, including four toasts (15 g each), four processed cheese cubes and orange juice (200 ml), totaling 435 kcal (including 24 g of fat, of which 14 g of saturated fat and 47 g of fast absorbing carbohydrates). The option for industrialized food was due to the fact that it does not need refrigeration, it presents a long validity, it is not necessary to prepare it at the IC and it can be bought with the same facility at all the centers. The idea of testing a food load in the diabetics was based on recent evidence according to which the alterations of the post-prandial period are important factors in the development of atherosclerosis.[9,10,13]

A sequence for collection in tubes was established, so that the first three enabled the performance of all the tests. The following tubes ensured the variety of the materials necessary to compose the ELSA biobank. In addition to the tests of each participant, 42 samples of 0.5 ml were stored locally and at the central biobank.

Urine collection in the ELSA was planned to evaluate glomerular filtration by creatinine clearance and to estimate the ingestion of electrolytes (Na, K) by renal excretion. Therefore, in the ELSA, urine collection was performed during 12 hours in the nightly period, when the majority of people are at home, thus reducing collection errors and increasing adherence. At night the temperature is lower, and this reduces the loss of electrolytes via sweat. A parallel study conducted with a population that was similar to ELSA's analyzed the correlation between urine collected over 24 h and over 12 h (7p.m. to 7 a.m.), and it was found that the creatinine clearance was similar in the two periods.[31]

Thirty minutes after the end of blood collection, at the most, all tubes were centrifuged under refrigeration during 15 minutes. Then, the aliquots were separated for tests in an ice bath, using cryotubes previously identified with barcodes.

To enable the implementation of the central laboratory, it was necessary to create a structure for transportation and short-term storage of samples at the centers, so that they could be subsequently sent to São Paulo on a monthly basis. The aliquots for the performance of the tests were stored in freezers at -80ºC until the date of transportation to the Central Laboratory. The samples collected at the University Hospital of USP were also stored in a freezer at -80ºC for up to 30 days for the subsequent performance of the tests, in the same way as in the other ELSA ICs.

[a] Programa Nacional de Controle da Qualidade. [cited Mar 4, 2013]. Available from: http://www.pncq.org.br/
[b] Proficiência em Ensaios Laboratoriais. [cited Mar 4, 2013]. Available from: http://www.sbpc.org.br/?C=133
[c] College of the American Pathologists. Laboratory Accreditation Program. Northfield (IL): CAP; 2012 [cited Mar 28, 2011]. Available from: http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets%2FcontentViewer%2Fshow&cntvwrPtlt%7BactionForm.contentReference%7D=laboratory_accreditation%2Faboutlap.html&_pageLabel=cntvwr
[d] Sociedade Brasileira de Patologia Clínica, Medicina Laboratorial. Recomendações da SBPC/ML para coleta de sangue venoso. São Paulo; 2005.

The transportation of biological material to the central laboratory also required the definition of a strategy. One of the options was the transportation of biological material in the primary tubes after centrifugation. This strategy would have the advantage that the local laboratory team at each center would be composed of few members, with the function of collecting and centrifuging the material. The largest part of the procedures would be performed at São Paulo. The negative aspect is that the weight of the sent material would increase very much with the transportation of the primary tubes to São Paulo, and the costs would become unfeasible. The second option was that each IC would have a team for the collection and processing of the biological material, which would be stored in cryotubes at -80ºC for subsequent transportation. The negative aspect of this strategy would be the need of centralized training and certification of the IC teams, and the positive aspect, the lower costs. Finally, it was decided that local teams would be hired and trained to perform the processing of the biological material.

The method chosen to perform the DNA extraction was salting-out, due to the lower cost and larger amount of extracted DNA. The solutions necessary for the extraction were prepared in São Paulo and sent by a transportation company.[24]

To control the quality of the collection and processing of biological samples in the ELSA, 10% of the participants' identification codes were randomly drawn. The drawn participants had a duplicate collection tube that generated an extra test aliquot which was used as blind control.

After it was decided to centralize the tests, a specific room was created for the processing of biological materials of research projects. The study renovated a room of the laboratory in the University Hospital to install the equipment, which, besides facilitating the study's logistics, qualified the hospital to receive other large research projects.

The strategy of performing the tests at a central laboratory aimed to ensure the uniformity of the utilized methodology, avoiding the variations between laboratories that would inevitably happen if the tests had been decentralized. All the defined procedures were described in detail in specific manuals for each stage of the process: (1) "Collection of biological samples"; (2) Processing of biological samples"; (3) "Tests and methodology" and (4) "Storage and transportation of biological samples".

Another important result was the centralized training of all the laboratory professionals. The entire team that worked at the six centers was trained in São Paulo by the Central Laboratory team. After the training and before the beginning of the fieldwork, the central laboratory team made a certification visit to each ELSA IC to check the adherence to the study's protocols. Periodically and whenever necessary, visits of the central laboratory team were scheduled to the ELSA centers. Furthermore, during data collection, and whenever necessary, the central laboratory received new team members from all the centers for training or retraining.

Table 1 lists the fasting tests and those performed after 2 h. Table 2 describes the methodology and the equipment used to perform the tests, with their reference values. The blood cell counts were carried out locally at each center. Slide reading was not performed, due to the difficulty in the inter-laboratory standardization of this procedure.

All the study's centers started with one or two participants scheduled per day until the maximum capacity of

Table 1. Fasting collection and collection after ingestion of a glucose solution or food load.

1st collection after 12-hour fasting	03 tubes (8.5 mL) with clot activator gel; 03 EDTA tubes (4 mL); 01 fluoride/oxalate tube (2 mL); 02 tubes (4,5 mL) containing citrate as anticoagulant; and 01 tube (4 mL) with heparin.
Fasting tests	Blood glucose, glycated hemoglobin (HbA1c), creatinine, sodium, potassium, uric acid, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GT), total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, TSH, insulin and free T4 only for the individuals who presented altered TSH, ultrasensitive C-reactive protein, and Serology for Chagas Disease.
Post-collection procedure	Non-diabetics: ingestion of a glucose solution to be submitted to the oral glucose tolerance test; Diabetics: food load composed of four toasts, four cheese cubes.
2nd collection 120 min after the beginning of the ingestion of the glucose solution for the non-diabetics and of the food load for the diabetics	Second collection two hours after the end of the ingestion of the glucose solution or food load in which the following tubes were collected through venipuncture with vacuum scalp: 01 tube (8,5 mL) with clot activator gel; 01 tube (4mL) with heparin; 01 fluoride/oxalate tube (2 mL).
Post-collection procedure	Snack

Table 2. Types of performed tests, their purpose, utilized methodology and reference values.

Test	Objective	Method	Equipment	Reference value
Blood glucose	Determination of diabetes	Hexokinase method (enzymatic)[25]	ADVIA 1200 Siemens®	Fasting: 70 to 99 mg/dL 120 minutes after ingestion of glucose solution: < 140 mg/dL: normal tolerance to glucose 140 to 199 mg/dL: glucose intolerance ≥ 200 mg/dL: diabetes
Total cholesterol	Lipid metabolism	Cholesterol oxidase method (enzymatic colorimetric)[1]	ADVIA 1200 Siemens®	Desirable: < 200 mg/dL Borderline: 200-239 mg/dL High: > 240 mg/dL
HDL-cholesterol	Lipid metabolism	Homogeneous, colorimetric, without precipitation[34]	ADVIA 1200 Siemens®	Desirable values: Non-diabetics:> 40 mg/dL diabetics: > 45 mg/dL
Triglycerides	Lipid metabolism	Glycerol-phosphate peroxidase method according to Trinder (enzymatic colorimetric)[17]	ADVIA 1200 Siemens®	< 150 mg/dl
LDL-cholesterol Used when triglycerides ≤ 400 mg/dl.	Lipid metabolism	Friedewald Equation		Desirable for: High risk patients: < 100 mg/dL Medium risk patients: <130 mg/dL Low risk patients: < 160 mg/dL
LDL-cholesterol Used when triglycerides > 400 mg/dl	Lipid metabolism	Homogeneous, enzymatic, colorimetric method without precipitation[26]	ADVIA 1200 Siemens®	Desirable for: High risk individuals: < 100 mg/dL Medium risk individuals: <130 mg/dL Low risk individuals: < 160 mg/dL
Creatinine	Renal function	Jaffe's Method[22]	ADVIA 1200 Siemens®	Serum: 0.4 to 1.3 mg/dL 12-hour urine: not established
Uric acid	Purine metabolism marker	Uricase method (enzymatic colorimetric)[16]	ADVIA 1200 Siemens®	Men: 3.5 to 7.2 mg/dL Women: 2.6 to 6.0 mg/dL
Aspartate aminotransferase	Identifier for hepatic steatosis	Modified IFCC (enzymatic)[29]	ADVIA 1200 Siemens®	Men: 10 to 35 U/L Women: 10 to 31 U/L
Alanine	Identificador para esteatose hepática	Modified IFCC (enzymatic)[28]	ADVIA 1200 Siemens®	Men: 9 to 43 U/L Women: 9 to 36 U/L
γ-glutamyl-transferase	Identifier for hepatic and alcoholic steatosis	Szasz Persijn (kinetic colorimetric)[30]	ADVIA 1200 Siemens®	Men: 2 a 30 U/L Women: 1 a 24 U/L
Ultrasensitive C-Reactive Protein	Inflammatory marker	Immunochemistry through nephelometry[14]	nephelometer BN II Siemens®	For cardiovascular risk assessment: Low risk: < 1.0mg/L Medium risk: 1.0 a 3.0 mg/L High risk: > 3.0 mg/L
Glycated hemoglobin	Determination of diabetes	High-pressure chromatography (HPLC)[20]	Variant Bio Rad®	< 5.7% Normal tolerance to glucose
TSH	Etiological investigation	Immunoenzymatic assay[23,35]	Centaur Siemmens®	0.55 – 4.78 mcUI/mL
Free T4	Etiological investigation	Immunoenzymatic assay[26,35]	Centaur Siemmens®	0.89 to 1.76 ng/dL
Insulin	Carbohydrate metabolism	Immunoenzymatic assay[23,35]	Centaur Siemmens®	Fasting:3.0 - 25.0 mUI/L After ingestion of glucose solution: Not established
Urine Na	Food intake	Potentiometry with ion-selective electrode	ADVIA 1200 Siemens®	Not established

Continue

Continuation

Test	Objective	Method	Equipment	Reference value
Urine K	Food intake	Potentiometry with ion-selective electrode	ADVIA 1200 Siemens®	Not established
Urine calcium	Food intake	Potentiometry with ion-selective electrode)[18]	ADVIA 1200 Siemens®	Not established
Microalbuminuria	Endothelial function	Immunochemistry through nephelometry	nephelometer BN II Siemens®	Normal: < 20 µg/m in Microalbuminuria: 20 to 200 µg/min Macroalbuminuria: > 200 µg/min
Serology for Chagas Disease	Differential diagnosis in cardiomyopathies	ELISA with solid-phase microplates – reagent WIENER (CHAGASTEST)[15]	Microplate incubator/ reader	Not reactive

IFCC: International Federation of Clinical Chemistry and Laboratory Medicine

each center was achieved, which varied from four to 20 participants per day. This number increased only when the team was totally capable of performing the task. In the case of problems, the instruction was to decrease the number of participants; it would only be increased again when the team was ready. This extended the period of data collection, but ensured the quality of the procedures.

In relation to the tests, the delivery of the 24-hour urine sample was the most complex point. Approximately 10% of the participants did not deliver it on the day of the tests. This occurred at the six centers in a similar way. Among the women, the most common cause was menstruation. In these cases, the participant was instructed to return on another day to deliver the material, but this sometimes did not occur. In the cases of difficulty in collecting blood, the protocol followed by the team guaranteed that the samples for the tests were always the first to be collected; therefore, there was always material to perform the tests. What was compromised, in these cases, were the samples that would be stored.

In relation to transportation, although the study used a company specialized in transportation of biological material, the few options of flights constituted a difficulty over time. To minimize these problems, it was necessary to create a distance support to the laboratory. A network of four cell phones with rotation of the team worked 24 h at a distance to solve problems related to transportation, like a delay in the delivery of the samples by the transportation company due to traffic problems in São Paulo, among others. This network contributed to solve problems and, with it, there was always a member of the team to receive the material and process it as soon as possible. The network, which had not been planned at the beginning of the study, was fundamental and brought tranquility during the fieldwork. A local problem experienced by some centers was difficulties in the supply of dry ice, which many times delayed the sending of the material.

During the 26 months of data collection, the laboratory received and processed 375,295 blood and urine tests of the participants included in the study, in the six centers. There was no loss of biological material due to transportation and no samples had to be discarded due to inadequate transportation.

CONCLUSIONS

The ELSA-Brasil is a multicenter cohort composed of six research and higher education institutions that aims at searching for new associations of diabetes and cardiovascular disease in the Brazilian population. The option to have one central laboratory ensured the uniformity of the methodology that was used to perform the tests, avoiding the inevitable variations among laboratories. Only the blood cell count was performed locally, with automatic readings, due to the impossibility of standardizing slide reading in the six centers. The centralization of glucose dosage was successful. The ELSA-Brasil is one of the few studies that, in the next years, will be able to make the diagnosis of diabetes based on the glucose tolerance test and on glycated hemoglobin, which will open a new perspective for research into diabetes in long-term studies.

The calculations made before the beginning of the study showed that the most cost-effective procedure proved to be the processing of the biological material locally at each center, with subsequent transportation to São Paulo. Sending the material already processed reduced the weight, which was fundamental to reduce transportation costs. However, the chosen strategy also required the formation of local teams at each center to collect and process the samples. The formation of local teams, in turn, implied the creation of a strategy of centralized training of these teams, increasing the expenses with transportation and stay in São Paulo. Comparing the costs related to the need of centralized training to the reduction in the volume of the

transported material, it was evident that processing the samples locally was much more cost-effective.

There were many bureaucratic restraints to hire the transportation companies and this jeopardized the transportation of biological material. Successful transportation depended more on bureaucratic restraints and on the traffic in São Paulo than on technical issues related to the collection and processing of biological samples. It is important to highlight that there is no insurance policy for loss of biological material, as it is considered irreparable and irreplaceable loss, which hinders, from the juridical point of view, the writing of the transportation agreement. Another important point is that there are no airlines with tradition in the transportation of this material. During the initial stage of the study, the company that concentrated a large part of this transportation stopped operating on trade routes. In addition, the reduction in the number of flights also affected the logistics, a problem that was minimized with the creation of a distance support network to the laboratory, based on cell phones, available 24 hours a day, seven days a week. The justification for its need, which was questioned many times, is that without it, the conduction of the study could have been negatively affected. This network also supported the local laboratories at each investigation center, 24 h per day. The difficulty in the supply of dry ice was another problem that many times delayed the sending of the material, changing the routine of the central laboratory.

In the planning of the study, many needs were underestimated, implying new costs. Although clinical research has advanced a lot in recent years in Brazil, the transportation of biological material is performed in small amounts and on specific dates. The ELSA-Brasil, for the first time, planned the transportation of material for a period of two and a half years. The absence of loss of material shows that the solutions that were found were successful and open good perspectives for long-term studies with transportation of biological material.

To conclude, the ELSA-Brasil has shown that it is possible to perform multicenter tests in the national territory with centralized analysis, and acceptable quality and cost, allied to the strategy of permanent custody of biological material for future studies.

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