Clin Chem Lab Med 2007;45(10):1390–1395 © 2007 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2007.160

Serum biobank certification and the establishment of quality controls for biological fluids: examples of serum biomarker stability after temperature variation[1)]

Christine Chaigneau, Thomas Cabioch, Katy Beaumont and Fotini Betsou*

Biobanque de Picardie, Centre Hospitalier Universitaire d'Amiens, Salouel, France

Abstract

Background: One of the main issues in biobanking is the establishment of standard operating procedures for specimen collection, preparation and storage to control for pre-analytical variation. For biological fluids such as serum, there is currently a lack of sensitive biomarkers for the quality control of cryopreservation conditions.

Methods: The process approach was used to establish an ISO 9001:2000 quality management system. Immunoenzymatic and functional assays were used to assess the stability of the following candidate quality control biomarkers: secretory phospholipase A2, matrix metalloprotease 7, transforming growth factor β1 and anti-HBs immunoglobulin.

Results: Five product processes and their corresponding indicators were identified. In the preparation-aliquoting-storage process, no quality control indicator for serum was identified. Only matrix metalloprotease 7 showed moderate susceptibility to freeze-thaw cycles.

Conclusions: Biomarkers that have an on-off response to temperature variation could serve as quality indicators for the core processes of biobanking, which are the preparation and storage of biological fluids. The identification of such biomarkers is needed.

Clin Chem Lab Med 2007;45:1390–5.

Keywords: biobank; pre-analytical variation; quality control; serum.

Introduction

One of the main issues concerning biobanks is the establishment of standard procedures for specimen collection, preparation and storage (1, 2). The Biobanque de Picardie is an on-site Biological Resource Center (BRC). Documented and aliquoted cryoconserved biospecimens mostly include serum and plasma. The Biobanque de Picardie undertook an ISO 9001:2000 certification procedure to guarantee the quality and performance of biological samples, compatible with a high level of security and the bioethical and legal framework.

Usually, quality control (QC) and proficiency testing programs are considered to be the central components of a quality assurance system. However, QC and proficiency testing serve as checks only for the end-use analytical processes. QC programs evaluate testing for repeated analyses of pooled material and are designed primarily to identify systematic changes in an analytical process. Other measures that are necessary to monitor and ensure proper performance of the end-use testing process include the development of procedures for specimen collection, processing and storage. An alternative process for the QC of procedures is to perform repeated evaluation of individual patient results. This may help to detect changes in the preanalytical process, and may ultimately define preanalytical process biomarkers.

Sample quality, as well as the quality of the analysis obtained from a sample, depends on the biological and preanalytical variations that exist in the sample (3). Biological variation includes sex, age, hormonal status, morphometry, biological rhythms, exercise, xenobiotics, nutrition, smoking and genetic factors. Biological variation is beyond the control of the biobank. However, the biobank does have control over specimen-associated preanalytical variation. Preanalytical variation can be classified into patient-associated, specimen-associated and sample-associated factors, as shown in Table 1 (4).

The impact of preanalytical variation is diverse. The time delay and storage temperature before processing influence the extent of clot formation, cross-linking and fibrinolysis. Centrifugation time, force and temperature influence the efficiency of cell separation. Knowledge of the preanalytical variation in samples used for mRNA expression analysis is crucial. The activation status of blood cells may change upon exposure to extracellular factors. Environmental solution modifications and contact with synthetic surfaces, during and after phlebotomy, may modify gene expression levels (5). Polymeric components are released by many types of collection tubes and in some cases quantitative immunoassays can be unpredictably affected by components released from blood collection tubes (6). There is no blood collection procedure that will universally block protease activities. Prolongation of sampling time may induce the secretion of prolactin or an increase in red blood cell (RBC) number (7). Orthostatic position of the patient during sampling may induce an increase in the concentration of certain analytes due to an orthostatic

[1)]This article is based on a contribution at the 3rd Santorini Biologie Prospective Conference, Sep 29–Oct 2, 2006.

*Corresponding author: Fotini Betsou, Biobanque de Picardie, Centre Hospitalier Universitaire, avenue René Laënnec, 80480 Salouel, France
Phone: +33-3-22331150, Fax: +33-3-22331465,
E-mail: fay.betsou@biobanque-picardie.com

Table 1 Preanalytical variation factors.

Patient-associated	Specimen-associated	Sample-associated
Time	Specimen type	Sample type
Dietary intake	Site of collection	Hemolysis
Alcohol	Rate of collection	Bilirubinemia
Exercise	Tourniquet time	Lipemia
Posture	Type of collection tube	Dilution
Medication	Tube additive	Detergent
	Time of contact with clot	Heating
	Transport	Blood cells
	Centrifugation	Aliquoting (tube, straw)
		Storage:
		Time between sampling-freezing
		Storage temperature and protocol
		Duration of storage
		Number of freeze-thaw cycles

decrease in plasma volume (8). Time of sampling has an influence on hormone levels, subject to diurnal or menstrual cycles.

Storage-associated preanalytical variation includes the time between sampling and freezing, the storage temperature and protocol, the duration of storage, and the number of freeze-thaw cycles. Previous biospecimen research studies have shown that micromolecular markers such as amino acids, free fatty acids, sodium, cholesterol, triglycerides and vitamin E do not vary significantly after 30 freeze-thaw cycles (9). Hormones such as estradiol, prolactin, and free and total testosterone are stable in frozen serum or plasma for 3 years at –80°C (10). Vitamins such as retinol, β-carotene and α-tocopherol are slightly sensitive to freeze-thaw cycles (11). The delay between sampling and centrifuging, as well as the storage duration and temperature, have an influence on catecholamine (12) and certain fatty acid measurements (13). DNA in whole blood degrades more rapidly at 4°C than at ambient temperature, probably due to increased granulocyte lysis at 4°C (14). Biobanks, which cryoconserve biological samples, need to control this preanalytical variation and perform appropriate QC on their samples.

Therefore, stability studies are important in assessing appropriate QC parameters and procedures. One part of the Quality Assurance (QA)/QC program in a biobank is to follow changes in different markers in serum according to storage conditions, such as repeated freeze-thaw cycles. We need to identify serum biomarkers that are sensitive enough to variations in storage temperature and to the presence of freeze-thaw cycles so that such biomarkers can be used as QC indicators to attest to the quality of sample cryopreservation. For a serum biomarker to be used for QC, it should be ubiquitous and show 100% loss of activity upon inadequate storage conditions and temperature variations.

We focused on four potential macromolecular biological markers belonging to different molecular classes: secretory phospholipase A2 (PLA, an enzyme), matrix metalloprotease 7 (MMP7, a protease), transforming growth factor β1 (TGF-β1, a cytokine) and anti-HBs IgG (an immunoglobulin).

Materials and methods

Quality management system

We used a process approach, where a process is defined as all correlated or interactive activities that transform entry elements into exit elements with an added value. The process approach was based on the identification of activities, objectives and indicators and used application of the ISO 9001:2000 norm requirements (15). These requirements include the following obligatory items: document control, records control, internal audits, non-conformity control, corrective actions and preventive actions. The certification scope included all BRC actions: contracts, transport, reception/registration, preparation/aliquoting, storage and sample distribution. Laboratory activities, other than the preparation of samples and sample derivatives prior to storage, were not included in the certification scope because they did not influence BRC activities in any way. Process efficacy was measured by quantitative indicators. An indicator was an objective value that provided quantitative descriptions of a situation. Indicators were financial, human or methodological.

Biological assays

We tested two serum specimens, both from healthy blood donors who gave informed consent. One specimen was from a 48-year-old male donor with 40% hematocrit, and another specimen was from a 38-year-old female donor with 31% hematocrit. The two specimens were taken on the same day and under the same sampling conditions, and were treated in parallel afterwards. Serum was aliquoted in 0.5-mL aliquots in polypropylene tubes, stored at –80°C and thawed 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 times before analysis. Samples were thawed in a water bath for 15 min at 37°C and refrozen at –80°C. All testing was performed within 1 h of each thawing. Other serum aliquots were stored at 37°C for time intervals of between 1 and 10 days.

The stability of PLA was studied using an enzyme activity test, sPLA2 Enzyme Assay (R&D Systems Europe, Lille, France) (functional assay), while the stabilities of MMP7, TGF-β1 and anti-HBs IgG were studied using the following sandwich ELISAs: Quantikine human MMP-7 total (R&D Systems), Quantikine TGF-β1 ELISA (R&D Systems) and Monolisa anti-HBs ELISA (Bio-Rad, Marnes la Coquette, France), respectively. We followed the manufacturers' protocols for the assays. For each of the above methods, the inter-assay

precision, expressed as the coefficient of variation (CV) was as follows: 2.3%–23.7% for the sPLA2 assay, 4.1%–4.6% for Quantikine MMP-7, 9.8%–12.8% for Quantikine TGF-β1 and 6.3%–9.3% for Monolisa anti-HBs. The same batches of reagents and calibration materials were used throughout the study. All parameters were measured at time zero, and then at all test points. Results are expressed as percentage changes from the time-zero baseline value.

Results

BRC ISO 9001:2000 certification

The processes and their quality indicators were:

1) The process of establishing contracts for biospecimen storage. Three types of storage were initiated: BRC storage, forensic storage and deposit storage. Indicators of this process included the number of signed contracts in relation to the number of contract projects, and the number of Biobanque citations in scientific publications, otherwise called the Biobank Impact Factor (BIF) (16).
2) The transport process. Three types of transport were initiated: transport of samples from the University Hospital, transport of samples from the client to the biobank, and transport of samples as a service to a third party. The process indicators were the number of round trips and the number of rotations in relation to the number of samples received.
3) The process of sample reception and registration. A biospecimen of human origin may either be accompanied by informed consent and a medical card and processed, or put into quarantine. The process indicators included the number of external non-conformities and the number of registrations in the computer system in relation to the number of samples received.
4) The process of preparation and aliquoting. This process included all the preparation protocols according to the nature of each sample and the specific prescriptions of the scientific manager. The sole indicator was the number of non-conformities due to internal errors of manipulation.
5) Transversal processes, such as staff processes (training provided was the indicator), and the quality management process (client satisfaction was the indicator), were also implemented (Figure 1).

Table 2 shows the BRC processes identified and their indicators. There was no QC indicator corresponding to the preparation/aliquoting/storage process because, to the best of our knowledge, a sensitive enough serum biomarker is not yet available.

Biomarker stability in frozen serum

PLA activity diminished from the baseline value by 24%–43% (sample 1, S1) and 18%–35% (sample 2, S2) for up to 10 freeze-thaw cycles. After 15 freeze-thaw cycles, PLA enzyme activity increased by 350% (S1) and 290% (S2) and remained stable thereafter for up to 100 freeze-thaw cycles. PLA activity increased by 190% (S1) and 220% (S2) over 10 days at 37°C.

IgG anti-HBs immunoglobulins were structurally stable, since their measured concentration and avidity showed a uniform 3%–30% increase from the baseline value for 1–100 freeze-thaw cycles. When incubated at 37°C for 6 days, S1 exhibited a mean 40% increase, while S2 deviated by 4%–11% from the baseline value. This prolonged serum incubation at 37°C did not negatively affect IgG concentration and avidity.

In both serum samples, TGF-β1 showed <5% deviation from the baseline value upon successive freeze-thaw cycles. When exposed to 37°C, TGF-β1 in S1

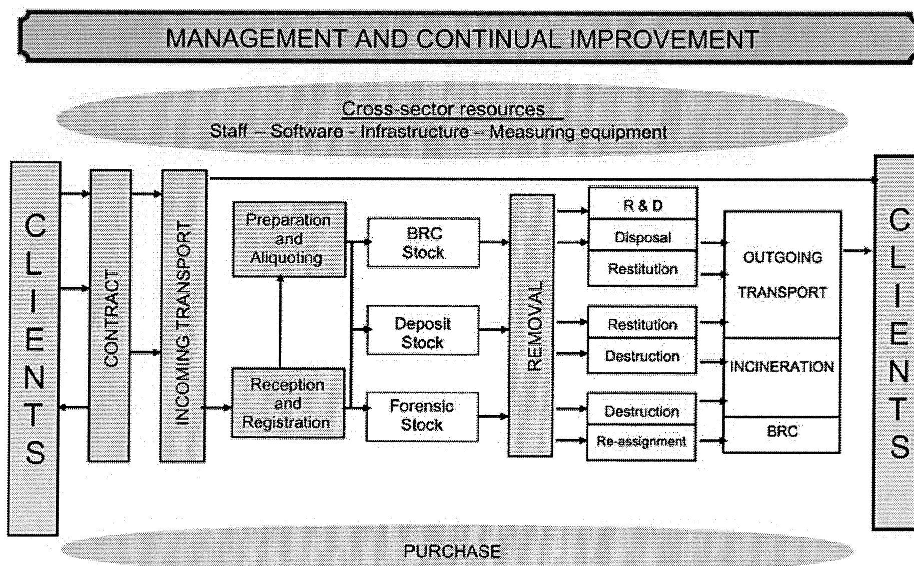

Figure 1 Process flow of Biological Resource Center activities.

Table 2 Certified processes and their quality indicators.

Process	Indicators
Establishment of contracts for biospecimen storage	Time delay; number of signed contracts; number of Biobanque citations in scientific publications
Transport	Number of round trips and number of rotations in relation to the number of samples received
Reception and registration of samples	Number of external non-conformities and number of registrations in the computer system in relation to the number of samples received
Preparation, aliquoting and storage	Number of non-conformities due to internal errors of manipulation
Restitution or distribution of samples	Number of samples distributed
Transversal processes (staff process and quality management process)	Training provided; client satisfaction

increased by 40% and S2 deviated by 4%–8% from the baseline value.

The only marker that showed an on/off susceptibility to freeze-thaw cycles was MMP-7. The immunoreactive epitopes of this serum protease exhibited <10% deviation from baseline values in S1 during 20 freeze-thaw cycles, and MMP-7 was completely degraded after 30 freeze-thaw cycles. In S1, MMP7 deviated by 15%–25% from baseline values after 8-day incubation at 37°C. In S2, a more progressive degradation of immunoreactive epitopes was observed. A 29% decrease was observed after two freeze-thaw cycles, a 67% decrease after 15–20 cycles, a 90% decrease after 20 cycles and complete degradation was observed after 30 cycles. In S2, incubation at 37°C for 7 days induced a 3%–75% decrease and complete

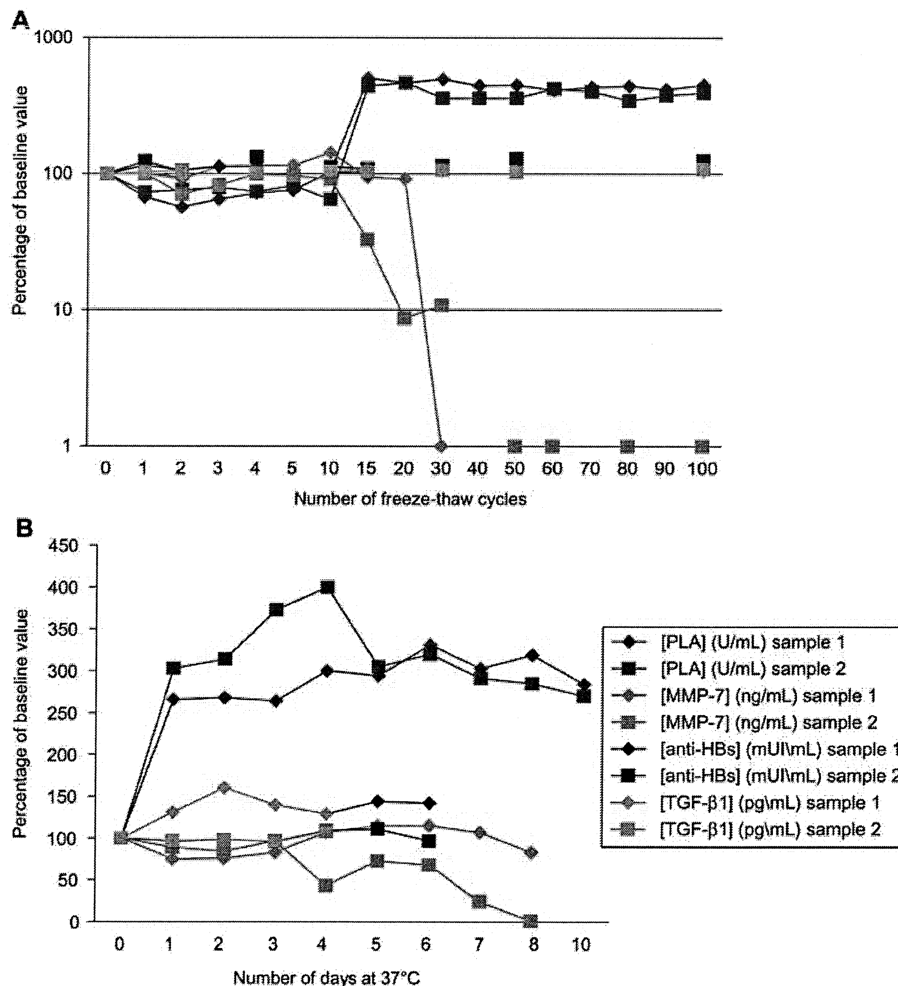

Figure 2 Stability data for biomarkers in frozen serum subjected to (A) freeze-thaw cycles or (B) 37°C. Data are expressed as the percentage change in value at each test point.

Table 3 Baseline values of parameters tested.

	Sex	Hematocrit, %	PLA, U/mL	MMP-7, ng/mL	anti-HBS, mU/mL	TGF-β1, pg/mL
Serum sample 1	M	40	51	2.43	210	3044
Serum sample 2	F	31	33	2.12	33	4750

Table 4 Summary of data showing the variation in results from the baseline value (100%) with the number of freeze-thaw cycles, and with time at 37°C.

	PLA, %		MMP-7, %		anti-HBS IgG, %		TGF-β1, %	
	S1	S2	S1	S2	S1	S2	S1	S2
Baseline value	100	100	100	100	100	100	100	100
No. of freeze-thaw cycles								
1	67	73	99	100	115	124	101	103
2	57	76	91	71	106	103	95	106
3	65	79	113	82	113	ND	ND	ND
4	72	74	115	100	113	133	102	102
5	76	82	115	97	ND	ND	ND	ND
10	103	65	143	92	104	112	103	104
15	505	441	95	33	98	109	110	103
20	466	467	92	9	ND	ND	ND	ND
30	496	359	0	11	106	115	108	107
40	445	359	ND	ND	ND	ND	ND	ND
50	447	359	0	0	111	130	106	103
60	412	423	0	0	ND	ND	ND	ND
70	431	403	ND	ND	ND	ND	ND	ND
80	441	344	0	0	ND	ND	ND	ND
90	417	376	ND	ND	ND	ND	ND	ND
100	449	394	0	0	113	125	105	108
Days at 37°C								
1	266	303	75	89	131	96	131	97
2	268	314	76	84	160	98	160	98
3	264	373	83	97	140	96	140	96
4	300	400	107	43	129	109	129	108
5	294	305	115	73	144	111	ND	ND
6	331	320	115	68	142	97	ND	ND
7	303	291	107	24	ND	ND	ND	ND
8	319	285	83	0	ND	ND	ND	ND
10	284	270	ND	ND	ND	ND	ND	ND

S1, serum sample 1; S2, serum sample 2; ND, not determined.

degradation was observed on the 8th day (Figure 2, Tables 3 and 4).

Discussion

The primary objective of biobanks is not merely archiving, but also distributing conserved and documented biological samples for research (17). The quality of biological samples is crucial for the success, in terms of validity and reproducibility, of immunological, biochemical or molecular biology methods and for the validity of results from clinical epidemiological studies. Sample quality is directly related to biological and preanalytical variation.

The environmental conditions experienced by a biological sample, including the initial sampling and storage conditions, may influence the results of its subsequent analysis by specific biological techniques. The impact of preanalytical variation on the "quality" of a sample depends on its end use, the type of parameter analyzed (DNA, RNA, antibodies, peptide, hormone, cytokine, enzyme, or vitamin), and the method applied (radio- or immuno-enzymatic, enzymatic, PCR, RT-PCR, hybridization, electrophoresis, mass spectrometry).

Freezing generally slows protein degradation, but freeze-thaw cycles may also lead to protein denaturation, aggregation, and loss of function. Some serum proteins may form a cryoprecipitate in frozen samples, resulting in a selective loss of components.

Quality controls capable of assessing the storage conditions depend on the type of biospecimen. Appropriate quality controls can be applied to cell suspensions (cell viability assays, contamination assays), to DNA and RNA (quantification and purity, function of DNA-modifying enzymes) and to tissues (morphology). However, no appropriate quality controls exist for liquid biosamples (serum, plasma, urine, saliva, cerebrospinal fluid, synovial fluid). The ideal QC serum biomarker should be ubiquitous and show 100% loss of functional or immunological activity upon variation in storage temperature.

Surprisingly, induction of PLA activity was observed after 15 freeze-thaw cycles or following

incubation at 37°C. This could be due to protein release by plasma membranes, as PLA is not only a circulating enzyme, but is also located among plasma membrane glycerophospholipids.

IgG isotype immunoglobulins were not affected by either freeze-thawing or elevated temperature stress. IgM isotype immunoglobulins are probably more sensitive and unstable, but there is no IgM specificity that could be used as a ubiquitous serum marker.

Cytokines have a reputation for molecular instability. However, storage conditions that included freeze-thaw cycles and incubation at 37°C showed that TGF-β1 was the most stable parameter studied.

The least stable parameter tested was MMP-7. MMP-7 structure was influenced by freeze-thaw cycles and it completely degraded after 30 cycles. However, a 30-freeze-thaw-cycle sensitivity is not sensitive enough for QC assessment of serum samples in a biobank, where samples are never supposed to thaw. The protease MMP-7, measured by an immunological assay, was more useful (more unstable) as a biomarker than PLA, measured by a functional assay. Proteolytic degradation or denaturation of MMP-7 immunoreactive epitopes could explain this observation.

The serum used was obtained from healthy donors, and the stability of some factors may be affected by physiological stresses, recent dietary intake or medications used. The variability in different parameters was not associated with the precision of the methods, as CVs were lower than the variabilities observed. Changes in various parameters may depend to some degree on their initial concentration, the greatest decline being observed in the higher or lower range of values for a given parameter.

In conclusion, enzymatic, cytokine and immunoglobulin markers tested in this study showed immunological or enzyme activity that was resistant to cryopreservation conditions in the serum milieu. Even when no on/off response was observed for these parameters, there were significant deviations from baseline values. These deviations further highlight the potential importance of preanalytical variations in storage conditions on later analytical test results.

Ubiquitous markers that are more sensitive, that tolerate only two or three freeze-thaw cycles and that have an on/off response to variations in storage conditions should be identified, probably by 2D electrophoresis or mass spectrometry of the whole serum proteome. Such biomarkers would be useful for assessing sample quality, and would thus represent important tools for biobanks.

Acknowledgements

We thank Gerard Luzergues (CEISO, France) for excellent quality management assistance. This study was financed by Conseil Régional de Picardie.

References

1. OECD. Best Practice Guidelines for Biological Resource Centers. DSTI/STP/Bio (2007)9/Rev1.
2. OECD. Biological Resource Centers – Underpinning the future of life sciences and biotechnology. Paris: OECD, 2001.
3. Bernard AM. Biokinetics and stability aspects of biomarkers: recommendations for application in population studies. Toxicology 1995;101:65–71.
4. Siest G, Henny J, Schiele F. Références en Biologie Clinique. Collection OptionBio. Paris: Elsevier, 1990.
5. Pahl A, Brune K. Gene expression changes in blood after phlebotomy: implications for gene expression profiling. Blood 2002;100:1094–5.
6. Bowen RA, Chan Y, Cohen J, Rehak NN, Hortin GL, Csako G, et al. Effect of blood collection tubes on total triiodothyronine and other laboratory assays. Clin Chem 2005;51:424–33.
7. Statland BE, Winkel P, Harris S. Evaluation of biological sources of variation of leukocytes count and other hematologic quantities using very precise automated analyzers. Am J Clin Pathol 1978;69:48–54.
8. Kieldsen SE, Eide I, Leren P. Effects of posture on serum cholesterol fractions, cholesterol ratio and triglycerides. Scand J Clin Lab Invest 1983;43:119–21.
9. Paltiel L, Meltzer HM, Hoppin JA, Skjerden T, Baker S, Magnus P, et al. QA/QC in the Norwegian Mother and Child Cohort study – assessment of freeze-thaw cycles on markers in plasma. In: Proceedings of the International Society for Biological and Environmental Repositories 2004 meeting, Perugia, Italy, Oct 17–20, 2004: 20.
10. Bolelli G, Muti P, Micheli A, Sciajno R, Franceschetti F, Krogh V, et al. Validity for epidemiological studies of long-term cryoconservation of steroid and protein hormones in serum and plasma. Cancer Epidemiol Biomarkers Prev 1995;4:509–13.
11. Wahrendorf J, Hanck AB, Munoz N, Vuilleumier JP, Walker AM. Vitamin measurements in pooled blood samples. Am J Epidemiol 1986;123:544–50.
12. Boosma F, Alberts G, van Eijk L, Man in't Veld AJ, Schalekamp MA. Optimal collection and storage conditions for catecholamine measurements in human plasma and urine. Clin Chem 1993;39:2503–8.
13. Unemura U, Koike KA, Sato S, Iso H, Shimamoto T, Komachi Y. The effect of storage on serum fatty acids. Nippon Eiseigaku Zasshi 1991;46:976–83.
14. Cuypers HT, Bresters D, Winkel IN, Reesink HW, Weiner AJ, Houghton M, et al. Storage conditions of blood samples and primer selection affect the yield of cDNA polymerase chain reaction products of hepatitis C virus. J Clin Microbiol 1992;30:3220–4.
15. ISO 9001:2000 Quality management systems – requirements. Geneva: ISO, 2000.
16. Cambon-Thomsen A. Social and ethical issues of post-genomic human biobanks. Nat Rev Genet 2004;5: 866–73.
17. Le Roux N, de Montgolfier S, Di Donato JH, Boccon-Gibod L, Teillac P, Hervé C, et al. Les collections de ressources biologiques humaines pour la recherche: de la réglementation à la mise en place d'un guide de bonnes pratiques du collectionneur. Rev Med Intern 2003;24: 803–14.

Received November 29, 2006, accepted March 27, 2007