

BIOMARKER NAME	COMMENTS ON STABILITY	MAX #FREEZE-THAWS ?	REFERENCE
			Proteases: trypsin, chymotrypsin, kallikrein, thrombin REFERENCE Protein degradation during prolonged storage represents a unique problem that may introduce bias when existing biobank resources are applied to future putative biomarker analyses. Plasma samples should be stored at -70 C or lower for LTS.
Albumin	"Most susceptible biomarkers"		Presentation at BRN Symposium 2010. <a href="http://biospecimens.cancer.gov/meeting/bmsymposium/2010/docs/Zimmerman%20BRN%20Protein%20Stability%20Studies.pdf">http://biospecimens.cancer.gov/meeting/bmsymposium/2010/docs/Zimmerman%20BRN%20Protein%20Stability%20Studies.pdf</a> Assessment of protein stability in whole blood.
Fibrinogen C-reactive protein, D-dimer, plasmin-alpha2-antiplasmin complex, plasminogen activator inhibitor-1, protein C, protein S, and tissue plasminogen activator, factor VII and fibrinogen	Measured in frozen plasma samples stored at -70 degrees C on LN2 for months or up to 6 years	Some fibrinogen peptides are stable up to F/T, others decrease  Measured monthly, no evidence of sample degradation over time for the factors studied, F/T not examined per se	Thromb Haemost. 2001 Dec;86(6):1495-500. Longitudinal stability of coagulation, fibrinolysis, and inflammation factors in stored plasma samples.
Fibrinogen; factors V, VII, VII; fibrin monomers, D-dimers; alpha-1 antiplasmin, and protein S	Fresh plasma stored at -40C for at least 8 wk. Thawed and samples 1,2,4,6 hr measured	From 1-6 hr after thawing, no significant difference in marker levels. In FVII activity significantly decreased immediately after thawing; FM significantly increased.	Anesth Analg 2006; 103(4):969-74. Thawing procedures and the time course of clotting factor activity in fresh-frozen plasma: controlled laboratory investigation.
Vitamin K-dependent coagulation factors (prothrombin, F VII, F IX, F X) and fibrinogen in fresh-frozen plasma	20 units collected, initially measured, kept 1 dat @ 4 C, stored 1 wk @ -20 C thawed, stored another wk @ -20 C, thawed Breast cancer biomarker generated in complement activation; all specimen handling operations should be carried out at 4 C to avoid generating C3a. Freeze immediately, preferred frozen for < 3 D before analysis. Store at -80 C LTS.	Mean levels not statistically different. Levels remained stable through 2 F/T cycles.	Transfusion 2003; 43:873-7. Vitamin K-dependent coagulation factors and fibrinogen levels in FFP remain stable upon repeated freezing and thawing.
human analytotoxin C3a des arg in plasma		Avoid repeated F/T, "becomes irreversibly denatured"	<a href="https://www.cambridgebiomedical.com/media/PDFLibrary/TechBriefs/C3a%20des%20Arg%20in%20Plasma%20by%20ELISA%2010-03-12.pdf">https://www.cambridgebiomedical.com/media/PDFLibrary/TechBriefs/C3a%20des%20Arg%20in%20Plasma%20by%20ELISA%2010-03-12.pdf</a> . Cambridge Biomedical kit insert. a number of stabilizers used for prepared LDH solutions, e.g., trehalose and borate, glycine, polyethylene glycols, sucrose, maltodextrin Clin Chem 1983;29(5):832-5. Creatine Kinase and lactate dehydrogenase: Stability of isoenzymes and their activity in stored frozen plasma and prostatic tissue extracts and effect of sample dilution.
LDH			
Creatine Kinase and LDH isoenzymes in plasma	Storage @ -90 C has no effect on total or isoenzyme activity for LDH or CK as long as not thawed to 37 C and held. (differs with previous refs)	Only 1 F/T examined	
Creatine Kinase and LDH isoenzymes in mini-pig serum	Collected serum subdivided into 3 parts: 1st analyzed immediately, 2nd stored @ -20 C and analyzed after 30 d; the 3rd stored @ -20 C and analyzed after 50 d. The total CK and LDH activities were stable in frozen samples; thawed results equivalent to initial	Only 1 F/T examined	Scand J Lab Anim Sci Suppl 1 1998; 25: 2059. Stability of Ck- and Ldh-isoenzyme values in minipig serum under different storage conditions.
metalloproteinases (MMP)-7, TIMP-1, vascular growth factors (VEGF) and VEGF-R2	Serum samples were frozen @ -20 & -70 C, and then thawed up to six times. According to the Arrhenius calculation, MMP-7 showed excellent stability, at least 5 years at -20°C and several 100 years at -75°C. The VEGF-receptor maintains 90% of its initial concentration at -20°C over 3 months, and decades at -75°C. TIMP-1 and VEGF showed poor stability with cryopreservation, even at -75°C. The stability of MMP-7, TIMP-1, VEGF or VEGF-receptor in biobanking is highly variable, and this should be taken into account in the interpretation of results. A temperature -20°C is unsuitable for prolonged storage of the biomarkers investigated, and repeated thawing of sera is not recommended. <b>VEGF is especially unstable and should be quantitated using serum that has never been frozen.</b>	The average concentration of TIMP-1 was stable, even after six freeze/thaw cycles. One thawing did not change the concentration of MMP-7 and VEGF-receptor. However, repeated freeze/thaw cycles increased the measured values significantly. Decreases in VEGF concentrations were dramatic, even after the first freeze/thaw cycle.	Clin Chem Lab Med. 2011 Feb;49(2):229-35. Epub 2010 Dec 1. Impact of cryopreservation on serum concentration of matrix metalloproteinases (MMP)-7, TIMP-1, vascular growth factors (VEGF) and VEGF-R2 in Biobank samples.
Polymeric proteins such as transthyretin in CSF	Delayed storage of CSF led to changes in prostaglandin D-synthase derived peptides as well as to increased levels of certain amino acids and metabolites. The changes of metabolites, amino acids and proteins in the delayed storage study appear to be related to remaining white blood cells. Our recommendations are to centrifuge CSF samples immediately after collection to remove white blood cells, aliquot, and then snap-freeze the supernatant in liquid nitrogen for storage at -80 degrees C. serum and cerebrospinal fluid carrier of the thyroid hormone thyroxine (T4); also 1:1 complexes with RBP. Store at -20 or -80 LTS.	Repeated freeze/thawing introduced changes in transthyretin peptide levels (due to trypsin digestion). F/T J Proteome Res. 2009 Dec;8(12):5511-22. The effect cycles should be avoided if at of preanalytical factors on stability of the proteome and selected metabolites in cerebrospinal fluid (CSF). <a href="http://www.uscncn.us/pdf/20091014105437.pdf">http://www.uscncn.us/pdf/20091014105437.pdf</a> . Uscn Life Sciences ELISA Kit insert for Rat Serum Transthyretin/Albumin	
transthyretin [originally called prealbumin] in serum	Other markers: Interleukin-6 (IL-6) and TNF-alpha levels remained stable for at least 6 h in timely separated plasma but not in unseparated plasma 4 h after blood draw, in which IL-6 was decreased by mean 14.3% and TNF-alpha increased by mean 9.6%. Leptin was unchanged in both conditions, presumably due to the involvement of blood cells in the release and clearance of IL-6 and TNF-alpha but not of leptin. Interleukin-6 and leptin were not affected by freezing and thawing for their stable alpha-helical structure, while TNF-alpha concentration increased by 17.0% after 3 cycles and by 23.9% after 6 cycles due to the unstable beta-pleated sheet structure.[8] Delayed (overnight) separation and short-term frozen-storage affected plasma but not serum IL-7 concentration.[9] Hepatocyte growth factor (HGF), also a glycoprotein of 190 kDa heterodimer, remained stable in serum after 20 freeze-thaw cycles, or after 4-months of frozen storage, but increased by 20% after 10-months of frozen storage, which was possibly ascribable to interassay variation, release of HGF from binding serum proteins, and some mechanism of Tg is stable for at least 24 hr in unseparated sera @ 4 C or separated sera @ RT, but not in sera undergoing even a short period of frozen storage.	Tg is stable at least within 24 h in unseparated sera stored at 4°C or in separated sera stored at room temperature, but fragile on freezing and thawing with a small decrease after 3 short cycles and a large decrease at subsequent cycles after 3 months of frozen storage.  F/T resulted in significant decreases in Tg conc.	<a href="http://www.medscape.com/viewarticle/564090_4">http://www.medscape.com/viewarticle/564090_4</a> . Medscape Today: Serum Thyroglobulin Stability for Immunoassay: Discussion <a href="http://labmed.ascpjournals.org/content/38/10/618.full.pdf">http://labmed.ascpjournals.org/content/38/10/618.full.pdf</a> (Science) Serum thyroglobulin stability for immunoassay.
Thyroglobulin (and other biomarkers)			
Thyroglobulin			
Insulin Glycoproteins [ e.g., alpha-1 acid glycoprotein, thyroglobulin] Folate	store @ -20 or -70 CLT.	Lab has determined that insulin is stable 5X F/T [other kits all say avoid multi-X R/T	<a href="http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/glu_e_met_insulin.pdf">http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/glu_e_met_insulin.pdf</a> . Human insulin immunoassay.

RBC & serum Folate	[Given that RBCF must be stabilized with ascorbic acid preservative] Whole blood hemolysates less stable than intact whole blood; plasma folate less stable than serum folate:	Sensitive to freeze/thaw cycles in a -20 C frost-free freezer [but not @ -80 C], no loss up to 3 freeze/thaw cycles (exposed to ambient temperature for 1 hour)	Clinical Laboratory News, Jan 2011, 8-10. Folte: Clinical Utility of Serum & Red Blood Cell Analysis
D-dimer in platelet-poor plasma	Samples split & stored at -20 & -70 C for 1,3,6, & 12 mo. Stable on storage at either temperature.	Avoid multi-X F/T	Various assay kit inserts. Also: <a href="http://repository.unm.edu/bitstream/handle/1928/6875/Norman_Ornelas%20Final%20Paper.pdf?sequence=1">http://repository.unm.edu/bitstream/handle/1928/6875/Norman_Ornelas%20Final%20Paper.pdf?sequence=1</a> . Stability of abnormal D-dimer levels in platelet-poor plasma stored at -20 and -70 C.
D-dimer in plasma	In vitro D-dimer stability in plasma is widely assumed, but has not yet been documented by systematic studies using samples covering a wide range of D-dimer. We investigated the short- and long-term stability of D-dimer in clinical citrated plasma samples with normal and pathological levels. The short-term stability was analysed by measuring D-dimer fresh, after storage of plasma for 4 hours at room temperature (RT) and after an additional 24 h storage at +2 to +8 degrees C (n=40). Long-term stability samples (n=40) were measured fresh and after storage for 19, 25 and 36 months at < or =-60 degrees C. The effect of repeated freezing was analysed by measuring samples (n=50) fresh and after four consecutive freeze-thaw cycles. D-dimer was measured on the BCS System using the INNOVANCE D-Dimer assay (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). D-dimer values at baseline ranged from 0.23-22.2 mg/l FEU. The mean percentage change after storage for 4 hours at RT and additional 24 hours at +2 to +8 degrees C was +3.8% and +2.7%, respectively. The n	The effect of repeated freezing was analysed by measuring samples (n=50) fresh and after four consecutive freeze-thaw cycles. Repeated freezing did not significantly alter D-dimer values (mean change degrees C was +3.8% and +2.7%, respectively. The n	Thromb Haemost. 2010 Feb;103(2):461-5. Epub 2009 Nov 13. Long- and short-term in vitro D-dimer stability measured with INNOVANCE D-Dimer.
Thyroid hormones (Endogenous LH, FSH, TSH, growth hormone, prolactin and insulin) in plasma	Endogenous LH, FSH, TSH, growth hormone, prolactin and insulin were measured by radioimmunoassay in human plasma samples stored at 4°, 20° and 37° for up to 8 days or repeatedly frozen and thawed. At 4°, the concentrations of all hormones were stable for at least 8 days; at 20° only LH, FSH and TSH were stable for 8 days; at 37° only TSH was stable for 8 days.	All the hormones except insulin were stable during 5 freeze-thaw cycles.	Clinical Biochemistry 1980 13(4):151-5. Effect of time, temperature and freezing on the stability of immunoreactive LH, FSH, TSH, growth hormone, prolactin and insulin in plasma
TSH, FT4 and FT3 in LTS serum	All contemporary assays detected significantly lower TSH and increased FT4 and FT3 concentrations in the stored samples after 8-11 yr @ -80 C.		Clinical Chemistry and Laboratory Medicine, Volume 48, Issue 3, Pages 409-412 Stability of serum thyroid hormones following 8-11 years of cold storage
ascorbic acid, cholesterol, dehydroepiandrosterone, epiandrosterone sulfate, retinol, carotenes, xanthophylls, estrone, estradiol, LH, progesterone, SHBG	store at -70 C LT	3X F/T no effect on CHOL, retinol & carotenoids, & most hormones. Estrone, estradiol, & SHBG some effect, but less than method CV variability. [Driskell 17X F/T OK]	Clinical Chemistry 47: 139-142, 2001. Effects of repeated freeze-thaw cycles on concentrations of cholesterol, micronutrients, and hormones in human plasma and serum.
sodium, cholesterol, triglycerides, vitamin E, aspartate aminotransferase (AST), and free-fatty acids	A majority of analytes showed no significant changes until 30 freeze-thaw cycles. After 30 freeze-thaw cycles, the largest percent change was observed for free fatty acids (+32%), AST (+21%), and triglycerides (-19%). Human plasma can go through several freeze-thaw cycles before analysis without influencing sample integrity for the selected analytes. average decreases of 2.0% per year for total cholesterol over 7 years and 2.8% per year in triglycerides for the first 5 years. HDL-cholesterol decreased by 1.3% per year, but this change was not statistically significant.		Cell Preservation Technology, Volume: 6 Issue 3: September 6, 2010. Evaluation of Freeze-Thaw Cycles on Stored Plasma in the Biobank of the Norwegian Mother and Child Cohort Study  Clin Chem. 2000 Mar;46(3):351-64. Estimating the long-term effects of storage at -70 degrees C on cholesterol, triglyceride, and HDL-cholesterol measurements in stored sera.
cholesterol, triglyceride, and HDL-cholesterol measurements in stored sera			
Albumin, apolipoprotein A-1, apolipoprotein B, cholesterol, creatinine kinase, creatinine, K fibrinogen, HDL-C, LDL-C, TCHOL, TP, TRIG	Stored at -20, -40, -80, -180 C for up to 6 years. Degradation detected in some samples at -20 & -40, no such effect at -80 and -180. simultaneously investigated the stability of 24 analytes (a) after prolonged contact of plasma and serum with blood cells and (b) after immediate separation of plasma and serum (centrifuged twice at 2000g for 5 min). We verified biochemical mechanisms of observed analyte change by concomitant measurement of pH, PCO <sub>2</sub> , and PO <sub>2</sub> .		Int J Epi 2008;37:234-44. The UK Biobank sample handling and storage protocol for the collection, processing, and archiving of human blood and urine. (citing Susan Clark's work at Oxford)
Alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALK), aspartate aminotransferase (AST), direct bilirubin, total bilirubin, calcium, total carbon dioxide (TCO <sub>2</sub> ), chloride, total cholesterol, creatinine, creatine kinase (CK), -glutamyltransferase (GGT), glucose, lactate, lactate dehydrogenase (LD), Mg <sup>2+</sup> , P, K <sup>+</sup> , Na <sup>+</sup> , total protein, triglycerides, uric acid, and urea	Conclusion: Storage of uncentrifuged specimens beyond 24 h caused significant changes in most analytes investigated because of (a) glucose N=25 stored at -20 C and reassayed on days 3,4,8,16,22. N=74 specimens stored at 4 C and reanalyzed on day 2.	F/T not examined	Clinical Chemistry. 2002;48:2242-2247. Stability Studies of Twenty-Four Analytes in Human Plasma and Serum. Clin Chem 1984; 30(1), 114-5. Freeze-thaw stability of transferrin and reference values obtained by kinetic nephelometry <a href="http://www.tricitieslab.com/Files/TestUpdates/Gliadin%20Antibodies.pdf">http://www.tricitieslab.com/Files/TestUpdates/Gliadin%20Antibodies.pdf</a> . Anti-gliadin test for gluten-sensitive enteropathies
Transferrin		Stable to multi-X F/T	Ca Epi Biomarkers Prev 2005: 141899-907. Design Options for Molecular Epidemiology Research Within Cohort Studies  Demography, 44(4) Nov 2007: 899-925. What a drop can do: Dried Blood spots as a minimal invasive method for integrating biomarkers int population-based research.
Anti-gliadin antibodies IgA, IgG in serum Lipoprotein A 19% in serum stored 3 yr @ -70C; 30% increase in serum testosterone in samples stored @ -80 C for 2 yr	Lpa 25% decrease after 2X F/T to -20C, 23% decrease after 4X F/T to 080C.	Avoid multi-X F/T	
C-reactive protein, Epstein-Barr virus, Transferrin receptor Ab in DBS	most stable < -20 C	no evidence of deterioration through 6 F/T	

CRP in serum	CRP was measured in serum samples at the baseline and in thawed plasma samples after an average storage period of 13.8 years. Geometric means of CRP were 0.25 mg/L and 0.59 mg/L before and after storage, respectively. The CRP values were significantly higher after long-term frozen storage than at the baseline (p<0.0001).		J Epi Vol. 17 (2007) , No. 4 pp.120-124. Comparison of C-reactive Protein Levels between Serum and Plasma Samples on Long-term Frozen Storage after a 13.8 Year Interval: The JMS Cohort Study
CRP in serum	Comparable results were obtained for plasma (heparin and EDTA treated) and serum samples, and levels were unaffected by delays in sample processing and storage temperature. CRP levels were also unaffected by up to seven freeze-thaw cycles.	7X +	Clin Diagn Lab Immunol. 2003 July; 10(4): 652-657. Analytical Performance of a Highly Sensitive C-Reactive Protein-Based Immunoassay and the Effects of Laboratory Variables on Levels of Protein in Blood Ann Clin Biochem 2008;45:575-584 . Simultaneous determination of guanidinoacetate, creatine and creatinine in urine and plasma by un-derivatized liquid chromatography-tandem mass spectrometry
guanidinoacetate, creatine and creatinine	.... stable in <b>urine</b> for up to seven <b>freeze thaw</b> cycles. creatine was not		Br J Nutr. 2010 Sep;104(5):629-32. Epub 2010 Apr 26. Temporal reproducibility of taurine measurements in frozen serum of healthy postmenopausal women. H. pylori Test. <a href="http://www.fishersci.com/wps/downloads/segment/Healthcare/pdf/HpIloriWBSPLpI23900535.pdf">http://www.fishersci.com/wps/downloads/segment/Healthcare/pdf/HpIloriWBSPLpI23900535.pdf</a>
Taurine	is not affected by <b>freeze-thaw</b> cycles		The Journal of Clinical Endocrinology & Metabolism Vol. 90, No. 11 6323-6331; Circulating Osteoprotegerin and Receptor Activator for Nuclear Factor- $\kappa$ B Ligand: Clinical Utility in Metabolic Bone Disease Assessment <i>Proteome Science</i> 2009, 7:15. Development of reverse phase protein microarrays for the validation of clusterin, a mid-abundant blood biomarker
H. Pylori	<b>Serum</b> or <b>plasma</b> samples may be ... frozen and <b>thawed</b> repeatedly. OPG is stable at -20 C in serum and in EDTA, citrate, and heparin plasma, and also at 4 C for up to 14 d; Three freeze-thaw cycles did not affect recovery of sample from serum, EDTA plasma, or citrate plasma, although recovery was significantly reduced in heparinized plasma. In one study, sRANKL was stable at up to four freeze-thaw cycles in serum and heparinized plasma (41). Another study showed that collection of sample on Li-heparin and storage for over 6 months at -70 C led to significant loss of recovery of sRANKL.		<i>Advances in Chromatographic Techniques for Therapeutic Drug Monitoring, Immunoassays for Therapeutic Drug Monitoring</i> . CRC Press 2010 Current standards for the storage of human samples in biobanks. <i>Genome Medicine</i> 2010, 2:72 (5 October 2010) <a href="http://genomemedicine.com/content/pdf/gm193.pdf">http://genomemedicine.com/content/pdf/gm193.pdf</a>
Osteoprotegerin and Receptor Activator for Nuclear Factor- $\kappa$ B Ligand	no change in clusterin levels after five freeze/thaw cycles		Post-collection, pre-measurement variables affecting VEGF levels. <a href="http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2367114">http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2367114</a> Comparison of Two Methods to Determine Plasma Bile Acid in Healthy Birds. <i>J Avian Med Surg</i> . 17(1), 1115-17, 2003
Clusterin	<b>freeze-thaw</b> cycles demonstrate protein interference from fibrins		J Lipid Res. 2010 Oct;51(10):3074-87. Epub 2010 Jul 21. Blood sphingolipidomics in healthy humans: impact of sample collection methodology.
Immunoassays for TDM, e.g., $\beta$ HCG			Coupling of Proadipocyte Growth and Arrest. <a href="http://jcb.rupress.org/content/94/2/394.full.pdf">http://jcb.rupress.org/content/94/2/394.full.pdf</a> . Aug 1, 1982 <i>Cancer Epidemiol Biomarkers Prev</i> . 2006 Sep;15(9):1578-81. Collection, processing, and storage of biological samples in epidemiologic studies: sex hormones, carotenoids, inflammatory markers, and proteomics as examples. <i>Informa Healthcare - Biomarkers</i> - 16(1):83. <a href="http://informahealthcare.com/doi/abs/10.3109/1354750X.2010.533285">http://informahealthcare.com/doi/abs/10.3109/1354750X.2010.533285</a>
metabolic analysis of human <b>serum</b> and <b>urine</b> for large scale population studies	Measuring VEGF in the <b>urine</b> , as opposed to <b>serum</b> or <b>plasma</b> , is preferable		JAMA. 1995;274:1926-1930. Serum Gonadotropins and steroid hormones and the development of ovarian cancer.
VEGF levels	Two <b>freeze-thaw</b> cycles decreased activity by over 45%.		<i>Cancer Epidemiol Biomarkers Prev</i> . 1995 Jul-Aug;4(5):509-13. Validity for epidemiologic studies of long-term cryoconservation of steroid and protein hormones in serum and plasma.
Bile acids	...examined the effect of freeze-and-thaw cycles on the stability of sphingolipids in serum and plasma freeze-thawing human plasma either had no effect or slightly ... storage at -20 C not acceptable for sex hormones - -70 C acceptable. Carotenoids drop substantially at -20 C after 6 months, up to 97% over 10 yrs; -70 C storage stable up to 1'0 yr. Store inflammatory markers or proteomic assay materials <-70 C.	Some IFs and proteomes sensitive to F/T - avoid F/T if possible	<i>Clinical Chemistry</i> . 2007; 53(4). Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications
Blood sphingolipids	No effects on serum hormone levels of repeated freeze-thaw cycles, up to three cycles were observed (another ref. "decades") estradiol, prolactin, and total testosterone had fairly good performance for both serum and plasma. Serum-free testosterone increased in time up to 30%, whereas progesterone decreased by about 40% of the initial concentration.	3+	<i>Steroids</i> 2001;66:737-41. Stability of salivary steroids: the influences of storage, food, and dental care.
Plasma Proadipocyte	Urine hepcidin is more affected by multiple freeze-thaw cycles and storage .... analytical, and biological variations that effect serum and urine differently.	Urine hepcidin is more affected by multiple freeze-thaw cycle	<i>Clinical Chemistry</i> 38: 1873-1877, 1992; Effect of freezing and thawing of serum on the immunoassay of lipoprotein(a) Long-Term Fatty Acids Stability in Human Serum Cholesteryl Ester, Triglyceride and Phospholipid Fractions. <a href="http://www.jlir.org/content/early/2010/05/06/jlr.D007534.full.pdf">http://www.jlir.org/content/early/2010/05/06/jlr.D007534.full.pdf</a> High Stability of Markers of Cardiovascular Risk in Blood Samples. <i>Clinical Chemistry</i> 49: 652-655, 2003; 10.1373/49.4.652
Sex hormones, ascorbic acid, and carotenoids, inflammatory markers, proteomics			<i>Clinical Chemistry</i> 49: 652-655, 2003; 10.1373/49.4.652
Biomarkers in NMR spectroscopy of urine, plasma, serum and tissue extracts			<i>Urology</i> . 1996 Dec;48(6A Suppl):33-9. Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions.
Serum gonadotrophins & steroid hormones (FSH, LH, estrone, estradiol, progesterone androstendione, dehydroepiandrosterone			
estradiol, free and total testosterone, and prolactin in serum and plasma samples over 3 years of cryoconservation			
Hepcidin (iron-regulating peptide) in serum & urine			
Cortisol & progesterone in saliva	CT should be analyzed within 2X FT; PGT more stable concentrations by ELISA that were significantly lower than those of fresh samples after one freeze-thaw cycle. By ITA the decrease was significant only after two cycles. In specimens frozen at -70 degrees C, Lp(a) concentrations determined by ELISA decreased after two cycles, and by ITA after three freeze-thaw cycles. Serum samples subjected to quick freezing at -70 degrees C and thawing did not show significant decreases in Lp(a) immunoreactivity during four cycles. Immunoreactivity of Lp(a) in samples stored at 4 degrees C decreased after 6 days but fell faster in serum samples subjected to freezing and thawing		
Serum lp(a)			
Fatty acids stability	no significant decrease after 10 yr @ -80C		
Glucose, TCHOL, HDL, TRIG, CRP	high stability during storage Approximately 1% of the free PSA was lost per hour of clotting time. Between 2% and 3% of the free PSA was lost per day of storage at 4 degrees C or 23 degrees C. About 0.9% of the free PSA was lost per month of storage at -20 degrees C compared with about 0.4% per month at -70 degrees C. Total PSA appeared to be stable throughout these studies. Tested up to 5X F-T	Repeated F/T do not affect free or total PSA, or % free PSA	
PSA			

salivary immunoglobulin A and lysozyme.	stable for up to 3 months when stored at -30 degrees C.	repeated freeze-thawing did not affect s-IgA and albumin	Clin Chim Acta. 2003 Dec;338(1-2):131-4. Effects of storage time on stability of salivary immunoglobulin A and lysozyme.
Anticardiolipin antibody IgG and IgM	decline appeared to occur most often between the second and third freeze-thaw cycle. Eight anticardiolipin IgG and three IgM-containing samples which had been positive initially became negative by the third freeze-thaw cycle		Am J Clin Pathol. 1994 Nov;102(5):586-8. Effects of repeated freeze-thaw cycles on anticardiolipin antibody immunoreactivity. Clinical Chemistry 43: 2281-2291, 1997; Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry
cotinine in serum	After >4 years, we have seen no evidence of instability in any of these serum pools during storage at -60 °C.		
cross-linked N-telopeptides of type I collagen (NTX), and linear C-telopeptides of type I collagen (CTX)	All the urine samples for the biological variability studies were stored at <=20 °C until testing.	All three analytes showed stability through five freeze-thaw cycles.	Clinical Chemistry. 1997;43:1570-1576 Comparison of analytical performance and biological variability of three bone resorption assays
Bone sialoprotein (BSP), tartrate-resistant acid phosphatase (TRAP), cathepsin K (CK), osteocalcin (OC) and alkaline phosphatase (AP), N-terminal (NTX) and C-terminal (CTX) collagen type I telopeptides	Both the free and conjugated forms of PYD and DPD have been shown to be stable in urine samples kept at room temperature for several weeks. Several reports show that pyridinium crosslinks can be stored at -20°C for years. Similar stability has been reported for urinary N-terminal (NTX) and C-terminal (CTX) collagen type I telopeptides, while ICTP in serum loses up to 12% of the signal when stored at room temperature for 5 days. The activity of serum tartrate-resistant acid phosphatase (TRAP) declines rapidly during storage at room temperature or even at -20°C but is stable when stored at -70°C or lower. Serum levels of BSP appear rather stable, both at room temperature, 4°C and -20°C.	Repeated freeze-thaw cycles of urine samples have no effect on the concentrations of PYD and DPD. Multiple freezing-thaw cycles usually have a deleterious effect on serum TRAP activity. BSP levels have been shown to not change significantly during repeated freeze-thaw cycles.	Clin Biochem Rev. 2005 November; 26(4): 97-122. Biochemical Markers of Bone Turnover Part I: Biochemistry and Variability
C-Reactive Protein, Retinol, Ferritin, Folic Acid, and Fatty Acids in whole blood	Samples mailed. Aliquots (1 mL) of plasma and serum were stored at -80 °C and transferred on dry ice to collaborating laboratories for analysis within 1 week. For CRP, retinol, and ferritin, changes in concentration during the 96-h storage period were small and not significant (≤10%; P ≥0.1). For folic acid, the mean concentration changed significantly over time (P = 0.037), with the sharpest decrease (8.7%) in the first 2 h of storage.		Clinical Chemistry 51: 230-232, 2005. Whole-Blood Samples Be Stored over 24 Hours without Compromising Stability of C-Reactive Protein, Retinol, Ferritin, Folic Acid, and Fatty Acids J Clin Lab Anal. 1999;13(4):166-72. Insulin-like growth factors (IGF-I, free IGF-I and IGF-II) and insulin-like growth factor binding proteins (IGFBP-2, IGFBP-3, IGFBP-6, and ALS) in blood circulation.
IGF-1, free IGF-1, IGH-II, IBFBP-2, IGFBP-3, IGFBP-6, & ALS	Freeze-thaw treatment up to five cycles had little impact on plasma levels of IGFs and IGFBP-3. aliquots stored as plasma or whole blood at 4, 21, or 30 degrees C for 1-5 days and after 1-5 freeze-thaw cycles. stability duration in plasma was 5 days for sVCAM-1 and sICAM-1 and at least 2 days for sE-selectin at 4, 21, and 30 degrees C and 5 days for CRP at 4 and 21 degrees C and 1 day at 30 degrees C. Stability duration in whole blood was 5 days for sVCAM-1 and sICAM-1 and at least 2 days for sE-selectin at 4, 21, and 30 degrees C and 5 days for CRP at 4 and 21 degrees C and 2 days at 30 degrees C.	5X+	
Soluble vascular cell adhesion molecules, soluble selectins, and C-reactive protein	After completing 10 freeze-thaw cycles, we found no clinically or statistically significant effect on measured antibody levels and found no discernible detrimental effect on the ability to measure these antibodies by enzyme-linked immunoassays.	sP-selectin was not stable in plasma or whole blood. sICAM-1, sVCAM-1, CRP, and sE-selectin were stable after 5 freeze-thaw cycles. sP-selectin is not stable and therefore requires immediate assay.	Clin Chem. 2007 Oct;53(10):1858-60. Epub 2007 Aug 3. Stability of soluble adhesion molecules, selectins, and C-reactive protein at various temperatures; implications for epidemiological and large-scale clinical studies.
Measles, Mumps, and Rubella Virus Antibodies in serum	One week storage at +4 degrees C did not significantly affect the serum apo E concentration. At -20 degrees C or -80 degrees C no significant change in apo E concentration occurred during up to three months of storage. Moreover, the concentration of apo E was not modified after long-term storage of serum samples kept at -196 degrees C in liquid nitrogen for up to four years. 15 freeze-thaw cycles, over a 3-week period, did not affect the apo E concentration in serum. A similar freeze-thaw procedure applied to purified human recombinant apo E showed that apo E2 isoform was the most stable in comparison with the apo E3 and apo E4 isoforms.	10x+	Clinical and Diagnostic Laboratory Immunology, January 2003, p. 19-21, Vol. 10, No. 1. Effect of Multiple Freeze-Thaw Cycles on Detection of Measles, Mumps, and Rubella Virus Antibodies
apolipoprotein (apo) E concentration in serum	Samples were stable for up to 10, <1, and at least 100 days for whole blood stored at 4, -20, and -80 °C. Samples may be kept under refrigeration up to 10 days and at -80 °C if long-term storage is required. Compared with its initial value, urine albumin, creatinine and UACR all did not show any significant differences (p > 0.05).	Urine samples can be safely frozen and thawed at least five times	Clin Chem Lab Med. 2000 Jun;38(6):525-8. Effect of short- and long-term storage on human serum and recombinant apolipoprotein E concentration.
Glycohemoglobin			Clinical Biochemistry, Volume 37, Issue 9, September 2004, Pages 836-839. Effect of pre-analytical variables on glycohemoglobin measurements in routine clinical care.
Urinary albumin & creatinine			Scand J Clin Lab Invest. 2009;69(8):886-8. Effect of repeated freeze-thaw cycles on urinary albumin-to-creatinine ratio
plasma albumin		Plasma albumin values were not affected (P 0.05) by the first two freeze thaw cycles; up to 5X (> decreases Ab levels)	http://linkinghub.elsevier.com/retrieve/pii/S0921448895006658 WHO/CS/CSR/EDC2001.16 Guidelines for Using HIV Testing Technologies I Surveillance Bioactive Diagnostica kit insert, http://www.bioactiva.com/resources/RUBM0400BAen/g107082009.pdf
HIV Ag, Ab	LTS @ -70 C		
Rubella Virus in human serum	store LT at -20 to -70 C.	avoid repeated F/T	
Hepatitis C Virus (HCV) RNA	HCV TMA exhibited robust performance in detecting HCV RNA in samples subjected to various conditions commonly encountered in a clinical laboratory, including long-term storage, multiple freeze-thaw cycles, different collection tubes, and the presence of endogenous substances, commonly prescribed drugs, or other microorganisms and viruses. samples stored @ -70C gave identical spectra to those from fresh urine samples.	OK in multiple F/T [Hepatitis C Virus Antibody (RIBA) stable 3X F/T]	J Clin Microbiol. 2003 January; 41(1): 310-317. Performance Evaluation of the VERSANT HCV RNA Qualitative Assay by Using Transcription-Mediated Amplification
Urinary biomarkers by MS/MS		F/T had little effect up to 4X Up to 5X FT do not affect urinary proteome or salivary proteome.	Molecular & Cellular Proteomics 5.10; 1760-71, 2006. Discovery of Urine Biomarkers J Biomed Biotech 2010 article ID 906082, 16 pp. Challenges for Biomarker Discovery in Body Fluids using Seldi-TOF-MS
Plasma, serum, urine, saliva for protein biomarkers	Store urine & saliva at -80 C. DBS for blood samples? plasma preferred ver serum; EDTA-plasma pref over hep & citrate		International Collaboration in Proteomics & Informatics, Oct 2007, G. Omen Clin Adv Hemat Oncol; 4(7): 2006, 541-9. Clinical Proteomics: The Promises and Challenges of Mass Spectrometry-Based Biomarker Discovery Comb Chem High Throughput Screen. 2005 Dec;8(8):725-33. Prerequisites for peptidomic analysis of blood samples: I. Evaluation of blood specimen qualities and determination of technical performance characteristics.
Plasma proteomic		"minimize F/T" Studies have shown significant variation in multiple F/T spectra	
plasma proteome			
low molecular weight proteome (peptidome)	plasma vs serum, ULT processing after thrombocytes have been removed		

plasma proteome	EDTA plasma stable on LTS at -70 C platelet-depleted plasma preferable, -80 C good, LN2 storage best, addition of protease inhibitors recommended but should be incorporated early & used judiciously as some form non-specific protein adducts & others interfere with peptide studies	1% of the proteins changed by 67% after 1 F/T. 2nd, 3rd, 4th, 5th F/T changed a given peak intensity by a median of 1.7, 2.4, 3.5, and 3.1 %. 5X F/T - repeatedly F/T leads to protein degradation.	Cancer Informatics 2005:1, 98-104. Impact of freeze-thaw cycles and storage time on plasma samples used in mass spectrometry based biomarker discovery projects.	
plasma proteome		The number of F/T cycles should be minimized to an absolute limit of 2X	Proteomics 2005, 5, 3262-77. HUPO Plasma Proteome Project specimen collection & hndling: Towards the standardization of parameters for plasma proteome projects. Expert Review of Proteomics. Review article Plasma/serum proteomics: pre-analytical issues. June 2007, 4(3), 363-70.	NEED THIS PAPER
plasma & serum proteins for proteomes		Differences caused by F/T cycles found to be relatively small, but keep # F/T to a minimum	Anal Chem 2006, 78, 4307-18. Impact of analytical bias in metabolonomic studies of human blood serum and plasma	
serum and plasma metabolomics	LiHep plasma used since EDTA produces strong signals for CaEDTA and MgEDTA as well as free EDTA	all are sensitive to degradation after sequential F/T	Cancer Informatics 2005:1, 98-104. Impact of freeze-thaw cycles and storage time on plasma samples used in mass spectrometry based biomarker discovery projects.	
Macromolecules (> 5000 Da, e.g. amino acid bpolymers or multimers, monoclonal Abs, recombinant proteins, vaccines)				
Urinary biomarkers (soluble urinary proteins & exosomes) measured by SELDI-TOF	Storage at 4 °C resulted in altered spectra over a 3-day period reflecting sample degradation. Samples stored at -70 °C, however, gave identical spectra to those from fresh urine samples. Freezing and thawing had little effect on the spectra for up to four freeze-thaw cycles, although evidence of degradation began to be apparent after five freeze-thaw cycles.		Discovery of Urinary Biomarkers* October 1, 2006 Molecular & Cellular Proteomics, 5, 1760-1771. PATH June 2005. RBP-EIA: Collecting, processing, and handling venous capillary, and blood spot samples	
retinol binding protein (RBP)	very stable in pH1 Acidic conditions, higher concentration in serum than in milk. Milk has more forms.	multi-X F/T should be avoided as samples may deteriorate	Silence 2010, 1:7, 1-7. microRNA as a new immunoregulatory agent in breast milk.	
microRNA in breast milk & serum		Subjected to 3X F/T; human breast milk miRNAs very stable.		
Cytokines in plasma or serum	freeze at -80 C until assayed. Most cytokines are stable for up to 2 yr stotage. Degradation of IL-13, IL-15, IL-17 & CXCL8 appear within 1 yr of storage, whereas IL-2, IL-4, IL-12, & IL-18 are stable for up to 3 yr. IL-1α, IL-1β, IL-5, IL-6, & IL-10 are degraded up to 50% with 2-3 years of storage.	Most of the cytokines are stable for up to 3X F/T. However, levels of certain CKs like TNK-α increase with each successive F/T, becoming significant after 3X.	Curr Opin Clin Nutr Metab Care. Digital Commons @ UConn, 9-1-2010. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. Clin Diagn Lab Immunol 1999; 6:89-95. Stability of plasma levels of cytokines and soluble activation markers in patients with human immunodeficiency virus.	
Cytokines in plasma (β2M, sIL-2R, neopterin, IFN-γ, sTNF-RII, TNF-α)	measured as stored at ambient to -70 C over 20 days: -70 C storage most stable			
15 cytokines measured	LTS showed cytokines are stable for a period up to 2 yr at -80 C. After 4 yr IL-1α, IL-1β, IL-10, IL-15, CXCK8 degraded up to 75%.	Only 2 of 15 cytokines remained stable after several F/T cycles. Although most cytokines are stable in a high protein matrix such as plasma during the 1st F/T, the second+ F/Ts should be avoided.	BMC Immunology 2009, 10:52 (e-paper). Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays.	QC for multiplex assay: coupled Abs on microspheres
C-peptide in urine	UCPCR was unchanged at room temperature for 24 h and at 4 degrees C for 72 h even in the absence of preservative.	UCPCR remained stable after 7 freeze-thaw cycles but decreased with freezer storage time and dropped to 82%-84% of baseline by 90 days at -20 degrees C.	Clin Chem. 2009 Nov;55(11):2035-9. Epub 2009 Aug 27. Stability and reproducibility of a single-sample urinary c-peptide/creatinine ratio and its correlation with 24-h urinary c-peptide.	
C-peptide in serum	stable @ -70 C	avoid multi-X F/T	multiple product inserts from assay kits for C-peptide	
Thyroglobulin, Interleukin-6, TNF-α, leptin, hepatocyte growth factor	HGF stable through 4 months of frozen storage, but increased by 20% after 10 months.	Tg small decrease after 3 F/T and a large decrease after 3 months of frozen storage. IL-6 and leptin not affected by F/T (stable α-helical structure), TNF-α increased by 17% after 3 F/T, 23.9% after 6 F/T; HGF stable in serum after 20 F/T	Medscape Today discussion, Nov. 9, 2007. Serum thyroglobulin stability for immunoassay: discussion.	
Bovine non-esterified fatty acid & β-hydroxybutyrate	BHBA in humans is unaffected by storage temp & time; NEFA are far less stable - affected by anticoagulants, increase with time esp @ higher storage temps	BHBA did not change after 1 F/T	J Dairy Sci 2005; 88 (9): 3139-44. Effect of anticoagulant, storage temperature, and duration of storage on non-esterified fatty acid and β-hydroxybutyrate concentrations from dairy cattle.	
sodium, potassium, calcium, chloride, inorganic phosphate, magnesium, creatinine, urea, uric acid, bilirubin, cholesterol, HDL- and LDL-cholesterol, triacylglycerols, creatine kinase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, alpha-amylase, lactate dehydrogenase and cholinesterase.	When separated serum was stored at + 9 degrees C for seven days, the mean changes in inorganic phosphate and lactate dehydrogenase exceeded significantly (p < 0.05 or 0.001, respectively) the maximum allowable inaccuracy according to the Guidelines of the German Federal Medical Council; all other quantities were sufficiently stable. In serum at room temperature, inorganic phosphate, uric acid, HDL-cholesterol and triacylglycerols increased continuously, whereas bilirubin, LDL-cholesterol, creatine kinase and aspartate aminotransferase decreased more than the guidelines permit during the storage period (p < 0.05 for aspartate aminotransferase, p < 0.001 for the other analytes mentioned). In whole blood stored for 7 days at + 9 degrees C, only the following serum analytes satisfied the stability requirements of the guidelines: calcium, urea, cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerols, creatine kinase, gamma-glutamyltransferase and cholinesterase. When stored at room temperature, only sodium, uric acid, bilirubin, cholesterol, triacylglycerols, aspartate aminotransferase: F/T not examined			
glucose, urea, creatinine, total proteins, sodium, potassium, chloride, calcium, phosphates, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and alkaline phosphatase (ALP) in canine serum	One aliquot was considered as the reference aliquot and used immediately for the assay of all of the biochemical constituents. All of the other aliquots were stored at -20°C. Three aliquots underwent 1, 2, or 3 freeze-thaw cycles during a 1- to 3-day period. The last aliquot remained at -20°C throughout the study and was thawed on the third day.	Repeated freeze-thaw cycles do not cause changes in the biochemical constituents studied in canine plasma.	Eur J Clin Chem Clin Biochem. 1995 Apr;33(4):231-8. Storage of serum or whole blood samples? Effects of time and temperature on 22 serum analytes.	
			Vet Clin Pathol 35:339-40. Effect of repeated freeze-thaw cycles on routine plasma biochemical constituents in canine plasma	

RNA in tissue	RNA quality depends on tissue handling prior to RNA isolation. This article examines the effect of freeze thaw cycles prior to RNA isolation on RNA. Never-frozen plasma, freeze-thawed plasma, and thawed plasma left at room temperature for 1 h showed no significant differences in RNA concentration. Plasma RNA is stable in uncentrifuged EDTA blood stored at 4 °C, but to obtain a stable serum RNA concentration, uncentrifuged clotted blood should be stored at 4 °C and processed within 6 h.	F/T process can disrupt cellular compartments where RNases are stored, giving them access to the RNA. Keep tissue frozen at all times prior to homogenization.	http://www.ambion.com/techlib/tn/93/9314.htm Effect of Freeze-Thawing of Tissue on RNA Integrity - Ambion, Inc
RNA in serum		No significant difference was observed for freeze-thawed serum	Clin Chem. 2002 Oct;48(10):1647-53. Stability of endogenous and added RNA in blood specimens, serum, and plasma.
isolated RNA	need protease inhibitors for LTS, store in RNase free materials; must be stored at -80 C	repeat cycles detrimental	Toxicol Appl Pharmacol 2005;206:261-8. Molecular epidemiology biomarkers - sample collection and processing considerations.
DNA and RNA	both DNA & RNA must be stored LT @ -80 C, although -20C may be adequate for DNA for up to 5 months		Epi Rev 19 (1997) 156-62 Profiling of microRNA in blood serum/plasma. Guidelines for the miRCURY LNA™ Universal RT microRNA PCR System.
microRNA in serum & plasma	high stability of microRNA in EDTA plasma samples	up to 6 F/T cycles with no significant effect (N=5 samples)	
Serum Thyrotropin, Thyroid Hormones, and Thyroid Autoantibodies	TSH, FT4, and FT3 can reliably be analyzed in samples stored for 23 years at -25 °C, and that TPO-Ab and TG-Ab are also stable for 14 years of storage. Choline concentrations with heparin in 5 of 12 volunteers were >10% higher than with EDTA; For freshly collected samples stored at ambient temperature, choline concentrations in all types of samples increased with storage time. For EDTA whole blood, EDTA plasma, and heparin plasma, the choline concentration increased for the first 60 min and then stabilized. For heparin whole blood, the choline concentration continued to increase linearly with storage time for >4 h, at which time the choline concentrations were increased by approximately 50%.	There were no differences in TSH, FT4, TPO-Ab, or TG-Ab concentrations when 50 frozen and thawed serum samples were compared with 50 fresh serum samples. FT3 concentrations were significantly higher (Student t-test, P <0.001) in frozen samples but remained within reference intervals.	Clin Chem. 2007 Nov;53(11):1986-7. The effect of freezing, thawing, and short- and long-term storage on serum thyrotropin, thyroid hormones, and thyroid autoantibodies: implications for analyzing samples stored in serum banks.
Choline in Whole Blood and Plasma human transforming growth factor-β1 (TGF-β1)		One freeze-thaw cycle led to significant mean (SD) increases in choline concentrations in heparin whole blood; effect was not significant for EDTA.	Clinical Chemistry. 2008;54:590-593. Choline in Whole Blood and Plasma: Sample Preparation and Stability
IGF-I and its main binding protein (IGFBP-3 in serum)	2X previously F/T samples from NHANES used routine serum preparation and refrigerated storage of samples for up to 24 hours is acceptable for the measurement of both free and total PSA. Samples that are to be retained for longer than 24 hours should be frozen. Samples stored for extended periods should be kept at -70°C.	avoid repeated F/T cycles	Quantikine human TGF-β1 immunoassay kit insert
PSA in serum	Urine samples from 24 h collections were portioned into 50 ml plastic and stored at -80°C until analysis. For freeze-thaw stability, triplicate samples at each concentration for synthetic urine samples and authentic urine samples were subjected to three complete freeze-thaw cycles with freezing at -20°C and thawing at room temperature.	Samples included in the 12 plates selected for reanalysis for both IGF-I and IGFBP-3 indicated that an additional freeze-thaw cycle did not influence levels of either analyte, confirming previous studies of these assays (19) and suggesting that extended time spent at room temperature may be more important for IGF degradation than an additional freeze thaw cycle.	Cancer Epidemiol Biomarkers Prev May 2007 16: 1017. Serum Levels of Insulin-like Growth Factor-I and Insulin-like Growth Factor-I Binding Protein-3: Quality Control for Studies of Stored Serum
Isoprostane isomers in human urine ( iPF2α-III and 15-epi-iPF2α-III, 2,3-dinor-iPF2α-III and 8,12-iso-iPF2α-VI, PGF2α. )		Looked at F/T 5X	Urology 1996; 48(6):S1:33-9. Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions
Hemostasis agents (Vitamin K, clotting factors, prothrombin)	The temperature at which archived samples are stored affects their shelf-life. For most coagulation tests storage at -35°C or less gives a shelf life of several years but storage at -20°C is inadequate.	The endogenous analytes in authentic urine samples were ±15% of the fresh concentration after freeze-thaw, short-term, and long-term storage.	need article
plasma TIMP-1, steroids		Repeated freeze-thaw cycles may affect factor level, for example, a reduction in vWF:CB activity and FXII levels. Freeze-thawing may also produce phospholipid rich membrane microvesicles from platelet damage which may then mask the presence of a lupus anti-coagulant.	Practical-Haemostasis.com: a practical guide to laboratory haemostasis
25(OH)-Vitamin D3 in Human Blood or Serum	A mean decrease of 2.3% was noted after 72 h storage of whole blood on the bench at room temperature, and a mean decrease of 3.4% after 24 h and 8.5% after 7 days storage of serum on the bench in daylight. Mean decreases of 4.5% after 3 days and 8.1% after 7 days storage of serum in the dark at room temperature were noted, whereas a mean decrease of 1.8% was observed after 7-day storage of serum in the refrigerator. A 4.0% decrease in the mean concentration was seen following storage at -20 °C for up to 2 months.	more than six cycles of repeated freezing and thawing significantly changed the TIMP-1 concentrations; steroids stable to multi-X F/T	Molecular & Cellular Proteomics 2008; 7: 2061-6. Banking of Biological Fluids for Studies of Disease-associated Protein Biomarkers
1,25 dihydroxy vitamin D	Three different patient sample pools were prepared by serial dilution with Zero Standard, and aliquots individually frozen at -20°C to accommodate multiple testing. Each sample and dilution were assayed in multiple replicates each day over three different assay dates. All data are based on a single freeze-thaw of each aliquot. Additionally, seven serum samples were subjected to three freeze/thaw cycles and assessed against fresh results.	Exposure to 4 freeze-thawing cycles had no important effect on 25(OH)-vitamin D3 concentrations; mean of 8 samples incr 2.6% ; can be attributed to evaporation or freeze-drying processes. A 4.0% decrease in the mean concentration was seen following storage at -20 °C for up to 2 months.	Clinical Chemistry. 2009;55:1584-1585. Preanalytical Stability of 25(OH)-Vitamin D3 in Human Blood or Serum at Room Temperature: Solid as a Rock
		The equivalence of fresh, frozen, serum or plasma sample values over as much as 6 days and repeated freeze/thaw cycles provides the laboratory with considerable flexibility in the logistics of sample processing.	Clinical Biochemistry 2002; 35(7): 517-21. Analytical and clinical validation of a radioimmunoassay for the measurement of 1,25 dihydroxy vitamin D

plasma kisspeptin	Kisspeptin is a peptide product of the KISS-1 gene and a key regulator of the hypothalamo-pituitary-gonadal axis. Pregnancy is associated with raised plasma kisspeptin concentrations. Kisspeptin-IR was poorly preserved in serum samples, but was relatively stable in plasma. When Plasma Separating II gel tubes were kept at +4°C or at ambient temperature for up to 24 hours before centrifugation, ribavirin concentrations decreased by 1% to 8% and 12% to 18%, respectively	Freeze-thaw cycles did not significantly influence plasma kisspeptin-IR levels.	Endocrine Abstracts (2008) 15 P281. Pre-analytical factors affecting measurement of plasma kisspeptin by radioimmunoassay
Ribavirin		Decrease after 3X F/T F/T significantly increased in platelet rich, but not platelet-poor, plasma.	Therapeutic Drug Monitoring: April 2010 - Volume 32 - Issue 2 - pp 237-241. Stability of Ribavirin Concentrations Depending on the Type of Blood Collection Tube and Preanalytical Conditions Clinical Science 2006, III: 341-7. Influence of pre-analytical and analytical factors on measurement of soluble CD40L
CD40L Cancer biomarkers (a-fetoprotein (AFP) for staging of non-seminomatous testicular cancer and monitoring of hepatocellular carcinoma; cancer antigen-125 (CA-125); and human epididymis protein 4 (HE4) for monitoring of ovarian cancer; thyroglobulin (Tg) for monitoring of thyroid cancer; prostate specific antigen (PSA) for screening and monitoring of prostate cancer, carcinogenic embryonic antigen (CEA) for monitoring of pancreatic cancer; and CA15-3/CA27-29 and HER2/neu for monitoring of breast cancer.)	proatherogenic mediator. Serum levels higher than plasma.	Multiple freeze/thaw cycles of clinical samples often used in biomarker discovery may also contribute to a lack of reproducibility during the validation phase. Thawing and refreezing of whole salivary specimens up to 3 times in the pre-analytical period does not appreciably affect the concentrations of salivary progesterone and estradiol	Medical Laboratory Observer On-line March 2011 ( <a href="http://www.mlo-online.com/features/201103/cover_story.aspx">http://www.mlo-online.com/features/201103/cover_story.aspx</a> ) Cancer Biomarkers: Surviving the journey from bench to bedside Presentation #1045 at ADEA/AADR/CADR Meeting & Exhibition (March 8-11, 2006). Effects of Repeated Freeze-Thaw in Self-collected Salivary Hormone Specimens
Salivary estradiol and progesterone	Aliquot 1 was thawed once prior to analysis, aliquot 2 was refrozen and thawed twice and aliquot 3 was refrozen and thawed 3 times. Refrigerate or freeze samples as soon as possible after collection. Many analytes are not stable at room temperature, and keeping samples cold after collection is important. When samples remain at room temperature for periods of time longer than a few hours there is also opportunity for bacterial growth, which can compromise assay validity.(33) We advocate a conservative approach and advise that <b>all</b> samples should be maintained at 4°C for no longer than several hours before freezing them at or below -20°C (temperature of a regular household freezer).	However, freeze-thaw cycles should be minimized for some analytes.	<a href="http://www.salimetrics.com/spit-tips/publications/salivary-collection-handbook.php">http://www.salimetrics.com/spit-tips/publications/salivary-collection-handbook.php</a> . Salimetrics Spit Tips - Saliva Collection Handbook
Other salivary analytes		No significant differences were found when serum samples were tested against either pepEBNA1, pepPT, or pepOMP2 after 1, 2, or 10 freeze-thaw cycles	Clinical and Vaccine Immunology, May 2010, p. 735-740, Vol. 17, No. 5. Immunological Fingerprinting Method for Differentiation of Serum Samples in Research-Oriented Biobanks
synthetic peptides from the EBNA1 (EBV nuclear antigen 1) protein of Epstein-Barr virus (EBV), the Bordetella pertussis toxin (PT), and the outer membrane protein 2 (OMP2) of Chlamydia pneumoniae.	serum sample fingerprint based on IgG titers obtained with three different antigens. tested one aliquot after 1 freeze-thaw cycle, one aliquot after 2 freeze-thaw cycles, and one aliquot after 10 freeze-thaw cycles. These aliquots were tested against pepEBNA1, pepPT, and pepOMP2 in triplicate.	Not affected by multi-X F/T	Alpco MLPO kit insert: <a href="http://www.alpco.com/pdfs/30/30-6631.pdf">http://www.alpco.com/pdfs/30/30-6631.pdf</a>
Myeloperoxidase in serum/plasma amphetamines, amphetamine-derived, piperazine-derived, and phenethylamine-derived designer drugs, antidepressants, neuroleptics, anti-HIV drugs, anti-epileptics, cardiovascular drugs, and others )	part of the defense mechanism in polymorphonuclear leukocytes against invading antigens stored at least in the refrigerator and preferably at -20 °C or lower to avoid any degradation. Finally, results obtained from biosamples that have been stored at room temperature for a longer time should be interpreted with great care and partial degradation should always be considered.	three or five freeze/thaw cycles had no significant effect on methylphenidate concentration	Analytical and Bioanalytical Chemistry, 388(7), 1505-19. Stability of analytes in biosamples—an important issue in clinical and forensic toxicology?
Alzheimer's Disease biomarkers in CSF (Aβ1-42; P-tau and T-tau)	Storage up 72 hr @ 25 C did not affect any analyte. P-tau and T-tau stable for up to 4 yrs -20 C LTS. At least 2yr stability at -20 C for Aβ1-42	Multi-X F/T led to decreases in Aβ1-42; no decreases for P-tau and T-tau	In: Biomarker's for Early Diagnosis of Alzheimer's Disease. 2008. Nova Science Pubs. Alzheimer's Disease Biomarkers: From concept to utility. <a href="http://www.pdpi.com/resource_library/posters/Octreotide_ASMS_2010.pdf">http://www.pdpi.com/resource_library/posters/Octreotide_ASMS_2010.pdf</a> ) Assessment effects and <a href="http://www.basinc.com/library/presentations/pdf/rsum-05.pdf">http://www.basinc.com/library/presentations/pdf/rsum-05.pdf</a> . Method development & validation of cystine in white blood cell lysate using LC/MS/MS <a href="http://www.kodtech.com/docs/2101/pdfr11668.pdf">http://www.kodtech.com/docs/2101/pdfr11668.pdf</a> . Stability factors influencing the analysis of environmental organic chemicals
Octreotide in plasma	anti-neoplastic agent stored at -20 C prior to analysis	stable up to 5X F/T	AMERICAN COLLEGE OF MEDICAL GENETICS, Standards and Guidelines for Clinical Genetics Laboratories, 2006 Edition. <a href="http://www.acmg.net/Pages/ACMG_Activities/tds-2002/DS.htm">http://www.acmg.net/Pages/ACMG_Activities/tds-2002/DS.htm</a>
Cystine in WBCs Urinary phthalates, 2-naphtol, enviro. Phenols, 3,5,6-trichloro-2-pyridinol (TCPy), BDE 209, HBCD	stable at least 9 months stored as lysate matrix @ -80 C	stable through 3X F/T	
Prenatal screening for Down Syndrome	Various temps/comments provided stored at 4-8°C for days and at -20°C for years. µE3 is not stable in whole blood, samples should be promptly centrifuged in separator tubes or separated from the clot. µE3 is stable in sera stored at 4-8°C for days. In the past, some kits produced systematically different µE3 values after the sera were frozen and thawed. For optimal performance, shipping time should be minimized (e.g., express mail, courier service) and samples should not be exposed to high temperatures. Free beta subunit is not stable in serum when exposed to high temperatures (e.g., daytime summer temperatures in the southern United States), due to dissociation of intact hCG. If free beta is to be measured, samples must be protected from high temperatures (e.g., cool packs with overnight shipment in the summertime). Shipping samples in the form of blood spots can also result in improved [a selective, potent, H1-antihistamine compound indicated for the treatment of allergic rhinitis and chronic idiopathic urticaria] Freezer stability of the analytes in biomatrix was assessed by analyzing the QC samples stored at -20 °C for at least 30 days. The stability of analytes in biomatrix following repeated three freeze-thaw cycles (stored at -20 C between cycles) was assessed using QC samples spiked with analytes. Stable at -20 and -70 C.	If frozen samples are to be used to derive medians, possible freeze/thaw effects should be examined.	Biomirror August 2010. Determination of Levocetizine in human plasma by liquid chromatography electrospray mass spectrometry. <a href="http://www.bmjournals.com/index.php?option=com_content&amp;view=article&amp;id=147:determination-of-levocetizine-in-human-plasma-by-liquid-chromatography-electrospray-tandem-mass-spectrometry&amp;catid=51:august&amp;Itemid=143">http://www.bmjournals.com/index.php?option=com_content&amp;view=article&amp;id=147:determination-of-levocetizine-in-human-plasma-by-liquid-chromatography-electrospray-tandem-mass-spectrometry&amp;catid=51:august&amp;Itemid=143</a> Phthalates stable multi-X F/T (others not commented on)
Levocetizine		Stable to 3 X F/T	
Erlotinib in human plasma	[Monoclonal Ab Ca treatment, Tarciva] stable for 24-hr at ambient temp, and 227 days @ -20 or -70 C.	Stable for 3X F/T	supported liquid extraction (SLE) coupled with HILIC-MS/MS: An application to method development and
Paroxetine in dried plasma spots	[SSRI antidepressant, Paxil] Replicate (n = 6) 15 µL human plasma samples at 0.8 and 160 ng/mL were spotted onto 226 paper and stored desiccated at room temperature for 35 days. The measured concentrations were compared to those of the same samples extracted and analyzed immediately after initial spotting and drying. The samples were stable.	The freeze-thaw stability of paroxetine was determined from spiked human whole plasma samples after three freeze-thaw cycles from -20 °C to room temperature. The difference of stored samples compared to fresh samples was -1.6% and 1.4% at 0.8 and 160 ng/mL, respectively.	Anal. Chem., 2011, 83 (1), pp 118-124. Use of Dried Plasma Spots in the Determination of Pharmacokinetics in Clinical Studies: Validation of a Quantitative Bioanalytical Method

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pro-atrial natriuretic peptide in human plasma	Mean recovery in eight samples after 6 months of storage at -20 degrees C was 108%.	Four freeze-thaw cycles had no influence on the analyte concentration in eight samples (mean recovery after the first thawing, 100%; after the last thawing, 110%). Limited experiments showed that undiluted serum samples could be thawed and refrozen a few times without any change in analyte concentration. Diluted serum samples also appear to be stable to freeze-thawing. Only sTR seems to be altered by freeze-thawing. Diluted serum is, however, stable for 1 d at room temperature.	<a href="http://www.thefreelibrary.com/immunoluminometric-assay-for-the-midregion-of-pro-atrial-natriuretic-peptide-in-human-plasma">http://www.thefreelibrary.com/immunoluminometric-assay-for-the-midregion-of-pro-atrial-natriuretic-peptide-in-human-plasma</a>
Ferritin, Soluble Transferrin Receptor, Retinol Binding Protein, and C-Reactive Protein			J. Nutr. 134:3127-3132, November 2004. Combined Measurement of Ferritin, Soluble Transferrin Receptor, Retinol Binding Protein, and C-Reactive Protein by an Inexpensive, Sensitive, and Simple Sandwich Enzyme-Linked Immunosorbent Assay Technique Presentation at 57th ASMS Conference on Mass Spectrometry. <a href="http://www.qps-usa.com/Userfiles/Docs/QPS%202009-016.pdf">http://www.qps-usa.com/Userfiles/Docs/QPS%202009-016.pdf</a> . LC-MS/MS determination of Emtricitabine and Tenofovir in human plasma Presentation at 57th ASMS Conference on Mass Spectrometry. <a href="http://www.qps-usa.com/UserFiles/Docs/Posters%20Abstracts/QPS%202010-002%20Abstract.pdf">http://www.qps-usa.com/UserFiles/Docs/Posters%20Abstracts/QPS%202010-002%20Abstract.pdf</a>
Emtricitabine and Tenofovir in human plasma	(Combination medicines used for treatment of HIV.) Stability of the analytes evaluated at ambient, -20 and -70 C storage. Stable at all conditions.	Stable to F/T cycles.	
L-arginine, L-citrulline, and asymmetric dimethylarginine in human plasma	(NO regulation in CVS)	Stable through 3 F/T cycles	<a href="http://www.qps-usa.com/UserFiles/Docs/Posters%20Abstracts/QPS%202010-002%20Abstract.pdf">http://www.qps-usa.com/UserFiles/Docs/Posters%20Abstracts/QPS%202010-002%20Abstract.pdf</a>
morphine, codeine, morphine-3-β-D-glucuronide, morphine-6-β-D-glucuronide, and codeine-6-β-D-glucuronide in human urine	storage at multiple temps	Stable F/T 3 times	Journal of Mass Spectrometry 005; 40(11):1412-16. LC-ESI-MS/MS analysis for the quantification of morphine, codeine, morphine-3-β-D-glucuronide, morphine-6-β-D-glucuronide, and codeine-6-β-D-glucuronide in human urine J Chromatography B 8, 878 (2010) 169-77. Simultaneous determination of Tolbutamide, omeprazole, midazolam, and dextromethorphan in human plasma by LC-MS/MS - A high throughput approach to evaluate drug-drug interactions.
Tolbutamide, omeprazole, midazolam, and dextromethorphan in human plasma	Analyte stability was tested by using QC samples for multiple F/T cycles at ambient and -20 C storage. Stable in -20 C freezer at least 90 days.	Stable through 3 F/T cycles	
Six 1,4-benzodiazepines (alprazolam, bromazepam, clonazepam, diazepam, flunitrazepam, lorazepam) in human plasma, urine, and saliva	Stored for 180 days for plasma and urine, 120 days for saliva - both at -20 C.	Stable through 7X F/T	Chiang Mai J Sci 2010; 37(3) 451-63. Stability study of Six 1,4-benzodiazepines in bio-fluids stored at -20 C. J Chromatography B 857 (2007) 67-75. Stereoselective analysis of bupropion and hydroxybupropion in human plasma & urine by LC-MS/MS
bupropion and hydroxybupropion in human plasma & urine	(treatments for depression) Stored at -20 C for 45 days, stable	Stable through 3 F/T cycles	
Rosuvastatin in human plasma	(lipid-lowering drug Crestor) Stable at -70 ± 5 °C for 138 days (long term stability) in human plasma. Stable over 24.0 hours in human plasma at room temperature (23-30 °C).	Effect of freeze and thaw cycles on stability of plasma samples after three freeze and thaw cycles was also was determined. stable even after subjecting to three-freeze thaw cycles.	J. Braz. Chem. Soc. vol.16 no.5 São Paulo Sept./Oct. 2005. Estimation of rosuvastatin in human plasma by HPLC tandem mass spectroscopic method and its application to bioequivalence study Clinical Chemistry and Laboratory Medicine 2008; 46(11):1589-97. Rapid sample preparation and simultaneous quantitation of prostaglandins and lipoxigenase derived fatty acid metabolites by liquid chromatography-mass spectrometry from small sample volumes <a href="http://www.wctrials.com/UserFiles/Docs/Levodopa%208%20Carbidopa_1.2.pdf">http://www.wctrials.com/UserFiles/Docs/Levodopa%208%20Carbidopa_1.2.pdf</a> . Measurement of levodopa and cardidopa in human plasma by SPE and LC-MS/MS. The Journal of Lipid Research, 2006; 47, 2340-45. Validation of the LC-MS/MS method for the quantification of mevalonic acid in human plasma and determination of the matrix effect
Prostaglandins and lipoxigenase derived fatty acid metabolites (from human plasma)	(lipid oxidation products important in diabetes)	stable to F/T after rapid prep?	<a href="http://www.wctrials.com/UserFiles/Docs/Levodopa%208%20Carbidopa_1.2.pdf">http://www.wctrials.com/UserFiles/Docs/Levodopa%208%20Carbidopa_1.2.pdf</a> . Measurement of levodopa and cardidopa in human plasma by SPE and LC-MS/MS. The Journal of Lipid Research, 2006; 47, 2340-45. Validation of the LC-MS/MS method for the quantification of mevalonic acid in human plasma and determination of the matrix effect
Levodopa and cardidopa in human plasma	(Parkinson's Disease treatments) Stable LTS @ -70 C	stable through multiple F/T	
mevalonic acid in human plasma	(related to statin treatment of hyperlipidemia) MVA was found to be stable for up to 28 days of storage (plasma) below -50°C. (a synthetic estrogen antagonist used clinically to treat male and female estrogen receptor-positive breast cancer ) Specimens collected during clinical studies are generally stored frozen at -30 to -80°C	Stable through 3 F/T cycles	
Tamoxifen in blood/serum/plasma, tissue		TAM, NDTAM, 4OHTAM and endoxifen are stable in plasma over four freeze/thaw cycles	<a href="http://toxwiki.wikispaces.com/Tamoxifen">http://toxwiki.wikispaces.com/Tamoxifen</a>
angiopoietin-1 and angiopoietin-2 in plasma	(novel biomarkers of endothelial integrity ) Stability only examined for 24 hr at ambient and + 4 C.	four cycles of freezing (20 hours at -70°C) and thawing (4 hours at room temperature) induced no discernible loss of Ang-1 immunoreactivity (102% (97% to 107%) versus 100% at baseline) or of Ang-2 immunoreactivity (92% (85% to 105%) versus 100% at baseline) in tests of five serum samples.	Critical Care 2008, 12:R94. <a href="http://ccforum.com/content/12/4/R94">http://ccforum.com/content/12/4/R94</a> . Circulating angiopoietin-1 and angiopoietin-2 in critically ill patients: development and clinical application of two new immunoassays
total plasma antioxidant capacity (TAC)	To counteract these limitations, several methods have been proposed for determination of total plasma antioxidant capacity (TAC). They can be divided in two main classes: Either distinct antioxidant components are assayed (ex. Vitamin E, ascorbic acid, etc), or the total antioxidant potency is estimated by the combined reducing activities of a given body fluid (especially plasma)	the automated method produces stable results during three freeze thawing cycles	BMC Clinical Pathology 2002, 2:3. A new automated method for the determination of the Total Antioxidant Capacity (TAC) of human plasma, based on the crocin bleaching assay ( <i>locin is a carotenoid</i> )
Naloxone and its metabolite nornaloxone in human plasma, urine, and human liver microsomes	Analytes were stable in plasma and urine for up to 24 h at room temperature and in plasma after three freeze-thaw cycles	3+?	Determination of Naloxone and Nornaloxone (Noryxomorphone) by High-Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry. J Anal Toxicol. 2009 Oct;33(8):409-17.
320 compounds in DMSO	powder forms mixed with DMSO & stored in sealed microtiter plates under Ar2. LC-MS assay after every 5th F/T cycle, plus ambient and never Th controls	Greatest loss after 25X F/T, then ambient, no loss in never Th. No degradation - possible pptation loss?	J Biomole Scrn 8(2), 200321-5. The effect of freeze-thaw cycles on the stability of compounds in DMSO

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