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SIMPLIFYING ACTH PREANALYTICAL PROCEDURE

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BACKGROUND-AIM: Adrenocorticotropin (ACTH) concentration is unstable due to proteolytic degradation. In practice, recommended pre-analytical procedure that includes collecting samples in pre-cooled tubes, transporting on ice and using cooled centrifuge, can present major logistical problem. Recently, new test tubes containing EDTA and protease inhibitor aprotinin (Greiner Bio-One) have become available. Our aim was to investigate the effect of EDTA/aprotinin additive on ACTH concentration at room temperature (RT) analyzed at different time points in comparison with ACTH concentration obtained according to recommendations.

METHODS: Study was performed according to CLSI guideline GP-34A. Samples were collected from 20 volunteers in: (I) EDTA tubes using recommended procedure analyzed immediately (EDTAice); (II) EDTA/aprotinin tubes, centrifuged and analyzed immediately at RT (AprotininRT); (III) EDTA/aprotinin tubes, centrifuged and analyzed after 2 h at RT (AprotininRT2h) and (IV) EDTA/aprotinin tubes, centrifuged and analyzed after 4 h at RT (AprotininRT4h). ACTH measurements were performed with electrochemiluminescence method on Roche Cobas e601 analyzer. The difference between EDTAice and other three tubes was tested using paired samples t-test. ACTH concentration was expressed as mean (standard deviation).

RESULTS: ACTH concentrations in EDTAice, AprotininRT, AprotininRT2h, AprotininRT4h were 3.56 pmol/L (1.50), 3.72 pmol/L (1.55), 3.64 pmol/L (1.48) and 3.54 pmol/L (1.44), respectively. Paired samples t-test revealed significantly higher ACTH concentration in AprotininRT and AprotininRT2h (P < 0.001 and P = 0.020, respectively), while there was no difference in AprotininRT4h (P = 0.582) compared to EDTAice.

CONCLUSION: According to our results, ACTH can be sampled at RT in EDTA/aprotinin tubes and sample processing can be delayed for up to 4 h. Also, ice-chilling of sample is not effective as EDTA/aprotinin additive in stabilization of ACTH concentration, questioning if recommended procedure should be revised.

STABILITY OF URINE SPECIMENS KEPT AT ROOM TEMPERATURE AND IN REFRIGERATOR FOR FOUR HOURS

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BACKGROUND-AIM: Urine is a very important biological material for diagnosis, treatment and following of many diseases. It is preferred that the urine analysis is performed in fresh urine. However, in some cases, such as a device failure, analysis may not be possible immediately. It may take several hours to perform the analysis. In this study, we investigated the effect of delayed urine analysis on specimens kept at the room temperature and in the refrigerator for four hours.

METHODS: Fresh urine specimens (n = 50) separated three aliquots. One of them were immediately analysed on an Iris Diagnostics iQ200. The other aliquots were analysed after were kept at room temperature and in the refrigerator for four hours. The agreement between results at the room temperature and in the refrigerator and the initial values was evaluated with the use of concordance correlation coefficients (pc) and Cohen’s kappa coefficients (κ). A pc > 0.90 and κ > 0.80 was accepted as good agreement, indicating that the analytes were stable.

RESULTS: There was no agreement at room temperature and in the refrigerator and the initial values for protein, nitrite and pH.

CONCLUSION: Urinalysis must work with fresh samples. If a delay in analysis causes some changes in several parameters.

SHORT-THERM STABILITY OF ROUTINE HAEMATOLOGY PARAMETERS AFTER REFRIGERATION

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BACKGROUND-AIM: Storage time and temperature may significantly affect routine haematology results. There is no unambiguous criteria for whole blood stability at 2-8 °C. Our aim was to determine short-therm stability of routine haematology parameters in whole blood samples stored in different time intervals within 48 hours at 2-8 °C.

METHODS: Blood was collected from 20 patients using K3EDTA Vacuette tubes (Greiner Bio-One GmbH, Kremsmünster, Austria). Routine haematology parameters were measured using the Sysmex XT 1800i analyzer (Sysmex Corporation, Kobe, Japan) immediately upon receipt.
Whole blood samples where stored at 2-8 °C and re-analysed after a 6, 12, 24 and 48 hours after baseline measurements. Paired samples t-test was used for statistical comparisons to baseline results. The level of significance was set at P < 0.05. Mean biases from baseline results were compared to Ricos desirable biological specifications to define clinically relevant variations.

RESULTS: Statistically different results compared to baseline values were found for: WBC after 6, 12 and 48 hours (P = 0.008, P = 0.035 and P = 0.012, respectively); hematocrit after 48 hours (P = 0.031); MCV after 24 and 48 hours (P = 0.008 and P < 0.001); MCHC after 48 hours (P < 0.001); platelets after 6 hours (P = 0.038); MPV after 6, 12, 24 and 48 hours (P < 0.001); neutrophils (%), lymphocytes (%) and eosinophils (%) after 48 hours storage (P = 0.001, P = 0.016 and P = 0.015, respectively). MPV exceeded the predefined criteria of 2.3% for clinical significance in all the time points tested (4.9% after 6; 7.5% after 12; 10.3% after 24 and 14.1% after 48 hours). WBC, MCHC and lymphocytes (%) exceeded the criteria for desirable bias after 24 hours storage.

CONCLUSION: All the routine haematology parameters tested, except MPV, showed acceptable stability when stored at 2-8 °C within 24 hours, compared to predefined desirable specification for bias in our laboratory conditions.

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IS HEPARIN PLASMA THE ULTIMATE SAMPLE MATERIAL FOR ANALYSIS?

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BACKGROUND-AIM: In order to change the type of sample for analysis from serum to heparin plasma, we conducted a study to examine the quality of the samples and the stability of the analytes in the different types of sample.

METHODS: 55 samples were collected from randomly chosen patients on haemodialysis. Two types of tubes were used, serum with gel barrier, and heparin plasma with gel barrier. The tubes were carefully mixed, left on room temperature and later centrifuged according to the recommendations of the manufacturer. After the centrifugation, none of the samples had hemolysis, or were inconsistent in any way. All the samples were used as primary samples on the analytical system. The analyses were performed consecutively, on COBAS Integra 400 plus analytical system. All tests were performed with analytical precision and accuracy required by the MKC EN: ISO 15189 standard. The analytes tested were: Na, K, Ca, P, Fe, UIBC, Glucose, ALP, CRP and urea.

RESULTS: For the statistical evaluation we used Pearson Correlation and the t-test, calculated by excel. For Na and K Pearson Correlation was 0.92 and 0.87 and p < 0.001. For Ca, Fe, Glucose, and ALP Pearson Correlation was 0.95, 0.98, 0.99 and 0.99 and p < 0.01. For UIBC Pearson Correlation was 0.99 and p < 0.05. For P, CRP and urea Pearson Correlation was 0.98, 0.99 and 0.94 and p > 0.05.

CONCLUSION: No statistically significant difference was determined between heparin plasma and serum for Na, K, Ca, Fe, UIBC, Glucose and ALP. For P, CRP and urea the reason for the statistically relevant difference must be reconsidered, perhaps in the possible interaction of the analytes with the gel. Generally, heparin plasma proved to be a stable sample material for the analytes in question.

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EFFECTIVENESS OF LIQUID CITRATE BUFFER-FLUORIDE MIXTURE IN SARSTEDT S-MONOVETTE® GLUCOEXACT TUBES AS AN INHIBITOR OF IN VITRO GLYCOLYSIS

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BACKGROUND-AIM: Since glycolysis affects glucose determination in vitro, NACB and ADA recommend to immediately place sample tubes in ice-water slurry and to separate plasma within 30' or to use an effective glucose stabilizer.

For this reason a study was designed to evaluate glucose concentration in different Sarstedt S-Monovettes maintained at room temperature (R.T.) for 2h, compared to reference glucose according to NACB-ADA.

METHODS: Blood from 113 volunteers (36M, 77F), was collected into lithium heparin (LH), NaF/Na2EDTA (NaF) and NaF/citrate buffer (GlucoEXACT) tubes. GlucoEXACT tubes contain a liquid additive and requires a proper tube filling and the use of a correction factor (1.16). Reference plasma glucose was determined in LH tube placed in an ice-water slurry, centrifuged at 4 °C with plasma separation from the cells within 30'. Samples were maintained at RT for 2h after drawing. Glucose testing of all samples of the same subject was performed in duplicate in the same analytical run using an hexokinase method.