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POTENTIAL ROLE OF CSF FERRITIN AS AN EARLY DIAGNOSTIC MARKER IN DIFFERENTIATING PEDIATRIC MENINGITIS

P. Dabla[1], S. Sharma[1]
[1]*Department of Biochemistry, Chacha Nehru Bal Chikitsalya Hospital, Associated to Maulana Azad Medical College, Delhi University, Geeta Colony, New Delhi – 110031*

BACKGROUND-AIM

Bacterial meningitis is a medical emergency with a potential for serious neurological damage or even death. Rapid diagnosis is important and henceforth critical for the early intervention by antibiotic therapy to prevent complications. Therefore the aim of the present study was to evaluate CSF ferritin levels in children with different etiologies of meningitis.

METHODS

65 children (1-124 months) with suspected meningitis admitted at Chacha Nehru Bal Chikitsalya hospital were included in the study. CSF sample was analyzed for glucose, protein, cell count, ferritin, gram stain and culture.

RESULTS

Based on the laboratory findings the 65 children were classified into 3 groups: 21 cases had bacterial meningitis, 18 had aseptic (viral) meningitis and 26 cases as the no-meningitis group. A significant relationship was observed between age and ferritin level in the non-meningitis group ($p<0.05$). CSF ferritin in bacterial meningitis group was 34.80 ± 11.20 ng/mL and was significantly higher than the aseptic meningitis group. Cut off value of ferritin to differentiate meningitis vs. no-meningitis group was estimated at 18.2ng/ml with a sensitivity of 94.9% and specificity of 96.2 %. However on differentiating bacterial from aseptic meningitis cutoff value was 20.3 ng/mL with a sensitivity of 98% and specificity of 33.3%.

CONCLUSION

CSF ferritin levels were found to be significantly different between the meningitis and the no-meningitis groups. However due to low specificity it may not prove useful for the early differentiation of different types of meningitis. Further studies are required on a larger sample size before we can substantiate our findings.

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PROFESSOR, M.D., PH.D.

L.A. Khorovskaya[2], I.O. Schmidt[1], S.N. Kovalevskaya[2]
[1]*Central Clinical Diagnostic Laboratory, St. Luka Hospital, Saint-Petersburg, Russian Federation*
[2]*Department of Laboratory Medicine with course of Molecular Biology, First Pavlov State Medical University, Saint-Petersburg, Russian Federation*

BACKGROUND-AIM

Comparison of blood collection tubes is an actual tack to control preanalytical factor that may affect the quality of the results of laboratory investigations. The aim of this study was to compare K2 EDTA Lind-Vac vacuum tubes from Estonia with reference vacuum tubes Greiner (Austria) for hematological investigations in condition of routine medical laboratory.

METHODS

Comparisons between vacuum tubes from Lind-Vac (Estonia) with K2 EDTA addictive with those from Greiner (Austria) were carried out as described in CLSI GP34-A (Validation and Verification of Tubes for Venous and Capillary Blood Specimen Collection) and EP-9A (Method comparison and Bias Estimation using Patient Samples). Sample collections from 20 patients were made according CLSI H3-A6 (Procedures for the collection of Diagnostic Blood Specimens by Venipuncture) in two tubes of each type for each patient and analyzed in Hematology Analyzer Hemalit 5500 Corway (China) on 20 hematological parameters in duplicates from each sample during May-June 2014 in St. Luka Hospital, St.-Petersburg, Russian Federation. Bias calculated assuming that results from Greiner tubes were referent. Differences in results were assessed for statistical significance with the Student paired t-test. Coefficient of variation (CV%) from duplicates compared between tubes with estimating of significant difference by F-test.

RESULTS

Results of comparisons of tubes did not show any significant difference of the blood analyts between samples from Lind-Vac and Grainer tubes ($p>0,05$). Repeatability differs in 8 parameters. CV% was higher for samples received from Lind-Vac tubes for 6 hematological parameters: concentration of red blood cells, white blood cells and platelets, concentration and relative amount of neutrophils, hematocrit ($p<0,05$). CV% received from Greiner tubes were higher for concentration and relative amount of monocytes ($p<0,05$).

CONCLUSION

K2 EDTA Greiner and Lind-Vac vacuum tubes comparison for hematological investigations shows identical results. Revealed differences in Imprecision of 8 parameters of blood tests did not influence to test interpretation from both types of tubes and were of the same order of magnitude within quality goals based on biological variation.

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SUITABILITY OF ICTERIC INDEX AS FRONT-LINE TEST FOR THE IDENTIFICATION OF BLOOD SAMPLES WITH ABNORMAL BILIRUBIN CONCENTRATIONS

S. Pasqualetti[1], D. Szőke[1], C. Valente[1], M. Panteghini[1]
[1]*Clinical Pathology Unit, "Luigi Sacco" University Hospital, Milan, Italy*

BACKGROUND-AIM

The use of the icteric index (II) as a front-line test for the preliminary identification of blood samples with abnormal total bilirubin (TB) concentrations was recently proposed. In case, laboratories should validate this approach on their own analyzers. Aim of this study was to validate the diagnostic accuracy of II on the Abbott Architect c16000 platform recently installed in our laboratory.

METHODS

TB concentrations (diazo-based colorimetric assay) and corresponding II values (derived from absorbance measurements of samples diluted with saline) in heparinised plasma and serum samples were collected for a 3-month (April-June 2014) period. Linear regression analysis (LRA) (II vs. TB) was performed for both serum and plasma samples. The diagnostic performance of II to discriminate between abnormal (>1.2 mg/dL) and physiological TB concentrations was evaluated for both sample types using the ROC curve analysis. The optimal II cut-off was selected at a negative predictive value (NPV) >99% for detection of abnormal TB values.

RESULTS

TB and relative II were obtained from 18,486 serum and 3700 plasma samples. LRA showed a strong correlation between II and TB (serum: R^2=0.951; plasma: R^2=0.941), with the following regression equations: serum: II = 0.86 (CI: 0.857-0.863) TB + 0.40 (CI: 0.386-0.405); plasma: II = 0.79 (CI: 0.788-0.801) TB + 0.40 (CI: 0.390-0.417). ROC curve analysis gave the following areas under the curve: serum, 0.948 (CI: 0.945-0.951), and plasma, 0.922 (CI:0.913-0.930), showing the high accuracy of II on both sample types for detecting abnormal TB. An II cut-off of 0.8 reliably excluded abnormal (>1.2 mg/dL) TB concentrations [serum, prevalence 25.4%: sensitivity, 99.6% (99.3-99.7), NPV, 99.7% (99.5-99.8); plasma, prevalence 16.7%: sensitivity, 98,6% (97.3-99.3), NPV, 99.4% (98.9-99.7).

CONCLUSION

In our setting the optimal performance of II as screening test for abnormal TB concentrations was achieved by a cut-off of 0.8. The use of II $\leq$ 0.8 as front-line test should allow the accurate 'zero-cost' detection of samples with low-normal TB concentrations avoiding TB measurement in a substantial number of cases, i.e., in our population ~35% of serum and ~40% of plasma samples, achieving a 4120 € saving on a yearly basis.

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BIOLOGY ORGANIZATION ON AN FRENCH HEALTH TERRITORY : INTEREST OF KEEPING A LABORATORY IN A PUBLIC HOSPITAL INSTEAD OF OUTSOURCING BIOLOGY

M. Sarazin[3], N. Tayeb[1], T. Garaix[2]
[1]*Centre Hospitalier le Corbusier , Firminy*
[2]*Ecole nationale Supérieure des Mines , saint Etienne*
[3]*UMRS 1136 Inserm*

BACKGROUND-AIM

In France, new accreditation requirements, technologies and hospitals expenditures force laboratories to reconsider biology. Also, considering the data of a the public hospital of Firminy (CHF), a study was set up to involve the impact of samples path on biological analysis and hospital stays, different scenarios considering all territory hospitals, and evaluate hospital size from which a laboratory must be kept.

METHODS

Data : biological activity from the CHF and providers laboratories : nature, financial coefficient of the tests, sample path. Hospital stay characterisation from the CHF using french descriptive algorithm (GHS), dates of entrance and exit from the hospital.
Analysis : average time between collection, feedback and all the sample path calculated taking into account daily collection, type of test. Relationship between feedback , collection times, length of stay and GHS characteristics, technical time studied by a linear regression. Other organizations performed with a probabilistic Markov model.

RESULTS

CHF is a medium sized hospital corresponding to 85% of French public hospitals. CHF laboratory performs 12 million of biological acts per year: 85% made on-site and 15% on three other laboratories Time period average for an analysis done by the on-site laboratory: 0.320 +/- 0.002 days versus 2.831 +/- 0.169 days by an outsourced laboratory with less days for private providers ($p < 0.05\%$) These periods do not occur on the length of stay ($p<0.05\%$). Biology expenditure average is 3.5+/- 0.8% per stay and 7248 +/- 560 euros per bed each year including 118 +/- 25 euros depending on outsourced biology. Simulation showed that from 160 beds an hospital can keep an on- site laboratory.

CONCLUSION

Analytical and post analytical periods are both involved in sample path. Organizations and technologies in place explain these differences. Although length of stay is not affected by sample path, empirical antibiotherapy established waiting for biological results may be an additional factor of morbidity. An on-site laboratory has got two advantages: rapidity for all routine tests determining therapeutic orientation particularly if there is a strong emergency activity, and an overall analysis of the results regarding clinical findings by a biologist.

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NEW STOOL COLLECTION AND EXTRACTION TOOL: PERFORMANCE ANALYSIS AND STABILITY OF CALPROTECTIN IN STOOL EXTRACTS PREPARED BY THE CALEX® CAP DEVICE

J. Weber[1], M. Ueberschlag[1], M. Prica[1], P. Spies[2], T. Jermann[1], C. Rothen[3], D. Gygax[2], J. Rothen[3]
[1]*BÜHLMANN Laboratories AG, Schoenenbuch, Switzerland*
[2]*Fachhochschule Nordwestschweiz FHNW, Muttenz, Switzerland*
[3]*Labor Rothen, Basel, Switzerland*

BACKGROUND-AIM

Inflammatory Bowel Disease (IBD) is a chronic inflammation of the gut. IBD can be diagnosed and its disease course can be followed by biomarkers such as calprotectin which is measured in extracted stools. The objective was to validate a new stool preparation tool, CALEX® Cap, and to compare its performance for the extraction of calprotectin with "gold standard" weigh-in and commercial extraction tools.

METHODS

The reproducibility of i) stool sampling and ii) stool extraction using CALEX® Cap device was determined. The calprotectin concentrations measured in stool extracts prepared by CALEX® Cap were compared with extracts of the same stools prepared by a manual weighing method. 15 stools with calprotectin levels from 49 to 3147 µg/g were extracted with 4 commercial stool preparation tools. The resulting extracts were stored for 1,2,3, and 6 days at 23°C, then analyzed in the respective ELISA tests and compared to the CALEX® Cap results. All statistical analyses were carried out with Analyse-it for Microsoft Excel.

RESULTS

The reproducibility CV of stool collection with the 10-mg sampling pin was 11.7%. The reproducibility CV of stool extraction using CALEX® Cap was 13.0%, while extraction by manual weighing resulted in 13.5% CV. The linear regression analysis of calprotectin levels measured in CALEX® Cap extracts and compared with the levels in the same stools extracted by manual weighing resulted in slope 0.95, bias +25, R^2 0.94. The stability of calprotectin (≤±20% deviation for each single sample as compared to t_0) in 15 stool extracts stored for 3 days at 23°C was given for 87% of CALEX® Cap, 60% of IDK Extract®, 73% of Calpro EasyExtract™ and 33% of RIDASCREEN® extracts. The average calprotectin recovery after 6 days at 23°C determined by Passing-Bablok was 95, 86, 63, and 134% for the 4 methods.

CONCLUSION

The performance of the CALEX® Cap device to quantitatively extract calprotectin from stools is as reliable as extraction with conventional weighing. The stability of Calprotectin in stool extracts stored at ambient temperature is given for at least 3 days when kept in the CALEX® Cap. Therefore, CALEX® Cap devices containing 10.5±1.3 mg stool in buffer solution after sampling can by sent from the collection site (ie. patients' homes or GP's offices) to the testing lab via normal postal mail.

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THE EFFECT OF A LONG-TERM STORAGE AND SINGLE FREEZE/THAW CYCLE ON HBA1C VALUES ASSAYED BY NGSP/IFCC CERTIFICATED HPLC METHOD

K. Bergmann[1], K. Olender[1], L. Szternel[1], G. Sypniewska[1]
[1]*Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Department of Laboratory Medicine*

BACKGROUND-AIM

Glycated hemoglobin (HbA1c) is considered as a "gold standard" in monitoring of diabetes. According to NGSP and IFCC standardization programs high-performance liquid chromatography (HPLC) is one of the certificated methods of HbA1c assay. Although HbA1c concentration is stable in biological material, many IVD manufacturers recommend to storage whole blood samples in a refrigerator for no longer than 1-2 weeks and avoid freezing. The aim of study was to evaluate the effect of a long-term storage and single freeze/thaw cycle on HbA1c values measured by commercially available HPLC method.

METHODS

Study included 95 whole blood samples collected from diabetic patients (n=38) and healthy volunteers (n=57). Fasting venous blood was drawn using sterile plastic tubes with potassium-EDTA and stored at 2-8°C. HbA1c was assayed by HPLC method on Bio-Rad D-10 (Bio-Rad Laboratories, CA, USA) analyzer with reportable range 18-179 mmol/mol (3,8-18,5%) and total precision CV=1,16%. According to manufacturer's manual, whole blood samples may be stored up to 7 days at 2-8°C. HbA1c concentration in fresh whole blood samples were measured within 1 day after collection and were frozen at -80°C for no longer than 3 months (mean 8,8±2,2 weeks). Samples were thawed in the refrigerator, brought to room temperature and thoroughly mixed prior to assay.

RESULTS

Mean HbA1c concentration was 51±20.2 mmol/mol (6.82±1.85%) for fresh and 50.5±20.1 mmol/mol (6.77±1.84%) for frozen/thawed samples. Reproducibility was 47.4% (n=45), while 43.1% results were slightly decreased (n=41; mean 1.6 mmol/mol) and 9.5% were increased (n=9; mean 2.8 mmol/mol). However, no significance differences in HbA1c levels were found between fresh and frozen/thawed samples in whole group (p=0.87), as well as in healthy (p=0.68) and diabetic subjects (p=0.91). Samples were also divided according to storage time. No significant differences in HbA1c concentration were found between fresh and frozen/thawed samples stored ≤5 weeks (47.6 vs. 47.5 mmol/mol; p=0.98), 6-9 weeks (52.1 vs. 51.9 mmol/mol; p=0.97) and ≥9 weeks (51.2 vs. 50.0 mmol/mol; p=0.79).

CONCLUSION

Samples storage at -80°C and a single freeze/thaw cycle do not affect the concentration of HbA1c measured by HPLC method.

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IT IS TIME TO AVOID THE USE OF SYRINGE DURING BLOOD COLLECTION BY VENIPUNCTURE

M.D.R. Campelo[2], J.D.R. Campelo[1], F. Dos Santos Gomes[1], L.M.A. Meirelles[1], G.L. Salvagno[4], G. Lippi[3], G.C. Guidi[4], G. Lima-oliveira[4]

[1]*AESPI*

[2]*Clinical Laboratory Bioanalise, Teresina, Piaui, Brazil.*

[3]*Department of Pathology and Laboratory Medicine, Clinical Chemistry and Haematology Laboratory, Academic Hospital of Parma*

[4]*Laboratory of Clinical Biochemistry, Department of Life and Reproduction Sciences, University of Verona*

BACKGROUND-AIM

Syringes, needles, holders and vacuum tubes are collectively known as specimen collection apparatus, whilst each one has its own technical characteristics devised for specific use. Needles are obtainable by manufacturers for either evacuated systems or in connection to syringe – in South America phlebotomists prefer to use straight syringe with a needle than to use vacuum tubes system. The aim of this study was to monitor the daily practices of phlebotomists during diagnostic blood specimen collection by venipuncture using straight syringe with a needle to identify potential nonconformities due this procedure.

METHODS

To evaluate the frequency of nonconformity – inappropriate tubes filling, hemolyzed samples, and presence of micro clots or fibrin filament – three phlebotomists were observed during blood collection procedure on 100 outpatients, using exclusively straight syringe with a needle to fill three different tubes (i.e., EDTA-, sodium citrate-, and lithium heparin-tube). Hemolysis index were measured on the same instrument Cobas 6000 <c501> module (Roche Diagnostics GmbH, Mannheim, Germany); inappropriate tubes filled, and either presence of micro clots or fibrin filament were indentified due to visual inspection by an laboratory quality manager. The frequency of nonconformity was classified regarding laboratory section (i.e., hematology, coagulation, and immunochemistry).

RESULTS

The frequency of inappropriate filled tubes were: 62% in hematology, 4% in coagulation, and 75% in immunochemistry. Fibrin filament were observed in immunochemistry on 5% of lithium heparin-tubes, whereas in coagulation section 2% of sodium citrated-tubes were clotted, and 5% of EDTA-tubes at hematology section showed micro clots. Samples showing higher hemolysis index were observed at coagulation section on 23% of sodium citrate-tubes, and at immunochemistry section on 12% of lithium heparin-tubes.

CONCLUSION

Since the unhappy frequency of nonconformity observed we suggest to laboratory managers to employ vacuum tubes systems than syringes to perform blood collection. Vacuum tubes systems are considered able to ensure the appropriate combination of blood and additives (i.e., anticoagulants or clot activators), so itself could minimize the nonconformities observed.

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HEPARINATE BUT NOT SERUM TUBES ARE SUSCEPTIBLE TO HEMOLYSIS (H) BY PNEUMATIC TUBE TRANSPORTATION (PTT)

S. Pasqualetti[1], D. Szőke[1], C. Valente[1], M. Panteghini[1]
[1]*Clinical Pathology Unit, "Luigi Sacco" University Hospital, Milan, Italy*

BACKGROUND-AIM

PTT may induce H in blood samples. Having recently introduced PTT (Sumetzberger, operated with an average speed of 2.5 m/s) in our hospital, we aimed to compare the H degree before (hand-delivered samples) and after PTT implementation and to verify a possible difference in susceptibility to H in lithium-heparin plasma (P) vs. serum samples collected in tubes with silica clot activator (S).

METHODS

H indices (HI) derived from absorbance measurements of samples diluted with saline (Abbott Architect c16000) for all P (BD tubes, cod. 368884) samples drawn by the Emergency Department in 2-month periods were retrospectively collected and pre- (n=3579) and post-PTT (n=3469) results compared. Particularly, we investigated the impact of PTT introduction on the following tests: LDH [HI threshold (HIt), 25], conjugated bilirubin (cBIL) (HIt, 30), K (HIt, 100) and ALT (HIt, 125), for which an H above the corresponding HIt does not allow reporting numeric values, but just the 'Hemolysis' comment. In addition, HI were retrieved for P and paired S (BD, cod. 369032) samples from the same venipuncture and results compared in pre- (n=501) and post-PTT (n=509) periods.

RESULTS

Median (5-95th percentile) HI in P samples was slightly but significantly higher in the post-PTT period [7 (0-112) vs. 6 (0-82), P<0.001]. After PTT implementation, the total number of results reported as 'Hemolysis' in P samples significantly increased (5.5% in pre-PTT vs. 8.0% in post-PTT, P<0.001). Investigated tests gave the following figures (total performed test number in parentheses): LDH, 13.4% pre-PTT (n=3218) vs. 18.8% post-PTT (n=3021), P<0.001; cBIL, 9.4% pre-PTT (n=53) vs. 27% post-PTT (n=37), P<0.05; K, 3.7% pre-PTT (n=3446) vs. 5.6% post-PTT (n=3420), P<0.001; ALT, 2.9% pre-PTT (n=3203) vs. 4.4% post-PTT (n=3177), P<0.01. The slightly higher susceptibility to H of S compared to paired P samples found in the pre-PTT [9 (1-64) vs. 6 (0-85)] was not confirmed in the post-PTT period [7 (0-90) vs. 8 (1-72)], in which median HI in S samples was significantly lower (P<0.001) than in pre-PTT.

CONCLUSION

In our setting PTT promotes H in P samples, increasing the rate of rejected tests. The use of S appears to protect against the hemolysing effect of PTT.

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STABILITY OF NEPHELOMETRIC ASSAY OF HUMAN URINE SAMPLES AFTER DIFFERENT STORAGE CONDITIONS.

M. Pieri[1], F. De Gregorio[1], V. Dinallo[1], F. Duranti[1], R. Zenobi[1], S. Bernardini[1], M. Dessi[1]
[1]*Department of Experimental Medicine and Surgery, "Tor Vergata" University Hospital, Rome (Italy).*

BACKGROUND-AIM

Pre-analytical factors, including storage procedures, result to be crucial to obtain reliable clinical results by the hospital laboratories. We explore routine testing of Free Light Chain (FLC) on urine samples, stored at different times and conditions. When the production of the immunoglobulin chains is unbalanced, the light chains produced in excess remain free in the plasma, pass through the renal filter and can appear in the urine.

METHODS

Urine samples were collected from 100 patients and analyzed on a BN ProSpec® System (Siemens Healthcare Diagnostics) using a nephelometric assay based on a mixture of monoclonal antibodies (N Latex FLC kit). Bias and imprecision (representing variation from analysis and storage) were calculated from values at baseline and after storage at different temperature and times. The differences were tested by Anova with Bonferroni Test post hoc.

RESULTS

We observed no statistically significant bias and imprecision between baseline and stored samples when kept for 24, 48 and 72 hours at +4°C or at -80 °C. While if the sample stored at -20°C have a significant bias more than 15%.

CONCLUSION

We conclude that urine tubes stored for 24, 48 and 72 hours at +4° C and at -80°C, may be suitable for routine analysis without restrictions for the determination of FLC.
We observed a large average decrease (16.1%) in the FLC level after storage at -20° of urine tubes.

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STABILITY STUDY OF SERUM SAMPLES STORED IN AN AUTOMATED REFRIGERATED MODULE CONNECTED TO APTIO AUTOMATION. COMPARISON WITH CONVENTIONAL REFRIGERATED SYSTEM.

N. Rico Santana[3], M. Parra Robert[2], S. Sandalinas[2], J. Alcaraz Quiles[2], I. Falcón[1], M. Perez [1], J.L. Bedini Chesa[3]
[1] *Core Laboratory/ Hospital Clínic, Barcelona*
[2]*Biochemistry and Molecular Genetic/Hospital Clínic, Barcelona*
[3]*Core Laboratory/ Hospital Clínic, Barcelona*

BACKGROUND-AIM

A good storage system for archiving samples is required in Clinical Laboratories. It is necessary to know for how long the different magnitudes are stable in specific storage conditions. Our laboratory has a new Aptio Automation (Siemens Healthcare Diagnostics) where the finished samples are archived at 4#C in a refrigerated storage module (RSM) after being sealed with an aluminum foil.
The aim of the study was to evaluate the stability of serum samples with the RSM. Secondly, we compared the results with those obtained in a previous study using a conventional refrigerated system, where the samples were manually stored, without previous sealing, in a refrigerated chamber (Alcaraz J., Rico N. et al. Revista del Laboratorio Clínico, 7(1), 9-16).

METHODS

A total of 50 serum samples were collected in serum separator tubes. For each of these samples 27 biochemical magnitudes were analyzed at 0 hours by an ADVIA 2400 Chemistry System (Siemens H.D.) connected to Aptio Automation. The 50 samples were divided in 5 sets of 10 samples. Each set was re-analyzed at one of the following times: 24, 48, 72, 96 and 120 hours, to avoid the evaporating effect. Before each analytical series a quality control was measured to assure the results comparability. The variation (Xt%) in the results was calculated by comparing the value at each time (Xt) with the initial value (X0), and it was expressed as a percentage change: (Xt%)=(Xt/X0)*100. The mean percentage change (Xmt%) for every set was calculated. Stability was evaluated according to the Total Limit of Change (TLC), which combines both analytical and biologic variation, TLC = ±√((1.65*CVa)2+ (0.5*CVb)2

RESULTS

A total of 26 out of 27 magnitudes were stable at the end of the study according to TLC criteria. Lactate dehydrogenase (LDH) was not stable at 72 hours observing a decrease in its level that was maintained until the end of the study. In the previous study (manual storage system) 9 biochemical magnitudes were not stable with an increase of their levels due to the evaporation process.

CONCLUSION

Automatic refrigerated system connected to Aptio Automation improves the serum samples stability. This system avoids the evaporation process due to the sealing of samples. The instability of LDH was not related to the storage refrigerated system.

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PREANALYTIC PHASE IN CRYOGLOBULIN DETECTION: NEW METHOD FOR SAMPLE TRANSPORT

M.T. Dell'abate[1], E. Torti[1], L. Colacicco[2], F. Gulli[3], C. Zuppi[2], G.L. Rapaccini[3], U. Basile[1]
[1]*Department of Laboratory Medicine, School of Medicine -Catholic University of the Sacred Heart. Rome, Italy*
[2]*Institute of Biochemistry School of Medicine -Catholic University of the Sacred Heart. Rome, Italy*
[3]*Institute of Internal Medicine, School of Medicine of the "Catholic University of the Sacred Heart. Rome, Italy*

BACKGROUND-AIM

Cryoglobulins are immunoglobulins which undergo reversible precipitation upon exposure to temperatures below 37°C and redissolve when warmed again. Cryoglobulins have a pathogenic role and are associated with a wide range of symptoms and manifestations. Their quantification requires strict pre-analytical protocol adhesion. In particular, the sample should be kept at a stable temperature of 37°C. Failure to ensure these critical conditions from sample collection may result in misdetection of cryoglobulins, to the detriment of the patient.
Furthermore since cryoglobulinemia is often associated to hepatitis C virus (HCV) infection, the adoption of a safe and disposable method is preferable.
The aim of our study is to evaluate the diagnostic efficacy of two different transport devices so as to assess their effectiveness by determining the cryoglobulinemic fractions obtained from serum stocked with both systems.

METHODS

Blood samples from 30 patients with positive cryoglobulinemia and presenting typical clinical cryoglobulinemic disease manifestations were collected into pre-warmed, anticoagulant-free tubes and immediately split into two aliquots. The two tubes were placed in either a plastic cup containing warm water at 37°C or in a thermos container lined with filter paper and filled with warm water at 37°C. Samples were left standing for 1hour at room temperature. Temperatures were recorded at the end of the incubation. Cryocrits were assessed and then reported as percentage of total serum.

RESULTS

After 1 hour incubation, recorded temperatures values differed considerably among the two devices. In particular, the plastic cup with warm water only, does not guarantee an adequate insulation system, which implies a drastic temperature drop, so low cryocrits are completely undetected. The filter paper thermos system instead limits heat dispersion and offers a more efficient recovery of cryoglobulinemic material from serum.

CONCLUSION

In light of these results, we concluded that the filter paper thermos system is an inexpensive and disposable way of carrying potentially infective blood samples. It is also easily accessible to laboratories and it may be widely used without difficulties, guaranteeing an adequate heat retention.

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A QUESTION TO CLINICIANS: WHICH IS THE BEST FORMULA TO REPORT THE IONIZED CALCIUM?

M. Duque Alcorta[1], M. Segovia Amaro[3], M.J. Alcaide Martin[2]
[1]*Core Laboratory, La Paz Hospital, Madrid*
[2]*Emergengy Laboratory, La Paz Hospital, Madrid*
[3]*Laboratory, La Paz Hospital, Madrid*

BACKGROUND-AIM

In adult, calcium is distributed in the bones(99%)and the soluble calcium(1%)is in the extracellular fluid (EF). In a non-diseased state,40%is bound to proteins,10%is bound to anions and the other half of the soluble calcium exists as free(biologically active).The narrow calcium range in EFcould imply laboratory critical result. IFCC recommended ion-selectiveelectrode assay by directpotentiometry (ISEDP) to measure ionized calcium(Ca+2). However this technology is not available in all laboratories, is more expensive and implies several obligations than total calcium(Cat).Therefore, measurement of Cat is the test that the clinicians use to know the calcium status in patients, but sometimes Cat could cover up a hypocalcemia.So it is important that the laboratory report Ca+2 as close to real concentration.Our aim is evaluate the formula to calculated Ca+2 based on albumin and total proteins

METHODS

83 patients with Ca+2 petition who attended emergency room (age:19-93 years;mean:65).Ca+2 was measured by ISEDP (ABL90).Cat, albumin (alb) and total proteins (tp) were performed in DimensionVista1500(DV) and Advia2400(Ad).The principle of procedure; DV:alb:bromocresol(bc) purple and Cat:purple complex. Ad:alb:bc green and Cat:arsenazoIII. tp is a modification of the Biuret reaction in both.We chose these formula to calculate Cat:1.Based on alb: Catad=Catm+0.8(4-alb(g/dL));2.Based on tp:Catad=Catm/[0.55+(tp(g/dL)/16)]3.Ca+2ad(mg/dL)=[6*Catm-(0.19*tp(g/dL)+alb(g/dL)/)/3)]/(0.19*tp(g/dL)+alb(g/dL)+6). Catad:Cat adjusted (mg/dL);Catm Cat measured(mg/dL);Ca+2ad ionized calcium adjusted.The relationship between measured and the corresponding value calculated was studied with StatisPro and if attended to the minimum biological variation (confidence interval 95%) at clinical decision levels (CDL).Total error accepted Ca+2:3.1%.

RESULTS

CDL: 4, 4.8 and 5mg/dL. Allowed difference:0.124,0.149 and 0.155, respectively. Level 4, in DV calcium difference (Cad) 0.434,0.426,0.496 based on formula 1,2 and 3 respectively, and 0.356,0.310,0.329 in Ad. Level 4.8, in DV Cad -0.114,0.015,-0.153 and 0.029,-0.015,-0.042 in Ad. Level 5, in DV Cat -0.250,-0.187,-0.316 and in Ad -0.053,-0.096,-0.135

CONCLUSION

Based in our results we could conclude that in CL we can report the Ca+2 with all the formula but only in the CDL 4.8 and 5, because fulfil minimum biological variation, while in EL none of the formulas studied. Therefore, each laboratory should validate the formula that best adjust to the population attended

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PROZONE EFFECT: WHAT IS THE BEST SOLUTION?

R. Lamanna[1], M. Benecchi[1], S. Giuliodori[1], P. Zanelli[1]
[1]*Immunogenetics Laboratory, Unit of Medical Genetics, Parma University Hospital, Italy*

BACKGROUND-AIM

Human leukocyte antigen (HLA) antibodies develop in many allograft recipients, with associated graft loss that may occurs years later. Luminex®-based methodologies, including Single Antigen class I and II test (LSA I, II; One Lambda), have been adopted to identify HLA antibodies and to define forbidden donor antigens. Although Luminex® assays excellent sensitivity, sometimes false-negative reactions occur. This phenomenon is known as the "prozone effect", due to the presence of high-titer HLA antibodies or HLA-specific IgM antibodies. Recent experimental data have related this artifact to direct block of IgG detection by complement component C1, by a competition for the alloantibody between the fluorescent anti-IgG conjugate and serum complement. Sera treatment with ethylene-diamine tetraacetic acid (EDTA) abolishes the prozone effect. We explored this effect in our cohort of kidney transplant candidates and transplanted patients.

METHODS

We compared the median fluorescence intensity (MFI) values obtained performing LSA I and II test among our cohort of 40 serum samples from immunized renal waiting list patients and transplanted patients, in the EDTA-treated and non-treated conditions, to describe the impact of the prozone phenomenon on antibody profile.

RESULTS

Our results showed that EDTA treatment abolished the drop/rebound effect, both for HLA class I and II, as explained in literature. This is evident in immmunized patients with MFI value > 10000, while no differences have found in patients with lower MFI levels (<10000). The treatment substantially increased MFI values of both class I and II HLA antibodies, resulting in an overall increase of the proportions of IgG positive single reactions.

CONCLUSION

The prozone phenomenon potentially affects the results of solid-phase HLA antibody Luminex® detection. Falsely low or negative test result, especially in case of dense IgG binding, may impair accuracy of LSA test. Our study confirmed the role of complement activation as a key mechanism of the prozone phenomenon in LSA assays and reinforced the utility of EDTA treatment, expecially in particular kind of patients, such as immunized subjects. This provided a basis for the establishment of more selective strategies to contrast the prozone effect.

Patient management, biological sample management

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PATIENT EMPOWERMENT IN LABORATORY TESTING: OPINIONS AND EXPERIENCES OF PATIENTS AND GENERAL PRACTITIONERS

W.P. Verboeket-van De Venne[1], A.M. Hendriks-dybicz[1], W.P. Oosterhuis[1]
[1]*Department of Clinical Chemistry, Atrium-Orbis Medical Centre, Heerlen, the Netherlands*

BACKGROUND-AIM

Patient empowerment fits the trend that patients prefer to be better informed. This applies equally to the results of laboratory testing. Patients want to know which tests are done, and – more importantly – what the results of the tests mean with respect to their health condition. The direct provision of test results to patients by the laboratory is not common practice in the Netherlands. The aim of the study was to inform patients about their test results including interpretative comments, and to evaluate opinions and experiences regarding this procedure.

METHODS

Four general practitioners (GPs) participated in the study. They each randomly recruited 10 patients in whom blood tests were requested. The sample was analysed and an interpretative comment in layman's terms was added, as well as links to additional information on the internet. After approval of the GP, the printed laboratory report was sent to the patient by post. Subsequently, patients were interviewed using topic lists to determine how they perceived this procedure. The topic list contained 8 opinion questions on clarity and comprehensibility of the laboratory report, and overall experience with the procedure. Answers were scaled into three categories and processed quantitatively. The participating GPs were interviewed before and after the study.

RESULTS

Results were complete for 38 patients (21 m, 17 f), aged 18-86 years. In total, 89% of the patients were positive about this way of providing explanatory information on laboratory results. They indicated that they were better informed and would like to receive this information with blood sampling in the future. Before the study, GPs indicated that they frequently received questions from patients regarding the meaning of their test results. Consequently, GPs experienced an increased quality of the subsequent appointment with the patient, since interpretation of test results was already given by the laboratory.

CONCLUSION

By giving patients access to their results – including explanation and background information – control over their treatment will be enhanced. It is anticipated that patients that are well informed about their own health will participate more in treatment decisions and are often better motivated to adhere to treatment.

Patient management, biological sample management

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STABILITY EVALUATION OF IMATINIB, DASATINIB AND NILOTINIB IN DRIED PLASMA SPOTS FOR A FUTURE WIDESPREAD USE OF THERAPEUTIC DRUG MONITORING

A. Ariaudo[1], M. Simiele[1], L. Paglietti[1], S. De Francia[2], G. Di Perri[1], A. D'avolio[1]

[1]*Laboratory of Clinical Pharmacology and Pharmacogenetics. Department of Medical Sciences, University of Turin, Amedeo di Savoia Hospital, Turin, Italy*

[2]*Laboratory of Clinical Pharmacology. Department of Clinical and Biological Sciences, University of Turin, San Luigi Hospital, Orbassano (Turin), Italy*

BACKGROUND-AIM

Imatinib, dasatinib and nilotinib are potent anti-cancer drugs, belonging to the class of tyrosine kinase inhibitors (TKIs), effective in the treatment of chronic myeloid leukemia. TKIs show a wide inter-individual variability and a good concentration-effect relationship, then therapeutic drug monitoring (TDM) of these compounds represents an important tool for a better therapy management, improving therapeutic efficacy, avoiding hematological toxicity, often presented in treated patients.
The aim of our study was to evaluate stability of TKIs in dried plasma spots (DPS), developing also a new UHPLC MS/MS method for the quantification of the drugs.

METHODS

Stability of dasatinib, nilotinib, imatinib and desmethyl imatinib on DPS was evaluated at least for two months at four different storage temperature: -20°C, 4°C, room temperature and 35°C, for three different concentration of drugs, corresponding to each concentration of internal quality control (QC) for each analyte, and compared to fresh daily prepared DPS.
Briefly, for each QC, plasma were spotted on a DPS device, then it was inserted into a tube for extraction. Quinoxaline was used as internal standard. 1800 µl of extraction solution (75/25 Dichlomethane-TBME) and 200 µl of basic solution (15% ammonia solution) were added to each tube. After evaporation step at 60° C, samples were reconstituted with 200 µl of H2O/ACN 60:40. 10 µl were injected into an UHPLC coupled with an MS/MS detector and compounds separation was obtained using a gradient run at flow rate of 0.4 mL/min.

RESULTS

No significant degradation of the drugs was observed (below 20%) in all storage conditions and for all QCs concentrations. Data about intra-day and inter-day accuracy and precision of analytes for the developed and validated method were consistent to FDA guidelines.
The mean recovery were 86% for imatinib and 70% for desmethyl imatinib, 65% for dasatinib and 65% for nilotinib. No matrix effect was observed.

CONCLUSION

Our developed and validated method has allowed to demonstrate for the first time the stability of TKIs drugs on DPS. Widespread availability of TKIs TDM could be improved by the introduction of using DPS for samples collection, for a safe and low cost samples shipment.

Patient management, biological sample management

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ARE PATIENTS WELL INFORMED ABOUT THE INFLUENCE OF OTC DRUGS, FOOD SUPPLEMENTS AND PREANALYTICAL FACTORS ON LABORATORY TESTS RESULTS?

P. Filipi[1], A. Vrtaric[1], M. Miler[1], N. Nikolac[1], A. Simundic[1]
[1]*University Department of Chemistry, Sestre milosrdnice University Hospital Center*

BACKGROUND-AIM

Consumption of some over the counter (OTC) drugs and food supplements can affect laboratory results. Therefore, the aim of this study was to assess the frequency of consumption of these preparations and the level of knowledge of their influence on the laboratory tests results in an outpatient hospital setting.

METHODS

The study included 200 outpatients who were referred to University Department of Chemistry for laboratory testing and voluntarily agreed to participate in the study. The survey was anonymous and performed in the form of interviews. It included questions about the frequency of consumption of various products, awareness of the importance of informing physicians and laboratory staff about it, and information about influence of preanalytical variables on the laboratory test results. Statistical analysis was performed using Microsoft Excel and chi-square test in MedCalc (Mariakerke, Belgium). Data are presented as numbers and percentages.

RESULTS

Out of total number of participants, 66% were female, and the most common age group is 46-65 years (38%). Results showed that 81% of patients take some preparations, mostly minerals (50%), vitamins (47%) and cranberry extract or tea (33%). Women were taking preparations more frequently than men (86% vs. 69%, P=0.008), while there was no difference between age groups (P=0.117). Majority of patients (52%) consider that it is not necessary to notify the laboratory staff about the consumption of preparations. However, 72% patients think that it is necessary to inform their physicians, even though only 53% of them did that. Patients recognized that alcohol (83%), physical activity (44%), grapefruit (23%) and broccoli (12%) can influence laboratory results. However, 47% think that coffee can affect laboratory results if taken the day before blood sampling. Also, 53% patients think that consumption of any of various products and food supplements doesn't affect result.

CONCLUSION

A large number of patients is taking food supplements and various OTC drugs and they are not sufficiently informed and aware about its potential impact on the laboratory tests results. Low level of knowledge and awareness about the influence of some preparations and preanalytical factors showed an urgent need for additional education.