containing malignant cells have CKBB which appears different from CKBB of brain or prostatic fluid origin. This information suggests that each variety of CKBB may have unique antigenic determinants; thus, immunoassays developed against CKBB from malignant effusions might have the highest sensitivity for detecting cancer associated with metastatic pleural effusions. Similarly, antibodies to prostate CKBB might be particularly useful in detecting prostatic disease. In addition, this information may help to explain the increased incidence of autoantibodies to CKBB in patiencs with malignancies as compared to individuals with circulating CKBB from benign sources, such as infarcted bowel. Other similar applications of 2-D may change current concepts of laboratory testing of proteins based on immunochemical techniques.

055 FINDINGS WITH A SENSITIVE FLUOROMETRIC METHOD FOR THE REGAN ISOENZYME.

H. Muensch, W. Maslow, and F. Carlson (City of Hope National Medical Center, Duarte, CA 91010 (Sponsor: Peter Dewhurst).

The Regan enzyme, a heat stable alkaline phosphatase (HSAP), was first reported by Fishman in 1968, and has since been demonstrated in tumor tissue and in the serum of patients with a variety of neoplasms. In its immunological, kinetic, and electrophoretic properties the enzyme is related to the placental alkaline phosphatase of pregnancy.

A method was developed for the determination of HSAP, using the substrate naphthol-ASMX-phosphate, which gives a fluorescent reaction product.

1 ml aliquots of serum are heated for 7 minutes at 65°C in order to inactivate all other alkaline phosphatase isoenzymes. Residual activity is allowed to react in the presence of 5 mMol naphthol-ASMX-phosphate at pH 10.2 for 15 minutes at 37°C. The reaction is stopped by the addition of acetone. Precipitated protein is removed by centrifugation, and the liberated naphthol-ASMX is measured in the decanted supernatant against an individual blank for each sample fluorometrically.

In 31 day to day determinations of controls, the following results were obtained: Control I had a mean value of 1.48 + 0.06 IU/L with a c.v. of 4.3% and Control II 0.080 + 0.003 IU/L with a c.v. 5.7%. HSAP determined in 53 healthy non-smoking, non-pregnant adults accounted for a fraction of 0.02-0.75% of the total alkaline phosphatase activity in serum. The mean value of activity in this population was 0.067 IU/L serum with a range of 0-0.15 IU/L. A markedly elevated value was found in 23 healthy smokers who had a mean value of 0.44 IU/L and a range of 0.10-1.50 IU/L. Non-smoking patients with neoplasm, in which abnormal HSAP levels followed extent of disease ranged up to 30 IU/L serum. In a high percentage they overlapped with the levels, found in smokers. The enzyme activity found in non-smokers, smokers, and patients with tumors was compared with the placental enzyme. Immunologic, kinetic, and heat stability properties were found to be similar.

We conclude that the described assay has a sensitivity and precision making it suitable as a potential tumor marker assay in cancer patients. A well defined normal range can be established provided smokers and pregnant individuals are excluded.

056 USEFULNESS OF SERUM LD-1 ISOENZYME IN PATIENTS WITH EXTRAGONADAL GERM CELL TUMORS.

Frank J. Liu, Herbert A. Fritsche, Jose M. Trujillo, Melvin L. Samuels, Christopher J. Logothetis, and Antohio Trindade (Dept. of Lab. Med. and Dept. of Med., Univ. of Texas System Cancer Ctr., M.D. Anderson Hospital, Houston, Texas)

Serum LD isoenzyme electrophoretic patterns were determined serially in 22 patients with extragonadal germ cell tumors. We assessed the serum LD-1 activity in terms of both its absolute and relative values. An LD-1 value in absolute units greater than 52 IU/L with the LD-1/total LD ratio greater than 37% was considered to be criterion 1 elevation. An absolute value of LD-1 less than 52 IU/L but an LD-1/total LD value greater than 37% was classified as criterion 2 elevation. A criterion 3 elevation consisted of either an absolute value of LD-1 greater than 52 IU/L with the relative ratio LD-1/total LD less than 37% and the LD-5/LD-1 ratio less than 0.5, or the LD-5/LD-1 ratio greater than 0.5 but LD-1/LD-2 ratio greater than or equal to 1.

Using these interpretive criteria, we have observed abnormal serum LD-1 levels in 77% (17/22) of patients with extragonadal germ cell tumors. Abnormal serum LD-1 values were found in 60% (3/5) of patients with pure seminoma (2 mediastinal and 3 retroperitoneal), in 90% (9/10) of patients with pure embryonal carcinoma (6 mediastinal and 4 retroperitoneal), in 100% (4/4) of patients with teratoma mixed with embryonal carcinoma (3 mediastinal and 1 retroperitoneal) and in 50% (1/2) of patients with choriocarcinoma (1 mediastinal and 1 retroperitoneal). One patient with malignant teratoma did not show abnormal LD-1 values. All ten patients with liver metastases showed abnormal serum LD-1 values. Serum LD-1 values decreased with response to therapy and increased with progression of disease. In conclusion, serial measurements of serum LD-1 values reflect the response of patients to therapy and their disease activity.

057 SERUM LACTATE DEHYDROGENASE ISOENZYMES IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

Shih-Ching Lee, Ming-Ching Kao and Shih-Jiun Yin. Tri-Service General Hospital and National Defense Medical Center, Taipei, Taiwan, Republic of China

The distribution of lactate dehydrogense(LDH) isoenzymes in the serum of 31 patients with hepatocellular carcinoma was studied by polyacrylamide disc gel electrophoresis. Results showed that relative ratios of LDH4 and LDH5 were significantly increased in the patients. A significant increase in the LDH5 activity ratio was present in 26 out of the 31 patients(84%), and the LDH4 ratio in 22 of the 31 patients(71%). The proportions of LDH1-3 activity, correspondingly, showed slightly reduced.The percentage of patients with elevations of alpha-fetoprotein (AFP) and abnormal LDH5 isoenzymes was 74%, and the false negative and false positive rates were both 10% with respect to the presense of abnormal AFP level. An elevation of the activities of aspartate amiaotransferase, alkaline phosphatase, LDH and bilirubin content as well as a decrease in albumin content was also observed in the patient sera. The present study suggests that the electrophoretic analysis of serum LDH isoenzymes would be a useful aid to the diagnosis of hepatocellular carcinoma.

058 ATYPICAL CATHODICALLY MIGRATING CREATINE KINASE: A POSSIBLE TUMOR-ASSOCIATED ENZYME MARKER FOR DISSEMINATED MALIGNANCY.

Tsan Z. Liu (Ctr. for Adv. Med. Tech., San Francisco State Univ., S.F., CA 94132), Young S. Kim (GI Res. Lab. VA Med. Ctr, S.F., CA 94121), and Stephen B. Shohet (Cancer Res. Inst , Univ. of Calif., S.F., CA 94143).

We investigated the possibility that atypical cathodically migrating creatine kinase isoenzyme (MCK) might serve as a general tumor marker. Sera from 83 patients with various types of malignant diseases were examined. The atypical enzyme MCK was found in 87% (72) of these samples. MCK was present in 96% (22 of 23) of samples from patients with histologically proven metastatic carcinoma. In contrast, less than 2% (5 of 400) of random samples from hospitalized patients with nonmalignant disease showed the presence of MCK. The brain-related form of creatine kinase (CK-BB), which has been previously proposed as a tumor marker, was found in only 15% (13 of 83) of all samples of cancer sera tested. The presence of CK-BB in samples of sera from metastatic carcinoma was 30% (7 of 23).

In homogenates from cultured colon and pancreatic tumor cell lines, we found MCK in the cytosol fractions of 7 of 7 cultured lines studied, but not in the normal skin fibroblast control. This apparently tumor-related MCK was resistant to heat inactivation at 56°C. In contrast, the CK-BB enzyme, which was also found in these homogenates, was completely heat inactivated. Treatment with urea shifted the electrophoretic migration of MCK to a position just cathodic to the origin, supporting the concept that MCK is probably of mitochondrial origin.

Our data suggest that this new variant of serum creatine kinase, MCK, which is found in the majority of patients with various malignant disorders, together with CK-BB, may serve as a useful enzyme marker for disseminated malignancy.

16

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059 GLYCYLPROLINE DIPEPTIDYL AMINOPEPTIDASE ACTIVITY IN NORMAL CHINESE AND PATIENT WITH LIVER DISEASES

Kwang-Jen Hsiao, Shew-Jen Wu, and Wen-Kuo Ting. Dept. of Laboratory and Dept. of Medical Research; Veterans General Hospital; Taipei, Taiwan 112; R. O. C.

Glycylproline Dipeptidyl Aminopeptidase(GPDAP) is an enzyme which cleaves the N-terminal glycylproline from peptides. The serum GPDAP activity was shown abnormal in different pathological conditions. In the present study, we used glycylporline-p-nitroanilide (a gift from Dr. Y. Kasahara, Fujizoki Pharm. Co., Tokyo) as substrate to determine the serum GPDAP activity kinetically at 37°C and pH 7.9. The within-run and run-to-run precision of the test were 0.4-0.7% (C.V.) and 0.6-2.6% (C.V.) respectively.

The reference range for normal Chinese was determined to be 68.2±16.0 U/L (mean±SD; range 38.9-100.5) from 140 apparently healthy adults (age 24-79 years). There was no significant difference between male (70.2±15.1 U/L) and female (66.3±16.6 U/L), (p>0.1, n=70 each). These data generally agreed with values reported for other populations. The serum GPDAP activity

of patients with liver diseases was significantly ($p<0.001$) higher than the normal control: 125.1±40.5 U/L (range 69.1-200.2) in 10 acute hepatitis, 119±38.8 U/L (41.9-221.8) in 42 chronic active hepatitis, 93.5±38.5 U/L (34.0-228.5) in 45 cirrhosis, and 186.7±116.9 U/L (61.5-681) in 40 primary hepatoma. Its activity increased in the order of cirrhosis, hepatitis and hepatoma. Preliminary result also has shown the GPDAP content based on per mg of protein was increased in hepatoma tissue, which may be the cause of the elevation of serum GPDAP activity in hepatoma patients. Those results indicated that the elevation of serum GPDAP maybe used as a diagnositic marker for liver diseases.

060 PATTERNS OF SERUM TOTAL AND ISOENZYME LACTATE DEHYDROGENASE IN BURKITT'S LYMPHOMA PATIENTS.

Ronald J. Elin and Gyorgy Csako, Clin. Chem. Service, Clin. Pathol. Dept., Clinical Center, NIH, Bethesda, MD 20205

Burkitt's lymphoma is a relatively rare, rapidly growing malignant tumor. The tumor tissue contains a high quantity of the enzyme lactate dehydrogenase (LDH). We studied the patterns of serum total and isoenzyme LDH in 45 patients with the tissue diagnosis of Burkitt's lymphoma. The patients were grouped according to their disease status: presentation, first induction of remission, remission, relapse and preterminal relapse. Serum total LDH was determined with SMAC (Technicon) and LDH isoenzymes by electrophoretic separation on agarose gel (Corning).

Serum total LDH activity correlated with the tumor mass as determined by clinical staging at presentation or disease status. Chemotherapy greatly elevated the serum total LDH activity.

The isoenzyme pattern showed the largest relative increase in LDH3 and LDH4, but the highest isoenzyme activity (U/L) occurred with LDH2 and LDH3. Serial determination of serum LDH isoenzymes in patients during the first induction of remission or with preterminal relapse with chemotherapy showed that the isoenzyme changes are recognized better by expressing the fractions in enzyme activities rather than in relative percentages. For example, as the serum total LDH increased, LDH2 decreased when expressed as a relative percentage, but increased commensurate with the serum total LDH when expressed as enzyme activity. Thus, the results indicate that serum total and isoenzyme LDH activities are of value in following and assessing the tumor burden in patients with Burkitt's lymphoma. Furthermore, expressing the isoenzyme concentrations in terms of enzymatic activity rather than relative percentages provides a more accurate isoenzyme profile.

061 THE KEY CONTROL - A UNIQUE INTERLABORATORY APPROACH TO ASSESSING ACCURACY FOR RIA PROCEDURES. D.J. Hassemer, A. Stencel and R.H. Laessig; State Lab of Hygiene, Madison, WI 53706

Establishing an accuracy base is the most difficult problem when using RIA "kit" procedures. Concerns include: the integral nature of "kit" components; frequent lot changes; lack of reference methods; limited usefulness of periodic (e.g. quarterly) external quality control programs; and slow turn around of outside survey results. The Wisconsin RIA Survey offers over 100 labs a system for monitoring accuracy. Objectives include: (1) long term evaluation of performance by a single control material; (2) self-evaluation using "graded" results; (3) performance data summarized by individual "kits" and (4) rapid turn around. The use of the KEY control enables individual laboratories to better understand both aggregate survey results and their own data for use as an accuracy base. The KEY control, analyzed by every lab, every month, provides a baseline, enabling the participant to differentiate intra-lab problems (precision, bias) from changes in accuracy due to method failure or inter-lot variation in kits. Sample free T-4 data:

Month	3	6	9	12	15	18	
Method A (n=6)	2.25	2.08	2.30	2.30	2.71	2.70	ng/dl
Method B (n=6)	2.08	1.95	1.88	1.97	1.90	1.90	ng/dl

The mean shift in Method A (months 12-15) is not observed in Method B, implying a change in the "kit" supplied by the manufacturer. The intra group range eliminates the possibility of a single lot problem. The consistent performance of "B" verifies the stability of the control and eliminates possible specimen related effects. Hence the conclusion: a fundamental change in method. Conventional Survey techniques, without baseline data would make this conclusion difficult.

062 CORRECTION OF BLOOD GAS QUALITY CONTROL RESULTS TO STANDARD EQUILIBRATION TEMPERATURE. D. C. DeGuzman, N. L. Smith, and D. L. McCullough. Fisher Scientific Co., 526 Rt. 303, Orangeburg, N.Y. 10962.

PO_2 values for hemoglobin-based blood gas controls (measured at 37° in commercial analyzers) increase as temperature of the control decreases. This behavior is related both to O_2 solubility in H_2O and to change in hemoglobin's O_2 binding constant with temperature. Since many laboratories equilibrate their controls to ambient temperature, we investigated correcting PRIME (Fisher Scientific Co.) control results from 18° or 32° (extremes for ambient) to 25°. Ratios of mean PO_2 values (IL-813) for 3 lots of each level are presented. Mean slopes M for each parameter of each level were calculated from linear regression slopes.

PRIME LEVEL	RATIO OF OBSERVED PO_2 VALUES $18°/25°$	$32°/25°$	MEAN SLOPES M $\Delta PO_2/\Delta T$ (mmHg/C°)	$\Delta PCO_2/\Delta T$ (mmHg/C°)	$\Delta pH/\Delta T$ (units/C°)
I	1.063 ±0.006	0.921 ±0.016	-1.58	-0.0424	+0.000361
II	1.098 ±0.006	0.858 ±0.019	-1.77	-0.0926	+0.000805
III	1.217 ±0.046	0.815 ±0.025	-1.58	-0.267	+0.00152

For a value P obtained at a temperature T between 18° and 32°, calculate the expected 25° result using the relationship:

$$P(25°, corr) = P(T) + M\,(25° - T°C)$$

Comparing corrected PO_2 results to the observed 25° mean, values from 18° and 32° agreed ± 2 mmHg. Although not necessary for most proficiency testing, correction of 18° and 32° results for PCO_2 and pH values can be made with the same calculation.

063 MICROPROCESSOR CONTROLLED SPECTROPHOTOMETRIC PERFORMANCE CHECKS FOR USE WITH BECKMAN LIQ-QA-PAC™ QUALITY ASSURANCE KIT.

J. Anderson, W. Kaye, M. Matlack, and L. Sun. Beckman Instruments Incorporated, 200 S. Kraemer Boulevard, Brea, CA 92621.

Microprocessor controlled spectrophotometric check algorithms have been developed and incorporated into the Beckman Model 42 Spectrophotometer. They are designed to be used in conjunction with Beckman's Liq-QA-Pac™ Quality Assurance Kit or appropriate NBS standards.

Wavelength accuracy is verified by performing an automatic limited wavelength scan over an interval centered at the expected absorbance maximum of an appropriate reference material. Liq-QA-Pac wavelength test solution, a samarium and neodymium chloride soln, provides well isolated peaks at 401 and 576 nm.

Photometric accuracy and linearity performance checks are obtained by making measurements on replicate samples of at least 3 known absorbance levels of an appropriate reference material. The AVG. and CV of each set of replicates are determined. A complete analysis of variance, including linear regression and lack-of-fit components, is accomplished. Relative photometric accuracy is evaluated by comparing the AVG., slope, and intercept with their expected values. Photometric linearity is automatically evaluated by use of a statistical F-test comparing the lack-of-fit component of variance to the replicate variance. Liq-QA-Pac photometric check solutions contain cobaltous ammonium sulfate. Three absorbance levels are supplied. Expected values and performance ranges are assigned for each lot.

Instrument noise and stray light check algorithms are also presented. These performance checks provide an easy and complete method of Quality Assurance.

064 NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS PROPOSED GUIDELINE EP5-P: PROTOCOL FOR USER EVALUATION OF PRECISION PERFORMANCE OF CLINICAL CHEMISTRY DEVICES. John W. Kennedy, Chairman, Subcommittee on User Evaluation of Precision. (Medstat Consultants, Plainsboro, NJ 08536)

This document is the first in a series of User Evaluation Protocol Guidelines from the NCCLS. The members of the Subcommittee involved in this effort were C.Garber (U.Wisconsin), S.Bauer and J.Levine (Technicon), I.Osberg (U.Colorado), R.N. Carey (Peninsula Hosp.,Salisbury MD), M.McLean (Beckman), H. Lee (FDA/BMD), V.Leitz (Electronucleonics), E.Sylvestre and R.Coolen (Kodak), and S.Steindel (Piedmont Hosp.,Atlanta), along with other advisors from industry and professional organizations.

These guidelines are intended for the use of the individual