346 The enzyme immunoassay of adenosine cyclic 3',5'-monophosphate.

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Succinyl c-AMP,coupled to human albumin,was injected into rabbit to elicit antibodies. Conjugation is accomplished by a carbodiimide coupling procedure. The cyclic AMP was conjugated to porcine thyroglobulin with carbodiimide as conjugated antigen. Later on, this conjugate, captured to microplate with coating buffer and then incubated (blocked) with 1 % gelatin for 30 minutes, was bound by antibody in inverse proportion to free cyclic AMP in a sample or standard. Bound antibody was then quantified with horseraddish peroxidase-labelled goat anti-rabbit immunoglobulin and ABTS. Our results showed that both standard and sample cAMP concentrations could be measured as low as 0.5 pmol/well. The recovery of added cAMP in plasma and urine was in the range of 90% and 99%.The intra-assay coefficient of variation was 6% for tissue buffer samples. The coefficients of variation for samples were less than 8.9%.It was shown that the cross-reactivity of antisera with cGMP was 5 % , but not with ADP, 5'-AMP and ATP. The results from our study are well correlated with those from RIA study.

347 A RAPID SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE ANALYSIS OF ACETAMINOPHEN IN URINE.

T.-Z. Liu and L. Lin.* School of Medical Technology, Chang Gung Medical College, Taoyuan, Taiwan, R.O.C.

Although qunatitation of urinary acetaminophen may not constitute an emergency screening procedure, it can be of value in the confirmation of the presence of this drug in the poisoning cases.

A rapid spectrophotometric method for quantitative analysis of acetaminophen in urine has been developed. This method has advantage of eliminating the interference caused by urinary catecholamine metabolites. The proposed method is based upon the hydrolysis of the drug, in a heated acidic solution, to p-aminophenol. The latter compound is then allowed to react with p-dimethylaminocinnamaldehyde(DACA) in citrate-HCl buffer to yield an orange red colored compound, which can measured by a conventional spectrophotometer at 520 nm, Absorbance and acetaminphen concentration are linearly related from 50 to 1,000 mg/L. The method is accurate; day-to-day CV's for two pooled control specimen (95 and 195 mg/l L) were 4.2 and 3.6%. Correlation study with an established HPLC method and with o-cresol procedure, showed correlation coefficients of 0.993 and 0.994, respectively. The method is not subjected to interference by HVA, VMA, salicylate and salicylamide.

348 SPECTROPHOTOMETRIC ASSAY FOR CATALASE ACTIVITY BASED ON THE INHIBITION OF H_2O_2-DEPENDENT, PEROXIDASE-CATALYZED OXIDATIVE COUPLING REACTION.

Jamin Wang,L. Li,D.-T.Y.Chiu and T.-Z. Liu. School of Medical Technology, Chang Gung Medical College, Taoyuan, Taiwan, R.O.C.

Catalase activity has previously been determined by either monitoring the disappearance of substrate at 240 nm or by a chemiluminescent approach using luminol. These methods, though simple, require the use of highly specialized instruments.

We have developed a rapid and simple spectrophotometric method for the determination of catalase activity. The method is based upon the capability of catalase to inhibit H_2O_2-dependent, peroxidase-catalyzed oxidative coupling of phenolic compounds (e.g.,thymol) and 4-aminoantipyrine to form a pink-colored quinoneimine dye reaction. The degree of inhibition of the above-mentioned reaction has been shown to be linearly related to catalase activity from 0 to 40 U/ml. This linearity range substantially exceeds to that of the U.V. method. Within-run precision is satisfactory(CV= 5.0 %). Correaltion study with an established U.V. method showed a correlation coefficient of 0.96. The proposed method has been used to determine catalase activities in the red cells of normal and G6PDH-deficient RBCs and the performance has been shown to be satisfactory.

349 A MICROPLATE METHOD FOR DETERMINATION OF GLUCOSE IN PLASMA

MS Shih*[1], SJ Wu[1], CT Yeh[1], SH Chiang[1], KJ Hsiao[1,2]. Dept. of Med. Res[1]., Veterans General Hospital-Taipei; Inst. of Genetics[2], National Yang-Ming Med. College; Taipei, Taiwan, R.O.C.

Since measurement of absorbance in determination of plasma glucose in routine clinical chemical analysis is relatively slow (approx. 3 samples/min), a microplate method for fast determining blood glucose in plasma was developed. For the glucose determination, plasma and normal saline (1:3 , v/v) was mixed to microplate, then 10μl mixture was transferred to another microplate and 250μl hexokinase reagent was added by robotic diluter (Tecan 5031). This reaction mixture was incubated for 5 min at room temperature to reach reaction end point. The concentration of glucose in the reaction mixture was then determined by spectrophotometry (340 nm /620 nm) with ELISA reader (96 samples/min). The within-run and between-run imprecision were 3.7-5.5% (n=20) and 3.0-7.2% (n=10), respectively. The linearity of the analysis was good up to 350 mg/dl (r = 0.997) and the detection limit was estimated to be 8.4 mg/dl. The correlation of this microplate method and the routine clinical chemical method (Gilford 103 system) was good (r=0.926, n=553). This rapid and sensitive method is useful when large amount of specimens are required to be determined at the same time, such as mass screening and epidemiological study of diabetes mellitus.

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