immunofixation (2), and beta-mercaptoethanol reduction (3) of serum, with subsequent zone electrophoresis (Figure 1). In each instance, such reduction caused the band to disappear. The immunofixation and immunoelectrophoresis procedures confirmed the presence of polymeric, polyclonal IgM. One would expect that a discrete band would still be present on zone electrophoresis after reduction if IgM were other than polyclonal and polymeric.

This technique offers the advantage of savings in terms of expensive reagents and technologists' time, and provides a rapid screen for polymeric IgM.

References

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Laboratory-Prepared Reagent for the Beckman Creatinine Analyzer

To the Editor:

In the interests of economy, we investigated the possibility of using laboratory-prepared reagent with the Beckman Creatinine Analyzer 2 (Beckman Instruments, Inc., Fullerton, CA 92634).

Preparation of the reagent is according to principles published by Heinegard and Tiderstrom (1). An alkaline buffer containing 0.5 mol/L sodium hydroxide (1 L), 0.1 mol/L sodium phosphate (0.5 L), 0.1 mol/L sodium borate (0.5 L), and 40 g/L sodium dodecylsulfate (0.5 L) (Sigma Chemical Co., St. Louis, MO 63178; cat no. L-5750) is prepared. To prepare the working creatinine reagent, mix the alkaline buffer (2.5 L) with 0.626 L of saturated picric acid.

Linearity of the Analyzer was unchanged when we used the reagent described above; linearity to at least 2.2 mmol-L\(^{-1}\) was observed with either reagent. The following data show the correlation between creatinine determinations with the above reagent and the commercially prepared reagent.

No. data pairs = 93
Range of data = 0.04–0.88 mmol/L
Slope = 1.0333
y-intercept = 0.0034
RMS dev. = 0.0106
r = 0.9979

With the reagent prepared in the laboratory, precision was acceptable: CV = 4% at 0.13 mmol/L and 1.4% at 0.50 mmol/L. Similarly, based on the assay of commercial quality-control sera, accuracy could not be faulted.

Preparation of reagent suitable for the Beckman Creatinine Analyzer has resulted in substantial cost savings in this laboratory.

References

A Modified Quantitative Determination of Ampicillin in Biological Fluids

To the Editor:

Recently several procedures have been developed for ampicillin—spectrophotometric (1), fluorometric (2), and microbiological (3, 4)—to measure its concentration in biological fluids. The fluorometric procedure is based on the formation of a fluorescent product in acidic solution during hydrolysis at elevated temperature. Jusko (2) reported a simple fluorometric procedure for detecting concentrations as low as 50 \(\mu\)g/L. We made certain modifications to the original procedure because of the formation of an unstable fluorescence product in acetone-chloroform mixture.

On using a 1:1 mixture of acetone:chloroform as earlier reported by Jusko (2) we found that the fluorescent product formed was not stable, and that a light greenish-yellow was observed just after extraction of the drug. We also observed a good linearity in the fluorescence values of ampicillin with chloroform alone (concentrations of 400–2000 \(\mu\)g/L), whereas readings with acetone:chloroform mixture were erratic, and after 40 min the fluorescence of the concentrations mentioned above reached blank values. On comparing the fluorescence values of acetone:chloroform mixture with those of chloroform alone, we observed that ampicillin extracted into chloroform alone was stable for longer than 60 min.

It is reported (5) that chloroform adds onto the carbonyl group of ketones in the presence of alkali; e.g., with acetone it forms chloroacetone, and the reported possible mechanism is

\[
(CH_3)_2C:C=O + (CH_3)_2CCl_2 \rightarrow (CH_3)_2CCl_2\cdot(\cdot\cdot) + (CH_3)_2CO
\]

If it is assumed that ideal conditions for this reaction are not met under the present experimental conditions, even 1% reaction in the forward direction is likely to have a serious impact on the fluorescence. Owing to lack of instrumentation, we could not ascertain the nature of the compound producing the greenish-yellow color. The problem prompted a search for a better solvent mixture than the acetone:chloroform mixture. Jusko (2) reported on other solvents, such as ethylene dichloride, hexane, heptane, and carbon tetrachloride. We found acetone alone to be unsuitable because it mixes easily with the aqueous phase, and acetone in the presence of alkali decomposes the fluorescence product. We find chloroform alone was found to be an ideal solvent. Comparison of one set of fluorescence readings between chloroform alone and