

EVALUATION OF NEWLY MANUFACTURED KITS
"DIAMOND DIAGNOSTICS"

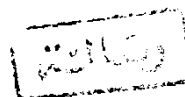
Thesis

Submitted in partial fulfilment of
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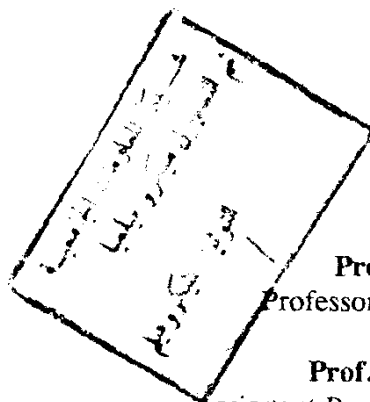
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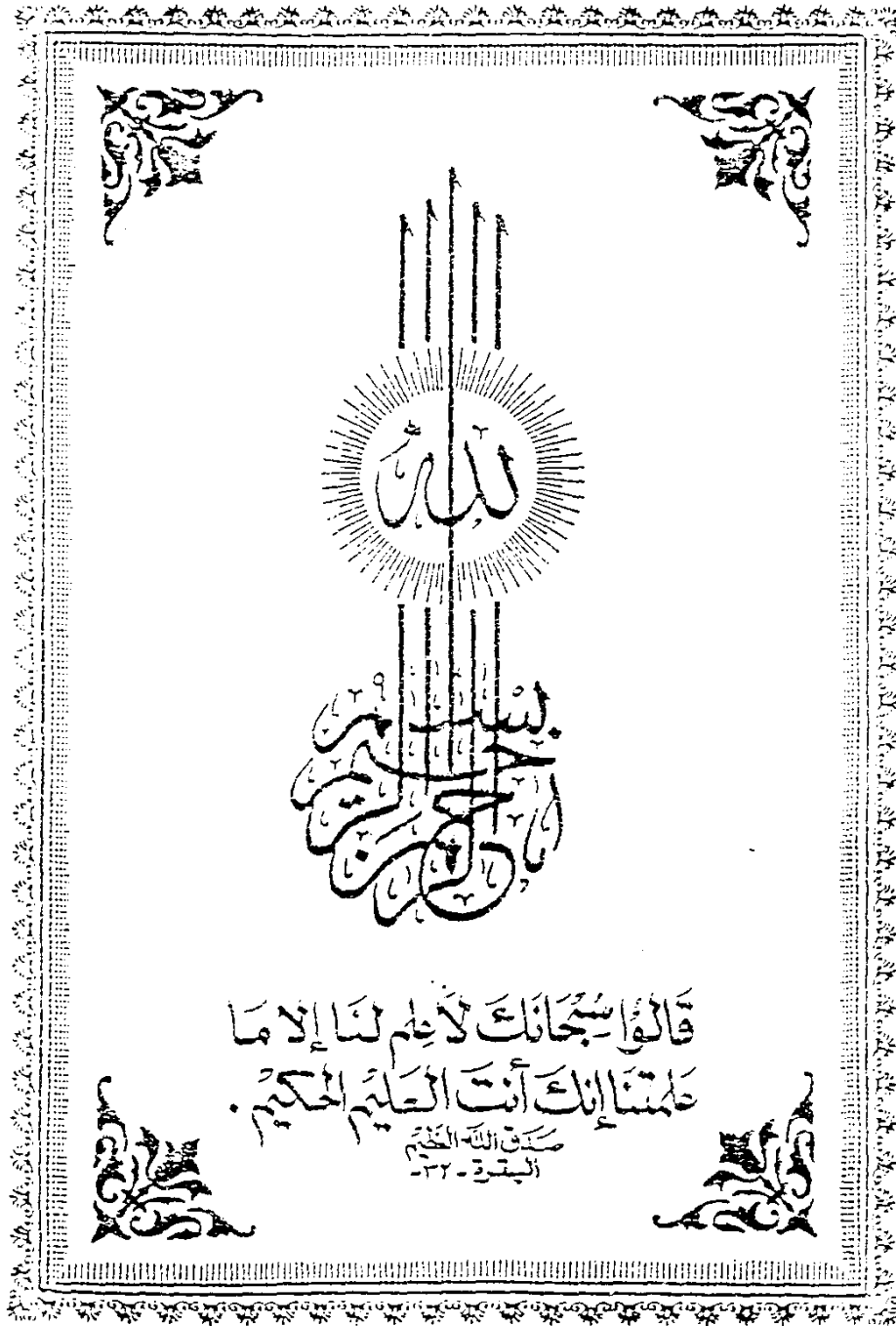
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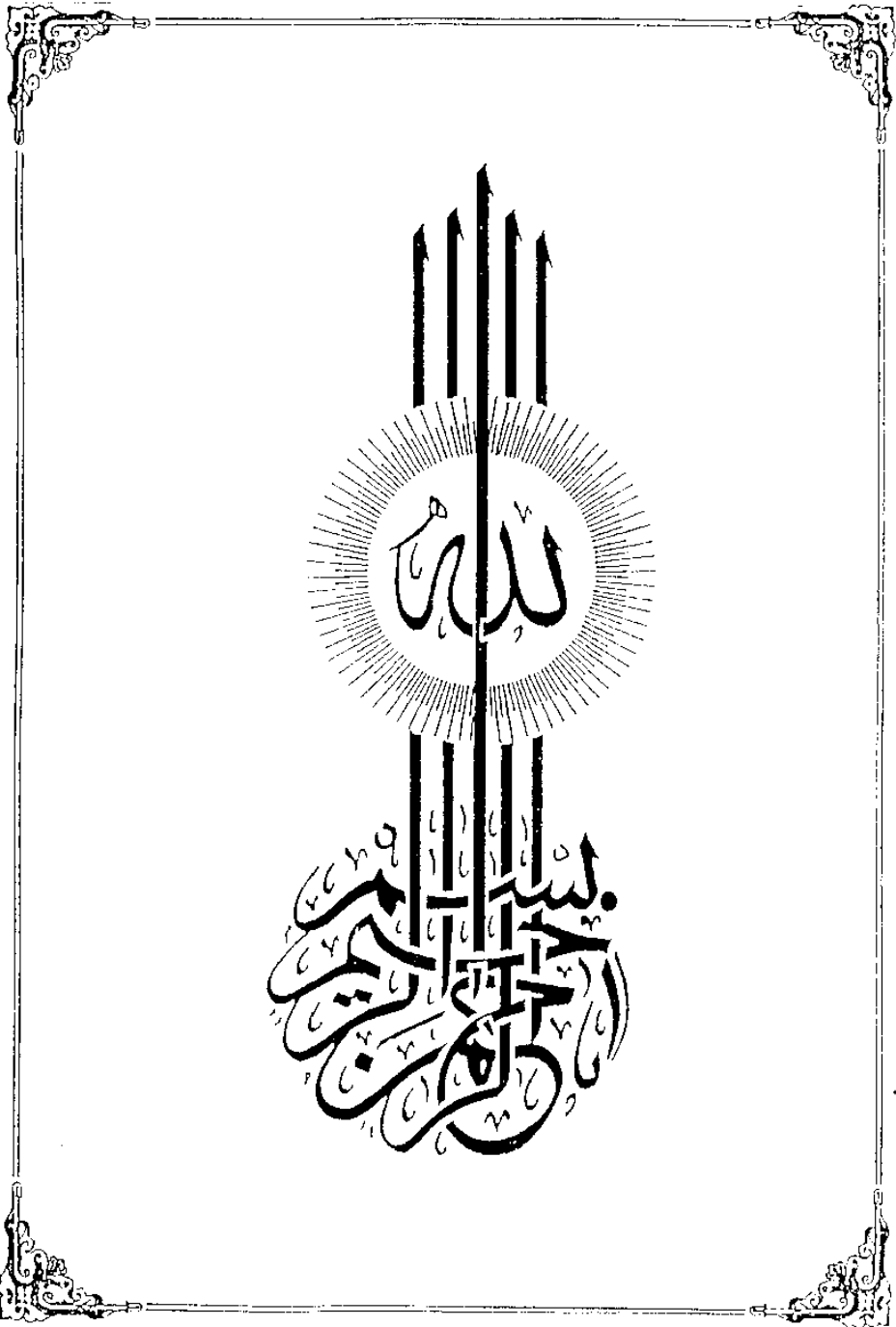
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Aim of the Work

Introduction and aim of the work

Method selection and evaluation are important steps in establishing high quality laboratory services. Before a new or improved method is introduced into a laboratory, it must be selected with care and its performance evaluated under laboratory conditions (Westgard et al., 1978).

Nowadays, instrumentation superimposed itself in most of the clinical chemistry laboratories, with it came the introduction of the so called reagent 'kits' or in other words, the "ready-made chemical reagents".

These reagents are relatively expensive for routine use in University Hospitals, where the work load is too big and the financial resources are limited. Moreover, importing these kits carries the risk of reagent deterioration due to bad storage conditions during transportation or delays in customs.

Fortunately, a number of Egyptian chemical pharmaceutical companies are now attempting to produce their own reagent kits. As a university hospital, which is dedicated for the service of the community, it is our role to evaluate these products before their widespread in the market for commercial purpose.

Aim of the work

The aim of the present study is to evaluate the performance characteristics and reliability criteria of a number of new locally produced chemical reagent products "Diamond Diagnostics¹". The chosen chemicals are those of glucose, urea, creatinine and calcium. Assessment of their precision, accuracy, specificity, sensitivity, linearity, short-term stability and batch-to-batch variation will be carried out and compared with that of their imported counterparts in a trial to evaluate the possibility of their subsequent use as a substitute for the more expensive imported ones.

¹Diamond Diagnostics: 40 Sadek El Rafaey st., Heliopolis, Cairo, Egypt.

Review of Literature

Glucose

Although the use of whole blood is easier for determination of glucose, the use of plasma or serum is highly supported because whole blood is a two-component system and the interpretations are less hazardous when applied to a single component system such as plasma. Also, whole blood must be mixed thoroughly before sampling which is inconvenient with automated analysis. Moreover, the value on whole blood tends to vary with hematocrit. Finally, specificity for glucose is improved with most methods when plasma is used for analysis.

Probably, the one major advantage in using whole blood is the convenience of measuring glucose directly on capillary blood especially from infants. In such cases, appropriate methodology will provide accurate and meaningful results (Folin, 1930).

(A) Principles of Analysis and Methods Used:

Chemically, glucose is an aldohexose, with the aldehyde form in equilibrium with the glucopyranose form which is the favoured structure at physiological pH. The aldehyde/endiol equilibrium allows glucose to be reduced and oxidized with facility.

1. Reduction Methods

a) Copper reduction reactions:

Most older established methods for measurement of serum glucose are based on the ability of glucose to directly reduce the cupric ions (Cu^{++}) to monovalent cuprous ions (Cu^+). In the presence of heat, the reduced cuprous ions can form cuprous oxide (Cu_2O) which can be detected by a variety of methods:

- (i) *Reduction of phosphomolybdate (Folin and Wu, 1920).*
- (ii) *Reduction of the arsenomolybdate (Smogyi, 1952 and Nelson, 1944).* Both will form a blue molybdenum compound.
- (iii) *Neocuprione (2,9-dimethyl-1,10-phenanthroline):*
Can also be reduced by Cu^+ ions to form a highly coloured complex. Although it is a more sensitive

procedure than the other copper reduction methods, it lacks specificity.

(iv) *Benedict's modification of copper reduction method:*

It is still used today but only as a semiquantitative method for estimation of urine glucose. This procedure which is sensitive to total reducing compounds present in urine, yields red Cu_2O and yellow CuOH precipitates. The greater the concentration of glucose, the redder the final colour.

In combination with a more specific enzymatic glucose assay, the Benedict reaction can be used to screen for genetic diseases of carbohydrate metabolism in newborns. A negative enzyme test and a positive Benedict reaction are suggestive of such diseases.

All copper reduction reactions are non-specific; compounds such as creatinine and uric acid interfere if a protein-free filtrate is used as the sample. The use of Benedict reaction for measuring urinary glucose is also restricted as many reducing compounds in urine can produce a false positive reaction (Pileggi and Szustkiewicz, 1974).

b) Other reduction reactions:

(i) Alkaline ferricyanide reaction:

The reaction involves the reduction of yellow ferricyanide $\text{Fe}(\text{CN})_6^{-3}$ to colourless ferrocyanide $\text{Fe}(\text{CN})_6^{-4}$, by glucose in alkaline conditions. When this method was automated, the reaction was monitored by measurement of the decrease in colour.

Later adaptations of the method utilized indirect measurements of ferrocyanide. The ferrocyanide was reacted with the excess ferric ions to form ferric ferrocyanide (Prussian blue).

The alkaline ferrocyanide reactions have largely been replaced by more specific methods because of significant positive interferences by creatinine and uric acid (Folin and Malmross, 1929).

(ii) The O-toluidine reaction:

This reaction is based on the ability of many aromatic amines in acid solutions to condense with the aldehyde group of glucose from glycosamines. The initial reaction product is more likely an unstable N-glucoside that is in equilibrium with the stable schiff base. The most widely used aromatic amine; O-toluidine, is believed to be a carcinogen.

Although the O-toluidine procedure has acceptable accuracy and precision, it has the disadvantages of noxious nature of its reagents and the non-specific reaction with urea and other hexoses, most notably mannose and galactose. It offers only an economic advantage over enzymatic methods, and its use has rapidly decreased over the past years (Marks, 1959).

2. Enzymatic methods

The most commonly used procedures for glucose analysis employ enzymes as reagents to increase analytical specificity. These are the hexokinase and glucose oxidase reactions. Both procedures have been automated with resulting high specificity and precision.

a) Hexokinase method:

It involves two coupled reactions; the hexokinase reaction which phosphorylates hexoses with ATP and then glucose-6-phosphate dehydrogenase "G6PD" reacts specifically with G6P produced by the hexokinase reaction to yield one mole of NADH or NADPH for each mole glucose oxidized.

The earlier hexokinase procedures employed mammal G6PD. Most assay today, however, use an enzyme derived