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Biochemistry

EVALUATION OF CLINICAL BIOCHEMISTRY KITS OF ENDPOINT METHOD

Dr. Kranti Sorte — Associate Professor, Biochemistry Department, Seth GS Medical College & KEM Hospital, Parel Mumbai-400012.

Dr. Pooja S. K. Rai — Associate Professor, Biochemistry Department, Lokmanya Tilak Municipal Medical College & General hospital, Sion Mumbai-400022

Dr. Vaishnavi Salunkhe — Assistant Professor, Biochemistry Department, Lokmanya Tilak Municipal Medical College & General hospital, Sion Mumbai-400022

ABSTRACT **Introduction:** A number of kits are available in market manufactured by different companies. Selection of best kit for laboratory by careful assessment is important. **Aims & Objectives:** Evaluation of kits (glucose kits as a model) by endpoint method of different companies to improve patient services. **Material & Methods:** Glucose of analytical grade, 08 different companies glucose kits based on GOD-POD method done on semi-autoanalyser on the basis of - physical examination of solution, absorbance of blank & standard, factor, standard concentration of new kit and after stability study, completion of reaction time, repeatability, finding out of upper & lower limit of linearity and stability of solution. **Results:** Different companies' kits showed different reaction time , repeatability, stability & reproducibility and ranking was done accordingly. **Conclusion:** Different kits must be evaluated properly using our developed model to select the best one for clinical laboratory to monitor its efficiency & give best service to the patients.

KEYWORDS : Kits Performance, Endpoint Method, Glucose Kit

INTRODUCTION

A number of Clinical Biochemistry kits are available in market manufactured by different companies. All the companies claim their product as best. But as Biochemist, we must assess performance of kits to select best one for laboratory to provide better service to the patients. 13As a model of endpoint kits, we examined glucose kits (GOD-POD method) of eight different companies'**.**

AIMS & OBJECTIVES

Evaluation of performance of end point-based kits (glucose kits as a model) of different companies' to select the best for patients' service.

MATERIALS & METHODS

A. Materials And Equipments: Glucose of analytical grade, 08 different companies' glucose kits based on GOD-POD method, Micropipettes (calibrated by gravidimetric method with electronic balance); Electronic balance (of 0.1mg sensitivity) of OhausCo., USA; Semi-autoanalyzer of Biotron, model: BTR-830, Italy and Double beam computerized spectrophotometer of ELICO Co.: model BL-198, India.

B. Reagents Preparations:

a) Stock glucose solutions in saturated benzoic acid solution were prepared using electronic balance of the following concentration (in mg/dL): 50, 100, 500 and 1000. Working glucose solutions were prepared of following concentrations (in mg/dL) by serial dilution of appropriate stock solutions: 5, 10, 20, 30, 75, 200, 300, 350, 400, 450, 600, 700, 800. Stock solutions were also used as working solutions.

b) **Glucose color reagents were reconstituted as per respective company's literature.**

c). Semi-autoanalyzer programming :

Programming parameters were as follows:
Unit: mg/dL,
Temperature: 37ºC,
Mode: endpoint with standard with blank,
Wavelength: 505 nm,
Standard concentration: 100 mg/dL
Result resolution: 0.1,
Aspiration volume: 400µL,

The instrument was calibrated by 100mg/dL glucose solution prepared by us.

d). Assay Procedure: Glucose color reagent 1000 ul mixed with 10 ul of standard glucose solution. Mix and wait at room temperature for 10/15mins (as per manufacturer's instruction). Then aspirated in the semi-autoanalyzer.

e). Study Design: This is based on evaluation of the following parameters in the kits. Different glucose kits were marked as 'A' to 'H' to code the company's name.

I. Physical examination: colour, transparency and any deposits in glucose color reagent
ii. Absorbance of blank and standard.
iii. Factor i.e.,100/Astd
iv. Concentration of glucose standards provided in kits: in new kits and after 02 weeks keeping at room temperature.
v. Completion of reaction time: in spectrophotometer with time scan mode.
vi. Repeatability: same glucose sample run for 05 times; if all the values were close then repeatability was good.
vii. Finding out of upper and lower limits of linearity: with working glucose solutions ranging from 5mg/dL to 1000mg/dL
viii. Stability of reagent: small volumes of color reagents were kept at room temperature and inspected for color development on day 3, 7, 10, 15.
ix. Rating of kits on the basis of performance (08-point scale).

Scoring System:

To award score to the kits depending on parameters 'performance, the following scoring system has been developed.

1) Physical examination: the color reagent must be colorless and transparent. Intensity of color was reflected by absorbance of blank and all the reagents were transparent. So, 01 point was awarded of the reagent's transparency and of no fungal growth.

2) Ablank and Astd: more the blank absorbance lesser is the quality of glucose kit (hypo phosphoric acid is to be added to protect phenol from oxidation). For absorbance up to 0.010, scoring of 01 point was assigned and thereafter for every increment of 0.010 absorbance –0.5 point was assigned. Higher the absorbance of standard, better is the kit and this reflects on factor. So, no points were awarded on this.

3) Factor: it is standard concentration of glucose (100mg/dL) divided by difference of absorbance of standard and blank. Lower the factor better is the kit and 01 point was assigned for factor between 290 - 310 which is ideal and thereafter –0.5 point for increment of each 20 value.

4) Standard concentration and stability: in all the kits standard glucose concentration were nearly 100mg/dL and keeping standard at room temperature for 02 weeks glucose molecules started degrading in all the standards. So, 01 point was assigned to each kit.

5) Completion of reaction time: it is very important parameter and within 15 minutes reaction must be completed for full color development. So, up to 15 minutes time 01 point was awarded and –01

point thereafter (instead of –0.5 point as it is an important parameter) for increment of every 05 minutes.

6) Reproducibility Study: it is an important parameter. For very very good 01 point and for very good point –01 point and thereof.

7) Lower And Upper Limit Of Linearity: for lower limit, no point was assigned as all the kits' performance was good, i.e., could detect up to 5 or 10 mg/dL glucose concentration which is not required in clinical practice. The upper limit of linearity is important. For glucose kit the optimum upper limit of linearity is 500mg/dL and 01 point was awarded for this. For decrement of every 50 mg/dL –01 point was awarded up to 300 mg/dL and thereafter -01 point for each decrement of 25 mg/dL.

8) Stability Study Of Color Reagent: glucose color reagent must be stable (i.e. no color development) at room temperature at least for 02 weeks. If the blank **absorbance was increased up to 0.010, then 01 was deducted and for each increment** of 0.010 absorbance thereafter, 01 points was deducted.

9) Setting: This work has been carried out in Biochemistry department and in Clinical Biochemistry Laboratory of tertiary care hospital.

RESULTS

Evaluation of different parameters of each kit was presented in tabular form in table-1. On the basis of parameters wise scoring system, points were awarded which was tabulated in table-2. Points were summed up to set total points on 08-point scale and the 08 different glucose kits were ranked from 1st to 8th.

DISCUSSION

This study was our attempt to rank in quantitative form the kits of end point method to select the best kit for laboratory. As far as in our knowledge, this study is the first attempt to rank the kits' performance. We admit that there might be some flaws in this study which needs to be improved for perfection. As per our scoring system kit-B was ranked 1st and kit-Ewas ranked last (8th). As per our experience, cost of best kit was not always the highest. Take the example of kits in this study – kit-B's cost was lesser compared to that of kit-E which was worst.

We encountered earlier one glucose kit, manufactured by a reputed foreign company, where the reaction continued even after 45 mins. Reaction completion time study does not require sophisticated instrument like computerized spectrophotometer; one Semiauto analyser is sufficient. 100 µL of standard glucose solution to be mixed to 10 ml of glucose color reagent and every 05 mins reading are to be taken to find out reaction completion time. This model can be extended to any endpoint-based kit to select the best one.

To face the stiff market competition every company tries to cut down manufacturing cost.

This holds true for the laboratory kits. We have the experience of deterioration of different kits' performance after 2-3 years which were initially used to give good results. As for example, in glucose kits the manufacturers add less quantity of the costly glucose oxidase enzyme. As a result, there is increase in reaction completion time, less absorbance of the standard glucose, repeatability and linearity problems.

RECOMMENDATION

Different kits must be evaluated properly using our developed model to select the best one for clinical laboratory to give service to the patients. Further, the selected kit is to be evaluated yearly to monitor its efficiency and if needed, is to be replaced by best one.

We find only one reference article related to this study in our search.

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