CLINICAL PROFICIENCY TESTING ESTABLISHES THAT THE DIAGNOSIS OF DIABETES MELLITUS CAN BE INFLUENCED BY DIFFERENT METHODOLOGY

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ABSTRACT

Background: *From 2003 to 2007, the ADA introduced several changes in the diagnostic criteria of diabetes mellitus. However, none of the published guidelines specified which glucose measurement methodology should be used in the clinical laboratory. Latin American laboratories utilize several methods, especially oxidase and hexokinase assays.*

Objective: *To evaluate if different methodology of glucose determination would change the diagnosis of diabetes mellitus in clinical laboratories.*

Methods: *This study received IRB approval and identification of all laboratories were preserved. To compare different methodology among the different laboratories the authors used data from the clinical proficiency testing of ControlLab, supported by the Brazilian Society of Clinical Pathology/ Laboratory Medicine (SBPC/ML). For the different methods the ControlLab calculates the robust mean, standard deviation and coefficient of variation (CV), employing the ISO 13528 (algorithm A). To calculate the acceptance range, ControlLab applies to the robust mean the limits recommended by Brazilian regulatory agencies. The rate of success is calculated by the formula: results in the acceptance range divided by all results. We evaluated glucose results reported by 1154 laboratories to ControlLab between July, 2003 and July, 2007, obtained by different methodology in Latin American laboratories (oxidase, colorimetric and UV hexokinase in several types of clinical analyzer systems). To evaluate if different methods of glucose determination would change the diagnosis we used ANOVA and Dunnett´s T3 tests.*

Results: *The rate of success is significantly higher using the UV hexokinase glucose determination method (Dunnett´s test p< 0,0001).*

Discussion and conclusion*: It is crucial to establish guidelines standardizing the methodology for the diagnosis and therapeutic monitoring of diabetes in clinical laboratories because several methods can be influenced by methodology used in open or closed clinical analyzer automation. This work demonstrates that oxidase and colorimetric hexokinase assays have higher coefficients of variation, and this can lead to diagnostic mistakes.*

BACKGROUND

In 2003 the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus of American Diabetes Association (ADA) introduced several changes to the diagnostic criteria for diabetes and for lesser degrees of impaired glucose regulation (IFG/IGT) established in 1997 by International Expert Committee. In 2004 the ADA published a new statement about diagnosis and classification of diabetes mellitus. (Table 1) However, there was no recommendations about specifics glucose measurement methods in clinical laboratory. Currently, Brazilian laboratories use on several methods, mainly, glucose oxidase (oxidase) and hexokinase. Taken together, one can argue if diagnosis of diabetes mellitus could be influenced by different methods and methodologies assay in Brazilian and Latin American clinical laboratories.

Table 1: PRINCIPAL CHANGES ON LABORATORIAL DIAGNOSIS OF DIABETES (mg/dL)

	before 1997	1997	2003	2004
normal FPG	<110	<110	**<100**	<100
FPG/2-h PG cut point separating diabetes from nondiabetes	≥140/ ≥200	**≥126/ ≥200**	≥126/ ≥200	≥126/ ≥200
casual plasma glucose plus symptons	—	—	—	**≥200**

• FPG: Fasting Plasma Glucose • 2-h PG: Two Hours Postload Glucose

OBJECTIVE

Based on those statements, we proposed to evaluate the influence of the application of different methods in Brazilian and Latin American laboratories.

METHODS AND PROCEDURES

From July 2003 to July 2007, 45 different serum samples for glucose proficiency testing were sent by ControlLab (4 times per year) to 1,154 laboratories, to test different methods and methodologies in Latin American laboratories.

All laboratories returned the result of the glucose proficiency testing with the information about method (oxidase, colorimetric hexokinase or UV hexokinase), reagent and equipment used to determination the concentration of glucose.

To compare different methodologies among the different laboratories the authors used data from the clinical proficiency testing of ControlLab, supported by the Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML).

This study was approved by our Internal Review Board (IRB) and identification of all laboratories was preserved.

Statistical methods applied by ControlLab´s Clinical Proficiency Testing Program

For the each different method, it was determined the robust mean, standard deviation and coefficient of variation (CV), employing the ISO 13528 (algorithm A). To calculate the acceptance range, the limit of 13% recommended by Brazilian regulatory agencies (ANVISA/REBLAS) was applied to the robust mean.

The rate of success was calculated by the following formula: results in the acceptance range divided by all results.

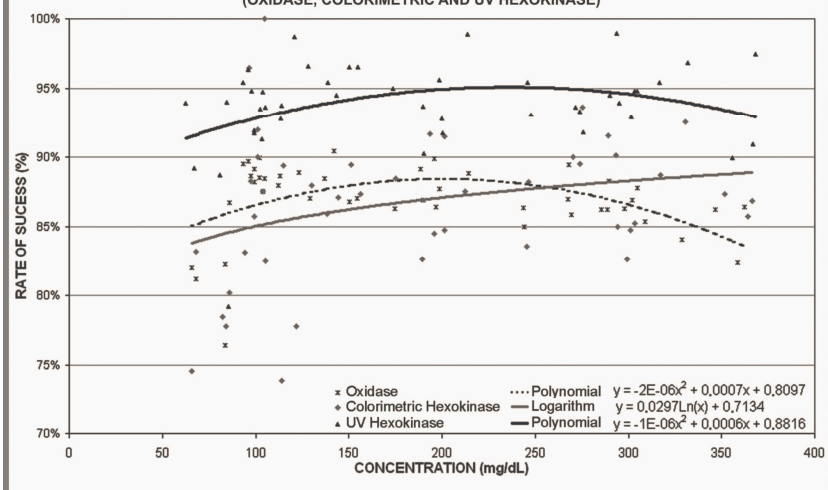
Statistical methods applied by our work group.

To evaluate if different methods of glucose determination (oxidase, colorimetric hexokinase or UV hexokinase) would change the diagnosis of diabetes mellitus we used ANOVA and Dunnett´s T3 tests.

To evaluate if different methodologies (opened or closed system) would change the diagnosis of diabetes mellitus were formed six groups and Mann-Whitney´s test was used. The groups formed are: (1) Oxidase – opened system; (2) Oxidase – closed system; (3) Colorimetric hexokinase – opened system; (4) Colorimetric hexokinase – closed system; (5) Ultraviolet hexokinase – opened system; (6) Ultraviolet hexokinase – closed system.

P values < 0.05 were considered statistically significant.

Figure 1: REGRESSION ANALYSIS OF THE RATE OF SUCCESS OF THE LABORATORIES AND GLUCOSE CONCENTRATIONS USING DIFFERENT METHODS (OXIDASE, COLORIMETRIC AND UV HEXOKINASE)



The regression establishes a mathematical relationship between variables and thus provides values of Y as a function of X.

RESULTS

The rate of success was significantly higher using the ultraviolet hexokinase glucose determination method (Dunnett´s test p< 0,0001), when not considering the methodology (Figure 1).

It was observed a difference between opened and closed system methodologies for each method (oxidase, colorimetric and ultraviolet hexokinase). No difference in performance was observed when we compared oxidase versus ultraviolet hexokinase by closed system and oxidase versus colorimetric hexokinase by opened system (Table 2 and Figure 2).

From 1,154 laboratories evaluated, 81% perfomed the laboratorial diagnosis of diabetes by opened systems (71% by oxidase method). Only 18% used closed systems (Table 3).

DISCUSSION AND CONCLUSION

It is important to establish guidelines standardizing the methodology for the diagnosis and therapeutic monitoring of diabetes mellitus in clinical laboratories. Several methods can be influenced by methodology, such as opened or closed clinical analyzer automation. Currently, the most frequent methodology used in Latin America to glucose determination is opened systems.

In the present study, we sought to compare the results of a proficiency testing of glucose of 1,154 laboratories and verify if different methods and methodologies could interfere in the diagnosis of diabetes mellitus.

Figure 2: RATE OF SUCESS IN LABORATORIES USING DIFFERENT METHODS (OXIDASE, COLORIMETRIC AND UV HEXOKINASE) AND METHODOLOGIES (OPENED AND CLOSED SYSTEM)

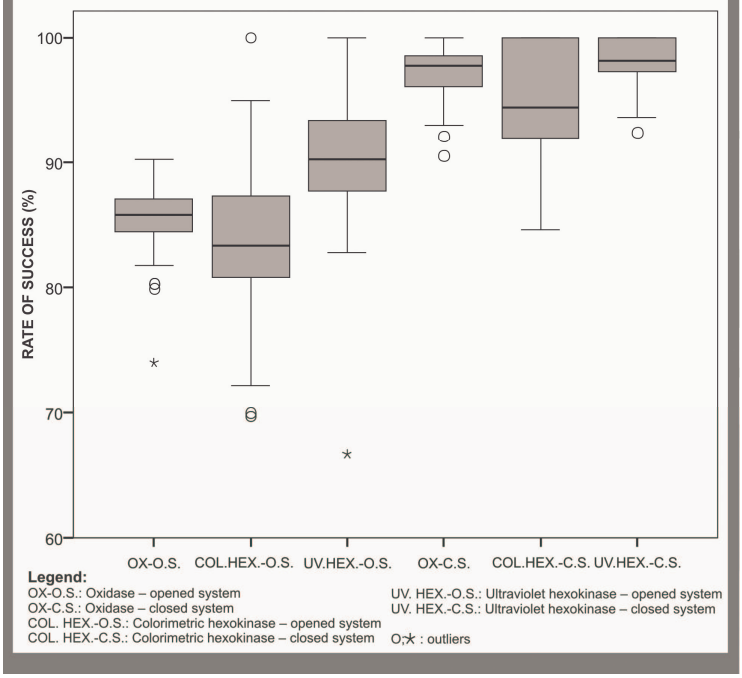


Table 2: COMPARATION THE RATE OF SUCCESS TO DIFFERENT METHODS AND METHODOLOGIES OF THE GLUCOSE ASSAY.

methods – methodologies	Colorimetric hexokinase – opened system	Ultraviolet hexokinase – opened system	Oxidase – closed system	Colorimetric hexokinase – closed system	Ultraviolet hexokinase – closed system
Oxidase – opened system	0.270	0.000	**0.000**	0.000	0.000
Colorimetric hexokinase – opened system	-	0.000	0.000	**0.000**	0.000
Ultraviolet hexokinase – opened system	-	-	0.000	0.000	**0.000**
Oxidase – closed system	-	-	-	0.048	0.944
Colorimetric hexokinase – closed system	-	-	-	-	0.002

Table 3: SUMMARY STATISTICS THE RATE OF SUCCESS OF DIFFERENT METHODS AND METHODOLOGIES OF THE GLUCOSE ASSAY.

methods – methodologies	Laboratories N - %	Sample N	Minimum	1°Q	Median	3°Q	Maximum
Oxidase – opened system	816 – 71%	45	74.0	84.5	85.8	87.1	90.2
Colorimetric hexokinase – opened system	55 – 5%	45	69.7	80.9	83.3	87.3	100.0
Ultraviolet hexokinase – opened system	71 – 6%	45	66.7	87.7	90.2	93.4	100.0
Oxidase – closed system	124 – 11%	45	90.6	96.1	97.8	98.5	100.0
Colorimetric hexokinase – closed system	25 – 2%	45	84.6	92.0	94.4	100.0	100.0
Ultraviolet hexokinase – closed system	63 – 5%	45	92.5	97.3	98.1	100.0	100.0

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The present analysis showed that the performance of a method is influenced by the methodologies application (opened or closed system). For example, the isolated analysis of figure 1 could lead to an erroneously interpretation of a superiority of only one method. A more careful analysis of Table 2 and Figure 2 lead us conclude that closed system yields to higher rate of success.

Taken together, our data provides interesting insights about different performance obtained with methods and methodology. It highlights concerns about methodology standardization.

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