Interferences of Different Concentration of Detergent Solution on Glucose Assays in Clinical Chemistry

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ABSTRACT

Background: The pre-analytical stage is so complex that a mistake at any step often becomes apparent in the analytical or post-analytical phase. Many medical laboratorians do not carefully evaluate the suitability of the pre-analytical challenges in laboratory testing especially in the area of washing test tubes by various forms of detergent in the wash-up room.

Objective: This study was then aimed at the effect of different concentration of detergent solution on glucose assay in the clinical chemistry laboratory.

Materials and Methods: A total of twenty (20) subjects who visited a clinic in Chemical pathology unit, Federal Medical Centre, Owo were randomly recruited for this case study. The recruited subjects were analyzed for glucose assays normally and also at a different concentration of the detergent solution.

Results: There was a statistically significant difference between glucose value with no dilution and detergent stock solution while others were not significantly different from normal statistically even though there is slightly different in their mean values.

Conclusion: Prevention of pre-analytical errors from detergent solution remains an ongoing problem in the wash-up room and ultimately affects the ability of clinical laboratories to produce accurate results. Therefore, it is important to establish close working relationships with laboratory attendants working in the wash-up room and also, developing a surveillance program should be considered to prevent such occurrences.

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1. Introduction:

An appropriate pre-analytical handling is essential because the influence of this step on laboratory services could not be overlooked. Proper washing of test tubes, blood collection and timely preparation of samples for laboratory processing are critical pre-analytical steps required for the integrity of laboratory reports (Bowen and Remaley, 2014).

However, many medical laboratorians do not carefully evaluate the suitability of the pre-analytical challenges in laboratory testing especially in the area of washing test tubes by various forms of detergent in the wash-up room.

Detergents are widely used in clinical biochemistry, cell biology or molecular biology. Cell lysis, protein solubilization, protein crystallization or reduction of background staining in blotting experiments is just a few of numerous applications (Caligur, 2008; AppliChem, 2008). Detergents can be classified for instance according to their chemical structure stating their constituent polar and non-polar group (glucosides, alkyl ionic detergents, polyoxyethylene alcohols, bile salts, sulphonates etc.), the charge character (anionic, cationic, zwitterionic = amphoteric and non-ionic) or simply whether they are mild or strong in terms of their

ability to solubilize and/or to denature proteins (AppliChem, 2008).

They all have in common that they are soluble amphipathic (amphiphilic) compounds, with both lipophilic (hydrophobic, non-polar) and lipophobic (hydrophilic, polar) sites within one molecule. The key to detergent function is an amphipathic structure. All detergents are characterized as containing a hydrophilic "head" region and a hydrophobic "tail" region (see Figure 1) (Caligur, 2008). Therefore, these structural characteristics allow detergents to aggregate in aqueous media.

At a sufficiently high concentration (called Critical Micellar Concentration (CMC) value), the polar hydrophilic region of each molecule is oriented toward the polar solute (water) while the hydrophobic regions are grouped together to form thermodynamically stable micelles with hydrophobic cores. The hydrophobic core region of the detergent micelle associates with the hydrophobic surfaces of proteins and results in soluble protein-detergent complexes (See Figure 2) (Garavito and Ferguson-Miller, 2001; Caligur, 2008; AppliChem, 2008). This value is specific to each detergent and different factors like temperature, chemical structure, salt concentration and pH value may influence it (Garavito and Ferguson-Miller, 2001).

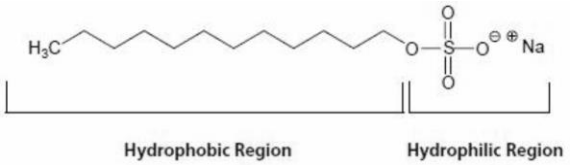

Figure 1. The structure of the anionic detergent sodium dodecyl sulphate (SDS), showing the hydrophilic and hydrophobic regions. (Source: Caligur, 2008)

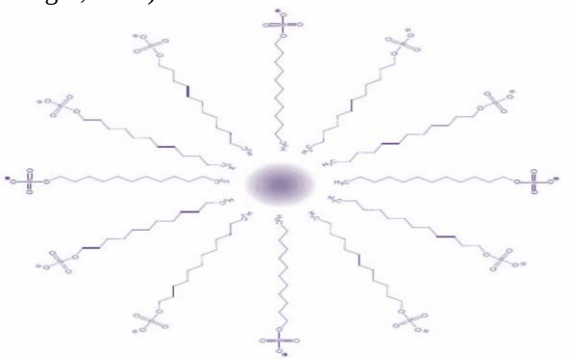

Figure 2. A simple illustration of a sodium dodecyl sulphates micelle. (Source: Caligur, 2008)

Although other authors have described the effects of endogenous substances on clinical assay results; the effects/impact of blood collection tube additives and components had also been well documented (Bowen and Remaley, 2014). But a large proportion, possibly a majority, of errors in laboratory medicine occurs in the pre-analytical phase of the testing process (Plebani, 2006; Livesey et al., 2008).

Accurate laboratory testing requires an understanding of the complex interactions between collection devices and blood specimens, samples analyzed and assay reagents and detergent washing solution used in recycling test tubes for subsequent analysis especially in developing countries. In this review, we discuss how different concentration of the detergent solution can alter glucose test results, with an emphasis on inappropriate rinsing recycled test tubes which may give different dilution detergent solution and possibly, way to minimize their effects on clinical chemistry assays.

2. Materials and Methods:

2.1. Subjects:

A total of twenty (20) subjects (both males and females) aged between 18 – 60 years from Chemical pathology unit, Federal Medical Centre, Owo was randomly recruited for this case study after obtaining an approval from the Federal Medical Centre (FMC) Joint Ethics Review Committee (FMC/OW/380/VOL.XXXVI/197) and obtained written informed consent (approved by the FMC Ethics committee) from each subject.

2.2. Inclusion and Exclusion Criteria:

Apparently healthy volunteers were randomly recruited for the study. Subjects who did not give their consent were excluded from the study; so also subjects that were less than 18years of age.

2.3. Blood Collection:

A Blood sample was obtained from each subject by applying a tourniquet around the arm above the elbow.

The antecubital fossa was disinfected with a 70 percent alcohol-soaked swab. Five milliliters (5mls) of venous blood was collected from each subject using aseptic procedure after 12 hours fast with all bio-safety precautions (Ray et al., 2006). The blood was dispensed into fluoride oxalate bottle and plasma was separated from the blood after centrifuging at 2000g/m for 10minutes in standard bench centrifuge to obtain plasma required for glucose estimation.

2.4. Chemical Substance of Detergent used:

Linear Alkyl Benzene sulfonate (LABS), Sodium Tripolyphosphate (STPP), Sodium Carbonate (Na2CO3), and Sodium Sulphate (Na2SO4) were dissolved in distilled water at the concentration of 10% (w/v) as working detergent solution (This would be referred to as 'neat' which is equal to 10g/dl).

2.5. Preparation of Serial dilution for working detergent solution:

A dilution series is a succession of step dilutions, each with the same dilution factor, where the diluted material of the previous step is used to make the subsequent dilution (Ochei and Kolhatkar, 2008).

To make a dilution series (Figure 3), use the following formulas:

Move Volume = Final Volume / (DF -1)

Diluent Volume = Final Volume – Move Volume

Total Mixing Volume = Diluent Volume + Move Volume

Working detergent solution was prepared as explained above at the concentration of 10% (w/v) ('neat' which is equal to 10g/dl). Thus, 10mls of this solution was pipetted into a test tube and was serially diluted at 1:10, 1:100 and 1:1000 as shown below. Then, five test tubes were arranged for each sample and 10uL of the sample was pipetted into all the test tubes. The detergent working solution was not added to the first test tube while 10uL of neat, 1:10, 1:100 and 1:1000 working detergent solution was added respectively to other arranged test tubes. This procedure was carried out for all the subjects recruited for the case study except blank and glucose standard.

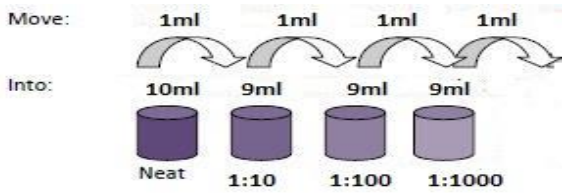

Figure 3. A dilution series.

2.6. Glucose Assay:

Glucose estimation was done using glucose oxidase method (enzymatic colorimetric) as described by Chessbrough, (2009).

3. Statistical Analysis:

A statistical package for social scientist (SPSS) 17.0 was used for the analysis of the data appropriately. The level of significance was taken at 95% confidence interval and P value less than 0.05 was considered significant.

4. Results:

Table 1 shows mean and Standard deviation of glucose estimated at different dilution detergent solution and no dilution (normal). In table 2, there is clear statistically significant difference between glucose value with no dilution (normal) and neat (detergent stock solution) while others are not significantly different from normal statistically even though, there is slightly different in their mean values. Representation of mean glucose estimated against their respective dilution was shown in Figure 4.

Table 1: *Mean and Standard deviation of glucose estimated at different dilution detergent solution and no dilution (normal)*

Reagent	Dilution	Mean	Std. Deviation	N
Glucose	Neat	136.74	34.54	20
	1:10	109.57	37.48	20
	1:100	103.88	35.67	20
	1:1000	103.24	32.52	20
	Normal	103.09	26.35	20
	Total	111.30	35.33	100

Table 2: *Contrast tests between glucose estimated at different dilution detergent solution and with no dilution (normal)*

	Contrast	Value of Contrast	Std. Error	t	df	p-Value
Glucose	Neat Vs Normal	33.66	10.60	3.17	95	0.002*
	1:10 Vs Normal	3.24	5.30	0.61	95	0.543
	1:100 Vs Normal	1.19	15.90	0.08	95	0.940
	1:1000 Vs Normal	0.02	1.06	0.02	95	0.988

* Significant at p≥0.05

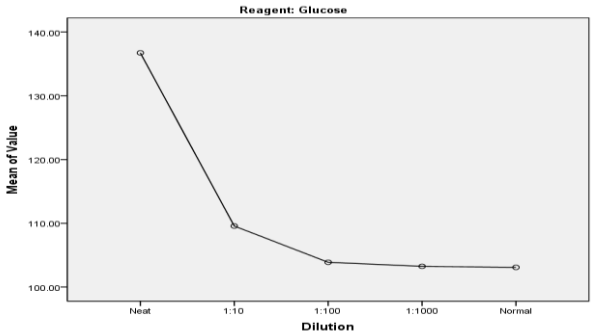

Figure 4. Representation of mean glucose estimated against their respective dilution

5. Discussion:

There are vast amounts of data, especially concerning the interaction of proteins with detergents using various techniques for more than three decades (Tsuge et al., 1984). All detergents are characterized as containing a hydrophilic region and a hydrophobic region which is the key to their function. (Caligur, 2008). These unique structural features allow detergents to aggregate in aqueous media. The

hydrophobic core region of the detergent micelle associates with the hydrophobic surfaces of proteins and results in soluble protein-detergent complexes (Garavito and Ferguson-Miller, 2001; Caligur, 2008).

The pre-analytical stage is so complex that a mistake at any step often becomes apparent in the analytical or post-analytical phase (Çuhadar, 2013). Total quality could be defined as the guarantee of a correctly performed activity throughout the total testing process (Delanghe and Speeckaert, 2014), providing accurate, precise and reliable medical diagnosis and efficient patient care in the medical laboratory. Various sampling methods, inappropriate specimen transport and detergent washing solution used in recycling test tubes for subsequent analysis can cause obvious pre-analytical errors. It is thus mandatory to focus on the pre-analytical phase in order to improve the reliability of test results (Delanghe and Speeckaert, 2014). This study was then aimed at the effect of different concentration of detergent solution on glucose assay in the clinical chemistry laboratory.

Table 1 shows increase mean glucose estimated at different serial dilution detergent solution from neat through 1:1000 dilution in comparing to mean of glucose obtained with no dilution. Similarly, there is clear statistically significant difference between glucose value with no dilution (normal) and neat (detergent stock solution) while others are not significantly different from normal statistically even though there is slightly different in there mean values. In contrast, Tsuge et al., 1984 demonstrated inactivation of glucose oxidase by the cationic detergent, while an anionic detergent did not produce measurable changes in the enzyme activity. Even though, this study could not ascertain the mechanism by which that glucose oxidase enzyme was protected against denaturation in different dilution of a detergent solution, activation of its activity might be due to zwitterionic nature of detergent used.

6. Conclusion and recommendations:

Prevention of pre-analytical errors from detergent solution remains an ongoing problem in the wash-up room and ultimately affects the ability of clinical laboratories to produce accurate results. Because it is not possible for laboratory personnel to assess the impact of their tubes on assay platforms because transparent residues other than the active ingredient may be present on the surface of test tubes and are not soluble in the cleaning solvent. It is thus important that they establish close working relationships with laboratory attendants working in the wash-up room for appropriate washing and rinsing. Also, developing a surveillance program should be also considered to quickly identify problems of such and prevent it.

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