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Effect of residual detergents in specimen collection containers on routine serum biochemical analytes

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ABSTRACT

The use of Teepol, Lysol and sodium hypochlorite are frequent in reusing of specimen collection tubes by the state hospitals in Sri Lanka. Detergent residue in collection tubes is thought to be the reason for uncertain results in the recent past. This raised a concern to evaluate the effect of washing of the specimen collection tubes by various detergents on serum creatinine, aspartate transaminase (AST), Na^+ and K^+. Three sets of newly purchased glass Khan tubes were washed using Teepol, Lysol (concentration of 1%), and sodium hypochlorite (0.1 %) adhering to World Health Organization protocol. Blood from a single donor was aliquoted to a detergent washed tubes (test) and to a newly purchased plain glass Khan tube (control). Both were tested for serum creatinine, AST, Na^+ and K^+. A sample size of 20 was analyzed for each detergent-washed tube. There was no significant difference in serum creatinine, AST and K^+ when Teepol, Lysol or sodium hypochlorite washed tubes were used in sequence ($p>0.05$). Na^+ concentrations measured in serum was significantly different in specimens collected to Lysol-washed tubes ($p<0.05$). The Lysol-washed tubes were significantly contaminated with detergent residues. In conclusion, domestic detergents (Teepol, Lysol and sodium hypochlorite) don't impart a significant effect on tested analytes if the cleansing is done according to guidelines by World Health Organization. However, newly purchased tubes are recommended in critical investigations such as serum electrolytes to improve the accuracy of laboratory reports.

KEYWORDS: *Teepol, Lysol, Sodium hypochlorite, creatinine, Na^+ and K^+*

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1 INTRODUCTION

The preanalytical phase is an important component of total laboratory quality and therefore ~~a~~ considerable attention has been given to the influence of preanalytical effects of laboratory results. A wide range of variables affect the results for a patient from whom a specimen of blood or body fluid has been collected. The type and the quality of the specimen container, procedure for collection, handling and processing before analysis constitute the preanalytical phase (Ahyayauch et al. 2010). Three types of specimen containers are used in chemical pathology laboratories for collection of specimens; plain tubes, fluoride - oxalate tubes, oxalate tubes (Bain et al. 2010). Fluoride – oxalate tubes are required in specimen collection for glucose assays while fibrinogen estimations require oxalate tubes. Ideally no anticoagulants or preservatives are required in all estimations of serum analytes. In addition, serum separator tubes (gel tubes) can be used to separate serum from whole blood and even plasma. They yield a greater volume of serum, are easy to process and a single centrifugation separates serum out of the clot, but are rather expensive (Bowen and Remaley, 2014).

The required volume of specimen for each analyte depends on the analytical procedure, either manual or using automated machines. Manual processing requires larger volumes while fully automated processing will perform the same with micro volumes (Bain et al., 2010).

The routine serum biochemical analytes in investigations include a wide variety of substances. They are enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), α – amylase, alkaline phosphatase (ALP), acid phosphatase, lactate dehydrogenase etc.,], proteins (albumin, total protein, etc.), lipids (triglycerides, high density lipoproteins and total cholesterol), hormones, ions (Na^+ , K^+ , Ca^{2+} , inorganic phosphates, etc.), pigments (bilirubin) and other metabolites (creatinine and blood urea nitrogen) (Caligur, 2008).

Owing to large number of specimens received each day, absence of waste disposal procedure, implementation cost, long waiting periods for purchasing new tubes and human power, most of the government sector hospitals in Sri Lanka cannot afford for new collection tubes. Hence, most of the specimen collection tubes are reused after washing with detergents. Re-cycled injection vials / penicillin bottles are widely used for specimen collection in Sri Lankan hospital sector. They are prepared by washing, boiling and drying. Heat-sensitive rubber stopper caps are washed and dried. Vacutainer tubes are used to collect blood in most of the laboratories in the state sector hospitals. These tubes are reused after several cycles of autoclaving, washing and drying. Ultimately, tubes with stoppers and sticker labels are re-issued by the laboratories though they are recommended for single use (Bain et al., 2010).

Detergents are used in washing step of the specimen collection containers. Detergents are classified as cationic, anionic, non –

ionic and zwitterionic detergents with respect to the hydrophilic groups they possess (Cheesbrough, 1999). Cationic and anionic detergents are harsh as they do protein modifications and denature proteins in large extents. Non – ionic detergents are mild and are less likely to denature proteins. Zwitterionic detergents have a net neutral charge and possess the properties of both ionic and non – ionic detergents. They efficiently denature proteins than non – ionic detergents (Cheesbrough, 1999).

According to World Health Organization (WHO) guidelines, ideally, the contaminated materials with potentially infectious substances such as blood, should be autoclaved first (Chhillar et al. 2011). Then they can be washed and reused. Chlorine releasing compounds (sodium hypochlorite, calcium hypochlorite, sodium dichloroisocyanurate, chloramines and chlorine dioxide), formaldehyde, glutaraldehyde, phenolic compounds and quaternary ammonium compounds can be used in appropriate concentrations to clean laboratory glassware (Chhillar et al. 2011).

In addition, there are commercially available laboratory grade detergents such as Fisherbrand ™ FL-70 ™ concentrate, Decon ™ Contrad ™ 70 liquid detergent, Thermo Scientific ™ RBS ™ 35 detergent concentrate, etc. where use of these in general practice is hardly ever found in Sri Lanka (Cornelis et al. 1995).

Specimen collection for certain serum analytes (total calcium, ionized calcium, iron and trace elements) needs specifically washed tubes with acids (Caligur, 2008). In the assay of serum aluminum, glass tubes should be avoided for collection of blood (Domingues et al. 2008). Use of Zn - doped stoppers must be prevented when determining Zn levels (Domingues et al. 2008). Plastic containers with Cd - based softeners should never be used in sample collection for serum Cd measurements (Domingues et al. 2008). Acid washed vials are required in total calcium and iron assays (Bain et al. 2010; Desmeules 2010). Also, hydrochloric and nitric acids under specific concentrations can be used in test tube washing for trace metal analysis (Gunatillaka et al. 1979).

Minor staff of the hospital is engaged in the cleaning of used specimen collection tubes. With the increased proportion of tubes to be reused with human labour alone, tube contaminations with detergents are possible. These contaminations contribute to errors in the pre-analytical phase.

Studies have shown that most of the laboratory-based errors arise in the pre – analytical phase (Gaehtgens and Benner 1974; Lam et al. 2005). Blood collection tube interferences (separator gels, clot activators, surfactants, order of draw and protease inhibitors) during pre – analytical phase affect adversely on laboratory testing (Bowen and Remaley 2014).

As investigated by Pakistan Medical Research Council, errors that occur during the pre – analytical phase account for a higher degree of laboratory-based errors and a proportion of 32 – 75 % errors were due to the pre-analytical errors (Larsen et al. 2006). Therefore, it is very important to minimize laboratory-based errors in the

pre - analytical phase and so are the errors in analytical and post - analytical phases.

The executed studies showed that detergent contaminations interfere with electrolyte assays, pH measurements, integrity of cell membranes, blood glucose measurements, coagulation studies and enzyme activities (Malinowska and Meyerhoff 1998; Moore et al. 1989; Naz et al. 2012; Plebani 2006; Parsi et al. 2008, Narayan, 2000; Samanga et al. 2011)

A survey in 2016 by our team revealed that most of the General and Base Hospitals of government sector of Sri Lanka utilizes detergent washed tubes for specimen collection (Figure 01). Detergents used in cleansing are Teepol, Lysol, 0.1% sodium hypochlorite and soap water which are not merely the laboratory grade detergents. Recently the hospital authorities have identified some factitious issues of test results of routine serum biochemical analytes which was suspected due to the detergent contaminations.

The effect of residual detergents in sample containers on routine serum biochemical analytes has not been scientifically studied in Sri Lanka and globally too there is a dearth of literature. However, there is a need to avoid pre-analytical errors in reports as each and every test result is vital in clinical diagnosis.

Therefore, this study has been planned to investigate the possible effects of residual detergents (Teepol, Lysol and sodium hypochlorite) on routine serum biochemical analytes; serum creatinine, AST, Na^+ and K^+**.** Four investigations were considered as a representation of routine biochemical analytes**.**

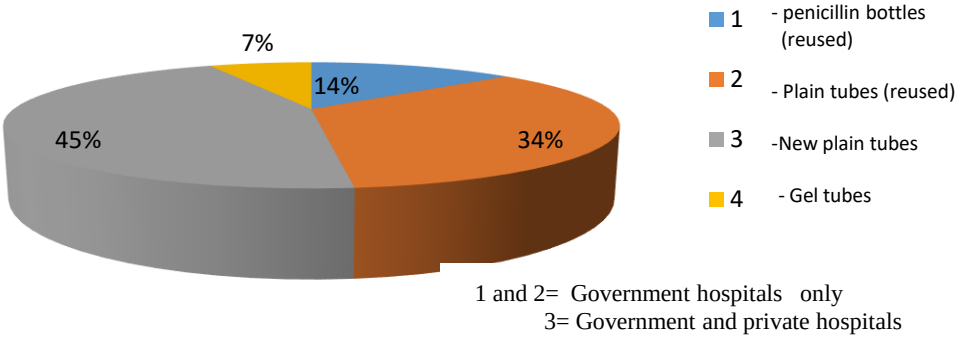

Figure 01: Graphical representation of specimen collection containers for serum biochemical analytes used in hospitals /institutions of Sri Lanka

2 MATERIALS AND METHODS

2.1 Subjects

This is an experimental study conducted at the National Hospital, Colombo, Sri Lanka. National Hospital, Colombo is the ultimate referral point and the main government institution pertaining to health issues in Sri Lanka. Sixty (60) participants visited to the hospital laboratory for any other prescribed investigation were explained the purpose of the study. If the participants consented (written consent), the remaining blood samples (after performing their prescribed investigation) were used for the study. The study was ethically approved by the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

2.2 Methods

Washing protocol for sample containers

Newly purchased glass Khan tubes were fully immersed in the detergent solution prepared (according to WHO guidelines) by a single person (to avoid individual variations), covered and placed for an overnight (Chhillar et al. 2011). On the following day, the tubes were taken out to a clean wash basin, washed twice using tap water and a separate test tube brush was used to wash detergent residues in tubes. The tubes were washed with tap water three times and the final washing was performed using distilled water. The tubes were inverted in a test tube rack to drain water for 2 hours. Then the tubes were oven dried (50^{o} C, 3 hours) and stored in a paper envelope and labeled with date, time, name of the detergent used until taken for the study.

Three sets of test tubes were prepared for each of the detergent; Teepol, Lysol and 0.1% sodium hypochlorite according to the above protocol and three set of control tubes (newly purchased plain glass Khan tube) were also supplied.

Blood (1 ml) was collected in to each control tube and respective washed test tube (plain glass Khan tubes washed by using Teepol, Lysol and sodium hypochlorite) from same individual. The blood of twenty (20) individuals were used in each group set as follows.

I. Control -1(C1) and Teepol washed
II. Control-2 (C2) and Lysol washed
III. Control-3(C3) and sodium hypochlorite washed

The detailed methodology is illustrated in figure 02. Four investigations (serum creatinine, AST, Na^{+} and K^{+}) were performed from each control and test by using Kornelab 60i auto-analyzer.

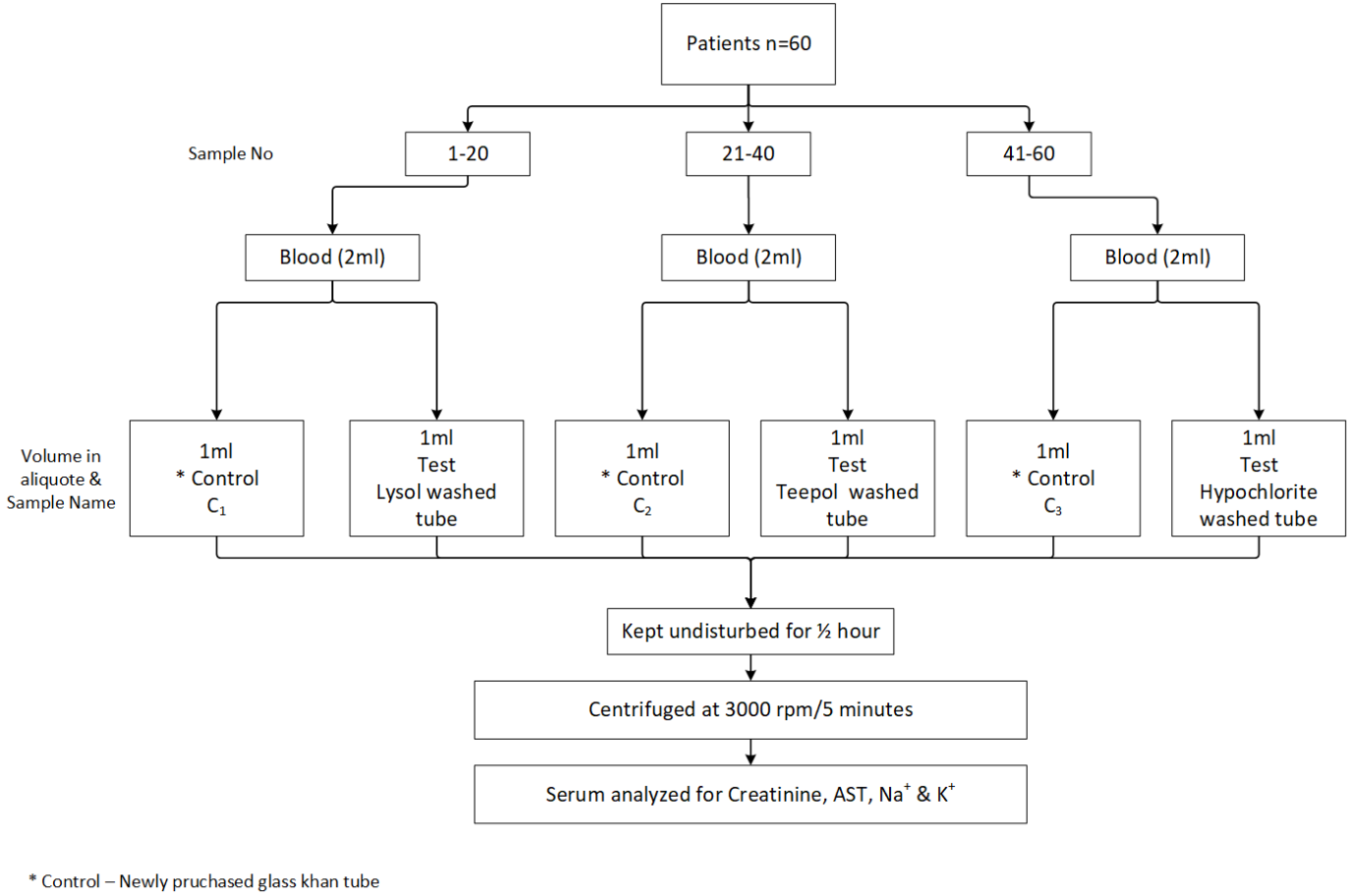

Figure 02: Detailed experimental procedure; preparation of controls (C_1, C_2 and C_3) and test samples (Lysol, Teepol and sodium hypochlorite washed tubes) proceeding to analysis

2.3 Statistical analysis

Statistical Package for Social Sciences; version 21.0 was used to estimate the mean for continuous variables. Paired t – test was applied for within group comparison of means. $p<0.05$ was considered statistically significant. Scatter plots and Pearson's Correlation Coefficient was used to assess the correlation between two continuous variables (control and test series of a single serum analyte) of data.

3 RESULTS & DISCUSSION

Mean serum values of test and control groups of analytes (serum creatinine, AST, Na+ and K+) are depicted in following Figures (Figure 03-06).

Mean serum creatinine, AST and K^+ levels obtained from newly purchased plain glass Khan tubes (control) was not significantly different ($p > 0.05$) from those values of the serum collected to Teepol washed, Lysol washed and sodium hypochlorite washed glass Khan tubes (Test).

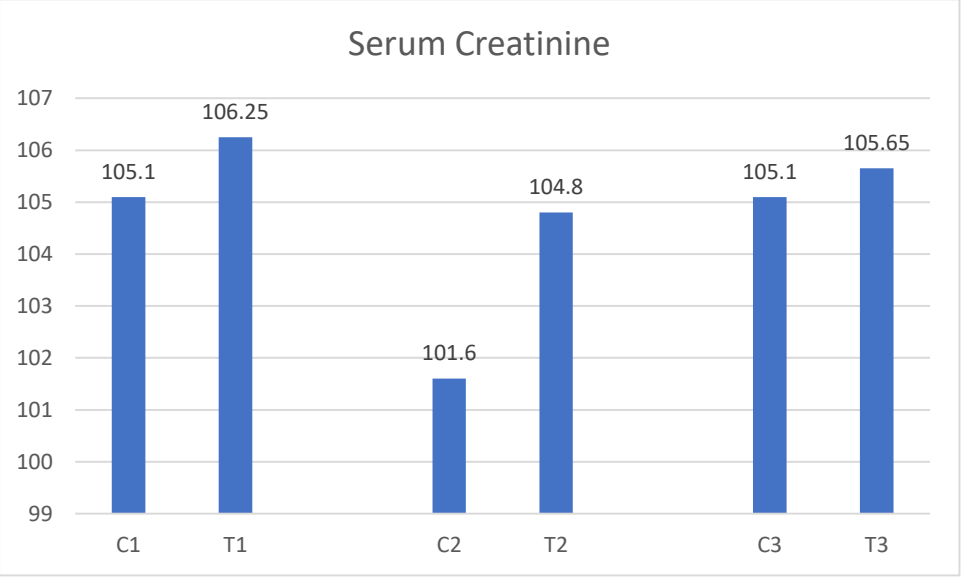

Figure 03: Mean serum creatinine levels of control (newly purcased plain glass Khan tubes) vs. test (detergent washed tubes)

C_1- control 1 vs. T_1- test 1 (Teepol washed plain glass Khan tubes)
C_2- control 2 vs. T_2 – test 2 (Lysol washed plain glass Khan tubes)
C_3- control 3 vs. T_3- test 3 (sodium hypochlorite washed plain glass Khan tubes)

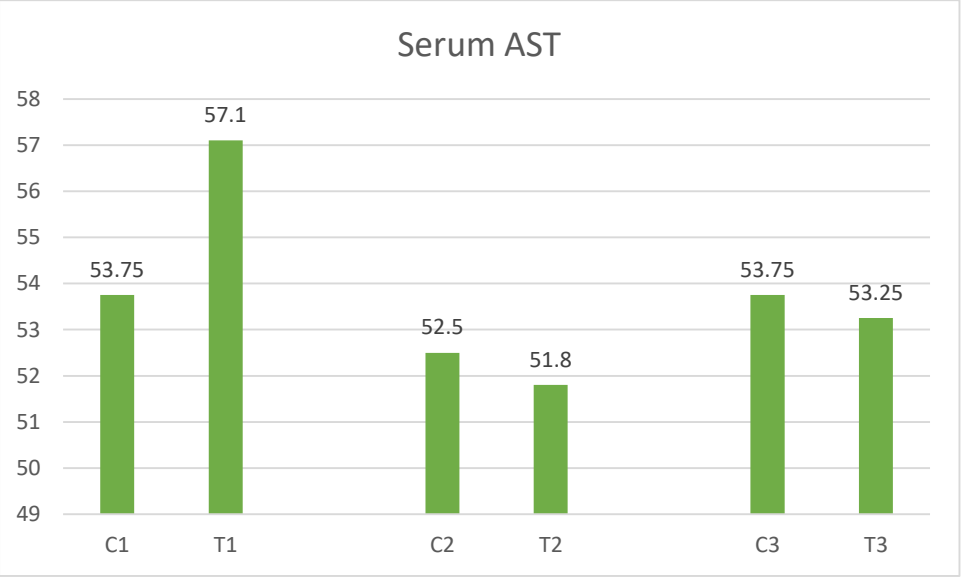

Figure 04: Mean serum AST levels of control (newly purcased plain glass Khan tubes) vs. test (detergent washed tubes)

C_1- control 1 vs. T_1- test 1 (Teepol washed plain glass Khan tubes)
C_2- control 2 vs. T_2- test 2 (Lysol washed plain glass Khan tubes)
C_3- control 3 vs. T_3- test 3 (sodium hypochlorite washed plain glass Khan tubes)

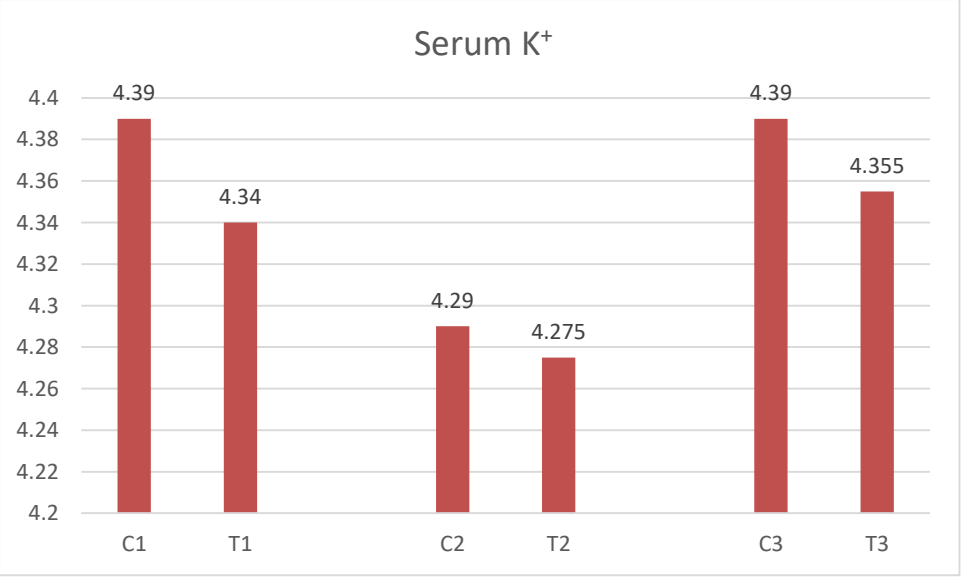

Figure 05: Mean serum K^+ levels of control (newly purcased plain glass Khan tubes) vs. test (detergent washed tubes)

C_1- control 1 vs. T_1- test 1 (Teepol washed plain glass Khan tubes)
C_2- control 2 vs. T_2 – test 2 (Lysol washed plain glass Khan tubes)
C_3- control 3 vs. T_3- test 3 (sodium hypochlorite washed plain glass Khan tubes)

However, serum Na^+ values exhibited a significant difference when specimens were collected into Lysol-washed plain glass Khan tubes when compared that with control values ($p<0.01$). In contrast, serum Na+ values were not significantly different when Teepol or sodium hypochlorite-washed plain glass Khan tubes were used for the collection of serum.

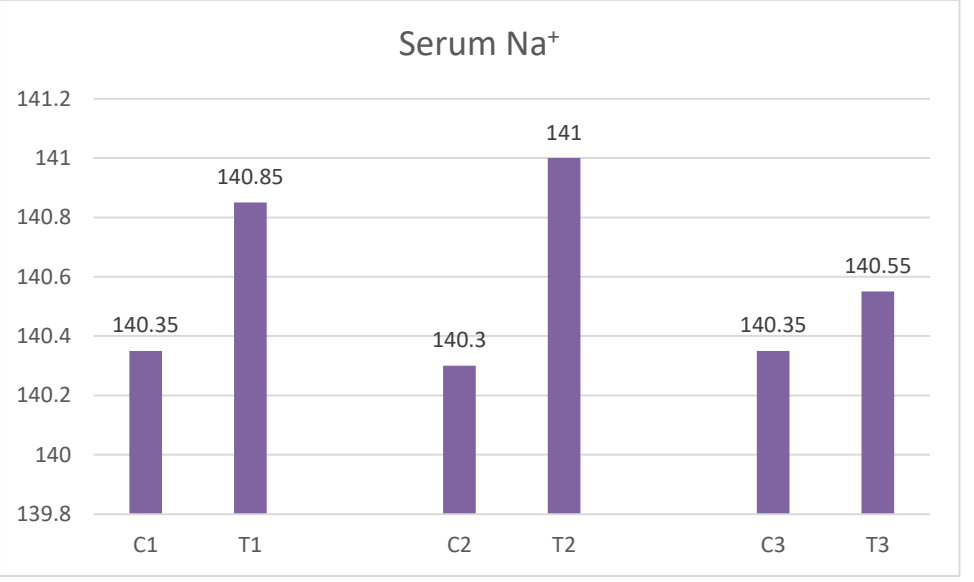

Figure 06: Mean serum Na^+ levels of control (newly purcased plain glass Khan tubes) vs. test (detergent washed tubes)

C_1- control vs. T_1- test 1 (Teepol washed plain glass Khan tubes)
C_2- control vs. T_2 - test 2 (Lysol washed plain glass Khan tubes)
C_3- control vs. T_3- test 3 (sodium hypochlorite washed plain glass Khan tubes)

Serum creatinine, AST, Na^+ and K^+ are critical investigations that influence on management of patients. The interference effects of detergent washed (Teepol, Lysol and sodium hypochlorite) specimen collection tubes on serum creatinine, AST, Na^+ and K^+ were not previously reported in literature. In state sector hospitals of Sri Lanka where free health care is offered, specimen collection containers are reused after washing with Teepol, Lysol and sodium hypochlorite. This study was designed to investigate the effect of detergent contamination on selected critical investigations. There are no reports of similar studies conducted elsewhere by using the detergents used in this study. However, previous research studies have ascertained detergents as contaminants which significantly change blood electrolyte measurements (Malinowska and Meyerhoff, 1998; Moore et al.1999).

The study revealed that there was no significant impact on serum creatinine, AST and K+ when serum was collected into Teepol, Lysol and sodium hypochlorite washed tubes. According to

the results, Lysol washed plain glass Khan tubes were found to formulate significantly different serum Na^+ values ($p<0.05$). In contrast, Lysol washed tubes didn't create a significant impact on the values for creatinine, AST or K^+ when compared with that of controls. Therefore, use of Lysol in cleansing of specimen collection containers creates ambiguity, especially in serum electrolyte assays. Also, more recent safety concerns have restricted the phenolic products in cleansing purposes of laboratory glassware as documented in the "Bio Safety Manual" published by WHO (Chhillar et al. 2011).

4 CONCLUSIONS

Sri Lanka is one such country where free health is offered in government sector hospitals. However, due to economic constrains, the health sector is unable to offer new sample collection tubes for all investigations requested by patients and thus reuse following washing. The evidences gathered reflect that the washing protocol followed by the state sector hospitals are satisfied to a greater extent and assure the cleanliness of reused containers.

Therefore, except electrolytes assays, recycled containers are satisfactory for the collection of blood specimens in routine laboratory testing if the containers are cleansed according to WHO washing protocol.

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