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Impact of sanitizers on quality of Lipase and Triglyceride analytes in clinical laboratory

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Use of sanitizers in diagnostic centres causes derangement in quality control values of lipase and triglycerides (TG) analytes during COVID-19. Our study provides a practical insight into the type of sanitizers to be used in a laboratory. Performance Verifier (PV) and reagents (lipase and TG) were contaminated with sanitizer and 70% isopropyl alcohol. Groups formed were- PVNet (G1), PV with Sanitizer (G2), PV with 70% Alcohol (G3), Sanitizer contaminated reagent (G4). Controls PV-1 and PV-2 were run. ANOVA and Tukey's test among groups and between groups were compared. Significant difference in mean PV-1and PV-2 values of TG [PV-1, PV-2 (P <0.0001)] and lipase TG [PV-1 P <0.0001) PV-2 P <0.001] among all tested groups were observed. Between-group analysis showed significantly higher PV-1 and PV-2 values in sanitizer contaminated PV group (P <0.001) compared to Neat PVs (P <0.001), and PVs contaminated with 70% alcohol (P <0.001). sanitizer contaminated PV-1 values were significantly higher when compared to Neat PV-1 (P <0.001)for lipase. It is advised that isopropyl alcohol (70%) should be preferred over glycerol containing sanitizers to reduce pre-analytical errors for lipase and TG estimation.

Keywords: Analytes, Dihydroxyacetone, Hypertriglyceridemia, Quinoneimine

With the onset of coronavirus disease (COVID-19), the way of working in a clinical biochemistry laboratory has also changed to prevent laboratory staff from getting infected by this highly contagious virus. Centre for Disease Control and Prevention (CDC) has provided detailed guidelines for general laboratory safety practices during the COVID-19 pandemic which states that employees must have access to personal protective equipment (PPE), soap, clean running water , and drying materials for hand washing, or alcohol-based hand sanitizers that contain at least 60% ethanol or 70% isopropanol. Further, there should be procedures for cleaning and sanitizing the commonly shared equipment and areas to ensure clean surfaces and equipment for all users[1].

World Health Organization (WHO) has developed two formulations that can be locally prepared by healthcare facilities. One formulation contains ethanol 80% (*v/v*), glycerol 1.45% (*v/v*) and hydrogen peroxide 0.125% (*v/v*), and the other isopropanol 75% (*v/v*), glycerol 1.45% (v/v) and hydrogen peroxide 0.125% (*v/v*). In both preparations, the addition of glycerol as an emollient aim to protect the hand skin against dryness and dermatitis potentially resulting from repeated use[2].

Prior to this pandemic, the availability of hand sanitizers in health facilities from low and middle-income countries such as India were inconsistent and such products were unavailable or inaccessible due to their high cost[3].The sudden increase in the demand for sanitizers and inadequate supply leads to the production of numerous types of sanitizers in India. This led to the use of different types of sanitizers as per availability in the laboratory set-up by healthcare professionals and support staff.

After starting the regular use of various types of sanitizers for hand and instrument disinfection by laboratory staff, derangement in internal quality control values of biochemical parameters, such as lipase, and triglycerides (TG) were observed, even

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Abbreviations: ABHS, alcohol-based hand sanitizer; CDC, Centers for Disease Control and Prevention; COVID-19, Coronavirus disease-2019; PPE, Personal protective equipment; PV, Performance verifier; TG, Triglycerides

after taking all necessary corrective and preventive measures. Other biochemical parameters, except lipase and TG, did not show any significant changes in their respective control values. The application specialist speculated that the error might be related to the use of sanitizer.

Analytical interference is defined as deviation from the actual value of the analysis due to the presence of some endogenous or exogenous substances. In a clinical laboratory setting, these interventions can be an important source of laboratory errors with major clinical outcomes[4].

The above hypothesis ignited our mind to conduct root cause analysis and investigate the effect of various sanitizer contaminations on these biochemical parameters. We assessed and compared the impact of different types of sanitizers frequently used in our laboratory, having a composition similar to most of the sanitizers are being used nowadays.

It was therefore decided to determine and compare the effects of different types of sanitizers often used in our laboratory. All clinical chemistry parameters were evaluated, and focussed specifically on serum TG and Lipase using performance verifiers (PV). The recognition and management of these issues is a crucial area for improvement to reduce laboratory errors that have cropped up in times of this COVID-19 pandemic.

This study aims to provide a practical and much-needed insight into the type of sanitizers to be used in a clinical laboratory setting without affecting the quality of test results.

Materials and Methods

The present experimental study was carried out in the Department of Biochemistry at Jaypee Hospital, Noida and was revalidated at All India Institute of Medical Sciences, Jodhpur, INDIA. The study was conducted in the month of July 2020. Performance verifier material from Ortho Clinical Diagnostics was contaminated with sanitizer and 70% isopropyl alcohol. In the 3 mL of PV solution, 0.25 mL of contaminant was added. We also contaminated the reagent cartridge surface with sanitizer in the hospital lab on a routine basis.

The composition of hand sanitizers used for study were: (a) isopropyl alcohol IP (75% *v/v*), hydrogen peroxide IP (0.125% *v/v*), emollient (glycerol IP-1.45% *v/v*) and purified water; (b) 70% Iso-propyl alcohol.

Four groups were formed- PV Neat (G1), PV +Sanitizer (G2), PV + 70% isopropyl alcohol (G3), contaminated reagent surface by sanitizer (G4) and PV-1 and PV-2 were run for all clinical chemistry parameters especially cholesterol, triglyceride (TG), amylase and lipase.

All Performance verifiers (G1, G2, G3 ,and G4) were run five times in different run in a day, and we repeated this exercise every day till five days. Cartridge contaminated on the surface with sanitizer used for NeatPVs run as above suggested protocol. All clinical chemistry parameters, along with TG and lipase, were analysed[5,6].

Statistical analysis

All the parameters were expressed as mean and standard deviation (SD). Analysis of quantitative data between a qualitative variable with more than two subgroups was done using one-way ANOVA. Tukey's Post Hoc test was then used for observations between individual groups of PVs. $P<0.05$ was considered statistically significant. All statistical analysis was performed using Graph Pad Prism software.

Results

The best approach for interference testing involves comparing the test method with the reference method. Interference by exogenous compounds has to be proved using a different approach. This consists of spiking native samples with some exogenous combination. In the present study, the interference caused by sensitizer was evaluated by comparing the results among G1, G2, G3 and G4 groups. Alcohol is the major component of all types of sanitizers, so alcohol contamination was also investigated by incorporating 70% isopropyl alcohol in one group.

Comparison of four groups: Performance verifier Neat (G1), Performance verifier with Sanitizer (G2), Performance Verifier with 70% Isopropyl alcohol (G3), contaminated reagent surface by sanitizer (G4), for meanPV-1 and PV-2 values of cholesterol and amylase parameters showed no significant changes in their mean value among groups and were within manufacteruer provided range. TGtest revealed a significant difference among the groups for both PV-1 (P <0.0001) and PV-2 (P <0.0001) (Table 1).

Comparison of four groups G1, G2, G3 and G4 for mean PV-1 and PV-2 of lipase test also showed significant difference among the groups for both PV-1 (P< 0.0001) and PV-2 (P< 0.001) (Table 1).

After applying Tukey's test for pairwise group comparison, the mean TG and lipasevalues of Level 1

Table 1 — Comparison of mean Triglycerides and LipasePV-1 and PV-2 values among different groups
[Values are mean ± SD of number of observations with upper and lower limits (range)]

Performance Verifier	Character (N)				F value	*P* value
	G1 (25)	G2 (25)	G3 (25)	G4 (25)		
Triglyceride (mg/dL) (Range: 95.9-129.9) (PV-1)	112.4 ± 2.6 (107.7-119.7)	133.7 ± 3.2 (129-141)	113.6 ± 2.3 (111-119)	111.7 ± 1.4 (110-114.3)	339.7	< 0.0001
Triglyceride (mg/dL) (Range: (261.9-270.9) (PV-2)	254.8 ± 2.9 (235.1-260.2)	274.1 ± 4.9 (266-284)	242.5 ± 4.6 (235-251)	240.2 ± 1.1 (238-242)	264.9	< 0.0001
Lipase (U/L) (Range: 148-188) (PV-1)	163.4 ± 9.3 (143.3-173.3)	199.9 ± 4.9 (195-215)	177.1 ± 4.3 (171-187)	174.9 ± 3.9 (170-183)	208.5	< 0.0001
Lipase (U/L) (Range: (591-723) (PV-2)	670.1± 9.1 (655.4-712.2)	658.5 ± 14.3 (641-690)	670.1 ± 6.4 (663-686)	666.6 ± 5.5 (660-674)	6.54	< 0.001
Cholesterol (mg/dL) (Range: (138.4-164.4) (PV-1)	152.8 ± 4.3 (148.1-157.2)	152.2 ± 4.1 (148-156.5)	150.7 ± 4.2 (146.1-154.5)	151.7 ± 4.3 (146.5-155.6)	1.106	0.251
Cholesterol (mg/dL) (Range: 231-263) (PV-2)	253.6 ± 5.9 (248.2-260.1)	250.2 ± 6.0 (244.0-256.4)	250.1 ± 5.9 (246.2-255.1)	252.4 ± 5.8 (247.3-258.0)	2.118	0.103
Amylase (U/L) (Range: 63-87) (PV-1)	77.0 ± 4.1 (71.0-80.2)	76.5 ± 4.0 (72.2-80.4)	77.3 ± 4.2 (72.5-81.3)	77.5 ± 4.1 (72.9-82.2)	0.28	0.84
Amylase (U/L) (Range: (266-350) (PV-2)	305.0 ± 6.4 (299.1-312.5)	303.8 ± 5.0 (298.3-309.3)	302.0 ± 3.3 (299.0-305.6)	303.1 ± 5.3 (298.4-310.1)	1.51	0.22

Performance Verifier Neat (G1) , Performance Verifier +Sanitizer(G2), Performance Verifier + 70% Alcohol(G3),Contaminated reagent surface with sanitizer(G4), PV- 1(Performance Verifier Normal), PV- 2 (Performance Verifier Abnormal)

The comparison was done using ANOVA (Analysis of Variance) test among the groups *(*P* <0.05) significant,** *P* <0.01) very significant,***(*P* <0.001) indicates that groups are responsible for variance in the measured variable and is highly significant & rest are not significant (*P* >0.05)

Table 2 — Post hoc comparison of mean Triglycerides and Lipase PV values between different groups

Parameters	Groups					
	G1-G2	G2-G3	G2-G4	G1-G3	G1-G4	G3-G4
Triglyceride (PV-1)	***	***	***	NS	NS	NS
Triglyceride (PV-2)	***	***	***	***	***	NS
Lipase (PV-1)	***	***	***	***	***	NS
Lipase (PV-2)	**	**	NS	NS	NS	NS

Performance Verifier NEAT (G1), Performance Verifier +Sanitizer (G2), Performance Verifier + 70% Alcohol (G3), Contaminated reagent surface with sanitizer (G4)

Comparison was done using Tukey's test between the groups *(*P* <0.05) significant, **P* <0.01) very significant, *** (*P* <0.001), NS= not significant

and 2 remained significantly higher in PV1+ sanitizer group (G2) when compared to Neat PVs group (G1) for both levels. Significantly higher values were observed in mean TG and lipase values of both levels of PVs contaminated with sanitizers compared with PVs contaminated with 70% isopropyl alcohol (Table 2).

Although for PV-2 significant difference was observed between mean values of alcohol contaminated control TG group (G3) and Neat control TG group (G1), but both mean values were within the control range provided by manufacturer (262 -271 mg/dL) and no significant difference was observed between both groups for PV-1 (Table 2).

Likewise, mean values of sanitizer contaminated PV-1 and PV-2(G2) were significantly higher when compared to Neat PV-1 (G1) in lipase. Although for PV-1 significant difference was observed between mean values of alcohol contaminated control group (G3) and Neat control group (G1), and both mean

values were within the control range provided by manufacturer (148 to 188 U/L), while no significant difference was observed between G3 and G1 values for PV2 (Table 2).

Both PV-1 and PV-2 levels were affected by sanitizer and a positive bias was observed for TG, whereas only mean PV-1showed positive bias and PV-2 values remained unaffected by sanitizer contamination for lipase. We had records of altered TG and Lipase contol values during the use of sanitizer in laboratory. Figure 1A & B shows deviated control values of TG PV-1 and PV-2, respectively. Figure 2A & B shows deviated control values of Lipase PV-1 and PV-2, respectively.

After conducting this study and starting the using 70% isopropyl alcohol, the control values of TG and lipase were resumed within manufacturer defined range. Figure 1A & B shows within range control values of TG PV-1 and PV-2, respectively. Figure 2A & B shows within range control values of Lipase PV-1 and PV-2, respectively.

Discussion

A large proportion of errors in the laboratory process occur in the pre-analytical phase of the testing process. Therefore in the evaluation of biochemical assays, pre-analytical factors need to be fully considered and investigated than more traditional direct analytical factors. During this COVID-19 pandemic, the use of various sanitizers with PPE kits was introduced for laboratory technicians while working in the laboratory.

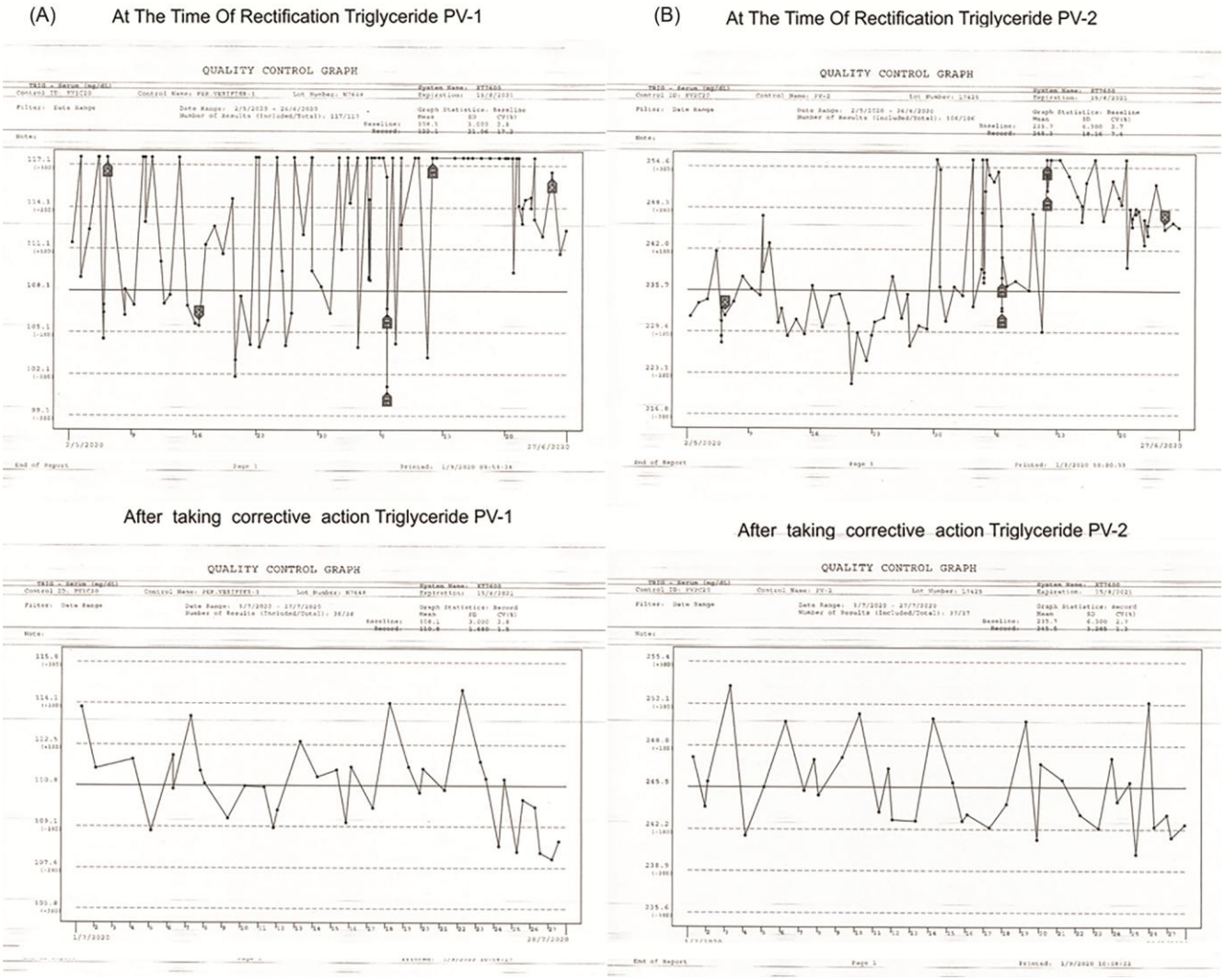

Fig. 1 — Shows deviated control values of TG PV-1 and PV-2

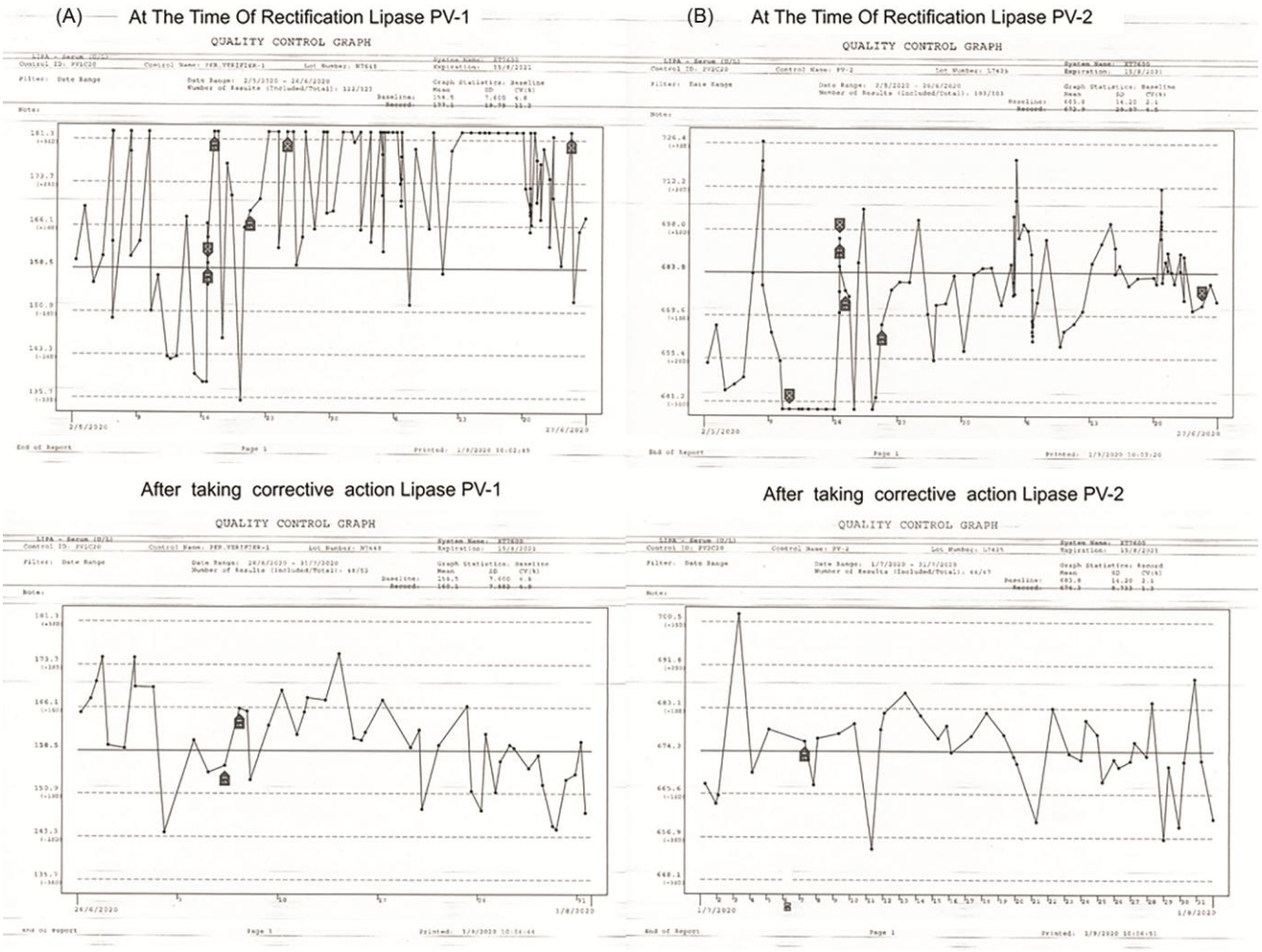

Fig. 2 — Shows deviated control values of Lipase PV-1 and PV-2

Our study indicated that the values of TG (PV1, PV2) and lipase (PV1) were significantly higher when quality control material was contaminated by sanitizer. Similarly, the mean values were within manufacturer defined ranges when contaminated with 70% isopropyl alcohol.

It indicated that some component other than alcohol in the sanitizer was interfering with the assay. The serum TG estimation principle involves the conversion of TG into glycerol and free fatty acids, and then glycerol is used as a substrate to produce final dye quinoneimine, which gives a quantitative estimation of serum TG[5]. Glycerol in emollient may have participated in the above reaction and contributed to the final dye formation and thus enhanced the values of PV-1 and PV2. Instructions provided by the manufacturer of the equipment also mentioned that collection tubes should be glycerol free as it can cause a false increase in TG levels.

Most reagents for TG estimation use an enzymatic method based on dihydroxyacetone phosphate from glycerol oxidation. The concentration of TG is proportional to the rate of glycerol oxidation. Therefore, an increased amount of glycerol in the sample would result in a false increased concentration of triglycerides. Several cases of pseudo-hypertriglyceridemia due to both exogenous and endogenous accumulation of glycerol have been described in the literature. A case of a patient with high triglyceride concentration (11.3 mmol/L) and a very low lipemic index due to exogenous glycerol contamination was reported[7].

The instruction manual of serum lipase estimation mentioned that highly elevated glycerol concentrations are usually caused by contamination and may interfere with lipase assay as glycerol is one of the intermediates formed during estimation[6]. As in our study, we used sanitizer as a contaminant which

contained glycerol (moisturizer). So we can assume that high Lipase results of PV-1 may be due to the glycerol present in the sanitizer. However, no significant difference in PV-2 results could be seen in Lipase estimation. This might be due to the low concentration of glycerol (present in the sanitizer) that is used for contamination for high control PV-2.

Isopropyl alcohol, particularly in solutions between 60-90% alcohol with 10-40% purified water, is antimicrobial against bacteria, fungi, and viruses. 70% isopropyl alcohol upholds critical requirements for use as a bactericidal in clean rooms or medical facilities, but also general purposes. Seventy percent isopropyl alcohol with 30% water solutions produces less vapour and odour, therefore reducing risks of toxic fumes or combustion[2].It is less flammable but also offers a more economical for general wipe down and large-surface disinfection.

The use of skin preparation pads containing 70% isopropyl alcohol was unlikely to generate false-positive blood ethanol levels using an enzymatic assay[8].These observations implied that the use of 70% isopropyl alcohol has minimal chances of interference in the estimation of biochemical analytes in patient samples compared to other sanitizers[8].

The incubators in the analysers are washed using de-ionized water, followed by a fresh 70% isopropyl alcohol. Cleaning solutions like bleach, ammonia, ammonia-containing compound, and any other oxidizing agents corrode unprotected metal parts of the incubator of the analysers and may cause erroneous results[9].

These observations suggest that70% isopropyl alcohol with 30% water should be preferred for instrument cleaning, instead of other sanitizers. Further, the sample cups and tips, which are handled with sanitizer contaminated gloves might have glycerol. Therefore, the gloves should also be washed in the same way. During the COVID-19 pandemic, it has become common practice to use sanitizer on gloves as a part of personal hygiene. Although the use of glycerol-based hand sanitizers is suitable for personal hygiene, its minute contamination adversely affects the results of TG and lipase testing. Our results advises laboratory personnel to use 70% isopropyl alcohol for instrument cleaning and hand hygiene with double gloves on while working in the clinical laboratory. By using this practice, both safety of laboratory workers, and results for the quality of TG and lipase parameters can be resolved.

Limitations

In the present study, the impact of sanitizer and alcohol was measured on only quality control materials. Further studies can be designed and conducted to evaluate the impact of sanitizer and alcohol on the patient's samples.

Conclusion

The findings of the present study reveals that the sanitizers containing glycerol as emollient may be avoided or used cautiously so that results of analytes like lipase and triglyceride are not affected.

Conflict of interest

All authors declare no conflict of interest.

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