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EXPERIMENTAL ANALYSIS OF LIQUID PHOTO STABLE BILIRUBIN FROM POOLED HUMAN SERUM AND ITS USAGE AS ENDOGENOUS BILIRUBIN CONTROL MATERIAL IN A CLINICAL LABORATORY

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ABSTRACT

Introduction: Quality control is an integral part of any clinical laboratory, which requires constant maintenance to deliver robust, reliable reports. Bilirubin in control material is an extremely photosensitive and unstable material; thus, we aimed to prepare endogenous bilirubin control material from pooled human serum and benchmark its stability against commercially available control materials at a fixed temperature range of 2°-8°C. **Material & Methods:** Endogenous bilirubin control material was prepared from the pooling of residual serum samples, where each total bilirubin level (Diazo method) was in the ≥3.0 mg/dl to ≤10.0 mg/dl range. Samples that were positive for HIV I/II, HbsAg, anti- HCV, Haemolyzed, Lipemic, SGOT, and SGPT >250U/L, Insufficient samples (<100 μL) were excluded. The baseline mean was established. Remnant serum was separated equally into a falcon tube and an amber vial shielded with aluminium zip-lock pouch, containing a silica gel packet enclosure. (A/AL). Commercial controls were reconstituted, and baseline means were settled. All control sets were stored at 2-8°C properly. Total bilirubin was measured daily from each control set for thirty days. A comparison of means, standard deviation, and CV% was done with ANOVA and post hoc Tukey HSD; a p value (< 0.05) was considered statistically significant. **Results:** Established baseline mean, final mean ±standard deviation (mg/dl), and CV% for total. bilirubin (mg/dl) from Falcon tube (8.0, 3.88±1.94,3.79), A/AL (8.0, 7.32±0.51,0.26), ERBA PATH (4.55, 3.21±1.10,1.22), RANDOX LEVEL 3 (5.43, 3.54±1.66,2.77), and TERSACO (4.64, 2.98±1.47,2.16) were observed, respectively. Statistically significant differences were recorded from the baseline means on the contrary to the final means of Falcon Tube, ERBA PATH, RANDOX LEVEL 3, and TERSACO (p <0.001), but there was no statistical difference between the baseline mean and final mean of A/AL (p 0.226). **Conclusion:** Our study concludes that endogenous bilirubin control material prepared from pooled human sera showed effective stability for thirty days at 2°-8°C when kept in an amber vial shielded within aluminium zip-lock enclosing a silica gel packet and can be used as in-house bilirubin control material. However, rapid deterioration was observed when stored in a falcon tube. Other commercial quality control materials showed acceptable analytical performance for 12-14 days.

KEYWORDS : Bilirubin, internal quality control, stability.

INTRODUCTION

Bilirubin, a product of heme metabolite, majorly formed from the heme degradation within haemoglobin during the erythrocyte breakdown. Unconjugated Bilirubin is generally insoluble with water but when conjuncts to plasma albumin with high affinity it results in formation of conjugated bilirubin [1]. Timely, assessment of the bilirubin level is quite essential for early diagnosis, both in adults (e.g., inflammatory, or obstructive lesions) and neonates (e.g., inborn errors, biliary atresia, or kernicterus) [2]. Robust quality control is an integral part of any clinical laboratory to keep a close performance check on all three phases, pre-analytical, analytical, and post-analytical with proper precision and accuracy [3].

Currently, most of the laboratories are utilizing commercial quality control materials, which is indeed a financial burden for several countries, hence pooled sera quality control material was considered an alternative to commercial quality control material, a cost-effective approach to combating the crisis. However, recent preparation of pooled sera for internal quality control purposes showed that total bilirubin (TBIL) control levels were highly unstable and unusable. Data from the study suggestive of even single freeze thaw leads to deterioration of bilirubin levels [4]. Lyophilised pooled sera material had similar instability post-reconstitution [5]. TBIL levels ultimately deteriorated and showed photodegradation, rendering it unfit for further quality benchmarks. The major culprit for the photo destabilization of bilirubin was either artificial light or ultraviolet, as per a few studies [1, 6, 7]. The NCCLS H18-A3 guideline suggests the use of amber vials or aluminium for protecting light sensitive materials [7]. One study revealed that the use of amber vials to store a photosensitive model drug of riboflavin was found to be effective [8], and a few previous studies [9, 10] have also shown that aluminium is an efficient shielding material with ultraviolet reflecting power.

Based on the current NABL guidelines, running quality control twice is indicated to eliminate errors and take corrective actions simultaneously maintaining the reliability of the results [11]. As per the updated protocol based on CLSI document C37, maintenance of aseptic conditions is strictly directed during the preparation of the pooled serum [12]. Our aim was to prepare a stable endogenous bilirubin control material from pooled human serum and benchmark it with other commercially available control materials at a fixed temperature range of (2° to 8°C).

MATERIAL AND METHODS

Study Design: Pilot Study, Experimental Type.

Study Setting and Area: This study was carried out at the Central Laboratory, Department of Biochemistry, Murshidabad Medical College & Hospital, West Bengal.

Study Duration: The study duration was thirty days (December 2023).

Our study was approved by the Institutional Ethical

Committee of Murshidabad Medical College and Hospital, West Bengal, India, bearing Registration No. ECR/1620/Inst/WB/2021 dated October 31, 2023.

Objective: To prepare endogenous bilirubin control material from pooled human sera and compare its stability against commercially available control materials within a fixed temperature range (2° to 8°C).

Inclusion Criteria

a) Residual serum samples received at the central laboratory whose TBIL (Diazo method) were within the ≥3.0 mg/dl (≥0.2 mmol/l) to ≤10.0 mg/dl (≤0.6 mmol/l) range from all age groups and all genders.
b) Samples were segregated within 2 hours of receiving them at the central laboratory.

Exclusion Criteria

a) Samples that rendered positive for HIV I/II, HbsAg, and anti-HCV.
b) Haemolyzed, Lipemic, and Insufficient samples (<100μL), respectively.
c) Samples that pertain to Serum Glutamic-Oxaloacetic Transaminase (SGOT) & Serum Glutamic Pyruvic Transaminase (SGPT) levels >250U/L.

Preparation Of Endogenous Bilirubin Control Material

A. Sample Separation And Segregation

Eighty-two residual serum samples in the Haemocheck™ Polymed Serum Tube, cap colour red, volume 3.0 ml, additive clot activator, received at the central laboratory were selected briefly whose TBIL (Diazo method evaluation with the EM360 Autoanalyzer based on NABL guidelines) was within the ≥3.0 mg/dl (≥0.2 mmol/l) to ≤10.0 mg/dl (≤0.6 mmol/l) range from all age groups and all genders were kept aside.

Selected samples were carefully examined for Haemolysis, Lipemic & any other visible artifacts. Each sample was pre tested with Rapid kits for HIV I/II (STANDARD Q HIV 1/2 Ab, SD Biosensor), HbsAg (HEPA™ CARD), Reckon Diagnostics, and anti-HCV (Oscar HCV Test Kit, Oscar Medicare).

B. Pooled Serum Preparation And Baseline Mean Establishment

A Sterile Falcon tube (TARSONS® 50 ML SPINWIN® CLEAR POLYPROPYLENE CENTRIFUGE TUBES WITH LIDS) was properly labelled as pooled serum along with date and time. 300μL from each sample was precisely drawn out into this sterile falcon tube, making its final net pool volume to be 24,600μL (24.6 mL).

The sterile Falcon tube was properly screwed with a cap, and it was gently swirled along with a couple of inversions. Violent agitation or vigorous shaking of this pooled serum is not recommended. From this prepared pooled serum, consecutively twenty pre-run TBIL estimations (Diazo method) were done for the establishment of the baseline mean value.

C. Pooled Serum Division And Storage

Pooled serum was finally checked again for HIV I/II, HBsAg, and anti-HCV using a rapid kit. Aseptically, carefully, this pooled serum was equally separated (12,300μL in each) into a New Sterile Falcon Tube (FT) and a sterile Amber vial (14.79 ml, Acme Vial & Glass Company). A sterile polypropylene orifice reducer (Acme Vial & Glass Company) with a sterile black polypropylene cap (Acme Vial & Glass Company) was specifically used to seal the amber vial. A silica gel (SORB-IT® (Silica Gel) GDTII) packet (not exceeding 2 grams) was placed inside aluminium packet (Micro-Tec Ziplock barrier foil storage bags, Labtech International), which enclosed this amber vial (A/AL). Both FT and A/AL were kept in the commercial fridge door in a fixed temperature range (2°-8°C). Temperature was monitored throughout the experiment using a pre-calibrated probe (Red LED Temperature Meter, -50~110 Degree C Detector Sensor Probe, 12V Digital Thermometer Monitor Tester, xCluma).

Reconstitution And Storage Of Commercial Controls

Lyophilised commercial controls considered were;

a) ERBA PATH (Transasia Bio-Medicals)
b) RANDOX LEVEL 3 (ACUSERA, Randox Laboratories)
c) TERSACO (multiCONTROL PATHOLOGICAL, Tersaco AG)

Each lyophilised commercial control (ERBA PATH, RANDOX LEVEL 3, TERSACO) was properly reconstituted with sterile distilled water (included with their kit). From each vial post reconstitution, serially twenty pre-run TBIL estimations (Diazo method) were done for each vial separately, and a baseline mean was established for each commercial control.

All the vials were stored in a commercial fridge door in a fixed temperature range (2°-8°C) along with FT and A/AL, Temperature was monitored throughout the experiment using a pre-calibrated probe (Digital Thermometer Monitor Tester, xCluma).

Endogenous Bilirubin Control Vs. Commercial Controls

There was no addition of preservatives in both FT and A/AL, nor were the samples spiked with artificial bilirubin materials to achieve the desired concentrations. Samples were not frozen. Daily TBIL was measured from FT, A/AL, ERBA PATH, RANDOX LEVEL 3, and TERSACO once, and the values were recorded until the 30th day.

Proper personal protection equipment (Nst Surgical Gloves, Surgicare) was used to maintain all the procedures aseptic. Sterile microtips were used for pipetting the serum for daily analysis. The well calibrated automated biochemistry analyzer EM 360 (Transasia Bio-Medicals) was used in accordance with the NABL guidelines, and the reagents used were Diazo, Linearity 23 mg/dl (Transasia Bio-Medicals), and appropriate Westgard multirules were applied. Any form of eccentricity found was corrected, and action was taken per se.

Containers were properly labelled with the batch number, serial number, date, time of pooling, and baseline mean value. Aseptic measures were always taken while preparing this pool. Cross-contamination was strictly observed. All the pooling processes were done at a normal laboratory temperature of 25°C and under normal laboratory illumination.

All vials must be considered biohazard materials and should be strictly handled by professionals only. A complete physical / digital record should be taken into consideration during data compilation and storage.

Statistical Analysis

The final data was drafted using IBM SPSS (Statistical Package for the Social Sciences) Statistics 20, Ver. 20.0.0.0.155, IBM Corp. 2006, 2011, US. For plotting the line graph, Origin 2022 Ver 9.9 was utilised. Mean, standard deviation (SD), and CV% were calculated using SPSS for FT, A/AL, ERBA PATH, RANDOX LEVEL 3, and TERSACO. The differences from the baseline mean (BM) in FT, A/AL, ERBA PATH, RANDOX LEVEL 3, and TERSACO were analysed with analysis of variance (ANOVA) with a post hoc Tukey HSD test, and a (p value) < 0.05 was considered statistically significant. The allowable total error for bilirubin was considered at 20% according to Clinical Laboratory Improvement Amendments (CLIA) criteria.

RESULTS

The established BM of TBIL for FT, A/AL, ERBA PATH, RANDOX LEVEL 3, and TERSACO were 8.0, 8.0, 4.55, 5.43, and 4.64 (mg/dl), respectively. The complete dataset from Day 1 to Day

30 is given in (Table 1). The final mean, standard deviation, and coefficient of variation (CV%) for FT and A/AL, ERBA PATH, RANDOX LEVEL 3, and TERSACO are given in (Table 2). There was a statistically significant difference between the baseline means of FT, ERBA PATH, RANDOX LEVEL 3, and TERSACO and the final means of FT, ERBA PATH, RANDOX LEVEL 3, and TERSACO (p value <0.001), but there was no statistical difference between the baseline mean of A/AL and the final mean of A/AL (p value 0.226). The dataset was also plotted in a line graph for FT, A/AL, ERBA PATH, RANDOX LEVEL 3, and TERSACO, showing TBIL concentration (mg/dl) vs. timeline (hrs/days) (Figure 1). On close observation, there was >20% of fluctuation from the baseline mean of TBIL levels within the Falcon tube on the 4th day, but for ERBA PATH, RANDOX LEVEL 3, and TERSACO, it was at the 13th, 14th, and 15th days, respectively. However, TBIL levels in A/AL had a 18.75% change from the baseline until the 30th day.

Table 1. Total Bilirubin Level (mg/dl) Per Day Data Point Observation In FT, A/L, ERBA PATH, RANDOX LEVEL 3, and TERSACO, Which Were Kept At A Fixed Temperature Range Of 2-8 °C. Falcon Tube*, Amber Vial Shielded With Aluminium Enclosing Silica Gel**

PARAMETERS	FT*	A/AL**	ERBA PATH	RANDOX	TERSACO
Baseline Mean	8.0	8.0	4.55	5.43	4.64
FINAL MEAN	3.88	7.32	3.21	3.54	2.98
SD	±1.94	±0.51	±1.10	±1.66	±1.47
CV%	3.79	0.26	1.22	2.77	2.16
P VALUE	<0.001	0.226	<0.001	<0.001	<0.001

(p value) < 0.05 was considered statistically significant

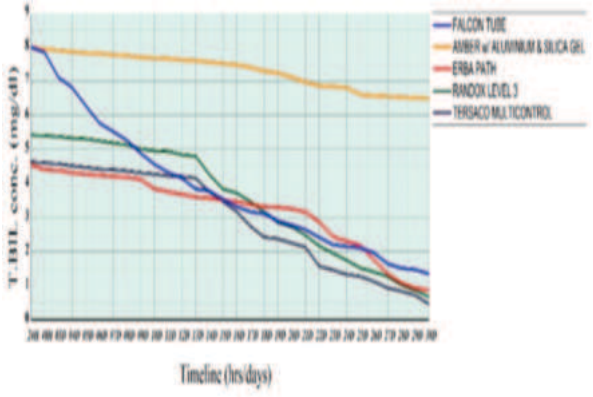

Figure 1. Line Plot Comparison Of Total Bilirubin Concentrations(mg/dl) In Falcon Tube, Amber Vial With Aluminium Packet And Silica Gel, ERBA PATH, RANDOX LEVEL 3, and TERSACO. The X-axis Plot Indicates The Timeline Trend, And The Y-axis Plot Indicates The Total Bilirubin Concentration (mg/dl).

Table 2. Baseline Mean, Final Mean, Standard Deviation, And Co-efficient Of Variation For FT, A/L, ERBA PATH, RANDOX LEVEL 3, and TERSACO Were Kept At A Fixed Temperature Range Of 2-8 °C. Falcon Tube*, Amber Vial Shielded With Aluminium Enclosing Silica Gel**

TIME	FT*	A/AL**	ERBAPATH	RANDOX	TERSACO
Day 1	8	8	4.55	5.43	4.64
Day 2	7.85	7.95	4.41	5.41	4.61
Day 3	7.12	7.91	4.39	5.38	4.59
Day 4	6.82	7.87	4.32	5.34	4.52
Day 5	6.28	7.83	4.28	5.31	4.48
Day 6	5.78	7.82	4.25	5.26	4.43
Day 7	5.53	7.78	4.21	5.19	4.41
Day 8	5.27	7.76	4.19	5.14	4.37
Day 9	4.89	7.7	4.13	5.02	4.32
Day 10	4.57	7.69	3.85	4.97	4.28
Day 11	4.33	7.67	3.76	4.94	4.23
Day 12	4.16	7.63	3.69	4.85	4.21
Day 13	3.85	7.61	3.61	4.81	4.17
Day 14	3.79	7.57	3.58	4.27	3.76
Day 15	3.54	7.52	3.52	3.84	3.48
Day 16	3.32	7.49	3.47	3.73	3.18
Day 17	3.18	7.41	3.39	3.47	2.75
Day 18	3.12	7.3	3.33	3.18	2.43
Day 19	2.91	7.25	3.31	2.86	2.38
Day 20	2.79	7.12	3.26	2.74	2.26
Day 21	2.65	6.98	3.17	2.49	2.14
Day 22	2.43	6.87	2.88	2.18	1.58
Day 23	2.21	6.85	2.45	1.96	1.47
Day 24	2.16	6.82	2.31	1.75	1.34
Day 25	2.12	6.61	2.19	1.53	1.28
Day 26	1.97	6.58	1.76	1.41	1.13
Day 27	1.65	6.56	1.35	1.28	0.94
Day 28	1.52	6.54	1.09	1.04	0.85
Day 29	1.48	6.51	0.94	0.87	0.73
Day 30	1.36	6.5	0.87	0.68	0.47

DISCUSSION

The reliability of an exceptional clinical laboratory depends on its core standardisation routine methods to be followed in accordance with the guidelines. Extensive quality control implications for each laboratory enable the reporting with better precision and accuracy, which includes total allowable error limits. The preparation and utilisation of pooled serum for the purpose of quality control has been tried, modified, and tested in several studies [2, 3, 4, 5, 11, 12].

In this new era, most of the commercially prepared bilirubin control material is either spiked with artificial bilirubin in a protein matrix or subsequently dissolved into synthetic substances for achieving desired levels of standards, where the absorbance peak along with absorptivity may yield significantly different results; hence, human serum was considered as a starting protein matrix for building the internal quality control material [2].

Sofronescu et al. [1], suggest that bilirubin is stable in the plasma without light exposure for up to 24 hours, and under normal laboratory conditions, a delay of until 8 hours does not significantly alter the results. In our experiment, we took the samples, which were segregated and reported within 2 hours of receiving them.

Damayanthi et al. [13] study found that the serum bilirubin levels were stable at -4 °C up to 24 h and at 33 °C up to 2 h with light protection. However, considering the baseline mean establishment and screening of each sample for HIV I/II, HbsAg, and anti HCV, the preparation of our endogenous bilirubin control material was done at a normal laboratory ambient temperature of 25°C and normal illumination.

Morishita et al. [14] investigated bilirubin in the samples for its stability under various temperature conditions (-20°C, 4°C, 25°C, and 37°C) with or without lighting for 7 days and found that the ratio of direct to total bilirubin does not significantly change at -20°C or 4°C. Suggesting a lesser fluctuation of bilirubin levels at -20°C or 4°C.

Mai NISHIOKA et al. [15] briefs in their study regarding shielded samples in micro blood collection tubes showed no fluctuation until 0.5 h, but later, the unbound bilirubin levels decreased by 18.5% within 5 hrs. These changes were significantly faster in the standard solution. In our study, the bilirubin levels in the A/AL showed better stability, may be due to the utilization of different materials for the purpose of shielding, there might be inconsistencies with the above study. Sabah et al. [8] suggested amber vials do provide photoprotective efficacy for photosensitive drugs, such as riboflavin (a model drug). Hence, in our study, we have utilized

the amber vial to store this prepared endogenous bilirubin control material. Coblentz et al. [9] mentioned the ultraviolet reflecting power of aluminium, and A. H. Taylor and Junius D. Edwards [10] suggested efficient ultraviolet reflecting properties in their studies. Henceforth we decided to include additional aluminium zip lock shielding for the amber vial, which will provide a good barrier.

We have also enclosed a silica gel packet not exceeding 2 grams in the aluminium zip lock to prevent moisture buildup as a few studies [16, 17] suggest using silica gel packets for effective moisture absorption. We enclosed a silica gel packet in the aluminium zip lock packet as it ensures minimal moisture buildup.

This endogenously prepared bilirubin control material should not be frozen or kept below 0°C, as freezing or thawing may lead to analyte deterioration and a rapid change in the bilirubin levels of this serum. It may also alter the chemistry for its further utility.

Even a single freezethaw cycle will lead to detrimental change; hence, this control material stays in liquid stabilized form at 2-8°C. No preservatives or additives should be added to this pooled serum, as they might alter the matrix and not yield the desired results. Negligible change was observed with respect to total bilirubin concentration within 24 hours in both storage containers (FT and A/AL). However, pooled serum kept in falcon tube experienced a detrimental colour change from a yellowish tinge to a greenish colour at the end of the 30th day of this experiment. Whereas there was no change in the colour from a yellowish tinge in the serum present in the amber vial within aluminium packet.

Apart from the in-house pooled serum control material where bilirubin is unstable [4], this prepared endogenous bilirubin control material kept in A/AL gives a better upper hand in terms of stability and commutability. Also, lyophilized in-house pooled serum is unstable in terms of bilirubin levels [5].

Limitations

The major disadvantage of this material is that its true value is already known to the user. As this was a pilot study done for 30 days using an autoanalyzer to determine its end results, more sensitive methods like HPLC and LC-MS/MS can be utilized to determine the stability of the bilirubin levels in the prepared control sera.

Prospects

Antibiotic and antifungal enhancement in this endogenously prepared pooled serum is yet to be done for its shell-life improvement and extension of the study period. Insights into the pH levels of this pooled sera are still to be explored.

Patent

A patent application was filed by the corresponding author with an international patent application bearing no. PCT/IN2023/051044 under the International Bureau (IB), WIPO (World Intellectual Property Organisation), and Indian Patent application no.202341062735, claiming the priority date 19/09/23, bearing the title "A PROCESS OF PREPARING ENDOGENOUS LIQUID PHOTOSTABLE BILIRUBIN CONTROL MATERIAL FROM POOLED HUMAN SERUM FOR ITS UTILITY IN A CLINICAL LABORATORY." This patent application is dynamic in nature and subjective; update accordingly with its timeline. Currently, this patent application does not have any funding support or rights that belong to any third-party members.

CONCLUSION

Our study concludes that endogenous bilirubin control material prepared from pooled human sera showed effective stability for thirty days at 2°-8°C when kept in an amber vial shielded within aluminium zip-lock enclosing a silica gel packet and can be used as in-house bilirubin control material. However, rapid deterioration was observed when stored in a falcon tube. Other commercial quality control materials showed acceptable analytical performance for 12-14 days.

Conflict of Interest: None

Financial Support: Nil

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REFERENCES

1. A.G. Sofronescu, T. Loebs, Y. Zhu, Effects of temperature and light on the stability of bilirubin in plasma samples, Clin. Chim. Acta 413 (3–4) (2012) 463–466.
2. Westwood A. The analysis of bilirubin in serum. Ann Clin Biochem. 1991 Mar;28 (Pt 2):119-30. doi: 10.1177/000456329102800202. PMID: 1859150
3. Kanagasabapathy AS, Swaminathan S, Selvakumar R. Quality control in clinical biochemistry. Indian J Clin Biochem 1996;11(1):17–25
4. Kulkarni S, Pierre SA, Kaliaperumal R. Efficacy of Pooled Serum Internal Quality Control in Comparison with Commercial Internal Quality Control in Clinical Biochemistry Laboratory. J Lab Physicians. 2020 Dec;12(3):191-195. doi: 10.1055/s-0040-1721151. Epub 2020 Nov 23. PMID: 33293781; PMCID: PMC7718080.
5. Jamtsho R. Stability of lyophilized human serum for use as quality control material in Bhutan Indian. J ClinBiochem. 2013;28(4):418–421
6. McDonagh AF. Photolysis and photoisomerization of bilirubin in serum specimens exposed to room lighting. Clin Chim Acta. 2008 Jul 17;393(2):130; author reply 131. doi: 10.1016/j.cca.2008.03.005. Epub 2008 Mar 20. PMID: 18397760.
7. NCCLS. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition. NCCLS document H18-A3 (ISBN 1-56238-555-0). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.
8. Sabah A, Shah A, Tanwir S, Arsalan A, Malik A, Ahmad I. Light transmission properties of pharmaceutical liquid bottles and evaluation of their photoprotective efficacy. Pak J Pharm Sci. 2020 Mar;33(2(Supplementary)): 877-885. PMID: 32863265.
9. Coblentz, W W, and Stair, R. Ultraviolet reflecting power of aluminum and several other metals. United States: N. p., 1930. Web. doi:10.6028/jres.004.013.
10. A. H. Taylor and Junius D. Edwards, "Ultraviolet and Light Reflecting Properties of Aluminum," J. Opt. Soc. Am. 21, 677-684 (1931)
11. Swaminathan S, Seshadri MS, Srikrishna G, Selvakumar R, Kanagasabapathy AS. Ethanediol stabilised quality control serum for measurement of zinc. Clin Chim Acta 1993; 215(1):119–121
12. Danilenko U, Vesper HW, Myers GL, Clapshaw PA, Camara JE, Miller WG. An updated protocol based on CLSI document C37 for preparation of off-the-clot serum from individual units for use alone or to prepare commutable pooled serum reference materials. Clin Chem Lab Med. 2020 Feb 25;58(3):368-374. doi: 10.1515/cclm-2019-0732. PMID: 31665109; PMCID: PMC7153737.
13. Damayanthi, Wickrama & Bandara, Atthanayaka & Priyadarshani, Buddhika. (2018). HOW DO LIGHT AND TEMPERATURE AFFECT LABORATORY INVESTIGATIONS ON SERUM BILIRUBIN?. Journal of Research in Health Science. 1. 4-13. 10.26739/2523-1243/-2018-1-2-1.
14. Morishita Y, Nakane K, Fukatsu T. Stability of Serum Bilirubin with or without Lighting at Various Temperatures. Japanese Journal of Clinical Chemistry. 1994;23(2):170–7.
15. Mai NISHIOKA, Masashi MIYOSHI, Takayuki NAKAO, et al. Effects of room-light exposure on unbound bilirubin measurement. Japanese Journal of Medical Technology. 2019, Vol.68, No.4, p.712.
16. Wiedower, A., Ondier, G., & Siebenmorgen, T. (2008). Investigating the utilization of silica gel packets in drying research-scale rough rice samples. Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences, 9(1), 96-104. Retrieved from https://scholarworks.uark.edu/discoverymag/vol9/iss1/16
17. Chirsty A, Sivarukshy P. Comparison of adsorption properties of commercial silica and rice husk ash (RHA) silica: A study by NIR spectroscopy. Open Chemistry. 2021;19(1): 426-431. https://doi.org/10.1515/chem-2021-0044