



**IMPLEMENTATION AND INDIGENOUS VALIDATION OF CLINICAL  
BIOCHEMISTRY INTEGRITY TESTING IN RETAINED SAMPLES**

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Article Received on 03/01/2018

Article Revised on 23/01/2018

Article Accepted on 13/02/2018

**ABSTRACT**

**Background:** Storage of serum is often necessary in laboratories because of technical issues or to stored samples for subsequent verification purposes. The aim of this study was implementation of sample integrity testing to determine whether the stability of biochemical analytes are affected by storage conditions. **Materials and Methods:** Clinical diagnosed samples were stored at 2–8°C for 48 hrs then analyzed for stability. The results of theoretical concentration obtained from fresh samples were compared with measured concentration obtained from retested sample or stored samples. This sample integrity acceptance % was compared with analysis of acceptable criteria as per standard guidelines of RCPA and CLSI. **Results:** Our results show that, creatinine, uric acid, total calcium, albumin, cholesterol, and triglyceride levels were within acceptable limit. Only exception of urea and total protein were beyond the acceptable limit. Acceptable limit was exceeds so we analysis the root cause and corrective and preventive action were taken. **Conclusion:** The samples on which the requested tests are done were retained/stored for a suitable period at temperature. For the purpose of cross verification and retesting if required later/for performing additional tests if required. The samples are maintained in suitable environment to protect the integrity of the sample and to protect the interest of the client and the laboratory. This saves a further vein puncture, but sample storage and sample integrity testing in our laboratory makes a query? This query leads to induce to do implementation of sample integrity testing.

**KEYWORDS:** RCPA, CLSI.

**INTRODUCTION**

Laboratory testing of patient samples is a complex process. The total testing process (or total testing cycle) is based on the original brain-to-brain loop concept described by Lundberg.<sup>[1,2]</sup> He outlined a series of activities, starting with the clinical question in the clinician's mind, leading to test selection, sample collection, transport to the laboratory, analysis, reporting back to the clinician, and final interpretation and decision making by the clinician. These activities have traditionally been separated into three phases (pre-analytical, analytical and post-analytical).<sup>[3,4]</sup> The term "laboratory error" is defined in International Organization for Standardization (ISO) 22367 as "failure of planned action to be completed as intended, or use a wrong plan to achieve an aim. Errors can occurring at any part of the laboratory cycle, from ordering examinations to reporting results and appropriately

interpreting and reacting to them" and is the preferred term.<sup>[5,6]</sup> So, laboratories must take steps to ensure reliable and accurate results are produced otherwise errors leads to incorrect or delayed patient results can affect medical decisions and compromise the efficacy of patient treatment. There is a spectrum of errors from very low to very high errors, and one can never achieve zero errors. No laboratory test or process is without errors. The laboratory should also determine how conditions unique to the laboratory, including testing personnel and environmental conditions, can impact risk and the probability of errors can be large. The laboratory must examine its processes for weaknesses or hazards where errors could occur and take action to detect and prevent errors before they affect test results.<sup>[6]</sup> This can be done by mapping the testing process or following a sample through pre-analytical, analytical and post-analytical stages of testing and examining each step in the process.

Errors were classified by Plebani *et al.*, as pre-analytical (68%) Analytical (12%) Post - analytical (20%).<sup>[7]</sup> However, the concepts may have value in shaping the laboratory's approach to error management is described by the accredited bodies.<sup>[8]</sup> Accreditation bodies such as CAP describes the definitions of the pre- and post-analytical processes and (items GEN.20348 and 20364) with the equivalent terms (pre- and post-examination procedures/ pre- and post-analytical phase) used in ISO 15189:2012 (items 3.10 and 3.11). The pre-analytical definitions are very similar but there are some differences in the post-analytical areas, with ISO 15189:2012 including "authorization for release" and "storage of samples" as post-analytical activities. These definitions illustrate the difficulties that can be encountered in discussions on extra-analytical phase errors and accounts for some of the variation in reported error rates.<sup>[9,10]</sup>

### Purpose of Storing the Sample

1. **Own controls:** Stored Patient samples can be used as their own controls through calculation of running averages of test results to indicate drift or shift in analyzer performance over time.
2. **Kit lot verification:** Manufacturers have started encoding expiration dates within reagent barcodes to prevent use post expiration date in this situations stored samples used as a tool for kit lot verification.
3. **Delta check:** Stored sample used for delta checks that detect significant differences between the current and previous test result for the same patient can flag and hold the result for operator review before release.
4. **Add on Diagnosis:** Addition/ repeat test on request (within 4hrs±30minutes for stable and particular test)
5. **Accuracy:** Evaluation and improvement of test accuracy
6. **Validation:** Method development and validation
7. **Error Eliminator:** Detect the pre-analytical errors such as mislabeled samples

### Sample storage analysis

Integrity means the stability of blood samples during storage was defined as the capacity of sample material to retain the initial value of the quantity measured within specified limits and under specified conditions.<sup>[11,12]</sup>

Integrity studies for different parameters are carried out on the retained samples re-testing for Clinical Biochemistry. The Indian Laboratory Accreditation Body, as per ISO 15189:2012 112<sup>[13]</sup> and CLSI<sup>[14]</sup> have certain instructions for the maintenance of quality of testing and procedures for accredited laboratories. The retention period of a sample after collection is 48 hrs. Sample integrity is critical to the safety of clinical laboratory services. If there is a problem with the sample, then test results are meaningless. Each time there is a problem with specimen integrity, patients experience wasted time in addition to anxiety and loss of faith in the

expertise of the phlebotomy staff. Patients may also experience harm, if harm is defined as delay in diagnosis, therapy, hospital admission or discharge.

The retained sample is preserved for the purpose addition/ repeat test on request. Preservation criteria are at 2°C-8°C for 48 hrs (from the time of collection). The total time interval for addition or repeat test is not less than 4 hrs (4hrs±30minutes). Laboratories face many challenges including equipment breakdown and the lack of reagents, certain factors like laboratory environment, time, lag between collection and processing by the laboratory, incompetency of laboratory personnel. All the above might be affect the quality of storage and retesting. These need further implementation of re-evaluation and corrective action by the laboratory. In a busy routine clinical biochemistry laboratory with approximate sample load 100/day collection is from the receipt of the laboratory to till processing. A time drift make maintaining and storage is difficult, rather impossible.<sup>[15]</sup>

### Problems experienced by Genesis laboratory

**Case 1:** We received a pre and post dialysis blood samples of a patient from outside standalone dialysis cum Renal Clinic. Physician requested total protein and albumin investigations from both samples. But the TRF mentioned as urea, creatinine, total protein and albumin test in pre-dialysis sample and urea and creatinine from post dialysis sample. The test was done and report has received by the patient attender. Physician called the laboratory and asks the report of post dialysis total protein and albumin report which is not requested in TRF too. Later we identified the errors which happen at the renal clinic itself. We are in the position that not able to help the patient and the physician. Later laboratory realized that if the sample is stored it can be supported both patient and the physician. The incident increases the suffering of patient added anxiety till the test was one after 10 days.

**Case 2:** Mr. X analyzed lipid profile in fasting state. The test was done; report was received on the next day evening by the patient's spouse. Hence the consultant suspect the patient may be a familial hyper cholesterolemia, and requested the laboratory to check LDL-cholesterol by direct estimation. Unfortunately sample was not available which is discarded on the same day evening. Later laboratory realized that if the sample is stored it can be supported both the patient and the physician.

**Case 3:** Lab received the cord blood and the blood sample from a maternity clinic. Test was processed for Bilirubin and TSH, on both samples. Since the serum TSH value is very low, the pediatrician requested the parent to check Thyroperoxidase antibody and thyroglobulin on the next day. As the lab failed to store the serum sample we are indent to take blood again for a new born of 3 days old. Hence the baby is a premature

one we face difficult to recollect the blood. These entire incidents happen in a week. So the laboratory decided to root cause the errors and plan to do corrective and preventive action. The gap analysis report reveals these type of errors can be easily eliminated by store the samples at least for 48 Hrs. Though the laboratory plan to implement and validate the retaining and testing of samples in biochemistry.

### Establishment of sample integrity testing based on ISO: Sample storage after testing

The samples on which the requested tests are done were retained/stored for a suitable period at temperature conditions as mentioned below in accordance with ISO Document 112 'Specific Criteria for Accreditation of Medical Laboratories' for the purpose of cross verification and retesting if required later/for performing additional tests if required. The samples are maintained in suitable environment to protect the integrity of the

sample and to protect the interest of the client and the laboratory. Samples which has to be tested again are stored with proper identification. (page3) again are stored with proper identification. Retention period of sample storage are given in the below table starts from the time of release of sample test reports dispatch.

### Final disposal of sample after retesting

The biomedical waste generated in the laboratory is segregated at the site of generation into color coded bags and disposed at the end of the day by biomedical agency with which the laboratory has entered into agreement. The disposal of biomedical waste is in accordance with the statutory regulations laid down by Pollution Control Board Rules 1998 as amended in 2000 & as per the Ministry of Environment and Forest, Government of India EIA notification 1984 under the Environment Protection Act 1986.

## IMPLEMENTATION OF SAMPLE INTEGRITY TESTING PROCEDURE

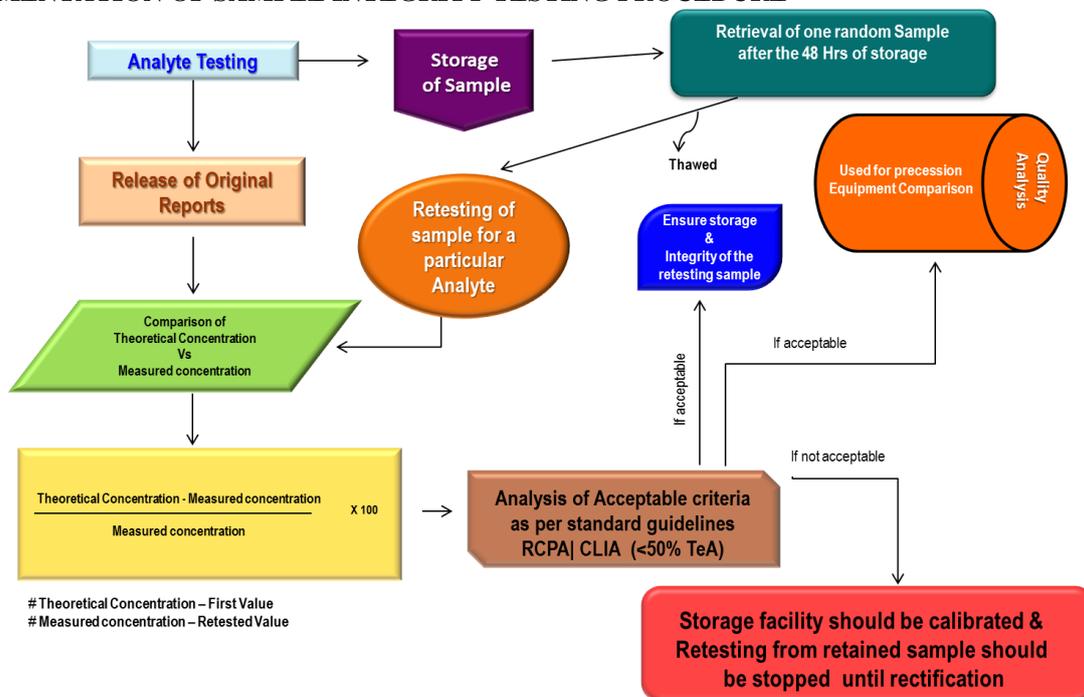


Fig. 1. Schematic representation of Integrity testing. Figure describes testing, storage, retesting, of a retained sample. Protocol established and performed as per the guidelines of ISO 15189:2012; Clause : 5.5.7

**FIGURE I:** Clinical biochemistry usually receives serum. They test the serum for biochemical substances present in blood. According to ISO 15189 norms, all results must be verified by a competent professional and dispatched by LIS (Laboratory Information System). After testing sample was stored in refrigerator. (2-8°C for 48 hrs). Retrieval of previous result of one random sample after the 48 hrs. Samples should be thawed only once. Retesting of retained sample for a anyone analyte per day. Compare the theoretical and measured concentration. Using the above mentioned formula for measuring. Acceptable criteria for integrity testing as per RCPA/CLIA 88 guidelines (>50 % total allowable error). Ensure the concentrations are within acceptable criteria for integrity testing .If integrity testing within acceptable limit it will be used for quality analysis. If integrity testing out of acceptable limit root cause analysis and corrective action should be taken.

Guidelines of Royal College of pathologist of Australia (RCPA) and Clinical laboratory standardization Institute (CLSI 88) which is followed as an Acceptance Criteria.

S.No	Analyte	Acceptable Performance / CLIA	Acceptable Performance / RCPA
01	Albumin	± 10%	
02	ALT	± 20%	
03	ALP	± 30%	
04	Amylase	± 30%	
05	Bilirubin Total	± 20%	
09	Calcium	± 1.0 mg/dL	
07	Chloride	± 5%	
08	Cholesterol total	± 30%	
09	HDL	± 30%	± 12 %
10	CK	± 30%	± 12%
11	Creatinine	± 15%	± 8%
12	Glucose	± 10%	± 8%
13	Iron	± 20%	± 12%
14	LDH	± 20%	± 8%
15	Magnesium	± 25%	± 8%
16	Potassium	± 0.5 mmol/L	± 5%
17	Sodium	± 4 mmol/L	± 2%
18	Protein total	± 10%	± 5%
19	Triglycerides	± 25%	± 12%
20	Urea	± 9%	±10%
21	Uric Acid	± 17%	
22	Ferritin		± 15%
23	Gama GT		± 12%
28	UIBC		± 8%

## RESULT

Table 1: Integrity Result for the Month of Sep – 2017.

S. No.	Patient ID No	Date of sample collection	Date of sample discard	Parameters	Original Value	Retested Value	Acceptable Limit %
1	314470	01/09/2017	03/09/2017	Urea	27	29	-6.9
2	314835	02/09/2017	04/09/2017	Albumin	3.80	3.80	-5.3
3	314958	03/09/2017	05/09/2017	Creatinine	0.60	0.63	-4.8
4	315195	04/09/2017	09/09/2017	Calcium	8.90	9.20	-3.3
5	315315	05/09/2017	07/09/2017	Cholesterol	160	164	-2.4
6	315555	09/09/2017	08/09/2017	Triglycerides	211	214	-1.4
7	315871	07/09/2017	09/09/2017	Urea	21	19	10.5
8	316085	08/09/2017	10/09/2017	Calcium	8.00	8.10	-1.2
9	316501	09/09/2017	11/09/2017	Creatinine	4.25	4.16	2.2
10	316696	10/09/2017	12/09/2017	Albumin	3.80	4.00	-5.0
11	316792	11/09/2017	13/09/2017	Urea	109	107	-0.9
12	316991	12/09/2017	14/09/2017	Uric acid	5.30	5.70	-7.0
13	317271	13/09/2017	15/09/2017	Creatinine	2.09	2.09	-1.4
14	317562	14/09/2017	16/09/2017	Urea	34	37	-8.1
15	31760	15/09/2017	17/09/2017	Calcium	9.80	9.70	1.0
16	317900	16/09/2017	18/09/2017	Albumin	3.70	3.90	-5.1
17	318144	17/09/2017	19/09/2017	Creatinine	1.08	1.13	-4.4
18	318324	18/09/2017	20/09/2017	Urea	23	21	9.5
19	318461	19/09/2017	21/09/2017	Total Protein	7.00	6.00	16.7*
20	318848	20/09/2017	22/09/2017	Urea	12	09	33.3*
21	318922	21/09/2017	23/09/2017	Creatinine	0.72	0.81	-11.1
22	319324	22/09/2017	24/09/2017	urea	33.00	32.00	3.1
23	319486	23/09/2017	25/09/2017	Albumin	2.50	2.30	8.7
24	319546	24/09/2017	26/09/2017	Urea	86	84	2.4
25	319848	25/09/2017	27/09/2017	Urea	62	60	3.3
26	319982	26/09/2017	28/09/2017	Creatinine	3.23	3.12	3.5
27	320241	27/09/2017	29/09/2017	urea	11	12	-8.3
28	320493	28/09/2017	30/09/2017	Creatinine	0.86	0.93	-7.5
29	320920	29/09/2017	31/09/2017	Urea	96	97	-1.0
30	320904	30/09/2017	01/10/2017	Calcium	9.20	9.20	0.0

We carried out implementation of Sample integrity testing is a comparison of theoretical concentration Vs measured concentration. Theoretical concentration refers an original value obtained from date of sample collection. Measured concentration refers a retested value obtained from date of sample discard. This sample integrity acceptance % was compared with analysis of acceptable criteria as per standard guidelines of royal college of pathologist of Australia (RCPA) and Clinical laboratory standardization Institute (CLSI) was given in the annexure.

Table -1 visualized the implementation and validation of sample integrity testing results for the month of September – 2017 was done by the genesis laboratory were the data's describes the theoretical and measured concentrations for the analytes of urea, creatinine, uric acid, calcium, cholesterol, triglyceride, total protein, albumin. The acceptable limits as per CLIA 88 guideline for following analytes are Calcium  $\pm$  1.0 mg/dL, Cholesterol total  $\pm$  30%, total Protein  $\pm$  10%, Triglycerides  $\pm$ 25% Urea  $\pm$ 9%, Uric Acid  $\pm$  17 %, Albumin  $\pm$  10%. The total allowable error (TeA) IS <50%. Table – 1 contain all parameters are within acceptable limit % or non-significance, only exception of total protein and urea at meticulous days (19<sup>th</sup> and 20<sup>th</sup> of Sept 2017), above the acceptable limit %.

Total protein as per guideline acceptable limit is  $\pm$ 10 % unfortunately 16.7%\* was obtained at 19<sup>st</sup> September. It is significantly increased then the acceptable limit. Similarly urea as per guideline acceptable limit is  $\pm$ 9 % unfortunately 33.3%\* was obtained at 20<sup>rd</sup> September. It is drastically increased then the acceptable limit. If acceptable limit not within limit should analysis the root cause and corrective and preventive action were taken.

The root cause of the study reveals that, the sample was improperly stored by a new staff, these error was categorise as personnel incompetency. The above mentioned total protein and urea was beyond the acceptable limit on particular days. Those days new staff handles the refrigerator mistakenly defrosting was done. Corrective action was taken on 22<sup>nd</sup> Sept by induction training for newly joined staff. Preventive action backup refrigerator was procured for maintaining and storage of sample.

## DISCUSSION

Maintaining retention samples assistances to achieve fulfillment with these requirements. It is a great deal of attention for sample storage and discarding in clinical laboratories. Most frequently there is a problem with specimen integrity, which creates a inconvenience in adding a test, to detect delta checks, using for kit lot verification, identifies the mislabeled samples, to analyze test accuracy and validation of own controls. All those events lead to inconvenience and bad experience of patients among lab diagnosis. Patients may also experience harm, which defined as delay in diagnosis,

therapy, hospital admission or discharge. Scientific literature proves the stability of serum, plasma. None of them have suggested the implementation of sample integrity process using retained sample. The authors referred some of the real time experience. The purpose of this experimental study was to implement the sample integrity testing process in stored sample which assists in add-on tests in existing sample are often requested by the clinician. This helps to avoid further vein puncture, but sample storage and the stability of the sample in our laboratory makes a query on above said cases? If we claim to be without sin (errors), we deceive ourselves and the truth (realization) is not in us. This gap analysis leads to do implementation of sample integrity testing in our laboratory. Sample integrity testing is a part of process control, one of the essentials of a quality management system.

The quality of the results is directly proportional to the quality of sample. The laboratory must be proactive in ensuring that the samples it receives meet all of the requirements needed to produce accurate test results. ISO and CLSI define a sample as “one or more parts taken from a system and intended to provide information on the system” (ISO 15189:2007). The term “specimen” is very commonly used in the laboratory to indicate a sample taken from the human body, but the terminology used throughout ISO documents is “primary sample”, or just “sample”. Importance of good management proper storage of samples is critical to the addition and reliability of testing, and, therefore, to the confidence in laboratory diagnosis. Improper sample storage influence patient's knowledge wasted time, in adding to anxiety. Patients may also knowledge mischief, if harm is defined as delay in diagnosis, therapy, therapeutic decisions hospital admission or discharge and can have significant impact on patient care and outcomes. It is important to provide accurate laboratory results in order to assure good treatment. Inaccuracies in testing can impact length of hospital stays, as well as expenses of treatment and the diagnosis. Values are more than the acceptable limit can also affect laboratory efficiency, leading to affect measured concentration with resultant waste of personnel time, supplies, and reagents. Set a laboratory policy for retention of each type of sample. Some samples can be quickly discarded, and others may need to be retained for longer periods. Monitor stored samples, and do not keep for longer than necessary, as refrigerator and freezer space may be limited. Sample storage must be monitored, as samples may deteriorate with these conditions. The inventory of stored samples should be reviewed at specified intervals to determine when they should be discarded.

Health care delivery is no longer a simple process of examining the patient and giving him a prescription. Over the years there has been rapid expansion in the various branches of health care services. As part of this expansion process and explosion of laboratory diagnosis has gained tremendous importance in today's practice.

Through the implementation of sample integrity testing the laboratory can ensure that the results being issued are reliable enough to allow additional test request and decisions to be taken with confidence. Sample integrity study of those errors which are the responsibility of the laboratory and of the procedures used to recognize and minimize them. Incorrect laboratory results may lead to wrong management decisions with possible fatal results. The reliability of laboratory results is therefore most important. It is not sufficient to 'think' that 'my' results are satisfactory. This has to be proved with scientific evidence.

### CONCLUSION

Researchers who assess the retest reliability of a newly developed measure should provide sufficient information for potential users to come to conclusions about the quality of both the measure and the evidence. With regard to establish and implement a policy for sample integrity testing for sample storage and disposal. Maintain sample integrity and assure that all regulations and requirements are met. The samples on which the requested tests are done are retained/ stored for a suitable period at temperature. For the purpose of cross verification and retesting if required later/for performing additional tests if required. The samples are maintained in suitable environment to protect the integrity of the sample and to protect the interest of the client and the laboratory. This saves a further vein puncture, but sample storage and sample integrity testing in our laboratory makes a query? The lab was improved a lot in quality testing and the patient care. After implementation the error has not been happen again which reveals from the continuous improvement program in the Quality management system.

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