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Original Research Article

Use of Quality Techniques in Improving Accuracy of C-Reactive Protein Quantitative Estimation in The Laboratory in A Tertiary Care Hospital

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Abstract: Quality in laboratory has huge impact on diagnosis and patient management as about 80% of all diagnosis is made on the basis of laboratory tests. The clinical measurement of C-reactive protein(CRP) in serum , therefore appears to be a valuable screening test for organic disease and a sensitive index of disease activity in inflammatory, infective and ischaemic conditions. Accurate result of CRP values is important to accurately predict the difference between viral and bacterial infections and also in predicting whether the patient is responding to antimicrobial therapy among others and therefore the need for its accurate reporting. This prospective study was carried out in Shaheed Hasan Khan Mewati, GMC College, A tertiary care centre from the period September 2015 to April 2016 for a period of six months to estimate accuracy of semi-Quantitative results of CRP testing and to improve the accuracy of CRP semi-quantitative screening by using six sigma techniques, if needed. The entire process was divided into Define, Measure, Analyze phase followed by suggestions for improvement for improving accuracy . 41 consecutive readings of CRP positive control by 2 operators was recorded as with a standard deviation of 3.86 with a mean of 6.21. c_{pk} was calculated as 0.26. Since it was less than 1 , hence The process did not meet its specification. Further there were approximately 390243 DPMO and hence the process was at less than 2 sigma level or approximately at 1.6 sigma equivalent .Furthermore Fish bone and pareto analysis followed by hypothesis testing by Chi square was done to cofirm the results which proved that pipetting error among technicians and dilution error due to bubble formation are significant causes for CRP reporting errors. This study suggests that by implementing quality techniques the accuracy of CRP testing could be improved by working on the the above mentioned root causes and similar practices could be implemented in other aspects of healthcare improve diagnosis and treatment.
Keywords: C-reactive protein, Six Sigma, Quality

INTRODUCTION

Quality in laboratory has huge impact on diagnosis and patient management as about 80% of all diagnosis is made on the basis of laboratory tests [1, 2]. International Organization for Standardization (ISO-15189) has recommended assessment and monitoring of quality management systems (QMS) in laboratory as quality improvement efforts towards quality laboratory services [3]. Quality laboratory management system has main objectives which are timely, precise and accurate results and meeting patients need and satisfaction. C reactive protein(CRP) the classic acute phase of human serum is synthesized by hepatocytes. Normally it is present only in trace amounts in serum, but it can increase by as much as 1000-fold in response to injury or infection. The clinical measurement of CRP in serum, therefore appears to be valuable screening test for organic disease and a sensitive index of disease activity in inflammatory, infective and ischaemic conditions. Measurement of C-reactive protein (CRP) and its quantitative testing , an acute-phase protein with a normal serum level of less than 1mg/dl, which may increase rapidly within hours of an inflammatory stimulus such as infection or tissue injury, is a reliable indicator of disease activity in various clinical conditions including distinguishing between viral and bacterial infections; indicating response to antimicrobial therapy; monitoring inflammatory conditions such as rheumatoid arthritis and systemic lupus erythematosus; and surveillance of malignant disease [4-10].

The study was conducted at Shaheed Hasan Khan Government Medical College, Nalhar to estimate accuracy of semi-Quantitative results of CRP testing and to improve the accuracy of CRP semi-quantitative screening by using Six sigma techniques, if needed. Accurate result of CRP values is important to accurately predict the difference between viral and bacterial infections and also in predicting whether the patient is responding to antimicrobial therapy among others and therefore the need for its accurate reporting. In our laboratory CRP is tested by Latex slide Method. The principle is based on immunological reaction between CRP antisera bound to biologically inert latex particles and CRP in the test sera. The study was undertaken to understand the accuracy of CRP reporting in the laboratory and also to undertake steps to improve quality in CRP reporting, if needed. Implement Qality standards is very critical in healthcare and patient safety. The study is first of its kind in India and the results could have great impact on the improvement of CRP testing in Medical Laboratory and thereby leading to better diagnosis. Similar approach can be adopted in others aspects of healthcare.

MATERIAL AND METHODS

This prospective study was carried out in Shaheed Hasan Khan Mewati, Government Medical College, A tertiary care centre from the period September 2015 to April 2016 for a period of six months. The entire process was divided into Define, Measure, Analyze phase . In the improvement phase suggestions based on analyze phase were given.

Define Phase

Define phase - In the define phase the problem statement and Mission statement was defined:

Problem Statement- It was observed that the CRP values is not accurate in about >= 40% of cases and falls below reference range .This was estimated by Clinical correlation of the test. This has been existing for the past 1 year leading to inability to predict difference between viral and bacterial infections and also inability to predict response to therapy in these 40 % cases.

Mission Statement-Goal was to predict the root causes for improving the accuracy CRP quantitative reporting.

Process Boundaries. These were set as follows

The experiment was started with assigning of Technicians for 20 measurement of CRP Positive control reference value followed with estimation of standard deviation and mean of 41 samples on consecutive days.

The Experiment was stopped with analyzing the factors for improvement of accuracy of CRP value.

The project Milestones and Process Description were defined[Fig1 and Fig2]

The following Constraints were encountered during the experiment

1. Lack of adequate Staff.
2. Lack of Motivation among staff members. As the test were conducted free of charge, The patients rarely complained. However there were complaints from the treating clinicians to improve the results.
3. Lack of EQAS for comparison.

Table 1: The Team Comprised of the members

Team Members	Role
Technician 1	1. CRP reporting of Positive Control values in measure phase 2.Brainstorming
Technician 2	1.CRP reporting of positive control values in measure phase 2.Brainstorming
Consultant 1	Project Development
Consultant 2	Brainstorming for root causes
Consultant 3	Six Sigma Black Belt

Measure Phase

Process Flow Chart- Blood specimen were collected in Red Plain blood collection tubes. Centrifugation was done at 5000 g for 1 minute to separate the serum .The samples were then processed using the Semi quantitative testing and CRP titre estimation by using positive controls. Reference standards of CRP positive control is at 1:8 or 6.4 mg/dl.

Five test tubes with a dilution of positive control at 1:2 , 1:4 ,1:8 ,1:16 were set up . Samples were diluted according to dilution factor on each test tube with normal saline solution. CRP latex reagent was gently resuspended by adding one drop to each test field. It was then mixed well with a stir stick and then gently rocking the slide for 3 minutes.[Fig 2]

Definitions

- Critical to Quality (CTQ'S)- A **CTQ** is a feature or characteristic that, if nonconforming, will result in a failure to meet a user, business, product, or component requirement. Furthermore, an item is critical to safety if a nonconformance may result in a failure and unsafe condition, per the established risk management documentation [11].
- Critical to processes(CTP)-These are the key process input variables. These are the process parameters which influences other critical approaches – Critical to Quality (CTQ), Critical to Delivery (CTD) and / or Critical to Cost (CTC) [12].

The Following CTQ'S Were defined(Table 2)

Table 2-Critical t o Quality paprameters in CRP Testing

S.no.	CTQ	Description	Type of Data
1.	Test Result	Semi quantitative testing with values ranging between 1.6 mg/dl to 512mg/dl	Continuous Data

The Following CTP'S Were defined:(Table 3)

Table 3-Critical to Processes parameters for CRP testing

S.no	CTP	Description	Data Type
1	Centrifuge	1000-5000g	Variable
2.	Operators	Determine training	Attribute
3	CRP Reagent		Attribute
4.	Normal Saline	0.9% concentration	Attribute
5.	Pippette	10-50microlitres	Attribute
6.	CRP Plate	6 circles	Attribute

Variation in data

The statistics were carried on Minitab 17 for Histogram and standard deviation calculation, Process Control and Hypothesis testing. In the Measure phase 41 samples of positive controls were analysed and their mean and standard deviation calculated. The CRP positive control reference value by calculating mean of 20 samples by trained laboratory personnel was 6.4 mg/dl.

After the calculation of standard deviation and mean, the process capability index was calculated. The following formula was used-

Min.-Minimum
CPK=Min[USL-μ/3σ ,μ-LS L/3 σ]

Where USL and LSL Were defined as Upper Specification and Lower Specification Limit.

Defects part per Million opportunity(DPMO) was then calculated.

Analyze phase-In the Analyse phase the variations of inputs were identified to identify root causes. We used the cause and effect or Ishikawa diagram(Fishbone analysis) to identify the probable root causes. Before the Fish bone diagram was filled, a brainstorming session took place to ascertain the possible causes for inaccurate CRP results. The various sources of variation in inputs were categorized into

Manpower- Skill , knowledge, health etc were taken into consideration

Machine- The machine's age, servicing conditions, make etc. were taken into consideration.

Material-variation in measurement and characteristic of raw material was considered

Environment –variation in temperature , humidity ,light etc

Method- Variation in process parameters, work instructions, settings etc. were considered. In the cause and effect analysis, fish bone analysis was done

Following this pareto analysis was done to find out the major contributors to the the hindrance of reproducibility of results .Hypothesis testing was further done using chi square to confirm the causes for pareto analysis. Null and alternate hypothesis were defined.

RESULTS AND DISCUSSION

41 consecutive readings of positive control by 2 operators was recorded as with a standard deviation of 3.86 with a mean of 6.21(Fig.4) c_{pk} was calculated as 0.26.Since it was less than 1 , hence The process did not meet its specification. The specification limit was defined by CLIA guidelines for C.R.P as 1dil.(Fig. 4). Further the Defects part per million opportunities was calculated was calculated There were approximately

390243 DPMO and hence the process was at less than 2 sigma level or approximately at 1.6 sigma equivalent .(Fig 4). In the Fish bone analysis ,the results are as shown in Fig. 5. This was followed by Pareto Analysis as in (Fig. 6,7) which indicated 3 main causes contributing to 80% of the reason for the low sigma levels in our study.

Name of Phase	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Define Phase								
Actual Time								
Measure phase								
Analyse Phase								
Improve								

Fig 1: The Project Milestones

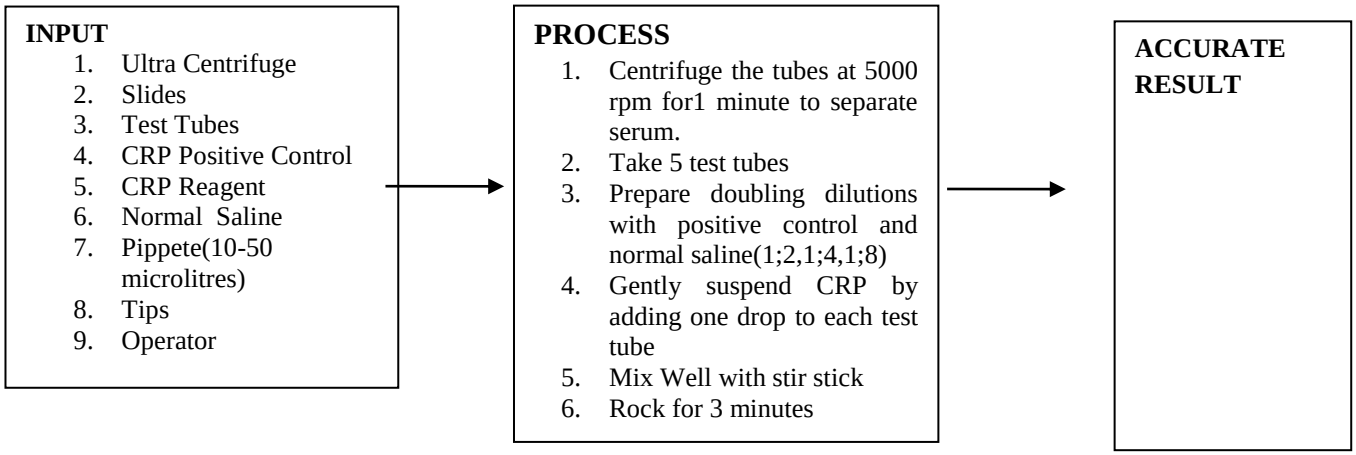

Fig 2: Process Description of CRP Testing

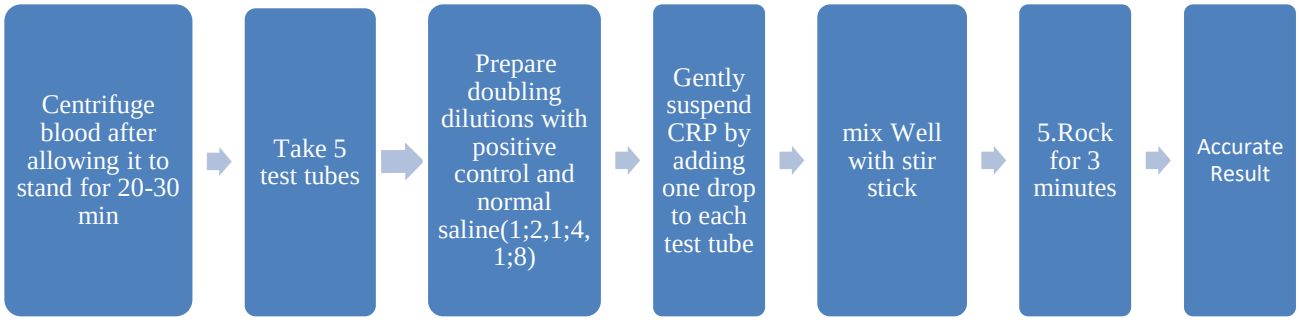

Fig 3: Flowchart CRP testing

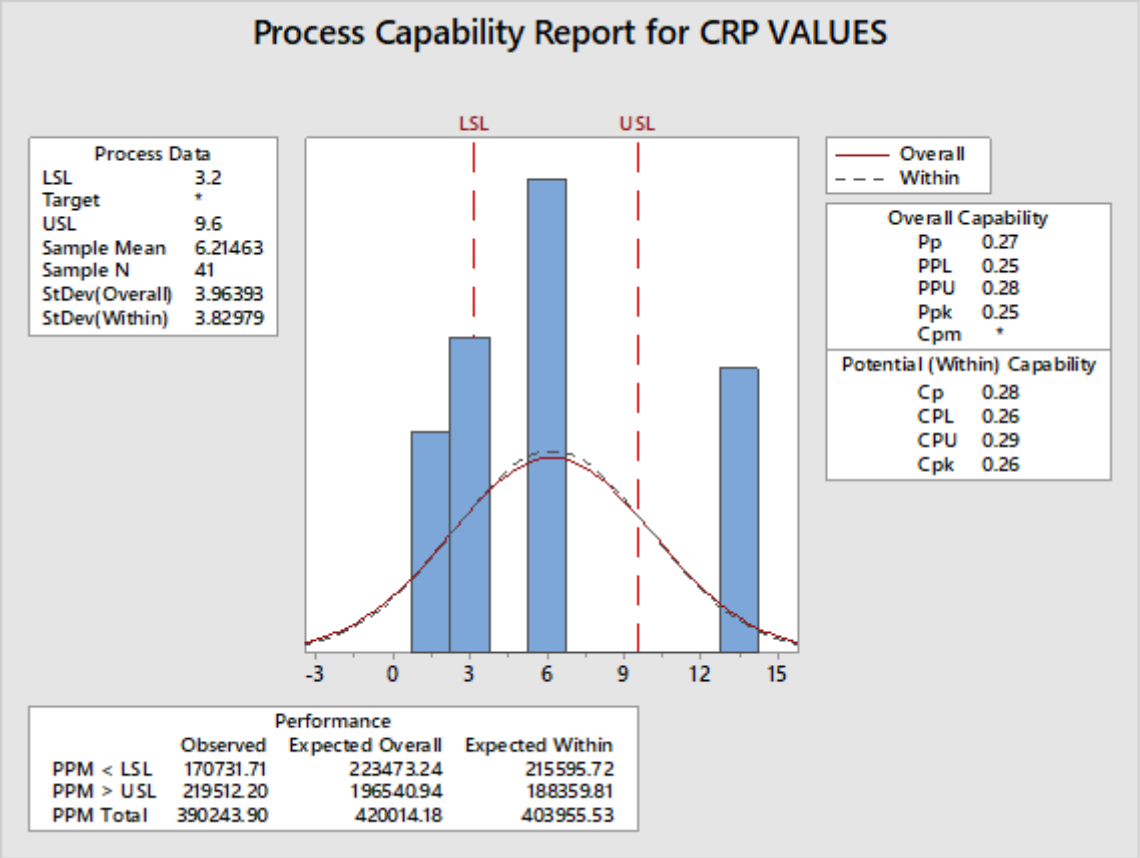

Fig 4: Process capability Report for CRP values

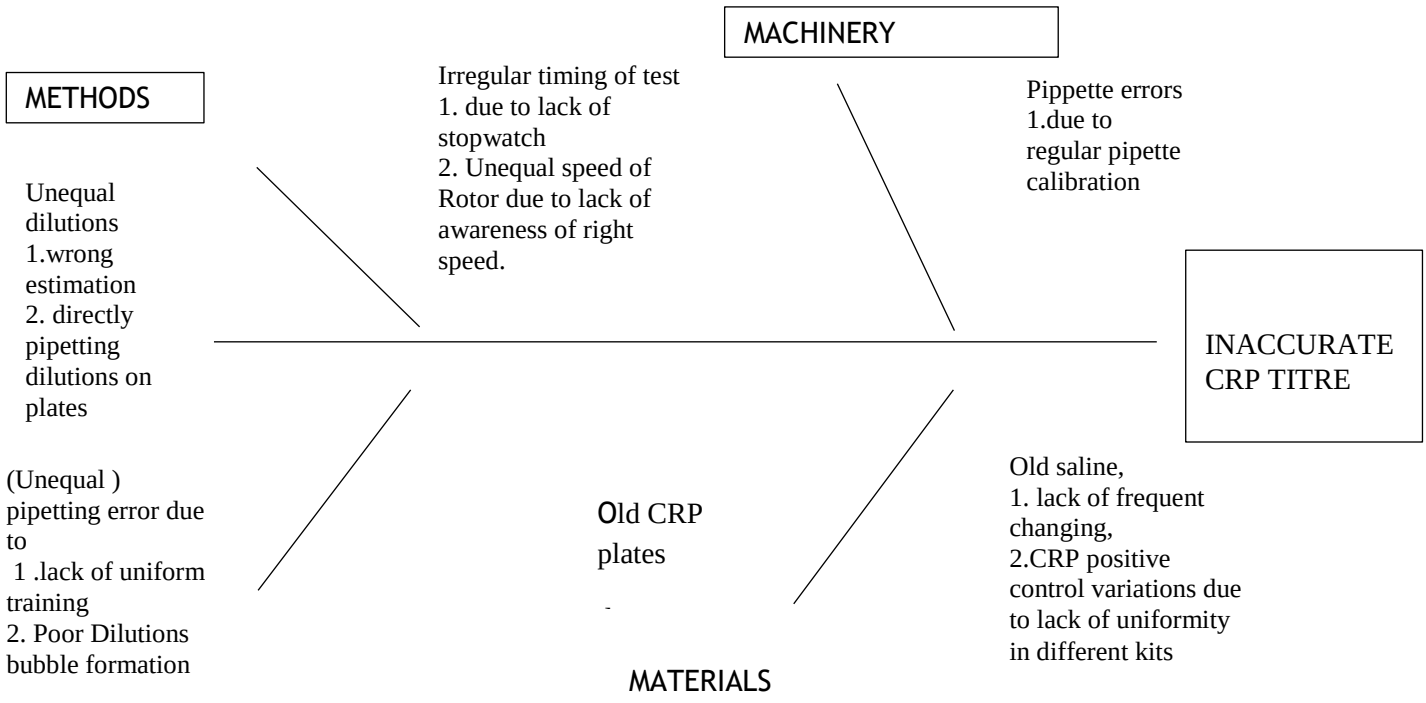

Fig 5: Identifying root causes for inaccurate CRP Results-Fish bone Analysis)

Fig 6: Pareto chart For CRP Testing

Pareto Chart	
Non conformity	No. of Reworks
(Unequal)Pipetting error among technicians due to lack of uniform training	10
(Unequal) dilution error due to direct preparation of dilution on to plates vs tube	8
Irregular timing of test due to lack of stopwatch	2
unequal speed of rotor due to lack of awareness of right speed	2
pipette errors due to lack of calibration	5
(Poor)Dilutions error due to mixing of saline and serum leading to bubble formation	7
old saline due to lack of frequent change	1
old CRP plates due to reuse and use of sodium hypo	2
CRP control variations due to lack of uniformity in kits	2

Fig 7: Pareto chart of non conformity

These were
1. pipetting error to lack of uniform training.
2. Unequal dilutions due to directly pipetting onto plates.
3. dilution errors due to bubble formation.

40 Samples were tested again by two operators and keeping in mind the above root causes and divided into good and bad quality according to mean and specification limits.

Hypothesis testing was done on the results to predict the root causes with null and hypothesis testing defined as-

1**. Null hypothesis for Root cause 1**=There is no effect on unequal pipetting due to operators on CRP results

Alternate Hypothesis –There is an effect of unequal pipetting on CRP results.

2.**Null Hypothesis for Root Cause 2**-There is no effect on unequal dilutions due to directly pippeting on to plates vs tube method

Alternate Hypothesis –There is an effect on unequal dilutions due to directly pipetting on to plates.

3.**Null Hypothesis for root cause 3**-There is no effect on bubble formation on CRP testing

Alternate Hypothesis testing – There is an effect on bubble formation on testing.

Further on hypothesis testing (Chi Square) for the above parameters the P value was calculated(Table 4,5,6). P value for hypothesis testing for comparison of operators affecting pipetting due to training was 0.047.Therefore Null hypothesis was rejected . P value for unequal dilutions for directly pipetting on to plates was 0.507.Therefore null hypothesis was accepted. Similarly P value for poor dilution due to bubble formation was 0.011. Therefore null hypothesis was rejected .

Table 4 -Comparison of Operators p value<0.047

OPERATORS	**GOOD QUALITY**	**BAD QUALITY**
A	4	16
B	10	10

Table 5 -comparison of Slide vs Tube method p value<0.507

METHOD	**GOOD QUALITY**	**BAD QUALITY**
Tube Method	8	12
Slide Method	6	14

Table 6-Comparison of Methodology with presence or absence of bubbles in the titre plate, P- Value= 0.010

METHOD	**GOOD QUALITY**	**BAD QUALITY**
Formation of bubbles	5	15
No bubbles	13	17

DISCUSSION

Measurement of C-reactive protein (CRP), an acute-phase protein with a normal serum level of less than 1 mg /dl which may increase rapidly within hours of an inflammatory stimulus such as infection or tissue injury, is a reliable indicator of disease activity in various clinical conditions including: distinguishing between viral and bacterial infections; indicating response to antimicrobial therapy; monitoring inflammatory conditions such as rheumatoid arthritis' and systemic lupus erythematosus; and surveillance of malignant disease. The accuracy of the test is therefore of paramount importance Inaccurate reporting of CRP in the Medical Laboratory can lead to misdiagnosis. Our Laboratory For CRP testing was operating at nearly 2 sigma which is considered nonacceptable.

Process performance at the 3-sigma level is considered as the minimum acceptable level of quality. The sigma metrics represent the correlation among numbers of product defects, wasted operating costs and customer satisfaction. Therefore, as sigma increases, the consistency, reliability, steadiness and overall performance of the test improves, thereby decreasing the operating costs.6 Therefore we needed to improve the laboratory reporting of six sigma to minimum of further 3 sigma followed by six sigma level. To achieve six sigma is considered as the gold standard for defining world class measure of quality. In clinical laboratory, six sigma methodology gives attention on regulating a process within 6 standard deviations which represents 3.4 defects per million opportunities. Therefore a cause and effect and pareto analysis was done to help anaylse the root causes. Cause and Effect Analysis was devised by professor Kaoru Ishikawa, a pioneer of quality management, in the 1960s. The technique was then published in his 1990 book, "Introduction to Quality Control.

We did a brainstorming session involving the Head of Department, consultants and technicians in the laboratory so as to identify the root causes as required for the the cause and effect analysis.The causes were then divided into Manpower, Material, Method ,Machines and measurement. Once this was done a pareto analysis was further undertaken. Pareto Analysis(G) is a statistical technique in decision making that is used for the selection of a limited number of tasks that produce significant overall effect. It uses the Pareto Principle (also know as the 80/20 rule) the idea that by doing 20% of the work you can generate 80% of the benefit of doing the whole job. Or in terms of quality improvement, a large majority of problems (80%) are produced by a few key causes (20%).

Accordingly 3 major causes among the numerous listed were identified. These were

1.(Unequal) Pipetting error among technicians to lack of uniform training

2.(Unequal)Dilution error due to directly pipetting dilutions onto CRP plates.

3.(Poor) Dilution error due to mixing of saline and serum leading to bubble formation in the plate. In order to predict the above mentioned as the right root causes, Hypothesis tests was done.

Null Hypthesis for root cause 1-

Therefore through this study we suggest that in order to improve accuracy and precision Medical laboratories need to implement quality techniques. Six sigma is one such study could help in improving the quality of reporting in laboratories. Also six sigma helps us to delineate among many factors,the most significant factors contributing to error. This helps in targeting efforts for improvement.

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MATERIALS