QUALITY CONTROL SIMPLIFIED

Quality control is a subject which is mainly difficult to digest, because of the mathematical figures and formulas contained in the calculations, and hence is many a times ignored by the pathologists. As an honest and sincere laboratory worker, everybody will be interested in providing correct results. In this connection, we should know the following words :

Quality Assurance : This describes all the steps taken , both in and outside the laboratory to achieve reliable results, starting with preparation of the patient and ending with correct interpretation of the results.

Quality Control describes the steps taken by the laboratory to ensure that the tests are performed correctly. In simple words, this refers to minimization of errors.

TYPES OF ERRORS : Two types of errors can occur while performing various tests :

1. Imprecision : Errors of scatter.

2. Inaccuracy : Errors of bias.

To understand these, the following experiment was done. Three different technicians were given a plasma to perform 20 tests on the same sample. They carried out glucose estimation on the same sample, with the same pipets and the autoanalyser, and the results were tabulated (Tables A , B, & C). In each case, the mean, bias and scatter were calculated. Bias is the difference between the actual level and the mean. Scatter means the standard deviation as calculated in the tables.

TABLE A : Glucose estimation by A

S NO	GL %	MG	DIFF FROM MEAN	DIFF SQ	S NO	GL MG%	DIFF FROM MEAN	DIFF SQ
1	131		2	4	**11**	136	3	9
2	130		3	9	**12**	139	6	36
3	127		6	36	**13**	139	6	36
4	130		3	9	**14**	128	5	25
5	129		4	16	**15**	132	1	1
6	142		9	81	**16**	129	4	16
7	134		1	1	**17**	131	2	4
8	135		2	4	**18**	135	2	4
9	142		9	81	**19**	133	0	0
10	131		2	4	**20**	130	3	9

Total of columns 2 & 4 .**2662 380**

Mean 133

Bias 0

Scatter 4.5

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SD = 380 / 19 = 20 = 4 . 5 2 SD = 9

CV = (4 . 5 / 133) X 100 = 3.38

TABLE B: Glucose estimation by B

S NO	GL MG%	DIFF FROM MEAN	DIFF SQ	S NO	GL MG%	DIFF FROM MEAN	DIFF SQ
1	123	0	0	**11**	124	1	1
2	122	1	1	**12**	122	1	1
3	122	1	1	**13**	123	0	0
4	124	1	1	**14**	122	1	1
5	126	3	9	**15**	122	1	1
6	122	1	1	**16**	123	0	0
7	123	0	0	**17**	123	0	0
8	123	0	0	**18**	122	1	1
9	123	0	0	**19**	124	1	1
10	123	0	0	**20**	124	1	1

TOTAL 2460 20

MEAN 123

BIAS - 10

SCATTER 1.02

2 SD = 2.04

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_____ ____

SD = 20 / 19 = 1.05 = 1. 02

CV =- (1.02 / 123) X 100= 0.83

TABLE C : GLUCOSE ESTIMATION BY C

NO	GL MG%	DIFF FROM			**S NO**	GL MG%	DIFF FROM	
		MEAN	DIFF SQ				MEAN	DIFF SQ
1	131	2	4		**11**	135	2	4
2	135	2	4		**12**	133	0	0
3	131	2	4		**13**	135	2	4
4	132	1	1		**14**	134	1	1
5	133	0	0		**15**	134	1	1
6	133	0	0		**16**	134	1	1
7	132	1	1		**17**	131	2	4
8	133	0	0		**18**	135	2	4
9	134	1	1		**19**	132	1	1
10	133	0	0		**20**	132	1	1

TOTAL 2660 36

MEAN 133

BIAS 0

SCATTER 1.37

2 SD 2.74

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SD = 36 / 19 = 1.89 = 1.37

CV =(1.37 / 133) X 100 = 1.03

Thus, to summerise,

TECHNICIAN	MEAN	BIAS	SCATTER
A	133	0	4.5
B	123	- 10	1.02
C	133	0	1.37

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A has ideal mean, good bias , but large scatter. B has very low mean (due to common pipeting error), large bias and very low scatter. C has all the factors ideal. So his performance is the best. When this experiment is repeated, the technicians come to know their errors, which are persistently reduced. Thus, this is the best practical experiment done constantly in my lab, especially when I get only one sample for glucose estimation (because of cheapness of the reagent).

ERRORS OF SCATTER , OR IMPRECISION : These are the irregular or random errors. The commonest causes of scatter (imprecision) are :

1. Faulty technique : incorrect and variable pipeting, inadequate mixing of samples with reagents,incubation of tests at inconsistent temperatures or for incorrect length of time.

Reliable test results can only be achieved if the methods are written clearly, and insufficient details, and followed exactly by all the members of staff.

2. Dirty glassware.

3. Too heavy a workload resulting in faulty technique, mistakes being made, or short cuts being utilized.

4. Too low a workload resulting in loss of concentration.

5. Faults in colorimeter / spectrophotometer / analyzer, and unreliable main supply.

6. Use of dirty or finger marked cuvettes, air bubbles in sample, vapor present in cuvettes.

7. Incomplete removal of interfering substances in serum, like RBCs, or protein.

ERRORS OF BIAS (INACCURACY) : These are regular or consistent errors. All the results differ from the correct result by approximately the same value (just like the technician B in above example). The commonest causes are :

1. Use of unsatisfactory reagents : impure chemicals,
    - wrong preparation,
    - improper storage,
    - use beyond working life / expiry date,

- improper balance,
- bad quality of distil water,
- uncalibrated volumetric apparatus.

2. Incorrect or infrequent calibration of a test method.

3. Poor controls / standards : wrongly prepared or stored controls or standards or used after expiry.

4. Readings on incorrect filter.

INTERNAL QUALITY CONTROL : This includes

1. Training laboratory workers to perform tests correctly,

2. Establishing performance standards for each test method,

3. Charting the results for bias and scatter,

4. Taking part in external quality control programmes,

5. Reporting results clearly and with minimum delay,

6. Making sure that equipment such as balance, colourimeters / spectrometers / autoanalysers, water baths are being used correctly and maintained adequately.

ESTABLISHING PERFORMANCE STANDARDS : (For doing this, I always use glucose reagent, as it is cheap, always present in stock in large quantity and is fairly consistent in results) The procedure is as follows :

1. Collect at least 2 ml clear plasma / serum, the value of which is around 130 160 mg / dl.

2. Perform 20 measurements on the sample very carefully.

3. Tabulate the results (column 2).

4. Calculate the mean.

5. Calculate the difference from mean for each value and tabulate in column 3.

6. Square this value in column 3 and write in column 4.

7. The total of column is made.

8. Standard Deviation is then calculated from the following formula :

$$SD = \frac{\text{Total of column 4}}{n - 1}$$

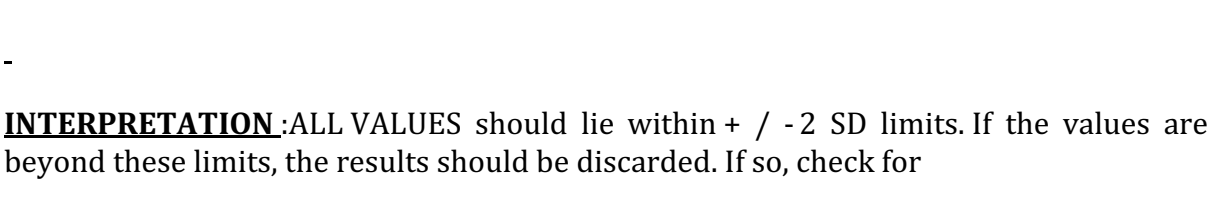
where n = number of readings

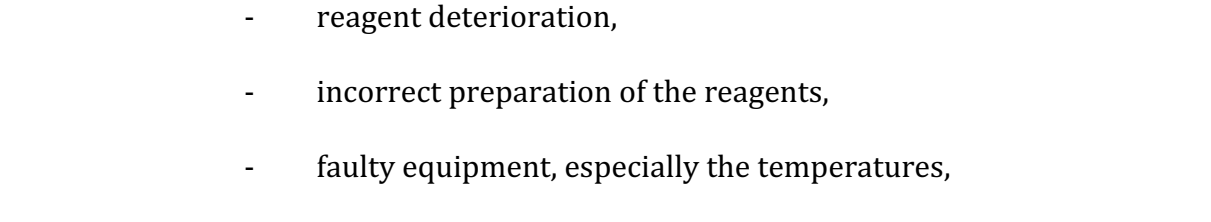
9. Coefficient of Variation (CV) is calculated from S D as

$$CV = \frac{SD}{\text{Mean}} \times 100.$$

10. The results are charted on graph paper as follows :

GRAPHS SHOWING 2 SD VALUES & THE MEAN :





INTERPRETATION :ALL VALUES should lie within + / - 2 SD limits. If the values are beyond these limits, the results should be discarded. If so, check for

- reagent deterioration,
- incorrect preparation of the reagents,
- faulty equipment, especially the temperatures,
- proper filters, dust over them.

QUALITY CONTROL SERA : As commonly available / self prepared standards are small in quantity, and also as their value may change after first use, they need not be used in each and every batch. Analyzed control sera are commercially available, but they are expensive. Hence sera are pooled together, and they are analyzed against such commercial sera, and then can be used as control sera

A control will detect errors in reagents and standards, but not individual pipetting or calculating errors in one or more of the patients specimens.

PREPARATION OF POOLED SERA :

1. Collect any unused tested sera at the end of days work. Exclude from them i. those with moderately to highly abnormal values, ii. icteric, cloudy or hemolysed sera and iii sera of patients with hepatitis or HIV.

2.Transer all the remaining sera in one screw-capped container. Freeze.

3. Add the next days samples till the bottle fills.

4 .Allow the serum to thaw completely. Transfer to another larger bottle and mix properly. Test for hepatitis and HIV. Add Sodium Fluoride, 100 mg / dl of serum.

5. Centrifuge to remove any fibrin or other debris.

6.. Analyse with maximum care for all values.

7. Disperse the supernate in 1 ml. amounts in penicillin bulbs and keep in deep freeze.

8. Remove one bulb at each time, thaw completely and use.

I hope this will help to understand the subject. In the next article, I will give some practical hints to achieve Quality Control in Biochemistry.

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