Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 10 ~ Issue 12 (2023) pp: 40-44
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Acceptability Criteria for Replicate Tests In ISO 17025:2017 Testing Laboratories

Dr Abhilash Babu G
Research Officer & ISO 17025 Technical Assessor
Government Analysts Laboratory , Vanchiyoor PO , Trivandrum-35 , Kerala , India.

***R**eceived 11 Dec., 2023; **R**evised 23 Dec., 2023; **A**ccepted 25 Dec., 2023*
P**ublished with open access at **www.questjournals.org

I. PURPOSE

The issue of reliability of repeatability data is a growing concern among testing laboratories.This document describes different ways to establish the acceptability of replicate analysis during quality assurance and measurement uncertainty calculations.

II. INTRODUCTION

There is, practically, always a certain variation among the measured values and also between the measured values and the actual or reference value.The variation is due to systematic error(bias) and random error.The precision ,which measures the random error,is divided into three levels;
Within-run Precision (Repeatability)
Between-run Precision (Intermediate Precision/Retesting/Within-laboratory Reproducibility) and
Between-lab Precision (Reproducibility).

This document discusses only the first level of precision,that is repeatability, which the ISO 17025 testing laboratory has to perform for several purposes.

III. ISO REQUIREMENTS

The testing laboratory which comply ISO 17025 is required to conduct replicate analysis for three purposes;

For assuring quality of test result under clause 7.7.1 (f)
For Measurement Uncertainty under clause 7.6 read with ILAC P 14
For the verification of Test methods under clause 7.2.1.5

The laboratory personnel usually perform replicate analysis ,but never evaluate the data.No body bothers whether all these data points are acceptable or not to be included in the calculation of arithmetic mean and standard deviation. However the laboratories have to define the acceptance criteria,analyze the data and evaluate them for improvements and/or corrective/preventive actions.

IV. DEFINITIONS

The definitions used in this document are directly referred to ISO standards.
1.Precision : The closeness of agreement between **independent test results** obtained under **stipulated conditions**.
"Independent test results" means results obtained in a manner not influenced by any previous result on the same or
similar test object.Repeatability and reproducibility conditions are particular set of "extreme conditions".Precision
should be investigated using homogenous , authentic samples.If a homogenous sample is not available , then artificially prepared samples (eg., spiked samples) or a sample solution can be used.Precision may be considered at three levels:Repeatability,Intermediate precision and Reproducibility

2.Repeatability : Represents precision under same operating conditions over a short interval of time .This means in repeatability the independent test results are obtained with same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.
3.Intermediate precision : Precision represented as within laboratory variations such as different days,different analysts,different equipment
4.Reproducibility : Represents precision between laboratories and are usually applied when standardizing methodology.
5.Outlier : A member of a set of values which is inconsistent with the other members of that set.

V. ABBREVIATIONS

1. ISO -International Organization for Standardization
2. ILAC – International Laboratory Accreditation Cooperation
3. CR-Critical Range (Repeatability Limit)
4. R-Range(Absolute difference between two extreme points)
5. RSD-Relative Standard Deviation
6. PRSD-Predicted Relative Standard Deviation
7. SD-Standard Deviation
8. ASTA -American Spice Trade Association
9. FSSAI-Food Safety and Standard Authority of India
10. AOAC-Association of Official Agricultural Chemists

VI. METHODOLOGY & DISCUSSION ON ACCEPTABILITY

A number of international protocols are available to check the acceptability of data points during repeatability studies and the laboratories can use any of them.This document explains three methods to evaluate repeatability data.These are Grubbs Method , Mean-Median Method and Horwitz method.Numerical examples are also provided for easy understanding.

6.1 :Method-1 Grubbs Method

This method involves determining the outlier using Grubbs equation.
Grubbs test is a simple technique to quantify the outlier in the set of data points.It is based on a normal distribution and a test statistic is calculated from the most extreme data point using the formula

$\mathbf{G_{exp} = (X_{max} - X_{mean}) / s}$ (for highest data point) $\mathbf{G_{exp} = (X_{mean} - X_{min}) / s}$ (for lowest data point)

A value can be regarded as an outlier if the statistic G value (G_{exp}) is greater than critical value $G_{(\alpha,n)}$ (Table-1).Then the mean and standard deviation are recalculated excluding the outlier.

Table-1 Critical values for Grubbs Test

n	$G_{(\alpha,n)}$ for α = 1 %	$G_{(\alpha,n)}$ for α = 5%	n	$G_{(\alpha,n)}$ for α = 1 %	$G_{(\alpha,n)}$ for α = 5 %
3	1.15	1.15	15	2.71	2.41
4	1.49	1.46	16	2.75	2.44
5	1.75	1.67	17	2.79	2.47
6	1.94	1.82	18	2.82	2.50
7	2.1	1.94	19	2.85	2.53
8	2.22	2.03	20	2.88	2.56
9	2.32	2.11	21	2.91	2.58
10	2.41	2.18	22	2.94	2.60
11	2.48	2.23	23	2.96	2.62
12	2.55	2.29	24	2.99	2.64
13	2.61	2.33	25	3.01	2.66
14	2.66	2.37	***α*** - probability of incorrectly rejecting the suspected outlier ***n***- number of samples in the data set.		

Example-1

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Test	Analysis of Curcumin in Turmeric Powder (ASTA Method)
Data (12 replicates)	3.72 , 3.44 , 4.15 , 3.61 , 4.28 , 4.19 , 3.93 , 4.86 , 5.17 , 3.09 , 3.36 , 4.05.
Arrange Descending order	5.17 , 4.86 , 4.28 , 4.19 , 4.15 , 4.05 ,3.93 ,3.72 , 3.61 , 3.44 , 3.36 , 3.09
Find Mean	3.9875
Find SD	0.605492
Find Grubbs Value G_{exp}	For highest data point (5.17), G_{exp} = 1.952958

	For lowest data point (3.09), G_{exp} = 1.482266
Critical Grubbs value $G_{(\alpha,n)}$ (n=12)	2.29
Remarks	Since both extreme values are less than the critical Grubbs value for n=12 , there is no outlier in the analysis.All data points can be included in the calculation of mean and standard deviation.

Example-2

Test	Analysis of Gluten content in wheat flour (FSSAI method)
Data (12 replicates)	13.16 , 15.78 , 13.82 , 14.08 , 13.99 , 13.77 , 13.96 , 14.19 , 13.93 , 14.26 , 14.05 ,13.45
Arrange Descending order	15.78 , 14.26 , 14.19 , 14.08 , 14.05 , 13.99 , 13.96 , 13.93 , 13.82 , 13.77 , 13.45 ,13.16
Find Mean	14.0366667
Find SD	0.629925
Find Grubbs Value G_{exp}	For highest data point (15.78) , G_{exp} = 2.767523 For lowest data point (13.16) G_{exp} = 1.391699
Critical Grubbs value $G_{(\alpha,n)}$	2.412
Remarks	The upper data value is more than critical value,so it is an outlier.
Recalculation	Recalculate the mean and standard deviation , excluding the outlier (15.78)
Recalculated Mean	13.87818
Recalculated SD	0.323939
Find Grubbs Value G_{exp}	For highest data point (14.26), Gexp = 1.17868 For lowest data point (13.16), Gexp = 2.21702
Critical Grubbs value $G_{(\alpha,n)}$ (n=11)	2.23
Conclusion	Since both extreme values are less than the critical Grubbs value for n=11 , there is no outlier in the analysis.

6.2 :Method-2 Mean-Median Method

Normal laboratory practice requires examination of the difference observed between two or more test results.The test results are compared with **repeatability limit ,CR = f(n) * s** , where s- standard deviation and f(n) -critical range factor(Table-2) and
n-number of replicates.

Table-2 Critical range factor ,f(n)

n	F(n)	n	F(n)
2	2.8	25	5.2
3	3.3	26	5.2
4	3.6	27	5.2
5	3.9	28	5.3
6	4.0	29	5.3
7	4.2	30	5.3
8	4.3	31	5.3
9	4.4	32	5.3
10	4.5	33	5.4
11	4.6	34	5.4
12	4.6	35	5.4
13	4.7	36	5.4
14	4.7	37	5.4
15	4.8	38	5.5
16	4.8	39	5.5
17	4.9	40	5.5
18	4.9	45	5.6
19	5.0	50	5.6
20	5.0	60	5.8
21	5.0	70	5.9
22	5.1	80	5.9
23	5.1	90	6.0
24	5.1	100	6.1

A stepwise description of the method

Step-1 : Start with ***n*** replicate data.Arrange them from maximum to minimum values.

Step-2 : Find arithmetic mean,standard deviation , absolute difference (X_{max} -X_{min}) between extreme values (called

Range , R) and the repeatability limit **(CR).**
Step-3 : Compare the Range (R) with repeatability limit (CR)
Step-4 : If the absolute difference does not exceed the repeatability limit , all data are acceptable and the final test result
can be reported as the arithmetic mean of all data.
Step-5 : If the absolute difference exceeds the repeatability limit,the laboratory should obtain ***n*** more test results.
Calculate the mean ,standard deviation of all the **2n** data and compare **R** with **CR**
Step-6 : If the difference is equal to or less than the critical repeatability limit, the final result is reported as **mean** of all
the test results.If the difference exceeds the critical repeatability limit,the **median** of the test results is reported
as the final quoted result.

Example-1

Test	Saponification Value of Coconut Oil
Data (2 replicates)	251.72 , 252.84
Arrange Descending order	252.84 , 251.72
Mean	252.28
SD	0.56
CR	2.8 x 0.56 = 1.568
Range , R	252.84 – 251.72 = 1.12
Remarks	Since , R < CR , both data are acceptable. The quoted final result = 252.28

Example-2

Test	Hardness of Drinking water
Data (3 replicates)	32.70 , 33.18 , 33.37 with stated SD of 0.21
Arrange Descending order	33.37 , 33.18 , 32.70
Mean	33.0833
SD	0.21 (stated)
CR	3.3 x 0.21 = 0.693
Range , R	33.48-32.70 = 0.78
Remarks	Since R > CR , data can't be reported. Obtain three more test results. 33.27 , 32.85 , 33.48
Arrange Descending order	33.48 ,33.37 , 33.27 , 33.18 , 32.85 , 32.70
Mean	33.142
SD	0.278
CR	4.0 x 0.278 = 1.112
R	33.48 – 32.70 = 0.78
Conclusion	Since R < CR , the quoted final result is the arithmetic mean of all the six data. The final quoted result = 33.142.

6.3 :Method-3 Horwitz Method
Horwitz equation is an empirical relationship between the concentration of the analyte and the precision of the method.
The repeatability relative standard deviation(RSD) varies with concentration , C , the dimensionless mass fraction.The approximate value for predicted standard deviation is calculated by the formula

$$\text{Predicted RSD, } \mathbf{PRSD} = C^{-0.15}$$

The maximum acceptable limit of RSD shall be twice the PRSD from Horwitz equation.
Example-1

Test	Protein content in Nutrimix
Data	16.37 , 16.64 , 17.08 , 15.92 , 15.72 , 16.93 , 15.41
Mean	16. 2957
SD	0.5854
RSD	3.592
PRSD	1.313
Max Limit (2PRSD)	2.626

Remarks	Since the estimated RSD is more than the Maximum limit ,the data points are unacceptable.

Example-2

Test	Tartrazine colour in Fruit Drink
Data	75.68 , 75.36 , 74.77 , 74.25 , 73.73 , 73.19 , 72.99 , 72.94
Mean	74.11375
SD	1.075479
RSD	1.45
PRSD	1.046
Max Limit (2PRSD)	2.092
Remarks	Since the estimated RSD does not exceed the Maximum limit,the data points are acceptable.

VII. CONCLUSION

The testing laboratory can use any of the above methods for determining the acceptability of data points for repeatability calculations.The evaluated data can be used for measurement uncertainty ,verification and quality control.The illustrations relate to food testing laboratories provide a better understanding of the application of methods.

CONFLICT OF INTEREST

The author has no technical ,financial and intellectual conflict of interest.

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