Estimation of Measurement Uncertainty in Analytical Chemistry Results

$$U = k * u_c$$

Equation 1

u_c - combined measurement uncertainty

$k = 2$ - expanded coverage factor

U – expanded measurement uncertainty

This presentation discusses within-laboratory variability and bias as the primary contributing factors.

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Analytical Method Should Produce Accurate and Precise data

- **The Accuracy** is the closeness of agreement between an analytical result and the true, or accepted reference value. When applied to a set of results, it involves a combination of random error (estimated as precision) and a common systematic error (trueness or bias)

- **The Bias** is the difference between the mean measured value and the true value.

- **The Precision** is closeness of agreement between independent analytical results obtained by applying the experimental procedure under stipulated conditions. A measure of precision (or imprecision) is the standard deviation.

Estimating Measurement Uncertainty based on Intra-laboratory Validation/QC data (u_c bias)

Uncertainty of measurement estimate the range around the reported result within which the true value can be expected to lie with a specified probability (confidence level, usually 95 %).
Uncertainty data should encompass trueness (bias) and precision.

$$u_c = \sqrt{u_c(\text{bias})^2 + u_c(\text{precision})^2}$$

Equation 2

u_c = measurement uncertainty

u_c (bias) = uncertainty component for the bias

u_c (precision) = uncertainty component for the precision

Estimating Measurement Uncertainty based on Intra-laboratory Validation/QC Data (u_c bias)

$$u_c = \sqrt{u_c\,(\text{bias})^2 + u_c\,(\text{precision})^2} \qquad \textit{Equation 2}$$

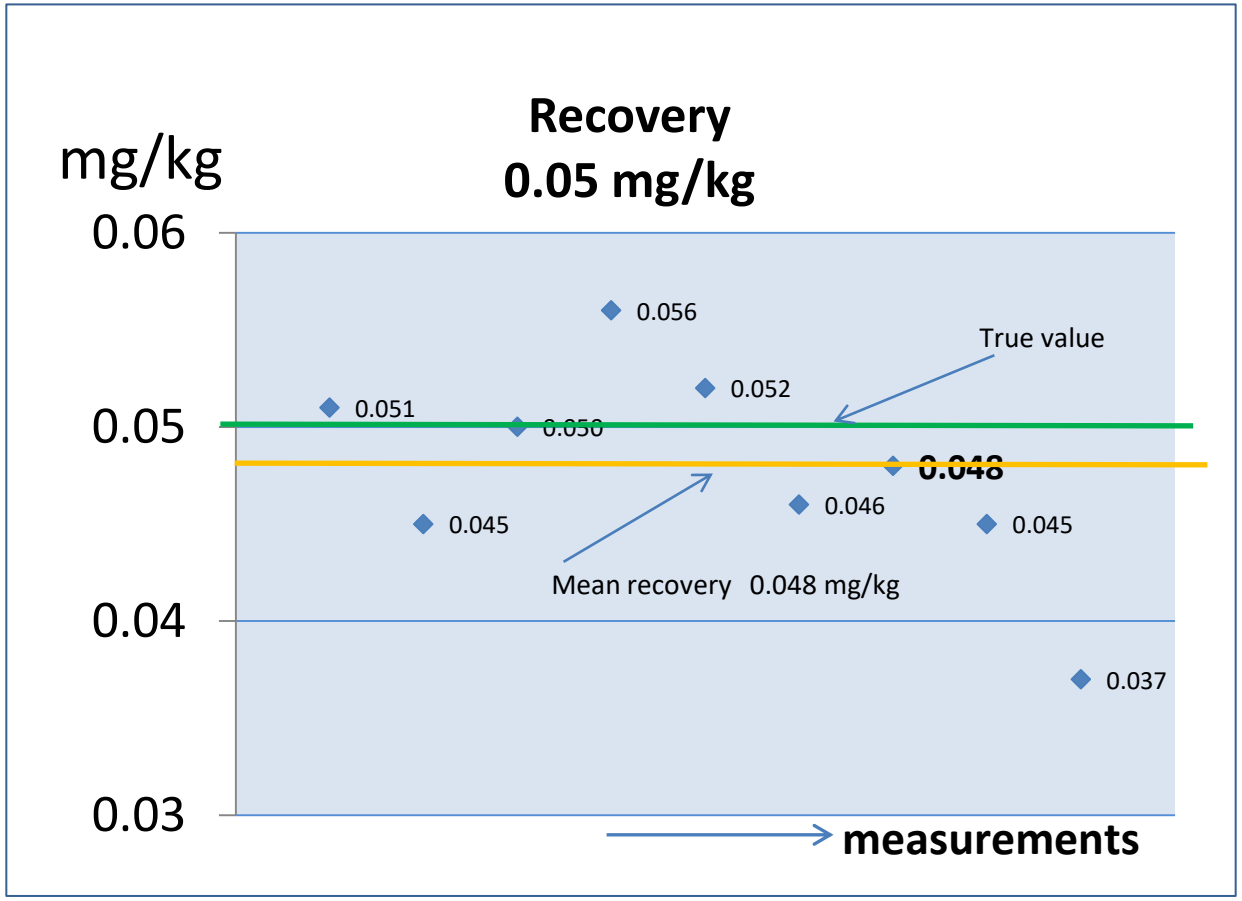
- u_c**(bias)** is the element of uncertainty associated with average (mean) of relative bias (r bias%), taken for a set of results, where **r** bias% is the recovery of the analyte in the certain matrix.

$$\text{r bias (\%)} = \frac{measured\ concentration - spiked\ concentration}{spiked\ concentration} \times 100\% \qquad \textit{Equation 3}$$

- Measured recovery may be affected by the matrix and the type of the material being tested, which can lead to results that are higher or lower than the spike concentration. **Mean relative bias *r* (bias)%, respectively, should be calculated using both positive and negative results of relative bias** (see examples on next slide).

Examples of Measured Recovery (pesticide X) and Mean Relative Bias

Date	spike(pesticide X) 0.05mg/kg	measured mg/kg	r bias % (Equation 2)
10-Jan	Apple	0.051	2
26-Yan	Pear	0.045	-10
4-Feb	Lettuce	0.050	0
8-Feb	Cauliflower	0.056	12
22-Feb	Cherries	0.052	4
28-Feb	Onion	0.046	-8
5-Mar	French beans	0.048	-4
6-Mar	Carrots	0.045	-10
22-Mar	Leek	0.037	-26
	mean (average)	0.0478	-4.44



Estimating Measurement Uncertainty based on Intra-laboratory Validation/QC Data (u_c bias)

$$\mathcal{U}_c \text{ (bias) \%} = \sqrt{meanr^{\,2}{}_{bias} + SD.P^2{}_{bias} + u_c(\text{Cref})^2}$$

Equation 4

$mean_{r\ bias}$ (%) = the mean of the relative bias

$SD.P^2{}_{bias}$= standard deviation of population for the relative bias

u_c **(Cref)** = uncertainty of the spiked concentration

When certified analytical standards and calibrated/verified volumetric material/balances are used to prepare the spiked samples, it can be assumed that the uncertainty associated with the spiking level u_c **(Cref)** is negligible, then:

$$\mathcal{U}_c \text{ (bias) \%} = \sqrt{meanr^{\,2}{}_{bias} + SD.P^2{}_{bias}}$$

Equation 5

Examples for the Estimation of Measurement Uncertainty based on Intra-laboratory Validation/QC Data (u_c bias)

Date	spike(pesticide X) 0.05mg/kg	measured mg/kg	r bias %
10-Jan	Apple	0.051	2
26-Yan	Pear	0.045	-10
4-Feb	Lettuce	0.050	0
8-Feb	Cauliflower	0.056	12
22-Feb	Cherries	0.052	4
28-Feb	Onion	0.046	-8
5-Mar	French beans	0.048	-4
6-Mar	Carrots	0.045	-10
22-Mar	Leek	0.037	-26
	mean (average)	0.0478	-4.444
	stdev.p (%) bias		10.232

$$u_c\ (\text{bias})\ \% = \sqrt{meanr^{2}_{bias} + SD.P^{2}_{bias}}$$ *Equation 5*

Determination of reproducibility for Initial validation based on **STDEV.P** (Standard Deviation of a **Population**)

Population	Sample
$\sigma = \sqrt{\frac{\sum(X - \mu)^2}{N}}$	$s = \sqrt{\frac{\sum(X - \bar{x})^2}{n - 1}}$
X - The Value in the data distribution μ - The population Mean N - Total Number of Observations	X - The Value in the data distribution $\bar{x}$ - The Sample Mean n - Total Number of Observations

The **STDEV.P** function applies STDEV for a population, whereas the **STDEV.S** does so for a sample, presumably less than the entire population.

Example for the Estimating Measurement Uncertainty based on Intra-laboratory Validation/QC Data (u_c bias)

Date	spike(pesticide X) 0.05mg/kg	measured mg/kg	r bias %
10-Jan	Apple	0.051	2
26-Yan	Pear	0.045	-10
4-Feb	Lettuce	0.050	0
8-Feb	Cauliflower	0.056	12
22-Feb	Cherries	0.052	4
28-Feb	Onion	0.046	-8
5-Mar	French beans	0.048	-4
6-Mar	Carrots	0.045	-10
22-Mar	Leek	0.037	-26
	mean (average)	0.0478	**-4.44**
Excel Equation	**stdev.p (%) bias**		**10.232**
Equation 5	**u_c (bias) %**		**11.155**

$$u_c(\text{ bias }) \% = \sqrt{meanr\,^2{}_{bias} + SD.P^2{}_{bias}}$$ *Equation 5*

u_c (bias) % calculation:

$$u_c(\text{ bias }) \% = \sqrt{(-4.444)^2{}_{bias} + (10.232)^2{}_{bias}} = 11.155$$

Definition given by EXCEL :

STDEV.P assumes that its arguments are the entire population. If your data represents a sample of the population, then compute the standard deviation using STDEV.
For large sample sizes, **STDEV.S** and **STDEV.P** return approximately equal values.

Estimating Measurement Uncertainty based on Intra-laboratory Validation/QC Data (u_c precision)

$$u_C \text{ (precision)} \% = u_C \text{ (Reproducibility)} \% = \text{RSD}_{wR}$$

Equation 6

- **Reproducibility** defined as precision (standard deviation) of measurement of an analyte (usually by means of recovery or analysis of reference materials) , obtained using the same method in a number of laboratories, by different analysts, or over a period in which differences in the materials and equipment will occur.
- The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result.
- **Within-lab-reproducibility (RSD_{wR})** is that produced in a single laboratory and may be calculated from **STDEV.S** or **STDEV.P .**

Examples for Estimation of Combined Measurement Uncertainty (Bias and Precision)

Date	spike(pesticide X) 0.05mg/kg	measured mg/kg	r bias %
10-Jan	Apple	0.051	2
26-Yan	Pear	0.045	-10
4-Feb	Lettuce	0.050	0
8-Feb	Cauliflower	0.056	12
22-Feb	Cherries	0.052	4
28-Feb	Onion	0.046	-8
5-Mar	French beans	0.048	-4
6-Mar	Carrots	0.045	-10
22-Mar	Leek	0.037	-26
	mean (average)	**0.0478**	**-4.444**
Excel Equation	**stdev.p (%) bias**		**10.232**
Equation 5	u_c **(bias) %**		**11.155**
Equation 6	u_c **(precision) RSD** $_{wR}$ **%**	11.357	
Equation 7	u_c **(combined) %**	**15.920**	

u_c (precision) % = u_c (reproducibility) % = RSD_{wR} *Equation 6*

$$u_c(\text{combined})\ \% = \sqrt{mean_r{}^2{}_{bias} + SD.P^2{}_{bias} + RSD^2{}_{wR}}$$ *Equation 7*

$$u_c\ (\text{combined})\ \% = \sqrt{(-4.444)^2{}_{bias} + (10.232)^2{}_{bias} + (11.357)^2{}_{precision}}$$

u_c (combined) % = 15.920

Estimating of the Expanded Measurement Uncertainty

Date	spike(pesticide X) 0.05mg/kg	measured mg/kg	r bias %
10-Jan	Apple	0.051	2
26-Yan	Pear	0.045	-10
4-Feb	Lettuce	0.050	0
8-Feb	Cauliflower	0.056	12
22-Feb	Cherries	0.052	4
28-Feb	Onion	0.046	-8
5-Mar	French beans	0.048	-4
6-Mar	Carrots	0.045	-10
22-Mar	Leek	0.037	-26
	mean (average)	**0.0478**	**-4.444**
Excel Equation	**stdev.p (%) bias**		**10.232**
Equation 5	**u_c (bias) %**		**11.155**
Equation 6	**u_c (precision) RSD $_{wR}$ %**	11.357	
Equation 7	**u_c (combined) %**	**15.920**	
Equation 1	**U` (expended MU)%**	**31.839**	

$$U = k * u_c$$ *Equation 1*

u_c - combined measurement uncertainty (MU)

***k* = 2** – expanded coverage factor

U – expanded measurement uncertainty **(expended MU %)**

u_c (combined) % = 15.920

U (%) = 2 * u_c = 2 * 15.920 = 32 %

Example A, pesticide X (low bias, good within-lab reproducibility

With each batch of samples, a sample spiked at 0.05 mg/kg is included in the batch. A different matrix is chosen each time to take the variability of matrices from this commodity group into account

Date	spike(pesticide X) 0.05mg/kg	measured mg/kg	r bias %
10-Jan	Apple	0.051	2
26-Yan	Pear	0.045	-10
4-Feb	Lettuce	0.050	0
8-Feb	Cauliflower	0.056	12
22-Feb	Cherries	0.052	4
28-Feb	Onion	0.046	-8
5-Mar	French beans	0.048	-4
6-Mar	Carrots	0.045	-10
22-Mar	Leek	0.037	-26
	mean (average)	**0.0478**	**-4.444**
Excel Equation	**stdev.p (%) bias**		**10.232**
Equation 5	**u_c (bias) %**		**11.155**
Equation 6	**u_c (precision) RSD $_{wR}$ %**	**11.357**	
Equation 7	**u_c(combined) %**	**15.920**	
Equation 1	**U (expended MU)%**	**31.839**	

Expanded MU = 32 %.

Example B, pesticide Y (high bias, good within-lab reproducibility)

This example is similar to example A, but for this pesticide a relatively high bias is observed. As can be seen, **while the RSD$_{wR}$ is the same as in example A, the higher bias results in high uncertainty**

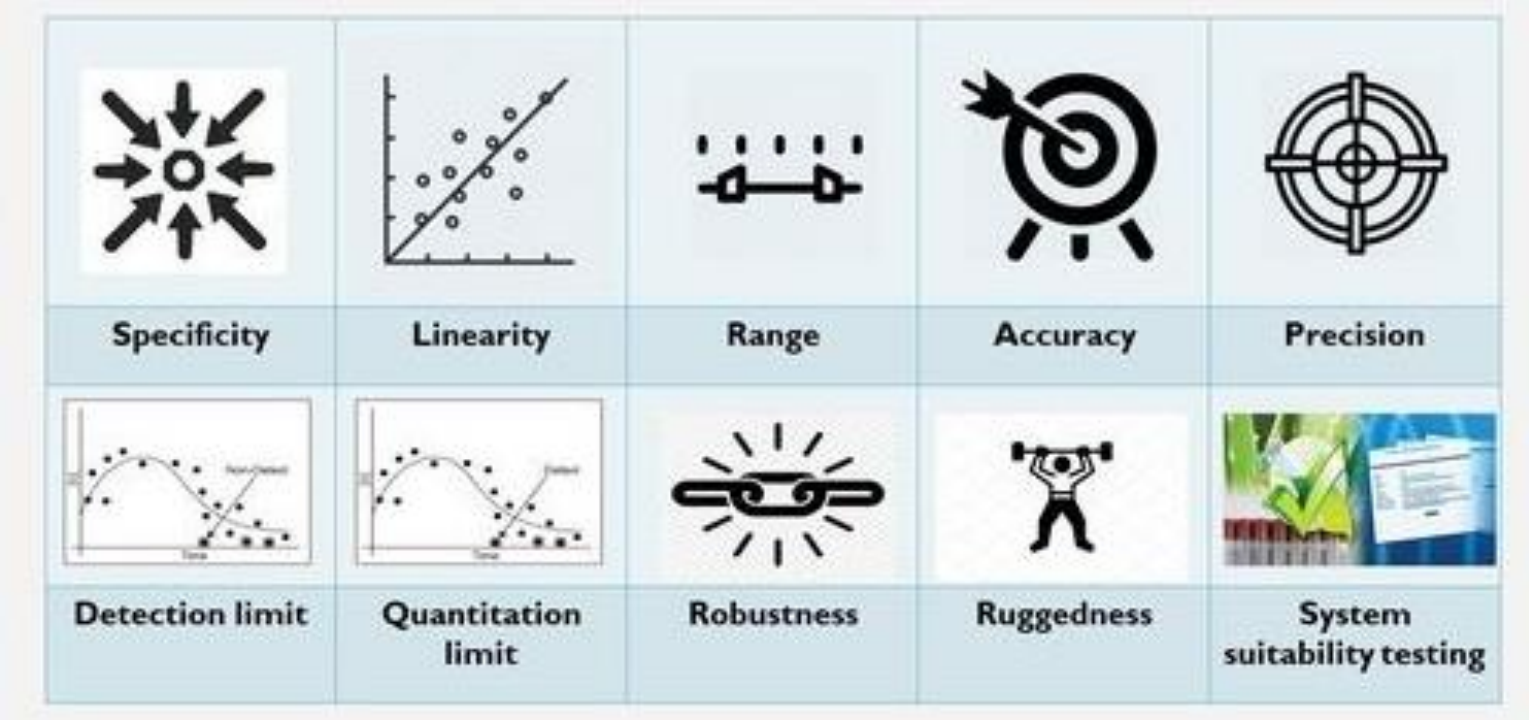
Date	spike (pesticide Y) 0.05mg/kg	measured mg/kg	r bias %
10-Jan	Apple	0.038	-24
26-Yan	Pear	0.034	-32
4-Feb	Lettuce	0.037	-26
8-Feb	Cauliflower	0.042	-16
22-Feb	Cherries	0.039	-22
28-Feb	Onion	0.034	-32
5-Mar	French beans	0.036	-28
6-Mar	Carrots	0.034	-32
22-Mar	Leek	0.028	-44
	mean (average)	**0.0358**	**-28.444**
	stdev.p (%) bias		**7.470**
Equation 5	**u_c (r bias) %**		**29.409**
Equation 6	**u_c (precision)=RSD $_{wr}$ %**	**11.073**	
Equation 7	**u_c(combined) %**	**31.424**	
Equation 1	**U (expended MU)%**	**62.849**	

Expanded MU = 63 %.

Conclusion: The higher bias results in high uncertainty

Initial Validation of Analytical Method

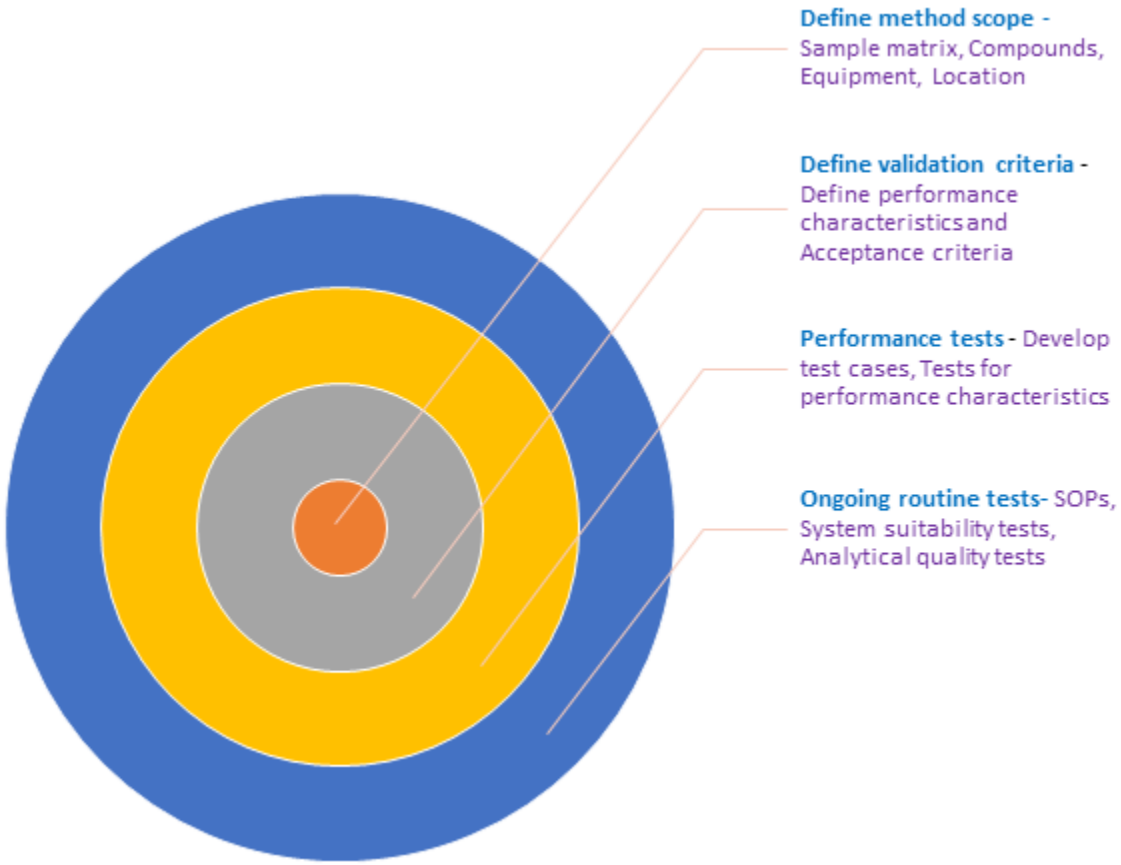
Initial validation establishes experimental evidence that analytical method consistently delivers reproducible, precise and accurate results using established and accepted methodology.



On-going Validation / Performance Verification

The purpose of on-going method validation is to:

- demonstrate robustness through evaluation of mean recovery and within-laboratory reproducibility
- demonstrate that minor adjustments made to the method over time do not unacceptably affect method performance
- determine acceptable limits for individual recovery results during routine analysis



Considering the Differences in Bias Reproducibility Definitions in Initial and On-going Validation

- Determination of bias reproducibility for Initial validation based on **STDEV.P** (Standard Deviation of a **Population**)

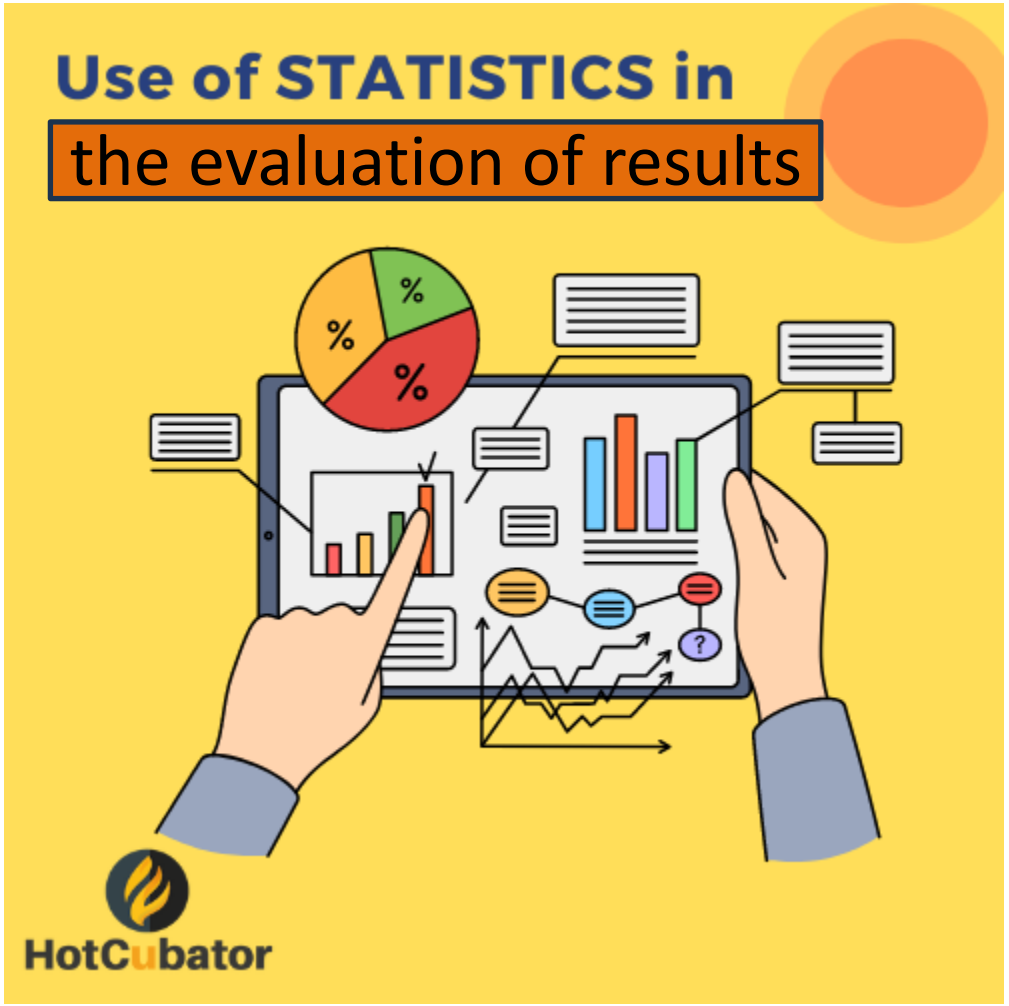
$$\mathcal{U}_c \text{ (bias) \%} = \sqrt{meanr^2_{bias} + STDEV.P^2_{bias}}$$

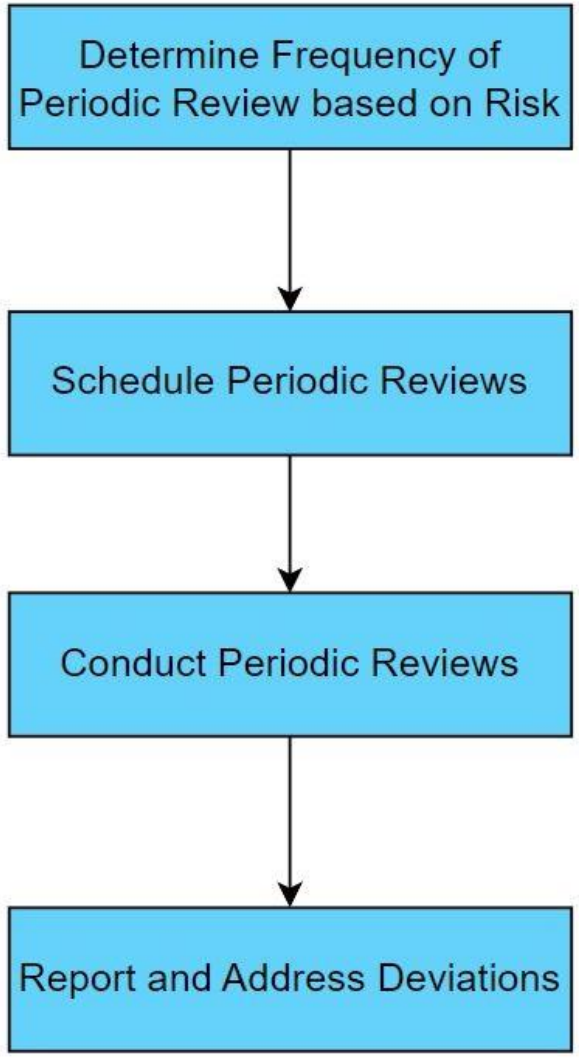
Population	Sample
$\sigma = \sqrt{\frac{\sum(X - \mu)^2}{N}}$	$s = \sqrt{\frac{\sum(X - \bar{x})^2}{n - 1}}$
X - The Value in the data distribution μ - The population Mean N - Total Number of Observations	X - The Value in the data distribution $\bar{x}$ - The Sample Mean n - Total Number of Observations

- Determination of bias reproducibility for on-going validation based on **STDEV.S** (standard deviation of sample data)

$$\mathcal{U}_c \text{ (bias) \%} = \sqrt{meanr^2_{bias} + STDEV.S^2_{bias}}$$

Evaluation of Results of the On-going Validation and Periodic Review





References

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- Codex Alimentarius Commission ,CAC/GL 59-2006 (Amendment 1-2011) Guidelines on Estimation of Uncertainty of Results, www.codexalimentarius.net/download/standards/10692/cxg_059e.pdf , Rome 2006 and 2011

- A. Valverde, A. Aguilera, A. Valverde-Monterreal, Practical and valid guidelines for realistic estimation of measurement uncertainty in multi-residue analysis of pesticides, Food Control 71 (2017) 1-9.

Significance of Bias

(EURACHEM/CITAC Guide, Quantifying uncertainty in analytical measurement, 3rd Edition, 2012)

7.16. **7.16.1.** It is a general requirement of the ISO *Guide* that corrections should be applied for all recognised and significant systematic effects. **7.16.2.** In deciding whether a known bias can reasonably be neglected, the following approach is recommended: i) Estimate the combined uncertainty without considering the relevant bias. ii) Compare the bias with the combined uncertainty. iii) Where the bias is not significant compared to the combined uncertainty, the bias may be neglected.	iv) Where the bias is significant compared to the combined uncertainty, additional action is required. Appropriate actions might: • Eliminate or correct for the bias, making due allowance for the uncertainty of the correction. • Report the observed bias and its uncertainty in addition to the result. NOTE: Where a known bias is uncorrected by convention, the method should be considered empirical (see section 7.8).