UNCERTAINTY MEASUREMENT IN A MICROBIOLOGY LABORATORY: A PRACTICAL APPROACH

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Introduction

- UoM is defined as a parameter associated with the result of a measurement that characterizes the dispersion of the values that can reasonably be attributed to the measurand.
- The word uncertainty means *doubt* thus one can define UoM as the doubt about the validity of the result or the exactness of the result
- R W followed an approach to determine UoM by combining the different approaches from available literature.

Introduction

- Implementation of UoM was done in 5 steps
  - Step1: Specification and modeling the measurement
  - Step2: Identification of Uncertainty sources
  - Step3: Evaluate standard uncertainty of the uncertainty components to Type A or Type B
  - Step4: Determine the individual, relative combined and expanded standard uncertainty
  - Step5: Reporting the uncertainty

1.Specification and modeling the measurement

- Give a clear statement of what is being measured, e.g
- Measurand
  - Total Coliform bacteria/100ml
- Matrix:
  - Any water sample including surface, ground water, effluent water and drinking water
- Method:
  - Membrane filtration and confirmation test

Specification and modeling the measurement

The model can be an analytical expression or a simple expression.

$$TC_{confirmed} = TC_{isolated} / volume_{filtered} * Percentage_{confirmed}$$

Where:

$TC_{isolated}$ = number of typical colonies isolated on M-Endo agar LES

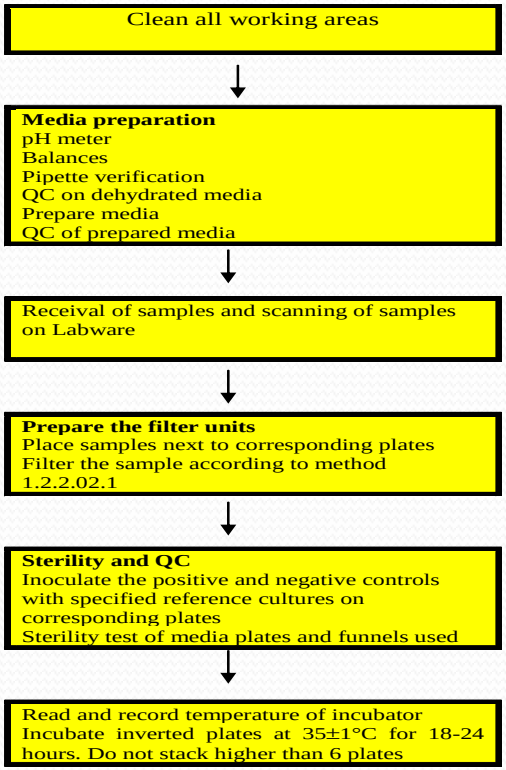
$volume_{filtered}$ = volume analysed

$Percentage_{confirmed}$ = part of the total number of isolated typical colonies confirmed as TC

$TC_{confirmed}$ = number of confirmed TC/100ml

Specification and modeling the measurement

Brief flow chart of the method to ensure that all steps are covered for UoM



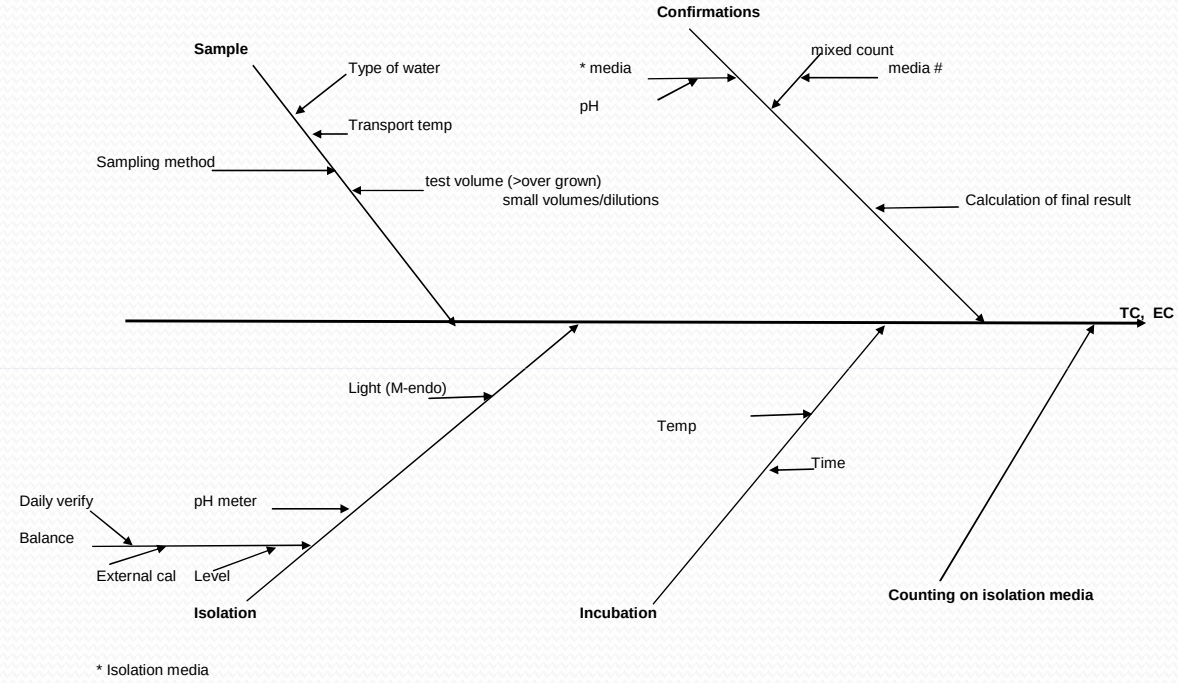
2.Identification of Uncertainty sources

Classification of sources of uncertainty

	Source	Type	Include in budget Yes/no	Reason for not including
1	Work instruction to clean work area	B	No	Work instruction must be compiled
2	**pH meter** Calibration specification verification standard	 B B A	 Yes Yes Yes	
3	**Conductivity** Specification Verification standard	 B A	Yes	
4	Balance	B	Yes	
5	Pipettes	B	Yes	
6	**Preparation of media** Volume of water Recovery of media	 **A** **A**	 No No	Verification must still be done (future project)
7	Sample homogeneity	A	No	Verification must still be done(future project)
8	Volume of sample Dilution	A	No	Verification must still be done (future project)
9	Sterile filter	-	No	QC step
10	Vacuum contribution	A	No	Verification must still be done (future project)
11	**Incubator** Temperature Calibration Time	 B B B	Yes	

Identification of Uncertainty sources

Structure the process by means of a cause and analysis effect



3.Evaluate standard uncertainty of the uncertainty components to Type A or Type B

The type of uncertainty and the probability of distributions for all sources of uncertainty included in the budget.

	Source	Type	Probability distribution
2	**pH meter** Calibration specification verification standard	 B B A	 Rectangular Rectangular Normal
3	**Conductivity** Specification Verification standard	 B A	 Rectangular Normal
4	Balance	B	Rectangular
5	Pipettes	B	Rectangular
11	**Incubator** Temperature Calibration Time	 B B B	 Triangular Rectangular Rectangular
18	Reproducibility (real data)	A	Normal
20	Temperature Fridge (prepared media) Room (dehydrated media)	 A A	 Normal Normal
22	Waterbaths	B	Rectangular
23	Autoclaves	B	Rectangular

4.Determine the individual, relative combined and expanded standard uncertainty

- Determine the combined standard uncertainty, relative standard uncertainty and the expanded uncertainty.
- According to the Eurachem Guide (2000), for all models involving a product and a quotient (e.g. y = p×q×r×….. or y = p/q×….) the combined standard uncertainty is calculated by expressing the uncertainty parameters as relative standard uncertainties as follows:

$$u_c(y) = \sqrt{\sum_{1}^{n} [u(xi)/xi]^2}$$

where: *u(xi)/xi* etc are the uncertainties in the parameters, expressed as relative standard deviations.

Determine the individual, relative combined and expanded standard uncertainty

Relative uncertainty

Standard uncertainty: $u(x_i)$

Symbol	Description	Type	Expected Value	Probability Distribution	Uncertainty Estimate (±) (i.e. semi-range for Type B)	Divisor	relative uncertainty	Standard uncertainty $u(x_i)$	units	Sensitivity coefficient c_i	Uncertainty contributor $u(y_i)$	Reliability	Degrees of Freedom v_i	Significance	$u(y_i)^2$	$u(y_i)^4$	$\frac{u(y_i)^4}{v_i}$
2	MB203	B	44.6	Rectangular	0.5	1.73205	0.00647	0.288675135	°C	1	0.006472537	50%	2	0.00%	4.18937E-05	1.75508E-09	9.E-10
3	MB118	B	45.2	Rectangular	0.5	1.73205	0.00639	0.288675135	°C	1	0.006386618	50%	2	0.00%	4.07889E-05	1.66373E-09	8.E-10
4	MB21	B	44.6	Rectangular	0.5	1.73205	0.00647	0.288675135	°C	1	0.006472537	50%	2	0.00%	4.18937E-05	1.75508E-09	9.E-10
5	MB125	B	44.8	Rectangular	0.5	1.73205	0.00644	0.288675135	°C	1	0.006443641	50%	2	0.00%	4.15205E-05	1.72395E-09	9.E-10
6	MB198	B	44.3	Rectangular	0.5	1.73205	0.00652	0.288675135	°C	1	0.006516369	50%	2	0.00%	4.24631E-05	1.80311E-09	9.E-10
7	MB199	B	44.4	Rectangular	0.5	1.73205	0.0065	0.288675135	°C	1	0.006501692	50%	2	0.00%	4.2272E-05	1.78692E-09	9.E-10
8	MB178	B	44.6	Rectangular	0.5	1.73205	0.00647	0.288675135	°C	1	0.006472537	50%	2	0.00%	4.18937E-05	1.75508E-09	9.E-10
9	MB126	B	44.5	Rectangular	0.5	1.73205	0.00649	0.288675135	°C	1	0.006487082	50%	2	0.00%	4.20822E-05	1.77091E-09	9.E-10
10	MB23	B	44.4	Rectangular	0.5	1.73205	0.0065	0.288675135	°C	1	0.006501692	50%	2	0.00%	4.2272E-05	1.78692E-09	9.E-10
11	MB117	B	44.8	Rectangular	0.5	1.73205	0.00644	0.288675135	°C	1	0.006443641	50%	2	0.00%	4.15205E-05	1.72395E-09	9.E-10
13	MB177	B	44.6	Rectangular	0.5	1.73205	0.00647	0.288675135	°C	1	0.006472537	50%	2	0.00%	4.18937E-05	1.75508E-09	9.E-10
14	Thermometer	B	refer to thermometer uncertainty budget								3.49388414				0722638	149.016376	7.E+01
										$u_c(y)$	3.493950039				12.20768688	149.016376	7.E+01
										v_{eff}	2.0						
										Level of Confider	95%						
										Coverage facto	1.96						
										U	6.848142077						

LOC

Combined standard uncertainty: $u_c(y)$

Expanded uncertainty: U

Coverage factor : *k*

Determine the individual, relative combined and expanded standard uncertainty

Symbol	Description	Type	Expected Value	Probability Distribution	Uncertainty Estimate (±)	Divisor	Standard uncertainty	relative uncertainty	Sensitivity coefficient	Uncertainty contributor	Reliability	Degrees of Freedom	Significance			
					(i.e. semi-range for Type B)		$u(x_i)$		c_i	$u(y_i)$		$veff$		$u(y_i)^2$	$u(y_i)^4$	$\frac{u(y_i)^4}{v_i}$
1	Imported Media prep	refer to media prep budget						0.003323622	1	0.003323622	50%	2.062329	0.00%	1.10465E-05	1.22024E-10	6.E-11
2	Imported Pipettes	refer to pipette budget						0.005608531	1	0.005608531	50%	21.6215	0.00%	3.14556E-05	1.09558E-10	5.E-11
3	Imported Incubator	refer to incubator budget						0.224734694	1	0.224734694	50%	2.347669	0.16%	0.050505683	0.002550824	1.E-03
5	Reproducibility (real data)	A	47.125	Normal		15.6205		4.3684875	1	4.3684875	100%	243	60.89%	19.08368304	364.1869583	1.E+00
6	imported Waterbath uncertainty budget	refer to waterbath budget						3.493950039	1	3.493950039	50%		38.95%	12.20768688	149.016376	7.E+01
7	Autoclave	refer to autoclave budget						0.015113939	1	0.015113939	50%	20.0	0.00%	0.000228431	5.21808E-08	3.E-09
									$u_c(y)$	5.598405713				31.34214653	513.2058852	8.E+01
									v_{eff}	12.9						
									Level of Confidence	95%						
									Coverage factor	1.96						
									U	10.9728752						

Imported uncertainty budget

Symbol	Description	Type	Expected Value	Probability Distribution	Uncertainty Estimate (±)	Divisor	Standard uncertainty	units	relative uncertainty	Sensitivity coefficient	Uncertainty contributor	Reliability	Degrees of Freedom	Significance			
					(i.e. semi-range for Type B)		$u(x_i)$			c_i	$u(y_i)$		ν_i		$u(y_i)^2$	$u(y_i)^4$	$\frac{u(y_i)^4}{\nu_i}$
1	balance MB200	B	10	Rectangular	0.0003	1.73205	0.000173205	g	1.73205E-05	1	1.73205E-05	50%	2	0.00%	3E-10	9E-20	5.E-20
2	Balance MB202	B	500	Rectangular	0.03	1.73205	0.017320508	g	3.4641E-05	1	3.4641E-05	50%	2	0.01%	1.2E-09	1.44E-18	7.E-19
3	pH meter 780 Calibration	B	7.002	Rectangular	0.04	1.73205	0.023094011	mV	0.003298202	1	0.003298202	50%	2	98.48%	1.08781E-05	1.18334E-10	6.E-11
	ph meter 780 resolution	B	7	Rectangular	0.0005	1.73205	0.000288675	mV	4.12393E-05	1	4.12393E-05	50%	2	0.02%	1.70068E-09	2.89231E-18	1.E-18
5	pH meter780 verification	A	6.99409091	Normal	0.013330627	4.69042	0.002842099		0.000406357	1	0.000406357	100%	21	1.49%	1.65126E-07	2.72667E-14	1.E-15
8	pH meter 691 verification	A	6.99590909	Normal	0.015934118	1.73205	0.009199567		0.001314992	1	0.001314992	100%	21	15.65%	1.7292E-06	2.99015E-12	1.E-13
9	Conductivity specification	B	1	Rectangular	1	1.73205	0.577350269	nS/cm	0.577350269	1	0.577350269	50%	2	3017656.97%	0.333333333	0.111111111	6.E-02
10	Pipettes (10 ml)	A	Refer to 10 ml pipette uncertainty budget									100%	35	0.00%	0	0	0.E+00
11	Temperature of coolroom	A	5.09090909	Normal	0.294244943	4.69042	0.062733233	°C	0.012322599	1	0.012322599	100%	21	1374.62%	0.000151846	2.30573E-08	1.E-09
12	Temp of room (dehydrated media)	A	20.3181818	Normal	0.994574024	4.69042	0.212043894	°C	0.010436165	1	0.010436165	100%	21	985.96%	0.000108914	1.18622E-08	6.E-10
										$u_c(y)$	0.003323622				1.10465E-05	1.18361E-10	6.E-11
										ν_{eff}	2.1						
									Level of Confider		95%						
									Coverage factc		4.30						
										U	0.014300392						

5.Reporting the uncertainty

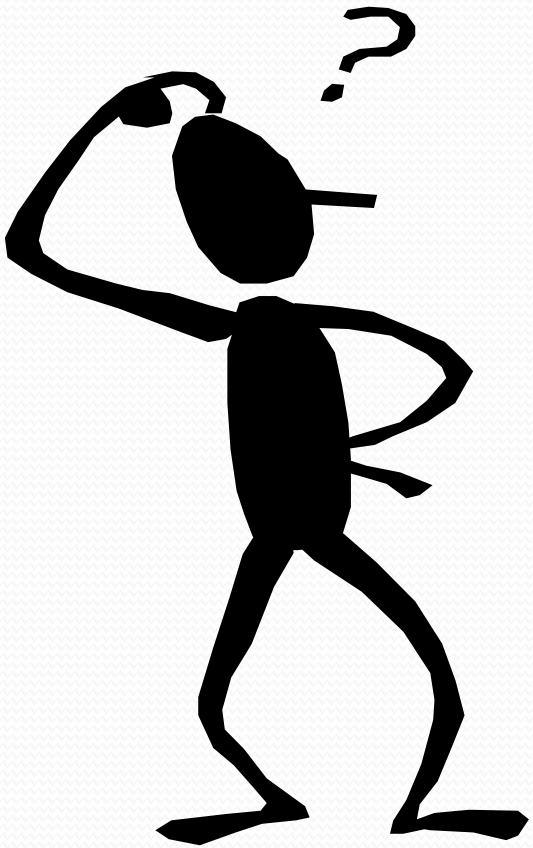
- Results must be rounded up.
- The coverage factor must be recorded
- The level of confidence must be stipulated

The expanded uncertainty is ± 11 at a 95% level of confidence with a coverage factor of 1.96.

Conclusions

- Not the perfect approach
- By calculating UoM:
  - Gives the opportunity to improve areas within the method with huge contributors to the uncertainty
  - Critically analyze all steps of the method to eliminate or reduce the uncertainty contributors
  - Vision to improve and reduce the expanded uncertainty over time
  - Gives an indication on where the possible problems within the method could be.

Thank you



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