

**Uncertainty Estimation using method
performance data of the method for
isolation and detection of
Cryptosporidium and *Giardia* (oo)cyts.**

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INTRODUCTION

- **Uncertainty of measurement is an indicator of the accuracy or quality of a measurement.**
- **Determination of uncertainty of measurement provides the following information:**
 - **Confidence in the technical competency of the laboratory.**
 - **Identification of major sources of error in a method.**
 - **Direction and opportunity for improvement.**

INTRODUCTION

- The uncertainty of measurement for the method of isolation and detection of *Cryptosporidium* and *Giardia* (oo)cyst was determined using a 6 step approach.
- 6 Step approach:
 - Step 1: Specification and modelling
 - Step 2: Identification of uncertainty components

INTRODUCTION

6 Step approach Continues:

- **Step 3: Quantification of sources of uncertainty (evaluation of standard uncertainty for Type A & Type B).**
- **Step 4: Determining the combined standard uncertainty.**
- **Step 5: Determining the expanded uncertainty.**
- **Step 6: Reporting the uncertainty.**

AIM

- To determine the uncertainty of measurement of the method for isolation and detection of *Cryptosporidium* and *Giardia* (oo)cysts in different types of water matrices.

Methodology

■ Step 1

- **Method: Filtration, elution concentration and enumeration.**
- **Measurand: Recovered (oo)cysts.**
- **Matrix: Reagent grade water, drinking water & raw water.**

Methodology

- **Step 1: Model**

- **$(oo)cysts_{\text{filtered}} = (oo)cysts_{\text{recovered}}$**

- **$(oo)cysts_{\text{filtered}}$ = number of (oo)cysts filtered in a water sample**

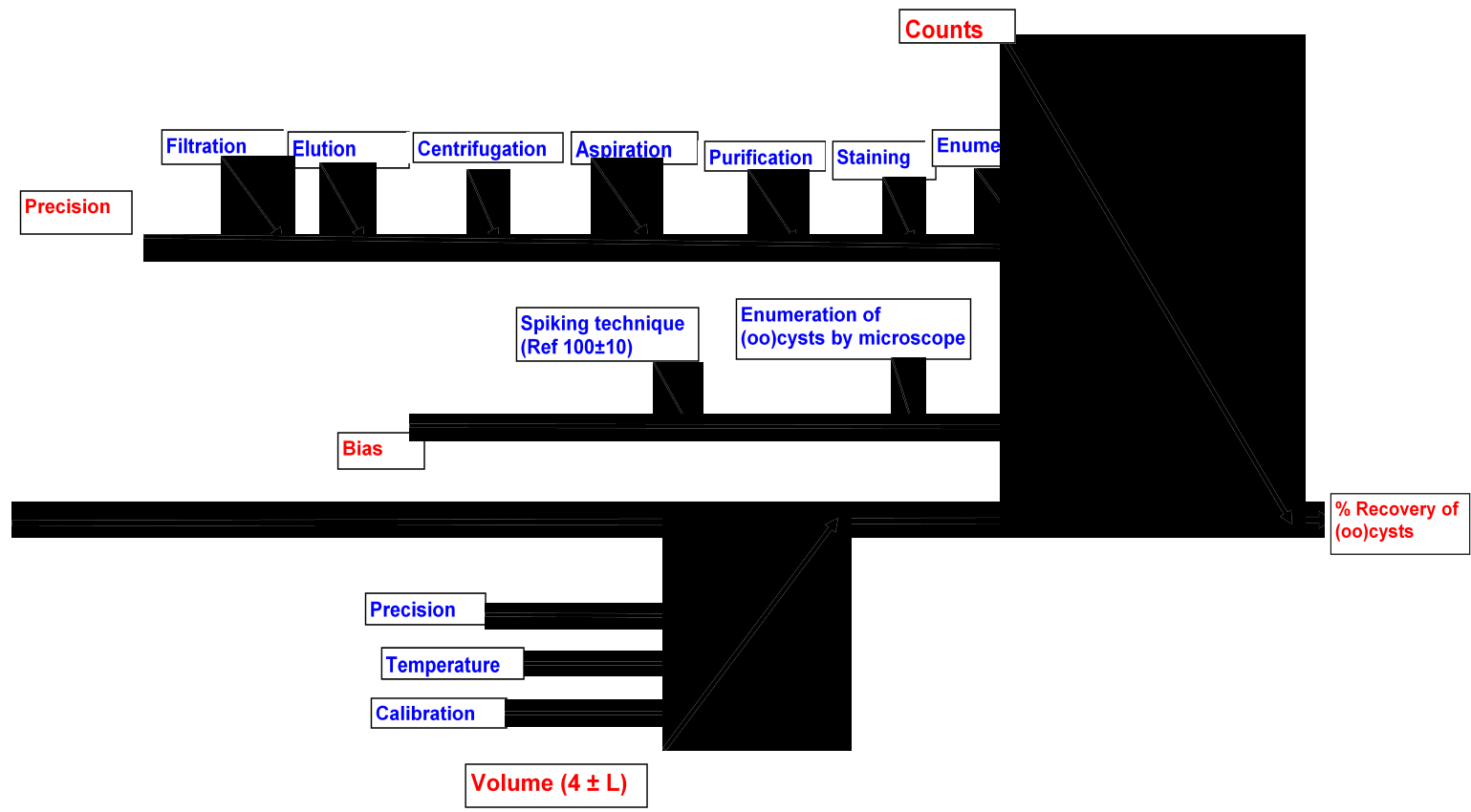
- **$(oo)cysts_{\text{recovered}}$ = number of (oo)cysts recovered after sample processing**

Methodology

Step 2: Identification 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	Filtration at 2L /minute	A	Yes	
3	Media preparation Balances Volume	B A	Yes Yes	
4	Wrist action shaker speed	A	Yes	
5	Centrifugation	A	Yes	
6	IMS	A	Yes	
7	Staining QC FITC stain Incubator Thermometer	B B B	No Yes Yes	QC step. Not quantifiable
8	Reproducibility <i>Cryptosporidium</i> in drinking water	A	Yes	
9	Reproducibility <i>Cryptosporidium</i> in raw water	A	Yes	
10	Reproducibility <i>Giardia</i> in drinking water	A	Yes	
11	Reproducibility <i>Giardia</i> in raw water	A	Yes	

Fishbone for the method of isolation and detection of *Cryptosporidium* and *Giardia* (oo)cysts



Methodology

Step 3: Evaluation of standard uncertainty of components to Type A or Type B uncertainty

	Source	Type	Probability distribution
1	Filtration at 2L /minute	A	Normal
2	Media preparation Balances Volume	B A	Rectangular Normal
3	Wrist action shaker speed	A	Normal
4	Centrifugation	A	Normal
5	IMS	A	Normal
6	Staining Incubator Thermometer	B B	Rectangular Rectangular
7	Reproducibility <i>Cryptosporidium</i> in drinking water	A	Normal
8	Reproducibility <i>Cryptosporidium</i> in raw water	A	Normal
9	Reproducibility <i>Giardia</i> in drinking water	A	Normal
10	Reproducibility <i>Giardia</i> in raw water	A	Normal

Results

- **Step 6: Reporting the uncertainty**
 - The expanded uncertainty (U) for both *Cryptosporidium* and *Giardia* in drinking water was derived as ± 8 oocysts and cyst at 95% level of confidence (LOC) with a coverage factor of 1.96.
 - (U) for *Cryptosporidium* in raw water = ± 23 oocysts at 95% with a coverage factor of 1.96
 - (U) for *Giardia* in raw water = ± 32 cyts at 95% with a coverage factor of 1.96

Discussion

- **Reproducibility was identified as the greatest contributor to the uncertainty of the method as indicated in the uncertainty budget:**
 - *Cryptosporidium* in drinking water = 99.40%
 - *Cryptosporidium* in raw water = 99.94%
 - *Giardia* in drinking water = 99.46%
 - *Giardia* in raw water = 99.97%
- **The fluorescence intensity of the FITC stain was identified as another contributor to the method uncertainty but could not be quantified.**

Conclusion

- **The uncertainty of measurement for this method is relatively high as a result of the reproducibility which had a major contribution.**
- **Therefore the uncertainty of the method will be improved by reducing the uncertainty of reproducibility over time.**

Conclusion

- **A 6 step approach is a good structured process to follow when determining the uncertainty of measurement for the method.**
- **This approach also helped to highlight the areas that need to be improved upon in the method.**



THANK YOU!