# Measurement Uncertainty and the CDS Method

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***Andrew Bowen Southern.IML Pathology Wollongong NSW Andrew.Bowen@southernpath.com.au***

## Introduction:

Since 2003 the standard used in the assessment of medical testing laboratories has been *ISO 15189 (Medical Laboratories – particular requirements for quality and competence)*. Under this standard laboratories are required to implement the principles of Measurement Uncertainty (MU) [1]. Furthermore, the National Association of Testing Authorities (NATA) has stated in NATA Technical Circular # 2 December 2003 that specialised antibiotic procedures are classified as quantitative tests and thus require MU to be calculated [2].

MU provides a quantitative estimate of expected result variability when measurements are repeated. Laboratories should be satisfied that the material used to calculate MU behaves in the measurement procedure in a similar way to that of patient samples [1]. Antibiotic susceptibility determination by the CDS method generates numerical values which are reported according to preset dichotomous values. For such methods the laboratory is required to estimate an MU for the part of the procedure that generates the numerical value, which is the antibiotic zone size.

A general approach to the estimation of uncertainty of measurement is suggested:
1. Define what is being measured.
2. Identify and list all potential sources of uncertainty.
3. Quantify the uncertainty associated with the significant sources.
4. Combine the individual uncertainties according to established rules [2].

## Method:

From accumulated internal Antibiotic QC results recorded from April 2006 to May 2010 imprecision was estimated by calculating co-efficients of variation ($CV_1$) and 95% confidence intervals (95% CI) for each antibiotic/reference organism combination. The $CV_1$ are amalgamated with the uncertainty determined for the saline volume ($CV_2$) to determine the combined standard measurement uncertainty ($u_C$) for each antibiotic. To provide expanded measurement uncertainties ($U$) for a confidence level of approximately 95%, a coverage factor ($\kappa$) of 2 is applied to the $u_c$. As there are no recognised $CV_A$ goals available for antibiotic susceptibility, *fitness-for-purpose* is achieved by a comparison of the testing laboratory's 95% confidence intervals (95% CI) with those provided by The Antibiotic Reference Laboratory [3].

CV = 100 x sd/x %
Standard measurement uncertainty = $u_C = [(CV_1)^2 + (CV_2)^2]^{0.5}$
Expanded measurement uncertainty = $U = u_C \times \kappa$

## Measurand Definition:

Quantity intended to be measured – Antibiotic susceptibility
System – Bacterial growth/Calibrated Antibiotic discs
Kind of quantity – Antibiotic disc zone radius
Measurement unit – millimetres
Method – CDS test

### Possible sources of Uncertainty

| Variables | Within control of lab | Need to estimate uncertainty |
|---|---|---|
| Saline Volume | No | Yes |
| Agar plates | No | No |
| Antibiotic disc strength | No | No |
| Inoculum preparation | Yes | No |
| Zone Reading | Yes | Yes |
| Incubation time/temp | Yes | No |
| Reference Organism | No | No |

## Materials:

Sensitest Agar (Oxoid CM409)
90mm diameter plastic Petri dishes
2.5ml tubes of sterile isotonic saline (Lomb)
Disposable plastic inoculating needles (Sarstedt)
6mm diameter antibiotic discs (Oxoid)
Disc dispenser (maximum of 6 discs – Oxoid)
35°C aerobic Incubator
Clear plastic ruler, marked in millimetres
Reference Organism *Staphylococcus aureus* ACM5190
Reference Organism *Escherichia coli* ACM 5185

Southern IML Pathology performs antibiotic susceptibility according to the CDS method outlined by the Australian Reference Laboratory CDS Users Group. Preparation of inoculum, inoculation of saline, disc dispensing, incubation of plates and reading of zones are all performed according to CDS guidelines [3].

## Results:

Measurement Uncertainty of Saline volume:
Manufacturers volume = 2.5ml
n=30 x=2.42 s.d = 0.06
$CV_2$ = 0.06/2.42 x100 = 2.5

### Reference Organism *Staphylococcus aureus* ACM5190

| Antibiotic | n | X | s.d | 95%CI | Ref. range | $CV_1$ | $u_c$ | *U* |
|---|---|---|---|---|---|---|---|---|
| Penicillin | 200 | 11.5 | 0.9 | 9.7-13.3 | 8.7-13.5 | 7.6 | 7.9 | 15.8 |
| Erythromycin | 200 | 9.5 | 0.6 | 8.3-10.7 | 8.0-10.8 | 6.3 | 6.8 | 13.6 |
| Rifampicin | 200 | 11.0 | 0.7 | 9.6-12.4 | 9.3-12.5 | 6.4 | 6.9 | 13.8 |
| Vancomycin | 200 | 4.0 | 0.3 | 3.4-4.6 | 2.8-4.9 | 7.5 | 7.9 | 15.8 |
| Cefoxitin | 200 | 8.8 | 0.5 | 7.8-9.8 | 7.1-10.1 | 5.9 | 6.4 | 12.8 |

### Reference Organism *Escherichia coli* ACM 5185

| Antibiotic | n | x | s.d | 95%CI | Ref. range | $CV_1$ | $u_c$ | *U* |
|---|---|---|---|---|---|---|---|---|
| Ampicillin | 200 | 9.1 | 0.6 | 7.9-10.3 | 7.5-10.7 | 6.6 | 7.1 | 14.2 |
| Cephalexin | 200 | 8.4 | 0.6 | 7.2-9.6 | 6.9-10.9 | 8.3 | 8.7 | 17.4 |
| Trimethoprim | 200 | 10.4 | 0.7 | 9.0-11.8 | 8.8-13.6 | 7.7 | 8.1 | 16.2 |
| Nitrofurantoin | 200 | 7.7 | 0.6 | 6.5-8.9 | 6.3-9.5 | 7.8 | 8.2 | 16.4 |
| Norfloxacin | 200 | 13.4 | 0.9 | 11.6-15.2 | 10.4-16.4 | 6.7 | 7.2 | 14.4 |

## Discussion:

No documented biological goals exist for antibiotic susceptibility testing and Measurement Uncertainty. This is in contrast to disciplines such as Haematology and Biochemistry where the Westgard QC database [4] provides an excellent resource for MU calculations. Microbiology has access to no such reference material, due primarily to the relative lack of automation in this area of clinical pathology. To help satisfy the NATA requirement that Uncertainty of Measurement be applied to specialised antibiotic procedures it would be useful to have a website established which provided a compilation of desirable standard and expanded uncertainty measurements for each antibiotic/reference organism combination.

In the absence of such a database, another method to determine *fitness-for-purpose* can be utilised. The Antibiotic Reference Laboratory provides acceptable zones of inhibition for reference strains for appropriate antibiotics, referred to as reference ranges in the above tables. These zones of inhibition are calculated from >120 measurements under differing conditions [3]. Although this reference procedure will have its own bias the sample size is large enough to use as a comparison [5]. If the calculated 95% CI are within those stated by the Reference Laboratory then *fitness-for-purpose* has been established and subsequent patient sample results are fit for clinical application.

The calculation of CV's is firmly entrenched in medical testing as the preferred statistical parameter of measurement uncertainty. As a statistical tool the CV is used to compare the variability between 2 or more sets of data representing different quantities with different units of measurement, using a numerical summary measure. Since it is independent of measurement units, it can be used to evaluate the relative variation between any two sets of observations, or different laboratories MU calculations. Most statisticians believe the statistical properties of CV measurement make it less than ideal in the field of medical testing and that its use should be discouraged [6].

## Bibliography:


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