# Uncertainty of Measurement (UoM) for Susceptibility Testing in Diagnostic Laboratories.

## Introduction

Uncertainty of Measurement (UoM) relates to the margin of doubt that exists for the result of any measurement, as well as how significant the doubt is.

In diagnostic microbiology laboratories the main methods employed for susceptibility testing are:

| Method | Reading result |
|---|---|
| EUCAST disk diffusion | Zone diameter (mm) |
| Gradient strip | MIC (mg/L) |
| Automated instrument | MIC (mg/L) |
| Broth Microdilution | MIC (mg/L) |

For any of the above methods there is the inherent error of the method plus the error which may occur during reading of the test.

*The inherent error of the method:*

For any test employed in any laboratory there is an inherent (in-built) error rate, meaning that it may not give the same result 100% of the time. The inherent error for Minimum Inhibitory Concentration (MIC) methods is ±1 $\log_2$ dilution, i.e. If the true MIC of an isolate is 1mg/L, then testing in the laboratory, using any MIC method, may result in MICs of 0.5, 1 or 2mg/L. The MIC results of 0.5mg/L and 2mg/L are not incorrect, they are the result of a natural variance, probably caused by small differences in media, incubation conditions and set up. These natural ranges are clearly seen in the MIC ranges of Quality Control organisms. Below is the EUCAST QC version 10 table for E. coli ATCC 25922:

***Escherichia coli* ATCC 25922**
**(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)**

**See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.**

| Antimicrobial agent | MIC (mg/L) | | Disk content (µg) | Inhibition zone diameter (mm) | |
|---|---|---|---|---|---|
| | Target[1] | Range[2] | | Target[1] | Range[2] |
| Amikacin | 1-2 | 0.5-4 | 30 | 22-23 | 19-26 |
| Amoxicillin | 4 | ***2-8*** | - | - | - |
| Amoxicillin-clavulanic acid[3,4] | 4 | 2-8 | 20-10 | 21 | 18-24[5] |
| Ampicillin | 4 | 2-8 | 10 | 18-19 | 15-22[5] |
| Ampicillin-sulbactam[4,6] | ***2*** | ***1-4*** | 10-10 | 21-22 | 19-24[5] |
| Aztreonam | 0.125 | 0.06-0.25 | 30 | 32 | 28-36 |
| Cefadroxil | - | - | 30 | ***17*** | ***14-20*** |
| Cefalexin | ***8*** | ***4-16*** | 30 | ***18*** | ***15-21*** |
| Cefazolin | 2 | 1-4 | IP | IP | IP |
| Cefepime | 0.03-0.06 | 0.016-0.125 | 30 | 34 | 31-37 |
| Cefixime | 0.5 | 0.25-1 | 5 | ***23*** | ***20-26*** |
| Cefotaxime | 0.06 | 0.03-0.125 | 5 | ***28*** | ***25-31*** |
| Cefoxitin | 4 | 2-8 | 30 | 26 | 23-29 |
| Cefpodoxime | 0.5 | 0.25-1 | 10 | 25-26 | 23-28 |
| Ceftaroline | 0.06 | 0.03-0.125 | 5 | ***27*** | ***24-30*** |
| Ceftazidime | 0.125-0.25 | 0.06-0.5 | 10 | ***26*** | ***23-29*** |

For ampicillin the MIC range (highlighted in the red box) spans 2x $log_2$ dilutions (2mg/L to 8mg/L). This means that ideally the result should be 4mg/L (target MIC) but MICs ±1x log2 dilution (i.e. 2mg/L and 8mg/L) are acceptable. For ampicillin disc testing the zone diameter (ZD) range spans 15-22mm, with an ideal (target) ZD of 18 or 19mm. The ZD ranges correlates with the MIC: roughly 2-3mm per $log_2$ dilution.

Internal error varies for different antibiotic / organism combinations. For those combinations EUCAST mitigate this error by having an Area of Technical Uncertainty (ATU), where inherent errors are prominant and could affect the S/I/R interpretation.

In summary, the inherent error within the susceptibility testing method has already been calculated during the development of the QC MIC and ZD ranges.

*The "external" error in measuring zone diameter or MIC i.e. Uncertainty of measurement:*

In most laboratories, multiple staff members will perform and read the susceptibility tests of control organisms and clinical isolates. These staff members will not read the same test exactly the same every time – this variance is called the "external" error. Therefore, it is important that all staff members which read susceptibility tests on clinical isolates can do so within acceptable limits. It is well established that slight variances occur in MIC or ZD results when different staff members read the same test, even with calibrated reading instruments. This may be due to light sources, natural eyesight ability etc.

These differences (UoM) in reading, when added to the "inherent" error of the test, can have a major impact when reading clinical isolate susceptibility testing. If ZD/MIC reading variance is high in a laboratory then incorrect reporting of the tests will be increased. This is especially important when the true ZD/MIC of a clinical isolate is near the breakpoint. This isolate would be reported resistant or susceptible depending upon who has read the test. This variance should be as small as possible to ensure good quality susceptibility testing reporting.

The impact of a high reading variance can be as follows:

<u>False susceptible results (Very Major Error):</u> reporting "Susceptible" when the true result is "Resistant". The patient may be treated with the drug for which the isolate has been reported as susceptible. The clinical outcome for this patient will be treatment failure, leading to longer hospital stay or even morbidity.

<u>False Resistance (Major Error):</u> reporting "Resistant" when the true result is "Susceptible". This leads to the treatment options for a patient being restricted. This may mean that the patient does not receive the best treatment option because it has been reported as falsely resistant.

So it is important that the UoM is calculated in each laboratory and kept as low as possible.

## When to perform Uncertainty of Measurement (UoM) investigations

UoM investigations should be performed as part of a verification process before implementing a new susceptibility testing method (e.g. EUCAST disc diffusion method).

For laboratories with established susceptibility testing methods, UoM investigations should be performed every 12 months, usually when updating to the current version EUCAST breakpoint tables.

Regular testing will ensure accuracy of reading and will fulfil UKAS 15189 standards

## How to perform UoM investigations

This document will describe how to perform UoM when using EUCAST disc diffusion testing and MIC determination using gradient strip, broth microdilution or any other method which involves a human reading element.

1. One staff member to perform 1x set of QC testing on ALL local antimicrobial disc test sets and routine MIC methods using appropriate QC organisms.
2. All staff who read susceptibility testing should be included in the UoM testing. Depending upon the number of staff members in your laboratory, obtain readings (ZDs/MICs) from as many staff members as possible. For smaller laboratories, all staff may be included on one occasion whilst larger laboratories should rotate at least 10 staff on each occasion.
3. All staff members should measure the ZDs/MICs within 2 hours, if a longer time is required then the QC set plates should be stored at 4°C. Do not exceed 6 hours.
4. In larger laboratories, all staff should be included in the UoM investigation; split staff over 2x 6 month investigations.
5. All ZD/MICs should be added to the UoM tool (see below) and compared.
6. All ZDs should be within 3mm; all MICs should be within ±1 log2 dilution. Any ZD/MIC outside of the range should be investigated as stated in the UoM tool.

For the UoM investigations, the mm or mg/L difference between staff readings is the most important factor to investigate laboratory variance when performing susceptibility testing. Because QC isolates are being used for the UoM investigations all ZS/MICs should be within range. If any staff member reading is outside the acceptance criteria (3mm for ZD and ±1 $\log_2$ dilution for MIC) but within the QC range, this should still be cited as unacceptable.

## Uncertainty of Measurement tool

Please find below the UoM tool for recording all aspects of the UoM investigation for susceptibility testing methods. Please read the instructions on the ”Performance process”.


EUCAST DD UoM tool.xlsx


MIC UoM tool.xlsx

## How to record clinical impact

If some staff members continue to read outside the acceptance criteria you may need to assess the clinical impact of this variance. This can be performed according to the processes detailed within the UoM tool.