Quality Control of Antimicrobial Susceptibility Testing

1. Control strains

Control strains include susceptible strains to monitor test performance (not for the interpretation of susceptibility), and resistant strains to confirm that the method will detect particular mechanisms of resistance, for example, *Staphylococcus aureus* ATCC 29213 is a cefoxitin susceptible strain whilst *S. aureus* NCTC 12493 has the mecA gene, which confers resistance to cefoxitin (see Routine & Extended Quality Control as Recommended by EUCAST.pdf).

Control strains can be purchased from the National Collection of Type Cultures (http://www.phe-culturecollections.org.uk/collections/nctc.jsp) or from other commercial manufacturers. Identify whether the control strain is a **reference strain** (i.e. passage 0) or **reference material** (i.e. passage 1) as this affects how it is used within the laboratory. Manufacturers documentation should include how many passages have taken place before receipt.

If using a "passage 0" strain, it should be cultured using standard laboratory media and checked for purity. This should be stored at -80^{0}C on beads in glycerol broth, or another method which minimises the risk of mutations. Ideally, two or more vials of each control strain should be stored, one as an "in-use" supply, the others for archiving. These are the REFERENCE STOCKS.

Every week a bead from the "in-use" REFERENCE STOCK vial should be sub-cultured onto appropriate non-selective media and checked for purity – This is "passage 2" or STOCK CULTURE. From this pure culture, prepare one sub-culture for each of the following 7 days – this is "passage 3" or WORKING CULTURE.

For fastidious control organisms, sub-culture the from the REFERENCE STOCK more than once a week.

2. Calculation of control ranges for disc diffusion tests

The acceptable ranges for the control strains have been calculated by collation of results from different laboratories around Europe.

3. Frequency of routine testing with control strains

When implementing any new susceptibility testing method daily testing is required until there are acceptable readings from 20 consecutive days (this also applies when new agents are introduced or when any test component changes).

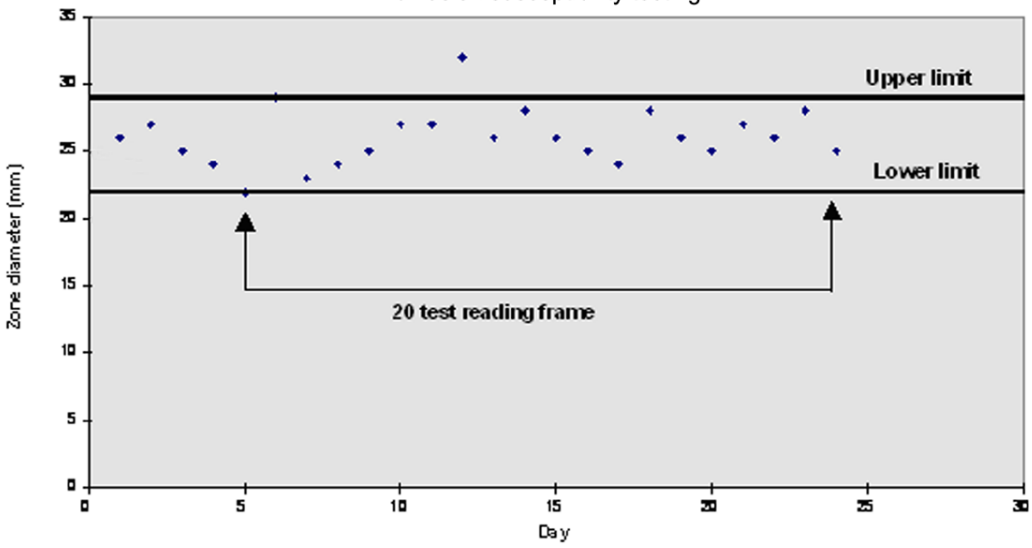
Once in routine use, the EUCAST disc diffusion method requires daily (or at least 4 times per week) internal quality assurance testing.

4. Use of control data to monitor the performance of disc diffusion tests

Use a reading frame of 20 consecutive results (remove the oldest result when adding a new one to make a total of 20) as illustrated in Figure 1. Testing is acceptable if no more than1 in every 20 results is outside the limits of acceptability. If 2 or more results fall out of the acceptable range this requires immediate investigation.

Look for trends within the limits of acceptability e.g. tendency for zones to be at the limits of acceptability; tendency for zones to be consistently above or below the mean; gradual drift in zone diameters. Quality Assurance will often pick up trends before the controls go out of range.

Figure 1. Use of control strain to monitor performance of disc diffusion susceptibility testing.



5. Recognition of atypical results for clinical isolates

Atypical results with clinical isolates may indicate problems in testing that may or may not be reflected in zone diameters with control strains.

- An organism with inherent resistance appears susceptible e.g. *Proteus* spp. susceptible to colistin or nitrofurantoin.

- Resistance is seen in an organism when resistance has previously not been observed, e.g. penicillin resistance in Group A streptococci.

- Resistance is seen in an organism when resistance is rare or has not been seen locally, e.g. vancomycin resistance in *Staphylococcus aureus*.

- Incompatible susceptibilities are reported, e.g. a methicillin resistant staphylococcus reported susceptible to a β-lactam antibiotic.

In order to apply such rules related to atypical results it is useful to install an `expert' system for laboratory reporting to avoid erroneous interpretation. See EUCAST Expert rules.pdf.

6. Investigation of possible sources of error

If the control values are found to be outside acceptable limits on more than one occasion during a reading frame of twenty tests, investigation into the possible source of error is required. Possible problem areas are indicated in table 1.

Table 1: Potential sources of error in disc diffusion antimicrobial susceptibility testing.

Possible source of error	Detail to check
Test conditions	Excessive pre-incubation before discs applied Excessive pre-diffusion before plates incubated Incorrect incubation temperature Incorrect incubation atmosphere Incorrect incubation time Inadequate illumination of plates when reading Incorrect reading of zone edges
Medium	Recommended susceptibility testing agar not used Batch to batch variation Antagonists present (e.g. with sulphonamides and trimethoprim) Incorrect pH Incorrect divalent cation concentration Incorrect depth of agar plates Agar plates not level Expiry date exceeded
Antimicrobial discs	Wrong agent or content used Labile agent possibly deteriorated Light sensitive agent left in light Incorrect storage leading to deterioration Disc containers opened before reaching room temperature Incorrect labelling of disc dispensers Expiry date exceeded
Control strains	Contamination Mutation Incorrect inoculum density Uneven inoculation Old culture used

7. Reporting susceptibility results when controls indicate problems

Microbiologists must use a pragmatic approach, as results from repeat testing are not available on the same day. If results with control strains are out of range the implications for test results need to be assessed.

Control results out of range
If control zones are below range but test results are susceptible, or control zones are above range but test results are resistant, investigate possible sources of error but report the test results. Otherwise it may be necessary to suppress reports on affected agents, investigate and retest.

Atypical results
If results are atypical with clinical isolates, the purity of the isolate and identification should be confirmed and the susceptibility repeated. Suppress the results for individual agents and retest.