# National Department of Health

## Title: Disc diffusion AST quality control

ID: G_90_SOP_3_A

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| **Reviewed by:** | **J Ferguson, C Allen** |
| **Authorized by:** | **W Porau** |
| **Issued:** | **18/11/21** |
| **Review:** | **2 years** |

**Changes to the last authorized version:**

| Version | Date issued | Changes |
|---|---|---|
| A | 19/11/21 | N/a- New document |

**Certification of printed copy:**

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| Date | |

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1. **Purpose and Scope**

   ATCC QC bacterial strains are used to monitor the performance of antibiotic discs and media used for disc diffusion so that reliable antimicrobial susceptibility results are reported.

2. **Principle/Clinical application**

   Properly stored ATCC strains exhibit predictable antimicrobial resistance properties and serve as a reliable test of the entire AST system.

## 3. Responsibilities

| Role | Responsibility |
|---|---|
| Quality officer or delegate | Prepares weekly QC runs and records results<br>Highlights problems that arise without delay in the QC summary sheet<br>Follows up on agreed corrective actions |
| Micro Lab manager | Reviews summary results with the QO and pathologist - discusses necessary corrective action(s) if required<br>Signs off on results and actions before filing<br>Tables QC records at the next Staff Meeting |
| Pathologist | Reviews summary QC record, signs and agrees necessary corrective actions |

## 4. Specimen

Not applicable

## 5. Equipment/Materials

- stock cultures of ATCC strains renewed monthly
- agar plates
- antibiotic disc sets
- ruler for measurement

## 6. Procedure

**Performed once a week:**

**Monday**: subculture each ATCC organism from your current working culture as follows:

- *E. coli* ATCC 25922 –blood agar
- *S. aureus* ATCC 29213 –blood agar
- *E. faecalis* ATCC 29212 –blood agar
- *S. pneumoniae* ATCC 49619 –blood agar
- *H. influenzae* ATCC 49766 – chocolate agar

**Tuesday (at 2pm)**: set up disc diffusion for each combination of antibiotics using ATCC strains as follows:

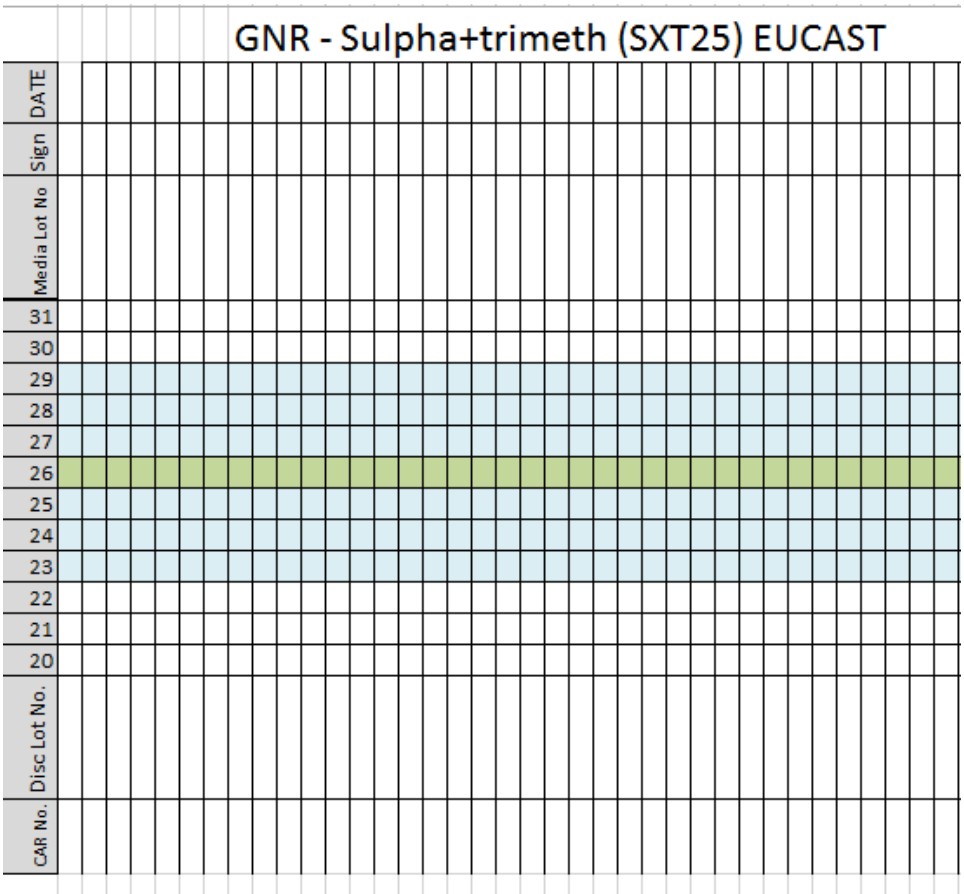
| Stamp | STAPH | ENC | GNR | MRGN | SSV | HAEM | STREP |
|---|---|---|---|---|---|---|---|
| Control strain | ATCC 29213 S. aureus | ATCC 29212 E. faecalis | ATCC 25922 E. coli | ATCC 25922 E. coli | ATCC 25922 E. coli | ATCC 49766 H. influenzae | ATCC 49619 S. pneumo |
| Media | MH | MH | MH | MH | MH | MH-F | MH-F |

**Wednesday (morning):**

- Read zone diameters in mm for each antibiotic/organism combination using a ruler
- Plot each zone diameter on the disc diffusion QC chart, along with the date, the disc batch number, and your initial (See Figure 2).
- If the zone diameter falls within the acceptable range (blue or green shaded areas), it is acceptable to use that disc to test patient samples.
- If the zone diameter does not fall within the acceptable range, you cannot use your disc and/or media to reliably test patient isolates. If your disc is out of range, refer to Section 8 for possible actions.
- Prepare the weekly summary QC zone sheet (Appendix for example)
- Meet with the lab manager and/or pathologist ASAP if necessary to discuss non-conforming (out of range) results

## 7. Recording of results


**Figure 2** – Example of how to record disc diffusion QC data. This is *E. coli* ATCC 25922. The zone diameter for SXT25 from the gram negative stamp is 26mm (photo above). An "X" is plotted at 26mm on the QC chart. This disc is in the acceptable QC range, and is therefore OK to use.



## 8. Interpretation

### Factors affecting zone sizes: zones too small

| Observation | Probable cause | Corrective action |
|---|---|---|
| AMP, AUG, CRO | Disc has lost potency | Check storage conditions and package integrity. |
| Any zones too small | Contamination | Pick well isolate colonies when preparing inoculum. Subculture/ID inner zones to check purity and repeat if necessary |
| Any zones too small | Inoculum too heavy | prepare 0.5 McFarland. |
| Any zones too small | Too long between plate inoculation and putting discs on | Adhere to 15-15-15 minute rule |
| Any zones too small | Incorrect endpoint reading. E.g use of magnification | Follow EUCAST recommedations for endpoint read. i.e. in most cases measure zone diameter with unaided eye |
| Any zones too small | Discs not applied properly | Make sure discs are pressed firmly against the agar. Do not move a disc once applied |

### Zones too large

| Observation | Probable cause | Corrective action |
|---|---|---|
| Penicillins, tetracycline, quinolones | pH of media too low | Avoid $CO_2$ incubation for MHA (clear plates)– lowers pH of media |
| Any zones too large | Inoculum too light | prepare inoculum to 0.5 McFarland standard. Make sure your lawn of growth is confluent with no spaces |
| Any zones too large | Incorrect endpoint read | Take into account double zones, colonies within the zone. Read at point of complete inhibition with fuzzy zone edges |
| Any zones too large | Inoculum source plate too old and contains too many non-viable cells | Use overnight growth – subculture if necessary. When reading sensitivity plates, should have a confluent lawn of growth with no spaces. |

## 9. Safety

As per standard precautions.

## 10. Quality Control

Not applicable.

## 11. Related documents

For access, refer to https://path-png.org/microbiology-sops-fleming-fund/

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|---|---|
| Weekly AST QC summary | G_90_WS_3 |
| Weekly AST QC Worksheets | G_90_WS_4 |
| Antibiotic disc susceptibility testing | G_90_SOP_6 |
| Maintenance of cultures used for quality control testing | G_90_SOP_2 |

## 12. Reference


European Committee on Antimicrobial Susceptibility Testing (EUCAST) QC tables: https://www.eucast.org/ast_of_bacteria/quality_control/

## Appendix: Weekly Disc AST QC summary results (example)

The Fleming Fund — Worksheet (form) — Burnet Institute — PRIDA

Document G_90_WS_3_A Author: F Courtney Authorised: W Porau 04/08/2021 Review: 04/08/21

**Weekly AST QC Summary**

Week commencing: 15/7/20

Prepared by: V Fabila Signed: [signature]

Summary assessment: _____

*(Tick cells where zone size is within control limits; otherwise indicate zone size readings with expected results in brackets)*

| Stamp | STAPH | ENC | GNR | MRGN | SSV | HAEM | STREP |
|---|---|---|---|---|---|---|---|
| Control strain | ATCC 29213 S. aureus | ATCC 29212 E. faecalis | ATCC 25922 E. coli | ATCC 25922 E. coli | ATCC 25922 E. coli | ATCC 49766 H. influenzae | ATCC 49619 S. pneumo |
| Media | MH | MH | MH | MH | MH | MH-F | MH-F |
| P1 | ✓ | | | | | ✓ | ✓ |
| 0X1 | | | | | | | ✓ |
| E15 | ✓ | | | | | | ✓ |
| TE30 | ✓ | | | | | ✓ | ✓ |
| SXT25 | ✓ | | ✓ | | ✓ | ✓ | ✓ |
| C30 | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| CRO30 | | | ✓ | | ✓ | ✓ | |
| FOX30 | ✓ | | | | | | |
| AMP2 | | ✓ | | | | | |
| F100 | | ✓ | ✓ | | | | |
| VA5 | | ✓ | | | | | |
| AMC30 | | | ✓ | | | | |
| CN10 | | | 17 (19-26) | | | | |
| CIP5 | | | ✓ | | | | |
| TOB10 | | | | ✓ | | | |
| MEM10 | | | | ✓ | | | |
| CAZ10 | | | | ✓ | | | |
| TZP30/6 | | | | ✓ | | | |
| AK30 | | | | ✓ | | | |
| AMP10 | | | | | ✓ | | |
| PEF5 | | | | | ✓ | | |
| AZ15 | | | | | ✓ | | |

Action recommended (lab manager):

Repeat gent. with new batch.

Action required (supervisor):

Supervisor name: Dr Joseph Signed [signature] Date: 20/7/20