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IQC In Microbiology Testing

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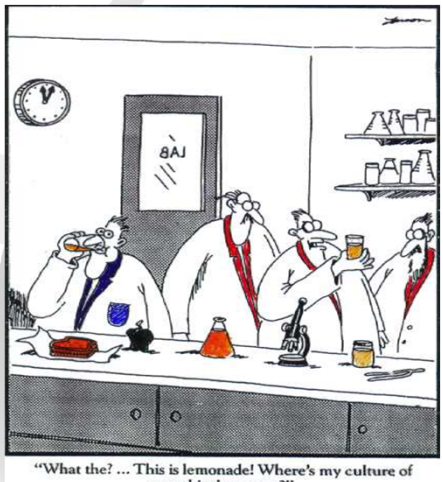

"What the? ... This is lemonade! Where's my culture of amoebic dysentery?"

Microbiological results help appraise safety, quality and legal compliance of materials.

Users of laboratories need to have confidence day to day performance of method

Laboratories must be able to demonstrate reliability of results

Operating a robust IQC Programme contributes to this.

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External Proficiency Test Schemes

Benefits:

- Contributes to demonstrating reliability of results.
- Shows performance of laboratory compared to peers
- Independent , impartial “blind” tests

Limitations:

- May not cover all organisms
- Frequency variable
- Not representative of routine samples or competing flora
- May not be tested in same manner as routine samples
- **Alone** insufficient to demonstrate “day to day” control of methods

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Microbiological IQC

- Robust IQC essential for day to day control of methods and reliability of results
- Should be **planned**, **documented** and **criteria defined** to appraise results.
- Common approaches include:
  - Media QC Checks
  - Verifying Proprietary Kits/Commercial Confirmation Tests
  - Daily Controls
  - Regular In House Method efficacy checks

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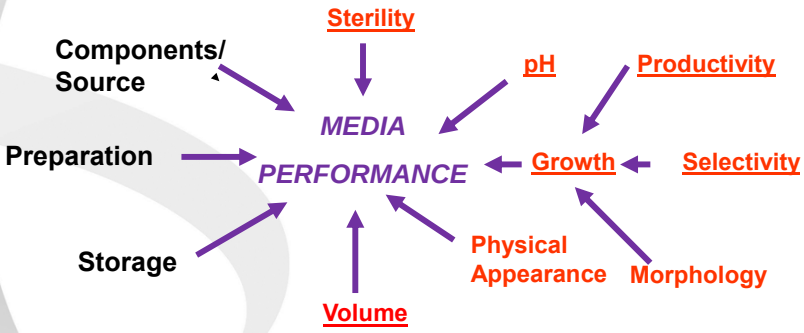
Media Quality Control



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Media QC

- **Post sterilisation pH** (cooled + post supplement addition)
- **Post sterilisation volumes** (critical volumes if dispensed before autoclaving, take into account evaporation loses)
- **Sterility**
- **Performance/Growth Checks**
- **Frequency:**
  - In House Prepared Media - Each laboratory prepared batch
  - Commercial Pre-Prepared Media - Each manufacturers batch code

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Media Sterility pH and Volume Checks

- Quick and easy.
- Typically do not present challenges or problems.
- Easy to integrate into routine working practices

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Performance and Growth Checks – The Challenge

- Frequently pose challenge to labs
- Time consuming
- Labs can find difficult
- Approaches do not easily integrate/fit into routine practices and difficult to combine with testing of samples
- Require cultures of known/defined concentrations to be regularly prepared

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Media Performance/ Growth Checks

- Approaches:
  - Quantitative
  - Semi-quantitative
  - Qualitative
- Dependent upon:
  - Type of Media - agar or broth
  - Use of media - enumeration or detection
  - What level of control labs wants
  - If seeking accreditation to ISO17025

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Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media

- **ISO/TS 11133-1 (2000) :** General guidelines on quality assurance for the preparation of culture media in the laboratory
- **ISO/TS 11133-2 (2003) :** Practical guidelines on performance testing of media

Quantitative, semi-quantitative and qualitative checks, type of cultures and appraisal of results

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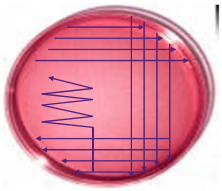
ISO 111322-2 Cultures for Performance Checks



- **Stationary phase cultures in non-selective broth from reference stock culture**
  (different techniques may be used but must guarantee purity of inoculum + its standardisation which allows it to be used at a later stage)
- **Productivity Testing** of target organism
  - semi-quantitative + qualitative tests inoculum level to obtain 10-100cfu per plate/tube
- **Selectivity Testing**
  - non-target organism to contain 10^4 to 10^6 cfu/ml inoculated onto plate/into tube

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QC of Commercial Kits/Confirmation Tests

- **If possible**:
  - Obtain information on manufacturers QC checks
  - Obtain evidence each batch has passed manufacturers checks
- **In-house checks** to verify performance of kits (positive +/- negative control cultures)
- **Frequency of in-house QC checks**:
  - Each use / Each batch code
  - Influenced by frequency of use, manufacturer's instructions, method requirements, control required by lab

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Daily Method Controls

- Sterility checks
- Positive (and negative) control cultures:
  - normally a non specific level of a pure culture inoculated into media and taken through tests to end point of samples.

Benefits

- Shows no contamination with storage melting handling
- Helps interpret results from samples.
- Provides ready source of controls for any subsequent confirmation steps if required

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In-House Method Efficacy Checks

- Duplicate/replicate samples
- Spiked samples
  - Levels to be known, appropriate to samples, method, organism
  - Reflect anticipated contamination levels, specs, legislation
- Reference materials (if available/appropriate)
- Frequency: Decided by the individual laboratory

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Spiked Samples

Benefits:

- Method performance checked with real samples
- Takes into consideration normal competing background flora
- Checks ongoing detection limit
- Checks ongoing linearity and accuracy of recovery at levels relevant to lab
- Note: Performance of method is normally hecked under 'ideal' conditions (typically fresh unstressed organisms used to spike samples and samples tested immediately after inoculation)

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Selection of Cultures

- Cultures grown in house–traditional approach
  - Culture organism in non-selective broth, incubate overnight under appropriate growth conditions, use fresh cultures (e.g. 18-24h stationary cells)
- Commercial Alternatives:
  - Non-quantitative e.g. Cultiloop; Selectrol discs
  - Quantitative e.g. Bioball; Lenticules; Quantiloop
- Ensure traceable to recognised culture collection (unless stated otherwise in method)

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Benefits and Issues to be Aware of:

In-House Prepared Cultures:

- Cheap, wide availability of many strains/cultures
- Broth cultures containing high numbers handled - increased risk of cross contamination

Commercial Preparations:

- Stable; easy to use; ? less risk of cross contamination.
- Expensive for day to day use.
- May not be available at required levels
- May not be acceptable to local Accreditation Body as either reference stock culture or working culture (depends on number of growth passages from NC)

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Storage and Handling of Cultures

Issues to be Aware of:

- Potential risk of cross contamination to samples
- Minimise by:
  - Store cultures (reference/working stocks) separate from media/samples
  - Set up controls/spikes and sub after samples tested, incubate away from samples
  - Use dedicated automatic pipettes and/or filtered tips
  - Handle/process in designated areas of lab
  - Sanitise hands/disinfect surfaces after handling
- Use an uncommon salmonella as control strain and check any positives from samples with antisera specific to lab control strain.

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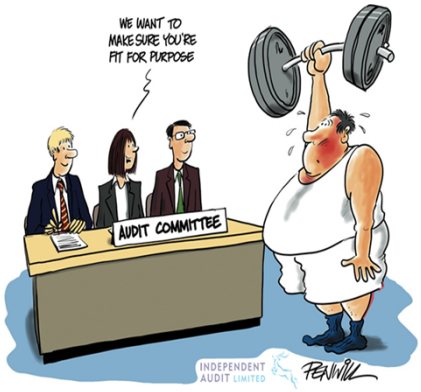

Conclusions

IQC important to verify day to day control of methods

Combination of Approaches Essential to consider
(Some easy others more a challenge)

Handling and using control cultures will be involved

Appropriate measures needed to prevent (and identify) cross contamination

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Additional Guidance Notes on
ISO11133-2
For media QC checks

Not covered in presentation

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Solid Media: Quantitative

- **Inoculation:** spread, modified Miles/Misra, spiral, or pour plate.
- **Inoculum:** 10-100cfu of target micro-organism.
- **Media:** test medium and reference medium (e.g. BP and TSA).
- **Calculate recovery**
- **Assess colony morphology**
- **Appraise results:** Productivity (target organism) and Selectivity (ability to inhibit competitor)

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Productivity: Quantitative Assessment

Productivity Ratio (Pr)

Pr = Ns/No

Ns = total colony count on test culture medium
No = total colony count on reference medium and shall be ≥ 100cfu

Pr of non selective medium is at least 0.7 for organisms that grow easily.

Pr of target organisms on selective medium should be at least 0.1.
Values generally achievable; but less rigorous criteria can be excepted for certain combinations of media/test organisms

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Selectivity Assessment

Quantitative: Calculation of Selectivity (S_f)
$S_f = D_o - D_s$

Do = highest dilution to show ≥ 10 colonies on reference medium
Ds = highest dilution to show comparable growth on test medium

Sf, Do, Ds expressed in log10 units

Semi-Quantitative and Qualitative
Growth of non target strain(s) shall be inhibited partly or completely.

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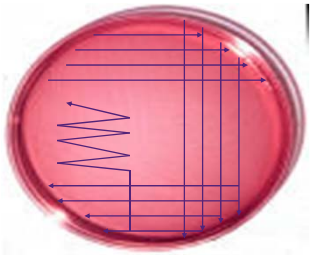
Solid Media: Semi-Quantitative
(Based on Ecometric test)



Applicable for spread and pour plate agars

1µl loop. Streak without recharging (unless low growth index expected) at an angle of 20 – 30º . Incubate as per method.

Calculate Gi (each streak with growth = 1; growth half way along streak = 0.5; streak with no/scant growth scored 0). Sum scores to obtain Gi

Interpretation of results:

Target culture(s) Gi ≥6 (non-selective media Gi normally higher). Colony appearance, size and intensity of growth to be as expected.

Non target culture(s) growth partly or completely inhibited

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Productivity: Semi-Quantitative and Qualitative Assessment

Semi-quantitative: Growth Index (Gi)

Gi = Sum of the consecutive sectors of an ecometric plate to yield growth.

Qualititative

Assessed by visually allocating growth scores

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Solid Media: Qualitative

Applicable for spread and pour plate agars

- Inoculation Technique: 1µl loop. Streak micro-organism(s) in parallel straight lines (can inoculate several M/O on one plate).

Interpretation of results:
0 = zero growth; 1= weak growth; 2= good growth

- **Target organism(s):** score 2 + show typical appearance/ size and morphology**.**
- **Non target organism(s):** partly or completely inhibited (score 0 or 1)

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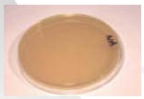
Liquid (Enrichment) Media: Quantitative

Target 10 –100 cfu
Non-target >1000 cfu

Test + reference broths

Incubate as per method.

Known volume/ dilution onto non-selective media: spread, spiral, M/M



Comparative Interpretations between test/reference broths

•Target M/O's: Pr ≥ 0.1

•Non Target M/Os: Sf ≥ 2

•In mixed culture inoculations target M/O to be dominant population and not inhibited by non-target M/O

Other cases:

Fixed minimum counts for target and maximum counts for non target M/Os suitable.

Target M/O: to reach 10^6 – 10^8 cfu/ml

Non target M/O: not exceed 10^4 cfu/ml

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Liquid Media: Semi-Quantitative

Target 10 –100 cfu Non-target >1000 cfu

Incubate as per method

Selective agar (10µl streak).

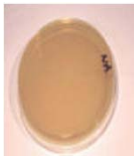
Acceptable if > 10 cfu target grow

Non-target >1000 cfu

Incubate as per method

Non-selective agar (10µl streak).

Acceptable if < 10 cfu or no growth of non-target



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Liquid Media: Qualitative Methods

(Turbid media (e.g. TET) cannot be tested by this approach)

Direct inoculation with 1µl loop + incubate as per method

Interpretation of results

0 = no visual turbidity
1 = very light turbidity
2 = good turbidity

Target M/O score = 2

Other characteristics such as gas, colour change can be assessed by this approach

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