FOREWORD

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Comments and considerations regarding the procedure are welcome and invited, and should be forwarded the General Secretariat of NMKL (address above).
1. **INTRODUCTION.**

In microbiological laboratories, many analyses and procedures are dependent on uniform culture media giving reproducible results. There are hundreds of commercially available media, and additionally, many more are described in the literature. In order to document the satisfactory quality of culture media, laboratories shall demonstrate a well-established quality assurance programme. This procedure lays down guidelines to the content of such a programme, and describes various control methods which may be used.

The laboratory’s quality control programme should be used to demonstrate:

- approval of every batch of medium prepared in the laboratory
- suitability of media using positive and negative control strains (qualitative control)
- reproducible results from each medium (quantitative control)

This procedure is aimed primarily at food laboratories. However, the described guidelines and control methods can be used in any microbiological laboratory.

The procedure has been elaborated for laboratories varying in size and resources. Most laboratories are accredited with respect to EN 45001 or ISO/IEC 17025, while others are incorporated in ISO-9000 companies, and some work with quality assurance without formal approval. The demands on control routines should therefore be evaluated individually according to the laboratory’s requirements.

2. **SCOPE.**

This procedure describes the control programmes and methods, which should be applied to the preparation of microbiological culture media in a quality-assured laboratory.

The procedure includes both solid and liquid culture media within the following categories:

- Purchased, ready-to-use culture media
- Culture media prepared from commercially available dehydrated media
- Culture media prepared from the individual components

3. **GENERAL.**

3.1 Quality assurance of ready-to-use and laboratory prepared culture media.

Culture media can be purchased ready for use or prepared from the individual components, by mixing dehydrated components or reconstituting complete dehydrated media. From a quality assurance perspective, the use of complete dehydrated media or ready-to-use media is recommended. The manufacturers of commercially available media can more easily minimize inter-batch variations than laboratories preparing media from the individual components.
Further, the purchase of both ready-to-use and dehydrated media from ISO-9000-approved manufacturers is recommended. In addition, the laboratory should ensure that the certification covers all relevant aspects which could affect the quality of the finished product, including storage and transport.

The following documentation shall be available from the manufacturers of ready-to-use and dehydrated media:

- Name and production code of the medium
- Batch code
- Batch pH value
- Storage information and expiry date
- Manufacturer’s control programmes, including qualitative and quantitative control
- Quality control certificates

### 3.2 Handling of dehydrated culture media.

Dehydrated culture media shall generally be stored in a dark area with low humidity. The temperature shall not exceed 25°C. Only products specifically labelled for refrigeration should be kept in a refrigerator. Unless the package is equilibrated, dehydrated media absorb moisture through condensation if taken out of the refrigerator too frequently. This shortens the shelf-life of the product.

Dehydrated culture media have a limited shelf-life. The manufacturer’s expiry date must be observed. The laboratory shall record both date of receipt and opening date on each package. Once the package is opened, the shelf-life can be reduced. The shelf-life of a product depends on its composition and storage conditions. Quality parameters such as colour change and formation of lumps should be noted on use. Dehydrated media showing such changes shall not be used.

On receipt, the laboratory shall register any physical damage to the package, and the package’s seal should be controlled.

### 3.3 Preparation of culture media.

NB: The preparation of a medium can be described differently in a method and in the manufacturer’s instructions. In general, the manufacturer’s instructions shall be used when preparing media from dehydrated media.

Variations in the end-product may occur when preparing media, due to poorly standardized routines (weighing, reconstruction, variable water quality, heat treatment, pH calibration, etc). It is therefore essential that the laboratory has well-established, written protocols for both preparation routines and control methods. It should be emphasized that a number of the chemicals and dehydrated media used contain harmful substances, and the protocols should include information, where necessary, on the use of protective equipment.
All equipment used during preparation of media (balances, volumetric equipment, autoclaves, dry sterilizing equipment) shall be adequately calibrated and controlled.

Glass equipment shall be clean and undamaged. Borosilicate glass is preferable to soda glass, to avoid alkaline leaching from the glass into solutions/media.

If metal containers are used in the preparation of media, they shall be made of stainless steel, to avoid the addition of metal ions, e.g. alumina, to the media.

Balances used in weighing dehydrated culture media shall be accurate to ± 0.1 g. It may be necessary to use balances of greater accuracy when preparing media from individual components.

Distilled or deionized water shall be used. It shall be free from any growth-stimulating or – inhibiting substances. Water shall be stored in containers made from inert materials (neutral glass, polyethylene, etc) which shall also be free of inhibitory substances. The use of freshly-distilled or –deionized water with a pH of 6.5-7.5 is recommended. Water which has been standing over a longer period may have become acidic, due to the presence of atmospheric CO₂. Tap water shall not be used, due to the risk of heavy metal content, which may inhibit growth or cause precipitation in the media. Bacterial content and conductivity of the water used shall be controlled. The maximum permissible level of bacteria is 50 CFU/ml (cultured at 22 ± 1°C). Conductivity should be less than 0.5 mS/m.

3.4 Handling of ready-to-use and laboratory prepared culture media.

Prepared culture media should, in general, be stored at 2-8°C, in the dark and protected from desiccation. Certain media, e.g. those with a high content of bile salts, should be stored at a higher temperature (10-15 °C). Purchased ready-to-use media shall be stored in accordance with the manufacturer’s instructions.

All batches of prepared media shall be labelled with the expiry date. Documentation of the laboratory’s accepted storage limit shall be available. Storage limits for purchased ready-to-use media are normally provided by the manufacturer. Storage limits for prepared media varies, and it is difficult to generalize any time limit for storage. Documentation of storage limits for media prepared within the laboratory may be found in the following publications:

- Standard methods
- Production manuals from manufacturers of dehydrated culture media
- ISO 7218
- ISO/TS 11133 – part 1
- GLP handbooks (eg NMKL report no. 5, 1994)
- DIN – Taschenbuch, 222

If the laboratory is unable to document the storage limits currently in use, they may need to be validated.
Re-melting of media should be done as gently as possible, to avoid over-heating. The media should then be cooled as rapidly as possible to 45 ± 2°C. Media should not be maintained at this temperature more than 4 hours after cooling.

4.  CONTROL METHODS.

In general, all data from the controls described below should be recorded.

On receipt, purchased ready-to-use media should be controlled as follows:

- Control of packaging (e.g. physical damage, labelling for storage conditions)
- Physical control of the medium
- Inspection of accompanying certificates of analysis
- Evaluation of other controls the laboratory may carry out

Every batch of medium prepared in the laboratory shall be controlled. Minimum 1% of all production units shall be randomly sampled and controlled for all the parameters described below. Samples shall be taken at different sites from larger batches, and as a minimum, from the batch’s first and last units.

In the event of unexpected results, a fault-finding process should be initiated. All factors, which potentially may have affected the prepared medium, should be evaluated (weighing, pH, autoclaving, addition of supplements, etc). Hints in fault-finding may be found in Appendix 2 (Possible errors in preparation of microbiological culture media). Batches, which fail to satisfy the laboratory’s demands, shall be discarded immediately.

4.1  Physical appearance.

Each batch of culture medium prepared in the laboratory shall be controlled with respect to:

- Colour
- Gel strength
- Homogeneity
- Surface

Colour, homogeneity and surface are inspected visually. Gel strength may be controlled by applying light pressure to the surface using an inoculating loop or glass rod, and should be documented as normal/not normal. In the event of deviations, the whole batch should be discarded.

4.2  pH value.

pH shall be controlled in every batch of culture medium prepared in the laboratory. A small amount should be sampled after autoclaving, and pH measured once the temperature of the medium has stabilized at 20-25°C. Some manufacturers of dehydrated media recommend that
the medium should stand for 12-18 hours prior to pH measurement, but in practice, it has been shown that 0.5-2 hours are sufficient (unpublished data from National Veterinary and Food Research Institute (EELA) and Norwegian Institute for Food and Environmental Analysis). Both immersion and surface electrodes may be used to measure pH.

Maximum permitted deviation from the stipulated value is 0.2 pH units, unless otherwise specified in the method.

Adjustment of pH is generally only carried out in media prepared in the laboratory from individual components, and this is most easily done prior to autoclaving. In addition, the pH shall be controlled after autoclaving. If media are prepared from commercially available dehydrated culture media, and the water and equipment are of satisfactory quality, normally pH should not need to be adjusted. If the pH has to be adjusted, it should be carried out aseptically, using 1M NaOH or 1M HCl.

4.3 Sterility.

Petri dishes, flasks or test tubes containing the culture medium are tested for sterility by incubation using the time and temperature normally used as standard in the laboratory.

In the event of growth, the whole batch is discarded.

4.4 Volume.

In situations requiring an accurate volume, routine control of volume shall be carried out. Examples include the use of diluents in flasks or tubes, and certain culture media used in the control of residual antibiotics. Due to evaporation during autoclaving, flasks or tubes shall contain the appropriate volume, plus the expected volume lost to evaporation.

The control is carried out by weighing a given number of labelled flasks or tubes containing liquid, both before and after autoclaving. The final volume is then calculated, taking into account the density of the liquid (volume = weight/density). Peptone water (0.85% NaCl + 1% peptone) weighs, for example 1.01 g/ml.

Where the liquid is dispensed into flasks or tubes after autoclaving, the containers are first weighed empty, and then again after the liquid has been dispensed.

Maximum permitted deviation is 2%.

4.5 Growth performance.

Growth performance of culture media consists of qualitative and/or quantitative control. In order to carry out this type of control, the laboratory requires a set of reference strains. In accredited laboratories, these reference strains must be traceable to recognized culture
collections. Reference strains must be stored and handled in such a manner that their characteristics do not alter (Appendix 1).

Qualitative control shall be carried out on every batch of culture medium that the laboratory purchases or prepares. This applies to all the media used in the laboratory. Control may be carried out on receipt, production or analysis series.

Quantitative control shall be carried out at a minimum after the following criteria:

- **Ready-to-use media from ISO-9000-approved manufacturers and media prepared in the laboratory from dehydrated powdered media from ISO-9000-approved manufacturers:**
  Control is carried out when a new type of medium is introduced into the laboratory and when changing to a new manufacturer.

- **Ready-to-use media from non-ISO-9000-approved manufacturers and media prepared in the laboratory from dehydrated powdered media from non-ISO-9000-approved manufacturers:**
  Every new batch of medium/powdered medium is controlled (one defined production lot with the same production no.)

- **Culture media prepared from individual components:**
  Every batch of medium prepared in the laboratory is controlled.

For non-selective media, it is sufficient to carry out the qualitative and quantitative controls using only one reference strain. For selective and indicative culture media, a minimum of one positive and one negative control strain shall be used. The positive strain should be robust and show typical characteristics. The negative strain should show medium to poor growth, or even no growth with the use of strongly selective culture media. For selective and indicative culture media, these strains may also be supplemented by more sensitive and/or biochemically atypical reference strains, which show different fermentation or fluorescence reactions. This applies particularly where several selective/indicative principles are employed in the culture medium. The Working Party on Culture Media (WPCM) of the International Committee for Food Microbiology and Hygiene has proposed a validated reference strain collection for qualitative and quantitative control of culture media (see Corry et al, section 5: References)

### 4.6 Qualitative control.

A given number of dishes are randomly sampled from the batch being controlled. Reference strains as specified in the laboratory’s procedure are streaked out onto the dishes, which are then incubated using the time and temperature normally used as standard in the laboratory. After incubation, the following parameters are recorded:

- Growth of the strains – good/dense, medium, poor
- Colony morphology – appearance and size
- Possible reactions in the media – colour changes, precipitates, haemolysis, etc.
The positive strain should show good, dense growth with characteristic colonies and reactions. The negative strain should show medium to poor growth, or even no growth with strongly selective media.

4.7 Quantitative control methods.

4.7.1 Solid agar media.

This procedure describes four methods for quantitative control:

1. Use of reference material
2. Ecometric method
3. Modified streaking method
4. Modified Miles and Misra method

The ecometric method and the modified streaking method are semi-quantitative. Both these methods are sensitive to operator differences: two technicians carrying out the procedure in parallel can obtain differing results. It is therefore essential that the laboratory has established their own standard routine for these methods. Technicians with little or no experience should not carry out controls using these methods.

The following general points apply to all the methods:

- If quantitative control is being carried out on a new medium, a non-selective medium should also be used, as a reference medium. Tryptone Soy Agar or blood agar may be used for this purpose.
- Reference medium (non-selective agar) should be used for the three first times a culture medium is controlled. After this, later results are compared with previous data.
- If a known type of medium but from a new manufacturer is being controlled, the earlier known medium is used as a reference medium.
- At each control point, the media being controlled should be of the same age, i.e. the time elapsed from preparation to control.
- Petri dishes of 9 cm diameter are used for all four methods.

4.7.1.1 Reference materials (reference samples).

Reference materials or reference samples, containing known quantities of microorganisms, may be used for quantitative control. A number of suppliers are to be found, supplying both certified and non-certified material, as both pure and mixed cultures.

Certificates/instructions supplied with the reference materials should, as a minimum, include the following information:

- Manufacturer’s name and address
- Packaging information
- Physical form
- Storage conditions and limits
• Measurement results with the associated measurement uncertainty
• Information on estimation of the measurement uncertainty
• Information on the certification process
• Methods used during certification
• Number of accepted measurements for the estimation of the certified value and measurement uncertainty

Procedure:

1. The medium of interest is prepared in accordance with the laboratory’s protocol and poured into dishes when cooled to a suitable temperature. The dishes are allowed to set.

2. The reference material or reference samples are homogenized in accordance with the supplier’s instructions. Their dilutions are selected on the basis of the materials’ given concentrations. 0.1 ml of the selected dilutions are sown onto the dishes, and the cultures spread over the surface using sterile glass or plastic rods.

3. The dishes are incubated using the time and temperature normally used as standard in the laboratory.

Reading and interpretation of results:

Following incubation, the number and morphology of the colonies is registered.

It is essential that limit values are established, on the basis of the information supplied with the material and the laboratory’s own results. It is important that these results are followed up, using control chart or similar system, such that deviations can be rapidly detected.

4.7.1.2 Ecometric method

Evaluation of productivity is based on an absolute growth index (AGI).

Procedure:

4. Fresh pure cultures of the reference strains are prepared on a non-selective agar, e.g. Tryptone Soy Agar or blood agar. Incubation at 37 ± 1°C for 24 ± 3 hours, or other conditions if necessary.

5. After incubation, 10 ml non-selective broth is inoculated with the reference strains. Tryptone Soy Broth or Brain Heart Infusion Broth may be used. Incubation at 37 ± 1°C for 24 ± 3 hours, or other conditions if necessary.

6. The medium of interest is prepared in accordance with the laboratory’s protocol and poured into dishes when cooled to a suitable temperature. The dishes are allowed to set.
7. The reference strains in broth culture are carefully mixed, and sown using a 1 µm inoculating loop, dipping only the loop of the inoculating loop into the culture, ensuring that it is filled with culture. Excess culture is removed by touching the side of the tube gently three times.

8. Randomly selected dishes are streaked out as shown in fig.1. Each dish is divided into four quadrants, and five parallel lines are streaked in each quadrant, in the order given. Finally, one line is streaked in the centre. The inoculating loop should not be changed or flamed between the streaks. As the streaks are drawn, the angle between the inoculating loop and agar surface should be 20-30°.

9. The dishes are incubated using the time and temperature normally used as standard in the laboratory.

*Figure 1: Ecometric method.*

*Reading of results:*

Following incubation, the growth intensity and colony morphology are determined. Growth along a whole line gives weighting of 0.2 points, whereas no growth on a line gives weighting 0 points. Growth in the centre line gives weighting 1 point.
The weighting numbers are summed up for all 21 lines. The maximum possible for the whole dish is 5 points. The sum is the absolute growth index, and is documented together with additional relevant information, such as colony morphology (typical/atypical) etc.

**Interpretation of results:**

In general, a culture medium is approved when the AGI is within the following limits:

- **Non-selective substrates**
  - AGI $\geq 3.0 \quad 1)$

- **Selective substrates**
  - Positive strains
    - AGI $\geq 2.5 \quad 2)$
  - Negative strains
    - AGI $\leq 2.0 \quad 3)$

1) 80% of the strains AGI shall not < 4.0
2) 80% of the strains AGI shall not < 3.0
3) 80% of the strains AGI shall not > 1.0

### 4.7.1.3 Modified streaking method.

Evaluation of productivity and selectivity is based on an absolute growth index. The streaking technique used resembles that normally used in bacterial seeding. The size and turbidity of the inoculum must be standardized to allow comparison of results obtained at different testings.

**Procedure:**

1. Fresh pure cultures of the reference strains are prepared on a non-selective agar, e.g. Tryptone Soy Agar or blood agar. Incubation at $37 \pm 1^\circ C$ for $24 \pm 3$ hours, or other conditions if necessary.

2. After incubation, 10 ml non-selective broth is inoculated with the reference strains. Tryptone Soy Broth or Brain Heart Infusion Broth may be used. The inocula should be small. One colony may be used to inoculate in strains producing small colonies. If the bacteria produce larger colonies, part of a colony may be used. Incubation at $37 \pm 1^\circ C$ for 5 hours, or other conditions if necessary.

3. The medium of interest is prepared in accordance with the laboratory’s protocol and poured into dishes when cooled to a suitable temperature. The dishes are allowed to set.

4. The reference strains in broth culture are carefully mixed, and sown using a 1 µm inoculating loop, dipping only the loop of the inoculating loop into the culture, and ensuring that it is filled with culture. Excess culture is removed by touching the side of the tube gently.

5. Randomly selected dishes are streaked out as shown in fig.2. Four parallel lines are drawn in sector A, 0.5 cm apart from one another. Use a fresh inoculating loop (or flame)
for the remaining sectors. Sectors B and C are drawn as sector A. Sector D is drawn as a continuous line.

6. The dishes are incubated using the time and temperature normally used as standard in the laboratory.

Figure 2: Modified streaking method.

Reading of results:

Following incubation, the growth intensity and colony morphology are determined. Growth along a whole line gives weighting of 1 point, whereas no growth on a line gives weighting 0 points. Growth in only half of a line gives weighting 0.5 points.

The weighting numbers are summed up for all 16 lines. The maximum possible for the whole dish is 16 points. The sum is the absolute growth index, and is documented together with additional relevant information, such as colony morphology (typical/atypical) etc.

Interpretation of results:

The growth index for a positive strain is normally 6 or higher. The method is semi-quantitative, and therefore the growth index will only be indicative, and will vary relative to the culture medium being tested. The growth index is compared with earlier data, to check that variation is not too great. A medium’s variation can be determined when the laboratory has measured a sufficient number of growth indices. The morphology of the reference strains should be typical for the medium of interest.

The growth of a negative reference strain should be partially or completely inhibited in selective culture media.
4.7.1.4 Modified Miles and Misra method.

1. Fresh pure cultures of the reference strains are prepared on a non-selective agar, e.g. Tryptone Soy Agar or blood agar. Incubation at 37 ± 1°C for 24 ± 3 hours, or other conditions if necessary.

2. After incubation, 10 ml non-selective broth is inoculated with the reference strains. Tryptone Soy Broth or Brain Heart Infusion Broth may be used. Incubation at 37 ± 1°C for 18 ± 2 hours, or other conditions if necessary.

3. The medium of interest is prepared in accordance with the laboratory’s protocol and poured into dishes when cooled to a suitable temperature. The dishes are allowed to set.

4. The reference strains in broth culture are carefully mixed, and a tenfold serial dilution to $10^{-8}$ is prepared.

5. Randomly selected dishes are sown as shown in fig. 3. A minimum of two replicates from the reference strains should be sown. A calibrated dropper is used, to apply one drop in each quadrant, starting with the highest dilution and using the same pipette. A sterile inoculating loop, or sterile glass or plastic rod is used to spread the drops.

6. The dishes are incubated using the time and temperature normally used as standard in the laboratory.

Figure 3: Modified Miles and Misra
Reading of results:

Following incubation, the number and morphology of the colonies is determined. Counting is done in the quadrant with the lowest dilution that gives easily read colonies. The productivity factor is calculated using the following formula: \( PR = \frac{N_8}{N_0} \) where \( N_0 \) is the mean number of colonies counted on the reference medium, and \( N_8 \) is the mean number of colonies counted on the medium of interest. The number of colonies and productivity factor are documented together with additional relevant information, such as colony morphology (typical/atypical) etc.

Interpretation of results:

In general, a culture medium is approved when the PF is within the following limits:

<table>
<thead>
<tr>
<th>Substrates</th>
<th>PF Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-selective</td>
<td>( \geq 0.7 )</td>
</tr>
<tr>
<td>Selective</td>
<td></td>
</tr>
<tr>
<td>Positive strains</td>
<td>( \geq 0.5 ) 1)</td>
</tr>
<tr>
<td>Negative strains</td>
<td>( \leq 0.00001 ) 2)</td>
</tr>
</tbody>
</table>

1) 80% of the strains PF shall not < 0.3  
2) 80% of the strains PF shall not > 0.00001

4.7.2 Liquid media

Liquid culture media can be analysed using several different dilution methods. Either the MPN value can be determined, or the highest dilution permitting growth in the medium of interest, and these values are compared with the reference medium.

As in the case solid agar media, it is necessary to include reference media only when introducing a new type of broth, or when changing to a new manufacturer. The results are later compared with earlier data for the same type of medium.

Procedure

1. Fresh pure cultures of the reference strains are prepared on a non-selective agar, e.g. Tryptone Soy Agar or blood agar. Incubation at 37 ± 1°C for 24 ± 3 hours, or other conditions if necessary.

2. After incubation, 10 ml non-selective is inoculated with the reference strains. Tryptone Soy Broth or Brain Heart Infusion Broth may be used. Incubation at 37 ± 1°C for 18 ± 2 hours, or other conditions if necessary.

3. The medium of interest is prepared in accordance with the laboratory’s protocol, dispensed into 9 ml test tubes and allowed to cool to a suitable temperature.
4. The reference strains in broth culture are carefully mixed, and a tenfold serial dilution to $10^{-12}$ is prepared.

5. 5 tubes of the medium of interest and 5 tubes of reference medium are inoculated with each dilution of the reference strains, in a volume of 1 ml.

6. The tubes are incubated using the time and temperature normally used as standard in the laboratory.

Microtitre plates may be used instead of tubes, dilution being carried out directly in the culture medium after dispensing into the microtitre plate. 150 µl culture medium per well is sufficient to achieve growth.

**Reading of results:**

Following incubation, the number of tubes with growth is counted, and the MPN value determined (Appendix 3). The productivity factor is calculated using the following formula:

$$PR = \frac{N_{12}}{N_0}$$

where $N_0$ is the MPN value for the reference medium, and $N_{12}$ is the MPN value for the medium of interest. The number of positive tubes and productivity factor are documented together with additional relevant information, such as colour change in the medium (typical/atypical) etc.

In cases where the MPN value cannot be used, the highest dilution permitting growth may be noted, and used to calculate the productivity factor.

**Interpretation of results:**

A culture medium is approved when the PF lies is within the limits defined in the Modified Miles and Misra method (4.7.1.4)
5 REFERENCES.

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Modified striking method in performance testing of microbiological culture media – experiences of the National Veterinary and Food Research Institute (EELA)  
Finnish Veterinary Journal 101:700-703
APPENDIX 1: Use of reference strains (reference cultures)

REFERENCE STRAINS
(from recognized culture collections)
Control of:
purity
biochemistry, serology, etc.

REFERENCE STOCKS
(freeze-dried, -70°C or similar)
Control of:
purity
biochemistry, serology, etc.

WORKING STOCKS
Cultured on non-selective medium.
Storage time dependent on the organism. Recommended storage of storage-tolerant organisms for max. 3-4 weeks under optimal conditions. May be sub-cultured for a defined number of passages. Recommended max. 3 passages.

Control of purity on use (inspection)

REGULAR/DAILY QUALITY CONTROL

The laboratory shall have procedures for the handling and storage of reference strains. In addition, all stages of the process should be recorded.
APPENDIX 2: Possible errors in preparation of microbiological culture media

1. Lumps in the dehydrated medium
   - The dehydrated medium is too old
   - The dehydrated medium has been incorrectly stored (exposed to humidity)
   - The package has not been sealed properly
   - The package has been open for too long

2. The pH deviates from the stipulated value
   - The dehydrated medium is too old
   - The package has not been sealed properly
   - Error in weighing the dehydrated medium or other ingredients
   - Poor water quality
   - Chemical contamination
   - The medium has been exposed to incorrect heat treatment
   - pH has been measured at too high temperature
   - The pH-meter has been incorrectly calibrated

3. The medium contains a precipitate
   - Poor water quality
   - Error in weighing the dehydrated medium or other ingredients
   - The pH of the medium is incorrect
   - The equipment used was not clean
   - The medium has been exposed to incorrect heat treatment

4. The medium sets at too high temperature
   - Error in weighing the dehydrated medium or other ingredients
   - The agar quality has deteriorated

5. The gel strength of the medium is too low
   - Error in weighing the dehydrated medium or other ingredients
   - The agar quality has deteriorated
   - The dehydrated medium/agar is not completely dissolved
   - The medium has been exposed to incorrect heat treatment
   - The pH of the medium is incorrect (low pH may cause acid hydrolysis)
   - The medium was not stirred sufficiently before dispensing

6. The medium has the wrong colour
   - Error in weighing the dehydrated medium or other ingredients
   - The pH of the medium is incorrect
   - The medium has been exposed to incorrect heat treatment
   - The equipment used was not clean
   - Poor water quality

7. The medium is contaminated
8. Poor growth
- Growth-inhibiting substances from water/equipment used in preparation
- Error in weighing the dehydrated medium or other ingredients
- The pH of the medium is incorrect
- The medium has been exposed to incorrect heat treatment
- The agar temperature was too high on addition of the culture
- The prepared medium was too old
- The prepared medium has been incorrectly stored
- The culture conditions were incorrect

9. Excessive growth
- The medium has been exposed to incorrect heat treatment
- Error in weighing the dehydrated medium or other ingredients
- The prepared medium has been incorrectly stored
- The culture conditions were incorrect

10. The colonies are spreading out
- The agar surface is too wet
- The volume used in sowing was too high
- The medium has been exposed to incorrect heat treatment

11. Atypical growth
- The dehydrated medium is too old
- The medium has been incorrectly prepared
- The prepared medium is too old
- The prepared medium has been incorrectly stored
- The culture conditions were incorrect
### APPENDIX 3: Selected MPN values and 95% confidence intervals

<table>
<thead>
<tr>
<th>Positive tubes</th>
<th>MPN/g</th>
<th>95% confidence intervals</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>1.0</td>
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<td>0</td>
<td>2</td>
<td>3.7</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>5.5</td>
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- **MPN/g**: Most Probable Number per gram
- **95% confidence intervals** are calculated based on the number of positive tubes.
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