Perhitungan Bakteri dg Haemacytometer dan MPN

Penggunaan Haemacytometer

Menggunakan haemacytometer atau Petroff Houser Counter

- Misal Diket Huas Kotak pada haemacytometer = 4 X 10^2 mm² Tinggi = 2 X 10^2 mm
- Maka volume daerah kotak = 8 X 10^-4 mm³ = 8 X 10^-7 ml
- Jumlah sel dihitung 14 Maka jumlah mikrobia

\[
\text{Number of bacteria/ml} = \frac{\text{number of cells counted}}{\text{volume of area counted}}
\]

\[
\frac{14}{8 \times 10^{-7}} = 17,500,000
\]
Methods to Detect Coliforms

- **MPN**
  - Based on Probability Statistics
  - NOT a direct Count
  - Liquid Technique

- **Solid**
  - Direct Count on Media

Most Probable Number (MPN)

- Begin with LST Broth
- **Dilution**
  - 1 - 2 - 3 - 4 - 5 - 6
- 3 Tubes/Dilution
- 1 ml of Each Dilution into Each Tube
- **Figure 1**: Three-tube design for MPN (unincubated)
- **Figure 2**: Three-tube design for MPN (incubated)
- Durum Tube Detects Gas Production
- POSITIVE TUBES Have GROWTH and GAS PRODUCTION
MPN - Continued

- Recording Results
  -1 -2 -3 -4 -5 -6
  3 3 2 1 0 0 - # of (+) Tubes
- Objective is to "DILUTE OUT" the organism
- Select Most Dilute Sample with All Positive and Take Next Two
  - 3,2,1 – Our Combination
- Determine MPN Using MPN Table

MPN

- Calculations
  - If 3, 2,1 Then Result is: 150/ml or g
  - This is IF we used the the –1, -2, -3 Dilution
  - WE used the –2,-3,-4 Dilutions so we must multiply by 10
  - OUR RESULT
    - 1500 cfu/g or ml
    - 1.5 x 10^3 cfu/g or ml

MPN

- If none of the tubes have 3 positive, take most dilute with a positive and take next two ABOVE it
- Examples
  - -1 -2 -3 -4 -5 -6
  - a. 3 3 3 3 1 1
  - b. 2 2 1 0 0 0
  - c. 3 3 2 1 1 0 ?????

MPN

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MPN

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- Examples
  - -1 -2 -3 -4 -5 -6
  - a. 3 3 3 3 1 1
  - b. 2 2 1 0 0 0
  - c. 3 3 2 1 1 0 ?????
MPN

- For C, use 3 2 2
  - There is a tube that has a positive result “below” the –4 tube (most dilute tube used in the result)
  - If a tube that is more dilute than the tubes in the 3 tube combination has a positive result then combine that number with the last tube of the 3 tube combination!!

Contoh lain

<table>
<thead>
<tr>
<th>Sample quantities (g or ml)</th>
<th>Reported positive values</th>
<th>MPN estimate/g or ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>a</td>
<td>3/3</td>
<td>1/3</td>
</tr>
<tr>
<td>b</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>d</td>
<td>3/3</td>
<td>3/3</td>
</tr>
</tbody>
</table>

MPN

- Also can do a 5 Tube MPN – Increases Accuracy
- ACCURACY – How close to true values
- PRECISION – How close numbers are to each other
- Be sure you are using appropriate TABLE
Contoh tabel MPN untuk seri pengenceran

Dengan jumlah inkulum 10 mL, 1 mL dan 0,1 mL

<table>
<thead>
<tr>
<th>Combination of Positives</th>
<th>MPN Index per g (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-0-0</td>
<td>0.21</td>
</tr>
<tr>
<td>3-0-1</td>
<td>0.34</td>
</tr>
<tr>
<td>3-0-2</td>
<td>0.64</td>
</tr>
<tr>
<td>3-1-0</td>
<td>0.41</td>
</tr>
<tr>
<td>3-1-1</td>
<td>0.75</td>
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<td>1.2</td>
</tr>
<tr>
<td>3-2-0</td>
<td>1.6</td>
</tr>
<tr>
<td>3-2-1</td>
<td>0.91</td>
</tr>
<tr>
<td>3-2-2</td>
<td>1.5</td>
</tr>
<tr>
<td>3-2-3</td>
<td>2.1</td>
</tr>
<tr>
<td>3-3-0</td>
<td>2.9</td>
</tr>
<tr>
<td>3-3-1</td>
<td>4.6</td>
</tr>
<tr>
<td>3-3-2</td>
<td>11.</td>
</tr>
<tr>
<td>3-3-3</td>
<td>&gt;11.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Combination of Positives</th>
<th>MPN Index per g (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-0-0</td>
<td>0.092</td>
</tr>
<tr>
<td>2-0-1</td>
<td>0.14</td>
</tr>
<tr>
<td>2-0-2</td>
<td>0.02</td>
</tr>
<tr>
<td>2-1-0</td>
<td>0.15</td>
</tr>
<tr>
<td>2-1-1</td>
<td>0.20</td>
</tr>
<tr>
<td>2-1-2</td>
<td>0.27</td>
</tr>
<tr>
<td>2-2-0</td>
<td>0.21</td>
</tr>
<tr>
<td>2-2-1</td>
<td>0.28</td>
</tr>
<tr>
<td>2-2-2</td>
<td>0.35</td>
</tr>
<tr>
<td>2-3-0</td>
<td>0.29</td>
</tr>
<tr>
<td>2-3-1</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Contoh tabel MPN untuk seri pengenceran

Dengan jumlah inkulum 1 mL, 0,1 mL dan 0,01 mL

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<thead>
<tr>
<th>Combination of Positives</th>
<th>MPN Index per g (ml)</th>
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<tbody>
<tr>
<td>1-0-0</td>
<td>0.036</td>
</tr>
<tr>
<td>1-0-1</td>
<td>0.072</td>
</tr>
<tr>
<td>1-0-2</td>
<td>0.11</td>
</tr>
<tr>
<td>1-1-0</td>
<td>0.074</td>
</tr>
<tr>
<td>1-1-1</td>
<td>0.11</td>
</tr>
<tr>
<td>1-2-0</td>
<td>0.11</td>
</tr>
<tr>
<td>1-2-1</td>
<td>0.15</td>
</tr>
<tr>
<td>1-3-0</td>
<td>0.16</td>
</tr>
<tr>
<td>0-0-0</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>0-0-1</td>
<td>0.030</td>
</tr>
<tr>
<td>0-1-0</td>
<td>0.030</td>
</tr>
<tr>
<td>0-1-1</td>
<td>0.061</td>
</tr>
<tr>
<td>0-2-0</td>
<td>0.062</td>
</tr>
<tr>
<td>0-3-0</td>
<td>0.094</td>
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## Combination of MPN Index per g (ml)

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<tbody>
<tr>
<td>2-0-0</td>
<td>0.92</td>
</tr>
<tr>
<td>2-0-1</td>
<td>1.4</td>
</tr>
<tr>
<td>2-0-2</td>
<td>2.0</td>
</tr>
<tr>
<td>2-1-0</td>
<td>1.5</td>
</tr>
<tr>
<td>2-1-1</td>
<td>2.0</td>
</tr>
<tr>
<td>2-1-2</td>
<td>2.7</td>
</tr>
<tr>
<td>2-2-0</td>
<td>2.1</td>
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<td>2-2-1</td>
<td>2.8</td>
</tr>
<tr>
<td>2-2-2</td>
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</tr>
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<td>3-1-1</td>
<td>7.5</td>
</tr>
<tr>
<td>3-1-2</td>
<td>12.</td>
</tr>
<tr>
<td>3-1-3</td>
<td>16.</td>
</tr>
<tr>
<td>3-2-0</td>
<td>9.3</td>
</tr>
<tr>
<td>3-2-1</td>
<td>15.</td>
</tr>
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<td>21.</td>
</tr>
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<td>29.</td>
</tr>
<tr>
<td>3-3-0</td>
<td>24.</td>
</tr>
<tr>
<td>3-3-1</td>
<td>46.</td>
</tr>
<tr>
<td>3-3-2</td>
<td>110.</td>
</tr>
<tr>
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<td>&gt;110.</td>
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## Positive Tubes

<table>
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<tr>
<th>Positive Tubes</th>
<th>MPN</th>
<th>95% Confidence Range</th>
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Assumptions of MPN

- Random Distribution of Bacteria
- Organisms do not occur in clusters
- If microorganism is present, it will grow
- Proper conditions for growth exist

Advantages over Direct Plating

- Larger Sample Sizes can be used
- Better for low numbers
- Liquid is less damaging than heat
- Prepare Media ahead of time

MPN Summary

- LST
- LST + MUG
- Gas Positive Tubes
- Fecal Coliforms
- Pres. MPN Coliforms
- BGB
- Confirmed Coliforms
- EC
- Long Wave UV for Fluorescent +
- E. coli MPN
**Media - LST**

- Lauryl Sulfate Tryptose Broth
- PRESUMPTIVE test for coliforms
  - Report as presumptive coliforms/g

**Ingredients**
- Lauryl Sulfate – Wetting agent and some bacteria don't do well - Selective
- Lactose – Gas Production – Differential w/durum tube
- NaCl – Increase Reproduction Rate
- Tryptone – Nitrogen Source
- Incubate at 35 C for 48 hours and read

**Media – EC Broth**

- E. coli Broth
- Test for FECAL COLIFORMS
- Incubate in Water Bath
  - 44 C +/- 2 C
  - 24 hours

**Ingredients**
- Lactose – Gas Production
- Bile Salts – Coliforms are Bile Tolerant
  - Bile inhibits Gram + cocci and spore formers

**Media - BGLB**

- Brilliant Green Lactose Bile
- Incubate 35 C for 48 hours
- Gas Production – Positive

**Ingredients**
- Oxgall – form of bile
- Brilliant Green Dye – Inhibits Gram +

**Results are Confirmed Coliforms**
- Don’t go Directly into BGLB – Too Inhibitory
Media – EMB Agar
- Eosine Methylene Bule Agar
- Pre-poured plates
- Streak for isolation from MPN
- Ingredients - Differential
  - Peptone – Protein
  - Lactose – Acid Production
  - Eosine Dye – Inhibitory
  - Methylene Blue – pH Indicator
- E. coli and Enterobacter
  - Nucleated, dark centered, with or without a metallic green sheen – CONFIRMED COLIFORMS

Media – MPN
- Standard Plate Count (SPC) agar slants
  - Streak to get pure colony for IMViC
- IMViC
  - I – Indole
  - M – Methyl Red
  - V – Vogues-Proskauer
  - C – Citrate

IMViC Media
- Tryptone Broth
  - Production of Indole from Tryptophane
  - Enzyme is Tryptophanase
  - KOVAC’s Reagent
    - P-dimethylamino benzaldehyde
    - If Indole is present it will be +
    - Test after 24 h at 35 C
IMViC Media

- **MR-VP Broth**
  - Methyl Red – Vogues-Proskaur
    - 48 h at 35 – VP Test
    - 72 at 35 – Methyl Red
  - V-P
    - Glucose, Acetymethycarbinol
    - 1 ml + 0.6 ml alpha-napthol solution and 0.2 ml KOH
    - Pink - Positive

- **IMViC Media**
  - MR Test
    - Test
      - Glucose, Acid
      - Acid is Yellow
    - Add 5 drops of MR Indicator

- **Citrate Broth**
  - Koser’s Cirtate
  - Test
    - Ability to use citrate as sole C Source
  - 98 Hours at 35 C
  - If Turbid - Positive
Typical IMViC Reactions

- **E. coli**
  - ++- or -++

- **Enterobacter aerogenes**
  - -++

Distinguishes Enterobacter from E. coli

Solid Method

- **VRBA**
  - Violet Red Bile Agar
    - Peptone – Nitrogen Source
    - Lactose – Acid Production
    - Neutral Red – Indicator (6.8 red, 8.0 yellow)
    - Crystal Violet – Inhibits Gram +
    - NaCl – Increase Growth Rate
    - Bile Salts

VRBA

- Typical – Purplish-Red, Elliptical Colonies, Might be a Zone of Precipitate
- Countable Plates 10-100 – Larger Colonies
- Overlay Agar after first layer solidifies
- Results – Presumptive Coliforms

---

5/21/2012
E. Coli and Coliforms

- **MUG**
  - Methylumbeliferyl beta-D-glucuronide (MUG)
  - MUG → 4-methylumbellifurone
    - Beta-glucuronidase** *
    - Fluorescent at 366 nm
  - **Most E. coli produce this enzyme**
    - NOT O157:H7

MUG

- **Uses**
  - LST Broth
    - Presumptive E. coli – Gas and Fluorescence
    - Presumptive Coliforms – Gas and NO Fluorescence
  - VRBA Agar
    - Fluorescent colonies are presumptive E. coli
  - E. coli Petrifilm
    - Red Colonies and Purple – Total Coliforms
    - Purple Colonies with Gas – Presumptive E. coli

- **Some Salmonella and Shigella can give false Positive**