



waag society

institute for art, science and technology



Microbiological techniques

Biohack Academy #3



Today's Programme

Morning

- Make agar
- Sterilise agar
- Pour plates

Afternoon

- Contamination test
 - Environment
 - Sterile hood



What is agar?

- Yelly substance from algae
- Sugar
- Used for:
 - Food (subtitute gelatin)
 - Microbiology



History of Agar

- Angelina Fanny Hesse
- Walter Hesse
- **Robert Koch**



Koch's Postulates

1. Microorganism causing disease must be found in abundance in all organisms suffering from disease, but not in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture
3. The cultured organism should cause disease when introduced into a healthy organism
4. The microorganism must be reisolated from the inoculation, diseased experimental host and being identical to the original host.



History of Agar

- Angelina Fanny Hesse
- Walter Hesse
- **Robert Koch**
- Julius Richard Petri

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC374482/>

wikipedia



Types of Agar



Nutrient Agar

- Non-selective medium suitable for bacteria
- 1000 mL Demi-water contains (pH ~4.7):

| Compound | Amount (g) |
|---------------|------------|
| Yeast extract | 2.0 |
| Peptone | 5.0 |
| NaCl | 5.0 |
| Agar | 15.0 |

- **Alternatives:**
 - Yeast extract → beef extract
 - Peptone (*amino acid, peptides, proteins*) → peptic digest of animal tissue
 - NaCl (*salt*) → Table salt
 - Agar (*jelling agent / sugar*) → Gelatin



Malt Agar

- Non-selective medium suitable for yeasts, fungi
- 1000 mL Demi-water contains (pH ~5.5):

| Compound | Amount (g) |
|--------------|------------|
| Malt extract | 30.0 |
| Agar | 20.0 |

- **Alternatives:**
 - Malt extract → boil malt in water
 - Agar (jelling agent / sugar) → Gelatin



Setting the pH

- Normal pH: 7.0
- Acid (low pH): 0-7
- Basic (high pH): 7-14

| | Acid | Base |
|-------------------------|--|--|
| <i>What does it do?</i> | Release a proton or hydrogen ion (H ⁺) | Release a proton or hydrogen ion (H ⁺) |
| <i>Chemical</i> | HCL | NaOH |
| <i>Alternatives</i> | Citric Acid | NaHCO ₃ |
| | Aquarium shop (pH/KH minus) | Aquarium shop (pH/KH plus) |



Calculations



Calculations

Nutrient Agar (per 1000 ml)

| Compound | Amount (g) |
|---------------|------------|
| Yeast extract | 2.0 |
| Peptone | 5.0 |
| NaCl | 5.0 |
| Agar | 15.0 |

250 ml

| Compound | Amount (g) |
|---------------|------------|
| Yeast extract | 0.5 |
| Peptone | 1.25 |
| NaCl | 1.25 |
| Agar | 3.75 |



Calculations

Malt agar (per 1000 ml)

| Compound | Amount (g) |
|--------------|------------|
| Malt extract | 30.0 |
| Agar | 20.0 |

250 ml

| Compound | Amount (g) |
|--------------|------------|
| Malt extract | 7.5 |
| Agar | 5 |



Performing the practical



Before you start

1. Tie your hair
2. Wash your hands
3. Put on lab coat
4. Put on glasses
5. Clean your work space with alcohol



Performing the practical

1. Dissolve Agarmix in water
- 2.



Performing the practical

1. Dissolve Agarmix in water
2. Autoclaving for 20 min





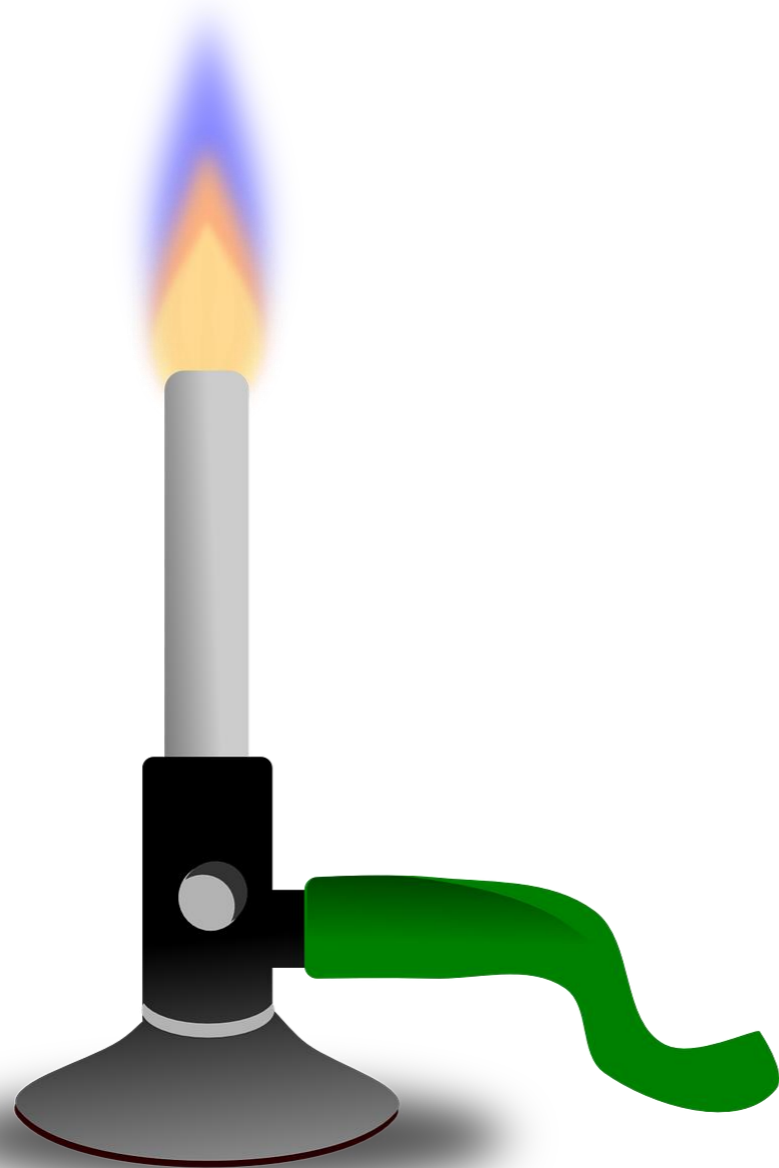
Performing the practical

1. Dissolve Agarmix in water
2. Autoclaving for 20 min
3. Let agar cool down to room temperature



Performing the practical

1. Dissolve Agarmix in water
2. Autoclaving for 20 min
3. Let agar cool down to room temperature
4. Pour the plates



Pixabay - Public Domain



<https://www.youtube.com/watch?v=PiWwnBbCrNs>



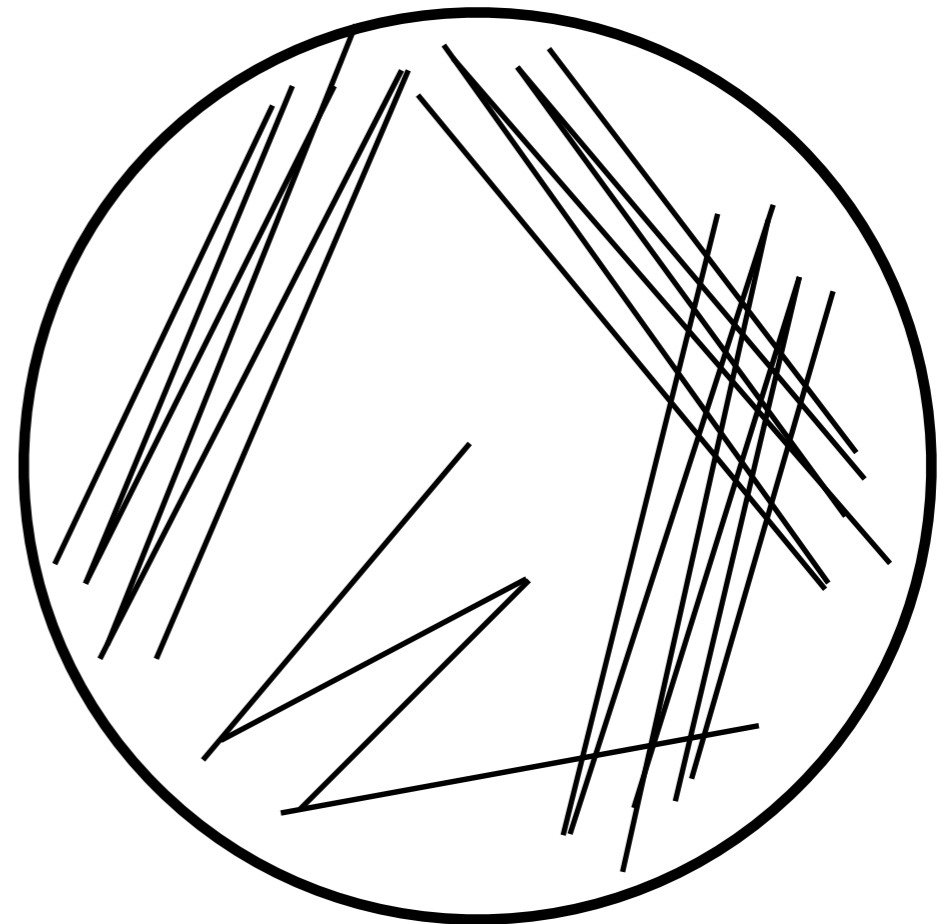
Performing the practical

1. Dissolve Agarmix in water
2. Autoclaving for 20 min
3. Let agar cool down to room temperature
4. Pour the plates
5. Let plates dry



Performing the practical

1. Dissolve Agarmix in water
2. Autoclaving for 20 min
3. Let agar cool down to room temperature
4. Pour the plates
5. Let plates dry
6. Inoculation of plates





Tue 8/3 - Project Meeting (14:30 - 15:30)

1. What is your project idea?
2. Documentation site: show your documentation site
3. Plan for coming week
4. Crazy cool stuff? / Other comments



some

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