

# **TEM Sample Preparation for Life Science**

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# Safety Precautions



**Almost all chemical reagents used in the preparation for EM observation are toxic**

- **Fixatives are poisonous and volatile**
- **Osmium tetroxide vapor is very hazardous to eye and the respiratory system**
- **During preparation and use resins, it is advisable not to breathe the vapor and to avoid spills and contact with skin as they are toxic and some are carcinogenic**
- **Waste resin may be disposed in a closed container in a fume hood and when full can be cured to polymerize in an oven at 70 °C**

# Safety Precautions



**Fume hood**



**PPE**

# TEM vs Light Microscope



Sample Block  
Spurrs Resin



Ultramicrotome



Diamond knife  
50 nm - 70 nm

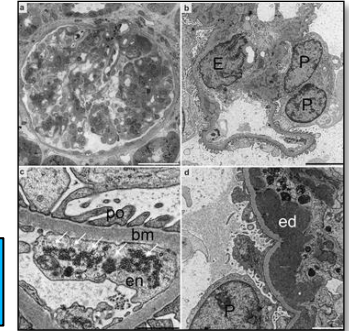


Grid 200Mesh

Electron



Screen



Sample Block  
Paraffin



Microtome  
(1  $\mu\text{m}$  - 5  $\mu\text{m}$ )



Microtome  
Knife

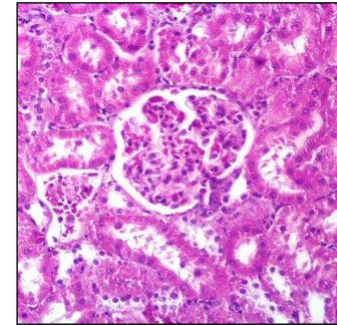


Object glass

Screen

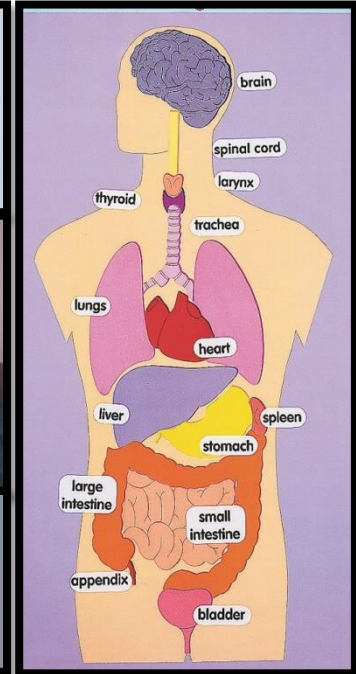
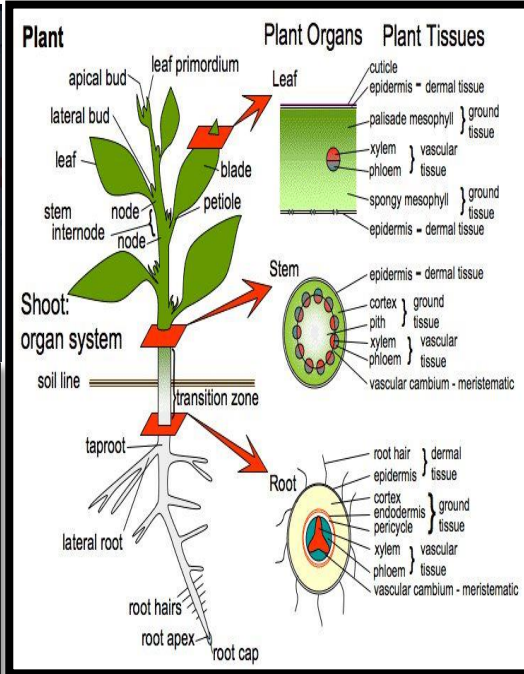


Light



H&E Staining

# Life Science Samples Source



# Materials and Tools

**Glutaraldehyde  
Cacodylate  
Osmium tetroxide  
Ethanol  
Propylene oxide  
Spurrs resin  
Ro Pure**

**Ultra-microtome  
Glass Knife  
Diamond knife  
Grids  
Nano pure  
Object glass  
Toluidine blue**

**Uranyl acetate  
Lead citrate  
Lead nitrate  
Lead acetate  
Sodium citrate  
Crystal NaOH**

# Technique of Preparation

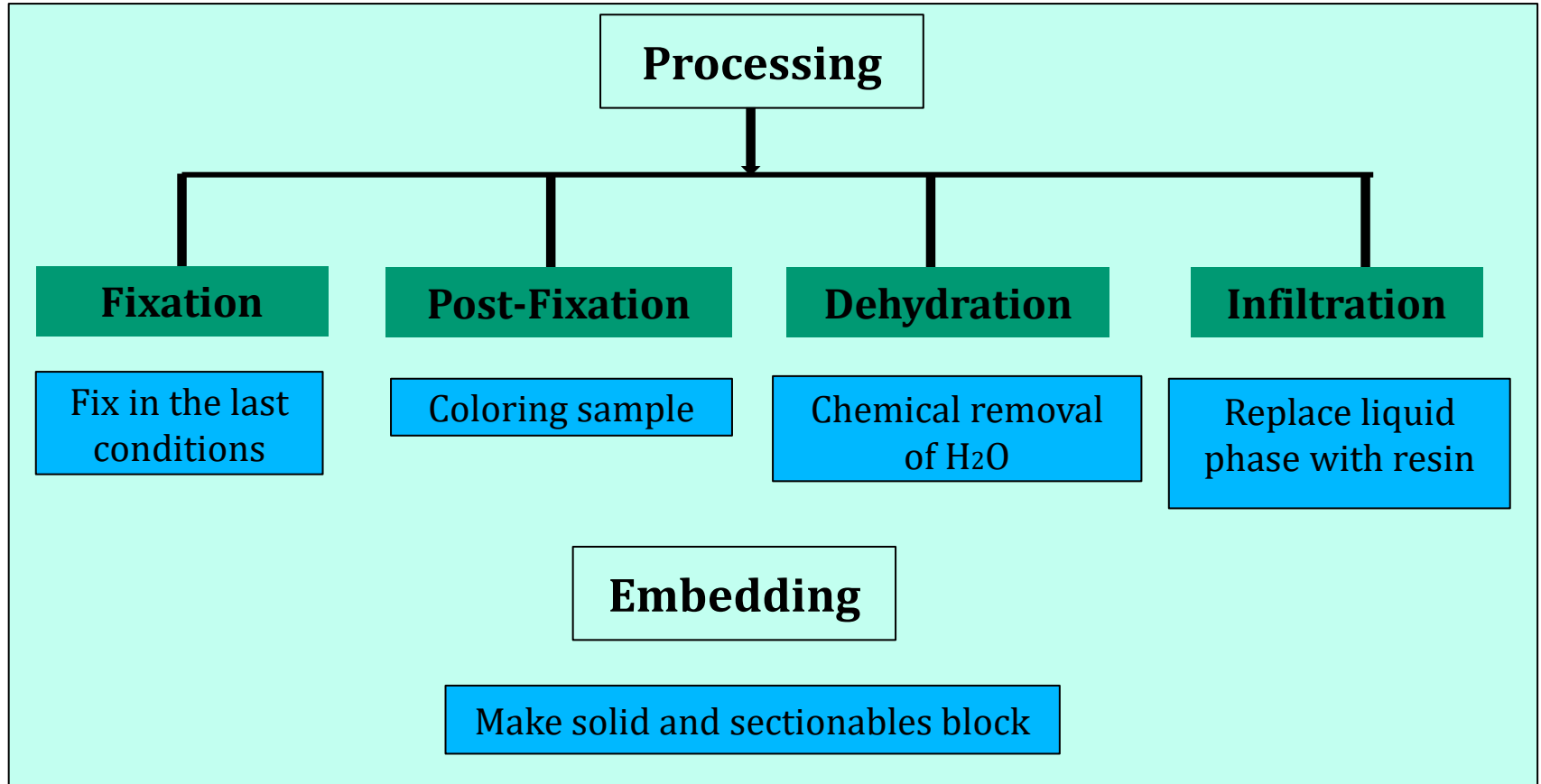
**Room Temperature**

**Negative Staining**

# **Room Temperature**



# Block Sample Preparation



# TEM Sample Preparation

**DAY 1**

**Tissue**

## Fixatif I

2.5% Glutaraldehyde  
in 0,1M Cacodylate  
Buffer

## Rinse 3 times

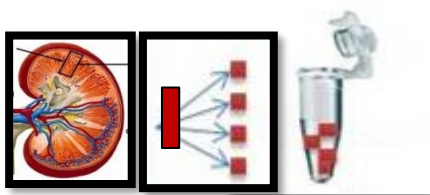
(Cacodylate buffer)  
@15 minutes  
Store at 4°C  
By shaking

## Fixatif II

2% Osmium tetroxide  
+  
2.5 %  $K_3FeC(N)_6$   
In buffer  
Incubate for 2h  
at 4°C by shaking

## Rinse 3 times

(Cacodylate buffer)  
@15 minutes  
store at 4°C  
By shaking



1mm<sup>3</sup>

**Cells ?**

## Centrifuge

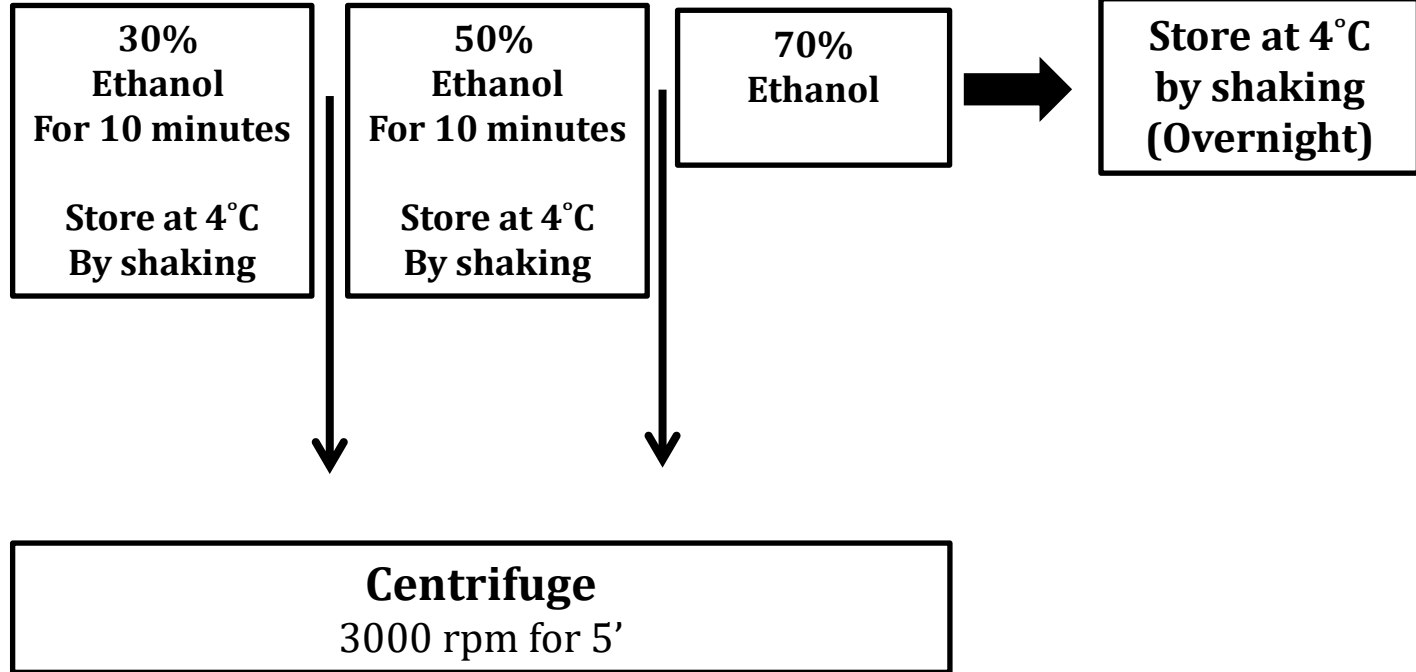
3000 rpm for 5'

# TEM Sample Preparation

**DAY 1**

## Dehydration

**Tissue**



# TEM Sample Preparation

**DAY 2**

## Dehydration

**Tissue**

**80%  
Ethanol  
For 10 minutes**  
  
**Store at 4°C  
By shaking**

**90%  
Ethanol  
For 15 minutes**  
  
**Store at 4°C  
By shaking**

**Ethanol  
Absolute I  
For  
20 minutes**  
  
**Store at 4°C  
By shaking**

**Ethanol  
Absolute II  
For  
20 minutes**  
  
**Store at 4°C  
By shaking**

**Cells**

**Centrifuge**  
3000 rpm for 5'

# TEM Sample Preparation

**DAY 2**

**Infiltration**

**Tissue**

2 Ethanol absolute : 1 Propylene oxide

1 Ethanol absolute : 1 Propylene oxide

1 Ethanol absolute : 2 Propylene oxide

Pure Propylene oxide

**Cells**

Centrifuge  
3000 rpm  
for 5'

@30 minutes at **room temperature** by Shaking



# TEM Sample Preparation

**DAY 2**

**Embedding**



1:1  
P. oxide : Spurr's



Incubate at Room Temperature for 30 minutes  
by shaking



Pour half  
change with Spurr's



Pure Spurr's resin



Vacuum for 1 hour

Vacuum condition for overnight



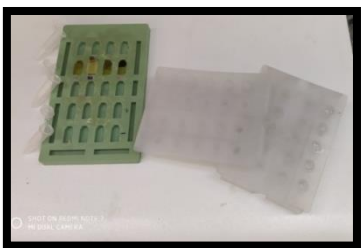
# TEM Sample Preparation

DAY 3



Pour off  
change with new  
Spurr's mixture

Vacuum for 2 hours

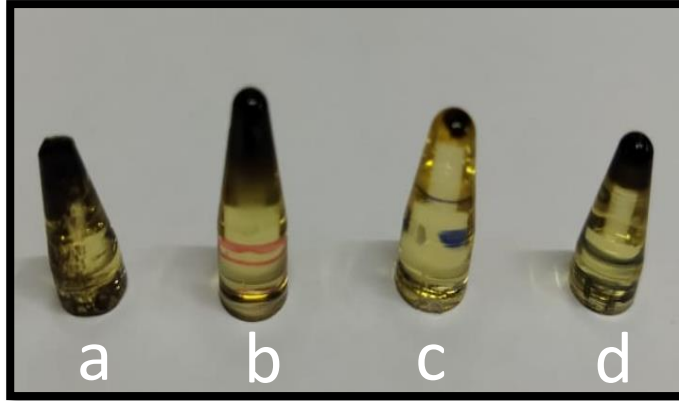


Incubate the sample in 58-70°C for 12-24 hours

# Block Samples



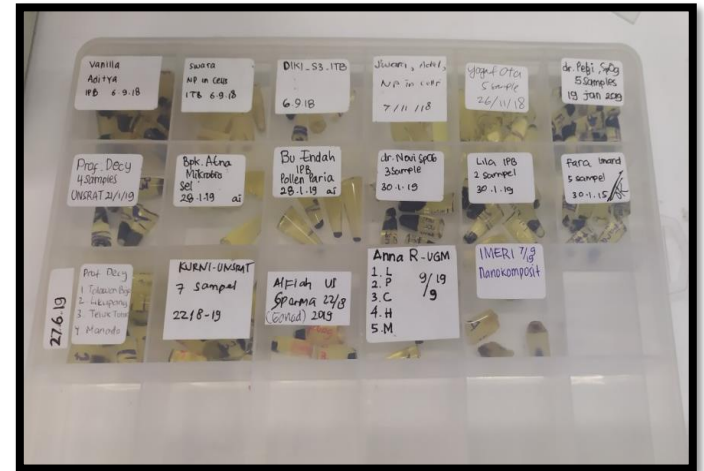
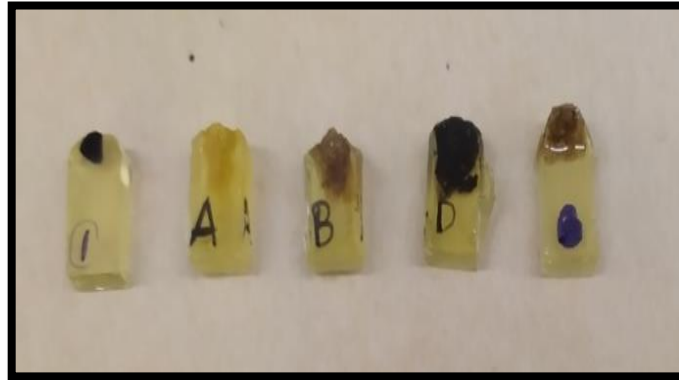
Eppendorf  
500µl



- a. Human heart mitochondria
- b. *Diatome* culture cells
- c. CMF-7
- d. *Streptococcus pneumoniae* cells



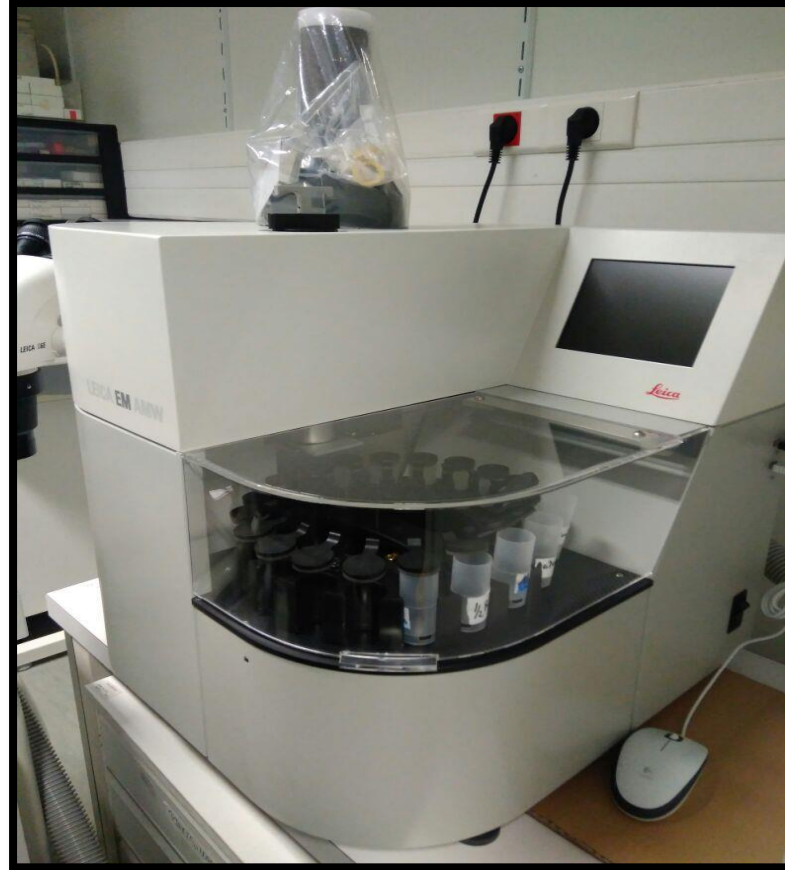
Flat Embedding  
Mold



Vanilla Acitya PB 6-9-18	Swara NP in GCS ITB 6-9-18	DIKI_S3_ITB 6-9-18	Juwana, Nekt NP in GCS 7-11-18	Yogurt OTex 5 sampel 26/11/18	dr. Perti, SpGg 52 sampel 19 Jan 2019
Prof. Dedy 43 sampel UNSRAT 21/1/19	Bpk. Alna Mikrobi Sel 28-1-19 ai	Bu. Indah IPB Pollen Baria 28-1-19 ai	dr. Noni SpCB 3 sampel 30-1-19	Lina IPB 2 sampel 30-1-19	Fara Unswat 5 sampel 30-1-15
Prof. Dedy 1. Taksony 2. Hapung 3. Teuk Tani 4. Manado 27-6-19	KURNI-UNSWAT 7 sampel 22/8-19	AlEidh U SPAMA 27/8 (Gard) 2019	Anna R-UGM 1. B 2. P 3. C 4. H 5. M 9/19 /9	INERI 7/9 Nanocomposit	



# Tissue Processor

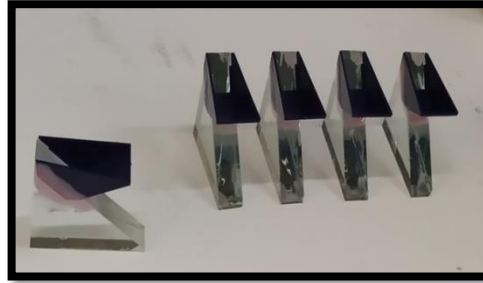


**Pitie Salpetriere Hospital, Paris**

# Trimming and Fast staining



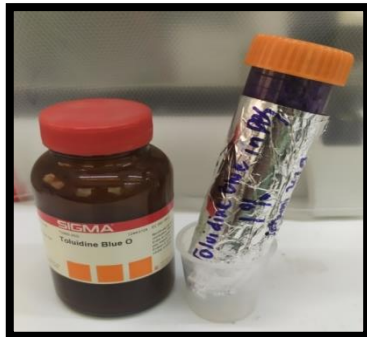
**Ultra-Microtome**



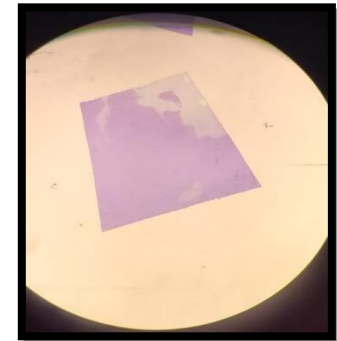
**Glass knife**



**Hot plate**



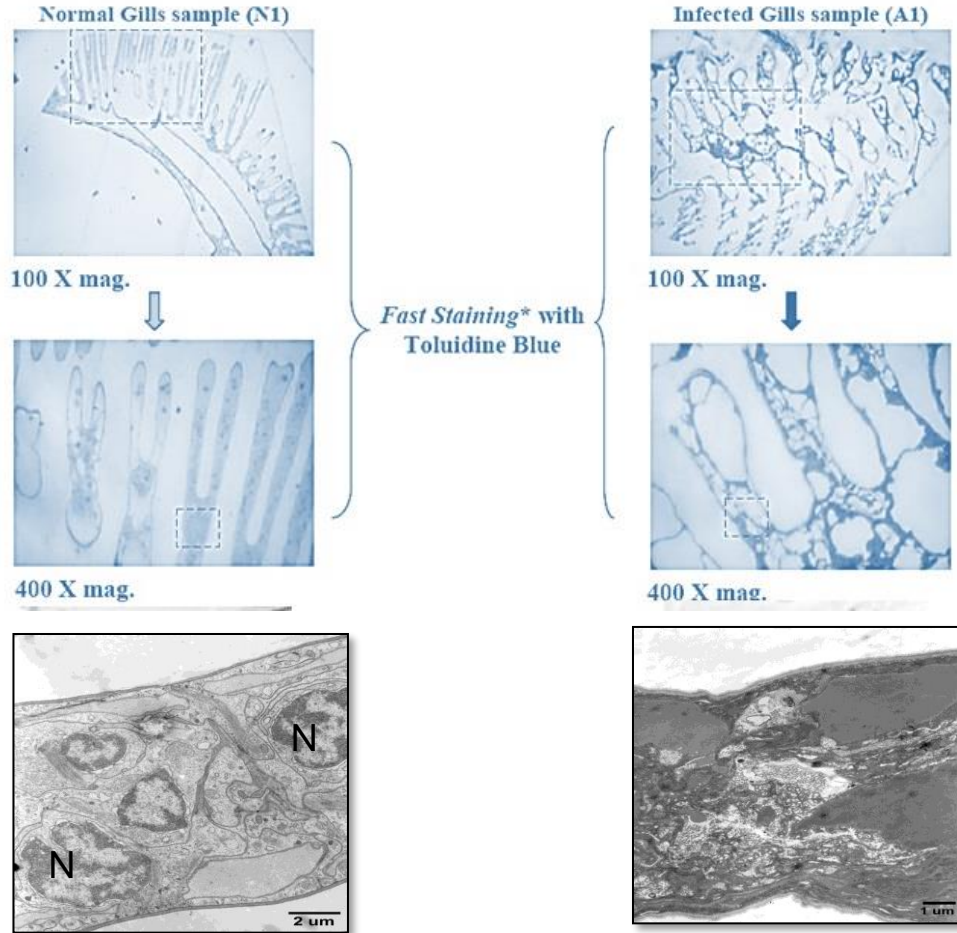
**1% Toluidine blue  
in Phosphat buffer**



**Check the sample area to find target area**

# Fast staining

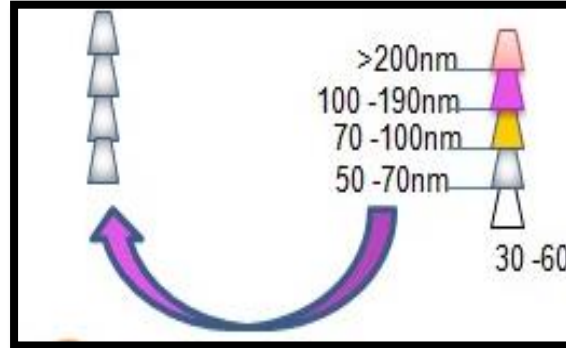
## How to Obtain the TEM Images



# Cutting & Fishing



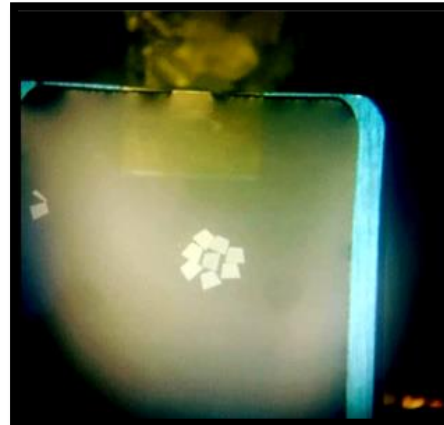
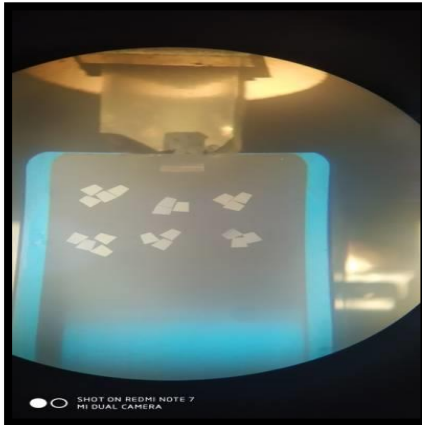
Trimming and cutting



Sample Thickness 50-70nm

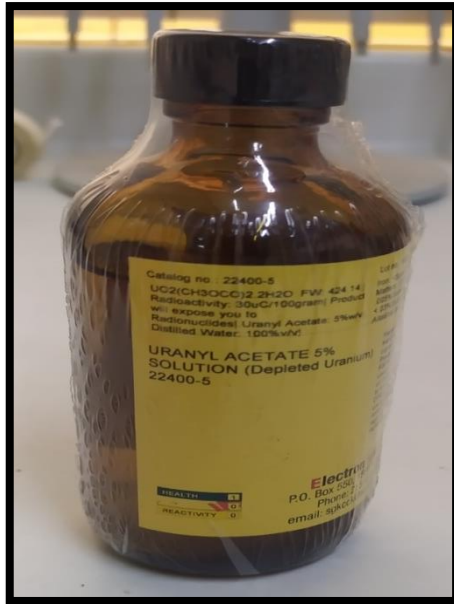


Grid 200 Mesh

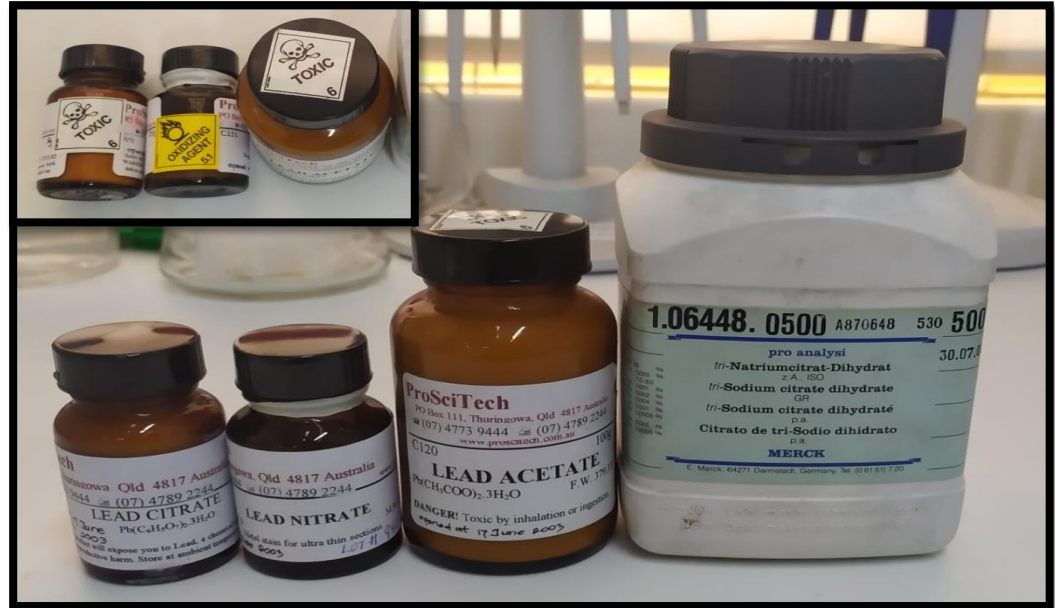


Collecting slices and transferring to the grid (Fishing)

# Staining Sample Grids

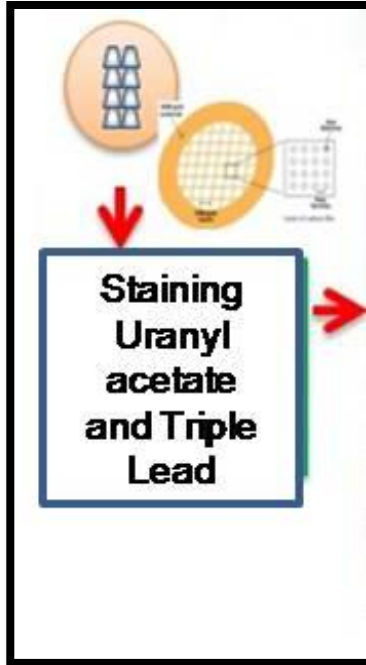


2% Uranyl acetate



Lead citrate, Lead nitrate, Lead acetate and Sodium citrate

# Positive Staining



**Step I : Uranyl acetate 30 minutes**

Washing  
30 dips  
In ddH<sub>2</sub>O

**Step II : Triple lead citrate**

Washing  
5-10 dips  
In 0,01 M NaOH

Washing  
30 dips  
In ddH<sub>2</sub>O

# *Staphylococcus aureus*

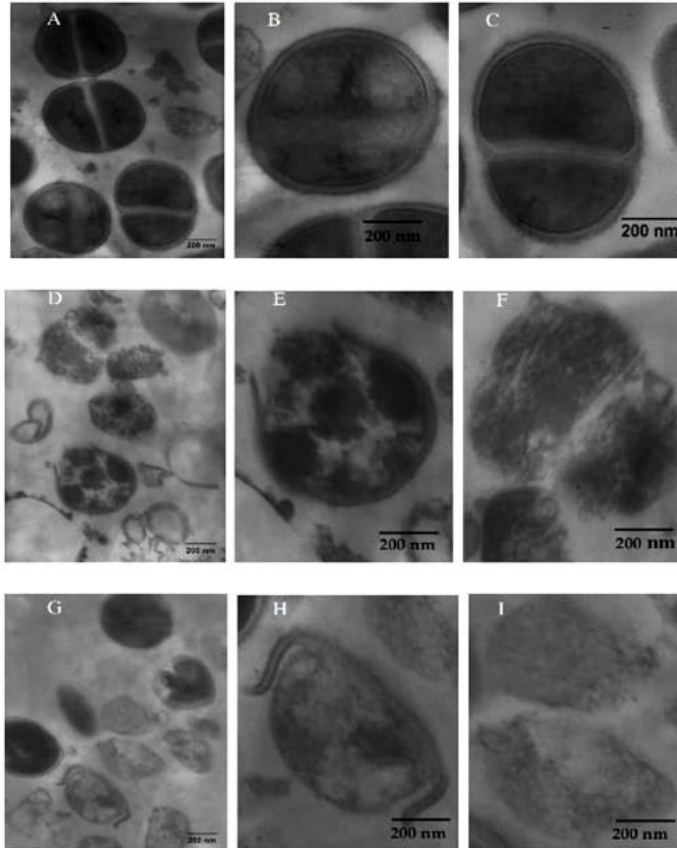
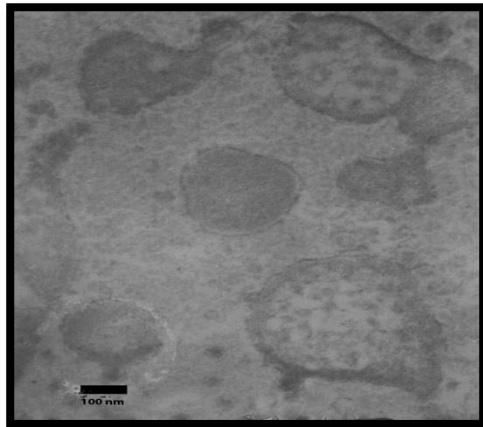
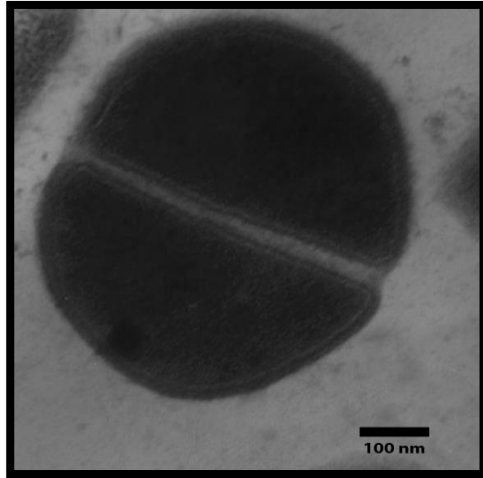
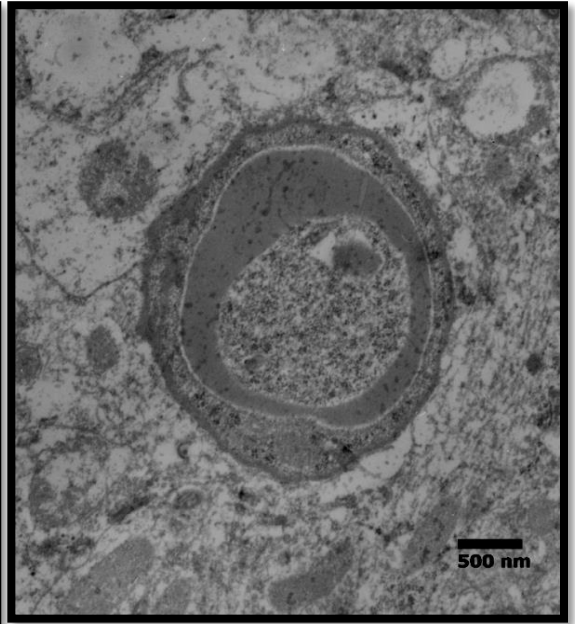
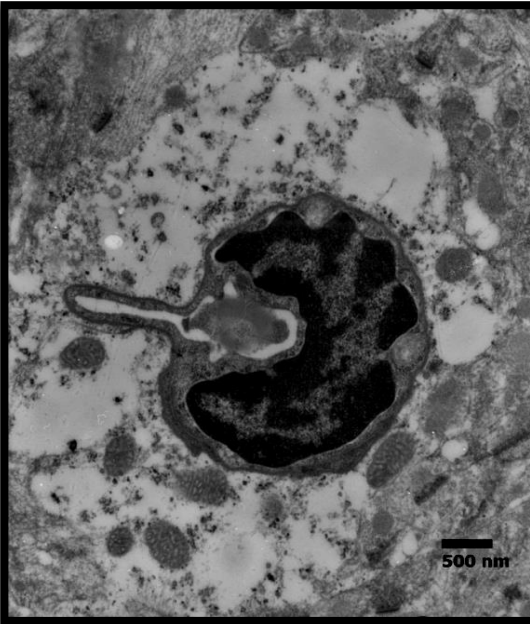
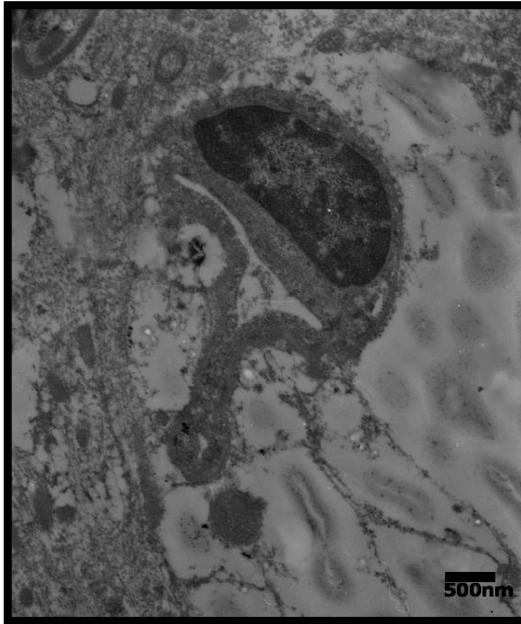


Figure 4. Morphologic observations of *Staphylococcus aureus* with Transmission Electron Microscopy (TEM). (A-C) untreated cell. (D-F) treated cells with 2000µg/mL compound 1. (G-I) treated cells with 8µg/ amoxicillin. They were incubated in Mueller-Hinton broth for 18 h at 37°C

# Mouse Brain

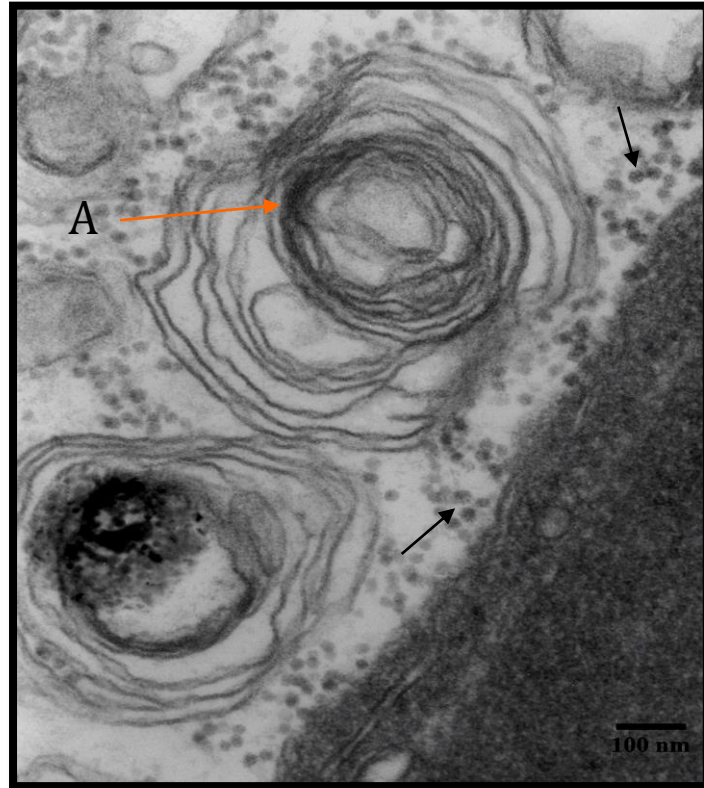
## Cerebral Malaria



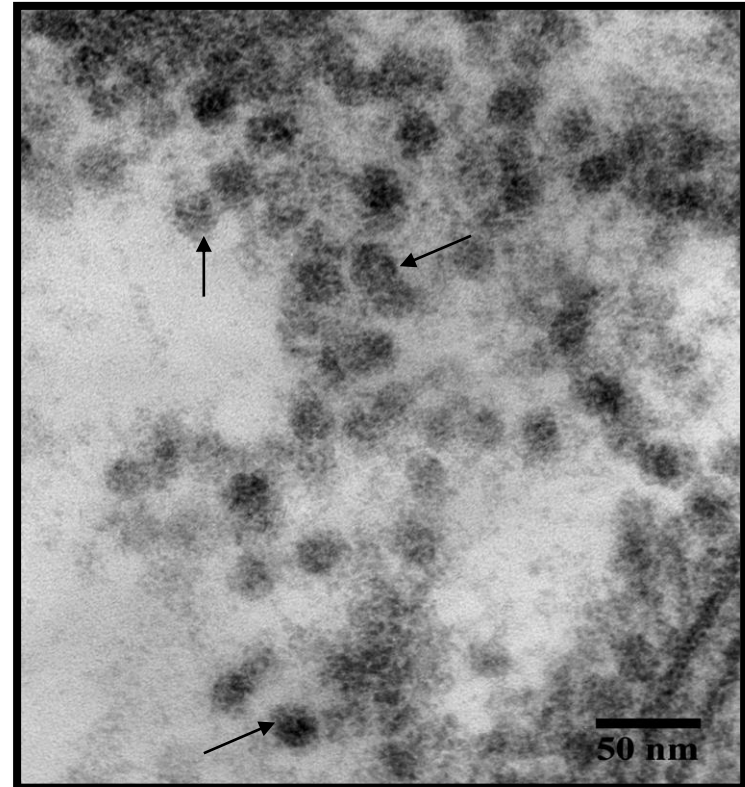


# Infectious Myonecrosis Virus (IMNV)

Shrimp Gill



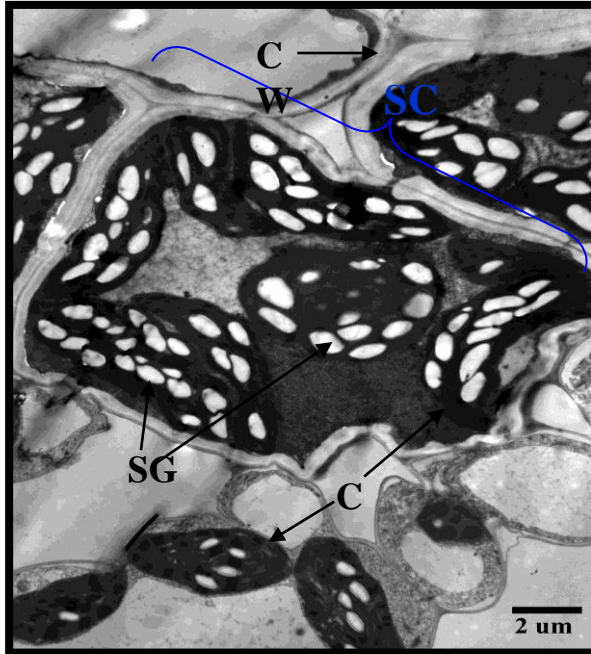
Mag 50.000 X



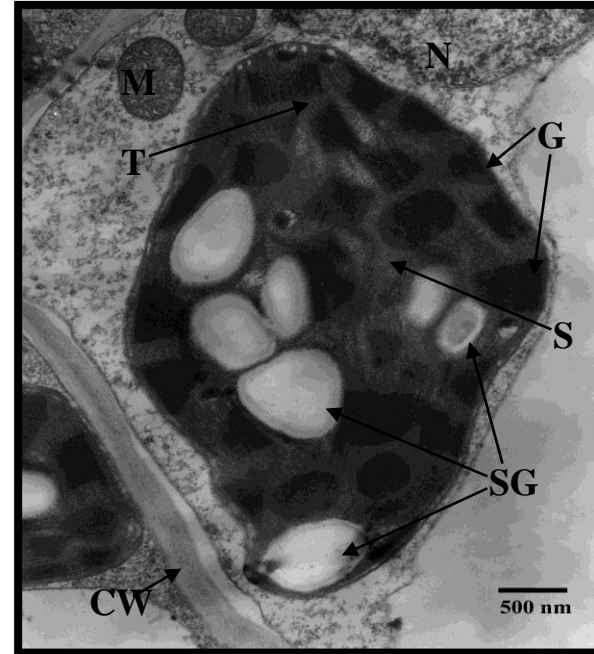
Mag 150.000 X

# Chloroplast

Magnification 3.000X



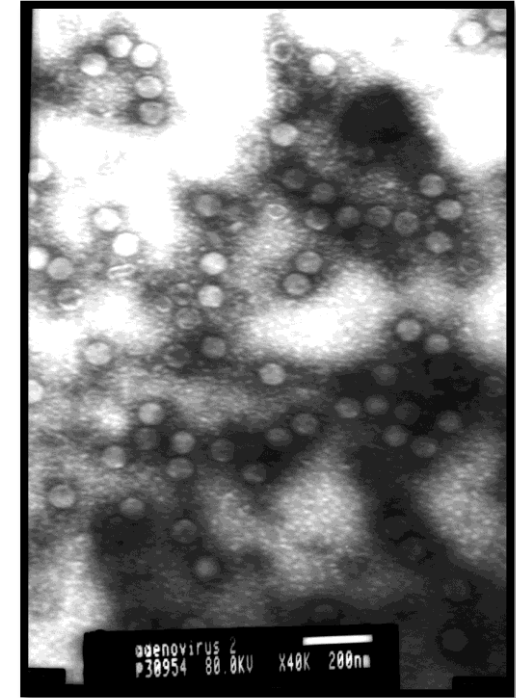
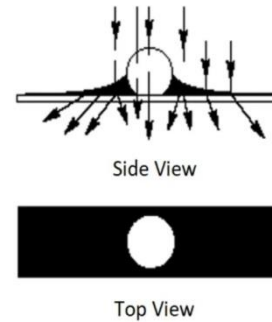
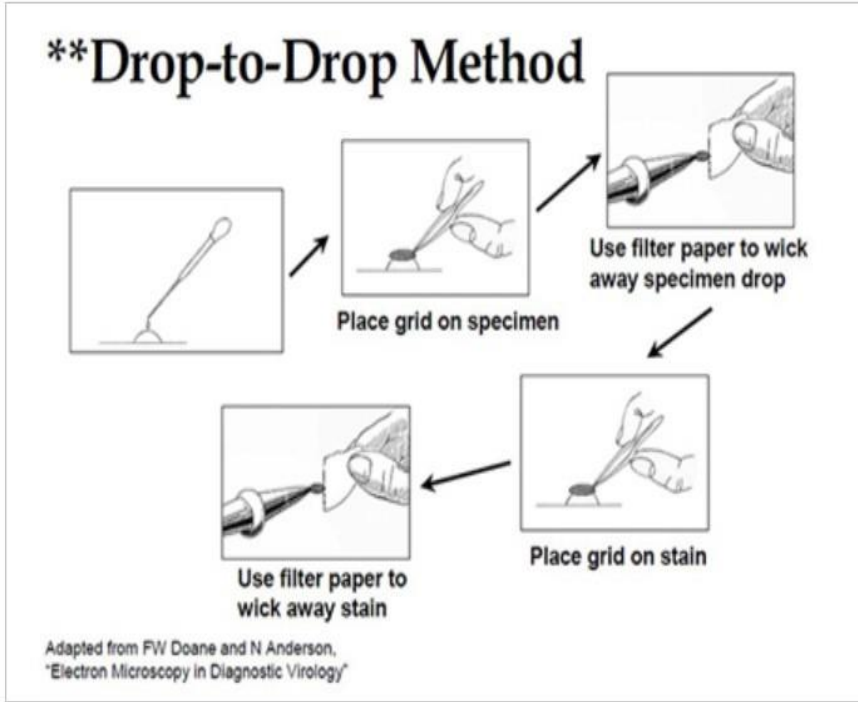
Magnification 12.000X



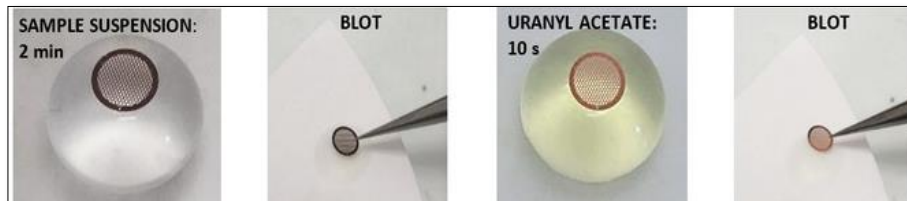
**N:** Nucleus, **C:** Chloroplast, **CW:** Cell Wall, **SC:** Sheath Cell **SG:** Starch Grain, **M:** Mitochondria, **G:** Grana, **T:** Tylakoid Membrane, **S:** Stroma

# **Negative Staining**

# Negative Staining



Adenovirus, Biofarma



## Negative Stain

1. Uranyl acetate
2. Phosphotungstic acid

# Negative vs Positive Staining

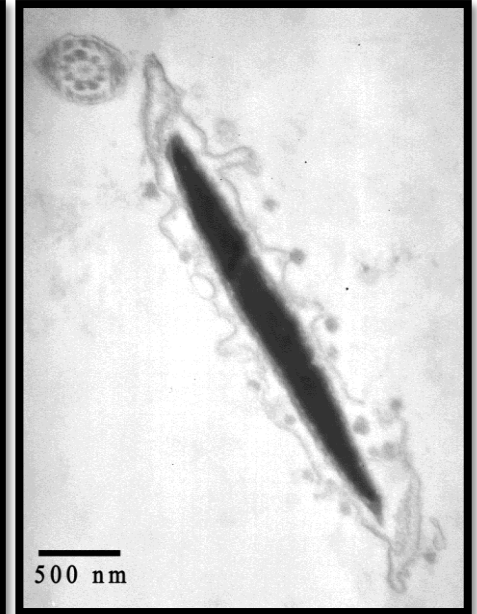
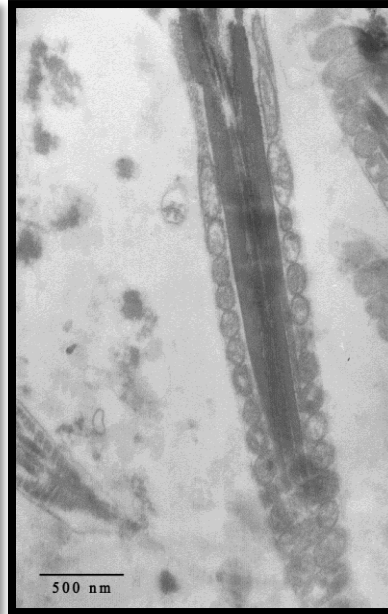
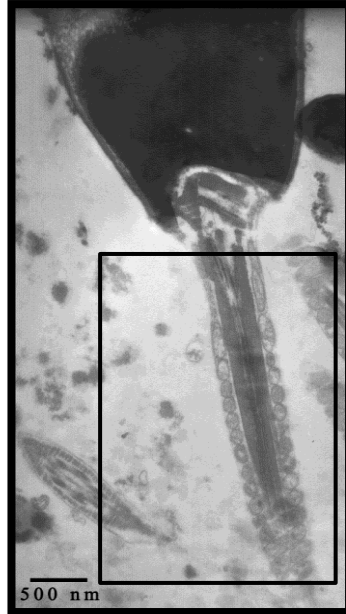
## Negative Staining

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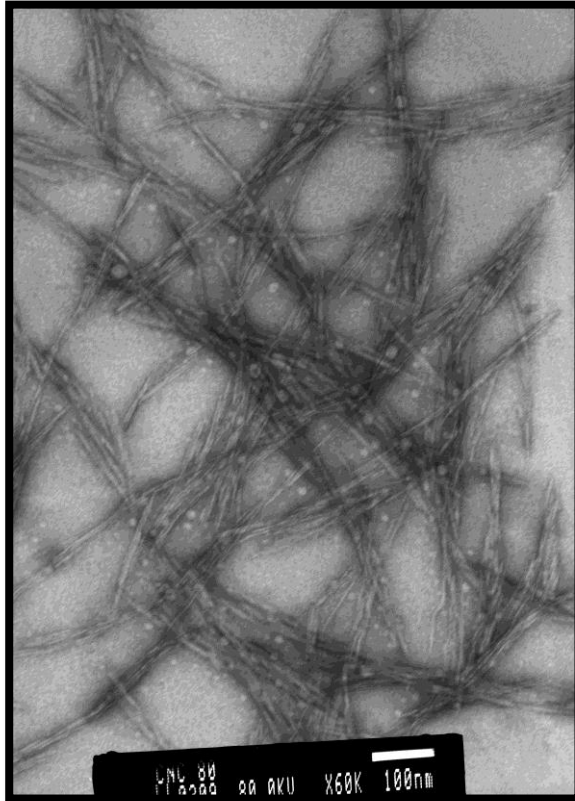
## Positive Staining

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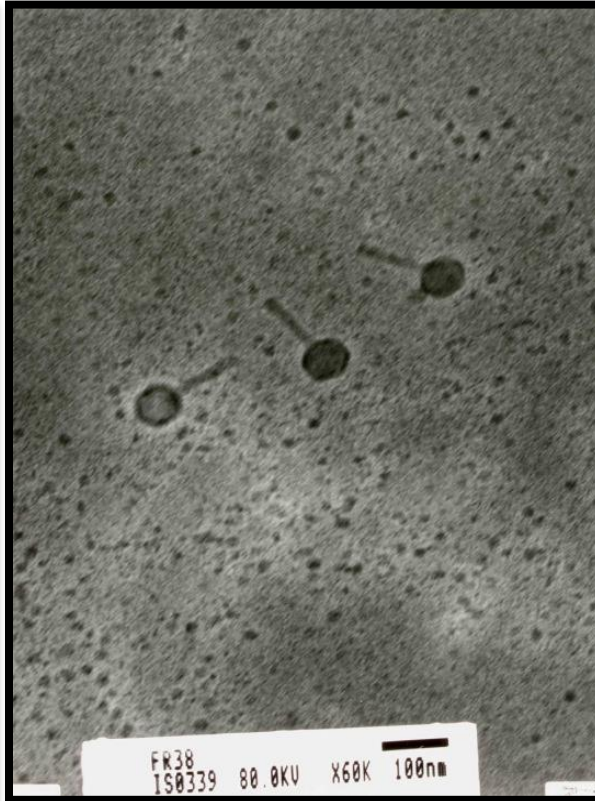
# Negative staining

Nano-cellulose



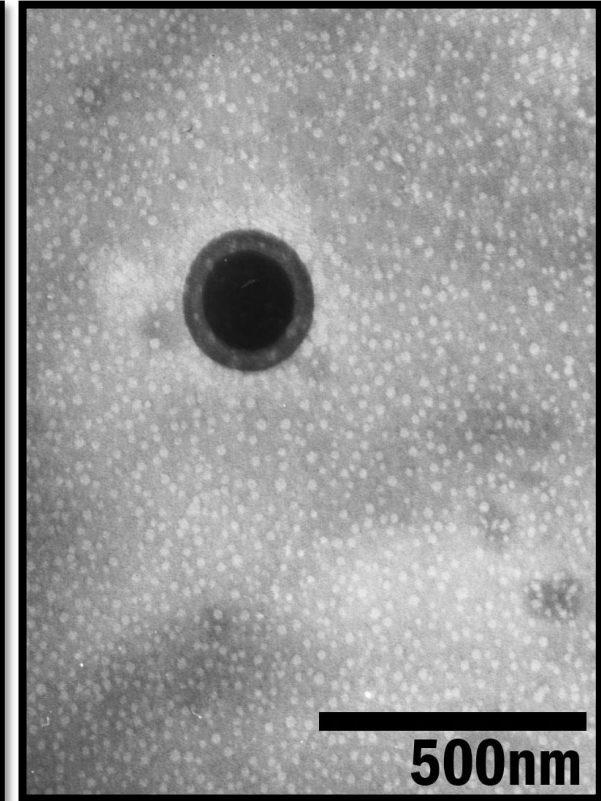
Mag 60.000x

Bacteriophage

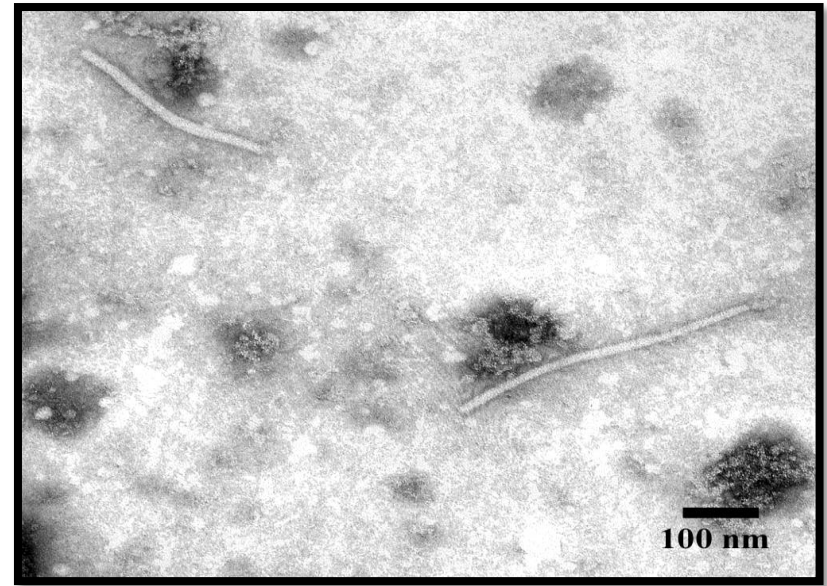
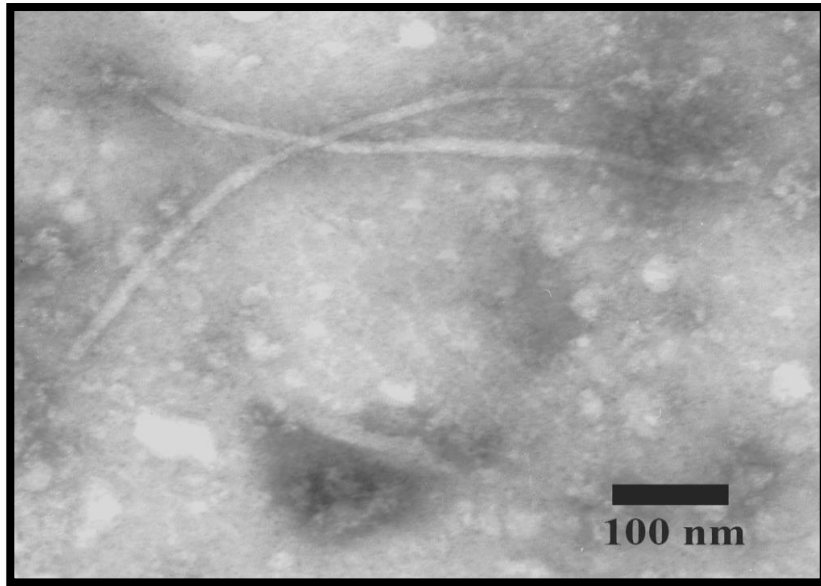


Mag 60.000x

Nano Kitosan



# Sugarcane Streak Mosaic Virus



**Magnification 50.000X**

# **Jurnal Review**



# Human Kidney

Aditjaningsih *et al. BMC Anesthesiology* (2020) 20:37  
<https://doi.org/10.1186/s12871-020-0956-7>

BMC Anesthesiology

RESEARCH ARTICLE

Open Access

Effects of low versus standard pressure pneumoperitoneum on renal syndecan-1 shedding and VEGF receptor-2 expression in living-donor nephrectomy: a randomized controlled study

Dita Aditjaningsih<sup>1,2\*</sup>, Chaidir Arif Mochtar<sup>3</sup>, Aida Lydia<sup>4</sup>, Nuryati Chairani Siregar<sup>5,6</sup>, Nur Ita Margyaningsih<sup>6</sup>, Amir Sjarifuddin Madjid<sup>1</sup> and Suhendro Suwanto<sup>7</sup>

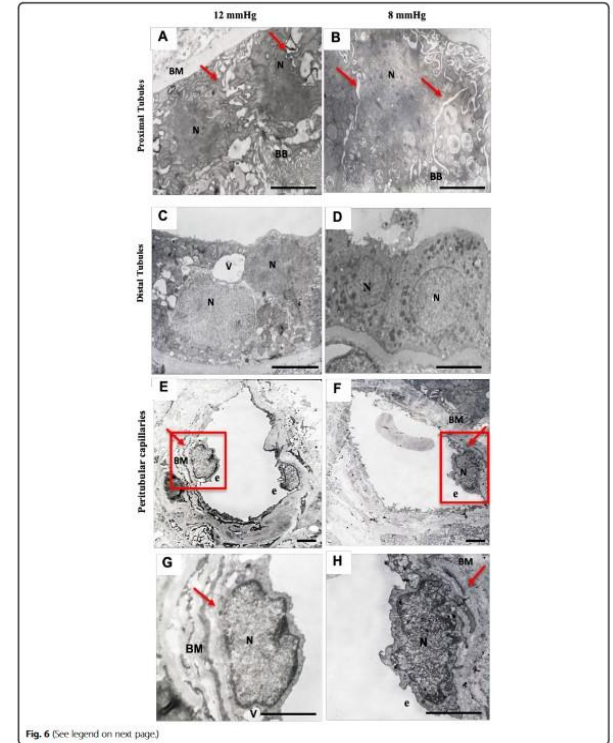


Fig. 6 (See legend on next page.)

# Human Spleen

PLOS MEDICINE

RESEARCH ARTICLE

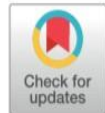
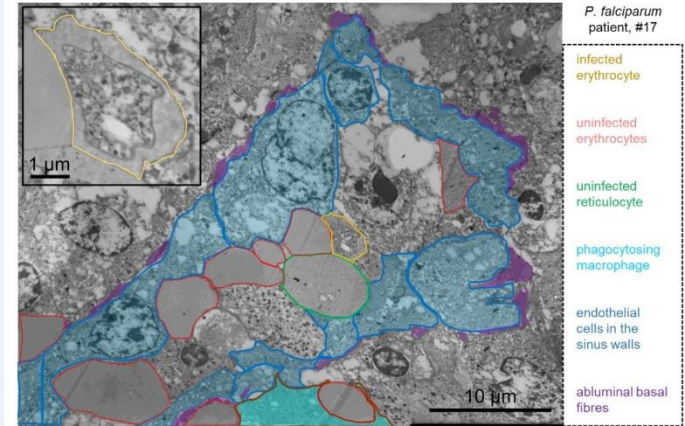
## Evaluation of splenic accumulation and colocalization of immature reticulocytes and *Plasmodium vivax* in asymptomatic malaria: A prospective human splenectomy study

Steven Kho<sup>1</sup>, Labibah Gotrunnada<sup>2</sup>, Leo Leonardo<sup>3</sup>, Benediktus Andries<sup>3</sup>, Putu A. I. Wardani<sup>4</sup>, Aurelie Fricot<sup>5</sup>, Benoit Henry<sup>5</sup>, David Hardy<sup>6</sup>, Nur I. Margyaningsih<sup>2</sup>, Dwi Apriyanti<sup>2</sup>, Agatha M. Puspitasari<sup>2</sup>, Pak Prayoga<sup>3</sup>, Leily Trianty<sup>2</sup>, Enny Kenangalem<sup>3,4</sup>, Fabrice Chretien<sup>6</sup>, Valentine Brousse<sup>5</sup>, Innocent Safeukui<sup>7</sup>, Hernando A. del Portillo<sup>8,9,10</sup>, Carmen Fernandez-Becerra<sup>8,9</sup>, Elamaran Meibalan<sup>11,12</sup>, Matthias Marti<sup>11,13</sup>, Ric N. Price<sup>1,14,15</sup>, Tonia Woodberry<sup>1</sup>, Papa A. Ndour<sup>5</sup>, Bruce M. Russell<sup>16</sup>, Tsin W. Yeo<sup>1</sup>, Gabriela Minigo<sup>1</sup>, Rintis Noviyanti<sup>2</sup>, Jeanne R. Poesoprodjo<sup>3,4,17</sup>, Nurjati C. Siregar<sup>2,18</sup>, Pierre A. Buffet<sup>5\*</sup>, Nicholas M. Anstey<sup>1\*,\*</sup>

**1** Global and Tropical Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, Northern Territory, Australia, **2** Eijkman Institute for Molecular Biology, Jakarta, Indonesia, **3** Timika Malaria Research Program, Papuan Health and Community Development Foundation, Timika, Papua, Indonesia, **4** Rumah Sakit Umum Daerah Kabupaten Mimika, Timika, Papua, Indonesia, **5** UMR\_S1134, BIGR, Inserm, Université de F-75015 Paris, and Laboratory of Excellence GR-Ex, Paris, France, **6** Institut Pasteur, Experimental Neuropathology Unit, Paris, France, **7** Department of Biological Sciences, Notre Dame University, Notre Dame, Indiana, United States of America, **8** ISGlobal, Hospital Clinic-Universitat de Barcelona, Barcelona, Spain, **9** Germans Trias I Pujol Research Institute, Badalona, Spain, **10** Catalan Institution for Research and Advanced Studies, Barcelona, Spain, **11** Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts, United States of America, **12** Center for Excellence in Vascular Biology, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts, United States of America, **13** Wellcome Center for Integrative Parasitology, University of Glasgow, Glasgow, United Kingdom, **14** Center for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, **15** Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,

b

Transmission electron microscopy



**OPEN ACCESS**

**Citation:** Kho S, Gotrunnada L, Leonardo L, Andries B, Wardani PAI, Fricot A, et al. (2021) Evaluation of splenic accumulation and colocalization of immature reticulocytes and *Plasmodium vivax* in asymptomatic malaria: A prospective human splenectomy study. PLoS Med 18(5): e1003632. <https://doi.org/10.1371/journal.pmed.1003632>

# *Streptococcus pneumoniae*

PLOS ONE

RESEARCH ARTICLE

## Antibacterial activity of medicinal plants in Indonesia on *Streptococcus pneumoniae*

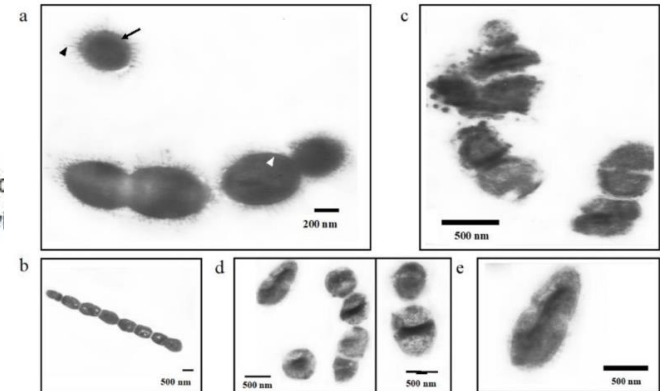
Wisnu Tafroji<sup>1,2\*</sup>, Nur Ita Margyaningsih<sup>1</sup>, Miftahuddin Majid Khoeri<sup>1,3</sup>, Wisiva Tofriska Paramaiswari<sup>1</sup>, Yayah Winarti<sup>1</sup>, Korrie Salsabila<sup>1,2</sup>, Hanifah Fajri Maharani Putri<sup>1</sup>, Nurjati Chairani Siregar<sup>1,4</sup>, Amin Soebandrio<sup>1,4</sup>, Dodi Safari<sup>1</sup>

**1** Eijkman Institute for Molecular Biology, Jakarta, Indonesia, **2** Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, **3** Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, **4** Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

\* [wisnutafroji@gmail.com](mailto:wisnutafroji@gmail.com)

### Abstract

*Streptococcus pneumoniae* is a human pathogenic bacterium able to cause pneumococcal diseases. Some studies have reported medicinal plants having



*Thank  
you*

