FMIC Negative Stain information & procedures

Materials; Carbon & formvar coated grid, fume hood, lab coat and gloves

Uranyl acetate - 

Viruses, bacteria, cell fractions, macromolecules (DNA, actin, enzymes, etc.)

0.5% to 2% aqueous

Only use on samples that are stable in acidic conditions

Phosphate & Cacodylate buffers will precipitate UA - avoid contact

Phosphotungstic Acid (PTA) - 

Viruses, bacteria, cell fractions, macromolecules (DNA, actin, enzymes, etc.)

0.5% to 2% aqueous

Less contrast than UA

Adjust pH to 5 – 7 with 1M KOH, below 6 most stable, above 7 unstable

Ammonium Molybdate - 

Enzyme subunits, membranes or subcellular organelles

1% or 2% aqueous

Anionic negative stain, relatively unreactive

Less contrast than UA, similar to PTA but not as stable

Finer grain structure so better resolution of fine details

Methods;

* 1. Drop Method;
		1. hold grid with self-closing forceps
		2. 4 – 5 ul sample on grid
		3. wait 1 minute,
		4. add 4 – 5 ul negative stain
		5. blot grid🡪touch edge of grid with filter paper
		6. dry 15 minutes under heat lamp
		7. store in grid box in desiccator prior to viewing
	2. Alternate Drop Method;
		1. hold grid with self-closing forceps
		2. mix equal portions of stain in epi-tube or on paraffin sheet
		3. apply mixture onto grid
		4. blot grid
		5. dry 15 minutes under heat lamp
		6. store in grid box in desiccator prior to viewing
	3. Flotation Method
		1. Float grid on drop of sample on Parafilm for 1 minute
		2. Transfer grid to drop of negative stain for 30 seconds
		3. blot grid
		4. dry 15 minutes under heat lamp
		5. store in grid box in desiccator prior to viewing

References:

**UA** – Va Bruggen et al., 1960, **PTA** - Valentine & Horne, 1962; Horne, 1967,

**Ammonium Molybdate** - Muscatello and Horne, 1968 et. al., 1974 13