

Developments in Nucleic Acid Extraction Methods

Neisha Jeoffreys



Institute of Clinical Pathology
and Medical Research

Nucleic acid Extraction

- **Race to improve the quality of PCR**
- **NA extraction often overlooked**
- **Critical for success**
- **Without good quality & quantity DNA or RNA you may be doomed to PCR failure!**

Extraction methods

- **Crude preparations**
- **Phenol chloroform (PCI)**
- **Alkaline Lysis**
- **Anion exchange resin**
- **Spin Columns**
- **Magnetic particles**

DNA Extraction Chemistry

Cell Lysis



Capture of DNA



Washing steps to remove protein & inhibitors



Elution of DNA

Cell Lysis

- **Guanidinium thiocyanate**
- **Detergent & Proteinase K**
- **Pre-processing**
 - **Sonication or manual breakdown**
 - **Deparaffinisation**
 - **Decontamination**
 - **Lyticase**

Capture of Nucleic acid

- **Magnetic particles**
(total nucleic acid)



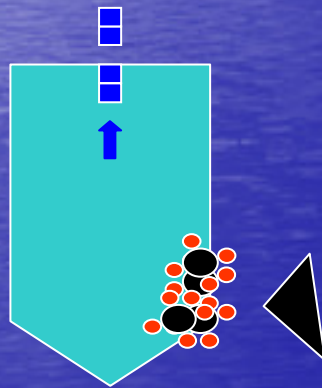
- **Silica membranes**
(DNA, RNA or total NA)



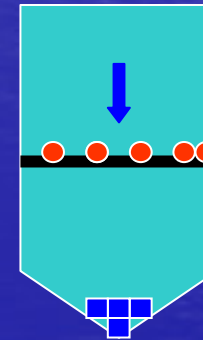
- **Similar principle of binding but different format**

Washing

- Similar for both methods
- Removal of cell debris & protein components



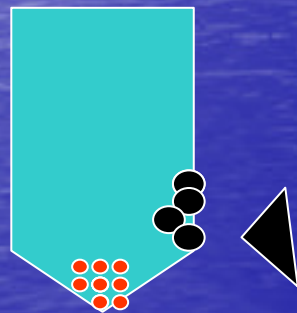
Magnetic particles



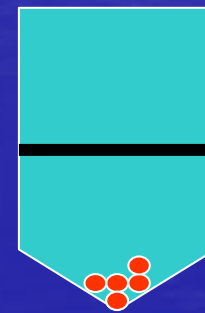
Silica Membrane

Elution & Storage

- **Elution with a low salt buffer**
- **Small volume, high concentration**
- **Correct storage conditions essential**



Magnetic
particles



Silica
Membrane

The Move to Automation

Magnetic Particles

- On board or off board addition of lysis reagent
- Robotic pipetting of reagents & waste in and out of cartridges
- Magnetic bars for the collection of particles

Silica Spin Columns

- On board or off board addition of lysis reagent
- Robotic pipetting of reagents
- Vacuum manifold or spin

Advantages of Automation

- **High throughput**
- **Faster TAT for larger numbers of specimens**
- **Reduced workload**
- **Less operator based error**

Disadvantages of Automation

- **Higher cost**
- **Loss of sensitivity**
- **Extraction Failures**
 - **Tip blockage**
 - **Vacuum failure**
- **Not all specimen types ideal**

Considerations

- **Number of samples and TAT per run**
- **Types of samples on one run and kits needed**
- **Starting volume & elution volume**
- **Detection and monitoring of failures**

Further Considerations

- **Tracking and tracing of samples/reagents**
- **Addition of internal controls**
- **Contamination levels**
- **Validation**

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