MOLECULAR SPECIMEN PROCESSING

Aims of specimen processing are:

- Release nucleic acid from the organism
- Maintain integrity of nucleic acid
- Render the sample non-infectious
- Remove inhibitors
- Concentrate the specimen

Want to make specimen processing as uncomplicated as possible: complex methods are time consuming, can lead to loss of target nucleic acid and may result in contamination between substances.

Inhibitors can include substances such as phenol or alcohol used in the extraction step.

Extraction methods
Different methods available. RNA and DNA require different methods. RNA can be difficults, since RNases are difficult to inactivate. RNases can be endogenous to the host organism, or can be introduced from laboratory materials or human hands. Disposable plastic ware, designated reagents and a separate area for RNA extraction can help.

Method also depends on the organsism – viruses are easy to lyse, while mycobacteria, staphylococci and fungi and be difficult: enzyme digestion or harsh lysis conditions may be required to distrupt the cell walls of the organsism.

For DNA, often use a **detergent** to lyse the cell wall, **protease** such as proteinase K to digest enzymes and **EDTA** to chelate the divalent cations that are required for nuclease activity. The resulting lysate can be used directly in amplification assays, or first **extracted** with phnenol and chloroform-isoamyl alcohol and **precipitation** of the nucleic acids by ethanol. This step remove proteins and organic solvents and concentrate the specimen.

If the crude lysate is to be used, high concentrations of DNA and minimal concentrations of inhibitors are required in the sample – if this is not met, then extraction and precipitation is required.

Another method, often used for RNA, is disruption of cells with **guanidinium thiocyanate** and a detergent, then **precipitation** with isopropanol. The guanidinium thiocyanate both denatures proteins and inhibits RNases. Can also be used for DNA. Can also use in combination with silica or glass particles to bind the nucleic acid, followed by eluting in a low-salt buffer after washing.

For tissue specimens, need to homongenise first. For paraffin embedded tissue, need to de-paraffin and slice into fine sections.

Removal of inhibitors
A key function of the extraction process. Simple boiling can be effective in relatively acellular specimens such as CSF, but not all inhibitors are inactivated by boiling. Silica extraction as described above can be efficient at removing inhibitors.

Urine samples for *N. gonorrhoea* and *C. trachomatis* PCR processed by commercial methods often use a crude detergent lysis step as processing. This can lead to inhibition in 3-7% of samples. Common inhibitory substances are haemoglobin, crystals, β-HCG and nitrates.

Small volumes of whole blood (1%) are inhibitory to Taq polymerase: samples from whole blood must be adequately purified.

Heparin has been shown to be inhibitory to amplification and should be avoided in the collection of blood samples: EDTA is preferred.