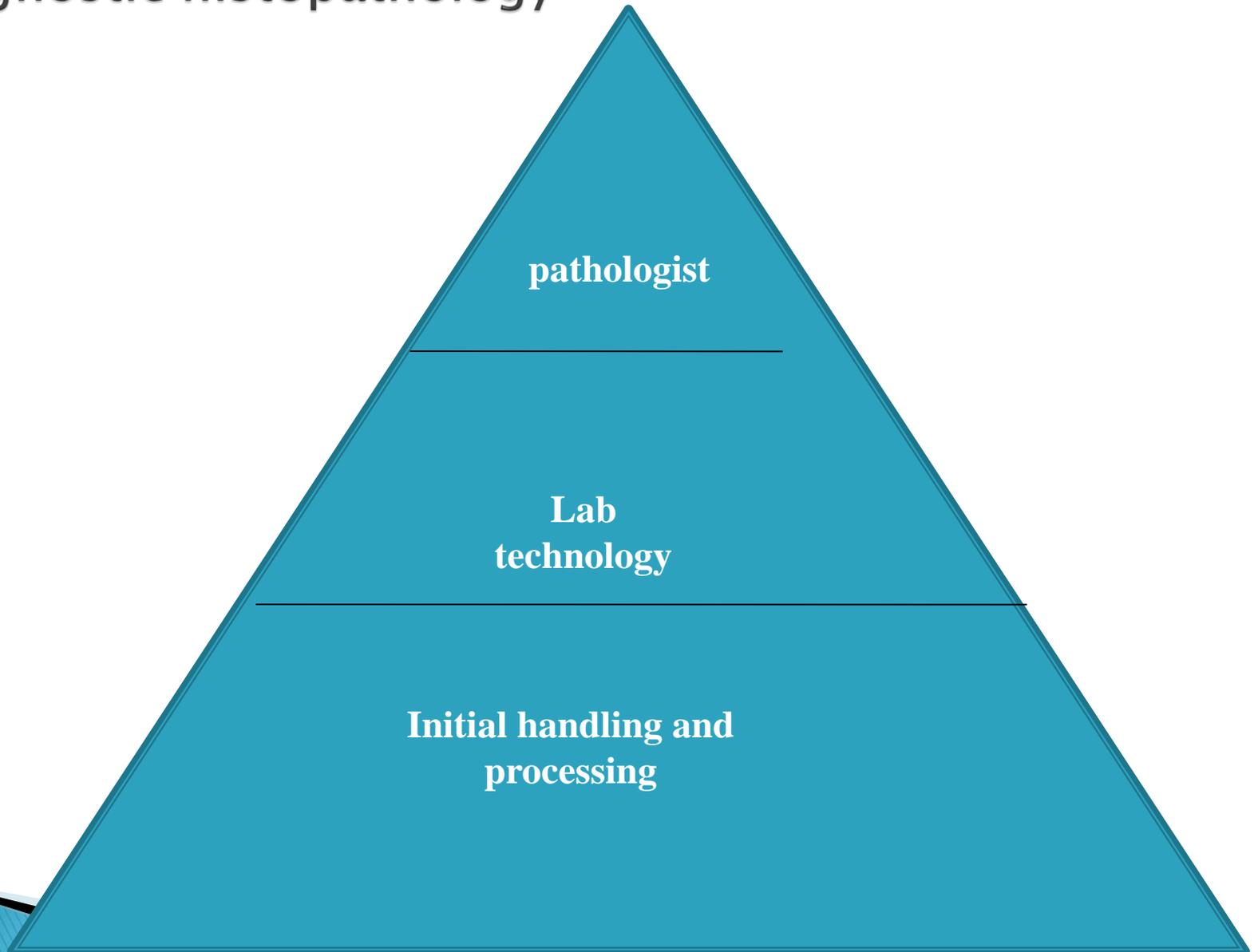


# **Immunohistochemistry in the sri Lankan scenario**

# Diagnostic histopathology



# Tissue fixation

- ▶ Must be seriously addressed
  - ▶ Large specimens should be adequately handled.
  - ▶ **Mastectomy specimens) invariably IHC required**
  - ▶ **Large lymph nodes )**
  - ▶ **Autolysis of varying degree, sometimes not readily apparent on H&E. IHC results are not optimal. Also FISH is affected**
- 

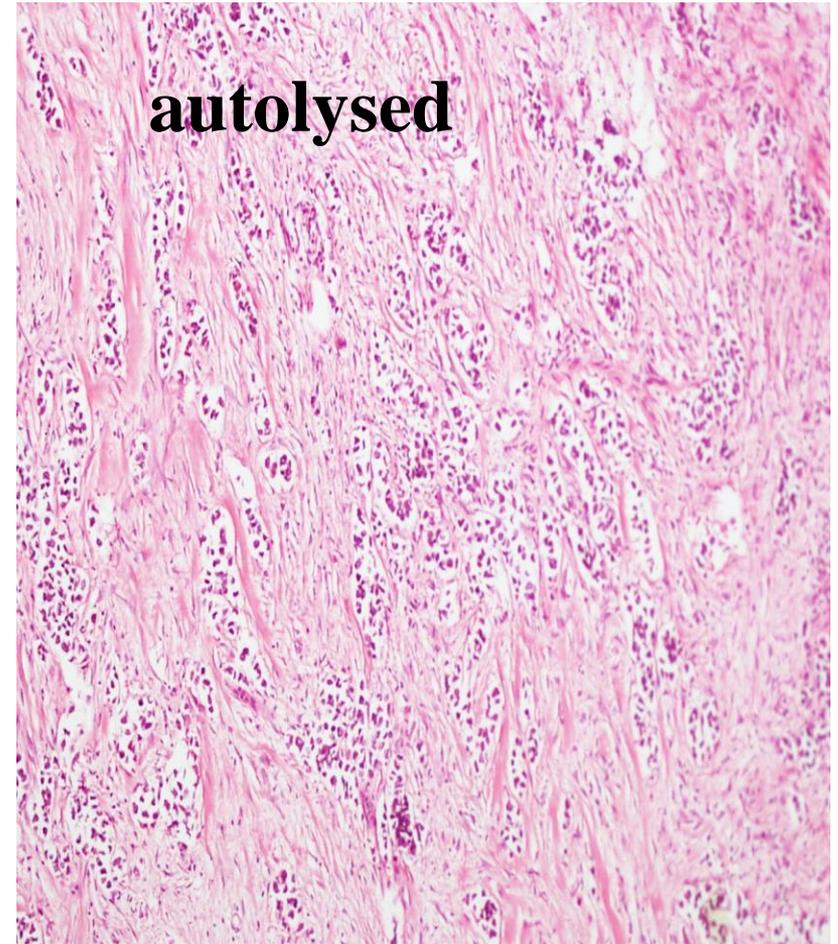
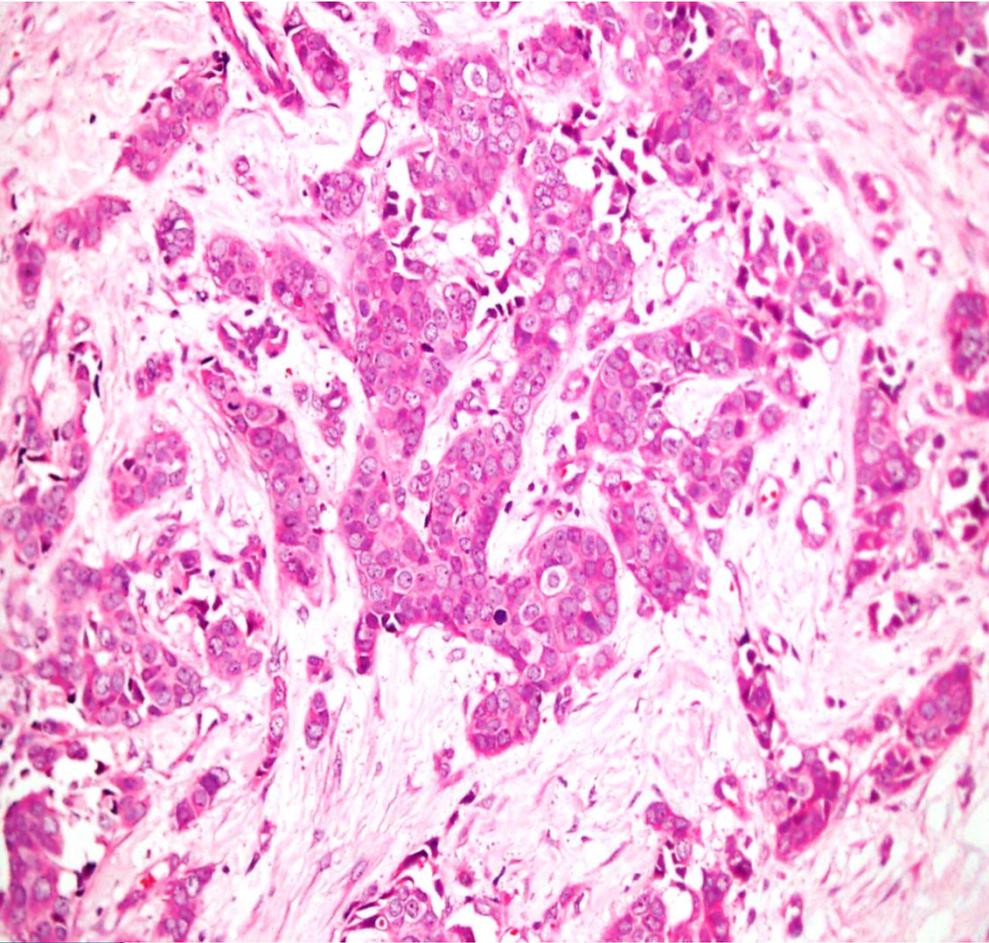
# Mastectomy is a big specimen with fat – floats

**Now the nightmare begins for the pathologists**

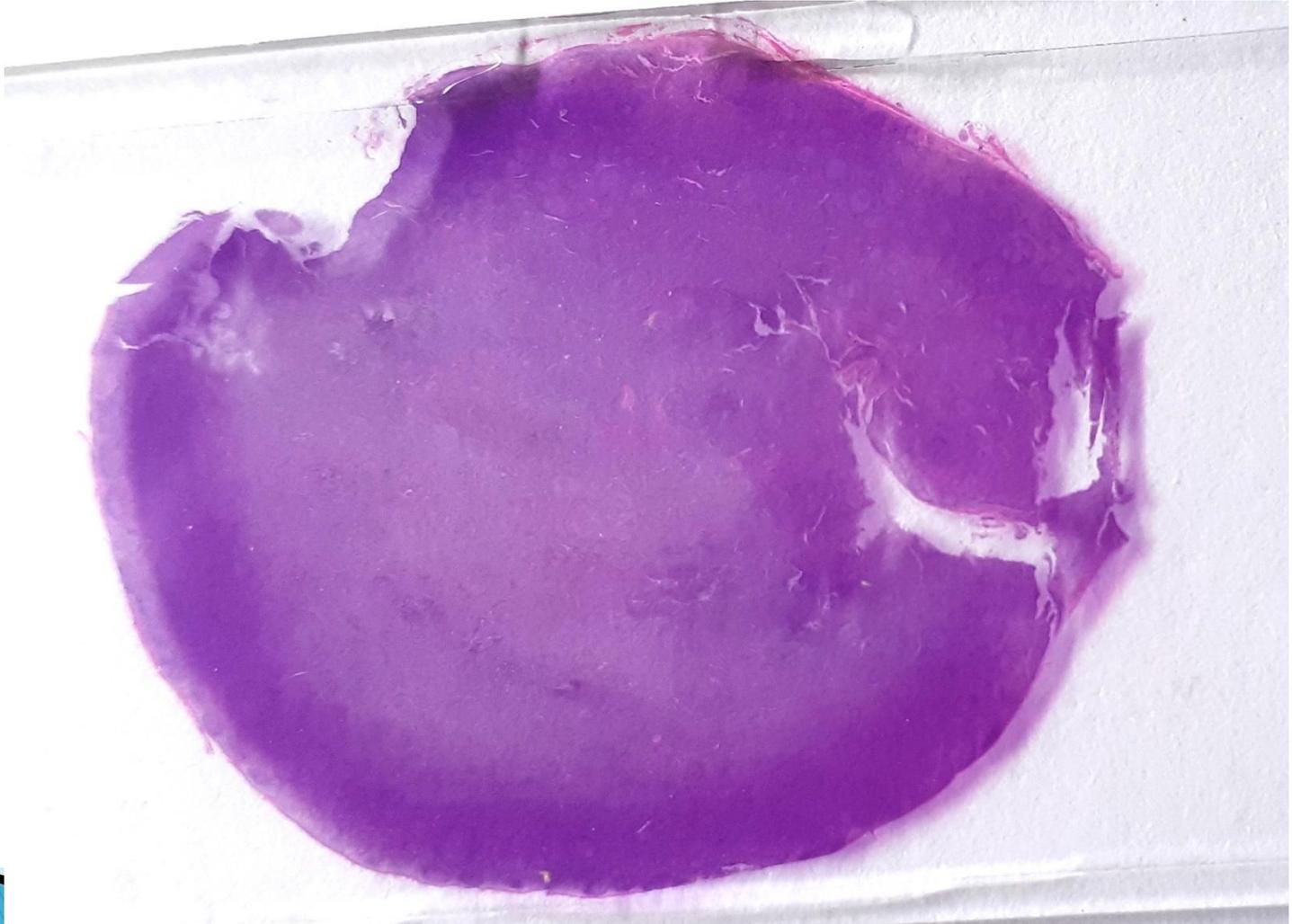


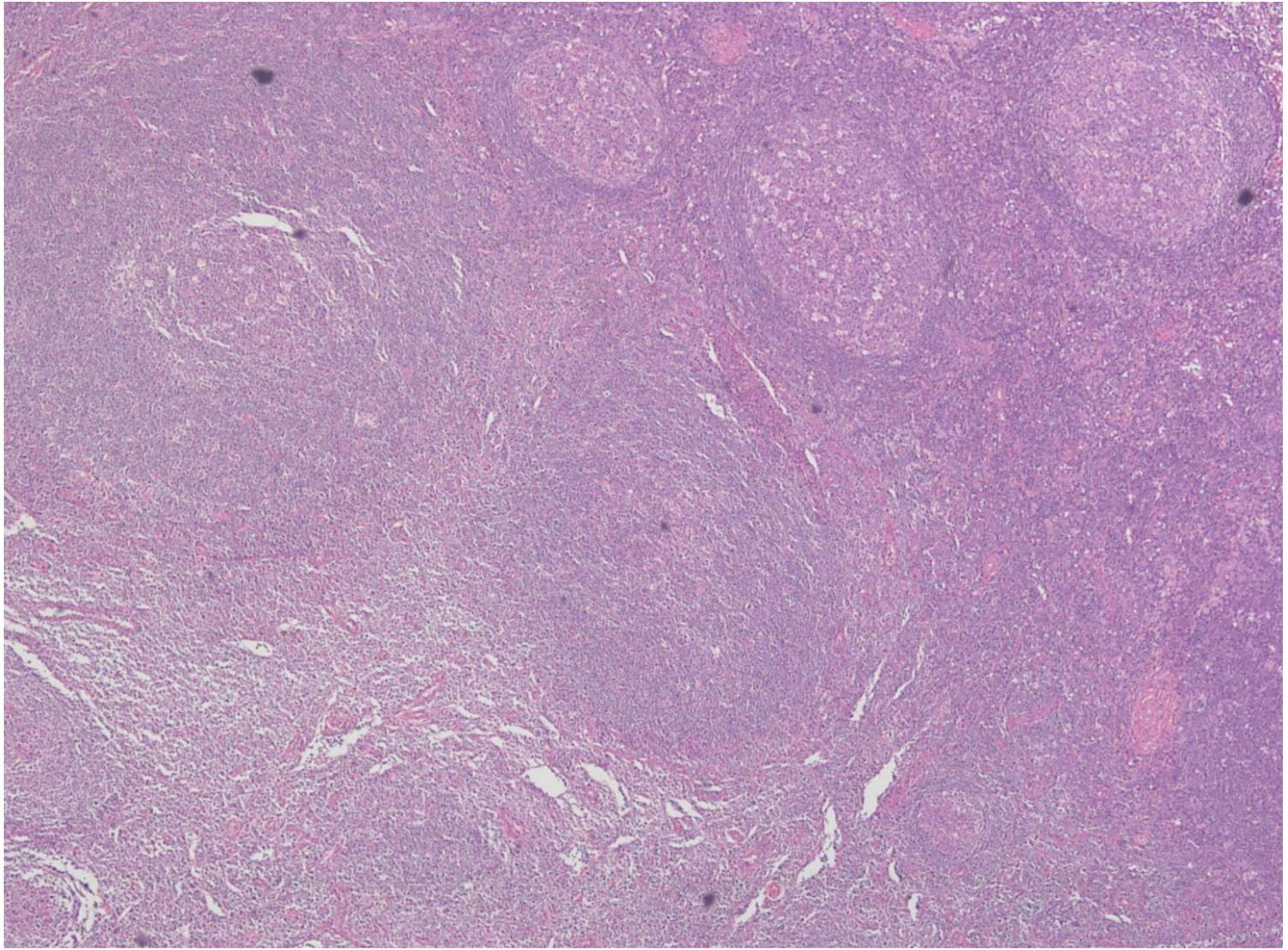


# Carcinoma breast-

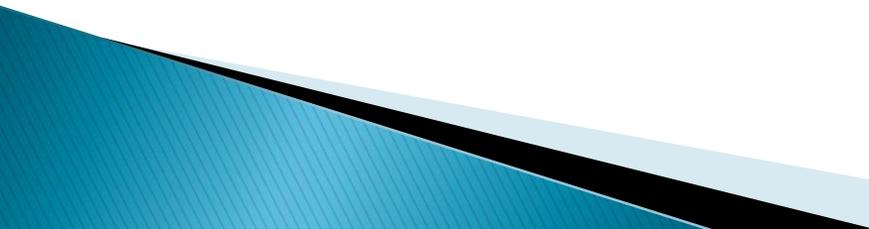


**Lymph node – outer zone well fixed. Autolysed center**





# Possible solutions

- ▶ Send specimen immediately to lab.
  - ▶ Send a person from the lab to bring the specimens after every theatre list
  - ▶ MO path to be on call and check the theatre lists and bring specimen to lab
  - ▶ Authorised person-Take a slice of the tumour ASAP and put into separate container. Grossing can be done later
- 

# Formalin fixation

- ▶ Formalin – neutral buffered advisable- but several chemicals required. expensive
- ▶ Concentration and fixation time –important *antigen retrieval has to be adjusted for these factors*
- ▶ Possible Solutions
- ▶ Have strict SOP,s to be followed by all labs.
- ▶ Enlighten the theatre staff/ pharmacy - not very practical
- ▶ Prepare the formalin in the lab & distribute
- ▶ *Discuss with reference lab re: fixation protocols*
- ▶ *Newer molecular markers may be developed in the future which might require archival tissue to be tested. Optimal fixation and processing advisable*

# Tissue processing into paraffin

- ▶ Tissue processing must be optimal
- ▶ the tissue processors must be properly maintained
- ▶ Solutions should be replaced appropriately
- ▶ If processing/dehydration and paraffin impregnation is sub optimal
- ▶ Section cutting-thick sections, several layers which stain interfering with results
- ▶ H&E DD is compromised – leading to unnecessary IHC

## Storage of antibodies/chemicals

- ▶ Lets assume the cold chain is maintained during flight and storage in stores and suppliers
  - ▶ Once in our labs – temperature fluctuation in refrigerators should be monitored. Frost free refrigerators can be a problem
  - ▶ Refrigerators cleaned and maintained adequately
  - ▶ Most labs are hot and humid. Wide fluctuation in temperature
  - ▶ Deterioration of antibodies -with all the associated problems- specially low turnover antibodies
- 

## Possible solutions

- ▶ All refrigerators must have UPS and voltage stabilisers
- ▶ Laboratory temperatures must be maintained at appropriate levels
- ▶ Reference labs should inform of poorly performing antibodies no sooner it is discovered. Otherwise false interpretations can occur.

## Reference labs and communication with referring labs

- ▶ *The present practice is for IHC stained slides to be sent back to the referring pathologist for reporting. This affords a wonderful opportunity for the pathologists report on ones own case and also help keep updated.*
- ▶ **In this context it is important for the pathologists to be also kept updated on the performance of the antibodies. Under performing tests, and those giving false positives should be duly informed regularly.**

# Preservation of archival material

- ▶ Important to retain all archival material.
- ▶ There is no organised archival storage system.
- ▶ Water content, oxidisation, extremes of temperature are thought to cause deterioration of blocks. Including cut sections on storage
- ▶ Protect from insects / pests
- ▶ Should we hand over the tissue to the patient for safe keeping for future analysis if and when required????
- ▶ Or preserve them ourselves for future investigations and research

## supplier of immunochemicals

- ▶ Access difficult
  - ▶ Replacing antibodies at short notice is virtually impossible
  - ▶ Companies reluctant to quote for immunochemicals because the market is small
- 

# Controls

- ▶ Must have
  - ▶ Positive controls of varying intensity
  - ▶ Negative controls
  - ▶ This becomes expensive
  
- ▶ Control blocks and slides lose antigenicity with time. Possibly because of temperature fluctuation and may be not using high quality reagents for tissue processing

# Servicing of equipment

- ▶ Proper maintenance of apparatus, instruments and equipment
  - ▶ micro pipettes
  - ▶ Ph meters
- 

# The future pathologist and personalised diagnostics

## A new paradigm

- ▶ Molecular classification –mandatory
- ▶ Molecular and genetic profiling- personalised targeted therapies
- ▶ Molecular prognostic tests- indices will be required
- ▶ Commercialised multigene assay techniques will become fashionable- mamma print, oncoType DX
- ▶ Many ethical issues - gene profiling is being used
- ▶ Pathologists to advise the government on the above issues, and maintain highly disciplined laboratories
- ▶ Maintaining Biobanks-for high quality research material for translating basic science discoveries into clinical applications

- ▶ Have sop's uniform for the country.
  - ▶ Strictly adhere to these
  - ▶ Aim for highly disciplined laboratories
- 