ACKNOWLEDGEMENTS

I thank Dr. Mathilde Boon of Leids Cytology and Histopathology centre, Leiden, Netherlands and Dr. Lambrecht Kok, Physicist at Groeninghen University Netherlands for sharing all their knowledge.
Role of Microwave in Cytology, Cell Block and Quick HP Diagnosis: An alternative to frozen section.

Dr. Meera Govindarajan
Properties of microwave

- Electro magnetic waves.
- Frequency 2.45GHz
- Dipolar molecules oscillate at 2450 million times a second
- They move in a straight line as waves
- They show reflection, refraction and absorption
Wave pattern if waves moved in single direction - a two dimensional representation
Properties of Microwave

- Microwave transparent material: eg. Glass, Plastic used as containers.
- Microwave resistant materials: eg. Metals
- Microwave absorbable materials: eg. Biological tissue, water, Alcohol etc.
Penetration depth of Microwave in chemical mediums

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>Depth per cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water at 37°C</td>
<td>4.4</td>
</tr>
<tr>
<td>at 45°C</td>
<td>5.4</td>
</tr>
<tr>
<td>at 55°C</td>
<td>6.6</td>
</tr>
<tr>
<td>Paraffin wax</td>
<td>15,000</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>1.5</td>
</tr>
<tr>
<td>Ethyl Alcohol</td>
<td>7</td>
</tr>
<tr>
<td>Propyl Alcohol</td>
<td>3.5</td>
</tr>
<tr>
<td>Butyl Alcohol</td>
<td>5</td>
</tr>
</tbody>
</table>
Penetration depth of Microwave into Tissue mediums

<table>
<thead>
<tr>
<th>Tissue Medium</th>
<th>Depth per cm at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>2</td>
</tr>
<tr>
<td>Skin</td>
<td>1.5</td>
</tr>
<tr>
<td>Liver</td>
<td>2.1</td>
</tr>
<tr>
<td>Lung</td>
<td>2.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>2</td>
</tr>
<tr>
<td>Brain</td>
<td>2.5</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>10</td>
</tr>
<tr>
<td>Fat</td>
<td>6</td>
</tr>
<tr>
<td>Bone</td>
<td>12</td>
</tr>
</tbody>
</table>
Cycle time of Microwave. Once the Magnetron is heated it releases 2.45 hertz MW with a 800Watt heating capacity till the Magnetron switches off.
Comparison of routine Tissue Fixation procedures with Microwave induced fixation

- Fixation - in formalin for all tissues.
- All tissues need 10% Formalin
- Time depends on size and may vary from 2-3 hours to 8hours.
- Fixation not required for small biopsies.
- Rest of the tissue needs 5% formalin
- Time depends on size and may vary from 3mins to 30 mins
COMPARISON OF ROUTINE DEHYDRATION PROCEDURE WITH MICROWAVE HEATING

- Graded Alcohol is used.
  - Usually either Ethyl or Isopropyl Alcohol are used.
  - Procedure is slow. 2 to 6 hours
  - Speed of the procedure depends on size/load of tissue/temperature and Vacuum

- Two different Alcohols are used sequentially
  - The two alcohols used are E.g. Ethyl/Isopropyl or Methyl/Isopropyl
  - Procedure is fast. 10 mins to 30 mins.
  - Time can be further shortened by use of Vacuum
Use of Clearing agents and their role in the two procedures

- Xylene or chloroform is essential
- The tissue may get hardened
- A minimum of 1-2 hours of processing in two changes is needed
- Not required
- Costly chemicals like Xylene can be avoided.
- Isopropyl Alcohol acts as the intermediate.
IMPREGNATION WITH PARAFFIN WAX - Comparison of the two procedures

- Paraffin Wax takes a longer time for impregnation.
- Good impregnation is dependent on clearing and dehydration
- The wax quality is altered as xylene mixes with it
- The tissue in the block is comparatively harder
- The impregnation time is shortened to 10-30 minutes.
- Good impregnation is achieved fast and depends on MW
- The wax is reused as the quality does not deteriorate.
- The tissue in the block is softer and good quality sections are easily obtained.
Tissue Processing: (Total time 21 - 24mins) for Rapid processing of less than 1mm thick tissue/or cell block only

- Immerse the Cassettes in Methyl Alcohol and microwave for 4 mins at 450 W.
- Drain the alcohol from the cassettes and immerse in Isopropyl Alcohol. Microwave for 4 min at 450 W.
- Drain the alcohol from the cassettes.
- Immerse cassettes in hot liquid paraffin wax and microwave for 7 mins at 750-800 W.
- Embed the tissues in wax. Keep the block on ice for cooling.
- Cut 5 micron sections and stain with Haematoxylin and Eosin.

**CAUTION:** If the tissue is very soft and 1mm thick bit is not easy to gross then fix the bit in 10% Formalin for 30 secs at 450W MW before proceeding to step 1.
Various advantages of Microwave processing

- Cost varies between Rs. 60,000 to Rs. 5 lakhs depending on the instrument purchased i.e. Tissue processor/cryostat.
- Time consuming. Large area is used.
- Frozen sections need more experienced Pathologists. Routine HPE may not allow enough time for discussions.
- Cost of setting up the processing unit – less than Rs. 10,000/- for routine as well as Quick diagnosis if MW is used.
- Technicians find it an easy, quick and clean procedure.
- Pathologist is more familiar with HPE sections. Also abundant time is available for discussions and analysis.
Various advantages of Microwave processing (contd)

- Frozen sections have to be refrigerated for storing. Remaining tissue is to be stored in liquid nitrogen.
- Technical support of the Engineer is essential in case of breakdown. Magnetron/Electronic/Mechanical parts need to be imported
- Wax blocks are available for Immunohistochemistry, special stains / Other studies. Storage is simple
- Technical support is easily available and costs less for repair. A new instrument may be purchased immediately when the magnetron life is over.
Various disadvantages of Domestic Microwave oven

- The time taken for quick diagnosis is around 30 minutes. An experienced department can give frozen section reports in 5-15 minutes.
- The domestic oven lacks temperature/output controls as compared to Microwave histology processors.
- Vacuum facility is not present
- Hotspot
Wave pattern in three dimensional representation with the formation of hot spots

Figure 5.4. Electric-field strength at height $z = 3.5$ cm inside the microwave oven according to Lorenson (1989). (courtesy Dr. C. Lorenson).
Hot Spot

- A hotspot is formed when the temperature rises to 140-200 degrees C.
- The spot shows signs of excess heating as in the adjacent figure.
- If the area covered in a hotspot is crucial for diagnosis then destain the slide. Heat the slide in MW at 400 W in Xylene for 5mins. Remove from Xylene and Stain.
62 Year old man with 25 years history of smoking/pan parag consumption came with complaints of difficulty in swallowing liquids for 2 days. Endoscopy showed total obstruction of the Esophagus at the 25cm level. Emergency surgical intervention was essential. More than 70 sections at the end of 42 mins, helped in diagnosis.
Factors which influence Tissue processing

- Size of the bits
- Methodology of packing in the beaker
- Total processing load / position in the Microwave oven
- Type of tissue and its absorption/penetration qualities
- Output of Microwave oven - lower 800W/900W output better than 1200W
- Power supply - specially if less than 200 Volts.
Hazards of Microwave Oven

A. Accidental malfunction of the door may lead to:
   1. burn injury of the hand – skin/ deep muscle injury with sparing of fat
   2. Anesthesia of the injured part
   3. Dysthesia

B. Injury secondary to inhalation of fumes

C. Potential to develop Cataract
Various practical applications of Microwave processing in Histopathology department

- Fixation of large specimen
- Quick diagnosis of any soft tissue biopsy material
- Cell block study
- H&E and other special stains of Cytology and histology material.
- Decalcification

- Large macro tissue processing.
- Immunohistochemistry
- Antigen retrieval
- In situ hybridisation study
- Immunofluorescence staining
- Electron microscopy processing/ staining
36 Year old male with history of smoking and drinking for 17 years was brought with complaints of haematemesis. A Endoscope was cautiously passed and revealed a bleeding ulcer with necrotic debris in the stomach. The gastric contents were removed. No biopsy taken.
A 62 year old male with obstructive jaundice with an ulcerated growth at the Ampulla of vater. Attempt to biopsy the growth caused bleeding. Only brush smears sent. Benign and malignant cells in a background of necrosis and Mucinous material seen. Patient operated at some other centre was diagnosed to have Adenocarcinoma of the Ampulla.
A 94 Year old female presented with blood and mucus in stool with diarrhea. Patient was uncooperative. Colonoscope could not be passed beyond rectum because of spasm. Only necrotic tissue could be removed. A cell block preparation of the necrotic tissue
Inguinal mass in a 76yr old man suspected to have metastatic deposits from tumor arising in a non healed ulcer of foot. FNAC revealed pleomorphic cells with large pleomorphic nuclei.
Histology revealed a pleomorphic spindle cell sarcoma.
33 year old healthy female presenting with hard gritty mass 5x5 cms in the inner quadrant of breast. Mammogram suggested a malignancy. FNAC done. Fungal infection was suggested.
FNAC from Breast mass with biopsy of the mass for quick diagnosis
FNAC of breast mass another case with biopsy of the mass for quick diagnosis
Pleural effusion metastasis
Ovarian Cyst aspiration
1. Follicular cyst aspirate
2. Cell block - papillary serous adenoma
3. Mucinous carcinoma aspirate
62 year Old female with history of back pain and non-healing wound at the L2-L4 level following a road Traffic accident showed an osteolytic lesion in the vertebra. Necrotic debris from the wound was reported as caseous material and a slide sent for second opinion.
PAS stain of the same slide was carried out. The foamy cells revealed fungal spores of Histoplasma Capsulatum
75 Year Old female with a discharging sinus tract in the abdominal wall was unfit for surgery due to cardiac problems. Discharge sent for culture and cytology. A cell block of the discharge revealed dense clusters of fungal hyphae and necrotic tissue.
Fungal stains were carried out. A picture of the pigmented conidiophore (fruiting bodies) seen in the block and dichotomous branching of Hyphae suggested Aspergillus Fumigatus.
52 year old Male with an inguinal node 2x2 cms. FNAC done. 2cc of haemorrhagic aspirate received showed brown sediments. The cell bock sections are shown below. Smears were haemorrhagic.
A melanin bleach was carried out on cell block sections. Biopsy of the node was carried out. Sections of the node are shown below.
Ultra sound guided Para Aortic mass aspirate with evidence of granulomatous reaction probably Tuberculous. Note the langhan’s giant cells.
FNAC from a Para Aortic mass in a 50 year old male - Clinical suspicion of Tuberculosis. A diagnosis of Massive Lymphadenopathy with sinus histiocytosis was suggested.
FNAC was carried out on 58 Year old Male with pain abdomen and left sided Para Aortic mass with necrosis. Clinical diagnosis was Renal Cell carcinoma with metastasis.
CT and Ultrasound study ruled out renal Pathology. The cytology on smear and cell block section of necrotic material is shown below.
A diagnosis of metastatic deposits probably from left Testis was considered based on clinicopathological analysis.
Clinical examination of Testis was normal. Ultrasound revealed a 1.5 diameter mass at one pole of the Testis with necrosis. Orchidectomy sections showed Teratocarcinoma.
40 Year old Male came with the history of testicular enlargement. FNAC of the mass sent. Seminoma was suggested. Orchidectomy carried out. Sections revealed sheets of round cells negative for LCA & CD 20.
OTHER APPLICATIONS OF MICROWAVE

Special stains
SPECIAL STAINS:
1. AFB- Zeil Neilson  
2. Liver – Masson’s Trichrome  
3. Gastric mucosa – Methylene Blue for H.Pylori  
4. Liver - Reticulin stain
Decalcification

- For large bits 1-2 cubic cms.
  1. Fixed bone tissue is MW in 5% Formic acid at 650 - 700W power for 30 to 45 minutes depending on the size of the bone tissue. Washed for 6 hours in running water. Large bit grossed and 2-3 mm thick bone cut.
  2. The 2-3 mm bone tissue is left overnight in 10% Formic acid. Washed for 6 hours in running water. Usually decalcification is complete.
  3. In case further decalcification is required step one is repeated for 10 to 45 minutes depending on the size and hardness of the bone.
QUICK IMMUNOHISTOCHEMISTRY

- Step 1: Blocking Endogenous Peroxide. MW the slide for 5 secs on 300W power in 3% Hydrogen peroxide. Wash in PBS and MW for 10 secs at 300W in PBS.
- Step 2: Add Blocking antibody and MW for 10 secs at 300W. Remove excess serum.
- Step 3: Add primary Antibody and MW for 15 secs at 300W. Wash with PBS as above.
- Step 4: Add link antibody and MW for 15 secs at 300W. Wash as above with PBS.
- Step 5: Add StreptAvidin Peroxidase and MW for 15 secs at 300W power.
- Step 6: Chromogen DAB substrate is added and incubated at room temperature for 4-6 mins.

CAUTION: THE SLIDES MUST BE KEPT IN MOISTURE CHAMBER THROUGHOUT THE PROCESS
34-year-old lady presenting with altered behavior and severe frontal headache of 3 months duration. CT scan showed a frontal partly solid, partly necrotic tumor. ST biopsy sent for quick diagnosis.
The sections revealed a lymphoma. Quick IHC carried out showed CD20 and CD45 (LCA) positivity.
Quick Immunohistochemistry carried out to assess the pancreatic function of Islet cell donor - Sections of the tail of the pancreas in a case of death due to road traffic accident.
Modifying a poorly processed block

- Old autopsy blocks
- Poorly processed Blocks
- Poorly fixed tissue
MODIFYING A OLD/ BADLY PROCESSED BLOCK:
Slide and Poorly processed block sent for opinion. AIDS patient with L2-L3 Osteolytic bone lesion. The sections were thick and Lymphoid morphology was not clear.
Sections after modification
Procedure for modifying block

- Blocks were reprocessed in paraffin wax.
- 450W for 5 minutes.
- 800 W output for 5 minutes.
- 10 minutes of 600W of MW.
ANTIGEN RETRIEVAL

The exposure of Antigen or Epitope which are hidden due to Fixation induced cross linkages is termed as Antigen retrieval.

Various methods of antigen retrieval are:
1. Enzymes like Trypsin, Pronase.
2. Microwaving of tissue sections in Ionic solutions like Citrate at PH 6.0 and at 9.5, Aluminium chloride, Lead Thiocynate, Zinc salt solutions etc.
3. Heating and boiling in Ionic solutions specially with pressure cookers.
MiB-1 activity in Protoplasmic Astrocytoma grade 2 and in Anaplastic Astrocytoma Grade 3 following Antigen retrieval.
Oestrogen positivity in Duct carcinoma of breast, Prostatic carcinoma and in Meningioma following Antigen retrieval
Use of microwave in in situ hybridisation (TUNEL) using Fluorescein dye to demonstrate apoptosis (Programmed cell death) based on labeling of DNA strands

- MW slide in citrate buffer for Antigen retrieval - 5mins
- Wash thrice in Tris, each wash for 15 mins
- Incubate in Reactive mixture for 2 hours at 37 Degrees.
- Wash thrice in Tris, each wash for 15 mins
- Mount and observe

- MW slide in citrate buffer for antigen retrieval - 5mins
- Wash thrice in Tris, MW at 400W for 1 min each
- Incubate in MW with Reactive mixture for 2 mins, at 400W
- Wash thrice in Tris, MW at 400W for 1 min each.
- Mount and observe
Picture on left shows positive Fluorescence after routine incubation technique. Picture on right shows Fluorescence following incubation in MW. Note the lack of background autofluorescence due to Formalin.
Picture on the right shows the same case as seen in a confocal microscope
THANK YOU