STRATEGIES TO IMPROVE QUALITY OF IHC TEST RESULTS / REPORT

Adetola Daramola, MBBS, FMCPath
Associate Professor of Pathology
College of Medicine University of Lagos &
Consultant Pathologist, Lagos University Teaching Hospital
The only strategy to improve any activity that involves more than one individual is TEAM WORK
All hands on deck

- Improving the quality of IHC tests therefore requires the cooperation of:
  1. Clinicians who request for the test – adequate information/communication
  2. Clinicians who send in the sample – appropriate sample, adequate and well labelled; fixed properly and in good time
  3. Laboratory staff – well trained, efficient and effective
  4. Administrative staff
  5. Others
Definition of Immunohistochemistry (IHC)

- IHC is a specialized test that allows for visualization of specific molecules (antigens) in tissues or cells based on antibody-antigen recognition.

- It detects antigens in histological sections through processes that revive the antigens after fixation (antigen retrieval) making them available for several specific antigen/antibody interactions.

- This allows them to become detectable by light microscopy resulting in biological identification of these antigens and characterisation of the tissue.
IHC aids personalised patient management

More than just differentiating between benign and malignant neoplasms, IHC provides prognostic and predictive information useful for the management of neoplastic disease e.g.

- Tumor classification
- Determining the origin of unknown primary tumors
- Detecting micrometastatic and microinvasive disease
- Tumour stage

All of which help tailor treatment to suit the patient’s tumour characteristics - Personalised patient management
What are the components of the IHC test?

Three Components, Several steps

- Components
  1. Slide preparation
  2. IHC procedure
  3. Interpretation.
HER-2
IHC Procedure

1. Antigen Retrieval

2. Quenching/Blocking Endogenous Target Activity

3. Blocking Non-Specific Sites

4. Immunodetection: Antibody – mediated antigen detection approaches are separated into direct and indirect methods. These methods both use antibodies to detect the target antigen.

5. Chromogenic detection of target antigens based on the activities of enzymes eg horseradish peroxidase

6. Counterstains give contrast to the primary stain and can be cell structure – specific.

7. Sealing the Stained Sample by mounting a coverslip with an appropriate mountant stabilizes the tissue sample and stain.

8. Sample Visualization by light or fluorescent microscopy.
Figure 1. Peroxidase Anti-Peroxidase (PAP) Complex Method.

Figure 2. Avidin-Biotin Complex (ABC) Method.

Figure 3. Labeled Streptavidin-Biotin (LSAB) Method.
Cellular Organization

Levels of Organization:
- Atom
- Molecule
- Macromolecule
- Organelle
- Cell
- Tissue
- Organ
- Organ System
- Organism
TYPES OF CELLS

NERVE CELL

MUSCLE CELLS
- Striated (voluntary)
- Smooth (involuntary)
- Cardiac

BONE CELL

GLAND CELLS

BLOOD CELLS
- White blood cells
  - Neutrophil
  - Lymphocyte
  - Monocyte
  - Eosinophil
  - Basophil

REPRODUCTIVE CELLS
- Sperm
- Ovum
Examples of some commonly used markers include:

- Cytokeratins: used for identification of carcinomas but may also be expressed in some sarcomas.
- CD15 and CD30: used for Hodgkin's disease
- Alpha fetoprotein: for yolk sac tumors and hepatocellular carcinoma
- CD117 (KIT): for gastrointestinal stromal tumors (GIST) and mast cell tumors
- CD10 (CALLA): for renal cell carcinoma and acute lymphoblastic leukemia
- Prostate specific antigen (PSA): for prostate cancer
- Estrogens and progesterone receptor (ER & PR) staining are used both diagnostically (breast and gyn tumors) as well as prognostic in breast cancer and predictive of response to therapy (estrogen receptor)
- Identification of B-cell lymphomas using CD20
- Identification of T-cell lymphomas using CD3
Why improve?

- “Up to 20% of current IHC determinations of ER and PR testing worldwide may be inaccurate (false negative or false positive). Most of the issues with testing have occurred because of variation in preanalytic variables, thresholds for positivity, and interpretation criteria”

Figure 1. A schematic representation of various factors which may influence the standardization and reproducibility of the IHC process.

Strategies to improve IHC test/report
Quality Assurance (QA)

1. Assessment of performance of test to promote excellence.
   - Assessment without the aim of improving is not QA

2. It involves the management of processes that ensure that the required quality is obtained and maintained
   - These processes should detect, correct and reduce errors
The aim of QA will be to produce an IHC test/report that is

- Accurate
- Timely
- easily understood by the oncologist

These three elements require the existence of a structured laboratory environment with excellent QA processes
Strategy no 1: Quality Management System in the laboratory

- Organization
- Personnel
- Equipment
- Purchasing & Inventory
- Process Control
- Information Management
- Documents & Records
- Occurrence Management
- Assessment
- Process Improvement
- Customer Service
- Facilities & Safety
Some Requirements…

1. Adequately trained staff: pathologists and laboratory scientists (at least one of each to run the service)
2. Appropriate and reliable equipment
3. Reliable reagents and a good reorder system to prevent out of stock syndrome
4. Good technical backup - maintenance agreement with equipment vendors and a trained technician less than 24hrs away if needed
5. Good administrative support
6. Continuous training
7. QA
Adequately trained staff

- Pathologists
- Laboratory scientists (at least one of each to run the service)
- Other staff specifically trained to support the service e.g. laboratory technicians

- Regular periodic trainings/to update staff on advances in IHC as well as competency/proficiency tests e.g.
  1. Ring Project
  2. EQA workshops
Appropriate and reliable equipment

- Manual
- Automation
- Good technical backup. Laboratories should aim to have
  - a maintenance agreement and
  - a trained technician less than 24hrs away if needed
Reagents

- Reliable reagents
- Routine validation and optimization of reagents
- Good reorder system to prevent out of stock syndrome
QA & Audit
QA

- **Preanalytic**
  - Good fixation & transport time
  - accurate labelling, processing, slide production etc

- **Analytic**
  - Antigen retrieval procedure
  - Reagent validation
  - Training

- **Postanalytic**
  - Accurate scoring and interpretation
  - Accurate data input (no mix ups)
Some strategies to eliminate preanalytic sources of error

- Effective communication between the laboratory and operating rooms
- 10% neutral buffered formalin (NBF) should be the standard fixative. Old stock (> 3 months) should not be used
- Samples should be placed in the appropriate amount of fixative 1:10 ratio
- Adequate sized containers should be used
- Gauze should be placed on top and even at the bottom of the sample to allow for proper fixation and preventing undue exposure of parts of the sample.
Some strategies to eliminate preanalytic sources of error

- Samples should be sent promptly to the histopathology laboratory within one hour of removal.
- If delay envisaged, samples should be sliced serially as per agreed protocol.
- Specimens should be properly labelled and oriented by the surgeon e.g. suture in axillary tail. Diagrams indicating lesion site may be helpful.
- Bloody fixative (seen immediately after a harvested sample is immersed in fixative) should be replaced with fresh fixative hourly until fixative becomes clear.
Some strategies to eliminate preanalytic sources of error

- Time and date of sample harvest should be recorded appropriately on sample container, request form and report/record books.

- Duration of fixation should be recorded for each sample correctly. Time of placing sample in fixation should be noted.

- The time from tumor removal to fixation should be kept to # 1 hour.

- The warm and cold ischemic times are widely accepted as important variables in the analysis of labile macromolecules such as proteins, RNA, and DNA from clinical tissue samples. The pathologist should effectively communicate this priority to all members of the breast care management team so processes are put in place to make sure these times are routinely recorded.
Other Strategies

1. Inter laboratory network for trouble shooting and QA
2. Automation
The greatest advantage of automated immunohistochemistry is improved quality, reproducibility, speed and standardisation. It consequently leads to the achievement of new levels of quality with reduced labour, reagent costs, and also permits the improvement of intra and inter-laboratory reproducibility of assays (Grogan, 1992; Markin, 1992).
Strategy no 2: Team meetings

Multidisciplinary team meetings.

- All individuals directly involved in patient management should meet to discuss the patient, his/her disease, the investigations and the treatment plan.
- Useful feedback is received that will improve our processes

Departmental/Unit meetings
In summary ........
IHC

Has revolutionized the field of surgical pathology by

- Markedly enhancing the diagnostic strength of the H&E slide;
- Improving diagnostic accuracy even with smaller biopsies
The following factors amongst others may affect the outcome of IHC

- Tissue handling
- Antigen retrieval
- Storage and handling of tissue sections
- Choice of antibody
- Detection method
- Interpretation procedure
QMS

Adoption of Laboratory QMS will ensure standardization, reliability and reproducibility of IHC reports
MDTs
Some References

- Marc A, IHC Staining Methods, Fifth Edition. Chapter 9, Immunohistochemistry Staining Methods
- O’Hurley G et al. Garbage in, garbage out: A critical evaluation of strategies used for validation of immunohistochemical biomarkers Molecular Oncology 2014; 8:783 -798
Thank you for your attention
Credits for IHC at LUTH

- Prof SO Elesha
- Prof AAF Banjo
- Prof FB Abdulkareem
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- Mrs Tokunbo Adeyemo
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- Prof Abeer Shaaban
- TSL-WADIAP EQA Workshop Team
- The Ring Project Team
MAUFACTURER’S TROUBLE SHOOTING SITES

- Thermofisher
- ABCAM
- GIBCO
- Etc