Immunohistological Diagnosis

An antibody (Ab) is also known as an immunoglobulin (Ig).

Antibody, also called **immunoglobulin**, a protective protein produced by the immune system in response to the presence of a foreign substance, called an antigen.

Antigen, substance that is capable of stimulating an immune response, specifically activating lymphocytes.

- Pre-Analytical Steps
- 1. A Biopsy (surgically removed tissue specimen or needle
- biopsy) from the surgery room arrives in fixative at the
- pathology laboratory.
- 2. In the Accessioning room the sample details are entered
- into the laboratory information system (LIS). A barcoded
- label can ensure track and trace capabilities.
- 3. During Grossing, the specimen is visually examined for
- suspicious areas that require further examination. Samples
- from the specimen that require further microscopic testing are
- excised as tissue blocks and placed in barcoded cassettes.

Pre-Analytical Steps

- 4. Tissue processing and embedding are the steps where
- the tissue block is processed into a form and condition suitable
- for making ultrathin microscopic sections. Typically, the tissue
- is fixed in formalin then dehydrated before it is embedded
- in paraffin.
- 5. Sectioning is the fine art of cutting the paraffin-embedded
- tissue blocks into ultrathin (~4 µm) sections and placing them
- onto glass slides. A barcode on the slide can ensure traceability and may also contain protocol information for the
- requested test for that particular section.

Analytical Steps

6. Staining is the analytical part of the IHC process. It encompasses antigen retrieval, application of the primary antibody

and visualization system, ending with counterstaining:

a. Antigen retrieval is performed to recover the antigens that may have been altered by fixation;

b. Endogenous enzymes are blocked (this step can also be performed after primary antibody incubation);

c. A primary antibody is applied that specifically binds to the antigen of interest;

Analytical Steps

6.

d. The secondary antibody carries the label (enzyme); upon application it binds to the primary antibody;

e. Chromogen is applied to visualize the antibody/antigen complex;

f. Counterstaining is performed to visualize nuclei and overall tissue architecture;

g. Sections are dehydrated, mounted and coverslipped.

Post-Analytical Steps

7. In the post-analytical process, the pathologist interprets the stains in context with positive and negative tissue controls, using bright field microscopy.

8. The results are reported to the oncologist for treatment decision.