



# Immunohistochemical and H&E Testing of Breast Tumor Biomarkers Estrogen and Progesterone Receptor to Evaluate Malignancy

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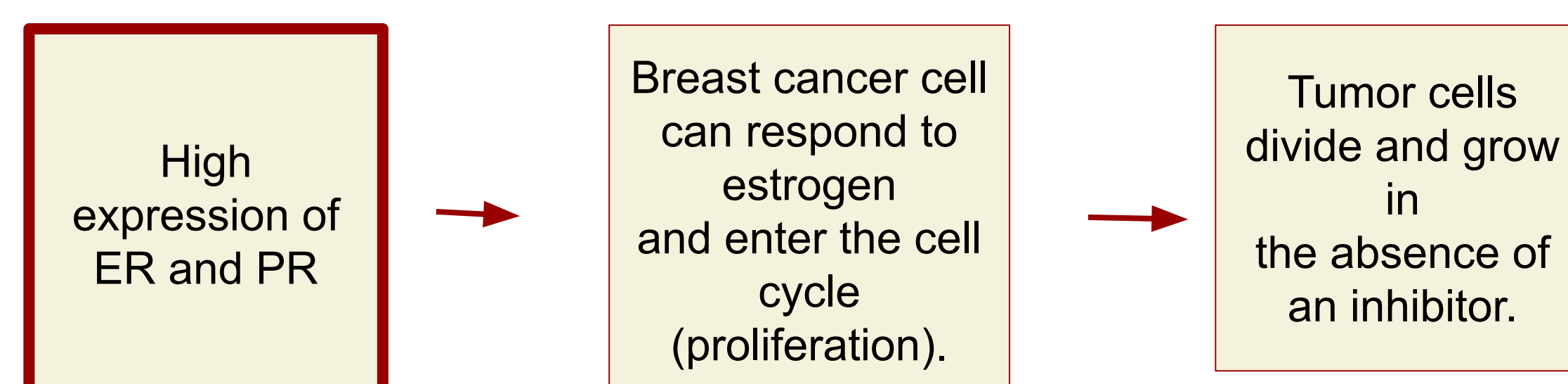
## Abstract

Breast cancer is the most common type of cancer among females in the United States. Molecular profiling of each histologic type is imperative to understand the molecular drivers of the disease. For example, ductal carcinoma in different patients may have similar histologic appearances, but may not have the same molecular profile. While one may be positive for the expression of estrogen and progesterone receptors, another with similar histologic appearance may be negative for expression of those receptors. The purpose of this study was to characterize tumor cells through histologic observation and determine the expression of biomarkers Estrogen Receptor and Progesterone Receptor (ER and PR). In order to do so, we conducted both Hematoxylin and Eosin (H&E) staining as well as Immunohistochemical (IHC) tests on a set of slides with patient tissue cut from two separate cases sourced from surgeries and embedded in paraffin blocks. Results of these tests showed that Case 1 exhibited intense immunostaining after applying an anti-estrogen receptor primary antibody on one slide of tissue and anti-progesterone receptor primary antibody on another slide of the same tissue section. Case 2 showed little to no immunostaining after applying an anti Estrogen Receptor primary antibody and anti Progesterone Receptor primary antibody during IHC. While pathologic review of the H&E staining facilitated characterization of the histology, the results of the IHC test showed that only the Case 1 patient will be eligible for hormone suppressive treatment. These tests are important to provide patients with individualized treatment, ultimately to rid them of only the cancerous cells rather than their healthy cells to maximize recovery.

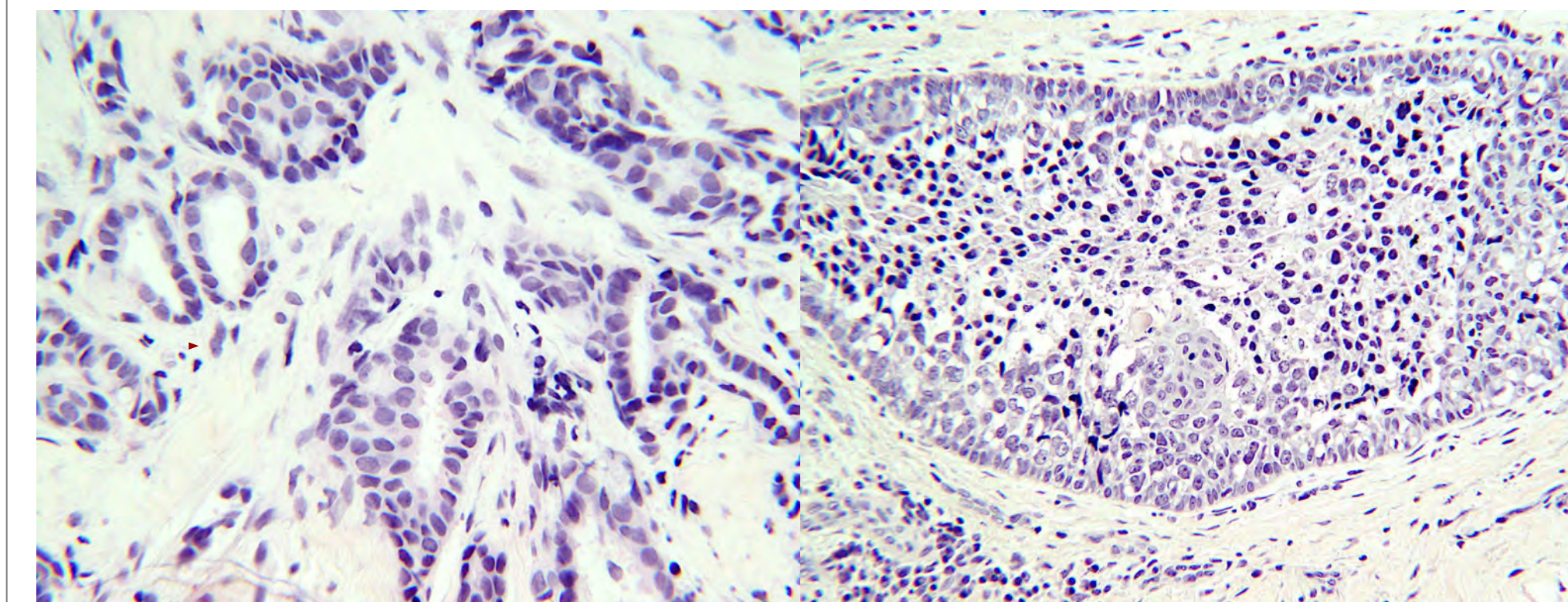
## Introduction

Cancer biomarkers are measurable substances (DNA, RNA, proteins) in cancer cells that are correlated with disease behavior or responsiveness to a particular form of therapy. Cancer biomarkers, generally, are of two types: prognostic biomarkers of clinical outcome or predictive biomarkers of treatment outcome. Prognostic markers are correlated with patient outcomes in the absence of systemic treatment, while predictive biomarkers are correlated with tumor response to specific systemic therapy. Estrogen receptor expression in breast carcinomas is associated with a significantly longer disease-free survival and longer overall survival of women whose cancer was treated with surgery alone and whose cancers expressed estrogen receptor compared to women with similar surgery whose cancers do not express this receptor (prognostic marker). Since estrogen receptor is a transcription factor that is activated by binding the hormone estradiol (the most common female estrogen hormone), expression of this hormone in breast cancer cells permits these tumor cells to respond to estrogenic stimulation. Administration of estrogen blocking drugs, like tamoxifen, interfere with the response of ER-positive breast cancer cells to estrogenic stimulation, hence ER is a predictive marker of breast cancer patient response to anti-estrogen therapy. Estrogen receptor and progesterone receptor are identified in breast cancer tissue samples using a laboratory test, known as immunohistochemistry. Immunohistochemistry determines which breast cancer patients receive anti-estrogen therapy to inhibit growth of residual breast cancer cells after surgery (ER-positive breast cancers) and which patients receive some other form of systemic adjuvant therapy (ER-negative breast cancers).

## ER & PR High Expression



## Hematoxylin & Eosin Staining (H&E)



Case 1

Case 2

**Hematoxylin** shows components within the nucleus such as chromatin or genetic material, etc.

### Process Overview:

**Staining Mechanism:** Most reactions which involve staining contain both physical and chemical factors. For example, staining fat is only a physical stain, where the dye is simply absorbed by and dissolved in the lipid.

However, most stains such as the H&E stain involves the attraction of minute particles from the surrounding solution, where the dye is then bound to the tissue by ionic, covalent, or hydrogen bonds.

**Eosin** is an acidic dye which shows components of the cytoplasm.

## Immunohistochemical Staining (IHC)

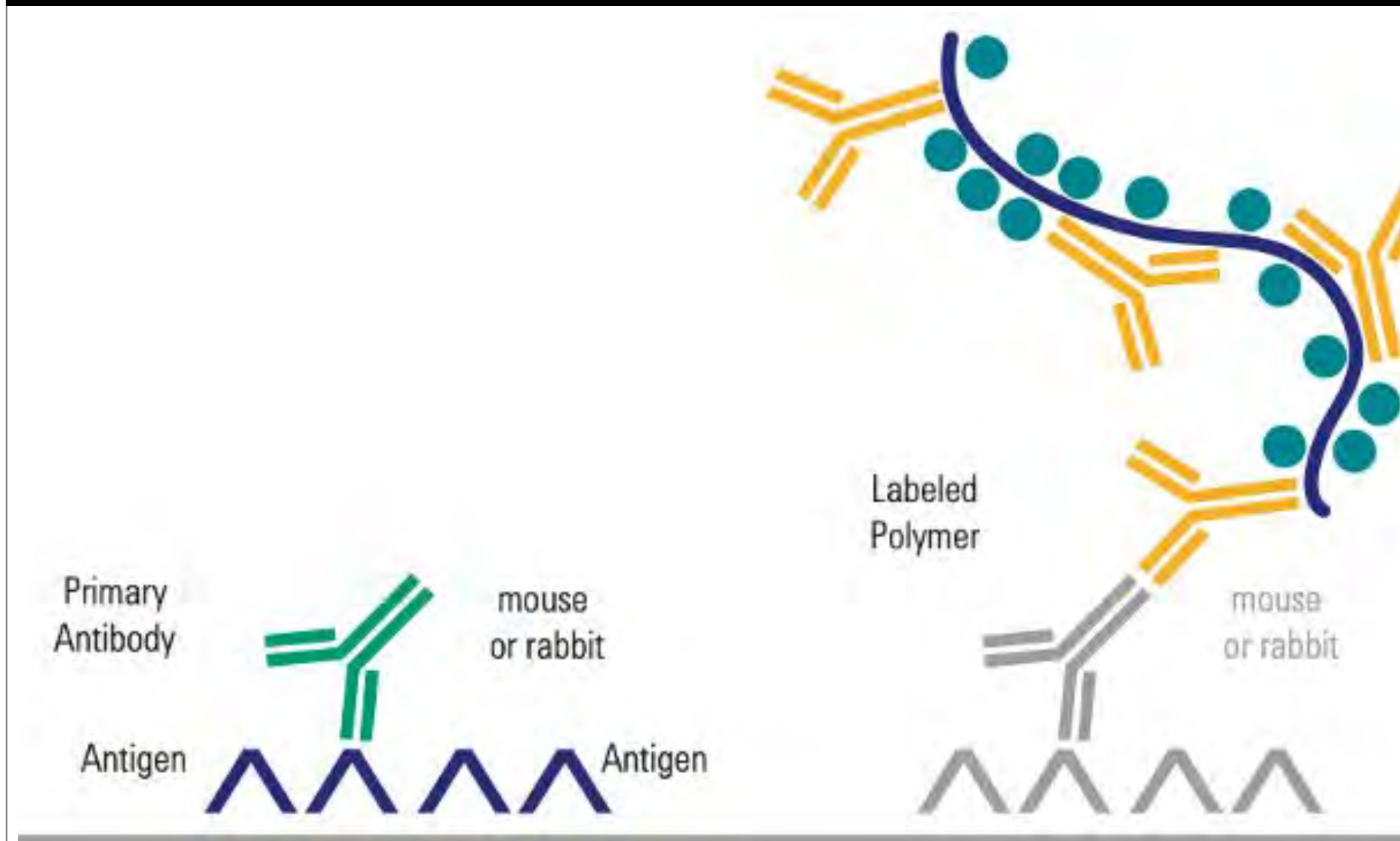


Figure 1

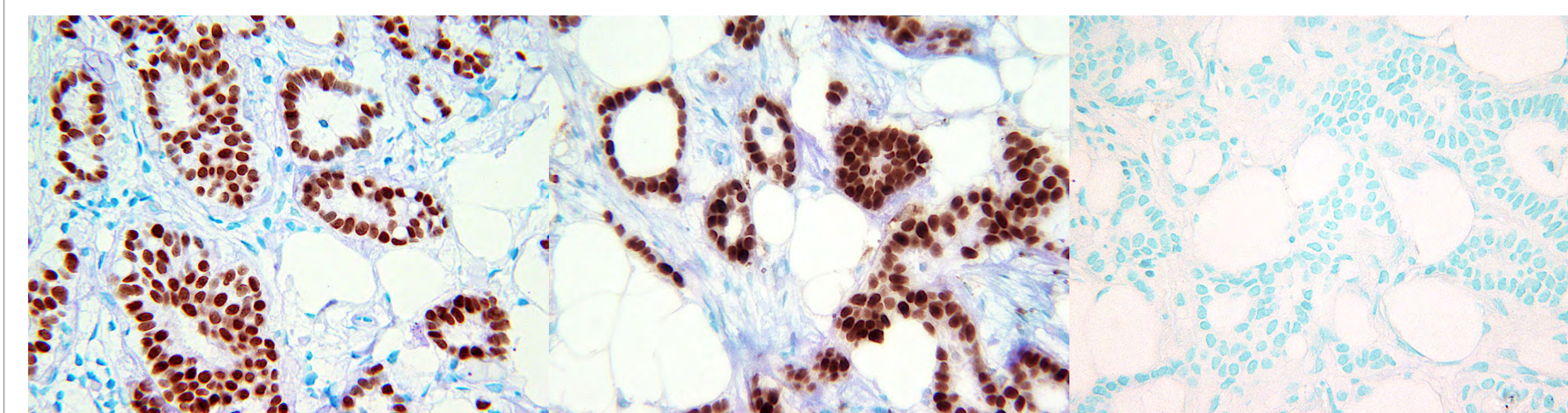
### Process Overview

Immunohistochemistry could be done using 4 different methods

- Direct
- Indirect
- Unlabeled or Soluble Enzyme Immune Complex
- Avidin-Biotin

The method I used was the Indirect Method (shown on the left). This is a 2-part method where a the primary antibody is added to the tissue antigen, then the labeled polymer must be bound to localize the first antibody.

## Result



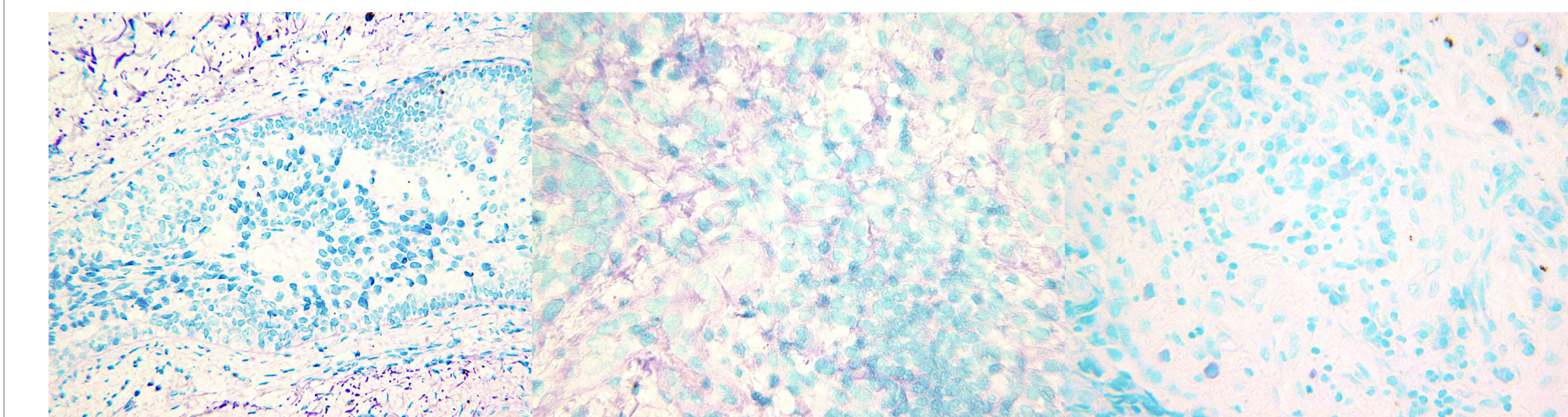
Case 1

ER+ highly expressed

PR+ highly expressed

NlIgG negative control

Case 2



ER negative not highly expressed

PR negative not highly expressed

NlIgG negative control

## Method/Procedure

### Hematoxylin and Eosin Stain (H&E):

Purpose: histologic review

Procedure Overview:

1. Paraffin slides are prepared then dried in 60°C oven for 1 hour
2. Slides are placed in xylene to deparaffinize (2 minutes each), then placed in 100% ethanol to dissolve the xylene, 95% and 70% to rehydrate the slides
3. Slides are stained in hematoxylin solution for 7 minutes, then rinsed in distilled water
4. Slides are differentiated in acid alcohol for 15 seconds, then rinsed
5. Slides are dipped in scott's tap water, rinsed for a last time, and then stained in eosin for 5 minutes
6. Slides are rinsed in 2 changes of 95% ethanol and one rinse 100% ethanol, then placed in 3 changes of xylene
7. Newly stained tissue is covered in permount, coverslipped, and left to dry



Set of slides stained in hematoxylin and ready to be rinsed with distilled water.

### Immunohistochemical Stain (IHC):

Purpose: To see expression of certain biomarkers

Procedure Overview:

1. Steps 1-2 of H&E staining repeat
2. Pre-circle slides with PAP pen
3. Antigen Retrieval: transfer slides from PBS to coplin jar with DAKO antigen retrieval solution and place in water bath at 97°C for 30 minutes. After water bath, rinse slides out with PBS for 5 minutes twice
4. circle tissue with PAP pen and flood tissue section with 10% normal goat serum for 10 minutes
5. Add 2-4 drops of primary antibody and let incubate for 1 hour at room temp
6. Rinse in PBS 3 x 5 minutes, remove excess saline, then add 2 drops of Envision+ labelled polymer to the sections
7. Make up DAB (diaminobenzidine)
8. Rinse in PBS 3 x 5 minutes, remove excess saline, then add DAB and incubate for 10 minutes
9. Rinse each slide individually
10. Stain slides in 0.1M Sodium Acetate buffer 5 min, Ethyl Green Solution 5-10 min, Distilled water 3 times; 10 dips, 10 dips, 30 seconds, 1-butanol 3 times; 10 dips, 10 dips, 30 seconds, Xylene 3 times; 2 min each, then mount



Applying primary antibody to set of slides (Case 1 and Case 2).

## Summary

1. The results of the Immunohistochemical and H&E test of case 1 show that the patient is positive for high expression of Estrogen and Progesterone Receptor. Therefore, the patient is eligible for hormone suppressive treatment to specifically target tumor cells.
2. The results of the Immunohistochemical and H&E test of case 2 show that the patient is negative for high expression of Estrogen and Progesterone Receptor. Due to the lack of expression of ER and PR biomarkers, this patient would not benefit from hormone suppressive treatment.

## CONTACT US

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