How to Bring New Antibodies into Your Clinical IHC Laboratory

Debra Horton MT(ASCP) QIHC
University of Alabama at Birmingham
Birmingham, AL
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Encourage your teammates to participate and become the top-notch facility in the Aurora Diagnostics family to show case the best well-trained laboratorians and recognized for excellence in performance.
Objectives

- Search the antibody
- Review package insert
- Know what is needed to cover CAP guidelines and what is required by the CAP checklist.
- Developing a process that will be easy to follow for any future antibody request.
Search for Antibody

How often will the Pathologist order this antibody?

- Once a day, week or month?
- Are there other Pathologist that will use this antibody for diagnostic testing?
Search Antibody

See if it would be more cost effective to send to reference lab or test in house.

In-house:
Antibody cost + detection system + labor = cost

Send-out:
Cost of test + shipping = cost to send out
Search Antibody

Try to buy from a vendor you already order antibodies.

◦ Ventana
◦ Dako
◦ Cell Marque
◦ Biocare
◦ Other vendors
Review Package Insert

Check to see if the antibody is produced in the animal you can test or is it compatible with your detection system.

- The polymer that we use in our laboratory is compatible with mouse or rabbit antibodies. If it is a goat antibody you will need to add a secondary before adding the polymer step.
Review Package Insert

Is the antibody a RUO (research use only), ASR (antigen specific reagent), or IVD (in vitro diagnostic)

RUO - will need to become a LDT (lab developed test) before you can use them in your clinical lab.
Antibody request Gata 3

- Package insert: Cell Marque
  - IVD
  - Clone
  - Animal type
  - Dilution (not a predilute)
  - Suggested positive control tissue
GATA3 (L50-823) Mouse Monoclonal Antibody

**PRODUCT AVAILABILITY**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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<tr>
<td>39606-14</td>
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<tr>
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<td>0.5 ml concentrate</td>
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<tr>
<td>39606-16</td>
<td>1.0 ml concentrate</td>
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<td>39606-17</td>
<td>1.0 ml, prostate ready-to-use</td>
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<tr>
<td>39606-18</td>
<td>2.0 ml, prostate ready-to-use</td>
</tr>
<tr>
<td>39805</td>
<td>Ready-to-use, 5 vials/pack</td>
</tr>
</tbody>
</table>

**SYMBOL DEFINITIONS**

- [ ] predilute
- [ ] concentrate
- [ ] dilute to desired concentration
- [ ] store at -20°C
- [ ] reconstitute
- [ ] key

**PRINCIPLES AND PROCEDURES**

This primary antibody (GAMM serum) may be used as the primary antibody for immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical staining in conjunction with a commercially available kit allows the visualization of anti-CD34 via the sequential application of a specific antibody (primary antibody to CD34) and a secondary antibody (secondary antibody to the primary antibody) and a chromogenic substrate with subsequent washing steps. Alternatively, a one-step polymer detection system may be used. The enzymatic activity of the chromogen results in a visible reaction product at the antigen site. The specimen should then be counterstained and a coverslip applied. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiologic processes, which may or may not be associated with a particular antigen.

**MATERIALS AND METHODS**

See product label for additional information for the following:

1. Antibody immunoglobulin concentration
2. Source detail

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GATA3 expression is primarily seen in breast carcinomas and uterine carcinomas and is rarely found in tumors from other organs, such as gastrointestinal or hematopoietic malignancies. GATA3 is expressed in all breast ductal carcinomas and 91% of invasive ductal carcinomas (grade I, II, III, and grade IV). GATA3 expression seems to be lower in lobular and tubular breast carcinomas. It has also been reported that GATA3 expression is associated with the status of ER, PR, and HER2 in breast carcinomas. GATA3 expression is found in uterine carcinomas, especially in metastatic and high-grade tumors. Therefore, anti-GATA3 can be used in a panel of antibodies for diagnosis of unknown primary carcinoma, where carcinoma of the breast or ovary is a possibility.
Reagents Provided

The optimal immunohistochemical concentration range for this product is 0.3 - 2.5 µg/mL.

Concentrated: The stated primary antibody product (150M-14, 390M-15, 390M-16) contains concentrated reagent.
Both the prediluted and concentrated forms of this antibody are diluted in 1X Buffer pH 7.3-7.7, with 1% BSA and -0.1% Sodium Azide. The concentration immunohistochemical concentration range for this product is 100 - 185 µg/mL.

Storage and Handling
Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody after every use, the cap must be replaced and the bottle must be immediately placed in the refrigerator in an upright position.

Every antibody reagent in expiration does. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date for the prescribed storage method.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Contact CELL MARQUE customer service if there is a suspected indication of reagent instability.

Specimen Collection and Preparation for Analysis

Routine protocols, neutral-buffered formalin-fixed, paraffin-embedded, tissues are suitable for use with this primary antibody when used with CELL MARQUE detection kits (see Materials, Reagents, and Equipment Needed But Not Provided section). Note: CELL MARQUE routinely performs with tissue sections. The recommended tissue fixation is 10% neutral-buffered formalin. Variability results may occur as a result of prolonged fixation or special processes such as dehydration of bone marrow preparations.

Each section should be cut to the appropriate thickness (approximately 3 µm) and placed on a positively charged glass slide. Tissue sections should be transferred to the tissue section may be kept for at least 2 hours (but not longer than 24 hours) at 50-60°C or 37°C over.
Gata 3 Antibody dilution

Here we have 3 dilutions. A 1:100, 1:200 and 1:400 if breast cancer. The Pathologist review the slides and chose the 1:200 dilution to then test 10 positive and 10 negative cases.
Antibody request Gata 3

Documentation:
Antibody Request: Napsin A

Package insert: Biocare Medical
- IVD
- Clone
- Animal type
- Dilution (not a predilute)
- Suggested positive control tissue
Napsin A
Prediluted Polyclonal Antibody
Catalog Number: PP 45A AA
Description: 6.0 mL, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use.

Summary and Explanation:
Napsin A is a peptide-like aspartic protease. It is expressed in type II pneumocytes and in adenocarcinomas of the lung and kidney. Studies have shown that Napsin A is both a more sensitive and specific marker than TTF-1 and is extremely specific for lung adenocarcinoma. Most studies show Napsin A is 100% specific for lung adenocarcinomas versus lung SqCC.
When used in combination, the Desmoglein 3 + Napsin A antibody cocktail is very sensitive and specific markers for discriminating between squamous cell carcinoma and lung adenocarcinoma. Used as a panel with TTF-1 and CK5, this antibody cocktail is extremely accurate and may be used as a first-line screener for lung cancer.

Protocol Recommendations:

Expected Incubation Times:

Optimal: Incubate for 5-10 minutes at RT with Biocare's Background Fuzzer; Primary Antibody: Incubate for 30 minutes at RT;
Probe: N/A
Polymer: Incubate for 30 minutes at RT with a Polymer;
Chromagen: Incubate for 5 minutes at RT when using Biocare's DAB - OR - Incubate for 5-7 minutes at RT when using Biocare's War H Red.

Counterstain: Counterstain with Hematoxylin
Rinse: with distilled water. Apply Tacha's Bluing solution for 1 minute. Rinse with distilled water.

Technical Note:
This antibody has been standardized with Biocare's MACH 4 detection system. It can also be used on an automated staining system and with other Biocare polymer detection kits. Use TRIS buffer for washing steps.

Performance Characteristics:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:
Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about tissue controls.

Precautions:
This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC.
Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing.

Speakers, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions.
Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.

Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If typical results occur, contact Biocare's Technical Support at 1-800-542-0002.

Limitations and Warranties:
There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.
Antibody Requested: Napsin A

Here we have 3 dilutions. A 1:50, 1:100 and 1:200 if breast cancer. The Pathologist review the slides and chose the 1:200 dilution to then test 10 positive and 10 negative cases.
Antibody Request: TCR C Gamma M1

Package Insert: ThermoFisher Scientific

- RUO
- Clone
- Animal type
- Dilution (not a predilute)
- Suggested positive control tissue
# Product Data Sheet

**TCR C gamma M1 Antibody (gamma 3.20)**

<table>
<thead>
<tr>
<th>Application</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemistry (Frozen)</td>
<td>Assay Dependent</td>
</tr>
<tr>
<td>Immunohistochemistry (FF)</td>
<td>Assay Dependent</td>
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<tr>
<td>Flow Cytometry (FACS)</td>
<td>2 ug/ml</td>
</tr>
<tr>
<td>Western Blot (IP)</td>
<td>Assay Dependent</td>
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</table>

**Published Applications**

- See publications
- See publications

**Form Information**

- Form: Liquid
- Concentration: 0.2 mg/ml
- Purification: Protein G
- Storage Buffer: PB with 0.05% BSA
- Preservative: 0.1% sodium azide
- Storage Conditions: -20°C, Avoid Freeze/Thaw Cycles

## Product Specific Information

**TCR1153 targets TCR C gamma M1 in IHC (F), FACS and IP applications and shows reactivity with human samples.**

The TCR1153 immunogen is human TCR gamma chain constant region.

TCR1153 detects TCR C gamma M1 which has a predicted molecular weight of approximately 18 kDa.

## General Information

**T Cell Receptors -** The ability of T cell receptors (TCRs) to discriminate foreign from self-peptides presented by major histocompatibility complex (MHC) class II molecules is essential for an effective adaptive immune response. TCR recognition of self-peptides has been linked to autoimmune disease. Mutant self-peptides have been associated with tumors. Engagement of TCRs by a family of bacterial toxins known as superantigens has been responsible for toxic shock syndrome (TSS). Autoantibodies to V beta segments of T cell receptors have been isolated from patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The autoantibodies block TCR mediated immunological synapse formation and are believed to be a method by which the immune system compensates for disease (ref 5).

**T Cell and TCR Diversity** Most human T cells express the TCR alpha-beta and either CD4 or CD8 molecule (double positive, DP). A small number of T cells lack both CD4 and CD8 (double negative, DN). Fractionated percentages of alpha-beta DN T cells have been identified in some autoimmune and immunodeficiency disorders (ref 5). Gamma-delta T cells are primarily found within the thymus. They show less TCR diversity and recognize antigens differently than alpha-beta T cells. Subsets of gamma-delta T cells have shown autonomy and transmembrane activity (ref 5).

**Class I and II TCR Specificity** - The Thermo Scientific T cell receptor offering includes pan alpha/beta and pan gamma/delta antibodies as well as V alpha, V beta, V delta, V gamma, Delta P1, Alpha P1, and C gamma-specific antibodies. All antibodies have been QC tested for use in flow cytometry and immunohistochemistry. Most clones are available in purified and FITC labeled formats.
Antibody Request: TCR C Gamma M1

Here we have 3 dilutions. A 1:40 with a 60min. Incubation of the antibody, 1:40 with a 40 min. incubation of antibody and a 1:60 with a 60 min. incubation of antibody. The Pathologist chose the 1:60 with 60 min. incubation.
# VALIDATION OF ANTIBODY

| DATE: 
| ANTIBODY: 
| VENDOR: 
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<tr>
<th>DAKO</th>
<th>RETRIEVAL</th>
<th>INCUBATION</th>
<th>TITER</th>
<th>RESULTS</th>
<th>COMMENTS</th>
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<table>
<thead>
<tr>
<th>Case # or tissue type</th>
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<tbody>
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<td>NEG TISSUE</td>
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| REPRODUCABILITY       |
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| 2)                     |
| 3)                     |

Directors signature Date___
Tech signature Date___
### Validation Specificity and Reproducibility

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<td>Source:</td>
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<tr>
<td>Lot No:</td>
<td>Expiration Date:</td>
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</table>

**Fixative / Time**

1. Sections are cut at 4 microns unless otherwise instructed
2. Sections are mounted on validated slides
3. Slides are labeled with the appropriate Ventana Label
4. Slides are dried for at least 30 minutes and no longer than one hour at 60 degrees C
5. Slides are loaded on stainer following operator manual instructions
6. When run is complete follow protocol instructions for washing, dehydration and clearing
7. Coverslip
8. Print Run Report, fill out run report and validation form
9. Submit paper work and slides for Pathologist evaluation and signature

<table>
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<tr>
<th>Case(s)</th>
<th>Tissue</th>
<th>Diagnosis</th>
<th>Comment</th>
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**Evaluation of antibody specificity:**

**Evaluation of assay reproducibility:**

**Additional Comments:**

This validation study has been reviewed and the performance of the method is considered acceptable for patient testing. **CPA.GEN.103.0 (F-1)**

**Evaluation Date**

Evaluating Pathologist:

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Example of validation worksheet from Cunningham Pathology: Jackie Brooks
CAP Guidelines

What are the guidelines for IHC antibodies?

What is required by CAP and what is suggested?

ihcval_summary.pdf

Summary of Recommendations
Principles of Analytic Validation of Immunohistochemical Assays

Summary of Recommendations

Guideline Statements

1. **Recommendation**: Laboratories must validate all IHC tests before placing into clinical service.
   **Note**: Such means include (but are not necessarily limited to):
   - Correlating the new test’s results with the morphology and expected results;
   - Comparing the new test’s results with the results of prior testing of the same tissues with a validated assay in the same laboratory;
   - Comparing the new test’s results with the results of testing the same tissue validation set in another laboratory using a validated assay;
   - Comparing the new test’s results with previously validated non-immunohistochemical tests; or
   - Testing previously graded tissue challenges from a formal proficiency testing program (if available) and comparing the results with the graded responses.

2. **Recommendation**: For initial validation of every assay used clinically, with the exception of HER2/neu, ER, and PgR (for which established validation guidelines already exist), laboratories should achieve at least 90% overall concordance between the new test and the comparator test or expected results. If concordance is less than 90%, laboratories need to investigate the cause of low concordance.

3. **Expert Consensus Opinion**: For initial analytic validation of nonpredictive factor assays, laboratories should test a minimum of 10 positive and 10 negative tissues. When the laboratory medical director determines that fewer than 20 validation cases are sufficient for a specific marker (e.g., rare antigen), the rationale for that decision needs to be documented.
   **Note**: The validation set should include high and low expressors for positive cases when appropriate, and should span the expected range of clinical results (expression levels) for markers that are reported quantitatively.

4. **Expert Consensus Opinion**: For initial analytic validation of all laboratory-developed predictive marker assays (with the exception of HER2/neu, ER and PgR), laboratories should test a minimum of 20 positive and 20 negative tissues. When the laboratory medical director determines that fewer than 40 validation tissues are sufficient for a specific marker, the rationale for that decision needs to be documented.
   **Note**: Positive cases in the validation set should span the expected range of clinical results (expression levels). This recommendation does not apply to any marker for which a separate validation guideline already exists.

5. **Recommendation**: For a marker with both predictive and nonpredictive applications, laboratories should validate it as a predictive marker if it is used as such.

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4. **Recommendation:** When possible, laboratories should use validation tissues that have been processed using the same fixative and processing methods as cases that will be tested clinically.

7. **Expert Consensus Opinion:** If IHC is regularly done on cytologic specimens that are not processed in the same manner as the tissues used for assay validation (e.g., alcohol-fixed cell blocks, air-dried smears, formalin-fixed specimens), laboratories should test a sufficient number of such cases to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative cases and the number of predictive and nonpredictive markers to test.

8. **Expert Consensus Opinion:** If IHC is regularly done on decalcified tissues, laboratories should test a sufficient number of such tissues to ensure that assays consistently achieve expected results. The laboratory medical director is responsible for determining the number of positive and negative tissues and the number of predictive and nonpredictive markers to test.

9. **Recommendation:** Laboratories may use whole sections, TMAs and/or MTBs in their validation sets as appropriate. Whole sections should be used if TMAs/MTBs are not appropriate for the targeted antigen or if the laboratory medical director cannot confirm that the fixation and processing of TMAs/MTBs is similar to clinical specimens.

10. **Expert Consensus Opinion:** When a new reagent lot is placed into clinical service for an existing validated assay, laboratories should confirm the assay’s performance with at least 1 known positive case and 1 known negative case.

11. **Expert Consensus Opinion:** Laboratories should confirm assay performance with at least 2 known positive and 2 known negative cases when an existing validated assay has changed in any one of the following ways:
   - Antibody dilution;
   - Antibody vendor (same clone);
   - Incubation or retrieval times (same method).

12. **Expert Consensus Opinion:** Laboratories should confirm assay performance by testing a sufficient number of cases to ensure that assays consistently achieve expected results when any of the following have changed:
   - Fixative type;
   - Antigen retrieval method (e.g., change in pH, different buffer, different heat platform);
   - Antigen detection system;
   - Tissue processing or testing equipment;
   - Environmental conditions of testing (e.g., laboratory relocation);
   - Laboratory water supply.
   - The laboratory medical director is responsible for determining how many predictive and nonpredictive markers and how many positive and negative tissues to test.

13. **Expert Consensus Opinion:** Laboratories should run a full revalidation (equivalent to initial analytic validation) when the antibody clone is changed for an existing validated assay.

14. **Expert Consensus Opinion:** The laboratory must document all validations and verifications in compliance with regulatory and accreditation requirements.
CAP CHECKLIST QUESTIONS:

**REVISED**       04/21/2014

ANP.22750

Antibody Validation

Phase II

The laboratory has documented validation of new antibodies, including introduction of a new clone, prior to use in patient diagnosis.

NOTE: The performance characteristics of each assay in the immunohistochemistry laboratory must be appropriately validated before being placed into clinical use. The initial goal is to establish the optimal antibody titration, detection system, and antigen retrieval protocol. Once optimized, a panel of tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation is at the discretion of the laboratory director and will vary with the antibody. For a well-characterized antibody with a limited spectrum of antigenic targets, like chromogranin or prostate specific antigen, the validation can be limited. A panel of 10 positive and 10 negative neoplasms would be sufficient in this setting. For validation of predictive markers, consideration should be made for a larger validation set. For an antibody that is not well characterized and/or has a wide range of reported reactivity, a more extensive validation is necessary. The number of tissues tested should, in this circumstance, be large enough to determine whether the staining profile matches that previously described.

An exception to the above requirements is that studies may not be feasible for antigens that are only seen in rare tumors.

This checklist has additional validation requirements for HER2 and estrogen/progesterone receptor testing in breast carcinoma. Please refer to the subsection "Predictive Markers," below.

Evidence of Compliance:
✓ Written procedure for the evaluation/validation of new antibodies

REFERENCES


If the laboratory performs immunohistochemistry for estrogen receptor (ER) and/or progesterone receptor (PgR) as a prognostic/predictive marker on breast carcinoma, the laboratory has documented appropriate validation for the assay(s).

NOTE: Initial test validation should include a minimum of 40 cases (20 positive and 20 negative cases) for FDA-cleared/approved tests; laboratories should consider using higher numbers of test cases if a Laboratory Developed or Laboratory Modified Test is to be validated. Validation should be performed by comparing the laboratory's results with another assay that has been appropriately validated. Acceptable concordance levels are 90% for positive results and 95% for negative results.

If significant changes are made to the testing methods (e.g. antibody clones, antigen retrieval protocol or detection system), revalidation is required.

This requirement is applicable to both new and existing assays. If review of the initial validation does not meet the current standard, it must be supplemented and brought into compliance. It is possible to do this retroactively by review and documentation of past proficiency testing challenges or by sending unstained slides from recent cases to a reference laboratory for correlation. If no documentation exists from the initial validation, the assay must be fully revalidated and documented.

REFERENCES


If the laboratory performs HER2 testing (HER2 protein over-expression by immunohistochemistry or HER2 gene amplification by in situ hybridization [e.g. FISH, CISH*, SISH*, etc.]), the laboratory has documented appropriate validation for the assay(s).
NOTE: This requirement applies to both new and existing assays. Initial test validation must be performed on a minimum of 20 positive and 20 negative samples for FDA-cleared/approved assays; or 40 positive and 40 negative samples for laboratory-developed tests (LDTs). Equivocal samples need not be used for validation studies. If the initial validation of existing assays does not meet the current standard, it must be supplemented and brought into compliance. It is permissible to do this retroactively by review of performance on past proficiency testing challenges or by sending unstained slides from recent cases to a reference laboratory for correlation. If no documentation exists from the initial validation, the assay must be fully revalidated and documented.

Validation may be performed by comparing the results of testing with a validated alternative method (i.e. IHC vs. FISH) either in the same laboratory or another laboratory, or with the same validated method performed in another laboratory; validation testing must be done using the same set of cases in both labs. The validation documentation should identify the comparative test method(s) used.

The validation data should clearly show the degree of concordance between methods (e.g. for IHC: 0, 1+, 3+; for FISH, CISH, SISH: pos**REVISED** 04/21/2014
CAP CHECKLIST

The characteristics of the cases used for validation should be similar to those seen in the laboratory's patient population (i.e. core biopsies vs. open biopsy material, primary vs. metastatic tumor, etc.).
1.1. **Validation of Antibodies**

1.1.1. The laboratory has documented appropriate validations for the IHC estrogen receptor (ER) and/or progesterone receptor (PgR) as a prognostic/predictive marker on breast carcinoma assays.

1.1.1.1. Initial test validation should include a minimum of 40 cases (20 positive and 20 negative cases) for FDA-approved/cleared tests;
1.1.1.2. Validation should be performed by comparing the laboratory's results with another assay that has been appropriately validated.
1.1.1.3. Acceptable concordance levels are 90% for positive results and 95% for negative results.
1.1.1.4. If significant changes are made to the testing methods (e.g. antibody clones, antigen retrieval protocol or detection system), revalidation shall be performed.

1.1.2. The laboratory has documented appropriate validations for HER2 testing (HER2 protein over-expression by IHC or HER2 gene amplification by in situ hybridization [e.g. FISH, CISH*, SISH*, etc.]), assay(s).

1.1.2.1. Initial test validation must be performed on a minimum of 25 cases (recommended 25-100). Validation may be performed by comparing the results of testing with a validated alternative method (i.e. IHC vs. FISH) either in the same laboratory or another laboratory, or with the same validated method performed in another laboratory; validation testing must be done using the same set of cases in both labs.

1.1.2.2. If specimens are fixed in a medium other than 10% neutral buffered formalin, the validation study must show that results are concordant with results from formalin-fixed tissues.

1.1.2.3. If significant changes are made in testing methods (e.g. antibody clone, antigen retrieval protocol or detection system, FISH probe or pretreatment protocol), revalidation is required.
What is written in your procedure?

1. PURPOSE: To establish guidelines for monitoring quality and of antibody, probes and polymers from lot to lot.

2. PRINCIPLE: It is our belief that reagents should be checked prior to use to confirm reagent quality.
   
   2.1. BACKGROUND INFORMATION:
   
   2.1.1. College of American Pathology (CAP) requires antibody, probe validation and new reagent lot verification.

3. STANDARDS:
   
   3.1. Before new antibodies can be performed in the IHC lab, the following steps must be completed.
   
   3.1.1. New antibodies have to be approved by the Director of the IHC Lab and a Reagent or Method Change/Addition form must be submitted.

   3.1.2. Once the form is approved by the Administrative Director and Pathology Division Chief, a method validation procedure for hospital labs must be completed.
   
   3.1.2.1. Use the titer range and type of tissue recommended by the manufacturer to decide what titer range and control tissue should be tested.

   3.1.2.2. A minimum of 3 dilutions should be tested to find the optimum conditions using the recommended retrieval. In some cases there may be a need to extend the dilution or change retrieval methods.

   3.1.2.3. The Director will need to approve the titer before continuing.

   3.1.2.4. Once the titer has been established, recommendation of 10 positive tissues and 10 negative tissues will need to be tested. However, the Pathologist can choose to do a less than 20 cases be sufficient. Results should show positive tissue specific for that antibody and negative tissue showing no staining. One positive tissue should also be tested 3 times in one run to show reproducibility. Document all results on the antibody validation form and put in antibody notebook.

   3.1.2.5. A method validation form is completed on-line with documentation of validation and written procedure.

3.2. Lot to lot verification of polymer detection kits should be completed as follows.
In Summary

1. Research your antibody before buying to make sure it provides you with the results you are needing and that the antibody will work easily with your system.

2. Make sure you validate our antibody to meet CAP requirements.

3. Your written procedure explains how you validate your antibodies in the laboratory.

4. You can answer CAP questions involved in validation of antibodies.

5. Make the process as easy as possible and use the same process for all antibodies.
References:

www.cap.org
www.biocare.com
www.cellmarque.com
www.thermofisher.com

Jackie Brooks- Cunningham Pathology Birmingham, AL
Questions: