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# HistoTEX*as Newsletter

INSIDE THIS ISSUE:



## Regulatory Requirements for Gross Examination of Tissues

**By** Dale Telgenhoff, PhD, HTL(ASCP)CM Histotechnology Program Director, Tarleton State University

The activities of the histotechnician in the gross room have been primarily focused on assisting the pathologist or pathologist assistant in the performance of their grossing duties. This has included the accessioning of specimens, labelling cassettes, preparing specimens for review, stocking, cleaning, and disinfecting the instrumentation. Due to the demand for faster turn-around-times on pathology reports the pathologist has largely stepped out of the gross room, passing the duties on to the pathologists' assistant. It is important to note that these highly skilled individuals are in high demand, especially considering there are only eleven NACCLS accredited pathologists' assistant schools in North America, with the closest one to Texas being in Indianapolis, Indiana. Considering the difficulty of a pathology practice to staff the gross room, it is not surprising that many practices have begun training histotechnicians and histotechnologists to take on some of the grossing duties (Dimenstein 2013).

The College of American Pathologists (CAP) issues regulations on the gross examination of tissues (CAP 2014), as does the Centers for Medicare and Medicaid Services (CMS) which are based on the CLIA regulations of 1988 (CMS 2003). Specifically, the CAP Laboratory Accreditation Checklist for the Anatomic Pathology (ANP) laboratory states that gross examination of tissues must be performed by a pathologist or under the supervision of a qualified pathologist. When grossing is performed by a non-pathologist there must be a documented protocol that dictates the extent of the non-pathologist's duties (ANP.11605), and the non-pathologist must qualify for "High Complexity Testing" under the CLIA regulations (ANP.11610).

Continued on page 2.

## Nominations for 2015-2017 TSH Executive Board Members

2015 is an election year and the TSH Nominating Committee is preparing a slate of candidates to run for office for the term of 2015 to 2017. The nominating committee is looking for members who are willing to share their time, talents and efforts in continuing support of our society. We need members who will continue the development and promote the Texas Society for Histotechnology to the level of quality and excellence it has enjoyed for many years. For qualifications and duties of all positions, check the TSH website, www.txsh.org. Please note that nominees will need to be a member in good standing, so be sure to get your membership applications and fee turned in by December 31. If you have any questions please call or email me. Fax/email/mail completed nominations forms to: fax 903-315-2044; email bedakaye@yahoo.com , mail to 1413 Centenary Drive, Longview, TX 75601. Nominations must be received by **December 10, 2014**. The ballots will be mailed on December 15, 2014. The Nominations form can be found at the end of this newsletter on page 14.

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# Grossing Requirements (continued from page 1)

CLIA'88 regulations for High Complexity Testing include the following requirements: current state license in states requiring licensure (not Texas), and an associate's degree in laboratory science from an accredited institution or 60 semester hours from an accredited institution that includes 24 hours of medical laboratory courses or 24 hours of science courses (biology and chemistry) and a minimum of three months documented training in grossing (Subpart M, CFR 493.1441-1495).

Histotechnicians who were grossing prior to September 1st, 1997 were grandfathered in, and were only required to have earned a high school diploma or equivalent and documented training in grossing duties. This rule was changed in 2011, and now all non-pathologists performing grossing duties must meet the current high complexity testing requirements as outlined above. The exception to this is that if an individual has been grossing specimens prior to 1997 they may continue to do so providing that they are "performing the exact same duties in the exact same way" (Mateski 2013). If their duties have changed in any way since September 1, 1997 (new format for dictations, different types of specimens, etc.), then they must meet the requirements for high complexity testing.

Another source of confusion was the differentiation some labs placed on the types of tissue specimens entering the laboratory. Small specimens that only required 2-3 sentences of dictation and were placed in cassettes either "in toto" or bisected were defined as specimens for "processing", not grossing. This allowed laboratories to circumvent the requirement for high complexity testing personnel processing these tissues. What made the matter even more confusing was the endorsement by CAP of this practice. To clear the confusion CMS issued a formal statement in 2010 declaring the separation between "processing" and "grossing" was contrary to the CLIA regulations, and that no clear distinction should exist (Yost 2010). CAP agreed, and in 2011 changed their accreditation checklist to include both practices as high complexity testing.

Although the distinction between processing and grossing has been discontinued from a regulatory standpoint, it still serves as an important division when considering tissues for grossing by a non-pathologist or pathologists assistant. Programs training histotechnicians to gross begin with these tissues which previously fell into the processing category, as they have a straightforward dictation template and minimal opportunity for misinterpretation of the gross findings (Telgenhoff 2014). They include specimens such as GI biopsies, breast cores, bronchial biopsies, endometrial biopsies, and other small tissues from uncomplicated cases. For some labs, this can significantly reduce the number of tissues being grossed by the pathologists' assistant, leaving them more time for complex cases and other duties. It also gives the histotechnician another area to rotate, and opens the possibility for greater career satisfaction and advancement. Although the regulatory requirements for histotechnicians grossing have been clearly defined, it is also important to consider the training required when moving staff into these positions. Most hospitals with training programs for histotechnicians grossing recommend a course in surgical pathology or in-house training equivalent to a course in surgical pathology with study materials and competency assessment. Additional training should include job shadowing, a review of grossing protocols for each tissue, and continuing education. All of these trainings should be carefully documented in the individual's personnel file, and available for CAP and CLIA inspection. Implementation of grossing by histotechnicians should follow an orderly progression which begins with discussions among all interested parties, a careful review of the CAP and CLIA guidelines, generation of grossing protocols for all potential tissues encountered, and a documented training program with periodic review and continuing education (Cortinas and Lamphere 2014) .

References continued on next page

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**IHC Validation Guidelines and the Role of Tissue Microarrays**
**Written By Andre Jordan Sanchez, B.S., M.B.A**
**Edited by Dr. Alfonso Heras and Dr. Regan Fulton**

**Background**

It is no secret by now that immunohistochemistry has experienced a tremendous amount of growth since its initial applications as a research tool in the 1960's. Immunohistochemistry (IHC), is often referred to as the "gold standard" in tissue-based diagnostics. IHC is so clinically accepted that since 1963, the number of publications that include the term "immunohistochemistry" has increased 40 fold to over 122,000(1). Additionally, it is expected that by 2018, "tissue based diagnostics" technologies (the bulk of which is IHC) will grow about 7% annually (2).

A lot of recent growth can be attributed to recent advances in personalized medicine and immunotherapy. Human genome analysis has given researchers a better understanding of how genes play a role in cancer biology, and has led to explosive growth in the fields of proteomics, genomics, and bioinformatics. Easy access to genomic information has pushed clinics to integrate new diagnostic IHC testing into the laboratory setting, and subsequently pressured manufacturers to produce larger portfolios of biomarkers validated for use in IHC.

**IHC Laboratories Today**

Despite the tremendous growth in IHC as a vital clinical diagnostic technology, many challenges remain. Recent Medicare payments for anatomic pathology have drastically changed (3), with an average decline of reimbursement at about 24%(4). In the wake of recent reimbursement cuts, there has been a continued industry emphasis of "doing more with less", which has put pressure on pathology labs to become more efficient.

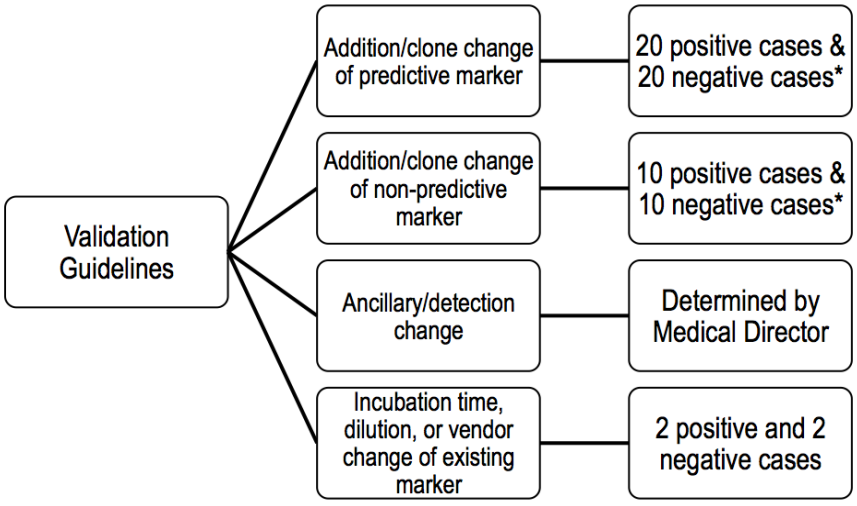
The increased pressure to implement larger portfolios of clinically validated biomarkers while taking reduced reimbursement has lead anatomic pathology labs to consider switching to more cost-effective reagents. Switching vendors, adding new antibodies, and changing other variables in the IHC process led to a growing question; what guidelines should be followed when validating a new biomarker or reagent in the clinical laboratory? How does a lab ensure that it is following a standardized protocol to integrate said products? How many patient samples should be used when validating reagents for use in IHC?

**IHC Validation Guidelines**

To address these concerns, The College of American Pathologists (CAP) released new IHC Validation guidelines earlier this year (5). The new validation guidelines are meant to improve patient care, ensure accurate testing and standardize previous ambiguous requirements for IHC validation.

How were the new CAP validation guidelines established? An expert panel of pathologists and histotechnologists conducted a systematic review of more than 125 publications covering almost 1500 citations to see how IHC validation standards could be improved. Additionally, the new CAP guidelines may help provide a framework for validating molecular and genomic-based assays, which is particularly important as diagnostics (and IHC) becomes increasingly focused on immunotherapy and companion diagnostics technologies. (6)

**Figure 1 (Below) – Chart displaying IHC Validation Guidelines**




* Justification for less than the noted amount of cases must be documented by lab director/manager.

So what are the Guidelines, and when should they be implemented? CAP recommends that **all** IHC tests be validated using one of the recommended guidelines before placing a product (antibody, reagent or ancillary) into clinical service (See Figure 1). The only exceptions to the CAP IHC validation guidelines are the ER, PgR and HER2 antibodies (which already have well defined guidelines set by ASCO & CAP).

Although the CAP guidelines are very thorough, there are some key take-away points for labs performing IHC in anatomic pathology. (7)

- 90% overall concordance should be achieved between a new test/biomarker and the old test/biomarker. A rate below 90% needs to be met with a laboratory investigation.
- If the marker is a non-predictive assay, 10 positive and 10 negative tissues should be tested. Should there be less than 20 cases (particularly for rare antigens), then the decision to use fewer cases should be documented.
- If the marker is a predictive assay, 20 positive and 20 negative cases should be tested. If there are less than 40 cases, the decision should be documented.
- If the marker has predictive and non-predictive characteristics, then treat the marker as a predictive assay and defer to the 40 case requirement (20 negative, 20 positive) as indicated.
- When switching a clone, revalidation should be treated as a new predictive (40 case) or non-predictive (20 case) assay.
- Incubation time, dilution, or manufacturer change (same clone) should be re-validated using 2 positive and 2 negative cases.
- Fixative, antigen retrieval, detection chemistry, tissue processing, equipment, relocation, and water supply changes require the laboratory medical director to establish the quantity of positive and negative cases to be used.
- **Tissue Micro Arrays can be used when appropriate.**

Initially, these changes may intimidate some lab managers, particularly those who operate smaller labs that are already adjusting to a changing reimbursement landscape. However it is important to highlight a point made by the CAP IHC validation guidelines; Tissue Microarray's **can** be used to help laboratories meet the new guidelines.

**What is a Tissue Microarray?**

So what is a Tissue Microarray? A Tissue Microarray (TMA) is a formalin-fixed paraffin-embedded tissue block comprised of several different tissues, or "cores". Each "core" represents a segment of tissue (usually chosen by the pathologist) taken from a tissue "donor block". Multiple cores can be affixed to a slide, and generally vary in size from 0.6 to 7 mm in diameter. The quantity of cores selected for TMA's can vary tremendously, from as little as two to as many as hundreds of tissue cores on one slide. Tissue microarrays let a researcher, pathologist or technician improve workflow by the testing of several cores on one slide, instead of the more traditional one sample per slide. Additionally, cell lines can also be substituted for tissues, leading to the construction of a cell line microarray. Such a microarray may prove to be useful for labs that may need to validate infectious reagents where traditional tissue cases may be hard to procure, such as Helicobacter Pylori, Adenovirus, or SV-40 (See Figure 2).

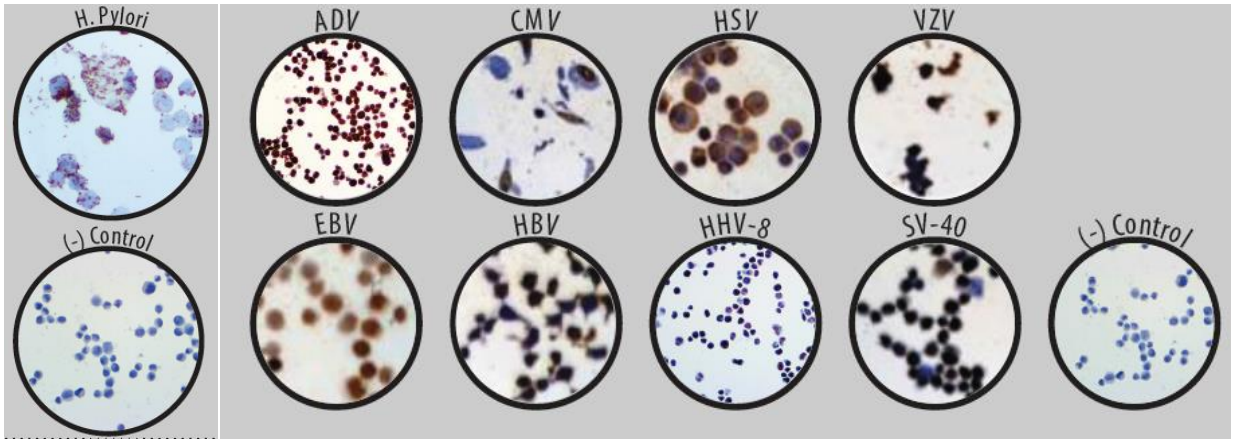



**Figure 2 (Left) – Cell Line Microarray designed for use with Infectious reagent validation in Immunohistochemistry. Cell line Microarrays should include a negative control to avoid false staining.**

**TMA Advantages**

The advantages of TMA integration into an IHC laboratory can be readily seen, as it allows for a more robust workflow. Additionally, incorporation of TMA's also helps lab conserve often-expensive reagents, as a TMA with multiple cores requires a similar amount of reagent as a similar size whole tissue sample.

Another advantage of TMA's is their flexibility. Due to the fact that TMA's are composed of tissues from selected "donor blocks", the variables and permutations allowed by TMA construction are almost endless. Tissue microarrays can be customized to certain specifications and include any number of tissue samples per lab requirements. Donor tissues for TMA construction can be composed of one tissue type (e.g. a TMA comprised solely of breast tissue) or a variety of different tissue types (e.g. multi-normal Breast, Prostate, Liver, etc.). Different types of TMA's may prove to be useful in different capacities. TMA's of varying tissue types are ideal for research or clinical biomarker discovery, while a TMA constructed from one tissue type may be ideal for quality control and validation (See Figure 3).

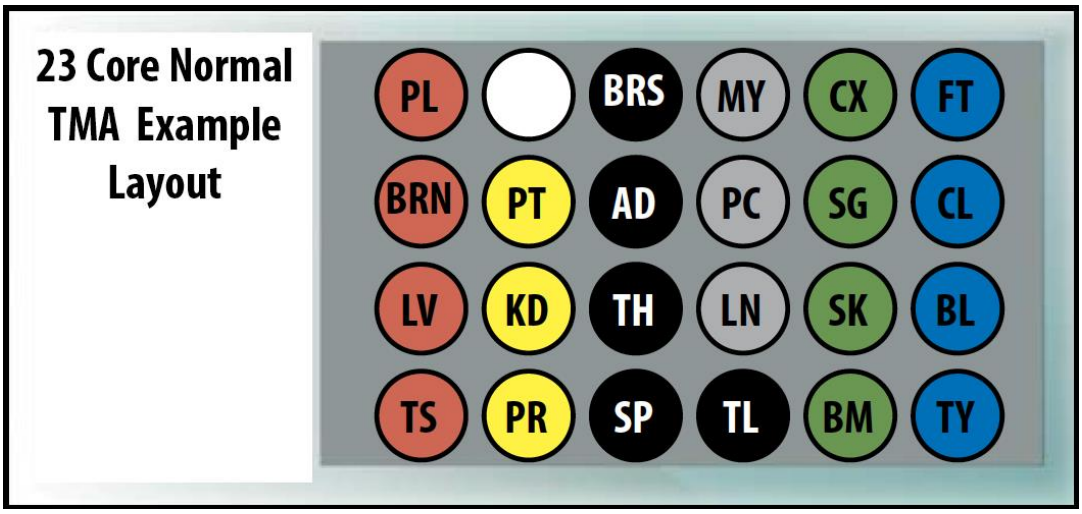



**Figure 3a (Above) - 23 Core Normal TMA Layout composed of multiple tissues. This TMA is ideal for research or clinical biomarker discovery.**

| | | | | | |
|---|---|---|---|---|---|
| PL - Placenta | Blank | BR - Breast | MY - Myometrium | CX - Cervix | FT - Fallopian Tube |
| BR - Brain | PT - Pituitary | AD - Adrenal | PC - Pancreas | SG - Salivary | CL - Colon |
| LV - Liver | KD - Kidney | TH - Thyroid | LN - Lung | SK - Skin | BL - Bladder |
| TS - Testis | PR - Prostate | SP - Spleen | TL - Tonsil | BM - Bone Marrow | TY- Thymus |

**Figure 3b (Above) - Corresponding TMA Map to Figure 3a. Above Normal Tissues validated with over 100 biomarkers used in IHC.**

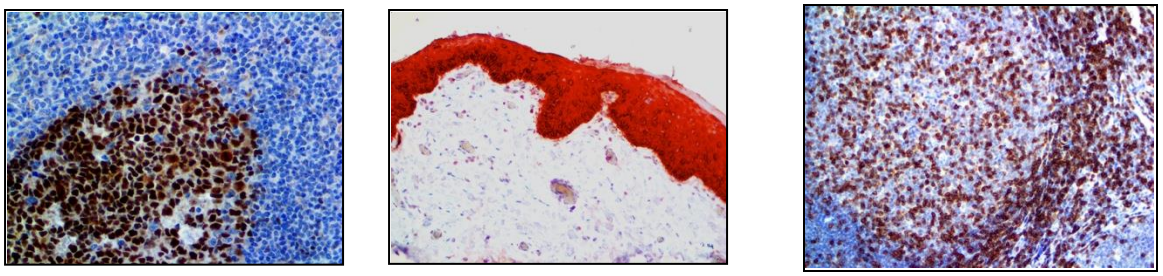


**Figure 4 (Above) - Example images of IHC Stains taken from tissue microarray slides. From left to right: bcl-6 on tonsil core (DAB), CK 5/6 on skin core (AEC), and CD3 on tonsil core (DAB).**

TMA's can also help reduce the burden on laboratories that are short-staffed yet have a need to re-evaluate IHC antibodies & reagents to cut costs. For example, if a new antibody released to the market is to be incorporated into a lab using CAP Guidelines, a technician could use two 20-core TMA slides (with 20 positive and 20 negative cases), instead of using 40 tissue slides that use whole tissue sections. This not only helps reduce reagent use and put less pressure on technical staff, but also reduces validation workload by about 95%.

**Addressing TMA Concerns**

However, there are some concerns that typically arise when discussing the integration of tissue microarrays. Pathologists and technicians are accustomed to working with whole tissue sections, and the idea of using TMA's in a clinical setting can sometimes raise questions, particularly with tumor heterogeneity. Can a "core" of a tissue serve as a viable substitute for a whole tissue section? Although TMA cores are smaller in size than whole tissue sections, many studies have shown that tumor heterogeneity is not a significant concern with most cancers (8,9). Of course, it is important to note that there are some exceptions to this rule (such as Glioblastomas) (10).

Another concern is the effort that goes into constructing a TMA. TMA's require a sometimes-burdensome workload on already overworked anatomic pathology laboratories. Constructing a TMA block that serves a specific purpose involves a lot of communication between a pathologist, histotechnician and staff. The amount of time, equipment, effort and expertise required to construct a TMA can prove to be a challenge for an already pressured lab.

**TMA Integration and Conclusion**

So how can a lab overcome challenges presented by validation and integrate TMA's into the laboratory? Commercially available TMA's that are cost-effective, IHC validated, and meet validation requirements present an opportunity for the modern anatomic pathology lab.

Not only will such products allow anatomic pathology labs meet IHC CAP validation guidelines, but will help address concerns of tissue supply and quality control, particularly when trying to incorporate IHC reagents from vendors that may supply unique or cost-effective products.

Additionally, TMA's allow laboratories to conduct more thorough biomarker discovery research, and see reactivity across several different tissue types on one slide. Tissue Microarrays already play a pivotal role in drug discovery, and with the list of clinically validated biomarkers in IHC growing, TMA's will undoubtedly play a greater role in today's diagnostic laboratory. (11)

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Insert: Dr. Alfonso Heras is president and CEO of Bio SB. Andre Sanchez B.S., M.B.A. is Director of Marketing at Bio SB, an FDA and ISO 13548:2003 approved facility located in Santa Barbara, California. Bio SB performs R&D, production, distribution and marketing of unique products for molecular pathology including Tissue and Cell Line Microarrays, IVD antibodies, detection systems and ancillaries for use in Immunohistochemistry. Cancer Diagnostic, Inc. distributes BIO SB's IHC products in the United States.

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