WHITEPAPER

Simplifying the Process of ‘Switching’ Antibodies and Verifying the Performance of a Modified IHC Procedure

BIOCARE MEDICAL

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Has your laboratory ever considered 'switching' from one vendor's antibody to another to obtain improved results and/or reduce reagent costs, but wondered what doing so might mean in terms satisfying various regulatory-agency standards? If so, you probably know that all immunohistochemistry (IHC) procedures employed for clinical-diagnostic purposes must be appropriately validated prior to placing them into service. However, a relatively new approach advocated by the College of American Pathologists (CAP) may simplify the verification of procedures in which use of the same antibody clone is an integral part of the 'switch'.

The process of validating all laboratory procedures – including IHC – is outlined in U.S. Clinical Laboratory Improvement Amendments (CLIA) regulations[1], and, for laboratories that are voluntarily accredited by the CAP, outlined in that organization's IHC-specific standards. As most individuals who work in the IHC area of a diagnostic pathology lab will agree, the process of validating an IHC procedure is time-consuming, relatively expensive, and often requires that several pathologists – who may not agree on the microscopically-observed reactivity of a given IHC procedure – review and approve the applicable staining protocol. Clearly, subjectivity to the interpretation of IHC assays can affect how well a modified procedure might be accepted.

Another reason that the validation process can be challenging is because it requires a significant amount of effort to identify positive- (and negative-) 'control' specimens that should be stained in order to confirm that the assay is capable of identifying different biological patterns of expression for a given protein. In other words, finding enough of the 'right' positive/negative control tissues can be difficult, especially in labs that only receive a few types of specimens, such as in gastroenterological, dermatological, and urological environments. So, if ways can be identified in which to reduce the quantity of specimens/slides that must be tested, doing so is a very worthwhile objective.

One of the most common and long-held beliefs is that initial IHC procedure validation must involve testing 10 to 20 'positive' specimens, each with varying degrees of reactivity. The truth is that the quantity of positively reactive and negatively reactive specimens that are tested during the validation process is at the discretion of each lab's medical director and must be described in the lab's policy/procedure manual. So, what about changing from one source (vendor) of a given antibody to another? Does this require a lab to "start from scratch" and determine the effectiveness of the modified procedure in the same manner as they would for an altogether new assay (antibody)? The answer to that question is "maybe" and depends on the nature of the procedural modification, as described in more detail below. To reduce any confusion that may exist with the associated terminology, the process of confirming the effects of modifying a previously validated IHC assay should be referred to as "verification" rather than "re-validation".

Although a great deal of information is available in the published literature on the process of initial procedure validation[2-4], until fairly recently, very little guidance was available on the steps that should be taken when only one or two parameters of a previously-validated procedure – such has heat-induced epitope retrieval (HIER) reagents/methods or the antibody – are modified. Prior to the publication of procedure-verification standards[5] by the CAP in 2014, each laboratory had to decide, and describe in policy form, the procedural changes that triggered the need to verify the performance of a modified IHC assay. Then, through its updated standards, the CAP began recommending that:

A) "When an existing validated assay has changed in any one of the following ways – antibody dilution, antibody vendor (same clone), or incubation or retrieval times (same method) – the laboratory should confirm assay performance with at least 2 known positive and 2 known negative cases; and

B) 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), or laboratory water supply – laboratories must confirm assay performance by testing a sufficient number of cases to ensure that assays consistently achieve expected results".

Based on these new guidelines, it should be clear that a laboratory is not required to "start from scratch" when verifying the performance of a modified procedure, especially when doing so involves the same antibody clone as was used in the previously validated procedure. And what if your lab is not accredited by the CAP? In this case, since CLIA regulations do not specifically address the verification of modified IHC procedures, it is simply a good practice to follow well-established guidelines such as those developed by the CAP[5,6].

So, what exactly does 'post-modification' procedure verification involve? The most important steps include:

1) Testing a small set of positive and negative specimens and comparing the staining results of the modified assay with the staining results of the existing assay – with the goal being that the modified assay is 'as good as' (if not better) than the existing assay;

2) Documenting the parameters that were modified and the outcome of the testing; and

3) Documenting the approval of the modified and verified assay by the lab's medical director before it is placed into routine clinical use.

An example of the information that might be gathered during the procedure verification process is shown in sample form shown on next page:

Table 1

Facility:_____ Target Antigen/Clone:_____

Performed/Completed By: _____ Date: ____/ ____/ ____

	Original Protocol Parameters			Modified Protocol Parameters		
Step-wise Procedure	**Reagent**	**Time**	**Temp.**	**Reagent**	**Time**	**Temp.**
Fixation						
Pretreatment						
Primary Antibody						
Secondary / Link						
Teritiary / Label						
Substrate-Chromogen						

The procedural changes described above resulted in: _____ Comparable Results _____ Better Results

Approved By: _____ Date: ____/ ____/ ____

In conclusion, verifying the performance of a modified IHC procedure is not as difficult as once thought The keys are understanding the expected effects of the proposed procedural change before proceeding, carefully evaluating the actual effects during the testing and verification process, and ensuring the laboratory's medical director approves the procedural change. For additional insight into the validation and verification of IHC procedures, please review the guidelines outlined in the publication that led to the new CAP standard[6].

If your lab is considering modifying an existing IHC procedure to obtain improved staining results and/or reduce reagent costs, the table below outlines the similarities and differences between the antibody clones offered by Biocare Medical and several other leading antibody vendors. Please call Biocare Medical at 1-800-799-9499 or visit our website at www.biocare.net for additional information.

1. Clinical Laboratory Improvement Act (CLIA) Regulations: 42CFR493.1253 through 493.1281. (http://wwwn.cdc.gov/clia/regs/toc.aspx).

2. Taylor CR and Cote RJ, eds. Theoretical and Practical Aspects of Test Performance. In Immunohistology: A Diagnostic Tool for the Surgical Pathologist, 3rd. ed., Volume 19 In Major Problems in Pathology. Philadelphia, PA: W.B Saunders, 2005.

3. Goldstein, NS, *et.al*. Recommendations for improved standardization in immunohistochemistry. Appl Immunohistochem Molec Morph 2007;15:124-133.

4. Quality Assurance for Design, Control, and Implementation of Immunohistochemistry Assays: Approved Guideline, Second Edition; Vol. 31, No. 4, 2011. Clinical and Laboratory Standards Institute (CLSI), Wayne PA, USA; Publication code: I/LA28-A2.

5. Anatomic Pathology Checklist, 2014. Laboratory Accreditation Program. College of American Pathologists. Waukegan, IL.

6. Fitzgibbons, *et.al*. Principles of analytic validation of immunohistochemical assays: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Arch Pathol Lab Med 2014;138:1-12.

Antibody Clones Offered by Different Vendors		Biocare Medical	Agilent	Leica	Cell Marque	Roche
Antibody Name	**Clone**	**SKU / Code**	**SKU / Code**	**SKU / Code**	**SKU / Code**	**SKU / Code**
Actin, Muscle Specific	HHF35	PM079AA	IR70061-2	PA0258	201M-98	760-2601
Actin, Smooth Muscle	1A4 (or ASM-1)	PM001AA	IR61161-2	PA0943	202M-98	760-2833
Adipophilin	Polyclonal	API3138AA			393A-18	
ALK	5A4	API3041AA		PA0306		
Arginase-1	EP261	API3058AA			380R-28	
bcl-2	100/D5	PM003AA		PA0117		
bcl-6	LN22	PM410AA		PA0204		
Ber-EP4	Ber-EP4	PM107AA	GA63761-2		248M-98	760-4383
Calcitonin	Polyclonal	PP072AA		PA0406	229A-18	760-2611
Calponin	CALP	PM172AA			231M-18	
Calretinen	Polyclonal	PP092AA			232A-78	
CD3	LN10	API3152AA		PA0553		
CD4	4B12	API3148AA	IR64961-2	PA0427		
CD8	SP16	PRM311AA			108R-18	
CD8	C8/144B	API3160AA	IR62361-2		108M-98	
CD15	MMA	PM029AA		PA0473	115M-18	760-2504
CD20	L26	PM004AA	IR60461-2	PA0359	120M-88	760-2531
CD30	Ber-H2	PM031AA	IR60261-2		130M-98	790-4858
CD34	QBEnd/10	PM084AA	IR63261-2	PA0212	134M-18	790-2927
CD45 (LCA) [Cocktail]	PD7/26 + 2B11	PM016AA			145M-98	GA75161-2
CD68	KP1	PM033AA	IR60961-2		168M-98	790-2931
CD99	EP8	PME392AA			AC-0013	
CD117 (c-Kit)	EP10 or Y145	PME296AA			AC-0029	
CD163	10D6	PM353AA		PA0090		
Chromogranin A [Cocktail]	LK2H10 + PHE5	PM010AA			238M-98	
CDX2	EP25	API3144AA		PA0375	AC-0008	
CEA	COL-1	PM058AA		PA0848		
Cyclin-D1	EP12	PME432AA	IR08361-2		241R-48	PA0046
Cyclin-D1	SP4	PRM307AA			241R-18	
Cytokeratin 7	OV-TL 12/30	PM061AA	IR61961-2			
Cytokeratin-LMW (AE1)	AE1	PM081AA				760-2521
Cytokeratin-LMW (8/18)	EP17 + EP30	API3061AA	IR09461-2			
Cytokeratin-HMW (34BE12)	34BE12	PM127AA	IR05161-2	PA0134		760-2637
Cytokeratin 18	DC10	API3061AA	IR61861-2			
Cytokeratin 20	Ks20.8	PM062AA	IR77761-2	PA0022		

Antibody Clones Offered by Different Vendors		Biocare Medical	Agilent	Leica	Cell Marque	Roche
Antibody Name	**Clone**	**SKU / Code**	**SKU / Code**	**SKU / Code**	**SKU / Code**	**SKU / Code**
Cytokeratin, Pan	AE1/AE3	PM011AA	IR05361-2	PA0094		
D2-40	D2-40	PM266AA	IR07261-2			760-4395
E-Cadherin	EP700Y	API3012AA				760-4440
EMA	E29	API3038AA			247M-98	790-4463
GATA-3	L50-823	PM405AA			390M-18	760-4897
GFAP	GA-5	PM065AA		PA0026		
Glypican-3	1G12	PM396AA			261M-98	760-4442
HMB45 (Melanosome)	HMB45	PM057AA	IR05261-2	PA0027	282M-98	790-4366
Inhibin	BC/R1	PM171AA	IR05861-2		271M-18	
Kappa	L1C1	API3149AA			274M-98	
Ki-67	MIB-1	API3156AA	IR62661-2			
Mammaglobin	1A5	PM269AA		PA0802	280R-18	760-4263
MART-1 (Cocktail)	M2-7C10 + M2-9E3	PM077AA			281M-98	
Melan-A	A103	API3114AA	IRR63361-2	PA0233	281M-88	790-2990
Melanoma Cocktail (HMB45 + MART-1)	HMB45 + M2-7C10 + M2-9E3	PM078AA			903H-08	
MiTF	34CA5	PM423AA		PA0803		
MOC-31	MOC-31	PM403AA	M352501-2	PA0797	248M-18	790-4561
MSH2	FE11	PM219AA	M363901-2			
MSH6	44	PM265AA			287M-18-ASR	
Myeloperoxidase	Polyclonal	PP023AA	IR51161-2		289A-78	760-2659
Napsin A (P)	Polyclonal	PP434AA			352A-78	760-4446
Neurofilament	2F11	PM066AA	IR60761-2		302M-18	760-2661
NKX3.1	EP356	API3189AA			441R-18	
p40	BC28	AVI3066KG				790-4950
p63	4A4	VP163G				PM163AA
PAX8	Polyclonal	PP379AA			363A-18	
PD-1	NAT105	API3137AA			315M-98	760-4895
PD-1	EP239	API3162AA			315R-18	
PMS2	A16-4	PM344AA				790-5094
PSA	EP109	PME390AA			324R-18	
S100	4C4.9	API3237AA		PA0820	330M-18	790-2914
TTF-1	8G7G3/1	PM087AA	IR05661-2		343M-98	790-4398
Vimentin	V9	PRM312AA	IR63061-2	PA0640	347M-18	790-2917
Vimentin	SP20	PM048AA			347R-18	