Validation and verification process for IHC

What, why and how ?

Donald Van Hecke
Dept. Anatomo Pathology Lab
AZ St. Lucas – Brugge
Belgium

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Validation – Verification : WHAT ?

Validation and Verification : What ?

Validation :

Demonstrate by means of objective evidence that performance-characteristics fullfill predefined criteria or specifc demands for a certain purpose or intended use.

Which implies :

- Validation performed by “manufacturer”
- (Full) validation done by the lab

Validation and Verification : What ?

Verfication :

Confirmation by providing objective evidence that a test fullfils specifications (specific demands) or specified performance characteristics/parameters.

Which implies :

- Specific demands/perfomance characteristics are defined and validated by manufacturer
- Verification of performance characteristics performed by lab

Validation and Verification : What ?

Validation

Demonstrate by means of objective evidence that performance-characteristics fullfill predefined criteria or specifc demands for a certain purpose or intended use.

Verification

Confirmation by providing objective evidence that a test fullfils specifications (specific demands) or specified performance characteristics.

Validation and Verification : What ?

Validation

Demonstrate by means of objective evidence that performance-characteristics fullfill predefined criteria or specifc demands for a certain purpose or intended use.

Verification

Confirmation by providing objective evidence that a test fullfils specifications (specific demands) or specified performance characteristics.

What is the difference ?

Validation and Verification : What ?

1 Validation or verification : which one ? → Determined by type - source : FDA/CE-IVD or LDT

In essence you need to proof “a test does what it needs to do” or “what it is intended for”.

FDA / CE – IVD according to Instructions For Use (IFU)

↓

Verification

↓

Performance characteristics and acceptance criteria

Any deviation from IFU / use of non FDA/CE-IVD (LDT)

↓

Validation

↓

Performance characteristics and acceptance criteria

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT and thus if verification or validation needed

What is "according to IFU" ?

- Type sample and purpose/intended use defined
- Clear instructions :
  - Pretreatment
  - Dilution of Ab
  - Incubationtimes Ab, detection system
  - Enhancingstep (linker)
  - Preprogrammed or full description of method ?

→ Always that clear – obvious – well described ?????

→ Know if you work according to IFU or not ?????

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

VENTANA®

PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody

REF 790-2991

05278368001

IVD Σ 50

INDICATIONS AND USE

Intended Use

This antibody is intended for in vitro diagnostic use.

Ventana Medical Systems, Inc.'s (Ventana) PATHWAY anti-HER-2/*neu* (4B5) Rabbit Monoclonal Primary Antibody (PATHWAY HER2 (4B5)) is a rabbit monoclonal antibody intended for laboratory use for the semi-quantitative detection of HER2 antigen in sections of formalin-fixed, paraffin-embedded normal and neoplastic tissue on a VENTANA automated immunohistochemistry slide staining device. It is indicated as an aid in the assessment of breast cancer patients for whom Herceptin treatment is considered.

Table 2. Recommended Staining Protocols for PATHWAY anti-HER-2/*neu* (4B5) with *ultra*View Universal DAB Detection Kit.

Procedure Type	Platform or Method	
	BenchMark XT instrument	BenchMark ULTRA instrument
Baking	None	None
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild	ULTRA CC1, mild
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 16 minutes, 37°C	Approximately 12 minutes, 36°C
Counterstain (Hematoxylin)	Hematoxylin II, 4 minutes	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes	Bluing, 4 minutes

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

Dako

FLEX
Monoclonal Mouse
Anti-Human
BCL6 Protein
Clone PG-B6p
Ready-to-Use
(Dako Omnis)

Code GA625

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human BCL6 Protein, Clone PG-B6p, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry (IHC) together with the Dako Omnis instrument. Results aid in the classification of diffuse large B-cell lymphoma, follicular lymphoma and Burkitt's lymphoma (1). Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Specimen preparation

Paraffin sections: The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of 4 µm.

Pre-treatment: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections with heat-induced epitope retrieval (HIER) is required. Pretreating tissues with HIER using diluted EnVision FLEX Target Retrieval Solution, High pH (50x) (Dako Omnis), Code GV804, is recommended. Deparaffinization, rehydration and target retrieval are performed onboard Dako Omnis. Please refer to Dako Omnis Basic User Guide.

Staining procedure overview*

Step		Comments
Fixation/embedding	Formalin-fixed, paraffin-embedded	Onboard deparaffinization
Pre-treatment	EnVision FLEX, High pH (Code V804)	30 in HIER
Antibody	Ready-to-use	12.5 min incubation
Negative Control	FLEX Negative Control, Mouse (Code GA750)	12.5 min incubation
Visualization	EnVision FLEX (Code V800) + EnVision FLEX+ Mouse LINKER (Code V821)	Block: 3 min; Link: 10 min; Polymer: 20 min; Chromogen: 5 min
Counterstain	Hematoxylin (Code C808)	3 min incubation
Control Tissue	Tonsil	Nuclear staining
Slides	FLEX IHC Microscope Slides (Code 8020)	Recommended for greater adherence of tissue sections to glass slides
Mounting	Non-aqueous, permanent mounting required	After staining, the sections must be dehydrated, cleared and mounted using permanent mounting medium
Instrumentation	Dako Omnis	Reagents are provided in instrument-specific vials

**The user must always read the package insert for detailed instructions of the staining procedure and handling of the product.*

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

Novocastra™ Liquid Mouse Monoclonal Antibody Prostate Specific Antigen

Product code: NCL-L-PSA-431

Intended Use

For in vitro diagnostic use.

NCL-L-PSA-431 is intended for the qualitative identification by light microscopy of human prostate specific antigen in paraffin sections. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Specimen Preparation

The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

Recommendations On Use

Immunohistochemistry on paraffin sections.

Suggested dilution: 1:100 for 30 minutes at 25 °C. This is provided as a guide and users should determine their own optimal working dilutions.

Visualization: Please follow the instructions for use in the Novolink™ Polymer Detection Systems. For further product information or support, contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems Web site, www.LeicaBiosystems.com

The performance of this antibody should be validated when utilized with other manual staining systems or automated platforms.

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

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Specimen Preparation

Do I use 10 % NBF ?

The recommended fixative is 10% neutral-buffered formalin for paraffin-embedded tissue sections.

Recommendations On Use

Immunohistochemistry on paraffin sections.

Incubation temperature on my staining platform ?

Suggested dilution: 1:100 for 30 minutes at 25 °C. This is provided as a guide and users should determine their own optimal working dilutions.

Visualization: Please follow the instructions for use in the Novolink™ Polymer Detection Systems. For further product information or support, contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems Web site, www.LeicaBiosystems.com

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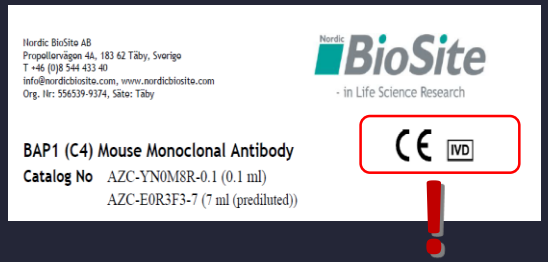
Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT



Materials Provided

BAP1 (C4) Mouse Monoclonal in concentrated form or prediluted

Antibody Specifications Antibody as Purified antibody diluted in Tris-HCI buffer containing stabilizing protein and

Host Mouse

Isotype IgG1 /κ

Immunogen Synthetic peptide against 430-729 of human BAP1

Cellular Localization Nuclear

Concentrate Dilution Range 1:100

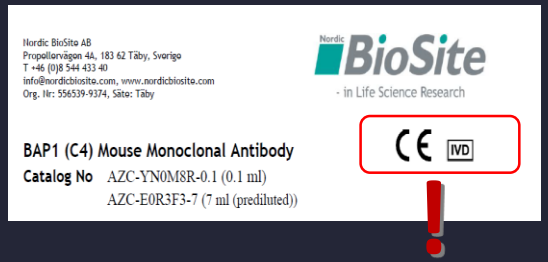
Positive control Malignant Mesothelioma

Optimization :
1: 50 or 1: 200 ?

According to IFU ?

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT



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Antibody Specifications Antibody as Purified antibody diluted in Tris-HCI buffer containing stabilizing protein and

Host Mouse

Isotype IgG1 /κ

Immunogen Synthetic peptide against 430-729 of human BAP1

Cellular Localization Nuclear

Concentrate Dilution Range 1:100

Positive control Malignant Mesothelioma

4. The user must validate incubation times and temperatures.
5. The prediluted, ready-to-use reagents are optimally diluted, and further dilution may result in loss of antigen staining.
6. The concentrated reagents may be diluted optimally based on validation by user. Normal Antibody Diluent (Nordic BioSite Normal Antibody Diluent [Tris Buffered]) is recommended. Any diluent used that is not specifically recommended herein must likewise be validated by the user for both its compatibility and effect on stability.

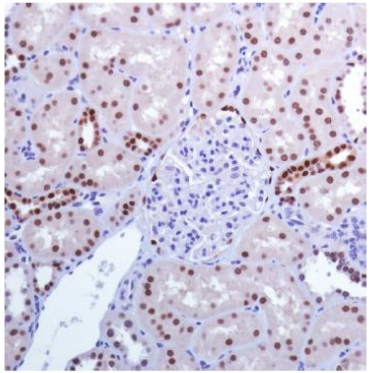
Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

abcam

Product datasheet

Anti-PAX8 antibody [SP348] - BSA and Azide free
ab242429
RabMAb



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PAX8 antibody [SP348] - BSA and Azide free (ab242429)

Application	Species
IHC-P	Human

Application	Abreviews	Notes
IHC-P	Ab Concentration ??? !	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with EDTA buffer pH 8.0 before commencing with IHC staining protocol. Primary antibody incubation for 10 minutes at room temperature.

General notes	FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.

Formalin-fixed, paraffin-embedded human kidney tissue stained for PAX8 using ab227707 at 1/100 dilution in immunohistochemical analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, and sodium azide (ab227707).

RUO !

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

Test used/**performed according to IFU** or not : when reading the IFU …

- IFU : varying from well to not well described
- "No info on pretreatment, detection, etc" **- vs -** easy to work IFU because less defined ?
  Easy to work according to less defined IFU, hence "only"verification ?? **= RISK !**

- Changes to IFU ?
  - Minor changes ? Not defined yet !
  - Major changes = validation

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

- Standard protocol (IFU) vs modified method ? **Depends on Ab/detection/staining platform**
  - Examples EQC : e.g. NORDIQC :
    - Standardprotocol recommended as start/first choice – however :
      - Standardprotocol sometimes, but not always best choice
      - Off label sometimes, but not always best - recommended
  - Patient interest : best method, best result, treatment = most important !
- Time a company needs to adapt IFU :
  - Documentary approval – timeframe can be long
  - Application specialist : method pool that is off label, but approved by manufacturer (good experiences)

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

2

Test used for **same purpose** as indicated in IFU ?

- Same purpose -> according to IFU : verification
- Change purpose = change test : validation

So the purpose of a test can also determine verification vs validation

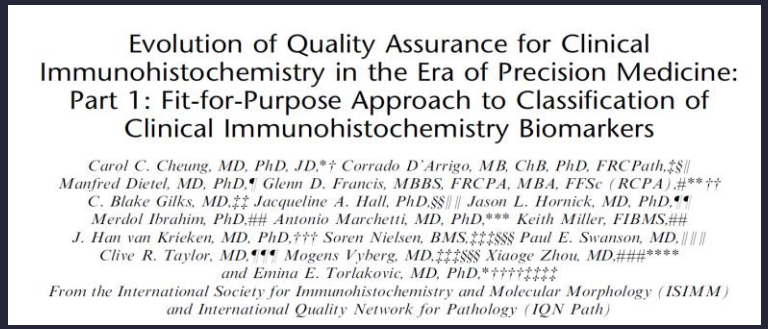
Purpose = **intended use at time test was developed**

- May or may not be the same as the clinical (intended) use
- Fit for purpose if a test has been validated for intended use at the time the test was developed (both lab/technical assay and clinical use)

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

- Purpose of a test :
  - Related to "3D" : Disease – Diagnostic Test -Drug
  - Classification based upon risk to patient



Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 1: Fit-for-Purpose Approach to Classification of Clinical Immunohistochemistry Biomarkers

Carol C. Cheung, MD, PhD, JD,† Corrado D'Arrigo, MB, ChB, PhD, FRCPath,‡§‖ Manfred Dietel, MD, PhD,¶ Glenn D. Francis, MBBS, FRCPA, MBA, FFSc (RCPA),#**†† C. Blake Gilks, MD,‡‡ Jacqueline A. Hall, PhD,§§‖‖ Jason L. Hornick, MD, PhD,¶¶ Merdol Ibrahim, PhD,## Antonio Marchetti, MD, PhD,*** Keith Miller, FIBMS,## J. Han van Krieken, MD, PhD,††† Soren Nielsen, BMS,‡‡‡§§§ Paul E. Swanson, MD,‖‖‖ Clive R. Taylor, MD,¶¶¶ Mogens Vyberg, MD,‡‡‡§§§ Xiaoge Zhou, MD,###**** and Emina E. Torlakovic, MD, PhD,*†††‡‡‡‡*

From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path)

TABLE 2. The Classification of Immunohistochemistry (IHC) Tests From the Perspective of the End User

Test Category	User-Based Classification	Example
Diagnosis in symptomatic patients (diagnostic)	Pathologist (type 1-IHC)	S100, VIM, CD45 and PAN-CK in diagnosis of unknown primary tumor
Disease screening (for an additional disorder) in symptomatic patients (diagnostic)	Treating physician (type 2-IHC)	MLH1, MSH2, MSH6 and PMS2 in colorectal cancer patients being screened for Lynch syndrome
Prognosis of a diagnosed disease (prognostic)	Treating physician (type 2-IHC)	CD10, Bcl-6, and MUM1 for cell of origin in diffuse large B-cell lymphoma
Predictive of treatment response or adverse reaction (predictive)	Treating physician (type 2-IHC)	ER, PR, HER2 for breast cancer, HER2 for gastric cancer

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

- Purpose of a test :
  - Classification based upon risk to patient : different terminology

TABLE 3. Comparison of Immunohistochemistry (IHC) Classification Schemes of IHC Tests by Regulatory Agency and Classification Based on Clinical Practice

Regulation of Test Manufacturers	Intention	Class	Clinical Practice (Nearest Correlate)
FDA	For regulating manufacturers of tests	Class 1	Type 1-IHC
		Class 2	Type 2-IHC
		Class 3	Type 2-IHC
Health Canada	For regulating manufacturers of tests	Class 1	Type 1-IHC
		Class 2	Type 2-IHC
		Class 3	Type 2-IHC
		Class 4	Type 2-IHC
EU In-vitro Diagnostic Regulation	For regulating manufacturers of tests	Class A	NA
		Class B	NA
		Class C	Type 1-IHC, type 2-IHC
		Class D	Type 2-IHC
Canadian Association of Pathologists	Guidance for clinical practice	Class 1	Type 1-IHC
		Class 2	Type 2-IHC

FDA indicates Food and Drug Administration in the United States; NA, not available.

Validation and Verification : What ?

Validation or verification : according to IFU determines CE-IVD or LDT

❖ Purpose of a test = intended use : indicated in the Instructions For Use

Dako

Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein

Code A0485

Intended use

For in vitro diagnostic use.

Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein is intended for use in immunohistochemistry. The antibody labels normal epithelial cells, which generally express c-erbB-2 protein at a very low level. It is a useful tool for the identification of overexpression of c-erbB-2 oncoprotein in a variety of epithelial neoplasms, for example subsets of breast carcinomas, pulmonary adenocarcinomas, colorectal adenocarcinomas, pulmonary squamous and gastric adenocarcinomas (1), transitional cell carcinomas of the urinary bladder (2), and endometrial adenocarcinomas (3). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Dako

FLEX Monoclonal Mouse Anti-Human Cytokeratin 7
Clone OV-TL 12/30
Ready-to-Use
(Dako Omnis)

Code GA619

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human Cytokeratin 7, Clone OV-TL 12/30, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry (IHC) together with the Dako Omnis instrument. This antibody labels glandular and transitional epithelial cells and is a useful aid for the classification of adenocarcinoma of the lung (1), breast and endometrium, thyroid gland (2) and ovary (3), as well as chromophobe renal cell carcinoma (4). Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Validation and Verification : What ?

Types of validations/verifications ?

- In general there are 3 kinds of initial validations :
  - Clinical validation of predictive tests/biomarkers
  - Diagnostic validation :
    - Diagnostic validation of **diagnostic tests/biomarkers** :
      diagnostic sensitivity and specificity of new marker vs golden standard (morphology, other biomarker, etc)
    - Indirect clinical validation of **prognostic and predictive tests/biomarkers** :
      requires a reference method e.g. ISH, NGS, PCR
  - Technical validation of IHC protocols
- Revalidation

Summary Part 1

What ?

- Definitions validation & verification
- Determining questions :
  - What kind of test ?

FDA/ CE-IVD (IFU)	verification
Other	validation

  - What is the purpose ?

Purpose – intended use	verification
Other	validation

  - How is the test used ?

FDA/ CE-IVD within IFU	verification
Not according to IFU	validation

02

Validation – Verification : WHY ?

Validation and Verification : Why ?

1. Legal or accreditation requirements, guidelines, etc :

- Requirements by Federal Agencies, national regulations, etc
  E.g. Belgian Practice Guideline for Pathology lab's
  = ISO15189 based practice guideline describing requirement for an operational quality management system, including validation of test, instruments, etc
- Guidelines by professional organisations (e.g. College of American Pathologists)
- Accreditation requirements (or similar) :
  - ISO 15189 Medical laboratories – Requirements for quality and competence (Chapter 5.5)
  - ASCP, ILAC, CLIA
  - etc

Validation and Verification : Why ?

2. EU Directive EU2017/746 In Vitro Diagnostics :

- IVD (old) vs IVD-R (new)
- Effective May 2022
- Classes of tests based upon risk :

Class	Risk profile	Application
A	Low individual risk and low public risk	Basic stains (e.g. H&E), histochemical stains
B	Moderate individual risk and low public risk	Some IHC stains like e.g. HP
C	High individual risk and/or moderate public risk	IHC stains, ISH, molecular testing
D	High indicvidual risk and high public risk	/

EU countries : class D (minimal requirement) ; Belgium : class C & D

Validation and Verification : Why ?

2. EU Directive In Vitro Diagnostics : **only 2 categories of tests**

- CE-IVD Tests used according to Instructions For Use (IFU)
- All other :
  - CE-IVD NOT used according to IFU
  - Non CE-IVD (e.g. Research Use Only (RUO) (not within IVDR)
  - Laboratory Developed or Home Brew test

=> NO specific terminology, but a definition : "*Devices manufactured and used within health institutions within EU*"; commonly used term = Laboratory Developed Test (LDT)

- CE-IVD according IFU = verification, LDT = validation
- Terminology used ? Laboratory Modified Test, Home Brew Test, CE test with minor / major modifications, In House IVD, etc

Validation and Verification : Why ?

2. EU Directive In Vitro Diagnostics : **only 2 categories of tests**

❖ CE-IVD Tests used according to Instructions For Use (IFU)

❖ Definition : "Devices manufactured and used within health institutions" (LDT)

Laboratory becomes a "manufacturer" of the LDT :

- Manufacturing from raw materials/parts/components
- Combining devices or products for a medical purpose (no CE mark or combination not in line with original intended purpose) (E.g. Ab on other staining platform)
- "**Significantly**" modifying an existing device : not intended by the manufacturer and has impact on product conformity

= changing a method, intended use, etc

➡ But, what's the impact of the changes, especially to patient safety and test performance ?

Significant or major -> validation as LDT

Non significant or minor -> verification as CE- IVD

Validation and Verification : Why ?

2. EU Directive In Vitro Diagnostics : Minor – major changes not defined yet !

Significant ?

Category	Description	Examples
Intended purpose - use	Extension of intended purpose	Use of a test for a purpose not intended or specified in IFU and not approved by application of manufacturer. *E.g. Agilent Dako SK006 PDL-1 IHC 22C3 pharmDx kit (= closed kit) used on another platform from another vendor e.g. Leica or Roche*
	New use or patient population	PDL-1 clone 28-8 is intended to be used on gastro specimen (=IFU). Lab uses other PDL-1 clone 22C3 on gastro, while the IFU does not state that use or is not yet adapted for that use.
	Change in clinical use	Leica CD117 (C-kit) clone not used for the intended use (e.g. GIST). *E.g. Use of Leica CD117 clone EP10 for the detection of Giardia lamblia.*
Performance specifications	Change in operating principle of a test	Addi[illegible]plification step to a detection method using reagents fr[illegible]nother vendor then the initial detection kit.
Interpretation of results		
Materials	Change in human materials (e.g. [illegible] on other tissues)	
	Change in m[illegible]	Using a test designed for frozen sections on FFPE samples. The use of a method for cytology specimens (monolayer) when the IFU only state the use on FFPE samples.
	Change in reagents if reagents are described as mandatory in the IFU	Change in detection kit for a PharmDx / companion diagnostic type 2 test were the IFU specifically state the intended use and required reagents and which one can NOT be substituted. *E.g. Agilent Dako SK006 PDL-1 IHC 22C3 pharmDx kit used on another platform from another vendor e.g. Leica or Roche* Change in reagents included in a closed RTU kit. *E.g. substitution of reagents in Herceptest or Oracle HER-2 staining kit other then allowed in IFU*
	Change in reagents to another type in relation to the IFU	E.g. Ab developed for LSAB detection kit, but used with IFU adaptation to polymer technology detection (so difference in strength of the detection kit).

Validation and Verification : Why ?

2. EU Directive In Vitro Diagnostics : Minor – major changes not defined yet !

Non -Significant ?

Category	Description	Examples
Intended purpose	Extension of intended purpose	Use of a test for a purpose not intended or specified in IFU, but approved by application of manufacturer. *E.g. use of a Agilent Dako Autostainer Ab (IR series) on the Omnis staining platform. (IFU states "for use on Autostainer", but application specialist of manufacturer approves use on Omnis).*
Performance specifications	Change in operating principle of a test	Changing the chromogen of a detection kit (e.g. from DAB to Magenta).
Materials	Change in human materials	Application on other tissues. *E.g HER-2 on breast vs stomach*
	Change in matrix	The use of a method to prepare cellblocks from cytology specimens (FFPE celblock) when the IFU only state the use on FFPE tissues. (Both use Formalin Fixed, Paraffin Embedded as matrix).
???	Change in reagents if reagents are NOT described as mandatory in the original IFU	Any modification of the staining protocol used for optimization of the staining assay in the patient's best interest and for which the improved staining results can be demonstrated in an objective way (e.g. scoring system, EQC, etc) . Use is covered by a (ISO15189 based) active QMS. • Change in dilution rate for the primary Ab outside IFU recommended range • Change in primary Ab incubation time outside IFU recommended range • Change in endogen blocking step : reagent, incubation time or protocol step outside IFU recommendations • Change in pre-treatment outside IFU recommended solutions, incubation times, etc • Adding linker or enhancement step (from same vendor) • Changing detection kit incubation time outside IFU recommendations (same working principle/strength e.g. 2 step polymer) • Changing chromogen incubation time outside IFU recommendations • Changing counterstain incubation time outside IFU recommendations

Draft to Belgian inspection agency

Depending on Ab – Detection – Staining platform !

! Significant/ non significant implies if these changes make the test an LDT (major) or if it stays within CE-IVD (minor).
LDT = additional documentation workload !

Validation and Verification : Why ?

EU Directive In Vitro Diagnostics & use of LDT :

- Laboratory Developed Test (LDT) :
  - First choice = CE-IVD test
  - LDT may be used if no “valid” CE-IVD available and :
    - Clinical and analytical performance demonstrated
    - Risk analysis for impact changes on IFU

    - OR -

    - Art. 5.5. c) lab fullfils requirements of EN ISO 15189 or – when applicable – appropriate national requirements, e.g. national guidelines regarding accreditation (requirements for legal recognition of lab)

+ Annex 1 IVDR

Validation and Verification : Why ?

EU Directive In Vitro Diagnostics & use of LDT : additional requirements
(documentation **per test**)

IVDR

- Used under Quality Management System
- Fullfils ISO15189 requirement or appropriate national guidelines regarding accreditation
- Justification of the use of LDT
  - Specific needs patient population
  - No CE-IVD available that fullfils requirements or performance criteria (EUDAMED database) + periodic review of availability CE-IVD

+ Declaration to competent authority
+ Incident reporting

IVDR Annex 1

- General Safety & Performance Requirements (GSPR)
- Risk management for “production” & usage
- Risk mitigating actions
- Performance of LDT (analytical and clinical performance is demonstrated + continuously evaluated upon use + CAPA)
- Documentary requirements :
  - Information on “production”
  - Instructions for Use (IFU)
  - Labelling (specific requirements !)

Validation and Verification : Why ?

3. Technical considerations :

- Test Developed by manufacturer under certain conditions and for a certain purpose/intended use
- IFU contains (well) described method
- IFU contain sample usage : e.g. FFPE (Formalin Fixed, Paraffin Embedded), Frozen sections, cytology
- IFU contains info on staining platform to be used on – or no remarks about staining platform :
  - Ab applied on staining platform recommended by manufacturer or not
- etc

Validation and Verification : Why ?

3. Technical considerations :

- Test Developed by manufacturer under certain conditions and for a certain purpose/intended use
- IFU contains (well) described method
- IFU contain sample usage : e.g. FFPE (Formalin Fixed, Paraffin Embedded), Frozen sections, cytology
- IFU contains info on staining platform to be used on – or no remarks about staining platform :
  - Ab applied on staining platform recommended by manufacturer or not
- etc

However …

Validation and Verification : Why ?

Manufacturer – development test

- Developed under certain conditions and for a certain purpose – intended use
- IFU contains (well) described method
- IFU contains sample usage e.g. FFPE, frozen sections, cytology
- Test development : samples used
- IFU contains info on staining platform to be used
- etc

Customer/lab using test

- Test correctly **performed as prescribed** by manufacturer ?
- In case usage on **FFPE** noted in IFU :
  - Tissue processing protocol used ?
  - Fixative used ? Concentration ? Time to fixation ? Fixation time ?
  - Processing protocol (type of reagent (e.g. Methanol vs Ethanol), temperature ?
  - Preparation of slides : section thickness (IHC, ISH), waterbath/stretchtable/oven,
  - Type of glass used (recommended or not) ?

Influencing results and therefore calibration/optimalization and validation/verification is needed before use on patiënt samples in daily routine

Validation and Verification : Why ?

Manufacturer – development test

- Developed under certain conditions and for a certain purpose – intended use
- IFU contains (well) described method
- IFU contains sample usage e.g. FFPE, frozen sections, cytology
- IFU contains info on staining platform to be used
- etc

Customer/lab using test

- Performed according IFU or with modifications?
- In case usage on **FFPE** noted in IFU :

E.g. 10% Formalin :

lab : 10 % ?

10 % in reality or 9 %? 8%? 11% 12 %

10 % using fresh Formalin ?

Acceptance testing (vs certificate) ?

Degradation during processing ?

(e.g. 4,5 % fresh -> end of run 4,3%)

→ impact on IHC ???? (Little to no data available)

Influencing results and therefore calibration/optimalization and validation/verification is needed before use on patiënt samples in daily routine

Summary Part 2

Why ?

- Determining questions :
  - Legal requirements ?
  - IVDR ?

CE– IVD according to IFU	**verification**
Other	validation

  - Technical considerations ?

03

Validation – Verification : HOW ?

Validation and Verification : what do we need ?

Validation and verification definitions:

- Validation : Demonstrate by means of objective evidence that performance-characteristics fullfill predefined criteria or specifc demands for a certain purpose or intended use.

- Verification : Confirmation by providing objective evidence that a test fullfils specifications (specific demands) or specified performance characteristics.

Validation and Verification : what do we need ?

Validation and verification definitions:

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Validation and Verification : How ?

In order to “demonstrate” or “ confirm”, a comparison of performance characteristics is needed between new test and comparator or reference/standard

- Comparator test /reference or standard needed to compare results :
  - Types of samples with known morphology and expression of an antigen (e.g. iCAPC (Immunohistochemistry Critical Assay Performance control))
  - Confirmed samples or controls stained with allready verified or validated method (own lab or other lab)
  - Reference in e.g. IFU about staining patterns, performance, etc
  - EQA samples
  - etc

Validation and Verification : what do we need ?

Validation and verification definitions:

- Validation : Demonstrate **by means of objective evidence** that performance-characteristics fullfill predefined criteria or specifc demands for a certain purpose or intended use.
- Verification : Confirmation **by providing objective evidence** that a test fullfils specifications (specific demands) or specified performance characteristics.

Objective evidence :

- tests performed, evaluated needs to be demonstrated and documented
- raw data, evaluation can be traced back to stains, predetermined performance and acceptance criteria

Validation and Verification : what do we need ?

Validation and verification definitions:

- Validation : Demonstrate by means of objective evidence that performance-characteristics fullfill predefined criteria or specifc demands for a certain purpose or intended use.

- Verification : Confirmation by providing objective evidence that a test fullfils specifications (specific demands) or specified performance characteristics.

Performance characteristics and acceptance criteria need to be defined

Validation and Verification : what do we need ?

- Performance characteristics often used :
  - Accuracy
  - Sensitivity
  - Specificity
  - Reproducibility
  - Overall concordance
  - Other
  - ….

Validation and Verification : what do we need ?

❖ **ACCURACY** : correctness & precision

- **Correctness :**
  - Comparison with known results from validated tests (reference samples, validated testsamples (own or another lab)
  - Comparison with other validated technique (e.g. ISH vs PCR), other validated instrument or other reagents (other manufacturor)
  - Third line control (EQC or interlabcomparison)
  - Populationstudy

Validation and Verification : what do we need ?

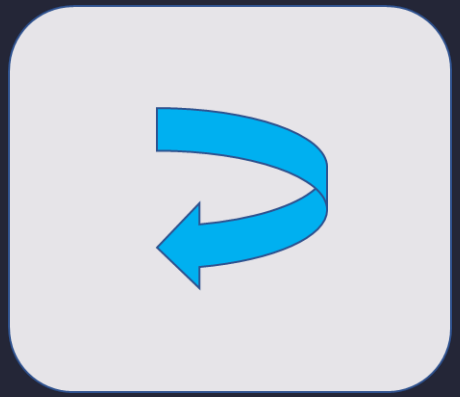
❖Accuracy : correctness & precision

- **Precision :**
  - Repeatability : intra run / within run tests
  - Intermediate precision : interrun / in between run tests
  - Reproducability : inter-lab reproducability

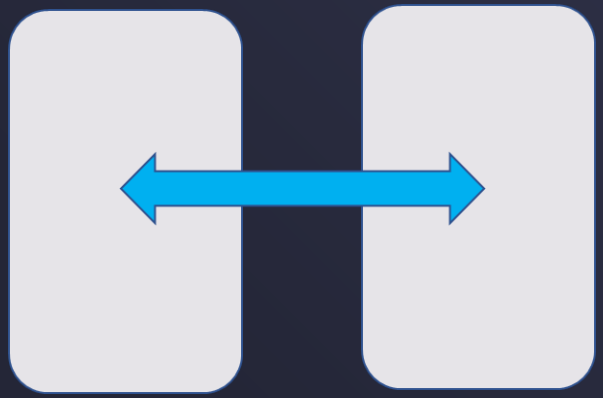
  - 1 or more staining platforms : precision determined on all platforms !

Validation and Verification : what do we need ?

WITHIN RUN



INTER RUN



! Multiple stainers !

Demonstrate stains have same quality, **independent of place** in stainer or on which stainer loaded/stained.

Validation and Verification : How ?

Within and in between run

BATCH LOADING

Carousel

Benchmark GX

Slide trays

Autostainer

CONTINUOUS LOADING

Fixed slide position

Bond MaX & III

Intellipath

NON Fixed slide position

Omnis

Single piece loading

Benchmark Ultra

Genie

Validation and Verification : How ?

Within and in between run

BATCH LOADING

Carousel

Benchmark GX

Slide trays

Autostainer

CONTINUOUS LOADING

Fixed slide position

Intellipath

NON Fixed slide position

Omnis

Single piece loading

Benchmark Ultra

Genie

How would you organise this ?

Validation and Verification : How ?

❖ Performance characteristics : **SENSTIVITY**

Sensitivity :

- ❖ Analytical : ability of a test to detect small amounts of a substance (e.g. antigen)

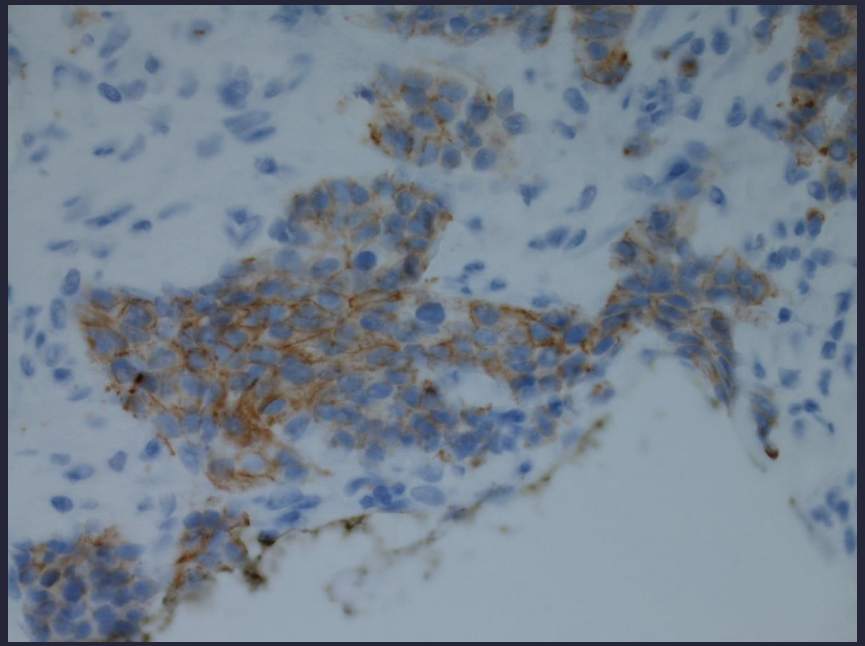
  dilutionrange for detectionlimit
- ❖ Diagnostic : evaluation of true positive staining vs false negative staining

	Reference (+)	Reference (-)	Total
New (+)	TP	FP	
New (-)	FN	TN	
Total			

Sensitivity : TP/ (TP + FN)

Validation and Verification : How ?

❖ Performance characteristics : SENSTIVITY



IFU :
Ab dilution 1:600 – 1:800 HIER low pH
- OR –
Ab dilution 1:1000 – 1:1200 HIER high pH

HER-2 clone poly A485
Dilution 1 : 1000
TRS High
Envision Flex detection
Breast tumor 2+
Omnis staining platform

Validation and Verification : How ?

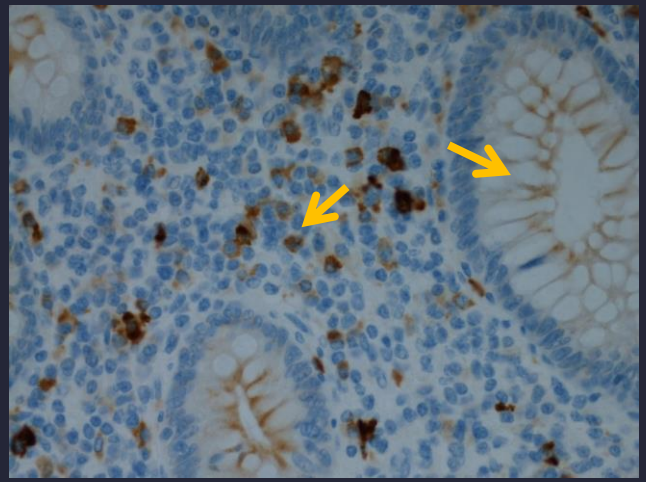
- Performance characteristics : **SPECIFICITY**
- Specificity :
  - Analytical : ability of a test to detect a substance (e.g. antigen) without interference of cross reacting substances
    → interferention study
  - Diagnostical : evaluation of true negative staining vs false positive staining

	Reference (+)	Reference (-)	Total
New (+)	TP	FP	
New (-)	FN	TN	
Total			

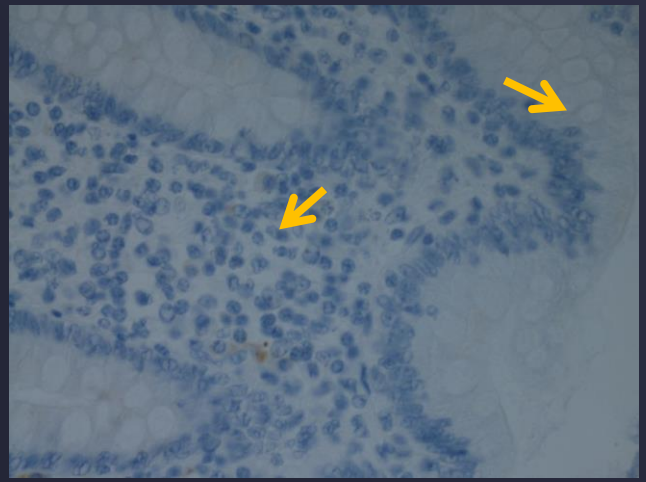
Specificity : TN/ (TN + FP)

Validation and Verification : How ?

❖ Performance characteristics : SPECIFICITY



PSA clone ER-PR8 – appendix crtl (non specific)



PSA clone 35 H9 – appendix crtl (specific)

Plasmacells and epithelial cells

Validation and Verification : How ?

- Performance characteristics : **OVERALL CONCORDANCE**

  Overall concordance :

  - Analytical : the degree of agreement between new test and reference

    ± = Correctness

  - Diagnostical : evaluation of true positive and negative staining vs total of true and false positive and negative staining

	Reference (+)	Reference (-)	Total
New (+)	TP	FP	
New (-)	FN	TN	
Total			

Concordance : TN + TN/ (TP +TN + FP + FN)

Validation and Verification : How ?

- Performance characteristics : **ROBUSTNESS**

  Robustness : what influences result ?

  - Ischemic time : time to fixation ?
  - Fixation time :

    Minimal and maximal fixation time ?

    Daily practice : different fixation times ?
  - Section thickness (IHC, ISH)
  - Stability antigen : How long can pre-cut sections be stored ?? *How ? RT ?* (*Patientsamples, controls)*
  - Stability reagents : How long can a diluted concentrated Ab be stored *?* *(e.g. dilutions from MSI Ab -> max 3 months)*
  - *Decal, etc*

Validation and Verification : How ?

- Performance characteristics : **READOUT**
  - *Type 1 (pathologist) vs type 2 (pathologist provides for treating physisian)*
  - *Validation/verification - initial :*
    - *Training of pathologists in e.g using scoring system by e.g. application specialist, professional organisations, etc*
    - *Readout new test from different pathologists vs expected results known cases/controls :*
      - *Verified by e.g. application specialist, expert panel, etc*
      - *Determine diagnostic sensitivity & specificity for different pathologists*
      - *Compare results pathologists and evaluate vs formulated acceptance criteria (e.g. >90% concordance)*
    - *Inter-observer tuning between different pathologists vs expected results*

Validation and Verification : How ?

❖ Performance characteristics : **READOUT**

❖ *Ongoing validation :*

Type 1	**IQC** **EQC / proficiency testing**
Type 2	IQC EQC / proficiency testing Interobserver **periodically reviewed** (e.g. review breast cases) **Correlationstudy IHC – ISH** (over- or underscoring) Education (e.g. online teaching aid) (e.g. CBQA-PCAB Readout Proficiency Testing)

Digitalisation & Artificial Intelligence = completely different story – more complex verification/validation !

Validation and Verification : How ?

❖ Performance characteristics : **STAINING QUALITY**

Stain quality : Scoringsytem to evaluate in an objective way, e.g. IHC* :

Stainingcriteria	Score			
Intensity	0 (none)	1 (weak)	2 (average)	3 (strong)
Uniformity	0 (none)	1 (uniform)	-	-
Specificity	0 (none)	1 (specific)	-	-
Absence of	0	1	2	
backgroundstaining	(strong)	(average)	(none)	-
Counterstaining	0	1	-	-
	(inadequate)	(adequate)		
Totaal				

Score :

0 - 4 :	unacceptable
5 - 6 :	borderline
7 - 8 :	optimal

"Basic" evaluation of (analytical) sensitivity and specificity

(*) Reference : Audit and internal quality control in immunohistochemistry, P. Maxwell and W G McCluggage, J Clin Pathol 2000 53: 929-932

Validation and Verification : How ?

❖ Which performance characteristics used with validation or verification ?

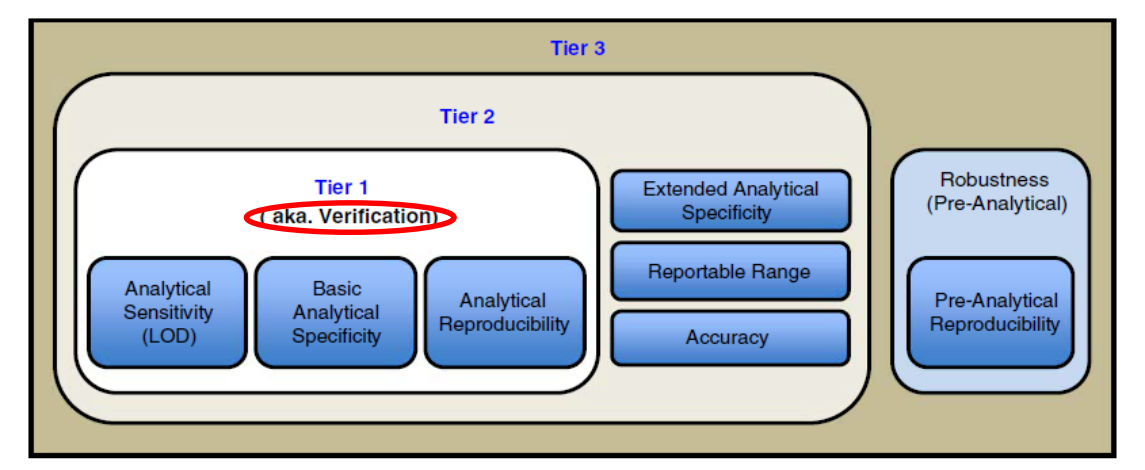
CAP guidelines

Type of test	Non Predictive	Predictive	Criteria
Unmodified FDA cleared/approved (each Ab)	✓	✓	Accuracy Precision Reportable range Overal concordance ≥ 90%
Non FDA cleared/approved *(= Laboratory Developed Tests)* Modified FDA cleared/approved *(= Laboratory Modified Tests)*	✓	✓	Accuracy Precision Analyt sensitivity Analyt specificity

Validation and Verification : How ?

❖ Which performance characteristics used with validation or verification ?

International Society for Immunohistochemistry and Molecular Morphology



E. Torlakovic et al *Appl Immunohistochem Mol Morphol* • Volume 25, Number 3, March 2017

FDA/CE-IVD : tier 1= verification
LDT : tier 1,2,3 = validation

Validation and Verification : How ?

❖ Which performance characteristics used with validation or verification ?

	Verification	Limited validation	Validation		
Performance characteristics	**CE-IVD test**	**Modified CE-IVD with reference**	**Modified CE-IVD without reference**	**Non CE-IVD (RUO) with reference**	**Non CE-IVD without reference (In house developed)**
Trueness	x	x	x	x	x
Precision	x	x	x	x	x
Sensitivity			x	x	x
Specificity			x	x	x
Robustness	x*	x*	x*	x*	x*
Inter operator variability	(x)	(x)	(x)	(x)	(x)

x*: The performance of a risk analysis can be a useful tool to determine which parameters can be verified in the context of the robustness.
(x): Mainly applicable to semi-quantitative testing

Belgian guidelines for Quality Management in Pathology labs.
Includes verification and validation of IHC methods
Federal Agency of Health – Sciensano

(Published 04/2022)

For Verification – LMT with reference :
"Basic" evaluation of sensitivity and specificity included in evaluation stain quality

Validation and Verification : How ?

❖ **Which acceptance criteria** for the selected performance characteristics ?

❖ Refered in guidelines :

e.g. CAP 90% overall concordance for every test used clinically

❖ Defined by number of samples in validation :

Number of samples	Acceptance criteria
10	≥ 90%
20	≥ 95%

Table 4. Validation Using 10- and 20-Tissue Validation Sets Against a 90% Concordance Benchmark

No.	Concordance Estimate, % (95% CI)		
	0 Discordant	1 Discordant	2 Discordant
10	100 (68–100)	90 (57–100)	80 (48–95)
20	100 (81–100)	95 (75–100)	90 (69–98)

Abbreviations: CI, confidence interval; No., number of validation tissues.

Arch Pathol Lab Med—Vol 138, November 2014 *Analytic Validation of Immunohistochemical Assays*—Fitzgibbons et al

Validation and Verification : which samples ?

- **Which samples** used in validation/verification set ?

- Related to purpose – intended use of test (IFU)
- Controls (preferably “in house”)
- Known patient cases
- Different expression levels(high, low), different tissue types, etc
  - Type 2 : e.g. 20 (+) = % weak – moderate – strong (+)

- Single piece samples
- Sausage blocks or mult-tissue blocks , TMA (Tissue Micro Array)
  Other
- Same processing as clinical samples/daily routine (preferably)

Validation and Verification : How many tests ?

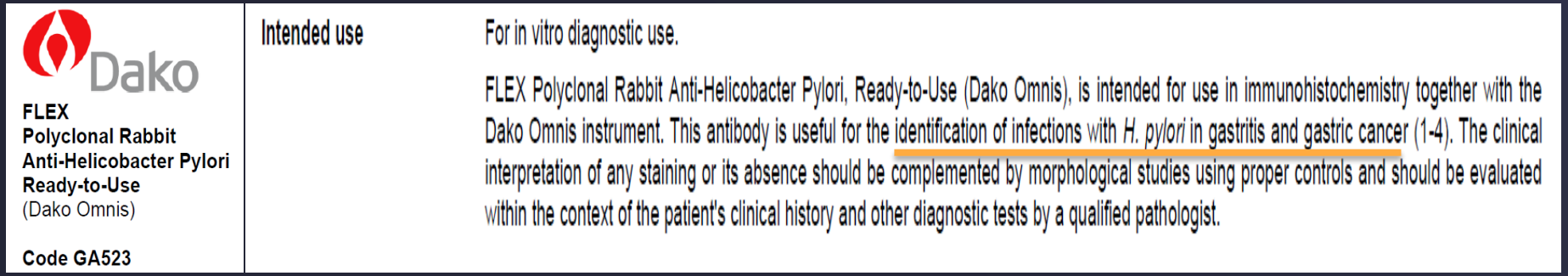
- **How many** test to be performed ?

- Determined by type of test :
  - FDA/ CE-IVD according to IFU or not
  - Type 1 vs Type 2

- Determined by the way test is used :
  - Performed according to manufacturers instructions (**IFU**) or not
  - Used for the same **purpose / intended use** or not ?

Validation and Verification : How many tests ?

❖ **How many** test to be performed ? **Purpose – Intended use**



Dako

FLEX
Polyclonal Rabbit
Anti-Helicobacter Pylori
Ready-to-Use
(Dako Omnis)

Code GA523

Intended use

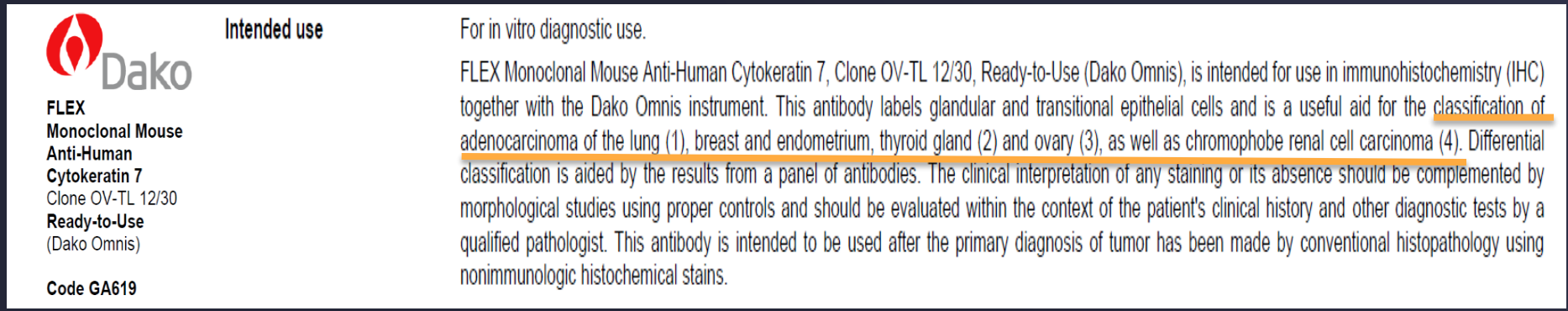
For in vitro diagnostic use.

FLEX Polyclonal Rabbit Anti-Helicobacter Pylori, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry together with the Dako Omnis instrument. This antibody is useful for the identification of infections with *H. pylori* in gastritis and gastric cancer (1-4). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

= 1 well determined purpose

Validation and Verification : How many tests ?

❖ **How many** test to be performed ? **Purpose – Intended use**



Dako

FLEX
Monoclonal Mouse
Anti-Human
Cytokeratin 7
Clone OV-TL 12/30
Ready-to-Use
(Dako Omnis)

Code GA619

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human Cytokeratin 7, Clone OV-TL 12/30, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry (IHC) together with the Dako Omnis instrument. This antibody labels glandular and transitional epithelial cells and is a useful aid for the classification of adenocarcinoma of the lung (1), breast and endometrium, thyroid gland (2) and ovary (3), as well as chromophobe renal cell carcinoma (4). Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

= 4 well determined purposes

Validation and Verification : How many tests ?

❖ **How many** test to be performed ?

Some Ab used for different purposes !

Do I need to validate seperatly for each purpose ??

- Difference type 1 vs type 2 :
  - Type 1 : validation set/cases including cases for different purposes
  - Type 2 : validation according to purpose e.g. HER-2 on breast and gastric

    = separate validation/verification per purpose/intended use

- Experience with test/biomarker
- Experience of lab and pathologist
- Implementation of IQC
- etc

Validation and Verification : How many tests ?

❖ **How many** test to be performed ? **Number of slides ?**

Available references referring to actual number of cases needed for validation or verification is limited.

Under revision !

Principles of Analytic Validation of Immunohistochemical Assays

Guideline From the College of American Pathologists Pathology and Laboratory Quality Center

Patrick L. Fitzgibbons, MD; Linda A. Bradley, PhD; Lisa A. Fatheree, BS, SCT(ASCP); Randa Alsabeh, MD; Regan S. Fulton, MD, PhD; Jeffrey D. Goldsmith, MD; Thomas S. Haas, DO; Rouzan G. Karabakhtsian, MD, PhD; Patti A. Loykasek, HT(ASCP); Monna J. Marolt, MD; Steven S. Shen, MD, PhD; Anthony T. Smith, MLS; Paul E. Swanson, MD

Arch Pathol Lab Med

Accepted for publication February 3, 2014.

= Evidence based : English language published literature from 2004 – 2013

Validation and Verification : How many tests ?

CAP guidelines

Type of test	Non Predictive	Predictive	Criteria
Unmodified FDA cleared/approved (each Ab)	10 (+) & 10 (-) - OR - Labdirector	≥ 20 (+) & 20 (-) Different expression levels ER/PR/HER-2 guidelines	Accuracy Precision Reportable range Overal concordance ≥ 90%
Non FDA cleared/approved *(= Laboratory Developed Tests)* Modified FDA cleared/approved *(= Laboratory Modified Tests)*	10 (+) & 10 (-) - OR - An appropriate tissue set	ER/PR/HER-2 : ≥ 40 (+) & 40 (-) Other : 20 (+) & 20 (-)	Acuracy Precision Analyt sensitivity Analyt specificity

Validation and Verification : How many tests ?

From a statistcal point of view : “the more the better “

“The more samples run in a validation set, the higher the likelihood that the concordance estimate reflects the “true” performance of a test.”

However…

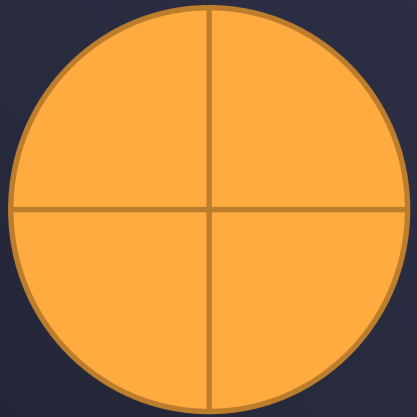
Validation and Verification : How many tests ?

❖ **Things to consider …**

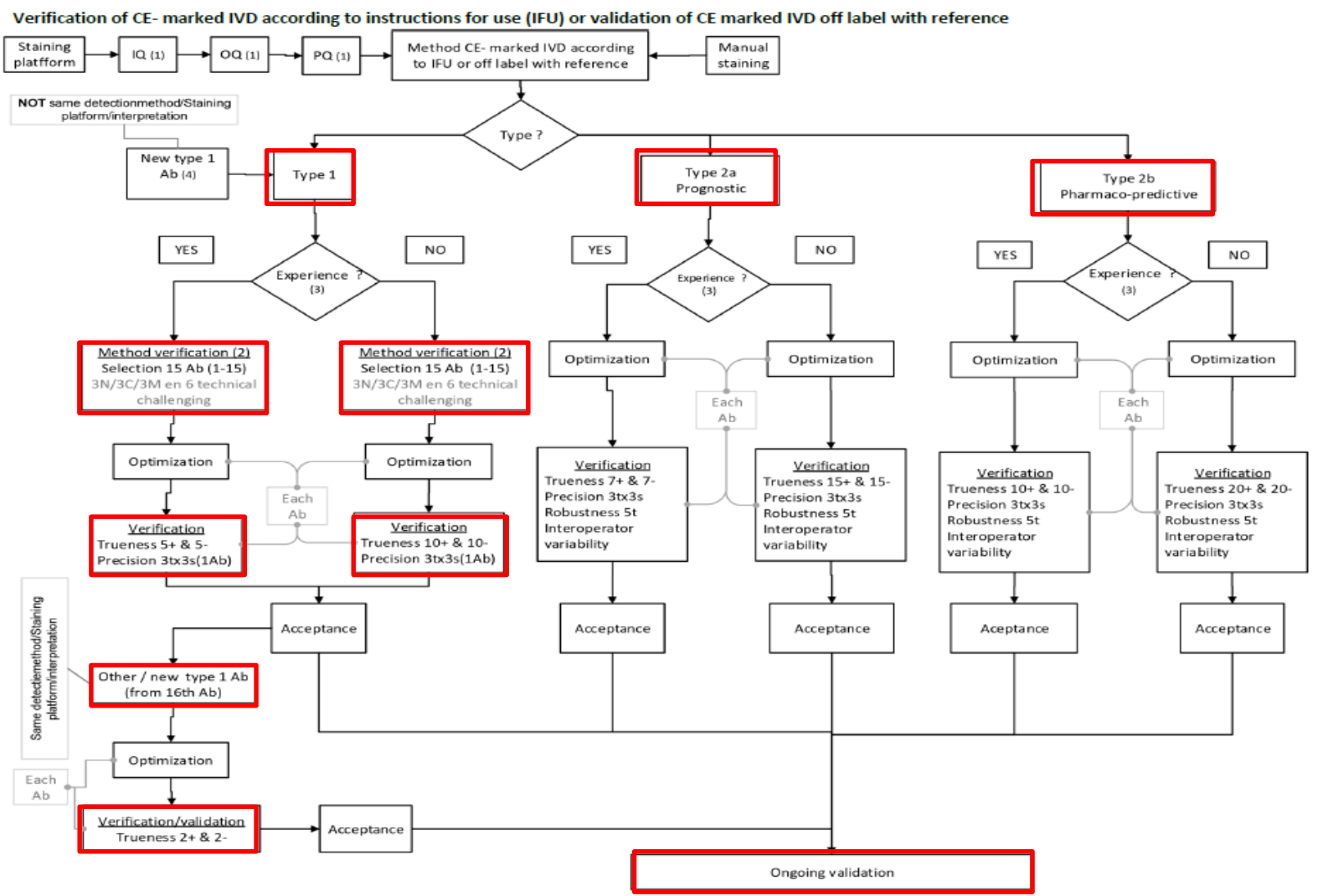
- ❑ Little or no information on how to perform optimalisation, use of controls, continuous/ongoing validation
- ❑ Predominatly a literature study and statistical analysis/approach. (Academic)
- ❑ Focus on enough samples in validationset and less on ongoing validation
- ❑ Input daily practice ? Other performance characteristics ? Execution of validation ?
- ❑ Availability of sufficient and appropriate cases, controls
- ❑ Ab in panel vs stand alone, IQC ?
- ❑ Necessary to use 10+/- for basic Ab like e.g. S-100, SMA,CD3, etc ?
- ❑ Experience of a lab with test/Ab ?
- ❑ Achievable ? Cost : 10 +/- controls / Ab, workload (Labtech's & pathologists)
- ❑ IVD-R compliant ?

Belgian guidelines

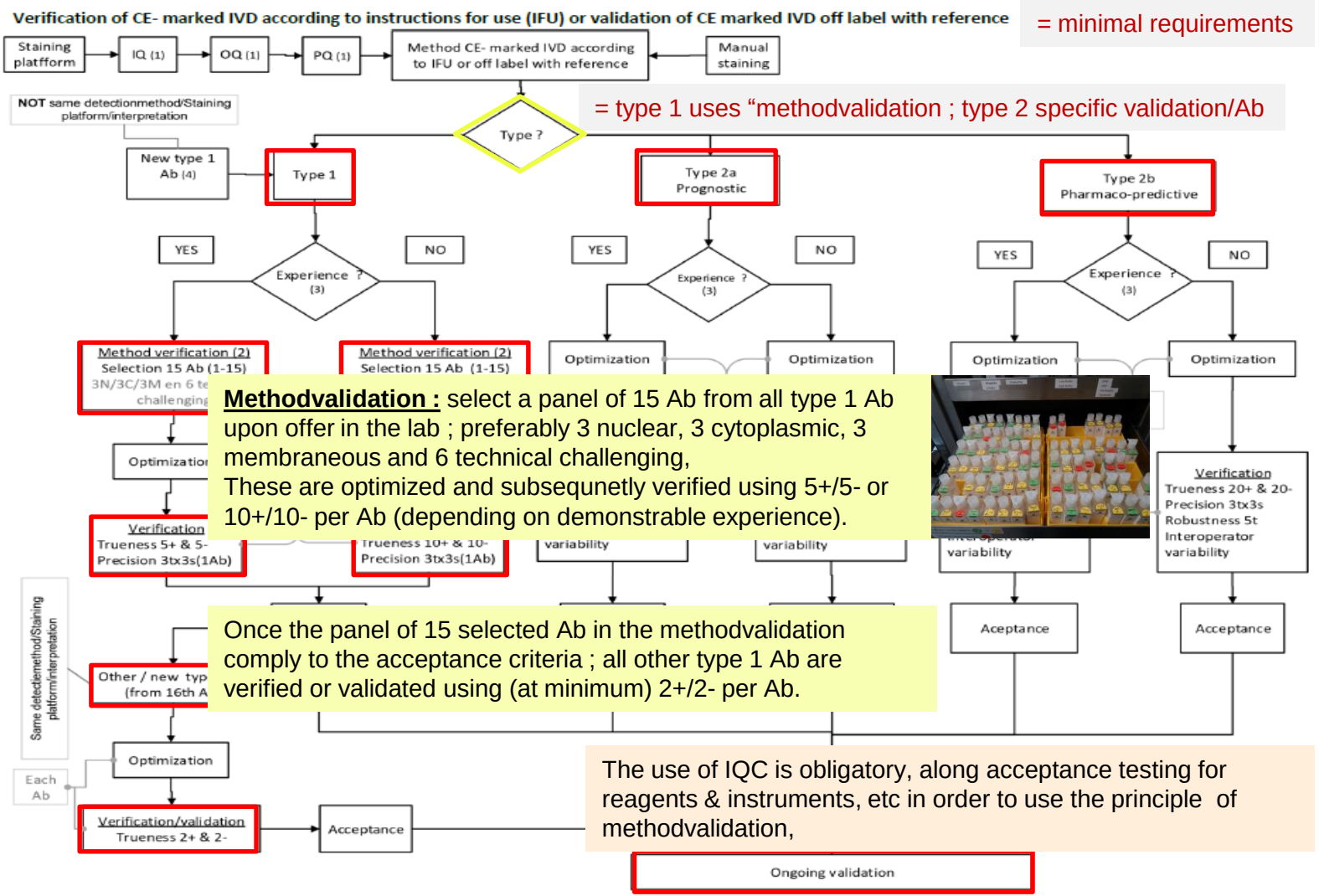
- Practice guideline for the implementation of a quality management system for Belgian Pathology labs
    - ISO15189 based
    - Emphasis shift to ongoing validation
- Results from a documentary audit on validation procedures and results from EQA
- Commision of Belgian Pathologists : task force to rewrite Practice Guideline
- Literature study
- Risk analysis
- Expert group panel consensus
- Taking into account :
    - Experience of a lab
      Availability controls, achievability, costs, etc



Belgian Guidelines



Belgian Guidelines



Summary Part 3

How ?

- Determining questions :

- What kind of test ?
- What is the purpose ?
- How is the test used ?

⇒ Validation or verification

- Comparator test – reference
- Performance characteristics
- Acceptance criteria for performance characteristics

- Which samples ?
- How many ?

Validation and Verification : How to proceed ?

Validation and verification process principle :

1. Question : which type of test (FDA/CE-IVD) and used according to IFU or not ?
   Used as intended (same purpose) ?
   ⟹ Verification or validation ?

2. Formulate :
   - ❖ Selected performance characteristics and acceptance criteria
   - ❖ Number of samples and type of samples

3. Prepare slides/cases according to manufacturers instructions

Validation and Verification : How to proceed ?

Validation and verification process principle :

4. Starting point : stain slides/cases according to IFU/standard/default protocol

5. Evaluate stain (stain quality – performance characteristics applied) :
   - ❖ Stain/method ok : proceed to verification/validation
   - ❖ Stain/method not ok : optimize/calibrate untill ok

When optimizing/calibration :

- ○ Method within IFU = verification
- ○ Method outside IFU = LDT = validation (change in performance characteristics)

Validation and Verification : How to proceed ?

Validation and verification process principle :

6. Optimized/calibrated method used to stain validation/verification set/cases
7. Evaluate stains vs reference/comparator
   - ❖ List results in 2x2 matrix

	Reference (+)	Reference (-)	Total
New (+)	TP	FP	
New (-)	FN	TN	
Total			

8. Determine/calculate performance characteristics
9. Acceptance criteria met ? **YES** -> OK for use in daily /clinical practice
10. Sign of by e.g. lab director, quality manager

Validation and Verification : How to proceed ?

POSITIEF	REK	Staalnr + MCB HER-2	Intensiteit (0-3)	Uniformiteit (0-1)	Specificiteit (0-2) (*)	Afwezigheid Achtergrond (0-2)	Tegenkleuring (0-1)	TOTAAL	Initialen beoordeler	HER-2 score Initieel	HER-2 score patholoog 1	HER-2 score patholoog 2	Correlatie
1	20	18B11057	3	1	1	2	1	8	AVC	2	2		ok
2	20	18B11252	3	1	1	2	1	8	AVC	2	2		ok
3	20	18B11382	2-3	1	1	2	1	8	IDA	2	2		
4	19	18B20661	3	1	1	2	1	8	AVC	3	3		
5	19	19B728	2-3	1	1	2	1	8	IDA	3	3	3	ok
6	25	19B4440	3	1	1	2	1	8	AVC	3	3		ok
7	19	18B10322	2-3	1	1	2	1	8	IDA	3	3		ok
8	19	18B8433	3	1	1	1	1	7	AVC	3	2		ok
9	27	19B3153	2-3	1	1	2	1	8	IDA	3	3		ok
10	27	19B5782	3	1	1	2	1	8	AVC	3	3		ok
11	25	19B5404	2-3	1	1	2	1	8	IDA	foc 3	foc 3	foc 3	ok
12	25	18B8292	3	1	1	2	1	8	AVC	3	3		ok
13	25	19B4287	2-3	1	1	2	1	8	IDA	3	foc 3		ok
14	27	19B7113	2-3	1	1	2	1	8	IDA	2	2		ok
15	25	20B2961	2-3	1	1	2	1	8	IDA	2-3	2-3		
16	27	19B7431	3	1	1	2	1	8	IDA	3	3		
17	21	19B7029	2-3	1	1	2	1	8	IDA	3	2 (ISH+)		ok
18	20	18B10069	2	1	1	2	1	8	AVC	2			
19	20	19B6737	2-3	1	1	2	1	8	IDA	2			
20	21	17B8490	3	1	1	1	1	8	AVC	2			

Stain quality evaluation

Readout validation pathologists

True positive (TP)

Comparator/reference

Verification HER-2 Breast

- Omnis HER-2 poly
- CE-IVD according to IFU
- Reference Roche HER-2 (4B5)

Validation and Verification : How to proceed ?

NEGATIEF	REK	Staalnr + MCB HER-2	Intensiteit (0-3)	Uniformiteit (0-1)	Specificiteit (0-3) (*)	Afwezigheid Achtergrond (0-2)	Tegenkleuring (0-1)	TOTAAL	Initialen beoordeler	HER-2 score Initieel	HER-2 score patholoog 1	HER-2 score patholoog 2	Corelatie
1	19	19B3995											
2	27	19B 338	2-3						IDA				
3	22	19B 5948	2-3						IDA				
4	23	19B 1324	2-3						IDA				
5	22	19B 4689	2-3						IDA				
6	23	19B 4685	2						AVC				
7	23	19 4439	2-3						IDA				
8	23	19B 3745	3						AVC				
9	23	193 3679	2-3						IDA				
10	22	19B 2441	2-3						AVC				
11	18	193 2582	2-3						IDA				
12	21	18B 9620	3						AVC				
13	15	18B 10825	2-3						IDA				
14	15	18B 11493	2						AVC				
15	15	193 1067	2-3						IDA				
16	15	18B 9210	3						AVC				
17	21	193 9579	2-3						IDA				
18	15	18B 9371	2						AVC				
19	21	193 10100	2-3						IDA				
20	22	19B 4304	2-3						IDA				

Stain quality evaluation

Readout validation pathologists

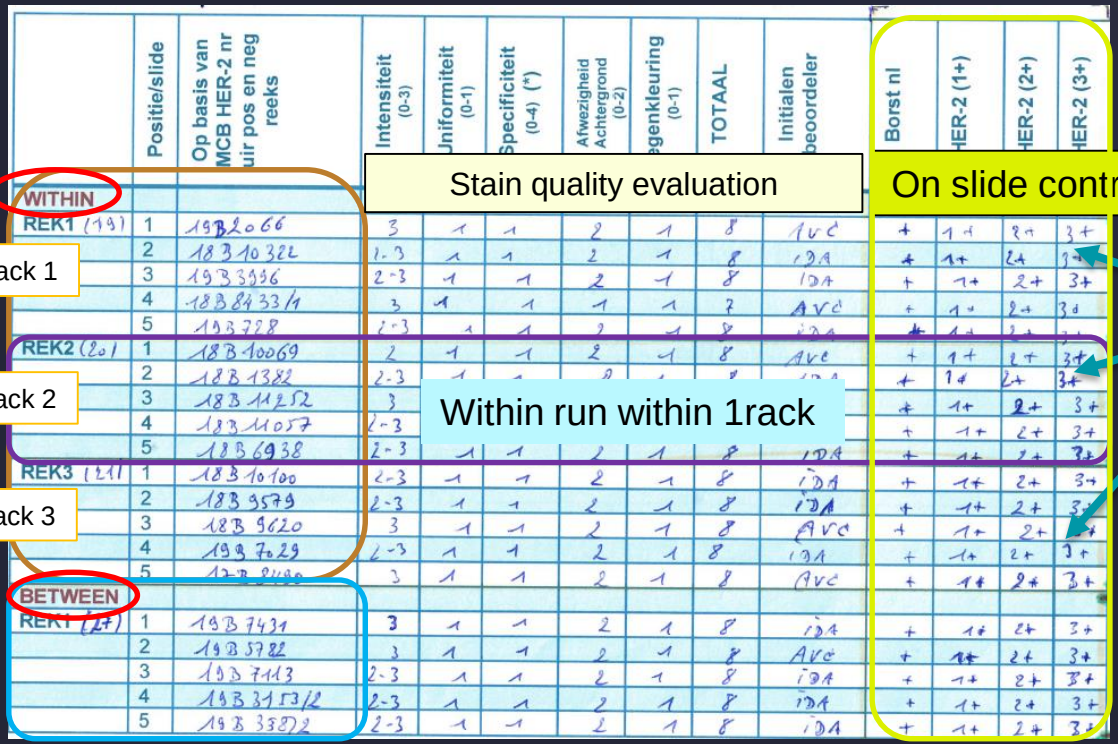
Comparator/reference

True negative (TN)

Verification HER-2 Breast

- Omnis HER-2 poly
- CE-IVD according to IFU
- Reference Roche HER-2 (4B5)

Validation and Verification : How to proceed ?



Verification HER-2 Breast

- Omnis HER-2 poly
- CE-IVD according to IFU
- Reference Roche HER-2 (4B5)

Validation and Verification : How to proceed ?

	Omnis HER-2 Poly (+)	Omnis HER-2 Poly (-)
Roche HER-2 4B5 (+)	TP	FN
Roche HER-2 4B5 (-)	FP	TN

	Omnis HER-2 Poly (+)	Omnis HER-2 Poly (-)
Roche HER-2 4B5 (+)	20	0
Roche HER-2 4B5 (-)	0	20

	Stain Quality Mean
20 (+)	7,95
20 (-)	7

	Reproducibility
Within run	100%
In between run	100%

Other : robustness, etc

Verification HER-2 Breast

- Omnis HER-2 poly
- CE-IVD according to IFU
- Reference test Roche HER-2 (4B5)

Evaluation :

Sensitivity : TP/ (TP + FN)
20/ (20 + 0) = 1 = 100%

Specificity : TN/ (TN + FP)
20/ (20 + 0) = 1 = 100%

Code	Expected result	Result	Evaluation
FLEX – 15	Stain quality - accuracy	Results accurate (equal to reference technique)	OK
FLEX – 16	Precision : Correlation within en between run >95%	HER-2 poly - Omnis : precision 100%	OK
FLEX – 17	Sensitivity : >90 % (diagnostic positive vs false negative)	HER-2 poly – Omnis : sensitivity = 100%	OK
FLEX – 18	Specificity : >95% (diagnostic negative vs false positive)	HER-2 poly – Omnis : specificity = 100%	OK
FLEX – 19	Stain quality score ≥ 7	Stain quality mean score 7,95 (positive) and 7 (negative)	OK
FLEX – 20	Readout – interpretation >90%	Correlation with reference technique = 100%	OK
FLEX – 21	Interobserver tunng	Correlation between pathologists = 100%	OK
FLEX – 22	Available internal qualitycontrol (IQC)	System for IQC available and operational	OK
FLEX – 23	Instructions For Use available (IFU)	IFU online available	OK
FLEX – 24	Cold ischemic time <1h	Cold ischemic time < 1u in >90% of cases	OK
FLEX – 25	Fixationtime between 6 – 72h	Verified fixationtime >90% of cases between 6-72h	OK
FLEX – 26	Robustness technique for fixationtime >72u	Technique can handle >72u fixation	OK
FLEX – 27	Storage of parafin sections	Storage of prepared parafin sections limited to 1 week at RT°.	OK
FLEX – 28	Mounting sections	Drying time and temperature max 1u op 60 °C (or overnight at RT°)	OK

Verification HER-2 Breast

- Omnis HER-2 poly
- CE-IVD according to IFU
- Reference Roche HER-2 (4B5)

Signed of for clinical use in daily practice

VALIDATION

Validation and Verification : How ?

Validation and verification process : **implementation**

- Education of staff :
  - New method/instrument, etc – by manufacturer or by allready trained staff
  - Competence & performance of staff (ISO15189)
- Procedures :
  - Performing method
  - Maintenace instrument
  - Etc
- IQC – appropriate controls
- EQC / Proficiency testing (if applicable)

Validation and Verification : How ?

Validation and verification process : **implementation**

- Communication to prescribing clinicians e.g. in case of changed method with impact on interpretation of results, impact on treatment, etc
- Logistics :
  - Ordering information, stock, etc
  - Switch in software staining platform to validated status/ diagnostic use, etc
  - Laboratory Information System (LIS) : e.g. bidirectional connection to (software) staining platform for automatic entering of task

Validation and Verification : How ?

Validation and verification process : **completion !?**

- Performed stains and compared results
- Met acceptance criteria for performance characteristics
- Implemented the validated method succesfully

- **Job done !?**

What have we proven so far ?

Validation and Verification : How ?

Validation and verification process : what has been proven so far ?

- Validation = captures a moment in time when limited to the initial validation :

It only prooves a test fullfilled the requirements

- *at a certain point in time,*
- *using certain lotnumbers of reagents,*
- *on a staining platform in a certain condition (e.g. new),*
- *performed by certain staff,*
- *etc*

Validation and Verification : How ?

Validation and verification process : what havs been proven so far ?

But :

- Staining platforms evolve over time (impact maintenance, wear, defects,etc)
- Lotnumbers of reagents and consumables differ over time
- Change in labtech's, pathologists
- Change in samples, fixation and processing (pre-analytics)
- etc

So **NO** 100% guarentee on daily quality if limited to the initial validation !

Validation and Verification : How ?

Ongoing validation safeguards daily quality :

- Internal quality control (daily – on slide/batch)
- Acceptance testing for critical reagents & consumables
- Acceptance testing of critical instruments after maintenance or repairs with possible impact on results
- Participation in EQC programmes / Proficiency testing
- Inter observer tuning, especially for type 2 IHC/ISH
- Correlationstudies with other methods

  E.g. correlation HER-2 IHC and ISH

04

CHANGES IN AN ALLREADY VALIDATED METHOD

Validation and Verification : How ?

Changes in **allready validated** method/test ?

What changes ?

- Preanalytical phase :
  - Fixation type
  - Decalcifiying reagent
  - Tissue processing : instrumentation/method/reagent

- Postanalytical :
  - Interpretation of readout for a particular intended use
  - Test used for other purposes ?

Validation and Verification : How ?

Changes in allready validated method/test ?

What changes ?

- Analytical phase :
  - Primary antibody : clone, dilution, lotnumber
  - Pretreatment : pH
  - Detection system
  - Readout
  - Staining platform
  - Water supply (when critical to stain)

Changes trigger additional verification/validation or an initial verification/validation

Validation and Verification : How ?

Changes in allready validated method/test ?

e.g. CAP guidelines – guidelines for number of samples

Change	Verification - Validation
New reagent lot for existing validated assay	Confirm assay performance using 1 (+) and 1(-)
Antibody dilition, antibody vendor (same clone), Incubation or retrieval times (same method)	Confirm assay performance using at least 2 (+) and 2(-)
Fixation type, antigen retrieval method (change pH, diferent buffer, different heat platform), detection system, tissue processing or testing equipment, relocation, water supply	Confirm with sufficient number of cases to ensure assay consistently achieves expected results Labdirector deecides on how many predictive and non predictive markers, how many (+) and (-) tissues to test
Antibody clone	Full revalidation

Summary

- Definitions validation & verification
- Determining questions :
  - What kind of test ? FDA/ CE- IVD or not ?
  - What is the purpose – intended use ? Used accordingly or not ?
  - How is the test used ? According to IFU or not ?

➡ Validation or verification

- Performing validation – verification :
  - Comparator test – reference
  - Performance characteristics
  - Acceptance criteria for performance characteristics
  - Which samples ?
  - How many ?

- Staining validation set of cases/controls
- Evaluate performance characteristics & acceptance criteria + document !
- If OK : implementation
- Ongoing validation

- Changes in allready validated test

THANKS!

I wellcome any questions or comments

Email : donald.vanhecke@stlucas.be