NordiQC
Seminar in Diagnostic Immunohistochemistry
Aalborg University Hospital, September 17-19 2025

Validation and verification process for IHC

What, why and how ?

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Note

- Purpose

The purpose of this presentation is to give background information about the verification and validation process for IHC. The point of view is that of the current regulations. Please check how this is reflected in your own national regulations, as they may vary from country to country.

- Disclaimer

This presentation has been prepared based on available information upon date.
Some slides contain documents that are the intellectual property of the Pathology Lab AZ St. Lucas Brugge (B). They cannot be used for commercial purposes.

- Informative slides

Some slides in this handout were not handled during the actual presentation and were added to this handout for informative reasons. They are indicated by the “i” in the upper right corner.

Agenda

1. Validation – Verification : WHAT ?

   Terms/definitions, difference in terminology

2. Validation – Verification : WHY ?

   Main reasons why we should perform validation/verification

3. Validation – Verification : HOW ?

   How can we perform validations/verifications ?

4. Changes in validated methods ?

Validation & verification : WHAT ?

Terms/definitions, different terminology

Validation & verification : definitions

Validation

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

Verification

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

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

Validation & verification : critical elements of the definitions

Validation

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Validation & verification : definitions

- In order to "demonstrate" or "confirm", a comparison of performance is needed between

NEW TEST ⟷ COMPARATOR TEST / REFERENCE / STANDARD

- <u>Comparator test/reference or standard performed on :</u>
  - *Types of samples with known morphology and expression of an antigen (e.g. iCAP (Immunohistochemistry Critical Assay Performance control))*
  - *Confirmed samples or controls stained with allready verified/validated method (own lab or other lab)*
  - *Reference in e.g. IFU, literature about staining patterns, performance, etc*
  - *EQA samples (if available), other techniques, etc*

Validation & verification : definitions

Validation

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

Verification

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

Objective evidence :

- Tests performed and evaluated need to be demonstrated and documented
- Raw data of staining and evaluation are tracable :
  - Sample numbers, lotnumbers reagents
  - Staining and evaluation date
  - Person performing staining, evaluation
  - Evaluation and conclusion
  - Etc

Validation & verification : definitions

Validation

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

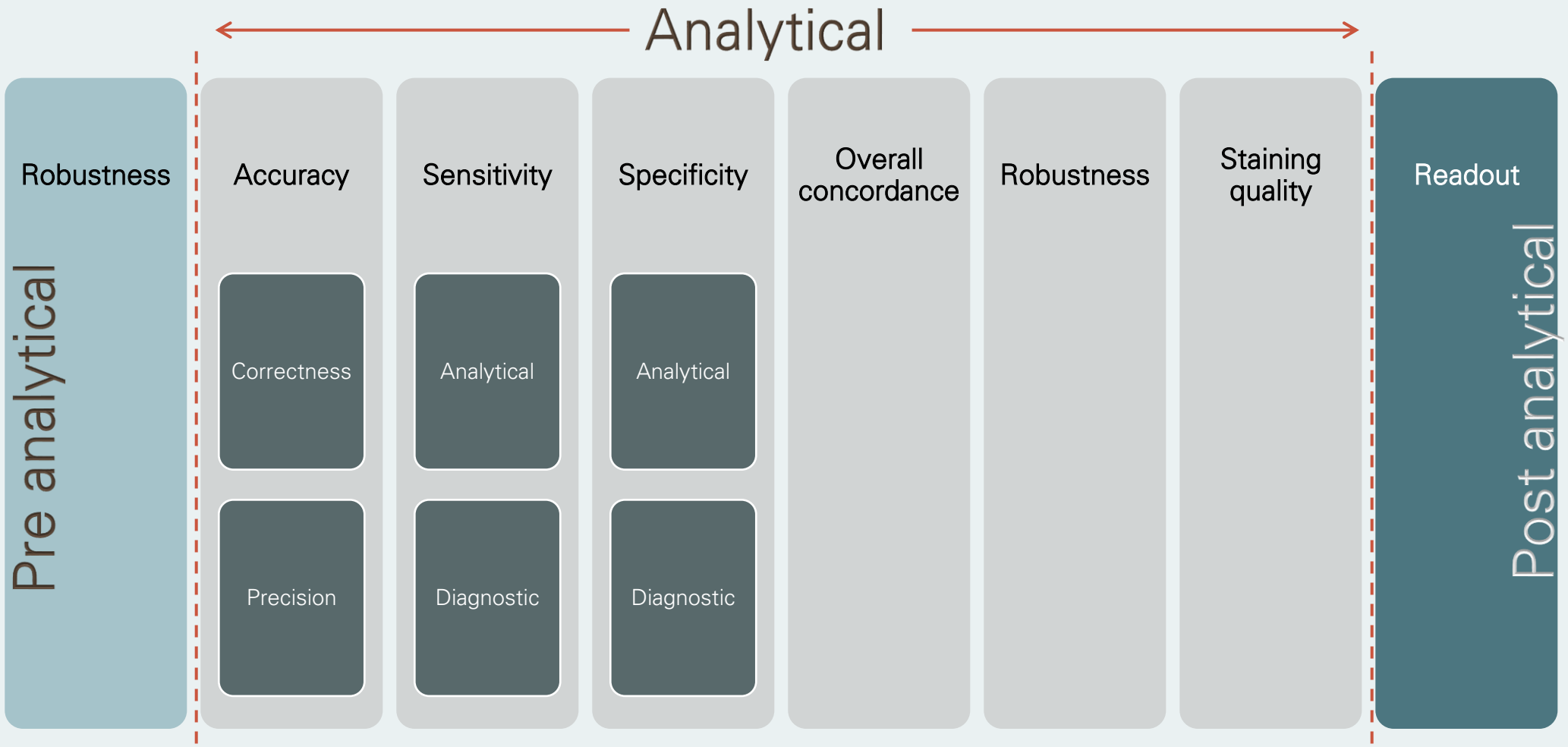
Verification

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

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

Validation & verification : definitions

Performance characteristics often used :



Validation & verification : definitions

- Performance characteristics often used :
  - **ACCURACY** : correctness & precision
    - **<u>Correctness :</u> does it stain the correct items ?**
      - Comparison with <u>known results</u> from validated tests (reference samples, validated testsamples (own or another lab)
      - Comparison with <u>other validated technique</u> (e.g. ISH vs PCR), other validated instrument or other reagents (other manufacturor)
      - Third line control (EQC or interlabcomparison)
      - Populationstudy

Validation & verification : definitions

- Performance characteristics often used :
  - **ACCURACY :** correctness & precision
    - **<u>Precision :</u> How “repeatable” are stains in the same or different runs ?**
      - 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 <u>all</u> platforms !

Validation & verification : definitions

- Performance characteristics often used :

  - Performance characteristics : **SENSTIVITY**

    - Analytical : ability of a test to detect small amounts of a substance (e.g. antigen)
      = dilutionrange for detectionlimit or e.g. detecting weak expressors
    - 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 & verification : definitions

- Performance characteristics often used :
  - Performance characteristics : **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 & verification : definitions

- Performance characteristics often used :

  - Performance characteristics : **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 & verification : definitions

- Performance characteristics often used :

  - Performance characteristics : **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 & verification : definitions

- Performance characteristics often used :

  - 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 & verification : definitions

- Performance characteristics often used :

❖ <u>Performance characteristics :</u> **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 & verification : definitions

- Which performance characteristics to be used ? Guidelines available ?

COLLEGE of AMERICAN PATHOLOGISTS

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

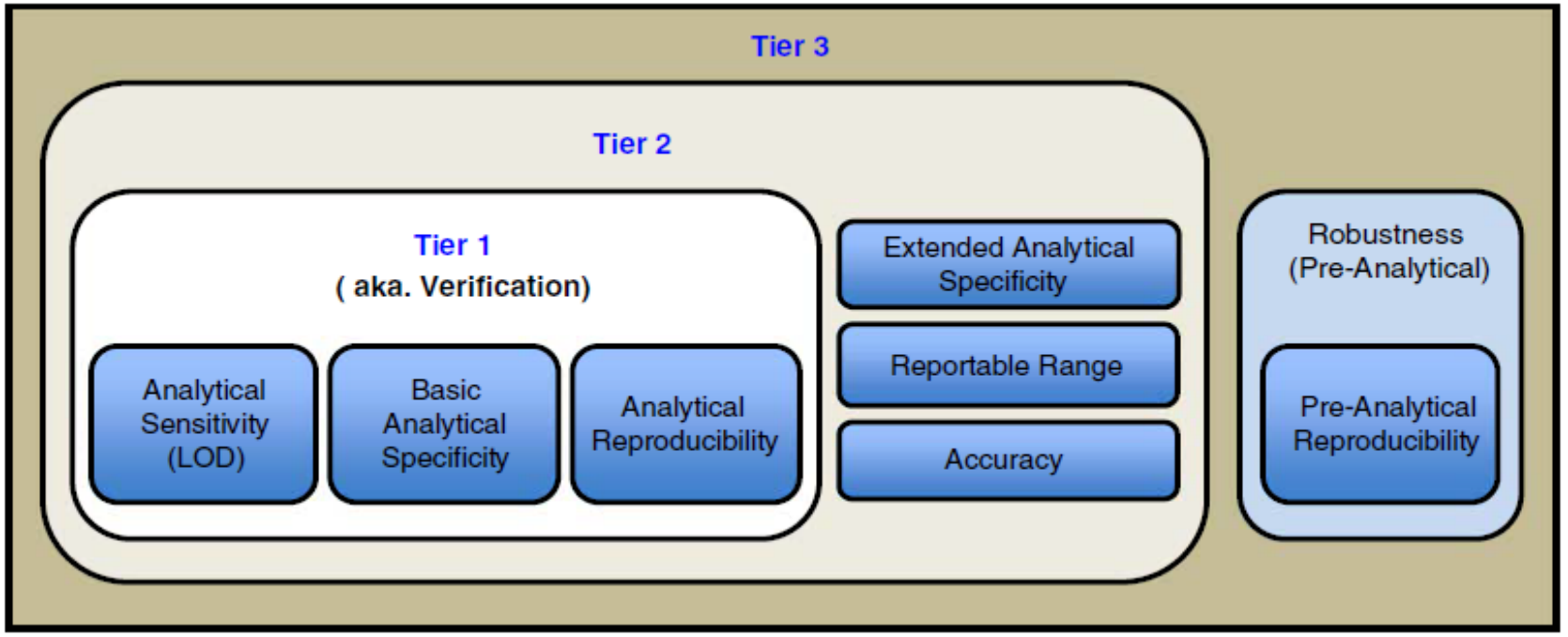
Validation & verification : definitions

- Which performance characteristics to be used ? Guidelines available ?

ISIMm
International Society for IHC and Molecular Morphology

	Tier 1	Tier 2	Tier 3
FDA / CE-IVD	✓	-	-
LDT	✓	✓	✓
Re- validation *	✓	✓	✓

Depending on reason for revalidation



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

Validation & verification : definitions

- Which performance characteristics to be used ? Guidelines available ?

	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

Volksgezondheid
Veiligheid van de Voedselketen
Leefmilieu

sciensano .be

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 & verification : definitions

- Which acceptance criteria for the selected performance characteristics ? Guidelines ?
  - E.g. CAP : 90% overall concordance for every test used clinically
  - Defined by the number of samples in the validation :

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

	Concordance Estimate, % (95% CI)		
No.	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

The higher the number of samples, the "easier" to get a high concordance

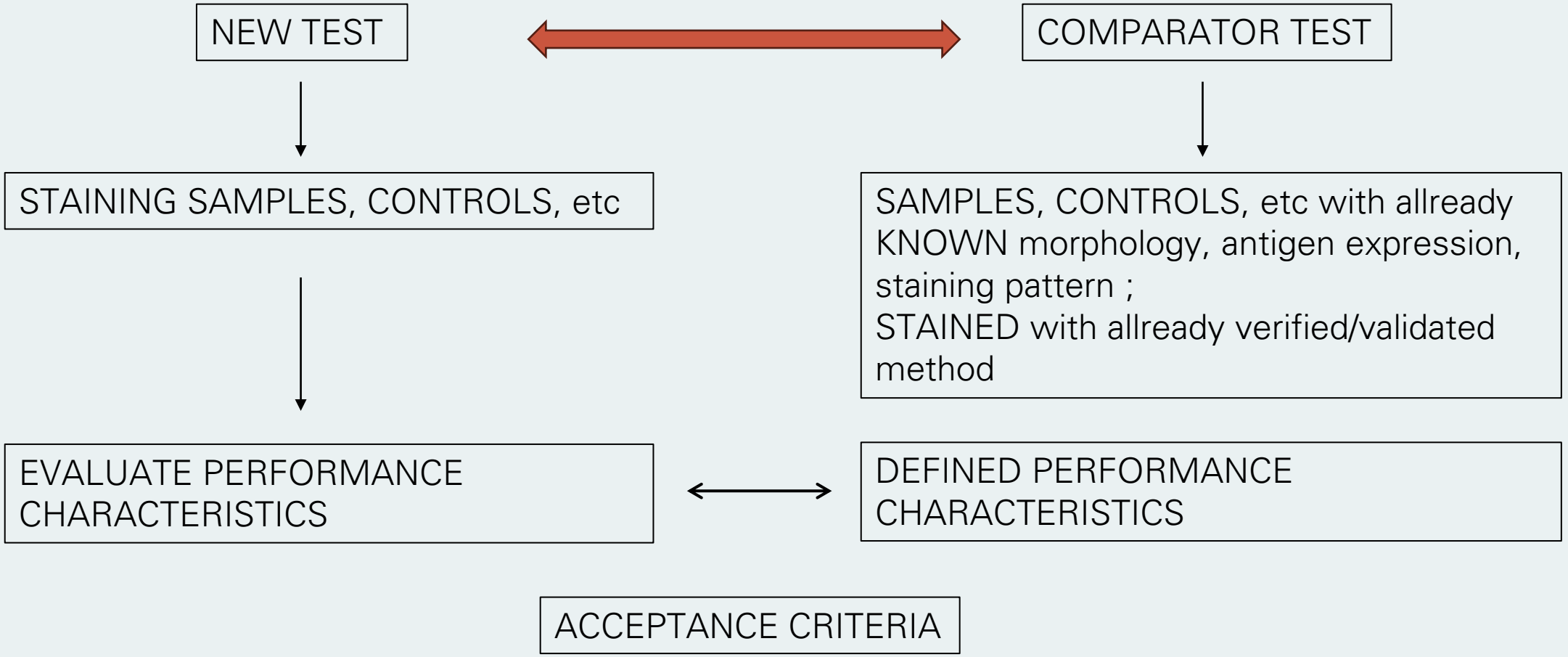
Validation & verification : definitions

Essential elements within these definitions :

Validation	Verification	What does this mean ?
Demonstrate	Confirmation	Comparator test, reference, standard, etc
Objective evidence	Objective evidence	Document
Performance characteristics	Performance characteristics	Various performance characteristics & Acceptance criteria

Validation & verification : principle

Comparison of performance :



The Key Difference Between Validation and Verification ?

Difference between validation & verification in :

- The **extent** of type of tests performed and evaluated,
- **Performance criteria** used,
- Degree of evidence, documentation, etc required

Validation & verification : difference ?

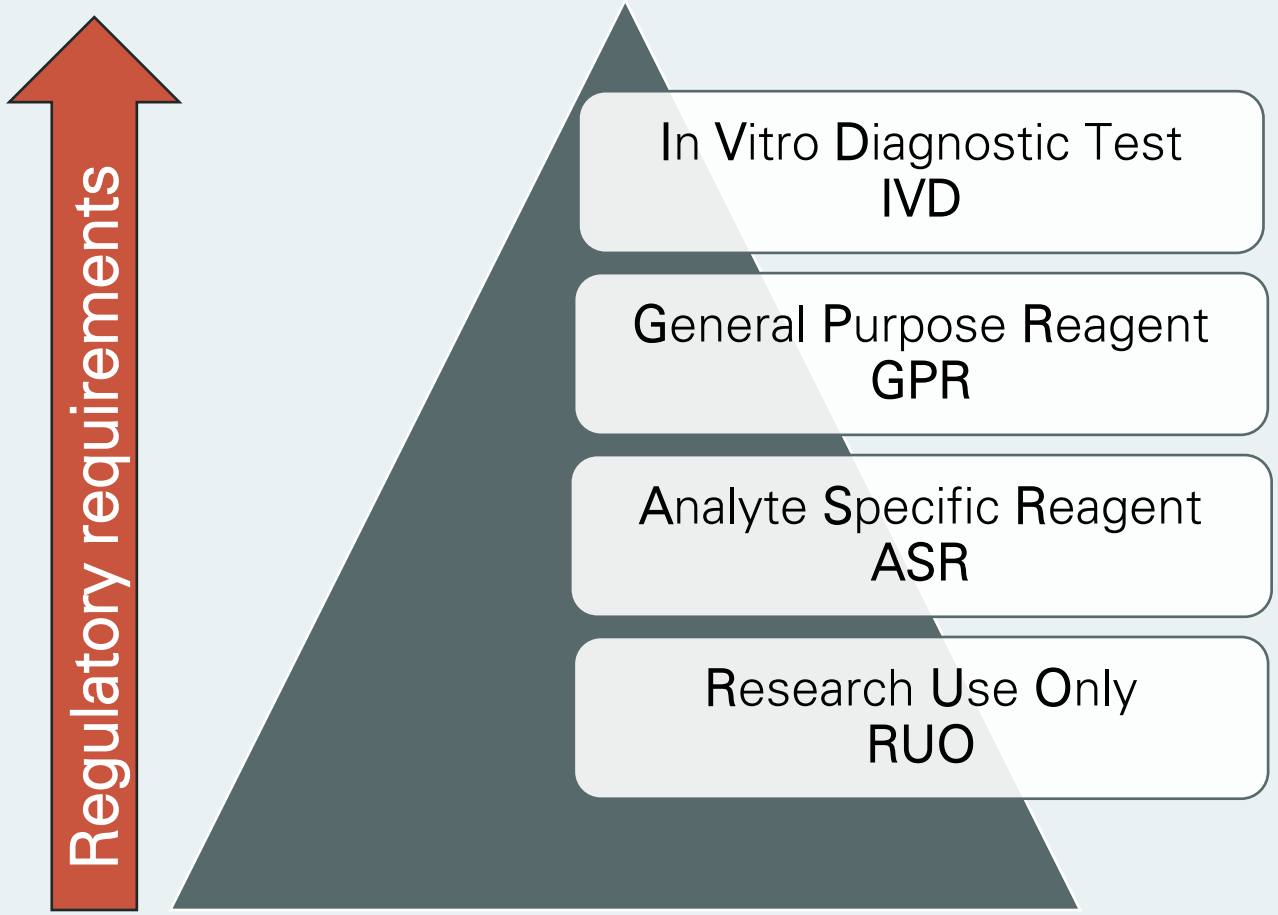
- What determines the use of “verification” or “validation” ?

Key questions ?	Verification	Validation
Type of test ?	CE – IVD Instructions For Use	All other (*)
Used according to IFU ?	YES	NO
Used for intended purpose ?	YES	NO

(*) Other include CE-IVD not according to IFU, non CE-IVD (e.g. RUO)

Validation & verification : difference ?

Question 1 : What type of test ?



EU-IVDR

FDA
U.S Food and Drug Administration

CE
IVD

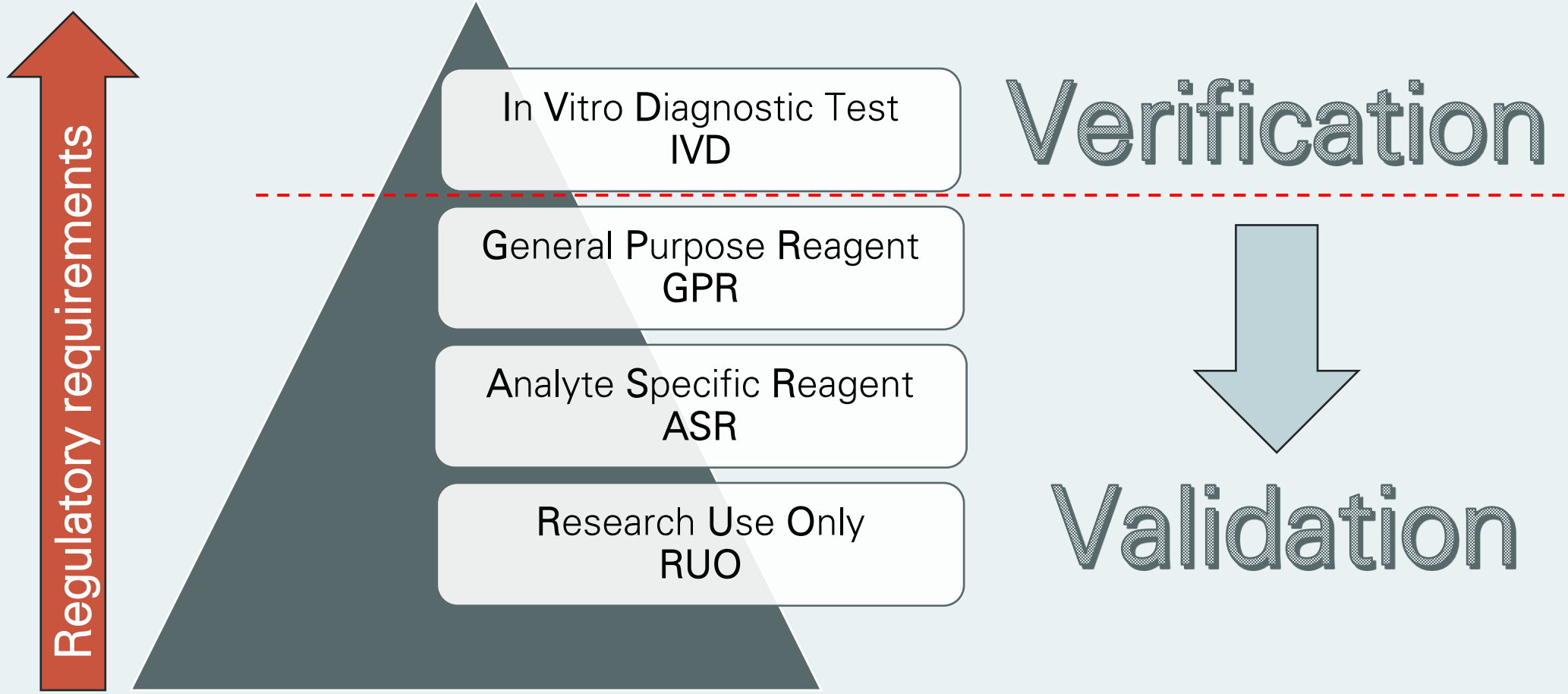
IVD

ASR

RUO

Validation & verification : difference ?

Question 1 : What type of test ?



Validation & verification : difference ?

Question 1 : What type of test ?

❖ In Vitro Diagnostic Test (IVD) :

- EU Regulation 217/746 : In vitro diagnostic medical devices (IVDs) are tests used on biological samples to determine the status of a person's health. There is a broad range of IVDs. Some examples are self-tests for pregnancy, COVID-19 tests, cancer genetic tests or high-throughput testing of blood donations for infections such as HIV, performed in clinical laboratories.
- FDA : In vitro diagnostic products are those reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body. These products are devices as defined in section 201(h)(1) of the Federal Food, Drug, and Cosmetic Act (the act) and may also be biological products subject to section 351 of the Public Health Service Act, including when the manufacturer of these products is a laboratory. [21 CFR 809.3]
  Like other medical devices, IVDs are subject to premarket and postmarket controls. IVDs are generally also subject to categorization under the Clinical Laboratory Improvement Amendments (CLIA '88) of 1988.

Validation & verification : difference ?

Question 1 : What type of test ?

❖Research Use Only (RUO) :

- EU Regulation 217/746 : No definition available. IVDR does not regulate the use of RUO reagents according to their Instructions For Use.
  (= For research use, not for clinical use)
- FDA : RUO stands for For Research Use Only, designating a product intended for use in laboratory research, not for diagnostic or therapeutic purposes. While the FDA regulates products for human use, RUO products are generally unregulated, provided they are clearly labeled "For Research Use Only. Not for use in diagnostic procedures".

Validation & verification : difference ?

Question 1 : What type of test ?

❖Investigational Use Only (IUO) :

- EU Regulation 217/746 : No definition available.
- FDA : Investigational Use Only" (IUO) means a product, typically an in vitro diagnostic (IVD) or medical device, is still in clinical studies and has not yet been approved or cleared by the FDA for commercial marketing or sale. Such products are labeled with a specific disclaimer, such as "For Investigational Use Only. The performance characteristics of this product have not been established," and are used to gather data to support future FDA approval. The FDA regulates these products under an Investigational Device Exemption (IDE) to ensure that they are used in clinical studies to collect

Validation & verification : difference ?

Question 2 : What is "used according to Instructions For Use" ?

- Instructions For Use (IFU) :
  - Purpose/ intended use and type of sample defined
  - Clear instructions :
    - Pretreatment
    - Dilution of Ab (concentrated Ab)
    - Incubation times Ab, detection system, enhancing step, etc
    - Preprogrammed or full description of method ?

Always that clear – obvious – well described ?
Know if you work according to IFU or not ?

Validation & verification : difference ?

- What is “used according to Instructions For Use” ?

VENTANA

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

REF 790-2991

05278368001

IVD

INDICATIONS AND USE

Intended Use

This antibody is inten

Ventana Medical Syste
Monoclonal Primary An
intended for laboratory
of formalin-fixed, paraffi
automated immunohist
assessment of breast c

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

Dako

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

Code

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 & verification : difference ?

- What is “used according to Instructions For Use” ?

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 & verification : difference ?

- What is “used according to Instructions For Use” ?

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Do I use 10 % NBF ?

Specimen Preparation

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

Suggestion/recommmendation vs “No changes allowed” ?

Recommendations On Use

Incubation temperature on my staining platform ?

Immunohistochemistry on paraffin sections.

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Validation & verification : difference ?

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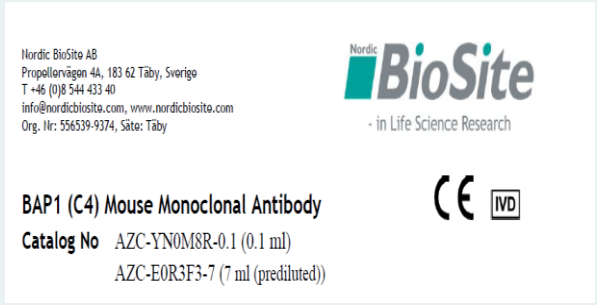


Materials Provided

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

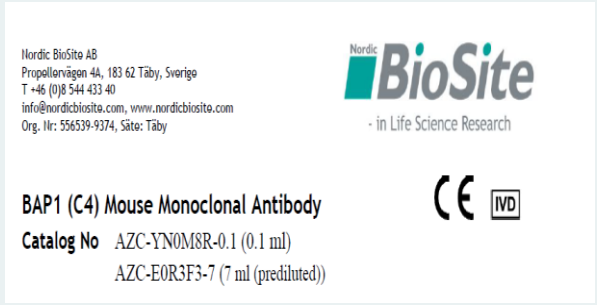
Positive control Malignant Mesothelioma

Optimization :
1: 50 or 1: 200 ?

According to IFU ?

Validation & verification : difference ?

- What is “used according to Instructions For Use” ?



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Antibody Specifications Antibody as Purified antibody diluted in Tris-HCl 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.

Optimization :
1: 50 or 1: 200 ?

According to IFU ?

Validation & verification : difference ?

Question 3 : What is "used for the intended purpose" ?

- Purpose of a test = intended use at the time test was developed (manufacturer)
- May or may not be the same as (clinical) intended use (lab – user)
- "Fit for Purpose" = test has been validated for an intended use at the time the test was developed (both lab/technical assay and clinical use)

Relation between intended purpose and verification/validation :

Same purpose	According to IFU	Verification
Change purpose (= change test)	Not according to IFU	Validation

Purpose of a test can also determine if a verification or validation needs performed

Validation & verification : difference ?

Question 3 : What is “used for the intended purpose” ?

- Purpose of a test :
  - Related to “3D” : Disease – Diagnostic test – Drug
  - Classification upon risk for patient :

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

Diagnostic	Prognostic	Predictive
Type 1	Type 2a	Type 2b

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)*

Validation & verification : difference ?

Question 3 : What is “used for the intended purpose” ?

❖ Purpose of a test ? Indicated in the Instructions For Use (IFU)

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

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 & verification : difference ?

Question 3 : What is “used for the intended purpose” ?

- Purpose of a test ? Indicated in the Instructions For Use (IFU)

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 & verification : WHY ?

Main reasons why we should perform a validation/verification

Validation & verification : WHY ?

Initial drivers for verification or validation ?

Requirements	Regulations	Technical
• Legal • Accreditation • Professional organisations	• EU IVDR • FDA	• Reagents • Instruments • Methods

N°1 = Patient best interest !

Validation & verification : WHY ?

1. <u>Legal or accreditation requirements, guidelines, etc</u>

   - Requirements by Federal Agencies, National regulations, etc

   - Guidelines by professional organisations : E.g. College of American Pathologists

   - Accreditation requirements : E.g. ISO 15189 (2022) Medical laboratories – Requirements for quality and competence


INTERNATIONAL STANDARD

ISO 15189

Fourth edition
2022-12

Medical laboratories — Requirements for quality and competence

Laboratoires de biologie médicale — Exigences concernant la qualité et la compétence

<u>Requirements under chapters :</u>

7.3.2 Verification of examination methods

7.3.3 Validation of examination methods

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

- Revised IVD regulation, effective May 2022
- Guidelines for manufacturing and use of IVD

L 117/176 EN Official Journal of the European Union 5.5.2017

REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 5 April 2017

on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU

Subject matter and scope

1. This Regulation lays down rules concerning the placing on the market, making available on the market or putting into service of *in vitro* diagnostic medical devices for human use and accessories for such devices in the Union. This Regulation also applies to performance studies concerning such *in vitro* diagnostic medical devices and accessories conducted in the Union.

= guidelines for " manufacturers"

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

Only 2 categories of tests :

CE- IVD	Used according to Instructions for Use	
CE- IVD	Used according to Instructions for Use	Verification
All other	CE- IVD NOT used according to Instructions For Use Non CE- IVD NOT used according to IFU (E.g. Research Use Only) Laboratory Developed or Home Brew test	Validation

"Other" : NO specific terminology in EU IVDR Directive, but a definition :

" Devices manufactured and used within health institutions within EU"

- Commonly used term : Laboratory Developed Test (LDT)
- Other terminology ? Laboratory Modified test (LMT), CE-IVD with minor/major modifications, Home Brew Test, In House test

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

- LDT definition : " Devices manufactured and used within health institutions "

  ➡ Laboratory becomes a "manufacturer" of the LDT

  - *Manufactured ?*

    - 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 manufacturer 1 on staining platform manufacturer 2))
    - Significantly modifying an existing device : not intended by the manufacturer and has impact on product conformity (= changing a method, intended use, etc)

What is "significantly modifying ?
What is the impact of the changes, especially to patient safety and test performance ?

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

- Significant (major) vs non significant (minor) changes ? (MDCG 2022-6)

			Y	N
☐	**Intended purpose**	☐ **Limitation** ☐ **Extended use** ☐ **Other** (specimen type, etc)	☐ NS ☐ S ☐ S	☐ NS ☐ NS ☐ NS
☐	**Design**	☐ **Testprinciple changed** ☐ **Negative effect on safety/performance or risk/benefit ratio**	☐ S ☐ S	☐ NS ☐ NS
☐	**Ingredients or materials**	☐ **Essential for testprinciple** ☐ **Negative effect on safety/performance or risk/benefit ratio**	☐ S ☐ S	☐ NS ☐ NS
☐	Software	Software - driver, database (no bugfix, security update, etc)	☐ S	☐ NS
☐	Sterility or packiging	☐ Impact on sterility of the product ☐ Changed packaging with impact on product	☐ S	☐ NS
☐	**CAPA**	☐ **CAPA evaluated and accepted by competent autority**	☐ NS	☐ S

Source : MDCG 2022 – 6 Guidance on significant changes regarding the transitional provision under article 110(3) of the IVDR)

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

- Significant (major) vs non significant (minor) changes ? (MDCG 2022-6)

			Y	N
☐	**Intended purpose**	☐ **Limitation** ☐ **Extended use** ☐ **Other** (specimen type, etc)	☐ NS ☐ S ☐ S	☐ NS ☐ NS ☐ NS
☐	**Design**	☐ **Testprinciple changed** ☐ **Negative effect** on safety/performance or risk/benefit ratio	☐ S ☐ S	☐ NS ☐ NS
☐	**Ingredients or materials**	☐ Essential for testprinciple ☐ **Negative effect** on safety/performance or risk/benefit ratio	☐ S ☐ S	☐ NS ☐ NS

- Effect of changes on performance/safety ? ➡ **Validation**
- Effect of changes vs risk/benefits ratio for patients ? ➡ **Risk analysis**

Extended use :
- E.g. : Leica C-kit CD117 for Giardia staining (= significant)

Other (specimen type) :
- Ab used on cytology (when not indicated in IFU) (= significant)

Ingredients/materials :
E.g. : Ab used on another platform then described in IFU
Changes to method outside IFU

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

- If a test is an LDT or not an LDT is about :

Changes on default method/materials/reagents or intended use	vs	Information in IFU

&

Effect of changes on performance/safety – or – risk/benefits ratio for patients

LDT is NOT about the use of an RTU or a concentrated Ab as such :

- The use of a concentrated Ab does not make it by default an LDT, but it can, depends on the IFU
- The use of an RTU reagent/Ab does not by default clear it from being an LDT, depends on the IFU

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

- Are lab's allowed to use LDT ? (Health Institutions within EU)

- EU IVDR Directive : CE-IVD test = first choice
  LDT may be used if no "valid" CE- IVD is available and :

  - Clinical and analytical performance demonstrated

  - Risk analysis for the impact of changes in IFU

OR

- Art 5.5c : Lab fullfils requirements of ISO15189 or – when applicable – appropriate national requirements (e.g. national guidelines regarding accreditation, requirements for certification of lab, etc)

+ Comply with Annex 1 of IVDR

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

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 !)

+ Post – market evaluation of performance of tests (both CE-IVD and LDT)

Validation & verification : WHY ?

2. EU Directive EU2017/746 In Vitro Diagnostics

-- Classification of IVD tests based upon risk for patients :

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	/

Inspection by competent authority : class D or C&D (depending on country)

Validation & verification : WHY ?

3. Technical considerations

- IHC test by manufacturer:
    - Developed under certain conditions
    - For a certain purpose / intended use
- Instructions For Use :
    - Contains (well) described method
    - Sample usage : e.g. Formalin Fixed, Paraffin Embeded (FFPE), frozen section, etc
    - Staining platform to be used on (recommended or not)
    - etc

Validation & verification : WHY ?

3. Technical considerations

- IHC test by manufacturer:
    - Developed under certain conditions
    - For a certain purpose / intended use
- Instructions For Use :
    - Contains (well) described method
    - Sample usage : e.g. Formalin Fixed, Paraffin Embeded (FFPE), frozen section, etc
    - Staining platform to be used on (recommended or not)
    - etc

However…

Validation & verification : WHY ?

3. Technical considerations

Manufacturer – development of a test

- Developed under certain conditions and for a certain purpose / intended use
- Instructions for use :
  - Sample usage : e.g. FFPE
  - Described method
  - Staining platform

Customer/lab using test

- Used for intended purpose or use ?
- Performed as prescribed by manufacturer ?
- Instructions for use :
  - Sample usage : e.g. FFPE
    - Tissue processing protocol ?
    - Fixative used ? Fixation times ?
  - Section thickness ?
  - Section preparation ?
  - Etc

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

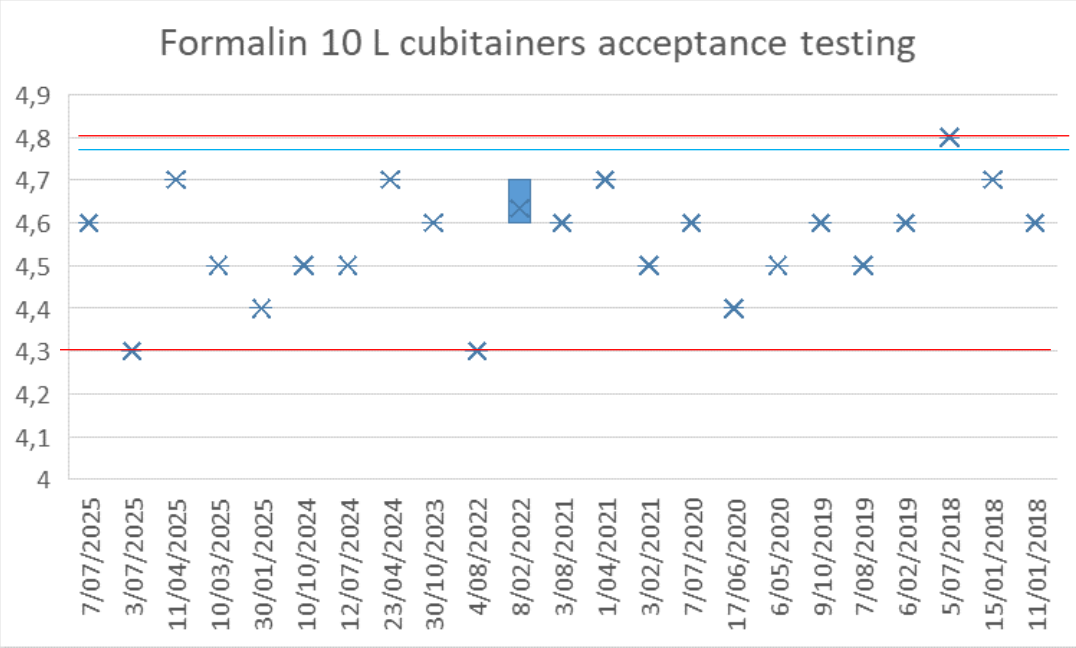
Validation & verification : WHY ?

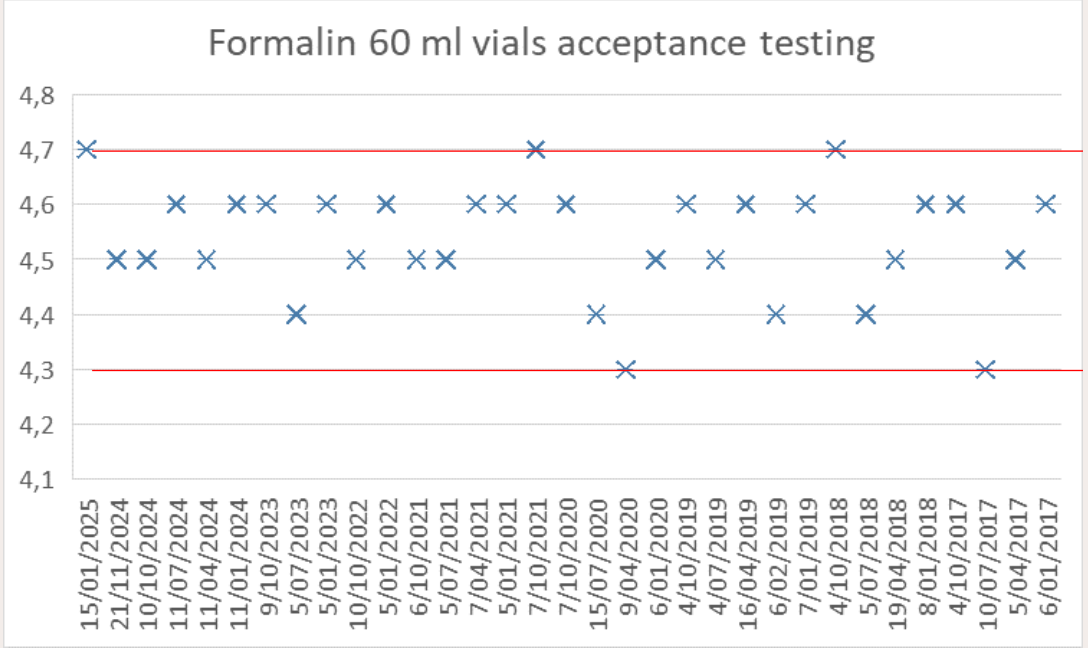
3. Technical considerations

Novocastra™ Liquid
Mouse Monoclonal Antibody
Prostate Specific Antigen
Product code: NCL-L-PSA-431

Specimen Preparation

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

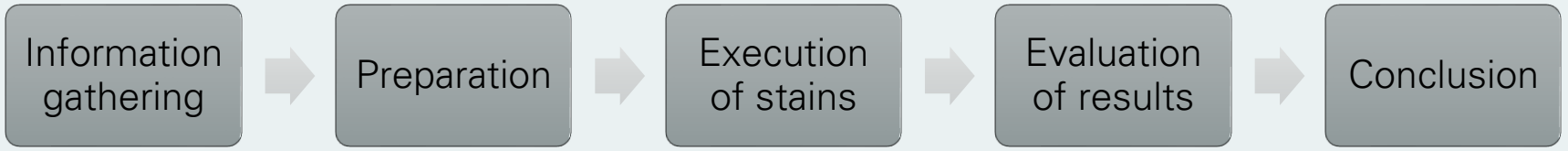




Validation & verification : How ?

Main reasons why we should perform a validation/verification

Validation & verification : How ?



Validation & verification : How ?

Step 1 : Information gathering

- Selection of the antibody and clone

	Verification	Validation
Type of test	CE – IVD according to Instructions For Use	All other (*)
Used according to IFU	YES	NO
Used for intended purpose ?	YES	NO

- Available information ?
  - Instructions For Use or other information from manufacturer , literature references, EQA results ?
  - Information about stainng patterns, tissue elements stained, etc (non specific staining)
    - Information about appropriate controls, weak expressors (iCAP's), etc
    - Guidelines about validation/verification ? National guidelines, professional organisations

Determines verification or validation strategy

Validation & verification : How ?

Step 2 : Preparation

- Formulate verification/validation strategy/plan :
  - Verification or validation ?
  - Select comparator test/reference
  - Select appropriate performance characteristics
  - Determine acceptance criteria for the selected performance characteristics

  - Type of samples/cases/controls
  - Number of cases/slides

Validation & verification : How ?

Step 2 : Preparation

- Determine **type of samples/cases/controls**
  - Related to purpose – intended use of test (IFU)
  - Controls (preferably "in house" = same processing)
  - Known patient cases
  - Different expresion levels (weak/moderate/strong), different tissue types, etc
  - Single piece samples
  - Sausage blocks or multi-tissue blocks, Tissue Micro Array (TMA), etc
  - Same processing as clinical samples in daily routine (preferably)

Slides/cases preparation according to manufacturer/guidelines + storage before and during validation !

Validation & verification : How ?

Step 2 : Preparation

- Determine **number of samples/cases/controls**
  - Determined by type of test :
    - CE-IVD or not
    - Type 1 vs type 2 IHC
  - Determined by the way test is used
    - Performed according to IFU or not (method, type of sample, staining platform)
    - Used for the same purpose/intended use or not

Validation & verification : How ?

Step 2 : Preparation

- Determine **number of samples/cases/controls**

Available references referring to actual number of cases needed is limited.

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
Regan S. Fulton, MD, PhD; Jeffrey D. Goldsmith, MD; Thomas S. Haas, DO; Rouzan G. Karaba
Patti A. Loykasek, HT(ASCP); Monna J. Marolt, MD; Steven S. Shen, MD, PhD; Anthony T. Smith, M

Accepted for publication February 3, 2014.

COLLEGE of AMERICAN PATHOLOGISTS

Principles of Analytic Validation of Immunohistochemical Assays

Guideline Update

Jeffrey D. Goldsmith, MD; Megan L. Troxell, MD, PhD; Sinchita Roy-Chowdhuri, MD, PhD; Carol F. Colasacco, MLIS, SCT(ASCP); Mary Elizabeth Edgerton, MD, PhD; Patrick L. Fitzgibbons, MD; Regan Fulton, MD, PhD; Thomas Haas, DO; Patricia L. Kandalaft, MD; Tanja Kalicanin, MLS(ASCP)^{CM}; Christina Lacchetti, MHSc; Patti Loykasek, HTL(ASCP); Nicole E. Thomas, MPH, CT(ASCP)^{CM}; Paul E. Swanson, MD; Andrew M. Bellizzi, MD

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Published online February 23, 2024.

Validation & verification : How ?

Step 2 : Preparation

- Determine **number of samples/cases/controls**

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 (-)	Accuracy Precision Analyt sensitivity Analyt specificity

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

Availability of controls/cases, use in panel or stand alone, experience of lab, cost ?

Validation & verification : How ?

Step 2 : Preparation

- Determine **number of samples/cases/controls** : Belgian guidelines CE-IVD

Experience

No experience

CE-IVD volgens bijsluiter of gewijzigde CE-IVD MET referentie												
INITIEEL MET ERVARING[1] OF HISTORISCH							INITIEEL ZONDER ERVARING[1]					
		Juistheid[2]	Precisie[2,3]		Robuustheid[2]	Andere[2,4]		Juistheid[2]	Precisie[2,3]		Robuustheid[2]	Andere[2,4]
			Intra	Inter					Intra	Inter		
3 IHC/ISH Type 1 panel 15 AL	per AL	5s (+) 5s (-)	3s x 3gl[9] (1 AL)	3s x 3gl[9] (1 AL)		I/U/S/A/T : > X	per AL	10s (+) 10s (-)	3s x 3gl[9] (1 AL)	3s x 3gl[9] (1 AL)		I/U/S/A/T : > X
(methodevalidatie)	AC[6]	≥ 90%	≥ 90%	≥ 90%			AC[6]	≥ 90%	≥ 90%	≥ 90%		
IHC/ISH Type 1 vanaf 16de AL	per AL	2s (+) 2s (-)				I/U/S/A/T : > X	per AL	2s (+) 2s (-)				I/U/S/A/T : > X
	AC[6]	≥ 75%					AC[6]	≥ 75%				
4 IHC/ISH Type 2a	Initiële matrix	7s (+) 7s (-)	3s x 3gl	3s x 3gl	5s(+) 5s(-)	I/U/S/A/T : > X 5s(+) en 5(s-) interobserver	Initiële matrix	15s(+) 15s (-)	3s x 3gl	3s x 3gl	5s(+) 5s(-)	I/U/S/A/T : > X 5s(+) en 5(s-) interobserver
(specifieke verificatie/validatie)	AC[6]	≥ 90%[10]	≥ 90%	≥ 90%	≥ 90%	≥ 90%	AC[6]	≥ 93%[10]	≥ 90%	≥ 90%	≥ 90%	≥ 90%
IHC/ISH Type 2b	Initiële matrix	10s (+) 10s (-)	3s x 3gl	3s x 3gl	5s(+) 5s(-)	I/U/S/A/T : > X 5s(+) en 5(s-) interobserver	Initiële matrix	20s(+) 20s (-)	3s x 3gl	3s x 3gl	5s(+) 5s(-)	I/U/S/A/T : > X 5s(+) en 5(s-) interobserver
	AC[6]	≥ 90%[10]	≥ 90%	≥ 90%	≥ 90%	≥ 90%	AC[6]	≥ 95%[10]	≥ 90%	≥ 90%	≥ 90%	≥ 90%

- ISO15189 based quality management system
- Emphasis shift to ongoing validation (e.g. IQC)

Taking into account : experience of a lab, availability controls, achievability, costs, etc

(*) Belgian Recommendations for Analytical Verification and Validation of Immunohistochemical Tests in Laboratories of Anatomic Pathology - PMC (nih.gov)
App Immunohistochem Mol Morphol - 2023

Validation & verification : How ?

Step 3 : Execution of stainings

- Starting point : default protocol according to IFU + selection of controls/cases
- Evaluation of staining :

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				

Use of a scoring system can help guide further optimization/calibration

Optimal	
Good	Comment
Weak	Comment

- Stain/method OK : proceed to verification/validation
- Stain not OK : optimize/calibrate untill ok
  - Method optimization within IFU = verification
  - Method optimization outside IFU = validation

No additional info to guide optimization

Validation & verification : How ?

Step 3 : Execution of stainings

- Default protocol according to IFU or optimized protocol used to stain verification or validation set/cases
  - Select cases, controls, etc
  - Prepare necessary cases according to IFU (Time, temperature, storage)
  - Perform stains required to evaluate performance characteristics
    - Staining runs (accuracy, sensitivity, specificity, etc)
    - Staining runs within run
    - Staining run in between run
    - Robustness, etc

Validation & verification : How ?

Step 4 : Evaluation of stainings

- Compare staining results from default protocol according to IFU or optimized protocol with staining of reference / comparator
  - Document staining results :
  - List up, use 2 x 2 matrix, etc
  - Paper, Excel, etc
- Evaluate / determine / calculate performance characteristics

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

Step 5 : Conclusion

- Acceptance criteria for performance characteristics met ?
  - YES = OK for use in daily / clinical practice
- Sign of by e.g. lab director, pathologist, quality manager

Validation & verification : How ?

Practical example : HER-2 conc Ab

- Select reagent and determine intended use (e.g. breast, stomach)
- Check literature – Instructions For Use – recommended controls, etc
- Determine comparator test
- Validation plan : determine performance critertia & acceptance criteria

Code	Performance criteria – breast tissue	Expected result
FLEX – 15	Accuracy	Stain quality – accuracy >95% (equal to comparator)
FLEX – 16	Precision	Precision : Correlation within en between run >95%
FLEX – 17	Sensitivity	Sensitivity : >90 % (diagnostic positive vs false negative)
FLEX – 18	Specificity	Specificity : >95% (diagnostic negative vs false positive)
FLEX – 19	Staining quality	Stain quality score ≥ 7
FLEX – 20	Interpretation - readout	Readout – interpretation >90%
FLEX – 21	Interobserver	Interobserver tunng > 95%
FLEX – 22	Ongoing – Internal Quality Control	Available internal quality control (IQC) system
FLEX – 23	Documentation - IFU	Instructions For Use available (IFU)
FLEX – 24	Robustness – Cold Ischemia Time	Cold ischemic time <1h
FLEX – 25	Robustness – Fixation time	Fixationtime between 6 – 72h
FLEX – 26	Robustness - Overfixation	Robustness technique for fixationtime >72u
FLEX – 27	Robustness – storage of sections	Storage of parafin sections determined, minimal 1w
FLEX – 28	Robustness – Mounting of sections	Mounting sections : max 1h at 60°C

Validation & verification : How ?

Practical example : HER-2 conc Ab

- Select appropriate number of cases/controls
- Prepare slides/cases/controls
- Check default method

Antilichaam	Kloon	Fabrikant	Referentienummer	⊗ Nieuw	○ Gewijzigd, reden
HER-2	c-erb-B2 POLY	DAKO	A0485 (picone-AL) c-erbB-2	Omnis	

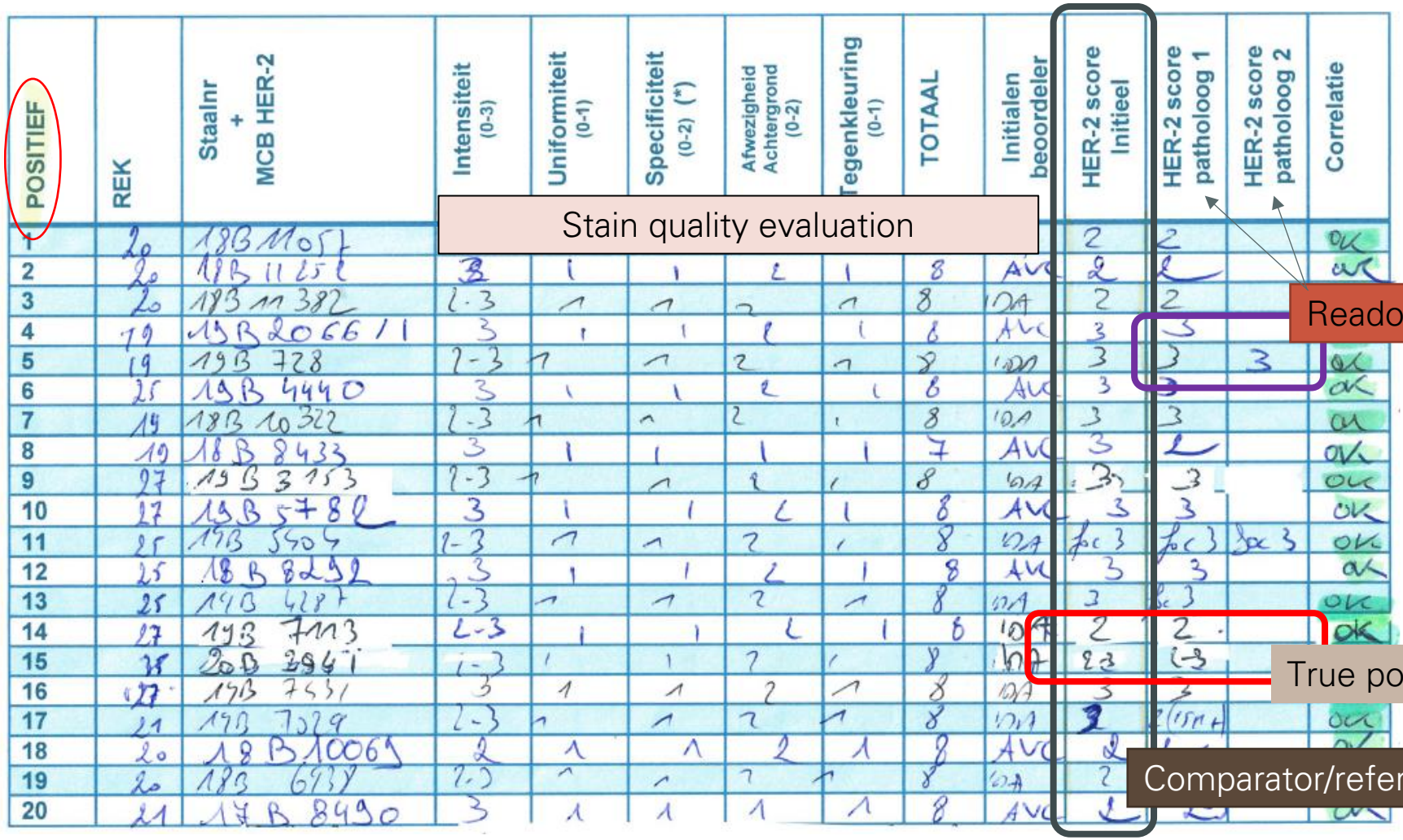
STANDAARDPROTOCOL BORST Uitvoerder : ..... Datum : 17.06.12a. Controleblok : HER-2 MC3 2019-06

Antilichaam				Voor-behandeling			Detectie kit		Intensiteit (0-3)	Uniformiteit (0-1)	Specificiteit (0-1)(*)	Afwezigheid Achtergrond (0-2)	Tegenkleuring (0-1)	Totaal
Lotnummer	Vervaldatum	Verdunning	Tijd	Oplossing	Tijd	Temperatuur	Lotnummer	Vervaldatum						
Lot 73789	[illegible]	1/1000	20'	TRS High	30'	97°C	Lot 73770	2021/12/28	3	1	1	2	1	8

Acceptance : score ≥ 7

Meets Acceptance criteria = method to be used for verification

Validation and Verification : How ?



Verification HER-2 Breast

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

Readout validation pathologists

True positive (TP)

Comparator/reference

Validation and Verification : How ?

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								1	1		ok
2	27	19B 338	2-3	1	1	2	1	8	IDA	1	1-2		ok
3	22	19B 5948	2-3	1	1	2	1	8	IDA	2	1-2		
4	23	19B 1324	2-3	1	1	2	1	8	IDA	1-2	2	1-2	
5	22	19B 4689	2-3	1	1	2	1	8	IDA	2	2	2	ok
6	23	19B 4685	2	1	1	2	1	7	AVC	2	1-2		ok
7	23	19 44 39	2-3	1	1	2	1	8	IDA	2	2 (3?)	2	ok
8	23	19 B 3745	3	1	1	2	1	8	AVC	2	2		ok
9	23	19B 3479	2-3	1	1	2	1	8	IDA	2	2	2	ok
10	22	19B 2441	2-3	1	1	2	1	8	AVC	2	2		ok
11	18	19B 2582	2-3	1	1	2	1	8	IDA	1	1	1	ok
12	21	18B 9620	3	1	1	2	1	8	AVC	1	1		ok
13	15	18B 10827	2-3	1	1	2	1	8	IDA	1			
14	15	18B 11493	2	1	1	2	1	7	AVC	1			
15	15	19B 10617	2-3	1	1	2	1	8	IDA	1	1		ok
16	15	18B 9210	3	1	1	2	1	8	AVC	1	1		ok
17	21	19B 9579	2-3	1	1	2	1	8	IDA	1	1	1	
18	15	18B 9371	2	1	1	2	1	7	AVC	1	1		
19	21	19B 10100	2-3	1	1	2	1	8	IDA	1	1		ok
20	22	19B 5304	2-3	1	1	2	1	8	IDA	2	2		ok

Stain quality evaluation

Readout validation pathologists

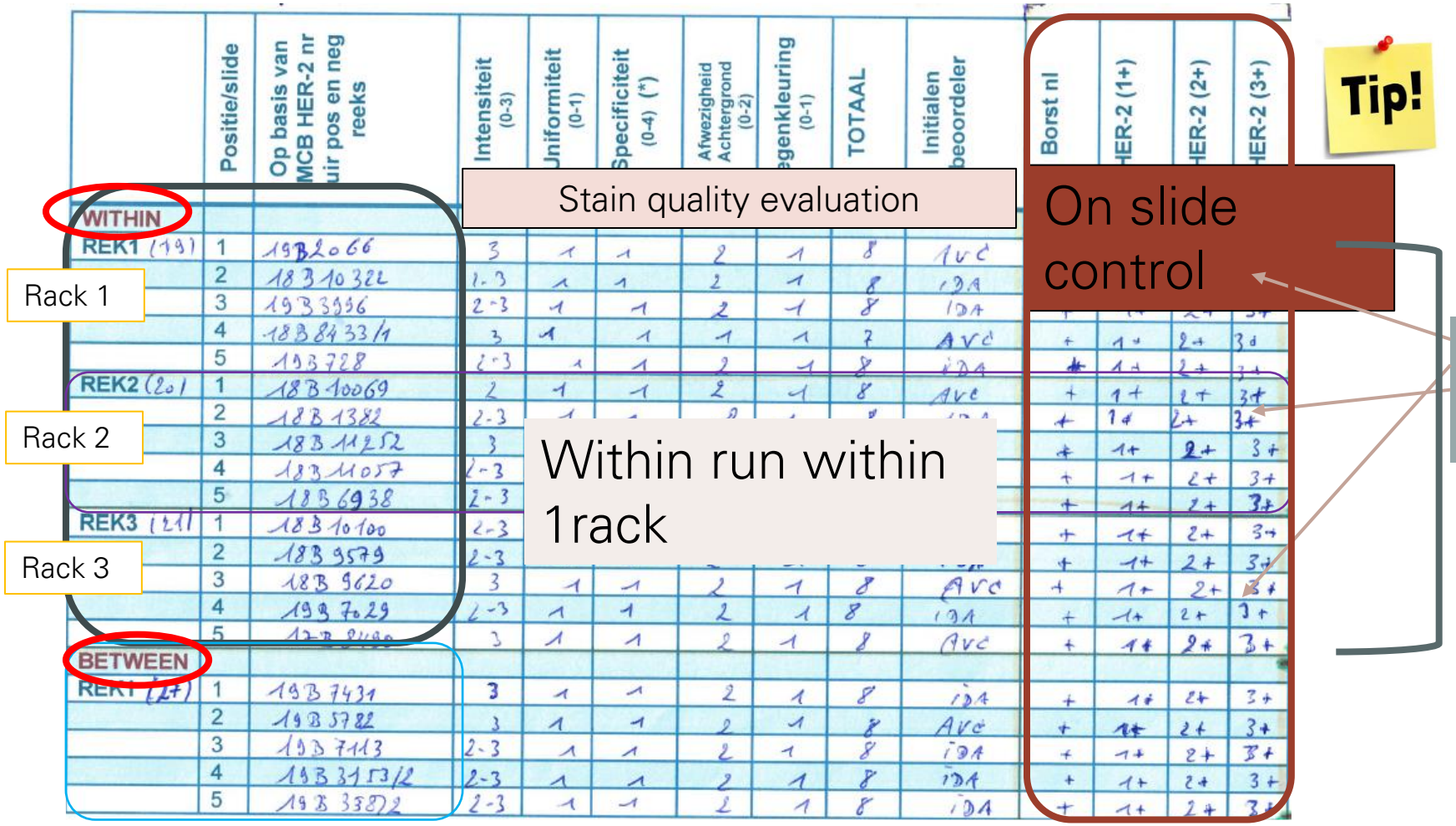
Comparator/reference

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 ?



Verification HER-2 Breast

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

Validation & verification : How ?

	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%

Validation & verification : How ?

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 & verification : How ?

- <u>Implementation :</u>
  - Education of staff by manufacturer or previously trained staff
  - Procedures
  - 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 & verification : How ?

- Validation and verification process : completion !?
  - Performed stains and compared results with comparator test ✅
  - Met acceptance criteria for performance characteristics ✅
  - Implemented the validated method succesfully ✅

Job done ?

What have we proven so far ?

Validation & 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 fullfills the requirements
    - At a certain point in time
    - Using certain lotnumbers of reagents
    - Performed on a staining platform in a certain condition (e.g. new)
    - Performed and evaluated by certain staff
    - etc

Validation & verification : How ?

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

  HOWEVER :

  - Reagent lotnumbers vary
  - Staining platforms evolve over time (impact maintenance, wear, defects, etc)
  - Change in samples : pre-analytics, fixation, processing
  - Change in staff, pathologists
  - Etc

  ➡ These changes may impact IHC quality

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

Validation & verification : How ?

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

  THEREFORE :

  - Implementation of ongoing validation : safeguards daily quality to a higher degree
    - Internal quality control : daily – on slide (batch)
    - Participation in EQA programs
    - Inter observer tuning – especially for typ 2 IHC
    - Correlation studies with other methods : e.g. HER-2 IHC vs HER-2 ISH

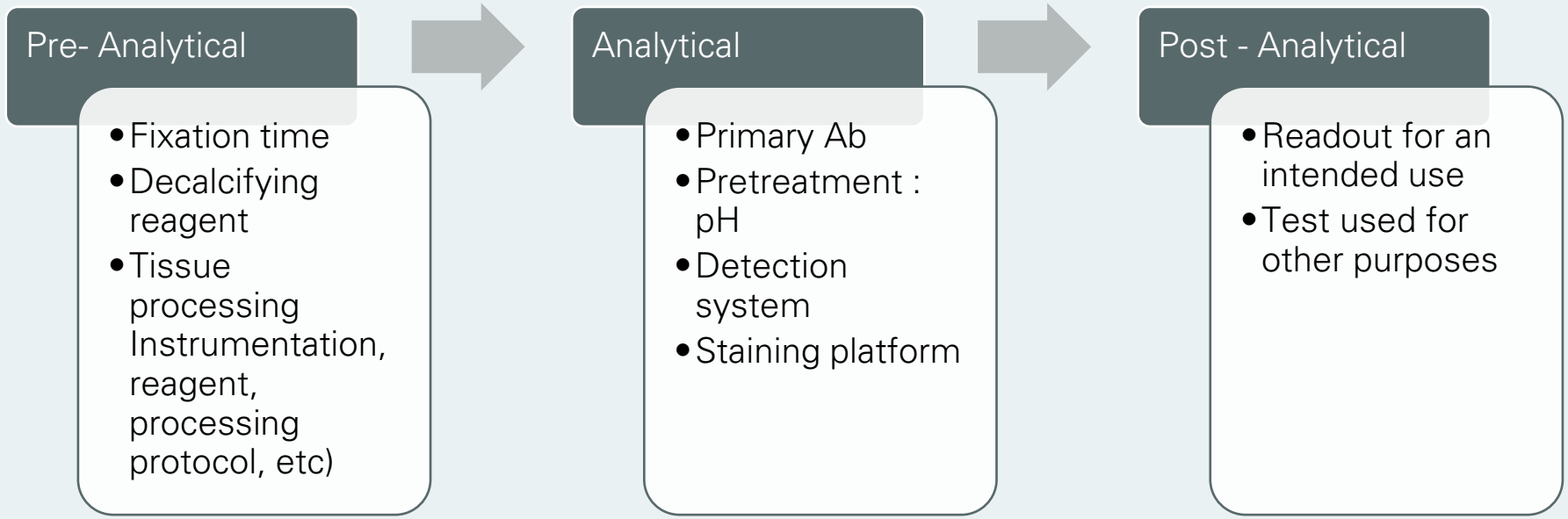
    - Acceptance testing for critical reagents & consumables
    - Acceptance testing for instruments after repair, maintenance

Changes in validated methods?

Which changes require a re-validation/verification ?

Changes in an allready validated method ?

- Changes that can trigger additional verification/validation :



Changes in an allready validated method ?

- IHC Ab/method re- validation : guidelines ?
  - E.g. CAP guidelines for number of samples in re-validation set :

Change	Verification - Validation
New reagent lot for existing validated assay (*)	Confirm assay performance using 1 (+) and 1(-)
Antibody dilution, 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, different 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 decides on how many predictive and non predictive markers, how many (+) and (-) tissues to test
Antibody clone	Full revalidation(equivalent to initial analytic validation)

(*) Equals acceptance testing (ISO15189)
(**) Ab vendor – same clone : Ab concentrations may vary !

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Antibody clone	Full revalidation(equivalent to initial analytic validation)

(*) Equals acceptance testing (ISO15189)
(**) Ab vendor – same clone : Ab concentrations may vary !

Changes in an allready validated method ?

- <u>IHC Ab/method that are verified/validated and used in daily practice :</u>
  - IQC
  - EQC

  - Acceptance testing reagents (new lotnumber/shipment)
  - Acceptance testing instruments (e.g. after repair)

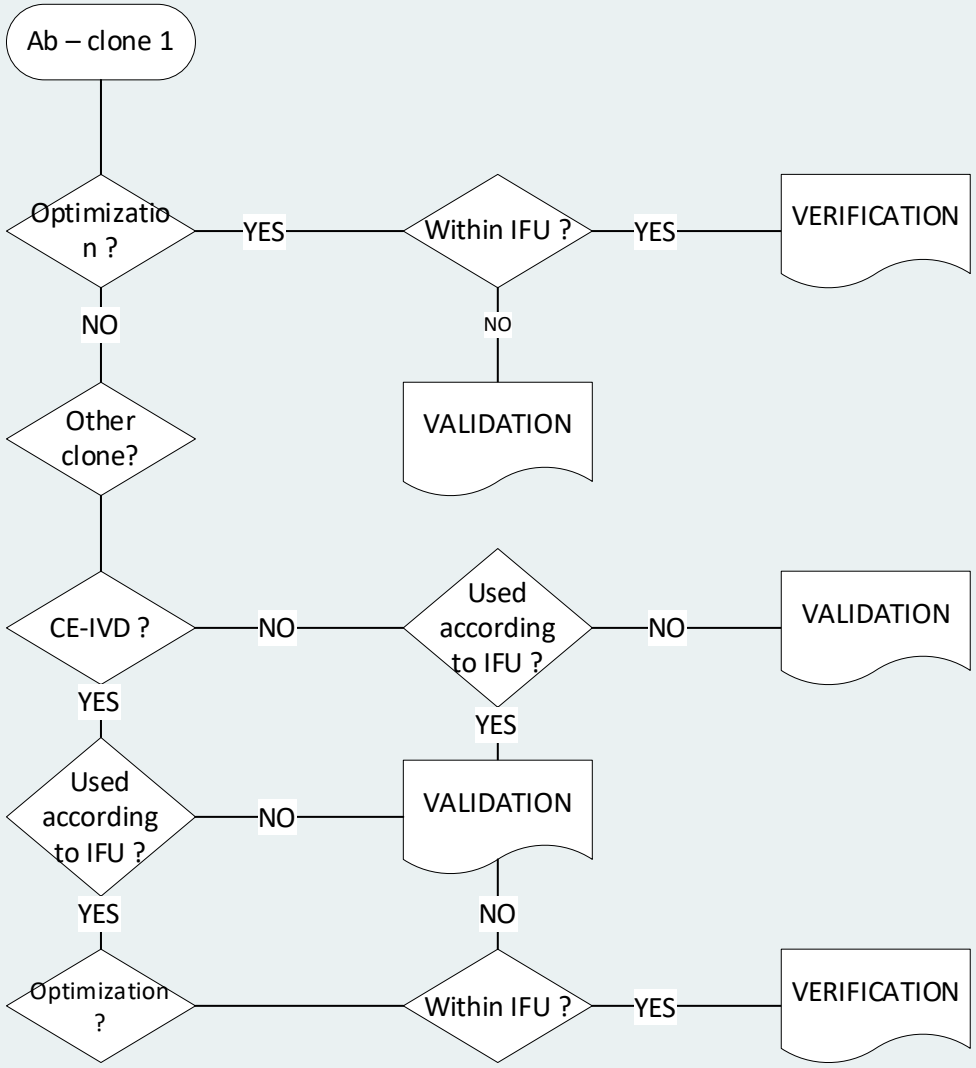
➡ Fail to meet acceptance criteria ➡ new optimization

⬇

verification/validation

Changes in an allready validated method ?

- My Ab clone fails on EQA ?



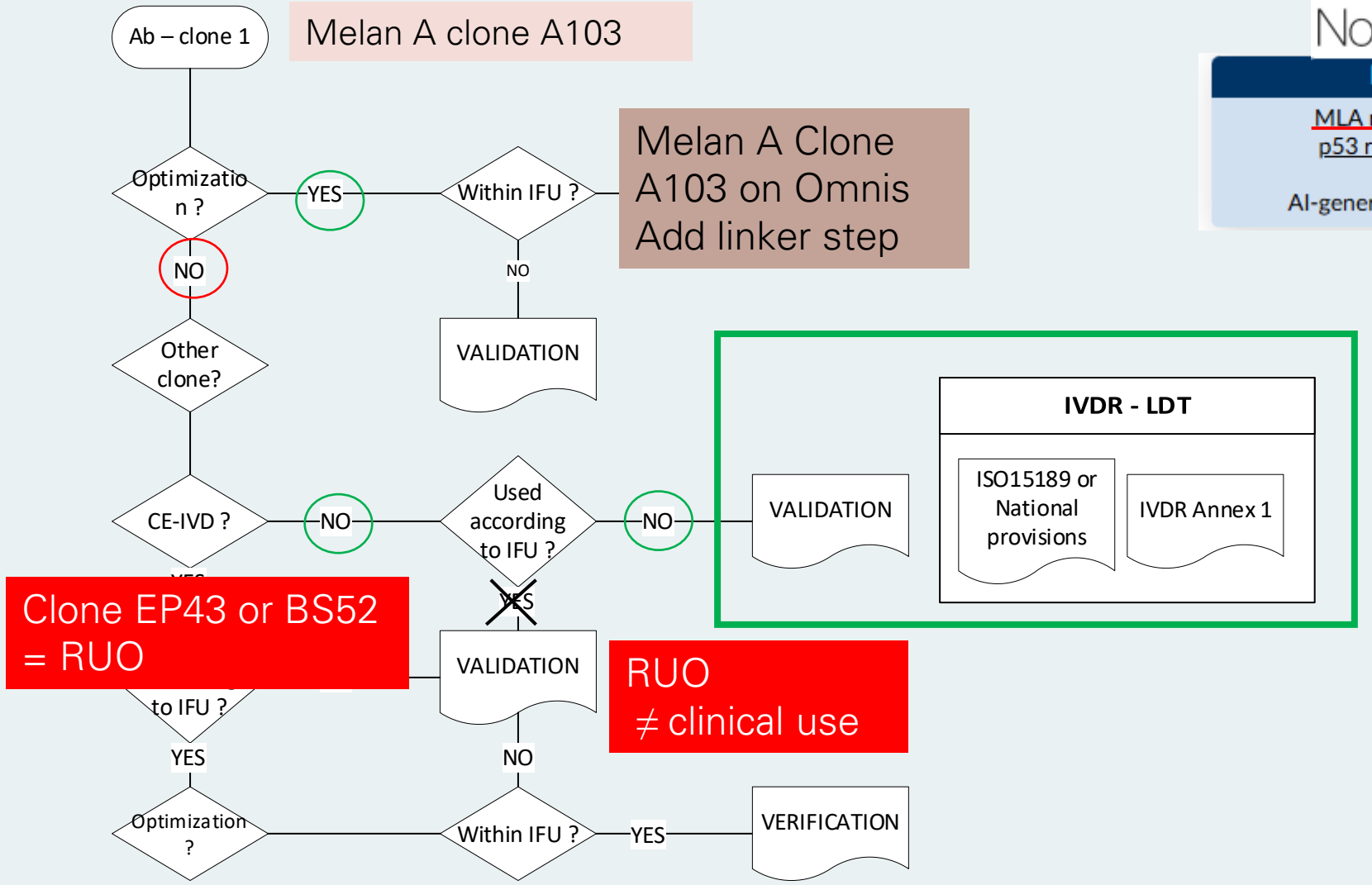
IVDR - LDT

ISO15189 or National provisions

IVDR Annex 1

Changes in an allready validated method ?

- My Ab clone fails on EQA ?



NordiQC

Podcasts

MLA run 74 podcast
p53 run 70 podcast

AI-generated by NordiQC

Changes in an allready validated method ?

- My Ab clone fails on EQA ?
  - EU - IVDR : CE-IVD = first choice reagent !
  - Re-validation of test with change from CE-IVD to RUO
  - Used in clinical setting ≠ Instructions For Use of RUO reagent

    → Lab Developed Test according to IVDR
  - Requires validation and additional documentation & registration
  - Validation : preferably on same cases/samples used for CE-IVD or split sample on both CE-IVD & RUO
    - Demonstrate performance RUO vs CE-IVD
    - Document

Changes in an allready validated method ?

1 No transfer to another legal entity

2
- Used under Quality Management System
- Fullfils ISO15189 requirement or appropriate national guidelines regarding accreditation
- Information of manufacturing, modification, use upon request to competent authority
- Public declaration to competent authority
- Documentation on manufacturing process, performance data, intended purpose to comply to **Annex 1 – for class D or C & D**
- Review experience + corrective actions

3 Justification of the use of LDT
- Specific needs of patient population
- No CE-IVD available that fullfils requirements of performance criteria

Periodic review of availability CE-IVD (EUDAMED database)

IVDR Art 5. 5- LDT

Annex 1 (Class C or D)
- Risk management plan
- Risk management (risk/benefit ratio)
- Performance + follow up
- Documentation manufacturing process
- Labelling

IVDR – LDT : PLAG-1 (RUO)

Validation (perfomance) + Art 5.5 requirements (summary)

ISO 15189 : 2022 & National guidelines

Organisme belge d'Accréditation
Belgische Accreditatieinstelling
Belgische Akkreditierungsstelle
Belgian Accreditation Body

EA MLA Signatory

Certificat d'Accréditation n° 580-MED

En application des dispositions de l'arrêté royal du 31 janvier 2006 créant BELAC, le Bureau d'Accréditation atteste avoir délivré une accréditation conformément aux exigences de la norme EN ISO 15189:2022 à:

A.Z. SINT-LUCAS vzw
Sint-Lucaslaan 29
8310 Brugge

BELAC

sciensano

Praktijkrichtlijn voor het opzetten van een kwaliteitssysteem in de erkende laboratoria voor pathologische anatomie werkzaam binnen het k van het Erkenningsbesluit

(in toepassing van het KB van 5 december 2011)

Risk management plan + risk/benefit assessment

Code	Processtap	Falen	Gevolg	Kans*	Effect*	Risico graad	Detectie*
	Wijziging beoogd gebruik/doel						
	❖ Ander beoogd gebruik dan vermeld in bijsluiter						
IVDR 1A	Uitbereiding met referentie						
IVDR 1B	Uitbereiding zonder referentie						
IVDR 1C	Beperking met referentie						
IVDR 1D	Beperking zonder referentie						

Public declaration & registration competent authority

Justification use LDT

FORMULIER Pathologische anatomie

IVDR sjabloon - motivering gebruik LDT
Middelen

Naam reagens	Fabrikant	Referentie

❖MDCG 2022-6 classificatie wijziging

(Aankruisen)

			JA	NEE
☐	**Beoogd doel**	☐ Beperking ☐ Uitbereiding ☐ Andere (specimen type,	☐ NS ☐ S ☐ S	☐ NS ☐ NS ☐ NS
☐	**Ontwerp**	☐ Testprincipe gewijzigd ☐ Negatief effect op veiligheid/performantie of risico/voordeel verhouding	☐ S ☐ S	☐ NS ☐ NS
☐	**Ingrediënten of materialen**	☐ Essentieel voor testprincipe ☐ Negatief effect op veiligheid/performantie of risico/voordeel verhouding	☐ S ☐ S	☐ NS ☐ NS
☐	**Software**	Besturingssysteem, database (geen bugfix, security update, etc)	☐ S	☐ NS
☐	**Steriliteit of verpakking**	☐ Impact op steriliteit van het product ☐ Gewijzigde verpakking met impact op steriliteit product	☐ S	☐ NS
☐	**Gevolg van CAPA**	☐ CAPA beoordeeld en geaccepteerd door competente autoriteit	☐ NS	☐ S

☐ Significante/ majeure wijziging (minimaal 1 x JA (S))	☐ Niet significante / mineure wijziging (alles NS)

❖Wijziging beoogd doel of type staal

Beoogd doel volgens originele bijsluiter	**Beoogd doel als LDT**
Type staal volgens originele bijsluiter	**Type staal als LDT**
☐ FFPE ☐ Andere :	☐ FFPE ☐ Andere

Conformity IVDR Annex I

FORMULIER Pathologische anatomie

IVDR – Checklijst conformiteit Annex 1
Middelen

Reagens	Referentie	Fabrikant	Klasse
			☐ A ☐ B ☐ C ☐ D

❖Algemene eisen

Item	JA	NEE	Opmerking
Risicomanagement			
☐ Risico management plan			
☐ Risicobepaling			
Risico beheersende maatregelen			
☐ Risicobeperkende maatregelen			
☐ Mitigeren of veilig ontwerp/productie			
☐ Evaluatie bv IQC, EQC, etc			
☐ Bijkomende veiligheidsinfo of opleiding			
☐ Informatie voor gebruiker i.v.m. restrisico			
☐ Opvolging efficiëntie maatregelen			
☐ Restrisico aanvaardbaar ?			

IVDR – LDT : PLAG-1

ISO 15189 : 2022 & National guidelines

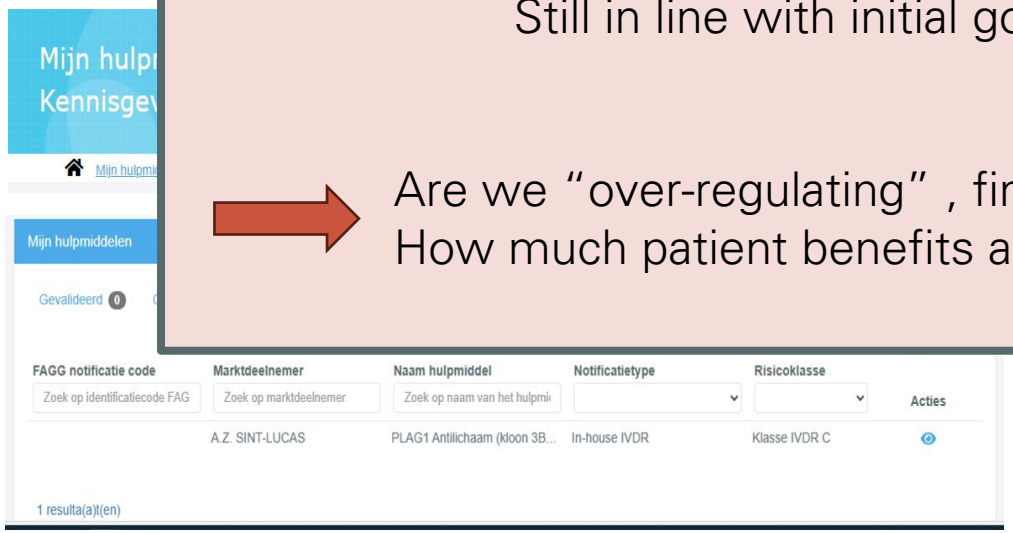
Risk management plan + risk/benefit assessment

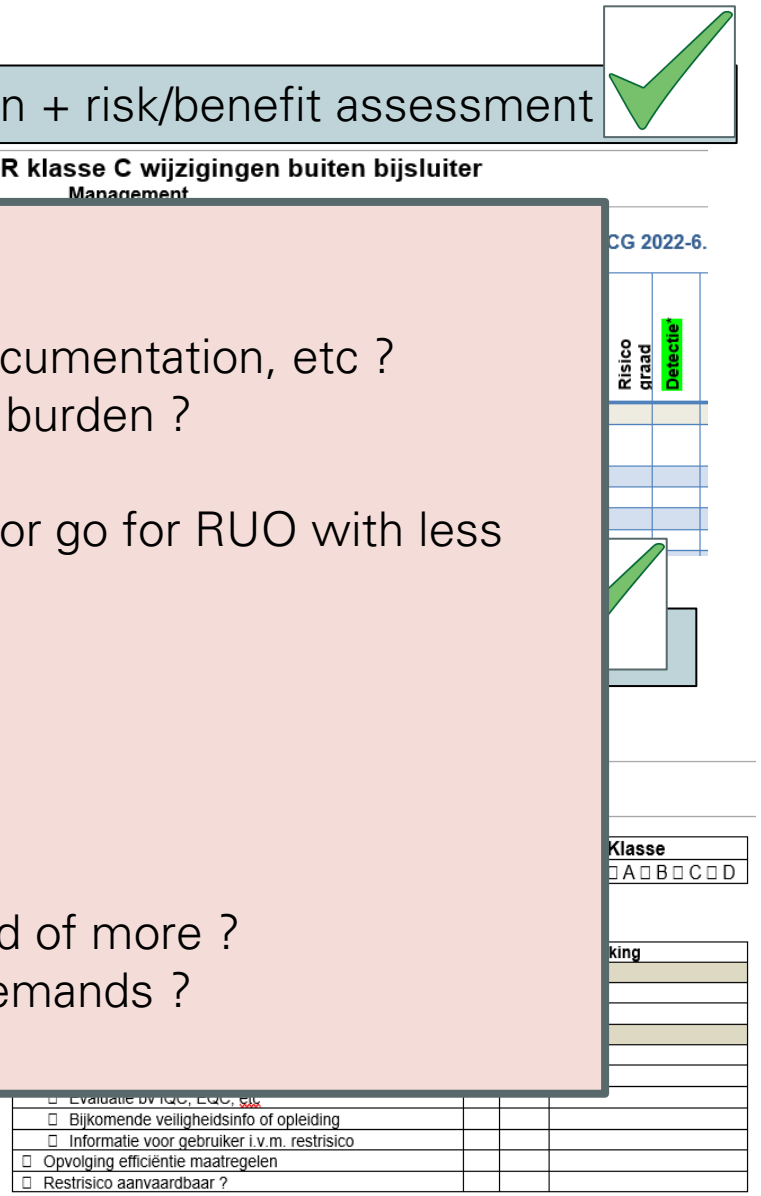
Public registr

Some remarks with RUO and IVDR …

- Lab : Switch from CE – IVD to RUO + additional requirements as to documentation, etc ?
  Does performance for patient overcome additional documentary burden ?
  Knowledge, dedicated staff, means to do so ?
- Manufacturer : Taking the effort of creating a CE-IVD certified reagent or go for RUO with less requirements or get the product out of the market ?
- EU – IVDR : IVDR still not fully operational
  Many "grey areas", uncertainties, etc
  Still in line with initial goal to improve quality with IVD ?

→ Are we "over-regulating" , finally resulting in less quality instead of more ?
How much patient benefits are left in the current regulations/demands ?





Take home messages

Validation – Verification : WHAT ?

- Demonstrating a test performs as it needs to or is intended for
- Key elements : Comparator/reference – performance characteristics – acceptance criteria
- Difference verification – validation determined by : Type of test – used according to IFU – for intended purpose

Validation – Verification : WHY?

- Legal/ISO15189 accreditation – IVDR - Technical

Validation – Verification : HOW?

- Type of test – intended use : verification or validation
- Select cases/controls, prepare slides & perform staining
- Evaluate performance characteristics & acceptance criteria
- Ongoing validation (IQA, EQA, etc)

Validation – Verification : Changes?

- Pre-analytical – Analytical
- Additional optimization – verification/validation
- Additional documentation (IVDR – LDT)

Thank you !

I wellcome any questions or comments

Email : donald.vanhecke@stlucas.be

IVDR – Checklist conformity Annex 1

Reagent	Reference	Manufacturer	Class
			☐ A ☐ B ☐ C ☐ D

❖ General

Item	Y	N	Remarks
Risk management			
☐ Risk management plan			
☐ Risk determination			
Risk management measures			
☐ Risk limiting measures			
☐ Mitigation or safe design/production			
☐ Evaluation e.g. IQC, EQC, etc			
☐ Additional safety info or education			
☐ Information for users vs residual risk			
☐ Follow up effectivenes of measures taken			
☐ Residual risk acceptable ?			

❖ Performance, design and manufacture

Item	Y	N	Remarks
Performance characteristics			
☐ Verification/validation			
☐ IQC or EQC			
Chemical, physical and biological properties			
☐ Negative influence used specimen type			
☐ Contamination risk and residuals			
☐ CMR reagent			
Infection risk	N.A.	N.A	
Sterility – sterile packkaging	N.A.	N.A	
Packaging retains integrity and purity			
Biological safety (human or animal source)			
Construction and interferance with environment			
☐ Use in combination with other devices or equipment			
☐ No negative effect on specified performance			
☐ Restrictions indicated on label or IFU			
☐ Limitation of risks during production or use			
☐ Risk of injury (physical and ergonomic features)			
☐ External influences			
☐ Potential reaction with other substances			
☐ Negative interaction software and IT environment			
☐ Potential incorrect specimen identification			
☐ Interference with other devices			
☐ Safe removal of waste			
☐ Measuring function	N.A.	N.A	
☐ Protection against radiation	N.A.	N.A	
☐ Programmable software	N.A.	N.A	
☐ Energy source	N.A.	N.A	
☐ Mechanical or thermal risks	N.A.	N.A	
☐ Self-testing or "near- patient" testing	N.A.	N.A	

❖ Labels and instructions for use

Item	Y	N	Remarks
Label			
☐ Identification of product			
☐ Identification intended purpose			
☐ Identification of manufacturer			
☐ Indication "in vitro diagnostic medical device"			
☐ Indication lotnumber and expiry date			
☐ Specific storage requirements (e.g. T°)			
☐ Warnings and precautions (e.g. GHS)			
☐ Self testing or 'near patient' testing	N.A.	N.A	
☐ Sterile packaging and additional demands	N.A.	N.A	
Instructions for Use			
☐ Identification of the product			
☐ Identification intended purpose and other information			

N.A. = Non Applicable

Conclusion :

☐ Product fullfills Annex 1	☐ Product does not fullfill Annex 1	☐ Remarks

Date : Name :

IVDR Template - Justification use of LDT

Reagent	Manufacturer	Reference

❖ MDCG 2022-6 classification

			Y	N
☐	**Intended Purpose**	☐ Limitation ☐ Extension ☐ Other (E.g. specimen type),	☐ NS ☐ S ☐ S	☐ NS ☐ NS ☐ NS
☐	**Design**	☐ Change of operational principle ☐ Negative effect on safety/performance or risk/benefit ratio	☐ S ☐ S	☐ NS ☐ NS
☐	**Ingrediënt or material**	☐ Essential for testprinciple ☐ Negative effect on safety/performance or risk/benefit ratio	☐ S ☐ S	☐ NS ☐ NS
☐	**Software**	Operating systeem, database (no bugfix, security update, etc)	☐ S	☐ NS
☐	**Sterility or packaging**	☐ Impact on sterility of the product ☐ Change in packaging with impact on sterility of the product	☐ S	☐ NS
☐	**CAPA**	☐ CAPA evaluated and accepted by competent autority	☐ NS	☐ S

☐ Significant/ major modification (minimal 1 x Y (S))	☐ Non significant / minor modification (all NS)

❖ Change in intended purpose

Intended purpose Instructions for use	**Intended purpose as LDT**
Type sample (Instructions for use)	**Type sample as LDT**
☐ FFPE ☐ Other :	☐ FFPE ☐ Other

❖ Modification method vs.Instructions for use Version :

Protocol step	IFU method	Modified Method
Pretreatment		
Blocking (Peroxidase)		
Primairy antibody		
Secundary reagent (linker)		
Labeled polymer (detection/visualisation)		
Substrate chromogen		
Counterstaining		

❖ Change/modification ingredient/material

☐ None	☐ Composition	☐ Pretreatment	☐ Other detection	☐ Instrument

❖ Other modifications :

Software	☐ Y	☐ N	☐ N.A.	Sterilitity	☐ Y	☐ N	☐ N.a.

❖ Information on production :

LDT based on raw material

☐	CE - IVD reagent, RTU, not according to IFU for method used
☐	CE – IVD reagent, concentrated, not according to IFU for dilution
☐	CE – IVD reagent, concentrated, not according to IFU for method used
☐	Non CE – IVD reagent, concentrated, not according to IFU for dilution
☐	Non CE – IVD reagens, concentrated, not according to IFU for method used

Production and storage criteria

RTU	☐ No change in composition ☐ Transferred to other packaging (E.g. vial for specific staining platform) ☐ Log data creation and use in software (E.g. staining platform)
Conc. Ab	☐ Diluted according to standardprocedure (similar as CE-IVD) ☐ Log data creation and use in software (E.g. staining platform)
Storage	☐ Storage according to manufacturers recommendations ☐ Deviation from manufacturers recommendations

❖ Performance :

	IFU method	Modified method
Performance and acceptance criteria fullfiled ?		
Validation requirements dullfilled ?		

❖ Riskmanagementplan

Risk identification and – evaluation

Bv IVDR 1A								

Risk/benefit ratio after risk mitigation :

☐ No negative effect if LDT is used	☐ Negative effect if LDT is used

Decision :

☐ Motivated use of LDT	☐ LDT does not meet criteria

Post market surveilance and periodic review availability of CE-IVD alternative.

AZ St. Lucas Brugge (B) - Dept Anatomo Pathology Lab