IHC and ISO1518

A Practical Approach

Donald Van Hecke

Aim of this presentation

Can a structural, managed approach to immunostaining provide a framework in order to help improve standardization and quality ?

IHC standardization ?

- Difficult !
- National / regional regulations, guidelines
- Professional organisations
- International guidelines, literature, EQC programs, etc…

- How to manage ?
  - Quality management
  - Framework ?

IHC standardization ?

- International Organization for Standardization (ISO)
- International Laboratory Accreditation Cooperation (ILAC)
- International Laboratory Accreditation Cooperation (IAF)

ISO, ILAC and IAF streamline quality management requirements for medical laboratories

IHC standardization ?

- ISO 17025 : General requirements for the competence of testing and calibration laboratories
- **ISO 15189** : Medical laboratories -- Requirements for quality and competence (2012)


INTERNATIONAL STANDARD

ISO 15189

Third edition
2012-11-01

Medical laboratories — Requirements for quality and competence

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

IHC standardization ?

- ISO 17025 : General requirements for the competence of testing and calibration laboratories
- **ISO 15189** : Medical laboratories -- Requirements for quality and competence (2012)

1	2	3	4	5
• Scope	• Normative references	• Terms & definitions	• Management requirements	• Technical requirements

ISO 15189 and IHC ?

5.1	Personnel
5.1.1	General
5.1.2	Personnel qualifications
5.1.3	Job descriptions
5.1.4	Personnel introduction to the organizational environment
5.1.5	Training
5.1.6	Competence assessment
5.1.7	Review of staff performance
5.1.8	Continuing education and professional development
5.1.9	Personnel records

5.2	Accommodation and environmental conditions
5.2.1	General
5.2.2	Laboratory and office facilities
5.2.3	Storage facilities
5.2.4	Staff facilities
5.2.5	Patient sample collection facilities
5.2.6	Facility maintenance and environmental conditions

5.3	Laboratory equipment, reagents, and consumables
5.3.1	Equipment
5.3.1.1	General
5.3.1.2	Equipment acceptance testing
5.3.1.3	Equipment instructions for use
5.3.1.4	Equipment calibration and metrological traceability
5.3.1.5	Equipment maintenance and repair
5.3.1.6	Equipment adverse incident reporting
5.3.1.7	Equipment records
5.3.2	Reagents and consumables
5.3.2.1	General
5.3.2.2	Reagents and consumables – reception and storage
5.3.2.3	Reagents and consumables – acceptance testing
5.3.2.4	Reagents and consumables – inventory management
5.3.2.5	Reagents and consumables – instructions for use
5.3.2.6	Reagents and consumables – adverse incident reporting
5.3.2.7	Reagents and consumables – records

5.4	Pre-examination processes
5.4.1	General
5.4.2	Information for patients and users
5.4.3	Requests form information
5.4.4	Primary sample collection and handling
5.4.4.1	General
5.4.4.2	Instructions for pre-collection activities
5.4.4.3	Instructions for collection activities
5.4.5	Sample transportation
5.4.6	Sample reception
5.4.7	Pre-examination handling, preparation, and storage

5.5	Examination processes
5.5.1	Selection, verification, and validation of examination procedures
5.5.1.2	Verification of examination procedures
5.5.1.3	Validation of examination procedures
5.5.1.4	Measurement uncertainty of measured quantity values
5.5.2	Biological reference intervals or clinical decision values
5.5.3	Documentation of examination procedures

5.6	Ensuring quality of examination results
5.6.1	General
5.6.2	Quality control
5.6.2.2	Quality control materials
5.6.2.3	Quality control data
5.6.3	Interlaboratory comparisons
5.6.3.1	Participation
5.6.3.2	Alternative approaches
5.6.3.3	Analysis of interlaboratory comparison samples
5.6.3.4	Evaluation of laboratory performance
5.6.4	Comparability of examination results

5.7	Post-examination processes
5.7.1	Review of results
5.7.2	Storage, retention and disposal of clinical samples

5.8	Reporting of results
5.8.1	General
5.8.2	Report attributes
5.8.3	Report content

5.9	Release of results
5.9.1	Automated selection and reporting of results
5.9.2	Revised reports

5.10	Laboratory information management
5.10.1	General
5.10.2	Authorities and responsibilities
5.10.3	Information system management

ISO 15189 and IHC ?

5.1	Personnel
5.1.1	General
5.1.2	Personnel qualifications
5.1.3	Job descriptions
5.1.4	Personnel [illegible] the organizational environment
5.1.5	Training
5.1.6	Competence assessment
5.1.7	Review of staff performance
5.1.8	Continuing education and professional development
5.1.9	Personnel records

Staff

5.2	Accommodation and environmental conditions
5.2.1	General
5.2.2	Laboratory and office facilities
5.2.3	[illegible]
5.2.4	Staff facilities
5.2.5	Patient sample collection facilities
5.2.6	Facility maintenance and environmental conditions

Means

5.3	Laboratory equipment, reagents, and consumables
5.3.1	Equipment
5.3.1.1	General
5.3.1.2	Equipment acceptance testing
5.3.1.3	[illegible]
5.3.1.4	Equipment calibration and metrological traceability
5.3.1.5	Equipment maintenance and repair
5.3.1.6	Equipment adverse incident reporting
5.3.1.7	Equipment records
5.3.2	Reagents and consumables
5.3.2.1	General
5.3.2.2	Reagents and consumables – reception and storage
5.3.2.3	Reagents and consumables – acceptance testing
5.3.2.4	[illegible] laboratory [illegible]
5.3.2.5	Reagents and consumables – instructions for use
5.3.2.6	Reagents and consumables – adverse incident reporting
5.3.2.7	Reagents and consumables – records

Equipment

Reagents

5.4	Pre-examination processes
5.4.1	General
5.4.2	Information for patients and users
5.4.3	Requests form information
5.4.4	[illegible] sample collection and handling
5.4.4.1	[illegible]
5.4.4.2	Instructions for pre-collection activities
5.4.4.3	Instructions for collection activities
5.4.5	Sample transportation
5.4.6	Sample reception
5.4.7	Pre-examination handling, preparation, and storage

Pre-exam

5.5	Examination processes
5.5.1	Selection, verification, and validation of examination procedures
5.5.1	Verification of examination procedures
5.5.1	[illegible]
5.5.1.4	Measurement uncertainty of measured quantity values
5.5.2	Biological reference intervals or clinical decision values
5.5.3	Documentation of examination procedures

Examination

5.6	Ensuring quality of examination results
5.6.1	General
5.6.2	Quality control
5.6.2.2	Quality control materials
5.6.2.3	Quality control data
5.6.3	Interlaboratory comparisons
5.6.3.1	Participation
5.6.3.2	Alternative approaches
5.6.3.3	Analysis of interlaboratory comparison samples
5.6.3.4	Evaluation of laboratory performance
5.6.4	Comparability of examination results

QC

5.7	Post-examination processes
5.7.1	Review of results
5.7.2	[illegible] samples

Post-exam

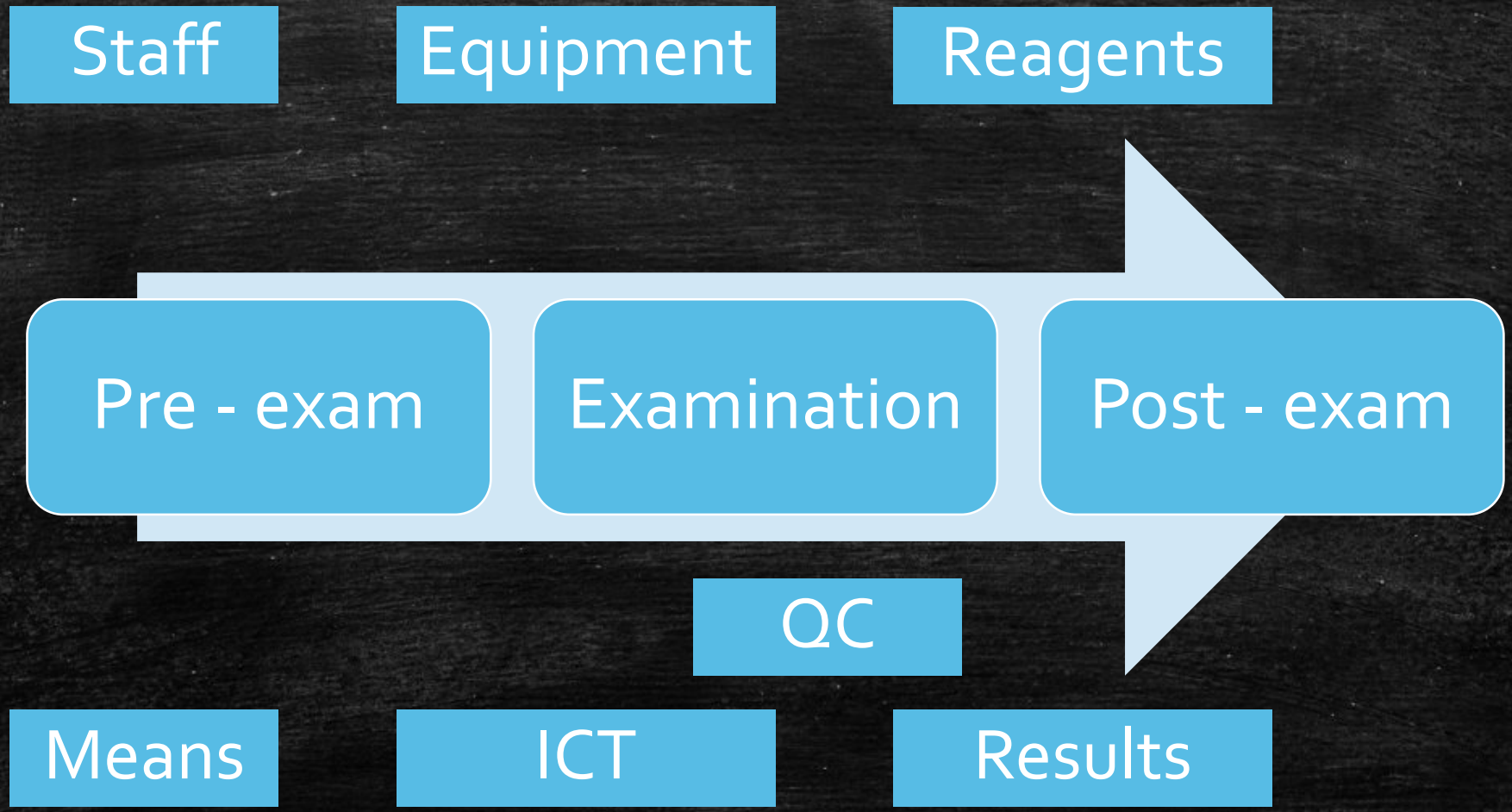
5.8	Reporting of results
5.8.1	General
5.8.2	[illegible]
5.8.3	Report content
5.9	Release of results
5.9.1	Automated selection and reporting of results
5.9.2	Revised reports

Results

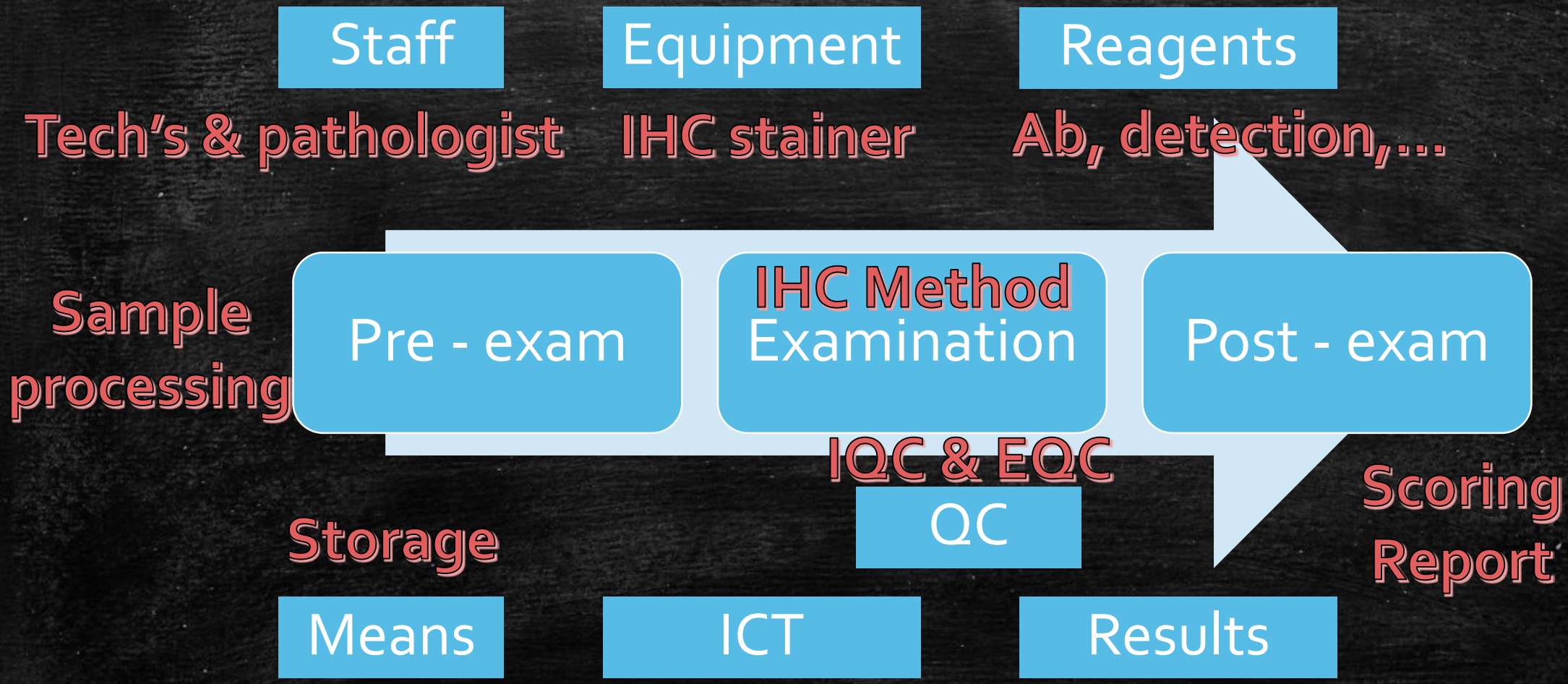
5.10	Laboratory information management
5.10.1	General
5.10.2	Authorities and responsibilities
5.10.3	Information system management

ICT

ISO15189 and IHC ?



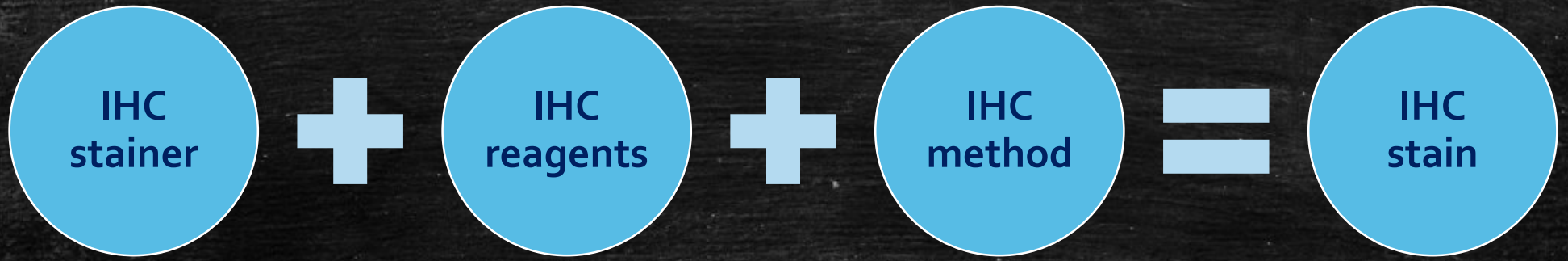
ISO15189 and IHC !



Pre - analytical

- Samples :
  - Collection : time to fixation, transport
- Processing :
  - Fixative
  - Fixation times
- Preparing slides : time & temperature

Equipment



Equipment : IHC stainer validation

- Acceptance testing – validation/verification IHC stainer :

→ criteria for acceptance ?

Equipment : IHC stainer validation

- Acceptance testing – validation/verification IHC stainer :

Equipment : IHC stainer validation

Installation qualification (IQ)

- Done by manufacturer/company
- Pre-check installation
- Installation according guidelines manufacturer
- Test initial working of instrument
- Certificate of installation

Operational qualification (OQ)

- Done by manufacturer/company and lab
- Check performance as set by manufacturer
- Check critical parameters :
  - Hardware : T°, volumes, etc…
  - Software : login, protocols, reagents
  - Staining : control tissues company

Equipment : IHC stainer validation

Performance validation (PQ) :

= Objective proof of stainer performance

- Using lab samples/controls (fixation & processing)
- Optimalisation protocols :
  - Reagents : Ab (RU/conc – clone) ; detection system
  - Standard/factory protocol
  - Protocol optimized : antigen retrieval – RTU/dilution – incubation times – detection system

Equipment : IHC stainer validation

Performance validation (PQ) :

- Stainer performance :
  - Different positions : WITHIN RUN
  - Different times/runs : BETWEEN RUN
- Don’t forget :
  - Software : user levels + protection against changes
  - Critical items : power supply, safety issues

Meeting acceptance criteria : validation confirmed

Equipment : IHC stainer validation

Maintaining validated state :

- Maintenance performed as prescribed
- Maintenance & repair records : history - trends
- Acceptance testing after intervention
- Ongoing evaluation staining performance

Equipment : Calibration & metrological traceability

Calibration & metrological traceability

- Microtomes : section thickness vs staining quality (ISH !)
- T° :
  - Paraffin stations
  - Waterbath/hotplate : mounting sections (max 1h @ 60°C)
  - HIER : waterbath, heatingplates/thermopads

Equipment : IHC stainer & LIS - Workflow

Validation !

TASK

LIS

Task information

Task processing

Automation

Labelprinter

Bi-directional

VIP-VLM

Ebar printer

Bond software

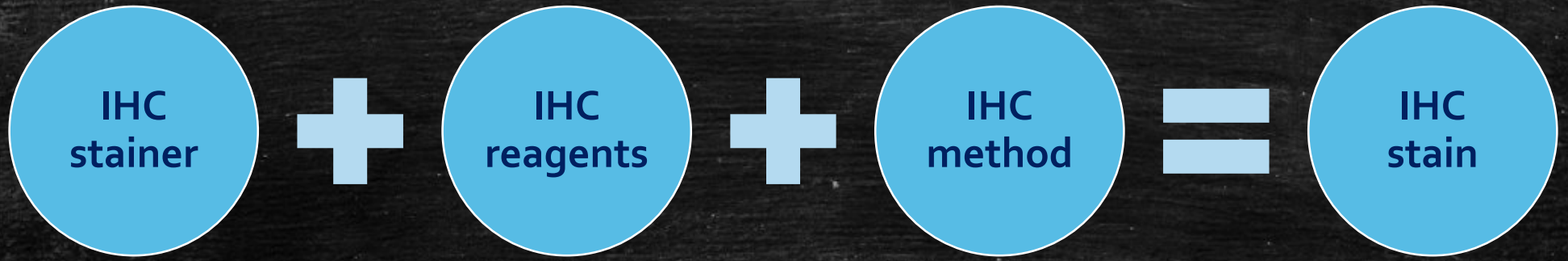
Roche software

Traceability

Equipment : IHC stain & digitalization

- Digitalized systems = Laboratory Information System + all digitalized items used
- Scanning slides : validation/verification :
  - Guidelines (Royal College of Pathologists, College of American Pathologists)
  - Instrument validation/verification (calibration slides)
  - Scanning vs conventional : at least 60 cases
  - Be aware of difficulties !

Equipment



Reagents & consumables

- Reagents & consumables critical for optimal staining !
- Reagents & consumables should perform as desired :
  - Instructions for use – critical updates
  - Records
  - Inventory management :
    - Segregation inspected vs not inspected
    - Optimal stock vs use (Expiry date !)
  - Storage as prescribed by manufacturer :
    - Storage facilities : RT – cooled – frozen

T° + humidity ! (Fluidics)

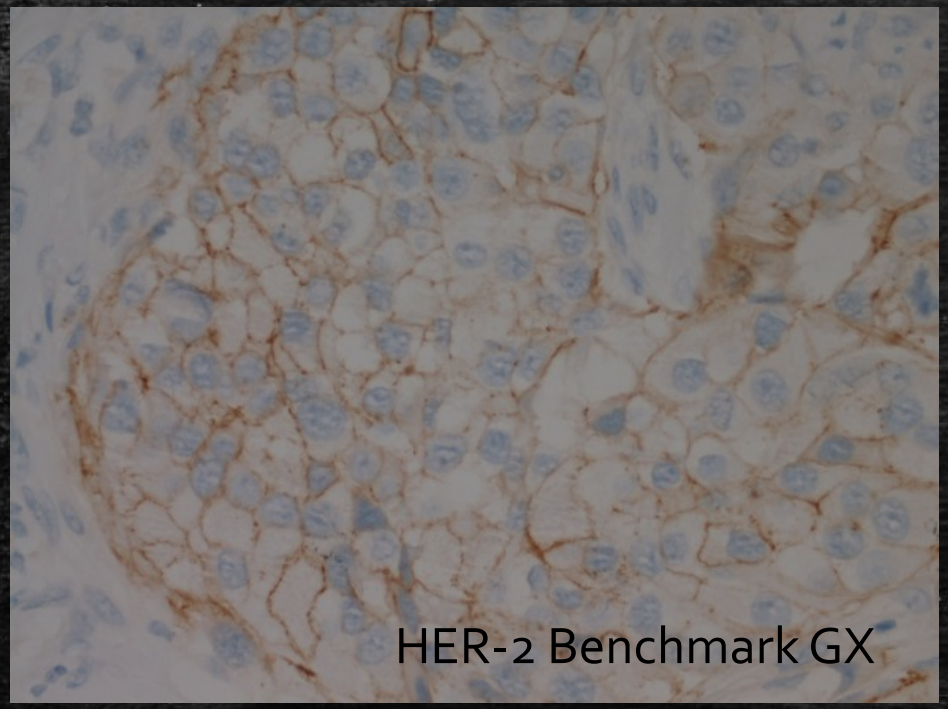
Reagents & consumables

Storage conditions	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Time outside original package	0	3m	3m	6m	6m	2w
Humidity	-	Silicate	RH 75%	RH 75%	RH 75%	RH 75%
Material	Original	Plastic	Plastic	Plastic	Glass	Plastic
Temperature	25°C	25°C	25°C	25°C	25°C	45°C
Fluidics						

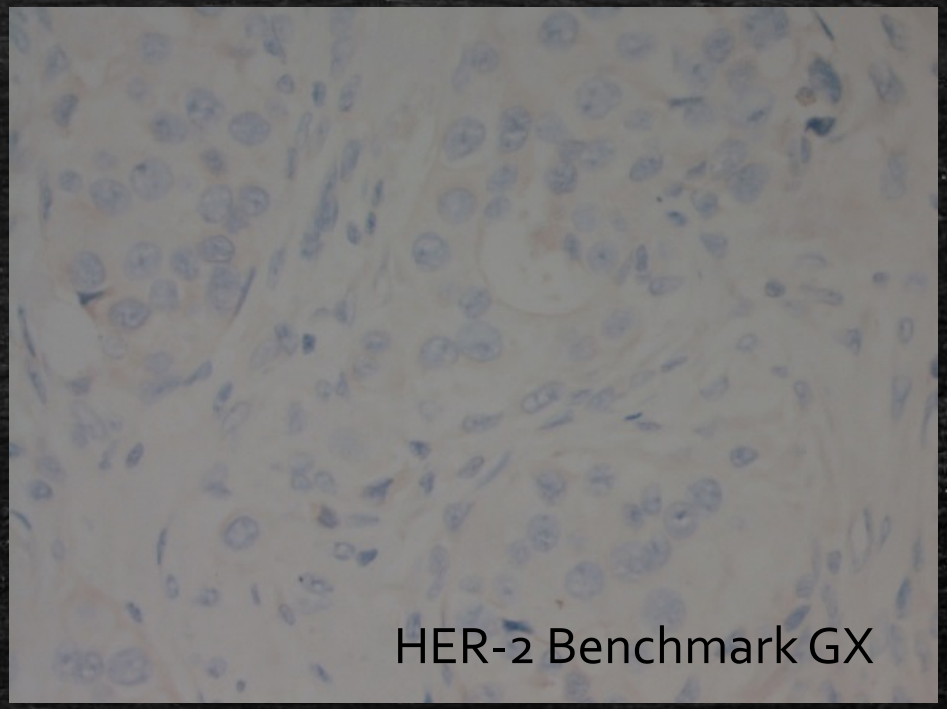
©Roche/Ventana recommendations for slide storage.

Reagents & consumables

Xtra slide white (freezer)



Xtra slide green (freezer)

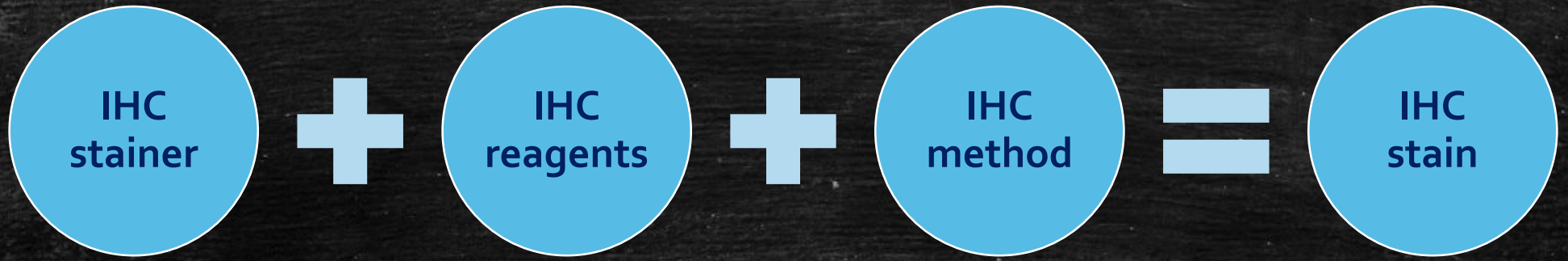


Reagents & consumables

Acceptance testing

- New lot or shipment
- New dilution made
- Before use in routine
- Acceptance criteria pre-determined
- Reagents (Ab, detection) + critical consumables (coated slides)

Equipment



Examination processes : IHC staining method

Validation

- Providing objective evidence that method fullfils requirements as prescribed by company
- In house, changed IVD
- For intended use ! (FFPE, cyto)

Verification

- Providing objective information to confirm method fullfils predescribed requirements
- Validation by company
- IVD
- For intended use ! (FFPE, cyto)

Examination processes : IHC staining method

Validation / verification guidelines :

- National/regional – Professional organisations

E.g. :

CAP – Principles of Analytic Validation of Immunohistochemical Assays

- Guidelines upon initial validation of IHC test/methods
- Class 1 (diagnostic) – Class 2 (therapeutic)

Examination processes : IHC staining method

Validation / verification criteria : class 1 – class 2 Ab

- Optimalization protocol :
  - Starting = default factory protocol (data sheet, software)
  - Ab clone, dilution, incubationtime
  - Detection systems used
  - Antigen retrieval method
  - Evaluation : using scoring system (= objective)
  - Acceptance criteria :
    - Criteria ok
    - Criteria not ok : adapt protocol, change clone, etc

Examination processes : IHC staining method

Validation / verification criteria : class 1 – class 2 Ab

- Optimized protocol – method validation/verification class 1
  - Accuracy : new method vs reference method
  - Precision – reproducibility : within and between run
  - Specificity

Examination processes : IHC staining method

Validation / verification criteria : class 1 – class 2 Ab

- Optimized protocol – method validation/verification class 2
- Specific requirements per test :
  - ER/PR : 10 pos + 10 neg ; correlation >90% pos and >95% neg
  - HER-2 : 25-100 samples (CAP) ; Belgian Guidelines 20 pos +20 neg
    > 95% correlation with reference method

Examination processes : IHC staining method

Validation / verification criteria : class 1 – class 2 Ab

Maintaining validated state :

- IQC
- EQC
- Correlation with reference method (e.g. IHC – ISH)
- Inter-observer tuning
- Instruments
- Reagents

Examination processes : IHC staining method

Changes in validated/verified IHC method

- Ab clone – different vendor
- Ab dilution
- Incubation or retrieval times (same method)

➡ Confirm performance with at least 2 pos & 2 neg cases

Examination processes : IHC staining method

Changes in validated/verified IHC method

- Fixative type
- Antigen retrieval (pH, buffer, heating)
- Detection system
- Relocation lab

➡ Confirm performance with sufficient cases

Quality control

- IQC :
  - Apppropriate controls
  - Standardisation preparation controls
  - Management controls
- EQC :
  - Similar to routine testing
  - Feedback & actions

Post - analytical

- **Scoring systems :**
  - Predetermined and according to guidelines
  - Periodical inter- observer evaluation
- **Reports :**
  - Content according to guidelines
  - Same for all reporting pathologists
- **Samples :**
  - intermediate storage (wet archive)
  - Archive blocks & slides

Conclusion

- Validation of instruments and methods are common for most labs
- ISO 15189 requirements adds value to quality/standardisation by ensuring staff, instruments, reagents and consumables are monitored. Requirements for samples, analytical processing and reporting ensure the whole IHC process is covered.