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Annex to the guideline of the Pathology/Neuropathology Sector Committee for the validation of examination methods in immunohistology

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Note:

This annex originated by the take-over of comments by the “Immunohistology” sub-commit-tee of the DAkkS. The following were active on this sub-committee (alphabetical order):

Ass Prof. Dr. med. S. Falk (Frankfurt); Prof. Dr. med. F. Hofstädter (Regensburg); Dr. med. O. Kaufmann (Cottbus); Ass. Prof. Dr. med. A. Lebeau (Lübeck); Prof. Dr. med. P. Schirmacher (Heidelberg); Prof. Dr. med. Dr. h. c. H. Stein (Berlin); Dr. med. S. Wagner and colleagues; Dr. med. S. Bartho, Dr. med. U Kerlikowski, Dr. med. K. Zels (Königs Wusterhausen).

Figures 1-14 were produced by Ass. Prof. Dr. med. F. Bataille (Amberg) and presented within the framework of the DAkkS event on this subject on 08.03.2013.

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The references in the annex follow the structuring of guideline 71 SD 4 028.

1 Purpose / scope of application

2 Terminology and Abbreviation

2.1 Abbreviation

2.2 Terminology

Note 1:

Antibody class I entails the antibodies evaluated in the context of histomorphology and clinical data/information. These antibodies serve determination of cell differentiation. The majority of immunohistochemical examinations is assigned to this category.

The immunohistochemical examinations, the result of which is observed alone with a view to a prognostic or predictive statement, are assigned to antibody class II. The result of the immunohistochemical examination with a class II antibody represents independent diagnostic information and influences the individual therapy management of the patient in question, partly also on the basis of required semi-quantitative assessments of the immune responses. A classical example is determination of the hormone receptor status in mamma carcinomas and the Ki67 index in neuroendocrine tumors.

Between antibody class I and antibody class II, there is an overlap on antibodies belonging to class I or class II as a function of their interpretation. For example, an antibody can be used for the oestrogen receptor for determination of the primarius in a metastasis - and would be classified as a class I antibody in this function. On the other hand, it acts as a class II antibody in the determination of the oestrogen receptor positivity of a mamma carcinoma with the objective of stratification of the therapy in the patient in question.

Note 2:

The term verification is used in this document in the sense of the definitions from the standards EN ISO 9000:2005, ISO/IEC 17025:2005 and EN ISO 15189:2012.

“Validated examination procedures used without modification shall be subject to independent verification by the laboratory before being introduced into routine use...” (EN ISO 15189:2012, 5.5.1.2)

The term verification is **not** used in this document for the performance assessment test or conformity assessment of the manufacturer of IVD.

3 Description

3.1 Introduction, fundamentals

Note 3:

There are antibodies available commercially which do not react, react less well or even differently from the description in the data sheet. In addition, a possible contamination must be ruled out. As a matter of principle, the Consultant for Pathology/Neuropathology has the responsibility for a mode of procedure secure in the sense of diagnostics.

Note 4:

The antibodies must be examined for an optimum result of the immunostaining with the detection system established in your own laboratory. In this context, there can be considerable deviations from the manufacturer's information. The objective must not be uncritical obeying of manufacturer's information, but an "optimum portrayal of the antigen", leading to a correct answer to the question.

Her2/neu is an antigen with peculiarities. Here, as in every semi-quantitative evaluation, there must be a validation relating to standards with a known content of molecules.

3.2 Statutory requirements

3.3 Responsibilities

Note 5:

Precise knowledge of the expression or the variety of expression (reactivity with more than only one kind of cell) and of the expression pattern (core, membrane, cytoplasm) of the antigen (Ag) to be detected in diagnostics is presupposed for the responsible Consultant for Pathology. Precise knowledge also includes knowledge of the strengths and weaknesses of an antibody and its tendency to artefacts.

3.4 Devices, materials, ancillaries

3.5 Implementation of the validation / verification

3.5.1 General notes on the validation and verification of the method

Note 6:

As a result of the specifics of pathological-anatomical cell and tissue diagnostics, this method is more theoretical. The reference to standards and the morphological assessment of the quality of the outcome are decisive. The extent to which one can proceed precisely in accordance with the manufacturer's requirements and whether this mode of procedure is even possible must be decided by the Consultant for Pathology/Neuropathology. Manufacturers emphasise that they only assume liability if their conditions are strictly complied with.

Note 7:

The responsible Consultant for Pathology/Neuropathology decides on the scope of the validation (e.g. how many differing test tissues are used for the validation) and on the output characteristics of a method. The scope of validation depends on various factors such as the kind of antibody (CE-marked antibody or antibodies intended for research purposes), the kind of tissue examined, the fixation, the embedding processes, the removal from the bed (paraffin temperature and quality), drying process etc..

Even antibodies exclusively declared for research ("for research use only") can be used in routine diagnostics following a matching validation. The important thing is the precise knowledge of the expression and the variety of expression (reactivity with more than only one kind of cell) and of the expression pattern (core, membrane, cytoplasm) of the antigen (Ag) to be detected in diagnostics and also knowledge of the strengths and weaknesses of an antibody and its tendency to artefacts.

3.5.2 Validation of the immunohistochemical methods in in-house processes

Fig. 1

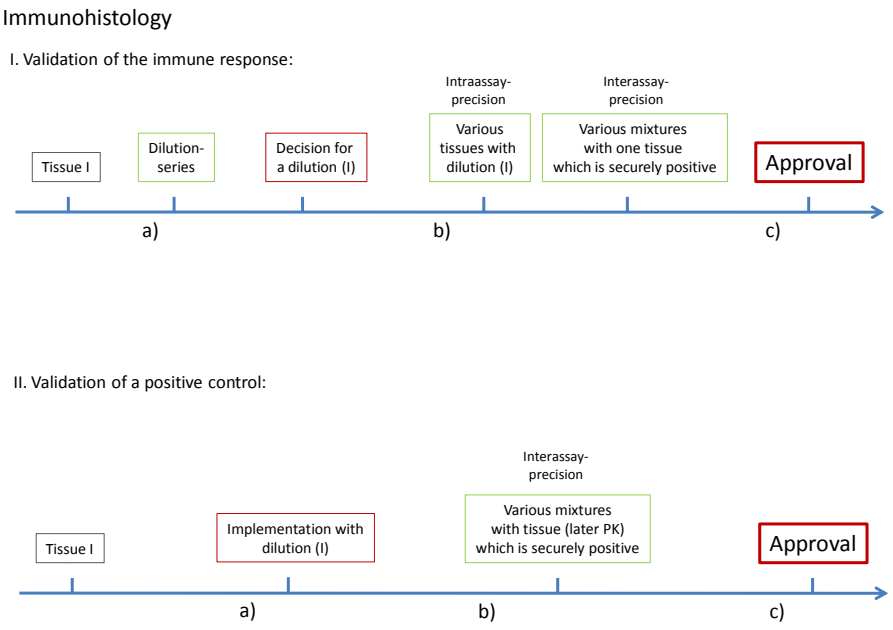


Fig. 2: If an antibody is to be introduced in routine diagnostics, it requires validation to start with. The test tissue used for this purpose must be suitable and representative for the question.

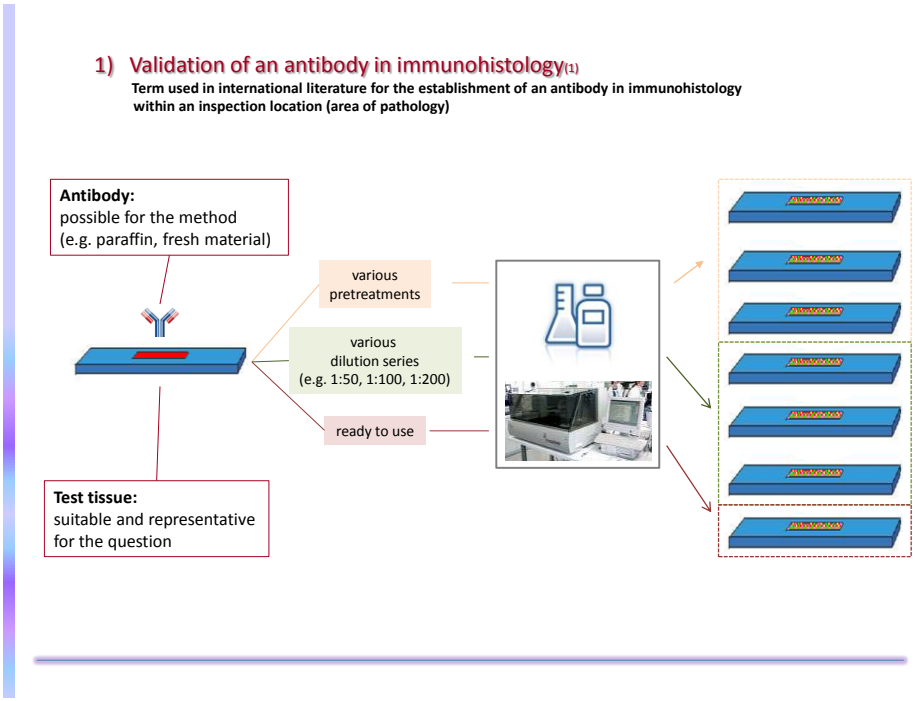


Fig. 3:

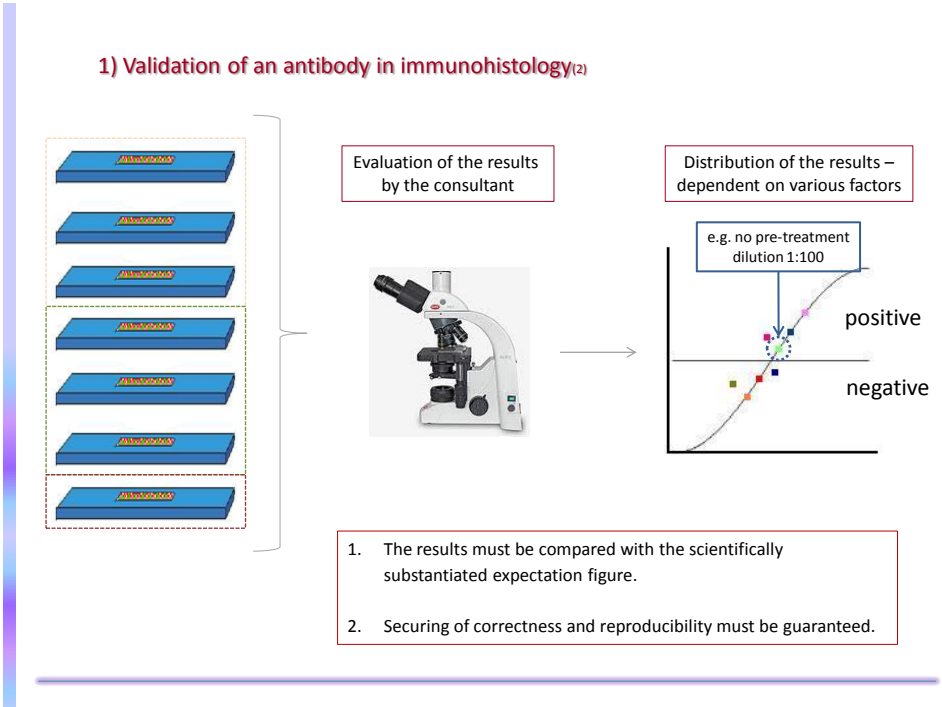

1) Validation of an antibody in immunohistology(2)
Evaluation of the results by the consultant
Distribution of the results – dependent on various factors
e.g. no pre-treatment dilution 1:100
positive
negative
1. The results must be compared with the scientifically substantiated expectation figure.
2. Securing of correctness and reproducibility must be guaranteed.

Fig. 4: The interassay and interassay precision are output characteristics of validation - they serve to verify the pre-analytical and analytical variables within an immunohistochemical reaction.

Pre-analytical, analytical and post-analytical methods influence the outcome of the immunohistochemical reaction and result interpretation.

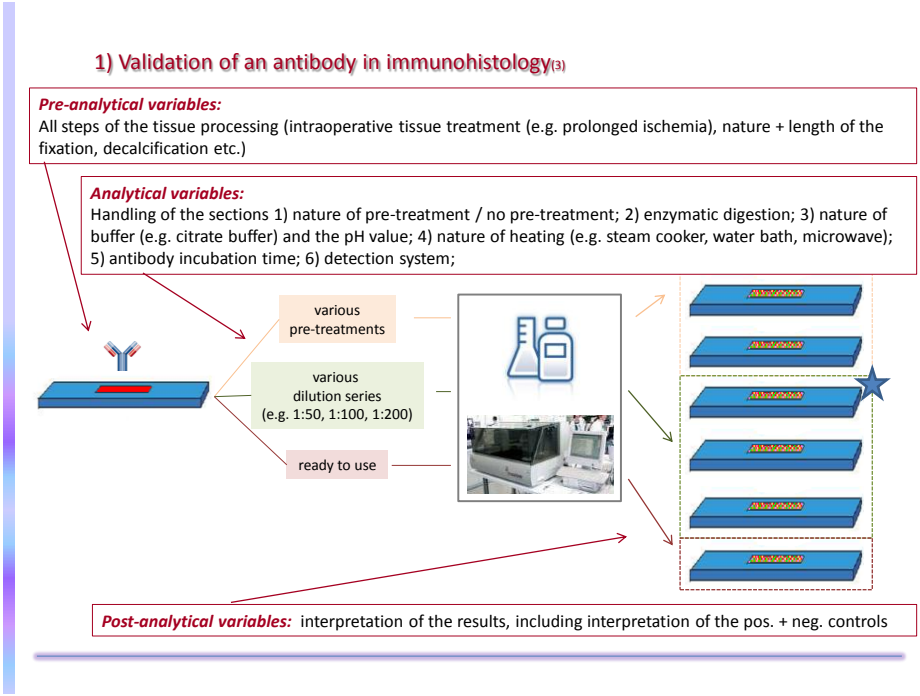


Fig. 5:

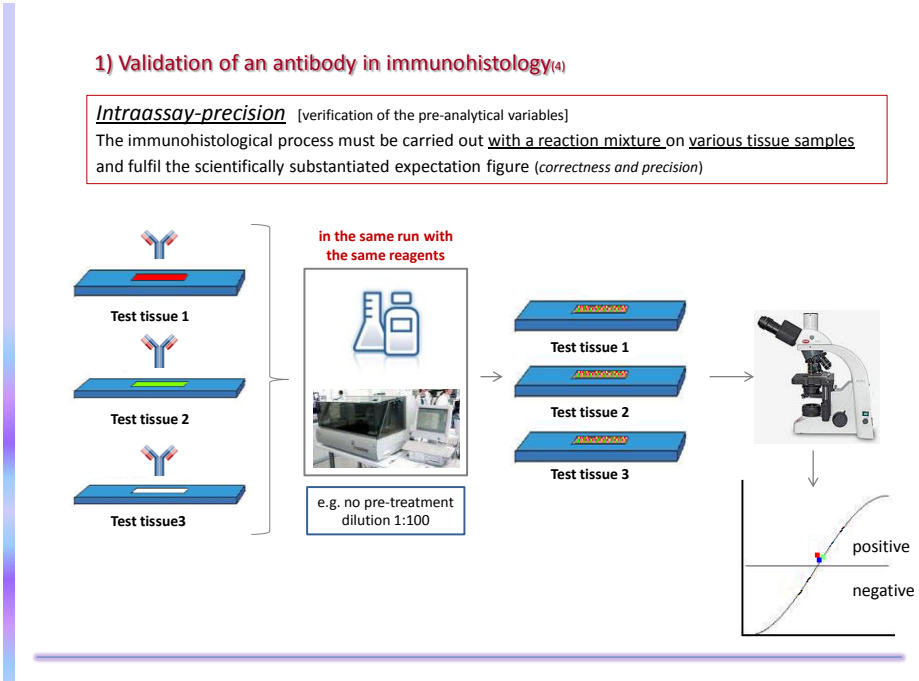
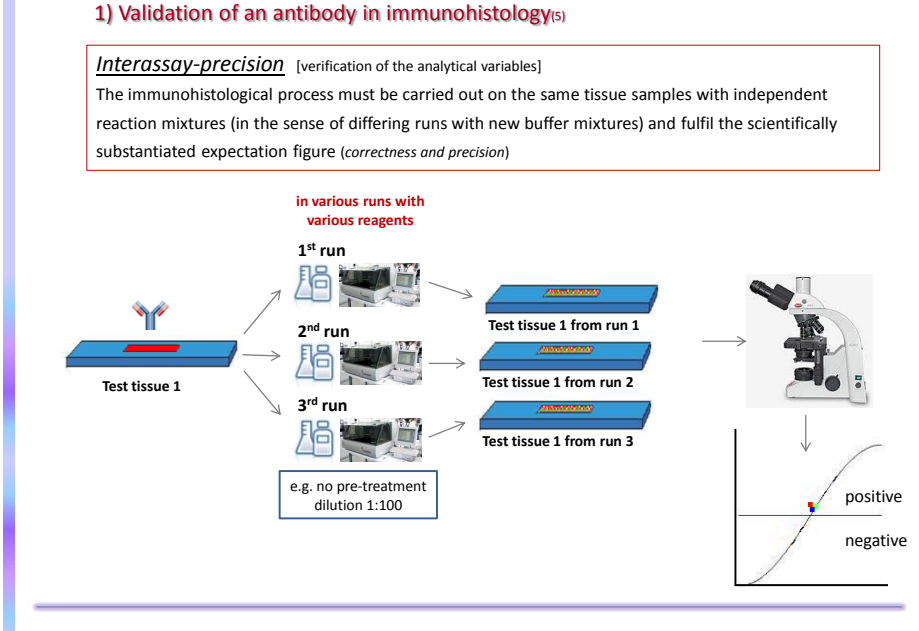
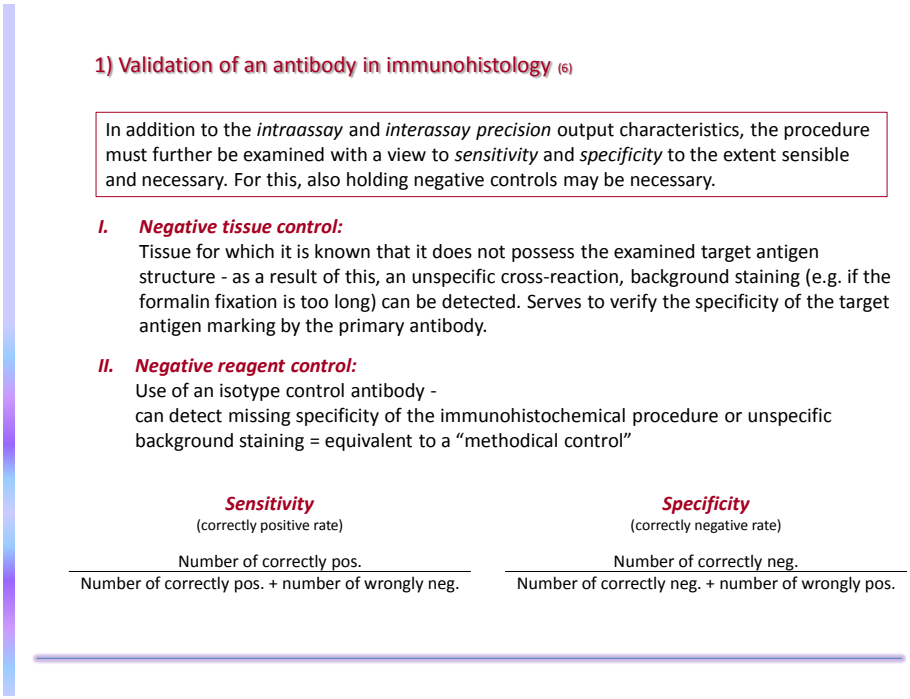


Fig. 6: The more closed the used immunohistological staining procedure is, the less parameters vary between the independent reaction mixtures.



As a result of the individual of the antibodies, the scope of the output characteristics to be verified (e.g. how many differing test tissues are used for the intraassay precision, on how many differing reaction mixtures is the interassay precision done) can hardly be set in general. The decision must be made for each individual antibody. In this context, the responsibility for a mode of procedure secure in the sense of diagnostics and for all decisions with a view to the implementation of the validation/verification proceedings is with the responsible Consultant for Pathology/Neuropathology.

Fig. 7:



Note 8:

There are a series of parameters which influence the examination process and partly can only be controlled conditionally:

- The variety of possible variables from the phase of sample preparation: fixation, embedding processes (formulations, duration, reagents, temperature), removal from the bed (paraffin temperature and quality), drying process et al..

- The immunohistological staining process itself: antigen demasking, antibody properties, quantity, concentration, boundary layer phenomena, electrostatic influences, incubation time, properties of the detection systems and chromogenes and similar.

Note 9:

The responsibility and all decisions with a view to the implementation of the validation/verification process are with the Consultant for Pathology/Neuropathology.

Note 10:

Isotype controls are the preferred choice for monoclonal antibodies. For polyclonal antibodies, dilutions of the immunoglobulin fraction of non-immunised animals of the same species or the pre-immune serum of the same animal should be used.

Keeping of negative controls for assessment of sensitivity and specificity can be done without in the following cases:

- If the test tissue and also the control tissue for the external on-slide controls are selected such that positive and negative structures are contained.

- Negative reagent controls in which the primary antibody is replaced by buffer are dispensable in systems such as BenchMark Ultra, as the system works with a single secondary antibody which recognises mice and rabbits and it thus the same for all primary in-house antibodies and is used and verified day in day out.

In a change of species also connected with a change of the secondary antibody, a negative reagent control is necessary.

3.5.3 Validation in changes in the process, e.g. short-term change of an antibody (manufacturing company, batch etc.) or replacement of a device

Fig. 8:

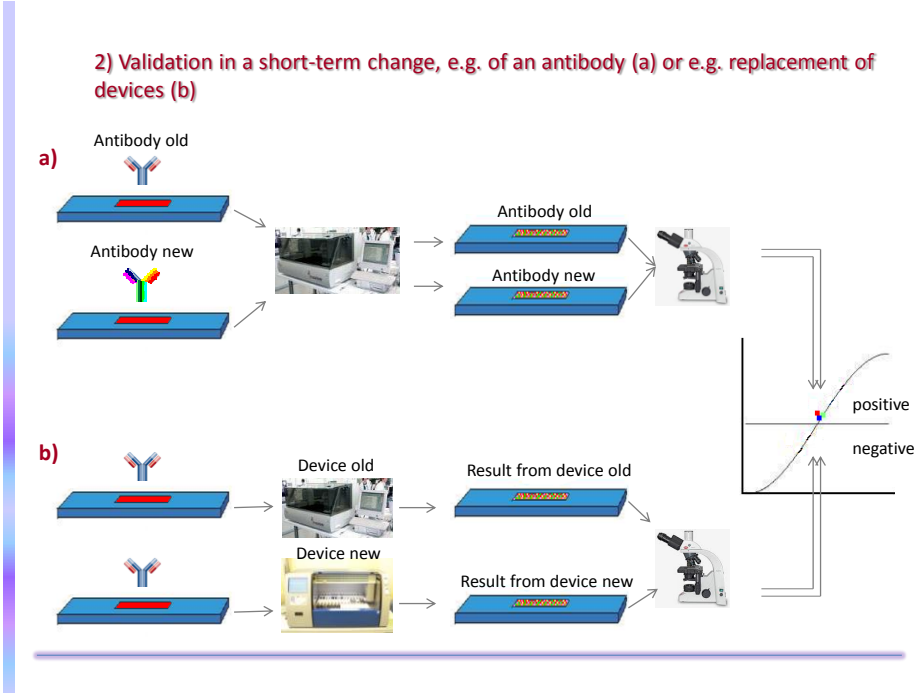
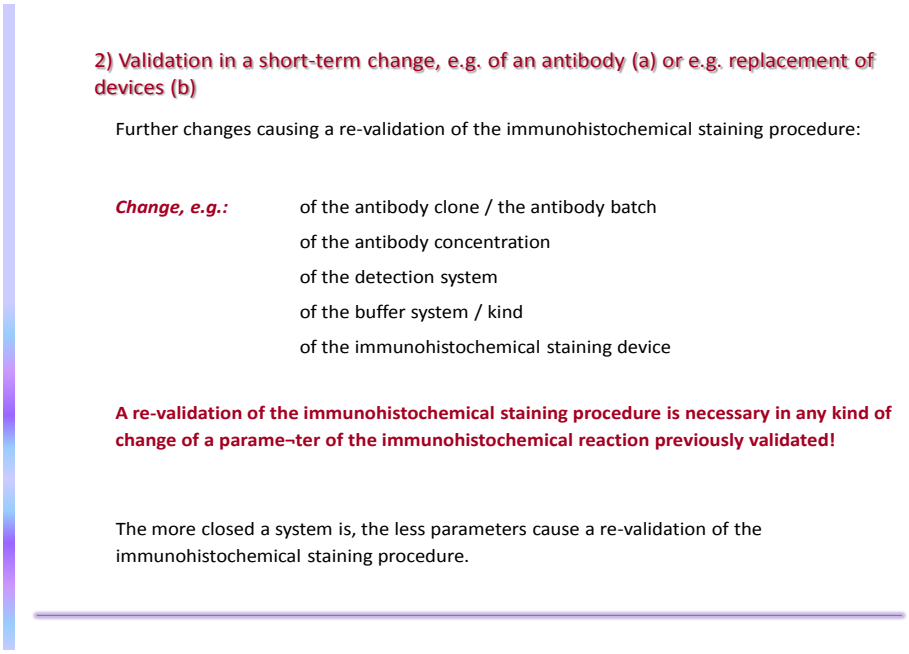


Fig. 9:



Note 11:

In practice, a validation should take place in the following cases, for example:

- with a new antibody
- with a new antibody clone
- if the antibody is purchased from a new manufacturer
- if the type of device is changed.

Verification could be sufficient in the following cases, for example:

- with a new antibody batch
- if a new antibody concentration is provided by the manufacturer
- if a new device, albeit of the same type, is purchased
- if a new kind of detection system is introduced, only verification is possible to start with, if applicable individual antibodies must be re-validated
- if a new buffer system is introduced, individual antibodies must be re-validated if applicable.

3.5.4 Verification of the validated immune staining procedure in routine diagnostics

Fig. 10:

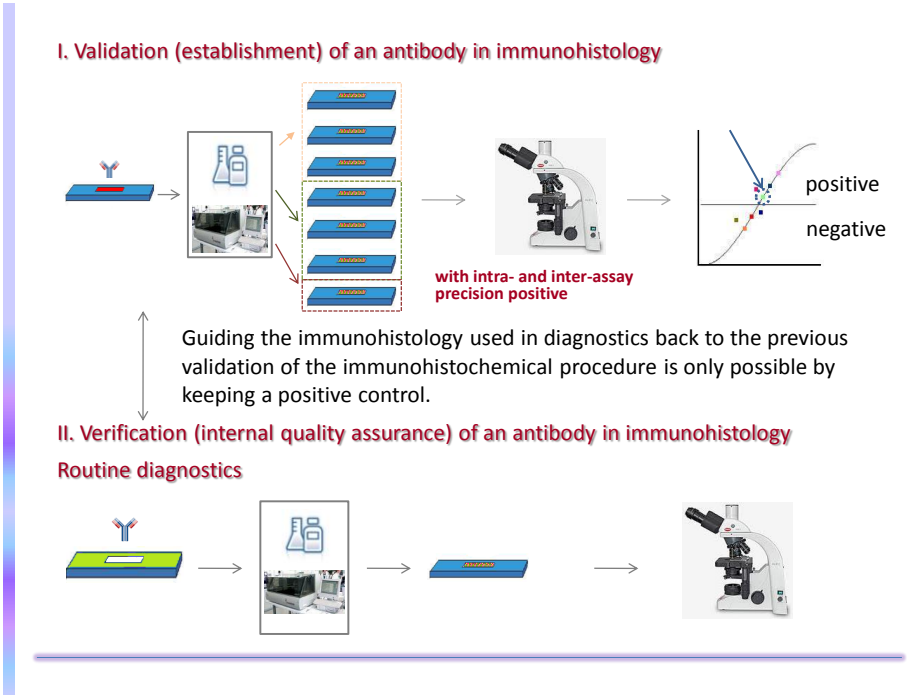


Fig. 11:

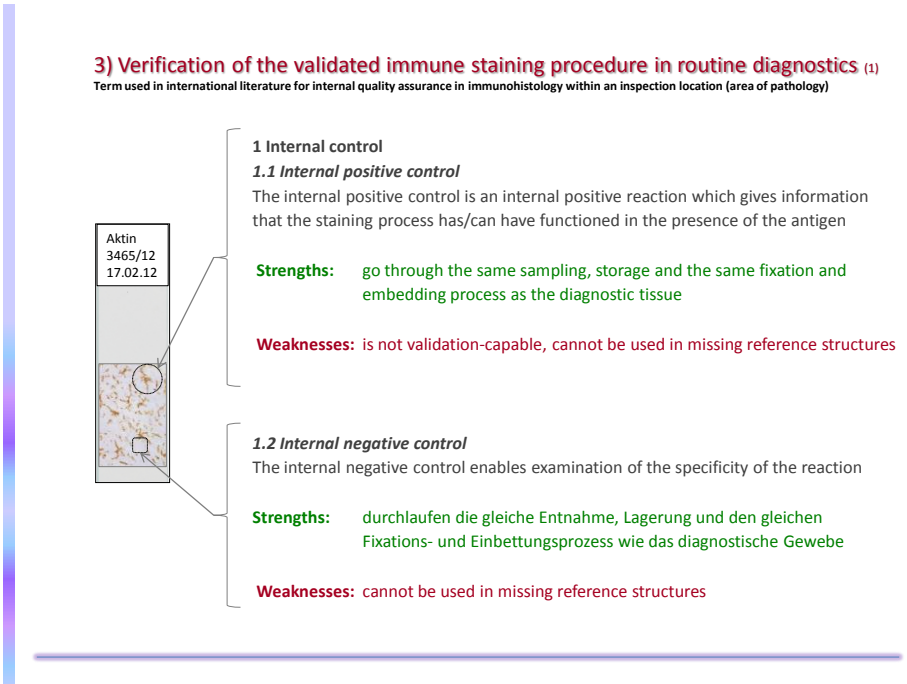
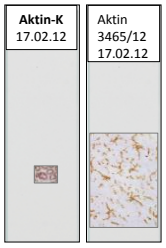


Fig. 12:

3) Verification of the validated immune staining process in routine diagnostics (2)



2 External control

Strengths: can be validated

Weaknesses: do not go through the same sampling, storage and the same fixation and embedding process as the diagnostic tissue

2.1 On-slide control

The on-slide control is a validated external (positive or negative) control also held on the same OT as the diagnostic tissue.

Strengths: has accurately been validated for the entire immune staining procedure, has passed through all the steps like the diagnostic tissue at best

Weaknesses: more difficult securing of a constant immune staining reaction on the entire OT surface (e.g. device specifics, pipette errors, flat storage of the OT, drying out).

2.2 External control on separate OT

This is a validated external control (positive or negative) which is also done on a separate (not the same) OT parallel to the diagnostic tissue

Strengths: Less positioning problems on the OT
larger tissue samples (TMAs) possible
a number of positive and negative controls possible at the same time

Weaknesses: not the same immune staining procedure as with the diagnostic tissue

Fig. 13:

3) Verification of the validated immune staining process in routine diagnostics(3)

int. pr. con.	*diagn. tiss.*	*Outcome*
+	+	IH reaction has possibly functioned, wrongly pos. reaction poss., cannot be fed back to or compared with the outcome of the validation
+	-	IH reaction has possibly functioned, wrongly pos. reaction in PC poss., cannot be fed back to or compared with the outcome of the validation
-	+	IH reaction has probably not functioned, wrongly pos. reaction in the diagn. tissue poss., not evaluable, repeat
-	-	IH reaction has not functioned, not evaluable, repeat

ext. pr. con.	*diagn. tiss.*	*Outcome*
+	+	IH reaction has functioned, outcome evaluable, can be fed back to and compared with the outcome of the validation
+	-	IH reaction has functioned, outcome evaluable, can be fed back to and compared with the outcome of the validation
-	+	IH reaction has probably not functioned, wrongly pos. reaction in the diagn. tissue, not evaluable, repeat
-	-	IH reaction has not functioned, not evaluable, repeat

Note 12:

Knowledge of the properties of the antibodies (cross-reactivity etc.) and the expression pat-tern of antigen/target structures is presupposed. If knowledge is missing (possibly also as an expression of a lack of scientific data basis), false assessments are possible despite good quality of the immune staining.

In the assessment of staining intensities, tissue standards with a known content of molecules are decisive.

Note 13:

The use of patients' material as control material has been regulated in § 24 of the German Medicinal Devices Act (MPG). This section enables the use of residual tissue which would have to be disposed of and is no longer diagnosis-relevant for the patient or has to be archived for other purposes (for the patient).

§§ 20 to 23 b:

§ 20 General preconditions for the clinical trial

§ 21 Specific preconditions for the clinical trial

§ 22 Procedures with the Ethics Commission

§ 22 a Approval procedures with the senior federal authority

§ 22 b Withdrawal, revocation and suspension of the approval or the approving assessment

§ 22 c Changes after approval of clinical trials

§ 23 Implementation of the clinical trial

§ 23 a Reports concerning the ending of stoppage of clinical trials

§ 23 b Exceptions for the clinical trial

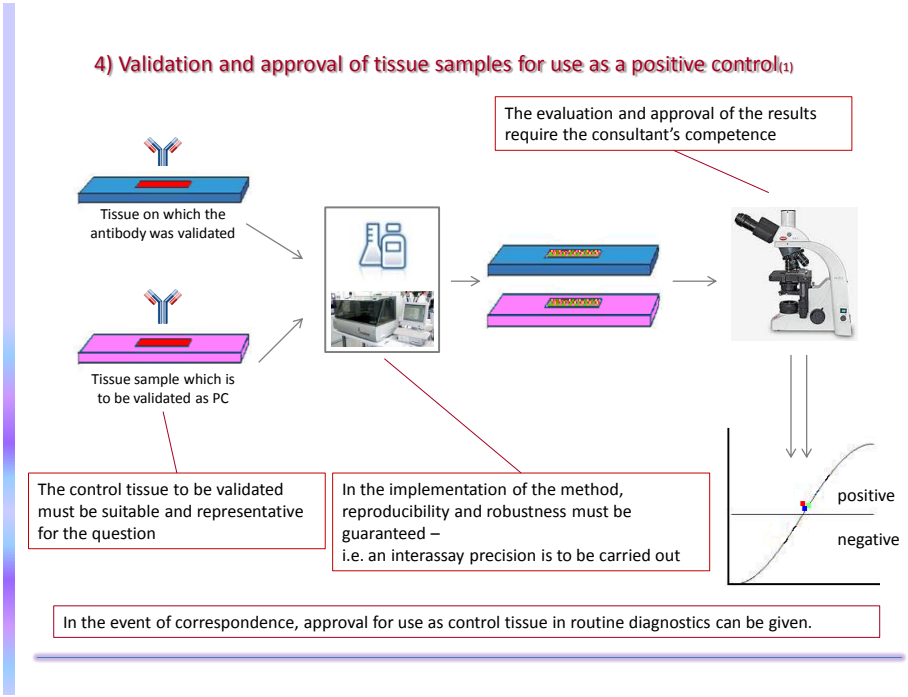
Are only to be taken into account in the 3 cases stated in § 24. In the final sentence, § 24 clearly states that the patient's approval is only necessary if the patient's personality right or commercial interests are affected. Neither is applicable to material which is to be disposed of.

Note 14:

The multi-block technique can eliminate the possible problem of the lack of positive or negative control structure in the test tissue in many cases thanks to a corresponding selection of tissues.

3.5.5 Validation and approval of tissue samples for use as a positive control

Fig. 14:



Note 15:

As a rule, using normal tissue with foreseeable antigen expression as a positive control is to be preferred instead of tumor tissue with variable expression.

Using positive controls also containing cells or tissues not expressing the antigen according to expectations is recommended.

Examples with a view to the importance of the correct test tissue for validation of an antibody and of the limited meaningfulness of internal positive controls:

1. CD 117

- The expression of CD 117 in GIST varies from negative to highly positive.
- Mast cells within GIST and in the neighbouring musculature show a strong CD 117 expression (often higher than in the neighboring GIST)
- Sensitivity for CD 117 should be selected such that also weakly CD 117-positive GIST are recognised.
- If strongly CD 117-expressing GIST or mast cells are used as a positive control, there is the risk that weakly CD 117-positive GIST behave in an immune-negative way.

2. Cyclin D1

- The expression of Cyclin D1 can be low in mantle cell lymphomas, on the other hand highly positive in benign glands of the mamma parenchyma
- Same problems: if benign parenchyma is used for validation of the antibody or as an internal positive control, mantle cell lymphomas can be falsely interpreted as being negative.

3. CD 10

- The expression of CD 10 is lower in follicular lymphomas than in a number of carcinomas.
- If a follicular lymphoma is taken for validation, the expression, for example in renal cell carcinomas or biliary ducts can be too strong in hepatocellular carcinomas and may no longer be evaluable.

Note 16:

If the tissue is subsequently stamped, a repeated immunohistochemical staining ought to confirm the suitability as a positive control for tumor tissue.

Monitoring of the quality of positive controls in the course of time (individual antigens are lost if sections are produced too early) must be guaranteed.

3.5.6 Documentation and archiving of validation data

3.6 Further quality assurance measures

4 Documents also valid