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Guideline of the Sector Committee Pathology/Neuropathology for the validation of examination methods in immunohistochemistry

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Scope of application:

This guideline applies to all accredited pathologies/neuropathologies or those aiming for accreditation in which immunohistochemistry examination methods are applied and the results are used for providing a diagnosis and a decision on a therapy.

This document gives technical and scientific instructions for the fulfilment of the requirements made of validation and verification of immunohistochemistry examination methods.

In these guidelines, the measures for validation and verification of immunohistochemistry examination methods which are to be introduced or replaced are described (i.e. in-house methods).

Date of confirmation by the Accreditation Advisory Committee: 31 August 2016

Relevant amendments to the previous review have been marked by a line at the side if it is a question of an entire section and in addition by italics for parts within a section.

In this document, the male form of function designations is used as a matter of principle in the interest of legibility; it also includes the female form.

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1 Purpose / Scope of application

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2 Terminology and abbreviations

2.1 Abbreviations

AK	Antibody
Ag	Antigen
MPG	German Medicinal Devices Act
MPV	German Medicinal Devices Ordinance
NWG	Detection limit
IVD	In-vitro diagnostics
OT	Slide

2.2 Terminology

Class I Antibody	AK used in immunohistochemistry/immunocytochemistry tests and interpreted in the context of histomorphology, cytomorphology and clinical data. They serve qualitative differentiation (e.g. cell kind and differentiation, tissue composition, detection of pathogens).
Class II Antibody	AK which have a direct therapy relevance in immunohistochemistry/immunocytochemistry tests as a result of their semi-quantitative evaluation (e.g. oestrogen receptor, progesterone receptor, Her2/neu e.g. with mamma carcinomas)[1].
“CE antibody”	Antibody with CE mark (CE-marked AK). The validation data have been recorded by the manufacturer and exist.

[1] Some antibodies can be in both classes depending on the question (see remark 1 in the annex).

External control on separate OT	This is a validated control (positive or negative) also done on a separate (not the same) OT parallel to the diagnostic tissue.
External on-slide control	The on-slide control is a validated external control (positive or negative) which is cultivated on the same OT with the diagnostic tissue and also carried out.
External positive control	Ag in external (a diagnostic tissue section other than the one to be assessed in immunohistochemistry) material known to be suitable and approved (e.g. other tissue samples, cell cultures or material provided by the manufacturer) which expectedly enters into an immune reaction with the applied AK.
"In-house methods"	Methods developed by the institution of pathology/neuropathology (=inspection body) itself or established on the basis of external scientific work (e.g. changed dilution of a CE-marked antibody, change of the fixation time of sample material). The institution of pathology/neuropathology (=inspection body) is responsible for the detection of the suitability of the method in the application in question (see statutory requirements), in which context the scope of the validation of the methods may be different).
Internal negative control	Ag within the diagnostic tissue section to be assessed in immunohistochemistry which expectedly does not enter into an immune reaction with the AK applied.
Internal positive control	Ag within the diagnostic tissue section to be assessed in immunohistochemistry which expectedly enters into an immune reaction with the AK applied.
In-vitro diagnostics from own manufacture	"In-vitro diagnostics from own manufacture" are in-vitro diagnostics produced in laboratories of health institutions and applied in these laboratories or in the rooms in their immediate vicinity without being placed on the market. For in-vitro diagnostics produced on an industrial scale, the directives concerning own production are not applicable ..." (§ 3, no. 22, MPG).

IVD (in vitro diagnostic medical device)	"...any medical device which is a reagent, reagent product, calibrator, control material, kit, instrument, apparatus, equipment, or system, whether used alone or in combination, intended by the manufacturer to be used in vitro for the examination of specimens, including blood and tissue donations, derived from the human body, solely or principally for the purpose of providing information: • concerning a physiological or pathological state, or • concerning a congenital abnormality, or • to determine the safety and compatibility with potential recipients, or • To monitor therapeutic measures." (Directive 98/79/EC)
Conformity assessment	Demonstration that specified requirements relating to a product, process, system, person or body are fulfilled (DIN EN ISO/IEC 17000:2005).
Performance characteristics	Sensitivity, specificity, robustness, precision etc.
Precision interassay	Precision between differing reaction measures
Precision intraassay	Precision within a reaction mixture
Precision/reproducibility	Degree of correspondence between the individual independent results
Reaction mixture	Implementation of the complete immunohistochemistry reaction with the same reagents (e.g. AK batch, buffer mixture etc.) and devices.
Correctness	Comparison of the results with the evidence-based expectation figure, determination and assessment of the systematic deviations of the results
Robustness	The robustness of a method is a degree of its ability to remain uninfluenced by small, but deliberate changes of the method parameters and shows its reliability during the normal application (e.g. fixation).
Semi-quantitative	Approximate determination of the quantity of an antigen (e.g. hormone receptor) with statement of a value which is less precise than a quantitative test and the result of which is expressed in categorical figures (e.g. slight, moderate, high degree) as a rule.

Sensitivity	Rate of the genuinely positive results. The sensitivity is a measure of the number of correctly positive results compared with the total number of positive results (number of correctly positive + number of wrongly negative).
Specificity	Rate of the genuinely negative results. Specificity is a measure of the number of correctly negative results compared with the total number of negative results (number of correctly negative + number of wrongly positive).
Validation	Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. (ISO 9000:2005, 3.8.5., DIN EN ISO 15189:2014)
Verification	Confirmation, through provision of objective evidence, that specified requirements have been fulfilled (ISO 9000:2005, 3.8.4., DIN EN ISO 15189:2014) (see note 2 in the annex)

3 Description

3.1 Introduction to fundamentals

The inspection body must validate all examination procedures used and self-developed which are standardised, standardised with modification, standardised outside the planned scope of application.

The nature and scope of the validation are to be set by the inspection body.

This guideline gives recommendations for the validation of the examination procedures in immunohistochemistry.

The immunohistochemistry examination is an inspection activity. The evaluation of immunohistochemistry reactions requires profound technical knowledge and professional experience.

Validation and verification of the immunohistochemistry procedure ensure the reproducibility and robustness of the procedure in (routine) diagnostics as well as possible, despite factors which are not to be influenced (structure of the tissue, fixation times etc.).

In principle, all the examination procedures are to be examined for their capability, i.e. there must be proof that the performance characteristics of an examination procedure fulfil the applier's (consultant for pathology) requirements with a view to precision, correctness, specificity etc.

Validation and verification of the procedure are to be observed as processes flowing into one another. In the in-house procedures, the validation relates to the establishment and determination of the precise procedure before approval for routine diagnostics.

Continuous verification of the applied procedures in routine diagnostics is to be observed as one of the most important in-house quality assurance measures. Verification must ensure that the performance data determined in the validation of the procedure are continuously achieved and the procedure thus runs reproducibly and stably.

For the procedures already validated by the manufacturer and used precisely according to the manufacturer's requirements, verification starts with the examination of the performance characteristics which have been stated in the institute before approval of the procedure for routine diagnostics.

Examination procedures applied precisely according to the manufacturer's requirements:

For CE-marked antibodies and kits and with precise implementation of the manufacturer's requirements (the methods for use of the antibodies and kits have been validated by the manufacturer), the applier must ensure that the performance characteristics stated by the manufacturer for precision and correctness can also be proven to be achieved in his institute (verification) (see note 3 in the annex).

Examination procedures in which there is a deviation from the manufacturer's requirements (in-house procedures):

In this case (if, for example, own AK dilutions not recommended by the manufacturer are applied), it is a question of in-vitro diagnostics from in-house production according to law (see note 4 in the annex):

Special productions may only ... be taken into operation if the fundamental requirements according to § 7, which are ... applicable to them, have been fulfilled and the conformity assessment procedure planned for them ... has been carried out ... . For the commissioning of medicinal devices from in-house production ..., the directives of sentence 1 (*for IVD: fulfilment of the fundamental requirements according to Annex I of Directive 98/79/EC, conformity assessment procedure in analogy to special productions*) are applicable accordingly (cf. § 12, sub-section 1, MPG).

The conformity assessment procedure is to be equated to the validation procedure in this context.

Further, the accreditation standards demand a complete validation (including documentation of the validation results and archiving of the validation data) of these procedures.

3.2 Statutory requirements

- German Medicinal Products Act in the version valid at the time in question, § 3, nos. 21 and 22, MPG
- Directive 98/79/EC
- § 5, sub-section 6, MPV

3.3 Responsibilities

Responsible person	Activity
MTA/BTA/CTA or similar	Technical implementation of the validation/verification and recording of the validation data
Consultant for Pathology/Neuropathology	Requirements for validation/verification, examination and assessment of all validation/verification results including the approval of the methods and introduction into the diagnostics (see note 5 in the annex)

The management of the inspection body is responsible for the fact that only validated methods are used for the diagnostics. It must set the nature and scope of the performance characteristics to be examined and decide whether the reliability and capability of the procedures can be guaranteed with the determined results in such a way that valid results are achieved reproducibly. The procedure is only approved by the Consultant for Pathology/Neuropathology following examination and assessment of all validation results.

3.4 Appliances, materials, ancillaries

Appliances, materials, ancillaries must be defined and listed.

3.5 Implementation of the validation / verification

3.5.1 General comments on the validation and verification of the method

If CE-marked antibodies and kits are used, the procedure must be precisely in accordance with the manufacturer's requirements (supplied product information; "package leaflet"). In routine, verification is to be carried out by the applier in advance. This includes the examination of the performance characteristics:

- interassay and intraassay precision
- correctness

(Note: This procedure is an exception in pathology/neuropathology.)

(See note 6 in the annex)

In pathology/neuropathology, in-house procedures are mainly used (partly also with CE-marked antibodies, e.g. with AK dilution deviating from the manufacturer's requirements, deviating fixation time of the sample tissue to be examined or other deviations from the manufacturer's requirements, cf. 3.1). An "in-house procedure" must be completely validated in advance. For this, the following performance characteristics are to be determined:

- intraassay and interassay precision
- correctness
- specificity
- adequate sensitivity on suitable test tissue

(See note 7 in the annex)

3.5.2 Validation of the immunohistochemistrymethods in in-house procedures[2]

All methods may only be approved for routine diagnostics when they have been validated (see also point 1.).

In the selection of a suitable antibody, the usefulness of the antibody for the method (e.g. paraffin passage, frozen section) must be examined. The diagnostic value of the antibody should generally be scientifically (evidence-based) proven or substantiated (e.g. subject-specific further training, subject literature).

The test tissue must be suitable and representative for the question.

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[2] See Annex: Figs. 1-7

Recommendations for the tissue selection for use as test tissue for Class I (qualitative) and Class II (quantitative/semi-quantitative) antibodies:

Reference material/validated control material with required and reproducible strength of the target antigen expression. Own test tissue can be used, the results must be compared with or balanced against the scientifically substantiated expectation value (evidence-based).

Negative controls should also be held at the validation.

In the implementation of the method (e.g. fixation, embedding, pre-treatment, dilution, incubation times, detection system), ensuring reproducibility and robustness must be guaranteed as well as possible (see note 8 in the annex).

Evaluation and approval of the results require the competence of the consultant who compares and balances the result with the scientifically substantiated expectation value (evidence-based, see above) as well as possible and grants the approval for the use in routine diagnostics in the event of correspondence following examination and guaranteeing of the matching performance characteristics (see note 9 in the annex).

The following performance characteristics must be guaranteed and determined before the approval:

Intraassay precision

The procedure must be carried out on various tissue samples with a reaction mixture and fulfil the scientifically substantiated expectation value (correctness and precision).

Interassay precision

The procedure must be carried out on the same tissue samples with independent reaction mixtures (in the sense of differing runs, e.g. with new buffer mixtures) and fulfil the scientifically substantiated expectation value (correctness and precision). The more closed the immunohistochemistry staining precision used is, the less parameters vary between the independent reaction mixtures.

After this, the deviations within (intraassay) and between (interassay) the series are to be assessed.

In addition to the intraassay precision and interassay precision performance characteristics, the procedure must be examined with a view to sensitivity and specificity, to the extent sensible and necessary. For this, holding of negative controls[3] is possibly 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[4]

If, for example, a device and/or an AK kit/AK batch is/are to be replaced, an anti-body/staining result comparison (comparison of the immunohistochemistry result between “old” and “new procedure) must be held. For this purpose, tissue samples are to be analysed and evaluated immunohistochemistry in various mixtures.

The examination of tissue samples with the old and new immune staining procedure (primary, secondary AK, kit, detection system, automatic immune stainer) must lead to comparable or scientifically substantiated better results. In the event of deviations, the Consultant for Pathology/Neuropathology must decide whether the new immune staining procedure is approved and substantiate this briefly in the document (if relevant for later traceability). If the tissue samples do not manifest any matching results in the immunohistochemistry reaction, the immune staining procedure is not to be approved and, if applicable, the comparison repeated.

(See note 11 in the annex)

3.5.4 Verification of the validated immune staining procedure in routine diagnostics[5]

As a result of the variability of the sample material (tissue), the validated procedure must be verified in routine diagnostics by adequate quality controls. This means, as a function of the AK, at least one known positive tissue sample, if required (e.g. Her-2/neu) one known weakly positive/borderline, one positive and one negative tissue sample must be held. (See note 12 in the annex)

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[3]

I. Negative tissue control:
Tissue for which it is known that it does not possess the examined target antigen structure - as a result of which an unspecific cross-reaction, background staining (e.g. with excessively long formalin fixation) can be detected. Serves to verify the specificity of the target antigen marking by the primary antibody.

II. Negative reagent control:
By leaving the primary antibody away, a missing specificity of the immunohistochemical procedure or unspecific background staining can be detected; equivalent to a “methodical control” (see note 10 in the annex).

[4] See Annex, Figs. 8-9

[5] See Annex, Figs. 10-13

The following controls are imaginable:

1. Internal control

1.1 Internal positive control

Strengths: go through the same sampling, storage and the same fixation and embedding process (including the pre-cutting of the controls) as the diagnostic target structure

Weaknesses: cannot be used in missing reference structures

1.2 Internal negative control

Strengths: go through the same sampling, storage and the same fixation and embedding process (including the pre-cutting of the controls) as the diagnostic target structure

Weaknesses: not validation-capable

2. External control

Strengths: can be validated

Weaknesses: does not go through the same sampling, storage and the same fixation and embedding process (including the pre-cutting of the controls) as the diagnostic target structure

2.1 External on-slide control

Strengths: can accurately be 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

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

As a quality control for the verification of the validated immune staining procedure, only one validated tissue sample can be used. In this, application of an external on-slide positive control offers the highest possible degree of quality within the routine diagnostics in most systems, as it passes through all the process steps within the validated immunohistochemical procedure parallel to the diagnostic tissue to be assessed.

For quality assurance in the performance of the immunohistochemistry *and immunocytochemistry examination procedures, external on-slide positive controls are also to be held as a matter of principle for Class II antibodies (see 2.2).*

If this mode of procedure is not possible immediately for technical reasons (e.g. appliance properties, diagnostic tissue, control tissue), at least a validated external control (on a separate OT) per antibody, run and appliance is to be done as a positive control as a transition.

For Class I antibodies, an internal control is sufficient if the balance with the test material (which was used in the establishment of the antibody) is possible and accountable. This balance is to prove the same reaction of the internal control with the test tissue and thus be valid as validation of internal control. If there is no internal positive control in the diagnostic section, at least a validated external control (on a separate OT or on-slide control) per antibody, run and appliance is also to be held as a positive control.

All controls (both external and also internal) are to be archived accordingly for traceability of the validation/verification procedure.
(This italic section portrays a resolution passed by the Pathology/Neuropathology Sector Committee and is thus binding.)
(See notes 13 and 14 in the annex)

If the results for the quality controls are within the admissible range, the examination results can be approved.

To the extent available, internal positive and negative controls must always be taken into account. This must match the scientifically substantiated experience value (evidence-based), but cannot be used for the verification of the validated immunohistochemistry procedure as an individual control.

3.5.5 Validation and approval of tissue samples for use as a positive control [6]

Tissue samples may only be used as a positive control for immunohistochemistry in routine diagnostics if they have been validated.

The control tissue to be validated must be suitable and representative for the question.

In the implementation of the method (e.g. fixation, embedding, pre-treatment, dilution, incubation times, detection system), ensuring reproducibility and robustness must be guaranteed as well as possible (see note 8 in the annex).

Evaluation and approval of the results require the competence of the consultant who compares and balances the result with the scientifically substantiated expectation value (evidence-based, see above) as well as possible and grants the approval for the use in routine diagnostics in the event of correspondence following examination and guaranteeing of the matching performance characteristics (see notes 15 and 16 in the annex).

3.5.6 Documentation and archiving of validation data

Performance of the method validation is to be documented and archived with all the raw data and the resulting outcomes. Likewise, general remarks or references possibly helpful for performance of the method should be recorded and archived.

The documents are to be archived for 5 years. After this period, the Consultant for Pathology/Neuropathology decides on further archiving.

The microscope slides, which are used for the traceability of the validation process (including batch change, validation of control material etc.), must be archived for 5 years. A storage in digitized form is possible. There is no obligation to archive the test immune responses that were discarded during the validation process.

All slides, which are used for the traceability of the verification process (controls, which are carried out in the routine) must be archived (assigned to the case) for 10 years.

[6] See Annex, Fig. 14

3.6 Further quality assurance measures

Internal quality assurance measures can, amongst other things, contain the following:

- use of acknowledged reference materials, control material supplied by the manufacturer or cell lines from strain collections or from reference laboratories
- repeat inspections or assessment of a case by a further colleague from the same institute text

External quality assurance measures can, amongst other things, contain the following:

- participation in suitability examinations (e.g. in proficiency tests)
- benchmarking
- participation in quality circles
- assessment of a case by an external colleague or the expert analysis within the framework of the accreditation procedure

4 Documents also valid

1. The Total Test Approach to Standardization of Immunohistochemistry (Arch Pathol. Lab Med, Vol 124, 2000)
2. A Practical Approach for Evaluation New Antibodies in the Clinical Immunohistochemistry Laboratory (Arch Pathol Lab Med Vol 125, 2001)
3. Recommendation for improved Standardization of Immunohistochemistry (Appl Immunohistochem Mol Morphol Vol 15, Nr 2, 2007)
4. Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories (J Vet Diagn Invest 20: 393-413, 2008)
5. Canadian Association of Pathologists-Association canadienne des pathologistes National Standards Committee/Immunohistochemistry (Am J Clin Pathol 2010; 133:354-365)
6. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunhistochemical Testing of Estrogen and Progesteron Receptors in Breast Cancer (Journal of Clinical Oncology; 2010 Vol.28, Nr. 16)
7. Recommendation for Validating Estrogen and Progesteron Receptor Immunhistochemitry Assays (Arch Pathol Lab Med, Vol 134, 2010)
8. Effects of Preanalytic Variables on the Detection of Proteins by Immunhistochemistry in Formalin-fixed, Paraffin-Embedded Tissue (Arch Pathol Lab Med, Vol 135, 2011)
9. Antibody validation (Jennifer Bordeaux, Allison W. Welsh, Seema Agarwal, Elizabeth Killiam, Maria T. Baquero, Jason A. Hanna, Valsamo K. Anagnostou, and David L. Rimm/Department of Pathology, Yale University School of Medicine, New Haven, CT, USA; BioTechniques, Vol. 48, No. 3, March 2010, pp. 197–209)