These standard operating procedures define the regulations and responsibilities in connection with the

**validation/verification of methods employed by and/or developed and used in medical laboratories for the detection of infectious agents[[1]](#footnote-2)**

**This SOP is valid:**  14 days after it has been approved

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**Notes on changes:** Regulation (EU) 2017/746 taken into consideration

*Changes are highlighted in grey in the text. All changes to the previous version are visible when both documents are compared in the document control system (XY).*

**To the attention of the following persons/professional groups:**

 all employees in the accredited field (IM, DM, LM, TA, QMO)

 *(IM = institute management, DM = department management, LM = laboratory management, TA = technical assistant, QMO = quality management officer)*

**Distribution:** Document control system XY

This document was prepared by the IVDR subgroup of the Ad Hoc Commission IVD at the Association of the Scientific Medical Societies in Germany (AWMF). The document is not legally binding and does not constitute guidance in the legal sense.

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| Authors of the sample SOP: | Representing the: |
| Prof Dr Holger F. Rabenau Institute of Medical VirologyUniversity Hospital FrankfurtPaul-Ehrlich-Str. 40, 60596 FrankfurtEmail: rabenau@em.uni-frankfurt.de | Society for Virology (GfV) |
| Prof Dr Jörg HofmannLabor Berlin – Charité Vivantes Services GmbHBerlin | Society for Virology (GfV) |
| Dr Axel SchubertVaricon, Ulm | Society for Virology (GfV) |
| Prof Dr Klaus-Peter HunfeldCentral Institute for Laboratory Medicine,Krankenhaus Nordwest, Frankfurt | German Society for Hygiene and Microbiology (DGHM),INSTAND e.V., Düsseldorf |
| Prof Dr Udo ReischlInstitute of Medical Microbiology and Hygiene University Regensburg Hospital | German Society for Hygiene and Microbiology (DGHM),INSTAND e.V., Düsseldorf |
| Prof Dr Folker SpitzenbergerTechnische Hochschule Lübeck | German Society for Pharmaceutical Medicine (DGPharMed) |

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# 1. Objective/Purpose

These standard operating procedures (SOP) outline the measures for method validation/verification in the field of virology and microbiology and take into account the requirements of Regulation (EU) 2017/746 (In-Vitro Diagnostic Medical Device Regulation, IVDR).

This SOP therefore serves to fulfil the requirements of Article 5, point g) of the IVDR (“drawing up documentation that makes it possible to have an understanding…of the design and performance data of the devices…that is sufficiently detailed”).

For ease of reading, reference is only made throughout the text to the field of “virology” - however, analogous or specifically adapted specifications also apply to the field of “microbiology” and/or in general to infectious disease diagnostics.

Also, to facilitate understanding and at the same time ensure completeness, this document covers not only in vitro diagnostic medical devices (IVDs) that have been developed and manufactured by the laboratory itself (so-called laboratory developed tests [LDTs]) -- but also IVDs that have been commercially manufactured (and validated by the manufacturer) and for which the trueness and precision (verification) are to be verified before being implemented in the laboratory.

A wide range of medical laboratories often use laboratory developed tests (LDTs) due to a lack of suitable commercially available diagnostic medical devices and to safeguard patient care. Various aspects can justify the use of LDTs, such as the performance characteristics of the tests (e.g., sensitivity, specificity, stability, quality control mechanisms), the turn-around time, multiplex versus monoplex tests, amount of material required (reagents, sample material), type of sample material/matrix, etc.). This SOP does not include a justification for why an LDT is used, which must be presented or documented elsewhere (see also Point 10). It is also to include a so-called justification for the use of an equivalent LDT, which demonstrates that the intended purpose of the LDT-IVD cannot be achieved or cannot be achieved at the desired level by a similar, CE-marked IVD. It must also determine the type and scope of the documentation for the “product monitoring” required by the IVDR (also for LDTs).

Also not covered in this SOP:

* LDT-IVD classified as Class D devices according to Regulation (EU) 2017/746 - Annex VIII (Classification Rules) and for which CS (Common Specifications) or CTS (Common Technical Specifications) are also available.
* Specifications for manufacturing the LDT-IVD as well as quality assurance measures (i.e., batch control, shelf life, storage conditions). A separate SOP must be prepared for this and reference made to it. In addition, the requirements set forth in Annex I, Sections 9.2 and 9.3[[2]](#footnote-3) of the IVDR must be ensured, e.g., by continuously maintaining and documenting appropriate quality controls in the sense of “robustness controls”.
* Specifications for classifying LDT-IVDs; a separate SOP must also be drawn up for this and reference made to it.

The commissioning of the LDT continues to be permitted under Regulation (EU) 2017/746 without the involvement of a notified body and without affixing a CE marking. In-house manufactured tests are exempt from the requirements of Regulation (EU) 2017/746 however they must comply with the requirements of Annex I of the IVDR and some of the requirements listed in Article 5(5) of the IVDR (e.g., be manufactured “on a non-industrial scale”).

This SOP describes the measures that are necessary - wherever possible and essential - to check the performance of a test (method) that is to be newly introduced or for which a modification is planned:

* Analytical performance characteristics include:
	+ - Precision (repeatability and reproducibility)
		- Trueness (bias), accuracy (resulting from trueness and precision)
		- Linearity, analytical sensitivity (limits of detection and quantification) and
		- Analytical specificity (control of known relevant endogenous and exogenous interference and cross-reactions). These shall be tested using appropriate statistical tools.
* Also to be considered (where applicable):
	+ - Classification of the LDT-IVD according to the IVD Risk Classes as per Annex VIII of the IVDR in conjunction with document MDCG 2020-16 and, where applicable, validation that includes risk management within its scope
		- Pathogen significance/incidence (where necessary, also establishing whether, for example, very rare pathogens are involved)
		- Orphan analyte/rare disease[[3]](#footnote-4)/emerging (new) disease
* In addition - where applicable - clinical performance is to be determined, such as “diagnostic sensitivity, diagnostic specificity, positive predictive value, negative predictive value, likelihood ratio, expected values in normal and affected populations”.

The scope of the assessment depends on the requirements placed on the method, e.g., whether it is completely new, newly developed, or represents a modification of the examination procedure.

It must also be considered whether the “LDT” has been developed by the laboratory itself and is described in the scientific literature (which has been subjected to peer review), or whether it is a procedure recognised by expert opinions or statements or by expert societies, uses study findings to demonstrate its operating principle, or is based on clinical performance studies. In such cases, it must be assumed that there is adequate evidence for the scientific validity of the marker/pathogen under consideration. The IVDR (Annex XIII, Part A) states: “Its depth and extent shall be proportionate and appropriate to the characteristics of the device including the risks, risk class, performance and its intended purpose.”

Additional regulations and specifications may be required for special, methodologically complex (LDT) IVDs such as sequencing analyses or NGS (next generation sequencing), however these are not covered by this SOP. This also applies to other special issues, such as the combined use of CE-marked test components (e.g., a PCR) with test components (e.g., an extractor) that have not been explicitly validated /approved by the manufacturer for this purpose. As such extraction systems may constitute a so-called IVD accessory, they must therefore be, in principle, subjected to an assessment/validation as per Article 5 (5) of the IVDR (see also the guidance of the Medical Devices Coordination Group (MDCG) “MDCG 2020-16 - Guidance on Classification Rules for In Vitro Diagnostic Medical Devices under Regulation (EU) 2017/746”, in particular the comments on Classification Rule 5). In the case of such “CE/non-CE IVD” combinations, an assessment/validation can, where justified, be carried out in accordance with “LDT Light” specifications.

Patient results may not be obtained nor disclosed until the method has been validated and/or verified and approved. In individual cases, it is possible to deviate from this and from the specifications listed below, for example, if there is an extremely low prevalence of disease and thus a low sample frequency, or if it cannot be performed due to a lack of controls. The laboratory management (if necessary, together with the QMO and the senior technical assistant) then determines the modified procedure and justifies this in writing.

The number of samples listed below as part of a validation and/or verification, as well as comparative test regimes apply primarily to those parameters that are usually performed daily or several times a week. This applies in particular to the required number of positive samples, which may not always be available to the extent stated for understandable reasons (see similar regulations for so-called “orphan diseases”). The number of samples may be less than the stated number for tests that are performed relatively infrequently but which are nevertheless essential for a laboratory’s spectrum of diagnostic testing and reliable patient care (e.g., highly pathogenic agents, special long-standing and well-established issues, etc.). However, each case must be documented systematically and understandably by laboratory management.

According to the IVDR’s definition and the requirements outlined in Annex XIII, special attention must be paid - where possible and applicable - to the “scientific validity” of LDTs in the so-called “technical documentation” - as well as to the data on analytical and clinical performance. An evaluation of these data and the clinical evidence derived from them must be documented in a report. The data may need to be updated during the lifecycle of the product.

# 2. Terms/abbreviations/definitions

The following definitions shall apply within the context of this SOP

|  |  |
| --- | --- |
| **Analytical performance** | The ability of a device to correctly detect or measure a particular analyte (Source: IVDR, Article 2, point 40). |
| **Coefficient of determination (r2 = B)** | The ratio of the scattering of the points on the regression line to the total deviation. It can be used as a measure of the sharpness of the line and is thereby a measure of the dependency of the two measurement series. Ideally, B = 1.  |
| **Blandt-Altman’s method comparison**  | Statistical method for comparing two measurement procedures. This method is used to graphically illustrate the differences between the pairs of values of the two procedures against the mean values of the two measurement procedures.  |
| **CE Tests** | Test systems marked “CE” are tests developed elsewhere or sold as a commercial reagent kit with a CE marking. The manufacturer has validated the method and the method’s key validation data are available. The user only needs to perform a “*verification*”.  |
| **Diagnostic sensitivity** | The ability of a device to identify the presence of a target marker associated with a particular disease or condition (Source: IVDR, Article 2, point 50). |
| **Diagnostic specificity** | The ability of a device to recognise the absence of a target marker associated with a particular disease or condition (Source: IVDR, Article 2, point 49). |
| **Clinical performance** | The ability of a device to yield results that are correlated with a particular clinical condition or a physiological or pathological process or state in accordance with the target population and intended user (Source: IVDR, Article 2, point 41). |
| **Clinical evidence** | Clinical data and performance evaluation results pertaining to a device of a sufficient amount and quality to allow a qualified assessment of whether the device is safe and achieves the intended clinical benefit(s) when used as intended by the manufacturer (Source: IVDR, Article 2, point 36). |
| **Correlation coefficient (r)** | Determines the degree of correlation of the random samples, in the ideal case = 1. The calculation of r is done using the formula: r = √ byx x bxy |
| **Interlaboratory comparisons** | **- Measurement of the trueness/correlation of two different or identical tests in detecting the same analyte in two different laboratories (e.g., by detecting known analyte concentrations in EQAS samples or reference material. A balanced level of positive and negative samples should be tested).**  |
| **LDT** | **- Laboratory developed tests (LDT)** are methods developed by the laboratory itself or established on the basis of external scientific work. LDTs are thus defined as test methods which are developed in-house and validated, and in which reagents, kits, controls, equipment without a CE marking or with a combination of CE and non-CE marked components are used for in vitro diagnostic purposes. This also includes the use of CE-marked IVDs that are used beyond the specifications of the manufacturer’s product information (“off label use”) and tests without a CE-marking labelled “research use only” (RUO devices). In this case, laboratory management is responsible for demonstrating the suitability of the method for the corresponding application (“*validation*”). The scope of validation can be reduced for methods established on the basis of external scientific work (see Fig. 4). The data on the scientific validity of the test and its analytical and clinical performance, as well as its assessment and the clinical evidence derived from this shall be documented in a report. This shall form part of the documentation and shall be updated as necessary during the lifecycle of the device based on the data.  |
| **“LDT light”** | **- The term “LDT light”** includes (commercially available) IVDs that are used in modified form and/or with modifications and therefore may only need a lower degree of validation. Modifications are usually considered additional sample materials that correspond in composition and complexity to the intended material but are not listed by the manufacturer. Where applicable, commercial RUO tests can also be grouped under the category “LDT light” or, for example, the combined use of CE-labelled test components (e.g., a PCR) with test components (e.g., an extractor) that have not been explicitly validated/approved for this purpose by the manufacturer. The scope of the validation may then need to be individually adapted and justified - e.g., depending on the scope of validation already carried out by the manufacturer/provider, when an “RUO test” was previously commercially distributed as a CE-IVD, and/or when there are corresponding data (e.g., through internal controls) on the combined use of the test components.  |
| **Performance of an IVD** | The ability of a device to achieve its intended purpose as claimed by the manufacturer. It consists of the analytical and, where applicable, the clinical performance supporting that intended purpose (Source: IVDR, Article 2, point 39). |
| **Linearity** | **- Determination of a test’s linear measuring range.**In laboratory medicine, biological and biochemical reactions often form an S-curve with 3 areas: 1. exponential increase, 2. linear area, 3. saturation.  In addition to complex “curve modelling procedures”, the regression line is usually calculated for the linear section and the deviating curves are indicated as end points. The resulting b (b = regression coefficient = slope of the curve) is optimal at a factor of 1 - values between 0.999 and 0.95 (PCR 0.90) are permissible.  |

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| **Method comparison**  | **Measurement of the trueness/correlation of the (quantitative) individual results of two different tests for detecting the same analyte (classical method is correlation analysis) (see Fig. 1).** **Fig. 1:** Illustration of correlation when the two methods are compared

|  |  |
| --- | --- |
| Ideale Korrelation | **Ideal correlation**The results of methods 1 and 2 are identical and therefore all results lie along the identity line. This basically does not occur in reality. (A red point is generated by plotting the result of method 1 on the X-axis and the result of method 2 on the Y-axis).  |
| Gute Korrelation | **Good correlation**The results lie close to and parallel to the identity line. The **correlation coefficient r** is often given as a measure of the agreement between the methods. This is ideally 1 and is usually above 0.95 when laboratory methods are compared.  |
| Proportionaler Fehler | **Good correlation but Method 2 is proportionally too high (divergent regression)**The values of method 2 lie above the identity line, to a lesser extent for low values and a greater extent for high values. The deviation is therefore proportional to the value. The proportional deviation is determined as follows: A straight line (regression line) is drawn (calculated) through the red points and the slope of the straight line is measured (= regression coefficient or slope which is calculated by dividing the rise by the run). Ideally, the slope equals 1; in this example it is greater than 1.  |
| Konstanter Fehler | **Good correlation but Method 2 is too high by a constant value (parallelly displaced regression)** Both low and high values of Method 2 lie above the identity line. The deviation is therefore not proportional to the value. The non-proportional deviation is determined as follows: A straight line (regression line) is drawn (calculated) through the red points and the height at which this line intersects the Y-axis is measured (deviation). This height corresponds to the constant deviation. This value is called the y-intercept.  |

 |
| **Negative predictive value** | The ability of a device to separate true negative results from false negative results for a given attribute in a given population (Source: IVDR, Article 2, point 53). |
| **Positive predictive value** | The ability of a device to separate true positive results from false positive results for a given attribute in a given population (Source: IVDR, Article 2, point 52).  |
| **Predictive value** | The probability that a person with a positive device test result has a given condition under investigation, or that a person with a negative device test result does not have a given condition (Source: IVDR, Article 2, point 51). |
| **Precision** | - **Reproducibility** - **Repeatability** of a lab test- **Intra-assay variance**- **Inter-assay variance**Degree of agreement between the different independent measurement results (see Fig. 2). The degree of precision is usually indicated by the statistical measure of “standard deviation” and “relative standard deviation (coefficient of variation)” of the individual values from the mean, which is inversely related to precision. The “precision” of an analytical procedure is split into the following conditions of precision. **“Repeatability”** refers to essentially unchanged conditions and is often referred to as **“precision in the series” (intra-assay)**. The **“precision from analytical series to analytical series” (inter-assay)** reflects the variations of one or more of the factors occurring in a laboratory. Such factors include time, calibration, investigator and measuring device. The standard deviation or relative standard deviation (**coefficient of variation - CV**) is calculated to determine and evaluate the random measurement deviation (assessment of precision) for quantitative measurement methods. For qualitative methods, the repeatability and/or reproducibility are considered. **Fig. 2:** Precision and trueness: Connotations such as “exact”, “true” and “precise” are commonly used interchangeably. An archer’s accuracy is used to illustrate these differences below.

|  |  |
| --- | --- |
| präzis und richtig | **Precise and true**The archer shoots precisely (always around the same point) and true (in the centre of the black target area)  |
| präzis aber unrichtig | **Precise but untrue**This archer shoots precisely (always around the same point) but untrue (always to the lower right of centre)  |
| richtig aber unpräzis | **True but imprecise** This archer shoots true (the hits are located all around the black target area) but imprecisely (the hits are considerably scattered)  |
| unpraezis und unrichtig | **Untrue and imprecise** The archer shoots both untrue (the hits are next to and below the black target area) and imprecisely (the hits are considerably scattered)  |

taken from: <http://www.med4you.at/laborbefunde/allgemeines/lbef_qualitaet.htm#Pr> |
| **Trueness** | **- Comparison of the measurement values with the reference values** **- Determination and evaluation of a systematic error** Closeness of agreement between a large number of measurement results as well as their mean value and a true value (recognised reference value) (see Fig. 3). This is usually expressed numerically by the systematic error, which is inversely related to the trueness. The deviation of the mean value of these measurements from the true value is defined as the systematic error; if it is low, trueness is high. This reveals nothing about the degree of scattering of the individual values.  |
| **RUO** | “Research use only” - a reagent that is essentially to be used for research purposes and not for in vitro diagnostic purposes. However, RUO does not mean that quality is limited. RUO reagents do not bear a CE marking. The scope of validation often does not correspond to that of an IVD. In addition to RUO, other terms with similar but non-identical meanings are sometimes used (e.g., “investigational use only” (IUO) or “analyte specific reagent” (ASR)).  |
| **Semi-quantitative** | **-** The term “semi-quantitative” is used here in accordance with the RiLiBÄK - - i.e., when there is an order relation for values (ordinal scale): e.g., titre level, + to +++, indication of a value range. In contrast, nominal characteristics are qualitative characteristics when there is no order relation for their values (nominal scale): e.g., detectable, not detectable.However, values are usually quantitative characteristics if they can be assigned to a scale with defined intervals (metric or cardinal scale). In the case of Ct values (in the field of PCR testing), for example, this “quantitative characteristic” is assigned a semi-quantitative value. The deciding factor is how the result is indicated in the findings (measurement scale).  |
| **Sensitivity** | **- Rate of true positive results**Sensitivity is a measure of the number of true positives compared to the total number of positives. It is determined as follows: Sensitivity = a / a + c (see also Fig. 3). Frequently, the term sensitivity is expanded to include the determination of an analyte’s limit of detection (e.g., through endpoint titration). To determine this, a dilution series of the positive control or reference material is carried out in negative sample material (serum, plasma, bronchial lavage, etc.). If it is possible to quantify the value at which 95% of the samples used with this concentration of analyte are still positive, this is called the limit of detection. Known positive samples are tested in order to eliminate false-negative results.  |
| **Specificity**  | **- Elimination of false-positive results**Specificity is a measure of the number of true negatives compared to the total number of negatives. It is determined as follows: Specificity = d / d + b (see also Fig. 3).  Often potentially cross-reactive reaction partners (viruses of the same family, sera with rheumatoid factors and/or autoantibodies) and known negative samples are incorporated in the testing.**Fig. 3:** Sensitivity and specificity - the following cross table and formulas illustrate the  differences. *Sensitivity* of the testing method = a / a + c  *Specificity* of the testing method = d / d + b Reference method (= “true”)

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | pos. | neg. |
| Testing method | pos.  | a | b |
| neg.  | c | d |

 |

|  |  |
| --- | --- |
| **Validation /verification** | In routine diagnostic testing, test validation/verification is differentiated as follows (see Fig. 4). In the interest of completeness, this document covers LDT-IVDs as well as those that have been commercially produced and validated by the manufacturer (CE-marked tests). Before starting the validation procedure, a correspondingly detailed validation plan must be drawn up: **Fig. 4:** Classification of diagnostic testing methods in medical laboratories based on the requirements for method validation/verification. Method validation\*Justification LDT equivalence Individual validation plan (not a component of this SOP)\*\*VerificationjustificationExtensive med. justificationExtensive med. Validation can be shortened\*YESYESYESYESNONONONONOPublished?/ ext. validated protocolModification of a CE testRisk class DCE-IVD availableCE test introduction reasonable/possible?Process: verification and validationEntry \* See also Section 10 “Sample validation plan” and Section 5 “Procedure/approach” \*\* See also Section 1 “Aim and Purpose”  |
| **Scientific validity of an analyte**  | The association of an analyte with a clinical condition or a physiological state (Source: IVDR, Article 2, point 38) |

# 3. Responsibilities

|  |  |  |
| --- | --- | --- |
|  | **Responsible** | **Supporting** |
| Ensuring that only validated/verified methods are used to conduct the test | LM | QMO, DM  |
| Determining the type and scope of performance characteristics to be evaluated - creation of a validation plan  | LM | QMO, DM |
| Creation of a risk assessment | LM | QMO, DM |
| Decision on whether the results indicate that the reliability and performance of the method can be ensured in such a way that valid analytical results can be reproducibly achieved, thereby fulfilling the requirements of Annex I of the IVDR  | LM | QMO, DM |
| Establishment of acceptance criteria  | LM | QMO, DM |
| Review and assessment of all validation/verification results  | LM | QMO, DM |
| Approval of the method for routine diagnostic testing  | LM |  |
| Monitoring of the method | LM | QMO, DM |

# 4. Risks related to the procedure

A risk management system is to be established, implemented, documented and maintained. Risk management is to be understood as a continuous iterative process throughout the lifecycle of a device which requires regular systematic updating.

|  |  |
| --- | --- |
| **Risk** | **Measures for reducing risk**  |
| Generation of invalid results  | Verification/validationSupporting activities: Implementation concept, creation of SOPs, running IRCs, monitoring test equipment  |
| Non-comparability of results  | Verification/validation |
| Weaknesses caused by test/manufacturer not recognised  | Verification/validationSupporting activities:running IRCs, monitoring test equipment |
| Unfamiliarity with how to conduct the test  | Supporting activities: Implementation concept, creation of SOPs, running IRCs, monitoring test equipment |
| IVD risk class incorrectly assigned, potentially resulting in insufficient validation documentation.  | Review of the assignment of the IVD risk class according to Annex VIII of the IVDR, the existing CS where available, and MDCG 2020.16 using the four eyes principle, to be approved by the QM department and/or RA department, where available.  |

For this purpose:

a) a risk management plan is to be defined and documented for each device

b) the known and foreseeable hazards associated with each device are to be identified and analysed

c) the risks associated with, and occurring during, the intended use and during reasonably foreseeable misuse are to be estimated and assessed

d) an evaluation is to be conducted on the impact of the information gathered during the production phase and, in particular, from the post-market surveillance system on hazards and their frequency, on estimates of their associated risks, as well as on overall risk, the risk-benefit ratio and risk acceptability.

# 5. Procedure/approach

Before “**CE Tests”** are put into routine use, a **verification process** needs to be conducted by the user (see Fig. 4). This is to ensure that the method is mastered by the user.

The following performance characteristics are to be verified:

* Trueness and
* Precision (intra-assay and inter-assay)

If individual points deviate from the manufacturer’s instructions when implementing the method, these are to be recorded in the verification report, the effect of this deviation is to be documented, and any performance characteristics influenced by the change shall be re-determined and re-validated.

Note: In justifiable cases, examination procedures can be approved without undergoing full verification. These include, for example, exceptional epidemiological cases or special circumstances[[4]](#footnote-5) (e.g., simultaneous adjustment of more than 10 testing procedures - in the case of equipment change/failure or change in location/new premises). In these cases, verification is completed after approval has already been given. Significant deviations from the verification plan must be justified in writing. This includes a risk analysis as well as a technical explanation issued by the institute management.

A risk analysis/risk assessment is to be carried out before introducing any “**LDT**”, and, if necessary, appropriate preventive measures are to be taken. The LDT is to be continuously monitored by means of an established error and procedure management system. The experience gained for each LDT is to be assessed as part of a management review.

When validating an “LDT”, a validation plan must be drawn up that describes the procedure as well as the acceptance criteria (see Fig. 4).

The following aspects are to be examined:

* Precision (intra-assay and inter-assay)
* Trueness (bias)
* Accuracy (resulting from trueness and precision)
* Linearity (quantitative test systems) and/or the limit of detection (qualitative tests)
* Specificity (control of known relevant endogenous and exogenous interference and cross-reactions)
* Sensitivity (limits of detection and quantification)
* In addition - where applicable - clinical performance is to be determined, such as “diagnostic sensitivity, diagnostic specificity, positive predictive value, negative predictive value, likelihood ratio, expected values in normal and affected populations”.

**The following applies to both the verification of CE tests and the validation of LDTs:**

When determining the reproducibility of “negative” samples, a statistical evaluation of the “negative” absolute values (e.g., S/CO values) is - at least in many cases - not meaningful. Therefore, only the qualitative result and repeatability are to be assessed.

At the same time, quantitatively defined internal run controls (IRC) should be conducted (where possible and reasonable) with all routinely used tests in order to monitor the *robustness* of the procedure and the *reproducibility* of the measurement results. Such test kit-independent IRCs are particularly useful during batch changes. Reference material as well as other samples with defined target values or target value ranges can act as IRCs.

A summary of the respective requirements is provided as a guide in Tables 1 - 3.

If failings occur with regard to the validated/verified parameters, laboratory management must decide whether the test should be halted or restarted after methodological improvements are made, or whether it should be continued after (justifiably) extending the limits.

Justification must always be given in cases where a deviation from the specifications of this SOP during the validation/verification process occur. Laboratory management must then determine the procedure and justify this in writing based on the current literature or obvious/logical conclusions.
Reasons for such a deviation may include:

* Control samples are difficult to obtain (e.g., anti-HDV-IgM),
* Limited significance of the parameter (scope of the test therefore not economically justifiable),
* Change of method: If the parameter was previously determined with another method or test, priority should be given to direct comparison of the tests by parallel testing of patient samples. In this case, the scope of validation can be limited.

Furthermore, for serological and cell culture-based test systems, all (quantitative) results obtained during method validation/verification should have a CV ≤ 50% for inter-assay precision, and intra-assay precision should not exceed 15-30%.

In the case of semi-quantitative and qualitative “CE tests”, in which a result is determined within certain value classes (e.g., titre levels, negative/positive), the results should, as a rule, be congruent for purely qualitative tests, while deviations of ± one titre level is acceptable for semi-quantitative tests whose values are given in “titres”. Discrepancies are to be assessed by the laboratory management.

It should also be considered that significant, method-related differences in the results may arise during comparative determinations from inhomogeneous sample material (e.g., wound swabs, biopsies, BAL, biofilm on prosthetic material, catheters, etc.) and/or with pathogen groups that are usually inhomogeneously distributed in the native sample material (e.g., mycobacteria, parasites, etc.).

International standards and/or reference materials should, where possible, be used for quantitative IVDs.

For (semi)quantitative molecular test systems, inter-assay precision should not exceed ± 1 log10 (or 3.3 CT/CP/CQ) and the intra-assay precision should not exceed ± 0.8 log10 unless otherwise specified.

If, during the evaluation phase, the method being introduced can be assessed as part of an EQAS, this result should also be included in the validation report to establish trueness.

*Switching methods for semi-quantitative and qualitative tests*

If the parameter under investigation has already been determined using another method, a *test comparison* must be carried out - in addition to the requirements listed under Points 5.1 - 5.3. For this purpose, a statistically relevant number of patient samples (usually approx. 20; or usually 5 samples in the case of qualitative, closed, mechanised molecular genetic test systems [e.g., unit-use test cartridges] for detecting pathogen-specific nucleic acid) are measured in parallel using the old and new methods and the (semi-quantitative) results are compared. For tests with two or more levels of results, a 4-field test is useful for comparing the two methods.

If multiple levels of results are possible, e.g., in the case of titres, it makes sense to limit the results to the most important range. The horizontal row indicates the levels of results for the previous method, the vertical column those of the method to be tested. The result pairs are assigned to the corresponding sub-squares as a tally and counted. When selecting the patient samples, care should be taken that they indicate weakly positive, strongly positive, negative and borderline (preliminary) results.

*Switching methods for quantitative tests*

If the parameter under investigation has already been determined using another method, a *test comparison* must be carried out - *in addition to* the requirements listed under Points 5.1 - 5.3. For this purpose, a statistically relevant number of patient samples (usually approx. 20 samples [or usually 5 samples in the case of quantitative, closed, mechanised molecular genetic test systems (e.g., unit-use test cartridges) for detecting pathogen-specific nucleic acid,]) are measured in parallel using the old and new methods and the results are compared. It is helpful to use a regression analysis to compare the two methods.

When selecting patient samples, care should be taken that they cover the critical range but also the upper and lower measurement ranges. The correlation coefficient (depending on the value range), the standard deviation and, if necessary, the differences between the value pairs, their mean and their standard deviation are to be calculated. In addition, the regression lines are to be displayed in graph form.

## 5.1 Serology tests

### 5.1.1 “CE tests”: Semi-quantitative[[5]](#footnote-6) and qualitative serology tests

Semi-quantitative and qualitative “CE test” results fall within certain value classes. Here the method is verified as follows:

To verify reproducibility:

Intra-assay *precision*: At least one known positive, one known negative and one weakly positive/ borderline[[6]](#footnote-7) sample is analysed in triplicate on the first day. The matrix of these controls should correspond to the patient samples.

Inter-assay *precision*: The same samples are singly determined on two additional days.

Afterwards, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and - where possible - precision.

### 5.1.2 “CE tests”: Quantitative serology tests

Quantitative “CE test” methods are verified as follows:

To verify reproducibility:

Intra-assay *precision*: At least 10 different samples (reference material, patient samples or pool serum), i.e., 3 negative, 3 weakly positive, 3 more highly positive and 1 strongly positive sample(s) are analysed in triplicate on the first day. The matrix of these controls should correspond to the patient sample.

Inter-assay *precision*: One of the samples from each of the different measurement ranges tested on day one is singly determined on two additional days.

Afterwards, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision. Other performance characteristics determined by the manufacturer (e.g., linearity) should also be verified if significant for the analysis.

### 5.1.3 “LDT”: Qualitative serology tests

The method validation measures described below apply to laboratory-developed semi-quantitative and qualitative methods and tests. In this case, methods are verified as follows:

To verify reproducibility:

Intra-assay *precision*: At least one known positive, one known negative and one weakly positive/borderline sample is analysed in triplicate on the first day. The matrix of these controls should correspond to the patient samples.

Inter-assay *precision*: The same samples are singly determined on two additional days.

*Sensitivity*: - Testing of at least 10 known positive and at least 10 known weakly positive or borderline samples.

*Specificity*: - Testing of at least 20 known negative samples.

- Testing of potentially cross-reactive analytes (sera exhibiting antibodies against other viruses of the same family, rheumatoid factor (tissue autoantibodies) positive sera, sera with other autoantibodies - for antigen tests: samples with viruses of the same family): at least 3 samples per analyte if possible. Care should be taken to use samples that are strongly or highly positive for the potentially cross-reactive parameter in the test. Finally, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision. The results of the sensitivity and specificity tests are also evaluated.

### 5.1.4 “LDT”: Quantitative serology tests

Quantitative “LDT” test methods are verified as follows:

To verify reproducibility:

Intra-assay *precision*: At least 12 different samples (reference material, patient samples or pool serum), i.e., 3 negative, 3 weakly positive, 3 more highly positive and 3 strongly positive samples are analysed in triplicate on the first day. The matrix of these controls should correspond to the patient sample.

Inter-assay *precision*: One of the samples from each of the four different measurement ranges tested on day one is singly determined on two additional days.

*Sensitivity*: Testing of at least 10 known positive and at least 10 known weakly positive or borderline samples.

*Specificity*: - Testing of at least 20 known negative samples
- Testing of potentially cross-reactive analytes (sera exhibiting antibodies against other pathogens of the same family, rheumatoid factor (tissue autoantibodies) positive sera, sera with other autoantibodies. For antigen tests: samples with pathogens of the same family): at least 3 samples per analyte if possible. Care should be taken to use samples that are strongly or highly positive for the potentially cross-reactive parameter in the test.

*Linearity*: At least 2 samples (positive control samples) are tested in a (1:10 or 1:5) dilution series (with at least 4 dilution levels). The test is performed at minimum in duplicate.

Afterwards, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision. The results of the sensitivity, specificity and linearity tests are also evaluated.

## 5.2 Pathogen culture tests

### 5.2.1 “LDT”: Qualitative and semi-quantitative pathogen culture tests

When validating pathogen (virus, bacteria) isolation methods:

During cultivation it is important to test the culture media and cells to ensure they are suitable for the respective pathogens. New culture methods are not introduced into routine testing until the following parameters have been verified:

|  |  |
| --- | --- |
| *Susceptibility: (Susceptibility for pathogen)* | The cultivation method to be validated is infected on 3 days in triplicate with the respective reference strain and additionally - where available - two patient isolates. If possible, the pathogen should be titrated and a “multiplicity of infection” (MOI) of 0.01 - 0.1 should be used. Where applicable and reasonable, a *method comparison* should also be carried out as part of susceptibility testing. In this case, the method to be validated is inoculated in parallel with the patient samples as part of the ongoing routine in addition to the “routine method” and assessed daily.  |
| *Cytotoxic effects of substances or sample material on the culture (matrix effects):* | The evaluation is usually carried out as part of the method comparison (see above) and the logging of the cell viability. The scope of the test must include at least 20 (different) (patient) samples. |

Afterwards, any deviations are assessed and the results are compared with regard to *trueness*. If differences arise, laboratory management must decide whether:

- there has been an improvement in the tested reference strains and/or patient isolates

- to halt the test

- to restart the testing/validation after making methodological improvements or to continue it after (justifiably) extending the limits.

## 5.3 Molecular tests for pathogen detection (NAT)

### 5.3.1 “CE tests”

#### 5.3.1.1 “CE tests” - Qualitative molecular methods

In qualitative “CE tests”, a sample is verified for the presence of pathogen-specific nucleic acid (negative/positive). Method verification is performed to verify reproducibility:

Intra-assay *precision*: At least one known positive, one known negative and one weakly positive (possibly borderline) sample is analysed in triplicate on the first day. The matrix of these controls should correspond to the patient sample.

Inter-assay *precision*: The same samples are singly determined on two additional days.

Finally, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision.

#### 5.3.1.2 “CE tests”: Quantitative molecular methods

Quantitative “CE test” methods are verified as follows:

To verify reproducibility:

Intra-assay *precision*: At least 9 different samples (reference material, patient samples or pool serum), i.e., 3 negative, 3 weak and 3 more highly positive samples are analysed in triplicate on the first day. The matrix of these controls should correspond to the patient sample.

Inter-assay *precision*: One of the samples from each of the different measurement ranges tested on day one is singly determined on two additional days.

*Linearity*: One sample (positive control sample) is tested in a (1:10) dilution series (with at least 3 dilution levels). The test is performed at minimum in duplicate.

Afterwards, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision. The results of the linearity tests are also assessed.

### 5.3.2 “CE tests”: Unit-use test cartridges for the qualitative/quantitative detection of pathogen-specific nucleic acid

The method verification measures described below apply to closed, mechanised molecular genetic test systems (e.g., unit-use test cartridges - Cepheid Xpert and similar systems are provided as examples of test/method types) for the qualitative/quantitative detection of pathogen-specific nucleic acid. It must be generally ensured that sufficient process controls (e.g., extraction/purification and inhibition controls) are integrated into these systems. If, for example, not only one but two or three analytes are detected simultaneously in a unit-use test cartridge system (e.g., influenza A, influenza B and RSV), intra-assay verification can be omitted due to the “closed” nature of the test system (and to the lower possibility that the test result will be externally influenced as well as to the fact that the use of each test cartridge represents a separate test). The verification can be carried out on the basis of only one of the analytes. The functionality of the other detectable analytes is checked and documented as part of routine internal and external quality controls. If the device contains several testing modules (multi-module device), the different test modules must be used during the testing unless specified by the device. Method verification is carried out to assess reproducibility.

In principle, specific preanalytical requirements must also be fulfilled. A differentiation must be made between the use of pre-filled and pre-supplied buffers and “disposable pipettes” (to reduce the risk of contamination, e.g., through own pipettes or buffers) and the use of the laboratory’s own reagents (e.g., own buffers) with the sample material and reaction system, and/or the use of systems in which individual components (e.g., extraction buffer, lysis/protease reagent, master mix, primers, probes) are mixed/pipetted separately. If preanalytical changes are made (e.g., other materials, own buffers), laboratory management is to determine the scope of the verification.

In the case of qualitative “CE cartridge tests” (for detecting ≤ 5 different analytes), samples are analysed for the presence of viral nucleic acid (negative/positive):

Inter-assay *precision*: At least one known positive, one known negative and one weakly positive material are singly determined on three different days. The matrix of these controls should correspond to the patient sample.

Afterwards, any deviations from one day to the next are assessed and the results are compared with regard to *trueness* and precision.

In quantitative “CE cartridge tests” (for detecting ≤ 5 different analytes) the method is verified as follows:

Inter-assay *precision*: At least 5 samples (of which at least three should be from different patients or different origins [reference material, patient samples or pool serum]), i.e., 1 negative, 2 weak and 2 more highly positive samples are singly determined on three different days. The matrix of these controls should correspond to the patient sample.

*Linearity*: One sample (positive control sample) is tested in a (1:10) dilution series (with at least 3 dilution levels). A single determination must be conducted at minimum. If necessary, the test can be “combined” with the inter-assay test and the samples used for that.

Afterwards, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision. The results of the linearity tests are also assessed.

#### 5.3.2.1 “CE tests”: closed, mechanised multiplex molecular genetic test systems (e.g., unit-use test cartridges)

The method verification measures described below apply to closed, mechanised multiplex molecular genetic test systems (e.g., unit-use test cartridges for the qualitative detection of pathogen-specific nucleic acid, whereby often considerably more than 10 different pathogens (e.g., viruses, bacteria) or (sub)types of pathogens can be detected with one test cartridge - the bioMerieux Film Array and similar systems are provided as examples of test/method types).

It must be generally ensured that sufficient process controls (e.g., extraction/purification and inhibition controls) are integrated into these systems. Due to the “closed” nature of the test system (and to the lower possibility that the test result will be externally influenced as well as to the fact that the use of each test cartridge represents a separate test), verification can be carried out on the basis of only one of the analytes. The functionality of the other detectable analytes is checked and documented as part of routine internal and external quality controls. If the testing device has several test modules (multi-module device), different test modules must be used during the testing unless specified by the device.

Method verification is carried out to check reproducibility. Due to the fact that each test set can cost a considerable amount (ranging between 80 - 180 € at the time the document was written), extended verification is often not economically feasible. Nevertheless, these systems are able to provide quick and valuable diagnostic results and should therefore not be excluded from the range of possible test systems due to the reduced scope of verification.

In principle, specific preanalytical requirements must also be fulfilled. A differentiation must be made between the use of pre-filled and pre-supplied buffers and “disposable pipettes” (to reduce the risk of contamination, e.g., through own pipettes or buffers) and the use of the laboratory’s own reagents (e.g., own buffers) with the sample material and reaction system, and/or the use of systems in which individual components (e.g., extraction buffer, lysis/protease reagent, master mix, primers, probes) are mixed/pipetted separately. If preanalytical changes are made (e.g., other materials, own buffers), laboratory management is to determine the scope of the verification.

In the case of qualitative “CE multiplex cartridge tests”, a sample is analysed for the presence of different viral and/or bacterial nucleic acids (negative/positive):

Inter-assay *precision*: At least one known positive and one known negative sample are singly determined on two different days. The matrix of these controls should correspond to the patient sample.

Afterwards, any deviations from one day to the next are assessed and the results are compared with regard to *trueness* and precision.

#### 5.3.2.2 “CE tests”: not fully closed/“open” multiplex molecular genetic test systems

The method verification measures described below apply to not fully closed/“open” molecular genetic multiplex test systems for the qualitative/(semi-)quantitative detection of pathogen-specific nucleic acid, whereby often considerably more than 10 different pathogens (e.g., viruses, bacteria) or (sub-)types of pathogens can be detected with one test (the Luminex Multiplex Assay and similar systems are provided as examples of test/method types).

It must be generally ensured that sufficient process controls (e.g., extraction/purification and inhibition controls) are integrated into these systems. Due to the fact that each test set can cost a considerable amount, extended verification is often not economically feasible. Nevertheless, these systems are able to provide quick and valuable diagnostic results and should therefore not be excluded from the range of possible test systems due to the reduced scope of verification.

Due to the “non-closed” nature of the test system, more extensive testing is required than for “closed, mechanised multiplex systems”. Nevertheless, intra-assay verification can be omitted, and verification can be carried out on the basis of only three of the analytes. The functionality of the remaining detectable analytes is checked and documented as part of routine internal and external quality controls. Method verification is carried out to verify reproducibility.

In principle, specific preanalytical requirements must also be fulfilled. A differentiation must be made between the use of pre-filled and pre-supplied buffers and “disposable pipettes” (to reduce the risk of contamination, e.g., through own pipettes or buffers) and the use of the laboratory’s own reagents (e.g., own buffers) with the sample material and reaction system, and/or the use of systems in which individual components (e.g., extraction buffer, lysis/protease reagent, master mix, primers, probes) are mixed/pipetted separately. If preanalytical changes are made (e.g., other materials, own buffers), laboratory management is to determine the scope of the verification.

In the case of qualitative (or semi-quantitative) not fully closed/“open” multiplex molecular genetic test systems, a sample is tested for the presence of viral nucleic acid (negative/positive):

Inter-assay *precision*: Of at least three different analytes, at least one known positive, one known negative and one weakly positive sample each are to be singly determined on three different days. The matrix of these controls should correspond to the patient sample.

Afterwards, any deviations from one day to the next are assessed and the results are compared with regard to *trueness* and precision.

### 5.3.3 “LDT”: Semi-quantitative[[7]](#footnote-8) and qualitative molecular methods

The method validation measures described below apply to laboratory-developed, semi-quantitative and qualitative methods and tests. In terms of the qualitative performance of a PCR, approx. 10 genome copies per reaction mixture (approx. 1,000 copies/ml [e.g., plasma]) of a pathogen-specific nucleic acid should be detected in the control sample.

The practical implementation of a new “in-house” NAT method requires a comparison of the sequences used in Virus BLAST and/or the use of a publication with viral nucleic acid sequences to verify the amplification product by real-time PCR, sequencing, or other methods. Furthermore, an “internal control” should be used as an extraction control and PCR control.

A (quantified) inhibition control is usually used to eliminate the likelihood that different sample materials (e.g., urine, sputum) influence the sensitivity and specificity of the test. The Ct values should not vary by more than ± 3 Ct between the individual sample materials. In the absence of an inhibition control, the respective sample material must be spiked, e.g., with a reference material, so that the final dilution in the spiked material is approx. 1 log level above the limit of detection. “Spiked” and “unspiked” sample materials are simultaneously inserted into the testing device. The test mixture containing no pathogen must have a negative detection result, the “spiked” sample should be positive. The detection must be verified in two independent runs. If the “spiked” sample does not produce a positive result, detection in this sample material is not possible within the limit of detection.

#### 5.3.3.1 “LDT”: Qualitative molecular methods

The method validation measures described below apply to laboratory-developed, semi-quantitative and qualitative methods and tests. Here the method is verified as follows:

To verify reproducibility:

Intra-assay *precision*: At least one known positive, one known negative and one weakly positive/borderline sample is analysed in triplicate on the first day. The matrix of these controls should correspond to the patient sample.

Inter-assay *precision*: The same samples are singly determined on two additional days.

*Sensitivity*: Testing of at least 10 known positive and at least 10 known weakly positive or borderline samples, where available.

In addition, where applicable and useful, differences in the test’s sensitivity to closely related pathogens should be considered and assessed, e.g., in the case of a type-independent detection of HSV or influenza (same sensitivity to: HSV-1 and HSV-2 or influenza A H1 and H3).

*Specificity*: Where possible and available:

* Testing of at least 20 known negative samples
* Testing of potentially cross-reactive pathogens or analytes (e.g., samples that have tested positive for viruses of the same family or have been “spiked” with a potentially cross-reactive reference material). Where possible and/or available, one potentially cross-reactive analyte each should be tested. Care should be taken to use samples that are strongly or highly positive for the potentially cross-reactive parameter (usually at least 105 TCID50/ml or 105 copies/ml).

|  |  |
| --- | --- |
| *Linearity*:*(where clinically useful)* | At least 2 samples (a positive or positive “spiked” control sample) are tested in a (1:10) dilution series (with at least 4 dilution levels). The test should be performed on two different days and at least in duplicate.  |
| *Matrix effect*: | Testing of various sample materials containing the virus that make good sample materials for implementing the parameter by “spiking” them with a positive control sample or by comparing the ct values.  |

Afterwards, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision. The results of the tests for sensitivity, specificity and linearity are also evaluated.

In addition to the above-mentioned assessments, the *robustness* of the procedure and the *reproducibility* of the measurement results are usually monitored (quantitatively defined) on a continuous basis by internal run controls (IRC), which are produced from a reference material, where available. The IRC data can be included in the validation report to assess trueness. Control panels should also be tested where available.

#### 5.3.3.2 “LDT”: Quantitative molecular methods

Quantitative “in-house” methods are verified as follows:

To verify reproducibility:

Intra-assay *precision*: If available: at least 12 different samples (reference material, patient samples or pool serum), i.e., 3 negative, 3 weakly positive, 3 more highly positive and 3 strongly positive samples are analysed in triplicate on the first day. The matrix of these controls should correspond to the patient sample.

Inter-assay *precision*: One of the samples from each of the four measurement ranges tested on day one is singly determined on two additional days.

*Sensitivity*: Testing of at least 10 known positive and at least 10 known weakly positive or borderline samples, where available.

*Specificity*: Where possible and available:

* Testing of at least 20 known negative samples
* Testing of potentially cross-reactive analytes (samples that have tested positive for viruses of the same family or have been “spiked” with a potentially cross-reactive reference material). Where possible or available, one potentially cross-reactive analyte each. Care should be taken to use samples that are strongly or highly positive for the potentially cross-reactive parameter (usually at least 105 TCID50/ml or 105 copies/ml).

*Linearity*: At least 2 samples (positive or positively spiked control sample) are tested in a (1:10) dilution series (with at least 4 dilution levels). The test is performed on two different days and at least in duplicate.

*Matrix effect*: Testing of various sample materials containing the virus that make good sample materials for implementing the parameter by “spiking” with a positive control sample or by comparing the ct values.

Afterwards, any deviations within the series and from one day to the next are assessed and the results are compared with regard to *trueness* and precision. The results of the tests for sensitivity, specificity and linearity are also evaluated.

In addition to the above-mentioned assessments, the *robustness* of the procedure and the *reproducibility* of the measurement results are usually monitored (quantitatively defined) on a continuous basis by internal run controls (IRC), which are produced from a reference material, when available. The IRC data can be included in the validation report to assess trueness. Control panels should also be tested where available.

## 5.4 Method verification in the case of last-minute changes to a test

In cases where last-minute changes to a test are necessary for analytical or organisational reasons (e.g., due to batch stoppage or supply difficulties from the manufacturer), the following performance characteristics shall be assessed as part of the method verification:

*Changing methods:*

Where feasible, at least 10 patient samples that have already been analysed using the old method should be tested using the new method and should produce the same/comparable results. If comparable results are not produced in every case, laboratory management must decide whether the method can be approved. This must be briefly justified in the documentation. However, if no comparable results are produced in more than three patient samples, the test cannot be approved (unless the deviations are clearly due to a more sensitive/specific analysis). An exception can be made in justified cases.

Patient results can only be released if the quality control results are within the acceptable range. If the new method is retained, an extended validation/verification process must be carried out.

After verifying the method, participation in an EQA scheme or interlaboratory comparison is necessary.

## 5.5 Documentation

All analytical results obtained during method validation/verification are to be documented and evaluated. The corresponding raw data shall be attached to form 11.1. General comments or remarks, which might be helpful for performing the analysis, are also noted on this form. In addition, images, for example, of the agarose gels are attached to the protocol pages.

In cases where lengthy comments are needed, these shall be attached to the form.

All method validation/verification documents are retained by laboratory management for as long as the method is used (and five years beyond this). After this period, laboratory management can decide whether to continue archiving them.

# 6. Continuous monitoring of the LDT (after initial use)

All LDT procedures are validated before being commissioned and verified in the daily work routine by carrying out regular controls. These controls are also validated. Special attention is paid to newly introduced methods during the internal auditing process. Laboratories are to participate in external quality comparisons (where available) for the methods used. These include:

* EQA schemes
* Interlaboratory comparisons (when no EQA scheme is offered)

The internal and external data on quality control are monitored on a continuous basis. If, for example, an EQA scheme is not passed, a systematic root cause analysis is carried out and any corrective measures are initiated. The results from the monitoring of the device are evaluated by management as part of the annual management review. Any necessary measures are determined.

# 7. Documents/applicable documentation

|  |
| --- |
| **Document** |
|  |
|  |

# 8. Annexes

The requirements for the samples, which are needed to meet the respective performance characteristics, are presented in the tables below. These must also be considered in a corresponding validation plan.

The terms used in Tables 1 - 3 for the specifications of the sample materials to be analysed are defined as follows in this SOP: “borderline” is considered to be 2 to 5 times the lower limit of detection for the respective test system (e.g., a PCR), a sample is considered to be “weakly” positive if the amount of analyte (e.g., nucleic acid) is 20 to 50 times the lower limit of detection for the respective test system (e.g., a PCR). This definition can be modified by laboratory management where justified.

## 8.1 Outline of the scope of validation/verification (see Tab. 1- 3)

Tab. 1: Summary of the scope of testing as part of the validation/verification of *serological tests and assays for detecting viral antigens*. Indicated is the minimum number of samples to be tested and, where applicable, the number of replicates (marked with 0x).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Performance character-istics** | **Sample requirements** | **Qualitative CE** | **Quantitative CE**  | **Qualitative LDT** | **Quantitative LDT**  |
| **Sensitivity** | **positive** | nr | nr | 10 | 10 |
| **borderline/weakly positive** | nr | nr | 10 | 10 |
| **Specificity** | **negative** | nr | nr | 20 | 20 |
| **potentially cross-reactive** | nr | nr | 3 per potent. cross-reactive parameter  | 3 per potent. cross-reactive parameter |
| **Precision (intra-assay)** | **positive** | 1 (3x each) | 3 (3x each) | 1 (3x each) | 6\* (3x each) |
| **negative** | 1 (3x each) | 3 (3x each) | 1 (3x each) | 3 (3x each) |
| **borderline/weakly positive** | 1 (3x each) | 3 (3x each) | 1 (3x each) | 3 (3x each) |
| **Precision (inter-assay)** | **positive** | 1 (1x on 2 d\*\*) | 1\* (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 2\* (1x each on 2 d\*\*) |
| **negative** | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) |
| **borderline/weakly positive** | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) |
| **Linearity** | **positive** | nr | nr | nr | 2 (2x each) (1:10 or 1:5 dilution series; min. 4 dilution levels) |
| **Matrix effects** | **positive** (n = 3) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **negative** (n = 3) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **borderline/weakly positive** (n = 3) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **Method comparison** | **positive** (n = 7) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **negative** (n = 7) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **borderline/weakly positive** (n = 6) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **Total number of analyses to be conducted**  |  | n = ≥15 | n = ≥38 | n = ≥55 | n = ≥100 |

nr = does not need to be conducted/not required, d = days, \* from two different concentration ranges, \*\* i.e., the same sample is to be tested on two additional days in addition to intra-assay testing, \*\*\* additional tests are to be conducted depending on requirements and testing conditions (e.g., testing of different sample materials) – the requirements are defined by laboratory management, \*\*\*\* testing of the other parameters can be omitted or reduced through method comparison – this is decided by laboratory management and must be justified in writing.

Tab. 2: Summary of the scope of testing as part of the validation/verification process in the area of *virus isolation*. Indicated is the minimum number of samples to be tested and, if applicable, the minimum number of replicates (marked with 0x).

|  |  |  |
| --- | --- | --- |
| **Performance characteristics** | **Sample requirements** | **Qualitative and/or semi-quantitative LDT** |
| **Susceptibility and precision** (and testing for sensitivity to cytotoxic substances)  | **positive / weakly positive** (MOI 0.01 – 0.1) | 3 (3x each on 3 d) |
| **Samples from routine testing** | ≥ 20 |
| **Matrix effects** | **positive** (n = 3) | conduct if necessary \* |
| **negative** (n = 3) | conduct if necessary \* |
| **Total number of analyses to be conducted**  |  | n = ≥47 |

d = days, \* additional tests are to be conducted depending on requirements and testing conditions (e.g., testing of different sample materials) – the requirements are defined by laboratory management

Tab. 3: Summary of the scope of testing as part of the validation/verification of *molecular-virological tests*. Indicated is the minimum number of samples to be tested and, where applicable, the minimum number of replicates (marked with 0x).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Performance character-istics**  | **Sample requirements** | **Qualitative CE** | **Quantitative CE** | **Qualitative LDT** | **Quantitative LDT** | **Qualitative cartridges1** | **Quantitative cartridges1** |
| **Sensitivity** | **positive** | nr | nr | 10 | 10 | nr | nr |
| **borderline /****weakly positive** | nr | nr | 10 | 10 | nr | nr |
| **Specificity** | **negative** | nr | nr | 20 | 20 | nr | nr |
| **potentially cross-reactive**  | nr | nr | If possible or available, 3 per pot. cross-reactive parameter | If possible or available, 3 per pot. cross-reactive parameter | nr | nr |
| **Precision (intra-assay)** | **positive** | 1 (3x each) | 3 (3x each) | 1 (3x each) | 6\* (3x each) | nr | nr |
| **negative** | 1 (3x each) | 3 (3x each) | 1 (3x each) | 3 (3x each) | nr | nr |
| **borderline /****weakly positive** | 1 (3x each) | 3 (3x each) | 1 (3x each) | 3 (3x each) | nr | nr |
| **Precision (inter-assay)** | **positive** | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 2\* (1x each on 2 d\*\*) | 1 (1x each on 3 d) | 2 (1x each on 3 d) |
| **negative** | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x each on 3 d) | 1 (1x each on 3 d) |
| **borderline /****weakly positive** | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x on 2 d\*\*) | 1 (1x each on 3 d) | 2 (1x each on 3 d) |
| **Linearity** | **positive** | nr | 1 (2x) (1:10 dilution series – at least 3 levels) | 2 (2x each on 2 d) (1:10 dilution series – at least 4 levels) | 2 (2x each on 2 d) (1:10 dilution series – at least 4 levels) | nr | 1 (1x) (1:10 dilution series\*\*\*\*\* - at least 3 levels) |
| **Matrix effects** | **positive** (n = 3) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **negative** (n = 3) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **borderline /****weakly positive** (n = 3) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **Method comparison** | **positive** (n = 7) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **negative** (n = 7) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **borderline /****weakly positive**(n = 6) | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* | conduct if necessary \*\*\* |
| **Total number of analyses to be conducted**  |  | n = ≥15 | n = ≥39 | n = ≥87 | n = ≥116 | n = ≥9 | n = ≥15 |

nr = does not need to be conducted/not required, d = days, \* from two different concentration ranges, \*\* i.e., the same sample is to be tested on two additional days in addition to intra-assay testing, \*\*\* additional tests are to be conducted depending on requirements and testing conditions (e.g., testing of different sample materials) – the requirements are defined by laboratory management, \*\*\*\* testing of the other parameters can be omitted or reduced through method comparison – this is decided by laboratory management and must be justified in writing.\*\*\*\*\* the testing can be “combined” with the inter-assay test and the samples used with it. 1 applies to unit-use test cartridges - see 5.3.2

For qualitative “CE multiplex cartridge tests” (see 5.3.2.1), a sample is analysed for the presence of different viral and/or bacterial nucleic acids (negative/positive):

Inter-assay precision: at least one known positive and one known negative sample are singly determined on two different days. The functionality of the remaining detectable analytes is evaluated and documented as part of routine internal and external quality controls.

# 9. Literature

- ISO/TS 17822-1:2014-12-31: In vitro diagnostic test systems. Qualitative nucleic acid-based in vitro examination procedures for detection and identification of microbial pathogens. General requirements, terms and definitions

- BS ISO 17822-2. In vitro diagnostic test systems. Nucleic acid amplification-based examination procedures for detection and identification of microbial pathogens. Part 2. Laboratory quality practice guide

- Hoffmüller P, Brüggemann M, Eggermann T, Ghoreschi K, Haase D, Hofmann J, Hunfeld KP, Koch K, Meisel C, Michl P, Müller J, Müller C, Rabenau HF, Reinhardt D, Riemenschneider MJ, Sachs UJ, Sack U, Stenzinger A, Streichert T, von Neuhoff N, Weichert W, Weinstock C, Zimmermann S, Spitzenberger F, Ad-hoc-Kommission In-vitro-Diagnostika der AWMF (2021) Stellungnahme der Ad-hoc-Kommission In-vitro-Diagnostika der AWMF zur Umsetzung der Verordnung (EU) 2017/746 (IVDR) im Hinblick auf In-vitro-Diagnostika aus Eigenherstellung. GMS Zeitschrift zur Förderung der Qualitätssicherung in medizinischen Laboratorien 2021, Vol. 12, 1-10. ISSN 1869-4241

- Medical Device Coordination Group. MDCG 2020–2016. Guidance on classification rules for in vitro diagnostic medical devices under regulation (EU) 2017/746. November 2020.

- Rabenau HF, Kessler HH (2009) (Abstract) Test validation and accreditation in the clinical virology laboratory. J. Clin. Virol (46 Suppl. 1) S3

- Rabenau HF, Kessler HH, Kortenbusch M, Steinhorst A, Raggam RB, Berger A (2007) Verification and validation of diagnostic laboratory tests in clinical virology. J Clin Virol.;40(2):93-8

- Rabenau HF, Kessler HH, Raggam RB, Berger A (2010) Verification and Validation of Virological Laboratory Tests in the Routine Diagnostic Laboratory. In: Jerome KR (ed.): Laboratory Diagnosis of Viral Infections (4th edition) S 1-8. Informa healthcare, New York, London

- Rabenau HF, Kortenbusch M, Berger A, Steinhorst A (2007) Validierung von Untersuchungsverfahren im Bereich der Virusdiagnostik. Lab. Med. 31(2) 41-47

- Reischl U, Rabenau HF (2012) Die MIQ-1 "Nukleinsäure-Amplifikationstechniken (PCR/NAT)" in der dritten Auflage - was ist neu ?" - Beitrag der Qualitätssicherungskommission und der Ständigen Arbeitsgemeinschaft (StAG) "Diagnostische Verfahren" der DGHM. Mikrobiologe 22: 48-52

- Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen (Gemäß des Beschlusses des Vorstands der Bundesärztekammer in seiner Sitzung am 18.10.2019). Deutsches Ärzteblatt DOI: 10.3238/arztebl.2019.rili\_baek\_QS\_Labor20192312

- Schoerner C, Abele-Horn M, Albert F, Haase G, Leitritz L, Rabenau HF (2009) Qualitätsmanagement im Medizinisch-mikrobiologischen Laboratorium (pp 1-86). In: Podbielski A, Herrmann M, Kniehl E, Mauch H, Rüssmann H (Hrsg.), MiQ 30 Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik URBAN & FISCHER München. ISBN-13: 978-3-437-22648-9

- Spitzenberger F,·Patel J, Gebuhr I, Kruttwig K, Safi A, Meisel C (2021) Laboratory Developed Tests: Design of a Regulatory Strategy in Compliance with the International State of the Art and the Regulation (EU) 2017/746 (EU IVDR [In Vitro Diagnostic Medical Device Regulation]). Therapeutic Innovation & Regulatory Science https://doi.org/10.1007/s43441-021-00323-7

- Verordnung (EU) 2017/746 des europäischen Parlaments und des Rates vom 5. April 2017 über In-vitro-Diagnostika und zur Aufhebung der Richtlinie 98/79/EG und des Beschlusses 2010/227/EU der Kommission

- Wellinghausen N, Abele-Horn M, Donoso Mantke O, Enders M, Gärtner B, Rabenau HF, Reiter-Owona I, Tintelnot K, Weig M, Zeichardt H, Hunfeld KP (2016) MiQ - Infektionsserologische Methoden. Elsevier Verlag

# 10. Sample validation plan, e.g., when developing an LDT

A sample validation plan and corresponding form (see Point 11.1) can be found below. All of the essential aspects of the validation process are to be performed before commencement of the validation. The validation plan must be signed off by the laboratory manager and the technical staff members who generate the raw data.

The institute management is responsible for introducing methods and, if necessary, for choosing to establish LDT processes.

Before starting the validation process, a search is conducted on the common European market for a commercial CE-marked product that is suitable for the intended purpose and the matrix used.

All of the procedures carried out in the laboratory can be found in a table in which the procedures are categorised:

* **Category I**: IVD (commercially available), used according to the manufacturer’s specifications
* **Category II**: IVD (commercially available), modified[[8]](#footnote-9) (thus “LDT light”)
* **Category III:** A commercially available IVD is available but is not used due to a lack of LDT equivalence (e.g., performance characteristics of the test, turn-around time, type/amount of sample material/matrix)
* **Category IV:** No commercial (alternative) IVD is available

An LDT device may be developed if no “comparable” commercial CE-marked product is available on the market for the intended purpose. The device is classified in accordance with the regulations outlined in Chapter V Section 1 (Art. 47) of the IVDR.

The sample forms presented under Points 11.2.1 – 11.3.2 each have the following basic structure:

* 1. General
	2. Planning (with various “sub-aspects”)
	3. Measurement values
	4. Summary of the results
	5. Assessment
	6. Appendix(es)

# 11. Sample forms

## 11.1 Introduction of a new testing procedure

|  |
| --- |
| **Introduction of a new testing procedure**O **LDT test** O **CE test**  |
| Name of the test:(According to manufacturer/declaration of conformity) |  |
| Testing procedure/method: |  |
| Risk class (IVDR): |  |
| Name of device: |  |
| RiliBÄK & ISO standard & internal specifications |
| Result in the findings: | O quantitative O qualitative O \_\_\_\_\_\_\_\_\_\_ |
| Classification of the measurement procedure: | O quantitative O qualitative O \_\_\_\_\_\_\_\_\_\_  |
| Validation: | O quantitative O qualitative O \_\_\_\_\_\_\_\_\_\_  |
| Method comparison: | O yes O no  |
| Sample material:(According to manufacturer/declaration of conformity) |  |
| Target validation/verification criteria achieved? |
| Categorisation | O I O II O III O IV |
| Sensitivity & specificity: | O yes O no O n/a  |
| Intra- and inter-assay precision: | O yes O no O n/a  |
| Linearity: | O yes O no O n/a  |
| Method comparison: | O yes O no O n/a  |
| Approved for routine: |  |
| Quality requirements: | O met O not met |
| Remarks/limitations |  |
| Date & signature: |  |
| QMA |
| Internal designation: |  |
| Analyte number (EDV): |  |
| Name of SOP: |  |
| Quality assessment programme:(Planned EQAS/lab comparison etc.) |  |
| Parameters required by RiliBÄK: | O yes (O B1 O B2 O B3) O no  |
| Inclusion in list of services: | O yes O no  |

## 11.2.1 LDT test (Category II, III, IV): Method validation - qualitative

## Method validation – qualitative – LDT test

# 1 General:

Name of test:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(Designation in declaration of conformity)

Internal designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Analyte number (EDV):\_\_\_\_\_\_\_\_\_\_

Device name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ or: O manual

## Sample material (matrix):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# 2 Planning:

Planned by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ on:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

To be implemented by (date):\_\_\_\_\_\_\_\_\_\_ by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **2.1 Remarks on planning (question/objective) and information on publications, reference laboratories, preliminary results, special features, references as well as justification if protocol is abridged** (e.g.: CE method with matrix expansion, use of RUO tests)

Details on the measurement procedure’s measurement scale

 Quantitative (determination of intra-/inter-assay precision):

 O Cardinal scale (OD values, (rt-)qPCR, IU values etc.)

Qualitative (robustness, trueness):
 O Ordinal scale (titre, +/++, etc.)

 O Nominal scale (Immunoblot, positive/negative, detectable, etc.)

**2.2 Definition of value ranges (information/categorisation in the findings, if applicable):**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Information in the findings |  |  |  |  |  |

Highly positive :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Positive :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Weakly positive :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Borderline :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Negative :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

**Further information on value range, if applicable:**

(Value ranges can include: titre, measurement ranges (e.g., 500 – 1000 IU), band intensity, number of bands (WB), etc.)

**2.3. Reference values/decisive clinical values:**

**2.4 Potentially cross-reactive parameters/samples**

List of potentially, cross-reactive parameters (at least 3) and the value ranges to be analysed / considered. A list is only required when the respective samples are also (externally/internally) available.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Name | Value range | Name | Value range | Name | Value range |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**2.5 Sensitivity/specificity using:**

O external samples\*
\*EQAS samples, quality controls (of other manufacturers), reference institute etc., external lab

Comparison measurement with the expected values:

#\_\_\_\_\_\_highly positive / #\_\_\_\_\_\_ positive (usually at least 10)

 #\_\_\_\_\_\_weakly positive/borderline (usually at least 10)

 #\_\_\_\_\_\_negative (usually at least 20)

#\_\_\_\_\_\_negative (cross-reaction) (usually at least 3)

O internal samples\* (supplemental or alternative)
\*earlier samples, quality controls

Comparison measurement with the expected values:

#\_\_\_\_\_\_highly positive / #\_\_\_\_\_\_ positive (usually at least 10)

 #\_\_\_\_\_\_weakly positive/borderline (usually at least 10)

 #\_\_\_\_\_\_negative (usually at least 20)

#\_\_\_\_\_\_negative (cross-reaction) (usually at least 3)

 **Remarks and/or requirements for selecting samples:**

**2.6** **Intra-/inter-assay precision and/or robustness/trueness (repeatability):**

Usually a sample set with 3 samples in different (clinically relevant) value ranges. First, measured in triplicate (measurement series 1) and then singly determined in 2 independent measurement series.
O Sample 1: (highly) positive Value range, if applicable\*:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 2: weakly positive Value range, if applicable \*:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 3: negative Value range, if applicable \*:\_\_\_\_\_\_\_\_\_\_\_\_\_

\* Usually only indicated for quantitative values

**2.7 Linearity\***(\*usually for molecular tests (NAT) with qualitative information in the findings) **O n/a**

If required:

**Sample designation 1:**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Order number, if applicable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Concentration of initial sample: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Unit:\_\_\_\_\_\_\_\_\_\_

Dilution level: #\_\_\_\_\_\_\_\_\_\_(minimum 4) Dilution factor:\_\_\_\_\_\_\_\_ (usually: 1:10 or 1:5)
**Sample designation 2:**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Order number, if applicable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Concentration of initial sample: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Unit:\_\_\_\_\_\_\_\_\_\_

Dilution level: #\_\_\_\_\_\_\_\_\_\_(minimum 4) Dilution factor:\_\_\_\_\_\_\_\_ (usually 1:10 or 1:5)

# 3 Measurement values:

1. Sensitivity and specificity O see 6.1 (sheet 1 to \_\_\_\_) O see appendix
2. Intra- and inter-assay precision O see 4.2
3. Linearity O see 4.3

**3.1 Remarks on measurement** (changes, issues, software, etc.)**:**

# 4. Summary of the results:

**4.1** **Sensitivity and specificity**

O see Table 1 O see appendix(es)

Table 1: Summary of the sensitivity and specificity measurements

|  |  |
| --- | --- |
|  | TARGET |
|  ACTUAL |  | (Strongly) positive#\_\_\_\_\_\_\_ | Weakly positive #\_\_\_\_\_\_\_ |  Negative #\_\_\_\_\_\_\_ |
| (Highly) positive |  |  |  |
| Weakly positive  |  |  |  |
| Negative |  |  |  |

# = number of tested samples

**4.2 Intra- and inter-assay precision**

O see Table 2 O see appendix

Table 2: Summary of the results of intra- and inter-assay precision

|  |  |  |  |
| --- | --- | --- | --- |
| Sample designation | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 3: |  |  |  |  |  |

**4.3 Linearity**

O see Table 3 O see appendix

Table 3: Determining linearity

|  |  |
| --- | --- |
| **Sample designation:** |  |
| **Measurement series** | **V1** | **V2** | **V3** | **V4** | **\_\_\_\_** | **\_\_\_\_** | **\_\_\_\_** | **\_\_\_\_** |
| Day 1-1 |  |  |  |  |  |  |  |  |
| Day 1-2 |  |  |  |  |  |  |  |  |
| Day 2-1 |  |  |  |  |  |  |  |  |
| Day 2-2 |  |  |  |  |  |  |  |  |
| **Sample designation:** |  |
| **Measurement series** | **V1** | **V2** | **V3** | **V4** | **\_\_\_\_** | **\_\_\_\_** | **\_\_\_\_** | **\_\_\_\_** |
| Day 1-1 |  |  |  |  |  |  |  |  |
| Day 1-2 |  |  |  |  |  |  |  |  |
| Day 2-1 |  |  |  |  |  |  |  |  |
| Day 2-2 |  |  |  |  |  |  |  |  |

# 5. Assessment:

**5.1 Sensitivity and specificity:**

Table 4: Assessment of sensitivity and specificity:

|  |  |  |  |
| --- | --- | --- | --- |
|  | New procedure (in percent)  | Target\*(in percent) | Assessment |
| Sensitivity |  | (>95) |  |
| Specificity |  | (>98) |  |

\* Information in brackets reflects the laboratory’s internal specifications. RiliBÄK specifications and manufacturer specifications may also need to be taken into account. Information to be inserted in front of the brackets.

**5.2 Intra- and inter-assay precision and/or trueness/robustness**

Table 5: Assessment of intra- and inter-assay precision:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample designation | Intra-assay(in percent) | Target\*(in percent) | Inter-assay(in percent) | Target(in percent) | Assessment |
| 1: |  | (<15) |  | (<25) |  |
| 2: |  | (<15) |  | (<25) |  |
| 3: |  | (<15) |  | (<25) |  |

\* Information in brackets reflects the laboratory’s internal specifications for quantitative measurement procedures. RiliBÄK specifications and manufacturer specifications may also need to be taken into account. Information to be inserted in front of the brackets. For purely qualitative measurement methods (IB, titre), the repeatability results are given as a percentage (#ACTUAL/#Target \*100). The internal laboratory specifications are 100% in this case) - whereby deviations of ± one titre level are acceptable/regarded as meeting the target value.

**5.3 Linearity**

Table 5: Determination of linearity Graphic illustration, if available – O see appendix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample designation | Regression line r-value | Target | Linear range | Target value | Assessment |
|  |  | >0.95  |  | >3 dilution levels |  |
|  |  | >0.95  |  | >3 dilution levels |  |

**5.4 Remarks on assessment:**

Approved for release: O yes O no (if necessary, follow-up measures under 5.4)

Date:\_\_\_\_\_\_\_\_\_\_\_\_ Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# 6. Appendix(es) for test validation: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**6.1 Sensitivity and specificity** (example)**:**

Measurement period:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Conducted by: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Batch code, if applicable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Sheet \_\_\_\_ of \_\_\_\_

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| No: | Sample designation | Measured | Expected value\* | No.: | Sampledesignation | Measured | Expected value\* |
| 1 |  |  |  | 12 |  |  |  |
| 2 |  |  |  | 13 |  |  |  |
| 3 |  |  |  | 14 |  |  |  |
| 4 |  |  |  | 15 |  |  |  |
| 5 |  |  |  | 16 |  |  |  |
| 6 |  |  |  | 17 |  |  |  |
| 7 |  |  |  | **\_\_\_** |  |  |  |
| 8 |  |  |  | **\_\_\_** |  |  |  |
| 9 |  |  |  | **\_\_\_** |  |  |  |
| 10 |  |  |  | **\_\_\_** |  |  |  |
| 11 |  |  |  | **\_\_\_** |  |  |  |

\*Do not enter expected value until after measurement is made.

## 11.2.2 LDT test (Category II, III, IV): Method validation - quantitative

**Method validation - quantitative**

# 1. General:

Name of test:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(Name in declaration of conformity)

Internal designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Analyte number (EDV):\_\_\_\_\_\_\_\_\_\_

Device name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ or: O manual

## Sample material (matrix):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## 2. Planning:

Planned by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ on:\_\_\_\_\_\_\_\_\_\_\_\_ signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

To be implemented by (date):\_\_\_\_\_\_\_\_\_\_ by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **2.1 Remarks on planning (question/objective) and information on publications, reference laboratories, preliminary results, special features, references as well as justification if protocol is abridged** (e.g.: CE method with matrix expansion, use of RUO tests)

**2.2 Definition of value ranges**

a) Internal requirements (purpose/target) or manufacturer’s specifications (modification):

O Unit: \_\_\_\_\_\_\_\_\_\_\_ O Limit of detection (LOD): \_\_\_\_\_\_\_\_\_\_\_

O Lower and upper limits of quantification (LOQ): <\_\_\_\_\_\_\_\_\_\_\_\_\_\_ >\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Linear measuring range: from:\_\_\_\_\_\_\_\_\_\_\_\_\_ to:\_\_\_\_\_\_\_\_\_\_\_\_\_\_

O Negative O not detectable O below limit of detection (lower limit) O \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

b) Internally established value ranges

Highly positive: \_\_\_\_\_\_\_\_\_\_\_\_\_\_ positive:\_\_\_\_\_\_\_\_\_\_\_\_ weakly positive/borderline:\_\_\_\_\_\_\_\_\_\_\_

Questionably positive:\_\_\_\_\_\_\_\_\_\_\_\_\_\_ negative/not detectable:\_\_\_\_\_\_\_\_\_\_\_\_\_

 **Further information on value range, if applicable:**

 **2.3 Reference values/decisive clinical values:**

**2.4 Potentially cross-reactive parameters/samples**

List of potentially, cross-reactive parameters (at least 3) and the value ranges to be analysed/considered. A list is only required when the respective samples are also (externally/internally) available.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Name | Value range | Name | Value range | Name | Value range |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

**2.5 Sensitivity/specificity using:**

O external samples\*
\* EQAS samples, quality controls (of other manufacturers), reference institute etc., external lab

Comparison measurement with the expected values:

#\_\_\_\_\_\_highly positive / #\_\_\_\_\_\_ positive (usually at least 10)

 #\_\_\_\_\_\_weakly positive/borderline (usually at least 10)

 #\_\_\_\_\_\_negative (usually at least 20)

#\_\_\_\_\_\_negative (cross-reaction) (usually at least 3)

O internal samples\* (supplemental or alternative)
\* earlier samples, quality controls

Comparison measurement with the expected values:

#\_\_\_\_\_\_highly positive / #\_\_\_\_\_\_ positive (usually at least 10)

 #\_\_\_\_\_\_weakly positive/borderline (usually at least 10)

 #\_\_\_\_\_\_negative (usually at least 20)

#\_\_\_\_\_\_negative (cross-reaction) (usually at least 3)

 **Remarks and/or requirements for selecting samples:**

**2.6 Intra-/inter-assay precision and/or robustness/trueness (reproducibility):**

Usually 3 sample sets with 4 samples in various value ranges. First, measured in triplicate (measurement series 1) and then singly determined in 2 independent measurement series/days.

O Sample 1: Upper linear measurement range Value range, if applicable:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 2: Mean linear measurement range Value range, if applicable:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 3: Lower linear measurement range Value range, if applicable:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 4: Lower detection limit/negative Value range, if applicable \*:\_\_\_\_\_\_\_\_\_\_\_\_\_
\* Usually only indicated for quantitative values (e.g.: qPCR)

**2.7 Linearity**

**Sample designation 1:**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Order number, if applicable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Concentration of initial sample: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Unit:\_\_\_\_\_\_\_\_\_\_

Dilution level: #\_\_\_\_\_\_\_\_\_\_(minimum 4) Dilution factor:\_\_\_\_\_\_\_\_ (usually: 1:10 or 1:5)
**Sample designation 2:**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Order number, if applicable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Concentration of initial sample: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Unit:\_\_\_\_\_\_\_\_\_\_

Dilution level: #\_\_\_\_\_\_\_\_\_\_(minimum 4) Dilution factor:\_\_\_\_\_\_\_\_ (usually: 1:10 or 1:5)

# 3. Measurement values:

1. Sensitivity and specificity O see 6.1 (sheet 1 to \_\_\_\_) O see appendix
2. Intra- and inter-assay precision O see 4.2
3. Linearity O see 4.3

**3.1 Remarks on measurement** (changes, issues, software, etc.)**:**

# 4. Summary of the results:

**4.1 Sensitivity and specificity**

O see Table 1 O see appendix

Table 1: Summary of the measurements on sensitivity and specificity

|  |  |
| --- | --- |
|  | TARGET |
|  ACTUAL |  | (Strongly) positive #\_\_\_\_\_\_\_ |  Weakly positive #\_\_\_\_\_\_\_ |  Negative #\_\_\_\_\_\_\_ |
| (Highly) positive |  |  |  |
| Weakly positive |  |  |  |
| Negative |  |  |  |

# = number of tested samples

**4.2 Intra- and inter-assay precision**

O see Table 2 O see appendix
Table 2: Summary of the results of intra- and inter-assay precision

|  |  |  |  |
| --- | --- | --- | --- |
| 1. Sample set | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 3: |  |  |  |  |  |
| 4: |  |  |  |  |  |
| 2. Sample set | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 3: |  |  |  |  |  |
| 4: |  |  |  |  |  |
| 3. Sample set | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 3: |  |  |  |  |  |
| 4: |  |  |  |  |  |

**4.3 Linearity**

O see Table 3 O see appendix

Table 3: Determining linearity

|  |  |
| --- | --- |
| **Sample designation:** |  |
| **Measurement series** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** |
| Day 1-1 |  |  |  |  |  |  |  |  |
| Day 1-2 |  |  |  |  |  |  |  |  |
| Day 2-1 |  |  |  |  |  |  |  |  |
| Day 2-2 |  |  |  |  |  |  |  |  |
| **Sample designation:** |  |
| **Measurement series** | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** |
| Day 1-1 |  |  |  |  |  |  |  |  |
| Day 1-2 |  |  |  |  |  |  |  |  |
| Day 2-1 |  |  |  |  |  |  |  |  |
| Day 2-2 |  |  |  |  |  |  |  |  |

# 5. Assessment:

**5.1 Sensitivity and specificity:**

Table 4: Assessment of sensitivity and specificity:

|  |  |  |  |
| --- | --- | --- | --- |
|  | New procedure | Target\* | Assessment |
| Sensitivity |  | ( ) |  |
| Specificity |  | ( ) |  |

\* Internal laboratory targets for quantitative measurement procedures (see SOP Method Validation). If applicable: provide manufacturer’s specifications in brackets (e.g., for test modifications, RUO)

**5.2 Intra- and inter-assay precision**

Table 5: Assessment of intra- and inter-assay precision:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample designation | Intra-assay | Target\* | Inter-assay | Target \* | Assessment |
| 1: |  | ( ) |  | ( ) |  |
| 2: |  | ( ) |  | ( ) |  |
| 3: |  | ( ) |  | ( ) |  |
| 4\*\*: |  | ( ) |  | ( ) |  |

\* Information on RiliBÄK targets or, if not available, internal laboratory target criteria for quantitative measurement procedures (see SOP Method Validation). If applicable: provide manufacturer’s specifications in brackets (e.g., for test modifications, RUO). \*\* If no values are available for negative samples (e.g., (rt-)qPCR), the results pertaining to repeatability are given as a percentage (#ACTUAL/#Target \*100). The internal laboratory specifications are usually 100%.

**5.3 Linearity**

Table 5: Determination of linearity Graphic illustration – see appendix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample designation | Regression line r-value | Target | Linear range | Target | Assessment |
|  |  | >0.95  |  | >4 dilution levels |  |
|  |  | >0.95  |  | >4 dilution levels |  |

**5.4 Remarks on assessment**:

Approved for release: O yes O no (follow-up measures, where necessary, under 5.3)

Date:\_\_\_\_\_\_\_\_\_\_\_\_ Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# 6. Appendix(es) for test validation: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**6.1 Sensitivity and specificity** (example)**:**

Measurement period:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Conducted by: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Batch code, if applicable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Sheet \_\_\_\_ of \_\_\_\_

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| No.: | Sample designation | Measured | Expected value\* | No.: | Sample designation | Measured | Expected value\* |
| 1 |  |  |  | 12 |  |  |  |
| 2 |  |  |  | 13 |  |  |  |
| 3 |  |  |  | 14 |  |  |  |
| 4 |  |  |  | 15 |  |  |  |
| 5 |  |  |  | 16 |  |  |  |
| 6 |  |  |  | **\_\_\_** |  |  |  |
| 7 |  |  |  | **\_\_\_** |  |  |  |
| 8 |  |  |  | **\_\_\_** |  |  |  |
| 9 |  |  |  | **\_\_\_** |  |  |  |
| 10 |  |  |  | **\_\_\_** |  |  |  |
| 11 |  |  |  | **\_\_\_** |  |  |  |

\* Do not enter expected value until after measurement is made.

## 11.3.1 CE test (Category I): Method verification - qualitative

**Method verification – qualitative – CE test**

# 1 General:

Name of test:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(Name in declaration of conformity)

Internal designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Analyte number (EDV):\_\_\_\_\_\_\_\_\_\_

Device name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ or: O manual

## Sample material (matrix):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# 2 Planning:

Planned by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ on:\_\_\_\_\_\_\_\_\_\_\_\_ signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

To be implemented by (date):\_\_\_\_\_\_\_\_\_\_ by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **2.1 Remarks on planning (question/objective) and information on publications, reference laboratories, preliminary results, special features, references as well as justification if protocol is abridged**

Details on the procedure’s measurement scale

 Quantitative (determination of intra-/inter-assay precision):

 O Cardinal scale (OD values, (rt-)qPCR, IU values etc.)

Qualitative (robustness, trueness):
 O Ordinal scale (titre, +/++, etc.)

 O Nominal scale (Immunoblot, positive/negative, detectable, etc.)

**2.2 Definition of value ranges (information/categorisation in findings, if applicable):**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Information in findings  |  |  |  |  |  |

Highly positive :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Positive :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Weakly positive :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Borderline :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

Negative :\_\_\_\_\_\_\_\_\_\_\_\_\_ O O O O O

**Further information on value range, if applicable:**

Value ranges can include: titre, measurement ranges (e.g., 500 – 1000 IU), band intensity, number of bands (WB), etc.

 **2.3 Reference values/decisive clinical values:**

**2.4 Intra-/inter-assay precision and/or robustness/trueness (repeatability):**

Usually one sample set with 3 samples in different (clinically relevant) value ranges. First measured in triplicate (measurement series 1) and then singly determined in 2 independent measurement series.
O Sample 1: (highly) positive Value range, if applicable\*:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 2: weakly positive Value range, if applicable \*:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 3: negative Value range, if applicable \*:\_\_\_\_\_\_\_\_\_\_\_\_\_

\* Usually only for quantitative values

# 3. Measurement values:

Intra- and inter-assay precision O see 4.1 O see appendix

**3.1 Remarks on measurement (changes, issues, software, etc.):**

# 4. Summary of the results:

**4.1 Intra- and inter-assay precision**

O see Table 2 O see appendix
Table 2: Summary of the results for intra- and inter-assay precision

|  |  |  |  |
| --- | --- | --- | --- |
| Sample designation | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 2: |  |  |  |  |  |

# 5. Assessment:

**5.1 Intra- and inter-assay precision and/or trueness/robustness**

Table 5: Assessment of intra- and inter-assay precision:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample designation | Intra-assay(in percent) | Target\*(in percent) | Inter-assay(in percent) | Target(in percent) | Assessment |
| 1: |  | (<15) |  | (<25) |  |
| 2: |  | (<15) |  | (<25) |  |
| 3: |  | (<15) |  | (<25) |  |

\* Information in brackets reflects the laboratory’s internal specifications for quantitative measurement procedures. RiliBÄK specifications and manufacturer specifications may also need to be taken into account. Information to be inserted in front of the brackets. For purely qualitative measurement methods (IB, titre), results pertaining to repeatability are given as a percentage (#ACTUAL/#Target \*100). The internal laboratory specifications are 100% in this case) - whereby deviations of ± one titre level are acceptable/regarded as meeting the target value.

**5.2 Remarks on assessment:**

Approved for release: O yes O no (if necessary, follow-up measures under 5.2)

Date:\_\_\_\_\_\_\_\_\_\_\_\_ Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Additionally, where applicable*

# 6. Appendix(es) for test verification: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**6.1 Sensitivity and specificity** (example)**:**

## 11.3.2 CE test (Category I): Method verification – quantitative

**Method verification – quantitative – CE test**

# 1 1 General:

Name of test:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(Name in declaration of conformity)

Internal designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Analyte number (EDV):\_\_\_\_\_\_\_\_\_\_

Device name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ or: O manual

# Sample material (matrix):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# 2 Planning:

Planned by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ on:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

To be implemented by (date):\_\_\_\_\_\_\_\_\_\_ by:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **2.1 Remarks on planning (question/objective) and information on publications, reference laboratories, preliminary results, special features, references as well as justification if protocol is abridged.**

**2.2 Definition of value ranges**

a) Internal requirements (purpose/target) or manufacturer’s specifications:

O Unit: \_\_\_\_\_\_\_\_\_\_\_ O Limit of detection (LOD): \_\_\_\_\_\_\_\_\_\_\_

O Lower and upper limits of quantification (LOQ): <\_\_\_\_\_\_\_\_\_\_\_\_\_\_ >\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Linear measuring range: from:\_\_\_\_\_\_\_\_\_\_\_\_\_ to:\_\_\_\_\_\_\_\_\_\_\_\_\_\_

O Negative O not detectable O below limit of detection (lower limit) O \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

b) Internally established value ranges

Highly positive: \_\_\_\_\_\_\_\_\_\_\_\_\_\_ positive:\_\_\_\_\_\_\_\_\_\_\_\_ weakly positive/borderline:\_\_\_\_\_\_\_\_\_\_\_

 Questionably positive:\_\_\_\_\_\_\_\_\_\_\_\_\_\_ negative/not detectable:\_\_\_\_\_\_\_\_\_\_\_\_\_

 **Further information on value range, if applicable:**

 **2.3. Reference values/decisive clinical values:**

**2.4 Intra-/inter-assay precision and/or robustness/trueness:**

Usually 3 sample sets with 3 samples in various value ranges. First, measured in triplicate (measurement series 1) and then singly determined in 2 independent measurement series/days.

O Sample 1: Upper linear measurement range Value range, if applicable:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 2: Lower linear measurement range Value range, if applicable:\_\_\_\_\_\_\_\_\_\_\_\_\_

O Sample 3: Negative Value range, if applicable \*:\_\_\_\_\_\_\_\_\_\_\_\_\_
\* Usually only indicated for quantitative values (e.g.: qPCR)

**2.7 Linearity\***

(Usually only for molecular testing (NAT) **O n/a**

**Sample designation:**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Order number, if applicable: \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Concentration of initial sample: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Unit:\_\_\_\_\_\_\_\_\_\_

Dilution level: #\_\_\_\_\_\_\_\_\_\_ (minimum 3) Dilution factor:\_\_\_\_\_\_\_\_ (usually: 1:10 or 1:5)

**3. Measurement values:**

1. Intra- and inter-assay precision O see 4.1
2. Linearity O see 4.2

**3.1 Remarks on measurement (changes, issues, software, etc.):**

# 4. Summary of the results:

**4.1 Intra- and inter-assay precision**

O see Table 2 O see appendix

Table 2: Summary of the results of the intra- and inter-assay precision

|  |  |  |  |
| --- | --- | --- | --- |
| 1. Sample set | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 3: |  |  |  |  |  |
| 2. Sample set | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 3: |  |  |  |  |  |
| 3. Sample set | Measurement 1 (3x) | Measurement 2 | Measurement 3 |
| 1: |  |  |  |  |  |
| 2: |  |  |  |  |  |
| 3: |  |  |  |  |  |

**4.2 Linearity**

O see Table 3 O see appendix

Table 3: Determining linearity

|  |  |
| --- | --- |
| **Sample designation:** |  |
| **Measurement series**  | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** |
| Day 1-1 |  |  |  |  |  |  |  |  |
| Day 1-2 |  |  |  |  |  |  |  |  |

# 5. Assessment:

**5.1 Intra- and inter-assay precision**

Table 5: Assessment of intra- and inter-assay precision:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample designation | Intra assay | Target\* | Inter assay | Target\* | Assessment |
| 1: |  | ( ) |  | ( ) |  |
| 2: |  | ( ) |  | ( ) |  |
| 3\*\*: |  | ( ) |  | ( ) |  |

\* Information on RiliBÄK targets or, if not available, internal laboratory target criteria for quantitative measurement methods (see SOP Method Validation). If applicable: provide manufacturer’s specifications in brackets (e.g., for test modifications). \*\* If no values are available for negative samples (e.g., (rt-)qPCR), the results pertaining to repeatability are given as a percentage (#ACTUAL/#Target \*100). The internal laboratory specifications are 100% in this case).

**5.2 Linearity**

Table 5: Determining linearity Graphic illustration – O see appendix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample designation | Regression line r-value | Target | Linear range | Target value | Assessment |
|  |  | >0.95  |  | >3 dilution levels |  |

**5.3 Remarks on assessment:**

Approved for release: O yes O no (if necessary, follow-up measures under 5.3)

Date:\_\_\_\_\_\_\_\_\_\_\_\_ Signature:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

*Additionally, where applicable*

# 6. Appendix(es) for test verification: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**6.1 Sensitivity and specificity** (example)**:**

## 11.4 Sample declaration for LDT-IVDs and for the implementation of general requirements

Corresponding statements on conformity and on the “implementation of general requirements”, as well as standard operating procedures on risk management and a checklist on compliance with IVDR, Annex I and ISO 15189 have been prepared by other AWMF working groups and will be published separately.

1. The Medical Devices Act defines these “methods” as “in vitro diagnostic medical devices manufactured in-house”. Regulation (EU) 2017/746 does not contain its own definition of this, but refers to these laboratory-developed test systems as “devices manufactured and used exclusively within healthcare institutions established in the Union”. In the English-speaking world, these methods are referred to as “laboratory developed tests – LDT”. [↑](#footnote-ref-2)
2. “9.2. The performance characteristics of the device shall be maintained during the lifetime of the device as indicated by the manufacturer.
 9.3. Where the performance of devices depends on the use of calibrators and/or control materials, the metrological traceability of values assigned to calibrators and/or control materials shall be assured through suitable reference measurement procedures and/or suitable reference materials of a higher metrological order. Where available, metrological traceability of values assigned to calibrators and control materials shall be assured through certified reference materials or reference measurement procedures.” [↑](#footnote-ref-3)
3. EU: Regulation (EC) No 141/2000 can be consulted for guidance with respect to medicinal products if no more than five in ten thousand persons are affected by the condition. In the EU, there are still no binding definitions or quantitative descriptions in this regard for the medical device sector.

In the USA, orphan drugs are defined as: “Intended for the effective and safe treatment, prevention, or diagnosis of rare diseases with fewer than 200,000 people in the US; or which affect more than 200,000 people but where the costs of marketing and developing the products are not expected to be recovered.” [↑](#footnote-ref-4)
4. Testing procedures that fall under risk class D ((EU) 2017/746; IVDR) are excluded from this. [↑](#footnote-ref-5)
5. [↑](#footnote-ref-6)
6. 5 See also Section 2 “Terms/abbreviations/definitions”

The terms used in Sections 5.1, 5.3 and 8.1 for the specifications of the analysed sample materials are defined in this SOP as follows: “borderline” is defined as 2 to 5 times the lower limit of detection for the respective test system (e.g., a PCR). A sample is considered “weakly” positive if the amount of analyte (e.g., nucleic acid) is 20 to 50 times the lower limit of detection for the respective test system (e.g., PCR) - the definition can be modified with justification by laboratory management. [↑](#footnote-ref-7)
7. See Section “Terms/abbreviations/definitions” [↑](#footnote-ref-8)
8. See also Section 2 “Terms/Abbreviations/Definitions” – LDT Light [↑](#footnote-ref-9)