

Qualitative examinations – estimation and control of uncertainty in laboratory medicine

Introduction

Qualitative examinations are defined as tests where the result either identifies a component or indicates whether a specific component can be detected (classification). In the latter case, the result is binary (expressed as yes/no, positive/negative, etc.). Therefore, the format of the result differs between qualitative and quantitative examinations - the latter being expressed as a numerical value.

According to SS-EN ISO/IEC 17025 and SS-EN ISO 15189 [1,2], measurement uncertainty must be stated for accredited quantitative examinations. Measurement uncertainty for “quantitative examinations” is addressed in SWEDAC DOC 05:03 [3].

This document describes how the uncertainty of qualitative examinations can be estimated and controlled, as well as how it should be reported to clients.

The methods for evaluating and ensuring quality are not as well established for qualitative examinations as for quantitative ones, and often there is a lack of consensus on terminology. This document is based on the work “Quality assurance of qualitative analysis in the framework of the European project ‘MEQUALAN’” [4], which discusses the implementation of quality concepts in qualitative examinations.

In this document, the word “uncertainty” is used as a Swedish equivalent to the English “unreliability”. The term uncertainty will thus have a corresponding function for qualitative methods as the term measurement uncertainty for the quantitative ones – even though the terms are not directly comparable.

Different types of qualitative examinations

Qualitative examinations can be divided into different types. The two main groups are identification and classification. Classification can then be further divided into subgroups. Note, however, that the boundaries between different types are sometimes fluid. This applies both between different types of qualitative examinations and between qualitative and quantitative examinations.

Examples of different types of qualitative examinations are given in Appendix 1.

Due to differences depending on specialties, pathological/cytological examinations are described separately.

Estimation of uncertainty in qualitative examinations

The description of the uncertainty of a qualitative examination should provide the client with a basis to adequately interpret the result in relation to the patient's clinical symptoms. Information about the uncertainty of the result should allow for a comparison of results between different examination occasions, different examination methods, and different laboratories.

Since the uncertainty for qualitative examinations often cannot be stated with a number, various expressions must be used in parallel to describe the uncertainty.

In SWEDAC DOC 01:55, Validation/verification of quantitative and qualitative methods [5], several concepts describing a method's uncertainty are defined. Several of these are applicable to qualitative methods. The uncertainty is also affected by several pre-analytical factors that may be difficult to estimate (see Appendix 1), but nonetheless should be stated if they are significant.

An examination's uncertainty depends on the performance of the method used. One way to estimate the uncertainty is therefore to compare the method used with an international or national reference method through validation. The Swedish Institute for Infectious Disease Control, in collaboration with the Association for Medical Microbiology, publishes a series "Reference methodology for laboratory diagnostics in clinical microbiological laboratories; I. Infection Diagnostics." Below, some of the most important concepts describing the uncertainty for qualitative methods based on identification and classification are commented on.

Identification

Methods based on the identification of a component, such as bacterial typing, morphological assessment, pattern comparison, and sequencing, involve uncertainty both in the subjective recognition of a pattern and in its interpretation when related to known and accepted patterns in various comparison libraries. Therefore, the uncertainty depends on the reader's experience but also on the quality of the comparison material and the method used.

Classification

Sensitivity and specificity are two concepts used to describe a method's usefulness for diagnostics. Other important measures of a method's uncertainty are the positive and negative predictive values. These latter measures depend on the prevalence of a disease. With high prevalence, lower specificity of a testing method gives a higher positive predictive value than with low prevalence. In cases of low prevalence, a very high specificity is required for a positive result to be truly positive. The opposite relationship applies to the negative predictive value.

The uncertainty in qualitative examinations also depends on how reliably a method's reference range and cut-off can be determined, as well as the method's detection level. Information about the method's detection level is therefore another important measure to be able to assess the uncertainty of a positive or negative result.

In pathology/cytology, special circumstances justify that this area is assessed separately, see Appendix 1.

Control and monitoring of uncertainty in qualitative examinations

To ensure good quality, qualitative examinations need to be validated and/or verified and have internal and external control programs. Guidelines for this are developed in SWEDAC DOC [5,6].

Regarding internal controls for qualitative examinations, in addition to what is mentioned in SWEDAC DOC 01:31 Internal Controls [6], the following should also be considered:

- For methods based on identification, positive and negative controls are required. Furthermore, any equipment - such as photometers and other analytical equipment - must be regularly checked and the values of various measurement parameters documented.
- For classification methods based on the comparison of a measurement value with a predetermined cut-off, both a negative and a positive control should be used. The positive control is used as an operational control and should be slightly above the cut-off value to ensure quality in this area. It should be related to the cut-off value, e.g., by calculating the ratio of the operational control's measurement value to the cut-off value. The ratio should be monitored in a chart, and permissible variation limits for it should be established.
- For titrations, an operational control should be used, with the permissible variation usually set to no more than +1 dilution step. To accurately determine the size of the variation, it is recommended that the operational control is used in 4 titer steps, where the dilutions are chosen so that the outcome is usually the next lowest dilution. This means that the variation is unlikely to be more than one dilution step. To reduce the significance of inter-assay variation, if necessary, additional certainty in the assessment can be achieved by repeating the titration of previous samples along with the titration of the new sample. In pathology/cytology, national recommendations are developed within the framework of quality and standardization work for each accredited method on how the examination is conducted and uncertainty is reported.

Reporting uncertainty to clients

The uncertainty of qualitative examinations cannot be expressed as a numerical value for measurement uncertainty in the way it is for quantitative methods – instead, other information must be used.

The laboratory should determine which factors significantly affect the examination's uncertainty. Significant pre-analytical factors should always be clearly indicated in the sampling instructions.

For identification methods, information about standards, comparison libraries, and comparison methods, calibration techniques for equipment, and reference methods should be available. These should be provided to the client upon request.

For classification methods, corresponding information about standards, choice of cut-off value, permissible variation of internal controls, and reference methods should be documented and made available. Information about sensitivity and specificity as well as positive and negative predictive values should be indicated to the extent that such information can be obtained.

References:

1. SS-EN ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories, SIS, Stockholm, 2018
2. SS-EN ISO 15189, Medical laboratories – Particular requirements for quality and competence, SIS, Stockholm, 2022
3. SWEDAC DOC 05:03, Measurement uncertainty and comparison of results between methods/instruments in laboratory medicine
4. Ríos et al. Quality assurance of qualitative analysis in the framework of the European project Mequalan'. Accred Qual Assur 2003; 8:68-77
5. SWEDAC DOC 01:55, Validation/verification of quantitative and qualitative methods
6. SWEDAC DOC 01:31, Internal and external controls
7. Reference methodology for laboratory diagnostics in clinical microbiological laboratories. I 1 - 11.
8. Ellison SLR ET AL. Characterising the performance of qualitative analytical methods: Statistics and terminology. Trans in Analytical Chemistry 2005:24:468.

Appendix 1 – Definition and examples of different types of qualitative examinations and pre-analytical factors

1. Examinations within the clinical specialties of Bacteriology, Pharmacology, Immunology, Chemistry, Parasitology, Transfusion Medicine, and Virology

1. **Identification.** Identification of, for example, a chemical substance, a bacterial strain, a blood group antigen, or an antibody specificity. The important thing is the identification without regard to the substance's concentration.
2. **Classification (presence or absence of a substance).** Even though the result of this type of examination is given in binary form, the boundary with quantitative methods is not sharp. Often, even in qualitative methods, a measurement value is first obtained, which then forms the basis for the classification.
 - a. Test strips or “plus/minus tests” This type of examination does not provide a measurement value, but the result is assessed based on a colour change or similar. The sensitivity is set so that a “positive” outcome corresponds to a concentration that is clinically significant. However, these methods are often based on measurement value- based quantitative methods, as such can be used to set appropriate sensitivity and specificity when developing, for example, a kit.
 - b. “Cut-off examinations” Determining whether a sample is above a certain threshold (cut-off). These examinations are close to quantitative ones, as they primarily provide a measurement value. This measurement value is then compared with a predetermined (or calculated) threshold value (cut-off) to determine whether the outcome of the examination is positive or negative.
 - c. Semi-quantitative examinations Examples of this are examinations where an interval or a titer is determined. These examinations are essentially quantitative in nature, but the result is not on a continuous scale. Therefore, it is difficult to use the formulas for calculating measurement uncertainty that are used for true quantitative methods.

Table 1 provides examples of different qualitative examinations within some of the aforementioned specialties.

Table 1 – Examples of Classification of Qualitative Examinations in Bacteriology, Immunology, Parasitology, Transfusion Medicine, and Virology

Type of Examination	Identification: (CPE, typing, sequensification)	Classification: Test strips of “Plus/Minus tests”	Classification: Cut-off examinations” (based on data analysis)	Classification: Semi-quantitative examinations
Bacteriology	-Fecal culture -Salmonella - Shigella - Yersinia -Campylobacter	Pneumococcus antigen in urine	PCR Chlamydia trachomatis	- Urine culture Serology: Elisa - Complement binding (titration)
Immunology	HLA typing	Detection of cryoglobulin	Screening tests with Elisa	Titration of autoantibodies
Parasitology	Fecal microscopy	Malaria test	–	–
Transfusion	Blood grouping	Recipient-donor test	–	Titration of erythrocyte antibodies
Virology	Virus culture	- Antigen tests: influenza RSV - Antibody tests: Heterophile antibodies	Antigen/antibody tests: Hepatitis/HIV PCR: Norovirus CMV	- Serology: Elisa - Complement binding (titration)

Examinations in Pathology/Cytology

Morphological diagnosis is based on several parallel assessments. One such is to determine tissue and cell type but also type of biological process. Another is to grade deviation from the normal image and to quantify/estimate findings such as positive outcome in various stains, grade cell infiltration and presence of certain structures or cells. Often several assessments are compiled into a comprehensive classification according to an agreed system.

The table below summarises different assessments in pathology and cytology where quantifications are also included.

Table 2 – Examples of Different Assessments in Pathology and Cytology

Typing: Application of complex criteria system	Grading: Univariate grading	Grading: Multivariate grading	Quantification: <i>indicated in continuous numerical scale or as %</i>
• Tissue typing in histological sections • Cell typing tissues and cytological material • Process typing/ interpretation in tissue or cell material with pathological changes such as inflammation, atrophy or neoplasia • TNM classification • WHO classification of tumour types	• Degree of inflammation • Fibrosis in chronic hepatitis • Bacterial count in gastritis biopsies	• Dysplasia grading in tissue or cell samples • Malignancy grading according to Nottingham • Gleason grading of prostate cancer	• Hormone receptors in IH sections • Intraepithelial lymphocytes • Differential count of bone marrow sample (= both quantification and typing) • Mitoses/HPF • LAP-score

Comment: For quantifications that strongly affect treatment, it should be stated how the assessment was performed (e.g., number of cells or counted fields where the field diameter is specified) and the method's uncertainty. For typings and gradings, it should be commented in each individual case whether there are special assessment difficulties and thus uncertainty in the assessment (in borderline cases, poor fixation, sparse material).

3. Pre-analytical Factors and Their Significance

The pre-analytical factors that are significant for the test result can be roughly grouped into factors as follows:

Patient and Patient Preparation

The patient's identity must be ensured. Certain examinations require special preparation of the patient, such as bladder incubation time for urine culture, fasting before certain examinations, or sampling at given time intervals.

Sample Collection and Referral Information

The sample material, sampling technique, and sample volume must be adequate for the examination. The sample must be correctly labelled, and the information must match the referral. In pathology, it is important to specify the sampling site and, if necessary, to orient the preparation with appropriate markings. The time of sampling, storage temperature, and any fixation or other handling must be stated where it is significant. The same applies to important anamnesis data, some of which constitute mandatory information for conducting an examination (recent blood transfusion, pregnancy, ongoing treatment, travel abroad, date of onset of illness, etc.).

Sample Handling, Transport, and Reception

Transport time, temperature, and choice of transport medium can be crucial for the quality of the sample and the examination, as can the immediate handling at the laboratory. When the sample arrives at the laboratory, unpacking and handling must be correct with proper interpretation of the question and coding. The sample must also be properly prepared for the examination (centrifugation, cutting, weighing, measuring, marking, etc.).

In summary, it is difficult to state a measure of the significance of the pre-analytical factors. However, the laboratory should determine if any factors are critical to the final result and inform the client of these. The laboratory should then, in its quality work, contribute to minimizing variation due to these factors. In the result, it should be stated whether there are critical pre-analytical factors that are inadequate.



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