Assessment of qualitative analysis performance and uncertainty

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- Scope of the guide
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- Assessment of qualitative analysis performance
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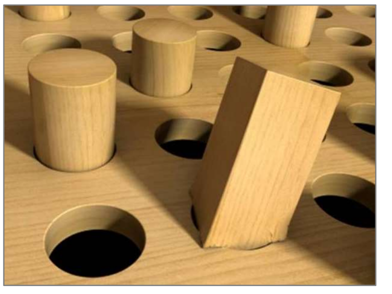
Relevance of qualitative analysis

- Many qualitative analyses have a relevant socio-economic impact, but some are unfit for the purpose.
- The assessment of analysis fitness for purpose can be based on the evaluation of results uncertainty
  - Not required by the test's accreditation although must prove analysis are adequately reliable
  - Different from measurement uncertainty

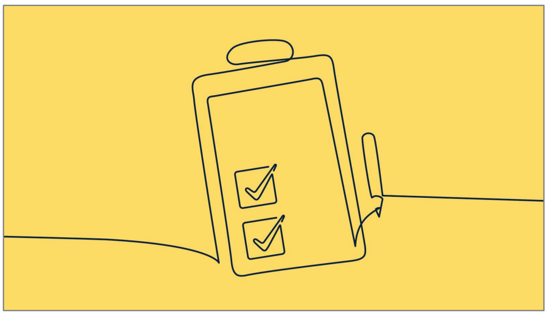


ISO, IEC, General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025), Geneva: ISO, 2017.
ISO, Medical laboratories — Requirements for quality and competence (ISO 15189), Geneva: ISO, 2012.

Scope of the guide

Assessment of performance and uncertainty in qualitative chemical analysis

- Assist laboratories in:
  - Setting and implementing appropriate methodologies for assessing the performance of qualitative analysis methods
  - Evaluating uncertainties in qualitative chemical analysis



Types of qualitative analysis

- Based on qualitative criteria:

  Example:

  (1) Detection of aliphatic aldehydes in a solution by colour change after the addition of Schiff's reagent.

  (2) Identification of human blood type by observation of agglutination.

- Based on quantitative criteria:

  Example:

  (1) Identification of a diuretic in urine from an athlete by GC-MS.

  (2) Identification of a virus in a clinical sample by qualitative real-time polymerase chain reaction (qRT-PCR).

(Conformity assessments with a quantitative limit are only briefly mentioned in the guide)

Assessment of qualitative analysis performance

The easy way of quantifying qualitative analysis performance is by the rate of true and false result rates:

$$TP = 1 - FN$$

$$TN = 1 - FP$$

True positive rate (TP); False negative rate (FN); True negative rate (TN); False positive rate (FP)

Assessment of qualitative analysis performance

The easy way of quantifying qualitative analysis performance is by the rate of true and false result rates:

$$TP = 1 - FN$$
$$TN = 1 - FP$$

If the performance is assessed experimentally, it can be reported as a contingency table:

		Case		
		Positive	Negative	Results total
Result	Positive	228	1	229
	Negative	5	300	305
	Cases total	233	301	

FP = 1/301 = = 0.33 %

Usually, rates are reference to the number of real cases.

Assessment of qualitative analysis performance

However, the *FP* and *FN* only characterise the method's performance.

It does not allow to estimate the probability of the sample's result being correct.

To estimate sample result reliability, it must be known the prevalence of positive and negative cases.

Assessment of qualitative analysis performance

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To estimate sample result reliability, it must be known the prevalence of positive and negative cases.

A positive COVID-19 infection result obtained from a fast test is more likely true in populations with a higher prevalence of the virus.

(prevalence information closes information gap)

Assessment of qualitative analysis performance

Challenges of *FP* and *FN* evaluation:

● After defining the analytical scope (property/ matrix/ method), it is necessary to test the method for all scope

● The reliability of *FP* and *FN* also depends on the number of tests:

Example of *FP* determination:

○ If *FP* of 2 % is estimated from 50 analysis of negative cases:

95% confidence interval of the *FP*: [0.35 % to 10 %]

○ If FP of 2% is tested from 200 analysis of negative cases:

95% confidence interval of the *FP*: [0.76 % to 4.9 %]

(200 analysis of negative cases allow proving that *FP* < 5 %)

Assessment of qualitative analysis performance

Challenges of *FP* and *FN* evaluation:

● After defining the analytical scope (property/ matrix/ method), it is necessary to test the method for all scope

● The reliability of *FP* and *FN* also depends on the number of tests:

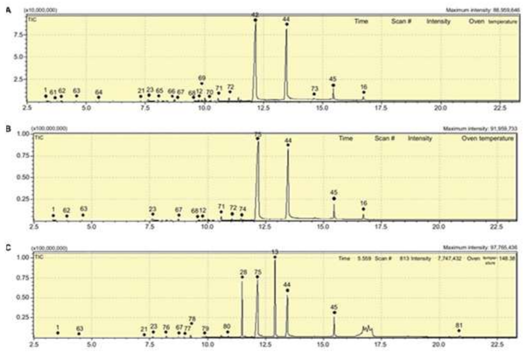
It can be acceptable to start using the analytical method with adequate results confirmation after an initial estimate of the *FP* and *FN* to be improved as more data is being collected.

Assessment of qualitative analysis performance

Challenges of *FP* and *FN* evaluation:

● After defining the analytical scope (property/ matrix/ method), it is necessary to test the method for all scope

● In instrumental methods of analysis, adequate models of signal used in the qualitative analysis can allow the simulation of very low *FP* and *FN*.



Expression of confidence in qualitative analysis

- **Likelihood ratio**

A convenient way of reporting qualitative analysis results is through a likelihood ratio, *LR*.

For positive results:

$$LR(+) = TP/FP$$

and for negative results:

$$LR(-) = TN/FN$$

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Expression of confidence in qualitative analysis

- **Likelihood ratio**

A convenient way of reporting qualitative analysis results is through a likelihood ratio, *LR*.

For positive results:

$$LR(+) = TP/FP$$

Assuming positive and negative cases are equally likely, a $LR(+)$ of 1000 suggests the positive result is 1000 times more likely true than false.

For forensic analysis: $LR(+) \geq 10^6$

For the identification of microplastics polymer type: $LR(+) \geq 19$

Association of Forensic Science Providers, Science and Justice 49 (2009) 161-164.
V. Morgado et al., Talanta 224 (2021) 121814.

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Expression of confidence in qualitative analysis

● **Likelihood ratio**

If the qualitative analysis is based on two independent tools (e.g. retention time and mass spectrum):

$$LR_{(1\&2)} = LR_{(1)} \cdot LR_{(2)}$$

Expression of confidence in qualitative analysis

● **Probability of result correctness**

If the probability of a positive result being correct, PP, is required, it is necessary to know the prevalence of positive $P(+)$ and negative $P(-)$ cases.

$$PP = \frac{\frac{P(+)}{P(-)} LR(+)}{\frac{P(+)}{P(-)} LR(+) + 1}$$

A similar expression is used for negative cases.

Expression of confidence in qualitative analysis

- **Probability of result correctness**

If the probability of a positive result being correct, PP, is required, it is necessary to know the prevalence of positive $P(+)$ and negative $P(-)$ cases.

Example:

$$PP = \frac{\frac{P(+)}{P(-)}LR(+)}{\frac{P(+)}{P(-)}LR(+) + 1} = \frac{\frac{0.5}{0.5}10^5}{\frac{0.5}{0.5}10^5 + 1} = 99.999\ \%$$

The determination of *PP* depends on the adequacy of $P(+)$ and $P(-)$.

Reporting qualitative analysis results

Currently, the accreditation does not request the reporting of the uncertainty of qualitative analysis.

Example:

Cocaine is present in sample 123
(identification with a likelihood ratio of 4.90×10^4 and considered a 'very strong' evidence of analyte presence)

Conclusions and recommendations

- It is crucial to check *FP* and *FN*
- *LR* is a convenient way of reporting performance
- Adequate references should be used
- Adequate analysis criteria should be considered
- Reporting qualitative analysis uncertainty should avoid misinterpretation

Examples

E1: Identification of compounds by low-resolution mass spectrometry using database searching

E2: Identification of purified compounds by infrared spectrometry

E3: Identification of drugs of abuse in urine by enzyme multiplied immunoassay technique

E4: Identification of human SRY gene in biological material by qPCR

E5: Identification of pesticide residues in foodstuffs by GC-MS-MS

E6: Identification of SARS-CoV-2 RNA by nucleic acid amplification testing

Working group members

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Guide voting

The guide was distributed for voting:

Comments and votes should be sent by July 5

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