rather typical today for most laboratories (e.g. 250 days per year, 15 h per day, 5 min per sample) about 500.000 sample could be analyzed during these 10 years. Taken together, in the highest through put scenario, overall expenses of about 2 € would result for one LC-MS/MS analysis, while about 6 € per sample are required in a standart throughput scenario.

State of the art HPLC-MS/MS instruments with one – or two dimensional chromatography set ups allow to run up to 20 analyses per hour. Consequently, within 24 h several hundred quantitative analyses can be performed with one HPLC-MS /MS system in a continuous work mode. In most clinical laboratories far smaller series are run in Daily routine, especially if switching from one assay to another requires hardware changes (e.g. of HPLC columns) causing significant down times.

LC-MS/MS is attractive for laboratory medicine for three main reasons

- The development of new methods is independent from the diagnostic industry
- Highly multiplexed analyses are feasible with very low current costs
- When applying the principle of isotope dilution internal standardisation, analyses on a reference method-level of accuracy can be performed in a routine laboratory setting.

Summarizing, it has to be stated, that present day routine HPLC-MS/MS assays are no "turnkey" applications. Both costs and response delays are significantly higher from MS industries.

Course-03

Validation and Verification Applications, Method Comparison and ROC Analysis on the Basis of CLSI Guidelines

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Introduction

Existing tests in clinical laboratories have gone through several stages before they were put into use, and after that they were used for diagnostic purposes. These steps refer to the same process in tests produced or modified in clinical laboratories as in commercial manufacturers. This process is examined in the CLSI evaluation protocol (EP) guidelines under two main headings: establishment stage and implementation stage.

CLSI guidelines play an important role in ensuring harmonization between clinical laboratories through standardization of laboratory procedures. During evaluation of measurement procedures, basic information about when and which CLSI guideline to use are defined in CLSI EP19. Establishment stage of the tests consists of feasibility, design, development and validation steps. Implementation stage includes preliminary evaluation, verification, measurement procedure launch and maintenance by the user and measurement procedure retirement (Figure 1) [1].

Establishment stage

1. Feasibility assessment step: It is a preliminary assessment of existing tests and alternative tests in a particular area, potential market, estimated production costs and new demands.
2. Design step: The manufacturer's commercial needs and the intended test performance are evaluated to meet the user's requirements.
3. Development step: The method is developed by making continuous improvements to all measuring procedure components.
4. Validation step: It is the validation of the product through a standardized process in a given population for the intended situation during the establishment stage. These performance characteristics for the measurement procedure are the performance claims that the manufacturer will provide for this method. The main elements and documents used in this scope can be listed as follows:

a. CLSI EP05 (Quantitative tests) and EP12 (Qualitative tests) provide useful information for precision evaluation. [2, 3].

b. Bias (accuracy): It is recommended to evaluate the accuracy using at least 100 patient samples in CLSI EP09 [4].

c. Measurement range (CLSI EP06): According to linearity, precision and quantitation limit values; the measuring range is determined [2, 5].

d. Reference range (CLSI EP28): It is determined within 90% confidence interval by using at least 120 samples, covering 95% of the reference individuals [6].

e. Analytical sensitivity (CLSI EP17): It is determined by limit of blank (LoB), limit of detection (LoD) and/or LoQ values [7].

f. Analytical specificity (CLSI EP14): It is the ability of a test to accurately identify and measure an analyte [8]. This ability is tested as described in CLSI EP07 with different sources of interference possible to affect the test. The effect of hemolysis, icter and lipemia indices and their end-user confirmation processes are described in CLSI C56 [9, 10].

g. Total analytical error (CLSI EP21): It is evaluated by comparisons with patient samples or reference materials; considering a predetermined error target [11].

h. Reagent stability (CLSI EP25): The stability of the reagent is assessed based on conditions such as storage conditions, packaging or transfer conditions [12].

i. Diagnostic sensitivity and specificity: In clinical trials, the diagnostic performance of the test is evaluated by determining the appropriate cut-off value using ROC analysis as specified in CLSI EP24 [13].

Implementation stage

1. Preliminary evaluations (CLSI EP10): In quantitative tests, bias, linearity, carry over and precision data are evaluated by repeated measurement of 10 samples. For qualitative testing, CLSI EP12 is used [3]. If the results are appropriate, the user passes to the verification step.
2. End-user verification: It is the verification of the manufacturer's presented data by the end-user (CLSI EP06, CLSI EP28, CLSI EP17, CLSI EP15) [5-7, 14].
3. Measurement procedure launch and maintenance: This is the process followed to ensure that the test performs properly. This process includes proficiency tests, internal quality control, periodic calibration and verification of calibration.
4. Measurement procedure retirement: It is the termination of the clinical use of a measurement procedure.

Conclusions

The CLSI evaluation protocol (EP) documents define and explain a variety of evaluation parameters for the establishment and implementation of a method. The relevant guidelines were briefly introduced in this text in order to manage and statistically evaluate the validation and verification process of the tests or measurement procedures produced by manufacturers or produced in our laboratories. These applications will also contribute to efficiency and quality management in clinical laboratories.

References

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Table 1. EP guidelines and evaluations during the establishment of measurement procedures (QMS=quality management system) [1]

CLSI Guideline	Establishment											
	1. Step: Feasibility and design	2. Step: Development	3. Step: Validation									
			Precision	Accuracy	Measuring Interval	Reference Range	Detection Capability	Analytical Specificity	Clinical Validation	Reagent/ Sample Stability	Risk Assessment	General
EP05[2]												QMS01; QMS02; QMS13
EP06[5]												
EP07[9]												
EP09[4]												
EP10[15]												
EP12[3]												
EP14[8]												
EP15[14]												
EP17[7]												
EP18[16]												
EP21[11]												
EP23[17]												
EP24[13]												
EP25[12]												
EP26[18]												
EP27[19]												
EP28[6]												
EP30[20]												
EP31[21]												

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Table 2. EP guidelines and evaluations during the implementation of measurement procedures (QMS=quality management system) [1]

CLSI Guideline	Implementation															
		5. Step: Verification								7. Step: Measurement Procedure Launch and Maintenance by the end User					
	4. Step: Preliminary Evaluation	Prec.	Acc.	Meas. Interval	Ref. Range	Detect. Capabil.	Risk Assess.	Qual.	6. Step: Preparation for Measurement procedure launch	Prec.	Acc.	Other	Results review and follow up	Gen.	8. Step: Measurement Procedure Retirement
EP05[2]															
EP06[5]															
EP07[9]															
EP09[4]															
EP10[15]															
EP12[3]															
EP14[8]															
EP15[14]															
EP17[7]															
EP18[16]															
EP21[11]															
EP23[17]															
EP24[13]															
EP25[12]															
EP26[18]															
EP27[19]															
EP28[6]															
EP30[20]															
EP31[21]															
													QMS01; QMS02; QMS13		

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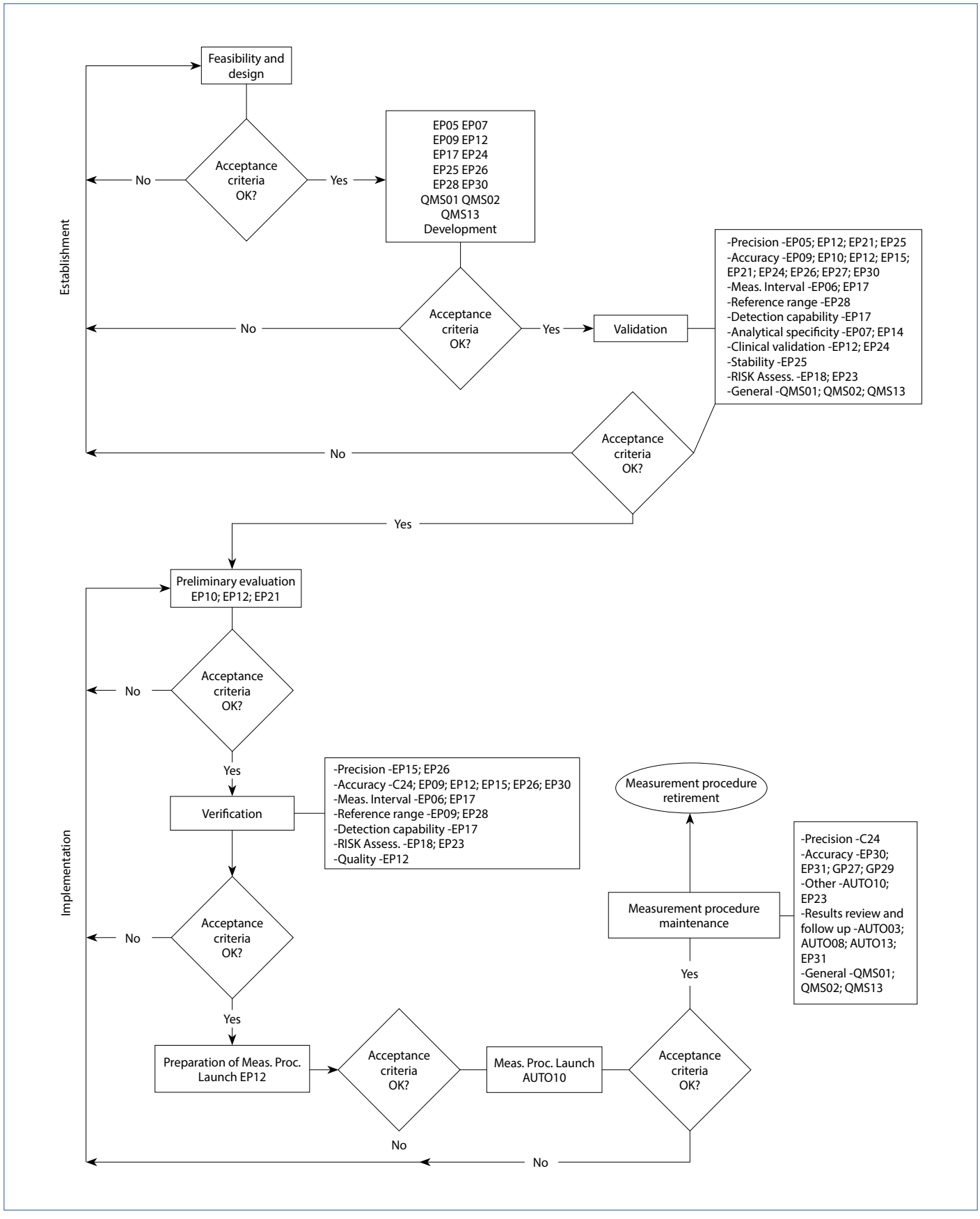

Figure 1. Flow chart of lifecycle of a measurement procedure [1].