Guidelines Molecular Diagnostics

ESCMID Postgraduate Education Course: Principles of Molecular Microbiological Diagnostics

M. Sc. Raymaekers Marijke marijke.raymaekers@jessazh.be

1

1. ISO15189: **the** standard for medical laboratories

Implement a quality management system:

- Quality of patient result is main concern: impact on patient care
- KIS-principal (Keep It Simple)
- Laboratory has to prove quality
- Based on ISO15189, **the** standard for medical laboratories

2

1. ISO15189: **the** standard for medical laboratories

ISO15189-*Particular requirements for quality and competence:*

- Specifies the **quality management system requirements** particular to **medical laboratories**.
- Includes:
  - provision of advice to users of the laboratory service
  - the collection of patient samples
  - the interpretation of test results
  - acceptable turnaround times
  - how testing is to be provided in a medical emergency
  - the lab's role in the education and training of health care staff
- Updated in 2012

3

1. ISO15189: **the** standard for medical laboratories

Chapter 5 of IS0 15189v2012:

§ 5.1 Personnel
§ 5.2 Accommodation and environmental conditions
§ 5.3 Laboratory equipment, reagents and consumables
§ 5.4 Pre-examination procedures
§ 5.5 Examination procedures
§ 5.6 Assuring quality of examination procedures
§ 5.7 Post-examination procedures
§ 5.8 Reporting of results
§ 5.9 Release of results
§ 5.10 Laboratory Information Management

4

1. ISO15189: **the** standard for medical laboratories

Chapter 5 of IS0 15189v2012 is very broad

- Interpretation is auditor dependent
- No suggestions concerning the implementation in daily practice
- Many aspects are topic of debate

Need for "translation" of ISO15189 to routine practice in guidelines

5

2. "Translate" to routine practice

Belgian working group:

- Discussion forum and platform for Molecular Biologists in Belgium affiliated to hospital laboratories and active in the field of molecular Diagnostics (www.moleculardiagnostics.be)
- Experts working in different sub disciplines of molecular diagnostics (microbiology, virology and haemato-oncology)
- Goal: to make a consensus interpretation of chapter 5 of ISO15189 **v2007**
- Acta Clinica Belgica 2011, 66(1):33-41

6

2. "Translate" to routine practice

§ 5.1 Personnel

- A **generalized structure** for a molecular diagnostics laboratory with the following functions:
  1. Routine molecular diagnostics testing
  2. Technical supervision
  3. Medical supervision

7

2. "Translate" to routine practice

§ 5.1 Personnel

- **Specific and adequate training** in the field of molecular biology based diagnostic techniques, especially on **contamination prevention**.
- **Need for ongoing education**, as the field is rapidly changing (review of literature, attend meetings and symposia).
- Records of the relevant educational & professional qualification, training and experience, and assessments of competence must be maintained.

8

2. "Translate" to routine practice

§ 5.1 Personnel

training plan: documents, theoretical, under supervision,…

↓

"test" for analyses with subjective interpretation (reference, EQC,..)

↓

authorization according to criteria

↓

competence declaration or corrective action (additional training), no analysis of patient samples before competency declaration

2. "Translate" to routine practice

§ 5.2 Accommodation and environmental conditions

Focus on **contamination prevention**:

- 2 separate working rooms: Pre-PCR and POST-PCR
- Handling of PCR products may never take place in the pre-PCR room
- Minimize traffic between Pre- and Post-PCR
- Emphasis on the implementation of good laboratory practice (GLP) (filter tips, disposable gloves, aliquoted solutions, …
- Monitoring of possible contamination by validated procedures and controls

10

2. "Translate" to routine practice

§ 5.2 Accommodation and environmental conditions

Focus on **contamination prevention**:
HOWEVER: future automated systems do not fit the Pre/POST PCR setting and therefore **emphasis on GLP and use of adequate controls** is required.

11

2. "Translate" to routine practice

§ 5.3 Laboratory equipment

Critical instruments in molecular diagnostics are :

- Thermal cyclers
- Pipettes
- Refrigerators and freezers

A traceable maintenance scheme and adequate nonconformity procedures should be present for ALL instruments (critical or non-critical).

12

2. "Translate" to routine practice

§ 5.3 Laboratory equipment

Critical reagents in molecular diagnostics are dNTPs, primers, probes and enzymes

- Avoid multiple freeze/thawing cycles : keep track of the amount of cycles or aliquot sufficiently
- Perform entry control

13

2. "Translate" to routine practice

§ 5.4 Pre-examination procedures

- A laboratory handbook, should be provided with clear instructions on kind of sample, sample transport, …
- A separate sample aliquot for molecular testing is preferred (prevention of cross-contamination and specific stability-transport conditions)
- procedure for accepting sample and keeping integrity of sample: amount of cells, transport time, reception, storage

14

2. "Translate" to routine practice

§ 5.5 Examination procedures

Following aspects should be taken into account when implementing a new diagnostic test:

- Scientific evidence found in peer reviewed articles, guidelines or expert opinions
- Target patient population (which might influence subsequent test selection criteria)
- Sample type (ease of collection, transport conditions, minimal volume, …)
- Required turn-around time
- Practical consequence for the laboratory
- Technical test performance should meet clinical needs

15

2. "Translate" to routine practice

§ 5.5 Examination procedures

- A blueprint was formulated which can be used as a template to guide the validation process
- Performance characteristics to be performed will depend on the type of examination procedure (CE/IVD or in-house developed)

	VERIFICATION	VALIDATION
	FDA/CE-IVD Peer review **multicentre** publications	Home brew Adapted FDA/CE-IVD Adapted peer review **multicentre** publications

2. "Translate" to routine practice

Verification/Validation in 4 steps:

1. Postulation of aims: description, method, purpose
2. criteria: expected clinical and technical performance

PLAN of validation: up front

3. results: includes raw data
4. conclusion: fit for purpose or not

RESULTS of validation

2. "Translate" to routine practice

- No consensus on the amount of samples that should be evaluated
- Validated procedures should be re-evaluated at defined intervals
- Reference material might be: Proficiency panels, commercial DNA/RNA panels, cell lines, NIBSC standards, clinical samples characterized by a second, alternative method (e.g. sequencing), spiked samples (negative matrix spiked with plasmids, reference material)

2. "Translate" to routine practice

§ 5.6 Assuring quality of examination procedures

Level 1: assay controls

- Negative amplification control (NTC): mandatory
- Negative extraction control
- Inhibition control/internal run control
- Positive sample control: mandatory, except for CE-IVD/FDA certified multiplex assays

2. "Translate" to routine practice

§ 5.6 Assuring quality of examination procedures

Level 2: quality of reported result depends on **2 parameters** which should be determined during primary validation and updated as a process of ongoing validation:

- **Total uncertainty/precision**: as established by inter-assay reproducibility experiments
- **Trueness/accuracy:** determined by use of reference material or inter-laboratory comparisons (EQC programs or ring controls)

2. "Translate" to routine practice

§ 5.7 Post-examination procedures

2 levels of reviewing and validating:

1. Technical validation:
   - Raw and analyzed data should be available
   - Manual result entry requires double-checking by 2 persons or at 2 different time points.
   - Result acceptance criteria, such as how to deal with non-complying run results of internal controls, should be documented in the analytical test procedure.
2. Medical validation should correlate results with clinical data and finally result in release of test results.

2. "Translate" to routine practice

§ 5.8 Reporting of results

1. The report should contain:
   - a reference to the used examination procedure
   - a medical interpretation of the test results
   - factors interfering with the procedure (e.g. sample criteria).
   - the reason of rejection of a sample for analysis.
2. When available, results should be communicated according to internationally proposed and published recommendations (e.g. t(9;22) BCR/ABL quantitative monitoring and HER2 amplification analysis by FISH)

22

3. Harmonization of current guidelines in 1 European guideline

Current existing guidelines:

- Reflections and proposals to assure quality in molecular diagnostics. *Consensus interpretation on chapter 5 of ISO15189,* Acta Clin Belg. 2011 66(1):33-41. Raymaekers M, Bakkus M, Boone E, de Rijke B, El Housni H, Descheemaeker P, De Schouwer P, Franke S, Hillen F, Nollet F, Soetens O, Vankeerberghen A; on behalf of MolecularDiagnostics.be working group. (www.moleculardiagnostics.be)
- Health Protection Agency. (2013). Guidance on the Development and Validation of Diagnostic Tests that Depend on Nucleic Acid Amplification and Detection. UK Standards for Microbiology Investigations. P 4 Issue 1.

23

3. Harmonization of current guidelines in 1 European guideline

Current existing guidelines:

- Nukleinsäure-Amplifikationstechniken (NAT), Mikrobiologisch-infektiologische Qualitaetsstandards (MiQ, 1/2011, 3. Auflage)
- Public Health England. (2013). Good Laboratory Practice when Performing Molecular Amplification Assays. UK Standards for Microbiology Investigations. Q 4 Issue 4.4
- Quality in the molecular microbiology laboratory, Methods Mol Biol, 2013;943:49-79. Wallace PS, MacKay WG.
- European medical laboratory accreditation. Present situation and steps to harmonisation, Clin Chem Lab Med. 2012 Jul;50(7):1147-52. Huisman W.

24

3. Harmonization of current guidelines in 1 European guideline

Role of ESGMD: make 1 consensus guideline document

Method:

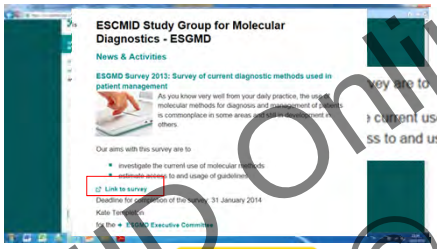
- Harmonize current existing guidelines in case of consensus
- If no consensus:
  - literature evidence
  - discussion with experts in the field

25

3. Harmonization of current guidelines in 1 European guideline

Role of ESGMD: make a consensus guideline document

- Survey of current diagnostic methods used in patient management, to be presented in business meeting ECCMID 2014

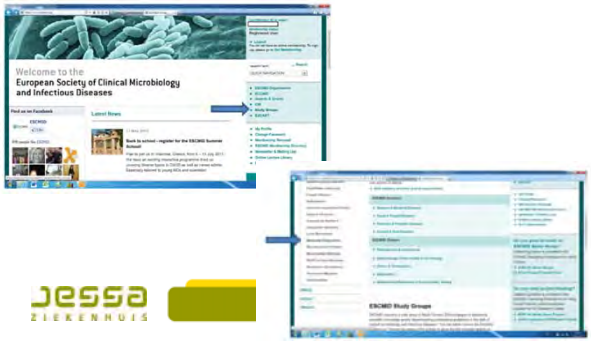


26

3. Harmonization of current guidelines in 1 European guideline

ESGMD contact

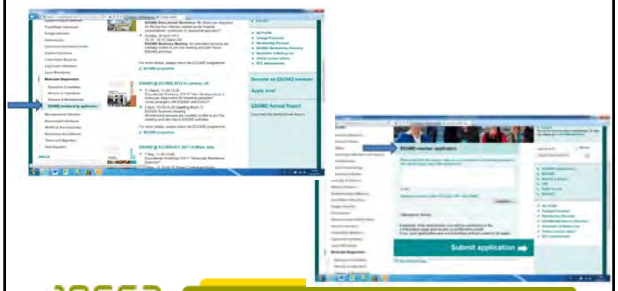
- Member ESCMID can become member by applying through website



3. Harmonization of current guidelines in 1 European guideline

ESGMD contact

- Member ESCMID can become member by applying through website



28

3. Harmonization of current guidelines in 1 European guideline

ESGMD contact

- Webpage ESGMD on ESCMID website:
  - ✓Forum ESGMD
  - ✓Information on past, current and future activities
  - ✓Download annual report

29

3. Harmonization of current guidelines in 1 European guideline

ESGMD contact

- Webpage ESGMD on ESCMID website:
  - ✓publications

30

3. Harmonization of current guidelines in 1 European guideline

ESGMD contact

- Linkedin page (search for "ESGMD groups")

3. Harmonization of current guidelines in 1 European guideline

ESGMD contact

- Mailings to members ESGMD
- Contact secretary ESGMD (marijke.raymaekers@jessazh.be) or board members
- Business meeting ECCMID (Barcelona 11/05/2014, 13h15-14h15)

4. Conclusion

- ISO15189 is the standard for medical laboratories
- The standard is very broad and prone to interpretation differences
- It is very important to make a **European consensus document by ESGMD.**