HCB1/24: Histological and Cytological Staining

This EQA round was accomplished according to the document ***EQA Plan 2024***.

Typing conventions: *We are using comma as a decimal separator and dates in day.month.year format.*

Samples

The samples for this round were prepared by the subcontractor. Each participant received:

- 2 histological slides (labelled A and B).
  Sample A: Lump on the neck, a 51-year-old man diagnosed with TB and AIDS.
  Sample B: Lump on the neck, a 51-year-old man diagnosed with TB and AIDS.
- 2 cytological slides (labelled C and D).
  Sample C: Chest puncture, woman 90 years old, basic dg. congestive heart failure.
  Sample D: Puncture of the pleural cavity, man 56 years old, basic dg. fluidothorax.

The staining to be performed by each participant was prescribed for each slide.

Assessment rules

The tasks of the participants were:

1. Perform staining using a standard procedure that is routinely used in the laboratory (or perform an alternative staining) and mark the staining really used in the result form.
2. Send both stained slides (EQA samples) and filled in result form back to SEKK.

Assessment of participant's staining is performed by a team of 3 experts. This team evaluates the staining quality for each slide separately. The experts evaluate **the quality of staining** on the scale from **0 to 2 points** for each individual slide as follows:

Score (points)	***Description***	***Criteria***
2	Excellent staining	Staining without comments from the experts.
1	Acceptable staining	For HE (sample A) and MGG/HE (samples C and D) staining, weak staining of the nuclei, still allowing to assess the details of the nuclear architecture. Sample B: for both the Ziehl-Neelson and Warthin-Starry methods, a level of staining that still allowed the detection of intracellular bacteria in macrophages as rod-shaped microorganisms in the samples.
0	Unacceptable staining	For HE staining (sample A), very weak staining of the cytoplasm of cells with hematoxylin, practically not allowing tissue evaluation, very weak staining of cell nuclei with eosin, not allowing detailed assessment of the architecture of the nuclei. Sample B: for both the Ziehl-Neelson and Warthin-Starry methods, a level of staining that no longer allowed the detection of intracellular bacteria in macrophages as rod-shaped microorganisms in the samples. For MGG/HE staining (samples C and D), very weak staining of the cytoplasm of cells, practically not allowing tissue evaluation, and very weak staining of cell nuclei, not allowing detailed assessment of nuclear architecture.

Virtually every routinely used staining has many variants that are used according to the local customs and traditions of workplaces. Whether or not individual experts like a particular staining is usually the subject of discussion during the evaluation, but it does not affect the scoring of individual preparations - a key parameter of the assessment is the applicability of the staining in routine operation.

The staining quality of a particular slide is not evaluated if an expert has marked the slide as not assessable, or if the participant used other than the prescribed or alternative staining, or has not done the staining at all.

Experts assess all samples anonymously, i.e. without knowledge of the participant that sent the sample.

Team of the experts	Jana Janková, MD assoc. prof. Tomáš Jirásek, MD, PhD Radoslava Tomanová, MD

Using several anonymous model cases, the experts verified their assessment criteria and discussed possible points of dispute in order to ensure the maximum possible objectivity in the interpretation among all experts.

The scores for individual samples from individual experts are summated, so the sums could range from **0 to 6 points** for each slide. The scores achieved were then evaluated as follows:

Score	***Evaluation***	***Recommendation***
6 or 5	Excellent result	Without comments.
4 or 3	Acceptable result	It is advisable to improve the staining (the staining is not optimal).
2 and less	Unacceptable result	It is a warning signal and an impulse for an immediate action

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If a participant's result is evaluated as “excellent result” or “acceptable result” on the basis of the scoring, then the result is evaluated as **successful** in the EQA.

Supervisor’s comment

There were 90 participants in this round, 7 of them from Slovakia, 1 from Poland and 1 from Romania.

Sample A (histology)

HE (success rate 100 %): Results do not require a comment.

Sample B (histology)

Ziehl-Neelson (success rate 97 %): The participants proved that they can detect acid-resistant rods in the material, for the few laboratories that did not receive the full number of points, we recommend adjusting the relevant methods (mycobacteria in their preparations could either not be detected at all, or the image was erased and the evaluators managed to find rod-shaped microorganisms only with great difficulty).

Warthin-Starry (success rate 38 %): Only 13 participants did the staining, but the success rate was extremely low, it was a replacement staining, usable only as a non-specific proof of bacteria in the sample, of course no one can use this staining as a proof of mycobacteria. A few sites have shown that their method stains bacteria in the cytoplasm of macrophages as rod-like formations; for the rest of the laboratories that did not pass the evaluation successfully, we recommend revising the method again on their own controls (usually a stomach and helicobacter test), because for some reason their method settings do not stain the bacteria as they should.

Sample C (cytology)

HE (success rate 100 %): Results do not require a comment.

MGG (success rate 100 %): Results do not require a comment.

Sample D (cytology)

MGG (success rate 99 %): The results basically require no comment. We leave isolated negative/weak staining without a recommendation, it could be, for example, a technical error during sample processing.

HE (success rate 100 %): Results do not require a comment.

Summary

With the above-mentioned exception in the case of Warthin-Starry staining, most of the specimens we received showed good staining and were, according to experts, usable in routine practice; the thickness of the sections is a matter of local custom, as is the intensity of tissue staining with hematoxylin and eosin. Samples that some laboratories consider to be excellent may be evaluated by another workplace as thick and unsatisfactory, or discolored (and vice versa). We repeat that the evaluation criterion is applicability in routine practice, not the "artistic tone" of the sample in front of the "jury".

Long term success rate

You can find in the following table the overview of the total success of the participants of this round over last 2 years. Individual ranges of success are defined in the column headers (0 % … no success; 50 % … success from 1 to 50 %; 75 % … success from 51 to 75 % etc.). Next 2 lines contain both absolute and relative number of participants that reached the success rate specified in the header.

Success		***0 %***	***1 - 74 %***	***75 - 79 %***	***80 - 89 %***	***90 - 94 %***	***95 - 99 %***	***100 %***
Success in words		unsatisfactory		acceptable	good	very good	excellent	
Count	absolute	0	0	2	1	14	0	73
	relative	-	-	2,2 %	1,1 %	16 %	-	81 %
Note: You can find your individual success over last 2 years in your result sheet.								

The table shows that the most participants in this round show a long-term success rate of over 90 %.

Long-term success in this round has touched the absolute optimum, which we consider a long-term success rate of 95 % or higher for all participants.

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Supplements

As a supplement to this report individual participants receive:

Name of the supplement	***Remark***
Confirmation of attendance	Issued only to those participants who have met the conditions for its issuance.
Result sheet	Issued only to those participants who sent us the results.

The supplements are labelled by its name, the code of the EQA round, and the code of the participant and are intended for the participant's private purposes only.

Also we return the slides that we received from the participants.

Additional information

The final report, with the exception of the supplements, is public. Further information is freely available to both participants and other professionals at www.sekk.cz, in particular:

- The summary of the results of this round, including this final report.
- The document ***EQA Plan*** (contains information that applies both to this round and also the EQA in general).
- Explanation of the content of the particular supplements mentioned above.
- Contact to the EQA provider and the EQA coordinator and the list of all supervisors, including contacts.