HCB2/23: Histological and Cytological Staining

This EQA round was accomplished according to the document ***EQA Plan 2023***.

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: Microscopic sections of bone marrow with varying degrees of myelofibrosis.
  Sample B: Microscopic sections of bone marrow with varying degrees of myelofibrosis.
- 2 cytological slides (labelled C and D).
  Sample C: Fluidothorax, female, born 1948.
  Sample D: Fluidothorax, male, born 1955.

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 staining (sample A) and MGG/HE (samples C and D) weak staining of the cores, which still allows to assess the details of the nuclear architecture. Sample B: For the method of histochemical detection of reticular fibers such a level of staining that still made it possible to detect reticular fibers in the samples and evaluate the degree of myelofibrosis. For the blue trichrome method, the staining of the tissue, which also made it possible to differentiate the collagen fiber from the surrounding tissue by its staining properties.
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 to assess in detail the architecture of the nuclei. Sample B: For the method of histochemical detection of reticular fibers such a level of staining that it was no longer possible to detect reticular fibers in the sample and evaluate the degree of myelofibrosis. For the blue trichrome method, the staining of the tissue, which no longer made it possible to distinguish the collagen fiber from the surrounding tissue by its staining properties. 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 to assess the architecture of the nuclei in detail.

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	Vít Campr, MD assoc. prof. Tomáš Jirásek, MD, PhD Inna Tučková, 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:

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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

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 87 participants in this round, 7 of them from Slovakia and 1 from Poland.

Sample A (histology)
HE staining (success rate 100 %): Results do not require a comment.

Sample B (histology)
Histochemical detection of reticular fibers (success rate 97 %): We noted unsatisfactory staining in 2 participants, when it was no longer possible to evaluate changes in the tissue as myelofibrosis, let alone to determine its degree. A relatively large number of workplaces achieved only an acceptable result - we also recommend to them to revise and optimize their established method of detecting reticular fibers in the tissue, we can focus on retesting the detection of reticular fibers in one of the next rounds of the program.
Blue trichrome (success rate 97 %): We noted unsatisfactory staining in 1 participant, when it was not possible to find the collagen fibers present in the samples (usually on the surface of the bone beams and in the intertrabecular spaces).

Samples C and D (cytology)
MGG staining (success rate 100 %): Results do not require a comment.
HE staining (success rate 100 %): Results do not require a comment.

Summary
In the opinion of the experts the vast majority of the submitted preparations demonstrated good quality staining and routine practice usability; section thickness is a matter of local habit, as is the intensity of a tissue staining with hematoxylin and eosin. Samples that some laboratories consider excellent may be evaluated by another workplace as thick and unsatisfactory, or discoloured (and vice versa). We reiterate that the measure of evaluation is usability in routine practice, not the "artistic impression" of the sample before 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	0	0	7	0	80
	relative	0 %	0 %	0 %	0 %	8 %	0 %	92 %
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 (qualitative results)	Issued only to those participants who sent us the results. In the result sheet you can find the scoring of the staining which was performed by a team of experts for individual glasses (the symbols are explained in the legend). Here you can compare your results with the anonymised results (points) of other participants.

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.