VIB1/23: General Immunohistochemistry - 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 (the slides bearing unstained TMA sections) for this round were prepared by the subcontractor.
Each participant received 5 slides (labelled A to E) and the staining to be performed by each participant was prescribed for each slide. In the event that a participant could not perform the prescribed staining, the participants had at their disposal other markers from which they could choose an alternative.
In the event that more samples on the slide (3 or more) were damaged during staining, the participant could request the replacement slide. **Therefore, it is necessary for participants to process the samples as soon as possible after the delivery** (only this way they have a chance to obtain a replacement glass before the deadline of the round).

Assessment of the participants' results

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	Low level of expected staining, strong background.
0	Unacceptable staining	Absolutely negative or very low level of staining at the expected location, little difference between weak signal and high background staining virtually impossible to assess. It should be noted that only those samples which, in the expert's opinion, cannot be used in the routine practice receive zero points.

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	Pavel Fabian, MD, PhD Alice Hlobilková, MD, PhD Daniela Skanderová, 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 (EQA sample). The achieved scores were then evaluated as follows:

Sum of points	***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 solution

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.

The design of this scheme is inspired by the NORDIQC system, the established European provider of EQA for immunohistochemistry. It is highly recommended to view the following pages when choosing primary antibodies and optimal protocols: www.nordiqc.org

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Supervisor's comment

There were 80 participants in this round, 13 of them from Slovakia, and 1 from Hungary.

Tissue selection both for EQA and IQA follows one general rule: a properly functioning method will stain well samples with low antigen expression levels. That is why the tissues are included where, with a sufficiently sensitive method, the staining result is weak. In this round, it is, for example, a weak positivity of p16, p40 and p63 in the stromal cells of the placenta, a weak to moderate positivity of CK 7 in the intercalary cells of the pancreas or a weak positivity of synaptophysin in the adrenal cortex.

The results in this round were very good. Unsatisfactory (eventually acceptable) results were usually conditioned by weaker than expected positivity, false positive were only sporadic, but if they occurred, they were very significant, such as p40 and p16 strongly staining nuclei in all cell types including lymphocytes. Any result in the "acceptable" category should be an incentive to optimize the method.
Note: In some appendix samples, a tumour lesion surprisingly appeared when cutting the blocks, this did not affect the evaluation of the IHC staining by the experts.

Some participants will find individual comments in their result sheets.
Please pay attention to them.

Achieved success rates (see the web statistics for a detailed overview including the summation of scores):

Sample A

p16 (success rate 99 %): Results do not require a comment.
p40 (success rate 75 %): The low success rate is due to the small number of the participants (succeeded 3 of 4). Results do not require a comment.
p63 (success rate 100 %): Results do not require a comment.

Sample B

S-100 (success rate 96 %): Results do not require a comment.
SOX10 (success rate 88 %): Results do not require a comment.

Sample C

CK 7 (success rate 99 %): Results do not require a comment.
CK AE1/AE3 (success rate 100 %): Results do not require a comment.

Sample D

PAX-8 (success rate 96 %): Results do not require a comment.
WT-1 (success rate 100 %): Results do not require a comment.

Sample E

chromogranin (success rate 97 %): Results do not require a comment.
synaptophysin (success rate 100 %): Results do not require a comment.
CD 56 (success rate 100 %): Results do not require a comment.

Long term success rate

You can find the overview of the total success of the participants of this round over last 2 years in the following table. Particular ranges of success are defined in the column headers (percentage of **the tests** on which the participant reported the correct result). Next 2 lines contain both absolute and relative number of participants **who** reached the success from 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	1	1	12	14	9	43
	relative	-	1,3 %	1,3 %	15 %	18 %	11 %	54 %
Note: You can find your individual success over last 2 years in your result sheet.								

Overall success of most participants of this round over the last 2 years is 80 % or higher.
Success rates below 80 % should be considered to be an impulse to improve.

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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 sent us the results.
Result sheet (qualitative results)	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 all 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.