Round: VIB1/20 – General Immunohistochemistry - Staining

This EQA round was accomplished according to the document **EQA Plan 2020**.

Note for the participants from the abroad
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 at the sub-contractor's site.
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 participant's results

The tasks of the participants are

1. Perform staining using a standard procedure that is routinely used in the laboratory (or perform a 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 a 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 unassessable, or if the participant used other than the prescribed or alternative staining, or has not done the staining at all.
We do not process the slides and the results sent by the participants after the expert group meeting.
Experts assess all samples anonymously, i.e. without knowledge of the participant that sent the sample.

Team of the experts	Pavel Fabian, MD, PhD Karel Veselý, MD, PhD Víta Žampachová, 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 this scheme is inspired by the NORDIQC system and website, the established European provider of EQA for immunohistochemistry. It is highly recommended that you review the following pages when choosing primary antibodies and optimal protocols: www.nordiqc.org

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Supervisor's comment

There were 73 participants in this round, 10 of them from Slovakia, and 1 from Hungary.

Tissue selection both for EQA and IQA follows a 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, weak positivity of smooth muscle actin in perisinusoidal liver cells, moderate positivity of CDX-2 in intercalted pancreatic duct cells, or weak positivity of WT-1 in stromal cells of the fallopian tube mucosa.

The results in this round were excellent. The overall success rate for all antigens exceeded 95 % and most of the results were even marked as optimal by experts. Only 6 individual staining out of a total of 327 performed was evaluated as unacceptable. Both "unacceptable" and "acceptable" results are mostly based on a weaker than expected positivity, false positive results are rare. Any result classified as "acceptable" should be considered as an impulse to optimise the method. Unsuccessful participants receive individual comments.

Please read the individual comments located in your results sheets.

Achievements (see the web statistics for a detailed overview including the summation of scores)

Sample A

GFAP – success rate 92 % - approximately 60 % of laboratories achieved week staining in all tissues in the TMA block and the experts agreed that the tissue immunoreactivity was probably affected by the time delay between sample preparation and staining itself. Therefore, we evaluated these weak GFAP results as acceptable. On the other hand, one third of the participants detected GFAP with excellent results. Only two participants did not succeed, their results were practically negative in all tissues and significantly worse compared to all others.

S-100 - success rate 98 % - results do not require a comment.

Sample B

Desmin - success rate 98 % - results do not require a comment.

Smooth muscle actin - success rate 94 % - results do not require a comment.

Sample C

CK 20 - success rate 100 % - results do not require a comment.

CDX-2 - success rate 100 % - results do not require a comment.

Sample D

PAX-8 - success rate 97 % - results do not require a comment.

WT-1 - success rate 100 % - results do not require a comment.

Sample E

IgG4 - success rate 100 % - results do not require a comment.

CD 138 - success rate 100 % - results do not require a comment.

CD 20- success rate 100 % - results do not require a comment.

Comments from the participants

Comments on lower quality of the samples were exceptional, some tissues were cut-off during preparation, according to valid rules this does not affect the usability of the samples for EQA testing. We apologize for these minor shortcomings.

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 %***	***50 %***	***75 %***	***80 %***	***85 %***	***90 %***	***95 %***	***99 %***	***100 %***
Count	absolute	0	0	3	3	3	10	25	0	29
	relative	-	-	4,1 %	4,1 %	4,1 %	14 %	34 %	-	40 %
Note: You can find your individual success over last 2 years in your result sheet.										

The table shows that almost all participants in this round show a long-term success rate of over 80 %.

A success rate of 80 % or less was achieved by 6 (i.e. 8,2 %) participants, which should be an impulse for the improvement.

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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 that fulfilled the criteria.
Result sheet (qualitative results)	Issued only to those participants that reported qualitative 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.
- List of all supervisors, including contacts.
- EQA Plan (contains information that applies both to this specific round and EQA in general).
- Explanation of the contents of the above-mentioned supplements.
- Contact to the EQA provider and the EQA coordinator.