VIB2/24: General Immunohistochemistry - 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 (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 Daniela Skanderová, MD, PhD Vladimír Židlík, MD, MBA, MIAC

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 EQA programme 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 82 participants in this round, 12 of them from Slovakia, 3 from Romania and 1 from Poland.

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 was, for example, weak to moderate positivity of hepatocytes in the detection of CK AE1/AE3 or moderate positivity of intercalary duct epithelia in the detection of CK 7 or CDX-2.

The results in this round were worse than we have been used to lately. The unsatisfactory (or acceptable) results were mostly due to weaker than expected positivity, sometimes on the contrary, strong background or even false staining of other than expected cells. Any result in the "acceptable" category should be a stimulus for method optimization.

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

Results of the participants (you can find a detailed overview, including the number of results, in the statistics on the web):

Sample A

BAP1 (success rate 75 %): This marker was included for the first time in the programme, out of twelve laboratories that stained it, three failed and three others succeeded with an acceptable, not optimal result. All of these sub-optimal images were conditioned by very weak nuclear positivity with simultaneous strong staining of the cytoplasm and extracellular matrix. On the contrary, some laboratories sent really beautiful slides for evaluation, so we approached one of them through SEKK and obtained their permission to publish the clone of the antibody used – it is clone/catalog number BAP1 (C-4): sc-28383, the complete protocol for the Ventana platform will be provided by the supervisor of this round upon request (also with the kind permission of the laboratory in question).

CK AE1/AE3 (success rate 84 %): The poor results in this very widespread staining are surprising and are almost without exception due to weak positivity – these are most noticeable in hepatocytes, where even weak positivity was often not achieved, and in these failing participants, part of the renal tubules, acinar cells of the pancreas and parabasal areas in the squamous epithelium of the tonsils were also weakly stained, so this is really a problem in the staining, not a problem of one potentially inappropriately selected liver sample. Only rarely did strong staining lymphocytes occur simultaneously with weak staining.

Sample B

SATB2 (success rate 97 %): Results do not require a comment.

CDX-2 (success rate 100 %): Results do not require a comment.

CK 20 (success rate 100 %): Results do not require a comment

Sample C

Napsin A (success rate 84 %): All unsatisfactory results are due to weak positivity - this was especially evident in kidney tissue, and to a lesser extent in lung tissue. Several participants achieved very strong false positive staining in pancreatic islet Langerhans cells.

CK 7 (success rate 94 %): Results do not require a comment.

Sample D

HMB 45 (success rate 99 %): It is clear that a well-selected angiomyolipoma as a "low expressor" is a suitable control tissue, while melanoma is of little use - most show too high expression of the antigen, while some melanomas are completely negative.

Melan A (success rate 89 %): The slide with false strong positivity in adipose tissue and in normal kidney was unsatisfactory. Acceptable results were characterized by expected but weak staining. It is clear that a well-selected angiomyolipoma as a "low expressor" is a suitable control tissue, on the contrary melanoma is of little use – most show too high expression of the antigen, on the contrary some melanomas are completely negative.

Sample E

p53 (success rate 95 %): Results do not require a comment.

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

PAX-8 (success rate 83 %): Most participants apparently use an antibody with cross-reactivity against other PAX family proteins, which is manifested in particular by strong positivity in B-lymphoid cells. This cannot be considered a failure (it is not a defect, it is a property of the method), but we consider it appropriate to point out to the participants that they should keep this fact in mind in practice. The unsuccessful participant even achieved a nice positivity only in lymphocytes, while the kidney tubules remained completely negative.

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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	3	0	7	21	11	40
	relative	-	3,7 %	-	8,5 %	26 %	13 %	49 %
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 90 % or higher.
A success rate of less than 90 % should be considered an impetus for improvement.

Scientific supervision: Pavel Fabian, MD, PhD
Masaryk Memorial Cancer Institute
Department of Oncological and Experimental Pathology
Žlutý kopec 7
Brno
e-mail: fabian@mou.cz

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	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 to be used by the participant.

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.