VIB1/26: General Immunohistochemistry - Staining

This EQA round was accomplished according to the document ***EQA Plan 2026***.

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).

By mistake, when preparing samples for this round, glass slides intended primarily for DAKO instruments were used, which are incompatible with VENTANA instruments. This led to very serious failures in some of the slides (false negative reactions on parts of the slide or on the entire surface of the slide). Some laboratories identified and reflected this fact already during the round. The experts took this fact into account when assessing - in slightly damaged slides, unstained areas were not assessed, in cases of more significant damage, these slides were marked as unevaluable and the participants were not assessed (marked as " Improper glass" in the results).
We apologize to all participants.
Of course, we have taken adequate measures so that this problem does not happen again.

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 (or not specified) 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 Jitka Kyclová, MD 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 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 87 participants in this round, 1 of them from Germany, 1 from Poland, 3 from Romania, 14 from Slovakia and 1 from United Kingdom.

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, for example, it is weak to moderate positivity in the intercalary ducts of the pancreas when detecting CK 7 or moderate positivity in spermatocytes when detecting SATB-2.

The results in this round were very good, with most stainings having a 100 % success rate, only for the CD 99 antigen the results were quite uneven and the success rate was the lowest. Acceptable results were mostly due to weaker than expected positivity. Every result in the "acceptable" category should be an impulse to optimize the method.

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

CA IX (success rate 100 %): Results do not require a comment.
PAX-8 (success rate 100 %): Results do not require a comment.
CK 7 (success rate 100 %): Results do not require a comment.

Sample B

TRPS-1 (success rate 90 %): Results do not require a comment.
GATA-3 (success rate 100 %): Results do not require a comment.
ER (success rate 100 %): Results do not require a comment.

Sample C

IFITM-1 (success rate 100 %): Results do not require a comment.
CD 10 (success rate 100 %): Results do not require a comment.
H-caldesmon (success rate 100 %): Results do not require a comment.

Sample D

CK 20 (success rate 100 %): Results do not require a comment.
SATB-2 (success rate 100 %): Results do not require a comment.
CDX-2 (success rate 100 %): Results do not require a comment.

Sample E

CD 99 (success rate 82 %): According to the literature, CD 99 expression in normal tissues is present on a large number of cell types, which we also observed during the assessment, where, contrary to the literature, we saw, for example, constant expression on pancreatic acinar cells or on endometrial gland epithelia. Most preparations showed a greater or lesser tendency to background staining. Where this phenomenon reached an extreme with strong positivity of completely all cells and non-cellular structures, we marked the preparations as unsatisfactory. In any case, basal epithelia in the squamous epithelium of the esophagus, islets of Langerhans of the pancreas and endothelia of the liver sinusoids are reliable positive controls.
CK AE1/AE3 (success rate 94 %): Results do not require a comment.
Pan-CK (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	3	1	9	6	15	53
	relative	-	3,4 %	1,1 %	10 %	6,9 %	17 %	61 %
Note: You can find your individual success over last 2 years in your result sheet.								

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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: pavel.fabian@mou.cz

Supplements

As a supplement to this report individual participant who reported the results gain:

Name	***Description***
Confirmation of attendance Result sheet	The conditions for issuing the relevant document specified in the EQA Plan must be met.

The supplements are identified by their name, EQA round identification and participant code and are intended for the needs of the participant.
If you reported the results in this round, you can find your individual evaluation (reports) in the **Cibule** application (https://www.eqa.cz/cibule). After login, select ***EQA Results - View*** in the menu and then click the ***Reports*** button for the particular round.
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.