VIB1/24: 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 Helena Hornychová, MD, PhD Petra Kašparová, MD, PhD

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 79 participants in this round, 12 of them from Slovakia, and 1 from Romania.

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 to moderate positivity of the liver perisinusoidal cells in the detection of smooth muscle actin or a moderate positivity of the epithelia of the intercalary ducts in the detection of CK 7.

The results in this round were very good. Unsatisfactory (or acceptable) results were mostly conditioned by weaker than expected positivity. We also recorded cases of false positives, which were very numerous in the case of SMA - see below. 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

TTF-1 (success rate 98 %): Results do not require a comment.
CK 7 (success rate 94 %): Results do not require a comment.

Sample B

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

Sample C

SMA (success rate 88 %): We observed a systematic error where a number of participants sent preparations with false positive results. In some cases, it was only a weak and limited staining of some structures (typically keratinocyte nuclei), in many cases it was positivity in all epithelia, lymphocytes, fibroblasts, etc., especially nuclear positivity, which we had to evaluate as unsatisfactory results. We have previously noted this problem within the VIB rounds, and it is also repeatedly documented in the rounds of the Scandinavian external quality control system NORDIQC (see the link below). Their conclusion, based on a comparison of a large number of participants, is - briefly - this: The mostly used clone 1A4 in combination with an inappropriate protocol (particularly, but not exclusively, with the Ventana instruments) systematically leads to these false positive results including nuclear staining. We therefore recommend all affected laboratories to consider modifying the protocol. Looking at the results of the last SMA run in NORDIQC, it is obvious that the most problematic is the use of the RTU antibody in Ventana, when using the concentrate (with the possibility of titre optimisation) the results are significantly better. A complete replacement of the primary antibody is also a possible solution, other clones (especially asm-1) have a higher success rate in NORDICQ, but are used by a minimum of laboratories, so the evaluation may be biased by small numbers.
https://www.nordiqc.org/downloads/assessments/134_6.pdf
Desmin (success rate 100 %): Results do not require a comment.

Sample D

MLH1 (success rate 82 %): Only half of the laboratories have optimal results, the others, without exception, stain weakly. For this antibody (taking into account the extraordinary clinical importance of MMR deficiency testing) we were stricter in the evaluation than for the others and marked glasses with an obvious but weak reaction as unsatisfactory. The optimal result is fluctuating weak to strong positivity of all cell nuclei except for hepatocytes and two MLH-1 deficient adenocarcinomas. Positivity limited only to the germinal centres in the tonsil indicates a low sensitivity of the reaction, which we consider clinically unacceptable. For comparison see NORDIQC:
https://www.nordiqc.org/downloads/assessments/171_81.pdf
Ki 67 (success rate 98 %): Results do not require a comment.

Sample E

CD 3 (success rate 98 %): Results do not require a comment.
CD 20 (success rate 100 %): Results do not require a comment.
LCA (success rate 100 %): Results do not require a comment.

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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	1	2	8	14	14	40
	relative	-	1,3 %	2,5 %	10 %	18 %	18 %	51 %
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	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.