Round: IHC1/20 – Immunohistochemistry - Detection of HER-2/neu

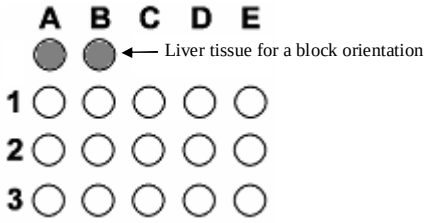
This EQA round was accomplished according to the document ***EQA Plan 2020***.
Typing conventions: *We are using comma as a decimal separator and dates in day.month.year format.*

Samples

EQA samples:

Each participant received one histological glass (TMA). All slides contained identically arranged samples from identical source tissue blocks. The TMA block map is displayed on the right. Source blocks were selected from the cases that had been previously tested with an immunohistochemically certified kit (Ventana PATHWAY anti-HER-2/neu) and some of them (especially those with 2+ positivity) also with the FISH method.

The samples were prepared by the subcontractor.



IQC samples **(internal quality control)**:

In addition to the EQA sample the participants also send their own routine IQC glass to the provider.

Assigned values and methodology of the assessment

The assessment of the results of the participants in this EQA scheme is divided into 2 parts.

1 In the first part, the results of **HER-2/neu expression** determined by the participants for individual TMA positions are evaluated.
The assigned values are determined at the laboratory that prepared the EQA samples.

2 In the second part, the assessment is performed by the team of 3 experts. This team evaluates the **quality of staining** by scoring:

- On a scale of 0 to 5 points for each TMA position of the EQA sample.
- On a scale of 0 to 3 points for a participant's IQC sample.

If a participant marks a sample as unevaluable at some TMA position - for objective reasons of reducing the amount of tumour tissue in the sample - then this sample will not be evaluated if the experts also mark the sample as unevaluable (otherwise the participant's missing result is considered erroneous).
The rules for assessment were defined in advance, i.e. when the scoring will be reduced. These were mainly the following factors: strong cytoplasmic positivity of staining (potentially increasing the difficulty or even making impossible to assess membrane expression), background staining, positivity in normal mammary gland, intensity and completeness of membrane staining higher or lower than it should be. The difference between staining rated as 0 and 1+ was considered insignificant - it is not a situation that would in any way change the further procedure (neither diagnostic, nor therapeutic).
The experts assess all samples anonymously, without knowing the identification of the participant or the kit that was used for the examination.

The team of the experts	Iva Babánková, MD Pavel Fabian, MD, PhD Rudolf Nenutil, MD, PhD

The points for individual samples (TMA positions) assigned by the individual experts are summed, thus the sums can reach 0 to 15 points for EQA glass and 0 to 9 points for IQC glass. The achieved sums of points are then evaluated as follows:

	EQA sample	***IQC sample***
Excellent result	13 to 15 points	8 and 9 points
Acceptable result	8 to 12 points	5 to 7 points
Unsatisfactory result	0 to 7 points	0 to 4 points

The participant's result is generally considered **successful** if it is "excellent" or "acceptable".

Comments from the experts

If the experts find shortcomings while assessing a specific glass, which the participant should pay attention to (even if they did not necessarily result in a reduction of the point evaluation), they will write a text note for the given participant. The note is then printed as part of the individual comment in the participant's result sheet.
The purpose of these verbal comments is to provide the participant with a feedback to help identify which of the steps of the analytical phase could be the cause of the suboptimal result. Experts often commented despite the fact that the final result of all staining was flawless - verbal evaluation allows for finer feedback than simply subtracting the points. If, for example, the laboratory has all samples a bit more strongly stained, it does not necessarily lose points (samples with their positivity still "fit" into the evaluation categories), but when comparing the slide with glasses from other laboratories it is clear that positivity is across individual sub-samples higher and in real life this could in some cases lead to a potential error. Similarly, when comparing samples from all participants, it is possible to identify, for example, samples with signs of too aggressive unmasking of epitopes ("boiled"), with non-specific background staining, etc.

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Supervisor's comment

There were 44 participants in this round, 9 of them from Slovakia, and 1 from Hungary.

EQA samples

Several participants stated in the note that some samples had been cut-off, this phenomenon (for which we apologize) cannot be completely prevented in a practice.

Negative and strongly positive samples

We did not encounter cases where the laboratory would produce false positive results, i.e. all negative samples were interpreted correctly as negative (the difference between 0 and 1+ is not significant and is not penalized in any way in the evaluation). Only one participant identified negative sample A1 as weak positive.

Also, the interpretation of strongly positive (3+) samples did not cause major difficulties for the participants, although there were slightly more errors. Specifically, 14 % of participants identified the sample at position A2 as negative.

Thus, if the TMA block contained only samples with these two expression levels, the results would be excellent.

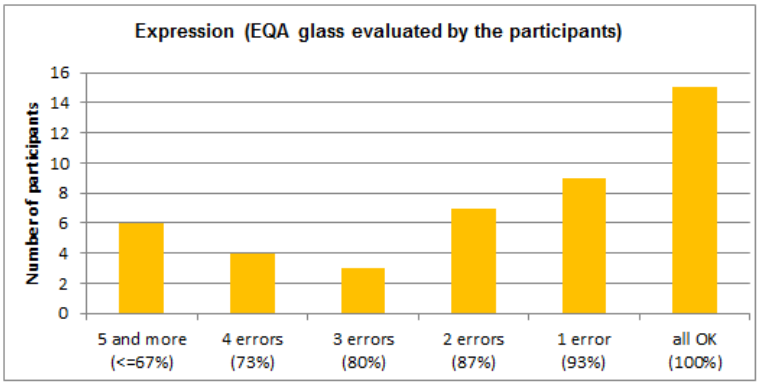
As can be seen from the above, most laboratories stain and evaluate well both negative (0/1+) and positive (3+) samples.

Weak positive samples

Weak positive (2+) samples were located at the positions A3, B2, C1, C2, C3 and E1, samples with verified Her-2 gene amplification were placed at the positions B2, C3 and E1. With the exception of position C1, where 93 % of the participants reported the correct result, these samples made difficulties to the participants. It was these samples that caused a large proportion of participants to experience 1 to 3 errors (and some more) in the expression evaluation.

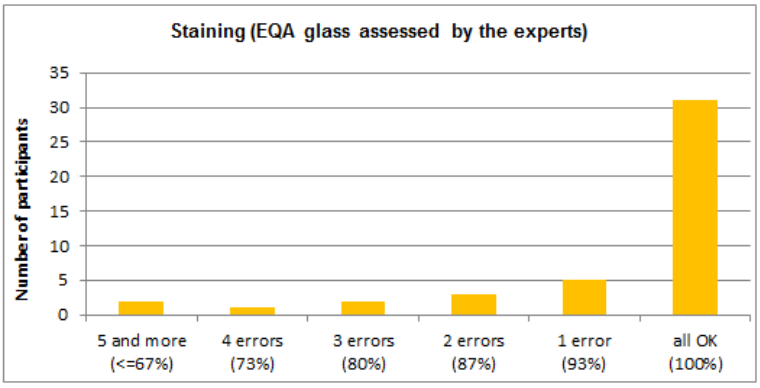
The overall success distribution for **the interpretation of 15 TMA samples by the participants** is shown in the figure on the right.

15 participants achieved 100 % success,
9 participants achieved 93% success
(i.e. 1 error), etc.



The overall success distribution for **the assessment of staining of 15 TMA samples by the experts** is shown in the figure on the right.

31 participants achieved 100 % success,
5 participants achieved 93 % success
(i.e. 1 error), etc.



In the case of the samples that had 2+ expression during preparation, the participants' results were as follows:

- A3: 48 % of participants determined it as 2+
- B2: 73 % of participants determined it as 2+
- C1: 93 % of participants determined it as 2+ or 3+
- C2: 77 % of participants determined it as 2+ or 3+
- C3: 73 % of participants determined it as 2+ or 3+
- E1: 68 % of participants determined it as 2+

Even when using the same methodology that was used in sample preparation and with proven Her-2 gene amplification, there was no consensus among the participants (2+ was reported by only 77 % of these participants in the sample B2, only 68 % in C3, and only 68 % in E1). The good news is that the evaluation of these "problematic" samples by the

laboratories and experts is not fundamentally different, i.e. what the laboratory determined as 1+, the experts mostly also considered 1+. In some cases, it is clear that the participants are adapted to their "weak" staining and the samples, which the experts rated as 1+, are referred to by the laboratory as 2+. This is not a procedure we could recommend, it is certainly better to tune the staining to the correct sensitivity, on the other hand it means that the laboratory sensitively perceives its own problem in staining and catches up with it in a stricter evaluation.

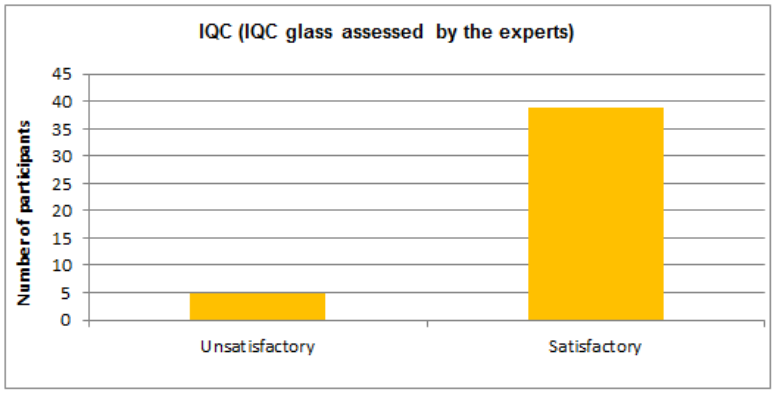
Thus, we repeatedly register a problem in the sufficient sensitivity of the detection of 2+cases, although to a lesser extent than in previous years. In case of the samples with proven amplification of the Her-2 gene, the patient would be harmed in clinical practice.

A recurring problem we observe in certain technical shortcomings in sample processing. In EQA samples as well as in the internal quality control preparations (IQC), we occasionally noticed significant signs of unnecessarily aggressive antigen unmasking, but there were fewer of these cases than in previous rounds. In some participants, we also noticed too intense hematoxylin staining, sometimes to a degree that significantly complicated the evaluation of the expression. Technical problems in the quality of staining (which do not necessarily result in the loss of points) are brought to the attention of the laboratories concerned in the form of individual comments (part of the results sheet). Please pay attention to them, relatively easy measures can lead to a clear improvement in the quality of staining, and thus to facilitate the interpretation of the immunohistochemistry.

IQC samples

The overall success distribution for **the assessment of the IQC samples by the experts** (IQC glass is evaluated by the experts as a whole, i.e. it is evaluated as one sample) is shown in the figure on the right.

39 participants (i.e. 89 %) succeeded.
The experts classified IQC glass as unsatisfactory in 5 participants (i.e. 11 %).

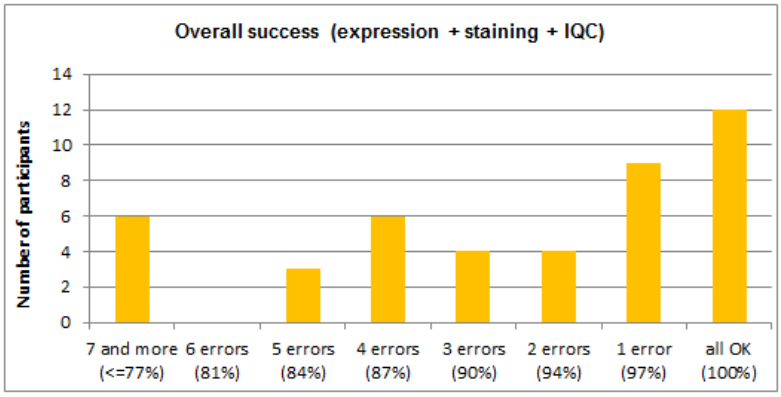


Basal analysis of the internal quality control results is fully sufficient to identify most problems in detection, as long as it is performed continuously and samples are selected properly. The laboratory can easily detect poor staining quality long before participating in the EQA - the EQA usually only confirms the problem in the laboratory.
In general, we rated the quality of participants' internal controls as good, almost all use compound tissue blocks with intensities of 0/1+/2+/3+. The use of needle biopsy specimens has completely vanished.

Overall success

The distribution of **the overall success** (including the interpretation of the EQA sample by the participant + scoring of the staining of the EQA sample by the experts + scoring of the VKK sample by the experts: i.e. a total of 31 tests) of the individual participants is shown in the graph on the right.

Each participant will find their own overall success at the end of their result sheet.



Cumulative sums of deviations from assigned values

Explanation of the term: These sums are calculated only for **the evaluation of the expression by the participants**. For each participant, the deviations from the assigned values for the samples at all positions of the TMA block are cumulatively summed, both respecting the sign (deviations downwards with a minus sign, deviations upwards with a plus sign) and in absolute value. The difference between the negative (0) and negative (1+) ratings is calculated as zero.

Example: the laboratory evaluated sample X1 (which was to be determined as 3+) as 0 and sample X2 (which was to be determined as 0) as 2+. The sum of the deviations with respect to the sign is therefore (-3) + (+2) = -1, and the sum of the absolute values of the deviations is 3 + 2 = 5.

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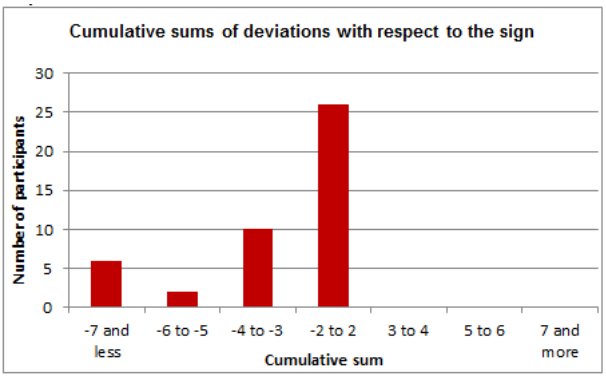
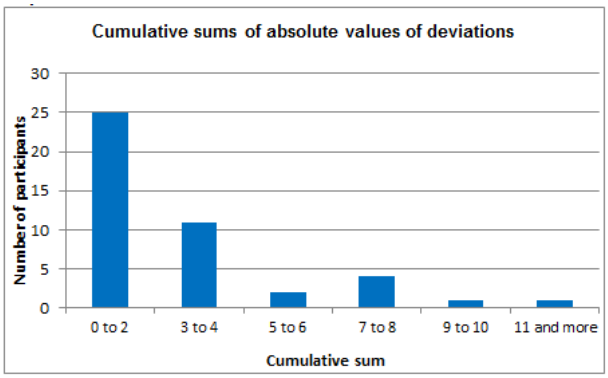
This view to the results can identify the laboratories that tend to overestimate (sum of deviations with respect to the sign is positive), or underestimate (sum of deviations with respect to the sign is negative), and those that have completely inconsistent results of interpretation (and usually staining) - sum of deviations with respect to the sign approaches 0, but the sum of the absolute values of the deviations is 10 or more.
The participants will find their own cumulative sums of deviations (respecting the sign and the sums of absolute values) in their result sheet as part of an individual comment.
To allow comparisons and to see if your cumulative totals are in the mean range or if they deviate in any way, you can find histograms of these deviations for all participants of this round in the figures below.
As we can be seen in the graphs below, the sum of the deviations from the assigned values:

- respecting the sign reached -5 and less in 8 laboratories, while in 26 participants it was in the range of -2 to +2.
- in case of the absolute values it reached 5 and more in 8 laboratories, while in 25 participants it was a maximum of 2.





A comprehensive view of the results
For a complex evaluation of how the laboratory performed in the EQA, 6 sources of the information can be used, which we described in detail above. None of them can be interpreted in isolation, but all need to be considered together. They are:

1) EQA sample - success of the interpretation
2) EQA sample - success in the assessment of the staining by the experts
3) IQC sample - success in assessment by the experts
4) Overall success in the round (includes the 3 categories above)
5) Cumulative sums of deviations from assigned values (respecting the sign and absolute values)
6) Comments from the experts (text notes)

Conclusion
For unsuccessful participants, appropriate corrective action should be taken in accordance with good laboratory practice.
However, it should be borne in mind that even repeated success in the EQA is not an automatic guarantee of the lasting quality of the laboratory's work. Therefore, I ask all participants to pay constant attention to quality control in daily operation. Problems have occurred, occur and will occur in all laboratories. The point is to identify them - as soon as possible after they occur - and to take corrective action to eliminate errors. Only in this way will we be able to provide consistently high-quality results and thus help patients with breast cancer.

Please pay attention to the individual comments that you will find in your result sheets.

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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)	At the beginning, the cumulative sums of the deviations are given as a part of the individual comment. Further in the result sheet you will find (symbolism is explained in the legend): a) Results of the interpretation of individual samples (these are tests named **A1 expression HER-2/neu**, etc.). Each sample also shows how it was evaluated by other participants. b) Scoring of the staining performed by a team of the experts (these are tests named **A1 sample staining**, etc.). Again, you can compare your results with the anonymized results (scores) of the other participants. c) Scoring of internal control preparation (test named **IQC**). Due to the fact that the type of internal controls used differs between the laboratories, the quality of the staining and its interpretation are only summarized for the glass as a whole, not for the particular samples. At the end of the results sheet, each participant will find their overall success - that is the percentage of the successful test results.
Summary of the results - overview	Displays a summary of the assigned values, participant results, and score obtained from the experts in a format that graphically corresponds to the positions of the samples in the TMA.

The supplements are identified by their name, EQA round identification and participant code and are intended for the needs of the participant.
We return to the participants all the glasses they sent us.

Additional information

The final report, with the exception of the supplements, is public. Further information is freely available to the 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.