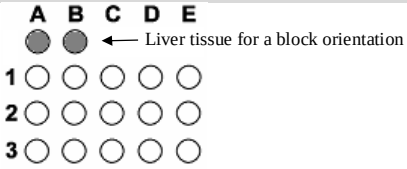
IHC2/21: Immunohistochemistry - Detection of HER-2/neu

This EQA round was accomplished according to the document ***EQA Plan 2021***.
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.
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 (AV) and methodology of the assessment

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

Part 1

In the first part, the results of **HER-2/neu expression** reported by the participants for individual TMA positions are evaluated.

General methodology of the AVs determination

AVs are determined as the consensus of the expert laboratories. The list of the expert laboratories for the IHC programme is available at the www.sekk.cz (EQA button and the link Expert laboratories). In fact this is a group of so called Reference laboratories for Her-2 diagnostics.
The consensus of expert laboratories that participated in the round is reached if **at least 80 % of the experts** agree on the result. The agreement of the experts is examined over the following groups of results:

- negative (0) and negative (1+)
- positive (2+)
- positive (3+)

These rules are applied:

- If the experts agree on one particular result then this result is marked as the expected result and complementary result from appropriate pair (0/1+ and 2+/3+) is marked as the acceptable value.
- If the results of the experts are spread inside the pair 0 and 1+ the way in which 80 % of the experts conclude on whole pair but not on one value from the pair, then both values are marked as expected results.
- If there is not consensus of the experts then the particular TMA position is not assessed (AV is missing).
- The consensus cannot be reached “across” the pair 0/1+ and other results. For example the consensus on the results 1+ and 2+ together is not possible.

This procedure of the AV determination eliminates cases in which the samples could be labelled as "inconclusive" or "questionable".

Overview of the AVs in this round

Test		Number of the results from the expert laboratories				AV
		Negative (0)	Negative (1+)	Weak positive (2+)	Strong positive (3+)	
601	A1 expression HER-2/neu	10				**0**
602	A2 expression HER-2/neu				10	**3+**
603	A3 expression HER-2/neu	10				**0**
606	B1 expression HER-2/neu		6	4		**missing**
607	B2 expression HER-2/neu	5	5			**0 and 1+**
608	B3 expression HER-2/neu		4	6		**missing**
611	C1 expression HER-2/neu				10	**3+**
612	C2 expression HER-2/neu	9	1			**0**
613	C3 expression HER-2/neu	2		2	4	**missing**
616	D1 expression HER-2/neu		2	8		**2+**
617	D2 expression HER-2/neu	2	7	1		**1+**
618	D3 expression HER-2/neu	7	3			**0**
621	E1 expression HER-2/neu	7	3			**0**
622	E2 expression HER-2/neu	6				**missing**
623	E3 expression HER-2/neu	10				**0**

The table shows that AV could not be determined at the positions B1, B3, C3 and E2.

At the positions where the consensus of the experts was not reached neither the results of the expression reported by the participants nor the scores from the experts are evaluated.

Part 2

In the second part, the assessment is performed by the team of 3 experts. This team evaluates the **quality of staining of the EQA sample and the quality of the IQC sample** by scoring on a scale **0 to 2 points** (where 0 is the worst result):
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	Pavel Fabian, MD, PhD assoc. prof. Zdeněk Kinkor, MD, PhD Dušan Žiak, MD

The points assigned by the individual experts for individual samples (TMA positions at EQA slide and IQC slide) are summed, thus the sum (score) can reach 0 to 6 points. The sums of points achieved are then evaluated as follows:

Score	***Description***	***Recommendation***
6 or 5	excellent result	
4 or 3	acceptable result	It is advisable to improve the staining results (there is room for improvement).
2 or less	unacceptable result	It is a warning signal and an impulse for an immediate solution.

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

Reminders from the experts – individual comments
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.

Supervisor's comment

There were 48 participants in this round, 11 of them from Slovakia, and 1 from Hungary.

EQA samples (TMA block)
As follows from the description of AV above, **11 samples** (positions in the TMA block) were evaluated in this round.

Samples without problems
Samples (TMA positions) for which the experts agreed on a consensus of 0 or 3+ can be described as completely problem-free. For these samples, erroneous results occur very rarely.

Problematic samples
Problematic samples (meaning: erroneous results are not sporadic to them) are usually found among those where expert laboratories have agreed on the assigned value of 1+ or 2+. However, it cannot be said that all of these samples are problematic, as the following table shows:

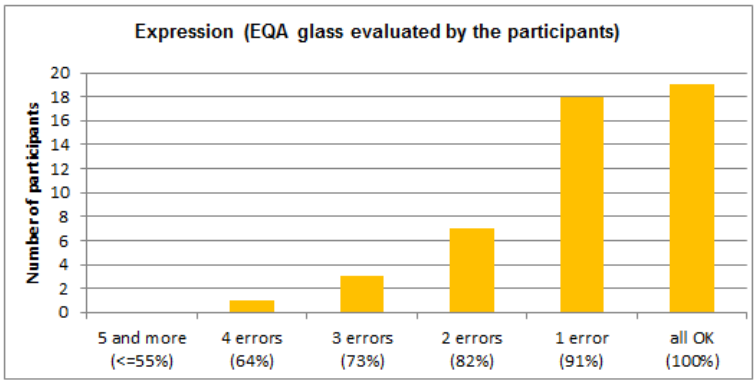
Position	***AV***	***Note***
D1	2+	But 40 % of the participants classified it as negative.
D2	1+	But 19 % of the participants classified it as 2+.

The problematic differentiation of the samples 1+ and 2+ may be influenced by the inhomogeneous distribution of a positivity in the sample (60 sections are cut from one TMA block for EHK, so this phenomenon cannot be completely

ruled out), as well as by demonstrable interpersonal variability in physician evaluation. The deviation 1+ versus 2+ in an individual case can therefore be caused by these phenomena and does not necessarily indicate a reason to change the setting of the IHC methodology. In the case of a systematic phenomenon, on the other hand, it is recommended to adjust the staining methodology. The best way to recognise a systematic error is from the cumulative sum of the deviations: the over-staining laboratory will have an arithmetic sum of the deviations "+8" and a sum of the absolute values of the deviations "8", i.e. all deviating results go in one direction to stronger staining; the laboratory that under-stains will have the arithmetic sum of the deviations "-8" and a sum of the absolute values of the deviations "8", i.e. all deviating results go in one direction to weaker staining (see the paragraph *Cumulative sums of deviations from the assigned values* below).
A recurring problem we observe in certain technical shortcomings in the 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. 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.

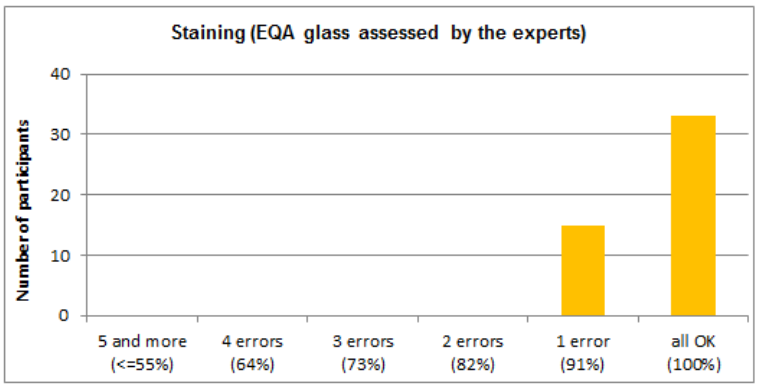
The success distribution for **the interpretation of 11 TMA samples by the participants** is shown in the figure on the right.

19 participants achieved 100 % success,
18 participants achieved 91 % success (i.e. 1 error), etc.



The success distribution for **the assessment of staining of 11 TMA samples by the experts** is shown in the figure on the right.

33 participants achieved 100 % success,
15 participants achieved 91 % success (i.e. 1 error), etc.

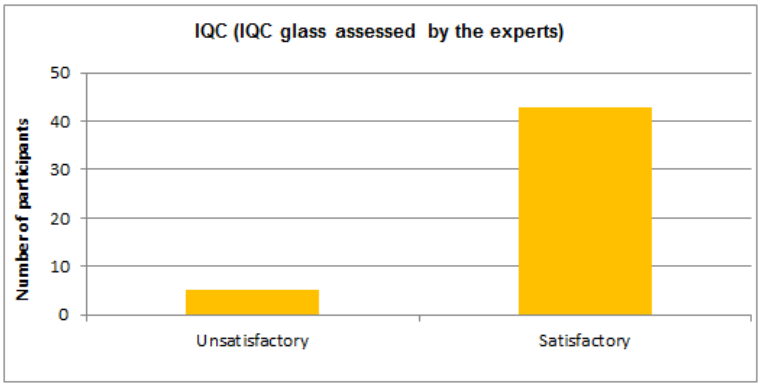


IQC samples
Basal analysis of the internal quality control results is fully sufficient to identify most problems in detection, as long as it is performed continuously and samples are selected properly. The laboratory can easily detect poor staining quality long before participating in the EQA - the EQA usually only confirms the problem in the laboratory. Also in this round we observed cases where a laboratory whose IQC slide was assessed as unsatisfactory by the experts also achieved worse results in other tests.
In general, we rated the quality of the most 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 almost vanished.
Such IQCs were assessed as unsatisfactory, which, in the opinion of experts, could not be used to set the sensitivity of the IHC method correctly. As an example we can use a case where the laboratory sent a composite sample, according to their own description with four tissues with intensities of 0, 1+, 2+ and 3+, but during the evaluation the experts assigned two samples with an intensity of 0 and two samples with an intensity of 2+, one of which was questionable, rather plasma positivity.

IHC2/21: Immunohistochemistry - Detection of HER-2/neu

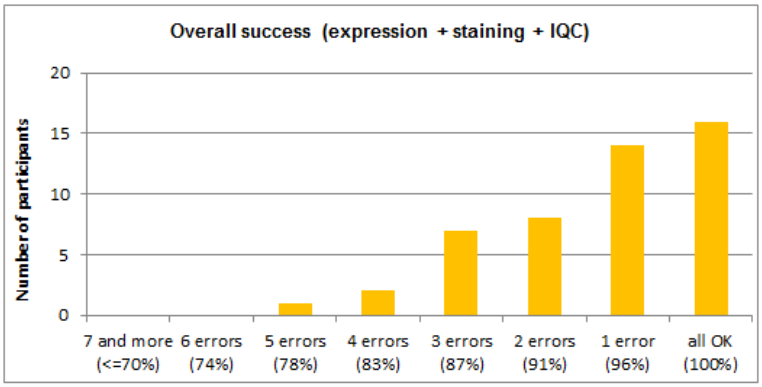
The success distribution for **the assessment of the IQC samples by the experts** (IQC glass is evaluated by the experts as a whole, i.e. it is evaluated as one sample) is shown in the figure on the right.

43 participants (i.e. 90 %) succeeded.
The experts classified IQC glass as unsatisfactory for 5 participants (i.e. 10 %).



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 23 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.

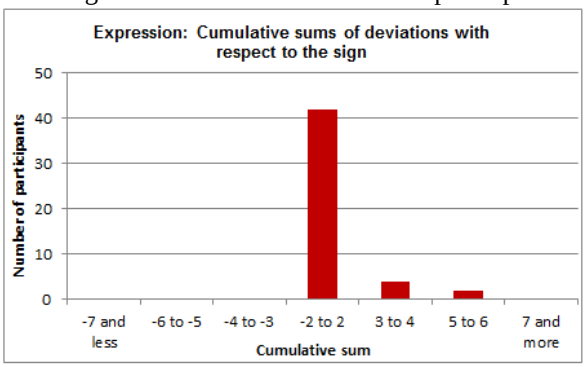


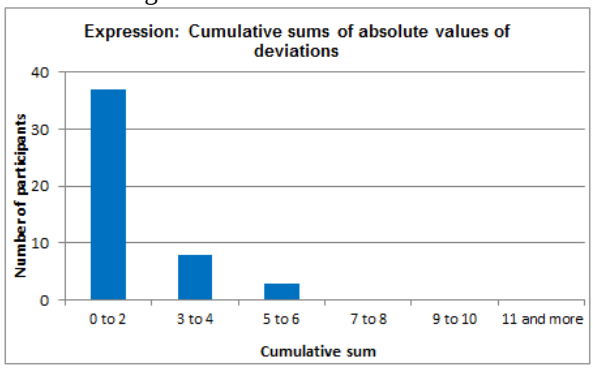
Cumulative sums of deviations from the assigned values

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 assessed 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.

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.





IHC2/21: Immunohistochemistry - Detection of HER-2/neu

Complex view on 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 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)

You will find all these data in your result sheet.

Conclusion
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 attention to the 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 can find in your result sheets.

Scientific supervision: Pavel Fabian, MD, PhD
Masaryk Memorial Cancer Institute
Brno, Czech Republic
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 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.