# Round: IHC2/20 – Immunohistochemistry - Detection of HER-2/neu

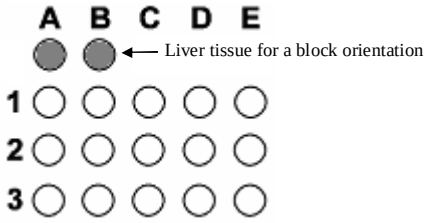
This EQA round was accomplished according to the document ***EQA Plan 2020***.
***Typing conventions:*** *We are using comma as a decimal separator and dates in day.month.year format.*

## 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 (AV) and methodology of the assessment

The assessment of the results of the participants in this EQA scheme 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 determining the AVs**

We improved the methodology of the AVs determination starting this round. Up to now the AVs were determined in the laboratory that prepared the samples – from the point of view of the ISO 17043 it is *known value* (KV) type of the AV. As there are many factors that should influence the results of the participants (namely spatial heterogeneity of the Her-2 expression within the sample) we have added on the recommendation of the CSP committee another source of the information which is the consensus of the expert laboratories - from the point of view of the ISO 17043 it is *consensus from experts* (CVE) type of the AV.

**The list of the expert laboratories** for the IHC programme is available at the web www.sekk.cz, EQA button and link *Expert laboratories*. In fact this is a group of so called *Reference laboratories* for Her-2 diagnostics.

The consensus of expert laboratories (CVE) 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+)

The final **AV is based on 2 sources of the information (KV and CVE)** this way:

- If the consensus of the experts (CVE) is not reached or the CVE does not agree to the value known from the samples preparation (KV) then the particular TMA position is not assessed (AV is missing).
- If the CVE exists and agrees to the KV, then:
  - If the experts agree on one particular result then this result is marked as the assigned value 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 does not conclude on one value then both values are marked as acceptable results,

**The procedure of the AV determination described above eliminates cases in which samples could be labelled as "inconclusive" or "questionable".**

**AVs in this round**

10 expert laboratories participated in this round and AVs were determined as follows - the table shows both the results of the expert laboratories and their consensus (CVE), as well as known values from samples production (KV) and final AVs:

| Test | | Expert laboratories | | | | | KV (samples production) | AV |
|---|---|---|---|---|---|---|---|---|
| | | Number of the results | | | | CVE | | |
| | | Negative (0) | Negative (1+) | Weak positive (2+) | Strong positive (3+) | | | |
| 601 | A1 expression HER-2/neu | 9 | 1 | | | 0 | | |
| 602 | A2 expression HER-2/neu | | | | 10 | 3+ | 3+ | **3+** |
| 603 | A3 expression HER-2/neu | 4 | 5 | 1 | | 0, 1+ | 2+ | **missing** |
| 606 | B1 expression HER-2/neu | 10 | | | | 0 | 0 | **0** |
| 607 | B2 expression HER-2/neu | | 6 | 4 | | missing | 2+ | **missing** |

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| Test | | Expert laboratories | | | | | KV (samples production) | AV |
|---|---|---|---|---|---|---|---|---|
| | | Number of the results | | | | CVE | | |
| | | Negative (0) | Negative (1+) | Weak positive (2+) | Strong positive (3+) | | | |
| 608 | B3 expression HER-2/neu | 9 | 1 | | | 0 | 1+ | **0** |
| 611 | C1 expression HER-2/neu | | | 10 | | 2+ | 2+ | **2+** |
| 612 | C2 expression HER-2/neu | 1 | 7 | 2 | | 0, 1+ | 2+ | **missing** |
| 613 | C3 expression HER-2/neu | | 2 | 8 | | 2+ | 2+ | **2+** |
| 616 | D1 expression HER-2/neu | | | | 10 | 3+ | 3+ | **3+** |
| 617 | D2 expression HER-2/neu | 10 | | | | 0 | 1+ | **0** |
| 618 | D3 expression HER-2/neu | 10 | | | | 0 | 0 | **0** |
| 621 | E1 expression HER-2/neu | | 4 | 6 | | missing | 2+ | **missing** |
| 622 | E2 expression HER-2/neu | 9 | 1 | | | 0 | 0 | **0** |
| 623 | E3 expression HER-2/neu | | | | 10 | 3+ | 3+ | **3+** |

The table shows that AV could not be determined at positions A3, B2, C2 and E1.
At these positions, the results of the expression reported by the participants are not evaluated.

## Part 2

In the second part, the assessment is performed by the team of 3 experts. This team evaluates the **quality of staining** by scoring (where 0 is the worst result):

- 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** | Pavel Fabian, MD, PhD<br>assoc. prof. Zdeněk Kinkor, MD, PhD<br>Dušan Žiak, MD |

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".
**Expert scoring is not performed on the samples (positions) where it was not possible to determine the AV for HER-2/neu expression** (see Part 1 above).
**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 46 participants in this round, 10 of them from Slovakia, and 1 from Hungary.

**EQA samples**

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

**Negative and strongly positive samples**

The cases where the laboratory reports false positive results occur very rarely. Thus almost 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).
Also, the interpretation of strongly positive (3+) samples did not cause major difficulties for the participants.
Thus, if the TMA block contained only samples with these two expression levels, the results would be excellent. Most laboratories stain and evaluate well both negative (0/1+) and positive (3+) samples.

**Weak positive samples**

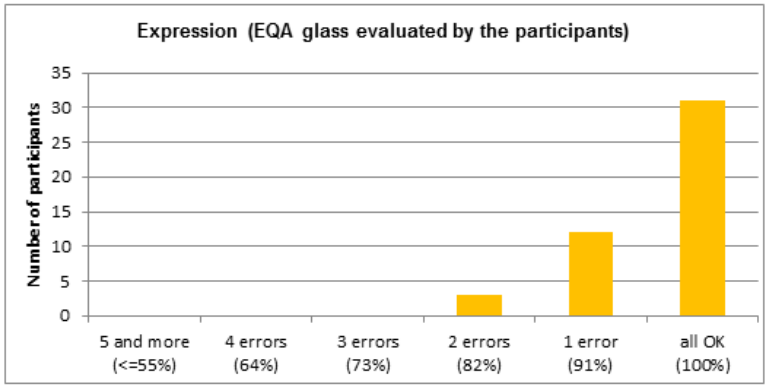
Weak positive (2+) samples were located at 6 positions A3, B2, C1, C2, C3 and E1, but only at 2 positions (C1 and C3) the AVs were determined successfully (remaining 4 positions were no assessed – see details above). In these 2 samples the results of the participants were as follows:

- C1: 93 % marked this sample as 2+
- C3: 74 % marked this sample as 2+

Even when using the same methodology that was used in sample preparation and with proven Her-2 gene amplification, there was not consensus among the participants. For example in the sample C3 the kit *Ventana PATHWAY anti-HER-2/neu* was used by 24 participants and they reported these results: 1x negative (0), 7x negative (1+) and 16x positive (2+)
The good news is that the evaluation of these "problematic" samples by the laboratories and experts is not fundamentally different - take a look at the table above, where the numbers of results of expert laboratories are given
**Thus, we repeatedly register a problem in the sufficient sensitivity of the detection of 2+cases. 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.

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

31 participants achieved 100 % success,
12 participants achieved 91 % success (i.e. 1 error), etc.



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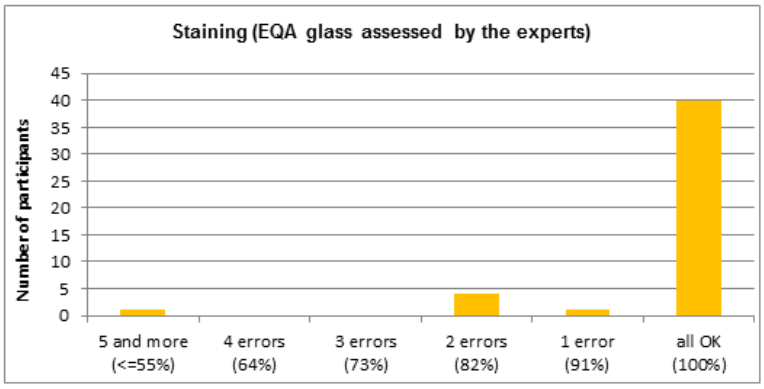
The overall success distribution for **the assessment of staining of 11 TMA samples by the experts** is shown in the figure on the right.

40 participants achieved 100 % success,
1 participant achieved 91 % success
(i.e. 1 error), etc.

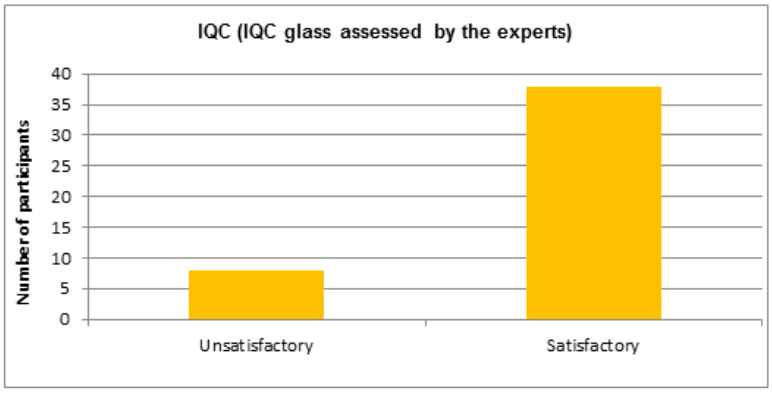



**IQC samples**

**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.
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 completely vanished.
Such IQCs were marked as unsatisfactory, which, in the opinion of experts, could not be used to set the sensitivity of the IHC method correctly. As an example - 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.

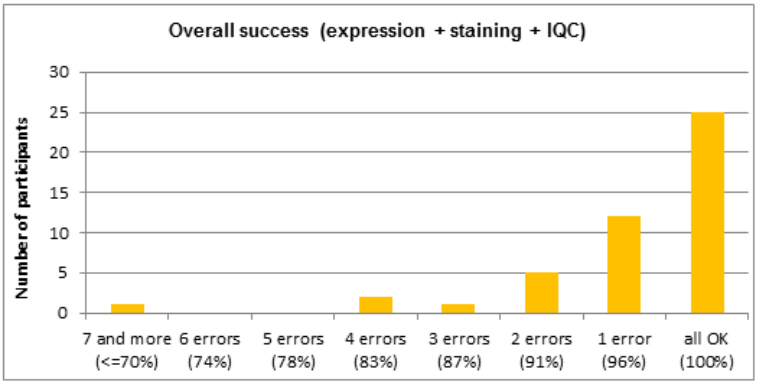
The overall success distribution for **the assessment of the IQC samples by the experts** (IQC glass is evaluated by the experts as a whole, i.e. it is evaluated as one sample) is shown in the figure on the right.

38 participants (i.e. 83 %) succeeded.
The experts classified IQC glass as unsatisfactory in 8 participants (i.e. 17 %).




**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 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.

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*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.*

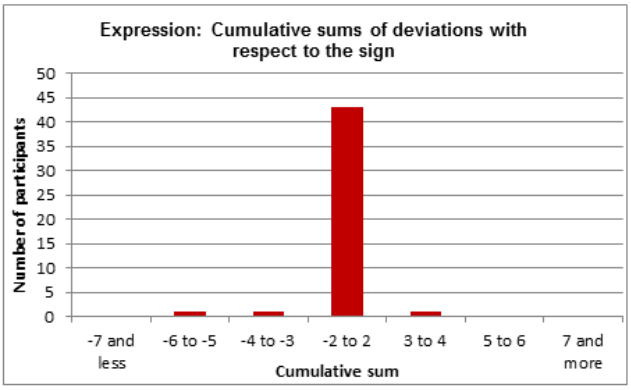
This view to the results can identify the laboratories that tend to overestimate (sum of deviations with respect to the sign is positive), or underestimate (sum of deviations with respect to the sign is negative), and those that have completely inconsistent results of interpretation (and usually staining) - sum of deviations with respect to the sign approaches 0, but the sum of the absolute values of the deviations is 10 or more.

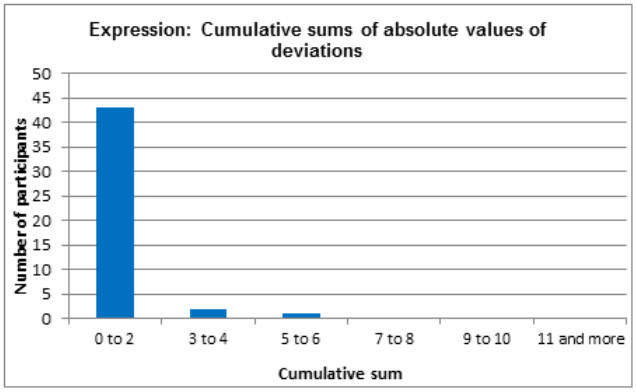
The participants will find their own cumulative sums of deviations (respecting the sign and the sums of absolute values) in their result sheet as part of an individual comment.

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.

As we can see in the graphs below, the sum of the deviations from the assigned values:

- respecting the sign reached -5 or less in 1 laboratory, while in 43 participants it was in the range of -2 to +2.
- in case of the absolute values reached 5 or more in 1 laboratory, while in 43 participants it was a maximum of 2.




**A comprehensive 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 the above 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
Masarykův onkologický ústav
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 that fulfilled the criteria. |
| Result sheet<br>(qualitative results) | At the beginning, the cumulative sums of the deviations are given as a part of the individual comment.<br>Further in the result sheet you will find (symbolism is explained in the legend):<br>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.<br>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.<br>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.<br>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.