CRC2/23: Colorectal Carcinoma

The terminology: *We adhere to the terminology of ISO 17043 and ISO 15189 wherever possible.*
Typing conventions: *We are using comma as a decimal separator and dates in day.month.year format.*

Please visit the web page
www.sekk.cz/CRC
to find complete information about CRC programme at one place.

Introduction

This EQA round was completed according to the document ***EQA Plan 2023***.

The scientific background of the CRC programme is under the control of the **European Society of Pathology Foundation** (ESPF, www.esp-pathology.org) by means of 2 scientific advisors (supervisors - see bottom of this report) nominated by the ESPF. Also expert laboratories (see the paragraph *Assigned values* on the next page) were selected on the basis of the recommendations of the ESPF.

The purpose of this EQA programme is to **identify and describe mutations** in genes which are **clinically relevant to the anti-EGFR therapy for colorectal carcinoma**. It is expected that:

- If the participant chooses to test *KRAS* or *NRAS* then at least: codons 12, 13 (exon 2)
  codons 59, 61 (exon 3)
  codons 117, 146 (exon 4) are tested
- If the participant chooses to test *BRAF* then at least: codon 600 (exon 15) is tested

The participant can choose any combination of *KRAS, NRAS, BRAF* testing. If the participant does not report the results for a particular gene in all samples then this gene is missing in their result sheet and it is not considered to be an error.

Participants

There were 44 participants in this round from 17 countries (the list of countries you can find in the evaluation of this round on the web).

Samples

The samples were formalin fixed paraffin embedded (FFPE) tissue sections from 5 invasive colorectal carcinomas (primary samples labelled A, B, C, D, E). Each participant received 3 sections from each primary sample. One section was intended for hematoxylin-eosin (HE) staining and the remaining 2 sections for DNA isolation and mutation detection.

The samples were prepared by the subcontractor.

The samples were shipped to the participants together with the documentation in one package via a courier service.

The participants were allowed to order spare samples in case of sample damage in their laboratory.

Virtual slides (HE staining) of all primary samples (tissue blocks) were available to the participants at our virtual microscopy website (https://www.eqa.cz/vm) to help the participants to optimally process the samples.

Assigned values (AV)

The AVs (expected results) are crucial and that is why great attention is paid to the process of their determination. AVs were obtained from the consensus of **3 expert laboratories**:

- Universitätsklinikum Carl Gustav Carus, Institut für Pathologie, Dresden, Germany
- Ipatimup Diagnostics, Porto, Portugal
- University Hospital, The Fingerland Department of Pathology, Hradec Králové, Czech Republic

In accordance with ISO 17043 classification we have used the CVE (consensus value from experts) type of AV.

Expert laboratories tested all primary samples as unknown. The task for each expert laboratory was to test the sample and report the identified mutations back to the SEKK (thus not only to confirm the mutation suggested by SEKK) and also report possible discrepancies. In other words: expert laboratories processed and tested the samples under the same conditions as regular participants.

Consensus of the results of the expert labs was required to establish the AV for particular sample.

Using the procedure described above these AVs (mutations confirmed by experts and thus expected to be found by participants) were determined:

Sample	Assigned values		
	KRAS	**NRAS**	**BRAF**
A	c.38G>A,p.G13D		
B			
C		c.181C>A,p.Q61K	
D			c.1799T>A,p.V600E
E	c.182_183delinsTC,p.Q61L		

Blank cells represent WT (wild type) status of the gene.

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Evaluation of the results

Participants had to report mutations they identified; in addition they were asked about their laboratory background (the answers to these questions did not influence the assessment of the participant's performance).

In principle, our standard practice is to sort the qualitative results of each test into 4 categories from the point of view of the performance assessment:

Category	Explanation
Expected (correct) result, marked >>> in the reports	This is the result that we expected to be found by the participants. This result is optimal for the patient's treatment. It is the result identical to the AV.
Acceptable (conditionally correct) result, marked > in the reports	A result that differs from the correct result only slightly, based on the laboratory procedure, the method used etc. The result should also be classified as "suboptimal" from the point of view of the patient's treatment. In case of the CRC programme, there are two possible scenarios to classify the result as acceptable: 1) a result obtained by a method that does not allow a particular mutation to be classified precisely 2) a result is missing, because mutation of another gene has been correctly identified and – according to routine clinical practice – the additional genes were not tested.
Incorrect result	Any result which is neither "Correct" nor "Acceptable".
Impossible to evaluate, result not assessed, marked ± in the reports	A category not used in this round. This is very special category indicating that it would not be possible to establish the AV. Without having the AV we are not able to classify the participant's result as "correct" or "incorrect". In the CRC programme this could only represent a very rare case where consensus of the experts would not be reached.

The questions not influencing participants' performance are not classified into the above mentioned categories – we present only the overview of the participants' answers and the commentary in these cases.

The participant's result is evaluated as successful if it falls into either expected or acceptable category.

General questions (educational, not assessed)

These questions have **no influence** on the evaluation of overall performance.

Question: **Do you estimate the percentage of neoplastic cells in the sample in your routine practice?**

Answer	Count
No	1
Yes	43

Comment: It is strongly recommended to make the estimation of neoplastic cell content as this step helps to decide whether neoplastic cells are present and their amount is sufficient for the method used in the laboratory. Vast majority of the participants follow this recommendation.

Question: **If so, who makes this estimate?**

Answer	Count
not specified	1
Another doctor/molecular geneticist	1
Laboratory technician / medical scientist	1
Pathologist	41

Comment: The answers confirmed that the participants pay great attention to this initial step of sample processing.

Question: **Describe the procedure of DNA isolation.**

As expected, the participants used a wide range of the methods (Biocartis, Promega, Qiagen, Roche atc.).

Question: **Did you perform dissection?**

Answer	Number of the participants				
	A	B	C	D	E
Not specified	2	2	1	1	1
No	21	24	21	22	23
Yes - macrodissection	18	15	19	18	17
Yes - microdissection	3	3	3	3	3

Comment:
- 18 participants processed all samples without dissection.
- 13 participants processed all samples using macrodissection.

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- 3 participants processed all samples using microdissection.

Question: **Specify the neoplastic cell content (NCC).**

2 participants did not answer.

The participants were instructed to report the NCC estimate (after dissection, if performed).

The summary of the values reported by the participants you can find in the table.

	[%]				
	A	**B**	**C**	**D**	**E**
All participants					
Minimum	15	10	20	10	20
Average	**40**	**57**	**51**	**44**	**49**
Maximum	75	95	80	80	80
Experts					
Average	43	77	65	63	57

Comment: The table shows a good agreement between the overall average and the average of expert laboratories. NCC in all samples was sufficient to perform the analyses by current routinely used methods.

Question: **Specify DNA concentration.**

12 participants did not specify a value.

The summary of the results reported by the participants (11 participants did not answer) you can find in the table (the numbers are rounded to the two significant digits).

	[mg/L]				
	A	**B**	**C**	**D**	**E**
All participants					
Minimum	0,8	1,5	1,3	0,7	0,4
Average	**15**	**22**	**26**	**19**	**10**
Maximum	78	93	128	91	59
Experts					
Average	36	64	53	50	29

Comment: The result of DNA concentration measurement strongly depends on the method used. Different methods target different parts/fragments of DNA (different entities are measured) and thus the results differ significantly. We did not ask the participants to describe the method as it was not our intention to assess the performance of the DNA concentration measurement.

Assessed tests

For details please see your result sheet or summary statistic on the web.

KRAS **mutation(s)**

We observed the mistakes only in sample E (the mutation was not detected by 2 participants and 1 participant did not report a result).

In case of the sample E the participants specified the mutation as a free text and – as expected – notations slightly differed based on the rules used in the particular laboratory. We sorted the "text" results into these groups:

- c.182_183delinsTC,p.Q61L: This is expected result determined by the expert laboratories.
- c.182A>T,p.Q61L: This result was mostly reported by the Oncomine users and it is acceptable.
- c.183A>C,p.Q61H : This result was mostly reported by the Biocartis Idylla users. This system does not allow identifying the mutation in detail. This mutation belongs to the group of the Exon 3 mutations (Exon 3 mutation reported many Idylla users and that is what we expected to receive from them – see the paragraph in the Conclusion chapter). We accepted this answer as "borderline acceptable" because from the clinical treatment point of view this result has not negative influence to the patient care.

KRAS **summary: 41 of 44 participants (93 %) succeeded.**

NRAS **mutation(s)**

Very good performance – only 3 errors (one participant reported Exon 2 mutation in sample B and one participant reported c.35G>A,p.G12D mutation in sample D and one participant omitted to report the result in the sample E).

NRAS **summary: 41 of 44 participants (93 %) succeeded.**

BRAF **mutation(s)**

Here we observed the best performance in this EQA round, only one mistake (one participant omitted to report the result in the sample E).

BRAF **summary: 43 of 44 participants (98 %) succeeded.**

Conclusion

Identifying the mutations

The participants demonstrated very good performance in this round.

Note to the users of the Biocartis Idylla system

This system has some limitations concerning the ability to identify particular mutation in detail. In these cases we strongly recommend to report the result in the form of *Exon XY mutation (unable to specify in detail)*. After selecting this item in the menu you are finished, no additional info (text note) is necessary.

This approach is far better than selecting the menu item *Other mutation* and adding complicated text note where the participant describes all possible mutations that they were not able to rule out or confirm.

Formal mistakes

Unfortunately some participants failed because they made trivial formal mistakes. The rules of thumb are:

- If you decide to test particular gene (say *KRAS*) then you have to test it and report the results in **all samples**. Missing result is assessed to be an error.
- If not able to specify exact mutation (this applies mainly to the users of the Biocartis Idylla platform) then select the answer *Exon XY mutation (unable to specify in detail)* and do not add any note (it is of no use).
- You must specify a text note only in case you found particular mutation that is not listed in the menu – in this case you have to select *Other* from the menu and describe the mutation in the note.
- If you specify exact mutation, never add a text note (it is of no use).

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Supplements

As a supplement to this report individual participants receive:

Name of supplement	Remark
Confirmation of attendance	Issued only to those participants who sent us the results.
Certificate	Issued only to those participants who passed successfully.
Result sheet (qualitative results)	Issued only to those participants who sent us the results.

The supplements are identified by their name, EQA round identification and participant code and are intended for the needs of the participant.

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.