CRC2/25: 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 2025***.
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 13 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 (AVs)

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			
B		c.181C>A,p.Q61K	
C			c.1799T>A,p.V600E
D	c.35G>T,p.G12V		
E			

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 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 result marked > in the reports	A result that differs from the expected 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 "Expected" nor "Acceptable".
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***
not specified	0
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
Pathologist	42
Another doctor / molecular geneticist	1

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, RBC Bioscience, Roche, Thermo Fisher etc.).

Question: **Did you perform dissection?**

Answer	***Number of the participants***				
	A	***B***	***C***	***D***	***E***
Not specified	0	0	1	0	0
No	15	15	14	18	16
Yes - macrodissection	25	25	25	22	24
Yes - microdissection	4	4	4	4	4

Comment:

- 14 participants processed all samples without dissection.

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- 19 participants processed all samples using macrodissection.
- 4 participants processed all samples using microdissection.
- 7 participants used different technics for different samples.

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

The participants were instructed to report the NCC estimate after dissection, if performed.
2 participants did not answer.
The summary of the values reported by the participants you can find in the table.

	[%]				
	A	***B***	***C***	***D***	***E***
All participants					
Minimum	10	25	10	8	15
Average	**58**	**60**	**54**	**34**	**50**
Maximum	90	90	90	70	90
Experts					
Average	63	65	50	43	53

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.**

9 participants did not specify a value.
The summary of the results reported by the participants you can find in the table.

	[mg/L]				
	A	***B***	***C***	***D***	***E***
All participants					
Minimum	1,0	0,5	0,3	1,0	0,8
Average	**45**	**35**	**55**	**21**	**43**
Maximum	200	186	200	119	200
Experts					
Average	139	76	161	81	128

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)**

Very good performance – only 1 error: one participant did not found a mutation in sample D.

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

***NRAS* mutation(s)**

We observed no error – great results!

***NRAS* summary: 44 of 44 participants (100 %) succeeded.**

***BRAF* mutation(s)**

In sample C, 2 participants (users of Biocartis Idylla) reported “other mutation” with text description. We checked these text descriptions carefully and all of them included expected mutation – thus we converted these results to “exon 15 mutation”.
The performance was excellent – no error.
One participant did not report the results for *BRAF* – this is not error, the participants are free to choose the genes they will be testing.

***BRAF* summary: 43 of 43 participants (100 %) succeeded.**

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Conclusion

Identifying the mutations

The participants demonstrated great performance in this round.

Note to (not only) the users of the Biocartis Idylla system

This system (and also some other systems) 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.

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	2	1	2	5	0	34
	relative	-	4,5 %	2,3 %	4,5 %	11 %	-	77 %
Note: You can find your individual success over last 2 years in your result sheet.								

Most participants of this round show an overall success rate of 80 % or greater over the past 2 years.

Formal mistakes

Sometimes we observe 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 then select the answer ***Exon XY mutation (unable to specify in detail)*** and do not add any note.
- 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 mutation*** 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 and is not taken into account during assessment).

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Supplements

As a supplement to this report individual participant who reported the results gain:

Name	***Description***
Confirmation of attendance Certificate Result sheet	The conditions for issuing the relevant document specified in the EQA Plan must be met.

The supplements are identified by their name, EQA round identification and participant code and are intended for the needs of the participant.
If you reported the results in this round, you can find your individual evaluation (reports) in the **Cibule** application (https://www.eqa.cz/cibule). After login, select ***EQA Results - View*** in the menu and then click the ***Reports*** button for the particular round.

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.