CRC2/22: 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 2022***.
The scientific background of the CRC programme is under the control of the **European Society of Pathology** (ESP, www.esp-pathology.org) by means of 2 scientific advisors (supervisors - see bottom of this report) nominated by the ESP. Also expert laboratories (see paragraph *Assigned values* on the next page) were selected on the basis of the recommendations of the ESP.
The purpose of this EQA programme is to **identify and describe mutations** (the participant can choose any combination of *KRAS, NRAS, BRAF* testing) 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

As mentioned above, the participants are not forced to test all genes. From the clinical point of view, the information about *KRAS+NRAS* status is the minimal requirement. But the motivation of the laboratories to participate in this programme may differ, for example:

- a standard clinical laboratory tests *KRAS+NRAS* at least
- an industry/research laboratory may select only the gene that they focus on
- a standard clinical laboratory which successfully participated in another EQA programme for e.g. *KRAS* and was not successful for *NRAS* can participate in our programme only to confirm their improvement in *NRAS*

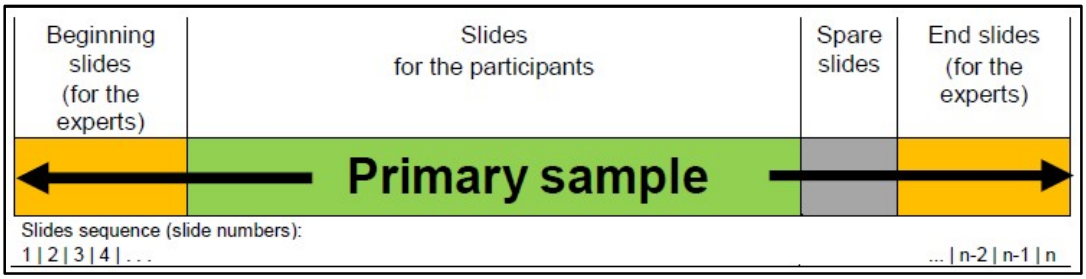
If the participant does not report the results for a particular gene in all samples then this gene is missing in their result sheet and it is not considered to be an error.

Participants

There were 52 participants in this round from 22 countries (the list of countries you can find 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 case (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. Each sample was labelled with the code of the round, the primary sample identification, and the position of the slide in the cutting sequence. The figure below shows the general arrangement of the cutting sequence of one tissue block.



Virtual HE slides of all primary samples (tissue blocks) were available to the participants at our virtual microscopy website to help the participants to optimally process the samples.

The samples were shipped to the participants together with the documentation in one package via a courier service. The time of the delivery ranged from 1 to 2 days in most cases (based on the participant's country), no damage or loss of the shipments occurred, all parcels were delivered.
The participants were allowed to order spare samples in case of sample damage in their laboratory.

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

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- 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 the sections from the beginning and from the end of the cutting sequence of each primary sample (orange areas in the figure above).
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 between slides from start and end of the sequence. In other words: expert laboratories processed and tested the samples under the same conditions as regular participants.
Full agreement of the results of all 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.1799T>A,p.V600E
C	c.38G>A,p.G13D		
D		c.38G>A,p.G13D	
E	c.183A>C,p.Q61H		

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

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. In the case of CRC programme, 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, 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 wold 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.

The results – part 1: General questions

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	2
Yes	50

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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*
Pathologist	47
Another doctor/molecular geneticist	2
Laboratory technician / medical scientist	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.**

Answer	*Count*
Biocartis Idylla	10
Promega products	10
Qiagen products	15
RBC Bioscience	2
Roche products	8
other manufacturers (AmoyDx, Exgene, Macherey-Nagel, ...)	7

Comment: As expected, the participants used a wide range of the methods.

The results – part 2: General questions concerning individual samples

These questions have no influence on the evaluation of performance.

Question: **Did you test the sample?**

Answer	*Number of the participants*				
	A	*B*	*C*	*D*	*E*
No	-	-	-	-	-
Yes	52	52	52	52	52

Comment: Almost all participants were able to test all samples.

Question: **Did you perform dissection?**

Answer	*Number of the participants*				
	A	*B*	*C*	*D*	*E*
Not specified	3	1	1	1	1
No	21	14	12	15	13
Yes - macrodissection	26	35	36	34	34
Yes - microdissection	2	2	3	2	4

Comment:

- 10 participants processed all samples without dissection.
- 23 participants processed all samples using macrodissection.
- 2 participants processed all samples using microdissection.
- 16 participants used different approaches in particular samples.
- 1 participant did not answer.

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

The participants were instructed to report the NCC estimate (after dissection, if performed).
5 participants did not specify a value.
The overview of the values reported by 47 participants you can find in the table.

	[%]				
	A	*B*	*C*	*D*	*E*
All results					
Minimum	25	20	15	5	12
Average	**63**	**50**	**42**	**31**	**59**
Maximum	90	90	90	90	95
Experts					
Average	65	55	55	20	70

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Comment: The table shows a good agreement between the overall average and the average of expert laboratories. We can also see that the NCC in all samples was sufficient to perform the analyses by current routinely used methods.
On the other hand there is a wide variation in the reported values. In particular the content less than 10 % seems to be underestimation of NCC (only 1 participant reported less than 10 % in any sample).

Question: **Specify DNA concentration.**
15 participants did not specify a value.
The table shows a wide range of the results obtained (the numbers are rounded to the two significant digits).

	[ng/µL]				
	A	***B***	***C***	***D***	***E***
All results					
Minimum	8,9	8,2	5,4	2,7	4,0
Average	**79**	**60**	**47**	**30**	**48**
Maximum	200	200	200	150	200
Experts					
Average	140	100	120	78	120

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.

Evaluation of the results – part 3: *KRAS* mutations

This is an assessed test. The participant **must** provide expected or acceptable result for all samples in order to be classified as successful.
The task: **Specify *KRAS* mutation(s) found and the method(s) used.**
52 participants reported the results (a mutation was present in the samples C, E - see the paragraph *Assigned values* above):

Results	*Number of the participants*				
	A	***B***	***C (mut)***	***D***	***E (mut)***
Expected result (>>>)	52	52	48	51	38
Acceptable result (>)			3		14
Incorrect result			1	1	

Comment:
- Samples A, B: All results correct.
- Sample C:
  3x acceptable result (these participants were not able to specify exact mutation due to limitations of their methods but reported correct exon containing the mutation - these results were accepted as from the clinical point of view these methods provide sufficient information for clinical management of the patient).
  1x wrong result (one participant did not detect the mutation).
- Sample D: One participant did not detect the mutation.
- Sample E: 14x acceptable result (either reported the correct exon or "other" mutation with adequate text note - this sample bore more mutations and the result depended on the threshold used in the particular laboratory).

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	≤ 1 %	≤ 5 %	≤ 10 %	≤ 20 %	> 20 %
Number of the participants	12	34	4	2	-

***KRAS* summary: 50 of 52 participants (96 %) succeeded.**

Evaluation of the results – part 4: *NRAS* mutations

This is an assessed test. The participant **must** provide expected or acceptable result for all samples in order to be classified as successful.
The task: **Specify *NRAS* mutation(s) found and the method(s) used.**
52 participants reported the results (a mutation was present in the sample D - see the paragraph *Assigned values* above):

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Results	***Number of the participants***				
	A	***B***	***C***	***D (mut)***	***E***
Expected result (>>>)	52	50	46	48	46
Acceptable result (>)			5	3	5
Incorrect result		2	1	1	1

Comment:

- Sample A: All results correct.
- Sample B: 2x wrong result (participant did not provide a result).
- Sample C:
  5x acceptable result (these participants did not test the sample as they found other mutations in it - these results were accepted because this approach reflects the routine practice).
  1x wrong result (participant did not provide a result).
- Sample D:
  3x acceptable result (these participants were not able to specify exact mutation due to limitations of their methods but reported correct exon containing the mutation - these results were accepted as from the clinical point of view these methods provide sufficient information for clinical management of the patient).
  1x wrong result (wrong mutation found).
- Sample E:
  5x acceptable result (the same reason as in the sample C).
  1x wrong result (participant did not provide a result).

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	≤ 1 %	≤ 5 %	≤ 10 %	≤ 20 %	> 20 %
Number of the participants	11	34	5	2	-

NRAS summary: 48 of 52 participants (92 %) succeeded.

Evaluation of the results – part 5: *BRAF* mutations

This is an assessed test. The participant **must** provide expected or acceptable result for all samples in order to be classified as successful.

The task: **Specify *BRAF* mutation(s) found and the method(s) used.**

52 participants reported the results (a mutation was present in the sample B - see the paragraph *Assigned values* above):

Results	***Number of the participants***				
	A	***B (mut)***	***C***	***D***	***E***
Expected result (>>>)	52	33	46	49	47
Acceptable result (>)		19	5	2	5
Incorrect result			1	1	

Comment:

- Sample A: All results correct.
- Sample B:
  19x acceptable result (either reported the correct exon or “other” mutation with adequate text note).
- Sample C:
  5x acceptable result (these participants did not test the sample as they found other mutations in it - these results were accepted because this approach reflects the routine practice).
  1x wrong result (participant did not provide a result).
- Sample D:
  2x acceptable result (the same reason as in the sample C).
  1x wrong result (the same reason as in the sample C).
- Sample E:
  5x acceptable result (the same reason as in the sample C).

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	≤ 1 %	≤ 5 %	≤ 10 %	≤ 20 %	> 20 %
Number of the participants	12	33	4	3	-

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BRAF summary: 51 of 52 participants (98 %) succeeded.

Conclusion

Identifying the mutations

The participants demonstrated very good performance in this round. We observed significant improvement compared to the 1st round of this year namely in BRAF testing. **Congratulation!**

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 results 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 (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).

Starting 2023 we will not require laboratory reports in any form and thus the CRC programme will by significantly simplified.

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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 of approval	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.