CRC2/21: 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 round of the accredited EQA programme was completed according to the document ***EQA Plan 2021***.
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*

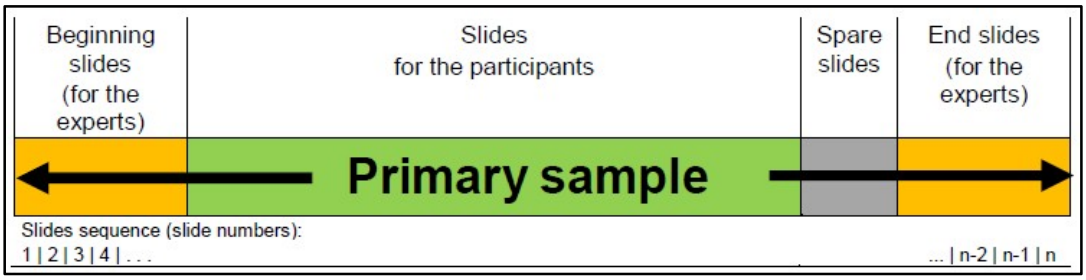
If the participant does not report the results for a particular gene in all samples then this gene is missing in their result sheet and it is not considered to be an error.

Participants

There were 46 participants in this round from 20 countries (the list of countries can be found in the report published on the web).
Additional 3 laboratories, which subscribed for the participation and received the samples, did not report the results.

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 (see the figure below).



Virtual HE slides of all primary samples 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 3 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.

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Assigned values (AV)

The AVs (expected results) are crucial and that is why we paid great attention 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
- Hôpital Saint-Antoine, service d'Anatomie et Cytologie Pathologiques, Paris, France
- 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 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			c.1799T>A,p.V600E
B	c.34G>A,p.G12S		
C			
D		c.35G>A,p.G12D	
E	c.436G>A,p.A146T		

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 the case of the CRC programme, there were two possible scenarios for such situation: 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 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.

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The results – part 1: General questions

These questions have no influence on the evaluation of the participant's performance.

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

Answer	*Count*
No	4
Yes	42

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 content 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	41
Another doctor/molecular geneticist	1
A person outside of our lab	1

Comment: Very good result confirming that the participants pay great attention to this initial step of sample processing.
One participant sent us confusing answer "*... we do not estimate ... and the estimate is made by the pathologist ...*".

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

Answer	*Count*
Biocartis Idylla	7
Promega products	9
Qiagen products	15
Roche products	9
other (MagCore, in-house)	2

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	46	46	46	46	46

Comment: Almost all participants were able to test all samples. Only one laboratory reported (as a text note): *Sample E failed no NGS data.* But they did not use the opportunity to request spare sample from SEKK.

Question: **Did you perform dissection?**

Answer	*Number of the participants*				
	A	***B***	***C***	***D***	***E***
Not specified	1	1	1	1	1
No	20	14	13	18	14
Yes - macrodissection	24	30	31	26	30
Yes - microdissection	1	1	1	1	1

Additionally:

- 9 participants processed all samples without dissection.
- 20 participants processed all samples using macrodissection.
- 1 participant processed all samples using microdissection.
- 15 participants used different approaches in particular samples.

Comment:
9 participants reported **no dissection** for all samples. All of them tested all 3 genes so we had 26 results of mutation testing (under "result" we mean the final result of the particular gene assessment, that is in all samples – to achieve a success, all 5 samples must be OK). Among these 26 results there was 1 failure, it is 4 %.

If we count errors the same way in the group of 21 participants who performed **a dissection** (micro- or macro-) in all samples we have 63 results with 8 failures, which is 13 %.
But the conclusion that "*a dissection leads to more mistakes*" is misleading – in this round the majority of mistakes raised from the formal mistakes, not analytical.

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

The participants were instructed to report the estimate (after dissection, if performed).
5 participants did not specify a value.
The overview of the values reported by 41 participants you can find in the table.

	[%]				
	A	***B***	***C***	***D***	***E***
All results					
Minimum	20	15	13	10	30
Average	**64**	**40**	**67**	**43**	**56**
Maximum	95	85	90	90	85
Experts					
Average	60	57	73	50	50

Comment: We can see a quite good agreement between the overall average and the average of expert laboratories. We can also see that the NCC in all samples was fully 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 20 % (or less) content seems to be clearly underestimation of NCC (16 participants reported 20 % or less in any sample).

Question: **Specify DNA concentration.**

The table shows a wide range of the results obtained (11 participants did not answer) – all numbers are rounded to the two significant digits.

	[ng/µL]				
	A	***B***	***C***	***D***	***E***
All results					
Minimum	0,99	1,32	2,85	1,44	1,1
Average	**17**	**29**	**38**	**44**	**20**
Maximum	46	90	198,2	172	71
Experts					
Average	21	23	31	20	33

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.
A good agreement between the overall average and the average of the experts is visible.
But the differences in the results reported for all samples (minimum vs. maximum) are enormous.

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.**

A mutation was present in the samples B, E - see the paragraph *Assigned values* above.

Results	***Number of the participants***				
	A	***B*** ***(mut)***	***C***	***D***	***E*** ***(mut)***
Expected result (>>>)	46	41	46	46	31
Acceptable result (>)		4			12
Incorrect result		1			3

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	≤ 1 %	≤ 5 %	≤ 10 %	≤ 20 %	> 20 %
Number of the participants	8	33	4	1	0

KRAS summary: 42 of 46 participants (91 %) succeeded.

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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.**

A mutation was present in the sample D - see the paragraph *Assigned values* above.

Results	***Number of the participants***				
	A	***B***	***C***	***D (mut)***	***E***
Expected result (>>>)	44	39	45	41	40
Acceptable result (>)		5		4	3
Incorrect result	1	1			2

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	≤ 1 %	≤ 5 %	≤ 10 %	≤ 20 %	> 20 %
Number of the participants	6	33	4	1	1

Comment: One participant reported the analytical sensitivity higher than 20 % - here we recommend to consider a method with better sensitivity, as in samples with limited neoplastic cell content might use of method with low sensitivity lead to falsely negative result.

***NRAS* summary: 42 of 45 participants (93 %) succeeded.**

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.**

A mutation was present in the sample A - see the paragraph *Assigned values* above.

Results	***Number of the participants***				
	A (mut)	***B***	***C***	***D***	***E***
Expected result (>>>)	33	42	46	46	41
Acceptable result (>)	11	3			3
Incorrect result	2	1			2

The distribution of the analytical sensitivity of the methods reported by the participants was very similar to the *KRAS* methods (please see the *KRAS* paragraph above).

***BRAF* summary: 43 of 46 participants (93 %) succeeded.**

Formal mistakes

In some cases the faulty result was very probably caused be the formal mistake of the participant. Let's discuss most frequent issues.

1) There is available in the list of the mutations the item *Exon 2 mutation (unable to specify in detail)* and similar items for other exons. But some participants – instead of selecting this item – selected *Other mutation* and added complicated text note (something like: *p.(Gly12Xaa) - unable to specify in detail etc.*). **Please do not use this approach. Select appropriate exon from the menu and text note is not necessary.** We assessed these results as acceptable.
2) There is available in the list of the mutations the item *Not tested because another mutation was found*. But some participants – instead of selecting this item – omitted to select the answer (in such case *Not specified* remains in the result form). **But this is wrong.** The answer *Not specified* menas: we have no answer, we do know, we have no opinion.

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Conclusion

Identifying the mutations

The participants demonstrated very good performance in this round. May be that some failures were caused by typing errors but we have seen also clear false negative results and wrong mutation identification.

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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.