CRC1/22: 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.

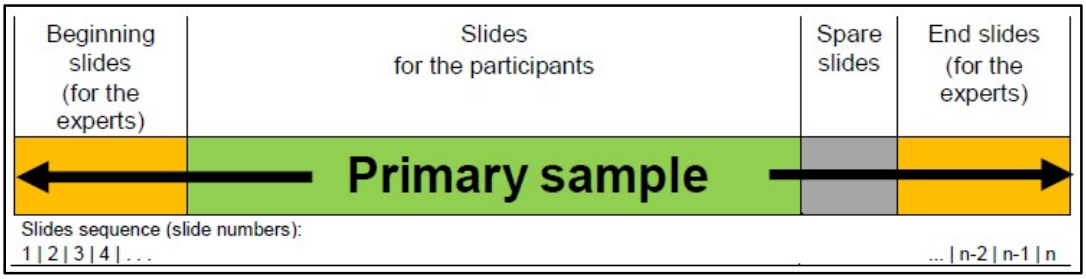
An integral part of this EQA round is also the evaluation of the **post-analytical phase** – the assessment of the routine laboratory reports of the participants. This part of the programme is entirely educational – it means that **reports are required**, although the analysis of the content of these reports does not influence the performance of the participant in any way, and the participants receive only verbal comments/recommendations (if any) to their reports.

Participants

There were 54 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.

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The participants were allowed to order spare samples in case of sample damage in their laboratory (one participant used this opportunity).

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 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	c.35G>C,p.G12A		
D		c.182A>G,p.Q61R	
E	c.35G>T,p.G12V		

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.

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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	3
Yes	51

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	49
Another doctor/molecular geneticist	1
Laboratory technician / medical scientist	1

Comment: Very good result confirming that the participants pay great attention to this initial step of sample processing.

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

Answer	*Count*
Qiagen products (QIAamp DNA FFPE Mini Kit, QIAamp DNA FFPE Tissue Kit, ...)	15
Promega products (Maxwell RSC DNA FFPE Kit, ...)	10
Biocartis Idylla	9
Roche products (cobas DNA Sample Preparation Kit, ...)	7
other manufacturers (AmoyDx, Exgene, MagCore, 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	-	-	1	-	-
Yes	54	54	53	54	54

Comment: Almost all participants were able to test all samples. Only one laboratory reported "*Sample C (method failure, sample damaged by mistake, etc.).*" The participant can request spare sample in such situation. But the participant in question did not request spare sample.

Question: **Did you perform dissection?**

Answer	*Number of the participants*				
	A	*B*	*C*	*D*	*E*
Not specified	1	1	1	1	3
No	26	24	15	29	28
Yes - macrodissection	23	25	33	20	20
Yes - microdissection	4	4	5	4	3

Comment:

- 13 participants processed all samples without dissection.
- 16 participants processed all samples using macrodissection.
- 3 participants processed all samples using microdissection.
- 21 participants used different approaches in particular samples.

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

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

3 participants did not specify a value.

The overview of the values reported by 40 participants you can find in the table.

	[%]				
	A	***B***	***C***	***D***	***E***
All results					
Minimum	10	20	10	25	10
Average	**67**	**54**	**41**	**61**	**41**
Maximum	95	90	85	95	85
Experts					
Average	80	63	57	60	50

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 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 content less than 20 % seems to be underestimation of NCC (only 9 participants reported less than 20 % in any sample).

Question: **Specify DNA concentration.**

17 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	0,3	0,9	0,4	0,2	0,1
Average	**28**	**32**	**29**	**40**	**17**
Maximum	95	120	200	200	55
Experts					
Average	51	64	95	110	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.

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.**

54 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 (>>>)	54	54	49	54	51
Acceptable result (>)			3		3
Incorrect result			2		

Comment:

- Samples A, B, D: 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).
  - 2x wrong result (one participant did not test the sample C at all due to method failure and one probably mistyped the results – wrote the result of *KRAS* testing into *NRAS* field of the result form).
- Sample E: 3x acceptable result (the same reason as in the case of sample C).

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	≤ 1 %	≤ 5 %	≤ 10 %	≤ 20 %	> 20 %
Number of the participants	11	36	4	3	-

KRAS summary: 52 of 54 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.**

54 participants reported the results (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 (>>>)	53	54	44	50	49
Acceptable result (>)			6	4	5
Incorrect result	1		2		

Comment:

- Sample A: 1x wrong result (participant did not provide a result).
- Sample B: All results correct.
- Sample C:
  - 6x 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).
  - 2x wrong result (please see the description in the *KRAS* paragraph above – sample C – it covers also these 2 erroneous results in *NRAS* testing).
- Sample D: 4x 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).
- Sample E: 5x acceptable result (the same reason as in the case of sample C).

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	≤ 1 %	≤ 5 %	≤ 10 %	≤ 20 %	> 20 %
Number of the participants	10	36	4	4	-

NRAS summary: 51 of 54 participants (94 %) 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.**

54 participants reported the results (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 (>>>)	35	54	48	52	48
Acceptable result (>)	12		5	2	5
Incorrect result	7		1		1

Comment:

We recorded the most errors in the *BRAF* testing of the sample A. We assume that most of these errors were caused by incorrect entry of the results rather than wrong analyses.
Thus we strongly recommend namely to the users of the Biocartis Idylla system to read the text below very carefully!

We expected to receive form the users of the systems that are not able to specify exact mutation:

- either the result ***Exon 15 mutation (unable to specify in detail)*** – this way is preferred
- or ***Other*** plus an appropriate text note (something like "*V600E/D*") - this way is not ideal, but borderline acceptable

But some participants reported very weird data, most frequently:

- The mutation ***c.1799T>A,p.V600E*** – yes, it is correct result, but how did they get it? Surprisingly in some cases a text note was added specifying other (and wrong) mutation(s).
- The mutation ***c.1799_1800delinsAA,p.V600E*** – it is wrong result, but how did they get it? Surprisingly in some case a text note was added specifying other mutation(s) and in some cases this note contained also correct result.

Let's summarize the basic rules:

- If you specify exact mutation, never add a text note – it has no influence to the result assessment.
- 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).
- A text note you must specify 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.

Based on the facts mentioned above we can conclude that:

- Sample A:
  12x acceptable result (these participants reported either ***Exon 15 mutation (unable to specify in detail)*** or ***Other + an appropriate text note***).
  7x wrong result (these participants reported wrong result ***c.1799_1800delinsAA,p.V600E*** either with or without a text note).
- Sample B: All results correct.
- 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 (one participant did not test the sample C at all due to method failure).
- Sample D: 2x acceptable result (the same reason as in the sample C).
- Sample E:
  4x acceptable result (the same reason as in the sample C).
  1x wrong result (false positive result).

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: 46 of 54 participants (85 %) succeeded.**

Evaluation of the results – part 6: Laboratory reports

Each participant **must** upload **real (routine) laboratory report** in order to be classified as successful.
The content of uploaded laboratory reports has no influence on the evaluation of the performance, it is only an educational (post analytical) part of the round and supervisors may comment on it if necessary. In other words: **uploading of the real laboratory report is required**, but content (possible defects found) has no influence on the scoring of the participant.

The participants were asked to:

Upload one randomly selected routine laboratory report (not related to EQA samples), not older than 3 months, in your mother tongue.
Recommended procedure: Take your laboratory report, blacken all personal information that identifies the patient, scan it, save it (PDF or JPG is preferred), and upload the file.
Upload a report that shows positive result (CRC sample with mutation in KRAS or NRAS or BRAF gene).

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Assessment method
As the participants uploaded the routine reports in their mother tongue, it was a bit challenging to assess the content (we used Google translate during the evaluation). But we are convinced that we were able to recognise most of the essential information necessary to assess the compliance of the report with the requirements of the international standard ISO 15189 (Medical laboratories — Requirements for quality and competence).
Each report was checked against these requirements (all selected from ISO 15189, chapter 5.8.3 – Report content):

a) identification of the laboratory that issued the report
b) patient identification on each page
c) identification of the examination and the examination procedure
d) name or other unique identifier of the requester and the requester's contact details
e) date of primary sample collection
f) type of primary sample
g) examination results including interpretation
h) identification of the person(s) reviewing the results and authorizing the release of the report
i) date of the report release
j) page number to total number of pages (e.g. Page 1 of 2)

Results
Two industry laboratories did not upload the report which was deemed acceptable (no patients' samples are tested in these labs).
One clinical laboratory rejected to upload the report, which was evaluated as not acceptable.
All other participants **successfully** uploaded the reports and thus we assessed **51 reports**.
Technical note: All reports are treated as a confidential and they are accessed only by SEKK staff and supervisors.

In general

- Many reports are very, very long (many pages!) and it is a bit difficult to find the most important and clinically relevant part – the result of the tests. Plenty of methodological details are not beneficial for the recipient of the information (the oncologist). The same we can say about large paragraphs verbosely stating that "*... results are valid in the time of sample analyses and these are subject to change in future based on the scientific development ...*". This type of disclaimer is not helpful for the oncologists, as this can be stated about any test in any medical laboratory. The oncologists need the information about the test result for treatment decision relevant for the patient now, not in future. **The shorter/better structured reports would be more appropriate to the needs of the oncologists.**
- On the other hand any – even very basic - clinical interpretation is missing in many reports. Simple conclusion like "*Mutation found ...*" should be supplemented by one or two sentences **concerning the clinical impact of the result from the point of view of the anti-EGFR therapy**. The spectrum of therapeutic options as well as markers used for the treatment decision is expanding enormously and it is essential to provide oncologists not only result of the test but also its correct and unbiased clinical interpretations. Only this way it can be ensured that the result of the test is optimally utilised in therapeutic decision making.
- We found wrong page numbering in many reports. In particular for extensive reports, there is a rather high risk of losing part of the report (or swapping the pages with potential impact on the message in the report). Therefore, **unequivocal page numbering** is of utmost importance.

In particular
Each participant can find a short comment concerning their laboratory report in their result sheet.

Future rounds
We are aware of the fact that any changes in the report layout are long-lasting and challenging process. In many cases the cooperation of the IT department and/or LIS provider is necessary. Thus we would like to provide all participants sufficient time to evaluate the feedback from this EQA round and eventually implement possible improvements in their reports. Therefore, upload of the reports will be not required in the future rounds.

Conclusion

Identifying the mutations
The participants demonstrated very good performance in this round. Most failures in *BRAF* results were caused by the errors in reporting but we have seen also clear false negative and false positive results.

Laboratory reports
There is certainly a room for improvement - namely if we consider the fact that many labs are ISO 15189 accredited in these days.

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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.