Round: CRC1/20 – 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 scheme at one place.

Introduction

This EQA round was accomplished according to the document ***EQA Plan 2020***.
The scientific background of the CRC scheme 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 target of this EQA scheme is to **identify and describe gene mutations** (the participant can choose any combination of *KRAS, NRAS, BRAF* testing) that are clinically relevant to the anti-EGFR therapy for colorectal carcinoma. It is assumed 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)
- If the participant chooses to test *BRAF* then at least: codon 600 (exon 15)
  codon 601 (exon 15)

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 scheme 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 scheme for e.g. *KRAS* and was not successful for *NRAS* can participate in our scheme only to "correct" the previous unsuccessful result of *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.

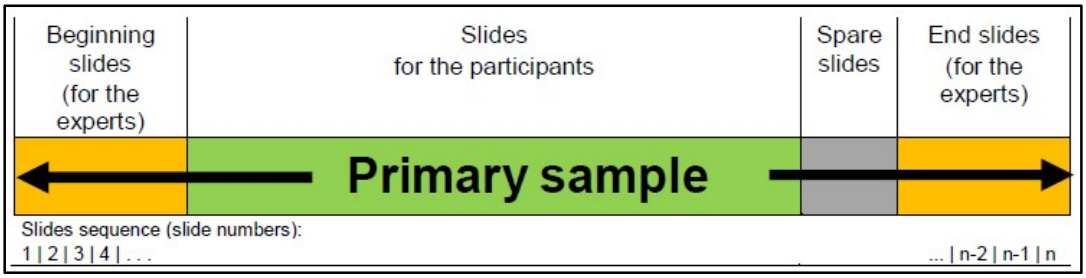
An integral part of this EQA scheme is also the evaluation of the **post-analytical phase** – the assessment of the routine laboratory reports of the participants. This part of the scheme is entirely educational – it means that **reports are required**, although the analysis of the content of these reports 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 45 participants in this round from 21 countries (the list of countries can be found in the report published on the web).
Additional two laboratories, which subscribed for the participation, received the samples, but did not upload the results and thus were not included in the evaluation.

Samples

The samples were formalin fixed paraffin embedded (FFPE) tissue sections from 10 invasive colorectal carcinomas (primary samples labelled A, B, C, ... J). Each participant received 3 sections (each 6 µm thick) 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.
We obtained the samples from 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 (starting from 1, see the figure below).



We shipped the samples and the documentation to the participants 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.

Round: CRC1/20 – Colorectal Carcinoma

The participants were allowed order spare samples in case of sample damage in their laboratory, but no participant used this opportunity.

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 (LRG_344t1)	***NRAS*** (LRG_92t1)	***BRAF*** (LRG_299t1)
A			
B			c.1799T>A,p.V600E
C			
D	c.35G>A,p.G12D		
E		c.181C>A,p.Q61K	
F	c.38G>A,p.G13D		
G	c.35G>T,p.G12V		
H			c.1799T>A,p.V600E
I		c.35G>A,p.G12D	
J			

Blank cells represent WT (wild type).

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 scheme, 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 scheme, there were two possible scenarios for such situation: 1) a result obtained by a method that does not allow a 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	The result is neither "Correct" nor "Acceptable".
Impossible to evaluate, marked ± in the reports	This is very special category indicating that we are not able 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 scheme this is very rare category indicating a case where expert consensus was not reached. It did not happen in this round.

Round: CRC1/20 – Colorectal Carcinoma

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 is either expected or acceptable.

Evaluation of 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	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. Most participants follow this recommendation.

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

Answer	*Count*
Pathologist	42

Comment: Excellent 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 (mostly GeneRead DNA FFPE kit and QIAamp DNA FFPE Tissue Kit)	19
Promega products (mostly Maxwell RSC FFPE Plus DNA Kit and Maxwell 16 FFPE Plus LEV DNA Purification Kit	11
Roche products (mostly Cobas DNA Sample Preparation Kit)	7
other manufacturers	6

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

Evaluation of 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*	*F*	*G*	*H*	*I*	*J*
No	-	-	-	-	-	-	-	-	-	-
Yes	45	45	45	45	45	45	45	45	45	45

Comment: All participants were able to test all samples.

Question: **Did you perform dissection?**

Answer	*Number of the participants*									
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
Not specified	1	0	0	0	0	0	1	1	3	1
No	7	11	21	8	17	10	22	17	18	14
Yes - macrodissection	31	30	19	31	22	29	19	24	21	24
Yes - microdissection	6	4	5	6	6	6	3	3	3	6

Comment:

- 7 participants processed all samples without dissection.
- 12 participants processed all samples using macrodissection.
- 2 participants processed all samples using microdissection.

The rest of the participants used different approaches in individual samples.

Round: CRC1/20 – Colorectal Carcinoma

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

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

3 participants did not specify the value.

The overview of the values reported by 42 participants you can find in the table.

	[%]									
	A	***B***	***C***	***D***	***E***	***F***	***G***	***H***	***I***	***J***
All results										
Minimum	20	20	30	20	25	15	25	30	15	20
Average	**41**	**58**	**62**	**48**	**58**	**37**	**64**	**71**	**56**	**52**
Median	**40**	**60**	**60**	**50**	**60**	**30**	**70**	**78**	**60**	**50**
Maximum	80	90	90	70	80	70	95	95	80	80
Experts										
Average	60	60	50	57	70	57	53	73	73	63

Comment: We can see a good agreement between the overall average and the average of expert laboratories. Also we can 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 20 % (or less) content seems to be clearly underestimated (only 9 participants reported 20 % or less in any sample).

Question: **Specify DNA concentration.**

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

	[ng/µL]									
	A	***B***	***C***	***D***	***E***	***F***	***G***	***H***	***I***	***J***
All results										
Minimum	1,8	2	0,8	1,1	5,1	1,0	0,4	3	2,9	0,7
Average	**17**	**27**	**50**	**21**	**48**	**23**	**45**	**45**	**53**	**52**
Maximum	55	79	200	100	200	72	170	200	200	200
Experts										
Average	18	34	42	19	49	28	53	43	58	69

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.

We highly appreciate very good agreement between the overall average and the average of the experts.

But the differences in the results reported for all samples (minimum vs. maximum) are enormous.

Moreover 2 participants noted (in the text comment) that they measured the concentration > 200 ng/µL (the Cibule app did not allow to enter such a high result). One participant reported such a high value in 3 samples and one in 7 (!) samples.

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.**

45 participants reported the results (a mutation was present in the samples D, F, G - see the paragraph *Assigned values* above):

Results	***Number of the participants***									
	A	***B***	***C***	***D*** ***(mut)***	***E***	***F*** ***(mut)***	***G*** ***(mut)***	***H***	***I***	***J***
Expected result (>>>)	44	45	45	42	45	42	40	45	45	45
Acceptable result (>)	1			3		3	3			
Incorrect result							2			

Comment:

- Most participants reported expected results in all samples.
- 3 participants were not able to specify exact mutations (due to limitations of their methods: *BioRad ddPCR KRAS G12/G13 screening kit, Roche: KRAS Mutation Test v2 LSR, EntroGen: RAS Mutation Analysis Kit*) but reported correctly exon containing the mutation; this is acceptable as from the clinical point of view these methods provide sufficient information for clinical management of the patient.
- 2 participants failed in the sample G (false negative results).

Round: CRC1/20 – Colorectal Carcinoma

The analytical sensitivity of the methods used by the participants:

Analytical sensitivity	0,1 %	1 %	2 %	3 %	4 %	5 %	7 %	10 %	20 %	25 %
Number of the participants	1	9	5	1	2	21	1	3	1	1

Only 2 participants reported the analytical sensitivity 20 % or more - here we recommend considering 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.

KRAS summary: 43 of 45 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.**

44 participants reported the results (a mutation was present in the samples E, I - see the paragraph *Assigned values* above):

Results	***Number of the participants***									
	A	***B***	***C***	***D***	***E (mut)***	***F***	***G***	***H***	***I (mut)***	***J***
Expected result (>>>)	44	42	43	41	41	41	41	42	41	44
Acceptable result (>)				3	3	3	3		3	
Incorrect result		2	1					2		

Comment:

- Most participants reported expected results in all samples.
- 3 participants were not able to specify exact mutation in the samples E and I (due to limitations of their methods: *BioRad ddPCR KRAS G12/G13 screening kit, Roche: BRAF/NRAS Mutation Test, EntroGen: RAS Mutation Analysis Kit*) but reported correct exons, which is acceptable as from the clinical point of view these methods provide sufficient information for clinical management of the patient.
- 3 participants did not test the samples D, F, G as these were *KRAS* positive. These results were accepted because this approach reflects the routine clinical practice.
- 2 participants failed in the samples B and H (reported *No result*) and 1 participant failed in the sample C (reported false positive result c.179 G>A, p.G60E).

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).

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

Please see the supplement dated 18.8.2020 at the end of this report.

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.**

44 participants reported the results (a mutation was present in the samples B, H - see the paragraph *Assigned values* above):

Results	***Number of the participants***									
	A	***B (mut)***	***C***	***D***	***E***	***F***	***G***	***H (mut)***	***I***	***J***
Expected result (>>>)	44	36	43	41	44	41	40	37	44	44
Acceptable result (>)		8		3		3	3	6		
Incorrect result			1				1	1		

Comment:

- Most participants reported expected results in all samples.
- 6 participants were not able to specify exact mutation in the samples E and I (due to limitations of their methods: *BioRad ddPCR BRAF V600 screening kit, Biocartis: Idylla NRAS-BRAF Mutation Test, Roche: BRAF/NRAS Mutation Test, Roche: cobas BRAF V600 Mutation Test, Idylla NRAS-BRAF Mutation Test, Qiagen: Therascreen BRAF RGQ PCR kit*) but reported mutations in correct exons, which is acceptable as from the clinical point of view these methods provide sufficient information for clinical management of the patient.

- 3 participants did not test the samples D, F, G as these had mutation in *KRAS*. These results were accepted because this approach reflects the routine clinical practice.
- 1 participant failed in the sample C (reported *No result*), 1 participant failed in sample H (reported c.1799_1800delinsAA,p.V600E), and 1 participant failed in the sample G (reported *Indeterminate mutation status*).

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: 41 of 44 participants (93 %) 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 report is required**, but content (possible errors found) has no influence on the scoring of the participant.

The task for the participants:

Upload scans of 2 randomly selected **routine laboratory reports** (not related to EQA samples), not older than 3 months, in your mother tongue.
Report 1: Negative result (CRC sample without mutations in *KRAS*, *NRAS*, and *BRAF* genes)
Report 2: Positive result (CRC sample with mutation in *KRAS* or *NRAS* or *BRAF* gene).
Instructions: Take your laboratory report, blacken all personal information identifying the patient, scan it, save it, and upload the file.

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 all the essential information necessary to assess the compliance of the report with the requirements of international standard for medical testing laboratories (ISO 15189).
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 is acceptable (no patients' samples are tested in these labs).
All other participants uploaded the reports and thus we assessed **43 pairs of reports**.
We would like to emphasise that we interpreted the assessment criteria mentioned above very "indulgently".

Example: ISO 15189 requires in 5.8.3., paragraph p): *page number to total number of pages (e.g. "Page 1 of 5", "Page 2 of 5", etc.)*. But we accepted also page numbering like: 1/5, 2/5 etc.

Round: CRC1/20 – Colorectal Carcinoma

Surprisingly, errors or non-conformities were found in vast majority (77 %) of the uploaded reports. The details are summarised in the table below:

Criterion	*Number of errors*		*Comment*
	absolute	*relative*	
Fake reports			
	17	40 %	**We classified report as a fake in all cases which did not fulfil original instructions (scan of a routine report with blackened patient data).** Many labs did not take into account that we required a scan of **the real laboratory report** with blackened sensitive patient data (like name, address etc.) and uploaded a "virtual" report prepared usually in Word. Moreover, even these fake reports contained many errors. One participant uploaded printscreen of the computer monitor content. One participant uploaded an output from the measuring system lacking most of the above required data. Several participants blackened so much data/areas that it was impossible to assess the reports (they blackened address of the lab, webpage, phones, dates etc.). **All above mentioned is unacceptable and we shall classify it as fatal error in the future EQA rounds!**
Real reports			
No error	10	23 %	Only 10 laboratories provided the reports that we can classify as conforming to all requirements - criterions a) to j). **Congratulations!**
a)	1	2 %	Very surprising that the identification of the laboratory was missing.
b)	2	5 %	We did not require patient name on each page, any unequivocal identification of the sample is sufficient.
c)	0	-	
d)	1	2 %	
e)	2	5 %	Very difficult criterion for many participants. There is usually mentioned (borderline acceptable) "date of the request" or "received", but clear "date of the primary sample collection" was missing in many reports (or was pre-printed, but left blank). **We do recommend paying attention to this criterion to all participants!**
f)	2	5 %	
g)	0	-	All reports presented a result but the interpretation was missing in many cases. **We do recommend paying attention to this criterion to all participants!** The spectrum of therapeutic options 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.
h)	3	7 %	This information is needed if the user wants to contact the authorizing person e.g. for clarification of some details in the report.
i)	2	5 %	
j)	8	19 %	Surprisingly the most critical point of the assessment. In extensive reports is the risk of loosing part of the report (or flipping the pages with potential impact on the message in the report) rather high. Therefore, correct pagination is of utmost importance. **We do recommend paying attention to this criterion to all participants!**

The participants will find a short comment concerning their laboratory report in their result sheets.

Conclusion

Identifying the mutations

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

Laboratory reports

To be honest we expected better performance in the content of the reports evaluation - namely if we consider the fact that many labs are ISO 15189 accredited in these days. A lot of participants sent us fake reports which is not useful and will be not accepted in future!

The CRC scheme in 2021 – what we are preparing for you

Based on the experience from this round of the CRC scheme, we have reviewed all aspects of the program and based on this ESP has decided to make some improvements to make the CRC scheme more user friendly and attractive to all participants. Here are the most important changes that you can expect in the next year:

- The CRC scheme will be organized in **2 rounds** (spring and autumn) with only 5 samples in each round. This step (splitting the 10 samples into 2 rounds) brings up **2 significant advantages**:
  1. The amount of work required from the participants per one round will be considerably reduced.
  2. In case of unsuccessful result the participant have the opportunity to demonstrate an improvement (effectiveness of corrective actions taken) sooner (the rounds will repeat every half year).

  The spring and autumn rounds are "independent" meaning that they are evaluated separately (e.g. in *KRAS* testing the participant can be unsuccessful in the spring, but successful in the autumn etc.). However, the participation in both rounds of the year will be required.
- **The virtual HE slides** of all primary samples will be available to the participants at our virtual microscopy website. This will help the participants to optimally process the samples.
- The requirement to upload the scan of routine laboratory report will remain, but the **procedure will be significantly simplified:** only one report will be required and the participant will receive comprehensive instructions (including an example). However, any fake reports (as mentioned above) will be considered unacceptable!
- The participants will be paying the participation fee directly to SEKK on the basis of the standard commercial invoice (VAT document). **This will decrease the participation fee significantly.**
- New items will be added to the list of the methods to make the data entry more convenient.

Scientific supervision:

Prof. Dr. med. Daniela E. Aust
Universitätsklinikum Carl Gustav Carus
Institut für Pathologie
Fetscherstraße 74
01307 Dresden, Germany
e-mail: Daniela.Aust@Uniklinikum-Dresden.de

Prof. Magali Svrcek, M.D., PhD.
Hôpital Saint-Antoine
service d'Anatomie et Cytologie Pathologiques
rue du Faubourg Saint-Antoine 184
750 12 Paris, France
e-mail: magali.svrcek@aphp.fr

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.

Round: CRC1/20 – Colorectal Carcinoma

Supplement dated 18.8.2020

This supplement was added to the final report on the basis the appeal of 2 participants. This supplement belongs to the evaluation of ***NRAS* results**.

We evaluated 2 participants as *not successful* as they reported *No result* for NRAS mutation in samples B and H (as described in the text above). These 2 participants informed us that they tested the samples in the order *KRAS – BRAF – NRAS* and this order of testing is their standard laboratory procedure.

These 2 participants did not test the samples B and H as these were *BRAF* positive. Thus we accepted additionally these results because this approach reflects the routine clinical practice (in principle this is the same as accepting missing *NRAS* results in samples with KRAS positivity).

After this adjustment, the new ***NRAS* summary is: 43 of 44 participants (98 %) succeeded**.

This supplement was approved by:

Prof. Dr. med. Daniela E. Aust and Prof. Magali Svrcek, M.D., PhD., scientific supervisors

and authorised by:

Marek Budina MSc., director of SEKK