

PDL12/22: Programmed Death Ligand 1

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.

Abbreviations used: TNBC ... triple-negative breast carcinoma
NSCLC ... non-small cell lung cancer
UC ... urothelial carcinoma
HNSCC ... head-and-neck squamous cell carcinoma

Please visit the web page
<http://www.sekk.cz/PDL1>
to find complete information about PDL1 programme in one location.

Introduction

This EQA round was completed according to the document *EQA Plan 2022*.

The scientific background of the PDL1 programme is under the control of the **European Society of Pathology** (ESP, www.esp-pathology.org). ESP recommended both scientific supervisor (see bottom of this report) and expert laboratories (see the paragraph *Expert laboratories*).

The tasks of the participants were to:

1. Perform immunohistochemical PD-L1 staining of the physical slides using the procedure they routinely use in their laboratory.
2. Examine all primary samples (both on physical and virtual slides) – this includes calculation of the required score and final result (negative/positive) determination.
3. Report the following information (using the web application):
 - The method used for staining.
 - Quantitative (the score) and qualitative (negative/positive) results. The **cut-offs** were prescribed and the participants were obliged to use these to divide the results into negative and positive.

Cut-offs prescribed

TNBC	1 % for SP142 clone (IC) and 1 for 22C3 clone (CPS)
NSCLC	1 % and 50 % (two cut-offs were prescribed) (TPS)
UC	10 (CPS)
HNSCC	1 (CPS)

Participants

There were 57 participants in this round from 19 countries (the list of countries you can find on the website).

Samples

The samples were prepared by the subcontractor. The samples were divided into 4 sets (subschemes), each set relates to one tumour type and contains one physical slide (unstained TMA section) and one virtual slide (PD-L1 stained TMA section).

The picture shows the map of the samples used for this round:

	Physical slide	Virtual slide																																													
Set 1: TNBC	<table border="1"> <thead> <tr> <th></th> <th>A1</th> <th>B1</th> <th>C1</th> <th>D1</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>2</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td>3</td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td></td> <td>●</td> <td>●</td> <td></td> <td></td> </tr> </tbody> </table>		A1	B1	C1	D1	1	○	○	○	○	2	○	○	○	○	3	○	○	○	○		●	●			<table border="1"> <thead> <tr> <th></th> <th>E1</th> <th>F1</th> <th>G1</th> <th>H1</th> </tr> </thead> <tbody> <tr> <td></td> <td>○</td> <td>○</td> <td>○</td> <td>○</td> </tr> <tr> <td></td> <td>○</td> <td>○</td> <td>○</td> <td>⊗</td> </tr> <tr> <td></td> <td>●</td> <td>●</td> <td></td> <td></td> </tr> </tbody> </table>		E1	F1	G1	H1		○	○	○	○		○	○	○	⊗		●	●		
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Each column in the TMA block represents one primary sample.

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Physical slides bore a minimum of 3 cores in the TMA block for each primary sample (this "redundancy" eliminates potential problems associated with missing or damaged tissue).

Virtual PD-L1 stained slides were available on our virtual microscopy website.

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 any sample damage in their laboratory.

Expert laboratories

All samples mentioned above were tested in 3 expert laboratories:

- University Erlangen-Nürnberg, Institut für Pathologie, Erlangen, Germany
- University Hospital Zurich, Department of Pathology and Molecular Pathology, Zurich, Switzerland
- Erasmus Universitair Medisch Centrum, Department of Pathology and Clinical Bioinformatics, Rotterdam, The Netherlands

Expert laboratories tested all samples as unknown. The task for each expert laboratory was to test the sample and report the results back to the SEKK (thus not only to confirm the results suggested by SEKK). In other words: expert laboratories tested the samples under the same conditions as regular participants.

We used the results of the expert laboratories to confirm the quality of the samples.

Assigned values (AVs)

The AVs (expected results) for the particular **primary samples** were obtained from the consensus of the participants. In accordance with ISO 17043 classification, we have used the **CVP** (consensus value from the participants) type of AV.

There are 2 rules applied in the process of establishing the AVs:

- Consensus is reached if **80 % or more** participants agree on a result.
- Minimal size of the group assessed is **n = 5** (in case of less than 5 results the group is not assessed).

Evaluation of the results

As mentioned above, the participants had to calculate and report the appropriate score (quantitative result) and using the prescribed cut-offs decide whether the sample is negative or positive (qualitative result).

The assessment is based on the qualitative results and consists of 2 steps:

Step 1)

The results of all primary samples (A, B H) were sorted into these categories from the point of view of the performance assessment:

<i>Category</i>	<i>Explanation</i>
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. It is the result identical to the AV (consensus of the participants).
Not assessed, marked ± in the reports	This category indicates that it would not be possible to establish the AV (the consensus among the laboratories was not reached). Without having the AV we are not able to classify the participant's result as "correct" or "incorrect". The sample is not assessed.
Incorrect result	Any result which is neither "Expected" nor "Not assessed".

Step 2)

On the basis of the primary samples assessment each slide (physical, virtual) of each set (TNBC, NSCLC, UC, HNSCC) was assessed (in EQA terminology there were 8 tests assessed: TNBC physical slide, TNBC virtual slide, NSCLC physical slide etc.).

The slide (one test) consists of 4 primary samples and the assessment of the slide depends on the number of the assessable primary samples on the slide this way:

- If all 4 primary samples are assessable then the slide is assessed as successful if the results of 3 or 4 primary samples are correct (i.e. an error in one primary sample is tolerated).
- If 3 or fewer primary samples are assessable then the slide is assessed as successful if the results of all assessed primary samples are correct (i.e. an error in any primary sample is not tolerated).

The results – each set (tumour type) and slide are discussed separately

When reading this report please view also your result sheet or summary statistics located on the web – you can find the complete overview of the results in these documents (including the primary samples where the consensus of the participants was not reached).

PDL12/22: Programmed Death Ligand 1**Set 1: TNBC**

The participants were free to choose either the SP142 or 22C3 clone to stain the physical slide.

Physical slide (samples A1, B1, C1, D1) – clone SP142

Number of the participants: 20

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
A1	negative	
B1	not reached	55 % of the participants reported negative
C1	negative	
D1	positive	

Assessment: 3 samples were assessable (consensus reached).

Quantitative results – overview [unit: %]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
A1	0	0,13	5	
B1	0	1,9	15	
C1	0	0,02	1	
D1	0,5	8,8	60	

Whole slide assessment: The slide assessment was based on the qualitative results of the samples A1, C1, D1 (as the sample B1 was not assessable) and only those participants succeeded who were successful in all the samples assessed. The total success rate was 75 % which is a good result.

Physical slide (samples A1, B1, C1, D1) – clone 22C3

Number of the participants: 9

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
A1	not reached	78 % of the participants reported negative
B1	not reached	67 % of the participants reported negative
C1	negative	
D1	positive	

Assessment: 2 samples were assessable (consensus reached).

Quantitative results – overview [unit: dimensionless number]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
A1	0	0	2	
B1	0	0,5	4	
C1	0	0	0,5	
D1	4	18	70	

Whole slide assessment: The slide assessment was based on the qualitative results of the samples C1, D1 (as the samples A1, B1 were not assessable) and only those participants succeeded who were successful in all the samples assessed. The total success rate was 89 % which is a good result.

Virtual slide (samples E1, F1, G1, H1)

Number of the participants: 28

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
E1	negative	
F1	not reached	79 % of the participants reported negative
G1	positive	
H1	not reached	57 % of the participants reported negative

Assessment: 2 samples were assessable (consensus reached).

Quantitative results – overview [unit: %]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
E1	0	0,07	20	
F1	0	0,33	20	
G1	1	30	80	
H1	0	0,89	5	

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Whole slide assessment: The slide assessment was based on the qualitative results of the samples E1, G1 (as the samples F1, H1 were not assessable) and only those participants succeeded who were successful in all the samples assessed. The total success rate was 93 % which is a good result.

Set 2: NSCLC**Physical slide (samples A2, B2, C2, D2)**

Number of the participants: 51

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
A2	negative (TPS < 1)	
B2	positive	both <i>positive (TPS 1 - 49)</i> and <i>positive (TPS ≥ 50)</i> were accepted
C2	positive	both <i>positive (TPS 1 - 49)</i> and <i>positive (TPS ≥ 50)</i> were accepted
D2	positive (TPS ≥ 50)	

Assessment: 4 samples were assessable (consensus reached).

Quantitative results – overview [unit: %]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
A2	0	0,17	2	
B2	10	59	95	the score was near 50 % and that is why both <i>positive</i> results were accepted (see table above)
C2	2	43	80	the same situation as in the B2 sample
D2	0	95	100	

Whole slide assessment: The slide assessment was based on the qualitative results of all samples and the rule “3 correct results of 4 must be achieved by the participant” was used. The total success rate was 100 % which is a great result.

Virtual slide (samples E2, F2, G2, H2)

Number of the participants: 50

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
E2	positive	both <i>positive (TPS 1 - 49)</i> and <i>positive (TPS ≥ 50)</i> were accepted
F2	negative (TPS < 1)	
G2	positive (TPS ≥ 50)	
H2	positive (TPS ≥ 50)	

Assessment: 4 samples were assessable (consensus reached).

Quantitative results – overview [unit: %]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
E2	5	45	80	the score was near 50 % and that is why both <i>positive</i> results were accepted (see table above)
F2	0	0,31	90	
G2	0	98	100	
H2	40	76	100	

Whole slide assessment: The slide assessment was based on the qualitative results of all samples and the rule “3 correct results of 4 must be achieved by the participant” was used. The total success rate was 96 % which is great result.

PDL12/22: Programmed Death Ligand 1**Set 3: UC****Physical slide (samples A3, B3, C3, D3)**

Number of the participants: 36

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
A3	negative	
B3	negative	
C3	negative	
D3	positive	

Assessment: 4 samples were assessable (consensus reached).

Quantitative results – overview [unit: dimensionless number]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
A3	0	0	0,5	
B3	0	0,16	5	
C3	0	1,1	10	
D3	1	20	62	

Whole slide assessment: The slide assessment was based on the qualitative results of all samples and the rule “3 correct results of 4 must be achieved by the participant” was used. The total success rate was 100 % which is a great result.

Virtual slide (samples E3, F3, G3, H3)

Number of the participants: 36

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
E3	positive	
F3	negative	
G3	negative	
H3	negative	

Assessment: 4 samples were assessable (consensus reached).

Quantitative results – overview [unit: dimensionless number]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
E3	15	63	100	
F3	0	3,0	13,5	
G3	0	0,83	20	
H3	0	0,32	7,4	

Whole slide assessment: The slide assessment was based on the qualitative results of all samples and the rule “3 correct results of 4 must be achieved by the participant” was used. The total success rate was 94 % which is very good result.

Set 4: HNSCC**Physical slide (samples A4, B4, C4, D4)**

Number of the participants: 36

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
A4	not reached	78 % of the participants reported positive
B4	not reached	58 % of the participants reported negative
C4	positive	
D4	positive	

Assessment: 2 samples were assessable (consensus reached).

PDL12/22: Programmed Death Ligand 1**Quantitative results** – overview [unit: dimensionless number]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
A4	0	6,3	50	
B4	0	1,2	20	
C4	0	11	100	
D4	5	88	100	

Whole slide assessment: The slide assessment was based on the qualitative results of the samples C4, D4 (as the samples A4, B4 were not assessable) and only those participants succeeded who were successful in all the samples assessed. The total success rate was 81 % which is a good result.

Virtual slide (samples E4, F4, G4, H4)

Number of the participants: 28

Qualitative results - assigned values (AV):

<i>Sample</i>	<i>Consensus (AV)</i>	<i>Notes</i>
E4	positive	
F4	not reached	67 % of the participants reported positive
G4	not reached	61 % of the participants reported negative
H4	positive	

Assessment: 2 samples were assessable (consensus reached).

Quantitative results – overview [unit: dimensionless number]:

<i>Sample</i>	<i>Minimum</i>	<i>Robust mean</i>	<i>Maximum</i>	<i>Notes</i>
E4	20	63	100	
F4	0	2,2	15	
G4	0	1	10	
H4	0,9	16	86	

Whole slide assessment: The slide assessment was based on the qualitative results of the samples E4, H4 (as the samples F4, G4 were not assessable) and only those participants succeeded who were successful in all the samples assessed. The total success rate was 94 % which is a good result.

Opportunities for improvement**Interpretation problems**

Similarly to the spring EQA round we observed some interpretation problems in this round. **Examples** demonstrating this problem:

- Virtual slide (UC), sample F3 (cut-off = 10): one participant reported the score = 3 and evaluated it as a *positive*.
- Virtual slide (HNSCC), sample F4 (cut-off = 1): one participant reported the score = 5 and evaluated it as a *negative* and one participant reported the score = 7 and evaluated it as a *negative*.
- At “borderline situations” (e.g. cut-off = 1 and reported score = 1) we observed a few cases where participants reported a *negative* result.

Recommendations

- Please consider the cut-off as a strict border (regardless of the fact that the qualitative result – score – has an uncertainty and this uncertainty is surely not negligible – we can try to consider the uncertainty in future).
- If you obtain the score “< 1” then specify “0” (zero) or any number less than 1 as a quantitative result (the web application does not allow to recognise the “less than” sign in the numerical result).

Large spread of scores calculated

You can notice in the tables above that the ranges of the scores reported by the participants were considerable in virtually all cases. **Examples** demonstrating this problem:

- Physical slide (NSCLC), sample D2: 48 participants reported the score > 30 %, but 1 participant reported 0 %.
- Virtual slide (NSCLC), sample F2: 43 participants reported the score ≤ 1 %, but 2 participants reported the score > 80 %.
- Virtual slide (NSCLC), sample G2: 46 participants reported the score ≥ 80 %, but 2 participants reported the score 0 %. The 2 participants from this and previous paragraphs were the same – did they mix up the samples?
- Physical slide (HNSCC), sample C4: 2 participants reported the score = 0 and one participant reported 100.

PDL12/22: Programmed Death Ligand 1**Recommendations**

It is necessary to pay attention to the identification of the samples to prevent confusion. In case of the virtual slides, these remain available for review, which in particular those participants who reported very outlying score may find helpful.

Conclusion

We have to congratulate the participants on their results because the total success in all slides was very good.

Physical slides

The performance of the participants was very good.

A few participants reported (as a text comment) that some cores in a TMA column were missing or folded – this can happen and that is why more cores of each primary sample were placed in each column of TMA (the cores were redundant).

Virtual slides

The wide spread of the scores was somewhat surprising. Despite this fact the performance was very good.

As a summary this round was very successful and the results achieved are very promising.

All virtual slides are available for free for everybody for the educational purposes till the end of the year 2022 at: <https://www.eqa.cz/vm>

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Supplements

As a supplement to this report individual participants receive:

<i>Name of supplement</i>	<i>Remark</i>
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.
Histograms (quantitative results)	Only for the quantitative 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 on 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.