PDL11/23: 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 2023***.
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 the 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 sort the results into negative and positive groups.
     Cut-offs prescribed

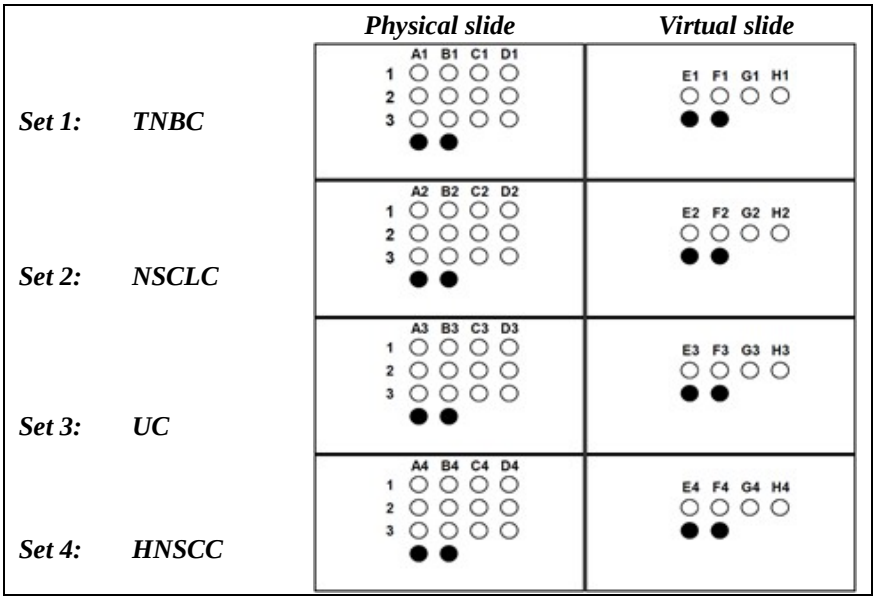
TNBC	**1 %** for SP142 clone (IC) and **1** for 22C3 clone (CPS)
NSCLC	**1 %** and **50 %** (two cut-offs were prescribed) (TPS)
UC	**10** (CPS)
HNSCC	**1** (CPS)

Participants

There were 55 participants in this round from 18 countries (the list of countries you can find on the website). These numbers are similar to those of the year 2022 (56 participants, 18 countries).

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-L1stained TMA section).
The picture shows the map of the samples used for this round:



Each column in the TMA block represents one primary sample. Black coloured cores represent tonsillar tissue.

PDL11/23: Programmed Death Ligand 1

Physical slides bore up to 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 a 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** (smaller groups are 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:

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. 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. no error in any primary sample is tolerated).

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

When reading this part of the report please view also your result sheet or summary statistics available 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).

PDL11/23: 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: 17

Qualitative results - assigned values (AV):

One participant did not report the result for the sample A1 and gave us no explanation why.

Assessment: 4 samples were assessable (consensus reached in all samples).

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 88 % which is a good result.

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

Number of the participants: 17

Qualitative results - assigned values (AV):

Assessment: 3 samples were assessable (consensus reached in the samples A1, C1, D1).

Whole slide assessment: The slide assessment was based on the qualitative results of 3 samples and only those participants succeeded who were successful in all the samples assessed. The total success rate was 65 % which is a poor result.

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

Number of the participants: 31

Qualitative results - assigned values (AV):

Assessment: 2 samples were assessable (consensus reached in the samples F1, G1).

Whole slide assessment: The slide assessment was based on the qualitative results of 2 samples and only those participants succeeded who were successful in all the samples assessed. The total success rate was 97 % which is an excellent result.

As mentioned above the consensus was not reached in 2 samples:

- E1: 13 participants reported the score < 1 and a negative result. 16 participants reported a score $\geq$ 1 and 13 of them evaluated it as a positive result, but – surprisingly – 3 laboratories evaluated this result as negative. Among the expert labs, two reported a negative and one reported a positive result.
- H1: Only 4 participants reported a score < 1 and a negative result. 25 participants reported a score $\geq$ 1 and 21 of these evaluated it as a positive result, but – surprisingly – 4 laboratories evaluated this result as negative. Among the expert labs, two reported a negative and one reported a positive result.

Set 2: NSCLC

In case of NSCLC two cut-offs are used and in case of positive samples both "1 % positivity" and "50 % positivity" are assessed as the correct results.

Physical slide (samples A2, B2, C2, D2)

Number of the participants: 50

Qualitative results - assigned values (AV):

Assessment: 4 samples were assessable (consensus reached in all samples).

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 98 % which is an excellent result.

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

Number of the participants: 50

Qualitative results - assigned values (AV):

Assessment: 3 samples were assessable (consensus reached in the samples F2, G2, H2).

Whole slide assessment: The slide assessment was based on the qualitative results of 3 samples and only those participants succeeded who were successful in all the samples assessed. The total success rate was 96 % which is an excellent result.

PDL11/23: Programmed Death Ligand 1

Set 3: UC

Physical slide (samples A3, B3, C3, D3)

Number of the participants: 39

Qualitative results - assigned values (AV):

Assessment: 3 samples were assessable (consensus reached in the samples B3, C3, D3).

Whole slide assessment: The slide assessment was based on the qualitative results of 3 samples and only those participants succeeded who were successful in all the samples assessed. The total success rate was 82 % which is a good result.

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

Number of the participants: 39

Qualitative results - assigned values (AV):

Assessment: 2 samples were assessable (consensus reached in the samples F3, G3).

Whole slide assessment: The slide assessment was based on the qualitative results of 2 samples and only those participants succeeded who were successful in all the samples assessed. The total success rate was 100 % which is excellent result.

As mentioned above the consensus was not reached in 2 samples:

- E3: 10 participants reported a score < 10 and 9 of these a negative result and one (surprisingly) inconclusive result. 28 participants reported a score $\geq$ 10 and 27 of these evaluated it as a positive result, but – surprisingly – one laboratory evaluated this result as inconclusive. All expert labs reported positive results.
- H3: 31 participants reported a score < 10 and 30 of these a negative result and one (surprisingly) an inconclusive result. 7 participants reported a score $\geq$ 10 and 6 of them evaluated it as a positive result, but – surprisingly – one laboratory evaluated a score of 95 as negative. All expert labs reported negative results.

Set 4: HNSCC

Physical slide (samples A4, B4, C4, D4)

Number of the participants: 35

Qualitative results - assigned values (AV):

Assessment: 4 samples were assessable (consensus reached in all samples).

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 an excellent result.

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

Number of the participants: 35

Qualitative results - assigned values (AV):

Assessment: 4 samples were assessable (consensus reached in all samples).

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 an excellent result.

Opportunities for improvement

Interpretation problems

Similarly to the previous EQA rounds we observed some interpretation problems. **Examples** demonstrating this problem:

- Virtual slide (TNBC, clone SP142), sample F1 (cut-off = 1 %): three participants reported the score as 1 % or 2 % and evaluated it as a *negative*.
- Physical slide (NSCLC), sample A2 (cut-off = 1 % and 50 %): two participants reported the score as 1 % or 2 % and evaluated it as a *negative*.
- Physical slide (UC), sample A3 (cut-off = 10): one participant reported the score = 5 and evaluated it as a *positive*.

PDL11/23: Programmed Death Ligand 1

- 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-offs provided in the documentation as a strict criterion (regardless of the fact that the qualitative result – score – has an uncertainty and this uncertainty is surely not negligible).
- If you obtain a score "< 1" then specify "0" (zero) or any number less than 1 as a quantitative result (the web application does not allow to enter the "less than" sign in the numerical result).

A few participants correctly pointed out that we should change the cut-off for TNBC clone 22C3 from 1 to 10. We would like to thank them for these comments and we will change the cut-off starting with the autumn EQA round.

All virtual slides are freely accessible for educational purposes untill the end of the year at:
https://www.eqa.cz/vm

Scientific supervision: Dr. Jan von der Thüsen MA MBBS PhD
Erasmus Universitair Medisch Centrum, Pathology
Dr. Molewaterplein 40
3015 GD Rotterdam, The Netherlands
e-mail: j.vonderthusen@erasmusmc.nl

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.
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.