PDL11/26: 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
www.sekk.cz/PDL1
to find complete information about PDL1 programme at one place.

Introduction

This EQA round was completed according to the document ***EQA Plan 2026***.
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

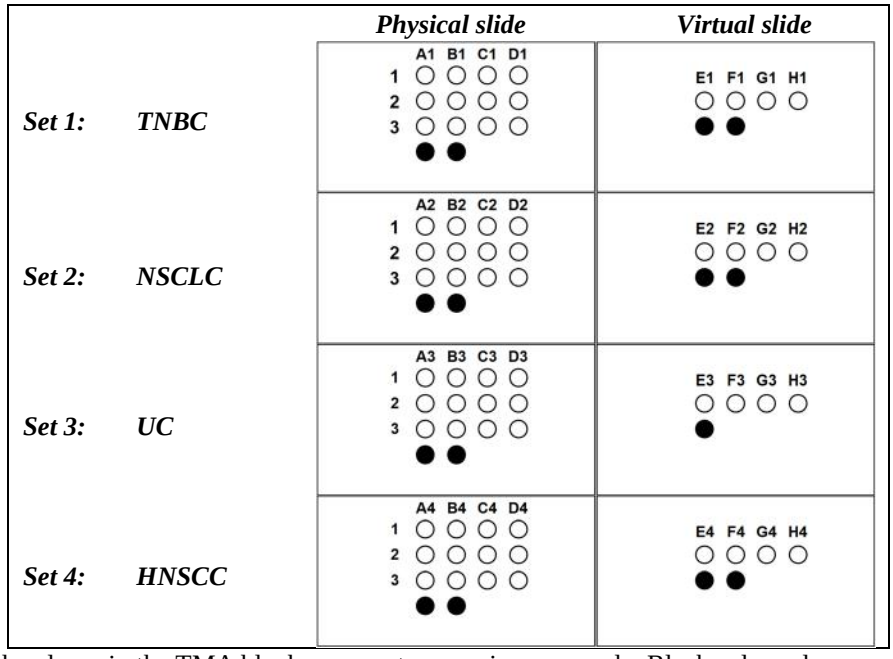
TNBC	**1 %** for SP142 clone (IC) and **10** for 22C3 clone (CPS)
NSCLC	**1 %** and **50 %** (two cut-offs were prescribed) (TPS)
UC	**10** (CPS)
HNSCC	**1** (CPS)

Participants

There were 61 participants in this round from 18 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, 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:



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

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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). One core is sufficient to proceed with the particular primary sample analysis.
We used DAKO glasses for the set 3 (UC) samples and a few participants reported incompatibility of these glasses with Ventana system. In these cases we sent the new slides to these participants and we prolonged the deadline for them (to compensate the time of the delivery of a new slide).
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), all parcels were delivered.
The participants were allowed to order spare samples in case of a sample damage in their laboratory.

Expert laboratories

The task for each expert laboratory was to test the sample and report the results the same way like other participants.
We used the results of the expert laboratories to monitor the quality of the samples.
List of the expert laboratories:

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

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.
Consensus is reached if **80 % or more** participants agree on a result.

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.
Acceptable result, marked > in the reports	The result is suboptimal, but acceptable and assessed as "correct".
Not assessed, marked ± in the reports	This category indicates that it is not 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 "Acceptable" nor "Not assessed".

Step 2)
On the basis of the primary samples assessment of 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).

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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 participants: 16
Consensus was not reached on the sample A1.
We observed no errors in the interpretation of the quantitative results.

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

Number of participants: 32
Consensus was not reached on the samples A1 and C1.
One participant reported wrong results of the interpretation – their quantitative results seemed to be OK but they probably overlooked that cut-off for CPS is 10 (they probably used 1 and thus they interpreted all samples as positive).

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

PD-L1 stained, clone SP142.
Number of participants: 45
Consensus was not reached on the sample G1.
Unfortunately we observed many errors in the interpretation of the quantitative results. It is possible that some participants **overlooked** the information that virtual slide was stained with SP142 clone (IC cut-off = 1 %) and used wrong cut-off.

- The most frequent error was the interpretation of the score = 1 % as negative or inconclusive result (6 cases).
- In other 6 cases the score higher than 1 % was interpreted as negative.

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 participants: 53
Consensus was not reached on the sample D2.
We observed no errors in the interpretation of the quantitative results.

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

PD-L1 stained, clone 22C3.
Number of participants: 53
Consensus was not reached on the sample E2.
We observed no errors in the interpretation of the quantitative results.

Set 3: UC

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

Number of participants: 44
Consensus was not reached on the samples A3, C3.
We observed a few errors in the interpretation of the quantitative results:

- sample B3: 1 participant classified the score 1 % as *positive*
- sample C3: 1 participant classified the score 3 % as *positive*

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

Number of participants: 44
Consensus was not reached on the sample G3.
We observed one error in the interpretation of the quantitative results:

- sample G3: 1 participant classified the score 5 % as *positive*

Set 4: HNSCC

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

Number of participants: 48
Consensus was reached on all samples.
We observed one error in the interpretation of the quantitative results:

- sample D4: 1 participant classified the score 8 % as *negative*

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Virtual slide (samples E4, F4, G4, H4)
Number of participants: 47
Consensus was not reached on the samples G4, H4.
We observed no errors in the interpretation of the quantitative results.

Opportunities for improvement

Interpretation problems

Similarly to the previous EQA rounds we observed some interpretation problems. Many **examples** demonstrating this problem are described in the text above.

Recommendations

- Pay attention to the cut-offs prescribed in the documentation of the EQA round and classify your quantitative results (scores) in accordance with these cut-offs.
- 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).

All virtual slides are freely accessible for educational purposes at: www.eqa.cz/vm

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Supplements

As a supplement to this report individual participant who reported the results gain:

Name	***Description***
Confirmation of attendance Certificate Result sheet	The conditions for issuing the relevant document specified in the EQA Plan must be met.
Summary overviews of quantitative results	These documents include a graphical overview of the results in the form of the complex statistics (this includes for each test histograms and summary statistics in graphical form).

The supplements are identified by their name, EQA round identification and participant code and are intended for the needs of the participant.

If you reported the results in this round, you can find your individual evaluation (reports) in the **Cibule** application (https://www.eqa.cz/cibule). After login, select ***EQA Results - View*** in the menu and then click the ***Reports*** button for the particular round.

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.