EuroFlow PIDOT EQA scheme 2025

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The Primary Immunodeficiency Orientation Tube (PIDOT) external quality assessment (EQA) scheme is intended for laboratories that use the EuroFlow PIDOT antibody panel and the relevant EuroFlow standard operating procedures (SOPs) in their routine diagnostics. It consists of a wet lab part and a dry part. The objective of the wet lab part is to evaluate the technical quality of sample preparation and measurement on the flow cytometer. The objective of the dry part is to evaluate the ability to analyze and interpret flow cytometry standard (FCS) files of patients with confirmed primary immunodeficiencies (PID).

In the wet lab part, peripheral blood samples of 3 healthy donors are taken locally at the participant's laboratory. The samples should be treated in the same manner as routine samples. They are stained with the EuroFlow PIDOT antibody panel and measured on the local flow cytometer, following the EuroFlow SOP for sample preparation and the EuroFlow SOP for instrument set-up and compensation. The FCS files are analyzed in BD Infinicyt™ software, using a provided EQA profile and a recommended gating strategy. Alternative analytical software can also be used.

In the dry part, participants are provided with 2 FCS files provided by the EQA provider. These FCS files were generated from PID, non-PID disease controls in whom PID diagnosis was ruled out (as defined by the treating physician based on standard clinical care), or healthy donor samples using the EuroFlow PIDOT antibody panel and standardized EuroFlow SOPs for sample preparation and bulk lysis. Participants analyze the provided FCS files and report their conclusions in terms of cell counts, interpretation of the cell counts against age-matched reference values, combined interpretation of the T and B cell maturation patterns, and the most compatible PID subtypes based on the immunophenotype and limited clinical information provided.

All EQA results, both for the wet lab and dry part, are submitted by the participants via an online datasheet in the ESLHO EQA Portal.

As EQA provider, ESLHO offers the PIDOT scheme in collaboration with the EuroFlow EQA Committee. The EuroFlow EQA Committee is composed of members of the EuroFlow Consortium (www.euroflow.org). Two unique rounds of the PIDOT scheme are offered in 2025: one in spring and one in autumn.

Data analysis and performance evaluation

Data analysis, performance evaluation, and preparation of the reports is supported by qualified and experienced experts within the EuroFlow Consortium.

Wet lab part

Numerical values of the MedFIs produced by flow cytometry are relative measurements that are not transferable to SI units. No artificial standards are available. Instead, distribution statistics is used by calculating D^{max} for each reagent on its target population (calculations made based on 96 measurements in 7 laboratories) (Neirinck et al., 2024).
MedFI values reported by the participants are compared to the EuroFlow reference data set using performance score metrics. The p-score for each reported numerical value is calculated using the below function,

$$p-score = \frac{\log_{10} MedFI - \log_{10} qaMedFI}{D^{max}}$$

where qaMedFI is the median of the MedFIs in the reference data set and D^{max} is the maximal allowed difference calculated based on the differences of MedFIs reported in the reference dataset from qaMedFI.

The absolute value of the p-score equals or exceeds the value '1' when the maximum allowed difference from the reference data set is exceeded. In such case, the particular result is considered incorrect. Based on D^{max}, it is expected that at least 90% of the p-scores fall within the acceptable range. The 'wet part overall score' is defined as the percentage of acceptable p-scores for each laboratory across all marker subset combinations per round. An overall score of 91% and above (at least 30 correct values out of 33 reported values) is considered as **successful with a perfect score**. Scorings higher than 76% and lower than 91% are considered as **successful with an acceptable score** (at least 26 correct values). Scorings equal to or below 76%, matching the 10^{th} percentile of the well-established EuroFlow LST EQA scheme, are considered **unsuccessful**.

In summary, performance in the PIDOT EQA scheme is scored as follows:

- **Successful (perfect score)**: 30, 31, 32, or 33 correct values
- **Successful (acceptable score)**: 26, 27, 28, 29 correct values
- **Unsuccessful**: 25 or less correct values

In case all three reported values for a given marker are incorrect, this indicates a systematic error in that marker.

Dry part

Performance evaluation is based on comparison of the participant's results, including cell counts and clinical interpretation, to the consensus reference interpretation of the 2 FCS files, which is defined by three experts. Where appropriate, the median, minimum, and maximum of the cell count values reported by the participants are provided so that participants can assess their results in comparison with the peer group.

Each participating laboratory will be provided with an EQA certificate that shows their performance in the wet lab and dry part, general information regarding the round, and an overview of common mistakes.

Educational meeting

All EuroFlow schemes' EQA rounds offered in 2025 will be concluded with an educational meeting, which will include all rounds performed throughout the year. Participation is free of charge for two representatives per participating laboratory. During the meeting, the rounds' results will be shown (anonymized), possible problems and pitfalls will be discussed, and there will be the opportunity to receive direct feedback. More information regarding the educational meeting will be announced on the ESLHO EQA Portal.

Timelines

Activity	**Date**
Registration for rounds 1 & 2 (spring & autumn)	7 Feb – 28 Feb 2025
Round 1: Distribution of round instructions	1 Apr 2025
Round 1: Reporting of results	1 Apr – 30 Apr 2025
Round 1: Distribution of certificates	Aug 2025
Round 1: Appeals period	3 weeks following distribution of certificates
Round 1: Distribution of final certificates	Sep 2025

Activity	**Date**
Registration for round 2 (autumn)	29 May – 31 Aug 2025
Round 2: Distribution of round instructions	30 Sep 2025
Round 2: Reporting of results	30 Sep – 31 Oct 2025
Round 2: Distribution of certificates	Jan 2026
Round 2: Appeals period	3 weeks following distribution of certificates
Round 2: Distribution of final certificates	Feb 2026

Registration

Registering for the EuroFlow PIDOT EQA scheme 2025 can be done via the ESLHO EQA Portal.

Appeals

Appeals regarding performance results can be submitted within 21 calendar days following the release of the EQA certificate via the ESLHO EQA Portal. A clear description of the appeal should be included and illustrating figures are recommended.

Complaints

Complaints related to ESLHO's EQA program, or specifically to the EuroFlow PIDOT EQA scheme, can be submitted at any time via the Complaints form that is available on the ESLHO EQA Portal.

Participation fee

ESLHO offers the PIDOT 2025 rounds free of charge to participants of the EuroFlow Consortium.

Organization

The laboratory at the University of Ghent, Ghent, BE, operates as the leading expert laboratory of the PIDOT scheme, with Prof. Dr. Carolien Bonroy in the role of lead subject-matter expert. In addition, other EuroFlow subject-matter experts provide support with case selection, determination of consensus results, data analysis, performance evaluation, and reporting.

ESLHO			
Name	**Organization/Institute**	**Role**	**Tasks**
Prof. Dr. Jacques J. M. van Dongen	ESLHO, Zutphen, NL	EQA Program Coordinator	Coordinator with final responsibility; authorizes the EQA certificate.
Dr. Bart Lubbers	ESLHO, Zutphen, NL	EQA Officer	Responsible for organization and operation of the PIDOT scheme by ESLHO.
Evelien Rijkers	ESLHO, Zutphen, NL	EQA Officer	Supports in the organization and operation of the PIDOT scheme.
Lead expert laboratory			
Name	**Organization/Institute**	**Role**	**Tasks**
Prof. Dr. Carolien Bonroy	Ghent University Hospital, Ghent, BE	Lead subject-matter expert	Pre-round: Case collection, clinical quality check of fcs data, case selection, PID case interpretation Round: Reference lab Post-round: Round summary report
Dr. Mattias Hofmans	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: PID case interpretation Round: Reference lab Post-round: Round summary report
Malicorne Buysse	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Case collection, technical/clinical quality check of fcs data, case selection, PID case interpretation Round: Reference lab Post-round: Round summary report

Jana De Wolf	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Technical quality check of fcs data
Pauline Breughe	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Technical quality check of fcs data
Other expert laboratories			
Name	**Organization/Institute**	**Role**	**Tasks**
Dr. Naděžda Brdičková	Charles University, Prague, CZ	Subject-matter expert	Pre-round: Supports preparation of the rounds. Post-round: Cleaning and analysis of the submitted results, preparation of the EQA certificate.
Prof. Dr. Tomáš Kalina	Charles University, Prague, CZ	Subject-matter expert	Case selection, expert analysis, input for determination of consensus results
Prof. Dr. Martín Pérez	University of Salamanca, Salamanca, ES	Subject-matter expert	Case selection, expert analysis, input for determination of consensus results

For more information or in case you have questions about the EuroFlow PIDOT scheme, or other EuroFlow EQA schemes, please contact EuroFlow.EQA@eslho.org.