EuroFlow PIDOT EQA scheme 2026

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The EuroFlow PIDOT EQA scheme

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 (note that no samples are provided by ESLHO). The samples should be treated in the same manner as routine samples. Participants stain the samples with the EuroFlow PIDOT antibody panel and measure them on the local flow cytometer, following the EuroFlow SOP for sample preparation and the EuroFlow SOP for instrument set-up and compensation, which can be accessed via https://app.euroflow.org/downloads/public. The FCS files are recommended to be analyzed in BD Infinicyt™ software, using a provided EQA profile and a recommended gating strategy. Participants report the median fluorescence intensity (MedFI) values of 11 cell subsets of each sample.

In the dry part, participants are provided with 2 FCS files by ESLHO. These FCS files are generated by EuroFlow-affiliated expert laboratories using the EuroFlow PIDOT antibody panel and standardized EuroFlow SOPs for sample preparation and bulk lysis. Peripheral blood was used 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. Participants are advised to analyze the files using BD Infinicyt™ software. Alternative analytical software may be used; however, files will only be

validated for analysis with BD Infinicyt™. Therefore, we cannot guarantee compatibility with other software. Participants who encounter issues analyzing files with software other than Infinicyt are advised to contact ESLHO, so we can look for a solution.

Participants 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 results form 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 rounds of the PIDOT scheme are offered in 2026: one in spring and one in autumn.

Data analysis, reference values, and participant performance

Data analysis, scoring participant performance, and preparation of the reports are carried out by qualified and experienced experts within the EuroFlow Consortium.

Wet lab part

MedFI values reported by the participants are compared to the EuroFlow reference data set (calculations made based on 96 measurements in 7 laboratories; Neirinck et al., 2024) 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 from qaMedFI. D^{max} is determined by calculating the 5^{th} and 95^{th} percentiles of the differences between all MedFI values in the reference dataset and qaMedFI. These two percentiles are expressed as absolute values, and the larger value is used as D^{max}.

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 out of range and therefore incorrect. Based on the calculation of D^{max}, it is expected that 90 – 95% 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% are considered **unsuccessful** (less than 26 correct values).

In summary, performance in the wet lab part of the PIDOT 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

The dry part of the PIDOT scheme does not include a performance scoring system. Instead, participants can compare their results to the reference values of the 2 PID cases to understand how they performed in comparison to the experts. The consensus reference interpretation is based on analysis by (typically) three experts per case and defined by consolidating the results and discussion during an experts meeting. The reference results are defined as follows:

- **Cell counts (% and cells/µL)**: median cell counts provided by the subject-matter experts.
- **Cell count interpretations, the combined interpretation of the T and B cell maturation patterns, and the combined interpretation of the most compatible PID subtype with the immunophenotype**: If all experts answer the same option, that option becomes the consensus. If one or more experts answer differently, a consensus is reached after discussion. If no consensus is reached, there is no consensus for that parameter.
- **Interpretation for the clinician**: The interpretation for a clinician is subject to variation across the experts. As a result, a formal consensus would be inappropriate and is therefore not established for this parameter. Instead, expert interpretations are provided to offer insight into the differences in expert judgment and highlight the variability in interpretation.

Where appropriate, the median, minimum, and maximum of the cell count values reported by the participants are provided so that participants can also compare their results to other participants in the round.

Each participating laboratory will be provided with an EQA certificate that shows their performance in the wet lab and dry part, a summary of the round's results, general information regarding the round, and an overview of common mistakes. Note that individual performance is specific to and only provided to the individual participant.

Educational meeting

All EuroFlow schemes' EQA rounds offered in 2026 will be concluded with an online educational meeting, which will include all rounds performed throughout the year. 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 from the experts involved. More information regarding the educational meeting, including dates and times, will be announced at the end of 2026.

Timelines

Activity	Date
Registration for rounds 1 & 2 (spring & autumn)	5 Jan – 30 Jan 2026 (23:59 CET)
Round 1: Release of round instructions	2 Mar 2026
Round 1: Reporting of results	2 Mar – 27 Mar 2026 (23:59 CET)
Round 1: Release of certificates (v1)	Jun 2026
Round 1: Appeals period	21 calendar days following release of certificates (v1)
Round 1: Release of certificates (final)	Jul – Aug 2026

Activity	Date
Registration for round 2 (autumn)	1 Jun – 28 Aug 2026 (23:59 CEST)
Round 2: Release of round instructions	28 Sep 2026
Round 2: Reporting of results	28 Sep – 23 Oct 2026 (23:59 CEST)
Round 2: Release of certificates (v1)	Jan 2027
Round 2: Appeals period	21 calendar days following release of certificates (v1)
Round 2: Release of certificates (final)	Feb – Mar 2027

Registration

Registering for the EuroFlow PIDOT EQA scheme 2026 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 (v1) via the ESLHO EQA Portal. A clear description of the appeal should be included and providing illustrating images is 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

- Participation in one PIDOT round: **€ 270,-**
- Participation in both PIDOT rounds: **€ 490,-**
- Participation is free for participants of the EuroFlow Consortium.

Organization

The PIDOT scheme is organized by ESLHO in collaboration with the EuroFlow EQA Committee. The laboratory at the University of Ghent, Ghent, BE, operates as the lead expert laboratory of the PIDOT scheme, with Dr. Mattias Hofmans in the role of lead subject-matter expert. The lead expert laboratory receives support from other EuroFlow subject-matter experts for case selection, expert analysis, determination of consensus reference results, data analysis, performance evaluation, and reporting.

Name	Organization/Institute	Role	Tasks
ESLHO			
Prof. Dr. Jacques J. M. van Dongen	ESLHO, Zutphen, NL	EQA Program Coordinator	Coordinator with final responsibility; authorizes the EQA certificate.
Evelien Rijkers	ESLHO, Zutphen, NL	EQA Officer (lead)	Overall responsible for organization and operation of the PIDOT scheme by ESLHO.
Dr. Bart Lubbers	ESLHO, Zutphen, NL	EQA Officer	Supports in the organization and operation of the PIDOT scheme.
Lead expert laboratory			
Dr. Mattias Hofmans	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
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
Julie D'Hondt	Ghent University Hospital, Ghent, BE	Subject-matter expert	Round: Reference lab Post-round: Round summary report
Pauline Breughe	Ghent University Hospital, Ghent, BE	Subject-matter expert	Pre-round: Technical quality check of fcs data
Additional expert laboratory			
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, support in performance evaluation and reporting.

Additionally, the following EuroFlow-affiliated laboratories provide support in case selection, expert analysis, and input for determination of consensus reference results:

- Charles University (Prague, CZ)
- University of Salamanca (Salamanca, ES)

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.