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External quality assessment for identification, antimicrobial susceptibility testing and molecular typing of *Streptococcus pneumoniae*, 2026

ECDC SURVEILLANCE AND MONITORING

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On behalf of the IBDLabNet surveillance network

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Abbreviations

AST	antimicrobial susceptibility testing
CSF	cerebrospinal fluid
Ct	cycle threshold
DNA	deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EQA	external quality assessment
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU/EEA	European Union/European Economic Area
H. influenzae	Haemophilus influenzae
IBDLabNet	Invasive Bacterial Diseases Laboratory Network
IPD	invasive pneumococcal disease
IQR	interquartile range
LINcode	lineage code
MALDI-TOF	matrix-assisted laser desorption ionisation time-of-flight
MIC	minimum inhibitory concentration
N. meningitidis	Neisseria meningitidis
NFP	national focal point
OCP	operational contact point
PCR	polymerase chain reaction
REDCap	Research Electronic Data Capture
S. pneumoniae	*Streptococcus pneumoniae*
ST	sequence type
WGS	whole-genome sequencing

Executive summary

Invasive pneumococcal disease remains a major public health issue in Europe, particularly among young children and older adults. Surveillance requires reliable identification of *Streptococcus pneumoniae (S. pneumoniae)*, serotype determination, AST and, increasingly, genomic characterisation. Serotype information is particularly important for monitoring vaccine-preventable disease, serotype replacement and the distribution of non-vaccine serotypes.

This EQA was organised within the IBDLabNet framework on behalf of ECDC to assess current EU/EEA laboratory capacity for the detection and characterisation of *S. pneumoniae* in both non-culture and culture-based settings. The exercise included a shared non-culture panel and an optional pneumococcal culture panel. Samples were shipped in December 2025, the online response form opened in January 2026, and the submission deadline was 15 April 2026.

A total of 34 EU/EEA laboratories from 24 countries were included in the EU/EEA analysis. All 34 EU/EEA laboratories received the non-culture panel, and 32/34 submitted at least one non-culture sample result in REDCap. The pneumococcal culture panel was requested by 24 EU/EEA laboratories, with 23 EU/EEA laboratories providing analysable isolate-level results.

Overall, performance was strong for culture-based species identification. Correct identification was reported by 23/23 laboratories (100.0%) for both P1 and P2 samples. Culture-based serotyping was also high: 19/23 laboratories (82.6%) correctly reported P1 sample as serotype 23F, and 21/23 (91.3%) correctly reported P2 sample as serotype 14. When restricted to laboratories reporting an interpretable serotype result, concordance was 19/21 (90.5%) for P1 and 21/21 (100.0%) for P2 samples. AST uptake was high, with MIC testing reported by 21/23 laboratories (91.3%) for P1 and 20/23 (87.0%) for P2. WGS was reported by 11/23 laboratories (47.8%) for each isolate; among WGS-positive submissions, sequence type was reported for 22/22 (100.0%) and LINcode for 16/22 (72.7%).

Non-culture species detection was also high for both pneumococcal non-culture samples. Correct pathogen identification was 28/31 (90.3%) for NC3, expected to contain serotype 14, and 28/31 (90.3%) for NC5, expected to contain serotype 23F. Serotype reporting from non-culture material was less complete. Correct serotype assignment among all laboratories with an interpretable pathogen response was 7/31 (22.6%) for NC3 and 6/31 (19.4%) for NC5. However, among laboratories reporting an interpretable serotype result, concordance was high: 7/8 (87.5%) for NC3 and 6/7 (85.7%) for NC5. These findings indicate that the main limitation was incomplete progression from pneumococcal detection to usable serotype information, rather than frequent incorrect serotype assignment among laboratories providing interpretable serotype results.

The EQA confirms strong isolate-based pneumococcal identification and generally high culture-based serotyping performance among participating EU/EEA laboratories. The main residual gap concerns completion and harmonisation of serotype reporting from non-culture material. Recommended next steps include continued regular EQA rounds, targeted feedback to participants, improved collection of methodological information for non-culture pneumococcal workflows, continued support for standardised MIC reporting, and further harmonisation of WGS-derived outputs.

1 Introduction

Streptococcus pneumoniae remains a major cause of invasive bacterial disease in Europe, particularly among older adults and young children [1]. In 2023, 24 567 confirmed cases of invasive pneumococcal disease were reported in the EU/EEA, with the highest age-specific notification rates in adults aged 65 years and above and infants under one year of age [1]. Although pneumococcal conjugate vaccines have reduced disease due to vaccine-covered serotypes, IPD epidemiology continues to evolve because of serotype replacement, persistence of non-vaccine serotypes and changes in vaccine recommendations and coverage [1-3].

For surveillance purposes, laboratory confirmation and further characterisation of *S. pneumoniae* remain essential. Species identification confirms invasive pneumococcal disease, while serotype determination supports monitoring of vaccine impact, serotype replacement, outbreak investigation and interpretation of trends over time. AST data contribute to monitoring of antimicrobial susceptibility patterns and support public health interpretation, while WGS is increasingly used for genomic typing, lineage assessment and integrated surveillance [1-4].

The present EQA was organised within IBDLabNet on behalf of ECDC as part of the first-year implementation of the EU invasive bacterial diseases laboratory network [4,5]. It aimed to assess the ability of participating laboratories to detect and characterise *S. pneumoniae* from both non-culture and culture-based materials, including species identification, serotyping, AST and, where available, WGS-derived characterisation. The exercise also aimed to document the routine methods currently used by participating laboratories and to identify needs for harmonisation, training or targeted support [5].

This EQA addressed a surveillance-oriented need in the EU/EEA context, namely the integrated evaluation of non-culture molecular detection, culture-based identification, serotyping, AST and optional WGS for IPD surveillance. The specific objectives were therefore to: (i) assess laboratory performance for the detection and identification of *S. pneumoniae* in shared panels; (ii) assess the concordance of pneumococcal serotyping results; (iii) describe AST practices and compare reported MICs across laboratories; and (iv) describe the uptake and outputs of WGS among laboratories performing sequencing.

2 Study design and methods

Organisation of the scheme

The EQA was organised by the IBDLabNet coordination team at Institut Pasteur, Paris, on behalf of ECDC, in accordance with the first-year workplan and EQA implementation plan of the network. ECDC provided oversight and approved the invitations, methodological documents, safety instructions and shipment materials. The organising team was responsible for panel design, preparation, quality control, shipment, data collection, analysis and reporting. The scheme was developed in line with ECDC principles for EU-level EQAs for public health microbiology laboratories [5,6].

Participants and recruitment

Invitation letters and pre-registration forms were disseminated through the ECDC disease network to National Focal Points and Operational Contact Points, who could participate directly or further relay the invitation to relevant national laboratories.

A total of 34 laboratories from 24 countries registered for the exercise. Denominators vary across sections according to panel allocation, sample-specific response and test-specific availability, and are indicated explicitly throughout the report.

Panel design

The scheme included one shared non-culture panel and optional culture panels. The non-culture panel comprised six lyophilised positive samples distributed to all participating laboratories; no negative non-culture sample was included in this edition of the EQA. Optional culture panels consisted of up to six isolates in total, corresponding to two isolates each for *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*, according to the pathogen-specific selections made at pre-registration.

All 35 registered laboratories received the non-culture panel by scheme design. Among EU/EEA laboratories included in the aggregate analysis, all 34 received the non-culture panel and 32/34 submitted at least one non-culture sample result in REDCap. Overall, 24 EU/EEA laboratories requested the pneumococcal culture panel; 23 EU/EEA laboratories provided analysable responses for P1 and P2 and were included in culture-based aggregate analyses.

This pathogen-specific report presents the pneumococcal components of the shared EQA scheme, namely the *S. pneumoniae*-positive non-culture samples and the two pneumococcal cultured isolates included in the exercise.

Distribution, testing window and instructions

Shipments started on 3 December 2025 and continued until 17 December 2025. The online response form was opened on 12 January 2026 and the submission deadline was 15 April 2026. Participants were instructed to perform only their routine methods. Non-culture samples were reconstituted according to the instructions included in the shipment package. Culture isolates were shipped in Amies charcoal transport swabs and were to be re-isolated on appropriate media upon receipt.

Laboratory procedures assessed

For non-culture samples, laboratories were asked to perform routine molecular detection of *S. pneumoniae* and, where feasible and relevant, serotype determination. Ct values for species detection and, when applicable, serotype determination were recorded.

For cultured isolates, laboratories were asked to perform routine species identification, serotyping, AST and molecular typing. Acceptable identification approaches included routine phenotypic methods, MALDI-TOF and validated molecular methods. AST methods reflected routine local practice. Participants were asked to report raw MIC values and interpretive standards where determined. Most participants reported use of EUCAST breakpoints [9].

Whole-genome sequencing

WGS was optional. Laboratories performing WGS were asked to report the sequencing technology used, whether assembly had been performed, and the genomic typing variables available in their routine practice. Because no complete predefined reference dataset had been established for all genomic outputs included in the questionnaire, WGS analyses were descriptive. A minimal analysis was performed for WGS uptake, platform and assembly practices, while an extended descriptive analysis was performed for the genomic variables actually submitted by participants, with variable-specific denominators reported.

Data capture and confidentiality

Data were collected and managed using REDCap hosted at Institut Pasteur, Paris [7,8]. Separate modules captured laboratory information, non-culture results and culture-set results. Laboratory identities were pseudonymised using laboratory identifiers. Aggregated results in the report are presented in pseudonymised form; full laboratory identification remained available only to the organising centre and ECDC.

Expected results and performance assessment

Expected species identification and serotype results were predefined by the organising centre on the basis of the panel composition. For the pneumococcal component, NC3 and P2 were expected to be serotype 14, and NC5 and P1 were expected to be serotype 23F.

For pneumococcal serotype analyses, three denominators were distinguished. The all-responder denominator was the number of laboratories with an interpretable pathogen response for a non-culture sample or an analysable isolate response for a cultured isolate. A serotype-field response was any non-empty serotype/type field entry, including non-informative entries such as Unknown or Untested. An interpretable serotype result was an actual serotype or interpretable serotype/category result. Unknown, Untested, Untyped/missing, blank and missing values were excluded from the interpretable-serotype denominator, while incorrect biological serotype/category calls were retained as interpretable but incorrect.

No predefined target MIC values or resistance-associated expected AST phenotypes were assigned for the pneumococcal panel. Species identification and serotyping were therefore assessed against the predefined expected results for each sample, whereas AST results were analysed comparatively across participants.

In accordance with the exercise protocol, MIC agreement was assessed against the modal participant result, and results within one doubling dilution of the modal MIC were considered concordant for comparative analyses. Ct values were analysed descriptively because no target Ct values had been predefined. Ct values equal to 0 were treated as missing. Results are reported as counts and percentages using the available denominator for each sample and variable. Because not all laboratories requested all culture panels and not all participants performed all methods, denominators vary across analyses and are indicated explicitly.

3 Results

3.1 Participation and laboratory methods

A total of 34 EU/EEA laboratories from 24 countries were included in the EU/EEA analysis. All 34 EU/EEA laboratories received the non-culture panel by scheme design. Of these, 32/34 submitted at least one non-culture sample result in REDCap. Performance analyses for individual non-culture samples used the number of laboratories with an interpretable response for the relevant sample as the denominator. The pneumococcal culture panel, containing isolates P1 and P2, was requested by 24 EU/EEA laboratories; 23 laboratories provided analysable isolate-level responses for P1 and P2.

Reported routine methods for *S. pneumoniae* detection and serotyping varied across participating laboratories. For non-culture molecular testing, 17/34 laboratories (50.0%) reported using *lytA*, and 18/34 (52.9%) reported other molecular targets. Multiple targets could be reported by the same laboratory.

For culture-based pneumococcal identification, molecular methods were reported by 12/23 laboratories (52.2%), other phenotypic methods by 11/23 (47.8%) and MALDI-TOF by 9/23 (39.1%). Serotyping relied on both phenotypic and molecular approaches. Phenotypic serotyping methods were reported by 15/23 laboratories (65.2%) for P1 and 14/23 (60.9%) for P2; molecular methods were reported by 14/23 (60.9%) for P1 and 13/23 (56.5%) for P2.

AST methods were heterogeneous. For P1, E-test was reported by 13/23 laboratories (56.5%), broth microdilution by 7/23 (30.4%), antimicrobial gradient methods by 3/23 (13.0%), automated instruments by 2/23 (8.7%), other methods by 2/23 (8.7%) and disk diffusion by 1/23 (4.3%). For P2, E-test was reported by 11/23 (47.8%), broth microdilution by 6/23 (26.1%), antimicrobial gradient methods by 3/23 (13.0%), automated instruments by 2/23 (8.7%) and other methods by 2/23 (8.7%). EUCAST was the main interpretive standard, reported by 19/23 laboratories (82.6%) for P1 and 17/23 (73.9%) for P2.

WGS was reported for both pneumococcal isolates by 11/23 laboratories (47.8%). Among WGS-positive laboratories, Illumina was the most frequently reported sequencing platform (9/11; 81.8% for each isolate), with Oxford Nanopore and other platforms each reported by 1/11 (9.1%). Assembly was reported as performed by 9/11 WGS-positive laboratories (81.8%) for each isolate. These data describe the range of methods reported across routine molecular detection, culture-based identification, serotyping, AST and WGS workflows.

Table 1. Overview of aggregated EU/EEA results for the *Streptococcus pneumoniae* component of the EQA

EQA component	Sample/isolate	Expected result	Denominator	Correct result, n/N (%)
Non-culture pathogen identification	NC3	*S. pneumoniae*	31	28/31 (90.3%)
Non-culture serotype among all responders	NC3	14	31	7/31 (22.6%)
Non-culture serotype among laboratories reporting an interpretable serotype result	NC3	14	8	7/8 (87.5%)
Non-culture pathogen identification	NC5	*S. pneumoniae*	31	28/31 (90.3%)
Non-culture serotype among all responders	NC5	23F	31	6/31 (19.4%)
Non-culture serotype among laboratories reporting an interpretable serotype result	NC5	23F	7	6/7 (85.7%)
Culture-based species identification	P1	*S. pneumoniae*	23	23/23 (100.0%)
Culture-based serotype among all responders	P1	23F	23	19/23 (82.6%)
Culture-based serotype among laboratories reporting an interpretable serotype result	P1	23F	21	19/21 (90.5%)
AST uptake	P1	MIC reported	23	21/23 (91.3%)
WGS uptake	P1	WGS performed	23	11/23 (47.8%)
Culture-based species identification	P2	*S. pneumoniae*	23	23/23 (100.0%)
Culture-based serotype among all responders	P2	14	23	21/23 (91.3%)

EQA component	Sample/isolate	Expected result	Denominator	Correct result, n/N (%)
Culture-based serotype among laboratories reporting an interpretable serotype result	P2	14	21	21/21 (100.0%)
AST uptake	P2	MIC reported	23	20/23 (87.0%)
WGS uptake	P2	WGS performed	23	11/23 (47.8%)

Denominators vary according to the number of laboratories providing an interpretable result for the relevant sample or isolate, and according to whether serotype assignment, MIC testing or WGS was reported.

3.2 Performance on non-culture samples

The pneumococcal non-culture component included two positive samples: NC3, expected to contain *S. pneumoniae* serotype 14, and NC5, expected to contain *S. pneumoniae* serotype 23F.

NC3

For NC3, 31 laboratories provided an interpretable pathogen response. Of these, 28/31 (90.3%) correctly identified *S. pneumoniae*. The remaining 3/31 laboratories (9.7%) reported a negative result.

A serotype-field response was available for 13 laboratories; however, 3 reported Untested and 2 reported Unknown. Among the 8 laboratories reporting an interpretable serotype result, 7/8 (87.5%) reported the expected serotype 14. This corresponded to 7/31 (22.6%) of all laboratories with an interpretable pathogen response. The remaining interpretable serotype result was Non-typable.

Species Ct values were available from 20 laboratories. The median species Ct was 31.75 (IQR: 30.3-36.14). Serotype Ct was available from one laboratory, with a value of 30.6.

NC5

For NC5, 31 laboratories provided an interpretable pathogen response. Of these, 28/31 (90.3%) correctly identified *S. pneumoniae*. The remaining 3/31 laboratories (9.7%) reported a negative result.

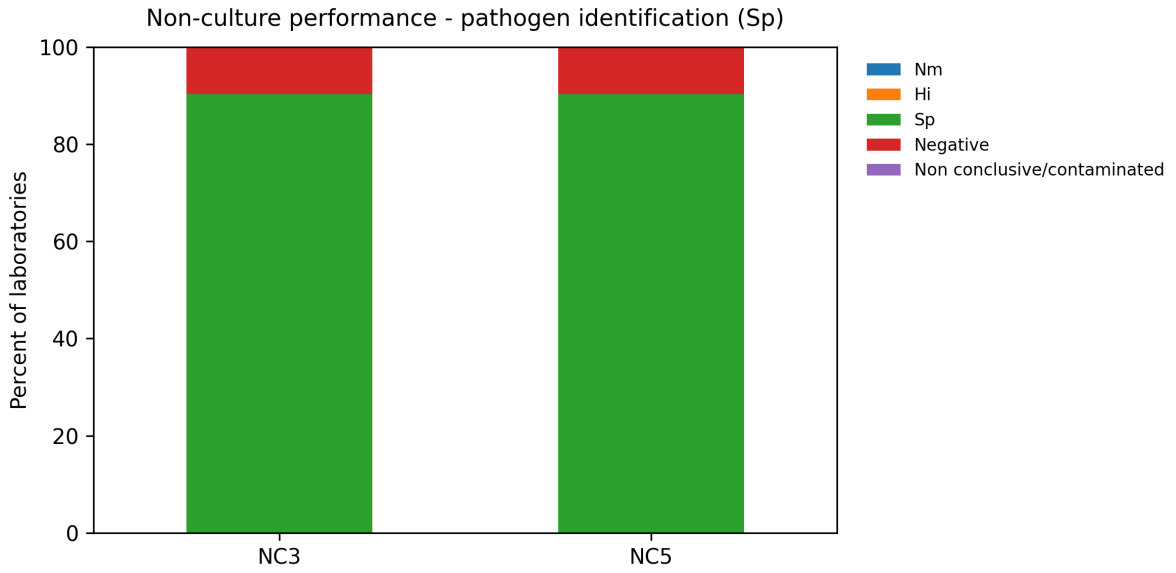
A serotype-field response was available for 12 laboratories; however, 3 reported Untested and 2 reported Unknown. Among the 7 laboratories reporting an interpretable serotype result, 6/7 (85.7%) reported the expected serotype 23F. This corresponded to 6/31 (19.4%) of all laboratories with an interpretable pathogen response. The remaining interpretable serotype result was Non-typable.

Species Ct values were available from 20 laboratories. The median species Ct was 34.0 (IQR: 30.6-35.0). Serotype Ct was available from one laboratory, with a value of 34.9.

Overall non-culture performance

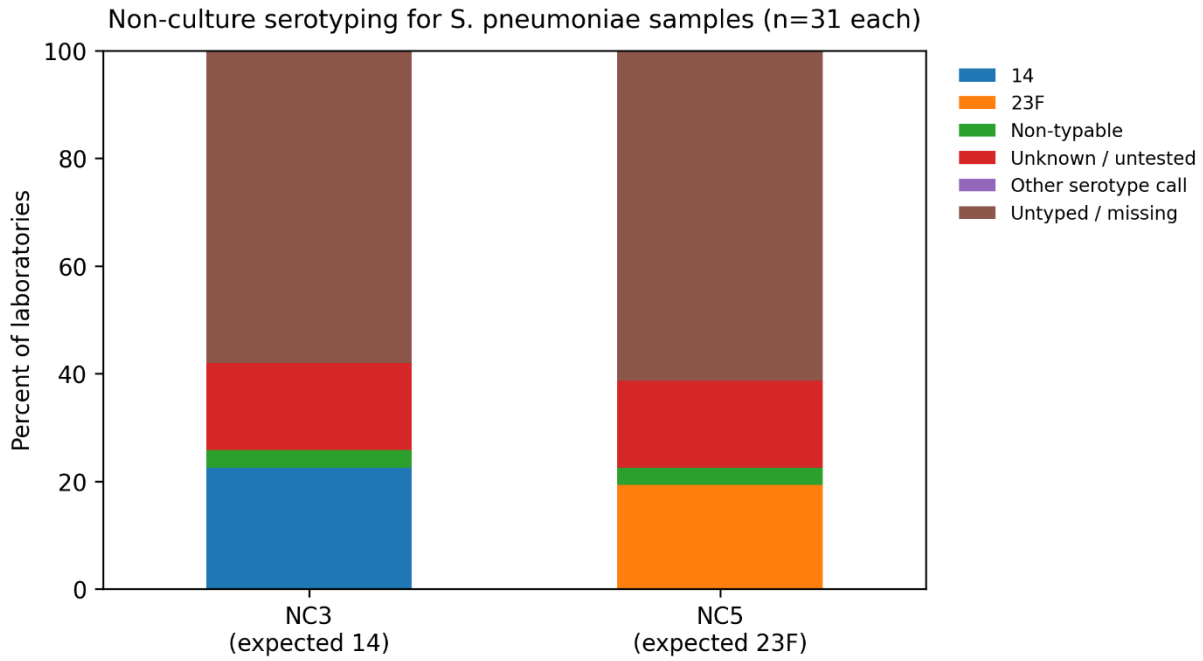
Across the two pneumococcal non-culture samples, correct pathogen identification was 28/31 (90.3%) for both NC3 and NC5. Serotype reporting was less complete than pathogen identification for both samples. Among laboratories reporting an interpretable serotype result, correct serotype assignment was 7/8 (87.5%) for NC3 and 6/7 (85.7%) for NC5. When expressed against all laboratories with an interpretable pathogen response, correct serotype assignment was 7/31 (22.6%) for NC3 and 6/31 (19.4%) for NC5.

Figure 1. Performance on pneumococcal non-culture samples: pathogen identification for NC3 and NC5 (*S. pneumoniae* expected in both samples)



Percentages are based on laboratories providing an interpretable pathogen response (NC3: n=31; NC5: n=31)

Figure 2. Performance on pneumococcal non-culture samples: serotype reporting for NC3 and NC5



Percentages are based on all laboratories providing an interpretable pathogen response; unknown/untested responses are grouped together and missing serotype results are shown separately from interpretable serotype calls.

3.3 Performance on cultured isolates

The pneumococcal culture panel included two isolates: P1, expected to be serotype 23F, and P2, expected to be serotype 14.

P1

Species identification for P1 was correct in 23/23 laboratories (100.0%). A serotype-field response was available for all 23 laboratories; however, 2 reported Untested. Among the 21 laboratories reporting an interpretable serotype result, 19/21 (90.5%) reported the expected serotype 23F. This corresponded to 19/23 (82.6%) of all isolate responders. The remaining interpretable serotype results were Other serotype in 1/21 laboratory and serotype 14 in 1/21.

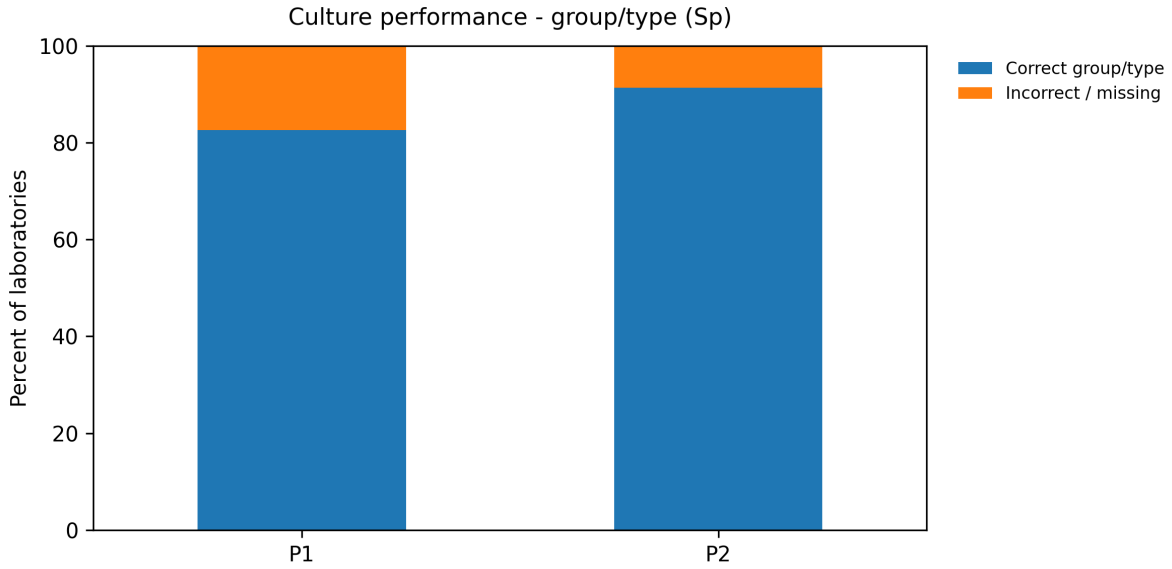
P2

Species identification for P2 was correct in 23/23 laboratories (100.0%). A serotype-field response was available for all 23 laboratories; however, two reported Untested. Among the 21 laboratories reporting an interpretable serotype result, 21/21 (100.0%) reported the expected serotype 14. This corresponded to 21/23 (91.3%) of all isolate responders.

Overall culture-based performance

Culture-based species identification was high for both pneumococcal isolates. Serotype assignment was 19/23 (82.6%) for P1 and 21/23 (91.3%) for P2 when expressed against all isolate responders.

Figure 3. Culture-based serotype performance for pneumococcal isolates P1 and P2 (expected: serotype 23F for P1 and serotype 14 for P2)



Percentages are based on all laboratories with an analysable isolate response (P1: n=23; P2: n=23); untested serotype results are included in the all-responder denominator.

3.4 Antimicrobial susceptibility testing

AST uptake was high for both cultured isolates. MIC testing was reported by 21/23 laboratories (91.3%) for P1 and 20/23 (87.0%) for P2. Because predefined target MIC values were not assigned for this exercise, MIC reproducibility was assessed comparatively against the participant modal MIC. Concordance was defined as a result within one doubling dilution of the participant modal MIC. No predefined resistance-associated expected phenotype was assigned for the pneumococcal isolates, and no EUCAST expected-phenotype recognition indicator was calculated for Sp.

P1

For P1, the participant modal MICs for selected antibiotics were 0.5 mg/L for benzylpenicillin, 0.12 mg/L for amoxicillin, 0.12 mg/L for cefotaxime, 0.12 mg/L for ceftriaxone, 256.0 mg/L for azithromycin and 8.0 mg/L for tetracycline.

Concordance within one doubling dilution of the participant modal MIC was 17/18 (94.4%) for benzylpenicillin, 7/14 (50.0%) for amoxicillin, 17/19 (89.5%) for cefotaxime, 10/12 (83.3%) for ceftriaxone, 2/3 (66.7%) for azithromycin and 7/11 (63.6%) for tetracycline.

P2

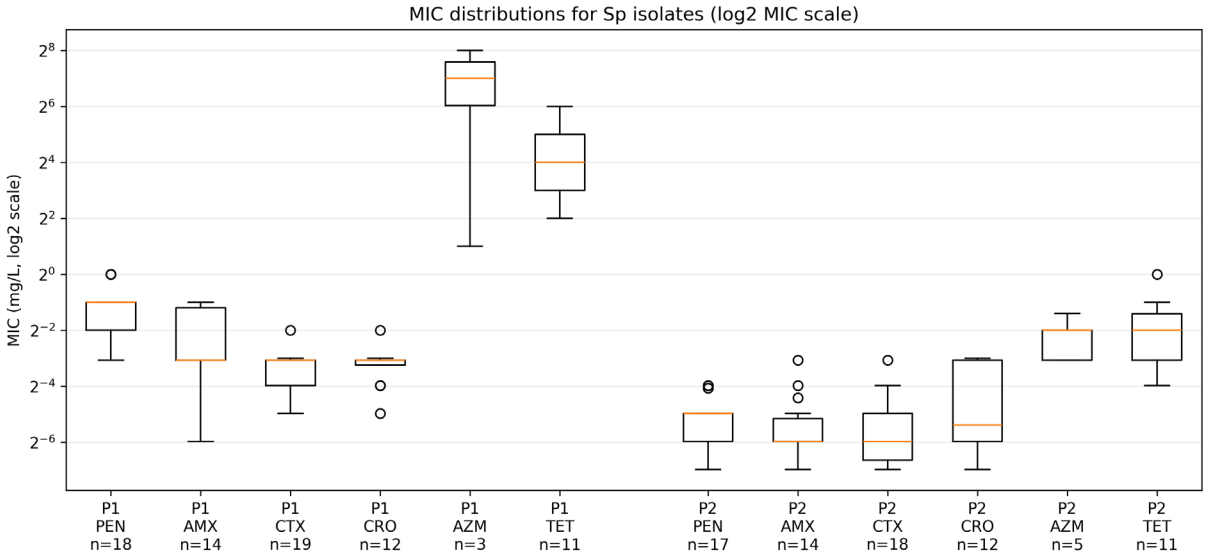
For P2, the participant modal MICs were 0.032 mg/L for benzylpenicillin, 0.016 mg/L for amoxicillin, 0.016 mg/L for cefotaxime, 0.016 mg/L for ceftriaxone, 0.25 mg/L for azithromycin and 0.25 mg/L for tetracycline.

Concordance within one doubling dilution of the participant modal MIC was 16/17 (94.1%) for benzylpenicillin, 11/14 (78.6%) for amoxicillin, 16/18 (88.9%) for cefotaxime, 7/12 (58.3%) for ceftriaxone, 3/5 (60.0%) for azithromycin and 6/11 (54.5%) for tetracycline.

Overall AST performance

MIC concordance was highest for benzylpenicillin for both isolates. Cefotaxime concordance was 17/19 (89.5%) for P1 and 16/18 (88.9%) for P2. Ceftriaxone results were available from fewer laboratories and showed 10/12 (83.3%) concordance for P1 and 7/12 (58.3%) for P2. Azithromycin MIC results were reported by few laboratories.

Figure 4. Distribution of reported MIC values for selected antibiotics for pneumococcal isolates P1 and P2, displayed on a log2 MIC scale



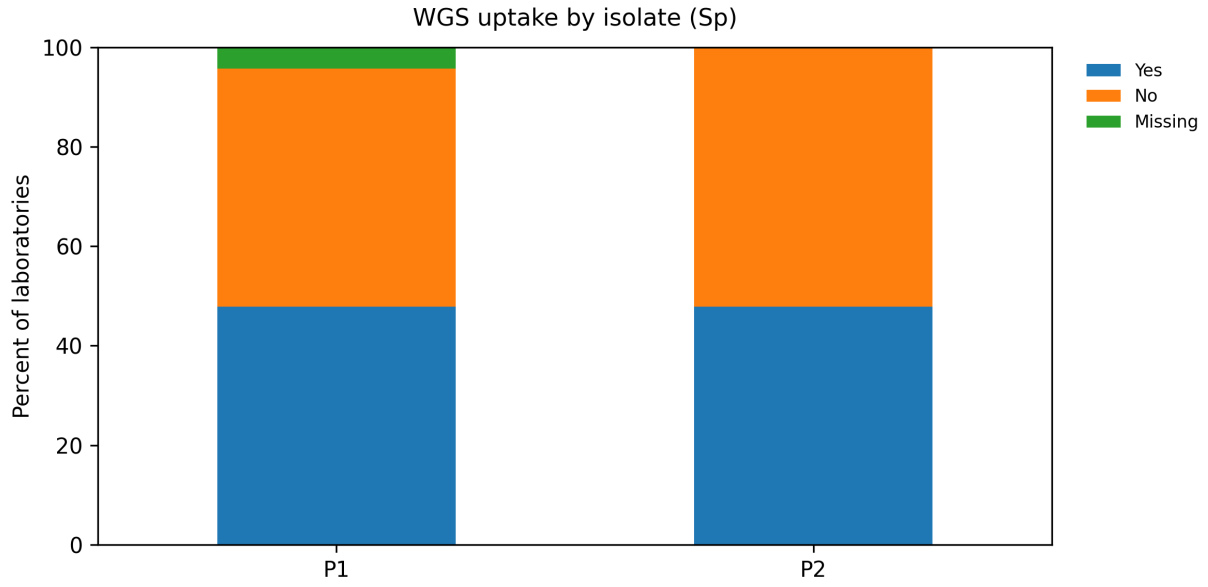
Only isolate-antibiotic combinations with available numeric MIC values are shown. The number below each antibiotic label indicates the number of numeric MIC values included for that isolate-antibiotic combination.

3.5 Whole-genome sequencing uptake and reported outputs

WGS was performed by 11/23 laboratories (47.8%) for P1 and 11/23 laboratories (47.8%) for P2. Illumina was the dominant reported sequencing platform among WGS-positive submissions, and assembly was reported as performed by 9/11 WGS-positive laboratories (81.8%) for each isolate.

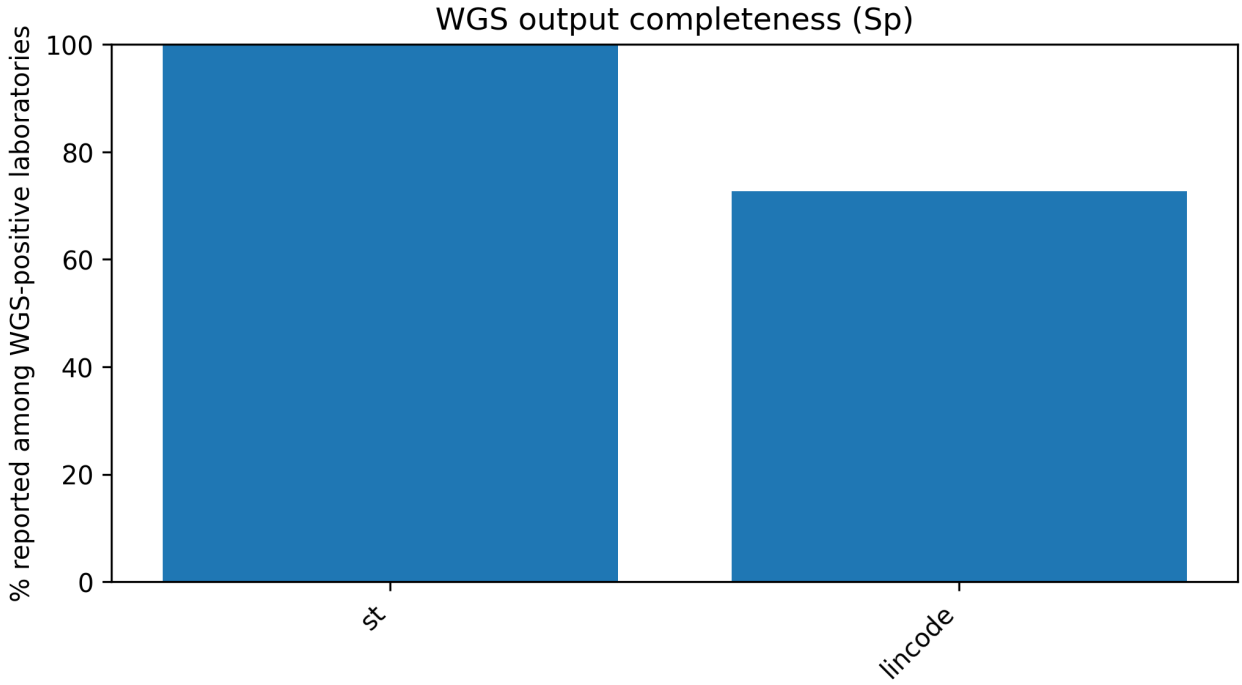
Among WGS-positive submissions, sequence type was reported for 11/11 (100.0%) submissions for P1 and 11/11 (100.0%) for P2. LINcode was reported for 8/11 (72.7%) submissions for P1 and 8/11 (72.7%) for P2. At pooled pathogen level, reporting completeness among WGS-positive submissions was 22/22 (100.0%) for sequence type and 16/22 (72.7%) for LINcode.

Figure 5. Uptake of WGS for pneumococcal isolates P1 and P2



Percentages are based on all laboratories reporting a result for the isolate (P1: n=23; P2: n=23).

Figure 6. Completeness of reported WGS outputs among WGS-positive submissions for pneumococcal isolates



Percentages are based on WGS-positive submissions only (pooled P1 and P2 submissions: n=22; P1: n=11; P2: n=11).

4 Discussion

This EQA provides an overview of current EU/EEA laboratory capacity for the detection, identification, serotyping, AST and genomic characterisation of *S. pneumoniae*. Overall, the results show strong performance for culture-based species identification, high culture-based serotype agreement, high uptake of AST among laboratories providing culture-panel results, and moderate implementation of WGS. In contrast, non-culture pathogen detection was high, but non-culture serotype reporting was much less complete. This pattern indicates that the main residual gap in the pneumococcal component is not species detection, but completion and harmonisation of serotype information from non-culture material.

The 2026 pneumococcal EQA should be interpreted in continuity with previous European EQA exercises, while recognising that direct comparison remains limited by differences in panel composition, participant networks, matrices, expected outputs and scoring approaches. In the 2012 ECDC *S. pneumoniae* EQA, five pneumococcal isolates and two simulated CSF samples were distributed to 29 reference laboratories, and the results already showed heterogeneity in the extent of characterisation performed, ranging from species identification to serotyping, genotypic characterisation and AST [10]. In the 2014 ECDC *S. pneumoniae* EQA, a panel of three viable isolates and two simulated CSF samples was sent to 30 laboratories in the IBDLabNet network [11]. That exercise showed generally strong culture-based identification and serotyping but also highlighted differences in the completeness and resolution of serotype characterisation, particularly when molecular methods were used [11]. The present exercise confirms this recurrent pattern: species identification is robust across the network, whereas full and harmonised serotype reporting remains more challenging, especially from non-culture material.

Culture-based species identification represented the strongest component of the exercise. All laboratories with analysable isolate-level responses correctly identified both P1 and P2 as *S. pneumoniae*. Culture-based serotyping was also high, with correct serotype assignment for 19/23 laboratories for P1 and 21/23 for P2 when all isolate responders were used as the denominator. If laboratories indicating that serotyping was not tested are considered separately, culture-based serotyping performance among laboratories providing an interpretable serotype result was even higher. This distinction is important because "untested" reflects incomplete characterisation rather than analytical misclassification. These findings are important for surveillance because pneumococcal serotype data are central to the interpretation of vaccine impact, serotype replacement and disease trends over time [1–4].

The present culture-based results are consistent with previous ECDC exercises. In the 2014 EQA, phenotypic species identification was performed by 26 laboratories; two of the three isolates were correctly identified as *S. pneumoniae* by all participants, with only one species-level error reported for the third isolate [11]. Phenotypic serotyping was generally strong, although errors occurred within serogroups and some laboratories reported only serogroup-level results rather than full serotypes [11]. This is directly relevant to the 2026 exercise, because serotype-level resolution remains critical for IPD surveillance. Correct identification of serogroup alone may be useful, but it is often insufficient for vaccine-impact assessment and for monitoring vaccine-type versus non-vaccine-type disease.

Non-culture testing showed a different pattern. Species detection was high for both NC3 and NC5, with 28/31 laboratories correctly identifying *S. pneumoniae* for each sample. However, serotype-field responses were reported by fewer laboratories, and correct serotype assignment against all interpretable pathogen responders was 7/31 for NC3 and 6/31 for NC5. Among laboratories that provided a serotype-field response, approximately half reported the expected serotype. These results should be interpreted carefully because this denominator included "untested" and "unknown" responses. Therefore, the main issue is not only discordant serotype assignment, but also incomplete progression from pneumococcal detection to usable serotype information. From a surveillance perspective, this is a key limitation: pathogen confirmation from non-culture material may be relatively robust, but incomplete serotyping reduces the epidemiological value of culture-negative cases.

Previous ECDC exercises provide useful context. In the 2014 EQA, all 22 laboratories attempting detection of *S. pneumoniae* in simulated CSF samples correctly detected pneumococcal DNA, while optional molecular capsule typing was attempted by only four laboratories and was fully successful for only a subset of samples [11]. The 2012 EQA similarly reported good non-culture detection performance, but also emphasised heterogeneity in extraction methods, detection methods and gene targets [10]. The 2026 findings are therefore consistent with earlier observations: non-culture species detection can perform well, but non-culture serotyping remains more dependent on local assay availability, serotype coverage, reporting algorithms and laboratory practice.

The Ct distributions provide descriptive context but should not be interpreted as quantitative comparisons across laboratories. Ct values are assay-dependent and influenced by extraction method, input volume, reagents, platform and positivity thresholds. Species Ct values were available for 20 laboratories for each pneumococcal non-culture sample, with median values of 31.75 for NC3 and 34.0 for NC5. Serotype Ct values were available from only one laboratory for each sample, limiting interpretation of serotype-specific amplification. This limitation is consistent with the 2014 ECDC report, which noted that Cq values cannot be compared directly between assays or runs, but can nevertheless indicate variability in DNA extraction efficiency and PCR sensitivity [11]. Future EQA rounds should therefore continue collecting Ct/Cq values, but with more structured method information to support interpretation.

The method survey highlights heterogeneity in routine pneumococcal workflows. Laboratories reported different combinations of molecular targets for non-culture detection, with *lytA* and other locally validated targets both commonly reported. For culture-based workflows, laboratories used molecular, MALDI-TOF and phenotypic identification methods, and both phenotypic and molecular approaches for serotyping. This heterogeneity is expected in a network of national and reference laboratories, but it has implications for comparability of surveillance outputs, particularly for non-culture serotyping. The 2014 ECDC report similarly recommended collecting more detailed information on PCR-based typing methods and on which serotypes could be distinguished by each protocol [11]. This remains highly relevant, because pneumococcal molecular serotyping schemes may provide different levels of resolution depending on primer or probe coverage, and some assays can distinguish serogroups but not individual serotypes [12,13].

The complexity of pneumococcal serotyping should also be considered. More than 100 pneumococcal serotypes have now been described, and closely related serotypes within the same serogroup may be difficult to distinguish by some molecular approaches [12]. CDC PCR-based schemes can support serotype deduction from isolates and sterile-site clinical specimens, but the range of serotypes detected and the resolution achieved depend on the assay format used [13]. In the 2014 ECDC EQA, some molecular methods were expected to identify certain isolates only to combined serotype or serogroup level, such as 9N/9L, 15A/15F or 33A/33F/37, rather than to a single serotype [11]. This methodological constraint is directly relevant to the 2026 results and supports the recommendation that future questionnaires should document not only whether serotyping was performed, but also the method, target range, resolution, and rules for reporting partial, unknown or untyped results.

AST participation was high and provided useful comparative information, even though no predefined reference MIC values were assigned. The modal-MIC approach gives a descriptive view of inter-laboratory comparability but should not be interpreted as formal susceptibility scoring against an independent reference standard. Concordance within one doubling dilution was high for benzylpenicillin for both isolates, while greater dispersion was observed for some antibiotic–isolate combinations. Azithromycin MIC results were reported by few laboratories and should be interpreted with caution. Because no predefined resistance-associated phenotype was specified for P1 or P2, no EUCAST expected-phenotype recognition indicator was added for the pneumococcal component.

The AST findings should be interpreted in light of previous ECDC pneumococcal EQAs. The 2012 and 2014 reports both emphasised that different interpretive standards, particularly EUCAST versus CLSI, complicated inter-laboratory comparison, especially for beta-lactams and pneumococci [10,11]. The 2014 EQA further noted that discrepancies could arise from differences in breakpoints, isolate source assumptions and interpretive categories [11]. In the present exercise, most laboratories reported EUCAST as the interpretive framework, which should facilitate greater comparability. However, because the pneumococcal panel was not designed around predefined resistance-associated phenotypes, the most appropriate use of AST data in this report is descriptive: raw MIC distributions, participant modal MICs and concordance around the modal value. Future rounds could include pneumococcal isolates with predefined resistance-relevant profiles, allowing complementary EUCAST-based phenotype recognition indicators to be calculated, as was done for selected meningococcal and *H. influenzae* combinations.

WGS uptake was moderate, with 11/23 laboratories performing WGS for each pneumococcal isolate. Among WGS-positive submissions, sequence type reporting was complete, while LINcode reporting was less complete. This suggests that sequencing is available in a substantial subset of laboratories, but that the set of routinely reported pneumococcal genomic outputs is not yet fully harmonised across the network. WGS-based approaches can support pneumococcal serotype prediction and lineage analysis, but different tools and reporting frameworks may differ in their outputs and in the level of serotype resolution achieved [14,15]. From a surveillance perspective, a future minimal common WGS reporting set for invasive pneumococcal isolates could include sequence type, lineage code where used, and capsule or serotype-related genomic interpretation.

The public health implications of these findings are directly linked to IPD surveillance objectives. Confirmation of *S. pneumoniae* is essential, but serotype information is needed to monitor vaccine impact, identify serotype replacement and assess changes in the distribution of vaccine and non-vaccine serotypes. Incomplete non-culture serotyping can reduce the usefulness of culture-negative case data for surveillance, especially where viable isolates are not available. Conversely, robust isolate-based identification, high AST uptake and growing WGS implementation strengthen the network's ability to monitor circulating pneumococcal lineages and susceptibility patterns. These priorities are particularly important in the context of changing pneumococcal epidemiology following widespread conjugate vaccine use [1–3].

Several limitations should be considered. First, the pneumococcal component included only two positive non-culture samples and two cultured isolates, which cannot capture the full diversity of pneumococcal serotypes, resistance profiles or clinical matrices encountered in routine surveillance. Second, no negative non-culture sample was included, limiting evaluation of specificity and false-positive reporting. Third, participation in the culture panel was optional and not all laboratories performed all requested tests, leading to variable denominators. Fourth, non-culture samples were lyophilised and simulated and may not fully reproduce the complexity of clinical CSF or blood specimens. Fifth, Ct values were collected descriptively and are not directly comparable across laboratories without assay harmonisation. Sixth, AST analysis relied on participant modal MICs rather than predefined reference MIC values, and no resistance-associated expected phenotype was predefined for the pneumococcal isolates. Finally, WGS outputs were analysed descriptively, and WGS reporting was not required from all participants.

Despite these limitations, the EQA provides a useful benchmark for individual laboratories and for the network as a whole. For participants, the results can support review of non-culture serotyping workflows, culture-based serotype discrepancies, MIC outliers and incomplete WGS reporting. At network level, the findings identify clear priorities for capacity strengthening: maintaining strong culture-based identification, improving completeness and harmonisation of non-culture serotyping, maintaining standardised raw MIC reporting, collecting more structured methodological information, and progressively harmonising pneumococcal WGS-derived outputs. These priorities are aligned with the objectives of ECDC EQA activities, which aim to support harmonisation, identify training needs and improve the comparability of surveillance data across the EU/EEA [5,6].

Overall, the 2026 pneumococcal EQA shows that EU/EEA laboratories participating in IBDLabNet have strong proficiency for isolate-based species identification and generally good culture-based serotyping capacity. The main residual gap concerns the completeness and harmonisation of serotype reporting from non-culture material. Regular EQA rounds, targeted feedback, more detailed method questionnaires and focused training on non-culture serotyping and WGS-derived reporting would help further strengthen the quality and comparability of IPD surveillance data across the EU/EEA.

5 Conclusions

The 2026 IBDLabNet EQA for *S. pneumoniae* showed strong performance among participating EU/EEA laboratories for culture-based species identification. All laboratories with analysable isolate-level responses correctly identified both pneumococcal isolates. Culture-based serotyping was also high, although not complete for all laboratories and isolates.

Non-culture pathogen detection was high for both pneumococcal non-culture samples, but serotype reporting from non-culture material was less complete. When expressed against all laboratories with an interpretable pathogen response, correct serotype assignment was 7/31 for NC3 and 6/31 for NC5. These findings indicate that non-culture serotyping remains the main area requiring further harmonisation in the pneumococcal component.

AST uptake was high, and MIC distributions provided descriptive information on inter-laboratory comparability. Because no predefined resistance-associated phenotype was assigned for P1 or P2, AST interpretation was limited to modal MICs and concordance within one doubling dilution. WGS was implemented by about half of laboratories providing pneumococcal culture-panel results, with complete sequence type reporting among WGS-positive submissions and less complete LINcode reporting.

The design of this EQA was appropriate for documenting current practice across the network and identifying surveillance-relevant gaps. At the same time, the limited number of pneumococcal samples, the absence of a negative non-culture sample, optional participation in the culture component, reliance on participant modal MICs for comparative AST analysis, absence of predefined resistance-associated AST phenotypes, and the descriptive nature of the WGS analysis should be taken into account when interpreting the findings.

6 Recommendations

Based on the findings of this EQA, the following actions are recommended for future rounds and follow-up activities:

1. **Prioritise improvement of non-culture pneumococcal serotyping.**
   Future capacity-strengthening activities should focus on improving completeness and reliability of serotype reporting from non-culture material after *S. pneumoniae* detection. This should include targeted technical guidance or training on DNA extraction, sample input volume, inhibition controls, serotype assay design, interpretation thresholds and reporting workflows.
2. **Collect more structured methodological information in future EQA rounds.**
   Future questionnaires should capture standardised information on pneumococcal molecular testing, including extraction procedures, input volume, internal controls, PCR targets used, Ct cut-off policies, and the algorithm used for progression from species detection to serotyping. These data would improve interpretation of inter-laboratory differences and help distinguish method-related variability from sample-related factors.
3. **Strengthen harmonisation of pneumococcal serotype reporting.**
   Given the surveillance importance of vaccine and non-vaccine serotype distributions, additional harmonisation work should address serotype nomenclature, reporting of unknown or untyped results, rules for partial or inconclusive serotype calls, and alignment between phenotypic, molecular and WGS-derived serotyping outputs.
4. **Maintain standardised AST reporting.**
   Laboratories should continue to report raw MIC values together with the test method and interpretive standard used. Future analyses should continue to present MIC distributions and concordance around participant modal MICs. If future pneumococcal panels include isolates with predefined resistance-relevant

phenotypes, these should be specified before analysis so that selected EUCAST-based phenotype recognition indicators can be added.

5. **Further harmonise WGS-derived reporting.**
   Future EQA exercises should continue to include WGS components, while progressively defining a minimal common reporting framework for sequenced invasive pneumococcal isolates. Sequence type, lineage code and capsule/serotype-related genomic interpretation appear particularly relevant as common outputs for surveillance.
6. **Increase the educational value of future pneumococcal panels.**
   Future panels could include a broader range of challenge materials, such as a negative non-culture sample, lower-signal non-culture samples, additional serotypes of epidemiological relevance, and isolates selected to represent distinct susceptibility profiles. Where feasible, predefined expected genomic and resistance-associated outputs should be documented to support more formal performance evaluation.
7. **Use EQA results for targeted feedback and training planning.**
   Regular EQA rounds should be maintained to monitor progress, document persistent gaps and assess the effect of follow-up actions. Results should be used to provide targeted feedback to participants and to guide future IBDLabNet training and harmonisation activities relevant to IPD surveillance in the EU/EEA.

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Annex 1. Participating laboratories and countries

Table 1A. EU/EEA laboratories included in the aggregated analysis

Country	Laboratory / institute	Non-culture panel	Pneumococcal culture panel
Austria	National reference center for meningococci, pneumococci and Haemophilus influenzae / Austrian Agency for Health and Food Safety	Yes	Yes
Bulgaria	National Center of Infectious and Parasitic Diseases	Yes	Yes
Belgium	LHUB-ULB - CNR Haemophilus influenzae	Yes	No
Latvia	Riga East University Hospital, Laboratory service, National Microbiology Reference Laboratory	Yes	Yes
Portugal	Laboratorio de Referencia de Infecoes Respiratorias - agentes bacterianos / Instituto Nacional de Saude Doutor Ricardo Jorge	Yes	Yes
Slovakia	National Reference Center for Meningococci, Public Health Authority of the Slovak Republic	Yes	No
Belgium	NRC Neisseria / Sciensano	Yes	Yes
Italy	Department of Infectious Diseases, Istituto Superiore di Sanita	Yes	No
Italy	Istituto Superiore di Sanita	Yes	No
Bulgaria	National Center of Infectious and Parasitic Diseases, Laboratory for Molecular Microbiology	Yes	Yes
Czechia	National Reference Laboratory for Streptococcal Infections, National Institute of Public Health	Yes	Yes
Sweden	Department of Laboratory Medicine, Clinical Microbiology, University Hospital Orebro	Yes	No
France	Centre National de Reference des Pneumocoques / Centre Hospitalier Intercommunal de Creteil	Yes	Yes
Norway	Norwegian Institute of Public Health	Yes	Yes
Germany	National reference center for meningococci and Haemophilus influenzae	Yes	No
Spain	Spanish Pneumococcal Reference Laboratory	Yes	Yes
Estonia	Republic of Estonia Health Board, Communicable Diseases Laboratory	Yes	Yes
Spain	Neisseria, Listeria and Bordetella Unit / National Centre for Microbiology / Instituto de Salud Carlos III	Yes	No
Poland	National Reference Centre for Bacterial Meningitis, National Medicines Institute	Yes	Yes
Romania	Bacterial Respiratory Infections Laboratory / Cantacuzino National Military Medical Institute for Research and Development	Yes	No
Hungary	National Center for Public Health and Pharmacy	Yes	Yes
Italy	Istituto Superiore di Sanita	Yes	Yes
Romania	INCDMM Cantacuzino	Yes	Yes
Finland	Finnish Institute for Health and Welfare	Yes	Yes
Denmark	Neisseria and Streptococci Reference Laboratory, Statens Serum Institut	Yes	Yes
Lithuania	National Public Health Surveillance Laboratory	Yes	Yes
Greece	Infectious Diseases and Chemotherapy Research Laboratory, National and Kapodistrian University of Athens	Yes	Yes
Cyprus	Microbiology Department, Nicosia General Hospital	Yes	Yes
Greece	Hellenic National Meningitis Reference Laboratory	Yes	Yes
Czechia	National Reference Laboratory for Haemophilus Infections, National Institute of Public Health	Yes	No
Malta	Bacteriology Laboratory, Mater Dei Hospital	Yes	Yes
Czechia	National Reference Laboratory for Meningococcal Infections	Yes	No
Netherlands	Netherlands Reference Laboratory for Bacterial Meningitis	Yes	Yes
France	Centre National de Reference des Meningocoques et Haemophilus influenzae	Yes	Yes

Annex 2. Pneumococcal panel composition and expected results

Table 2A. Non-culture samples

Sample code	Material type	Expected pathogen	Expected serotype	Purpose in the EQA
NC3	Lyophilised non-culture sample	*Streptococcus pneumoniae*	14	Assessment of molecular detection and serotype determination from non-culture material
NC5	Lyophilised non-culture sample	*Streptococcus pneumoniae*	23F	Assessment of molecular detection and serotype determination from non-culture material

Table 2B. Cultured isolates

Isolate code	Material type	Expected pathogen	Expected serotype
P1	Cultured isolate	*Streptococcus pneumoniae*	23F
P2	Cultured isolate	*Streptococcus pneumoniae*	14

Table 2C. Performance variables assessed for the pneumococcal component

EQA component	Variables assessed
Non-culture panel	Pathogen detection, serotype determination, reported Ct values for species detection and serotype determination
Culture panel	Species identification, serotype determination, AST, and, where performed, WGS uptake and reported outputs

Annex 3. Supplementary detailed result tables

Detailed results for non-culture pneumococcal samples

Table A3.1. Pathogen calls for NC3

Reported pathogen result	n	Denominator	%
S. pneumoniae	28	31	90.3
Negative	3	31	9.7

Table A3.2. Serotype calls for NC3

Reported serotype-field value	n	% among all responders (n=31)	Included in interpretable serotype denominator	Reason if excluded
14	7	22.6	Yes	
Non-typable	1	3.2	Yes	
Untyped / missing	18	58.1	No	Missing or blank
Untested	3	9.7	No	Non-informative response
Correct serotype among interpretable serotype results	7/8	87.5		

Table A3.3. Pathogen calls for NC5

Reported pathogen result	n	Denominator	%
S. pneumoniae	28	31	90.3
Negative	3	31	9.7

Table A3.4. Serotype calls for NC5

Reported serotype-field value	n	% among all responders (n=31)	Included in interpretable serotype denominator	Reason if excluded
23F	6	19.4	Yes	
Non-typable	1	3.2	Yes	
Untyped / missing	19	61.3	No	Missing or blank
Untested	3	9.7	No	Non-informative response
Correct serotype among interpretable serotype results	6/7	85.7		

Table A3.5. Summary indicators for pneumococcal non-culture samples

Sample	Interpretable pathogen responses, n	Correct pathogen identification, n (%)	Serotype-field responses, n	Interpretable serotype results, n	Correct serotype among all responders, n (%)	Correct serotype among interpretable serotype results, n (%)	Species Ct median (IQR)	Serotype Ct median (IQR)
NC3	31	28 (90.3)	13	8	7 (22.6)	7 (87.5)	31.75 (30.3–36.14)	30.6
NC5	31	28 (90.3)	12	7	6 (19.4)	6 (85.7)	34.0 (30.6–35.0)	34.9

Detailed results for cultured pneumococcal isolates

Table A3.6. Serotype calls for isolate P1

Reported serotype-field value	n	% among all responders (n=23)	Included in interpretable serotype denominator
23F	19	82.6	Yes
14	1	4.3	Yes
Other serotype	1	4.3	Yes
Untested	2	8.7	No
Correct serotype among interpretable serotype results	19/21	90.5	

Table A3.7. Serotype calls for isolate P2

Reported serotype-field value	n	% among all responders (n=23)	Included in interpretable serotype denominator	Reason if excluded
14	21	91.3	Yes	
Untested	2	8.7	No	Non-informative response
Correct serotype among interpretable serotype results	21/21	100.0		

Table A3.8. Summary indicators for cultured pneumococcal isolates

Isolate	Responses, n	Correct species identification, n (%)	Serotype-field responses, n	Interpretable serotype results, n	Correct serotype among all responders, n (%)	Correct serotype among interpretable serotype results, n (%)	MIC testing uptake, n (%)	WGS uptake, n (%)
P1	23	23 (100.0)	23	21	19 (82.6)	19 (90.5)	21 (91.3)	11 (47.8)
P2	23	23 (100.0)	23	21	21 (91.3)	21 (100.0)	20 (87.0)	11 (47.8)

Supplementary AST tables

Table A3.9. Participant modal MICs for selected antibiotics

Isolate	Antibiotic	n numeric MIC	Modal MIC	n with modal MIC
P1	Benzylpenicillin	18	0.5	8
P1	Amoxicillin	14	0.12	6
P1	Cefotaxime	19	0.12	9
P1	Ceftriaxone	12	0.12	7
P1	Azithromycin	3	256.0	1
P1	Tetracycline	11	8.0	3
P2	Benzylpenicillin	17	0.032	7
P2	Amoxicillin	14	0.016	8
P2	Cefotaxime	18	0.016	7
P2	Ceftriaxone	12	0.016	4
P2	Azithromycin	5	0.25	2
P2	Tetracycline	11	0.25	4

Table A3.10. Concordance within one doubling dilution of the participant modal MIC

Isolate	Antibiotic	n numeric MIC	Modal MIC	n within +/-1 doubling dilution	Concordance, %
P1	Benzylpenicillin	18	0.5	17	94.4
P1	Amoxicillin	14	0.12	7	50.0
P1	Cefotaxime	19	0.12	17	89.5
P1	Ceftriaxone	12	0.12	10	83.3
P1	Azithromycin	3	256.0	2	66.7
P1	Tetracycline	11	8.0	7	63.6
P2	Benzylpenicillin	17	0.032	16	94.1
P2	Amoxicillin	14	0.016	11	78.6
P2	Cefotaxime	18	0.016	16	88.9
P2	Ceftriaxone	12	0.016	7	58.3
P2	Azithromycin	5	0.25	3	60.0
P2	Tetracycline	11	0.25	6	54.5

Supplementary WGS tables

Table A3.11. Completeness of reported WGS outputs for pneumococcal isolates, pooled P1 and P2 submissions

WGS output	n reported	Denominator WGS-positive submissions	% reported
Sequence type	22	22	100.0
LINcode	16	22	72.7

Table A3.12. Isolate-specific completeness of reported WGS outputs

Isolate	WGS output	n reported	Denominator WGS-positive submissions	% reported
P1	Sequence type	11	11	100.0
P1	LINcode	8	11	72.7
P2	Sequence type	11	11	100.0
P2	LINcode	8	11	72.7

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