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# External quality assessment scheme for identification, antimicrobial susceptibility testing and molecular typing of *Haemophilus influenzae*, 2026

**ECDC** MONITORING

# External quality assessment for identification, antimicrobial susceptibility testing and molecular typing of *Haemophilus influenzae*, 2026

On behalf of the IBDLabNet surveillance network

This report was commissioned by the European Centre for Disease Prevention and Control, coordinated by Dr Maria Keramarou, and produced by Dr Samy Taha and Prof. Muhamed-Kheir Taha, Institut Pasteur, Paris, France, on behalf of the IBDLabNet consortium, as part of the implementation of Framework Contract ECDC/2025/001.

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# Contents



# Figures



# Tables

# Abbreviations

| | |
|---|---|
| AST | antimicrobial susceptibility testing |
| BLNAR | beta-lactamase-negative ampicillin-resistant |
| BLPAR | beta-lactamase-positive ampicillin-resistant |
| CLSI | Clinical and Laboratory Standards Institute |
| CSF | cerebrospinal fluid |
| Ct | cycle threshold |
| DNA | deoxyribonucleic acid |
| ECDC | European Centre for Disease Prevention and Control |
| EMGM | European Meningococcal and Haemophilus Disease Society |
| EQA | external quality assessment |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| EU/EEA | European Union/European Economic Area |
| Hia | *Haemophilus influenzae* serotype a |
| Hib | *Haemophilus influenzae* serotype b |
| Hie | *Haemophilus influenzae* serotype e |
| H. influenzae | *Haemophilus influenzae* |
| IBDLabNet | Invasive Bacterial Diseases Laboratory Network |
| LINcode | Life Identification Number code |
| MALDI-TOF | matrix-assisted laser desorption ionisation time-of-flight |
| MIC | minimum inhibitory concentration |
| NFP | National Focal Point |
| NTHi | non-typeable *Haemophilus influenzae* |
| OCP | Operational Contact Point |
| PCR | polymerase chain reaction |
| REDCap | Research Electronic Data Capture |
| ST | sequence type |
| WGS | whole-genome sequencing |

# Executive summary

Despite the success of conjugate vaccination against serotype b, invasive *Haemophilus influenzae* disease continues to pose a challenge to public health in Europe. Surveillance requires not only reliable species identification, but also accurate distinction between capsulated and non-capsulated strains, capsule typing, antimicrobial susceptibility testing (AST) and, increasingly, genomic characterisation.

This external quality assessment (EQA) was organised within the IBDLabNet framework on behalf of the European Centre for Disease Prevention and Control (ECDC) to assess current European Union/European Economic Area (EU/EEA) laboratory capacity for the detection and characterisation of *H. influenzae* in both non-culture and culture-based settings. The exercise included a shared non-culture panel and an optional *H. influenzae* 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, 32/34 submitted at least one non-culture sample result to the Research Electronic Data Capture (REDCap) platform, and 23 received the *H. influenzae* culture panel.

Overall, performance was strong for identification of culture-based species. Correct identification was reported by 22/23 laboratories (95.7%) for H1 and 23/23 (100.0%) for H2. Capsule typing was more variable: 16/23 laboratories (69.6%) correctly reported H1 as non-typeable *Haemophilus influenzae* (NTHi), and 18/23 (78.3%) correctly identified H2 as *Haemophilus influenzae* serotype b (Hib). AST uptake was high, with minimum inhibitory concentration (MIC) testing reported by 19/23 laboratories (82.6%) for H1 and 20/23 (87.0%) for H2. The pre-defined resistance-associated phenotypes were recognised by most laboratories with interpretable results. Whole genome sequencing (WGS) was reported by 12/23 laboratories (52.2%) for H1 and 13/23 (56.5%) for H2. Among the WGS-positive submissions, reporting completeness was highest for sequence type and lower for Life Identification Number code (LINcode) and some additional outputs.

In contrast, non-culture performance was more heterogeneous. NC2, expected to contain Hib, appeared to be a low-signal and more challenging sample. Among laboratories with an interpretable response, 17/31 (54.8%) correctly identified *H. influenzae*, and only 2/31 (6.5%) correctly identified Hib when expressed against all interpretable responders. For NC6, expected to contain *Haemophilus influenzae* serotype a (Hia), species identification was higher, at 25/31 (80.6%), but correct capsule typing remained limited overall at 5/31 (16.1%), despite 5/10 (50.0%) correct results among typed submissions. These findings indicate that non-culture molecular detection, and especially capsule typing, are still the main areas for inter-laboratory variability.

The EQA confirms that participating EU/EEA laboratories have strong capacity for isolate-based *H. influenzae* identification, high AST uptake and moderate WGS implementation. However, important gaps remain for non-culture detection and capsule typing, particularly for challenging samples and the completeness of usable surveillance outputs. The design of the exercise was appropriate to document current practice, but interpretation should take into account the limited number of *H. influenzae* samples, the absence of a negative non-culture sample, optional participation in the culture component, reliance on participant modal MICs for comparative AST analysis, restriction of European Committee on Antimicrobial Susceptibility Testing (EUCAST)-based interpretation to selected informative combinations, and the descriptive nature of the WGS analysis.

Recommended next steps include continued rounds of EQA, targeted feedback to participants, improved collection of methodological information on non-culture workflows, focused training on molecular detection and capsule typing from non-culture material, further standardisation of AST and beta-lactamase reporting, and progressive harmonisation of WGS-derived outputs. These actions would improve the quality and comparability of invasive *H. influenzae* surveillance data across the EU/EEA.

# 1 Introduction

Despite the major success of conjugate vaccination against serotype b, invasive *Haemophilus influenzae* disease remains an important public health issue in Europe [1-3]. In 2023, 5 234 confirmed invasive H. influenzae cases were reported in the EU/EEA, representing a continued increase since 2022 and a marked rise on pandemic-era levels [1]. While Hib disease has been substantially reduced by vaccination programmes, invasive disease due to non-typeable *H. influenzae* and non-b encapsulated strains persists and is now making a substantial contribution to the burden of disease, particularly among infants and older adults [1-3].

For surveillance purposes, laboratory confirmation and further characterisation of *H. influenzae* remain essential since species confirmation alone is insufficient to describe epidemiological trends. Capsule typing, distinction between encapsulated and non-encapsulated strains, and AST data are important for interpreting vaccine impact, monitoring the changing distribution of invasive disease, detecting unusual events and supporting the public health response [1-4]. Variability in routine laboratory methods between countries may therefore affect the comparability of surveillance data and hinder interpretation of changes over time.

This EQA was organised by IBDLabNet on behalf of ECDC as part of the first-year implementation of the EU invasive bacterial diseases laboratory network [4,5]. The aim was to assess the ability of participating laboratories to detect and characterise *H. influenzae* from both non-culture and culture-based materials, including species identification, capsule typing, 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].

The current EQA was designed to address a practical need for surveillance within the EU/EEA, namely the integrated evaluation of non-culture molecular detection, culture-based identification, capsule typing, AST and optional WGS for invasive *H. influenzae* surveillance, rather than the isolated assessment of a single methodological step [1,4,5]. The specific objectives were therefore to: (i) assess laboratory performance for the detection and identification of *H. influenzae* in shared panels; (ii) assess the concordance of capsule typing results; (iii) describe AST practices, compare reported MICs across laboratories, and provide complementary EUCAST-based phenotypic readings for pre-defined informative resistance-associated combinations, 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 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 34 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, 23 laboratories received the *H. influenzae* culture panel and were eligible for the culture-based *H. influenzae* analyses.

This pathogen-specific report presents the *H. influenzae* components of the shared EQA scheme, namely the *H. influenzae*-positive non-culture samples and the two *H. influenzae* 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 opened on 12 January 2026, and the submission deadline was 15 April 2026. Participating laboratories were instructed to use only the methods routinely available in their laboratories. Non-culture samples were reconstituted according to the shipment instructions. Cultured isolates were shipped in Amies charcoal transport swabs and were to be re-isolated on appropriate media after receipt.

## Laboratory procedures assessed

For non-culture samples, laboratories were asked to perform routine molecular detection of *H. influenzae* and, where feasible and relevant, capsule typing. Ct values for species detection and, when applicable, type determination were recorded.

For cultured isolates, laboratories were asked to perform routine species identification, capsule typing, AST, beta-lactamase testing where relevant, and molecular typing. Acceptable identification approaches included routine phenotypic methods, matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) and validated molecular methods. AST methods reflected routine local practice. Participants were asked to report raw MIC values, interpretive standards and beta-lactamase results where tested. 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. Since no complete pre-defined reference dataset had been established for all the 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 capsule type results were pre-defined by the organising centre on the basis of the panel composition. For the *H. influenzae* culture panel, isolates were selected to include relevant capsule and resistance-associated phenotypes, including one beta-lactamase-positive isolate and one isolate with reduced susceptibility to cefotaxime. No pre-defined target MIC values were assigned for the EQA panel. Species identification and capsule typing were therefore assessed against the pre-defined expected results for each sample, whereas AST results were analysed comparatively among the 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. For selected isolate-antibiotic combinations with informative expected resistance phenotypes, raw MIC values were also interpreted using EUCAST v16.0 breakpoints to describe the proportion of laboratories recognising the expected phenotype [9]. Beta-lactamase results were analysed separately among all isolate responders and among laboratories providing an interpretable Yes/No result.

Ct values were analysed descriptively because no target Ct values had been pre-defined. 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. Since 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 *H. influenzae* culture panel, containing isolates H1 and H2, was received by 23 laboratories included in the EU/EEA aggregate analysis.

Reported routine methods for *H. influenzae* detection and typing varied across participating laboratories. For non-culture molecular testing, 12/34 laboratories (35.3%) reported using *hpd*, 3/34 (8.8%) reported *bexD*, 2/34 (5.9%) reported *ompP2*, and 13/34 (38.2%) reported other molecular targets. Multiple targets could be reported by the same laboratory.

For culture-based identification, molecular methods were reported by 15/23 laboratories (65.2%), MALDI-TOF by 12/23 (52.2%) and other phenotypic methods by 6/23 (26.1%). Capsule typing relied on both molecular and phenotypic approaches, reported by 15/23 (65.2%) and 13/23 laboratories (56.5%), respectively.

AST methods were heterogeneous. E-test was reported by 14/23 laboratories (60.9%), broth microdilution by 5/23 (21.7%), antimicrobial gradient methods by 2/23 (8.7%) and disk diffusion by 1/23 (4.3%). EUCAST was the main interpretive standard, reported by 19/23 laboratories (82.6%).

WGS was reported for at least one *H. influenzae* isolate by 13 laboratories. Among these WGS-positive laboratories, Illumina was the most frequently reported sequencing platform (10/13; 76.9%), and assembly was reported as performed by 10/13 laboratories (76.9%). These data describe the range of methods reported across routine molecular detection, culture-based identification, capsule typing, AST and WGS workflows.

**Table 1. Overview of aggregated EU/EEA results for the *Haemophilus influenzae* component of the EQA**

| EQA component | Sample/isolate | Expected result | Denominator | Correct result, n/N (%) |
|---|---|---|---|---|
| Non-culture pathogen identification | NC2 | *H. influenzae* | 31 | 17/31 (54.8%) |
| Non-culture capsule typing among all responders | NC2 | Hib | 31 | 2/31 (6.5%) |
| Non-culture capsule typing among typed submissions | NC2 | Hib | 9 | 2/9 (22.2%) |
| Non-culture pathogen identification | NC6 | *H. influenzae* | 31 | 25/31 (80.6%) |
| Non-culture capsule typing among all responders | NC6 | Hia | 31 | 5/31 (16.1%) |
| Non-culture capsule typing among typed submissions | NC6 | Hia | 10 | 5/10 (50.0%) |
| Culture-based species identification | H1 | *H. influenzae* | 23 | 22/23 (95.7%) |
| Culture-based capsule assignment among all responders | H1 | NTHi | 23 | 16/23 (69.6%) |
| Culture-based capsule assignment among typed submissions | H1 | NTHi | 20 | 16/20 (80.0%) |
| Culture-based species identification | H2 | *H. influenzae* | 23 | 23/23 (100.0%) |
| Culture-based capsule assignment among all responders | H2 | Hib | 23 | 18/23 (78.3%) |
| Culture-based capsule assignment among typed submissions | H2 | Hib | 21 | 18/21 (85.7%) |
| AST uptake | H1 | MIC reported | 23 | 19/23 (82.6%) |
| AST uptake | H2 | MIC reported | 23 | 20/23 (87.0%) |
| WGS uptake | H1 | WGS performed | 23 | 12/23 (52.2%) |
| WGS uptake | H2 | WGS performed | 23 | 13/23 (56.5%) |

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

## 3.2 Performance on non-culture samples

The *H. influenzae* non-culture component included two positive samples: NC2, expected to contain Hib, and NC6, expected to contain Hia.

### NC2

For NC2, 31 laboratories provided an interpretable pathogen response. Of these, 17/31 (54.8%) correctly identified *H. influenzae*. Incorrect responses mainly consisted of negative results, reported by 12/31 laboratories (38.7%). Two laboratories (2/31; 6.5%) reported *N. meningitidis*.

Capsule typing information was available from nine laboratories. Two of the nine laboratories (22.2%) correctly reported Hib, corresponding to 2/31 (6.5%) of all laboratories with an interpretable pathogen response. Other reported capsule or type results included NTHi, type e, and off-pathogen meningococcal serogroup calls.

Species Ct values were available from 15 laboratories. The median species Ct was 36.0 (IQR: 33.15-37.82). Type Ct values were available from four laboratories, with a median of 33.5 (IQR: 32.75-34.25).

### NC6

For NC6, 31 laboratories provided an interpretable pathogen response. Of these, 25/31 (80.6%) correctly identified *H. influenzae*. Incorrect responses mainly consisted of negative results, with only occasional alternative pathogen calls.

Capsule typing information was available from 10 laboratories. Five of the 10 laboratories (50.0%) correctly reported Hia, corresponding to 5/31 (16.1%) of all laboratories with an interpretable pathogen response. Incorrect or non-expected type results mainly included NTHi or non-b *H. influenzae* reports.

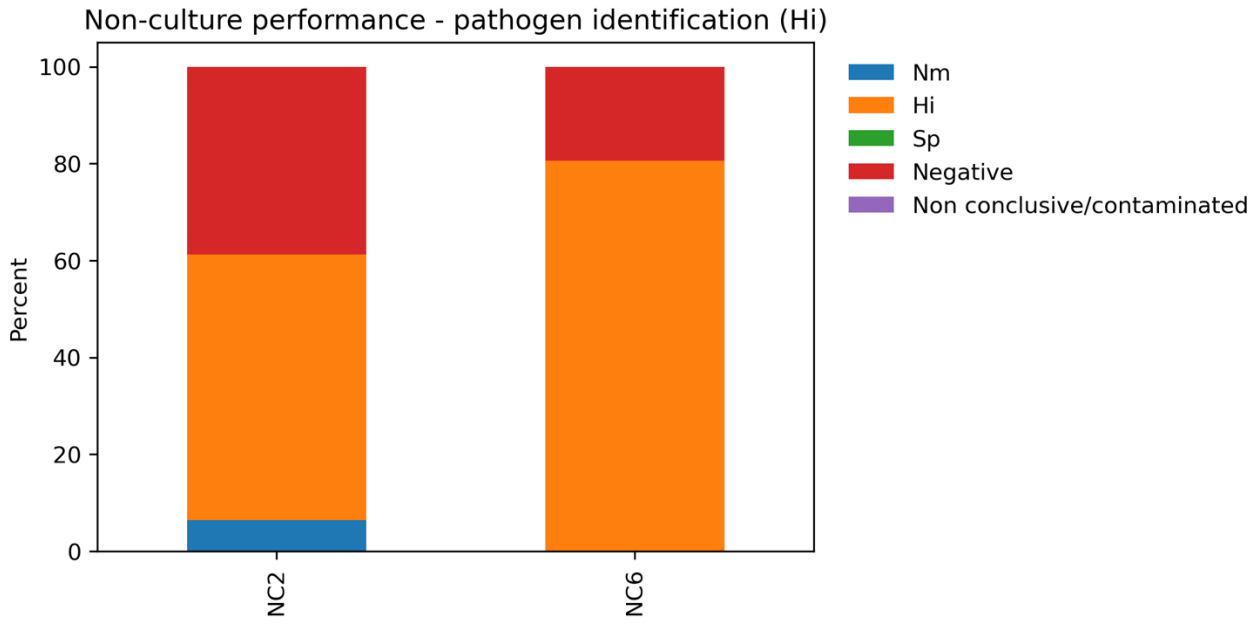
Species Ct values were available from 18 laboratories. The median species Ct was 32.0 (IQR: 30.25-33.0). Type Ct values were available from five laboratories, with a median of 32.3 (IQR: 29.0-34.9).

### Overall non-culture performance

Across the two *H. influenzae* non-culture samples, correct pathogen identification was 17/31 (54.8%) for NC2 and 25/31 (80.6%) for NC6. Capsule typing performance was lower than pathogen identification for both samples. Among laboratories reporting a capsule result, correct capsule assignment was 2/9 (22.2%) for NC2 and 5/10 (50.0%) for NC6. When expressed against all laboratories with an interpretable pathogen response, correct capsule assignment was 2/31 (6.5%) for NC2 and 5/31 (16.1%) for NC6.

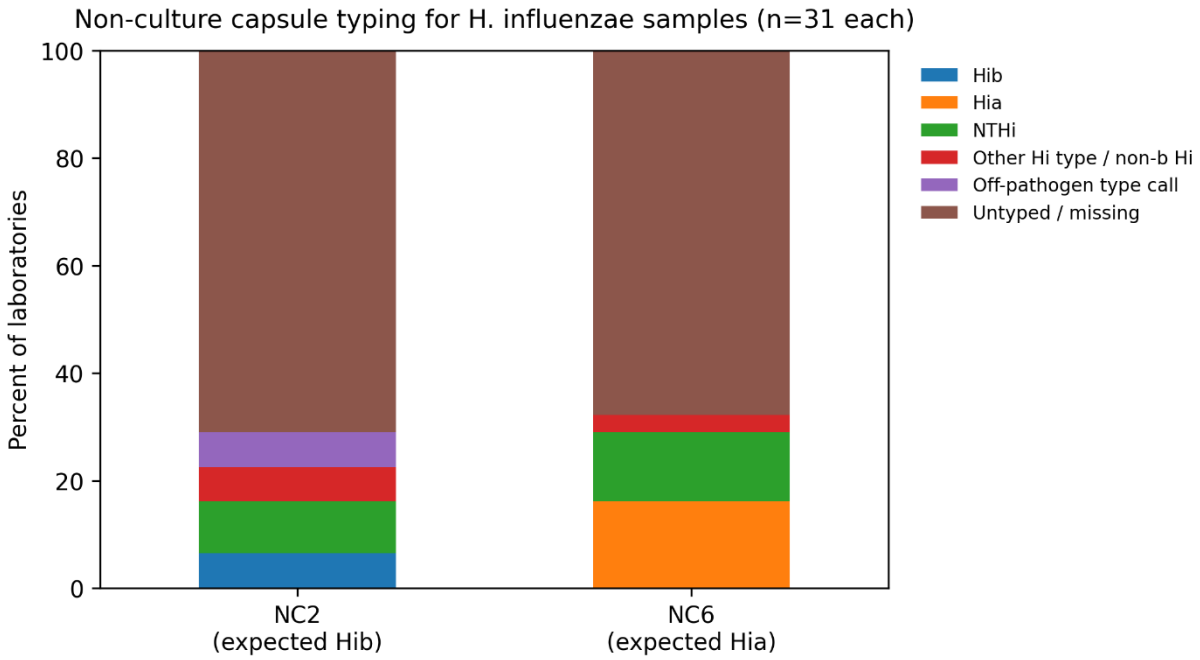
NC2 had lower pathogen detection and lower correct capsule assignment than NC6. NC2 also had a higher median species Ct than NC6.

**Figure 1. Performance on *Haemophilus influenzae* non-culture samples: pathogen identification for NC2 and NC6 (*H. influenzae* expected in both samples)**




*Percentages are based on laboratories providing an interpretable pathogen response (NC2: n=31; NC6: n=31).*

**Figure 2. Performance on *Haemophilus influenzae* non-culture samples: capsule typing for NC2 and NC6**




*Percentages are based on all laboratories providing an interpretable pathogen response; untyped or missing capsule results are shown as separate categories.*

## 3.3 Performance on cultured isolates

The *H. influenzae* culture panel included two isolates: H1, expected to be NTHi, and H2, expected to be Hib.

### H1

Species identification for H1 was correct in 22/23 laboratories (95.7%). Among all isolate responders, 16/23 laboratories (69.6%) correctly reported H1 as NTHi. Among laboratories that reported a capsule typing result, 16/20 (80.0%) reported the expected result.

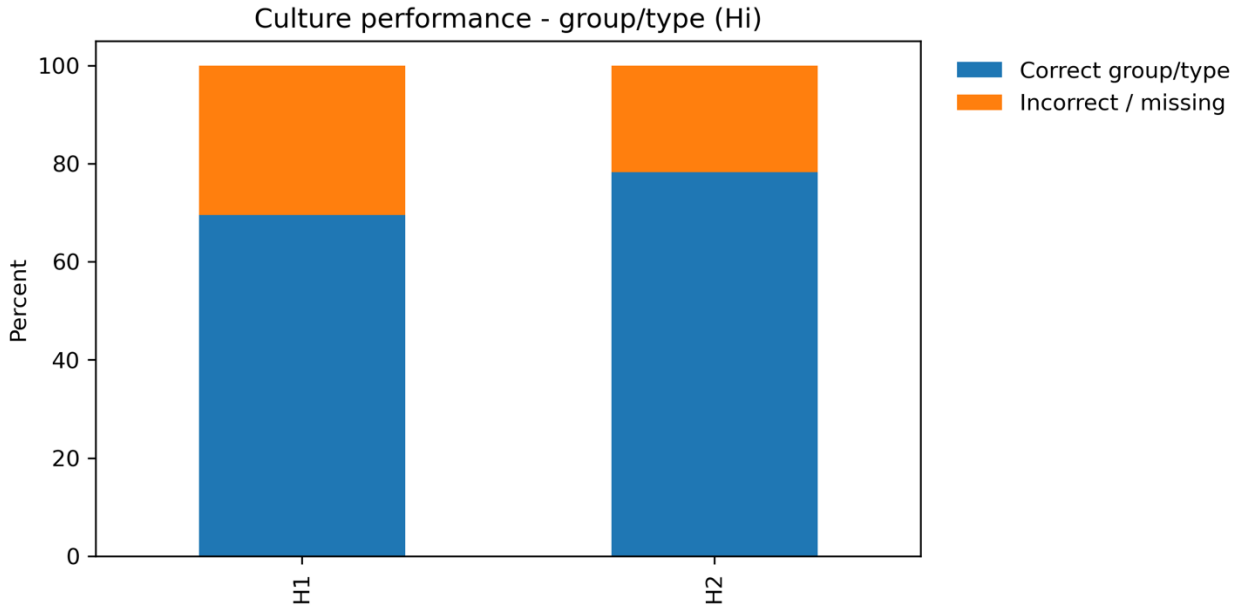
### H2

All 23 laboratories correctly identified H2 as *H. influenzae* (23/23; 100.0%). Among all isolate responders, 18/23 laboratories (78.3%) correctly reported Hib. Among laboratories that reported a capsule typing result, 18/21 (85.7%) reported the expected result.

### Overall culture-based performance

Culture-based species identification was high for both *H. influenzae* isolates. When expressed against all isolate responders, capsule typing was lower than species identification, but most laboratories that reported a capsule result provided the expected result.

**Figure 3. Culture-based capsule typing performance for *Haemophilus influenzae* isolates H1 and H2 (expected: non-typable for H1 and type b for H2)**




*Percentages are based on all laboratories reporting a result for the isolate (H1: n=23; H2: n=23).*

## 3.4 Antimicrobial susceptibility testing

AST uptake was high for both cultured isolates. MIC testing was reported by 19/23 laboratories (82.6%) for H1 and 20/23 (87.0%) for H2. Because pre-defined 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. In addition, EUCAST v16.0 criteria were applied to pre-defined informative isolate-antibiotic combinations to describe detection of expected resistance-associated phenotypes.

### H1

For H1, the participant modal MICs for selected antibiotics were 2.0 mg/L for ampicillin, 4.0 mg/L for amoxicillin, 0.25 mg/L for cefotaxime, 0.008 mg/L for ciprofloxacin and 0.25 mg/L for rifampicin.

Concordance within one doubling dilution of the participant modal MIC was 12/13 (92.3%) for ampicillin, 9/11 (81.8%) for amoxicillin, 14/18 (77.8%) for cefotaxime, 15/17 (88.2%) for ciprofloxacin and 11/15 (73.3%) for rifampicin.

For the pre-defined informative phenotype, cefotaxime resistance was identified in 15/18 laboratories (83.3%) with an interpretable numeric cefotaxime MIC.

Beta-lactamase testing for H1 was expected to be negative. When all isolate responders were used as the denominator, the expected result was reported by 15/23 laboratories (65.2%) and by 15/18 laboratories (83.3%) among laboratories providing an interpretable Yes/No beta-lactamase result.

### H2

For H2, the participant modal MICs were 4.0 mg/L for ampicillin, 2.0 mg/L for amoxicillin, 0.016 mg/L for cefotaxime, 0.008 mg/L for ciprofloxacin and 0.25 mg/L for rifampicin.

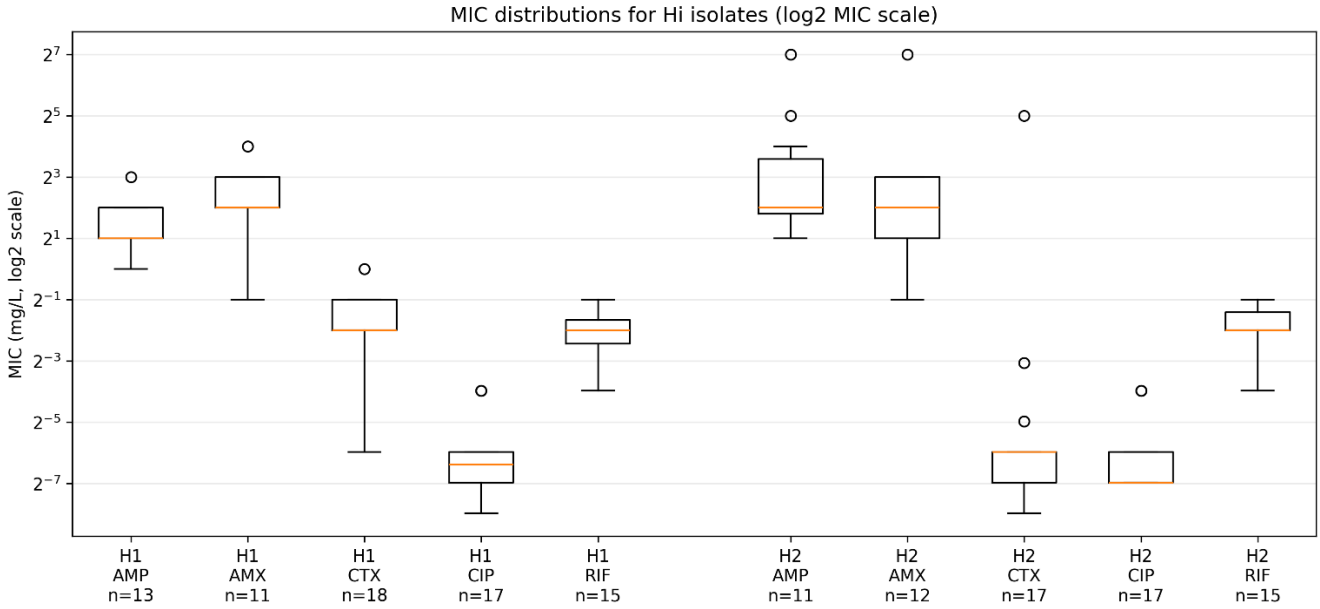
Concordance within one doubling dilution of the participant modal MIC was 8/11 (72.7%) for ampicillin, 7/12 (58.3%) for amoxicillin, 13/17 (76.5%) for cefotaxime, 15/17 (88.2%) for ciprofloxacin and 12/15 (80.0%) for rifampicin.

Ampicillin resistance was identified in 11/11 laboratories (100.0%) with an interpretable numeric ampicillin MIC. When all isolate responders were used as the denominator, beta-lactamase positivity was reported by 19/23 laboratories (82.6%), and by 19/19 laboratories (100.0%) among laboratories providing an interpretable Yes/No beta-lactamase result.

## Overall AST performance

For H1 cefotaxime, 15/18 numeric MIC results were categorised as resistant using EUCAST v16.0. For H2 ampicillin, 11/11 numeric MIC results were categorised as resistant. For beta-lactamase testing, H1 was reported as negative by 15/23 isolate responders and H2 was reported as positive by 19/23 isolate responders. Among laboratories with Yes/No results, the expected result was reported by 15/18 for H1 and 19/19 for H2.

**Figure 4. Distribution of reported MIC values for selected antibiotics for *H. influenzae* isolates H1 and H2, 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.*

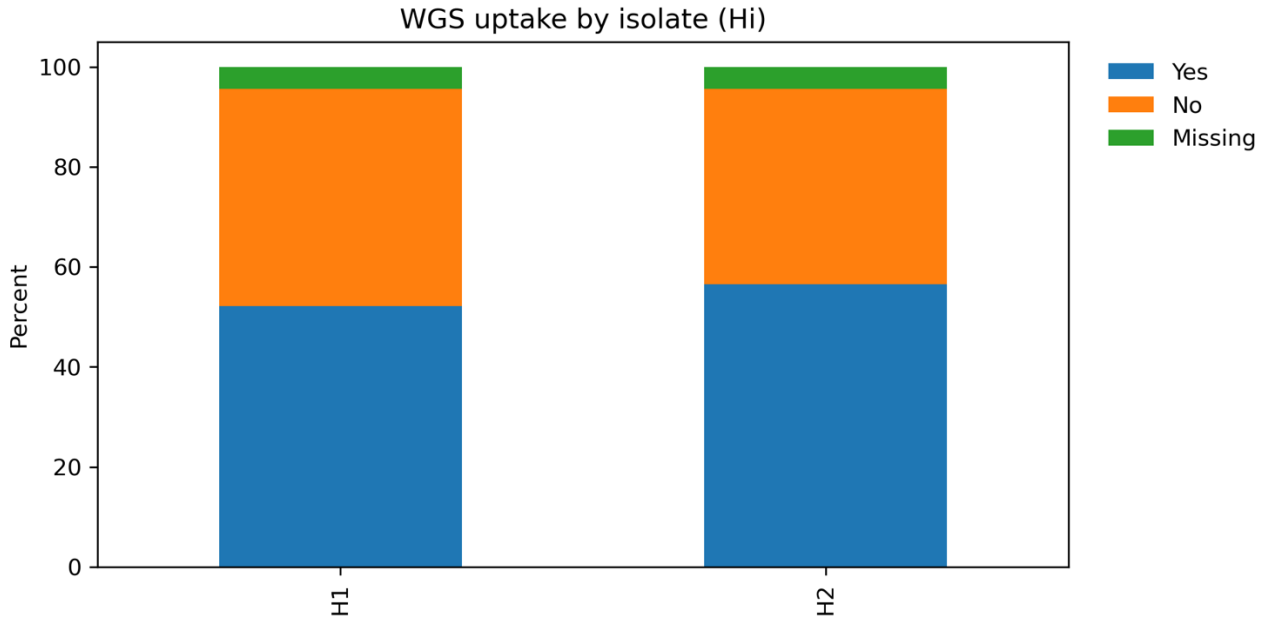
## 3.5 Whole-genome sequencing uptake and reported outputs

WGS was performed by 12/23 laboratories (52.2%) for H1 and 13/23 laboratories (56.5%) for H2. Illumina was the dominant reported sequencing platform, and assembly was reported as performed in most WGS-positive submissions.

Among WGS-positive submissions, reporting completeness varied by genomic output. For H1, the proportion of WGS-positive submissions reporting each variable was 10/12 (83.3%) for clonal complex, 12/12 (100.0%) for sequence type, 7/12 (58.3%) for LINcode and 10/12 (83.3%) for ftsI. For H2, reporting completeness was 12/13 (92.3%) for clonal complex, 13/13 (100.0%) for sequence type, 8/13 (61.5%) for LINcode and 11/13 (84.6%) for ftsI.

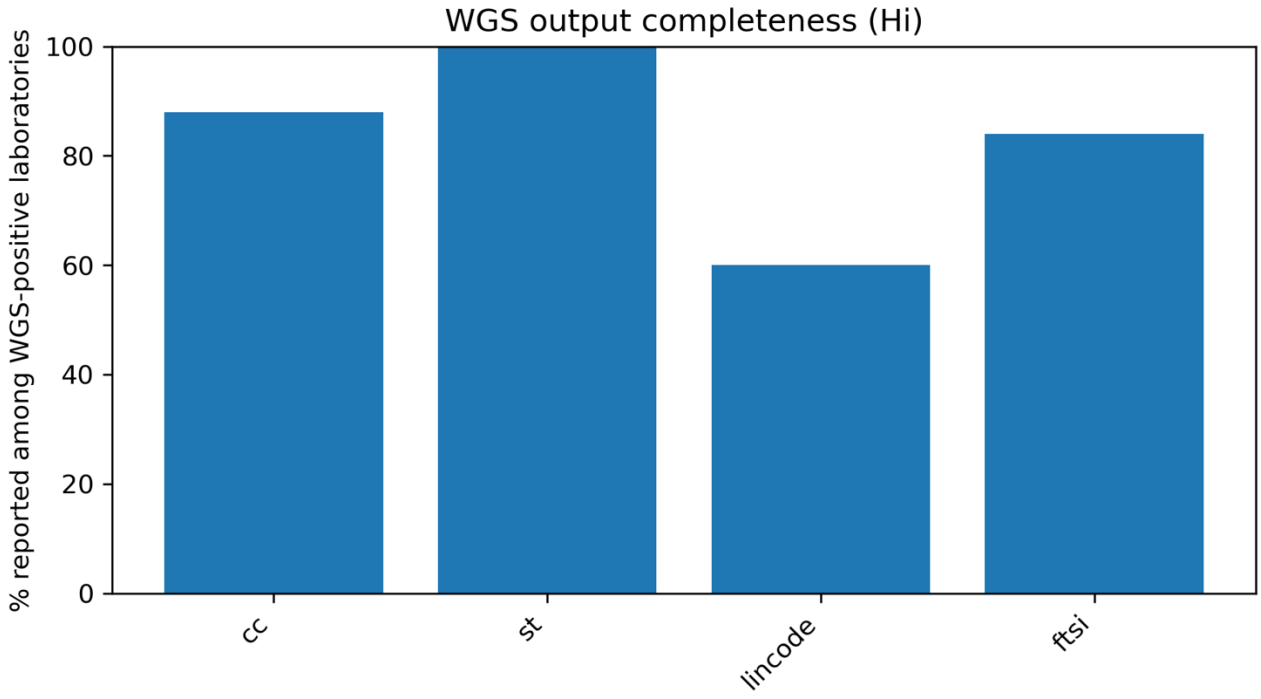
At pooled pathogen level, reporting completeness among WGS-positive submissions was 22/25 (88.0%) for clonal complex, 25/25 (100.0%) for sequence type, 15/25 (60.0%) for LINcode and 21/25 (84.0%) for ftsI.

**Figure 5. Uptake of whole-genome sequencing for *Haemophilus influenzae* isolates H1 and H2**




*Percentages are based on all laboratories reporting a result for the isolate (H1: n=23; H2: n=23)*

**Figure 6. Completeness of reported WGS outputs among WGS-positive submissions for *Haemophilus influenzae* isolates**




*Percentages are based on WGS-positive submissions only (pooled H1 and H2 submissions: n=25; H1: n=12; H2: n=13).*

# 4 Discussion

This EQA provides an overview of current EU/EEA laboratory capacity for the detection, identification, capsule typing, AST and genomic characterisation of *H. influenzae*. Overall, the results show very good performance for culture-based species identification, high uptake of AST among laboratories receiving the culture panel, and moderate WGS uptake. In contrast, performance on non-culture samples was more heterogeneous, particularly for species detection in NC2 and for capsule typing in both non-culture samples. This general pattern is consistent with the role of EQA in documenting inter-laboratory variability, identifying areas requiring harmonisation, and guiding targeted support activities [5,6].

The 2026 *H. influenzae* EQA should be interpreted alongside earlier European exercises, while recognising that direct comparisons remain imperfect because panel composition, matrices, expected outputs, participant networks and scoring approaches differed between exercises. The 2014 ECDC *H. influenzae* EQA included one Hib isolate, one non-typeable isolate, and two simulated cerebrospinal fluid (CSF) samples containing Hib and Hie [10]. The 2022 EQA led by the European Meningococcal and Haemophilus Disease Society (EMGM) indicated similar good overall performance for cultured *H. influenzae* isolates, with a mean species identification score of 89% across the panel and correct antibiotic susceptibility profiles reported for all isolates when tested [11]. Taken together, previous and current exercises suggest that culture-based identification is generally robust, whereas non-culture detection, and especially capsule typing, remain more method-dependent and variable.

Culture-based species identification represented the strongest component of the present exercise. Species identification was correct in 22/23 laboratories for H1 and 23/23 for H2. Capsule typing was less robust, especially for H1, where 16/23 laboratories correctly reported a non-typeable result when all responders were used as the denominator, and 16/20 laboratories reported a capsule result. This indicates that once an isolate is available, core identification capacity is well established across the network, but that distinction between non-capsulated strains and encapsulated types remains more susceptible to incomplete or discordant reporting. This remains epidemiologically important because surveillance of invasive *H. influenzae* increasingly depends on distinguishing Hib from non-b serotypes and NTHi, rather than on species confirmation alone [1–4]. In the 2014 ECDC EQA, both viable *H. influenzae* isolates were correctly identified by all participants and phenotypic serotyping was almost uniformly correct, although some errors or ambiguities remained for genotypic capsule typing of the NTHi isolate [10]. The results of this EQA are therefore consistent with sustained high proficiency for culture-based species identification, while confirming that capsule typing, particularly classification of non-typeable isolates, remains an area for harmonisation.

Non-culture testing showed more pronounced variability. NC2, expected to contain Hib, was the most difficult sample in the *H. influenzae* component. Only 17/31 laboratories correctly identified *H. influenzae*, and only 2/31 correctly identified Hib when expressed against all laboratories providing an interpretable pathogen result. NC6, expected to contain Hia, performed better for species detection, with 25/31 correct identifications, but capsule typing remained incomplete overall. This distinction is important for surveillance interpretation. Among laboratories that proceeded to capsule typing, performance was higher than when all responders were used as the denominator, but at sample level the combined effect of false-negative molecular detection and incomplete or discordant typing substantially reduced the amount of usable surveillance information.

The markedly lower performance observed for NC2 should be interpreted with caution. NC2 had the highest median species Ct among all non-culture samples in the exercise: 36.0, compared with 34.0 for NC1, 31.75 for NC3, 32.7 for NC4, 34.0 for NC5 and 31.98 for NC6. This suggests that NC2 may have been in a relatively low-signal range for routine molecular workflows. The very small number of available capsule/type Ct values for NC2 further limits interpretation of capsule typing performance for this sample. A post hoc linkage with the shipment sequence did not identify a clear shipment-date effect: Hi-positive results were observed across the sending period, and Hib-positive results were not confined to the first shipment wave. However, as for any lyophilised non-culture EQA material, variability related to sample preparation, reconstitution, DNA recovery or transport cannot be entirely excluded. These findings are compatible with observations from the 2014 ECDC *H. influenzae* EQA, where mixed success in additional typing of simulated CSF samples was noted and false-negative results were considered to possibly have been related to low DNA yield after extraction [10].

Previous exercises support the view that non-culture *H. influenzae* testing remains one of the most fragile components of surveillance-oriented laboratory practice. In the 2014 ECDC EQA, non-culture detection was generally good, but some real-time PCRs targeting *ompP2* had difficulty identifying one of the simulated samples, and further capsulation assessment gave mixed results [10]. In the EMGM 2022 exercise, *H. influenzae* species detection in the two non-culture samples reached 81% and 94%, whereas correct serotype identification was much lower, at 31% and 38%, respectively [11]. Together with the 2026 results, these findings indicate that species detection from non-culture material may be achievable in many laboratories, but that progression to reliable capsule typing remains inconsistent.

Variation in molecular targets and typing workflows may have contributed to the differences observed. In the present exercise, laboratories reported routine use of *hpd*, *bexD*, *ompP2* and other locally validated targets for non-culture detection. The 2014 ECDC report already showed that assay choice could influence performance in simulated CSF samples, and suggested that future EQA rounds should collect Cq values and more detailed methodological information to better interpret differences between laboratories [10]. The current dataset was not

designed to formally compare performance by target, extraction method or platform, and no causal attribution can therefore be made at laboratory level. Nevertheless, the results support the value of collecting more structured information in future rounds, including extraction procedure, input volume, internal controls, PCR targets used, Ct cut-off policies and the algorithm used for progression from species detection to capsule typing.

The method survey also highlights areas where routine practice remains heterogeneous. Laboratories reported different combinations of molecular targets for non-culture detection, a mixture of molecular and phenotypic approaches for isolate identification and capsule typing, and several AST methods. This heterogeneity is expected in a network of national and reference laboratories with different local workflows, but it has implications for comparability of surveillance outputs. Future EQA rounds would benefit from more detailed linkage between reported methods and sample-level results, while preserving the primary aim of the scheme as an assessment of routine practice rather than an evaluation of a single standardised protocol.

AST participation was high and provided useful comparative information, even though no pre-defined reference MIC values were assigned. The two *H. influenzae* isolates displayed distinct and epidemiologically relevant resistance-associated profiles. H1 was mostly reported as beta-lactamase negative and was selected to represent a cefotaxime-resistant phenotype, whereas H2 was beta-lactamase positive and ampicillin resistant. The expected cefotaxime-resistant phenotype for H1 was recognised by 15/18 laboratories with an interpretable cefotaxime MIC, and the expected ampicillin-resistant phenotype for H2 was recognised by 11/11 laboratories with an interpretable ampicillin MIC. Beta-lactamase positivity for H2 was reported by 19/23 laboratories when all isolate responders were used as the denominator, and by 19/19 among laboratories providing an interpretable Yes/No result.

The 2014 ECDC report is particularly informative for interpretation of the present AST findings. The report concluded that results were generally excellent for the beta-lactamase-positive isolate, whereas the low BLNAR isolate proved more challenging because ampicillin MICs could lie at or around the breakpoint and disc diffusion or even MIC determinations might fail to identify such strains reliably [10]. The present results show a broadly similar pattern: the beta-lactamase-positive phenotype was consistently recognised among laboratories reporting an interpretable beta-lactamase result, while MIC dispersion was more apparent for some beta-lactams. As in 2014, differences in methods and interpretive standards complicate inter-laboratory comparison. The present findings therefore reinforce the value of reporting raw MICs together with beta-lactamase results and of maintaining harmonised interpretive frameworks across the network [9,10].

WGS uptake for *H. influenzae* was moderate, with about half of participating laboratories performing sequencing for H1 and H2. Among WGS-positive submissions, reporting completeness was highest for sequence type and lower for clonal complex, LINcode and *ftsI*. This suggests that the main challenge is not only access to sequencing but also harmonisation of downstream analysis and reporting. Compared with meningococci, the range of WGS-derived outputs routinely reported for *H. influenzae* appears less standardised across laboratories. From a surveillance perspective, a realistic short-term objective may therefore be to define a minimal common reporting set for sequenced invasive *H. influenzae* isolates, including at least sequence type, capsule status or capsule-locus interpretation, and *ftsI* where relevant to beta-lactam susceptibility interpretation.

The public health implications of these findings are considerable. Surveillance of invasive *H. influenzae* requires more than species confirmation. Incomplete capsule typing can directly affect interpretation of vaccine-preventable disease burden, estimation of the residual contribution of Hib, and monitoring of non-b serotypes and NTHi. This is especially important in the post-Hib-vaccine era, in which invasive disease is increasingly dominated by non-typeable isolates and non-b serotypes [1–3]. False-negative non-culture results are also relevant because invasive cases may be culture-negative after antibiotic exposure, or when only clinical material is available. Conversely, robust isolate-based identification, broad AST participation and growing WGS use strengthen the network's ability to monitor changes in circulating strains and resistance-associated phenotypes.

Several limitations should be acknowledged. First, the *H. influenzae* component included only two non-culture samples and two cultured isolates, which cannot capture the full diversity of invasive *H. influenzae* epidemiology, capsule types and resistance mechanisms. 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 pre-defined reference MIC values, although EUCAST v16.0 criteria were applied to selected informative combinations to describe recognition of expected resistance-associated phenotypes. Finally, WGS outputs were analysed descriptively. A fully pre-defined reference dataset was not available for all genomic fields included in the questionnaire, and WGS reporting was not required from all participants. The inclusion of optional WGS fields was intended primarily to document implementation and identify areas for harmonisation rather than to provide formal performance scoring against pre-defined reference values.

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 internal review of false-negative molecular results, discordant capsule typing, AST outliers and incomplete WGS reporting, and may contribute to accreditation or quality-management documentation. At network level, the findings identify clear priorities for capacity strengthening: improving non-culture molecular detection and typing, harmonising interpretation of non-typeable isolates and resistance-associated phenotypes, maintaining standardised AST reporting, and progressively defining a minimal common set of WGS outputs. These priorities are aligned with the objectives of ECDC EQA activities: to support harmonisation, identify training needs and improve the comparability of surveillance data across the EU/EEA [5,6].

Overall, the 2026 *H. influenzae* EQA shows that EU/EEA laboratories participating in IBDLabNet have strong proficiency for isolate-based species identification and high AST uptake and moderate WGS implementation. The main residual gaps concern non-culture sensitivity, capsule typing completeness and harmonisation of genomic reporting. Continued EQA activity, structured feedback, more detailed method questionnaires and focused training on non-culture workflows and *H. influenzae* typing would help strengthen the quality and comparability of invasive *H. influenzae* surveillance data across the EU/EEA.

# 5 Conclusions

Overall, the 2026 IBDLabNet EQA for *Haemophilus influenzae* showed strong performance among participating EU/EEA laboratories for culture-based species identification. Capsule typing of cultured isolates was also generally good, although less complete than species identification, particularly when all isolate responders were used as the denominator. AST uptake was high among laboratories receiving the culture panel, and the main pre-defined resistance-associated phenotypes were recognised by most laboratories with interpretable results. WGS was implemented by about half of the laboratories that received the culture panel.

In contrast, the non-culture component showed more heterogeneous performance. The main limitations were reduced species detection for NC2, incomplete capsule typing and, less frequently, discordant capsule results, rather than frequent attribution to another pathogen. NC2 may have been a low-signal and more challenging sample, and its results should therefore be interpreted with caution. Overall, these findings indicate that isolate-based *H. influenzae* characterisation is relatively robust within the participating network, whereas non-culture molecular workflows are still the main area requiring further harmonisation.

From a surveillance perspective, the exercise confirms that participating laboratories have good capacity to support isolate-based confirmation and further characterisation of invasive *H. influenzae* disease. However, improving the completeness and robustness of non-culture detection and capsule typing remains important, particularly for culture-negative cases and for accurate monitoring of Hib, non-b serotypes and NTHi in the post-Hib-vaccination era.

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 *H. influenzae* samples, the absence of a negative non-culture sample, optional participation in the culture component, reliance on participant modal MICs for comparative AST analysis, restriction of EUCAST-based interpretation to selected informative combinations, and the descriptive nature of the WGS analysis should be taken into account when interpreting the findings. Overall, this exercise provides a useful baseline for future EQA rounds and for monitoring progress in harmonisation over time.

# 6 Recommendations

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

1. **Prioritise improvement of non-culture molecular workflows for *H. influenzae.***
   Future capacity-strengthening activities should focus on reducing false-negative detection and improving capsule typing from non-culture material. This should include targeted technical guidance or training on DNA extraction, sample input volume, inhibition controls, target selection, interpretation thresholds and capsule-typing workflows.
2. **Collect more structured methodological information in future EQA rounds.**
   Future questionnaires should capture standardised information on non-culture 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 capsule typing. These data would improve interpretation of inter-laboratory differences and help distinguish method-related variability from sample-related factors.
3. **Strengthen harmonisation of capsule typing and reporting of non-typeable isolates.**
   Given the surveillance importance of distinguishing Hib, non-b serotypes and NTHi, additional harmonisation work should address capsule typing interpretation, capsule gene detection, nomenclature for non-typeable isolates, and reporting rules for incomplete or discordant typing results.
4. **Maintain standardised AST and beta-lactamase reporting.**
   Laboratories should continue to report raw MIC values together with the test method and interpretive standard used. Beta-lactamase results should be reported systematically where tested. Where feasible, additional characterisation relevant to BLNAR or related beta-lactam resistance phenotypes should be encouraged.
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 *H. influenzae* isolates. Sequence type, capsule-related interpretation and *ftsI* reporting appear particularly relevant as common outputs for surveillance and resistance interpretation.
6. **Increase the educational value and interpretability of future *H. influenzae* panels.**
   Future panels could include a broader range of challenge materials, such as a negative non-culture sample, calibrated lower-signal non-culture samples, and isolates selected to represent distinct capsule and beta-lactam resistance mechanisms. Where feasible, documented stability or repeat-testing checks should be included for challenging non-culture samples to support interpretation of unexpected performance patterns.
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 invasive *H. influenzae* surveillance in the EU/EEA.

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# Annex 1. Participating laboratories and countries

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

| Country | Laboratory/institute | Non-culture panel | *H. influenzae* 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 | No |
| Belgium | LHUB-ULB - CNR *Haemophilus influenzae* | Yes | Yes |
| Latvia | Riga East University Hospital, Laboratory Service, National Microbiology Reference Laboratory | Yes | Yes |
| Portugal | Laboratório de Referência de Infeções Respiratórias - agentes bacterianos/Instituto Nacional de Saúde 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 Sanità | Yes | No |
| Italy | Istituto Superiore di Sanità | Yes | Yes |
| 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 | No |
| Sweden | Department of Laboratory Medicine, Clinical Microbiology, University Hospital Örebro | Yes | No |
| France | Centre National de Référence des Pneumocoques/Centre Hospitalier Intercommunal de Créteil | Yes | No |
| Norway | Norwegian Institute of Public Health | Yes | Yes |
| Germany | National Reference Center for Meningococci and *Haemophilus influenzae* | Yes | Yes |
| Spain | Spanish Pneumococcal Reference Laboratory | Yes | No |
| 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 | Yes |
| Hungary | National Center for Public Health and Pharmacy | Yes | Yes |
| Italy | Istituto Superiore di Sanità | Yes | No |
| 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 | No |
| Republic of 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 | Yes |
| 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 Référence des Méningocoques et *Haemophilus influenzae* | Yes | Yes |

# Annex 2. Haemophilus influenzae panel composition and expected results

## Annex 2A. Non-culture samples

| Sample code | Material type | Expected pathogen | Expected capsule type | Purpose in the EQA |
|---|---|---|---|---|
| NC2 | Lyophilised non-culture sample | *Haemophilus influenzae* | Hib | Assessment of molecular detection and capsule typing from non-culture material |
| NC6 | Lyophilised non-culture sample | *Haemophilus influenzae* | Hia | Assessment of molecular detection and capsule typing from non-culture material |

## Annex 2B. Cultured isolates

| Isolate code | Material type | Expected pathogen | Expected capsule type | Additional note |
|---|---|---|---|---|
| H1 | Cultured isolate | *Haemophilus influenzae* | NTHi | NTHi cefotaxime-resistant isolate |
| H2 | Cultured isolate | *Haemophilus influenzae* | Hib | Hib beta-lactamase-positive isolate |

## Annex 2C. Performance variables assessed for the *H. influenzae* component

| EQA component | Variables assessed |
|---|---|
| Non-culture panel | Pathogen detection, capsule typing, reported Ct values for species detection and type determination. |
| Culture panel | Species identification, capsule typing, antimicrobial susceptibility testing, beta-lactamase detection, and, where performed, WGS uptake and reported outputs. |

# Annex 3. Supplementary detailed result tables

## Annex 3A. Detailed results for non-culture *Haemophilus influenzae* samples

**Table A3.1 Pathogen calls for NC2**

| Reported pathogen result | n | Denominator | % |
|---|---|---|---|
| *H. influenzae* | 17 | 31 | 54.8 |
| Negative | 12 | 31 | 38.7 |
| *N. meningitidis* | 2 | 31 | 6.5 |

**Table A3.2 Capsule type calls for NC2**

| Reported capsule type result | n | % among typed submissions (n=9) | % among all responders (n=31) |
|---|---|---|---|
| Hib | 2 | 22.2 | 6.5 |
| NTHi | 3 | 33.3 | 9.7 |
| Hie | 2 | 22.2 | 6.5 |
| Nm B | 1 | 11.1 | 3.2 |
| None of the above | 1 | 11.1 | 3.2 |

**Table A3.3 Pathogen calls for NC6**

| Reported pathogen result | n | Denominator | % |
|---|---|---|---|
| *H. influenzae* | 25 | 31 | 80.6 |
| Negative | 6 | 31 | 19.4 |

**Table A3.4 Capsule type calls for NC6**

| Reported capsule type result | n | % among typed submissions (n=10) | % among all responders (n=31) |
|---|---|---|---|
| Hia | 5 | 50.0 | 16.1 |
| NTHi | 4 | 40.0 | 12.9 |
| Non-b Hi | 1 | 10.0 | 3.2 |

**Table A3.5 Summary indicators for non-culture *H. influenzae* samples**

| Sample | Interpretable pathogen responses, n | Correct pathogen identification n (%) | Capsule type reported n | Correct capsule type among all responders n (%) | Correct capsule type among typed submissions n (%) | Species Ct median (IQR) | Type Ct median (IQR) |
|---|---|---|---|---|---|---|---|
| NC2 | 31 | 17 (54.8) | 9 | 2 (6.5) | 2 (22.2) | 36.0 (33.2–37.8) | 33.5 (32.8–34.3) |
| NC6 | 31 | 25 (80.6) | 10 | 5 (16.1) | 5 (50.0) | 32.0 (30.3–33.0) | 32.3 (29.0-34.9) |

# Annex 3B. Detailed results for cultured *Haemophilus influenzae* isolates

**Table A3.6 Capsule type calls for isolate H1**

| Reported capsule type result | n | % among typed submissions (n=20) | % among all responders (n=23) |
|---|---|---|---|
| NTHi | 16 | 80.0 | 69.6 |
| Non-b Hi | 2 | 10.0 | 8.7 |
| Hid | 1 | 5.0 | 4.3 |
| Hib | 1 | 5.0 | 4.3 |
| Untyped / missing | 3 | – | 13.0 |

**Table A3.7 Capsule type calls for isolate H2**

| Reported capsule type result | n | % among typed submissions (n=21) | % among all responders (n=23) |
|---|---|---|---|
| Hib | 18 | 85.7 | 78.3 |
| NTHi | 2 | 9.5 | 8.7 |
| Non-b Hi | 1 | 4.8 | 4.3 |
| Untyped / missing | 2 | – | 8.7 |

**Table A3.8 Summary indicators for cultured *H. influenzae* isolates**

| Isolate | Responses n | Correct species identification n (%) | Capsule type reported n | Correct capsule type among all responders n (%) | Correct capsule type among typed submissions n (%) | MIC testing uptake n (%) | WGS uptake n (%) |
|---|---|---|---|---|---|---|---|
| H1 | 23 | 22 (95.7) | 20 | 16 (69.6) | 16 (80.0) | 19 (82.6) | 12 (52.2) |
| H2 | 23 | 23 (100) | 21 | 18 (78.3) | 18 (85.7) | 20 (87.0) | 13 (56.5) |

# Annex 3C. Supplementary AST tables

**Table A3.9 Beta-lactamase results for H1 and H2**

| Isolate | Expected beta-lactamase result | Reported result | n | Denominator | % |
|---|---|---|---|---|---|
| H1 | Negative | No | 15 | 23 | 65.2 |
| H1 | Negative | Yes | 3 | 23 | 13.0 |
| H1 | Negative | Missing | 5 | 23 | 21.7 |
| H1 | Negative | Expected result recognised among Yes/No results. | 15 | 18 | 83.3 |
| H2 | Positive | Yes | 19 | 23 | 82.6 |
| H2 | Positive | Untested | 1 | 23 | 4.3 |
| H2 | Positive | Missing | 3 | 23 | 13.0 |
| H2 | Positive | Expected result recognised among Yes/No results. | 19 | 19 | 100.0 |

**Table A3.10 Participant modal MICs for selected antibiotics**

| Isolate | Antibiotic | n tested | Modal MIC | n with modal value |
|---|---|---|---|---|
| H1 | Ampicillin | 13 | 2.0 | 5 |
| H1 | Amoxicillin | 11 | 4.0 | 5 |
| H1 | Cefotaxime | 18 | 0.25 | 9 |
| H1 | Ciprofloxacin | 17 | 0.008 | 7 |
| H1 | Rifampicin | 15 | 0.25 | 7 |
| H2 | Ampicillin | 11 | 4.0 | 3 |
| H2 | Amoxicillin | 12 | 2.0 | 4 |
| H2 | Cefotaxime | 17 | 0.016 | 8 |
| H2 | Ciprofloxacin | 17 | 0.008 | 10 |
| H2 | Rifampicin | 15 | 0.25 | 8 |

**Table A3.11 Concordance within one doubling dilution of the participant modal MIC**

| Isolate | Antibiotic | n numeric MIC | Modal MIC | n within +/-1 doubling dilution | Concordance, % |
|---|---|---|---|---|---|
| H1 | Ampicillin | 13 | 2.0 | 12 | 92.3 |
| H1 | Amoxicillin | 11 | 4.0 | 9 | 81.8 |
| H1 | Cefotaxime | 18 | 0.25 | 14 | 77.8 |
| H1 | Ciprofloxacin | 17 | 0.008 | 15 | 88.2 |
| H1 | Rifampicin | 15 | 0.25 | 11 | 73.3 |
| H2 | Ampicillin | 11 | 4.0 | 8 | 72.7 |
| H2 | Amoxicillin | 12 | 2.0 | 7 | 58.3 |
| H2 | Cefotaxime | 17 | 0.016 | 13 | 76.5 |
| H2 | Ciprofloxacin | 17 | 0.008 | 15 | 88.2 |
| H2 | Rifampicin | 15 | 0.25 | 12 | 80.0 |

**Table A3.12 Selected EUCAST v16.0 phenotype summary for informative H. influenzae AST combinations**

| Isolate | Informative combination | Result basis | No. with interpretable result | Expected phenotype | Expected phenotype recognised n/N (%) | EUCAST S, n/N (%) | EUCAST I, n/N (%) | EUCAST R, n/N (%) |
|---|---|---|---|---|---|---|---|---|
| H1 | Cefotaxime resistance | Numeric cefotaxime MIC | 18 | Resistant | 15/18 (83.3%) | 3/18 (16.7%) | 0/18 (0.0%) | 15/18 (83.3%) |
| H2 | Ampicillin resistance | Numeric ampicillin MIC | 11 | Resistant | 11/11 (100.0%) | 0/11 (0.0%) | 0/11 (0.0%) | 11/11 (100.0%) |

# Annex 3D. Supplementary WGS tables

**Table A3.13 Completeness of reported WGS outputs for *H. influenzae* isolates, pooled H1 and H2 submissions**

| WGS output | n reported | Denominator WGS-positive submissions | % reported |
|---|---|---|---|
| Clonal complex | 22 | 25 | 88.0 |
| Sequence type | 25 | 25 | 100.0 |
| LINcode | 15 | 25 | 60.0 |
| ftsI | 21 | 25 | 84.0 |

**Table A3.14 Isolate-specific completeness of reported WGS outputs**

| Isolate | WGS output | n reported | Denominator WGS-positive submissions | % reported |
|---|---|---|---|---|
| H1 | Clonal complex | 10 | 12 | 83.3 |
| H1 | Sequence type | 12 | 12 | 100.0 |
| H1 | LINcode | 7 | 12 | 58.3 |
| H1 | ftsI | 10 | 12 | 83.3 |
| H2 | Clonal complex | 12 | 13 | 92.3 |
| H2 | Sequence type | 13 | 13 | 100.0 |
| H2 | LINcode | 8 | 13 | 61.5 |
| H2 | ftsI | 11 | 13 | 84.6 |

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