

1 Supplementary Methods

2 Systematic review

3 A systematic review of the literature was performed to capture studies evaluating metagenomic
4 sequencing for human respiratory infection. The review was prepared following PRISMA guidelines
5 with the checklist available in the Appendix.

6 The inclusion criteria for published manuscripts were defined as follows i) original research studies ii)
7 must involve clinical action i.e. clinical reporting or interpretation of metagenomic NGS in respiratory
8 samples for pathogen detection or antimicrobial resistance prediction iii) studies must involve human
9 participants iv) include respiratory samples v) be published between Jan 1, 2020 and Sept 1, 2024. The
10 exclusion criteria were small case reports/series ($n < 20$ patients), conference abstracts, and secondary
11 sources such as narrative reviews or editorials.

12 The review utilised PubMed databases. An example search string: "metagenomic" OR "mNGS" OR
13 "NGS" OR "shotgun sequencing" AND "respiratory" OR "pneumonia" OR "bronchoalveolar lavage"
14 OR "sputum". References of included studies and relevant reviews were used to identify additional
15 studies. Searches were performed in January 2025 and included non-English publications.

16 After de-duplication, a two-step screening (title/abstract screening, then full-text screening) by two
17 reviewers (AAM and LBS) was used, with conflicts resolved by discussion. The document numbers
18 at each stage are shown in Figure 1. Data extraction fields were pre-specified to systematically capture
19 relevant information related to foreground questions from each included study (Appendix). The
20 reviewer assessed risk of bias using QUADAS-2 for diagnostic studies.

21 Delphi consensus

22 The Delphi consensus was registered (<https://osf.io/2f4zt>). Formal research ethics approval was not
23 required as the study did not involve patient data. A working group was convened and two virtual
24 workshops were held, informed by literature review, to help shape the draft statements and inform
25 development of the Delphi instrument. The working group comprised individuals responsible for the
26 interpretation and reporting of metagenomic results from all sites within the UK NHS Respiratory
27 Metagenomics Network (<https://metagenomics.nhs.uk>, 'the Network') and included clinical scientists,
28 microbiologists, and virologists from six Network sites. All working group participants were informed
29 of the study purpose and provided consent to participate. No compensation was provided. A steering
30 group (LBS, AAM, GN, JDE) curated the scope of the Delphi from the review and working group
31 discussions, and drafted candidate consensus statements. Evidence summaries were prepared as neutral
32 background material for panellists.

33 The survey was hosted on Microsoft Forms. Each statement was rated using a five-point Likert scale
34 ranging from strongly disagree (1) to strongly agree (5), with an additional "Unsure" option. Free-text
35 comment boxes accompanied each item. Three Delphi rounds were conducted. In the first round,
36 panellists rated all candidate statements, after which aggregate response distributions and a neutral

37 summary of free-text comments were shared with the panel. In the next rounds, participants re-rated
38 items which had been amended in light of this feedback. For each item and round, we calculated the
39 median score, interquartile range, and the proportion of panellists in agreement. Consensus was pre-
40 defined as at least 70% of panellists rating a statement as “agree” or “strongly agree” and fewer than
41 25% rating it as “disagree” or “strongly disagree.”

42 The guidance document was appraised using the AGREE II instrument by the Network programme
43 director (RB, Appendix) who was independent of the guideline development group, with the findings
44 used to refine the manuscript. The guidance has been endorsed by the NHS Respiratory Metagenomics
45 Network. The need for revision will be reviewed annually, and NHS network sites will be monitored
46 for adoption of recommendations.

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