**Supplementary Appendix for:**

**Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study**

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# **Supplementary Methods**

## **Study Sites**

The study was conducted at Aintree University Hospital NHS Foundation Trust, Bupa Cromwell Hospital, Chelsea and Westminster Hospital NHS Foundation Trust, City Hospitals Sunderland, Dudley Group NHS Foundation Trust, Great Ormond Street Hospital, Guy’s and St Thomas’ NHS Foundation Trust, James Paget University Hospitals NHS Foundation Trust, Norfolk and Norwich University Hospitals NHS Foundation Trust, North Middlesex University Hospital NHS Trust, Queen Elizabeth Hospital Kings Lynn NHS Trust, Royal Free Hospital, Royal Liverpool and Broadgreen University Hospitals NHS Trust, University College London Hospitals and University Hospitals of North Midlands. These sites were served by 11 different microbiology laboratories

## **Conventional culture and susceptibility testing**

Each respiratory specimen was initially cultured locally, at the laboratory serving the participating hospital, according to their standard operating procedures (SOPs). These SOPs were all based on the Public Health England (PHE) UK Standard.1 Prior to culture, specimens underwent quality control checks. Salivary specimens or those with excess epithelial cells were rejected. Except in the case of bronchoalveolar lavage (BAL) specimens, the PHE standard specifies initial homogenisation of the respiratory sample with 0.1% dithiothreitol, followed by a 10-5 dilution, and inoculation of the diluted and undiluted specimen onto chocolate agar with bacitracin (incorporated, or as a disc), cysteine lactose electrolyte deficient agar (CLED) or MacConkey agar, along with Sabouraud agar for fungi. Blood agar was added at some sites. In the case of BAL specimens, culture is performed on serial dilutions of a sample that has been concentrated by centrifugation.

Plates are incubated at 35-37°C in the presence of 5% CO2 (blood and chocolate agar) or in air (MacConkey and CLED agar) for 40-48h, with daily reading of results. Bacterial pathogens are identified to species level by MALDI-TOF or biochemical methods, followed by antimicrobial susceptibility testing using EUCAST or BSAC interpretive standards.

The PHE standards provide guidance on the interpretation of culture results for BAL samples, whereas interpretation and reporting are left to the discretion of individual laboratories for other sample types.

## **16S rRNA Analysis**

All specimens with a sufficient surplus (300 µl) after PCR testing underwent 16S rRNA analysis. Samples were inactivated by incubating for 30 minutes at 99°C, then DNA was extracted using the ZR Viral RNA/DNA kit and ZR BashingBead Lysis Tubes (Zymo Research). Briefly, 300 µl of sample were transferred into a bead tube, homogenized in a bead-beater for 30 seconds at 3,500 oscillations per minute, centrifuged for 1 minute at 21,000 ***g***. Next, 200 µl of the supernatant were transferred to a clean microcentrifuge tube and DNA was extracted following manufacturer's instructions. Illumina 16S rRNA sequencing was then performed according to the manufacturer’s protocol (Illumina, 15044223B). The V3-V4 16S rRNA region was amplified on a LightCycler 480 II instrument (Roche) and sequenced on an Illumina MiSeq system. The Illumina BaseSpace 16S rRNA pipeline was used to analyse the results. Only samples with at least 10,000 total reads were deemed eligible for analysis. For a genus to be considered significant, it had to comprise at least 1% of all reads.

## **Comprehensive Culture**

A sub-set of 103 specimens, selected at random or based on disagreement between culture and PCR for resistance detection, underwent additional culture-based analysis, termed ‘comprehensive culture’ at the UCL research laboratory, using methodology described previously.2 Briefly, a sweep of growth was taken across the plate of a fresh primary culture of the specimen on chocolate agar, and stored in MicrobankTM vials at -80°C until analysis. Ten microliters of neat sample and a 10-5 dilution in 0.9% saline were then plated onto chocolate agar, Columbia blood agar (CBA), Brilliance UTI agar (Oxoid, Basingstoke, UK) and Columbia colistin-nalidixic acid agar (C-CNA) (Oxoid). The CBA, UTI and C-CNA plates were incubated at 37°C in air for 18h; chocolate agar plates were incubated in 5% CO2 at 37°C for 18h. Representative bacterial colonies of different morphologies on each medium were identified by MALDI-TOF MS (Bruker GmbH, Mannheim, Germany), either directly from colonies or by using formic acid extraction where necessary

## **Characterisation of Antimicrobial Resistances**

Additional investigation of antimicrobial resistances, or the genes responsible, was performed on isolates found resistant in microbiology laboratories or by comprehensive culture, or when either of the two molecular systems detected key resistance genes.

 Gram-negative bacteria (i) reported resistant to cephalosporins or carbapenems in routine microbiology, or (ii) found to have ESBL or carbapenemase genes using the PCR systems, or (iii) grown in comprehensive culture were tested for resistance to ceftazidime, cefotaxime, ceftriaxone, ertapenem, meropenem and imipenem (Enterobacterales) or imipenem, meropenem, ceftazidime and piperacillin/tazobactam (*Acinetobacter* spp*.* and *P. aeruginosa*) by EUCAST disc diffusion methodology.3 Potential methicillin-resistant *Staphylococcus aureus* (MRSA) were screened for resistance to cefoxitin.

 When isolates had phenotypes consistent with the presence of antimicrobial resistance genes, genetic testing was performed. Enterobacterales resistant to a carbapenem or to oxyimino cephalosporins, *P. aeruginosa* resistant to both carbapenems and cephalosporins and *A. baumannii* resistant to imipenem or meropenem were tested with the Check-MDR CTX103XL kit (Checkpoints, Wageningen, the Netherlands) according to manufacturer’s instructions, following extraction of total genomic DNA using the Qiagen DNA Mini Kit (Qiagen). *S. aureus* isolates resistant to cefoxitin underwent in-house PCR (primers and conditions described previously)4,5 for detection of *mecA* and *mecC* using HotStartTaq PCR Mastermix (Qiagen) on DNA extracted with the Qiagen DNA Mini Kit.

## **Data collection**

Routine microbiology data available on the Laboratory Information Management Systems (LIMS) of each participating hospital were collected and managed using REDCap6 electronic data capture tools hosted at Norwich Clinical Trials Unit. For each included sample, we collected: (i) the culture result as reported to treating clinicians and (ii) details of significant organisms reported, and their full antimicrobial susceptibility profile. Any results for relevant respiratory pathogens detected by non-culture-based methods were also included. Hospitals’ routine virology data (by other PCR methods) were collected if testing had been performed on the same calendar day as collection of the lower respiratory tract sample for INHALE. We also collected details required to confirm patient eligibility and the times samples were collected, processed and results released. All PCR and supplementary data generated by study staff were also recorded in RedCap. All data were anonymised.

# **Supplementary Results**

**16S rRNA Analysis**16S rRNA analysis was originally included to act as an independent molecular reference method. Four-way BLC analysis including 16S rRNA results is shown in Table S5. However, he 16S technique was only able to distinguish organisms to genus level, so PCR and routine microbiology data are likewise grouped to genus level. Streptococci are omitted because of the high density of commensal streptococci found in the respiratory tract and the inability of the 16S method to distinguish these from each other and from pathogenic streptococci, including *S. pneumoniae*7. For this analysis only, *Klebsiella aerogenes* was grouped within the genus *Enterobacter* owing to its relatively recent re-classification. The results show that 16S rRNA analysis was less sensitive than PCR and so was not fit-for-purpose as an alternative molecular reference method; nonetheless, it had had greater sensitivity than routine microbiology. Further optimisation might yield better results.

## **Table S1.** Criteria for HAP/VAP diagnostic test progression to RCT phase of the inhale study (WP3)

|  |  |  |  |
| --- | --- | --- | --- |
| **Criterion** | **Description** | **Point Scoring** | **Maximum available points** |
| Concordance - essential criterion | Major discordance i.e. failures by the test to find pathogen(s) detected by routine microbiology must account for < 5% of all tests performed. | NA | NA |
| Overall Concordance | A measure of the overall accuracy of the test compared to the gold standard. | 1 point is awarded for every % point over 55% overall concordance | 45 points |
| Sensitivity  | Sensitivity for detection of common pathogens (i.e. *P. aeruginosa, S. aureus, K. pneumoniae, K. oxytoca*, *E. coli, E. cloacae, E. aerogenes, A. baumannii, H. influenzae* and *S. pneumoniae*) | 2 points for every ‘win’, i.e. the best sensitivity against a particular pathogen | 20 points |
| Breadth of panel | Each PCR test seeks some targets that the other cannot, principally resistance genes for Curetis and viruses for Biofire.  | Maximum points for most detections of unique targets, other tests awarded points as a proportion of unique detection | 15 points |
| Time to Result | Time to Result | 1 Point allocated for each 30 min less than 8h, the common dosage interval for antibiotics | 15 points |
| Cost of tests and equipment | Cost per test, A composite measure of both test and equipment cost. | Cheapest test is awarded the maximum points. One point is deducted from others for every 10% increase in price compared to the cheapest. | 15 points |
| Failure rate  | Failure rate of test and/or machine, full or partial. | 1 point deducted for each 0.5% of failures | 15 points |
| Footprint and space occupied | Amount of space required to host machine | Smallest machine awarded maximum points. Cheapest test is awarded the maximum points. One point is deducted from others for every 10% increase in price compared to the cheapest. | 5 points |
| Customer service | The quality and speed of customer service in the event of breakdown, ordering, installation etc. | . Average score based on assessment from individual users who have dealt with manufacturers during the study. | 5 points |
| Consumable logistics | Space required for storage of consumables, storage temperature, shelf life, delivery speed, delivery cost. | 1 point for best performing machine for each criterion | 5 points |
| Ease of use | User perception and experience | Average scored based on assessments from individual users who have operated machines during the study. | 10 points |
| **Total** |  |  | **150 points** |

## **Table S2.** Number of bacterial target organisms detected by routine microbiology or PCR in specimens from patients with HAP or VAP.

|  |  |  |  |
| --- | --- | --- | --- |
| **Target Organism** | **Routine Microbiology** | **Unyvero** | **FilmArray** |
| **HAP (n= 260)** | **VAP (n=392)** | **HAP (n = 240)** | **VAP (n = 366)** | **HAP (n = 247)** | **VAP (n = 373)** |
| *A. baumannii*  | 1 | 5 | 3 | 8 | 2 | 7 |
| *C. pneumoniae* | 0 | 0 | 0 | 0 | 0 | 0 |
| *C. freundii* | 0 | 2 | 4 | 5 | NA | NA |
| Coliform  | 2 | 4 | NA | NA | NA | NA |
| *E. cloacae* | 4 | 8 | 9 | 18 | 22 | 29 |
| *E. coli* | 15 | 28 | 45 | 51 | 51 | 62 |
| *H. influenzae* | 9 | 14 | 20 | 38 | 34 | 57 |
| *K. aerogenes* | 0 | 6 | 2 | 4 | 2 | 9 |
| *K. pneumoniae* | 7 | 19 | 21 | 33 | 31 | 43 |
| *K. oxytoca* | 3 | 9 | 13 | 27 | 13 | 27 |
| *K. variicola* | 0 | 0 | 2 | 3 | NA | NA |
| *L. pneumophila* | 0 | 0 | 1 | 1 | 0 | 0 |
| *M. catarrhalis* | 1 | 3 | 5 | 10 | 9 | 14 |
| *M. morganii* | 1 | 0 | 6 | 5 | NA | NA |
| *M. pneumoniae* | 0 | 0 | 0 | 1 | 0 | 1 |
| *Proteus* sp. | 2 | 8 | 13 | 16 | 14 | 17 |
| *P. aeruginosa* | 25 | 48 | 37 | 57 | 41 | 62 |
| *Pseudomonas* sp. | 0 | 6 | NA | NA | NA | NA |
| *S. marcescens* | 1 | 9 | 3 | 14 | 3 | 17 |
| *S, aureus* | 31 | 52 | 46 | 58 | 58 | 79 |
| *S. maltophilia* | 3 | 11 | 15 | 31 | NA | NA |
| *S. agalactiae* | 0 | 0 | NA | NA | 10 | 12 |
| *S. pneumoniae* | 2 | 4 | 9 | 13 | 14 | 26 |
| *S. pyogenes* | 1 | 1 | NA | NA | 2 | 7 |

## **Table S3**. Viral detections made by FilmArray (n=620 eligible samples) and sensitivity and specificity compared with routine virology (n = 102 samples with routine virology performed with eligible FilmArray result). 95% confidence intervals are given in brackets.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Virus** | **Number of Detections** | **% positive samples** | **Sensitivity %** | **Specificity %** | **PPV %** | **NPV %** |
| Rhinovirus | 55 | 8.9 | 100.0 (15.8 - 100.0) | 85.6(77.3 - 91.7) | 11.8 (1.5 - 36.4) | 100.0 (95.9 - 100.0) |
| Influenza A | 29 | 4.7 | 100.0 (59.0-100.0) | 97.0(91.4 - 99.4) | 70.0 (34.8 - 93.3) | 100.0 (96.2 - 100.0) |
| Influenza B | 25 | 4.0 | 100.0 (39.8 - 100.0) | 98.0 (93.1 - 99.8) | 66.7 (22.3 - 95.7) | 100.0 (96.4-100.0) |
| Parainfluenza | 17 | 2.7 | 75.0 (19.4-99.4) | 99.0 (94.7 - 100.0) | 75.0 (19.4-99.4) | 99.0 (94.7 - 100.0) |
| Coronavirus (229E, HKU1, NL63, OC43) | 16 | 2.6 | ND\* | 95.3 (89.3 -98.5) | 0.0 (0.0 - 52.2) | 100.0 (96.4-100.0) |
| Adenovirus | 7 | 1.1 | 50.0 (6.8-93.2) | 100.0(96.4 - 100.0) | 100.0(15.8 - 100.0) | 98.1 (93.2 - 99.8) |
| Respiratory Syncytial Virus | 6 | 1.0 | 66.7 (9.4 - 99.2) | 99.0 (94.7 - 100.0) | 66.7 (9.4 - 99.2) | 99.0 (94.7 - 100.0) |
| Human metapneumovirus | 5 | 0.8 | 100.0 (2.5 - 100.0) | 100.0(96.5 - 100.0) | 100.0 (2.5 - 100.0) | 100.0(96.5 - -100.0) |
| MERS coronavirus | 0 | 0 | ND | ND | ND | ND |

\*ND – not determined because routine virology did not report any positives.

## **Table S4.** Discordant samples, where one or both PCR tests did not detect potential pathogens which they sought and which were reported by routine microbiology.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample designationa** | **Sample type** | **Routine microbiology resultb** | **Unyvero Resultc** | **FilmArray resultc** |
| D001 | SPU | ***S. aureus*** | Negative | *S. aureus* |
| D014 | ETT | *M. catarrhalis****, S. aureus*** | *M. catarrhalis, H. influenzae, S. pneumoniae* | *M. catarrhalis, S. pneumoniae* |
| D019 | SPU | ***P. aeruginosa, S. marcescens*** | Negative | *P. aeruginosa, S. marcescens* |
| D022 | SPU | ***S. aureus*** | Negative | *S. aureus* |
| D037 | ETT | *H. influenzae****, S. aureus*** | *H. influenzae* | *H. influenzae, S. aureus* |
| D054 | SPU | *S. aureus,* ***H. influenzae*** | *H. influenzae, M. catarrhalis, S. aureus* | *M. catarrhalis, S. aureus* |
| D064 | SPU | *M. catarrhalis,* ***K. pneumoniae*** | *M. catarrhalis* | *M. catarrhalis, S. pyogenes* |
| D065 | SPU | ***E. coli,*** *H. influenzae* | *H. influenzae* | *E. coli, H. influenzae* |
| E005 | SPU | *P. aeruginosa,* ***K. pneumoniae*** | *P. aeruginosa, S. maltophila* | *P. aeruginosa, S. agalactiae* |
| F006 | Other | ***S. aureus*** | Negative | *S. aureus* |
| I012 | SPU | *P. aeruginosa,* ***S. aureus*** | *K. pneumoniae, P. aeruginosa* | Invalid result |
| I026 | SPU | ***P. aeruginosa*** | Negative | *P. aeruginosa* |
| I052 | SPU | *P. aeruginosa,* ***S. aureus****,* Coliform | *P. aeruginosa* | *P. aeruginosa, S. aureus* |
| I063 | ETT | ***K. pneumoniae*** | *K. variicola* | *K. pneumoniae* |
| I076 | SPU | ***E. aerogenes,*** *B. cepacia* | Negative | *E. aerogenes* |
| J002 | ETT | ***K. pneumoniae*** | *E, coli, K. oxytoca, S. aureus* | *E. cloacae, E. coli, K. pneumoniae, K. oxytoca, S. aureus* |
| J007 | SPU | ***P. aeruginosa,*** *S. maltophila* | Negative | Negative |
| K060 | ETT | ***E. aerogenes*** | Negative | *E. aerogenes*, *Proteus* sp. |
| K101 | BAL | ***S. aureus*** | Negative | Negative |
| L002 | ND-BAL | ***P. mirabilis*** | Negative | Negative |
| L011 | ND-BAL | ***E. coli*** | Negative | *E. coli* |
| L022 | ND-BAL | ***E. coli, S. aureus*** | Negative | *E. coli* |
| L034 | SPU | ***E. coli,*** *M. catarrhalis* | *K. pneumoniae, M. catarrhalis* | *K. pneumoniae, M. catarrhalis* |
| L039 | ND-BAL | ***K. oxytoca*** | Negative | *K. oxytoca* |
| M055 | ETT | ***E. cloacae*** | *E. cloacae* | Negative |
| N002 | SPU | ***E. coli*** | Negative | *E. coli, S. aureus, S. pneumoniae* |
| N018 | SPU | ***E. aerogenes,*** *H. influenzae* | *H. influenzae* | *E. cloacae, E. aerogenes, H. influenzae* |
| N054 | SPU | *E. cloacae,* ***S. aureus*** | *E. cloacae* | *E. clocae, S. aureus, S. agalactiae* |

a The prefix letter is an arbitrary code indicating samples were from the same site.

b Discordant pathogen(s) is shown in bold type.

c Shading indicates missed detections

BAL, bronchoalveolar lavage; ND-BAL, non-directed bronchoalveolar lavage; ETT, endotracheal tube aspirate; SPU, sputum.

## **Table S5.** Summary of frequency of potential pathogens reported by routine microbiology that are absent from the panels of one or both of the PCR tests.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Organisma** | **Frequency of detection by routine microbiology** | **Sample type (number)** |
| Organism absent from Unyvero panel | *Streptococcus pyogenes* | 2 | ETT (1) SPU(1) |
| Organisms absent from FilmArray panel | *Citrobacter freundii* | 1 | ND-BAL (1) |
| *Morganella morganii* | 1 | ETT (1) |
| *Stenotrophomonas maltophila* | 12 | ETT (6), ND-BAL (1), SPU(4) Other (1) |
| Organisms absent from both Unyvero and FilmArray panels | *Burkholderia* *cepacia* | 1 | SPU (1) |
| *Citrbacter koseri* | 5 | ETT (3), ND-BAL (1), SPU (1) |
| *Raoultella ornitholytica* | 2 | ETT (1), SPU (1) |
| *Achromobacter xylosoxidans* | 1 | ETT (1) |
| *Corynebacterium striatum* | 1 | ETT (1) |
| *Enterococcus faecium* | 1 | ETT (1) |
| Group G streptococcus | 1 | ETT (1) |
| *Pseudomonas putida* | 1 | ETT (1) |
| *S. pseudopneumoniae* | 1 | ETT (1) |

aIn addition, there were 3 cases (1 x ETT and 2 x SPU) where routine microbiology reported ‘coliforms’ not identified to species level, 1 (Other specimen type) where it reported *Pseudomonas* spp. and 2 (both SPU) where it reported *Streptococcus* spp. Without a species level identification is impossible to distinguish whether these represent cases where the PCR tests failed to detect organisms that they sought, or cases where the particular species was not sought by these tests.

## **Table S6.** Meannumbers of pathogens per eligible and valid sample detected by PCR tests in relation to sample type

|  |  |
| --- | --- |
| **PCR Test** | **Sample Type** |
| **BAL or ND-BAL**  | **ETT** | **Sputum** |
| **Unyvero** | 0.81 ± 0.96 (n = 63) | 1.08 ± 1.18 (n = 278) | 1. 13 ± 1.19 (n = 251) |
| **FilmArray** | 1.23 ±1.37 (n = 64) | 1.44 ± 1.26 (n = 285) | 1.63 ± 1.40 (n = 257) |

BAL, bronchoalveolar lavage; ND-BAL, non-directed bronchoalveolar lavage; ETT, endotracheal tube aspirate

## **Table S7.** Pathogen-specific performance of PCR tests when compared with routine microbiology as the gold standard, including 95% confidence intervals

|  |
| --- |
| **UNYVERO** |
| **Target Organism** | **Number of Detections** | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 94 | 95.3 | 86.9 | 99.0 | 93.9 | 91.6 | 95.8 | 64.9 | 54.4 | 74.5 | 99.4 | 98.3 | 99.9 |
| *S. aureus* | 104 | 87.2 | 77.7 | 93.7 | 93.2 | 90.7 | 95.2 | 65.4 | 55.4 | 74.4 | 98.0 | 96.4 | 99.0 |
| *K. pneumoniae* | 54 | 83.3 | 62.6 | 95.3 | 94.2 | 91.9 | 95.9 | 37.0 | 24.3 | 51.3 | 99.3 | 98.2 | 99.8 |
| *K. oxytoca* | 40 | 90.9 | 58.7 | 99.8 | 95.0 | 92.9 | 96.6 | 25.0 | 12.7 | 41.2 | 99.8 | 99.0 | 100.0 |
| *E. coli* | 96 | 87.8 | 73.8 | 95.9 | 89.4 | 86.5 | 91.8 | 37.5 | 27.8 | 48.0 | 99.0 | 97.7 | 99.7 |
| *E. cloacae* | 27 | 100.0 | 73.5 | 100.0 | 97.5 | 95.9 | 98.6 | 44.4 | 25.5 | 64.7 | 100.0 | 99.4 | 100.0 |
| *K. aerogenes* | 6 | 50.0 | 11.8 | 88.2 | 99.5 | 98.5 | 99.9 | 50.0 | 11.8 | 88.2 | 99.5 | 98.5 | 99.9 |
| *A. baumannii* | 11 | 100.0 | 47.8 | 100.0 | 99.0 | 97.8 | 99.6 | 45.5 | 16.7 | 76.6 | 100.0 | 99.4 | 100.0 |
| *H. influenzae* | 58 | 100.0 | 83.9 | 100.0 | 93.7 | 91.4 | 95.5 | 36.2 | 24.0 | 49.9 | 100.0 | 99.3 | 100.0 |
| *S. pneumoniae* | 22 | 100.0 | 54.1 | 100.0 | 97.3 | 95.7 | 98.5 | 27.3 | 10.7 | 50.2 | 100.0 | 99.4 | 100.0 |
| *M. catarrhalis* | 15 | 100.0 | 39.8 | 100.0 | 98.2 | 96.8 | 99.1 | 26.7 | 7.8 | 55.1 | 100.0 | 99.4 | 100.0 |
| *S. marcescens* | 17 | 77.8 | 40.0 | 97.2 | 98.3 | 96.9 | 99.2 | 41.2 | 18.4 | 67.1 | 99.7 | 98.8 | 100.0 |
| *C. pneumoniae* | 0 |  |  |  |  |  |  |  |  |  |  |  |  |
| *L. pneumophila* | 2 |  |  |  |  |  |  |  |  |  |  |  |  |
| *M. pneumoniae* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |
| *C. freundii* | 9 | 100.0 | 2.5 | 100.0 | 98.7 | 97.4 | 99.4 | 11.1 | 0.3 | 48.2 | 100.0 | 99.4 | 100.0 |
| *M. morganii* | 11 | 100.0 | 2.5 | 100.0 | 98.3 | 97.0 | 99.2 | 9.1 | 0.2 | 41.3 | 100.0 | 99.4 | 100.0 |
| *S. maltophilia* | 46 | 92.9 | 66.1 | 99.8 | 94.4 | 92.3 | 96.1 | 28.3 | 16.0 | 43.5 | 99.8 | 99.0 | 100.0 |

|  |
| --- |
| **FILMARRAY** |
| **Target Organism** | **Number of Detections** | Sensitivity | Specificity | PPV | NPV |
| % | 95% CI | % | 95% CI | % | 95% CI | % | 95% CI |
| *P. aeruginosa* | 103 | 98.5 | 91.8 | 100.0 | 93.1 | 90.7 | 95.1 | 63.1 | 53.0 | 72.4 | 99.8 | 98.9 | 100.0 |
| *S. aureus* | 137 | 96.2 | 89.4 | 99.2 | 88.9 | 85.9 | 91.4 | 56.2 | 47.5 | 64.7 | 99.4 | 98.2 | 99.9 |
| *K. pneumoniae* | 74 | 92.0 | 74.0 | 99.0 | 91.4 | 88.9 | 93.6 | 31.1 | 20.8 | 42.9 | 99.6 | 98.7 | 100.0 |
| *K. oxytoca* | 40 | 100.0 | 71.5 | 100.0 | 95.2 | 93.2 | 96.8 | 27.5 | 14.6 | 43.9 | 100.0 | 99.4 | 100.0 |
| *E. coli* | 113 | 97.6 | 87.4 | 99.9 | 87.5 | 84.6 | 90.1 | 36.3 | 27.4 | 45.9 | 99.8 | 98.9 | 100.0 |
| *E. cloacae* | 51 | 91.7 | 61.5 | 99.8 | 93.4 | 91.1 | 95.3 | 21.6 | 11.3 | 35.3 | 99.8 | 99.0 | 100.0 |
| *K. aerogenes* | 11 | 100.0 | 54.1 | 100.0 | 99.2 | 98.1 | 99.7 | 54.5 | 23.4 | 83.3 | 100.0 | 99.4 | 100.0 |
| *A. baumannii* | 9 | 100.0 | 54.1 | 100.0 | 99.5 | 98.6 | 99.9 | 66.7 | 29.9 | 92.5 | 100.0 | 99.4 | 100.0 |
| *H. influenzae* | 91 | 95.2 | 76.2 | 99.9 | 88.1 | 85.3 | 90.6 | 22.0 | 14.0 | 31.9 | 99.8 | 99.0 | 100.0 |
| *S. pneumoniae* | 40 | 100.0 | 54.1 | 100.0 | 94.5 | 92.3 | 96.1 | 15.0 | 5.7 | 29.8 | 100.0 | 99.4 | 100.0 |
| *M. catarrhalis* | 23 | 100.0 | 39.8 | 100.0 | 96.9 | 95.2 | 98.1 | 17.4 | 5.0 | 38.8 | 100.0 | 99.4 | 100.0 |
| *S. marcescens* | 20 | 100.0 | 66.4 | 100.0 | 98.2 | 96.8 | 99.1 | 45.0 | 23.1 | 68.5 | 100.0 | 99.4 | 100.0 |
| *C. pneumoniae* | 0 |  |  |  |  |  |  |  |  |  |  |  |  |
| *L. pneumophila* | 0 |  |  |  |  |  |  |  |  |  |  |  |  |
| *M. pneumoniae* | 1 |  |  |  |  |  |  |  |  |  |  |  |  |
| *S. agalactiae* | 22 | NA | 0.0 | 100.0 | 96.5 | 94.7 | 97.8 | 0.0 | 0.0 | 15.4 | 100.0 | 99.4 | 100.0 |
| *S. pyogenes* | 9 | 100.0 | 15.8 | 100.0 | 98.9 | 97.7 | 99.5 | 22.2 | 2.8 | 60.0 | 100.0 | 99.4 | 100.0 |

## **Table S8.** Pathogen specific performance of routine microbiology and PCR tests using independent BLC modelling, including 95% confidence intervals

|  |
| --- |
| **ROUTINE MICROBIOLOGY** |
| **Target Organism** | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 64.7 | 54.7 | 73.9 | 99.7 | 98.9 | 100.0 | 97.3 | 91.5 | 99.6 | 93.9 | 91.7 | 95.8 |
| *S. aureus* | 65.2 | 56.1 | 74.1 | 99.2 | 98.2 | 99.8 | 95.2 | 88.8 | 98.6 | 92.5 | 90.0 | 94.7 |
| *K. pneumoniae* | 37.8 | 26.0 | 51.4 | 99.5 | 98.6 | 99.9 | 89.3 | 73.2 | 97.7 | 93.5 | 90.9 | 95.6 |
| *K. oxytoca* | 30.2 | 18.3 | 45.5 | 99.9 | 99.3 | 100.0 | 94.3 | 73.0 | 99.8 | 95.5 | 93.7 | 97.0 |
| *E. coli* | 38.8 | 29.8 | 48.2 | 99.7 | 98.9 | 100.0 | 96.1 | 86.8 | 99.5 | 88.5 | 85.5 | 91.1 |
| *E. cloacae* | 42.9 | 25.6 | 61.3 | 99.9 | 99.3 | 100.0 | 94.6 | 71.6 | 99.8 | 97.2 | 95.5 | 98.4 |
| *K. aerogenes* | 68.7 | 32.1 | 94.7 | 99.9 | 99.4 | 100.0 | 88.9 | 54.4 | 99.6 | 99.6 | 98.5 | 99.9 |
| *A. baumannii* | 57.5 | 27.1 | 84.9 | 99.9 | 99.4 | 100.0 | 87.4 | 50.8 | 99.5 | 99.4 | 98.5 | 99.8 |
| *H. influenzae* | 36.3 | 24.8 | 49.1 | 99.9 | 99.3 | 100.0 | 96.8 | 84.5 | 99.9 | 93.5 | 91.0 | 95.4 |
| *S. pneumoniae* | 27.1 | 15.9 | 46.2 | 99.9 | 99.4 | 100.0 | 90.0 | 61.0 | 99.6 | 97.0 | 95.0 | 98.3 |
| *M. catarrhalis* | 27.6 | 15.8 | 50.4 | 99.9 | 99.4 | 100.0 | 86.7 | 50.5 | 99.4 | 98.0 | 96.5 | 99.0 |
| *S. marcescens* | 48.4 | 27.7 | 69.7 | 99.9 | 99.3 | 100.0 | 92.9 | 67.4 | 99.7 | 98.4 | 97.1 | 99.2 |

|  |
| --- |
| **UNYVERO** |
| **Target Organism** | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 95.8 | 89.6 | 99.0 | 99.9 | 99.2 | 100.0 | 99.2 | 95.8 | 100.0 | 99.2 | 98.0 | 99.8 |
| *S. aureus* | 91.1 | 82.9 | 96.1 | 99.8 | 99.2 | 100.0 | 99.3 | 96.4 | 100.0 | 98.0 | 95.9 | 99.2 |
| *K. pneumoniae* | 88.9 | 73.3 | 97.5 | 99.8 | 99.0 | 100.0 | 97.6 | 90.6 | 99.9 | 98.8 | 96.6 | 99.8 |
| *K. oxytoca* | 92.7 | 80.3 | 98.8 | 99.2 | 98.1 | 99.9 | 88.7 | 74.7 | 98.6 | 99.5 | 98.6 | 99.9 |
| *E. coli* | 89.6 | 80.5 | 96.4 | 99.7 | 98.9 | 100.0 | 98.6 | 94.4 | 99.9 | 97.8 | 95.8 | 99.3 |
| *E. cloacae* | 94.9 | 74.9 | 99.8 | 99.9 | 99.3 | 100.0 | 97.2 | 86.9 | 99.9 | 99.8 | 98.5 | 100.0 |
| *K. aerogenes* | 48.4 | 21.4 | 80.3 | 99.6 | 98.8 | 99.9 | 62.1 | 25.5 | 93.0 | 99.3 | 98.1 | 99.8 |
| *A. baumannii* | 92.6 | 66.2 | 99.7 | 99.5 | 98.6 | 99.9 | 70.9 | 39.7 | 94.6 | 99.9 | 99.4 | 100.0 |
| *H. influenzae* | 96.9 | 84.8 | 99.9 | 99.7 | 98.8 | 100.0 | 97.1 | 89.3 | 99.9 | 99.7 | 98.2 | 100.0 |
| *S. pneumoniae* | 90.8 | 63.2 | 99.6 | 99.9 | 99.3 | 100.0 | 96.7 | 83.8 | 99.9 | 99.6 | 97.8 | 100.0 |
| *M. catarrhalis* | 89.0 | 60.6 | 99.5 | 99.9 | 99.4 | 100.0 | 95.5 | 78.1 | 99.8 | 99.7 | 98.5 | 100.0 |
| *S. marcescens* | 83.9 | 64.1 | 95.6 | 99.9 | 99.4 | 100.0 | 95.7 | 78.0 | 99.8 | 99.5 | 98.7 | 99.9 |

|  |
| --- |
| **FILMARRAY** |
|  | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **Target Organism** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 99.2 | 95.9 | 100.0 | 99.3 | 98.3 | 99.9 | 96.6 | 91.1 | 99.6 | 99.9 | 99.2 | 100.0 |
| *S. aureus* | 99.3 | 96.5 | 100.0 | 95.6 | 93.3 | 97.5 | 83.9 | 76.2 | 90.7 | 99.8 | 99.1 | 100.0 |
| *K. pneumoniae* | 98.1 | 91.1 | 99.9 | 97.7 | 95.8 | 99.4 | 82.2 | 69.5 | 95.6 | 99.8 | 99.0 | 100.0 |
| *K. oxytoca* | 95.2 | 81.0 | 99.8 | 99.7 | 98.9 | 100.0 | 95.9 | 84.0 | 99.8 | 99.7 | 98.6 | 100.0 |
| *E. coli* | 98.9 | 95.3 | 100.0 | 98.7 | 96.8 | 99.9 | 94.2 | 86.1 | 99.6 | 99.8 | 99.0 | 100.0 |
| *E. cloacae* | 94.2 | 81.7 | 99.2 | 96.4 | 94.6 | 97.9 | 56.1 | 40.5 | 72.6 | 99.7 | 99.0 | 100.0 |
| *K. aerogenes* | 89.8 | 58.5 | 99.6 | 99.4 | 98.4 | 99.9 | 67.8 | 34.3 | 96.7 | 99.9 | 99.2 | 100.0 |
| *A. baumannii* | 89.4 | 55.6 | 99.5 | 99.9 | 99.4 | 100.0 | 91.3 | 62.5 | 99.6 | 99.8 | 99.2 | 100.0 |
| *H. influenzae* | 95.3 | 87.4 | 99.2 | 93.8 | 91.5 | 95.8 | 62.4 | 51.9 | 73.8 | 99.5 | 98.5 | 99.9 |
| *S. pneumoniae* | 97.1 | 85.8 | 99.9 | 97.1 | 95.4 | 98.8 | 57.9 | 40.8 | 81.7 | 99.9 | 99.3 | 100.0 |
| *M. catarrhalis* | 95.7 | 80.2 | 99.8 | 98.9 | 97.6 | 99.8 | 71.4 | 47.1 | 95.0 | 99.9 | 99.4 | 100.0 |
| *S. marcescens* | 96.1 | 81.8 | 99.9 | 99.8 | 99.2 | 100.0 | 94.2 | 76.3 | 99.8 | 99.9 | 99.3 | 100.0 |

## **Table S9.** Pathogen-specific performance of routine microbiology, PCR tests and 16S rRNA analysis using independent BLC modelling, showing 95% confidence intervals

|  |
| --- |
| **ROUTINE MICROBIOLOGY** |
|  | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **Target Genus** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *Acinetobacter* | 54.2 | 26.8 | 82.0 | 99.9 | 99.3 | 100.0 | 87.5 | 49.5 | 99.5 | 99.2 | 98.3 | 99.8 |
| *Escherichia* | 38.7 | 29.1 | 48.7 | 99.6 | 98.6 | 99.9 | 95.3 | 85.2 | 99.3 | 88.3 | 85.2 | 91.0 |
| *Enterobacter* | 46.4 | 30.2 | 63.3 | 99.8 | 99.2 | 100.0 | 95.9 | 79.0 | 99.9 | 96.2 | 93.7 | 97.8 |
| *Haemophilus* | 30.4 | 20.2 | 42.8 | 99.8 | 99.2 | 100.0 | 96.6 | 83.7 | 99.9 | 90.9 | 87.7 | 93.4 |
| *Klebsiella* | 38.0 | 27.8 | 48.5 | 99.4 | 98.3 | 99.8 | 92.1 | 79.4 | 98.1 | 89.6 | 86.6 | 92.3 |
| *Moraxella* | 23.9 | 15.4 | 43.4 | 99.9 | 99.3 | 100.0 | 85.7 | 50.4 | 99.5 | 97.5 | 96.0 | 98.6 |
| *Pseudomonas* | 75.3 | 64.8 | 83.8 | 99.5 | 98.5 | 100.0 | 96.6 | 89.8 | 99.8 | 95.8 | 93.7 | 97.4 |
| *Proteus* | 33.6 | 18.3 | 52.2 | 99.7 | 98.9 | 100.0 | 84.8 | 57.4 | 97.7 | 96.5 | 94.6 | 97.8 |
| *Staphylococcus* | 66.3 | 56.4 | 75.2 | 99.6 | 98.6 | 99.9 | 97.5 | 91.6 | 99.7 | 92.5 | 89.6 | 94.8 |
| *Serratia* | 51.8 | 30.3 | 73.7 | 99.8 | 99.2 | 100.0 | 92.4 | 65.7 | 99.7 | 98.4 | 97.1 | 99.3 |

|  |
| --- |
| **UNYVERO** |
|  | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **Target Genus** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *Acinetobacter* | 92.9 | 67.9 | 99.7 | 99.7 | 98.8 | 100.0 | 83.0 | 51.9 | 98.0 | 99.9 | 99.3 | 100.0 |
| *Escherichia* | 95.6 | 89.4 | 99.0 | 99.8 | 99.1 | 100.0 | 99.2 | 96.0 | 100.0 | 99.1 | 97.7 | 99.8 |
| *Enterobacter* | 80.4 | 60.6 | 94.3 | 99.8 | 99.2 | 100.0 | 97.6 | 87.7 | 99.9 | 98.6 | 96.4 | 99.6 |
| *Haemophilus* | 80.5 | 67.5 | 92.0 | 99.7 | 98.8 | 100.0 | 97.1 | 90.6 | 99.8 | 97.3 | 95.0 | 99.0 |
| *Klebsiella* | 90.7 | 81.8 | 96.6 | 99.0 | 97.6 | 99.8 | 94.2 | 86.5 | 98.8 | 98.3 | 96.5 | 99.4 |
| *Moraxella* | 80.0 | 56.0 | 95.3 | 99.9 | 99.3 | 100.0 | 95.2 | 76.8 | 99.9 | 99.3 | 98.2 | 99.9 |
| *Pseudomonas* | 95.7 | 89.4 | 99.0 | 99.8 | 99.2 | 100.0 | 99.1 | 95.2 | 100.0 | 99.3 | 98.1 | 99.8 |
| *Proteus* | 96.6 | 83.5 | 99.8 | 99.7 | 99.0 | 100.0 | 94.7 | 82.5 | 99.7 | 99.8 | 99.0 | 100.0 |
| *Staphylococcus* | 92.6 | 85.2 | 97.1 | 99.8 | 99.1 | 100.0 | 99.2 | 96.1 | 100.0 | 98.2 | 96.3 | 99.3 |
| *Serratia* | 85.1 | 64.5 | 96.3 | 99.9 | 99.3 | 100.0 | 95.5 | 78.0 | 99.8 | 99.5 | 98.7 | 99.9 |

|  |
| --- |
| **FILMARRAY** |
|  | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **Target Genus** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *Acinetobacter* | 83.2 | 53.5 | 98.4 | 99.9 | 99.2 | 100.0 | 91.4 | 61.8 | 99.7 | 99.7 | 99.0 | 100.0 |
| *Escherichia* | 99.2 | 95.9 | 100.0 | 97.8 | 96.0 | 99.0 | 90.9 | 83.7 | 95.9 | 99.8 | 99.1 | 100.0 |
| *Enterobacter* | 95.1 | 84.6 | 99.4 | 96.8 | 94.6 | 98.7 | 68.5 | 51.9 | 86.8 | 99.6 | 98.8 | 100.0 |
| *Haemophilus* | 96.8 | 89.8 | 99.5 | 95.5 | 92.9 | 97.6 | 75.8 | 63.2 | 86.7 | 99.5 | 98.5 | 99.9 |
| *Klebsiella* | 96.4 | 90.0 | 99.7 | 97.8 | 95.9 | 99.1 | 88.9 | 79.7 | 95.8 | 99.3 | 98.0 | 99.9 |
| *Moraxella* | 96.1 | 81.2 | 99.9 | 99.1 | 97.9 | 99.8 | 77.7 | 54.3 | 95.8 | 99.9 | 99.3 | 100.0 |
| *Pseudomonas* | 98.5 | 93.4 | 99.9 | 99.0 | 97.8 | 99.7 | 94.4 | 88.5 | 98.2 | 99.7 | 98.8 | 100.0 |
| *Proteus* | 96.9 | 84.9 | 99.9 | 99.3 | 98.3 | 99.9 | 88.9 | 74.1 | 97.8 | 99.8 | 99.1 | 100.0 |
| *Staphylococcus* | 99.3 | 96.4 | 100.0 | 95.6 | 93.2 | 97.5 | 84.5 | 76.4 | 91.0 | 99.8 | 99.1 | 100.0 |
| *Serratia* | 96.0 | 80.7 | 99.8 | 99.7 | 98.9 | 100.0 | 91.1 | 72.2 | 99.1 | 99.9 | 99.3 | 100.0 |

|  |
| --- |
| **16S rRNA Analysis** |
|  | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| **Target Genus** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *Acinetobacter* | 83.2 | 55.1 | 97.6 | 97.8 | 96.2 | 98.8 | 38.9 | 19.4 | 62.3 | 99.7 | 99.0 | 100.0 |
| *Escherichia* | 73.3 | 64.0 | 81.8 | 99.2 | 97.9 | 99.8 | 94.9 | 88.2 | 98.5 | 94.5 | 92.1 | 96.4 |
| *Enterobacter* | 21.1 | 15.3 | 34.9 | 86.5 | 83.2 | 89.4 | 10.6 | 6.1 | 18.6 | 93.7 | 90.8 | 95.9 |
| *Haemophilus* | 85.2 | 74.8 | 93.0 | 88.0 | 84.6 | 90.8 | 50.5 | 40.5 | 60.6 | 97.6 | 95.6 | 99.0 |
| *Klebsiella* | 66.8 | 56.0 | 77.2 | 95.7 | 93.5 | 97.4 | 74.0 | 63.5 | 83.6 | 94.0 | 91.4 | 96.1 |
| *Moraxella* | 58.0 | 34.5 | 78.4 | 99.9 | 99.2 | 100.0 | 93.1 | 69.7 | 99.7 | 98.6 | 97.3 | 99.4 |
| *Pseudomonas* | 89.0 | 81.0 | 94.5 | 94.7 | 92.3 | 96.5 | 74.5 | 65.1 | 82.5 | 98.0 | 96.4 | 99.0 |
| *Proteus* | 54.6 | 36.1 | 71.7 | 99.9 | 99.2 | 100.0 | 95.5 | 77.4 | 99.8 | 97.6 | 96.0 | 98.7 |
| *Staphylococcus* | 82.8 | 74.3 | 89.2 | 77.9 | 73.8 | 81.8 | 47.4 | 40.2 | 55.0 | 95.0 | 92.1 | 96.9 |
| *Serratia* | 90.6 | 72.7 | 98.6 | 87.8 | 84.8 | 90.5 | 19.8 | 12.1 | 29.8 | 99.7 | 98.8 | 100.0 |

## **Table S10**. Pathogen-specific performance of PCR tests compared with routine microbiology according to whether the sample was taken before or after antibiotic administration.

|  |
| --- |
| **UNYVERO** |
|  | **Before Antibiotics (n = 329)** | **After Antibiotics (n = 277)** |
| **Target organism** | **Sensitivity** | **Specificity** | **Sensitivity**  | **Specificity** |
| **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 100.0 | 89.1 - 100.0 | 93.3 | 89.8 - 95.8 | 90.6 | 75.0 - 98.0  | 94.7 | 91.1 - 97.1 |
| *S. aureus* | 91.5 | 79.6 - 97. 6 | 93.3 | 89.7 - 95.9 | 80.6 | 62.5 - 92.5  | 93.1 | 89.2 - 95.1 |
| *K. pneumoniae* | 83.3 | 51.6 - 97.9 | 94.6 | 91.6 - 96.8 | 83.3 | 51.6 - 97.9 | 93.6 | 89.9 -96.2 |
| *K. oxytoca* | 100.0 | 59.0 - 100.0 | 93.5 | 90.2 - 95.9 | 75.0 | 19.4 - 99.4 | 96.7 | 93.8 - 98.5 |
| *E. coli* | 81.0 | 58.1 - 94.6  | 89.9 | 86.0 - 93.1 | 95.0 | 75.1 - 99.9 | 88.7 | 84.2 - 92.3 |
| *E. cloacae* | 100.0 | 54.1 - 100.0 | 97.2 | 94.8 - 98.7 | 100.0 | 54.1 - 100.0 | 97.8 | 95.2 - 99.2 |
| *K. aerogenes* | 50.0 | 11.8 - 88.2 | 99.4 | 97.8 - 99.9 | ND | ND | 99.6 | 98.0 - 100.0 |
| *A. baumannii* | 100.0 | 15.8 - 100.0 | 98.8 | 96.9 - 99.7 | 100.0 | 29.2 - 100.0 | 99.3 | 97.4 - 99.9 |
| *H. influenzae* | 100.0 | 71.5 - 100.0 | 93.1 | 89.7 -95.6 | 100.0 | 69.2 -100.0 | 94.4 | 90.9 - 96.8 |
| *S. pneumoniae* | 100.0 | 47.8 - 100.0 | 97.8 | 95.6 - 99.1 | 100.0 | 2.5 - 100.0 | 96.7 | 93.9 - 98.5 |
| *M. catarrhalis* | 100.0 | 29.2 - 100.0 | 98.8 | 96.9 - 99.7 | 100.0 | 2.5 - 100.0 | 97.5 | 94.8 - 99.0 |
| *S. marcescens* | 85.7 | 42.1 - 99.6 | 98.4 | 96.4 - 99.5 | 50.0 | 1.3 - 98.7 | 98.2 | 95.8 - 99.4 |
| *C. freundii* | 100.0 | 2.5 - 100.0 | 97.9 | 95.7 - 99.1 | ND | ND | 99.6 | 98.0 - 100.0 |
| *M. morganii* | 100.0 | 2.5 - 100.0 | 98.5 | 96.5 - 99.5 | ND | ND | 98.2 | 95.8 - 99.4 |
| *S. maltophila* | 100.0 | 71.5 -100.0 | 93.1 | 89.7 - 95.6 | 66.7 | 9.4 - 99.2  | 96.0 | 92.9 - 98.0 |

**ND - no detections**

|  |
| --- |
| **FILMARRAY** |
|  | **Before Antibiotics (n = 337)** | **After Antibiotics (n = 283)** |
| **Target organism** | **Sensitivity** | **Specificity** | **Sensitivity**  | **Specificity** |
| **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 100.0 | 89.1 - 100.0 | 92.5 | 88.9 - 95.2 | 97.1 | 84.7 - 99.9  | 94.0 | 90.3 - 96.6 |
| *S. aureus* | 100.0 | 92.6 -100.0 | 88.6 | 84,3 - 92.0 | 90.6 | 75.0 - 98.0  | 89.2 | 84.7 - 92.8 |
| *K. pneumoniae* | 84.6 | 54.6 - 98.1 | 92.3 | 88.8 - 94.9 | 100.0 | 73.5 - 100.0 | 90.4 | 86.3 - 93.6 |
| *K. oxytoca* | 100.0 | 59.0 - 100.0 | 93.6 | 90.4 - 96.0 | 100.0 | 39.8 - 100.0 | 97.1 | 94.4 - 98.8 |
| *E. coli* | 95.5 | 77.2 - 99.9 | 88.6 | 84.5 - 91.9 | 100.0 | 83.2 - 100.0 | 86.3 | 81.6 - 90.2 |
| *E. cloacae* | 83.3 | 35.9 - 99.6 | 92.7 | 89.4 - 95.3 | 100.0 | 54.1 - 100.0 | 94.2 | 90.8 - 96.7 |
| *K. aerogenes* | 100.0 | 54.1 - 100.0 | 98.8 | 96.9 - 99.7 | ND | ND | 99.6 | 98.0 - 100.0 |
| *A. baumannii* | 100.0 | 29.2 - 100.0 | 99.4 | 97.9 - 99.9 | 100.0 | 29.2 - 100.0 | 99.6 | 98.0 - 100.0 |
| *H. influenzae* | 100.0 | 71.5 - 100.0 | 88.0 | 84.0 - 99.4 | 90.0 | 55.5 - 99.7 | 88.3 | 83.9 - 91.8 |
| *S. pneumoniae* | 100.0 | 47.8 - 100.0 | 96.4 | 93.8 - 98.1 | 100.0 | 2.5 - 100.0 | 92.2 | 88.4 - 95.0 |
| *M. catarrhalis* | 100.0 | 29.2 - 100.0 | 97.9 | 95.7 - 99.2 | 100.0 | 2.5 - 100.0 | 95.7 | 92.7 - 97.8 |
| *S. marcescens* | 100.0 | 59.0 - 100.0 | 98.5 | 96.5 - 99.5 | 100.0 | 15.8 - 100.0 | 97.9 | 95.4 - 99.2 |
| *S. agalactiae* | ND | ND | 97.3 | 95.0 - 98.8 | ND | ND | 95.4 | 92.3 - 97.5 |
| *S. pyogenes* | ND | ND | 98.2 | 96.2 - 99.3 | 100.0 | 15.8 - 100.0 | 99.6 | 98.0 - 100.0 |

ND - no detections

All samples had to be taken within 12h of antibiotic adminstration

## **Table S11.** Pathogen-specific performance of PCR tests compared with routine microbiology in relation to whether samples were fresh or had been frozen prior to PCR testing

|  |
| --- |
| **UNYVERO** |
|  | **Fresh Samples (n = 456)** | **Frozen Samples (n = 150)** |
| **Target organism** | **Sensitivity** | **Specificity** | **Sensitivity**  | **Specificity** |
| **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 98.0 | 89.6 - 100.0 | 93.8 | 91.0 - 96.0 | 84.6 | 54.6 - 98.1  | 94.2 | 88.8 - 97.4 |
| *S. aureus* | 84.0 | 70.9 - 92.8  | 92.6 | 89.6 - 95.0 | 92.9 | 76.5 - 99.1 | 95.1 | 89.6 - 98.2 |
| *K. pneumoniae* | 77.8 | 52.4 - 93.6 | 94.3 | 91.7 - 96.3 | 100.0 | 54.1 - 100.0 | 93.8 | 88.5 - 97.1 |
| *K. oxytoca* | 88.9 | 51.8 - 99.7 | 94.9 | 92.4 - 96.7 | 100.0 | 15.8 100.0 | 95.3 | 90.5 - 98.1 |
| *E. coli* | 87.9 | 71.8 - 96.6 | 90.1 | 86.8 - 92.7 | 87.5 | 47.3 - 99.7 | 87.3 | 80.7 - 92.3 |
| *E. cloacae* | 100.0 | 54.1 - 100.0 | 96.9 | 94.8 - 98.3 | 100.0 | 54.1 - 100.0 | 99.3 | 96.2 - 100.0 |
| *K. aerogenes* | 25.0 | 0.6 - 80.6 | 99.3 | 98.1 - 99.9 | 100.0 | 15.8 - 100.0 | 100.0 | 97.5 - 100.0 |
| *A. baumannii* | 100.0 | 47.8 - 100.0 | 98.9 | 97.4 - 99.6 | ND | ND | 99.3 | 96.3 - 100.0 |
| *H. influenzae* | 100.0 | 75.3 - 100.0 | 95.0 | 92.6 - 96.9 | 100.0 | 63.1 -100.0 | 89.4 | 83.2 - 94.0 |
| *S. pneumoniae* | 100.0 | 2.5 - 100.0 | 97.4 | 95.4 - 98.6 | 100.0 | 47.8 - 100.0 | 97.2 | 93.1 - 99.2 |
| *M. catarrhalis* | 100.0 | 39.8 - 100.0 | 98.2 | 96.5- 99.2 | ND | ND | 98.0 | 94.3 - 99.6 |
| *S. marcescens* | 83.3 | 35.9 - 99.6 | 98.2 | 96.5 - 99.2 | 66.7 | 9.4 - 99.2 | 98.6 | 95.2 - 99.8 |
| *C. freundii* | 100.0 | 2.5 - 100.0 | 98.7 | 97.2 - 99.5 | ND | ND | 98.7 | 95.3 - 99.8 |
| *M. morganii* | 100.0 | 2.5 - 100.0 | 98.0 | 96.3 - 99.1 | ND | ND | 99.3 | 96.3 - 100.0 |
| *S. maltophila* | 100.0 | 69.2 -100.0 | 95.3 | 92.9 - 97.1 | 75.0 | 19.4 - 99.4  | 91.8 | 86.1 - 95.7 |

|  |
| --- |
| **FILMARRAY** |
|  | **Fresh Samples (n = 476)** | **Frozen Samples (n = 144)** |
| **Target organism** | **Sensitivity** | **Specificity** | **Sensitivity**  | **Specificity** |
| **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** | **%** | **95% CI** |
| *P. aeruginosa* | 98.1 | 89.9 - 100.0 | 92.9 | 90.0 - 95.2 | 100.0 | 75.3 - 100.0  | 93.9 | 88.3 - 97.3 |
| *S. aureus* | 94.4 | 84.6 - 98.8 | 87.4 | 83.9 - 90.4 | 100.0 | 86.8 - 100.0  | 94.1 | 88.2 - 97.6 |
| *K. pneumoniae* | 89.5 | 66.9 - 98.7 | 91.9 | 89.0 - 84.2 | 100.0 | 54.1 - 100.0 | 89.9 | 83.6 - 94.3 |
| *K. oxytoca* | 100.0 | 66.4 - 100.0 | 94.6 | 92.2 - 96.5 | 100.0 | 15.8 - 100.0 | 97.2 | 92.9 - 99.2 |
| *E. coli* | 97.0 | 94.2 - 99.9 | 88.0 | 84.6 - 90.9 | 100.0 | 66.4 - 100.0 | 85.9 | 78.9 - 91.3 |
| *E. cloacae* | 100.0 | 54.1 - 100.0 | 92.6 | 89.8 - 94.8 | 83.3 | 35.9 - 99.6 | 96.4 | 91.7 - 98.8 |
| *K. aerogenes* | 100.0 | 39.8 - 100.0 | 98.9 | 97.5 - 99.7 | 100.0 | 15.8 - 100.0 | 100.0 | 97.4 - 100.0 |
| *A. baumannii* | 100.0 | 54.1 - 100.0 | 99.6 | 98.5 - 99.9 | ND | ND | 99.3 | 96.2 - 100.0 |
| *H. influenzae* | 92.9 | 66.1 - 99.8 | 89.6 | 86.5 - 92.2 | 100.0 | 59.0 - 100.0 | 83.2 | 75.9 - 89.0 |
| *S. pneumoniae* | 100.0 | 2.5 - 100.0 | 95.2 | 92.8 - 96.9 | 100.0 | 47.8 - 100.0 | 92.1 | 86.3 - 96.0 |
| *M. catarrhalis* | 100.0 | 39.8 - 100.0 | 96.6 | 94.6 - 98.1 | ND | ND | 97.9 | 94.0 - 99.6 |
| *S. marcescens* | 100.0 | 54.1 - 100.0 | 98.1 | 96.4 - 99.1 | 100.0 | 29.2 - 100.0 | 98.6 | 95.0 - 99.8 |
| *S. agalactiae* | ND | ND | 96.6 | 94.4 - 98.1 | ND | ND | 95.8 | 91.2 - 98.5 |
| *S. pyogenes* | 100.0 | 2.5 - 100.0 | 99.6 | 98.5 - 99.9 | 100.0 | 2.5 - 100.0 | 96.5 | 92.0 - 98.9 |

## **Table S12.** Antimicrobial resistance (%) to selected agents as determined by routine microbiology

|  |  |  |
| --- | --- | --- |
|  | **Antimicrobial** |  |
| **Organism** | **n a** | **AMC** | **3GC** | **CIP** | **MAC** | **METH** | **GEN** | **MEM** | **TZP** | **GLYC** | **MDR** |
| *P. aeruginosa & Pseudomonas* spp*.* | 79 | - | 21.6b | 17.3 | - | - | 10.8 | 23.6 | 24.3 | - | 20.3 |
| *S. aureus* | 83 | - | - | 18.9 | 25.0 | 14.6 | 10.0 | - | - | 0.0 | 13.3 |
| *E. coli* | 43 | 47.5 | 21.4 | 25.6 | - | - | 10.3 | 0.0 | 16.2 | - | 37.1 |
| *H. influenzae* | 23 | 22.2 | - | 0.0 | 41.7 | - | - | - | - | - | 13.0 |
| *Klebsiella* spp. | 44 | 30.8 | 20.7 | 10.5 | - | - | 7.7 | 3.3 | 13.5 | - | 15.9 |

* : Drug inherently inactive against species group

Antimicrobial abbreviations: AMC – amoxicillin/clavulanate, 3GC, third-generation cephalosporin (meaning ceftazidime, cefotaxime or ceftriaxone, as tested), CIP – ciprofloxacin, MAC – macrolide (erythromycin and clarithromycin depending on local laboratory), METH – detection of methicillin resistance (agent tested may be cefoxitin, flucloxacillin and oxacillin, depending on local laboratory)~~,~~ GEN – gentamicin, MEM – meropenem, TZP- piperacillin-tazobactam, GLYC, glycopeptide (vancomycin and teicoplanin depending on local laboratory)- MDR – multi-drug resistant, defined as resistant to ≥ 3 classes according rules described in Magiorakos et al.8

a Refers to the total number of isolates in the data set. The number tested for any given drug may be fewer.

b Ceftazidime only considered for these species.

## **Table S13.** Frequency of resistance gene detections by PCR tests among eligible samples (n = 606 for Unyvero, n = 620 for FilmArray)

|  |  |  |
| --- | --- | --- |
| Resistance Gene Target | Unyvero | FilmArray |
| **Carbapenemase**s |  |  |
| *bla*IMP | 0 | 1 |
| *bla*KPC | 1 | 1 |
| *bla*OXA-23 | 5 | NA |
| *bla*OXA24/40 | 0 | NA |
| *bla*OXA-48 | 0 | 0 |
| *bla*OXA-58 | 0 | NA |
| *bla*NDM | 2 | 0 |
| *bla*VIM | 3 | 1 |
| **Other genes relevant to resistance to b-lactams** |  |  |
| *bla*CTX-M | 14 | 32 |
| *bla*SHV | 55 | NA |
| *bla*TEM | 108 | NA |
| *mecA* | 92 | NA |
| *mecC* | 3 | NA |
| *mecA/C* and MREJ | NA | 32 |
| **Miscellaneous** |  |  |
| *ermB* | 68 | NA |
| *E. coli gyrA83* | 29 | NA |
| *P. aeruginosa gyrA87* | 35 | NA |
| *sul1* | 67 | NA |

# **Supplementary References**

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