Rapid Isolation and Identification of Pneumonia-Associated Pathogens from Sputum Samples Combining an Innovative Sample Preparation Strategy and Array-Based Detection

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INTRODUCTION

According to the World Health Organization (WHO), pneumonia is the most common infectious disease worldwide. It is associated with very high morbidity and mortality, especially among patients with a compromised immune system or small children. In 2015, pneumonia killed 92,016 children under the age of five, accounting for 16% of all deaths of children under five years. Pneumonia can be caused by bacteria, viruses, or fungi. Most often, bacterial pneumonia is induced by an infection with Streptococcus pneumoniae. Currently, the gold standard for diagnosing this disease is the microbiological cultivation of the pathogens along with an X-ray examination of the lung and a blood cell count. Unfortunately, this approach is very time consuming and not all pathogens can be successfully cultivated. Accordingly, for therapy, a broad-spectrum antibiotic is frequently administered before the result of the cultivation is even known. This unspecific and random use of antibiotics is highly problematic regarding the increase in drug-resistant bacteria. A delayed initiation of the appropriate therapy, however, is also not ideal as an effective treatment of pneumonia requires early intervention. At present, only one-third of children with bacterial pneumonia receive the right antibiotics. This illustrates that there is an urgent unmet medical need for fast and highly specific assays for diagnosing pneumonia.

In principal, this need already has been recognized, and efforts were made to develop alternatives to a culture-based diagnosis. Generally, assays based on the polymerase chain reaction (PCR) have a great potential for detecting pathogens causing pneumonia because of the speed and high specificity of this method. Kim et al. developed a multiplex PCR assay capable of differentiating between virulent S. pneumoniae and ubiquitous normal flora, Streptococcus mitis, and Streptococcus oralis in...
respiratory samples. Li et al. combined real-time quantitative PCR (qPCR) with an immunoassay for improving the detection of *Mycoplasma pneumonia* infections in children with pneumonia. Furthermore, a real-time PCR assay targeting eight different bacteria and six viruses relevant for respiratory tract diseases was introduced by Edin et al. Recently, Gadsby et al. established two real-time multiplex PCR assays enabling the quantification of eight respiratory pathogens. While qPCR provides both high specificity and sensitivity even with the possibility for quantitation, major obstacles preventing its use in routine clinical diagnostics are the high costs as well as the rather complicated associated experimental procedures. Regarding point-of-care applications, isothermal amplification has been proven to be less demanding in terms of equipment and financial burden. For example, Gotoh et al. successfully applied loop-mediated isothermal amplification (LAMP) for detecting *Mycoplasma pneumoniae*. Huang et al. integrated a LAMP-based detection scheme into a microfluidic system, allowing a parallel and automated nucleic acid analysis of 24 samples. Moreover, Renner et al. designed a user-friendly cartridge, in which isothermal amplification enables detecting various clinically relevant bacterial species. To the best of our knowledge, the BIOFIRE FILMARRAY (Pneumonia Panel) currently offers the widest range of respiratory pathogen identification based on multiplex PCR, including a fully automated sample preparation. Results will be available within only 1 h. Next to PCR-based approaches, matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF MS) can be used to identify bacterial species from blood cultures within 1 h. Even though MALDI-TOF MS is an attractive tool for the identification of bacteria, within the context of pneumonia diagnosis, it is a distinct disadvantage, that a precultivation step is necessary in order to generate sufficient amounts of biomass for the analysis. Alternatively, immunoassays are a further option for the specific detection of pneumonia pathogens. Li et al. developed an immuno-chromatographic assay, which allows a rapid and sensitive detection of *M. pneumoniae* from sputum samples or throat swabs. In practice, urinary antigen tests, such as ImmuView UAT and BinaxNOW, are frequently applied for detecting *S. pneumoniae* and *Legionella pneumophila* in urine samples. Furthermore, Wang et al. recently reported an electrochemical immunosensor to detect the pneumococcal surface protein A, enabling ultrasensitive detection of *S. pneumoniae*. While it is encouraging that various promising attempts for a fast diagnosis of bacterial pneumonia already have been made, some challenges still remain. For instance, many approaches only encompass the amplification and detection steps, omitting the sample preprocessing which is necessary for the application of real samples. Others only address single species. The aim of the present study is to cover the entire process chain, starting with an efficient isolation and enrichment of the bacteria from patients’ sputum samples up to the specific detection of eight pneumonia-relevant pathogens in parallel (Figure 1). With regard to the potential application in point-of-care testing, we made special efforts to incorporate cost-efficient materials and to keep the assay as simple and convenient as possible. For sample preparation, we employed expanded glass beads (EGB), which are an intermediate product occurring during the recycling process of glass. Our assay begins with the liquefaction of the sputum samples, followed by the isolation of the bacteria from the complex sample matrix using EGB. These feature an amine

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Scheme illustrating the different steps of the PCR-based pneumonia assay. (A) First, the pathogens are isolated from the sputum matrix using amine-functionalized particles. (B) After a lysis step, the target DNA is amplified via PCR. (C) Biotinylated PCR products are subsequently analyzed using array-based hybridization. The result is visualized by employing enzyme-induced silver deposition. (D) Finally, the gray values of the silver spots are measured, enabling the identification of the pathogens according to the capture probe positions.
modification, that allows a pH-dependent adhesion and desorption of cells. After a lysis step, the target DNA is exponentially amplified and labeled using specific primers. Finally, the PCR products are further analyzed using array-based hybridization and enzyme-induced silver deposition. The generated silver nanoparticles in case of a positive reaction served as robust endpoint signals allowing an immediate visual readout by the naked eye.

## RESULTS AND DISCUSSION

The main objective of this study was the development of a PCR-based assay enabling the fast and reliable detection of bacterial pneumonia from sputum samples. We addressed the whole process chain and considered sample preparation as well as a user-friendly detection scheme. Prior to the amplification of the target DNA, we utilized a bead-based approach for the isolation of the bacterial cells from the sputum matrix. As it is our goal to address multiple species, we chose amine-modified particles for the sample preparation. Based on electrostatic interactions between the bacterial cell wall and the bead surface, this type of surface modification allows directed binding or detachment of the bacteria depending on the pH value of the surrounding buffer. The particle surface will exhibit a positive charge because of protonated amine groups in the acidic to neutral pH range causing an attractive force for the bacteria, which generally have a negative charge on their surface. By raising the pH to the alkaline region, the amine groups will be deprotonated. Accordingly, the bacterial cells are no longer drawn to the particle surface and can be detached. This principle is highly interesting for assays, which aim at isolating several pathogens, because various bacterial species can be captured with only one type of surface modification.\(^{20,21}\) The expensive and often troublesome surface modification of particles with antibodies can be avoided.

### Array-Based Detection of Eight Bacterial Species Relevant for Respiratory Diseases

We tested two types of amine-functionalized particles, namely, EGB and PEI-modified PE particles, for the isolation of pneumonia-relevant bacteria from sputum samples. In order to optimize the protocol for the particle-based isolation of the pneumonia pathogens from sputum samples, we used an artificial sputum matrix spiked with defined cell numbers of eight different species (Acinetobacter baumannii, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphylococcus aureus, and S. pneumoniae) to a final concentration of approximately 10^5 cfu/mL. Each sample was divided into two equal volumes and the isolation was performed with both types of particles. After that, a heat lysis of the cells was performed, followed by the amplification of the target DNA. In order to verify each single step of the process chain, successful amplification of all pathogens was as well examined on agarose gels (exemplarily shown in Figure S1). Using the biotinylated primers (see Figure 1B) resulted in biotin-labeled PCR products. These were finally hybridized on polymer chips modified with specific capture probes for the pathogens of interest. By incubating the chips with a streptavidin enzyme conjugate, the successful hybridization can be visualized exploiting the enzyme-catalyzed reduction of silver ions: the streptavidin enzyme conjugate will bind to biotinylated-hybridized PCR products and induce the reduction of the silver ions to elementary silver resulting in the formation of silver nanoparticles (see Figure 1C). The silver nanoparticles are visible to the naked eye as black or gray spots on the chip surface and can be further investigated using gray value analysis (see Figure 1D).

The results of the corresponding analysis for the samples processed either with EGB or polymer particles are summarized in Figure 2. For clarity, we only displayed the gray values of the positions of the correct capture probes for each species. Both sample preparation methods enabled the PCR-based detection of all eight investigated species. For each sample, the results of the array-based hybridization were specific, meaning no false positive signals were obtained at the positions of the noncomplementary probes. The respective diagrams can be found in the Supporting Information (Figure S2 for EGB and Figure S3 for polymer particles). Generally, we found that the signal intensities were slightly higher for the EGB, with the exception of the samples with H. influenzae and S. aureus. With these results, we were able to demonstrate that our introduced assay enables detecting each of the eight investigated respiratory species from sputum samples. The time required for completing the whole process chain only accounts for 4 h. As the handling of the glass beads with the highly viscous sputum matrix turned out to be even more convenient than the use of the polymer beads, we decided to employ the glass beads for all future experiments.

### Detection of Patient Isolates

In order to investigate the applicability of the abovementioned assay, the optimized protocol was further tested for several strains of S. pneumoniae, S. aureus, and H. influenzae isolated from patients of the University Hospital Jena. For this purpose, artificial sputum was spiked with ten different strains to a final concentration of approximately 10^5 cfu/mL. Subsequently, the complete protocol starting from the isolation of the pathogens with EGB was conducted. The results of the gray value analysis from the polymer chips after performing the hybridization assay are depicted in Figure 3. Because no unspecific signals at the noncomplementary probe positions were detected, we only included the values of the correct species-specific probes for better comprehensibility. The detailed evaluation of each chip

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Figure 2. Comparison of the gray values from 16 individual chips measured at the positions of the species-specific capture probes for eight pneumonia-relevant pathogens for EGB and PEI-modified particles (PEI).
can be found in the Supporting Information (see Figure S4). Overall, the results are in good agreement with the previously investigated strains. We were able to unambiguously detect all of the ten patient strains. For the two isolates of *H. influenzae*, the intensities of the gray values were even more pronounced than those of the ATCC 9007 strain. The same trend was observed for the three *S. pneumoniae* isolates, the gray values of which all exceeded 80%, while the type strain DSM 20566 typically yielded intensities of approximately 55%. For the five isolates of *S. aureus*, we obtained signal intensities between 40 and 80%, while the type strain DSM 20231 displayed a mean intensity of 55% in comparable experiments. These results indicate that the established protocol is capable of covering several different pneumonia-relevant bacterial species with only one set of parameters for the isolation protocol. Even though the signal intensities vary, depending from the species and also from the strain, a distinct identification is enabled in each case. One factor impacting the gray values of the chip-based hybridization protocol is the isolation yield in the sample preparation. Depending on the composition of the bacterial cell surface and the presence of functional groups contributing to the surface charge of the cells, the attractive interaction between the particles and the cells will be influenced. The constitution of the cell surface of course is affected by the species (Gram-positive, Gram-negative bacteria), the strain, and even the growth conditions. Accordingly, it would be possible to optimize the isolation protocol for each species or strain. However, we refrained from this proceeding, as it was our goal to access multiple species with one protocol. Based on the presented results, it can be concluded that the introduced approach shows great promise to be robust enough for coping with the cell surface variations that occur within one species from strain to strain.

**Determination of the Limit of Detection.** A critical parameter for the application of an assay in a clinical setting is the sensitivity. Because *S. pneumoniae* is the most common causative agent of pneumonia, we performed the corresponding experiments with the type strain of *S. pneumoniae*. Briefly, samples with artificial sputum in the concentration range between $10^2$ and $10^5$ cfu/mL were prepared and the full process chain was completed. Down to a concentration of $10^3$ cfu/mL, we were able to detect the type strain of *S. pneumoniae* as can be referred from Figure 4. For the sample of $10^3$ cfu/mL, we did not obtain a significant gray value in the hybridization assay. In contrast, the analytical gel showed distinct bands only for $10^3$ and $10^5$ cfu/mL (see Figure S5). Once again, the results of the array-based hybridization were specific and no signals were detected at the noncomplementary probe positions (data not shown). The sensitivity of our assay is sufficient for investigating sputum samples as typical bacterial loads range from $10^3$ to $10^7$ cfu/mL.22 Comparable results were reported by Gillespie et al. They also targeted the autolysine gene *lytA* of *S. pneumoniae* and were able to detect the pathogen down to a concentration of $10^4$ cfu/mL in a simulated sputum matrix using conventional PCR.23 The sensitivity can be further improved by employing qPCR. Werno et al. achieved reproducible and quantifiable results to a level of $10^2$ cfu/mL also addressing the *lytA* gene.22 Ikegame et al. investigated clinical sputum specimens for evaluating the performance of an antigen kit for the C-polysaccharide of the bacterial cell wall of *S. pneumoniae*. They found that $10^5$ cfu/mL was required for a positive test result.24 Overall, the sensitivity of our assay is comparable or superior to other PCR-based detection schemes for *S. pneumoniae* from complex samples.

**Investigation of Real Sputum Samples.** Finally, we challenged our assay by processing actual patient samples. From the University Hospital in Jena, we received three leftover samples, which had been previously characterized via routine microbiological methods. In each of the samples, only the standard microflora was detected. We spiked each sample with the type strain of *S. pneumoniae* to a concentration of $10^6$ cfu/mL and performed the whole process chain from isolation of the bacterial cells to the array-based hybridization. In general, we were able to detect *S. pneumoniae* in all samples as can be seen in Figure 5. For the samples R002 and R003, we obtained signal intensities over 80%. For the sample R001, however, only a comparably low intensity of 20% was achieved. The lower yield can probably be ascribed to difficulties during sample preprocessing. Sample R001 displayed a very high viscosity compared to samples R002 and R003, accordingly the mixing of the beads and sample was apparently less efficient than in the other samples. Nevertheless, the outcome of the experiment is encouraging and proves the general applicability of our assay for real samples.

**CONCLUSIONS**

We developed a fast and convenient PCR-based assay enabling the detection of eight different pneumonia-relevant pathogens within 4 h. Our approach covers the complete chain of analysis.
from sample preparation to array-based detection. We included robust and cost-efficient materials, wherever possible, that is, EGB, which are a byproduct from glass recycling. Our sample preparation strategy relies on amine-functionalized particles, which are capable of isolating a broad range of different Gram-negative and Gram-positive species, as demonstrated in this study. The species-specific identification was achieved with a hybridization assay on low-cost polymer chips. An additional advantage of these chips is the simple functionalization with capture probes, without the need of any additional surface modification. The detection method based on enzyme-catalyzed silver deposition is not only robust, but also entails great potential for an automated readout and analysis, that is via smartphone. We successfully detected eight species in an artificial sputum matrix and could also show that the previously optimized parameters work well for different patient strains. Regarding sensitivity, we found a detection limit of $10^3$ cfu/mL for the type strain of *S. pneumoniae*, which is sufficient for the vast majority of sputum samples. Furthermore, we were able to successfully analyze real sputum samples spiked with *S. pneumoniae*. Overall, our results make an important contribution toward the development of culture-independent, sensitive, and specific point-of-care assays for the reliable detection of pneumonia. Future work will be aimed at an automated readout of the hybridization assay and implementing the sample preparation into a cartridge for more user friendliness.

**EXPERIMENTAL SECTION**

**Ethics Considerations.** The study was approved by the ethics committee of the Jena University Hospital (Germany), accession number: 4672-01/16.

**Cultivation of Bacteria.** In this study, we investigated eight different bacterial species, namely, *A. baumannii*, *E. coli*, *H. influenzae*, *K. pneumoniae*, *M. catarrhalis*, *P. aeruginosa*, *S. aureus*, and *S. pneumoniae*. The strains were either purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ, Brunswick, Germany) or isolates from patients’ samples provided by the Jena University Hospital (UKJ, Jena, Germany). All species, except from *H. influenzae* which was raised on chocolate agar with Vitox (Oxoid Deutschland GmbH), were cultivated on Columbia sheep blood agar (Oxoid Deutschland GmbH). The spiking of the sputum samples was carried out with cells from the exponential growth phase. To that end, 5 mL of the liquid growth medium (further information, see Table 1) was inoculated with a small amount biomass from an agar plate and incubated overnight at the appropriate temperature. On the next day, 2−3 mL of the overnight culture was added to 50 mL fresh growth medium and incubated until the exponential phase was achieved. The progress was monitored by measuring the optical density at 600 nm ($OD_{600}$) using a biophotometer (Eppendorf AG, Hamburg, Germany). Bacterial cells were harvested when $OD_{600}$ attained 0.4−0.6. Then 1 mL of the bacterial solution was centrifuged at 10 000 rcf for 3 min. The supernatant was discarded and the remaining cell sediment was dissolved in 1 mL phosphate-buffered saline (PBS). Afterward, a serial dilution from 1:20 up to 1:200 000 was performed and aliquots of 50 μL were plated in order to determine the colony-forming units (cfu) per mL.

**Expanded Glass Beads and Polymer Beads.** The EGB were purchased from Liaver GmbH & Co KG in Ilmenau, Germany. The size of the particles ranges from 0.5 to 1.2 mm and they have a density of 0.9 g/cm$^3$. Their surface was modified with primary amine groups. The modification was a three-stage coating. First, it was coated with 3-(aminopropyl)triethoxysilane (Sigma-Aldrich, Munich, Germany) followed by lysine diisocyanate ethyl ester (LDI, Actu-All Chemicals, Oss, The Netherlands) and finally with a polyetheramine (JEFFAMI-
Table 1. List of Investigated Bacteria Strains and Their Cultivation Conditions

| species            | strain/isolate | growth conditions |
|--------------------|----------------|-------------------|
| A. baumannii       | DSM 50007     | 30 °C at 120 rpm, lysogeny broth |
| E. coli            | ATCC 25922    | 37 °C at 120 rpm, lysogeny broth |
| H. influenzae      | ATCC 9007     | 37 °C at 120 rpm, brain heart infusion supplemented with nicotinamide adenine dinucleotide (NAD) and hemin |
| K. pneumoniae      | DSM 30104     | 37 °C at 120 rpm, lysogeny broth |
| M. catarrhalis     | ATCC 43617    | 37 °C at 120 rpm, brain heart infusion |
| P. aeruginosa      | ATCC 27853    | 37 °C at 120 rpm, lysogeny broth |
| S. aureus          | DSM 20231     | 37 °C at 120 rpm, lysogeny broth |
| S. pneumoniae      | DSM 20566     | 37 °C at 50 rpm, CASO broth with 3 g/L yeast extract |

*a*Type strain. Patient strains provided by the Jena University Hospital, all media and reagents were purchased from Carl Roth, Karlsruhe, Germany.

Specifically have a similar GC content, length, and melting temperature. Afterward, all designed sequences were reblasted against all available target sequences to rule out false negative or cross-reactive binding events. Subsequently, probes were modified with an amino-C6 linker and all forward primers were biotin-labeled for the staining procedure after hybridization on the chip. The primers and probes were synthesized by Eurofins Genomics GmbH (Ebersberg, Germany). Detailed information about the used primers and probes (sequences, melting temperature, and amplicon size) as well as the target genes (accession numbers) are provided in Table 2.

**Asymmetric PCR.** In the present study, an asymmetric PCR was conducted to amplify a fragment within the target gene region of the bacteria species (Table 2). The primer ratio between biotinylated and nonbiotinylated primers was 0.2:0.05 μM as determined experimentally (data not shown). A single reaction mix contained 1 μL cell lysate, 1X PCR buffer, 2.5 mM MgCl₂, 0.4 mM dNTPs, 0.2 μM of each primer, and 0.1 U/μL Taq Polymerase (innuTaq DNA Polymerase Kit, Analytik Jena AG, Germany) in a final volume of 30 μL. All PCR reactions were carried out with the thermocycler FlexCycler (Analytik Jena AG, Jena, Germany). The PCR was performed with the following cycle profile: initial denaturation at 95 °C for 300 s, 45 cycles of denaturation at 94 °C for 30 s, and annealing/elongation at 58 °C for 30 s.

Successful DNA amplification was verified on a 2% (w/v) agarose gel. For visualization, the DNA was stained with GelRed (Biotium, Fremont, CA, USA) according to the recommendations of the manufacturer. The molecular weight marker “GeneRuler 100 bp DNA Ladder” as well as “pUC19 DNA/ MspI (HpAll) Marker, ready-to-use” were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA USA).

**Array-Based Hybridization and Signal Detection.** White planar polypropylene (PP) sheets (Modulor GmbH, Berlin, Germany) were cut into chip size (17 × 17 mm) by laser, and successively cleaned in an ultrasonic bath with acetone, ethanol, and water for 10 min each.

The capture probes (Eurofins MWG Operon, Ebersberg, Germany) were dissolved in 1× micro spotting solution (ArrayIt Corporation, Sunnyvale, USA) to a final concentration of 20 μM and spotted (Nanoplotter 2.1, GeSim, Grosserkmannsdorf, Germany) in an array format on the PP chips (layout see Figure S7 of Supporting Information). A biotin-labeled noncomplementary probe was immobilized as a process control to verify the binding of streptavidin enzyme and the subsequent silver deposition (=positive control). Another noncomplementary probe was spotted to exclude unspecific binding (=negative control).

The specific biomolecule interaction on the chip was realized as previously described by Schwenkbier et al. Briefly, 70 μL of hybridization solution (20 μL asymmetric PCR product in 3× SSC/0.5% SDS) was incubated on the PP chip surface for 1 h at 50 °C, followed by 30 min of incubation with the streptavidin–horseradish peroxidase (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany; 1:1000 diluted in 1× PBS with TWEEN 20, PBST). Afterward, the PP chips were washed with PBST and tap water. Finally, the enzymatic silver deposition was performed by applying the EnzMetTM HRP detection kit (Nanoprobes Inc., Yaphank, USA; components A–C). The generated silver nanoparticles in case of a positive reaction served as robust endpoint signals allowing an immediate visual readout by the naked eye. In addition, the amount of silver deposits was...
Table 2. Primers and Capture Probes

| species                  | primers and probes     | sequence 5’ → 3’ | Tm (°C) | modification | amplicon length (bp) | target gene | accession number |
|--------------------------|------------------------|------------------|--------|-------------|----------------------|-------------|-----------------|
| A. baumannii             | A. ba. for             | ACTTGAATCGGGTTCTCTGC | 57.3   |             | 111                  | basC        | CP000521        |
| A. ba. for               | A. ba. for             | GCCCAATCTGACCATAGGCC | 57.3   | 5’-biotin   |                      |             |                 |
| A. ba. basC             | A. ba. basC            | AW7TTAGAAGCTGTGCTCGTGT | 54.7   | 5’-NH2-C6   |                      |             |                 |
| E. coli                  | E. coli for            | CGTGTGAAATCGATGAGTC | 57.3   | 5’-biotin   | 113                  | gad         | AE014075        |
| E. coli                  | E. coli rev            | ACCTGGAACACAGTTTCCCTG | 57.3   |             |                      |             |                 |
| Eco_gad                  | Eco_gad                | TCAAGGCGAATACCCGAGGC | 61.4   | 3’-NH2-C6   |                      |             |                 |
| H. influenzae            | H. inf. for            | ATGGGAGGACATCATAGTA | 54.5   | 5’-biotin   | 102                  | fucK        | CP000671        |
| H. influenzae            | H. inf. rev            | ACCGATAGAGGAAATGTTTGT | 57.9   |             |                      |             |                 |
| Hin_fucK                 | Hin_fucK               | ATGGATGCAATATACCGGG | 50.4   | 3’-NH2-C6   |                      |             |                 |
| K. pneumonia             | M. cat. for            | CGTGTGCGAGTACCTATACCC | 60.3   |             |                      |             |                 |
| K. pneumonia             | M. cat. rev            | CATAGAATGTAGCTACAGGACG | 60.6   | 5’-biotin   |                      |             |                 |
| M. cat. rev              | M. cat. rev            | ACCGAGATCAACCCCAAGTGGTG | 62.4   | 5’-NH2-C6   |                      |             |                 |
| P. aeruginosa            | P. aer. for            | AGCGTAGTCCCTGCACAAG | 59.4   | 5’-biotin   | 84                   | ecfX        | DQ996558        |
| P. aer. rev              | P. aer. rev            | TTTCCACCATGCTCGAGGAG | 59.4   |             |                      |             |                 |
| Pae_ecf22               | Pae_ecf22             | TTCCATGCGCGCTGCCACCTCGA | 67.8   | 3’-NH2-C6   |                      |             |                 |
| S. aureus                | S. au. for             | AGCATCTAAAAGGATAGGA | 57.6   |             | 87                   | nuc         | from Pichon et al.27 and McDonald et al.28 |
| S. aureus                | S. au. rev             | CTGCAATTTTTTTGAGTTTTGAC | 56.6   | 5’-biotin   |                      |             |                 |
| S. aureus                | S. au. rev             | TGGCCCTGAAGCAATGCAATGCTATTACAAA | 65.3   | 5’-NH2-C6   |                      |             |                 |
| S. pneumonia             | S. pn. for             | ACAGGACTTGCACGAGTAGGCA | 58.4   |             | 75                   | lytA        | AE005672        |
| S. pneumonia             | S. pn. rev             | TCCTGCGGTTTTAATTCAGCCT | 55.5   | 5’-biotin   |                      |             |                 |
| Spn_lytA                 | Spn_lytA               | TGGCCGAAACCTGTAACAGGGAG | 64.6   | 5’-NH2-C6   |                      |             |                 |
| positive control         | positive control       | ACTGACTGACTGACTGACTGCGGCGG | 5’-NH2-C6 |             |                      |             |                 |
| negative control         | negative control       | ACTGACGTAGACTGAGCTGAGCTGGCGG | 5’-NH2-C6 |             |                      |             |                 |

“Primer by Gadsby et al. 2015, *W = adenine (A)/thymine (T).”

Quantified by their gray values. The respective spots were scanned (ProScan 7200, reflecta GmbH, Rottenburg, Germany) and analyzed using the software ImageJ (National Institutes of Health, USA). After inversion of the image, the gray value could potentially range from 0 (white) indicating a clear chip surface without any deposition of silver to 255 (black). The gray value was determined by mean gray value calculation (at least 10 spots with a size of 2 pixels each), subtracting the measured background values from the sample values. The gray mean value of the biotin-positive control was set at 100% and the gray value signals of the capture probes were presented as gray value percentage of the positive control. The mean gray values of the negative controls plus three times the standard deviation were used to set the threshold for a positive test result.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00904.

Spotting layout for the capture probes, analytical agarose gel of the eight investigated respiratory species, results of gray value analysis for samples preprocessed with EGB and PEI beads, results of gray value analysis for patient strain samples, analytical agarose gel of the amplification of S. pneumoniae in a concentration range between 10³ and 10⁶ cfu/mL, SEM images of EGB and PEI beads (PDF)

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. S.P. and L.L. contributed equally to this work.

**Notes**

The authors declare no competing financial interest.

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