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ARTICLE

# Facemask Analyses for the Non-Invasive Detection of Chronic and Acute *P. aeruginosa* Lung Infections Using Nanoparticle-based Immunoassays

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David Delgado-Cano,[1] Antonio Clemente,[1,2,3]* Cristina Adrover-Jaume,[1,2,#] Andreu Vaquer,[1,2,#] Meritxell López,[4,5,6,†] Rocío Martínez,[4,6,†] Isabel M Roig,[6,†] Amanda Iglesias,[4,5] Borja G Cosío,[4,5,6] and Roberto de la Rica.[1,3]

*Pseudomonas aeruginosa* (*P. aeruginosa*) is a pathogen that persistently colonizes the respiratory tract of patients with chronic lung diseases. The risk of the acquiring a chronic *P. aeruginosa* infection can be minimized by rapidly detecting the pathogen in the patient's airways and promptly administrating adequate antibiotics. However, the rapid detection of *P. aeruginosa* in the lungs involves the analysis of sputum, which is a highly complex matrix that is not always available. Here, we propose an alternative diagnosis based on analyzing breath aerosols. In this approach, nanoparticle immunosensors identify bacteria adhered to the polypropylene layer of a surgical facemask that was previously worn by the patient. A polypropylene processing protocol was optimized to ensure the efficient capture and analysis of the target pathogen. The proposed analytical platform has a theoretical limit of detection of $10^5$ CFU·mL$^{-1}$ in aerosolized mock samples, and a dynamic range between $10^5$ and $10^8$ CFU·mL$^{-1}$. When tested with facemasks worn by patients, the biosensors were able to detect chronic and acute *P. aeruginosa* lung infections, and to differentiate them from respiratory infections caused by other pathogens. The results shown here pave the way to diagnose *Pseudomonas* infections at the bedside, as well as to identify the progress from chronic to acute infection.

## Introduction

For people living with chronic lung conditions such as chronic obstructive pulmonary disease (COPD), bronchiectasis, or cystic fibrosis, poorly managed lower tract respiratory infections can result in an irreversible loss of pulmonary function.[1-4] The chronic lung damage that these patients experience also makes them more susceptible to acquire an infection caused by a superbug called *Pseudomonas aeruginosa* (*P. aeruginosa*).[5,6] If not promptly eradicated with adequate antibiotics this pathogen can attain a hypermutable mucoid phenotype that accelerates the acquisition of new mechanisms of antimicrobial resistance.[7] This results in a chronic bronchial infection that is difficult to completely eradicate and that requires antibiotic therapy for life.[8,9] Providing adequate antibiotics before bacteria mutate is the best strategy to avoid this unresolved medical issue. Yet, detecting *P. aeruginosa* or biomarkers for infections caused by this pathogen in the lungs is challenging because it requires analyzing a sputum sample,[10,11] and many patients are not able to produce it spontaneously.[12-14] Furthermore, bacteriological culture is the standard approach to identify bacteria in hospitals. This method requires around 3 days to yield an answer. Because of that, antibiotics are often prescribed empirically. Furthermore, the diagnosis is often based on a yes/no result (above/below a certain threshold value) because a quantitative sputum culture takes even longer, and because of this, antibiotics are not personalized according to bacterial load.[15,16] In this scenario, inadequate antibiotic coverage risks selecting the hypermutable mucoid strains, whereas abusing last-generation antibiotic exacerbates antimicrobial resistant issues in the long term.

In this article we report a method for detecting *P. aeruginosa* in the patient's airway in under 40 minutes that does not require analyzing a sputum sample. It consists of analyzing a facemask worn by the patient with a rapid diagnostic test (Figure 1). In this approach, the patient wears a surgical mask for 30 minutes. The polypropylene layer captures the aerosols produced by the patient, which may contain *P. aeruginosa* from the lower respiratory tract. After a quick blocking step that renders the mask hydrophilic, the presence of bacteria adhered to the mask

[1]*Multidisciplinary Sepsis Group, Hospital Universitario Son Espases, Health Research Institute of Balearic Islands (IdISBa), Palma de Mallorca, Spain*
[2]*Department of Chemistry, University of the Balearic Islands, Palma de Mallorca, Spain*
[3]*CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III Madrid, Spain*
[4]*Inflamación, reparación y cáncer en enfermedades respiratorias (i-RESPIRE), Hospital Universitario Son Espases, Health Research Institute of Balearic Islands (IdISBa), Palma de Mallorca, Spain*
[5]*CIBER de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain*
[6]*Department of Respiratory Medicine, Hospital Universitario Son Espases, Palma de Mallorca, Spain*
#Contributed equally; †Contributed equally
*Corresponding author: antonio.clemente@uib.cat,
Electronic Supplementary Information (ESI) available: Procedures for wetting and blocking polypropylene by low and high pressure (Figure S1); Bacterial etiology of the respiratory infections in the patients included in the study (Table S1). See DOI: 10.1039/x0xx00000x

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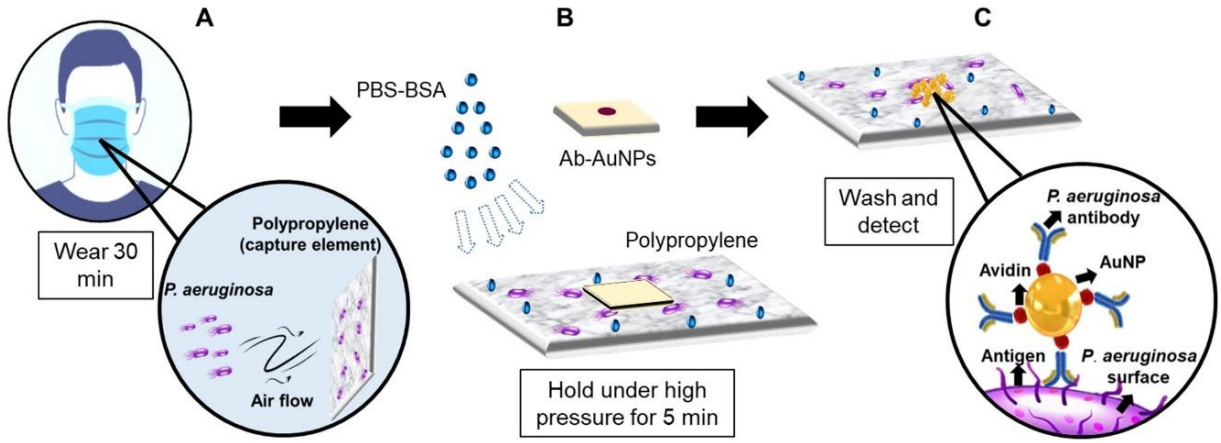


**Figure 1. Schematic representation of the nanoparticle-based immunoassay for detecting *P. aeruginosa* lung infections**. (A) Surgical facemasks are used for capturing airways bacteria from exhaled breath of patients with a *P. aeruginosa* respiratory infection. (B) A rapid blocking step by applying high pressure in the presence of PBS-BSA renders the polypropylene layer hydrophilic, which enables the efficient transfer of Ab-AuNPs from the paper reservoir to the facemask. (C) The transferred Ab-AuNPs interact with the *P. aeruginosa* cells captured by the surgical facemask, and after washing, a spot remains on the polypropylene layer whose color intensity depends on the bacterial load in the lungs of infected patients. Ab-AuNPs: antibody-decorated gold nanoparticles; PBS-BSA: phosphate buffered saline supplemented with bovine serum albumin.

is detected with a paper biosensor based on nanoparticle transfer in under 10 minutes. In this approach,antibody-decorated nanoparticles are transferred from a paper decal. After washing away excess nanoprobes, a colored spot remains whose pixel intensity correlates with the target concentration. While this approach has been successfully used to detect proteins (viral antigens, cytokines),[17,18] this is the first report of applying it for detecting bacterial cells. This brought new analytical challenges regarding facemask processing that have been successfully addressed here. Thanks to this, we have been able to detect *P. aeruginosa* with a limit of detection of $10^5$ CFU·mL$^{-1}$ in mock samples, and to successfully identify the pathogen in patients suffering an acute infection or whose lungs had been chronically colonized by the pathogen. Test results are dose dependent. This, along with the high specificity afforded by the use of antibodies as recognition elements, overcomes the qualitative detection of *Pseudomonas* with electronic noses[19-21] and paves the way to personalize antibiotics according to bacterial load.[15,16] The proposed technology makes it easy to screen patients for *Pseudomonas* infections, even when they cannot produce sputum spontaneously. In turn, this will allow clinicians to fine-tune antibiotics depending on the presence of this superbug, thus changing the current prescription paradigm from empirical to evidence-based.

## Experimental

### Materials

Gold (III) chloride hydrate, sodium citrate tribasic dihydrate, poly (ethylene glycol) 2-mercaptoethyl ether acetic acid (thiol-PEG-acid) 2100, N-Hydroxysulfosuccinimide sodium salt (sulfo-NHS), poly (sodium 4-styrenesulfonate, average Mw ≈ 70.000) 30 % solution, Tween-20, glycine, sodium phosphate dibasic dihydrate (98 %), Poly (ethylene glycol) 2-aminoethyl ether biotin (PEG-biotin), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and avidin from egg white were obtained from Sigma Aldrich. Whatman filter paper #1 was obtained from GE Healthcare Life Sciences. Rabbit *Pseudomonas* polyclonal biotin antibody was purchased from Invitrogen. Albumin from bovine serum (BSA, protease free) was obtained from VWR Chemicals. PBS refers to phosphate buffered saline pH 7.4. PBST refers to PBS supplemented with 0.1 % Tween-20. PBS-BSA refers to PBS supplemented with 5 mg·mL$^{-1}$ BSA.

### Antibody-decorated gold nanoparticles

A previously described citrate-reducing method was used to obtain 250 mL of gold nanoparticles (AuNPs) with a diameter of ca. 40 nm.[22] To obtain nanoprobes against *P. aeruginosa* the AuNPs were decorated with specific antibodies by using a covalent functionalization protocol to attach avidin to the colloids followed by the addition of biotinylated antibodies. In brief, the citrate around the AuNPs was substituted for thiol-PEG-acid and nanoparticles were then concentrated 250 times. Next, the pegylated AuNPs were functionalized with avidin by an acid-to-amine coupling protocol mediated by EDC and sulfo-NHS that has been previously described.[23] Then, the avidin-decorated AuNPs were capped and blocked by adding a BSA blocking solution (10 mg·mL$^{-1}$ BSA supplemented with 0.1 M glycine in 0.1 M phosphate buffer, pH 7) at a 1:1 volume ratio for 30 min. In this step, the unreacted sulfo-NHS esters react with the amine group of glycine and BSA. After a PBST wash, antibody-decorated AuNPs (Ab-AuNPs) were obtained by adding 10 μL of biotin polyclonal antibodies against *P. aeruginosa* developed in rabbit (at 5 mg·mL$^{-1}$) to 100 μL of avidin-decorated AuNPs for 1 h. Next, free biotin-binding sites were capped with 10 μL of 0.1 mM PEG-biotin for 30 min. Subsequently, excess reagents were washed away by 3 consecutive centrifugation steps at 7000 rpm for 6 min using PBST. Finally, the pellet was resuspended in 100 μL PBST and Ab-AuNPs were kept at 4 °C until use.


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**Fabrication of paper biosensors**

Paper biosensors were fabricated following our previously described polymer-infusing method.[18] Briefly, Whatman filter paper #1 was cut into 7 x 7 cm squares. Then, 3 mL of 18% PSS was added to a petri dish and the paper square was placed above the liquid. Finally, to obtain a polymer film, the petri dish containing the piece of paper was dried in an oven for 30 min at 37 $^{o}$C. The polymer-infused paper was cut into 0.5 x 0.5 cm squares and filled with 0.5 μL of Ab-AuNPs using a Hamilton syringe coupled to a microdrop dispenser.

**Surgical facemask processing**

Surgical facemask processing was studied in order to stablish a suitable method for rendering them hydrophilic while preserving the integrity of the analyte. 2 cm x 2 cm pieces of the polypropylene layer of the surgical masks were placed on top of three layers of 2.5 cm x 2.5 cm pieces of blotting filter paper that was previously soaked with 1.9 mL of PBS or PBS-BSA. The assembly was then pressed for 10 s to block them and make them hydrophilic. To investigate the role of pressure in this analytical step, we applied low pressure by lightly pressing with the finger or high pressure using a clamp tool (see supplementary Figure S1). Polypropylene pieces modified without applying any pressure were added as negative controls. Then we evaluated the hydrophobicity/hydrophilicity of the facemasks as well as their ability to receive antibody-decorated nanoparticles transferred from paper biosensors. To assess the hydrophilicity, 200 μL of MQ water was added and the contact angle was measured (i.e. the angle between the surface of the liquid and the outline of the contact surface). To assess the ability to transfer nanoparticles, the polypropylene pieces were placed on one layer of blotting paper that had been previously soaked with 200 μL of PBS and the nanoparticle biosensors were pressed against the assembly for 5 min under high pressure. Finally, the polypropylene pieces and the reservoirs were scanned with an MFC-1910W scanner-printer (Brother), and the colorimetric signal S was measured within the obtained images by densitometric analysis as described in previous publications.[24-26]

**Detection of *P. aeruginosa* attached to surgical masks**

*P. aeruginosa* suspensions in the range from $10^3$ to $10^8$ CFU·mL$^{-1}$ were prepared in PBS. The bacterial suspensions were transferred to a spray bottle. The polypropylene layer of the surgical facemasks was cut into 2 cm x 2 cm pieces. The spray bottle and the polypropylene piece were held by a 3D printed device that maintained a fixed distance between them.[18] Each bacterial suspension was sprayed thrice and, after drying at room temperature (RT), clipped with three layers of blotting filter that had been previously soaked with 1.9 mL of PBS-BSA assembled on a clamp tool. Next, the paper biosensor was placed above the polypropylene piece and pressed against it for 5 min under high pressure. Then, the reservoir was removed and the polypropylene substrate washed by adding 400 μL of PBST and softly pressing the polypropylene piece twice. Finally, the polypropylene substrates were scanned and the colorimetric signal S was measured within the obtained images by densitometric analysis.

***P. aeruginosa* autofluorescence and imaging**

A *P. aeruginosa* solution at $10^8$ CFU·mL$^{-1}$ was prepared in MQ water, immediately sprayed against pieces of the polypropylene layer of surgical facemasks as described above and left to dry at RT for 15 min. Next, the fluorescence was measured with a Typhoon FLA 9500 laser scanner (General Electric) by using the blue LD laser (470 nm) in the instrument. Then, we simulated the abovementioned protocol for hydrating polypropylene with PBS-BSA under high pressure (*Surgical facemask processing* section). Finally, the polypropylene pieces were washed, left to dry and scanned again. Surgical facemasks sprayed with MQ water were included as negative controls. The fluorescent signal S was measured by densitometric analysis of the obtained images as previously reported.[27]

**Detection of *P. aeruginosa* in surgical facemasks worn by patients**

Patients were recruited by nurses at the Department of Respiratory Medicine at Son Espases University Hospital (Balearic Islands). Six patients with a confirmed respiratory infection caused by *P. aeruginosa* were included, of which 5 were chronic infections and 1 an acute infection. Chronic infection was defined as the growth of *P. aeruginosa* in three cultures in one year, separated by at least one month.[9] Patients were asked to wear a surgical surgical mask for 30 min. The control group consisted in 5 patients with confirmed acute respiratory infections caused by bacterial pathogens different from *P. aeruginosa,* that also wore a surgical mask for 30 min. Bacterial etiology of the respiratory infections in the patients included in this study and their chronic lung condition can be found in supplementary Table S1. The surgical mask was stored at -20 $^{o}$C until it was analyzed. The study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and approved by the local ethics committee (protocol IB3537/17PI). All samples were collected after informed consent from patients or a family member was obtained.

In the surgical masks collected from patients four 2 cm × 2 cm pieces were cut in the center of each one. The hydrophobic polypropylene layer was then isolated and *P. aeruginosa* was detected with the protocol described above.



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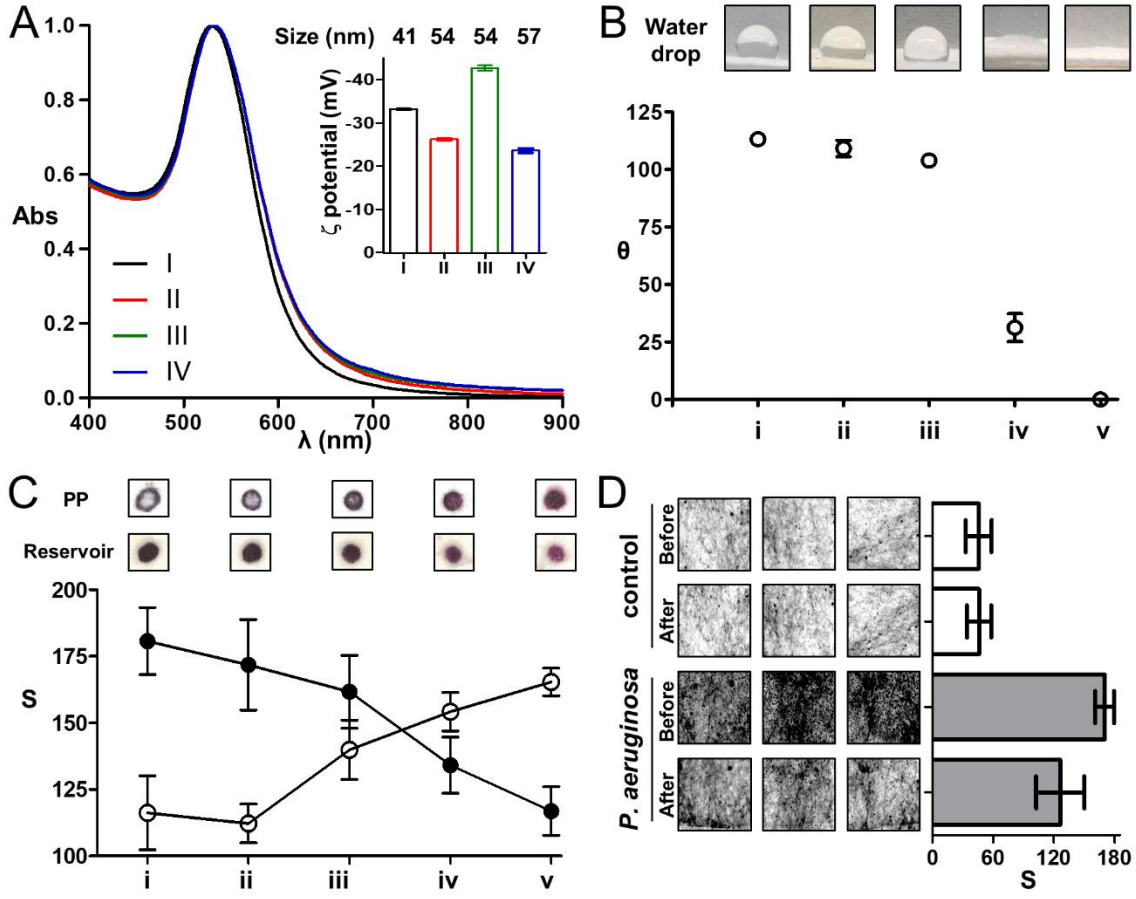


**Figure 2. Characterization of the different elements of the proposed biosensor.** (A) Vis-NIR spectra of gold nanoparticles (AuNPs), particle size determined by nanoparticle tracking analysis (size data in Inset) and ζ-potential measurements (bars in Inset) after the synthesis (I, black) and after each step of the functionalization protocol; addition of thiol-PEG-acid (II, red), modification with avidin and capping with glycine (III, green), and addition of biotinylated anti-*P. aeruginosa* antibodies followed by capping with PEG-biotin (IV, blue). (B) Photographs of water drops and measures of contact angle (θ) produced by polypropylene pieces without processing (i), and after processing with PBS at low (ii) and high (iii) pressure or with PBS-BSA at low (iv) and high (v) pressure for 10 s. (C) Images of scanned polypropylene (PP) and reservoirs, and colorimetric S signals yielded by the Ab-AuNPs released from reservoirs to PP (open dots) and those still stored within reservoirs (black dots) after nanoparticle transfer using polypropylene pieces (previously processed as in panel B) as receiving substrates. (D) Fluorescence images and S signals of polypropylene pieces sprayed with MQ water (white bars) or *P. aeruginosa* at $10^8$ CFU·mL$^{-1}$ (grey bars) before and after applying high pressure while blocking with BSA and washing. Error bars are the standard deviation of 3 independent measurements.

## Results and discussion

Figure 2 shows the characterization of the different elements of the proposed analytical platform, including the gold nanoprobes in the nanoparticle biosensor and the facemask capture element. Figure 2A shows the characterization experiments performed at each step of AuNPs modification protocol with anti-*P. aeruginosa*. In this Figure, the LSPR shifts from 529 to 532 nm, the ζ-potential increases (from -33.2 to -26.2 mV), and the nanoparticle size increases from 41 to 54 nm after adding thiol-PEG-acid, which demonstrates that AuNPs are coated with the polymer (Figure 2A-I and 2A-II). The ζ-potential of AuNPs falls up to -42.7 mV after being decorated with avidin and capped. This is attributed to the carboxylic acid of glycine contributing negative charges after the capping step, which transforms the amine into an amide bound to thiol-PEG-acid (Figure 2A-III). The addition of biotin anti-*P. aeruginosa* to avidin-decorated AuNPs increases again the ζ-potential from -42.7 to -23.6 mV and rises the particles size from 54 nm to 57 nm (Fig, 2A-IV). These results demonstrate that the AuNPs have been functionalized with avidin and antibodies. Next, we conducted a set of experiments aimed at optimizing the steps required to analyze the facemask with the proposed nanoparticle biosensors. This includes making it more hydrophilic so it can receive the antibody-nanoparticles transferred from the biosensors. The challenge here was to obtain this feature without damaging or detaching bacterial cells from the polypropylene layer, which would decrease the sensitivity. To make masks hydrophilic, mask pieces were placed on blotting paper previously soaked with PBS or PBS-BSA and pressed for 10 s with the finger (low pressure) or using a clamp tool (high pressure). After drying, the wettability of the polypropylene was assessed by measuring the contact angle (θ) produced by a drop of water (Figure 2B). Raw polypropylene produces



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θ>100°, which confirms that the material is highly hydrophobic (Figure 2B-i). The θ barely decreases if polypropylene is processed with the low-pressure procedure in the presence of PBS (Figure 2B-ii). Increasing the pressure with the clamp tool still renders the substrate highly hydrophobic (Figure 2B-iii). On the other hand, BSA dramatically increases the wettability of the polypropylene. In photographs depicted in Figure 2B-iv the drop of water is almost completely absorbed (θ≈25°) after the low-pressure treatment (Figure 2B-iv), and this effect is even more acute when using the high-pressure protocol (θ=0°, Figure 2B-v). These results are in line with previous studies reporting that BSA coatings increase the wettability of hydrophobic surfaces.[28] BSA is also commonly used as a blocking agent in biosensors.[29-31] Therefore, this step serves the dual purpose of making the surface more hydrophilic and blocking non-specific interactions.

Next, we studied how the polypropylene hydrophobicity influences nanoparticle transfer, which is crucial for detecting *P. aeruginosa*. In Figure 2C polypropylene pieces with varying degrees of wettability (i<ii<iii<iv<v, as in Figure 2B) were placed on soaked pieces of blotting paper and then nanoparticle biosensors were pressed against them for 5 min. Figure 2C demonstrates that the colorimetric signals S in the receiving substrate increases as the wettability of the polypropylene increases (Figure 2C). Conversely, S in the reservoirs decreases as the mask becomes more hydrophilic because the nanoparticles are being transferred. These results demonstrate that nanoparticle transfer depends on the degree of wettability of the receiving substrate, which in turn depends on the pressure applied during the blocking step with BSA (Figure 2C-v). In this context, applying high pressure while blocking with BSA ensures that highly concentrated nanoprobes are available for detecting the analyte.

We then studied whether the application of high pressure during the blocking step could damage or detach bacterial cells from the facemask, thus decreasing the sensitivity of the analytical platform. For this purpose, we studied whether bacterial cells remain attached to the facemasks after blocking and washing steps with fluorescence microscopy. We were able to do this without labelling the cells with a fluorophore by leveraging the intrinsic fluorescence of pyoverdine, a siderophore produced by *P. aeruginosa* that shows a maximal fluorescence emission at excitation wavelengths near 460 nm.[32] In Figure 2D we sprayed bacterial solutions on surgical masks in order to simulate exhaled breath, and the fluorescence of the polypropylene layers was subsequently evaluated. In this Figure, the fluorescence emission after spraying *P. aeruginosa* solutions is higher than in control experiments with no bacteria, which demonstrates that surgical masks efficiently capture pathogens within aerosols (Figure 2D). Furthermore, the fluorescent signal is highly preserved after blocking and washing, which demonstrates that the cells remain attached to the mask (Figure 2D). Altogether the results depicted in Figure 2 demonstrate the correct functionalization of AuNPs (Figure 2A), establish the methodology for blocking and transferring AuNPs (Figure 2B-C), and prove that this method ensures the retention of bacteria trapped in surgical masks (Figure 2D). Below we explore this analytical platform for detecting *P. aeruginosa* aerosolized onto the masks.

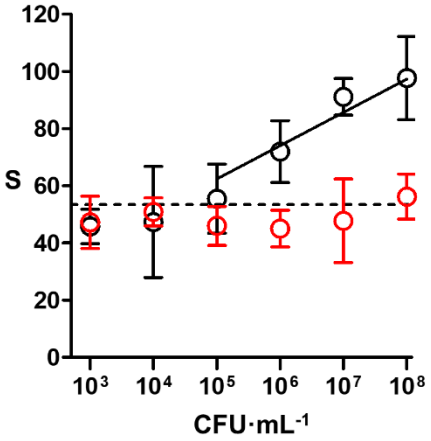


**Figure 3. Performance of the nanoparticle transfer biosensor for detecting *P. aeruginosa*.** Calibration plot representing the colorimetric signals S with respect to the concentration of bacterial suspensions of *P. aeruginosa* (black dots) and *Klebsiella pneumoniae* (red dots) sprayed onto surgical facemasks. Error bars are the standard deviation of 3 independent experiments. The dotted line shows the LOD, expressed as the signal above 2 times the standard deviation of the blank.

Figure 3 shows the calibration experiments performed to determine the analytical performance of the proposed nanoprobes against *P. aeruginosa*. For this purpose, we simulated the generation of aerosols by spraying *P. aeruginosa* solutions at increasing concentrations on surgical facemasks. In this Figure, the biosensors produce dose-dependent S signals (black dots in Figure 3). The limit of detection (LOD), expressed as the sample that yields a signal higher than 2 times the standard deviation of the blank (95% confidence), is $10^5$ CFU·mL$^{-1}$. Control experiments with surgical masks sprayed with *Klebsiella pneumoniae* yielded S signals that are below the limit LOD in all the concentrations assayed, except for $10^8$ CFU·mL$^{-1}$ (red dots in Figure 3). These results show that the signals observed during the analysis of surgical facemasks sprayed with *P. aeruginosa* are originated by the specific recognition of the pathogen with antibody-decorated AuNPs. These experiments also demonstrate that the proposed analytical platform can detect bacterial cells in a semi-quantitative fashion. The LOD corresponds to the clinical threshold value for diagnosing an infection through the analysis of sputum. Nevertheless, this threshold value may vary depending on the type of respiratory sample used for the analysis (sputum, bronchial aspirate, bronchoalveolar lavage),[33,34] and therefore a direct correlation between both methods cannot be established. Furthermore, the clinical

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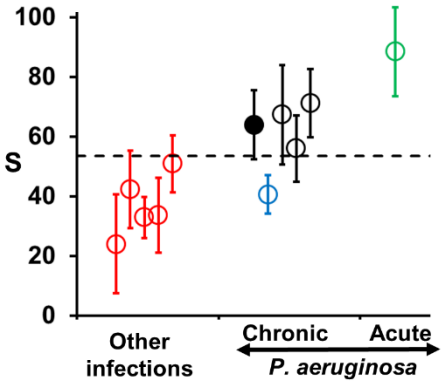


**Figure 4. Response of the nanoparticle transfer biosensor in surgical facemasks worn by patients with respiratory infections.** Colorimetric signal S in surgical facemasks worn by patients with respiratory infections caused by pathogens different from *P. aeruginosa* (red dots) and patients with a chronic (black and blue dots) or acute green dot) respiratory infection caused by *P. aeruginosa.* Filled black dot represents a patient chronically infected with *P. aeruginosa* that was acutely co-infected with *Staphylococcus aureus* at the time of recruitment. Blue dot shows a patient with a chronic infection by *P. aeruginosa* that yielded a bacterial load $<10^5$ CFU·mL$^{-1}$ in a sputum culture test. Error bars are the standard deviation of 4 independent experiments in the same mask. The dotted line shows the LOD obtained in the calibration plot of Figure 3.

threshold value is determined with bacteriological culture, which only detects live cells that proliferate in the culture. Finally, the reported LOD, dynamic range and error bars were obtained with a mock sample prepared using a spray bottle, which does not necessarily correlate to the way that aerosols are produced when breathing. Below we explore whether the reported LOD can be used to identify patients with either a chronic or acute infection in the lungs.

After demonstrating the suitability of our biosensors towards the recognition of *P. aeruginosa* in sprayed surgical masks, we sought to determine whether they could be used to detect a lung infection caused by this pathogen by analyzing the breath of infected patients wearing a surgical facemask. To this end, a collection of 11 surgical masks worn for 30 min by patients suffering from a respiratory infection (including acute or chronic infections caused by *P. aeruginosa* and other pathogens), were queried with our biosensors. All patients were enrolled at the Respiratory Medicine Department of Son Espases University Hospital (Balearic Islands) after signing an informed consent approved by the local Ethics Committee. In Figure 4 all masks worn by patients with an acute respiratory infection not caused by *P. aeruginosa* (red dots in Figure 4) yielded S signals below the LOD according to the calibration shown in Figure 3. Furthermore, 5 out of 6 surgical facemasks worn by patients who had a respiratory infection by *P. aeruginosa* produced S signals above this threshold (black and green dots in Figure 4). Among the mask samples with *P. aeruginosa* above the LOD, 4 were chronic infections in stable phase (black dots in Figure 4) and 1 was an acute infection (green dot in Figure 4). It should be noted that 1 patient with a *P. aeruginosa* chronic infection had an acute co-infection caused by *Staphylococcus aureus* at the time of mask sampling (filled black dot in Figure 4), which demonstrates that our biosensor is capable of identifying *P. aeruginosa* infections even in the presence of other pathogens that may interfere with its detection. Furthermore, the patient who provided the mask sample with *P. aeruginosa* below the LOD was receiving antibiotic treatment and had a bacterial load lower $10^5$ CFU·mL$^{-1}$ in a sputum culture test (blue dot in Figure 4), which indicates that the sensor signal in real samples is related to the bacterial load. These results show that the proposed analytical platform can identify chronic and acute infections as long as the bacterial load is above the LOD. In turn, this paves the way to diagnose chronic colonization after an acute infection, as well as to fine-tune antibiotics according to the biosensor signal, which is related to the bacterial load in the lungs.

## Conclusions

In this paper we have introduced an analytical platform for detecting bacteria in aerosols based in analyzing a facemask with nanoparticle biosensors. Making polypropylene hydrophilic using a high-pressure blocking procedure was the key to transfer antibody-decorated nanoparticles and detect the pathogen. The analytical platform was validated with aerosolized mock samples as well as with facemasks worn by 11 patients with chronic lung conditions that were suffering from a respiratory infection. Our results pave the way to diagnose bacterial infections without obtaining a sputum sample. In the context of *P. aeruginosa* infections this could provide the key piece of information required to guide anti-pseudomonal therapy during chronic and acute respiratory infections.

## Author Contributions

The manuscript was written through contributions of all authors. D.D-C: Investigation, Methodology, Writing Review and Editing; A.C: Data Curation, Formal Analysis, Investigation, Methodology, Supervision, Writing Original Draft, Writing Review and Editing; C.A-J: Investigation, Methodology; A.V: Investigation, Methodology; A.I: Investigation, Resources; M.L: Methodology, Resources; R.M: Methodology, Resources; I-M.R: Methodology, Resources; B-G.C: Investigation, Resources: R.R: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Writing Original Draft, Writing Review and Editing. All authors have given approval to the final version of the manuscript.

**Corresponding Author:** antonio.clemente@uib.cat



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## Conflicts of interest

A.C., C.A-J., A.V. and R.R. are founders of Nanodecal, a spinoff company that is exploiting the patent describing the method for storing nanoparticles in paper reservoirs (WO2021048087A1).

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