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Gold Nanoparticle-Based Breath Test Device for Rapid Detection of Infections

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Abstract: Respiratory tract infections represent a significant global health burden. We developed a gold nanoparticle-based breath test device that enables rapid detection of infections at the point-of-care. The device measures the colorimetric properties of gold nanoparticles, which change upon aggregation when exposed to specific antigens filtered from exhaled breath. The system integrates sampling and analysis in a portable unit, providing results within 30 seconds. In this proof-of-concept, we demonstrate the detection of SARS-CoV-2 antigens. Initial tests using NaCl-induced aggregation validate the device's functionality. The tests show the suitability for rapid, user-friendly infection diagnostics from exhaled breath. Further optimization is needed to enhance sensitivity and reliability.

Keywords: Point-of-care, Diagnostic, Infection Testing, Gold Nanoparticles, Breath Test

1 Introduction

Respiratory tract infections (RTI) are one of the most common diseases worldwide which highlights the need for diagnostics to detect RTI at the point-of-care. The World Health Organization defined, that those new diagnostics must be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable. [1], [2]

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The gold standard for SARS-CoV-2 testing are polymerase chain reactions (PCR) and lateral flow antigen tests. PCR is highly sensitive but requires specialized labs, making it expensive and time-consuming. Rapid antigen tests are quicker, cheaper but less sensitive. [3] Both tests rely on specimens from the proximal and distal respiratory tract such as nasopharyngeal swabs or tracheal aspirates. The sampling is often perceived as uncomfortable. [2], [3] Exhaled breath (EB) can be used as an alternative sample, it originates from the entire respiratory tract and correlates with the viral spread. EB can contain respiratory viruses including SARS-CoV-2, rhinovirus, influenza, and others. [4], [5] There are many methods and studies available for the detection of SARS-CoV-2 from EB. These methods rely on external molecular analysis and therefore lack improvements in turnaround time and applicability. [5], [6], [7], [8], [9]

Other devices with integrated EB analysis have disadvantages in terms of reusability, device preparation, sensitivity or specificity, or are expensive. [10], [11]

Colorimetric biosensors based on metal nanoparticles are being proposed as a more sensitive alternative for use in molecular diagnostics [12], [13], [14].

Gold nanoparticles (GNP), support plasmons localized at their surface (LSP). LSPs are electron clouds that can oscillate at a specific resonance frequency (LSPR) which causes strong absorbance or scattering of incident light. The oscillation frequency can be modulated by the shape, size and the interparticle distance of GNPs. Depending on the refractive index of the metal and its surrounding medium the LSPR wavelength changes. [14], [15], [16] When GNPs aggregate, their individual plasmon oscillations couple with each other, resulting in a collective oscillation of the electron clouds and a shift of the

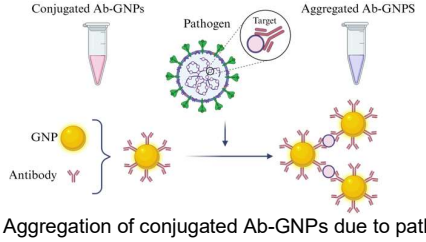

Figure 1: Aggregation of conjugated Ab-GNPs due to pathogen recognition. Colorimetric change from red to blue. Created with BioRender.com

LSPR to shorter wavelengths. This aggregation can be induced by antigen-antibody by coating with specific antibodies (Ab-GNPs) and presence of the antigen (see Fig. 1, where Ab-coated GNPs recognize their respective target protein). [17] The colorimetric change of plasmonic GNPs can be used in naked-eye tests to detect SARS-CoV-2 [13], [18], [19], [20], [21]. However, the limit of detection (LOD) lower to molecular analysis but turnaround time and test costs are improved.
A novel GNP-based RTI breath test has been developed. It provides results in 30 seconds, is portable, applicable to laymen, and combines sampling and analysis. This work demonstrates a measurement solution for SARS-CoV-2 detection and a proof-of-concept for the method.

2 Methods

2.1 Breath Test Device

The device consists of four main components: fluid reservoir, optical unit, display, and filter tube, all housed in a 3D-printed casing. The fluid reservoir (936101, Macherey-Nagel, Düren, Germany) holds 2 mL of test solution, can be interchanged and remains fixed during measurements. A 3D-printed probe port positions the fluid reservoir and enables the rinsing of the filter tube using a seal ring (4301250150, HUG Technik und Sicherheit, Ergolding, Germany).

The optical unit is a spectral sensor sensitive from 450 to 650 nm, a white LED (AS7262 Visible, SparkFun Electronics, Boulder, USA), a USB-C interface and push buttons. The optical unit is aligned and tangentially mounted to the fluid reservoir. The LED emits through the liquid, with a reflective surface (NC-1531, PEARL GmbH, Buggingen, Germany) behind it. For each measurement, the LED is activated ten times, and the average reflection signal is calculated.

The filter tubes are manufactured by thermally bonding polypropylene tubes (1E91.1, Ratiolab, Dreieich, Germany) to the filter (hydrophobic layer FFP2 mask (Strongfit, nordeko safety, Stapelfeld, Germany)). The mouthpiece (Shisha Set DEFSKAH, eVatmaster Consulting GmbH, Frankfurt, Germany) is attached to the filter tube before sample collection.

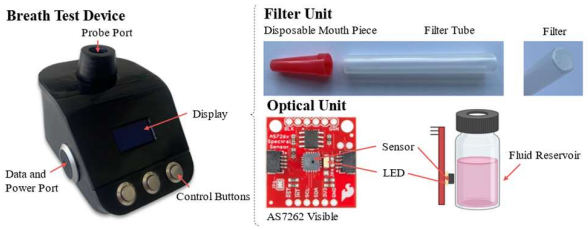

Figure 2: The breath test device, Filter tube and optical unit. Created with BioRender.com

2.2 Breath test procedure

The breath test procedure includes the steps shown in Fig. 3:

1. **Preparation of the system**: The probe port is screwed onto the fluid reservoir and mounted in the housing.
2. **Calibration Measurement**: The LED is switched on and the reflection signal of the test solution is measured.
3. **Sample collection**: The test person mounts the mouthpiece to the filter tube and exhales into the tube for 10s.
4. **Filter rinsing**: After removing the mouthpiece, the filter tube is inserted into the fluid reservoir, rinsed three times, and the reflection signal is measured with the LED on.
5. **Test result**: The filter tube is removed and disposed. The result is displayed within 10 seconds. The fluid reservoir is closed and disposed.

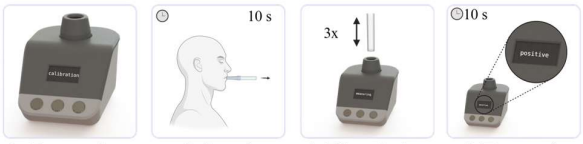

Figure 3: The five step procedure. Created with BioRender.com

2.3 Test solution

SARS-CoV-2 / 2019-nCoV nucleocapsid antibodies (NP-Ab, #40588-T62, SinoBiological, Beijing, China) are covalently conjugated to 40 nm PEG12-carboxylic acid coated GNPs (AUXR40, NanoComposix, San Diego, USA). 200 µg EDC (NanoComposix, San Diego, USA) and 400 µg S-NHS (NanoComposix, San Diego, USA) are added to 1 mL of GNP and incubated (30 min, room temperature (RT)). The solution is centrifuged (3.8k RCF, RT, 10 min) and washed twice with 1 mL of reaction buffer (5 mM potassium phosphate, 0.5% 20K MW PEG at pH 7.4, RXKR, NanoComposix, San Diego, USA). 50 µg of purified NP-Ab is added and incubated (1 hr, RT). To deactivate the NHS-esters, 10 µl 50 % (w/v) Hydroxylamine (438227, Sigma Aldrich, St. Louis, USA) is added. The washing with reaction buffer is performed twice before dilution with 1 mL of storage buffer (0.1X DPBS, 14040133, Gibco, New York, USA), 0.5% BSA, 9048-46-8, Carl Roth, Karlsruhe, Germany), 0.5% Tween 20 (9005-64-5, Carl Roth, Karlsruhe, Germany)).

2.4 Characterization of the measuring liquid

The Ab-GNPs are characterized using a spectrophotometer (NanoDrop One, Thermo Fisher Scientific, Waltham,

USA). For the breath test Ab-GNPs must aggregate with NP and remain stable against buffer changes caused by to contamination. Samples of 5 µl each of Ab-GNPs are combined with i) 2 µl of 15% NaCl in ultrapure water (#7647-14-5, Carl Roth, Karlsruhe, Germany) ii) 2 µl of artificial saliva (SAE0149, Sigma-Aldrich, St. Louis, USA) and iii) different NP (Recombinant SARS-CoV Nucleocapsid His-tag Protein, 10710-CV, R&D Systems, Minneapolis, USA) concentrations ranging from 75 to 2400 ng/mL in storage buffer. Each sample is brought up to 40 µl using storage buffer, mixed and incubated (1 min).

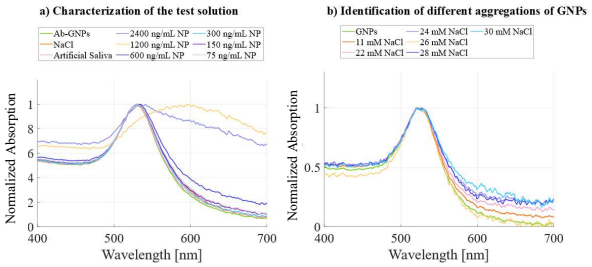

Figure 4: a) spectra of Ab-GNPs with the addition of a) NaCl, artificial saliva and NP n=5 b) NaCl concentrations ranging from 11 mM to 30 mM. n=1

2.5 Breath test device testing

To test the functionality of the breath test device, two initial proof-of-concept experiments are performed. Instead of using the expensive Ab-GNPs with limited LOD (see section 3.1), the experiments simulate the aggregation Ab-GNPs by reducing the surface charge of bare GNPs with NaCl. [22][30]

1. **Identification of aggregations of GNPs**: To assess the breath test's ability to detect changes of the size of bare GNPs, samples of 2 mL of 40 nm citrate-coated GNPs (AUCN40-50M, NanoComposix, San Diego, USA) are combined with 40 µl NaCl in ultrapure water (resulting NaCl concentrations: 0 to 86 mM). The fluid reservoirs are then sequentially interchanged in the device and the spectral changes are measured.
2. **Simulation of breath test procedure**: The pathogen detection via Ab-GNP aggregation is experimentally simulated. 2 mL of bare GNPs are added to the fluid reservoir. Onto the filter tube a) 20 µL of ultrapure water b) 20 µL of NaCl in ultrapure water (43 mM maximum concentration) c) 20 µL of NaCl ultrapure water (86 mM maximum concentration) are pipetted. The filter is then rinsed in the GNP solution. After each rinse, the filter is removed, and the spectral changes are measured.

3 Results

3.1 Characterization of the test solution

The results of the characterization of the Ab-GNPs are shown in Fig. 4a. The colorimetric change upon the addition of NaCl and artificial saliva is minimal. A shift of the peak wavelength or spectral distribution can be observed due to the addition of 600 ng/mL NP or more. This results in a LOD from 300 and 600 ng/mL NP. The biggest difference occurs at wavelengths higher than 600 nm.

3.2 Breath test device

1. **Identification of aggregations of GNPs:** Fig. 5b shows the prepared GNPs, with color changes more apparent at higher NaCl concentrations. At 32 mM, most GNPs form visible aggregates and a clear solution. Fig. 4b shows the measured absorption spectra. To improve clarity, the spectra with concentrations >30 mM are not shown. Changes in the spectral distribution can be observed in all samples. The biggest difference in absorption can be observed at wavelengths >600 nm. The measured reflection intensities of the GNPs with different NaCl concentrations for the detection wavelengths of the sensor are shown in Fig. 5a. The largest signal change occurs at 600 nm, which correlates to the measured absorption spectra of the GNP solution shown in Fig. 4b. The formation of large aggregates at concentrations >30 mM results in higher reflection intensities.
2. **Simulation of the breath test procedure:** Fig. 6a shows the mean measured intensities during the experimental simulation of the procedure. Rinsing a filter containing water results in the GNP solution in a minor signal change. Rinsing the filter containing NaCl results in an aggregation of the GNPs and the measured reflection intensity peaks shift to lower wavelengths due to the shift of absorbance (see Fig. 4).

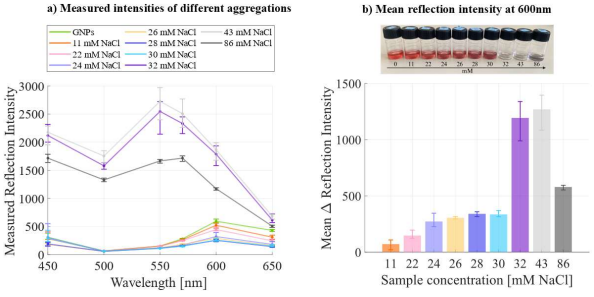

Figure 5: a) Measured reflection intensities of the test solution containing NaCl. b) Mean difference in reflection intensity at 600nm. n=7

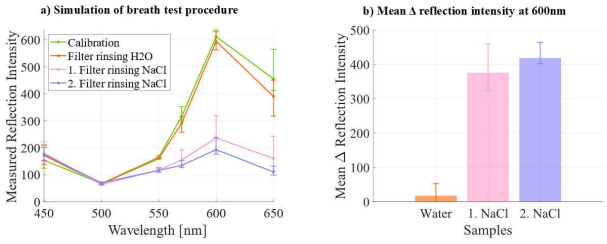

Figure 6: a) Measured reflection intensities of the procedure. Green: GNPs, orange: Filter rinsing with water, pink: 1. filter rinsing with NaCl, blue: 2. filter rinsing with NaCl. b) Mean difference in reflection intensity at 600nm. n=7.

4 Discussion

The article presents a proof-of-concept of pathogen detection using the colorimetric change in a GNP solution due to aggregation caused by antibody-antigen binding. The presented breath test device measures these colorimetric changes by using a spectral color sensor, to detect pathogen presence. First results for the detection of SARS-CoV-2 out of EB was shown. The LOD for the Ab-GNPs is in between 300 to 600 ng/mL NP (see Fig. 4), which is less sensitive compared to literature [18], [19]. Further testing is needed to determine and improve the exact LOD by optimizing antibody concentration, incubation times, alternative GNP designs and buffer agents. Using SARS-CoV NP instead of SARS-CoV-2 NP may reduce sensitivity. Pre-experiments confirm the breath test's adaptability for other pathogens. Further research is needed on its platform potential and cross-reactivity.

The functionality test used GNP aggregation via NaCl to mimic antigen-antibody interaction. While suitable for device design, future tests must use Ab-GNPs and antigens to test sensitivity and specificity against existing methods. The reflectance measurements showed promising results despite inaccuracies (Fig. 6). Reducing stray light and improving the optical unit may help. Some inaccuracies will not be avoidable due to the movement and sedimentation of aggregates in the fluid reservoirs. A key challenge is inconsistent NaCl-induced aggregation (Fig. 6), caused by variable NaCl dissolution and liquid retention in the filter tube.

Overall, this proof-of-concept successfully demonstrated the potential of the breath test device, yet further optimization and testing are necessary to enhance its sensitivity, reliability, and applicability for detecting actual pathogens.

Author Statement

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References

[1] Seok Y, Mauk MG, Li R, Qian C. Trends of respiratory virus detection in point-of-care testing: A review. Anal Chim Acta 2023;1264:341283.
[2] Basile K, Kok J, Dwyer DE. Point-of-care diagnostics for respiratory viral infections. Expert Rev Mol Diagn 2018;18(1):75.
[3] Lassaunière R, et al. Evaluation of nine commercial SARS-CoV-2 immunoassays. medRxiv 2020;2020.04.09.20056325.
[4] Nwanochie E, Linnes JC. Review of non-invasive detection of SARS-CoV-2 and other respiratory pathogens in exhaled breath condensate. J Breath Res 2022;16:24002.
[5] Malik M, Kunze A, Bahmer T, Herget-Rosenthal S, Kunze T. SARS-CoV-2: Viral loads of exhaled breath and oronasopharyngeal specimens in hospitalized patients with COVID-19. Int J Infect Dis 2021;110:105–110.
[6] Kurver L, et al. SARS-CoV-2 RNA in exhaled air of hospitalized COVID-19 patients. Sci Rep 2022;12(1):1–6.
[7] Ryan DJ, et al. Use of exhaled breath condensate (EBC) in the diagnosis of SARS-CoV-2 (COVID-19). Thorax 2021;76:86–88.
[8] Stakenborg T, et al. Molecular detection of SARS-CoV-2 in exhaled breath at the point-of-need. Biosens Bioelectron 2022;217:114663.
[9] Pramanik A, Gao Y, Patibandla S, Gates K, Ray PC. Bioconjugated nanomaterial for targeted diagnosis of SARS-CoV-2. Acc Mater Res 2022;3(2):134–148.
[10] Shan B, et al. Multiplexed nanomaterial-based sensor array for detection of COVID-19 in exhaled breath. ACS Nano 2020;14:12125–12132.
[11] Ghumra DP, et al. Rapid direct detection of SARS-CoV-2 aerosols in exhaled breath at the point of care. ACS Sens 2023;8:3023–3031.
[12] Tang L, Li J. Plasmon-based colorimetric nanosensors for ultrasensitive molecular diagnostics. ACS Sens 2017;2(7):857–875.
[13] Della Ventura B, et al. Colorimetric test for fast detection of SARS-CoV-2 in nasal and throat swabs. ACS Sens 2020;5(10):3043–3048.
[14] Jazayeri MH, Aghaie T, Avan A, Vatankhah A, Ghaffari MRS. Colorimetric detection based on gold nanoparticles (GNPs): an easy, fast, inexpensive, low-cost, and short-time method in detection of analytes (protein, DNA, and ion). Sens Biosensing Res 2018;20:1–8.
[15] Mayer KM, Hafner JH. Localized surface plasmon resonance sensors. 2011.
[16] Zhang L, Mazouzi Y, Salmain M, Liedberg B, Boujday S. Antibody-gold nanoparticle bioconjugates for biosensors: synthesis, characterization, and selected applications. Biosens Bioelectron 2020;165:112370.
[17] Chang CC, Chen CP, Wu TH, Yang CH, Lin CW, Chen CY. Gold nanoparticle-based colorimetric strategies for chemical and biological sensing applications. Nanomaterials 2019;9(6):861.
[18] Pramanik A, et al. The rapid diagnosis and effective inhibition of coronavirus using spike antibody attached gold nanoparticles. Nanoscale Adv 2021;6.
[19] Behrouzi K, Lin L. Gold nanoparticle-based plasmonic sensing for the detection of SARS-CoV-2 nucleocapsid proteins. Biosens Bioelectron 2022;195:113669.
[20] Karakus E, Erdemir E, Demirbilek N, Liv L. Colorimetric and electrochemical detection of SARS-CoV-2 spike antigen with a gold nanoparticle-based biosensor. Anal Chim Acta 2021;1182.
[21] Jin NZ, Anniebell S, Gopinath SCB, Chen Y. Variations in spontaneous assembly and disassembly of molecules on unmodified gold nanoparticles. Nanoscale Res Lett 2016