FIBRO-ON-THE-GO

The first at-home diagnostic test for Pulmonary Fibrosis

Meet the team:
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PULMONARY FIBROSIS

- Pulmonary fibrosis (PF) is an interstitial lung disease characterised by the **irreversible loss** of lung function and is marked by formation of **scar tissue** (fibrosis) in the lungs, blocking the movement of oxygen.
- **70,000** people in the UK are living with PF, with an average life expectancy of **only 3-5 years**.
- PF development is often preceded by **untreated acute lung inflammation**, often caused by viral/ bacterial infections, ionising radiation, chemotherapy, air irritants and pollutants.
- **Non-specific symptoms** such as tiredness, clubbing of the fingers/toes, shortness of breath, and dry coughs can be mistaken for other conditions. It is likely that by the time PF is diagnosed, **irreversible lung damage has already occurred**.
- Certain individuals are **more susceptible** to PF than others, namely workers exposed to dust, smokers, and those with family history.
- High-resolution CT scans, lung function tests, and biopsies are the current standard of diagnosis but are **impractical for widespread testing**, so are often done only **after symptoms worsen** and **scarring** has occurred.
- Whilst scarring to the lungs cannot be reversed, there are two available drugs which **slow the development** of PF (Pirfenidone and Nintedanib), making **early diagnosis** important. There are no cures/treatments available yet.
- Hence we have decided to develop a point-of-care diagnostic tool, which can be widely distributed in order to detect and subsequently manage PF in its early stages **before symptoms worsen.**

OUR PROPOSAL

Comparing individuals with and without pulmonary fibrosis, we found differences in the **concentration of biomarkers** in the blood and **gene expression** in nasal epithelium. This altered gene expression is observed in both the nasal and bronchial epithelium, but we solely focused on expression in the **nasal epithelium** as this requires minimally invasive sampling methods.

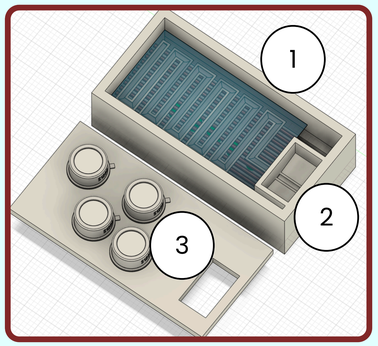
To detect these differences, and to investigate the presence and extent of PF in at risk individuals, we propose a two-pronged diagnostic approach combining:

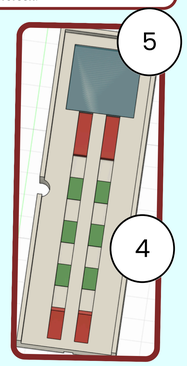
- A **quantitative lateral flow test** to measure the concentration of blood-based protein biomarkers.
- A **lab-on-a-chip RT-LAMP** (Reverse Transcriptase Loop-mediated Isothermal Amplification) test to assess nasal gene expression by measuring mRNA levels in nasal epithelial cells.
- A **central unit** Bluetooth module that transmits data from both tests to a smartphone app, which processes the results and calculates the likelihood of PF.

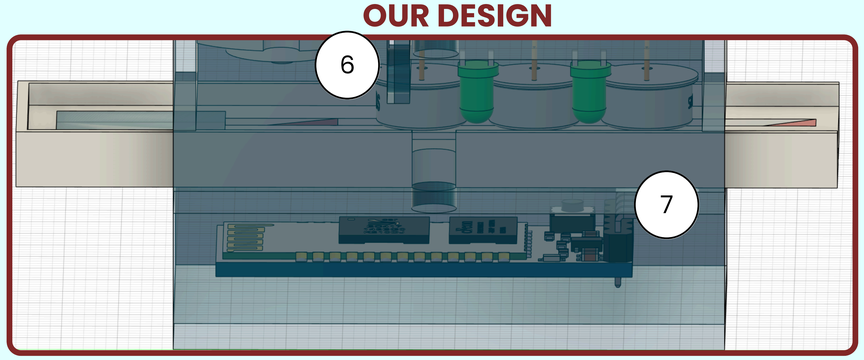
The central unit will be **reusable** whilst the lateral flow and lab-on-a-chip RT-LAMP test will be **disposable** cartridges. The test can be retaken every three months to monitor at-risk individuals' molecular signs of fibrosis.

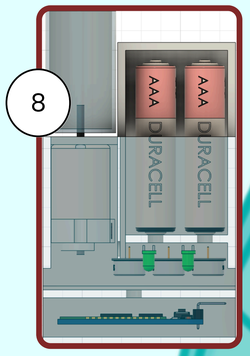
A quantitative lateral flow test is used instead of a qualitative test because the relevant biomarkers are present in both healthy and affected individuals. Measuring their concentration, rather than presence, is essential for accurately assessing the extent of pulmonary fibrosis. RT-LAMP is essential because gene expression cannot be measured directly from the tiny quantity of mRNA in a regular sample. RT-LAMP amplifies the gene exponentially, making it detectable via fluorescence. Embedding this process into a lab-on-a-chip platform allows us to miniaturise the assay, enabling accurate and rapid at-home testing.

OUR DESIGN









Annotations:

1. RT-LAMP chamber, **microfluidic channels** which are coated with the master mix powder. Buffer solution flows into the microfluidic channels, hydrating the master mix powder. Beneath the chamber are resistive heaters to heat the solution to 65°C for RT-LAMP. **Master mix powder** contains:
   - Thermostable **reverse transcriptase** to convert mRNA to cDNA- must be thermostable to be able to withstand 65°C temperature.
   - **Bst DNA polymerase** is the enzyme used in LAMP to copy identical strands of DNA.
   - dNTPs (building blocks of DNA) and Mg^{2+} (cofactor for reverse transcriptase and DNA polymerase)
   - **Gene-specific primers** which facilitates amplification of cDNA strands: Each gene (e.g., MUC5B, CCL4, LRG1) has a set of 6 unique primers (F3, B3, FIP, BIP, LoopF, LoopB)
   - Gene-specific molecular beacon probes: DNA strands bound to **fluorophores** (fluorescing molecules) complimentary to the replicated DNA.
2. **Buffer solution** for RT-LAMP - containing detergents (e.g., Triton X-100) to lyse cell membranes and RNase inhibitors - prevent RNA degradation e.g. guanidine salts to stabilize RNA and inactivate RNases.
3. For RT-LAMP test: **Emission filters** are placed in front of the photodiode to allow only the emission wavelength of the target fluorophore to reach the sensor. **Photodiode array** – detects the specific wavelength of light emitted from the fluorophore. LED excitation array is underneath the chamber.
4. Lateral flow assay- mobilised **antibodies** specific to each biomarker, bound to gold nanoparticles + rows of immobilised antibodies specific to each biomarker and a control line- to ensure test validity
5. **Buffer pad**- Blood is micro pipetted into the buffer solution. Buffer solution contains: Bovine Serum Albumin, sucrose, surfactant and PBS.
6. For Lateral Flow test: **Lateral flow strip reader**- LED to illuminate control and test lines and photodiodes to detect reflected light intensity from gold nanoparticles.
7. Reusable **central unit**- Contains Bluetooth chip which transmits signals to the app from the tests.
8. **Motor** on which buffer solution is placed in order to rotate the curette and buffer so that mRNA is released into solution.

NASAL TEST:
Our nasal test checks for signs of PF by measuring the **overexpression** of specific genes. The user collects a small sample of cells from inside the nose using a **curette** (tool with a scoop to remove tissue samples) that is spring-loaded (click once to move curette up and down inside of nose) to ensure a consistent sample. The swab is then mixed with buffer solution.
This liquid buffer flows into a compact testing device that heats the sample and starts a reaction to copy and detect the target genes. We use a method called **RT-LAMP**, which works quickly at a **constant temperature**, making it faster and more suitable for home use than traditional lab methods.
The device measures how brightly certain **fluorescent signals** glow, which reflects how much of each gene is present. Because each gene is tested in order, the system automatically ensures that every test is timed the same way to allow for accurate comparisons. Results are sent via Bluetooth to an app, which interprets them and compares the user's gene levels to healthy ranges (determined during clinical trials).
We currently are basing our test on three genes - MUC5B, CCL4, and LRG1 - but aim to include more in the future, to improve diagnostic accuracy.

BLOOD TEST:
Our blood test looks for protein markers of PF using a small finger prick of blood. A specific volume of blood is taken by a **micropipette** and mixed with a buffer. This solution is then added to a lateral flow test strip that filters the blood and detects key protein biomarkers including KL-6, SP-D, SP-A, MMP-7, MMP-1, and α-SMA, via **antibody binding**.
If these biomarkers are present, they create red lines on the strip. The darker the line, the higher the protein level. A reader inside the central unit shines light on the strip and uses sensors to measure the result, which is then analysed through an app. The system uses two test strips to allow for testing of multiple biomarkers without greatly increasing the test strip length - the longer the test strip, the longer the lateral flow takes. allowing for faster results. to test for multiple biomarkers in parallel at once for faster results, and tests multiple biomarkers on the same strip and the findings can be easily forwarded to healthcare providers.

FEASIBILITY

Strengths	Limitations	Improvements
1. The **first at-home diagnostic tool** for PF, offering early detection without requiring hospital visits. 2. Testing of multiple genes and biomarkers simultaneously to improve diagnostic accuracy. 3. Can be done regularly for self monitoring, relatively simple and **minimally invasive** sample collection. 4. App for user-friendly interpretation of results with results sent immediately to GP 5. Low power and **miniaturised**/designed for mass production and distribution.	1. Whilst single-use cartridges ensure sterility and prevent cross-contamination, they contribute to **plastic waste**. 2. User-collected nasal curette and fingerprick blood samples may vary in quality and quantity, affecting accuracy. 3. The current device targets a **small set** of biomarkers and gene transcripts, some of which **may not be fully specific** to pulmonary fibrosis. 4. While suitable for home use, the device's molecular quantification may be **less precise** than hospital-grade laboratory testing.	1. Potential design using **biodegradable** materials or implementing a cartridge return and recycling system. 2. Integrate step-by-step **video tutorials** via the app and easy-to-use sampling tools. Spring-loaded curette and micropipette ensure accuracy of sample collection. 3. Conduct **further research** to identify additional PF-specific biomarkers and gene expression to increase diagnostic specificity. 4. While hospital tests will remain the gold standard, our goal is to provide the **most accurate point-of-care molecular test** possible. This can be achieved through rigorous calibration curve testing, robust image processing algorithms, and stringent internal quality control mechanisms.

ACCEPTABILITY

'Fibro-on-the-go' aims to significantly change the conversation around pulmonary fibrosis. We aim to **raise awareness** and funding for new PF treatments while providing a short-term solution through our early detection.
This is as existing medications only work to slow down PF: so the earlier, the better.
Concerning public dissemination for our tester, we will focus on patient capability - working alongside individuals of all ages in clinical trials, using feedback to iterate the app tutorial. For opportunity, we will liaise with GPs and manufacturing companies to recommend our tester and issue them. For motivation, we aim to increase **public education** on pulmonary fibrosis, through NHS facebook videos, leaflets issued by mail and encouraging word of mouth conversations. With more clinical trials, we hope to tailor the app to our target audience and find even more genes and biomarkers for more accurate testing.

CLINICAL TRIALS

Pre-Clinical

Device Hardware and Software: 3 months
Test the **optimal lifespan** of the device to determine its re-usability. Ensure **Bluetooth** connection works in app to upload all data from quantitative lateral flow and RT-LAMP, giving accurate concentration values from results processing for both.

In vitro testing: 12 months
Test **known concentrations** of biomarkers using the device and the illuminance of the gold nanoparticles is detected. This is used to form a calibration curve. Test mRNA of MUC5B, CCL4, LRG1 individually to ensure that each fluorophore fluoresces and the binding of a quencher prevents this fluorescence. Test the genes together to ensure that the test **correctly quantifies** the gene expression level. Ensure concentration of amplified DNA is proportional to initial mRNA concentration.

System Algorithm: 3 months
Test that the app can correctly assign ranges (**healthy, slight PF, moderate PF, severe PF**) when test values are inputted into its algorithm.

MHRA Approval: 2 months
Apply for approval to begin clinical trials.

Phase I: Efficacy: 8 months
Test efficacy using a small sample group of **50** individuals. Half would be healthy and the other half would have pulmonary fibrosis. Run the RT-LAMP and quantitative lateral flow tests on the individuals, **not self-tested** so we can observe results under **optimal conditions**. Individuals must be tested for PF using high resolution CT (HRCT) scans before and after phase I, to compare actual disease progression to results from our device.

Phase II: Specificity and Accuracy: 18 months
Test specificity and accuracy and using a larger group of individuals of **150 people**. Three groups of 50 individuals with Chronic Obstructive Pulmonary Disease (COPD), Interstitial Lung Disease (ILD), Pulmonary Fibrosis (PF), to ensure the RT-LAMP and quantitative lateral flow test is **specific** to PF and only gives positive results for these 50.

Phase III: Larger Scale Feedback, 24 months
Conduct a study with a much larger group of **1000** individuals, 18+, including smokers, construction workers, miners, farmers, and manufacturers. Create a survey for individuals to ask about ease of testing with the app tutorial and how likely they are to recommend the test to coworkers/smokers.

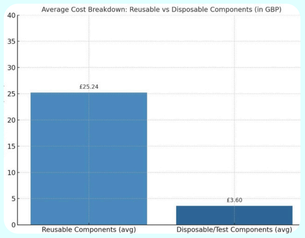
Clinical

COST

Reusable Components:
The device includes reusable electronics and structural parts such as a Bluetooth module (£3.16–£6.32), motor (£0.79–£3.95), AAA batteries (£1.58–£2.37), LEDs (£0.02–£0.40), photodiodes (£3.16–£31.60), general wiring (£0.08), and mounting hardware (£0.08–£0.16). A 3D-printed plastic casing (£1.19–£3.95) houses these components for repeated use.

Testing Parts:
Single-use elements include the plastic test strip (£0.40–£1.58), RT-LAMP reagents (£1.98–£2.77), lancet (£0.04–£0.24), curette (£1.58–£3.16), and absorbent materials (£0.08–£0.63). Additional consumables include buffer solution (£0.04–£0.20), copper wiring (£0.02–£0.25/m), and gold nanoparticles (£0.46/ml), along with sterile, preloaded reagents.

Affordability:
This system is designed with cost-efficiency in mind. The reusable components range from just **£10.27** to **£39.50,** depending on configuration, and can be used across many tests. Each individual test costs approximately **£1.58** to **£5.53**, making it a highly affordable solution for regular or widespread diagnostic use, especially in low-resource settings.



THE APP

Test Instructions:

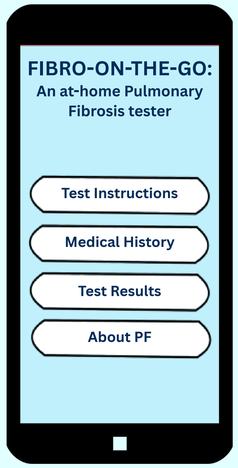
- App will detail step-by-step video instructions on how to carry out the test. E.g. how to use the curette, how to get a blood sample and put on the motor, etc.

Medical History:

- There will questions gathering a detailed medical history, e.g. any family history of PF, whether their occupation increases likelihood of developing PF (such as working around high levels of dust), smoking and what other conditions they have.
- It is important to find out any other conditions to prevent a false positive result.

Test Results:

- Results from the central processing unit will be transmitted to the app via Bluetooth, then calculations will be done, taking the raw data (light intensity from photodiode and colorimetric reader) and converting to original blood protein and mRNA concentrations via calibration curves.
- These values will then be compared to different ranges, associated with different levels of fibrosis (the values of the ranges being determined during clinical trials).
- Each range (healthy, slight PF, moderate PF, severe PF) will be associated with a number, the sum of the numbers will be found based on the results of all blood biomarkers and gene expression, and tell the user to what extent they are likely to have pulmonary fibrosis.
- Depending on the efficacy of each biomarker as a indicator of PF, scores can be weighted to give a more accurate overall score.
- The results are simultaneously sent to GP.



SCIENTIFIC DETAILS AND REFERENCES