

# AFA-based Sputum Liquification to Enable Downstream Microbial Analysis

Sputum is an important biological sample for diagnostic support of respiratory infections. As part of the host immune defenses, sputum is over-produced during infection. Analysis of sputum by direct staining and microscopy, culture, biomarker or molecular testing can reveal important information that can guide patient management and therapy decisions. Additionally, emerging lung microbiome research projects may start with sputum as the primary specimen.

However, the high viscosity and partial solubility of sputum samples often render them difficult to pipet and make the quantitative analysis difficult. High viscosity sputum samples can interfere with instrumentation flow, and visually sputum smears can be difficult to read in transmitted light for bright-field microscopy or fluorescence microscopy. Many false negatives could be turned into useful information if sputum were appropriately solubilized without damaging the target analytes.

Historically, processing raw sputum to a more manageable liquid has involved chemical treatments, sonication, vortexing and combinations.

## HOW AFA CAN HELP PROCESS A SPUTUM SAMPLE

Adaptive Focused Acoustics is well established for shearing DNA for Next Generation Sequencing and for extraction of peptides prior to MALDI-TOF MS. These applications are optimized by considering the initial starting material concentrations, volumes and desired outcomes. Then, a specific software-controlled program allows the researcher to conduct experiments with Peak Incident Power (PIP), Duty Factor (DF), Cycles per Burst (CPB), temperature control and process duration. Following recommendations from Covaris, a research Lab can optimize an extraction protocol by measuring changes in results while controlling a single variable. After each variable is considered, a final set of conditions can be locked in for reproducible results.

## PRESERVING CELL VIABILITY WHILE RENDERING CF SPUTUM LIQUID ENOUGH TO PIPET.

A key problem for patients with Cystic Fibrosis CF is the risk of developing infections from *Aspergillus fumigatus* and other filamentous fungi. Primary cultures require sputum to be inoculated

to solid agar media. Many microbiology labs would like to improve the sensitivity of primary fungal culture by getting more samples to actually grow, and to get a better separation on solid media of the yeast, bacteria and fungi.

Authors from Johns Hopkins Medical Institutes, Baltimore, MD (1) describe using the Covaris S220 Focused-ultrasonicator to liquefy sputum samples from patients with CF. Their objective was to treat the raw sputum sample from patients and compare cultures observed on solid agar. After optimization experiments, the Johns Hopkins team found the following AFA conditions to be effective, using a 2.0 ml tube and a Teflon fiber.

<b>PIP</b>	<b>100 watts</b>
<b>DF</b>	<b>50%</b>
<b>CPB</b>	<b>200</b>
<b>AFA duration</b>	<b>15 seconds</b>
<b>Temperature</b>	<b>18° C</b>

The Johns Hopkins researchers report that the Covaris treatment to liquefy sputum did not damage four fungi commonly isolated from CF patients.

## OTHER SONICATION METHOD CITED

Baxter et al (2) described homogenization of sputum in order to recover *Aspergillus fumigatus* from 1-10 ml of sputum diluted 1:1 with diluent using a probe sonicator with amplitude 295  $\mu$ m for 120 seconds in a plastic tube that was cooled by ice and using an audio baffle. "Sonication was performed using a Sonics® VC505 ultrasonic processor (Sonics and Materials Inc., Newtown, CT, USA) in a sound abating enclosure. Sonication produces a high pitched noise due to harmonics emanating from the fluid and container walls. An enclosure reduces this noise and protects the operator from accidental spillage."

## COMPARISON

#	Covaris AFA	Probe sonicator
Vessel materials	Glass with sealed cap	Open plastic tube
Contamination	Sealed tube, no aerosols	Probe directly contacts samples
Process duration per sample	15 seconds	120 seconds
Temperature control	Temp controlled bath, 18° C	Tube packed in ice
Power	100W/50%DF/200CPB	Amplitude 295 µm
Noise	Silent operation	High pitched – needs baffle

## CONCLUSION

Covaris AFA can be used to liquefy sputum from CF patients for downstream culture or other analysis. The process is safer, more precise, faster and quieter than using a probe sonicator. Due to the instrumentation and software provided, the AFA process is likely to offer more reproducible quantitative results and shorter time to clinical implementation.

## BIBLIOGRAPHY

1. ASM 2016 poster 221 “Adaptive Focused Acoustics Processing to Aid Recovery of Fungi from CF Patient Sputum” Miller, et al
2. Journal of Microbiological Methods 2011 v.85 p.75–81  
“Homogenisation of cystic fibrosis sputum by sonication — An essential step for Aspergillus PCR” Baxer, et al