Supplementary Materials

A Nanoparticle-Based Immunoassay on Facemasks for Evaluating Neutrophilic Airway Inflammation in COPD Patients

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SECTION 1. Raw Data of Colorimetric Detection of MPO on Facemasks.

Table S1. Raw data of pixel intensity (PI) and colorimetric signal (S) for MPO detected on facemasks using nanoparticles coated with anti-MPO antibodies. S = integral value of (255 - PI)" and PI was calculated in this way for each spot in the triplicates, and the same procedure was applied to the entire dataset

Log [MPO] µg/mL	MPO µg/mL	Pixel Intensity (PI)	Mean	SD	Colorimetric Signal (S)	Mean
-3.52	**0.0003**	205.13	204.75	2.24	49.87	50.25
		206.77			48.23	
		202.34			52.66	
-3.00	**0.001**	195.57	197.07	1.93	59.43	57.93
		196.40			58.60	
		199.25			55.75	
-2.52	**0.003**	186.89	184.32	2.47	68.11	70.68
		184.11			70.89	
		181.95			73.05	
-2.00	**0.01**	177.72	178.57	1.04	77.28	76.43
		179.73			75.27	
		178.28			76.72	
-1.52	**0.03**	173.79	173.99	2.11	81.21	81.02
		171.98			83.02	
		176.19			78.81	
-1.00	**0.1**	168.77	166.97	5.77	86.23	88.03
		160.51			94.49	
		171.62			83.38	
-0.52	**0.3**	161.29	157.90	3.21	93.71	97.10
		157.51			97.49	
		154.91			100.09	
0.00	**1**	153.65	151.10	2.62	101.35	103.90
		151.23			103.77	
		148.42			106.58	
0.48	**3**	147.83	145.88	1.71	107.17	109.12
		145.14			109.87	
		144.67			110.33	
1.00	**10**	137.64	137.73	1.37	117.36	117.27
		136.42			118.59	
		139.15			115.86	
1.48	**30**	132.46	131.98	1.70	122.54	123.02
		130.09			124.91	
		133.39			121.61	

SECTION 2. Example of MPO Levels in Respiratory Samples from COPD Studies.

Table S2. Summary of MPO levels studies in human respiratory samples across different COPD phases. The table includes sample type, disease condition, MPO values (mean or median) and ranges, detection methods used, and references. MPO values are reported as found in the literature. LPS: Lipopolysaccharide endotoxin challenge model; PEx: Exhaled breath particles; EBC: Exhaled breath condensate.

Respiratory Sample	COPD Phase	MPO Value	MPO Range	Detection Method	Reference
Sputum	Healthy Volunteers (non-smokers)	$3.0 \cdot 10^6$ pg·mL^{-1}	-	ELISA	[1]
Sputum	Healthy Volunteers (smokers)	$2.5 \cdot 10^6$ pg·mL^{-1}	-	ELISA	[1]
Sputum	Stable	$5.2 \cdot 10^6$ pg·mL^{-1}	0 - $1.6 \cdot 10^4$ pg·mL^{-1}	ELISA	[1]
Sputum	Exacerbated	-	$2.5 \cdot 10^3$ - $1.6 \cdot 10^4$ pg·mL^{-1}	ELISA	[1]
Sputum	Healthy Volunteers	$(6.58 \pm 1.87) \cdot 10^4$ pg·mL^{-1}	-	ELISA	[2]
Sputum	Stable	$(5.74 \pm 1.12) \cdot 10^5$ pg·mL^{-1}	-	ELISA	[2]
Sputum	Healthy Volunteers	$2.0 \cdot 10^5$ pg·mL^{-1}	-	Electrochemical immunosensor	[3]
Sputum	Stable	$9.1 \cdot 10^6$ pg·mL^{-1}	2.4-6.1 ng/mg	Electrochemical immunosensor	[3]
Sputum	Healthy Volunteers (non-smokers)	$3.0 \cdot 10^6$ pg·mL^{-1}	$2.0 \cdot 10^6$ - $1.6 \cdot 10^7$ pg·mL^{-1}	ELISA	[4]
Sputum	Healthy Volunteers (smokers)	$4.0 \cdot 10^6$ pg·mL^{-1}	$1.0 \cdot 10^6$ - $1.5 \cdot 10^7$ pg·mL^{-1}	ELISA	[4]
Sputum	Stable	$1.2 \cdot 10^7$ pg·mL^{-1}	$9.0 \cdot 10^6$ - $2.3 \cdot 10^7$ pg·mL^{-1}	ELISA	[4]
Pex	Healthy Volunteers	$1.0 \cdot 10^{-1}$ pg·mL^{-1}	$1.0 \cdot 10^{-1}$ - $8.0 \cdot 10^{-1}$ pg·mL^{-1}	LPS	[5]
EBC	Healthy Volunteers	3 mU/mL	0-9 mU/mL	Immunoassay	[6]
EBC	Exacerbated	20 mU/mL	0-70 mU/mL	Immunoassay	[6]

SECTION S3. Optimization of anti-MPO around the nanoparticles.

The ideal antibody concentration for coating nanoparticles was determined by analyzing signal responses across a range of antibody concentrations: 12.5, 25, 50, 100, and 200 μg·mL^{-1}. For each concentration, signal intensity was compared between a negative control (0 μg·mL^{-1} of MPO) and a positive control (3 μg·mL^{-1} of MPO). The optimal concentration was defined as the one that produced the greatest difference in signal between these two conditions. In the case of Myeloperoxidase (MPO) detection, 25 μg·mL^{-1} yielded the highest signal distinction and was selected as the optimal antibody concentration.

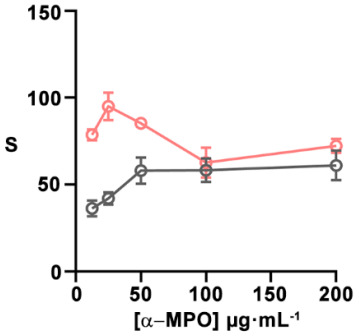

Figure S1. Testing antibody concentrations for the detection of Myeloperoxidase (MPO). Antibody performance was assessed by comparing signal intensities between negative controls (0 μg/mL MPO, displayed in gray) and samples with 3 μg/mL MPO (displayed in red). Error bars indicate the standard deviation based on three independent experiments.

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