Samples Submitted for Log in 32165

1 cotton-based mask
1 “gaiter”
1 t-shirt.
A blank mask (fresh and not worn) was provided as a control sample.

Methods

Protein Extraction

A 1 cm square was cut from the center region of the mask and placed in an Eppendorf tube. From that 1 cm square, the sample was cut into smaller pieces to increase the surface area. Roughly 5 mm square of each sample was used for further experiments. Each piece of mask was soaked in 100 mL of 0.2% Surfactant Enhancer (Promega, Madison WI) at 4°C overnight to extract protein. For the t-shirt samples, a 1 cm square was cut from the front bottom and from the front top (near the collar bone) and processed identical to the mask samples.

In Solution Digestion

Total protein was determined on a Qubit and the appropriate volume of each sample was taken to equal 20 μg total protein for digestion. The samples were digested with sequencing grade trypsin/lys C rapid digestion kit from Promega (Madison WI) using manufacture recommended protocol. Three times the sample volume of rapid digestion buffer (provided with the kit) was added to the samples. The sample was incubated at 56°C with 1 μl of dithiothreitol (DTT) solution (0.1 M in 100 mM ammonium bicarbonate) for 30 minutes prior to the addition of 0.54 μL of 55 mM Iodoacetamide in 100 mM ammonium bicarbonate. Iodoacetamide was incubated at room temperature in dark for 30 min. The trypsin/lys C was prepared fresh as 1 μg/μl in the rapid digestion buffer. 1 μl of enzyme was added and the samples were incubated at 70°C for 1 hour. The digestion was stopped with addition of 0.5% TFA. The MS analysis is immediately performed to ensure high quality tryptic peptides with minimal non-specific cleavage.

Q Exactive HF Orbitrap

Nano-liquid chromatography tandem mass spectrometry (Nano-LC/MS/MS) was performed on a Thermo Scientific Q Exactive HF Orbitrap mass spectrometer equipped with a EASY Spray nanospray source (Thermo Scientific) operated in positive ion mode. The LC system was an UltiMate™ 3000 RSLCnano system from Thermo Scientific. The mobile phase A was water containing 0.1% formic acid and the mobile phase B was acetonitrile with 0.1 % formic acid. The mobile phase A for the loading pump was water containing 0.1 % trifluoracetic acid. 5 μL of sample is injected on to a PharmaFluidics μPAC™ C18 trapping column (C18, 5 μm pillar diameter, 10 mm length, 2.5 μm inter-pillar distance). at 10 μL/ml flow rate. This was held for 3 minutes and washed with 1 %B to desalt and concentrate the peptides. The injector port was switched to inject and the peptides were eluted off of the trap onto the column. PharmaFluidics 50 cm μPAC™ was used for

chromatographic separations (C18, 5 μm pillar diameter, 50 cm length, 2.5 μm inter-pillar distance). The column temperature was maintained 40°C. A flowrate of 750 nl/min was used for the first 15 minutes and then the flow was reduced to 300 nl/min. Peptides were eluted directly off the column into the Q Exactive system using a gradient of 1% B to 20%B over 100 minutes and then to 45%B in 20 minutes for a total run time of 150 minutes:

Time (min)	% B	Flow Rate (nL/min)
0	1	750
3	1	750
15	5	750
15.1	5	300
100	20	300
123	45	300
130	95	300
135	95	300
135.1	1	300
150	1	300

The total run time was 150 minutes. The MS/MS was acquired according to standard conditions established in the lab. The EASY Spray source operated with a spray voltage of 1.5 KV and a capillary temperature of 200°C. The scan sequence of the mass spectrometer was based on the original TopTen™ method; the analysis was programmed for a full scan recorded between 375 – 1575 Da at 60,000 resolution, and a MS/MS scan at resolution 15,000 to generate product ion spectra to determine amino acid sequence in consecutive instrument scans of the fifteen most abundant peaks in the spectrum. The AGC Target ion number was set at 3e6 ions for full scan and 2e5 ions for MS^2 mode. Maximum ion injection time was set at 50 ms for full scan and 55 ms for MS^2 mode. Micro scan number was set at 1 for both full scan and MS^2 scan. The HCD fragmentation energy (N)CE/stepped NCE was set to 28 and an isolation window of 4 *m/z*. Singly charged ions were excluded form MS^2. Dynamic exclusion was enabled with a repeat count of 1 within 15 seconds and to exclude isotopes. A Siloxane background peak at 445.12003 was used as the internal lock mass.
HeLa protein digest standard is used to evaluate the integrity and the performance of the columns and mass spectrometer. If the number of protein ID's from the HeLa standard falls below 2700, the instrument is cleaned and new columns are installed.

All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific, San Jose, CA, USA; version IseNode in Proteome Discoverer 2.4.0.305). Sequest was set up to search Full Swiss Prot Database of all species (7/27/2020 475603 sequences) and the SARS2 Covid database (4/14/2021 855 sequences) assuming the digestion enzyme trypsin. Sequest was searched with a fragment ion mass tolerance of 0.020 Da and a parent ion tolerance of 10.0 PPM. Carbamidomethyl of cysteine was specified in Sequest as a fixed modification.

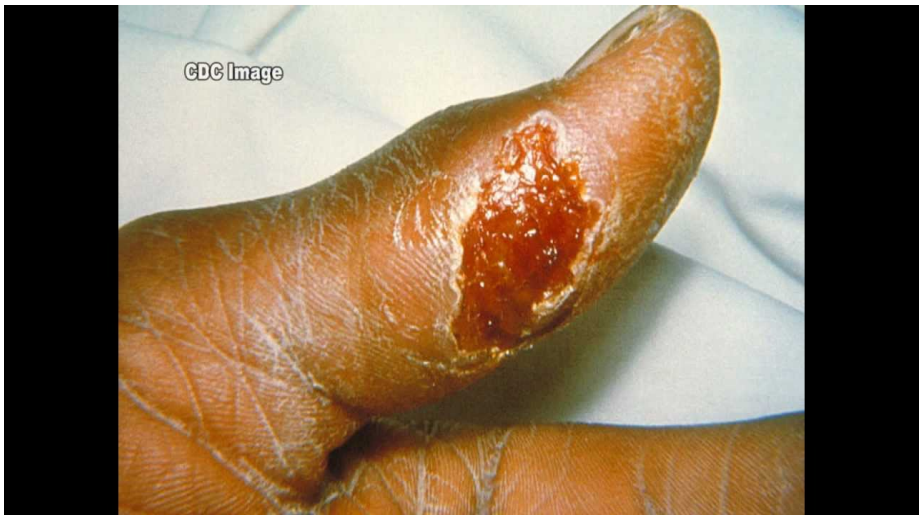
Met-loss of methionine, met-loss+Acetyl of methionine, oxidation of methionine and acetyl of the n-terminus were specified in Sequest as variable modifications.

Results

Black and white cotton mask:
Total of 36 proteins identified and listed in the Excel spreadsheet. The most abundant proteins detected are human proteins found in saliva and skin. The following bacteria proteins were detected.

Bacteria	**Comment**
Rhodococcus opacus	Soil dwelling
Bifidobacterium adolescentis	human gut microbiota
Pediococcus pentosaceus	Produces lactic acid – found in cheese and processed meats
Francisella tularensis	Pathogenic Causes tularemia, fever, skin ulcers, sore throat and pneumonia
Salinispora tropica	soil dwelling/sand
Actinobacillus pleuropneumoniae	Pathogenic - Respitory pathogen in swine
Cutibacterium acnes	Causes acne, blephartis and endophthalmitis
Borrelia burgdorferi	Cause lyme disease
Beutenbergia cavernae	soil dwelling
Escherichia coli	found in lower intestine and can cause food poisoning
Desulfotalea psychrophila	marine bacteria
Shewanella frigidimarina	marine bacteria

Not all bacteria are harmful or pathogenic, and many are a natural part of the human flora on skin, saliva, or in the gut; and natural to the environment in soil and water. However, 4 pathogenic bacteria, were detected and highlighted in yellow. There was also one bacteria that are harmful to livestock but not pathogenic to humans.
Here is an image of the infection francisella tularensis.



Black grey “gaiter” mask

Total of 130 proteins identified and listed in the Excel spreadsheet. The most abundant proteins detected are human proteins found in saliva and skin. The following bacteria proteins were detected. Mycolicibacterium paratuberculosis link to Crohn’s disease is controversial in the literature. Interestingly Pelotomaculum thermopropionicum is a bacteria that survives an oxygen free and a warm temperature environment.

Bacteria	**Comment**
kocuria rhizophila	Soil dwelling
shewanella piezotolerans	Marine bacteria
pelotomaculum thermopropionicum	Anaerobic and thermophilic bacteria
mycolicibacterium paratuberculosis	Link to Crohn’s disease known to be pathogenic to bovine
pseudarthrobacter chlorophenolicus	Soil dwelling
paenarthrobacter aurescens	Soil dwelling
rhodococcus erythropolis	Soil dwelling
kocuria rhizophila	Soil dwelling

Blank mask (Control)

A total of 10 proteins were identified and are all accounted for in the sample preparation steps. For example, trypsin and Lys C enzymes were detected because we add that to the samples digest the proteins. No bacterial proteins were detected.

T-shirt

A total of 47 proteins were identified from the “front-bottom” t-shirt sample. The most abundant protein was keratin which a protein in human skin and hair. Two

bacteria were detected but not pathogenic to human and found in the normal environment.

Bacteria	**Comment**
Mycoplasma arthritidis	Pathogen to rats
Schizosaccharomyces pombe	“fission yeast” used in traditional brewing (beer).

A total of 105 proteins were identified from the “front-top” t-shirt sample. The most abundant protein was keratin, which is a protein in human skin and hair. Only one soil dwelling bacteria was detected.

Bacteria	**Comment**
Rhodopseudomonas palustris	Soil dwelling