

## **Samples Submitted for Log in 32165**

1 pink tie-dye surgical mask (#8)

2 blue tie-dye surgical masks (#9 – third grade, #10 – fourth grade)

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.

### **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 % trifluoroacetic 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<sup>2</sup> mode. Maximum ion injection time was set at 50 ms for full scan and 55 ms for MS<sup>2</sup> mode. Micro scan number was set at 1 for both full scan and MS<sup>2</sup> 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 from MS<sup>2</sup>. 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

## Pink Mask (#8)

Total of 274 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.

methanothermobacter  
thermautotrophicus

interesting that this only grows in warm temperatures  
(55 oC - 65 oC and need carbon dioxide to grow

acinetobacter baumannii

Pathogenic pneumonia, blood stream infections,  
meningitis, wound and surgical site infections and  
urinary tract infections Resistant to antibiotics and very  
difficult to treat.

picophilus torridus

soil dwelling only grows in warm environments

listeria innocua serovar

non-pathogenic version

novosphingobium aromaticivorans

pathogenic gut microbe similar to e. coli

alcelaphine herpesvirus 1

Virus - natural hosts primarily cow but is fatal

streptomyces griseus

soil dwelling used to produce streptomycin, an  
antibiotic

frankia casuarinae

soil dwelling

saccharomyces cerevisiae

species of yeast - used for baking and making beer

paraburkholderia phytofirmans

found on pine trees - all the pollen in the air

corynebacterium kroppenstedtii

antibiotic resistant pathogen

corynebacterium glutamicum

soil dwelling

streptococcus pyogenes

Strep throat

encephalitozoon cuniculi

Pathogenic in immunocompromised people

prochlorococcus marinus

marine bacteria

streptococcus pneumoniae

porphyromonas gingivalis

significant human pathogen - major cause pneumonia  
Pathogenic Found in the oral cavity causing periodontal  
disease as well as upper gastrointestinal tract, respiratory  
infections

mycobacterium tuberculosis

Pathogenic Causes Tuberculosis

cupriavidus necator

soil dwelling capable of both aerobic and anaerobic  
growth

neisseria meningitidis

extremely pathogenic Causes meningitis and life  
threatening sepsis

staphylococcus aureus

Pathogenic range of illnesses from minor skin infections to life threatening pneumonia, meningitis and sepsis

brucella melitensis

infectious to livestock - mainly sheep

parabacteroides distasonis

Pathogenic

geobacillus stearothermophilus

soil dwelling causes food spoilage

corynebacterium jeikeium

Pathogenic infection in bone marrow transplant patients

polaromonas naphthalenivorans

found in water

nitrosomonas europaea

soil dwelling

actinobacillus pleuropneumoniae

Pathogenic to swine

staphylococcus epidermidis

Part of normal skin flora

mycolicibacterium vanbaalenii

soil dwelling

saccharomyces cerevisiae

species of yeast - used for baking and making beer

lactobacillus gasseri

gastrinointestinal tract bacteria

synechococcus sp

freshwater bacteria

neisseria meningitidis Serogroup C

menigocccal disease. About 1 in 10 people have these bacteria in their nose and throat without being ill. However when it invades the body casues serious disease with fever, headach and stiff neck

staphylococcus suis

infectious to swine but can cause severe infection in human

Bifidobacterium longum subsp. Intantis

Normal gut bacteria

buchnera aphidicola subsp

soil dwelling

laribacter hongkongensis

anaerobic bacteria potential human pathogen

eikenella corrodens

anaerobic bacteria severe human pathogen

neisseria meningitidis Serogroup B

menigocccal disease. About 1 in 10 people have these bacteria in their nose and throat without being ill. However when it invades the body casues serious disease with fever, headach and stiff neck

Corynebacterium efficiens

soil dwelling

Rickettsia rickettsii

Pathogenic causes Rocky Mountain Spotted Fever

Corynebacterium diphtheriae

Causes diphtheria - a serious infection - most are vaccinated

Clavibacter michiganensis subsp

Pathogenic to tomatos

chromobacterium violaceum

**Legionella pneumophila**

Altermonas mediterranea

Acidphilium cryptum

streptococcus salivarius

cunninghamella elegans

shewanella piezotolerans

Flavobacterium johnsoniae

Bacteriodes vulgatus

Bacteriodes thetaiotaomicron

rhodococcus erythropolis

Nostoc sp

Bacillus cereus

Bacteriodes fragilis

Sulcisa muelleni

mycoplasma mycoides subsp mycoides  
SC

**Corynebacterium aurimucosum**

**streptococcus agalactiae serotype III**

Paenarthrobacter aurescens

**streptococcus dysgalactiae subsp.**

**Equisimilis**

**staphylococcus pyogenes serotype M3**

beutenbergia cavernae

staphylococcus oralis

**staphylococcus saprophyticus**

Dechloromonas aromatica

Coxiella burnetii

Dichelobacter modosus

Acidovorax sp

soil dwelling. Disease to human is rare but mortality is high

**Pathogenic causes Legionnaires' disease**

Marine bacteria

soil dwelling

Found in the oral cavity - opportunistic pathogen.

Harmless unless it enters the bloodstream

fungus found in soil

marine bacteria

soil dwelling

human gut microbiota

human gut microbiota

soil dwelling

soil dwelling

soil dwelling

human gut microbiota

normal insect bacteria

Pathogenic to bovine

**causes UTI**

**invasive human infections**

soil dwelling

**human pathogen antibiotic resistant**

**Strep - severe invasive infection**

soil dwelling

Found in the oral cavity - opportunistic pathogen.

Harmless unless it enters the bloodstream

**common cause of UTI**

soil dwelling

Pathogenic to farm animals like goats, sheep, and bovine

Pathogenic to sheep

soil dwelling

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, 21 pathogenic bacteria, were detected and highlighted in yellow. Some are quite dangerous.

Blue Mask #9

Total of 150 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.

acaryochloris marina  
emenicella nidulans

bacteria found in water  
mold associated with numerous health problems

#### Blue Mask #10

Total of 68 proteins identified and listed in the Excel spreadsheet. The most abundant proteins detected are human proteins found in saliva and skin. The bacterial proteins detected are right at the threshold for a confident identification and is not considered significant.

#### 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.