

# Dangerous pathogens found on children's face masks

in [COVID-19](#)



06/16/2021

## 6 MASKS, WORN FOR AN AVERAGE OF 5.7 HOURS/DAY

83%

CONTAMINATED

96

UNIQUE STRAINS

21

PATHOGENIC STRAINS

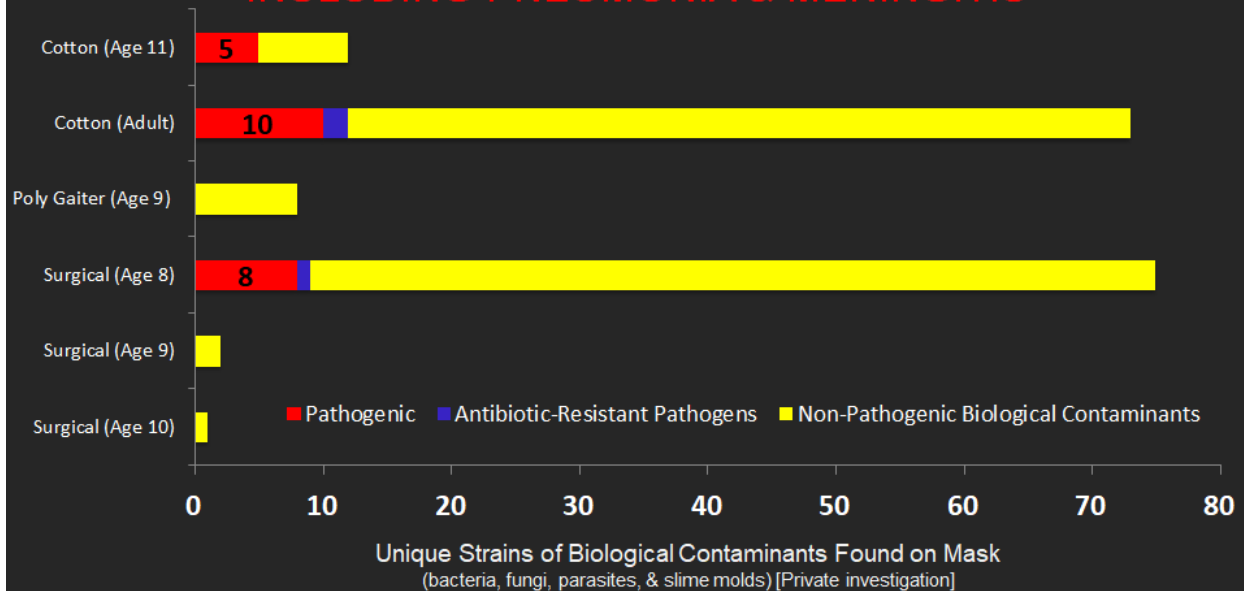
50%

MASKS CONTAMINATED W/PATHOGENS

33%

W/ANTIBIOTIC-RESISTANT STRAINS

MULTIPLE STRAINS FOUND CAUSING DANGEROUS DISEASES, INCLUDING PNEUMONIA & MENINGITIS



BY JENNIFER CABRERA

A group of parents in Gainesville, FL, sent 6 face masks to a lab at the University of Florida, requesting an analysis of contaminants found on the masks after they had been worn. The resulting report found that five masks were contaminated with bacteria, parasites, and fungi, including three with dangerous pathogenic and pneumonia-causing bacteria. Although the test is capable of detecting viruses, including SARS-CoV-2, only one virus was found on one mask (*alcelaphine herpesvirus 1*).

The analysis detected the following 11 dangerous pathogens on the masks:

- *Streptococcus pneumoniae* (pneumonia)
- *Mycobacterium tuberculosis* (tuberculosis)
- *Neisseria meningitidis* (meningitis, sepsis)
- *Acanthamoeba polyphaga* (keratitis and granulomatous amebic encephalitis)
- *Acinetobacter baumannii* (pneumonia, blood stream infections, meningitis, UTIs—resistant to antibiotics)
- *Escherichia coli* (food poisoning)
- *Borrelia burgdorferi* (causes Lyme disease)
- *Corynebacterium diphtheriae* (diphtheria)
- *Legionella pneumophila* (Legionnaires' disease)
- *Staphylococcus pyogenes serotype M3* (severe infections—high morbidity rates)
- *Staphylococcus aureus* (meningitis, sepsis)

Half of the masks were contaminated with one or more strains of pneumonia-causing bacteria. One-third were contaminated with one or more strains of meningitis-causing bacteria. One-third were contaminated with dangerous, antibiotic-resistant bacterial pathogens. In addition, less dangerous pathogens were identified, including pathogens that can cause fever, ulcers, acne, yeast infections, strep throat, periodontal disease, Rocky Mountain Spotted Fever, and more.

| PATHOGEN                                     | TYPE     | DESCRIPTION   |
|--|----------|---|
| <b>acinetobacter baumannii</b>               | Bacteria | pneumonia, blood stream infections, meningitis, wound and surgical site infections and urinary tract infections Resistant to antibiotics and very difficult to treat. |
| <b>alcelaphine herpesvirus 1</b>             | Virus    | Natural hosts primarily cow, but is fatal   |
| <b>Borrelia burgdorferi</b>                  | Bacteria | Causes Lyme disease   |
| <b>corynebacterium jeikeium</b>              | Bacteria | infection in bone marrow transplant patients  |
| <b>corynebacterium kroppenstedtii</b>        | Bacteria | antibiotic resistant pathogen   |
| <b>cutibacterium acnes</b>                   | Bacteria | Causes acne, blepharitis and endophthalmitis  |
| <b>encephalitozoon cuniculi</b>              | Bacteria | Pathogenic in immunocompromised people  |
| <b>Escherichia coli</b>                      | Bacteria | Found in lower intestine and can cause food poisoning   |
| <b>francisella tularensis</b>                | Bacteria | Causes tularemia, fever, skin ulcers, sore throat and pneumonia   |
| <b>mycobacterium tuberculosis</b>            | Bacteria | Causes Tuberculosis   |
| <b>neisseria meningitidis Serogroup A</b>    | Bacteria | Extremely pathogenic. Causes meningitis and life threatening sepsis   |
| <b>neisseria meningitidis Serogroup B</b>    | Bacteria | Extremely pathogenic. Causes meningitis and life threatening sepsis   |
| <b>neisseria meningitidis Serogroup C</b>    | Bacteria | Extremely pathogenic. Causes meningitis and life threatening sepsis   |
| <b>parabacteroides distasonis</b>            | Bacteria | Causes infections   |
| <b>porphyromonas gingivalis</b>              | Bacteria | Found in the oral cavity causing periodontal disease as well as upper gastrointestinal tract, respiratory infections  |
| <b>Rickettsia rickettsii</b>                 | Bacteria | Rocky Mountain Spotted Fever  |
| <b>staphylococcus aureus</b>                 | Bacteria | range of illnesses from minor skin infections to life threatening pneumonia, meningitis and sepsis  |
| <b>streptococcus pneumoniae</b>              | Bacteria | Major cause pneumonia   |
| <b>streptococcus pneumoniae serotype 19F</b> | Bacteria | Major cause of pneumonia  |
| <b>streptococcus pyogenes</b>                | Bacteria | Causes strep throat   |
| <b>streptococcus pyogenes serotype M3</b>    | Bacteria | Causes strep throat   |

Here is an image of the infection *francisella tularensis*, which causes tularemia, fever, skin ulcers, sore throat, and pneumonia:



The face masks studied were new or freshly-laundered before wearing and had been worn for 5 to 8 hours, most during in-person schooling by children aged 6 through 11. One was worn by an adult. A t-shirt worn by one of the children to school and unworn masks were tested as controls. No pathogens were found on the controls; samples from the front top and bottom of the t-shirt found proteins that are commonly found in skin and hair, along with some commonly found in soil.

A parent who participated in the study, Ms. Amanda Donoho, commented that this small sample points to a need for more research: "We need to know what we are putting on the faces of our children each day. Masks provide a warm, moist environment for bacteria to grow."

The parents contracted with the lab because they were concerned about the potential of contaminants on masks that their children were forced to wear all day at school, taking them on and off, setting them on various surfaces, wearing them in the bathroom, etc. This prompted them to send the masks to the University of Florida's Mass Spectrometry Research and Education Center for analysis.

[Click to view the mask reports.](#)

<https://rationalground.com/mask-reports-from-lab/>



06/16/2021



## **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<br>thermautotrophicus | interesting that this only grows in warm temperatures<br>(55 oC - 65 oC and need carbon dioxide to grow   |
| acinetobacter baumannii                   | Pathogenic pneumonia, blood stream infections,<br>meningitis, wound and surgical site infections and<br>urinary tract infections Resistant to antibiotics and very<br>difficult to treat. |
| picrophilus 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<br>antibiotic  |
| frankia casuarinae                        | soil dwelling   |
| saccharomyces cerevisiae                  | species of yeast - used for baking and making beer  |
| paraburkholderia phytofirmans             | found on pie 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                  | significant human pathogen - major cause pneumonia  |
| porphyromonas gingivalis                  | Pathogenic Found in the oral cavity causing periodontal<br>disease as well as upper gastrointestinal tract, respiratory<br>infections   |
| mycobacterium tuberculosis                | Pathogenic Causes Tuberculosis  |
| cupriavidus necator                       | soil dwelling capable of both aerobic and anaerobic<br>growth   |
| neisseria meningitidis                    | extremely pathogenic Causes meningitis and life<br>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

gastrointestinal tract bacteria

synechococcus sp

freshwater bacteria

neisseria meningitidis Serogroup C

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

staphylococcus suis

infectious to swine but can cause severe infection in human

Bifidobacterium longum subsp. infantis

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

meningococcal disease. About 1 in 10 people have these bacteria in their nose and throat without being ill. However when it invades the body causes serious disease with fever, headache 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 tomatoes

chromobacterium violaceum

**Legionella pneumophila**

Alteromonas mediterranea

Acidiphilium 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

bacteria found in water

emenicella nidulans

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.



## **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**

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chromatographic separations (C18, 5  $\mu\text{m}$  pillar diameter, 50 cm length, 2.5  $\mu\text{m}$  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.

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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.

| <b>Bacteria</b>                 | <b>Comment</b>  |
|---------------------------------|---|
| Rhodococcus opacus              | Soil dwelling   |
| Bifidobacterium adolescentis    | human gut microbiota  |
| Pediococcus pentosaceus         | Produces lactic acid – found in cheese and processed meats                    |
| Francisella tularensis          | Pathogenic<br>Causes tularemia, fever, skin ulcers, sore throat and pneumonia |
| Salinispora tropica             | soil dwelling/sand  |
| Actinobacillus pleuropneumoniae | Pathogenic -<br>Respiratory pathogen in swine                                 |
| Cutibacterium acnes             | Causes acne, blepharitis 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.

| <b>Bacteria</b>                    | <b>Comment</b>   |
|------------------------------------|--|
| 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.

| <b>Bacteria</b>                  | <b>Comment</b>                                      |
|----------------------------------|---|
| <i>Mycoplasma arthritidis</i>    | Pathogen to rats                                    |
| <i>Schizosaccharomyces pombe</i> | "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.

| <b>Bacteria</b>                   | <b>Comment</b> |
|-----------------------------------|----------------|
| <i>Rhodopseudomonas palustris</i> | Soil dwelling  |



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

1 cotton-based mask

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.

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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

Black and white cotton mask:

Total of 305 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<br>thermautotrophicus | interesting that this only grows in warm temperatures<br>(55 oC - 65 oC and need carbon dioxide to grow)  |
| acinetobacter baumannii                   | Pathogenic pneumonia, blood stream infections,<br>meningitis, wound and surgical site infections and<br>urinary tract infections Resistant to antibiotics and very<br>difficult to treat.   |
| microphilus torridus                      | soil dwelling only grows in warm environments   |
| listeria innocua serovar                  | non-pathogenic version  |
| novosphingobium aromaticivorans           | pathogenic gut microme similar to e. coli   |
| alcelaphine herpesvirus 1                 | Virus - natural hosts primarily cow but is fatal  |
| streptomyces griseus                      | soil dwelling used to produce streptomycin, an<br>antibiotic  |
| frankia casuarinae                        | soil dwelling   |
| saccharomyces cerevisiae                  | species of yeast - used for baking and making beer  |
| paraburkholderia phytofirmans             | found on pie 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<br>Pathogenic Found in the oral cavity causing periodontal<br>disease as well as upper gastrointestinal tract, respiratory<br>infections |
| mycobacterium tuberculosis                | Pathogenic Causes Tuberculosis  |
| cupriavidus necator                       | soil dwelling capable of both aerobic and anaerobic<br>growth   |
| neisseria meningitidis                    | extremely pathogenic Causes meningitis and life<br>threatening sepsis   |
| staphylococcus aureus                     | Pathogenic range of illnesses from minor skin infections<br>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 diptheria - a serious infection - most are vacinnated   |
| Clavibacter michiganensis subsp        | Pathogenic to tomatos  |
| chromobacterium violaceum              | soil dwelling. Disease to human is rare but mortality is high  |
| Legionella pneumophila                 | Pathogenic causes Legionnaires' disease  |
| Altermonas mediterranea                | Marine bacteria  |

|  |   |
|--|---|
| Acidphilium cryptum                          | soil dwelling   |
| streptococcus salivarius                     | Found in the oral cavity - opportunistic pathogen.<br>Harmless unless it enters the bloodstream |
| cunninghamella elegans                       | fungus found in soil  |
| shewanella piezotolerans                     | marine bacteria   |
| Flavobacterium johnsoniae                    | soil dwelling   |
| Bacteriodes vulgatus                         | human gut microbiota  |
| Bacteriodes thetaiotaomicron                 | human gut microbiota  |
| rhodococcus erythropolis                     | soil dwelling   |
| Nostoc sp                                    | soil dwelling   |
| Bacillus cereus                              | soil dwelling   |
| Bacteriodes fragilis                         | human gut microbiota  |
| Sulcisa muelleni                             | normal insect bacteria  |
| mycoplasma mycoides subsp mycoides           | Pathogenic to bovine  |
| SC   |   |
| <b>Corynebacterium aurimucosum</b>           | <b>causes UTI</b>   |
| <b>streptococcus agalactiae serotype III</b> | <b>invasive human infections</b>  |
| Paenarthrobacter aureus                      | soil dwelling   |
| <b>streptococcus dysgalactiae subsp.</b>     | <b>human pathogen antibiotic resistant</b>  |
| Equisimilis                                  |   |
| <b>staphylococcus pyogenes serotype M3</b>   | <b>Strep - severe invasive infection</b>  |
| beutenbergia cavernae                        | soil dwelling   |
| staphylococcus oralis                        | Found in the oral cavity - opportunistic pathogen.<br>Harmless unless it enters the bloodstream |
| <b>staphylococcus saprophyticus</b>          | <b>common cause of UTI</b>  |
| Dechloromonas aromatica                      | soil dwelling   |
| Coxiella burnetii                            | Pathogenic to farm animals like goats, sheep, and bovine  |
| Dichelobacter modosus                        | Pathogenic to sheep   |
| Acidovorax sp                                | 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.

#### 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