

Testing the Effectiveness of Five Widely Used Masks

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I. Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) virus, has swept the globe. Aerosols and droplets are thought to be the primary means of transmission for this virus. The main pathways of person-to-person transmission are droplet transmission and airborne transmission. Despite the ongoing scientific controversy over the major form of transmission, it has been determined that airborne transmission is most likely the dominant route of transmission, particularly in enclosed environments with little ventilation. Countries around the world have issued restrictions such as lockdowns as well as suggestions on social distancing, hand hygiene, and the use of personal protective equipment (PPE), use of face coverings/masks.

During the COVID-19 pandemic, the use and demand for facemasks skyrocketed since early 2020. The usage of masks has been demonstrated to aid in the prevention of COVID-19 infection and to reduce the transmission of the virus. According to studies, scientists discovered that wearing masks offered strong protection and reduced transmission instances by 47% in a study of non-health professionals. Face masks are referred to as personal protection equipment that is designed to reduce the spread of airborne particles or droplets. Masks are worn to prevent infected people from spreading the virus to others (source control) and/or to protect healthy people from infection (protection). Surgical masks, face filtering pieces (e.g. FFPs, N95, KN95), and non-medical or community face coverings are the three basic types of face masks.

Masks are currently being used worldwide as an urgent response to the COVID-19 pandemic. Throughout several months, masks have been developed in

different materials and sizes, some being up to \$30 each, and some as low as \$1. Which mask is the most effective against COVID-19, regardless of price? In this experiment, five of the most popular masks were collected; one-ply cloth, two-ply cloth, cheap surgical, (C.S.), expensive surgical (E.S.), and KN95. According to studies, KN95 masks are known to have electrostatic filtration and are the most effective of the five used in the experiment.

The initial hypothesis stated that if masks that are more expensive provide more safety and reduce the risk of getting a bacterial infection or a virus, then KN95 or [expensive] surgical masks should be used because they have been proved to be highly effective by medical experts and have better protection against bacteria or viruses trying to get in through the mask. The experiment was conducted twice to ensure repeatable results that would either support or disprove the initial hypothesis. However, the initial hypothesis was reformulated after observing the first trial because it was noted that the C.S. mask was the most effective possibly due to its material and low water absorption.

There were few experiments on the effectiveness of masks against bacteria online which prompted the experiment to discover which mask was the most effective against bacteria rather than viruses. Using a unique approach, five of the most popular masks were tested using bacteria instead of viruses to determine if prokaryotic cells could pass through the masks. Bacteria are larger in size than viruses, thus, assuming if bacteria can pass through, viruses should pass through more easily. Without assuming extraneous variables, the fewer bacteria that pass through the mask and onto the BHI agar, the fewer viruses will pass through as well. The initial expectation of this experiment was to determine if the KN95 mask offers the most protection as discovered in the research.

II. Materials and Methods

Independent Variable: The type of mask

Dependent Variable: The amount of respiratory bacteria (*Neisseria sicca*) that passed through the mask and grew on the BHI agar

Control Set-up: No mask on the BHI agar

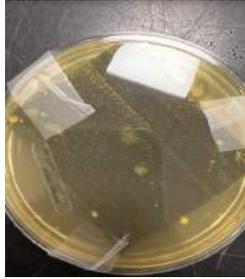
1. The materials were collected: Brain Heart Infusion Agar Plates, Live *Neisseria sicca*, five types of masks (one-ply, two-ply, cheap surgical, expensive surgical, KN95), incubator, ruler, map pins, wire cutter, 50 ml beaker of water, dropper, compound light microscope
2. All of the table surfaces were cleaned thoroughly with Envirocleanse A (a disinfectant).
3. The BHI Agar plates, *Neisseria sicca* tube, ruler, map pins, wire cutter, and masks were placed on the table.
4. Each mask was taken out of the plastic bag and cut in half (the extra went back into the bag) and then into 6" by 6" squares in the middle (sides, which had thick material, was avoided)
5. Twenty map pins were cut to fit the depth of the agar (about 1") and pinned through the corners of each mask (four pins were used for each mask).
6. Masks were carefully placed on the agar (inside part facing the agar) and pins were pushed through the agar to keep the mask in place.
7. Using an inoculating loop, about half of *Neisseria sicca* was collected on the inner part of the inoculating loop and spread on the surface of the mask.

8. Each Petri dish was taped securely using three pieces of tape.
9. The Petri dishes were then taped together, flipped over, and placed in an incubator for one week.
10. The results were measured by counting the number of colonies present on each dish. The length and width (or diameter) were measured to make a conclusion.
11. All materials were sterilized and sanitized after experimentation was done. The agar plates were cleaned with bleach, put in a plastic bag, and thrown in the trash.
12. Repeat steps 1-11 for the second trial.
13. The petri dish with the least bacteria colonies on the agar plate (not on the mask) was determined the most protective, and vice versa.
14. A sample of the bacteria was scooped from the agar and diluted in water; one drop of the solution was collected on a slide and observed under a compound light microscope (on 10x power and 40x power).

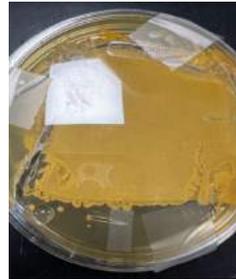
III. Results

Qualitative Data:

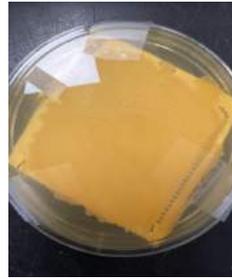
Trial 1:



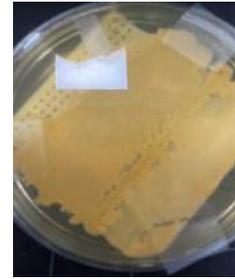
Cheap Surgical



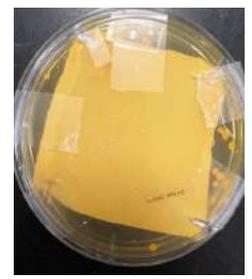
Two-Ply Cloth



KN95



Expensive Surgical



One-Ply



Cloth

All masks had bacterial spread outside the mask area and some condensation on the coverslip of the Petri dishes. The cheap surgical mask is observed to have bacterial colonies spread out over different distances. The two-ply cloth and KN95 mask bacterial “lawn” is thick and not as transparent as the other masks. The expensive surgical mask has a very long bacterial “lawn” near the top half of the mask. The one-ply mask seemed to absorb water and has bacterial colonies spread outside the mask area, there are more bacteria observed on the right rather than on the other sides.

Trial 2:



When compared to the previous models of Trial 1, the two-ply cloth and KN95 have a similar bacterial spread of thickness and transparency. All masks have some bacterial spread outside the mask area. The cheap surgical mask is observed to have a bacteria “lawn” instead of separate colonies as seen in the last trial. The bacterial “lawn” of the cheap surgical seems very transparent as the texture of the mask is clearly seen through the bacteria. The expensive surgical also has a wider spread of bacteria when compared to the last trial. Not only does the expensive surgical have a bacterial “lawn” on the top half of the mask, but also on the bottom half and around the sides.

Amount of Bacterial Growth on Five Different Masks After a Seven Day Period

Trial #	Measurement (average)	Control	One-Ply Cloth	Two-Ply Cloth	Cheap Surgical (C.S.)	Expensive Surgical (E.S)	KN95
1	Length (longest bacterial spread)	5 cm	Could not be determined	7.3 cm	(Diameter of largest colony) 0.5 cm	7.2 cm	7 cm
	Width (perpendicular to length)	3.7 cm	Could not be determined	5.4 cm	(Diameter of smallest colony) 0.2 cm	5.3 cm	5.5 cm
2	Length (longest bacterial spread)	Not tested	Not tested	9 cm	6.7 cm	6.5 cm	7.5 cm
	Width (perpendicular to length)	Not tested	Not tested	7.5 cm	6.5 cm	5.4 cm	5.3 cm

Trial 1: Length of Bacterial Growth (cm) on Five Different Masks After a Seven Day Period

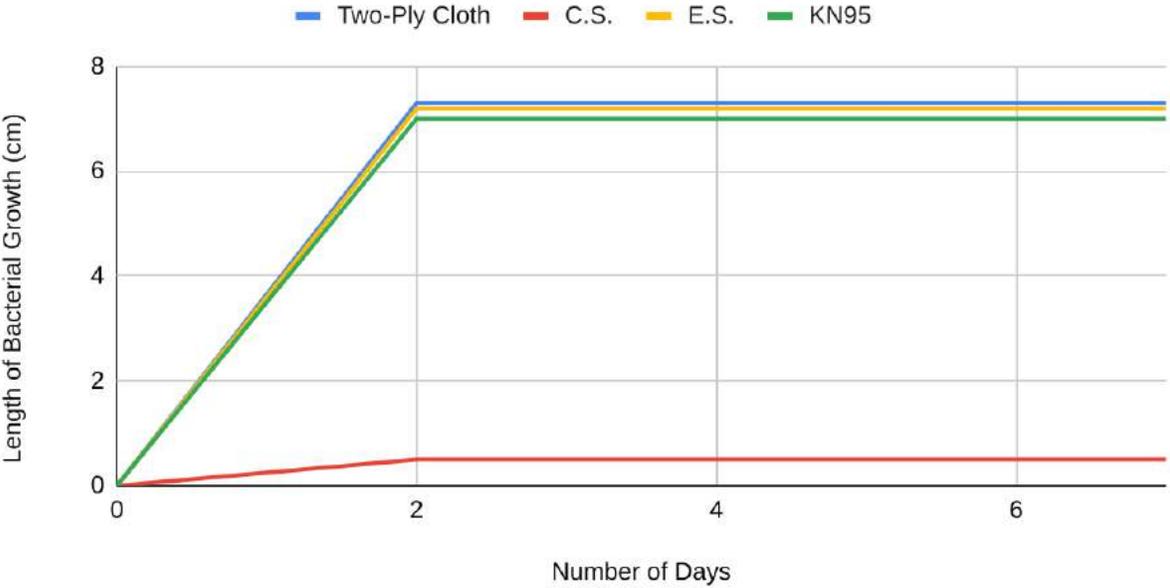


Figure 1: The length of the bacterial colony/"lawn" was measured by the longest spread seen.

Trial 1: Width of Bacterial Growth (cm) on Five Different Masks After a Seven Day Period

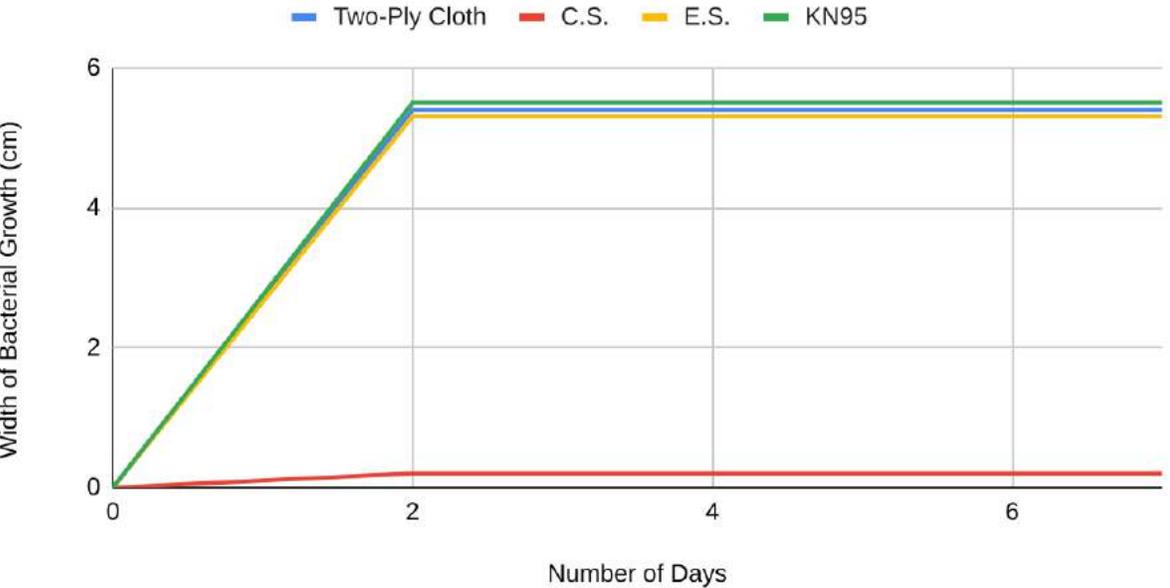


Figure 2: The width of the bacterial colony/"lawn" was measured perpendicular to the length.

Trial 2: Length of Bacterial Growth (cm) on Five Different Masks After a Seven Day Period

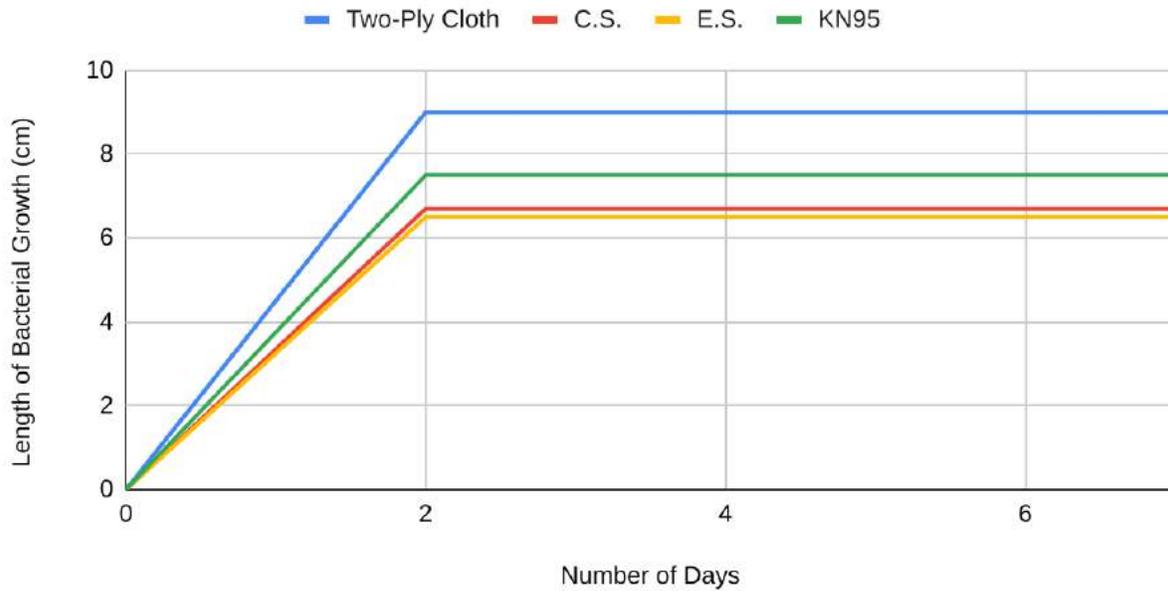


Figure 3: The length of the bacterial colony/"lawn" was measured by the longest spread seen.

Trial 2: Width of Bacterial Growth (cm) on Five Different Masks After a Seven Day Period

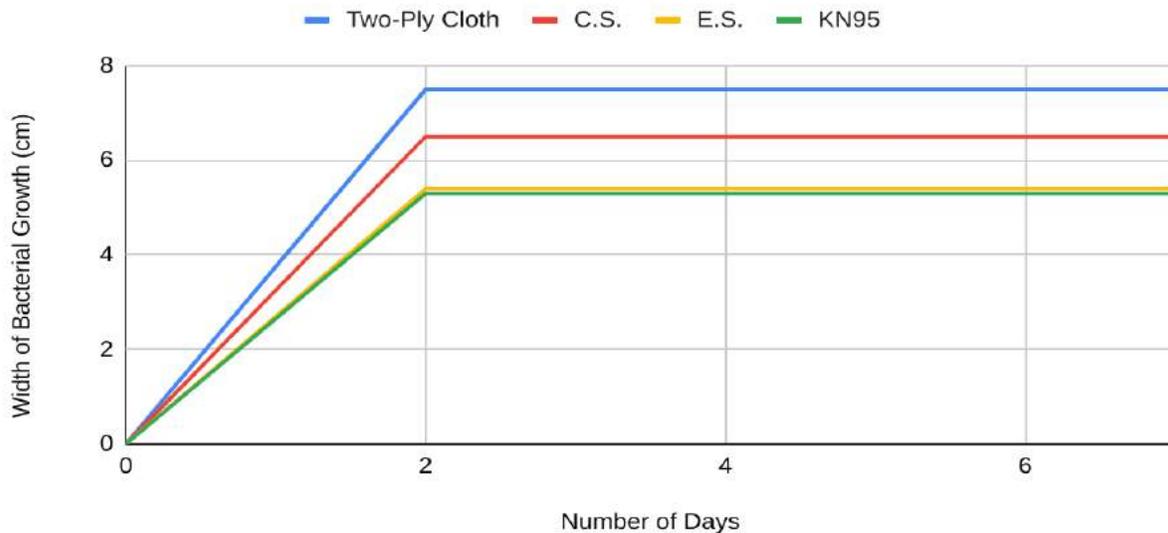


Figure 4: The width of the bacterial colony/"lawn" was measured perpendicular to the length.

Analysis:

After being incubated for one week, the bacterial growth on the Petri dishes was measured by length (longest bacterial spread) and by its width (perpendicular to the length). According to the data table and Figures 1 and 2, the C.S. mask was the most effective, because it had the least amount of bacteria passing through the mask after one week. However, according to Figures 3 and 4, the results are mixed. For example, while the KN95 has the second longest bacterial spread in Figure 3, it has the fourth widest bacterial spread in Figure 4. Another example is how the C.S. has the third longest bacterial spread in Figure 3 and the second widest bacterial spread in Figure 4. This also differs from the results of Trial 1, where the C.S. had the least long and wide bacterial spread as shown in Figures 1 and 2. The results of the experiment were ultimately determined by qualitative observations. In this case, the C.S. had the most transparent “lawn” of bacteria when compared to the rest. The results of the one-ply mask could not be determined due to high water absorption. However, there were some visible colonies on the surface area around the mask. Overall, the results stayed generally the same for the two-ply cloth, E.S., and KN95 for both qualitative and quantitative observations, the thickness and transparency of the bacterial “lawn” were similar and the measurements of the length and width were about the same in both trials. Alternatively, the C.S. mask had somewhat of a similar thickness and transparency of bacteria, but the measurements were completely different; instead of having a bacterial “lawn”, the first trial was observed as separate colonies, while the second trial had a bacterial “lawn.”

Each petri dish in the first trial reached its carrying capacity after two days, while the second trial reached its carrying capacity after three days. The temperature of the incubator was set to 35°C in both trials, an optimal temperature for bacteria growth. In the first trial, bacteria

did not spread as much and stayed in distinct colonies on the C.S. mask; the biggest colony measured 0.5 cm, while the smallest measured 0.2 cm. However, in the second trial, the bacteria spread out instead of staying in colonies. The length of the C.S. mask was measured to 6.7 cm and the width was measured to 6.5 cm. The second most effective was the E.S. mask, the third was the KN95, and the least was the two-ply cloth mask. In the first trial, the KN95 mask had a length of 7 cm and a width of 5.5 cm. In contrast, in the second trial, the KN95 mask had a length of 7.5 cm and a width of 5.3 cm.

Some qualitative observations that were noted were the thickness and transparency of the bacterial growth on each mask and the textures of the masks. The C.S. mask is ranked for having the thinnest bacterial growth because the texture of the mask is clearly seen through the bacteria. The E.S. is ranked second, the two-ply cloth is ranked third, and the KN95 had the thickest bacterial “lawn” in both trials. The material of the C.S. mask was quite different from the others. Streaking the plates on the C.S. using an inoculating loop due was difficult because the material was rough and shifted when trying to spread the bacteria. This material also had the least water absorption compared to the rest, while the one-ply had the most due to its elasticity and transparency. That is why the results could not be determined for that mask even though there was little bacterial spread on the surface area surrounding the mask.

IV. Discussion

The results of this experiment are unexpected, considering existing scientific studies and the initial hypothesis. Studies show that face filtering masks (N95 and KN95) are the most effective when compared to surgical and cloth masks. However, in this experiment, the observations and data suggest that the cheap surgical mask is the most effective due to its material and low water absorption. Additional variables may have caused errors. The variables that may have affected the data were the exact distribution of bacteria, the precise amount of bacteria scooped up by the inoculating loop from the culture tube, the time of mask exposure to air, the usage of a thin microbial one-ply mask, accidental splits in agar with the use of pins, the usage of masks with different water absorbency, the amounts of condensation in each plate, and the streaking technique. These errors could have affected the results in various ways, such as the material of the masks. In this experiment, one mask (one-ply cloth) absorbed too much water, making the results undeterminable; another mask (cheap surgical) absorbed almost little to no water making the results disparate from the others, especially in the first trial. During the experiment, specific measures have been taken to prevent introducing new variables. Some examples are keeping the size of the mask cut-outs the same, using a new inoculating loop for each petri dish, and sanitizing all surfaces before experimentation.

The conclusion of this experiment can be stated with confidence. Errors have been made and affected the outcome of the experiment. All factors were held constant and only one variable was changed: the type of mask. Between the two trials, the data varied slightly with the two-ply cloth staying the least effective in both trials and the C.S. staying the most effective for the first trial. In addition, the results were not only based on

the quantitative data but on the qualitative data and observations of thickness and transparency of the bacteria spread ("lawn") and the textures of the masks as well. However, these results were affected by uncontrollable variables such as bacteria and possibly virions present in the surrounding air during the experiment. These variables will forever exist and cannot possibly be rid of completely.

If this project were to be repeated, instead of testing five completely different masks, five similar masks could be tested from different manufacturers. Another variable that would be changed would be the one-ply cloth mask, which was microbial. This was an error that greatly affected the outcome of the experiment. It completely got rid of one variable due to the inability to get determinable results. The last variable that could be changed is getting a different "cheap surgical" mask, possibly one that has similar absorbency to the other masks that will be tested. Another experiment that could be conducted in relation to this experiment would be testing the survival rate of bioaerosols on different masks in order to determine their effectiveness by blocking the most bioaerosols. The same masks from the initial experiment could be used, as well as masks of similar material and texture from different manufacturers. This experiment can determine the effectiveness and the microbial viability of each mask.

V. Conclusions

The purpose of this experiment was to test the effectiveness of the five most popular masks: one-ply cloth, two-ply cloth, cheap surgical (C.S.), expensive surgical (E.S.), and KN95. The initial hypothesis stated that if masks that are more expensive provide more safety and reduce the risk of getting a bacterial infection or a virus, then KN95 or (expensive) surgical masks should be used because they have been proved to be highly effective by medical experts and have better protection against bacteria or viruses trying to get in through the mask. After testing the first trial, the alternate hypothesis suggested that the C.S. mask was the most effective due to its material and low water absorbency. The alternate hypothesis was supported by the results of both trials.

The relationship seen between the independent variable, type of mask, to the dependent variable, amount of respiratory bacteria (*Neisseria sicca*), that pass through the mask and grow on the BHI agar is almost entirely dependent on the material and water absorbency. This can be supported with data and observations. As it is seen, the results of the one-ply mask could not be determined due to its high water absorption and the fact that the company manufactured it as a microbial mask. A microbial mask kills bacteria and germs on the surface of the mask, this also contributed to the fact why results could not be seen. The one-ply mask was also very thin and elastic, which made it very absorbent. On the other hand, the C.S. mask was deemed the most effective due to its material and water absorbency. The material was very rigid and dry, it was hard to pull apart, and it showed little to no water absorption. Studies show that bacteria can easily pass through wet material compared to dry material. The thickness of the bacteria “lawns” and spread varied throughout the different mask types as seen in the data

analysis. In conclusion, the alternate hypothesis was supported by the results and the C.S. mask proved to be the most effective out of the five.

VI. Credits

I would like to acknowledge Corrinne Pelletier, a Cheshire High School biology teacher for assisting me with this project on the topic of microbiology and the experimentation process. I would also like to thank Julie Barker, CHS Science Department Chair for assisting me with the Connecticut Science and Engineering Fair registration and reminders.

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