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Microbe Penetration Levels on Facial Masks Fabricated at the University of Dodoma versus the Surgical Ones.

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Abstract

Background: Coronavirus disease 2019 is a pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The outbreak was first identified in the city of Wuhan, Hubei, China in December 2019, and was recognized as a pandemic by the World Health Organization on 11 March 2020. The virus primarily spreads among people via respiratory droplets from coughing, breathing, or sneezing. To reduce virus transmission, close contact between people is discouraged. In response to advice by health practitioners, individuals are advised to wear face masks, regularly wash their hands, and apply sanitisers. However, the effectiveness of locally manufactured masks against COVID 19 and other microbes has not been investigated.

Aims and methods: The current study aimed to experimentally determine and compare the effectiveness of two approved surgical masks and two face masks fabricated at the University of Dodoma (UDOM).

Results: The effectiveness of the UDOM-made mask was similar when compared to surgical masks (Mann- Whitney, $U = 390.000$, $p > 0.05$; Mean ranks: Japan fabric = 32.5; N95 surgical mask = 28.50). However, the Japan fabric mask made at UDOM was more effective than BBL surgical mask made in China (Mann-Whitney, $U = 270.000$, $p < 0.05$; Mean ranks: Japan fabric = 24.50; BBL surgical mask = 36.50). Whereas the handkerchief mask made at UDOM and BBL surgical mask had similar levels of effectiveness (Mann-Whitney, $U = 369.500$, $p > 0.05$; Mean Ranks: Handkerchief = 27.82; BBL surgical mask = 33.18). The results obtained suggest that the two UDOM types were as effective as the N95 and BBL masks in reducing virus spread.

Conclusion: The study recommends the determination of pore sizes of the materials used to make the mask to explain the effectiveness of the single layer, double layers, and double layers with cotton blends in the prevention of different microbes inhalable.

Keywords: Masks, COVID-19, UDOM, Tanzania

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Introduction

The 2019–21 coronavirus pandemic (abbreviated as COVID-19) in humans is an ongoing pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Zhu *et al.,* 2019). The outbreak was first identified in the city of Wuhan, Hubei, China in December 2019, and was recognized as a pandemic by the World Health Organization (WHO) on 11 March 2020. Global estimates on13thSeptember 2021, numbered 224,372,380 confirmed cases of COVID-19 including 4,625,006 deaths (WHO-COVID-19 Dashboard). The same source reported that more than 5.3 billion vaccine doses against the pandemic have been administered worldwide.

The virus primarily spreads among people via respiratory droplets from coughing, breathing, or sneezing (Lau *et al.,* 2004). It is considered most contagious when people are symptomatic, although the spread is possible even before symptoms appear (Wiersinga et al., 2020). The time between exposure and symptom onset is typically around five days but may range from two to fourteen days (Wiersinga et al., 2020). Common symptoms include fever, cough, fatigue, vomiting, dyspnea, myalgia, nausea, arthralgia, headache, diarrhea, arthritis, and shortness of breath (Cipollaro et al., 2020; Grant et al., 2020). Complications may include pneumonia and acute respiratory distress syndrome (Lovato et al., 2020). Currently, there is no specific antiviral treatment through some vaccines that have been developed, and are currently being administered to people in many countries. Treatment involves symptom alleviation and supportive therapy. Recommended preventive measures include the wearing of face masks, hand washing, covering the mouth when coughing, maintaining a distance of at least 1.8m from other people, and monitoring and self-isolation for people who suspect they are infected (WHO, 2020).

Efforts to prevent the global spread of the virus, especially during the outbreak in December 2019, included travel restrictions and border closure, incoming passengers restrictions, travel advice, screening in public places like airports and train stations, quarantines, curfews, event postponements, and cancellations, and facility closures. Schools, universities, and colleges in many countries were completely closed, affecting many national and international students (WHO, 2020).
According to WHO (2020), the pandemic not only affected academic but also caused global socioeconomic disruption, the postponement or cancellation of sporting and cultural events,https://en.wikipedia.org/wiki/2019%E2%80%9320_coronavirus_pandemic - cite_note-24 as well as widespread fear of supply shortages across various sectors including pharmaceuticals, electronics, and food, plus other negative effects such as the spread of false information, xenophobia, and panic buying of basic goods and provisions.

Measures to combat COVID-19 and their effectiveness

Governments worldwide have applied different measures to control the spread of COVID-19. These include reducing contact between individuals, thoroughly washing hands using running water and soap and/or use of sanitizers, and wearing a facial mask. Of these reactive measures, little information is currently available on the effectiveness of the use of sanitizers and facial masks. In the market, there are lots of brands of sanitizers and different types of masks made locally of different fabrics. In response to advice from health practitioners and the governments, people buy and wear masks.

Several studies have shown that wearing a facial mask reduces infections from airborne and droplets (Belkin, 1997; Karen & Cindy, 2003; Van de *et al*., 2008). However, some masks are made of single-layer; others are two or three layers and different fabrics are used. The current study aimed to determine the effectiveness of different approved surgical masks (N95 and BBL) and compared their performance to facial masks fabricated using locally available materials. The performance of these face masks was experimentally evaluated at the Microbiology Research Laboratory at the University of Dodoma (UDOM) in Tanzania. Microbes were blown into these different types of face masks to determine the efficacy at which the masks prevented their passage.

Materials and Methods

Study area

This study was conducted at Microbiology Research Laboratory, College of Natural and Mathematical Sciences (CNMS-UDOM) for twenty consecutive days from 4^{th} – 24^{th} April 2020.

Research design

The study was purely experimental. Experimental research is research conducted with a scientific approach using two sets of variables. The first set acts as a constant or control, which is used to measure the differences of the second set (Cash *et al.,* 2016). Surgical masks that were available at the supermarkets and/or pharmacies during the outbreak of COVID-19 became so expensive that most people could not afford to purchase them; hence, the alternative, relatively cheaper and locally available masks were required in large quantities to protect the large number of people who needed them during the outbreak of the epidemic. To investigate whether face masks help to prevent the spread of the virus, this project purchased N95 and BBL masks (N-95, Kimberly Clark Corporation, USA and BBL, China) and subjected these masks to experimental trials to test their effectiveness in filtering microbes.

These results were then compared to the performance of locally fabricated masks using the same methods. For each type of purchased mask, 90 pieces were used in blown-in microbial penetration experiments. The experiments were set along the gradient of distance from the mouth of test persons (i.e. 5cm, 50 cm, and 100 cm). For comparison purposes, UDOM purchased cotton fabrics that included handkerchiefs and, Japanese fabric (locally known as Sanda) that were labeled 100% pure cotton and stiff cloth, all of which were available in local markets. These fabrics were used to make 1080 facial masks of similar size as N-95 and BBL types (i.e., 15.5cm by 18cm). UDOM fabricated masks were made of one layer (without cotton wool), two layers (without cotton wool), and two layers with cotton wool. Each experiment was replicated 30 times, hence a total of 1320 replicates were performed.

Laboratory equipment/materials

This study was conducted in a room of 2.5m wide by 6.8m long and 4.2m high where an incubator, UV light equipment, and a microscope were maintained. Other equipment in the room included sterilizers (steam sterilizer or autoclave), Petri dishes, Bunsen burner, electric burner, wire loop, and operating protocol book. Chemicals used included universal growth media (agarose), alcohol (70% V/V), and distilled water. The temperature in the room was maintained at 25°C using wall-fixed air condition (LG model 9000 BTU).

The research protocol

Two technicians and one scientist were recruited to perform and supervise the experiments while observing the standard protocol. In total 60 individuals participated in this study including three project personnel and other 57 individuals (mainly UDOM staff, students, and the private guards at the CNMS), who voluntarily accepted invitations to participate in the blow-in-experiment. The purchased (180 pieces) approved surgical and the 1320 UDOM fabricated masks were sterilized using both steam and UV light sterilizer to remove any microbes present before the start of the blown-in-microbe experiment. To ensure that the researcher, the two technicians, and the participants were not contaminating the room, all were required to wash their hands thoroughly using clean water and soap and thereafter sanitized their hands and operating benches using 70% V/V alcohol. All individuals participating in the blow-in-microbe experiment were required to change their shoes and used only sterilized ones. The growth media were prepared following the standard protocol (Lee *et al.,* 2012).

Data collection techniques

The growth media were poured into Petri dishes and allowed to cool before the experiments. The experiments were conducted by one person (hereafter referred to as a test person) at a given time in the room but were required to perform at least three experiments (i.e. 5 cm. 50 cm and 100 cm) for consistency. To trap contaminants in the room, each day of the experiments, one Petri dish with growth media was left open during the experiments and thereafter was covered, labeled, and placed in an incubator; this was regarded as the first control experiment for the in-room microbial load. During the experiment, the test person: firstly, was required to blow in the air directly from their mouth (without putting on a mask) to the prepared agar at set distances from the mouth (i.e.5 cm, 50 cm, and 100 cm), this was considered as a second control. To standardize the procedure of the blow-in-air across all experiments, the test persons were asked to imitate coughing.

The number of microbes (i.e. the number of bacteria and fungi colonies forming the unit) obtained from the first control experiments and those from the mouth of coughing test persons counted from the Petri dishes after 24 hours incubation period was used for subsequent total microbe colonies analyses in the room and direct from the mouth of the test persons, respectively. This would mimic the situation in the normal environment where people live and interact. After coughing direct to the growth media as per procedure, the sample was immediately shifted to the incubator set at 37^{o}C.

The same procedure was repeated throughout the study for the control experiments. Secondly, the experimental procedure involving the wearing of different masks by a test person and coughing towards the growth media at a set distance gradient from the mouth was conducted as detailed above. The Petri dishes were immediately covered after coughing, labeled, and shifted to the incubator. The same procedure was repeated until all masks were tested. The results from the control experiments (first and second) were used to calculate the effectiveness of any mask included in the study by calculating the ratio between them and the colonies observed in each growth media performed by test persons.

Mathematically, the effectiveness of any masks was calculated as:

$E(\%) = (\Sigma noc / \Sigma ncc) \times 100$ Where;

E (%) = Percentage of the effectiveness of given masks, Σnoc = sum of the numbers of observed colonies forming units from the test persons after 24 hours of incubation. Σncc = sum of the numbers of observed colonies forming units from the control experiment after 24 hours of incubation.

Study limitations

The study had the following limitations, the experiments did not specifically measure the relative ability of face masks to capture SARS-CoV-2. The growth media used was for bacteria and fungi and not viruses. The individual who participated in the experiment were of different ages and sex hence might have different coughing pressure. Lastly, we did not ask the participants to take shower or to brush their teeth; hence different participants might have different levels of microbes in their skins and mouths.

Ethical consideration

Ethical approval was sought from the Directorate of Research and Publication, UDOM. This study, however, did not use human tissues; only air expelled from the mouth. All experiments were conducted in the laboratory following research and publication policy, guidelines, and regulations at UDOM.

Analysis procedures

SPSS package for the window was used (version 24) for all analyses. Mean microbial colony load was calculated using descriptive statistics and was presented as mean ± standard error of the mean. The numbers of colonies growth in each Petri dish were compared whereas the most effective fabric was the type with the least load in all categories (i.e., low penetration level). The statistical test applied was a Kruskal-Wallis one-way analysis of variance to demonstrate any significant difference among masks. To avoid statistical repetitions, the values at 5 cm from the mouth of test persons were used for comparisons.

At 5 cm from the mouth of the test person, the thrust is relatively high which can push microbe cells through the tiny pore of the mask. The variables used in analyses included: the distance of air blow/coughing/sneezing by a test person to the growth media, type of mask fabrics, layers of fabrics masks are made of and the presence or absence of cotton wool in between the layers. The statistical distribution adopted in the current study was two-tailed whereas for all statistics, $p < 0.05$ is considered significant.

Results

Overview

Overall, a total of 1320 experiments were conducted including 60 control experiments (30 direct coughing to the media without masks and 30 Petri dishes left open throughout) for 20 consecutive days. The first 15 days were used to conduct experiments in the unsterilized room to mimic what would be expected in a normal environment where people interact with nature and among themselves. Experiments performed during the last five days were conducted in the sterile chamber using UV light set for 3 minutes to sterilize masks and growth media before the conduct of the following experiments.

Effectiveness of the three types of UDOM-made masks in an unsterilized room

The mean number of microbial colonies counted after 24 hours of incubation is summarized in Table 1. Generally, results suggest that microbial loads were decreasing with distance from the mouth. However, the trends were opposite for Japanese fabric types whose microbial forming colonies, especially bacteria were increasing with distance from the mouth of the test persons.

Table 1: Mean microbial colonies counted (± S.E.M)from different treatments (n = 30 per treatment)

Type of treatment: distance from the mouth	Mean Bacterial Colonies			Mean Fungal colonies		
	5cm	50cm	100cm	5cm	50cm	100cm
Control (test persons + open Petri dish)	22.3 ± 7.7	17.1 ± 2.9	11.9 ± 2.1	5.8 ± 1.8	2.3 ± 1.0	2.3 ± 1.2
Handkerchief single layer	10.9 ± 3.3	8.8 ± 1.1	5.1 ± 3.6	2.7 ± 1.1	3.8 ± 1.8	0.4 ± 0.4
Handkerchief double layer	6.7 ± 2.1	4.7 ± 2.7	4.2 ± 3.1	2.8 ± 1.5	1.8 ± 1.1	2.2 ± 1.3
Handkerchief double layer with cotton	2.1 ± 0.8	3.0 ± 0.8	4.0 ± 1.7	0.5 ± 0.3	0.5 ± 0.3	1.1 ± 0.7
Japan fabric single layer	3.3 ± 1.2	4.1 ± 1.3	6.5 ± 2.8	0.8 ± 0.4	1.4 ± 0.5	3.7 ± 1.6
Japan fabric double layer	2.1 ± 0.5	2.4 ± 0.4	3.6 ± 1.5	2.4 ± 0.8	1.5 ± 0.7	4.0 ± 1.2
Japan fabric double layer with cotton	0.8 ± 0.1	1.6 ± 0.5	1.9 ± 0.6	1.0 ± 0.3	2.2 ± 0.6	2.2 ± 0.7
Stiff cloth single layer	13.9 ± 1.2	7.1± 1.2	8.3 ± 2.0	2.8 ± 0.5	3.3 ± 0.7	11.4 ± 0.8
Stiff cloth double layer	11.1 ± 2.0	6.5 ± 1.1	8.9 ± 1.2	2.5 ± 0.8	1.8 ± 0.6	5.1 ± 1.3
Stiff double layer with cotton	2.0 ± 1.0	3.6 ± 1.3	3.1 ± 0.9	2.1 ± 0.9	3.1 ± 0.9	4.2 ± 1.3

Effectiveness of the three UDOM-fabricated masks in the sterilized room

After experimenting in the unsterilized room, the double layers fabrics with cotton blended at a rate of 7.2×10^{-3} g/cm^2 performed better than those without cotton wool blended within. Hence, further experiments were performed in a controlled environment (sterilized chamber) using double layers with cotton wool blended for Japan, handkerchief, and stiff fabrics. The results (Table 2) indicated that masks made of double layers of Japanese fabric with cotton blended within had minimum penetration levels, followed by masks made of handkerchief double layer with cotton blend (Table 2). The least performing masks based on the mean number of microbe penetrations were those made of stiff cloth double layer with cotton blend (Table 2). Under a controlled environment, no fungus growth was observed.

Table 2: Mean microbial colonies counted (± S.E.M) from UV-light sterilized chamber (n = 30 per treatment) for three types of masks made at UDOM

Type of fabrics used to make masks	Bacteria colonies			Fungal colonies		
	5cm	50cm	100cm	5cm	50cm	100cm
Japan fabric double layer with cotton	**0.9 ± 0.1**	**0.3 ± 0.1**	0.2 ± 0.1	0	0	0
Handkerchief double layer with cotton	1.7 ± 0.2	0.9 ± 0.1	0.3 ± 0.1	0	0	0
Stiff cloth double layer with cotton	17.1 ± 1.4	9.8 ± 0.8	7.6 ± 0.6	0	0	0
Control (test person)	22.3 ± 1.5	17.1±1.1	11.9 ± 0.6			
Effectiveness rate (%) on preventing coughed microbes from the mouth of a test person						
Japan fabric double layer with cotton	96.0	98.2	98.3	100	100	100
Handkerchief double layer with cotton	92.4	94.0	97.5	100	100	100
Stiff cloth double layer with cotton	23.3	42.7	36.1	100	100	100

Effectiveness of surgical masks: surgical type1 (BBL) and surgical type2 (N95)

Results of the mean microbial forming units counted from the coughing experiments using surgically masked available in the local markets (Table 3) indicated that surgical mask type 2 had a low level of microbial penetration compared to type 1 surgical mask.

Table 3: Mean microbial colonies counted from two types of masks available at local markets. All these masks were sterilized using UV light following their purchase before coughing experimental infection (n = 30 per treatment)

Type of treatment	Bacteria colonies form units			Fungal colonies forming units		
		50cm	100cm	5cm	50cm	100cm
Surgical type 1 (BBL, China)	2.8 ± 0.5	1.3 ± 0.2	0.4 ± 0.1	0	0	0
Surgical type 2 (N-95, USA)	**0.9 ± 0.1**	**0.5 ± 0.1**	**0.1 ± 0.1**	0	0	0
Control	22.3 ± 1.5	17.1 ± 1.1	11.9 ± 0.6			
Effectiveness rate (%) of preventing coughed microbes from the mouth of a test person						
Surgical type 1 (BBL, China)	87.4	92.4	96.6	100	100	100
Surgical type 2 (N-95, USA)	96.0	97.1	99.2	100	100	100

Comparison of the effectiveness between UDOM-made masks and the two surgical ones

When the three masks that were fabricated at UDOM (double layers with cotton blend) were compared for their effectiveness in filtering the microbes particularly different types of bacteria at 5cm from the mouth of the test person, the result revealed a significant difference (Kruskal-Wallis = 67.175, df = 2, $p < 0.001$). Of these three, the Japanese fabric was the best for bacterial filtrations followed by handkerchief types and the least was stiff cloth masks (Mean Ranks: Japan fabric = 26.00; Handkerchief = 35.10; Stiff cloth = 75.00). In addition, we performed a further comparison of effectiveness between the two masks fabricated at UDOM; Japan fabric and Handkerchief (double layer with cotton blend) at 5cm from the mouth of the test person. This also showed significant difference (Mann-Whitney, $U = 315.000$, $p < 0.05$). Furthermore, the masks fabricated at UDOM using handkerchief materials and those made of stiff cloth were compared and revealed a significant difference in their effectiveness of bacterial filtration at a closer distance i.e. 5cm (Mann-Whitney, $U = 3.000$, $p < 0.001$).

After comparing the effectiveness of masks made at UDOM, we then compared the two; Japanese fabric and handkerchief masks with the two surgical masks purchased at the supermarkets and pharmacies. All showed different levels of filtering bacteria at 5cm from the mouth of the test person (Kruskal-Wallis = 304.942, $df = 3$, $p < 0.05$) 5cm from the mouth of the test person. Finally, it was important to compare the effectiveness of the two best effective masks that were made at UDOM (Japan fabric) and the surgical one purchased at the supermarket (N95). The two showed similar effectiveness (Mann-Whitney, $U = 390.000$, $p > 0.05$; Mean ranks: Japan fabric = 32.5; N95 surgical mask = 28.50). However, Japan fabric mask made at UDOM was more effective than BBL surgical mask made in China (Mann-Whitney, $U = 270.000$, $p < 0.05$; Mean ranks: Japan fabric = 24.50; BBL surgical mask = 36.50) whereas the Handkerchief mask made at UDOM and BBL surgical mask had similar levels of effectiveness (Mann-Whitney, $U = 369.500$, $p > 0.05$; Mean Ranks: Handkerchief = 27.82; BBL surgical mask = 33.18).

The effect of the quantity of cotton wool blended in between the layers of fabrics

How does the amount of cotton wool per square centimetre blend in between the layers of the fabric used to make a mask affect the ease of breathing and microbe penetration? Different weights of cotton wool were blended into different tailored masks during the preliminary experiments. In each trial, the results for microbe penetrations were recorded and compared to surgical masks (N95 and BBL-China). While performing the experiments, the test persons ($n = 30$) were asked about their breathing quality (difficult or easy). When the rate of cotton wool reached 7.2×10^{-3} g/cm^2 for Japanese fabric, the microbe penetrations were more or less the same as to surgical masks (N95) at the distance of 5cm from the mouth of the test person and at that amount of cotton wool; the breathing quality was considered normal.

This procedure revealed that an additional 25% of cotton weight above 7.2×10^{-3} g/cm^2 increased the effectiveness by 3.1% ($n = 30$) but 76% of test persons ($n = 30$) complained to have difficulty in breathing. Likewise, when the cotton wool was reduced by 25%, the effectiveness dropped by 12% but all test persons were breathing well. Thus, the set amount of cotton per square centimetre reported above was the tradeoff between ease of breathing and effectiveness of the masks in reducing the penetration of microbes as compared to surgical masks.

Discussion

The findings communicated here were gathered through the analyses of colony-forming units of different types of microbes trapped in a standard growth media from the environment and the mouth of different test persons. Thus, it is possible that some microbe's growths were inhibited by the environment in which the experiments were conducted or by the type of growth media. Moreover, different test persons might have different levels of microbial load and/or might have different coughing or sneezing pressures. However, since the aim was to determine the effectiveness of different types of masks that were made locally or purchased from the markets and used to protect people from COVID-19 and other respiratory infections, the findings obtained here serve the intended purpose because both UDOM-made and purchased surgical masks were subjected under similar conditions during the experiment; and they produced different results.

Single-layer masks of all types were ineffective in the prevention of microbes sneezing or coughed directly at a short distance from the mouth (Table 1) probably since microbes blown or coughed were mostly smaller than the pore size of the fabrics, hence were not retained in the fabric. Masks made of a double layer of fabric, without cotton wool blended in between, had reduced penetration probably due to reduced pore size resulting from misaligned layers of fabric. Masks made from double layers with cotton blended in between were the most effective, especially those made of Japanese fabric and handkerchief fabric. The blend of cotton wool maintained at a rate stated in the results section (i.e. 7.2 x 10^{-3}g/cm^2) was reached after several trials of additional or reduction of the weights of cotton wool during the preliminary experiments.

The relatively high effectiveness of double layers of cotton wool for Japanese fabric might have been contributed by the ability of the two layers to reduce blown or coughed pressure and the presence of a cotton wool blend that is known to have the ability to trap bacteria (Sikyta, 1995). Other studies conducted elsewhere revealed a similar conclusion (Van de *et al.,* 2008). Masks made of stiff cloths were not equally effective probably due to the relatively large pore sizes of the material.

When the experiment was run in an unsterile environment, the results did not follow the expected pattern; the decrease of bacterial forming colonies along the gradient of distance from the mouth of the test persons (Table 1). Rather, the microbial forming colonies, especially bacteria did not perform a defined pattern (i.e. increased, decreased, or remain more or less the same along the gradient of distance from the mouth of the test persons).

The possible explanation is that when the experiments were performed in an unsterilized environment, already the room might have been contaminated by the test persons introduced in the room to perform the experiments or by supervisors who were moving in and out of the room occasionally to invite and assist the test-persons. The observed high numbers of bacteria colonies therefore might not only come from the mouth of the test persons but also the surroundings. A similar case was observed from the colonies that were in the Petri dishes that were left open during the experiment. Repeating the experiments in a sterilized environment produced the expected patterns; the microbial forming colonies decreased with the increase of distance from the mouth of test persons (Table 2).

Conclusion

The current study concludes that the UDOM-made masks, those made of Japanese fabric double layer blended with cotton wool at the rate of 7.2 x 10^{-3}g/cm^2 were the most effective followed by masks made of handkerchief double layer with a cotton wool blend. The two masks were as effective as surgical masks N95 and BBL, respectively. To protect people from the COVID-19 pandemic and other microbes, it is better to use either of the two types when the surgical masks are not locally available or become so expensive that most local people fail to afford them.

From the study, we recommend the determination of the pore size of the materials used to make masks that were experimented with at UDOM. This would help to explain the penetration level of COVID-19 and other microbes in single, double, and double with cotton wool blend masks. Also, the study recommends a follow-up experiment to evaluate the effectiveness of the homemade and surgical masks after being used and sprayed with 70% V/V alcohol before being re-used. It is important to study in detail the quality of breath by people who use the UDOM and other homemade masks.

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