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Qualitative Assessment of Different Face Masks using Povidone-Iodine in Comparison to Standard Saccharin Method

Microbiology Section

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ABSTRACT

Introduction: The World Health Organisation (WHO) recommends that N95 masks should be used by healthcare workers and patients. Given the shortage, extended use and reuse of masks, it becomes imperative to test the filtration efficacy. Surgical masks and cloth covers should also be assessed for their protection factor, since their use is more prevalent among the general population, and these masks are not certified by National Institute for Occupational Safety and Health (NIOSH). Occupational Safety and Health Administration (OSHA) is the authority for the standard testing and certification of Filtering Face Pieces (FFPs) in the USA; its equivalent in India is the Bureau of Indian Standards (BIS). The BIS recently relaxed the certification guidelines for both N95 and Surgical Masks; thus, even certified masks may prove to be inefficient.

Aim: To assess different face masks using povidone-iodine in comparison to standard saccharin method.

Materials and Methods: A cross-sectional study was carried out in a tertiary care hospital in western India from August 2021 to October 2021. A novel, qualitative (and semi-quantitative) function test using an aerosol of iodine solution and detector strips of starch-iodide paper was studied to prove for efficacy of masks by 15 volunteers and various masks of different types (surgical, N95, cloth) over three months. This method provides a rapid, simple and cost-effective assessment of respirator efficacy.

Results: Thirty five masks (N95, Surgical, Cloth and Silver nanoparticle) were tested, of which N95 - 90% pass the test. A 2 out of 5 trials of the double layered cloth mask + surgical mask combination passed both the iodine and saccharin tests. The sensitivity and specificity of proposed iodine test in comparison to standard saccharin test were (10/10, 100%) and (22/25, 88%).

Conclusion: Povidone-iodine method is a qualitative assessment which is a crude indicator of mask function as the masks that fail both tests will provide some degree of protection from airborne particles, and concentrations of many respiratory organisms. The N95 masks along with the double masks (surgical and cloth) passed the tests proving their efficacy.

Keywords: Efficacy, Filtering face pieces, Healthcare, Novel

INTRODUCTION

One of the easiest and efficient methods to prevent the transmission of respiratory illnesses is through the use of face masks. Both the Centers for Disease Control and Prevention (CDC) and WHO recommend the use of FFP masks (Respirators) to reduce transmission of infections [1,2]. The requirement of such Personal Protective Equipment (PPE) has seen a drastic rise recently, due to the COVID-19 pandemic. From late 2019 and throughout 2020 to 2021, cases of COVID-19 caused by the novel coronavirus (SARS-nCoV2) continued to appear, leading to a shortage of PPE [3,4]. In order to make up for this deficit, the CDC put forth recommendations for the extended use and/or reuse of face masks [5]. Relaxations have been made in the certification of N95 [6] and surgical masks [7] by the BIS to speed up production. Despite decreasing cases, and the easing of quarantine, PPE remains an important commodity in high demand. The pandemic has caused an increase in use of all types of masks by both healthcare workers and the general public. Evaluation of masks will be helpful mostly for healthcare workers to optimise appropriateness of use.

Filtering facepiece respirators: A FFP respirator (face mask or dust mask) refers to a negative pressure particulate respirator with a filter as an integral part or with the entire mask composed of the filtering medium [8]. Respirator filters are rated as N, R, or P for their level of protection against oil aerosols- "N" if not resistant to oil, "R" if somewhat resistant to oil, and "P" if strongly resistant (oil proof). The numerical value describes the percentage of particles filtered [9]. In this study the following types of masks have been tested including N95, surgical masks, cloth masks, nano silver particle impregnated masks and combinations of either two surgical, cloth and surgical were included. A N95 Respirator (or KN95) type FFP respirator is capable of filtering 95% of all particulate matter >300 nm in the ambient air, but not gases or vapors and is not resistant to oil. It should be well fitting forming a tight seal around the mouth and nose [9,10]. Surgical masks are not liable to testing or certification have an uncertain and variable filtration efficacy [11]. They may not always be well fitting and may not form an efficient seal around the face.

Two varieties of cloth masks one single layer and another double layer of the same material was tested, these are mask types are commonly used by general population for protection against dust particles. They usually have poor filtration, but may also protect the community transmission of disease. Double surgical masks are used on the basis that two layers provide more protection than one. A combination of a cloth mask+surgical mask: double masking is frequently observed in the general population [12-14]. In this study, trials were conducted with both mask orientations, i.e., surgical covered by cloth mask, and vice versa. The two combinations did not show any significant difference, and have been regarded as the same unit. Nano-Silver particle impregnated (silver nanoparticle) cloth masks are masks claimed by the manufacturer to incorporate silver particles of the size 1-10 nm. While the antimicrobial properties of silver have been extensively studied and established [15], this variety of masks are not certified by NIOSH. A recent study does indicate that such masks may be effective in neutralising microbes on their surface, and thus can be used on top of N95 masks to extend their use [16].

The mechanism of action of FFP masks for filtering particles depends on the size of the particle [10,11]. Larger particles are removed by the processes of inertial impaction and interception, smaller particles are removed by diffusion, charged particles of appropriate size are

eliminated through electrostatic attraction. Most Penetrating Particle Size (MPPS) refers to the size of particles which cannot be filtered by mechanical forces (impaction, interception or diffusion) and will pass through with relative ease [9]. A measurement of particle collection by the filter at its MPPS is the best measure of its efficacy. A high filtration at MPPS means the mask will filter particles smaller as well as larger than the MPPS. WHO recommends that N95 masks should be used by both healthcare workers and patients [1]. Given the current shortage, and the resultant need for extended use and reuse of masks, it becomes imperative to test the filtration efficacy. Occupational Safety and Health Administration (OSHA) is the authority for the standard testing and certification of FFPs in the USA, its equivalent in India is the BIS. The BIS recently relaxed the certification guidelines for both N95 [6] and surgical masks [7]. This may compromise the efficiency of even certified masks. Both OSHA and BIS provide similar guidelines for the Quantitative Function testing (QNFT) of N95 respirators, such as the NaCl aerosol test [8]. However, the QNFTs prescribed, have several disadvantages like being time consuming and requiring complicated machinery like a Kr85 equilibrator, spectrophotometer or ELPI- electrical low-pressure impactor, High Efficiency Particulate Air (HEPA) filter, etc [8,17] and such testing cannot be performed at short notice.

Qualitative Function testing (QLFT) thus becomes the more feasible option for a rapid and approximate assessment of mask filtration and fit. These tests are usually based on the detection of a reagent by the subject, either by taste, smell or irritation (cough reflex). These are standard tests and reagents as per the OSHA. The reagents used include Isoamyl acetate (banana odour), Saccharin solution (sweet taste), Bitrex or denatonium benzoate (bitter taste), and Stannic chloride smoke (irritant). These methods have the disadvantage that they rely on a subject's sense of taste and smell. This is relevant to the outcome as Coronavirus Disease-2019 (COVID-19) infection is known to produce an impairment of theses senses. Subjects with normal senses will also show inter individual variation of thresholds of sensation to the same concentration of the reagents [18,19].

This study represents a novel, qualitative function testing method for the evaluation of face masks using an aerosol of iodine solution and detector strips of starch-iodide paper. Betadine (Povidone-iodine) is a safe and commonly used antiseptic in the hospital setting and is readily available as well as economical [20]. Iodine in the solution (orange-brown) interacts with starch-iodide (colorless) to form a black-blue complex. The interaction is quite sensitive, and scales with the concentration of iodine and starch interaction [21]. The intensity of coloration of the starch paper is inversely related to the protection factor offered by the mask, and can provide a semi-quantitative assessment method. A Povidone-iodine solution forms thin films of 500-900 nm [20] and can be aerosolised to similar particle sizes. The NIOSH certification process for N95 masks requires a 95% filtration efficacy for particles of size >0.3 µm (300 nm) or Count Median Diameter (CMD) 0.75 µm (750 nm) [5,10]. Therefore, the use of betadine for such a test is appropriate. This study was undertaken to create economical and quick methods for testing of masks in a hospital setting with a view to maximise use of PPE, especially face masks.

MATERIALS AND METHODS

This cross-sectional study was carried out at a tertiary care hospital in a metropolitan setting in Western India over a period of three months from August 2021 to October 2021. The study was commenced after obtaining approval from the Institutional Ethics Committee (IEC) and informed consent was taken from all the participants.

Inclusion criteria: Consenting adults (healthcare workers and medical students) aged between 18-60 years who passed the OSHA questionnaire and cleared the medical evaluation [8,22].

Exclusion criteria:

- ◊ Individuals under 18 years or over 60 years of age.
- ◊ Adults not willing to consent for the procedure.
- ◊ Current or past patients of COVID-19.
- ◊ Subjects with current or past history of respiratory infections or other diseases like bronchial asthma.
- ◊ Individuals with history of iodine allergy.
- ◊ Individuals with history of smell/taste dysfunction, particularly those failing the saccharin threshold test.
- ◊ Individuals with a beard.

Screening procedure: Twenty adult volunteers were screened by administering the OSHA mandatory fit test questionnaire and medical evaluation [8]. A total of 15 subjects were selected after applying the exclusion criteria.

Materials Required for the Procedure

- **Nebulizers:** Air compressor nebulizers were used for this study instead of DeVilbiss Model 40 Inhalation medication nebulizer. It has been demonstrated by a recent study that 'mist makers' provide accurate results when used as an alternative [22]. Three separate nebulizers in total were used while assessing the validity of the method- one for the iodine test, one for the saccharin threshold test and one for the concentrated saccharin test as per OSHA regulation [8].
- **Test hood:** This was constructed from 5 panes of 12 inch×12 inch acrylic sheets (Plexiglas), forming a cube with the base open. A 2 cm wide vent was created in the upper left corner of the anterior face of the hood, to snugly fit the nozzle of the nebulizer. A plastic drape with a small hole in the centre, was placed over the head of the subject before placing the hood and taped to the sides to create a secure enclosure. This plexiglass hood was used as a replacement of a standard 3M hood and has been found to be adequate [22] as well as much more economical [Table/Fig-1].

[Table/Fig-1]: Testing within the hood.
Strips are stuck inside the hood on the right of the subject and on the posterior surface of the hood

- **Masks to be tested:** N95, surgical masks, cloth masks and silver nanoparticle masks, double masks (cloth+surgical). All the above masks were BIS and NIOSH certified respirators.

  Multiple masks were used as representative specimens for each type, i.e., 10 masks for BIS/NIOSH certified N95 respirator (two commercial products), five each of surgical masks, double surgical masks, common cloth masks (single and double layered), combined cloth and surgical masks, and silver nanoparticle masks. Some images has been provided in [Table/Fig-2].

[Table/Fig-2]: Mask types used.

Solutions:

- **Povidone-iodine:** A 5% povidone-iodine solution in a water base was used as the reagent. To avoid bubbling and to ensure formation of uniform aerosols solutions with detergent base was not used.
- Starch iodide paper strips, moistened with distilled water.
- **Sodium saccharin:** The test and threshold solutions were made according to OSHA regulations [8]. Test solution is an 83% solution in distilled water; threshold solution: 0.83% solution in distilled water.

Preparation of masks and test hood: Each mask was removed from packaging immediately prior to testing and visually examined for any defects. Unsatisfactory masks were discarded and replaced. A test strip of starch-iodide paper, moistened with minimal quantity of distilled water was attached (with little adhesive, applied at the edges) to the inner surface of the mask. Two strips of moist starch-iodide paper were placed on the inner surface of the hood (which was stuck simply by virtue of surface tension), one on the anterior surface and one on the lateral surface to serve as positive control strips.

Procedure

The procedure was explained to the participants, following which their consent was obtained. The subject was instructed on appropriate fitting of the mask as per apparatus [22]. Masks were randomly provided to the subject, and donned without any assistance. Seal checks (positive and negative pressure, for N95 types only) were performed by the subject, and mask placement was inspected to ensure correct fit. Eye protection was also provided to avoid irritation.

Iodine test: A plastic drape of sufficient size was placed over the subject's shoulders by making a small slit. The test hood (12-inch× 12-inch×12 inch) was then placed over the subject's head and the edges of the drape secured to the sides. Using a nebulizer, aerosols of 5% solution of povidone-iodine was then passed into the chamber via the opening on the front surface. In order to create the aerosol, the nebulizer was turned on for 10 seconds, repeated at intervals of 15 seconds to replenish escaping reagent. To avoid a false reaction due to use of excess reagent, no more than 1 cc of povidone-iodine was used at a time. The subject was then instructed to perform the following exercises prescribed by OSHA Regulation 1910.134-A [8] each for 1 minute:

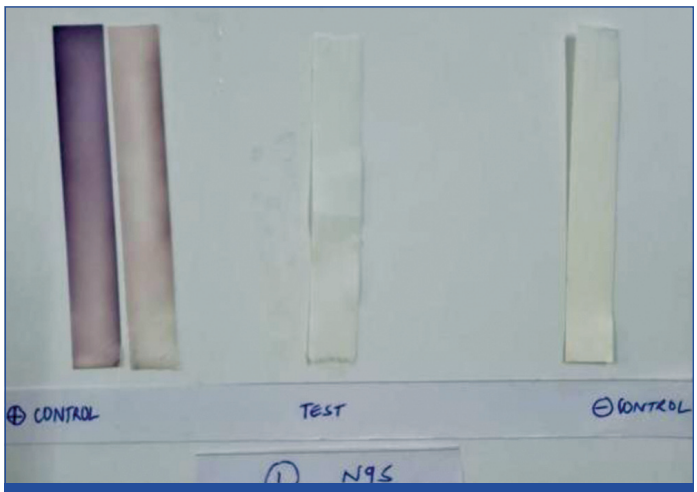
- Normal breathing. In a normal standing position, the subject breathes normally.
- Deep breathing: In a normal standing position, the subject breathes slowly and deeply, taking caution so as not to hyperventilate.
- Turning the head side to side: Standing in place, the subject slowly turns his/her head from side to side between the extreme positions on each side. Moving the head up and down by standing in place, the subject slowly moves his/her head up and down.
- Talking: The subject talks out slowly and loud enough so as to be heard clearly by the investigator. The subject can count backward from 100, or recite a memorised poem.

The subject then removed the hood and doffed the mask, and was questioned and examined for any irritation, difficulty breathing, discomfort, etc. and allowed to rest and drink water. The strips were removed from the mask as well as hood and placed on a plastic sheet over a white surface. The color was noted, and compared to the positive control (strip placed on the inner wall of the hood, reacting freely with the iodine aerosol) and a negative control (plain strip).

Saccharin test: In the same subject, using the same mask, after an appropriate rest for 15-20 minutes, the standard Saccharin test was performed by using the threshold and test solutions following the identical exercise protocol as above (Iodine Test). To simulate the 'puffs' of the recommended method, the nebulizer was switched on and off in rapid succession. The hood was wiped down with sterile cotton to clear any residual reagents, and the nebulizer solutions discarded and refilled for each subject.

First iodine procedure was done followed by saccharin procedure with threshold and testing of solutions was done after a 15-20 minute rest period. The subject was again questioned and examined for any irritation or difficulty breathing, following which they were allowed to take off the test hood.

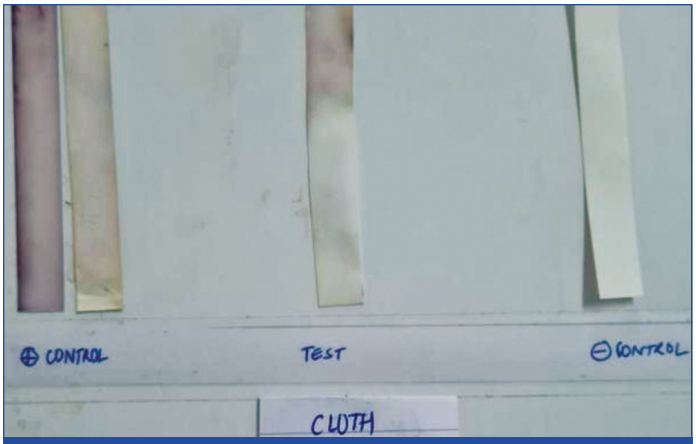
Interpretations: The test results were reported as 'pass' for no discoloration of the test strip (while making sure the control strips had adequately reacted) and 'fail' for diffusely blue-black colored strips [Table/Fig-3-6] and false positive where the mask actually gives protection but the strips get colored which may be due to imperfect seal or fit of the mask. Using the OSHA standardised Saccharin test for comparison, the sensitivity and specificity of the proposed iodine test in comparison to standard saccharin test were observed.



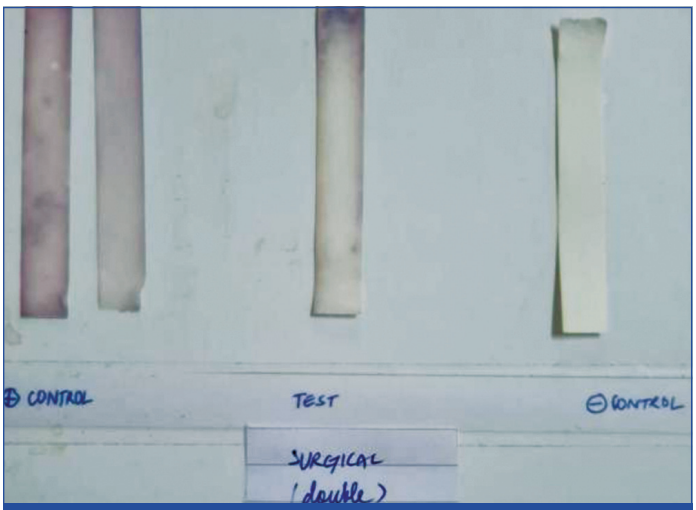
[Table/Fig-3]: Iodine test- N95 mask.

STATISTICAL ANALYSIS

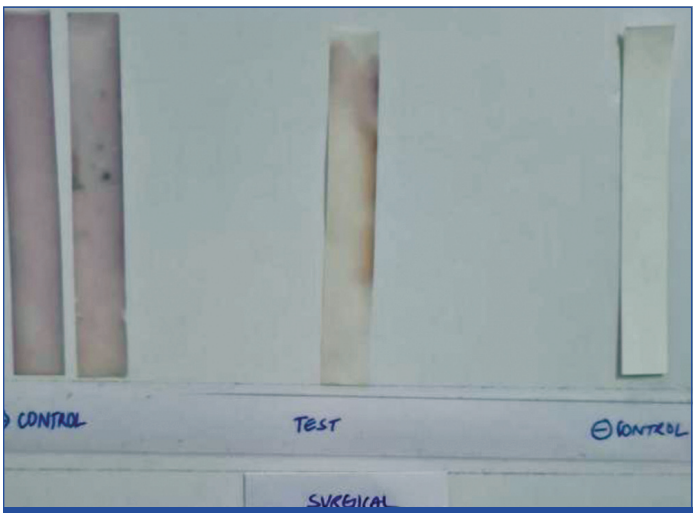
The data was processed and arranged into distribution tables and cross tables with the help of Microsoft excel.



[Table/Fig-4]: Iodine test- cloth mask.



[Table/Fig-5]: Iodine test- double layer surgical mask.



[Table/Fig-6]: Iodine test- surgical mask.

RESULTS

Of the 15 subjects, nine were males and six females, all between the age group 20-30 years. No issues were reported by any subject and the iodine aerosol was well tolerated. As per availability and comfort, each subject was given the opportunity to test multiple mask types after adequate rest and water intake, to avoid sensory fatigue and discomfort. This provides better comparability between the methods by eliminating some inter subject variation. The sensitivity and specificity of proposed iodine test in comparison to standard saccharin test were (10/10, 100%) and (22/25, 88%) [Table/Fig-7].

In all subjects, imperfect seal was observed in all masks except N95. Even the cloth and silver nanoparticle masks, which appeared to be tight fitting, revealed gaps around the bridge of the nose and under the chin.

	Saccharin test- PASS	Saccharin test- FAIL	Total
Iodine test- PASS	10	3	13
Iodine test- FAIL	0	22	22
Total	10	25	35

[Table/Fig-7]: Validity of the iodine test.

Interestingly, 2 out of 5 trials of the double layered cloth mask+surgical mask combination passed both the iodine and saccharin tests. Authors believe that the relatively tight fit of the cloth mask was able to provide a better seal with the surgical mask beneath it.

False positive iodine test was observed with a trial of one N95, a double surgical mask and a combination of cloth and surgical masks as stated in [Table/Fig-8].

	Mask type (N=35)	Iodine test Pass/Fail	Saccharin test Pass/ Fail	Saccharin test- Seconds fail time
1.	N95 - Product 1	Pass	Pass	N/A
2.	N95 - Product 1	Pass	Pass	N/A
3.	N95 - Product 1	Pass	Fail	<40s
4.	N95 - Product 1	Fail	Fail	<50s
5.	N95 - Product 1	Pass	Pass	N/A
6.	N95 - Product 2	Pass	Pass	N/A
7.	N95 - Product 2	Pass	Pass	N/A
8.	N95 - Product 2	Pass	Pass	N/A
9.	N95 - Product 2	Pass	Pass	N/A
10.	N95 - Product 2	Pass	Pass	N/A
11.	Surgical	Fail	Fail	<20s
12.	Surgical	Fail	Fail	<20s
13.	Surgical	Fail	Fail	<10s
14.	Surgical	Fail	Fail	<20s
15.	Surgical	Fail	Fail	<10s
16.	Double layered surgical	Fail	Fail	<10s
17.	Double layered surgical	Fail	Fail	<10s
18.	Double layered surgical	Fail	Fail	<20s
19.	Double layered surgical	Pass	Fail	<30s
20.	Double layered surgical	Fail	Fail	<20s
21.	Double layer cloth mask	Fail	Fail	<10s
22.	Double layer cloth mask	Fail	Fail	<10s
23.	Double layer cloth mask	Fail	Fail	<10s
24.	Single layer cloth mask	Fail	Fail	<20s
25.	Single layer cloth mask	Fail	Fail	<10s
26.	Single layer cloth+surgical	Pass	Fail	<30s
27.	Double layer cloth+surgical	Pass	Pass	N/A
28.	Double layer cloth+surgical	Pass	Pass	N/A
29.	Single layer cloth+surgical	Fail	Fail	<20s
30.	Single layer cloth+surgical	Fail	Fail	<30s
31.	Silver nanoparticle	Fail	Fail	<20s
32.	Silver nanoparticle	Fail	Fail	<30s
33.	Silver nanoparticle	Fail	Fail	<20s
34.	Silver nanoparticle	Fail	Fail	<20s
35.	Silver nanoparticle	Fail	Fail	<10s

[Table/Fig-8]: Iodine and saccharin test results.

DISCUSSION

The demand and shortage of PPE, including face masks has become an important issue in recent times; while healthcare workers are at risk due to a lack of adequate PPE. On the other hand, some populations refuse to wear masks [23] and/or follow quarantine requirements. In the current pandemic face masks have become a part of our daily lives hence it is important to ensure

their appropriate functioning to prevent the spread of respiratory illnesses. Masks have a double benefit of protecting one self and limiting the spread of respiratory infection to others hence efficacy testing along with a fit check proves highly beneficial for prevention of most of the respiratory diseases.

The pandemic though nearing towards the end phase, the world is currently reeling with bouts of increase in the infection rates of COVID-19 hence, there is a need to prioritise the use of the PPE for better utilisation of present resources. It is known that an N95 respirator is the most efficient amongst all in its filtering capacity and hence should be limited to use by healthcare workers in the hospital setting where the possibility of aerosol generation is greater. The other masks such as the surgical masks and cloth masks can be used by general population to avoid dissemination of the disease. Double masking with surgical or surgical and cloth may also provide sufficient degree of protection against the respiratory diseases. Previous data indicate that using multiple masks of any type can improve the filtration efficacy by some degree [14]. A comprehensive study of mask leak by particle flow visualisation confirms this finding [24,25].

In this study only the N95 masks adequately passed both tests thereby highlighting that only this category of face masks provide sufficient protection from aerosols in high exposure, a result which is consistent with findings of Bartoszko JJ et al., [25]. Cloth and surgical masks provide sufficient protection for general public, respirators of N95 certification should be reserved for healthcare personnel.

The false positive tests for iodine test may be attributed to manufacturing defects, improper fit or seal of the masks during testing. While the saccharin test false positive may be due to subjective error in taste as observed by the author during the study. The FFE of masks plays an important role for the spread of aerosols while wearing improperly fit masks. Simple modifications can improve the fit and filtration efficiency of surgical masks. The consumer masks worn by the common man also can be made more effective for prevention of community transmission and can be comparable with or better than their non N95 respirator medical mask counterparts [26]. Clapp PW et al., shows that masks (surgical masks) though have an FFP and Fitted Filtration Efficacy (FFE) lesser than 95% are effective in prevention of acquiring the SARS CoV-2 infection by healthcare workers, except at times for aerosol generating procedures a N95 would prove beneficial [26].

Cloth masks are an effective source control along with hygiene practices, social distancing and contact tracing for the limiting spread of the disease in public during the current pandemic situation. Current shortage/unavailability of medical masks have urged the common man to use cloth masks as an alternative for prevention from the disease [27]. Povidone-iodine method provides a rapid, simple and cost-effective assessment of respirator efficacy.

As compared to a 3M test kit, priced at $369.11 [22], this method requires minimal capital, common and easily accessible hospital or laboratory reagents are used. Also this method places reliance on visual interpretation rather than taste or smell unlike with other reagents, the mask remains usable after testing since 5% povidone-iodine is a known antiseptic/disinfectant.

Limitation(s)

Qualitative assessment is a crude indicator of mask efficacy as masks that fail both tests will still provide some degree of protection from airborne particles, and concentrations of any organism or particle in daily life. For a definite and accurate measure of mask function, quantitative assessment of FFE is recommended. Present study does not measure the FFE but it was observed that masks which fitted tightly passed the test than compared with those lacked a proper fit indicating that the proper fit of masks also augments to the level of protection offered by it.

The nebulizer used in the study may produce smaller particles than recommended by DeVilbiss Model 40 nebulizer; it is known that the particles required for the sensation of smell as well as its threshold are much smaller. The saccharin test, subjects almost immediately reported a vague ‘sweet taste in the back of their throat’ even when breathing through their nose, which differed from the taste sensation on the tongue, which was perceived properly when the subject was asked to break the seal and breathe through the mouth with the tongue slightly protruded (as per saccharin test protocol).

CONCLUSION(S)

The study is an economical, quick and feasible method for rapid testing of masks in a hospital setting. The povidone-iodine method may be used for qualitative testing of masks as an alternative method to those prescribed by the OSHA. By developing a scale for the colour developed on the test strip, it has the potential for serving as a semi-quantitative method too. This method can be considered comparable to the standardised saccharin qualitative test put forth by OSHA. This would help to optimise the use of the masks given the differential potential protective efficacy observed. It is thus prudent to ensure not only judicious use but also a priority based allocation of the FFP depending on the risk of exposure of the end users.

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