

Mask decontamination methods (model N95) for respiratory protection: a rapid review

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Research

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Abstract

Background: N95 respiratory protection masks are used by healthcare professionals to prevent contamination from infectious microorganisms transmitted by droplets or aerosols.

Methods: We conducted a rapid review of the literature analyzing the effectiveness of decontamination methods for mask reuse. The database searches were carried out up to September 2020. The systematic review was conducted in a way which simplified the stages of a complete systematic review, due to the worldwide necessity on reliable fast evidences on this matter.

Results: A total of 563 articles were retrieved of which 48 laboratory-based studies were selected. Fifteen decontamination methods were included in the studies. A total of 19 laboratory studies used Hydrogen peroxide, 21 studies used ultraviolet germicidal irradiation, 4 studies used ethylene oxide, 11 studies used dry heat, 9 studies used moist heat, 5 studies used ethanol, two studies used isopropanol solution, 11 studies used microwave oven, 10 studies used sodium hypochlorite, seven studies used autoclave, three studies used electric rice cooker, one study used cleaning wipes, one study used bar soap, one study used water, one study used multi-purpose high-level disinfection cabinet and another one used chlorine dioxide. Five methods promising: hydrogen peroxide vapor, ultraviolet irradiation, dry heat, wet heat/pasteurization, and microwave ovens.

Conclusions: We have presented the best available evidence on masks decontamination, nevertheless, its applicability are limited due to few studies on the topic and lack of studies on real environments.

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the coronavirus pandemic (COVID-19), has affected the world and changed the way we live.¹⁻⁵ Aerosols, droplets, secretions, and direct contact with nasal mucosa are the main respiratory transmission routes of these viruses between humans.⁶

Health professionals have been using respiratory protection masks; particularly model N95, in the care of infected patients for aerosol generating procedures (tracheal intubation, non-invasive ventilation, tracheotomy, cardiopulmonary resuscitation, manual ventilation, before intubation, collections of nasotracheal secretions and bronchoscopies). The recommendation is to discard them after close contact with a patient (single-use).⁷⁻⁹

During the current COVID-19 pandemic, governments have found it difficult to acquire adequate amounts of Personal Protection Equipment (PPE), including respiratory protection masks. This has been accompanied by a high level of infection of health professionals on the front lines of care provided for sick people.¹⁰⁻¹⁴ The COVID-19 pandemic has not only exposed how crucial effective PPE are for health professionals, but also exposed the inability of health systems to meet this demand. In this context,

based on the shortage of N95 masks, one of the alternatives found was the decontamination and reuse of PPE.¹⁵

Even though researches have been conducted on how decontamination may be conducted, there is concern about disease transmission as there are no best practices for mask decontamination and reuse.¹⁶ In such critical circumstances, as we go through, rapid analyses are recommended by WHO¹⁷ to provide guidance for timely decision making. Therefore, this rapid review aims to describe the effectiveness and safety of decontamination methods for N95 masks for reuse as protection against COVID-19.

A recent systematic review evaluated the efficacy and safety of N95 mask decontamination methods from SARS-CoV-2.¹⁸ Other reviews were carried out evaluating specific methods of decontamination, such as microwave and heat¹⁹, ultraviolet germicidal irradiation²⁰ and chemical disinfectants.²¹ Concerns about efficacy and safety of methods of decontamination of personal protective equipment is, and will continue to be, relevant for scientific evidence as there has been likelihood for a second wave of COVID-19 recently. Thus, planning for stockpiling and handling of personal protective equipment should be one of the priorities.^{22,23}

In this sense, providing a global view of all decontamination methods already tested is essential to support decision makers. Our first search, on April 15th 2020, recovered 166 studies, whereas a new search, on September 25th 2020, has located 552 studies, indicating growth of primary research on the topic.

Methods

This rapid review follows the recommendations outlined by Haby et al²⁴. A rapid review is a form to provide a knowledge synthesis through streamlining or omitting specific methods to produce evidence for stakeholders more briefly.²⁵ Challenging scenarios, such as the onset of coronavirus demand that decision makers receive the best evidence quickly and urgently, making traditional methods of systematic review unviable.²⁶

Criteria for considering studies for this review

Based on these recommended approaches,²⁴ we developed a specific protocol for this study (Supplementary material 1). We planned to include only primary researches that evaluated methods for decontamination of N95 masks for reuse and whose outcome was the effectiveness, safety, maintenance of protection or filtering characteristics of the evaluated decontamination method were included. Therefore, we could answer the question: “How effective and safe are decontamination methods for respiratory protection masks model N95/PFF2 against respiratory viruses?”

Search methods for identification of studies

We followed the limit main database searching recommended by Cochrane Rapid Reviews Methods Group.²⁶ Therefore, searches were conducted on Medline, Cochrane Library and EMBASE databases on September 25th, 2020. Search terms were related to decontamination (e.g., “Sterilization”, “Disinfection” and “Decontamination”), reuse (e.g., “Equipment Reuse” and “Reuse”), device failure (“Equipment Failure”) and masks (e.g., “N95” and “filtering facepiece respirators”). The review did not have date or language restrictions. The complete search strategies can be found in Table 1 of Supplementary material 2.

Searching other resources

In addition to searching the official databases, the reference lists of all studies selected for full text reading, as well as the review articles identified in official searches, were scrutinized to identify possible eligible studies.

Data collection and analysis

Two reviewers (LFP and AIQC) independently used a pre-specified data extraction sheet form, in duplicates, designed to obtain the specific data required for this review. The data extracted from the primary studies were: data related to the author, year, study objective, intervention, comparator, commercial mask model, target microorganism, results and conclusions of the study authors, limitations and detailed description of the decontamination process making its reproducibility in other scenarios possible. Moreover, authors of the included primary studies were also contacted to provide data that was not available in the manuscripts.

Data synthesis

The characteristics of each study (cycles, temperatures, protocols, densities, exposure time, technology used and results) are presented in Table 3 of Supplementary material 4. The differences before and after decontamination are shown in Table 4 of Supplementary Material 5 for outcomes: filter aerosol penetration, filter airflow resistance and filtration efficiency. Results of the studies were summarized based on decontamination method and results for the two following issues:²⁷

1. Whether the device maintains its structural characteristics and provides an adequate level of protection after the decontamination method, without any risk of exposure for the health professional (as inhalable chemical residues that may have remained after the method used). The penetration of 0.3 µm (aerodynamic mass mean diameter) of sodium chloride aerosols aerosol particles through a certified N95 respirator cannot exceed 5%.²⁸ Also inhalation and exhalation resistance to airflow of certified N95, i.e., filter airflow resistance utilizing a filter tester at 85 l/min of constant airflow, shall not exceed 35mm (343.2 Pa) water column height pressure and upon initial exhalation shall not exceed 25mm (245.1 Pa) water column height pressure. These are specifications required for certification as a 95% filtration efficiency level.²⁹

2. Whether the decontamination method used was effective in reducing or eliminating the infectious capacity of the target organism without any risk of exposure for the health professional to contamination. This criterion can be verified when the mean log reduction of the microorganisms allows us to state that the mask has reached non-infectious levels. Alternatively, using a quantitative molecular amplification assay (quantitative real-time polymerase chain reaction) that shows if there was a reduction in the levels of detectable viral RNA.²⁷

Results

Results of the search.

We identified 552 records through searches on databases (Medline: 381, Cochrane: 52 and EMBASE: 119) and 11 records detected through other resources. The complete search strategies with the results for each database can be found in Table 1 of Supplementary material 2. Mendeley citation management software was used for automatic removal of duplicate articles, leaving 301 remaining studies. Two reviewers (LFP and AIQC) independently screened the 301 studies using the Rayyan systematic review application to screen abstracts and titles.³⁰ Of these, 240 were excluded for not meeting the inclusion criteria. 61 studies full texts were screened by two reviewers (LFP and AIQC). At this stage, we excluded 13 full-text reports and provided the reasons for exclusions and their references in Table 2 of Supplementary material 3. Disagreements during the selection process were resolved by discussion with a third review (SMLVO). Finally, 48 studies were selected for the full review process. The results of these 48 included studies are summarized in the following narrative results and in details in Table 3 of Supplementary material 4 and Table 4 of Supplementary material 5. We provided a diagram of the search and study selection process in a PRISMA flow-chart (Figure 1).

Fifteen methods were assessed in the 48 papers: hydrogen peroxide, ultraviolet irradiation, ethylene oxide, dry heat, moist heat/pasteurization, ethanol, isopropanol solution, microwaving, sodium hypochlorite (NaClO), autoclaving, electric rice cooker, cleaning wipes, bar soap and water, Multi-Purpose High-Level Disinfection Cabinet (Altapure, Mequon, WI) and chlorine dioxide (ClO₂).^{27,31-77} Each method will be briefly analyzed bellow:

1. Hydrogen peroxide

Hydrogen peroxide was evaluated in its liquid, plasma and gas/vapor forms by nineteen laboratory studies.^{33,40-44,48,52,58-62,65,66,69,73,75,76}. The effect of hydrogen peroxide on the filtering capacity varied according to the method used. The average penetration was not significantly changed when the masks were submersed in 3% or 6% hydrogen peroxide liquid or treated with Vaporized Hydrogen Peroxide (STERRAD®) for one cycle⁶⁵. However, it resulted in mean penetration levels > 5% after 3-Cycles.⁴² The integrity and filtering capacity of the mask were preserved when Hydrogen Peroxide was used as steam.^{33,42,78}

Additionally, studies have shown Hydrogen Peroxide led to changes in the masks' metallic nasal clips.^{42,65,66} As for the ability to eliminate microorganisms, SteraMist™ Binary Ionization Technology® (BIT™) was effective against Influenza A virus subtype H1N1⁷⁵ and *Geobacillus stearothermophilus* spores⁷⁶ and STERRAD 100NX sterilization system eliminated SARS-CoV-2, *Staphylococcus aureus* and *Acinetobacter baumannii*.⁴⁰

On the other hand, vaporized hydrogen peroxide (VHP) was effective in eliminating SARS-CoV-2³³, *Geobacillus stearothermophilus* spores⁶², *Porcine respiratory coronavirus* (PRCV)⁵², *Escherichia coli*, *Mycobacterium smegmatis* and spores of *Bacillus stearothermophilus*⁶⁰ and 3 aerosolized bacteriophages: T1, T7, and Pseudomonas phage phi-6.⁴³

2. Ultraviolet germicidal irradiation (UVGI)

The effect of ultraviolet germicidal irradiation on N95 respirator masks was evaluated by 21 studies^{27,33,35,38,42,47,50–53,55,56,58,61,63–68,70} and there was a difference among studies in relation to UVGI doses and application time periods. In general, UVGI did not affect the integrity and ability of the masks to filter aerosols or adapt to the face, nor did it leave a smell, irritating/toxic residues, or create important changes in appearance even when multiple cycles were performed.^{27,33,35,42,47,50,51,53,58,61,65–67} However, different commercial brands of N95 models resisted differently in terms of performance penetration after multiple cycles and doses applied.⁵¹ The most efficient N95 brand in terms of long standing penetration performance was 3M 9210, but the mean penetration values for all brands were 5% or less both before and after exposure.⁵¹ The most efficient N95 brand in terms of long standing flow resistance performance was 3M 9210, but the mean flow resistance values was less than 1% of the initial value for all brands.⁵¹

Ultraviolet germicidal irradiation was effective against MS2 coliphage (ATCC 15597-B1)⁶⁸, the influenza virus H5N1²⁷ SARS-CoV-2^{33,56,63}, bacteriophages MS2^{35,70}, influenza virus H1N1^{38,55}, *Porcine respiratory coronavirus* (PRCV).⁵² However, at the same time, one study reported that even after 20 min of irradiation with 365 nm UVA the relative survival of *Bacillus subtilis* spores remained above 20%⁵⁰ and another one highlighted the UV-C technologies tested did not meet pre-established criteria for decontamination against *Methicillin-resistant Staphylococcus aureus* (MRSA), *Bacteriophage Phi6* and *Bacteriophage MS2*.⁶⁴, which is worrisome.

3. Ethylene oxide

Evaluated by four studies, the effectiveness of ethylene oxide (EtO) depended on the type of sterilization equipment used, whether there was a hot cycle, and exposure to EtO.^{42,61,65,66} The process did not affect the filtration, resistance, odor or appearance of the masks. The main limitations of the method were the processing time and the presence of toxic residues. It is also important to note that none of the studies

tested the effectiveness of EtO treatments against microorganisms. Thus, there is no evidence that EtO can eliminate any microorganism from N95 masks.

4. Dry heat

The use of dry heat was evaluated for by eleven different experimental studies.^{32,33,45–47,52,57,64–66,71} Temperatures between 70 and 85 °C did not affect the structural characteristics of the masks under various humidity conditions ($\leq 100\%$ RH).^{33,47} Also, filtering efficiency remained acceptable ($\geq 95\%$) up to 50 cycles at 85 °C and 30% of RH.⁴⁷ When an oven was used at 70°C, filtration performance was maintained if only one cycle was performed.³³ However, there were no detectable changes in aerosol filtration efficiency, even after three reprocessing cycles when the masks were subjected to the same temperature of 70°C when laboratory MINI/6 incubator (Genlab Ltd) was used to provide dry heat.³²

These results concerning dry heat reinstates that decontamination attempts must be conducted in identical manners and conditions to the ones performed in the laboratory. Not only regarding the temperature, but also regarding the device used. Nevertheless, the effect against different microorganisms varied greatly between different studies due to the decontamination cycle time.

Dry heat treatment (70°C for 60 min) was effective against SARS-CoV-2^{32,33}, however, dry heat (70°C for 30 min) had limited effectiveness against bacteriophages MS2 and Phi6 versus methicillin-resistant *Staphylococcus*.⁶⁴ Also, dry heat at 100°C for 15 minutes did not eliminate *Methicillin-resistant Staphylococcus aureus (MRSA)* and *Nonenveloped single-stranded RNA virus bacteriophage MS2*.⁴⁵

5. Moist heat / pasteurization

Nine studies^{27,31,32,38,42,47,53,54,67} evaluated the effect of moist heat between 60 and 100°C. The method did not alter mask fit, odor or comfort.^{27,38,42,47,67} In one study⁴⁷ filtration efficiency presented a significant drop after cycle 5 when stacked on top of a beaker with boiling water inside (around 15 cm above the water), although, filtration efficiency was not affected when masks were subjected to 5 cycles with a lower temperature – at 85°C + 60-85% humidity.³¹ Moist heat (65±5 °C for 3h) was effective in eliminating H1N1³⁸ and H5N1²⁷ viruses, in addition, when masks were steamed in boiling water the treatment was effective against the Avian infectious bronchitis virus H120 without changing.⁵⁴ Another study also has shown that a single heat treatment for thermal disinfection in cycles of 60 minutes at 70°C at 50% eliminated *SARS-CoV-2* and *Escherichia coli*³².

6. Ethanol

Different methods of decontamination with ethanol were tested: spray,³³ immersion,^{36,47,49} and pipette drips.⁵⁰ Results were divergent between methods. The filtration efficiency of masks was degraded to unacceptable levels when they were immersed in alcohol.^{36,47,49} Mask filtration performance was not significantly reduced after single ethanol sprays which were also effective in eliminating SARS-CoV-2.³³

Subsequent rounds of spraying caused significant decrease in efficacy in filtration performance.³³ Nonetheless, pipette drips were not effective in eliminating *Bacillus subtilis* spores.⁵⁰

7. Isopropanol solution

The filtering capacity of N95 masks was changed and the particle penetration through N95 mask exceeded 5% after they had been submerged in isopropanol solution.^{49,65} Although effects on microorganisms were not evaluated, this method could be further studied in order to check these effects.

8. Microwave oven

Eleven studies tested the use of microwave ovens in the disinfection of N95 masks.^{27,34,35,38,42,53,57,65–67,72} The type of commercial furnace, maximum temperature, and time protocols varied between the studies (Table 3, Supplementary material 4). When masks were placed directly on the rotating plate of the microwave without protection, two commercial models of the tested masks melted.⁶⁶ When the masks were placed in containers with water^{27,38,42,67,72} or in steam bags specifically marketed for microwave ovens no residual odor was observed.³⁵ In addition, there were no structural changes affecting adjustment on the face, filtration capacity, or resistance to airflow and none of the metal components melted or combusted. Microwaving the masks was effective in eliminating H5N1⁶⁶ and H1N1 influenza viruses⁶⁷, bacteriophage MS2^{35,72} and *Staphylococcus aureus*.⁵⁷

9. Sodium hypochlorite (NaClO)

Ten studies^{34,42,47,49,50,58,61,65,66,68} evaluated the use of hypochlorite at different concentrations and application methods (Table 3, Supplementary material 4). The maintenance of mask integrity and filtering capacity varied among studies. For instance, one round of disinfection drastically degraded filtration efficiency to unacceptable levels when samples were left to air dry and off-gas completely, hanging⁴⁷ or submersed in 0.5% sodium hypochlorite for 10 min.⁴⁹ Application of sodium hypochlorite discolored the metallic components of the masks which, unfortunately, caused a characteristic smell of bleach.⁶⁶

One of the study treatment caused the release of low levels of hydrochloric gas.⁶¹ Hypochlorite concentrations of 0.006%, 0.06%, and of 0.6% was not effective against Coliphage MS2³⁴. On the other hand, when higher sodium hypochlorite doses (>8.25 mg/liter) were used for the dilute solutions containing 2.75 to 5.50 mg/liter during 10-min decontamination period MS2 coliphage was inactivated.⁶⁸ When 5.4%, 2.7% or 0.54% NaOCl was used the method was effective in eliminating *Bacillus subtilis* spores.⁵⁰

10. Autoclave

Considered an accessible method, as it is an equipment present in all hospital environments, autoclave decontamination was evaluated by 7 studies.^{36,37,49,50,65,74,77} Autoclave disinfection was effective in eliminating *Bacillus subtilis* spores,⁵⁰ however, the integrity of the mask was altered.^{37,49,65} Two studies pointed that even though masks were able to retain their structural integrity and efficacy, no results were provided regarding the filtering capacity and only the fittest was performed.^{74,77} In addition, a survey conducted in 2020 showed the degree of integrity of the mask can be preserved depending on the model tested (Table 3, Supplementary material 4).³⁶

11. Electric rice cooker

The method showed 99–100% biocide efficacy against *Bacillus subtilis*⁵⁰ spores after using dry heat for 3 minutes (149–164 °C, without adding water). Also, the treatment for 13-15 minutes, including 8-10 minutes of heating and 5 minutes of steam resulted in a greater than 5 log₁₀ reduction in bacteriophage MS2 and methicillin-resistant *S aureus*.⁴⁵ However, the method visibly changes the mask's integrity.⁴⁹

12. Cleaning wipes

The effectiveness of commercial wipes containing 0.9% hypochlorite, benzalkonium chloride or no active antimicrobial ingredients was evaluated in masks contaminated with *Staphylococcus aureus* and mucin.³⁹ The three mask models, 3M-1860S, 3M-1870 and Kimberly-Clark-46727-PFR, withstood handling and abrasion during the disinfection process. All were successfully decontaminated against atypically high microbe levels by wipes containing antimicrobial agents, however, inert wipes did not produce adequate decontamination.

13. Bar soap and water

Average penetration has markedly increased for N95 respirators after being submerged. Authors have hypothesized the soap could have removed the charge on the fibers similar to the effect observed with isopropanol solution exposure.⁶⁵

14. Multi-Purpose High-Level Disinfection Cabinet (Altapure, Mequon, WI)

The treatment was effective against microorganisms and the researchers reported no visible changes in the masks. However, the efficiency of the filtration has not been confirmed.⁶⁴

15. Chlorine dioxide (ClO₂).

The method significantly changed the filtering efficiency of the tested masks which makes this method not worth using or testing in further studies.⁷³

Discussion

Fifteen decontamination methods were identified in the 48 studies^{27,31-77} included in this review. Ten methods have addressed the infectious capacity of microorganisms and all fifteen methods have investigated masks structural integrity after the decontamination process, also being based on laboratory tests

As different methodologies were used in each study, summarizing the results is a challenging task. Nevertheless, this review has uncovered the inadequacy of the evidence supporting the use of N95 mask decontamination methods. Therefore, it is important to highlight that even when a mask has retained its structural integrity after decontamination, if the elimination of an organism's infectious capacity has not been proven, it is still a potential vehicle of transmission. This concern is reinforced by a recent study which showed that pathogens may be present on the external surface of about 10% of used masks, and that the risk of infecting the user increases with prolonged use⁷⁹, as the number of viral particles and their survival time are key determining factors when consideration of reuse becomes necessary.⁸⁰

The most valuable data for decision making is to identify after which methods the filtration efficiency remained unchanged or within the established thresholds.²⁸ Although hydrogen peroxide vapor, germicidal ultraviolet irradiation, dry heat $\leq 85^{\circ}\text{C}$, moist heat/pasteurization, and microwaving seems not only promising but also has demonstrated these methods have preserved the integrity of the masks, so far, the evidence does not indicate a method that is consistently safe and effective to decontaminate N95 respiratory protection masks.

Particularly, hydrogen peroxide vapor and ultraviolet germicidal irradiation require specific equipment and environments increasing costs, which should be taken into account. Dry heat, wet heat/pasteurization, and microwave ovens also showed positive results in terms of effectiveness and safety and are more accessible methods in scenarios of scarce financial resources. Feasibility of these methods should be assessed with new studies, especially by health systems in developing countries.

The decontamination protocols that used hydrogen peroxide (solution and plasma), ethylene oxide, ethanol, sodium hypochlorite, autoclaves, electric rice cooker, and isopropanol solution interfered with the integrity of the masks within the test conditions used in the studies. Commercial cleaning wipes were effective antimicrobial agents and did not degrade the masks, however, they were evaluated by a single study and for only two microorganisms.

In the case of serious emerging infections such as COVID-19, the principle of universal precaution must be considered, that is, a balance must be made between the benefits and risks of the possible decontamination methods, in order to ensure maximum safety and real protection for the user, especially in the case of health professionals on the front line.^{15,81}

Using only three databases for the search conducted was one of the limitations of this study, even though this review was performed as a quick response in the midst of the COVID-19 pandemic. As a rapid review, another limitation concerns a series of methodological simplifications adopted, which may affect the

findings and our interpretations. Eliminating the evaluation of the studies' methodological qualities was among the simplifications and calls for caution in interpreting the results presented.

The strengths of this review, however, are the number of identified methods and its unfolding results which points to practices that may be adopted and further studied as they seem to present better results initially, the multiple approaches used to search for relevant studies, and the participation of a team of multi-disciplinary specialists in all stages of the project.

Agreements and disagreements with other Reviews

A previous systematic review that included 15 studies concluded that future studies are required in order to establish the efficacy and security the efficacy and safety of N95 decontamination methods.¹⁸ Others systematic reviews have reported that masks can be decontaminated with microwave irradiation and moderate-temperature heat (up to 90°C), in both moist and dry conditions¹⁹ and a single cycle of vaporized H₂O₂ can be used as chemical disinfectant to remove viral pathogens without degrading the masks.²¹ However there is not sufficient evidence concerning UVGI as a safe decontamination method.²⁰ To our knowledge, this is the first rapid review to assess 15 different decontamination methods identified in 48 studies, providing an overview of all available methods.

Implications for future researches

Whereas the current evidence is insufficient to determine a safe and widely accessible method, even for countries with financial limitations, our review points to an important gap in the evidence base, despite recent research efforts. In addition, considering the possibility of new challenging pandemic scenarios, investigating decontamination methods for reuse of protection professional equipment has an environmental, social appeal and economic aspect. Moreover, it is important invest in new fabric technologies that are prepared to be reused.

Conclusion

Access to effective PPE should be guaranteed for health care workers on the front lines of pandemics. However, there is currently insufficient evidence to recommend any method as being safe and effective for the decontamination and reuse of respiratory protection masks. Even though, there are several promising methods worth further study such as hydrogen peroxide vapor, germicidal ultraviolet irradiation, dry heat at temperatures $\leq 85^{\circ}\text{C}$, wet heat / pasteurization and the microwave oven, this rapid review has exposed all methods for decontamination need further evaluation and validation in real-life scenarios, also considering economic issues for implementation.

List Of Abbreviations

COVID-19 coronavirus disease

EtO ethylene oxide

SARS-CoV-2 severe acute respiratory syndrome coronavirus 2

PPE Personal Protection Equipment

Influenza A virus subtype H5N1

Influenza virus A subtype H1N1

NaClO sodium hypochlorite

UVGI Ultraviolet germicidal irradiation

UVA Ultraviolet A

MS2 bacteriophage

WHO World Health Organization

Declarations

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Availability of supporting data

All data are included in the manuscript

Competing interests

The authors declare that they have no competing interests

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All authors read and approved the final manuscript

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Figures

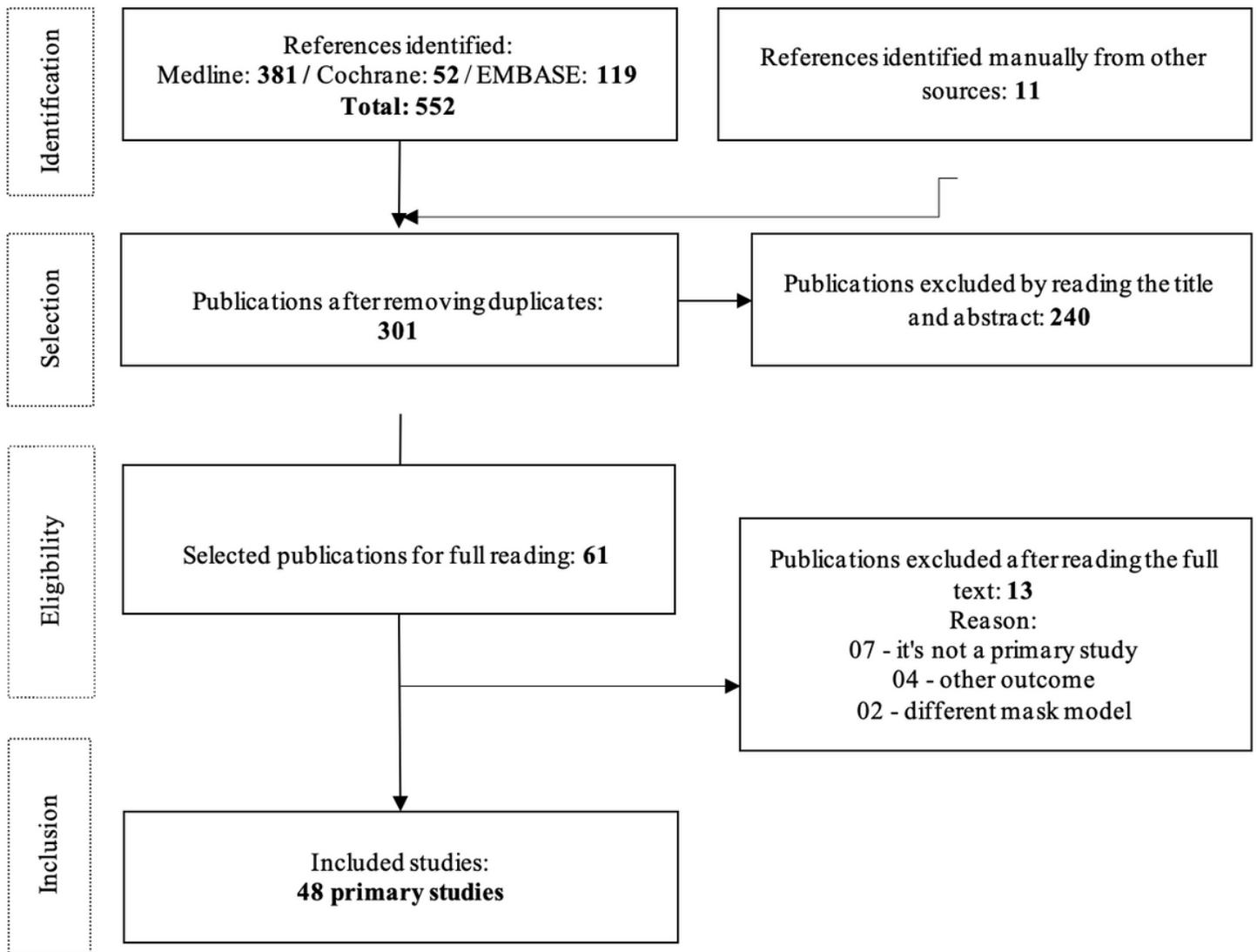


Figure 1

PRISMA flow-chart of the study selection process

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