STUDY PROTOCOL

PROTOCOL TITLE:

A study to explore the physiological impact of AIR^{+} KN95 Masks on children

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PRINCIPAL INVESTIGATOR:

Eugene Loy
Business Development Manager
Respiratory Protection
Urban Environment Solutions
ST Engineering Innosparks Pte. Ltd.
10 Tampines North Drive 4, #05-07
Singapore 528553
T: (65) 6873 2074
www.stengg.com

STUDY SITE:

School(s) designated by MOE

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1. BACKGROUND AND RATIONALE

1.1 General Introduction

There is potential for severe haze episodes to occur in Singapore, and the main air pollutant in the event of haze is particulate matter (PM). Short-term exposure (continuous exposure to unhealthy daily average PSI levels over a period of a few days) to haze like the pattern seen in Singapore may cause irritation of the eyes, nose, and throat in healthy individuals. Haze particles can also affect the heart and lungs, especially in people who already have chronic heart or lung disease e.g., asthma, chronic obstructive pulmonary disease (COPD), or heart failure.

Haze is a perennial problem in Singapore. Commercially available disposable particulate respirators are typically certified for surgical and occupational use. The test standards are specified according to adult breathing conditions and fit. As commercially available disposable masks designed for occupational use are mainly for adults, such masks do not fit well on children, they do not serve well as protection against Particulate Matter 2.5 (PM 2.5), an air pollutant found in transboundary smoke haze.

Commercially available disposable particulate respirators are typically certified under the NIOSH and CE EN 149:2001+A1:2009 standards for surgical and occupational use. These respiratory masks are tested to filter particulate matter in the air with varying degrees of filtering efficiencies according to the respective categories, e.g. NIOSH KN95 certifies respirators to filter at least 95% of non-oil particulates. The test standards are specified according to adult breathing conditions and fit.

Children's respiratory minute volume and rate are different from adults. As commercially available disposable masks designed for occupational use are mainly for adults, such masks do not fit well on children, they do not serve well as protection against Particulate Matter 2.5 (PM 2.5). As haze is a perennial problem, and there is potential for severe haze episodes to occur again, there is a need to consider the development of disposable particulate respirator with enhanced safety and comfort specific for use in children.

ST Engineering Innosparks Pte. Ltd. developed a new type of disposable particulate respirator (hereon referred to as AIR+ KN95 Mask) that is suitable for use in both adults and children, from ages 7 and above. The masks come in S, M and L sizes, and have been tested according to the KN95 protocol for respirators.

Studies have shown that prolonged wearing of disposable particulate respirators may lead to an increase in carbon dioxide (CO_2) levels in the dead space of the respirator as well as in the re-breathing of the expired air when wearing it. The increased CO_2 levels may result in headaches, increased irritability, and breathing difficulty. In addition, there is some discomfort due to the accumulation of heat and humid air in the dead space of between the respirator and face. However, the mask design is based on our previous AIR+ Smart Mask specifications, which was previously evaluated in a clinical trial performed in collaboration with NUH and the data published in a peer-reviewed journal[1], showing no significant increase in CO_2 retention and the mask was found to be comfortable at rest and on mild exertion. The mask was commercially launched in 2015.

As the masks were developed specifically for school-going children, this study is designed to explore whether the new disposable particulate respirator is safe and effective for use in healthy children aged 7 to 14 years of age. This is through measuring the variation of carbon dioxide levels in children whilst wearing the AIR+ KN95 Mask. End-tidal carbon dioxide pressure (ETCO2) is a good indicator of arterial carbon dioxide pressure (PaCO2) in healthy adults and children and has been used for continuous direct assessment of PaCO2 in clinical contexts. The normal range of PaCO2 is from 35 – 45 mmHg. As there is a slight margin of difference between ETCO2 and PaCO2 values during measurement of about 1.6 +/- 4.3mmHg, this study defines 30 to 50mmHg as the acceptable range for ETCO2 levels.

1.2. Rationale and justification for the Study

a. Rationale for the Study Purpose

Haze is a perennial problem in Singapore. There is a need to consider the development of disposable particulate respirator with enhanced safety and comfort specific for use in children.

ST Engineering Innosparks Pte. Ltd. developed a new type of disposable particulate respirator (AIR^{+} KN95 Mask) that is suitable for use in both adults and children, from ages 7 and above.

b. Rationale for Doses Selected (not applicable)

No drugs are used in the study.

c. Rationale for Study Population

Commercially available disposable masks designed for occupational use are mainly for adults, such masks do not fit well on children. As the masks under study were developed specifically for school-going children, this study is designed to explore whether the new disposable particulate respirator is safe and effective for use in healthy children aged 7 to 14 years of age.

d. Rationale for Study Design

This study entails the study of subjects doing various activities with and without donning on the mask. The baseline results of subjects without donning on the mask will serve as control.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

We hypothesise that the subject's measurements of end-tidal CO2 (ETCO2) levels of using AIR+ KN95 Mask will be within the acceptable range for ETCO2 levels of between 30 to 50mmHg.

2.2 Primary Objective

The primary objective is to evaluate the physiological impact of AIR+ KN95 Masks on end-tidal carbon dioxide (ETCO2) of children aged 7 to 14 years of age.

2.3. Secondary Objectives

The secondary outcomes include other physiological parameters such as oxygen saturation (SPO2), heart rate (HR) and respiratory rate (RR). In addition, we will assess the general well-being and comfort level of the child when wearing the AIR+ KN95 Mask.

2.4. Potential Risks and benefits:

a. End Points - Efficacy

There is no direct benefit in participating in this research. However, the knowledge gained would benefit other children and the public in the future. The results from this research will enable us to test the safety of the use of Air+ KN95 masks in children. This study will measure the carbon dioxide levels inside the mask and in the children as they wear it.

b. End Points - Safety

The study is designed to observe the impact of wearing the Air+ KN95 mask in children while at rest and on minimal exertion only. They may experience mild discomfort when wearing the mask whilst brisk walking on the treadmill. However, their vital signs will be monitored, and they will be encouraged to remove the mask if they feel uncomfortable.
Studies have shown that prolonged wearing of disposable particulate respirators may lead to an increase in carbon dioxide (CO2) levels which may result in headaches, increased irritability, or breathing discomfort. Usually, this is resolved by simply removing the mask.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

A total of 100 children will be recruited. Students aged 7 to 14 years old from two schools in Singapore [to be advised by MOE] will be invited to participate in this study. The participants will be randomly selected from the pool of qualifying subjects.

3.2. Criteria for Recruitment

Students aged 7 to 14 years old from schools as selected by MOE will be invited to participate in this study. Since subjects are minors, one of the parent or legal guardians will sign the informed consent form. There will be no restrictions based on race or gender. The subject must satisfy the inclusion and exclusion criteria to be eligible for the study. A Parental Screening Form will be attached to the **PARTICIPATION INFORMATION SHEET AND CONSENT FORM** for the parent or legal guardian to declare any past medical

condition(s) of the subject. Should parents have doubts about any of the questions in the Parental Screening Form, they will be able to contact the PI directly. Similarly, should the study team wish to clarify their concerns regarding parents' responses to the screening form prior to enrolling the subject, they will contact the parent directly. Any such communication between parent/legal guardian and PI will be documented in the study record.

3.3. Inclusion Criteria

The inclusion criteria are as follows:

1. Aged between 7 and 14 years of age (inclusive)
2. Subjects and their parents or legal guardian must provide their consent/ assent to take part in this study

3.4. Exclusion Criteria

The exclusion criteria are as follows:

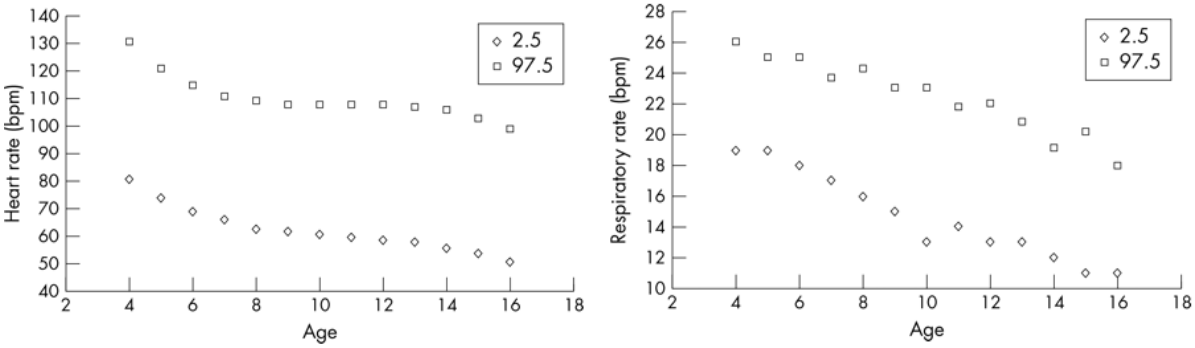
1. Subjects with any known cardiorespiratory conditions (including but not limited to the following: asthma, bronchitis, cystic fibrosis, congenital heart disease, emphysema)
2. Subjects with any known medical conditions that may be exacerbated by strenuous activity, including but not limited to the following: exercise-induced asthma, lower respiratory infection (including pneumonia, bronchitis) in the past 2 weeks, anxiety disorders, diabetes, hypertension, or epilepsy/ seizure disorder
3. Subjects with any physical disability from medical, orthopaedic, or neuromuscular disorders
4. Subjects who have an acute upper respiratory tract infection/ moderately severe rhinitis (i.e. blocked nasal passages) on the day of the study
5. Subjects who may compromise the integrity of the mask fit (e.g. those with excessive facial hair)

3.5. Withdrawal Criteria

Subjects may withdraw voluntarily from participation in the study at any time.

Also, subject will be withdrawn from the study if there is clinical suspicion of hypercarbia or the physiological parameters exceed their threshold:

- ETCO2 > 50mmHg for at least 3 minutes sustained
- SPO2 < 92%
- HR and RR within 2.5 and 97.5 centiles by age as proposed by Wallis LA *et al:*



- Subject complains of symptoms such as dizziness & confusion, even if ETCO2 or SPO2 levels remain within the specified normal range i.e., ETCO2 ≤50mmHg and SPO2 ≥92%

3.6. Subject Replacement

Subjects who drop out will be replaced.

4. TRIAL SCHEDULE

Screening will be screened through forms given out to students for parents to fill up together with the letter of invitation and study information sheet. Only eligible subjects whose parents consented to join in the research study will be shortlisted.

There is only one study visit. Subjects will be de-identified and coded at source. Data on subject's gender, age, race, height, and weight will be obtained. An on-site screening questionnaire will also be administered by the study team to check on the participant's current condition. Each will then undergo a mask fit test to determine the right mask size for the face, and test for leakage by performing the following exercises in sequence, which would take 12 minutes each: normal breathing, deep breathing, turning their heads sideways, up and down, speaking, and bending exercise as described in Section 6.1. Subject will be excluded if he/she is unable to be fitted with any of the mask sizes.

If the subject passes the fit test, he/she will don the monitoring equipment and athletic wear. The equipment will monitor breathing rate and heart rate. Oxygen and carbon dioxide concentrations will also be monitored.

The subjects will then be assessed while at rest and on mild exertion. Parameters obtained will be recorded in de-identified case report form. Please refer to figure 1 in section 5.1.

The test will be carried out in a closed and controlled environment, with temperature range of 26-32 degrees Celsius, relative humidity between 50-80%, and wind speed of <1.5m/s (light breeze).

The general well-being and comfort level of the children whilst wearing the mask will be self-rated using a visual analogue scale at the end of the study procedure.

5. STUDY DESIGN

5.1. Summary of Study Design

This study is designed to explore and determine whether the new disposable particulate respirator is safe and effective for use in healthy children aged 7 to 14 years of age.

Figure 1 shows the algorithm of the summary design.

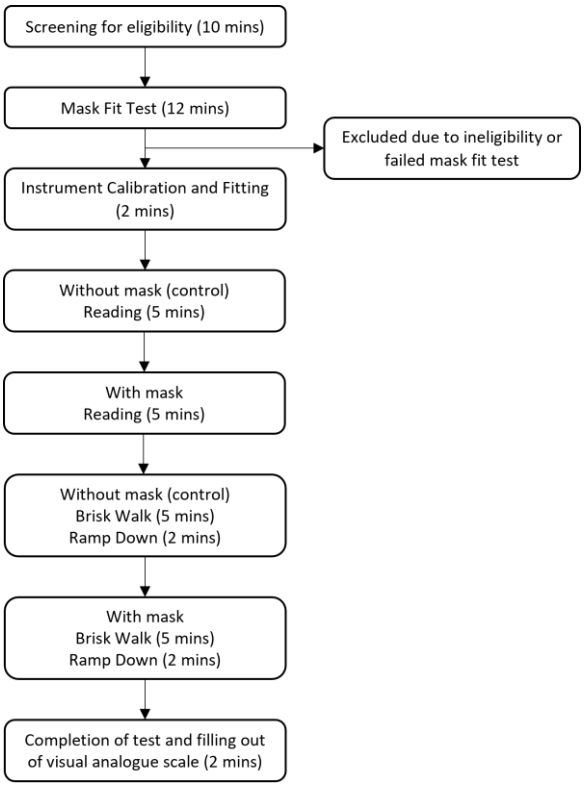


Figure 1. Study Design

6. METHODS AND ASSESSMENTS

6.1. Selection of Test Subjects

a. Screening Visit and Procedures

Pre-test actions
An invitation letter with study information sheet which contains the study's objectives, procedures, risk and benefits will be sent to all subjects aged 7 to 14 years old from the selected schools. Their parents will complete a questionnaire to ascertain the students' general well-being, and to ensure that the appropriate criteria for selection / exclusion are applied. Contact of the designated study team member will be provided for any queries.

Subjects who fulfil the eligibility criteria, whose parents return the Reply form expressing their willingness to participate, will proceed on to the study enrolment.

The total number of eligible students may exceed the stated sample size, in which case the participants will be randomly selected from the pool of qualifying subjects.

b. Study Visit and Procedures

On-site pre-screening
An on-site screening questionnaire will be administered by the study team to check on the participant's current well-being. Informed consent and/or assent if applicable will be obtained.

Mask fit test

Biometric data (gender, age, race, height, and weight) of the subjects will be recorded. De-identified case report form will be used to record data. Each will then undergo a mask fit test to determine the right mask size for the face, and test for leakage by performing the following exercises in sequence, which would take 12 minutes each: normal breathing, deep breathing, turning their heads sideways, up and down, speaking and bending exercise. The fit test will be performed using the Portacount Respirator Fit Tester which indicates the fit factor (ratio of ambient air particle concentration vs. in-mask particle concentrations). A high fit factor will indicate a good seal, and the passing fit factor is 12.5 (8% leakage) based on respirator certification EN 149:2001+A1:2009 FFP2 standards. Should a subject keep failing the fit test despite trying on all the sizes, they will be excluded from the study.

The study

Subjects who pass the fit test will don the monitoring equipment and athletic wear. The physiological monitoring equipment will comprise a pulse oximeter and an $ETCO_2$ monitor. The pulse oximeter will measure through the use of a finger sensor the percent SPO_2% as well as the HR (beats per minute/bpm). The $ETCO_2$ measurement (in mmHg) will be made through the use of a nasal cannula and will be used to determine the level of carbon dioxide within the test participant as well as the RR (breaths per minute) [1].

Impact on subject's physiological measurements for different interventions will then be assessed while at rest and on mild exertion.

Determining the parameters for mild exertion

Mild exertion will be achieved using treadmills at speeds that represent realistic children activity levels during play. As the subjects are of varying ages and heights, with correspondingly different levels of physiological reserves, it may not be feasible to assign a specific treadmill speed that would indicate a uniform level of mild exertion.
Based on the known normal resting heart rate of healthy children ages 7-14 years of 70 to 100 beats per minute, this study proposes to keep the actual speed of the treadmill flexible and primarily depend on the subject achieving a target heart rate zone of 50- 60% of maximum heart rate. The rationale for choosing this target is based on studies in adults were achieving a target heart rate zone of 50- 60% of maximum heart rate would yield an exercise intensity of about 40% of maximal oxygen consumption (VO2max), which is a good indicator of aerobic endurance. For adults, the anaerobic threshold is about 50-60% of VO2max. There is no literature on VO2max for children, hence, to be conservative, we have referenced adult threshold and taken 40% of VO2max as children's normal anaerobic threshold. As such, to attain a stable ETCO2 during aerobic exercise and before the subject crosses the anaerobic threshold, we aim to keep to 50-60% of predicted maximal heart rate.
The most common method to estimate maximum heart rate within the exercise community is to use the 220 – age formula. This study proposes a more conservative assumption of a maximum heart rate of 194bpm, and a target heart rate zone to be achieved on the treadmill of between 100 – 120bpm. As an additional safety measure, subjects should be encouraged to talk comfortably while on the treadmill. This would indicate that the activity level is appropriate. The child will be led to a target heart rate before 3 minutes of steady state data would be taken. Given the physiological differences between different children, as well as their varying levels of fitness, different times may be required to attain the requisite heart rate for the test.

The test will be carried out in a closed and controlled environment, with temperature range of 26-32 degrees Celsius, relative humidity between 50-80%, and wind speed of <1.5m/s (light breeze).

The general well-being and comfort level of the children whilst wearing the mask will be self-rated using a visual analogue scale at the end of the study period.

The study should take about 50 minutes and will be completed within the same day.

c. Final study Visit:

After the completion of the study procedure, there will be no follow-up or repeat visits required.

d. Post Study Follow up and Procedures

Not applicable

e. Discontinuation Visit and Procedures

Subjects may withdraw voluntarily from participation in the study at any time.

6.2 Study Visits and Procedures

There is only one study visit.

7. TRIAL MATERIALS

AIR+ KN95 Mask tested according to the KN95 protocol for respirators

- AE220L
- AE220M
- AE220S

8. TREATMENT

Not applicable

9. SAFETY MEASUREMENTS

9.1. Definitions

No adverse events are anticipated.

9.2. Collecting, Recording and Reporting of "Unanticipated Problems Involving Risk to Subjects or Others" - UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

NAD

9.3. Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

No drugs are used.

9.4. Safety Monitoring Plan

Data will be coded at source and identifiers will also be removed

9.5. Complaint Handling -

The PI is responsible for handling complaints and ensuring the safe conduct of the procedures listed above.

10. DATA ANALYSIS

10.1. Data Quality Assurance

Data collection will be performed by the study team members. Training on proper data collection and data entry methods will be provided to all staff involved in the study. A data quality assurance system which will include appointing designated supervisors to oversee the quality of the fieldwork, logic checks on data, etc. will be setup.

10.2. Data Entry and Storage

Data will be collected first in hard copies that will be stored in a locked drawer in the Pi's locked office. The data will subsequently be entered into password-protected stand-alone desk top computer in the PI's office as well as a password-protected laptop. All documents will be password protected. Access to the data will be controlled by passwords only known to the investigators and the passwords will be changed periodically.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

It is anticipated that a child at rest will have a mean ETCO2 of 40 mmHg with standard deviation of approximately 8 mmHg. Based on the proposed safety threshold of 50mmHg and below for the ETCO2, an increase of 10 mmHg after the subject wears a mask would indicate non-inferiority. A more stringent tolerance level of 5mmHg in ETCO2 will be assumed given the variability of the ETCO2 measurements among the children.

Assuming a one-sided test size of 5% and one-sided power of 80%, at least 80 subjects will be required. To account for the children who may withdraw during the study, a total of 100 children will be recruited, distributed across the age groups.

11.2. Statistical and Analytical Plans

a. General Considerations

The primary outcome of the study is ETCO2. The 95% upper confidence limit of the increase in ETCO2 after wearing a mask will be computed. The proportion and its associated 95% confidence intervals of children whose ETCO2 are > 50mmHg for at least 3 minutes sustained will also be presented. Similar analysis will be carried out for other physiological parameters such as SPO2, HR and RR. In addition, the change in mean of visual analogue scale after the child wears the mask will be compared using the paired-t test and adjusted for baseline imbalance using regression analysis.

These analyses will be repeated for physiological parameters collected while at rest and on mild exertion.

b. Safety Analyses

NAD

c. Interim Analyses

This will not be done.

d. Describe the types of statistical interim analyses and stopping guidelines (if any) that are proposed, including their timing.

NAD

12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

A written, informed consent, and assent if necessary, will be taken from eligible subjects and parents or legal gardian prior to administering the study proper. The consent acquisition process will be conversed in the language preferred by the subject's parents/legal gardian.

12.2. IRB review

This study has been reviewed by the Parkway Independent Ethics Committee (PIEC) and the Ministry of Education (MOE), Singapore.

12.3. Confidentiality of Data and Patient Records

Only members of the study team e.g PI, con-investigator, and study coordinator, will have access to the data. Data collected will be de-identified and coded at source. The patient identifier and assigned code will be kept in hard copy under lock and key in the PI's office. A back up soft copy will be maintained in a password-protected document in a stand-alone desk top computer in the PI's office. Confidentiality of the data will be maintained at all times.

13. PUBLICATIONS

The study findings can be published as manuscripts or in databases with the individual patient's identity and particulars kept anonymously. All manuscripts must acknowledge that the data are collected from the proposed study. The investigators are responsible for reviewing and agreeing to all the publications, ensuring that the samples and data are used in the manner outlined in the protocol, and disseminating results to the respective collaborators/co-authors in a timely manner.

14. RETENTION OF TRIAL DOCUMENTS

Data and identifiers that is collected in hard copies including assigned code will be kept in hard copy under lock and key will be stored in a secured storage room. The data will be stored in a stand-alone computer that will be password protected and stored in a secure location within their office premises. All documents will be password protected. Data that is entered into software will not contain any identifiers. It will be stored for 10 years following the end of the study and then deleted/destroyed.

References

[1] Daniel Yam Thiam Goh, Meng Wai Mun, Wei Liang Jerome Lee, Oon Hoe Teoh, Dimple D. Rajgor. A randomised clinical trial to evaluate the safety, fit, comfort of a novel KN95 mask in children. *Scientific Reports* (2019)