Protocol Amendment to

Trial protocol: Effect of physical distancing during vocal ensemble (choir) rehearsals: protocol for a pilot cluster-randomized controlled trial to investigate feasibility, acceptability, adherence, and outcome distribution

Trial registration: ISRCTN80062362

Amendment 1, 16 December 2025

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Additional virus testing by PCR

Background and rationale
We plan to include both symptoms of respiratory tract infection and infections detected by rapid antigen testing as a composite primary outcome. The inclusion of subjective symptoms reflects all symptomatic infections perceived by the individual participants and is not limited to the infections detected by the rapid antigen tests. The purpose of a composite outcome consisting of both self-reported symptoms and test results, is to achieve a more objective assessment of respiratory tract infections.

Although rapid antigen testing may work as a relatively easy and quick way to detect infections, it may also be problematic for several reasons: Firstly, rapid antigen tests are limited to only detecting three viruses, with a reported sensitivity at 80.8% for influenza A, 65.9% for influenza B, 77.8% for SARS-CoV-2, and 41.5% for RSV.[1] Sensitivity may be even lower in asymptomatic individuals, but is likely higher in those who are most capable of transmitting the disease. Secondly, since the participants are not blinded to the results of rapid antigen tests, the test itself may act as an intervention and influence participants' behavior. If participants in one arm are more likely to be affected by test results and stay home from choir practice in the case of a positive test, this could lead to biased estimates.

PCR testing allows for more sensitive detection of a large panel of pathogens and bypasses any issues related to unblinded test-results since these samples may be analyzed in the laboratory at a later timepoint, after the intervention period. To explore the feasibility and potential for later upscaling we plan to perform self-sampling with subsequent PCR-analysis on one of the choirs included in the study.

Methods
For PCR testing, we select a choir within proximity to laboratory facilities for practical reasons. Briefly, participants will receive swabs (FLOQSwabs®, COPAN Italia S.p.A) and tubes containing virus transport medium (UTM-RT, COPAN Italia S.p.A), together with instructions on how to

perform self-sampling (Appendix 1). Test tubes will be pre-labelled with a unique identifier key for each participant. Key linking test identifiers and personal identifiers will be stored at Services for Sensitive Data (TSD) at University of Oslo with access restricted to the project leader.

Samples will be taken in conjunction with the weekly choir practice, gathered by the choir coordinator, and collected by project members the following day. Participants who are unable to attend choir practice will be instructed to take the test at home on the same day as the choir practice, store the sample at 4ºC and bring it to next week's rehearsal. In the case where the participant is unable to provide the sample within a week, the participant is instructed to contact the project manager to coordinate pick-up.

Samples will be analyzed batch-wise at the Department of Virology at the Norwegian Institute of Public Health using a standard multiplex PCR-assay covering the following respiratory pathogens: Influenza A virus (Flu A), Influenza A-H1 (Flu A-H1), Influenza A-H1pdm09 (Flu A-H1pdm09), Influenza A-H3 (Flu A-H3), Influenza B virus (Flu B), Respiratory syncytial virus A (RSV A), Respiratory syncytial virus B (RSV B), Adenovirus (AdV), Enterovirus (H-EV), Metapneumovirus (MPV), Parainfluenza virus 1/2/3/4, Bocavirus 1/2/3/4 (HBoV), Coronavirus 229E (229E), Coronavirus NL63 (NL63), Coronavirus OC43 (OC43), Human rhinovirus (HRV), SARS-Cov-2 (three different targets), Bordetella parapertussis (BPP), Bordetella pertussis (BP), Chlamydophila pneumoniae (CP), Haemophilus influenzae (HI), Legionella pneumophila (LP), Mycoplasma pneumoniae (MP) and Streptococcus pneumoniae (SP).

Samples positive for these pathogens may undergo sequencing of the pathogen for detailed characterization and phylogenetic analysis.

To evaluate the potential for later upscaling in a main trial setting, we will explore pooled analysis of up to ten samples. If the pooled analysis is positive for one or more pathogens, samples will be reanalyzed separately.

The choir selected for PCR-analysis of samples will be allocated to the intervention arm and not undergo randomization. Participants will be informed that the samples are analyzed retrospectively and test results will be made available to participants upon request.

Statistical analysis
The selected choir will undergo PCR testing rather than rapid antigen tests; we chose not to also include rapid antigen testing to minimize the participants' burden. Hence, results from the selected choir may only be included in analyses of the secondary outcomes (time to a first self-reported respiratory tract infection and number of days with reported symptoms of respiratory tract infection).

Distribution of test results will be reported descriptively. The total number of positive test results for each pathogen will be described for each week and over the trial period, with the corresponding total number of tests as the denominator.

Agreement between PCR test results and symptomatic infections reported by participants will be evaluated.

Limitations
Testing the feasibility of PCR testing limits the number of choirs undergoing testing with rapid antigen tests. However, we believe the advantage of exploring the possibility of large-scale self-testing with PCR tests justifies this limitation. Although PCR tests are expected to outperform

rapid antigen testing, they both rely on the participants self-sampling. Detailed instructions will be provided to participants to ensure correct sampling procedure (Appendix 1).

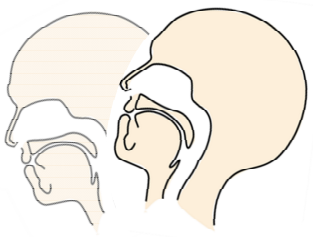
References

1. Bayart JL, Gillot C, Dogne JM, et al. Clinical performance evaluation of the Fluorecare(R) SARS-CoV-2 & Influenza A/B & RSV rapid antigen combo test in symptomatic individuals. J Clin Virol 2023;161:105419. DOI: 10.1016/j.jcv.2023.105419.

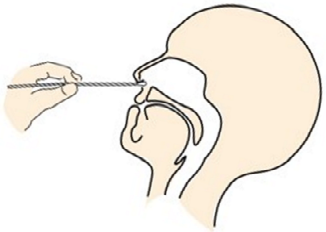
Appendix 1: Instructions for self-sampling.

How to perform a nasal swab

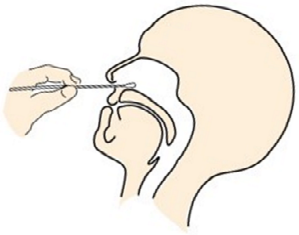
1. Tilt your head slightly backwards and calmly insert the tip of the swab into your nostril so that the entire absorbing part of the swab enters the nostril. Insert at a horisontal angle, not upwards, about 1-2 centimeters into the nostril.
2. Carefully twist and rub the swab along the inside of your nostril for about 10 seconds.
3. Repeat steps 1-2 in the opposite nostril using the same swab. Try not to touch the lower end of the swab.
4. Place the swab directly into the test tube and break the swab at the break point, labeled with a red dot.
5. Screw the cap of the tube on tigthly.
6. Label the tube with your surname and date.



Tilt your head back slighlty.



Swab 1-2 cm in, horizontally.



Twist and rub for about 10 seconds.

The choir leader will collect the samples at the choir rehearsals.
If you are unable to attend rehearsal, you can perform the swab at home:
Perform the swab on the same day as the choir rehearsal.
Put the test tube in the fridge and bring it to next week's choir rehearsal.
Contact project manager Erle Refsum at erle.refsum@fhi.no
to arrange pick-up if you are unable to attend choir practice the following week.

Remember to reply to the weekly questionnaire!
Scan the QR-code to get to the questionnaire:

www.fhi.no/korstudien

Thank you for contributing to this study!

FHI