*Online Supplementary 1: Study Protocol in brief:*

The protocol has been adapted and shortened from two previous publications:

Kheiroddin, P., Gründl, M., *et al.* (2021) ‘How to Implement Safe, Efficient and Cost-Effective SARS-CoV-2 Testing in Urban and Rural Schools within One Month’, *COVID 2021, Vol. 1, Pages 717-727*, 1(4), pp. 717–727. doi: 10.3390/COVID1040057.

Kheiroddin, P., Schöberl, P., *et al.* (2021) ‘Results of WICOVIR Gargle Pool PCR Testing in German Schools Based on the First 100,000 Tests’, *Frontiers in Pediatrics*, 9, p. 1162. doi: 10.3389/FPED.2021.721518/BIBTEX.

***Study Design and Population***

The objective of this proof-of-concept study was to show that regular gargle pool rRT-PCR testing is safe, efficient, and cost-effective in all school environments.

## ***Implementation***

On March 14th , 2021, Cham County decided to participate in the WICOVIR study, which marks the beginning of the pilot phase. In this phase, two pilot tests were conducted on March 22nd and 24th, 2021. With the successful completion of the second pilot test, the preparatory phase for the entire county of Cham with its 38 primary schools began. At the beginning of the project, only the fourth graders of the primary schools took part. However, after changes to the 12th BayIfSMV (Bavarian Infection Protection Measures Ordinance), first- to third-grade students were included. The regular gargle pool PCR testing – and thus marking the beginning of the study – started on April 12th, 2021, two days before school started after the Easter break on April 9th, 2021. This ensured with a relatively high probability that only students who tested negative would attend class after the holidays.

It was essential to build a stable infrastructure for the implementation, which extended over a four-week period. Therefore, a central warehouse for tubes and containers was set up at a secondary school in Cham, which was managed by the local school management. The participating schools placed their orders with the school authority and picked up the test equipment from the central warehouse. The schools then distributed the tubes to the students or their legal guardians. This usually took place on fixed days and via the class teachers. Finally, the students gargled at home on the morning of the test day and brought the two test tubes to school. The individual (second test tube) samples remained with the school administration, and a driver picked up the pools. In the event of a positive pool, the individual samples were collected from the school.

A safety workbench was set up in the laboratory of the SANA Clinic in Cham, where the samples were pipetted, and the containers were cleaned. A driver then drove the pools to the laboratory in Regensburg, where the entire pool underwent the PCR test procedure. If the pool turned out to be positive, the corresponding individual samples were brought to the laboratory in Regensburg and an individual medical test was carried out according to the instructions of the public health office in Cham.

Cham is a rural county thus, a local seminar was offered to school administrators and local authorities to promote and explain the study procedures. As a result, a regional organization board, including the school board, a doctor from the public health office and the county authorities was set up. Tasks were clearly divided in the beginning and meetings were held continuously and often spontaneously among various members of the organizational board.

***Exclusion of previously infected students***

Employees of the public health office checked the database for previously infected (in the past three months) cases for school-aged children (ages 6-10) and contacted their parents prior to the study via email to inform them about the exclusion of their child from the study.

***Pooling results and depooling process***

The pooling results were available the same evening, and in the case of a positive pool, WICOVIR's Regensburg laboratory conducted depooling the next morning.

Consecutively the headmaster of the respective school was informed, which then sent the class into temporary quarantine until the test result of the individual sample was resolved (maximum one day). If only one index person was identified, the quarantine for the other students could be revoked the following day due to the stringent testing regime.

***Data collection***

A questionnaire was developed to document data on viral load (Ct-values), symptoms, duration of symptoms, risk factors, and hospitalization within the family in the web-based management software, and the family of an index person (students/staff who tested positive) was contacted no later than the morning after the positive test. Data was entered into a web-based form and process management software (XIMA® FORMCYCLE, Dresden, Germany) designed by the public health office in October 2020 and tailored to track school outbreaks. Because the public health office is only authorized to contact and trace permanent residents of the county, detailed information on all index and contact persons could only be acquired on those living in Cham County.

Supplementary 2:Table on Direction of Transmission

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Date of test** |  | **Ct-values** | **Date ofprevious negative (pooling) test** | **Onset of symptoms**  | **Assumed direction of infection**  |
| **x** | **Student 1** | **26** | **1st test after holidays** | **none** | ND  |
| x | parent | negative |   | none |
| x | sibling | 19,9-20,0 |   | none |
| x | parent | 23,6-23,8 |   | x+2 |
| **x** | **Student 2** | **28** | **x-2** | **none** | S>F strong agreement |
| No test – fully vaccinated | parent | not tested |   | none |
| x+1 | parent | negative |   | none |
| x+3 | sibling | 17,7-17,9 |   | x+4 |
| **x** | **Student 3** | **27,09** | **none**  | **none** | ND |
| x+3 | parent | 36,6-33,4 |   | x+1 |
| x+3 | parent | 31,9-30,9 |   | none |
| **x** | **Student 4**  | **23,7 – 25,6** | **x-3** | **none** | F>S strong agreement |
| x | parent | 26 - 25 |   | x-4  |
| x | sibling | 17,5 - 23,8 |   | none |
| x | sibling | 17,5 - 23,8 |   | none |
| x+1 | sibling | 20,2 - 20,2 |   | none |
| x+2 | parent | 23 - 23 |   | x+1 |
| **x** | **Student 5** | **21,6 -23,6** | **x-1** | x+1 | S>F strong agreement |
| x+1 | parent | 30 - 29 |   | x+1 |
| x+1 | parent | 36 - 34 |   | x+1 |
| **x** | **Student 6** | **29,22** | **x-2** | **none** | Not relevant |
| No test – fully vaccinated | parent | not tested |   | none |
| **x** | **Student 7** | **25,47** | **1st test after holidays** | **none** | F>S strong agreement |
| x+2 | parent | negative |   | none |
| x+2 | sibling | 29,3-30,0 | x - negative | x+2 |
| x+2 | parent | 16,7-20,1 |   | x |
| x+8 | twin sibling | 20,5-20,8 |  | none |
| x+8 | sibling | 24,5-24,7 | x - negative  | none |
| x+8 | sibling | 17,4-17,5 |   | none |
| **x** | **Student 8** | **25,2 – 26,6 (local test center)**  | **x-1 pool negative, single Ct-38, Delta variant** | **none** | F>S strong agreement |
| x-2 | parent |  18, 3 – 20,3 | Delta variant  | none |
| x-2 | parent | PCR negative |  | none |

Family to student (F>S) or Student to Family (S>F) or not to be determined (ND)

Assumed direction of infection: if an agreement was found among all three reviewers, this was indicated as “strong agreement”