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Title: Evaluation of real-life use of point-of-care rapid antigen testing for SARS-CoV-2 in schools (EPOCRATES): a cohort study
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Reviewer 1: Dr. Aaron Campigotto
Institution: The Hospital for Sick Children, University of Toronto
General comments (author response in bold)

Thank you for the opportunity to review this interesting paper on the use of RADTs compared to PCR for detection of SARS-CoV-2 cases within schools and especially within exposed cohorts.

1. This study occurred in Jan to June 2021, would a description of the viral epidemiology be helpful to put these finding in perspective. The authors state this was pre-Delta (and Omicron) but the circulating virus was predominately alpha?
Thank you for this relevant comment. The study was conducted from January 2021 to June 2021. We have added the following sentence: "…the predominant circulating strains were ancestral SARS-CoV-2 and the Alpha variant as of April 2021" with a reference containing local epidemiological data, in the Methods, page 6. In addition, the following sentence: "The study was performed before the advent of the Delta variant in our region. Because RADT detects the N protein, we expect that its sensitivity and specificity would not be affected negatively, as viral loads of Delta variant infections are reported to be higher" (Discussion, page 13) is meant to highlight that the Delta variant was not yet circulating in Quebec during the study period.

2. Methods, 4th paragraph:

- Expand and consider separating this description as the asymptomatic surveillance and symptomatic case detection are very different groups. Difficult to follow with both under the same heading.
Thank you for this comment. We have modified the headings to make this clearer, as: a) surveillance screening in asymptomatic participants, versus b) symptomatic participant testing.

3. Methods, 5th paragraph:

- Any additional PCR at D21, D28 or only RATs? Were these included in the sensitivity analysis as they are quite distant from exposure and are they more in keeping with asymptomatic surveillance?
Thank you for this question. We did PCR in addition to RADT 3 days after the exposed participants' last contact with the index positive case in school, as well as 48h before their return to school (whether that was after a 7-day quarantine OR after a 14-day quarantine). We did RADT alone on day 10, day 21 and day 28, and did not repeat PCRs considering this was 'additional' testing than what would have been done by public health for individuals with significant exposures to a

positive case, in that time. The RADT at day 21 and 28 were not included in the sensitivity analysis.

Results

4. RAT - define equivocal and weak positive. Since this is a qualitative assay, is there interuser variability? PCR - define equivocal and weak positive results (based on Ct values?).
We have addressed this question by providing a description of what was considered positive (Ct under 33), weakly positive (Ct 33-36.9), equivocal (Ct 37-39.9) and negative (Ct over 40) for PCR, in the Methods, page 7. The inter user variability was not significant for a given sample.

Results

5. Clarify 37/57 cases on line 163. Were the 57 cases from the total 76 cases? Did the study team define the known exposure based on epidemiologic data or public health? Clearly state the exposures were based on clinical data and case definitions.
There were 76 SARS-CoV-2 PCR positive cases during the study period. There were 57 cases where the source was known; and there were 37 cases where the contact occurred with a known positive household member. Known exposures were based on what the Montreal Public Health considered a significant exposure.

Results (vaccination)

6. Was percentage of vaccination (by dose/time since dose) available for cases?
The percentage of vaccination (by dose / time since dose) was not available for cases. When the study started, vaccination was not yet available in the population and therefore, participants had not consented for us to capture this data.

7. Was there a difference in vaccination uptake between the schools? Were vaccination rates for staff/students available at the schools? Currently the time when vaccination was available is listed but this does not necessarily correlate with uptake.
Thank you for this comment. There may have been participants that received their first dose of vaccine against SARS-CoV-2 during the study period. However, the study period ended 2 weeks after vaccination began for children aged 12 years and older. In addition, the aims of this study were to assess the performance of RADT for SARS-CoV-2 infection in school settings, and to see if exposed students could safely return to school earlier than 2 weeks based on serial negative RADT. As the reviewers may know by now, being immunized (especially with 1 dose only at that time in 2021) does not fully prevent SARS-CoV-2 infection and transmission. Therefore, knowing if some participants had received or not 1 dose of vaccine during the very few weeks of the study period that were left was not a priority. In addition, because the study had started almost 6 months prior to vaccination, participants had not consented for us to collect this confidential data. We have added a sentence in Discussion, page 14 regarding this issue, as follows: "Some participants may have received their first dose of vaccine during the last few weeks of the study, however we did not collect data regarding vaccination, as infection and transmission could still occur despite immunization

and therefore the findings of this study related to the use of RADT to prevent outbreaks are valid and relevant."

Discussion, paragraph 3

8. The sensitivity of 78.6% in symptomatic individuals is not described in the results? Can this be clarified.
Thank you for this comment. This sensitivity of 78.6% is for symptomatic staff and students combined. We have clarified this in the text, as follows: "The overall sensitivity of RADT in symptomatic staff and students was 78.6% (95% CI 49.2-95.3)."

Discussion, paragraph 5

9. It is stated that despite vaccination, transmission is occurring but without vaccination rates and status, is this true? Please clarify. Is this a finding of this study?
As directly cited from the CDC's science brief on COVID-19 vaccines: "Vaccinated people can still become infected and have the potential to spread the virus to others" (https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/fully-vaccinated-people.html). We have added this reference where we state that the findings of this study are still relevant, despite not having documented whether participants had received the first dose of the COVID-19 vaccines series.

Reviewer 2: Dr. Jim Strong
Institution: National Microbiology Laboratory, University of Manitoba Max Rady College of Medicine
General comments (author response in bold)

In this paper the authors use RADTs in a school setting to determine feasibility of their use for school screening for SARS-CoV-2 comparing to the gold standard of PCR testing in a prospective observational cohort design. By evaluating 2099 students and 286 staff from two separate Quebec schools they found that the RADTs had a very good specificity (99.8-100%) but rather disappointing sensitivity (ranging between 28.6% in asymptomatic to 83.3% in symptomatic). Some secondary analysis showed that students that returned after a 7-day quarantine and negative PCR did not result in secondary cases. As well, of the cases where source of infection was known 72.5% were from household transmission, 25% were of intra-school origin and 0.01% (1 of 57) were from community contacts (*see comment below). These authors conclude that RADTs are reasonable for screening of symptomatic students but less so for asymptomatic. They also wanted to know if serial sampling of contacts would mitigate intra-school school spread.

Overall, the paper is well written and has reasonable science. It comes to very reasonable conclusions and the numbers are appropriate. As public health practitioners, we all rely on timely and reliable determination of cases to implement non-broadly applied interventions. Although PCR-based tests have served as the gold-standard for diagnostics, due to their requirements for specialized skills and lab setups, as well as longer turn-around-times, the hope that the recent supply of RADTs without most of these hang--ups could supplant. This paper suggests that RADTs could be used for screening of symptomatic students and staff.

1. I think it would be very important to include the data on the variants circulating throughout the time of this study. This may have impacted the results and deserves mention.

Thank you for this relevant comment. Please refer to our response to Reviewer #1, Question 1 for our detailed response and modifications.

2. Given that vaccinations began in these demographics (students and adults) I think a more thorough discussion of the impact this may have had to this study should be entertained. I am not suggesting that this needs to be included in the analysis but rather that this concept should be further developed as a confounder in the discussion.

Thank you for this comment. There may have been participants that received their first dose of vaccine against SARS-CoV-2 during the study period. However, the study period ended 2 weeks after vaccination began for children aged 12 years and older. In addition, the aims of this study were to assess the performance of RADT for SARS-CoV-2 infection in school settings, and to see if exposed students could safely return to school earlier than 2 weeks based on serial negative RADT. As the reviewers may know by now, being immunized (especially with 1 dose only at that time in 2021) does not fully prevent SARS-CoV-2 infection and transmission. Therefore, knowing if some participants had received or not 1 dose of vaccine during the very few weeks of the study period that were left was not a priority. In addition, because the study had started almost 6 months prior to vaccination, participants had not consented for us to collect this confidential data. We have added a sentence in Discussion, page 14 regarding this issue, as follows: "Some participants may have received their first dose of vaccine during the last few weeks of the study, however we did not collect data regarding vaccination, as infection and transmission could still occur despite immunization and therefore the findings of this study related to the use of RADT to prevent outbreaks are valid and relevant."

3. It is unclear why the authors chose to describe the demographic make-up of the two schools in the methods and neglect to provide any further analysis on the two different populations. If they chose to include this analysis, which school had participation rate of 78.5% and which 63.5%, 94.4% for students and 89.5% for staff? Furthermore, were there any other differences between these groups (male:female ratios, vaccine uptake, school social distancing policies, others)?

We decided to provide a brief description of the demographics of each school, because we wanted the study population to represent the multicultural nature of Montreal and varied socio-economic backgrounds, depending on the geographical area. However, as this was noted by the reviewers, it was not included in the analyses, therefore we have simplified this by changing the description of school enrollment in Methods, page 6 to: "Pensionnat du Saint-Nom-de-Marie (PSNM) is a private school and École secondaire Calixa-Lavallée (ESCL) is a public school.".

As mentioned in Methods, page 6: both schools followed the same Quebec provincial Ministry of Education recommendations, in terms of class groups (called bubbles), mandatory masking (as of October 8th, 2020) and distancing. We did not investigate biological sex as a determining variable, as we did not anticipate that RADT would perform differently in males than females, nor that males vs. female status would impact transmission in exposed contacts returning to school earlier.

4. The PCR cut-offs need to be defined. What is considered positive <40 cycles, 2 or more targets and are there quality controls of housekeeping gene(s). Why were some samples rejected – on what criteria? (see line 121). What is weak positive and is this included as positive in overall data?
There is only 1 viral target: the E gene, as described in the attached reference in Methods, page 7 (Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3).) β-actin is used as housekeeping gene to assess the quality of the sample.
A PCR was considered invalid if actin and SARS-CoV-2 were not detected, as this may mean that SARS-CoV-2 was not detected because the sample was not reliable, that RNA was degraded or that there were PCR inhibitors in the sample. We have addressed this by providing a description of what was considered positive (Ct under 33), weakly positive (Ct 33-36.9), equivocal (Ct 37-39.9) and negative (Ct over 40) for PCR, in the Methods, page 7. Weakly positive PCR were not included as positive in the overall data analysis.

5. Students returning after 7 day quarantine and negative-tested via PCR did not lead to subsequent outbreaks – given that this paper was designed to test the ability of RADT to triage students when compared to PCR, should these researchers have attempted to use RADT to assess appropriateness of student return (or at least compare PCR to RADT)?. This is especially true given that CDC recommends that individuals get tested five to seven days have passed since the last exposure and there is uncertainty whether RADTs can be effectively used in this capacity. Furthermore, there is reasonable evidence to suggest that RADTs can detect live SARS2 over non-viable virus that may more readily be detected by PCR. Authors should justify the reasoning for not including the RADT assessment of returning students after exposure in primary analysis.
As stated in the Methods section, on page 7: exposed contacts of a positive individual in a classroom were allocated to a 7- or 14-day quarantine. Both RADT and PCR were done 3 days after the contact. In addition, both RADT and a PCR were performed within 2 days of anticipated return to class date and then RADT alone was performed on day 14, 21 and 28 if the initial RADT and PCR was negative after contact. Therefore, we did include RADT assessment of returning students after exposure. The following sentence in the Methods, page 7 explains this: "Students were allocated to a 7- or 14-day quarantine, staffs were allocated to a 7-day quarantine, with tests (both nasal RADT and gargle PCR) three days after last contact with the known positive case, and up to two days before the end of quarantine. RADT alone was performed on day (D)14, D21 and D28, if the initial PCR was negative. If symptoms developed, both the RADT and PCR were performed."

Some minor comments:

6. Line 36 Please modify the statement that "RADT perform best in the early stages of infection, when viral load is generally high" to include the fact that the sensitivity is poor in asymptomatic patients.
Thank you for this comment. The fact that sensitivity is poor in asymptomatic patients is mentioned in the sentence following the one the reviewer is referring to: "Reported RADT sensitivity ranges from 28.9% to 98.3%, with improved RADT

sensitivity in samples with high viral loads and in symptomatic individuals", therefore we have not modified the sentence.

7. Line 50 I am wondering why the authors chose to define the demographic differences of the two schools involved and not include this difference in the final analysis or discussion. Did this have impact or if not, why should this info be included?
We decided to provide a brief description of the demographics of each school, because we wanted the study population to represent the multicultural nature of Montreal and varied socio-economic backgrounds, depending on the geographical area. However, as pointed out, we did not include this in the analyses and we do not believe that these demographic differences would impact RADT performance, therefore we are removed the demographic differences from the Methods, to describe this as: "Pensionnat du Saint-Nom-de-Marie (PSNM) is a private school and École secondaire Calixa-Lavallée (ESCL) is a public school."

8. Line 134 1491 RADT and PCR tests done on asymptomatic – were these paired?
That is correct. According to the study protocol (ref. Methods), asymptomatic participants were tested with both nasal RADT and water gargle PCR.

9. Line 153 76 PCR (gargle or nasal) positive cases – did they do nasal PCR? This was not described in the methods, rather only gargle. There is some inconsistency in this throughout the paper.
Thank you for this comment. The reason why we mention that participants may be PCR-positive on gargle or nasal specimens is because as described in Methods, page 6: asymptomatic participants were tested with nasal RADT and water gargle PCR. Symptomatic participants would undergo water gargle PCR as well as nasal RADT, with residual sample also tested by PCR.

10. Line 221 We cannot infer whether PCR positive individuals were contagious. I think this statement needs to be developed more including the literatures suggestion that RADTs may be good at determining live virus (better than PCR).
We have modified as follows: "We cannot infer whether PCR positive individuals were contagious, however we used PCR as the gold standard test to consider individuals infected – as was being done by the public health jurisdiction during the time that the study took place. Since then, there has been some evidence suggesting that RADT may correlate well with live viral culture (41)."
41 - Pekosz A, Parvu V, Li M, Andrews JC, Manabe YC, Kodsi S, et al. Antigen-Based Testing but Not Real-Time Polymerase Chain Reaction Correlates With Severe Acute Respiratory Syndrome Coronavirus 2 Viral Culture. Clinical Infectious Diseases. 2021;73(9):e2861-e6.

11. Figure 1 needs better more description of content. E.g., This is supposed to include students and staff although reader cannot discriminate. What are the sections divided into, what is the other, what is a reception class?
Figure 1 only displays student participation, which is why we indicated "The participation rate for students was 78.5% and 63.5% (Figure 1), and…". This is shown by school (PSNM versus ESCL), by level (Secondary 1, Secondary 2, etc.) with each color representing a year and each box representing a class within the

year. The reception class is a class for students newly arrived to Canada, and Other represented special education classes for students with learning disorders or other difficulties in school. We have clarified this by providing more details to the Figure legend.