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Preview

A dual antibody test for accurate surveillance of SARS-CoV-2 exposure rates

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Accurate population surveillance of SARS-CoV-2 infection has been hampered by limited testing and inadequate serological assays. In a recent issue of *Med*, Hippich et al.[1] describe a two-step antibody test with 100% specificity, revealing higher-than-reported SARS-CoV-2 exposure rates in children.

Nasal swabs followed by reverse-transcription polymerase chain reaction (RT-PCR) detection of SARS-CoV-2 have been at the forefront of screening programs to identify and isolate COVID-19 cases. However, the requirement for specialized laboratories and trained personnel limits testing capacity. The broad range of COVID-19 clinical manifestations, including asymptomatic infections, has further disrupted traditional epidemiological surveillance, with confirmed COVID-19 cases likely capturing only a subset of the true exposure rate. Population-based serological assays can aid the quantification of the proportion of the population presenting antibodies against SARS-CoV-2 and thus exposed and potentially immune.[2] Due to the delay in antibody production in response to infection, antibody testing is not a suitable screening tool.[3] However, antibody testing has a critical role in successful epidemic surveillance, implementation of public health and containment measures, and evaluation of the impact of these measures.

As a result, several antibody tests have been developed and a myriad of serological surveys have been conducted. Studies examining COVID-19 epicenters such as Wuhan,[4] and hard-hit cities (e.g., Los Angeles[5]) and countries (e.g., Spain[2]) have reported exposure prevalence of equal to or less than 5% in the population. However, the inadequate specificity of many antibody assays remains problematic, particularly in the surveillance of populations with low SARS-CoV-2 exposure incidence.[6] With children shown to have lower rates of SARS-CoV-2 infection than adults, a test with 99% specificity would present a frustrating rate of false positives.

In a recent issue of *Med*, Hippich et al. report the development of a two-tiered serology assay with 100% specificity applied to population-scale immune surveillance of children in Bavaria, Germany.[1] The dual screening approach consisted of a first sensitive detection of antibodies against the SARS-CoV-2 spike protein receptor binding domain (RBD), followed by, if positive, an orthogonal test for antibodies against the nucleocapsid antigen. By using samples from the Fr1da and Freder1k Bavarian public health studies for type 1 diabetes, Hippich et al. had a unique opportunity to track antibody prevalence in nearly 16,000 children and 2,000 neonates, spanning from the time before the first case of COVID-19 in Germany to a reopening post-lockdown.

Antibodies were measured using a luciferase immunoprecipitation system (LIPS). A threshold for positivity was established using over 3,000 samples collected from children in the latter half of 2019 and a cohort of 75 SARS-CoV-2-positive individuals. After validation of the definition of antibody positivity with samples collected in the spring of 2019, it was observed that none of the 3,887 children sampled prior to January 2020 were antibody positive, conferring 100% specificity. While the authors did not formally rule out the possibility of cross-reactivity with other coronaviruses, it is expected that the thousands of negative samples from 2019 would have included children previously infected by common cold coronaviruses. Antibody positivity was observed in 73 of the 75 positive individuals, resulting in 97.3% sensitivity.

The public health screening by Hippich et al.[1] during the pandemic showed an overall SARS-CoV-2 prevalence in children between April and July 2020 of 0.87%, which is 6-fold higher than the incidence of cases reported by health authorities. The ability to use separate samples, all collected under the same conditions, in the definition and validation of threshold positivity as well as for the pandemic immune surveillance, provides robust confidence in the findings and highlights the utility of population screening programs with consented biobanking.

Beyond the assessment of SARS-CoV-2 prevalence, this population-scale study addresses many questions around SARS-CoV-2 exposure, such as the influence of age and gender, the true extent of asymptomatic infections, as well as association with autoimmune diseases, specifically type 1 diabetes (Figure 1).

First, in contrast to reported virus-positive cases, there was no difference in antibody frequency between younger and older children. Furthermore, there were no differences in antibody frequency between boys and girls. Other studies, such as the nationwide seroepidemiological survey in Spain, have also reported no difference in seroprevalence by gender.[2] Still, while exposure may be similar, other evidence suggests that SARS-CoV-2-infected men have a consistently higher risk of hospitalization and death than women.[7]

Second, Hippich et al.[1] reviewed questionnaires on previous SARS-CoV-2 exposure and symptoms completed by the parents of nearly 5,000 children whose blood samples were collected between April and July 2020, revealing that almost half of the antibody-positive

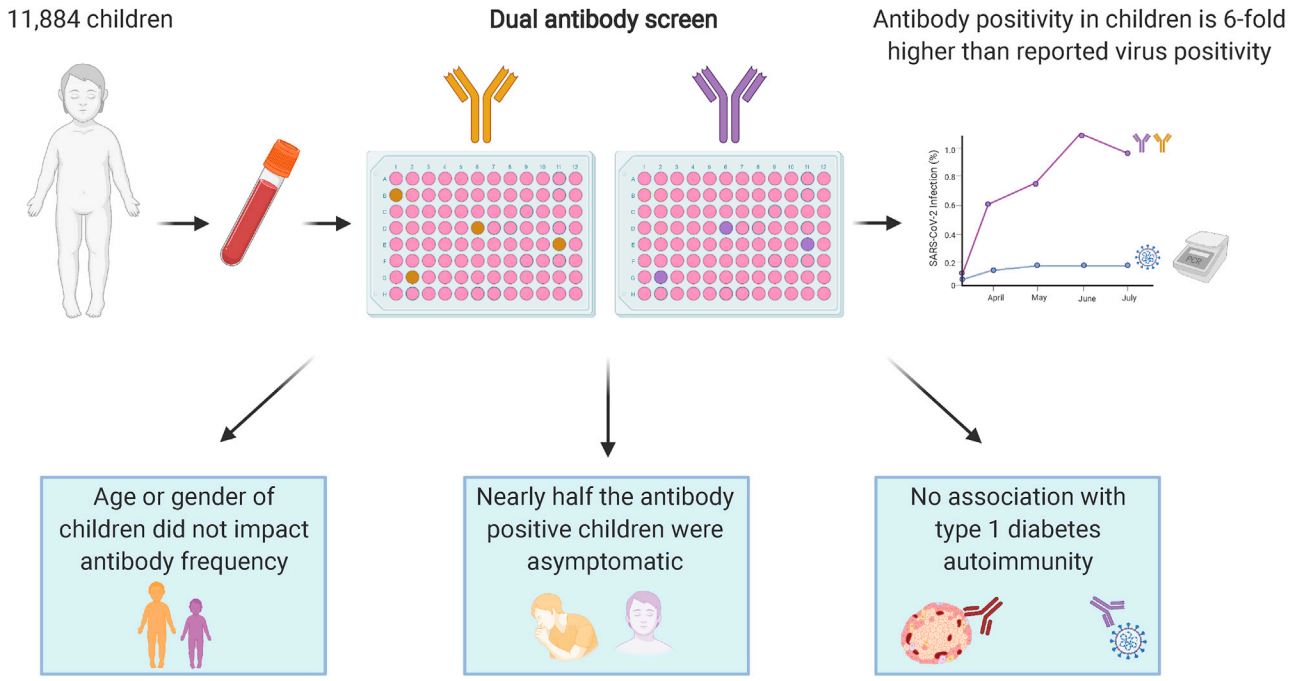

Figure 1. Graphical summary of the two-staged detection of SARS-CoV-2 antibodies

Graphical summary of the two-staged detection of SARS-CoV-2 antibodies by Hippich et al.[1] and their findings related to infection prevalence in children. Created with Biorender.com.

children were asymptomatic. Notably, half of these asymptomatic cases had reported a virus-positive family member. This finding strongly supports that the identification of SARS-CoV-2-positive children requires testing not only of children with symptoms, but also of children who may have had contact with virus-positive individuals. More effective testing strategies may help to inform the safe reopening of schools.

Finally, case reports of new-onset diabetes and severe complications with pre-existing diabetes in COVID-19 patients have generated interest in the investigation of the relationship between SARS-CoV-2 infection and diabetes.[8] Since the blood samples tested in this report were collected as part of the Fr1da study, a study designed to detect and follow children with pre-symptomatic type 1 diabetes, Hippich et al.[1] were well-situated to address this question. In brief, no association between SARS-CoV-2 antibody positivity and type 1 diabetes autoimmunity was observed.

Continued widespread surveillance requires an accurate assay amenable to high throughput screening. Hippich et al.[1] stress that neither the LIPS assay nor the antigens (i.e., RBD and nucleocapsid) are superior to existing tests, but that the strategic use of multiple assays with a different target antigen for confirmation is key to achieving 100% specificity. The authors extended this approach to testing nearly 2,000 dried blood spots from neonates and indicated that a semi-automated procedure currently enables the testing of over 1,000 samples daily.

Overall, the two-stage strategy presented by Hippich et al.[1] provides a useful advancement in accurate population-level immune surveillance for SARS-CoV-2. One question that remains to be addressed is the neutralizing activity of the antibodies detected. Further studies are needed to determine the extent of immunity in those individuals who have tested positive for antibodies. A combined knowledge of SARS-CoV-2 antibody prevalence and extent of immunity will be instrumental in implementing public health measures to manage the pandemic.

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