Challenges in interpreting SARS-CoV-2 serological results in African countries

A diagnosis of COVID-19 is based on a positive PCR test for SARS-CoV-2. Over the past year, PCR testing capacity has varied globally due to the availability of tests, and testing strategies have targeted mainly symptomatic individuals. Therefore, the spread of the virus is probably wider than the numbers reported by official surveillance systems that are based on PCR results. Serology tests detect antibodies against SARS-CoV-2, which start being measurable around 1–2 weeks after infection. They are used in seroprevalence studies to estimate the proportion of people in a population that has been infected, including asymptomatic infection. These studies are of particular importance in African countries, where reported testing and incidence are among the lowest in the world.

We did a cross-sectional serological survey of staff working in health-care facilities in Kinshasa, the capital of the Democratic Republic of the Congo, where the first COVID-19 case was reported on March 10, 2020, in a traveller returning from France. We preselected five health zones across the city, from which we included one hospital, two health-care centres, and one COVID-19 response team. Within each facility, health-care workers and other service staff from different departments were randomly selected. Those who provided written consent to participate were enrolled in the study and a blood sample was taken, which was further analysed in the virology laboratory of the Institut National de la Recherche Biomédicale in Kinshasa. The study was approved by ethics committees in Belgium (number B3002020000144) and the Democratic Republic of the Congo (189/CNES/BN/PMMF/2020).

Between July 17 and Aug 13, 2020, 562 blood samples were collected from 562 participants and five different SARS-CoV-2 serology tests were done on each sample: two in-house Luminex IgG based assays using recombinant nucleocapsid and spike protein 1, and three commercial assays targeting the receptor binding domain on the spike protein, all validated by the manufacturers with serum samples from Europe. These three commercial assays were the EUROIMMUN IgG ELISA (EUROIMMUN, Lübeck, Germany)—a widely used assay in Europe, with sensitivity of 94·6% and specificity of 99·8%—and Quickzen IgG and IgM (Zentech, Belgium), a lateral flow test that detects IgG and IgM antibodies on a single device with a specificity of 100% and sensitivity of 68·8% for IgM and 49·2% for IgG.[1] The blood samples were also tested for malaria with microscopy at the same facility.

The median age of participants was 42 years; 242 (43%) of 562 participants were male and 320 (57%) were female. 27 study participants declared having previously had a SARS-CoV-2 PCR test between March and July, of whom eight (30%) of 27 reported a positive result (1% of the total population).

202 samples (36%) were seropositive using the EUROIMMUN IgG, 171 (30%) using the Quickzen IgM and 72 (13%) using the Quickzen IgG. Only 46 (8%) of 562 samples were positive by all three tests. 89 (16%) samples were positive for malaria by microscopy. Two of eight participants who had previously tested positive by SARS-CoV-2 PCR were seronegative by all three commercial serology tests.

The Kappa coefficient, used to measure agreement between tests,[2] was 0·3 for the EUROIMMUN IgG assay and the Quickzen IgG assay, which is considered a minimal agreement. Because specificity for both tests is nearly 100%, but sensitivity is very different, we assessed whether all samples that were positive with the Quickzen IgG assay would be confirmed as positive with the EUROIMMUN IgG. Only 59 (82%) of 72 samples that were positive with the Quickzen IgG assay were also positive with the EUROIMMUN IgG assay.

Depending on the commercial assay used, seropositivity in health-care staff in our study varied between 13% and 36%, which is relatively high considering the low number of symptomatic and severe cases reported in Kinshasa by the end of the study period. In other African countries, SARS-CoV-2 seroprevalence has been estimated at 45·1% in frontline health-care workers in Nigeria (Elabscience ELISA IgG),[3] 40% in women attending antenatal clinics and people living with HIV in South Africa (Roche ECLIA IgG and IgM),[4] 12·3% in health-care workers in Malawi (Omega ELISA spike protein and nucleocapsid protein,[5] 5·6% in blood donors in Kenya (in-house ELISA IgG),[6] and 3% in the general population in Ethiopia (Abbott CMIA IgG).[7] Although these studies were done in different settings and using different serological tests, all of them tend to give a higher seroprevalence than expected on the basis of surveillance data of confirmed cases in the study period. Given our observations, part of this discrepancy could be due to the assays used.

One explanatory hypothesis for the higher than expected rate of seropositivity could be because of cross-reaction of the tests with other circulating viruses or parasites in the African subcontinent that could lower their specificity. The presence of pre-existing antibodies recognising SARS-CoV-2 in uninfected individuals due to seasonal coronaviruses was identified by Ng and colleagues,[8] whereas Tso and colleagues[9] showed that pre-pandemic samples from sub-Saharan Africa had higher cross-reactivity against SARS-CoV-2 than those from the USA. However, these observations need to be confirmed in larger sample sizes to assess whether prevalence of pre-existing antibodies

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For more on **EUROIMMUN ELISA systems** see https://www.coronavirus-diagnostics.com/antibody-detection-tests-for-covid-19.html

For more on **testing** see https://www.finddx.org/covid-19/test-tracker/

For the **WHO COVID-19 dashboard** see https://covid19.who.int/

are consistently higher in African countries. Cross-reactivity between SARS-CoV-2 and acute malaria infection was observed in a study in Benin by Yadouleton and colleagues.[10] Our data do not show any correlation between participants who tested positive for malaria and those testing positive with any of the commercial SARS-CoV-2 serological tests.

Notably, SARS-CoV-2 emerged only 1 year ago and antibody response according to severity of infection and the duration of antibody persistence are not yet completely understood. However, sensitivities and specificities of serological assays can vary across populations, as shown in the early 1990s with HIV serological tests, for which lower specificity in serum samples from African individuals was observed than in samples from European individuals.[11]

The potential lower specificity of SARS-CoV-2 commercial tests in African countries, together with the low reported prevalence of clinical cases, makes the interpretation of population surveys in this setting difficult. The seroprevalence results could be misleading and even report more false-positive cases than true-positive cases.[12]

On the basis of the low agreement between test results of our study and the available literature, we would like to warn the research community and policy makers to interpret with caution the results of seroprevalence studies done in African countries with commercial tests validated in Europe, the USA, or Asia. To establish whether these high seroprevalences are a sign of serious under-reporting of COVID-19 cases, a milder COVID-19 clinical presentation, or a cross-reaction with other circulating pathogens, we recommend in this context the use of a combination of serological tests, targeting two or more independent antigens, adjusting the cutoff values due to the overall higher background noise, or repeated serosurveys of the same population to better understand the serological profile dynamics. It will be necessary to develop standardised testing strategies, as was done in the past for serological diagnosis of HIV, and assess assays for sensitivity and specificity on reference panels that include samples from different geographical areas, including Africa.

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*Antoine Nkuba Ndaye, *Ana Hoxha, Joule Madinga, Joachim Mariën, Martine Peeters, Fabian H Leendertz, Steve Ahuka Mundeke, Kevin K Ariën, Jean-Jacques Muyembe Tanfumu, Placide Mbala Kingebeni , Veerle Vanlerberghe*
ahoxha@itg.be

Department of Virology (ANN, SAM, J-JMT) and Department of Epidemiology (JMad, PMK), Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo; Institut de Recherche pour le Développement, University of Montpellier, Montpellier, France (ANN, MP); Virology Unit, Department of Biomedical Sciences (JMar, KKA) and Tropical Infectious Diseases Unit, Department of Public Health (AH, VV), Institute of Tropical Medicine, 2000 Antwerp, Belgium (AH) ; Evolutionary Ecology Group (JMar) and Department of Biomedical Sciences (KKA), University of Antwerp, Antwerp, Belgium; Epidemiology of Highly Pathogenic Microorganisms Project Group, Robert Koch Institute, Berlin, Germany (FHL); Department of Medical Microbiology, University of Kinshasa, Kinshasa, Democratic Republic of the Congo (SAM, J-JMT, PMK)

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