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**Short communication**

**Performance evaluation of Abbott ARCHITECT SARS-CoV-2 IgG immunoassay in comparison with indirect immunofluorescence and virus microneutralization test**

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**ABSTRACT**

**Background:** Serological tests for anti-SARS-CoV-2 antibodies are becoming of great interest to determine seroprevalence in a given population, define previous exposure and identify highly reactive human donors for the generation of convalescent serum as therapeutic.

**Objectives:** We evaluated the diagnostic performance of the Abbott ARCHITECT SARS-CoV-2 IgG test, a fully automated indirect immunoassay that detects antibodies directed to a recombinant SARS-CoV-2 Nucleocapsid antigen.

**Study design:** Abbott ARCHITECT SARS-CoV-2 IgG immunoassay was compared to an indirect immunofluorescence assay (IFA) on sera from patients with COVID-19 collected at different days after symptoms onset or infected by other human coronaviruses. Comparison with neutralization test was also performed.

**Results:** After 7, 14 and > 14 days after onset ARCHITECT was positive on 8.3% ; 61.9% and 100% of the tested samples compared to 58.3% ; 85.7% and 100% by IFA. The sensitivity was 72% vs. IFA and 66.7% vs. a real-time PCR, the specificity was 100%. On 18 samples with neutralizing activity, 17 were positive by Abbott ARCHITECT SARS-CoV-2 IgG.

**Conclusions:** In our study, Abbott ARCHITECT SARS-CoV-2 IgG assay showed a satisfactory performance, with a very high specificity. IgG reactivity against SARSCoV-2 N antigen was detectable in all patients by two weeks after symptoms onset. In addition, concordance between this serological response and viral neutralization suggests that a strong humoral response may be predictive of a neutralization activity, regardless of the target antigens. This finding supports the use of this automated serological assay in diagnostic algorithm and public health intervention, especially for high loads of testing.

1. **Background**

The rapid spread of severe respiratory syndrome coronavirus-2 (SARS-CoV-2) [¹] has caused, as of June 20th, 2020, almost 8.5 million people infected worldwide and over 455,000 COVID-19 related deaths [²]. While viral RNA is the preferred marker for diagnosis [³,⁴], serological methods may help both to diagnose COVID-19 suspect cases and to assess total prevalence of the infection, contributing to plan public health measures [³-⁶].

2. **Objectives**

We report an evaluation of the Abbott ARCHITECT SARS-CoV-2 IgG assay on characterized serum samples from SARS-CoV-2 infected and uninfected patients in Italy.

3. **Study design**

Clinical sensitivity of the Abbott ARCHITECT SARS-CoV-2 IgG assay was verified on a panel of 140 sera obtained from patients diagnosed as SARS-CoV-2-infected (COVID-19 panel), based on molecular testing for

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SARS-CoV-2 RNA performed by real-time RT-PCR on respiratory secretions, or on clinical symptoms plus the presence of SARS-CoV-2-specific antibodies by the reference method employed in the laboratory. This method is an indirect immunofluorescence assay (IFA), established using home-made slides prepared with Vero E6 cells infected with SARS-CoV-2 isolate in the INMI BSL3 facility, as described elsewhere [7].

The panel included serum samples collected at different days from symptoms onset (DSO): 0–7 (n = 12); 8–14 (n = 21); > 14 (n = 27; range 15–82 DSO), unknown DSO (n = 80). All samples had been anonymized before use.

Specificity was checked on 20 samples from convalescent patients diagnosed with other human coronavirus infection: HKU1V (n = 12); NL63 V (n = 5); OC43 V (n = 2); 229 EV (n = 1) and on 17 samples from patients with no respiratory disease matched for age. All 37 samples have been collected before the SARS-CoV-2 epidemic in Italy.

Samples were tested by the Abbott ARCHITECT SARS-CoV-2 IgG assay (research use only -RUO- at the time of this study), which is a two-step fully automated, indirect immunoassay that detects antibodies directed to a recombinant Nucleocapsid (N) SARS-CoV-2 antigen. Results are reported as an Index (ratio of the chemiluminescent signal between the samples and a calibrator), with values >1.4 indicating a positive result. On 18 samples from COVID-19 patients, collected between 42 and 82 DSO, SARS-CoV-2 microneutralization test was also performed [8]. Briefly, patients’ sera were heat-inactivated, diluted 1:10 in serum-free medium, and titrated in duplicate in two-fold dilutions. Equal volumes of SARS-CoV-2 (100 TCID50/well) and serum dilutions were mixed and incubated at 37 °C for 30 min. Subsequently, 96-wells tissue culture plates with sub-confluent Vero E6 cell monolayers were incubated with 100 μl/well of virus-serum mixtures at 37 °C and 5% CO2. The endpoint titer for neutralizing activity was established by light microscopy inspection to assess the absence of cytopathic effect (CPE) after 72 h.

Positive concordance of Abbott ARCHITECT SARS-CoV-2 IgG assay in comparison with the DSO and sensitivity vs. IFA and RT-PCR were calculated by 2 × 2 contingency tables. Two-tailed 95 % confidence intervals were calculated. The overall agreement and correlation with microneutralization test results was also established. Data reduction and statistical analysis were performed by Microsoft Excel.

4. Results

Demographic data of the study population are reported in Table 1 and diagnostic criteria for the 140 COVID-19 samples in Table 2. The positivity for SARS CoV-2 RNA was 100 % until 7 DSO, 95.2 % from 8 to 14 DSO and 80.8 % after more than 14 days. On the same time intervals, the positivity rates for IFA and Abbott ARCHITECT assay were 58.3 % and 8.3 %; 85.7 % and 61.9 %; 100 % and 100 %, respectively (Fig. 1). The Abbott ARCHITECT IgG assay showed a 72 % (95 % CI: 64.3 %–79.6 %) sensitivity compared to IFA and 66.7 % (95 % CI: 56.4 %–76.9 %) compared to viral RNA. The positivity rate by Abbott ARCHITECT was directly related to the IFA titers, raising from 29.4 % on samples with a titre <1:40 to 88.6 % on samples with a titre >1:640.

Clinical specificity of the ARCHITECT on both SARS-CoV-2 negative groups resulted at 100 %. Furthermore, the negative Index results obtained on the two sets were totally comparable (p = n.s. by chi square). On the other side, 4 of the 20 samples (20 %) from patients with other Coronavirus infections were positive by IFA (Table 3) presumably due to a cross-reactivity phenomenon.

All 18 samples assayed by microneutralization showed titres ranging from 1:10 to 1:1280, and all but one, showing a high negative ARCHITECT Index (1.19) and neutralization titer of 1:10, were positive by IgG by Abbott ARCHITECT test, for an overall sensitivity vs. microneutralization of 94.4 %; however, the correlation between neutralization titers and ARCHITECT Index was low (R² = 0.0348, Fig. 2).

5. Conclusions

After an initial phase when only molecular assays were available to diagnose SARS-CoV-2 infection, serological assays for specific antibodies started to appear. The first ones were “rapid” lateral flow serological assays with a wide range of performance and unsuitable for testing large numbers of samples and for screening [3,6]. Automated serological assays will enable to enlarge the population base to be tested and should guarantee a more reliable performance compared to manual and rapid assays [9]. There is a common interest from scientists as well as from decision makers in the health care sector to have reliable evidences on the performance of those assays to decide on their adoption, either as a support for clinical diagnosis and for seroepidemiological surveillance programs [3,5].

The results from our evaluation of the automated assay for SARS-CoV-2 IgG on the Abbott ARCHITECT instrument were quite consistent with the performance characteristics declared by the manufacturer on the positive agreement relative to days after the disease onset (100 % patients detected after 14 days post-symptoms onset). Actually, the positive agreement we observed was lower than reported recently by

### Table 1

Demographic characteristics of the study population - all samples.

| Gender | N    | mean age | sd   | median | min | max |
|--------|------|----------|------|--------|-----|-----|
| Female | 64   | 51.0     | 18.9 | 52     | 3   | 91  |
| Male   | 121  | 53.3     | 18.7 | 55     | 0   | 89  |
| ND     | 1    | 41       | 0    | 41     | 41  | 41  |
| Total  | 186  | 52.5     | 18.8 | 54     | 0   | 91  |

sd = standard deviation; min = minimum; max = maximum; ND: not done; age is expressed in years.

### Table 2

Diagnostic criteria on 140 COVID-19 patients.

| Laboratory testing | N   | IgG+ |
|--------------------|-----|------|
| IFA IgG neg, RT-PCR pos | 8   | 0    |
| IFA IgG pos, RT-PCR ND | 39  | 23   |
| IFA IgG pos, RT-PCR pos | 73  | 54   |
| IFA IgG pos, RT-PCR neg | 20  | 18   |

pos = positive; ND: not done; neg = negative; IgG+ = positive by Abbott ARCHITECT SARS-CoV-2 IgG.

* All the patients met the clinical criteria, i.e. presence of respiratory symptoms and fever, plus the indicated laboratory criteria.
Bryan et al. [9], who obtained an 82.7 % positivity rate by ARCHITECT already after 10 days from the onset. Samples preselection may have played a role on this apparent discrepancy; in fact, samples in the present evaluation included many low-level IFA-positive. While the sensitivity of our reference IFA was higher, this latter assay is not suitable for testing large batches of samples and is also less specific, picking up as positive 4 out of 20 samples from patients infected with other coronaviruses. This finding was not unexpected, since whole virus antigens are used for IFA, and reduced specificity is coupled with increased sensitivity. On the contrary, we did not observe any positive results with samples from patients positive for other coronaviruses with the ARCHITECT assay. Finally, the results obtained in the comparison with a microneutralization assay confirm previous evidences generated with different assays that detect IgG towards N-derived antigens [10,11]. While those antibodies do not have neutralizing ability, the concordance between this serological response and viral neutralization suggests that a strong humoral response may be predictive of neutralization activity, regardless of target antigens.

The main limitation of this study is the number of samples, especially for the evaluation of specificity, we compensated that with a keen specimen’s selection as detailed before. Furthermore, the comparison with a highly sensitive immunofluorescent assay has not been reported so far; moreover, the additional findings here described, i.e. lack of cross-reactivity with other coronaviruses and concordance with neutralizing antibodies, add evidences on aspects that are not explored to a substantial extent yet with this assay.

On the clinical side, our data confirm that an IgG reactivity to antigens domains coded for by the N region of SARS-CoV-2 appears as early as one week after symptoms onset and is detectable in all patients by two weeks post-symptoms. This finding supports the use of serology as an adjunct to molecular biology to achieve an etiological diagnosis on probable COVID-19 cases whenever NAT yields a negative result [3,5] and potentially to surrogate NAT when the latter is not affordable or available.

**Table 3**

| Group          | N  | M/F | Age (mean ± sd) | Age (median) | IFA IgG pos | ARCHITECT IgG pos | IgG⁺ S/C (mean ± sd) | IgG⁺ S/C (median) |
|----------------|----|-----|-----------------|--------------|-------------|-------------------|----------------------|-------------------|
| Coronavirus     | 20 | 10/10 | 48.1 ± 19.8    | 53           | 4           | 0                 | 0.08 ± 0.13          | 0.00              |
| Pre-COVID-19    | 17 | 10/7  | 47.5 ± 12.6    | 52           | 0           | 0                 | 0.05 ± 0.11          | 0.00              |
| Total          | 37 | 20/17 | 48.0 ± 16.7    | 52           | 4           | 0                 | 0.06 ± 0.12          | 0.00              |

N = number; M = males; F = females; pos = positive; sd = standard deviation.

* IgG: Abbott ARCHITECT SARS-CoV-2 IgG positive.

**Declaration of Competing Interest**

Abbott Diagnostics supplied the materials employed for the purposes of this study. In no way this contribution influenced the study design and the analysis of the results. No additional conflict of interest or other competing relationships exist.

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