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Inhibition of receptor-binding domain—ACE2 interaction after two doses of Sinovac's CoronaVac or AstraZeneca/Oxford's AZD1222 SARS-CoV-2 vaccines

Valéria O. Silva[1] | Rosemeire Yamashiro[2] | Cintia M. Ahagon[1] |
Ivana B. de Campos[3] | Isabela P. de Oliveira[4] | Elaine L. de Oliveira[2] |
Giselle I. S. López-Lopes[1] | Elaine M. Matsuda[4] | Marcia J. Castejon[2] |
Luís F. de Macedo Brígido[1]

[1]Virology Center, Adolfo Lutz Institute, São Paulo, State of São Paulo, Brazil

[2]Immunology Center, Adolfo Lutz Institute, São Paulo, State of São Paulo, Brazil

[3]Santo André Regional Center, Adolfo Lutz Institute, Santo André, State of São Paulo, Brazil

[4]Infectious Diseases Outpatient Clinic, Santo André Health Secretary, Santo André, State of São Paulo, Brazil

Correspondence
Luís F. de Macedo Brígido, Centro de Virologia, Instituto Adolfo Lutz, Avenida Doutor Arnaldo, 355 - São Paulo, SP 01246902, Brazil.
Email: lubrigido@gmail.com

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Abstract

Practical laboratory proxies that correlate to vaccine efficacy may facilitate trials, identify nonresponders, and inform about boosting strategies. Among clinical and laboratory markers, assays that evaluate antibodies that inhibit receptor-binding domain (RBD) ligation to angiotensin-converting enzyme-2 receptor (receptor-binding inhibition [RBI]) may provide a surrogate for viral neutralization assays. We evaluated RBI before and after a median of 34 days (interquartile range [IQR]: 33–40) of the second dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Sinovac's CoronaVac (CN) or AstraZeneca/Oxford's AZD1222 (AZ) vaccines in 166 individuals. Both vaccines elicited high inhibitory titers in most subjects, 95% (158/166), with signal inhibition above 30% and 89% (127/143) with more than fourfold increase from prevaccination titers, but titers tend to decrease over time. Both postvaccination inhibitory titers (95%, IQR 85%–97% for AZ vs. 79%, IQR 60%–96% for CN, $p = 0.004$) and pre/post-titer increase (AZ 76%, IQR 51%–86% for AZ vs. 47%, IQR 24%–67% for CN, $p < 0.0001$) were higher among AZ vaccinees. Previous serological reactivity due to natural infection was associated with high prevaccination signal inhibition titers. The study documents a robust antibody response capable of interfering with RBD–angiotensin-converting enzyme binding. Evaluation of SARS-CoV-2 infection incidence in these populations is necessary to assess its association to protection and its duration.

KEYWORDS
Antibodies, COVID-19, Receptor-Binding Domain, SARS-CoV-2, Vaccine, Viral Neutralization

1 | INTRODUCTION

Vaccines are the best way to present pathogens to the immune system, such as priming innate defenses and eliciting an adequate T cell memory capable to trigger B and T cell protective responses. Some degree of protection is conferred by natural infection[1] but with an intolerable mortality cost. Moreover, documented reinfection,[2] and immunopathogenic potential[3] triggered by infection are worrisome and suggest that the immunity generated by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is suboptimal. Most vaccines tested at Phase 3 have shown efficacy in protection against severe disease,[4] and data from populations with a high proportion of fully vaccinated individuals suggest vaccine effectiveness and decrease in coronavirus disease-2019 (COVID-19) cases.[5] Neutralizing

antibodies are key to advance a SARS-CoV-2 vaccine candidate in the regulatory testing pipeline and its importance is supported by evidence for SARS-CoV-2[6] as well as other viral pathogens.[7] T cell response is necessary to support B cell maturation and may exert a direct antiviral effect.[4] However, techniques to detect T cell response tend to be more laborious, depend on more complex technology, and have limited standardization across laboratories, so it is tempting to identify relevant antibody-based assays. A key step in the viral life cycle is the binding of domains at spike 1 protein of the virus, the receptor-binding domain (RBD) to the main cellular receptor, the angiotensin-converting enzyme 2 (ACE2) protein.[3] To evaluate the presence of antibodies able to inhibit RBD–ACE2 binding, a potential proxy of protection, we tested binding inhibition in prevaccination blood and after the second dose among individuals vaccinated with Sinovac's CoronaVac (CN) or AstraZeneca/Oxford's anti-COVID-19 vaccines.

2 | MATERIALS AND METHODS

2.1 | Ethics statement, study population, and clinical characteristics

The study is registered and approved by the Institutional ethical committee (CAAE 43250620.4.1001.0059 and CAAE 31924420.8.0000.0059), and written informed consent was obtained from all subjects. Individuals were enrolled for humoral evaluation during 2020. These volunteers had one or more serological evaluations, had COVID-19 related clinical symptoms investigated with questionnaires, and SARS-CoV-2 RNA tests if symptomatic. Some asymptomatic cases with an epidemiological link to a patient with COVID-19 were also tested. Volunteers were asked to collect an additional blood sample before vaccination and after 1 month of the second dose of available SARS-CoV-2 vaccines at the time, either CN (Sinovac Life Sciences) or AZD1222 (AZ; AstraZeneca). The cohort, although mostly laboratory workers, includes relatives and health workers with direct contact with patients. However, most were not involved in direct COVID-19 care. Clinical disease followed the list of symptoms listed by WHO[8] except "altered mental state."

2.2 | SARS-CoV-2 diagnosis and serological tests

Confirmation of SARS-CoV-2 RNA was obtained for quantitative reverse transcription-polymerase chain reaction (RT-qPCR) from nasopharyngeal and/or oropharyngeal secretions collected with swab or saliva/gargle throat wash.[9] SARS-CoV-2 RNA was retro transcribed and amplified using available tests, in most cases, the commercial Allplex Kit (Allplex™ 2019-nCoV), but other tests, based on the Charité protocol[10] were additionally used. Positive cases had an RT-qPCR $C_t < 37$ in one of three viral targets (e.g., E, RdRP, and N). Study cases had a serological evaluation with one or more tests, performed following the manufacturer's instructions. Tests included (i) lateral flow immunochromatographic assay (Wondfo SARS-CoV-2 antibody test; Guangzhou Wondfo Biotech Co.,

Ltd.), that detects immunoglobulin G (IgG) and IgM to the SARS-CoV-2 binding domain of the spike protein (S); (ii) electrochemiluminescence immunoassay (Elecsys anti-SARS-CoV-2; Roche Diagnostics) that detects total antibodies to nucleocapsid (N) antigen, and (iii) Chemiluminescent microparticle immunoassay (SARS-CoV-2 IgG; Abbott Diagnostics) that detects IgG to N antigen, and (iv) Microarray enzyme-immunoassay (SARS-CoV-2 ViraChip® Test Kit; ViraMed Biotech AG) that detects IgG or IgA to SARS-CoV-2 spike protein 1 and 2 subdomain (S1, S2) and N domains.

2.3 | Inhibition of RBD–ACE2 binding

Antibodies able to interfere with RBD–ACE2 interaction were measured in sera with a competitive enzyme-linked immunosorbent assay (ELISA) test (cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit; GenScript), following the manufacturer's instructions. Sera samples were diluted with equal volume (vol/vol) of horseradish peroxidase-conjugated RBD incubated (37°C, 30 min), transferred to capture plate previously coated with human ACE2 receptor protein for 15 min at 37°C. After washing, a chromogenic substrate 3,3′,5,5′-tetramethylbenzidine solution was incubated at room temperature for 15 min, with the reaction stopped by the addition of a stop solution. The absorbance was measured using a spectrophotometer microplate reader at 450 nm. An inhibition titer (signal inhibition) was calculated based on the sample absorbance in relation to the average of negative controls absorbance (included in each reaction) as

$$\text{Inhibition}(\%) = \left(1 - \frac{\text{Sample O. D. 450 value}}{\text{Negative Control O. D. 450 value}}\right) \times 100.$$

The test, validated with conventional and pseudovirus neutralization assays,[11] was approved by the Food and Drug Administration as an emergency use authorization, considering the 30% signal inhibition cutoff for interpretation of the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit signal inhibition rate.[12]

Continuous variables were described as the median and 25th–75th interquartile range (IQR) unless noted, with the difference among groups evaluated with Mann–Whitney, Kruskal–Wallis rank tests, and Cuzick test for trend of ordered groups. Pearson χ^2 (or Fischer exact test, two-tailed) for categorical variables and Spearman's rank correlation coefficient for two continuous variables were tested with STATA 14 (Stata Corp).

3 | RESULTS

Samples from 166 volunteers were included, 132 (80%) vaccinated with CN and 34 (20%) with AZ. Demographic and other characteristics of volunteers are described in Table 1 according to serological status, previous COVID-19-related symptoms, and SARS-CoV-2 RNA detection. Volunteers without SARS-CoV-2 RNA detected no reactive serological

TABLE 1 Volunteers' characteristics, vaccine product used, RBD–ACE2 inhibition (RBI) according to COVID-19 related clinical condition, serology, and SARS-CoV-2 RNA

No. (%)	Total166 (100%)	No COVID-1946 (27%)	Suspected or confirmed COVID-19 infections						p
			Seronegative			Seropositive			
			Asymptomatic	Symptomatic		Asymptomatic	Symptomatic		
			RNA positive6 (4%)	RNA negative28 (17%)	RNA Positive24 (14%)	RNA positive5 (3%)	RNA negative21 (13%)	RNA positive36 (22%)	
Female gender	130 (78%)	35 (76%)	4 (67%)	23 (82%)	21 (88%)	5 (100%)	13 (62%)	29 (81%)	0.33
Age	49 (39–56)	52 (43–59)	50 (29–57)	46 (41–52)	41 (32–54)	58 (52–64)	37 (30–44)	53 (48–58)	0.0001
Prevaccine RBI (%)	13 (5–38)	8 (4–15)	5 (−2–13)	5 (0.6–10)	8 (4–14)	60 (18–97)	62 (32–75)	70 (58–91)	0.0001
Time Prevaccine[a]	21 (13–28)	21 (13–27)	23 (17–27)	21 (13–27)	26 (21–55)	32 (24–55)	23 (14–27)	21 (19–41)	0.18
CN Vaccines	132 (80%)	36 (78%)	4 (67%)	21 (75%)	18 (75%)	4 (80%)	18 (86%)	31 (86%)	0.84
AZ Vaccines	34 (20%)	10 (22%)	2 (33%)	7 (25%)	6 (25%)	1 (20%)	3 (14%)	5 (14%)	
Postvaccine RBI (%)	84 (62–96)	67 (53–92)	70 (66–85)	61 (51–81)	79 (67–92)	97 (96–97)	95 (79–97)	97 (95–97)	0.0001
RBI titer ≥ 30%	158 (95%)	42 (91%)	6 (100%)	26 (93%)	23 (96%)	5 (100%)	20 (95%)	36 (100%)	0.63
Delta RBI[b] (%)	53 (26–75)	61 (41–76)	65 (52–78)	57 (41–76)	72 (54–83)	37 (−0.5–79)	25 (12–38)	24 (6–33)	0.0001
Time postvaccine[c]	34 (33–40)	37 (33–42)	33 (33–35)	34 (33–42)	34 (33–38)	33 (32–36)	35 (33–39)	33 (31–37)	0.026

Note: An absolute number of cases (percentage) or median (interquartile 25th–75th) and *p*-value calculated with Kruskal–Wallis or Pearson as appropriate.

Abbreviations: ACE2, angiotensin-converting enzyme 2; AZ, AZD1222; CN, CoronaVac; COVID-19, coronavirus disease 2019; RBD, receptor-binding domain; RBI, receptor-binding inhibition; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Time (days) from pre-RBI determination to vaccine first dose.

[b]Delta RBI is postvaccination minus prevaccination inhibition titers.

[c]Time (days) from vaccine second dose to postvaccination RBI determination.

test and without compatible symptoms were considered as no COVID-19 cases.

3.1 | Prevaccination inhibition titers

Prevaccination signal Inhibition titers, available for 143 volunteers, were generally low, with a median of 13% inhibition titers (IQR, 5%–38%). However, some cases showed high titers, with 28% of the cases (40/143) with inhibition above 30%. Higher prevaccination titers were associated with the presence of antibodies to SARS-CoV-2 (Table 1), with seropositive cases showing higher prevaccination inhibition titers (69%, 38%–87%), as compared to seronegative individuals (7%, 3%–14%, $p < 0.0001$).

The presence of symptoms did not show association to binding (signal) inhibition titers, with similar prevaccination titers for seronegative asymptomatic, including individuals with no evidence of COVID-19, (8%, 4%–15%) and symptomatic cases (6%, 2%–13%, $p = 0.17$) as well as among symptomatic seropositive (69%, 48%–86%) or without clinical symptoms (60%, 18%–97%, $p = 0.87$).

3.2 | Postvaccination inhibition titers

Postvaccination inhibition titers, obtained at a median of 34 days (33–40) after the second dose of either vaccine product were generally high (84%, 62%–96%), with most individuals (95.2%, 148/166) with titers above minimal inhibition (30% receptor-binding inhibition [RBI]) and 78% (130/166), with titers above 60% inhibition (two times the cutoff). If only cases with results 3–6 weeks post the second dose are evaluated, 97% (125/129) have signal inhibition titers above 30%. Considering the 143 with both pre-/post-vaccine determinations, a fourfold or higher increase in titer was observed in 127 (89%) of volunteers. Titers tended to be associated with prevaccination values, and seropositive categories (Table 1) had higher inhibition titers (96%, 91%–97%) than seronegative individuals (70%, 54%–87%, $p > 0.0001$). There was no significant association of inhibition titers to age at Spearman correlation ($p = 0.1$) or by comparing age quartiles ($p = 0.16$).

3.3 | Response according to vaccine type

To compare RBD–ACE2 inhibition according to the vaccine used, we compared volunteers according to the serological status before vaccination. Table 2 describes these patients, showing that seronegative AZ vaccinees, having higher postvaccination inhibition titers (95%, 85%–97%), as compared to seronegative CN vaccinees (79%, 60%–96%, $p = 0.004$). This increase from prevaccination titers is also higher among AZ recipients, even if only cases with low prevaccination binding inhibition (signal inhibition < 30%) were analyzed (AZ 84%, 72%–88% vs. CN 57%, 43%–73%, $p < 0.0001$). No significant difference was observed for previously seropositive individuals.

TABLE 2 Volunteers' characteristics according to previous COVID-19 serological status and vaccine product used (CoronaVac or AZ)

No. (%)	Total 166 (100%)	Seronegative			Seropositive		
		CN vaccine 79 (76%)	AZ vaccine 25 (24%)	*p*	CN vaccine 53 (85%)	AZ vaccine N = 9 (15%)	*p*
Female gender	130 (78%)	62 (78%)	21 (84%)	0.55	39 (74%)	8 (89%)	0.32
No COVID-19	46 (27%)	36 (46%)	10 (40%)	0.93	0	0	–
Symptomatic	109 (66%)	39 (49%)	13 (52%)	0.82	49 (92%)	8 (89%)	0.72
Age	49 (39–56)	49 (39–55)	44 (39–53)	0.40	52 (42–58)	48 (47–50)	0.29
Prevaccine inhibition	13 (5–38)	6 (2–14)	9 (6–15)	0.15	69 (55–91)	48 (17–73)	0.14
Time prevaccine[a]	21 (13–28)	22 (14–28)	13 (13–57)	0.41	22 (20–28)	23 (13–74)	0.80
Postvaccine inhibition	84 (62–96)	63 (50–79)	94 (84–96)	0.0001	96 (91–97)	97 (96–97)	0.27
Postvaccine RBI titer ≥ 30%	158 (95%)	72 (91%)	25 (100%)	0.19	53 (100%)	8 (89%)	0.14
Time postvaccine[b]	35 (33–40)	36 (34–43)	33 (33–33)	0.0001	34 (31–39)	33 (33–33)	0.21
Delta RBI gain[c]	53 (26–75)	56 (34–72)	82 (72–88)	0.0001	23 (5–33)	28 (13–74)	0.20

Note: Absolute number of cases (percentage) or median (interquartile 25th–75th) with *p*-value calculated with Mann–Whitney, Pearson (or Fisher exact), as appropriate.

Abbreviations: AZ, AZD1222; CN, CoronaVac; COVID-19, coronavirus disease 2019; RBI, receptor-binding inhibition.

[a]Time (days) from pre-RBI determination to vaccine first dose.

[b]Time (days) from vaccine second dose to postvaccination RBI determination.

[c]Delta RBI is the postvaccination minus prevaccination inhibition titers.

As the time of postvaccination collection is longer for some CN recipients, and antibodies may wane with time,[6] we also analyzed only cases at the same time range (28–34 days, n = 73). We still observe a difference in postvaccination titers (AZ 95%, 85%–96% vs. CN 78%, 63%–96%, p = 0.05), especially if only seronegative cases (n = 45) are included (AZ 94%, 85%–96% vs. CN 66%, 53%–74%, p < 0.0001). It is important to note that the time between doses varied and CN users had a second dose generally in 3–4 weeks, whereas AZ second dose was used at the time of the study after 12 weeks.

3.4 | Persistence of inhibition postvaccination

A decrease in assay reactivity, indicative of lower binding inhibition, was observed with time after vaccination. Stratifying all cases results according to the number of weeks after vaccination, a test for trend of ordered groups show a significant tendency (p < 0.001) for decreasing binding inhibition. Similar results are obtained when the analysis is restricted only to those receiving the CN vaccine, the product used by the majority of vaccinees with longer time points. Accordingly, a comparable tendency (p < 0.001) is observed for those with no previous positive serological test. Moreover, testing paired samples from the same patient (n = 13) with two postvaccination collections at the median of 63 days (56–63) apart, 77% (10/13) show a median decrease of 32% (23%–42%) in inhibition titer, from 64% (51%–71%) to 42% (30%–51%) binding inhibition.

4 | DISCUSSION

Laboratory correlates of immunity, proxies of immunological protection to SARS-CoV-2 acquisition or for development of severe disease, are key for better handling of the pandemic. Although some scientists predict an end to SARS-CoV-2,[13] viral characteristics, along with the health inequalities among nations (as well as within some countries) suggest that control, rather than eradication seems a more reasonable goal. In that scenario, correlates of immunity to SARS-CoV-2 may be used to define protection and favor targeting of nonpharmacological restrictions, an intent for an early generation of antibodies tests that proved inadequate for this purpose.[8] These markers of protection are also useful in vaccine development, facilitating the selection of products for further testing or even used as secondary endpoints of vaccine studies. These markers may also help in the triage of volunteers at enrollment, as many volunteers in new trials will be previously infected and/or previously vaccinated. They may also be used to monitor the need for boosting doses and inform on better boosting strategies.

The interaction of the virus RBD to the main host protein that allows viral ligation to a permissive cell, the ACE2 molecule, is considered a key step in the viral life cycle.[6] We evaluated the titer of RBD–ACE2 binding inhibition (RBI) of pre and postvaccination sera

from a small cohort of well-characterized health workers and relatives; mostly working at laboratory facilities or in non-COVID-19 related outpatient healthcare. Regular serological testing, one to three times during 2020 using different testing platforms, access to RNA tests to symptomatic cases and some asymptomatic (as those with contact to COVID-19 cases) and documentation of COVID-19 related symptoms, allowed the discrimination of cases in subgroups based on clinical and laboratory evidence of previous COVID-19 infection to contextualize RBI response to vaccine products. Some groups were, however, too small to allow proper subgroup evaluation.

This assay to assess RBI was developed to mimic a standard neutralization assay that uses a preincubation of sera with coronavirus before infection in cell culture systems. An alternative to evaluate blocking of viral–host cell interaction, this ELISA assay facilitates eventual incorporation in general low complexity laboratory settings. The preincubation of viral RBD to the patient's serum allows the binding of antibodies that may prevent subsequent binding to ACE2, mimicking an inhibition of viral/host cell interaction (RBI) of neutralization assays. All but two fully vaccinated (over 21 days after a second dose) had detectable inhibition (signal inhibition above 30%[11,12]). One of the volunteers without detectable titers was tested about 1-month postvaccination (33 days) and another, that received CN vaccine during a trial in 2020, had available samples only after 100 and 180 days after the second dose, both samples with low inhibition titers (4.8% and 5.1%).

We observed a robust signal of receptor binding inhibition in most postvaccination sera, but these titers in many cases reflected previous, nonvaccine-related binding inhibition, probably due to natural infection. High prevaccination titers were significantly associated with previous SARS-CoV-2 serological reactivity. Although the study enrolled a small number of asymptomatic infections (positive RNA), they all show a similar pattern of higher prevaccine inhibition titers associated with previous COVID-19 serological reactivity. Therefore, most seropositive individuals (with or without symptoms or RNA detection), have high prevaccine signal Inhibition titers. The test, performed according to manufacture recommendations, did not allow discriminating potential titer change after vaccination for cases already with high prevaccination titers. Serial dilution of these samples with high titers may be necessary to document if vaccination leads to further increase in these titers, as have been suggested with other vaccine products.[14] However, it is important to note that this assay is not yet validated for a quantitative interpretation and further evaluations are recommended. Another important issue is the duration of these inhibitory antibodies. Although only a few cases had samples tested after a longer period, as 3 months from the second vaccine dose, we observed a trend suggesting diminishing titers with time. Paired samples tested 2 months apart also show a decrease in titers in most tested cases. The fact that titers may wane with time does not mean that a robust B cell memory, T cell, and innate immunity were not induced to respond adequately to future infections.[6]

Moreover, the duration and some other characteristics of antibodies generated after natural infection may differ from that of vaccine-induced receptor binding inhibition, but both vaccine-induced response and natural infection provide protection to infection in most individuals.[6,14]

Our study documented receptor binding inhibition generated by both vaccine products evaluated, AZ and CN, with the formed inducing titers higher after about 1 month of the second dose.

Although it is tempting to associate the higher postvaccination titers of AZ vaccinees with the vaccine product, it is important to highlight that the second dose of AZ was applied after 3 months, whereas CN's second dose was 21–28 days after the first dose. Some studies suggest that a longer interdose spacing may provide a more robust immunological response.[15] However, both products have been providing protection from severe disease and the actual role of antibodies in protection to current and emerging variants of SARS-Cov-2 will need prospective studies.

COVID-19 IAL WORKING GROUP

Amanda I. Portilho, Ana M. S. Afonso, André R. de Campos, Andreia M. dos Santos Carmo, Audrey C. Chirelli, Carmem A. de Freitas Oliveira, Daniela B. B. da Silva, Daniela R. Colpas, Edilene P. R. da Silveira, Elaine A. R. de Paula, Elizabeth De Gaspari, Fabiana C. P. dos Santos, Gabriela C. Bastos, Igor M. Hussein, Isabella P. de Oliveira, Jaqueline H. da Silva Santos, Margarete A. B. Pinho, Maria do C. S. T. Timenetsky, Marisa A. Hong, Monica Stofer, Paula M. Guimarães, Paula O. Rigato, and Stefanni R. F. Alves.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Conception and design were performed by Luís F. de Macedo Brígido. Volunteers recruitment and data collection were performed by Valéria O. Silva, Cintia M. Ahagon, Elaine M. Matsuda, Elaine L. de Oliveira, Giselle I. S. López-Lopes, Isabela P. de Oliveira, and Ivana B. de Campos. Execution of laboratory tests were performed by Rosemeire Yamashiro and Marcia J. Castejon. Samples management was performed by Valéria O. Silva, Elaine L. de Oliveira, Isabela P. de Oliveira, and Ivana B. de Campos. Statistical analysis was performed by Valéria O. Silva and Luís F. de Macedo Brígido. The manuscript was written by Valéria O. Silva and Luís F. de Macedo Brígido. All authors contributed to the study, commented on previous versions of the manuscript, and approved the final manuscript.

ORCID

Valéria O. Silva http://orcid.org/0000-0002-5346-9633
Rosemeire Yamashiro https://orcid.org/0000-0002-6932-8915
Cintia M. Ahagon http://orcid.org/0000-0001-8233-0369
Ivana B. de Campos http://orcid.org/0000-0003-0334-0572
Isabela P. de Oliveira https://orcid.org/0000-0003-2202-0443
Elaine L. de Oliveira https://orcid.org/0000-0002-1285-3532
Giselle I. S. López-Lopes https://orcid.org/0000-0002-4564-3208
Elaine M. Matsuda https://orcid.org/0000-0002-0254-4154
Marcia J. Castejon https://orcid.org/0000-0002-3525-740X
Luís F. de Macedo Brígido https://orcid.org/0000-0002-1022-7837

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