VITÓRIA TAVARES DE CASTRO

Detecção de anticorpos salivares anti-SARS-CoV-2 em adultos vacinados

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Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Ciências da Saúde pelo programa de Pós-Graduação em Ciências da Saúde da Universidade de Brasília.

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RESUMO

O presente trabalho foi proposto para avaliar a viabilidade da saliva como uma alternativa para detecção de anticorpos anti-SARS-CoV-2. Esse trabalho foi dividido em 2 estudos. O primeiro objetivou avaliar a detecção de anticorpos anti-SARS-CoV-2 na saliva após a vacinação usando a metodologia da revisão sistemática rápida. Foram incluídos 15 estudos, com aproximadamente 1.080 amostras de saliva de indivíduos vacinados e/ou convalescentes. As vacinas eram principalmente à base de mRNA e a principal técnica utilizada foi o ensaio de ELISA. A IgG foi frequentemente encontrada na saliva de vacinados anti-COVID-19, mas raramente a IgA. Embora os títulos de anticorpos sejam mais baixos na saliva que no soro, os resultados mostraram que a saliva é adequada para detecção de anticorpos. O segundo estudo foi do tipo experimental longitudinal e tinha o objetivo de detectar anticorpos anti-SARS-CoV-2 no soro e na saliva de adultos vacinados. Foram incluídos 13 participantes como controle negativo (não vacinados e não infectados) e 35 participantes vacinados com duas doses da vacina CoronaVac (Sinovac/Butantan) que posteriormente receberam a vacina BNT162b2 (Pfizer-BioNTech) como terceira dose. Os participantes vacinados foram avaliados aproximadamente dois meses após a segunda dose, um mês e cinco meses após a terceira dose, totalizando 118 amostras de saliva. Foi utilizado o ensaio ELISA para detecção de anticorpos neutralizantes (NAb), IgA e IgG. Ainda, eletroquimioluminescência para detecção de anticorpos totais (TAb) em 20 amostras iniciais. O TAb foi detectado em 10/10 amostras no soro (158.13±88.1 U/mL) e somente em 3/10 na saliva (0.63±0.46 U/mL) após a segunda dose. No soro, o NAb foi detectado em 34/35 participantes após a segunda dose (57,86±20,74%) e em 35/35 participantes um mês (95,6±3,34%) e cinco meses (95,03±1,17%) após a terceira dose ($p<0,0001$). Na saliva, o NAb foi detectado em 30/35 amostras após a segunda dose (6,54±5,54%), e em 35/35 amostras um mês (29,51±11,96%) e cinco meses (10,17±4,99%) após a terceira dose ($p<0,0001$). A IgA foi detectada em 19/34 amostras de saliva após a segunda dose (1,46±1,01 ratio), 18/35 amostras de saliva um mês após a terceira dose (1,71±1,65 ratio) e 30/35 cinco meses após a terceira dose (2,69 ±1,72 ratio) ($p<0,0013$). A IgG foi detectada em 1/34 amostras de saliva após a segunda dose (0,38±0,21 ratio), 33/35 amostras de saliva um mês após a terceira dose (3,08±1,63 ratio) e 20/35 amostras de saliva cinco meses após a terceira dose (1,44±0,76 rario) ($p<0,0001$). Houve correlação positiva

moderada entre NAb e TAb no soro (r=0,6634), NAb no soro e IgG na saliva (r=0,7896), e NAb e IgG ambos na saliva (r=0,6115). Observou-se excelente sensibilidade para o teste de NAb salivar (95%). O teste de IgG salivar apresentou excelente especificidade (100%) após segunda dose, um mês e cinco meses após a terceira dose, excelente acurácia (100%) um mês após a terceira dose e ainda boa acurácia (86,8%) cinco meses após a terceira dose. Os anticorpos NAb, IgA e IgG foram encontrados na saliva dos participantes vacinados. Concluindo, os estudos mostraram que anticorpos anti-SARS-CoV-2 podem ser encontrados na saliva de indivíduos vacinados para COVID-19.

Palavras-chave: COVID-19; SARS-CoV-2; saliva; anticorpos; anticorpos neutralizantes; IgG; IgA; vacinas anti-COVID-19.

ABSTRACT

The present work was proposed to evaluate the viability of saliva as an alternative for detecting anti-SARS-CoV-2 antibodies. This work was divided into 2 studies. The first aimed to evaluate the detection of anti-SARS-CoV-2 antibodies in saliva after vaccination using the rapid systematic review methodology. Fifteen studies were included, with approximately 1,080 saliva samples from vaccinated and/or convalescent individuals. Vaccines were mainly mRNA-based and the main technique used was the ELISA assay. IgG was frequently found in the saliva of anti-COVID-19 vaccinees, but rarely IgA. Although antibody titers are lower in saliva than in serum, the results showed that saliva is suitable for antibody detection. The second study was a longitudinal experimental type and aimed to detect anti-SARS-CoV-2 antibodies in the serum and saliva of vaccinated adults. Thirteen participants were included as negative controls (non-vaccinated and uninfected) and 35 participants vaccinated with two doses of the CoronaVac vaccine (Sinovac/Butantan) who subsequently received the BNT162b2 vaccine (Pfizer-BioNTech) as a third dose. Vaccinated participants were evaluated approximately two months after the second dose, one month and five months after the third dose, totaling 118 saliva samples. The ELISA assay was used to detect neutralizing antibodies (NAb), IgA and IgG. Also, electrochemiluminescence for detection of total antibodies (TAb) in 20 initial samples. TAb was detected in 10/10 samples in serum (158.13±88.1 U/mL) and only in 3/10 in saliva (0.63±0.46 U/mL) after the second dose. In serum, NAb was detected in 34/35 participants after the second dose (57.86±20.74%) and in 35/35 participants one month (95.6±3.34%) and five months (95.03±1.17%) after the third dose ($p<0.0001$). In saliva, NAb was detected in 30/35 samples after the second dose (6.54±5.54%), and in 35/35 samples one month (29.51±11.96%) and five months (10.17±4.99%) after the third dose ($p<0.0001$). IgA was detected in 19/34 saliva samples after the second dose (1.46±1.01 ratio), 18/35 saliva samples one month after the third dose (1.71±1.65 ratio) and 30 /35 five months after the third dose (2.69±1.72 ratio) ($p<0.0013$). IgG was detected in 1/34 saliva samples after the second dose (0.38±0.21 ratio), 33/35 saliva samples one month after the third dose (3.08±1.63 ratio) and 20 /35 saliva samples five months after the third dose (1.44±0.76 rare) ($p<0.0001$). There was a moderate positive correlation between NAb and TAb in serum ($r=0.6634$), NAb in serum and IgG in saliva ($r=0.7896$), and NAb and IgG both in saliva ($r=0.6115$). Excellent sensitivity was observed for the

salivary NAb test (95%). The salivary IgG test showed excellent specificity (100%) after the second dose, one month and five months after the third dose, excellent accuracy (100%) one month after the third dose and still good accuracy (86.8%) five months after the third dose. NAb, IgA and IgG antibodies were found in the saliva of vaccinated participants. In conclusion, studies have shown that anti-SARS-CoV-2 antibodies can be found in the saliva of individuals vaccinated for COVID-19.

Key words: COVID-19; SARS-CoV-2; saliva; antibodies; neutralizing antibodies; IgG; IgA; COVID-19 vaccines.

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LISTA DE ABREVIATURAS E SIGLAS

2019-nCoV = Novo coronavírus

Ab = Anticorpo

ACE 2 = Enzima conversora de angiotensina 2

BAU = Binding Antibody Units

BBIBP-CorV = Vacina Sinopharm

BBV152 = Vacina Covaxin - Bharat Biotech

BNT162b2 = Vacina Pfizer-BioNTech

CD8 T cell = Células CD8

ChAdOx1/AZD1222 = Vacina ChAdOx1-AZD1222 Oxford-AstraZeneca – Fiocruz

CLIA = Chemiluminescence immunoassay

COI = Cut off Index

CoronaVac = Vacina Sinovac/Biotec

COVID-19 = Doença do coronavírus 2019

Curva ROC = Curva característica do operador do receptor

d = Dias

ECLIA = Ensaio de Eletroquimioluminescência

ELISA = Ensaio de imunoabsorção enzimática

ELISpot = Enzyme-linked immune absorbent spot

FC = Citometria de fluxo

HIV = Vírus da imunodeficiência humana

Ig = Imunoglublina

IgA = Imunoglobulina A

IgG = Imunoglobulina G

IgM = Imunoglobulina M

IWV = Virion inteiro inativado

m = Meses

MB = Multiplex Bead

Mg = Micrograma

mL = Millilitro

MNA = Micro neutralização

mRNA-1273/MOD = Vacina Moderna

NA = Atividade neutralizante

NAb = Anticorpos neutralizantes

NC = Nucleocapsídeo

ng = Nanograma

NR = Não reportado

NS = Não significante

NVX-CoV2373 = Novavax's COVID-19 vacina Nuvaxovid

OD = Optical density

r = Coeficiente de correlação

RBD = Domínio de Ligação do Receptor

RNA = Ácido ribonucleico

S = Proteína spike

S1 = Subunidade 1 da proteína spike

S2 = Subuinidade 2 da proteína spike

SARS-CoV- 2 = Síndrome Respiratória Aguda Grave Coronavírus-2

SIgA = Imunoglobulina A Secretora

T cell = Células T

TAb = Anticorpos totais

Total Ig = Imunoglobulina Total

TS = Spike trimerico

U = Unidade

WD = tipo selvagem

WST = Whole spike timer

y = Anos

SUMÁRIO

1. INTRODUÇÃO

A Doença do coronavírus 2019 (COVID-19), causada pelo coravírus 2 da sindrome respiratória aguda grave (SARS-CoV-2), teve seu primeiro surto em Wuhan, na China, espalhou-se globalmente, e, em março de 2020, foi reconhecida pela OMS como uma pandemia (Sharma et al., 2021). Inicialmente, o vírus foi caracterizado como novo coronavírus 2019 (2019-nCoV), que após ter seu genoma viral estudado, foi denominado “síndrome respiratória aguda grave coronavírus-2” (SARS-CoV-2), pelo Comitê Internacional de Taxonomia de Vírus, por ter um genoma idêntico ao da infecção por coronavírus que causou o surto da SARS em 2003 (Chavda et al., 2022).

Os sintomas da COVID-19 variam de assintomáticos ou leves a graves, sendo os primeiros mais comumente identificados a febre ou calafrios, dor de cabeça, dores musculares ou corporais, tosse seca, mialgia ou fadiga, pneumonia e dispneia (Chilamakuri and Agarwal, 2021). Posteriormente, os distúrbios do paladar também foram considerados sintomas comuns associados à doença, com uma prevalência de 34% para hipogeusia, 33% para disgeusia e 26% para ageusia. Lesões na mucosa oral podem apresentar-se como manifestações secundárias, sendo o padrão clínico mais comum o tipo aftoso, seguido de lesões tipo herpes, candidíase, glossite/despapilação/língua geográfica, parotidite e queilite angular (Amorim dos Santos et al., 2021a, 2021b).

O SARS-CoV-2, um vírus de RNA de cadeia positiva envelopado, se liga ao receptor da enzima conversora de angiotensina 2 (ACE2) e entra nas células por meio das proteínas de pico de coronavírus 1 e 2. Essas proteínas de pico possuem diversos locais de clivagem, o que pode aumentar a patogenicidade do vírus. O acúmulo gradual de pequenas alterações genéticas nessas proteínas de pico viral resulta em deriva antigênica e diferentes variantes (Long et al., 2022).

Numerosas linhagens geneticamente distintas têm evoluído, e elas podem apresentar maior transmissibilidade, gravidade da doença ou capacidade de escapar de anticorpos, o que pode impactar negativamente nas estratégias de gerenciamento da pandemia (McLean et al., 2022). Entre essas variantes estão a Alpha (inicialmente reconhecida no Reino Unido), a variante Beta (inicialmente identificada na África do Sul), a variante Gamma (originalmente reconhecida no Brasil), a variante Delta

(inicialmente identificada na Índia) e a variante Ômicron (inicialmente identificada na África do Sul), além de um número crescente de outras variantes de SARS-CoV-2 recentemente identificadas (Tao et al., 2021).

A vacinação foi considerada a maneira mais significativa e viável de conter a pandemia da COVID-19, e a rapidez no desenvolvimento de vacinas eficazes é considerada uma conquista notável (Z. Huang et al., 2022). Entre as principais vacinas disponíveis estão as vacinas de subunidade de proteína, como a NVX-CoV2373 (Novavax); as vacinas de vetor viral (não replicantes), como a Ad26.CoV.S (Johnson & Johnson), a ChAdOx1/AZD1222 (Oxford-AstraZeneca) e a Sputnik V (Gamaleya); as vacinas à base de ácido nucleico (RNA), como a BNT162b2 (Pfizer-BioNTech), a mRNA-1273 (Moderna); e as vacinas de vírus inativados, como a BBIBP-CorV (Sinopharm), a CoronaVac (Sinovac/Butantan) e a BBV152 (Covaxin - Bharat Biotech) (Guiomar et al., 2022).

Embora a maioria das vacinas reduza a proporção de pessoas com COVID-19 sintomática confirmada e para algumas vacinas exista alta certeza de evidência para redução dos sintomas graves ou críticos (Graña et al., 2022), há um declínio de anticorpos em indivíduos vacinados que não foram previamente infectados por SARS-CoV-2 com o tempo (Ali et al., 2021; Khoury et al., 2021). A herdimunidade é alcançada quando uma proporção crítica da população está imune, proporcionando ao vírus menos chances de se espalhar localmente (Hussain et al., 2021). Para isso, e devido ao tempo rápido de produção e aplicação de vacinas em massa, o monitoramento dos níveis de anticorpos em pessoas vacinadas é essencial no contexto epidemiológico (Guiomar et al., 2022).

A detecção de antígenos em testes sorológicos tornou-se comercialmente disponível, podendo ser útil para estimar a produção de anticorpos em um indivíduo previamente infectado pelo virus, monitorar a imunidade coletiva e possivelmente também para detectar anticorpos após a vacinação anti-COVID-19 (Mueller, 2021). No entanto, estabelecer o nível sérico de anticorpos anti-SARS-CoV-2 envolve exames de punção venosa, o que pode ser invasivo e dolorido para os indivíduos, além de requerer profissionais treinados para realização. Para contornar esse problema, estratégias não invasivas, não dolorosas e realizadas pelo próprio individuo, como o uso de fluidos orais, representam alternativas desejáveis de

monitoramento personalizado da imunidade induzida por vacina (Seneviratne et al., 2020).

Nesse contexto, a saliva humana é um fluido corporal produzido pelas glândulas salivares, composto principalmente por água (94-99%), com moléculas orgânicas representando quase 0,5% e as inorgânicas por 0,2%. Exerce funções na digestão de alimentos, lubrificação da mucosa oral, limpeza, preservação da cavidade oral e influência na homeostase oral. A saliva também inclui partículas de alimentos, elementos séricos, microrganismos orais e seus metabólitos, leucócitos, células epiteliais esfoliadas e também anticorpos (Fini, 2020).

Sendo assim, a utilização da saliva como alternativa para detecção de anticorpos vem sendo explorada em diversas doenças, como por exemplo, o pênfigo vulgar mucoso (Ali et al., 2016), vírus da hepatite C (Flores et al., 2017), vírus da imunodeficiência humana (HIV) (Vohra et al., 2020), citomegalovírus (Riis et al., 2020), entre outros, além da detecção de proteomas na Síndrome de Sjögren (Jung et al., 2021).

O SARS-CoV-2 é capaz de infectar e se replicar nas glândulas salivares, razão pela qual a saliva representa uma amostra alternativa para detectar tanto o RNA viral quanto os anticorpos específicos anti-SARS-CoV-2. Os tecidos orais, incluindo as glândulas salivares e a mucosa, podem desempenhar uma dupla função: são locais de infecção precoce, desempenhando papel crítico na disseminação viral para os pulmões ou trato gastrointestinal via saliva; e também, representam a primeira linha de defesa contra uma infinidade de patógenos. Por isso, a imunidade humoral local na cavidade oral e sua relação com os níveis de anticorpos sistêmicos precisam ser melhor abordadas (Garziano et al., 2022).

As duas classes de anticorpos mais significativas presentes na saliva são a imunoglobulina A secretora (SIgA) e a imunoglobulina G (Brandtzaeg, 2013, 2007). SIgA é produzida como IgA dimérica por plasmócitos locais no estroma das glândulas salivares e é transportada da glândula secretora pelo receptor polimérico de imunoglobulina (Ig), também denominado componente secretor de membrana. A maior parte da IgG na saliva é derivada da circulação sanguínea por exsudação passiva principalmente via epitélio crevicular gengival, embora algumas possam ser produzidas localmente na gengiva ou nas glândulas salivares (Brandtzaeg, 2013).

A produção de anticorpos encontrados no fluído salivar pode ser intensificada por meio de vacinas mucosas, sendo um meio seguro e eficaz para a indução de imunidade sistêmica e mucosa duradoura anti-SARS-CoV-2, visto que as superfícies das mucosas oral e nasal servem como porta de entrada primária para o SARS-CoV-2 no corpo humano (Mudgal et al., 2020). Vacinas mucosas estão em desenvolvimento e há estudos que reportam altos níveis de anticorpos neutralizantes, respostas de imunoglobulina A (IgA) e de células T sistêmicas e mucosas, após uma dose de uma vacina intrasal, que foi também capaz de previnir quase inteiramente a infecção por SARS-CoV-2 em ambos as vias respiratórias superiores e inferiores (Hassan et al., 2020).

No entanto, apesar dos intensos esforços de pesquisa, vários determinantes da produção de anticorpos específicos anti-SARS-CoV-2 permanecem não completamente elucidados, como o conhecimento sobre anticorpos IgA e IgG específicos para SARS-CoV-2 em locais de mucosa e como seus títulos estão correlacionados com os parâmetros da COVID-19 (Cervia et al., 2021). Sendo assim, o objetivo do presente estudo é avaliar a viabilidade do fluído salivar como uma alternativa para detecção de anticorpos anti-SARS-CoV-2 em adultos vacinados.

2. PROBLEMAS E HIPÓTESES

A detecção de anticorpos anti-SARS-CoV-2 no sangue foi amplamente estudada, no entanto, a imunidade mucosa ainda não está clara, mesmo com a cavidade oral sendo a porta de entrada para o vírus SARS-CoV-2. Existem na literatura pesquisas acerca da detecção de anticorpos anti-SARS-CoV-2 na saliva de indivíduos vacinados, a grande maioria sendo relacionadas às vacinas de RNA e compostas de estudos do tipo transversal. Assim, fazem-se necessárias pesquisas acerca da imunidade mucosa proporcionada por outros tipos de vacinas, como por exemplo, a vacina CoronaVac, que foi amplamente utilizada no Brasil e ainda pouco estudada. Além disso, a condução de estudos longitudinais possibilita determinar se a saliva pode ser utilizada para monitoramento populacional de anticorpos anti-SARS-CoV-2. Diante desse contexto, formularam-se as seguintes perguntas:

Pergunta 1: A vacinação intramuscular é capaz de induzir anticorpos anti-SARS-CoV-2 salivares?

Hipótese: A vacinação intramuscular irá produzir anticorpos séricos que serão exsudados para a saliva.

Pergunta 2: Os testes disponíveis no mercado para detecção de anticorpos anti-SARS-CoV-2 desenvolvidos para o sangue são capazes de detectar anticorpos anti-SARS-CoV-2 salivares?

Hipótese: Os testes disponíveis no mercado para o sangue podem ser adaptados para detecção de anticorpos anti-SARS-CoV-2 na saliva.

3. ARTIGOS

3.1 MANUSCRITO 1

O artigo a seguir foi escrito de acordo com as normas da revista *Frontiers in Immunology*, ISSN 1664-3224 (versão online), classificada como periódico A1 no Qualis-Capes, com fator de impacto 8,78. O artigo foi publicado na mesma revista no dia 20 de setembro de 2022 sob o DOI: 10.3389/fimmu.2022.1006040. A escolha da revista foi influenciada pelo seu escopo, onde são publicadas pesquisas na área de imunologia.

Saliva is suitable for SARS-CoV-2 antibodies detection after vaccination: A rapid systematic review

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Abstract

Since the introduction of efficient vaccines anti-SARS-CoV-2, antibody quantification becomes increasingly useful for immunological monitoring and COVID-19 control. In several situations, saliva samples may be an alternative to the serological test. Thus, this rapid systematic review aimed to evaluate if saliva is suitable for SARS-CoV-2 detection after vaccination. For this purpose, search strategies were applied at EMBASE, PubMed, and Web of Science. Studies were selected by two reviewers in a two-phase process. After selection, 15 studies were eligible and included in data synthesis. In total, salivary samples of approximately 1,080 vaccinated and/or convalescent individuals were analyzed. The applied vaccines were mostly mRNA-based (BioNTech 162b2 mRNA/Pfizer and Spikevax mRNA-1273/Moderna), but recombinant viral-vectored vaccines (Ad26. COV2. S Janssen - Johnson & Johnson and Vaxzevria/Oxford AstraZeneca) were also included. Different techniques were applied for saliva evaluation, such as ELISA assay, Multiplex immunoassay, flow cytometry, neutralizing and electrochemical assays. Although antibody titers are lower in saliva than in serum, the results showed that saliva is suitable for antibody detection. The mean of reported correlations for titers in saliva and serum/plasma were moderate for IgG (0.55, 95% CI 0.38-9.73), and weak for IgA (0.28, 95% CI 0.12-0.44). Additionally, six out of nine studies reported numerical titers for immunoglobulins detection, from which the level in saliva reached their reference value in four (66%). IgG but not IgA are frequently presented in saliva from vaccinated anti-COVID-19. Four studies reported lower IgA salivary titers in vaccinated compared to previously infected individuals, otherwise, two reported higher titers of IgA in vaccinated. Concerning IgG, two studies reported high antibody titers in the saliva of vaccinated individuals compared to those previously infected and one presented similar results for vaccinated and infected. The detection of antibodies anti-SARS-CoV-2 in the saliva is available, which suggests this type of sample is a suitable alternative for monitoring the population. Thus, the results also pointed out the possible lack of mucosal immunity induction after anti-SARS-CoV-2 vaccination. It highlights the importance of new vaccination strategies also focused on mucosal alternatives directly on primary routes of SARS-CoV-2 entrance.

Keywords: SARS-CoV-2; Antibodies; IgG; IgA; Saliva; COVID-19 vaccines.

Introduction

Since the COVID-19 outbreak, the development of vaccines is the highest priority due to the rapid transmission and lethality of SARS-CoV-2. Although the development of a safe and effective vaccine is a long and complicated process that typically takes 10 to 15 years (1), the scientific community turned it into an active and powerful field to develop emerging vaccines at an unprecedented speed (2, 3). The main COVID-19 vaccine type currently available are messenger RNA (mRNA) based, including BioNTech 162b2 mRNA/Pfizer (BNT) and Spikevax mRNA-1273/Moderna (MOD). Soon there were also recombinant viral-vectored vaccines, as Ad26. COV2. S Janssen - Johnson & Johnson (JJ), Vaxzevria/Oxford AstraZeneca (AZD), and inactivated virus approaches, like CoronaVac (Sinovac/Butantan) and Covaxin (Bharat Biotech) (4).

From the urgent introduction of these vaccines anti-SARS-CoV-2 worldwide until now, more than 60% of the world population had already received a full initial protocol of vaccination (5). The public health effect was mostly in the reduction of symptomatic and severe cases, impacting also on proportionate mortality caused by COVID-19 (4, 6-8). In this regard, vaccines anti-COVID-19 proved to be effective in inducing humoral immunity (9). However, antibody titers declined over time after vaccination, with a subsequent reduction in neutralizing activity (10-13).

In this sense, frequent population follow-up on antibody quantification becomes increasingly useful for immunological monitoring and COVID-19 control after vaccination. Serological testing for SARS-CoV-2 antibody is the standard reference, being important to assess immunological responses after both vaccine and infection (14). Nevertheless, the invasive process needed for blood collection can limit its employment as a frequent method. As an alternative, saliva has been reported to be a rich biofluid in the assessment of immunity for several diseases, especially those in which the mouth is a route of infection (8, 15–17). Oral fluid, more commonly named “saliva”, is a complex mixture of salivary gland secretions, gingival crevicular exudate, oral microorganisms and food debris. Thus, oral fluid is a potential source of immunoglobulins, such as immunoglobulin G (IgG) issued from the blood and reaching the oral cavity by the gingival crevicular fluid and immunoglobulin A (IgA) issued from the salivary glands. The production of secretory IgA reflects mucosal immunity, which

may impact COVID-19 transmission in addition to the current reduction of symptomatic and severe cases (18–20). Moreover, saliva collection is easy, non-invasive, and requires relatively simple instructions, representing several advantages over blood samples (19, 21).

Thus, the characterization of coronavirus saliva-specific signatures could provide valuable information towards antibodies anti-SARS-CoV-2 in vaccinated individuals. So, this rapid systematic review aims to verify whether saliva is suitable for SARS-CoV-2 antibody (immunoglobulins) detection after vaccination.

Methods

A rapid systematic review was undertaken to evaluate whether saliva is suitable for SARS-CoV-2 antibodies detection after vaccination. Rapid systematic reviews are a knowledge generation strategy that provides high evidence in a short timeframe to support clinical and policy decision-making, especially during disease outbreaks (22, 23). Thus, the methodology was systematized as suggested by the PRISMA guideline (24) with some adaptations, such as a shorter search strategy, faster data extraction, and mostly qualitative synthesis (PROSPERO Protocol - CRD42022336968). The purpose is to provide urgent information with reference to the potential of saliva in assessing immunological response after vaccination anti-SARS-CoV-2. In addition, our evidence also contributes to the new discussion on the induction of mucosal immunity.

Search Strategy and Inclusion Criteria

Electronic search strategies were developed and applied to Embase, PubMed, and Web of Science (Appendix 1). The search included all articles published until May 8, 2022, without language restrictions. The inclusion criteria were based on the PICOS strategy, in which Population (P): Human vaccinated for SARS-CoV-2, infected or uninfected; Intervention (I): Anti-SARS-CoV-2 vaccine; Comparator/control (C): Humans not vaccinated for COVID-19; Outcomes (O): detection of SARS-CoV-2 antibodies in saliva, type of vaccine, type of antibodies, methods and techniques for detection; and Studies (S): observational studies.

Study Selection, Data Collection and Synthesis

The selection was completed in a systematic two-phase process by VTC and JAS. A third author (ENSG) was involved when required to make a final decision. Final selection was always based on the full text of the publication. VTC and JAS collected the required information from each selected article and ENSG cross-checked all data to confirm its accuracy. Primary outcome was the detection of antibodies in saliva, considering correlation analysis of saliva and serum/plasma, and detectable capacity using titers reference values and proportions. Secondary outcomes included comparison of titers levels among variable characteristics. The qualitative synthesis was conducted by grouping and comparing data reported in included studies in relation to primary and secondary outcomes. Additionally, graphics were conducted to better illustrate the outcomes analyzed in each study. For correlation data, the coefficient of each study reporting this analysis were collected and grouped. Then, the mean with standard deviation were calculated without comparison or statistical tests. The GraphPad Prism software version 9.4.0 (GraphPad Software, La Jolla California USA) was used to construct the graphics.

Results

Selection and Characteristics of Studies

In the first phase, 178 studies were identified through databases, and after removing duplicates, 134 references remained for screening titles and abstracts. From that, 97 records were excluded and 37 were selected to phase 2. A full-text reading was conducted on 35 references since two were not available. Based on inclusion criteria, 20 articles were excluded, and 15 studies were selected for the synthesis of results (12, 16, 25–37). The flow diagram summarizing the selection process is presented in Figure 1.

Of the included articles, 11 are longitudinal studies (12, 16, 25–28, 30, 32, 35–37), and four are cross-sectional studies (29, 31, 33, 34). All studies were published in English between 2021 and 2022. Five of them were conducted in Italy (25, 27, 28, 31,

32), three in United States (12, 16, 33), three in Germany (30, 34, 35), two in Canada (26, 36), one in Croatia (29), and one in Australia (37).

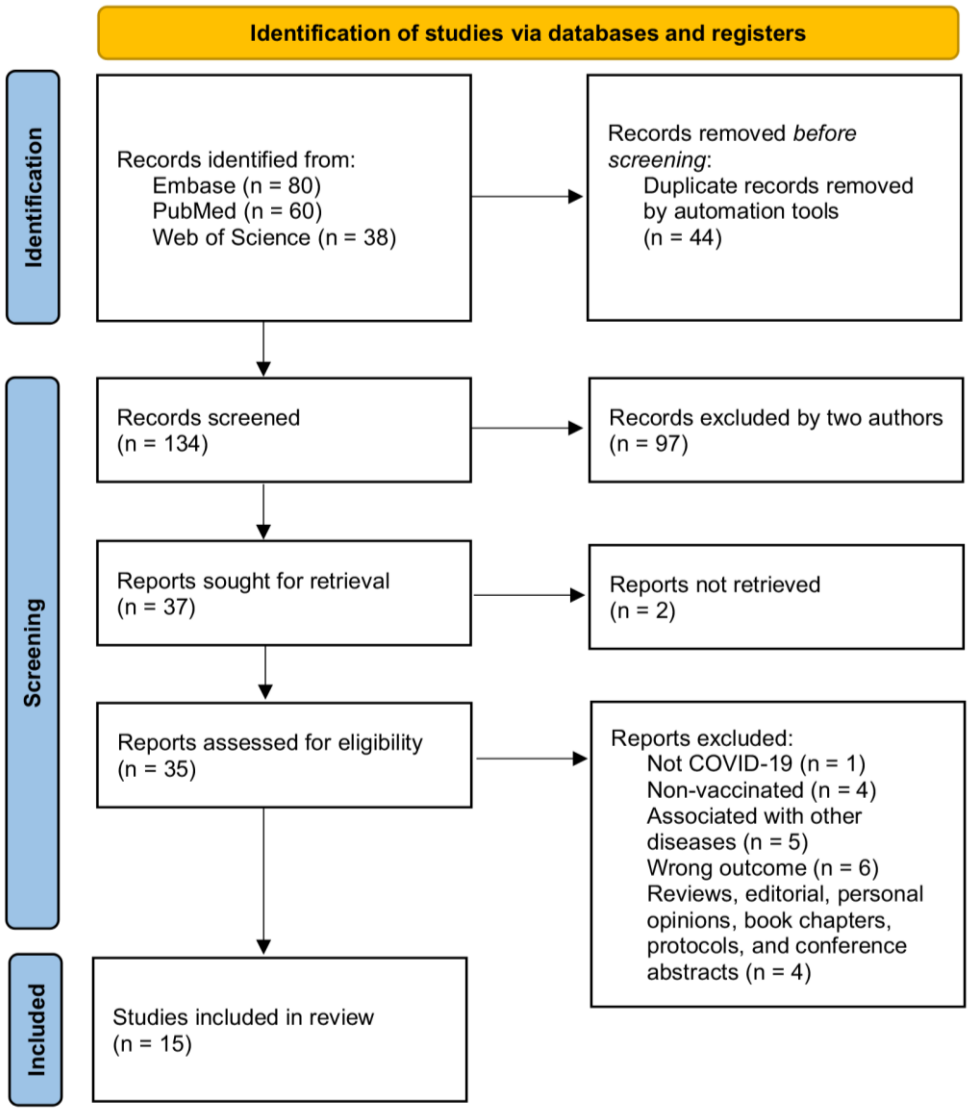

Figure 1. Flow diagram of literature search and selection criteria based on PRISMA 2020.

Summary of results

Considering the 15 included studies, approximately a total of 1,080 vaccinated and/or convalescent individuals were analyzed. The most evaluated vaccine was the BioNTech 162b2 mRNA/Pfizer (BNT) (15 studies, around 637 vaccinated), followed by

Spikevax mRNA-1273/Moderna (MOD) (five studies, about 77 vaccinated), Vaxzevria/Oxford AstraZeneca (AZD) (three studies, approximately 44 vaccinated), and Ad26. COV2. S Janssen (JJ) (one study, one vaccinated). Although the sample of main interest consisted of serum/plasma and saliva of vaccinated individuals, some studies also included healthy and previous infected ones as a comparison. From this, only two studies did not include participants previously infected with SARS-CoV-2 (25, 29).

Saliva samples were mainly collected using cotton devices (n=6), such as Salivette® and similar (26, 29, 32, 35–37), splitting methods (n=4) (12, 25, 30, 31) and aspiration (n=1) (27). Some papers did not report the collection method (n=4) (16, 28, 33, 34). The methods of analysis included Enzyme-linked immunosorbent assay (ELISA), Multiplex bead assays, Electro-Chemiluminescence immunoassay (ECLIA), Flow Cytometry (FC), and Chemiluminescence Immunoassay (CLIA). Additionally, five studies evaluated neutralizing activity anti-SARS-CoV-2 (Figure 2A).

Saliva samples were evaluated for IgA in 10 studies and for IgG in nine. Total Igs were assessed in five, while IgM was assessed only in two studies (Figure 2B). Concerning specific antigens for antibodies detection, 14 studies assessed the spike protein (S) subunits 1 and 2 or whole trimer, 10 studies used the RBD region, and three the nucleocapsid (NC) (Figure 2C). Detailed information can be found in Table 1.

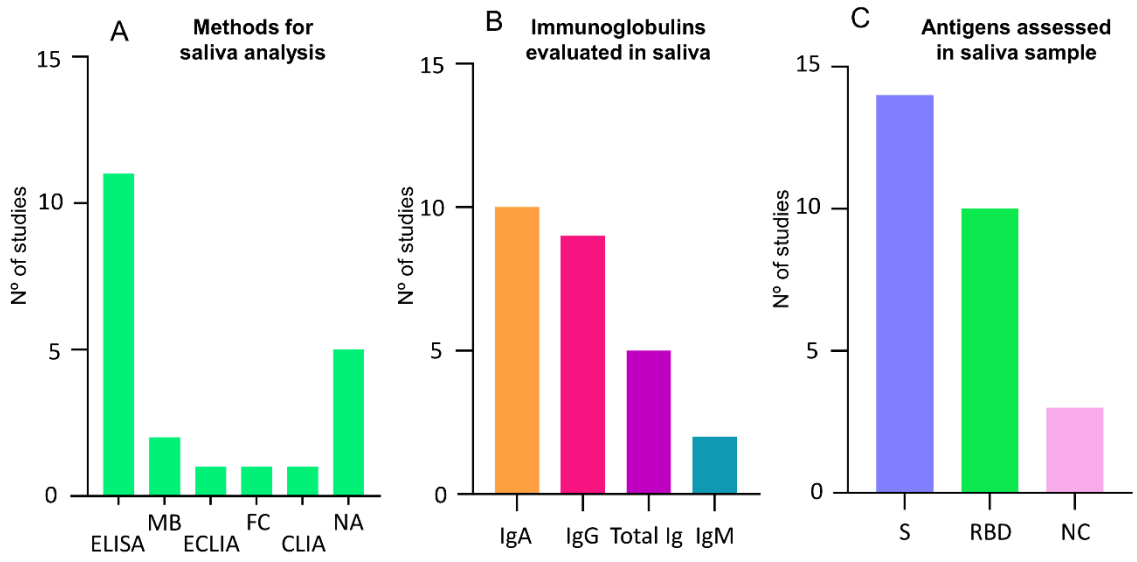

Figure 2. Characteristics of studies and antigen analysis in salivary samples. (A) Graphic showing the methods assessed and assays applied for saliva analysis and the number of studies using each type. (B) Graphic showing different immunoglobulins assessed and the number of studies assessing each type. (C) Graphic showing different antigen assessed for binding reaction and the number of studies assessing each type. CLIA, chemiluminescence immunoassay; ECLIA, electro-chemiluminescence immunoassay; ELISA, Enzyme-linked immunosorbent assay; FC, Flow Cytometry; IgA, Immunoglobulin A; IgG, immunoglobulin G; IgM,Immunoglobulin M; MB, Multiplex Bead; NA, Neutralizing activity; NC, nucleocapsid; NR, Not reported; BD, receptor binding domain; S, spike protein; Total Ig, Total immunoglobulins.

Correlation of immunoglobulins detection in serum/plasma and saliva

To assess the correlation between antibodies quantity in serum/plasma and saliva, seven analyses were reported for IgG, three for IgA, and two for Total Igs. All studies reported linear correlation; however, the results were heterogeneous. The mean coefficients reported for IgG were 0.55 (95% CI 0.38-9.73), indicating a moderated correlation that varies between weak and strong (Figure 3A). The mean correlation coefficient for IgA was 0.28 (95% CI 0.12-0.44), representing a weak correlation result (Figure 3A). A mean analysis for Total Igs correlation in serum/plasma and saliva was not feasible. The individual reports for Total Igs presented a strong correlation in Lapic et al. (29) study (Spearman correlation; r=0.66), and no correlation in Robinson et al. (36) study (Linearity analysis; p=0.90).

These results lead to a question of whether there is a similarity in correlation strongness when the different antigen-antibody reaction was detected. Thus, the correlations performed with anti-S and anti-RBD were separated, and the mean

coefficients were compared for IgG and IgA. The mean analysis showed 0.57 (95% CI 0.35-0.78) for anti-S IgG, 0.55 (95% CI 0.01-1.02) anti-RBD IgG, 0.32 (95% CI -0.06-0.70) for anti-S IgA, and 0.22 for anti-RBD IgA (Figure 3B), which suggests approximate results using different antigen binding.

Only three studies reported accuracy values for salivary analysis (Table 1). Sensitivity was higher than specificity in two studies (99% and 88%; 100% and 86.5%, respectively). The other study reported just the sensitivity value of 75% six months after the second dose.

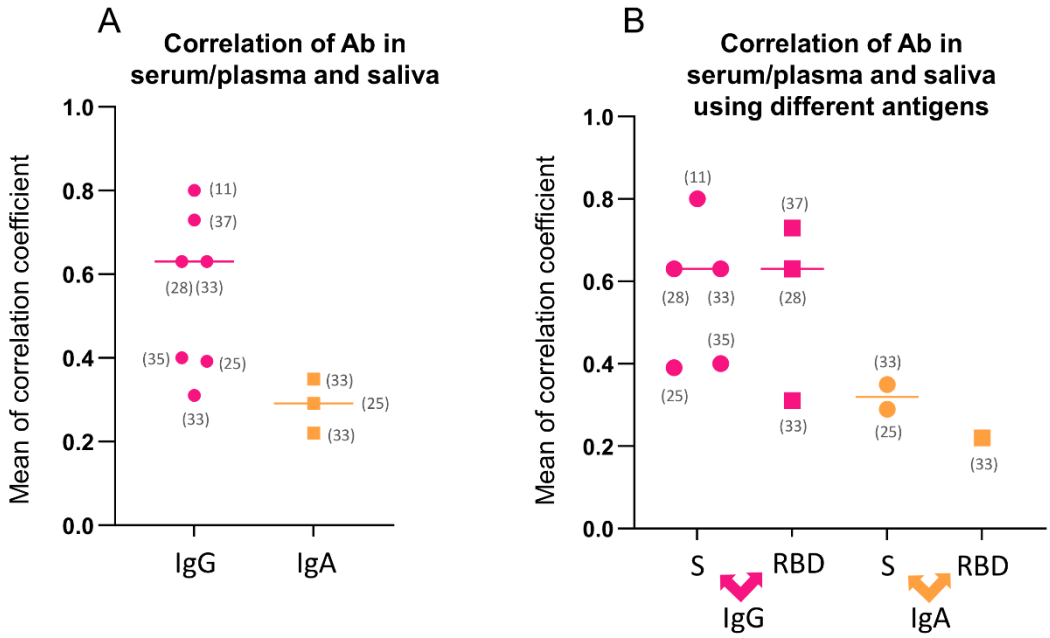

Figure 3. Correlations analysis regarding antibodies detection in serum/plasma and saliva. (A) Mean of correlation coefficients between antibodies detection in serum/plasma and saliva for IgG and IgA. (B) Mean of correlation coefficients between antibodies detection separated by antigenantibody reaction for IgG and IgA. IgA, Immunoglobulin A; IgG, immunoglobulin G; RBD, receptor binding domain; S, spike protein; Total Ig, Total immunoglobulins.

Immunoglobulins titers in saliva

Immunoglobulins levels in serum/plasma and saliva were assessed as an outcome in all included studies. Table 2 presents the summary of the nine studies that reported antibody titers or proportions detected in saliva versus serum/plasma. There were two ways of reporting: 1. Quantification of antibody titers, and 2. Proportion of individuals with positive detection. The studies presented a main increasing pattern of

titers before vaccination, and after first and second doses. On the contrary, Sheikh-Mohamed et al. (26) reported higher detection after one dose than after two doses. Most studies reported higher antibody titers in serum/plasma than in saliva. Six out of nine studies reported the numerical quantification of antibody titers, from which titers' level in saliva was able to reach the reference value for detection in four studies. The two reminding studies did not define an objective reference value for saliva standardized analysis. Three studies presented proportion values showing that saliva was suitable for antibody detection in all (100%) with superior percentages for IgG than IgA in two studies that assessed both immunoglobins.

Furthermore, eight studies performed the comparison of IgA titers in saliva between vaccinated and previous infected individuals with or without vaccine doses. Four studies reported lower IgA salivary titers in vaccinated without previous infection (50%), otherwise, two reported higher titers of IgA in those individuals (25%%). One reported conflicting results showing higher titers in vaccinated compared to mild/moderate COVID-19 cases and lower titers compared to severe ones (12.5%). In addition, one study failed to detect values for both groups of individuals (12.5%) (Figure 4A). Toward IgG, two studies reported high levels in saliva of only vaccinated individuals compared to previous infected ones (66%) and one reported similar results for vaccinated and infected (33%) (Figure 4B).

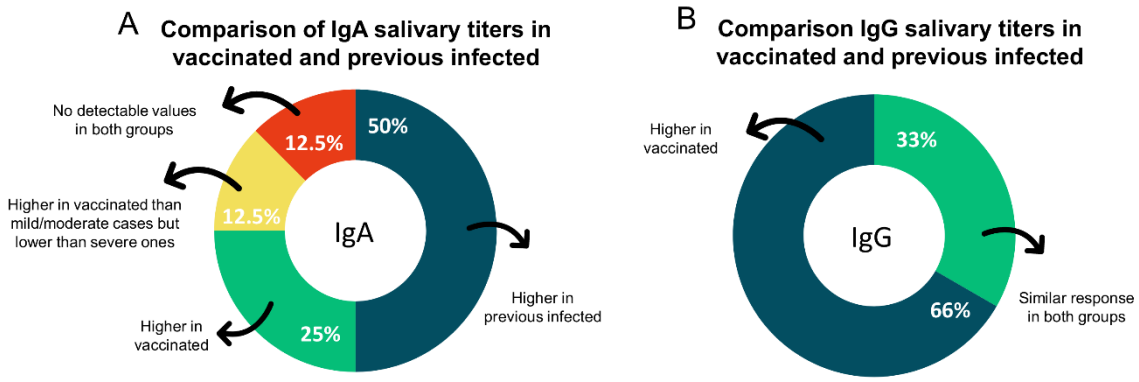

Figure 4. The proportion of studies on secondary outcomes. (A) Graphic showing the proportion of studies reporting results on IgA comparison between vaccinated and previous infected individuals (B) Graphic showing the proportion of studies reporting results on IgG comparison between vaccinated and previous infected individuals. IgA, immunoglobulin A; IgG, Immunoglobulin G.

Neutralizing activity

Two studies reported the main conclusions on neutralization correlations (Table 1). Garziano et al. (31) assessed the correlation of neutralizing activity in plasma and saliva. The reported results showed stronger coefficients for individuals who were previously infected with (r=0.52) or without (r=0.55) vaccination, compared to those uninfected and vaccinated (r=0.03). Meyer-Ardnt et al. (34) evaluated the correlation of salivary secretory IgA and neutralizing activity, showing weak results for elderly vaccinated individuals (r=0.46 after 28 days; r=0.44 after 49 days), very weak for middle-aged vaccinated (r=0.38 after 28 days; r=0.08 after 49 days), and moderate for previous infected ones (r=0.66 after 28 days; r=0.59 after 49 days). Additionally in neutralizing fields, Nickel et al. (35) was the only study reporting neutralization after the third dose of vaccine. The results provided evidence of stronger neutralizing activity in the group receiving heterologous vaccination protocol (AZD-BNT) compared to homologous one (BTN-BNT). Thus, a combination of different SARS-CoV-2 vaccine classes seems to lead to a stronger humoral immune response which may result in a better protective effect.

Table 1. Summary of overall descriptive characteristics of included studies (n= 15).

Author/ Year/ Country	**Groups (n)**	**Age (years)**	**Antigen tested/antibodies detected (Fluid of collection)**	**Method analyses (Fluid sublimed to analysis)**	**Main results**
Azzi et al. 2022 (25), Italy	Vaccinated BNT (60)	41.2 ± 10.4 (26-62)	Anti-S/ IgG, IgA (Serum and saliva) Anti-S1/S2 IgG (Serum)	ELISA (Serum and saliva) CLIA (Serum) Anti-RBD neutralizing assay (Serum and saliva)	Pearson correlation of IgG in serum and saliva (r=0.392) Pearson correlation of IgA in serum and saliva (r=0.291) Sensitivity: 99% Specificity: 88%
Darwich et al. 2022 (28), Italy	Vaccinated BNT (92) Control (19) Unvaccinated and previous infected (28)	38.35 (11.95) 42.8 (15.4) 47.3 (16.6)	Anti-S Total Ig (Saliva) Anti-RBD/S/N IgG, IgA, IgA1, IgA2 (Plasma and saliva)	ELISA (Saliva) Adapted dual-ELISA (Plasma and saliva)	Spearman correlation of anti-S IgG in serum and saliva (r=0.4) Sensitivity 100% Specificity 86.5%
Garziano et al. 2022 (31), Italy	Vaccinated AZD/BNT (40)	34.1 ± 11.5	Anti-RBD Total Ig (saliva)	ELISA (saliva)	Neutralizing activity titer: All groups

	Vaccinated previous infected (28) Previous infected (20)	41.36 ± 19.19 29.4 ± 20.5		Virus neutralization assay (Plasma and saliva)	Correlation in plasma and saliva (r^2=0.32) Vaccinated Correlation in plasma and saliva (r^2=0.03) Vaccinated previous infected Correlation in plasma and saliva (r^2=0.52) Previous infected Correlation in plasma and saliva (r^2=0.55)
Guerrieri et al. 2021 (32), Italy	Vaccinated BNT (28) Previous infected (18) Control (33)	52 49 (22-70) 52	Anti-S IgA (Serum and saliva) Anti–RBD IgG (Serum and saliva)	ELISA (Serum and saliva) CLIA (Serum and saliva)	Production of salivary anti-S1 IgA and anti-RBD IgG IgG and IgA production are higher after the vaccine second dose compared to subjects recovered from COVID-19
Johnson et al. 2022 (12),	Vaccinated MOD/ BNT/JJ and/or	NR	Anti-S IgG (Dried blood and saliva)	ELISA (Dried blood and saliva)	Repeated measures correlation of

United States	infected (13)				matrices in blood and saliva (r=0.80)
Ketas et al. 2021 (26), United States	Vaccinated BNT/MOD (85) Previous infected (10) Uninfected (7)	39.5	Anti-S/RBD IgM, IgG, IgA (Serum and saliva)	ELISA (Serum and saliva)	Anti-RBD IgA reactivities were higher in saliva than in serum. Anti-S IgG were detected in more participants with 2 doses than 1. Anti-S-IgA were present in 60.6% saliva samples after 2 doses
Klingler et al. 2021 (33), United States	Vaccinated BNT/ MOD (20) Previous infected (13) Control (4)	30-69 25-79 NR	Anti-S/S1/S2/RBD Total Ig (Plasma and saliva) Anti-S/RBD IgM, IgG1, IgG1, IgG3, IgG4, IgA1, IgA2 (Plasma and saliva)	Multiplex Bead Ab Binding Assay (Plasma and saliva)	Spearman correlation of anti-S and anti-RBD IgG1 in serum and saliva (r=0.63) No correlation found between serum and saliva to IgA1 and IgM
Lapic et al. 2021 (29), Croatia	Vaccinated BNT (43)	52 (27-63)	Anti-S Total Ig (Serum and saliva)	ECLIA (Serum and saliva)	Spearman correlation of Total Ig in serum and saliva (r=0.606)
Meyer-Ardnt et al. 2022 (34), Germany	Elderly vaccinated BNT (18) Middle-age vaccinated BNT (14) Previous infected (37)	83 ± 6 47 ± 10 36 ± 11	Anti-S1 IgA (Serum and saliva) Anti-S1 IgG (Serum)	ELISA (Serum and saliva) Anti-S1 neutralizing assay (Serum and saliva)	Spearman correlation of salivary sIgA and neutralization Elderly Vaccinated (r=0.46) in 28d, (r=0.44) in 49d Middle-age vaccinated (r=0.38) in 28d, (r=0.08) in 49d

					Previous infected (r=0.66) in 28d, (r=0.59) in 49d
Nickel et al. 2022 (35), Germany	Vaccinated BNT (104) AZD/BNT (11) Previous infected (57)	41 31 51	Anti-S1/RBD IgA (saliva) Anti-S1/NC/IWV IgA, IgG (serum) Anti-RBD Polyvalent IgGAM (serum)	Flow cytometry and neutralizing assay by flow cytometry (saliva) ELISA (serum) ELISpot (serum)	No increase of IgA production at the day of second dose (median 21d) or 14-28 days after second dose was observed in the vaccinated individuals. In contrast, most COVID-19 patients had detectable salivary IgA towards after 15-30 days after the onset of symptoms
Pinilla et al. 2021 (30), Germany	Vaccinated 1 dose BNT (22) MOD (7) AZD (13) Previous infected (72)	NR 29 (19–75)	Anti-RBD IgG (Serum and saliva)	ELISA (Serum and saliva) Anti-S1 neutralizing assay (Serum)	Pearson correlation of IgG in plasma and saliva (r=0.73)
Robinson et al. 2022 (36), Canada	Vaccinated BNT (10) Previous infected (10)	NR	Anti-S/NC Total Ig (Serum and saliva)	NR	Linearity of anti-S Total Ig in serum and saliva was insignificant 6 months after second dose (p=0.9) Sensitivity in 6 months after second dose: 75%
Selva et al. 2021 (37), Australia	Vaccinated BNT (15) Previous infected (16)	34 (25-57) 52 (22-76)	Anti-WST/S1/S2/RBD/NC IgA1, IgA2, IgG (Plasma and saliva)	Multiplex bead array (Plasma and saliva) Anti-S1 neutralizing assay	RBD-specific antibodies were detected in convalescent plasma, however RBD-specific antibodies were not detectable in convalescent saliva in comparison with healthy controls Vaccination induced high levels

				(Plasma and saliva)	of spike-specific IgG antibodies in tears, saliva and plasma, however no IgA1 and IgA2 responses were detected in saliva
Sheikh-Mohamed et al. 2022 (26), Canada	Vaccinated BNT (66) MOD (34)	48	Anti-S/RBD IgA, IgG (Serum and saliva)	ELISA (Serum and saliva)	Spearman correlation of anti-S IgG in plasma and saliva 2-4 weeks after second dose (r=0.63)
	Previous infected (11)	53		Anti-RBD neutralizing assay by flow cytometry (Serum and saliva)	Spearman correlation of anti-RBD IgG in plasma and saliva 2-4 weeks after second dose (r=0.31) Spearman correlation of anti-S IgA in plasma and saliva 2-4 weeks after second dose (r=0.35) Spearman correlation of anti-RBD IgA in plasma and saliva 2-4 weeks after second dose (r=0.22) Spearman correlation of anti-S IgA and secretory component in the saliva 2-4 weeks after second dose (r=0.42) Spearman correlation of anti-RBD IgA and secretory component in the saliva 2-4 weeks after second dose (r=0.45) Spearman correlation of anti-S IgA and

					secretory component in the saliva 6 months after second dose (r=0.53) Spearman correlation of anti-RBD IgA and secretory component in the saliva 6 months after second dose (r=0.85)
Terreri et al. 2022, Italy	Vaccinated BNT (34)	46.3 (12.15)	Anti-S IgA (Saliva)	ELISA (Saliva)	S-specific salivary IgA was very low in the majority of vaccinated. Anti-S IgA was still present in the saliva of individuals who had previous COVID-19 infection
	Previous infected (33)	39.9 (11.3)	Anti-NC IgA, IgG, IgM and anti-RBD Total Ig (Serum)	ECLIA (Serum)	
	Control (34)	46.3 (12.15)	Anti-TS IgG (serum)	CLIA (serum) Neutralizing assay by MNA (serum)	

Abbreviations: Ab – Antibody; NAb – Neutralizing Antibody; AZD – Vaxzevria/Oxford AstraZeneca; BNT – BioNTech 162b2 mRNA/Pfizer; CLIA – chemiluminescence immunoassay; COVID-19 – corona virus diseases 2019; ECLIA – electro-chemiluminescence immunoassay; ELISA – Enzyme-linked immunosorbent assay; ELISpot - Enzyme-linked immune absorbent spot; IgA – Immunoglobulin A; IgG – immunoglobulin G; Total Ig – Total Immunoglobulins; IWV – Inactivated Whole Virion; MNA – micro-neutralization assay; JJ - Ad26. COV2. S, Janssen, Johnson & Johnson; MOD - Spikevax mRNA-1273/Moderna; NC – nucleocapsid; NR – Not reported; r – Correlation coefficient; RBD – Receptor binding domain; S – Spike protein; S1 – Spike 1; S2 – Spike 2; SARS-CoV-2 – severe acute respiratory syndrome corona virus 2; TS – Trimeric spike; WST – Whole spike timer;

Table 2. Antibodies titers or proportion of detection in saliva versus serum/plasma of studies that expressly reported numerical data.

Author/ Year/ Country	Vaccinated group	Before vaccine or infection	1st dose (Period between dose and collection)	2nd dose (Period between dose and collection)	Reference
Azzi et al. 2022 (25), Italy	Saliva	IgG: 0.02 ng/mL IgA: 0.02 ng/mL	(2w) IgG: 0.07 ng/mL IgA: 0.05 ng/mL	(2w) IgG: 10.8 ng/mL IgA: 0.07 g/mL	IgG: 1.54 ng/mL
	Serum	IgG: 0.04 ng/mL IgA: 0.02 ng/mL	(2w) IgG: 432.1 ng/mL IgA: 1.71 ng/mL	(2w) IgG: 20373.65 ng/mL IgA: 49.59 ng/mL	IgG: 904.5 ng/mL
Garziano et al. 2022 (31), Italy	Saliva (ELISA)	NR	NR	(0.5-12m) Vaccinated Total Ig: 30.58% (0.5-12m) Vaccinated and previous infected Total Ig: 58.40%	Negative < 20%
	Serum	NR	NR	NR	
Guerrieri et al. 2021 (32), Italy	Saliva	IgG-RBD 1.19 BAU/mL IgA-S1 10.50 COI	IgG-RBD (<70d) Previous infected 1 BAU/mL IgA-S1 (<70d) Previous infected 13.75 COI	IgG-RBD (15d) 1.57 BAU/mL IgA-S1 (15d) 44 COI	CLIA > 1.19 BAU/mL ELISA Negative < 0.8 COI Positive >10.50 COI
	Serum	IgG-RBD 0.75 BAU/mL IgA-S1 29.29 COI	IgG-RBD (70d) Previous infected 109.10 BAU/mL IgA-S1 (<70d) Previous infected 169.70 COI	IgG-RBD (15d) 1711 BAU/mL IgA-S1 (15d) 739.30 COI	CLIA >4.33 BAU/mL ELISA Negative < 0.8 COI Positive >1.1 COI
	Saliva	NR	NR	(≤6m) Vaccinated	NR

Johnson et al. 2022 (33), USA				IgG: 29 ng/mL - Peak (≤6m) Vaccinated Previous Infected IgG: 982.5 ng/mL- Peak	
	Serum	NR	NR	(≤6m) Vaccinated IgG: 60.1 µg/mL- Peak (≤6m) Vaccinated Previous Infected IgG: 532.8 µg/mL- Peak	NR
Ketas et al. 2021, USA	Saliva	NR	Proportions of detection S-protein BNT IgA: 17% IgG: 33% IgM: 0% MOD IgA: 71% IgG: 86% IgM: 14%	Proportions of detection S-protein BNT IgA: 55% IgG: 100% IgM: 17% MOD IgA: 85% IgG: 100% IgM: 8% RBD BNT IgA: 83% IgG: 100% IgM: 4% MOD IgA: 77%	NR

				IgG: 100% IgM: 8%	
	Serum	NR	Proportions of detection S-protein BNT IgA: 38% IgG: 54% IgM: 17% MOD IgA: 100% IgG: 100% IgM: 71%	Proportions of detection S-protein BNT IgA: 100% IgG: 100% IgM: 71% MOD IgA: 100% IgG: 100% IgM: 62% RBD BNT IgA: 76% IgG: 100% IgM: 55% MOD IgA: 100% IgG: 100% IgM: 46%	NR
	Saliva	NR	NR	(71d) Total Ig: 2.5 U/mg proteins	NR

Lapic et al. 2021, Croatia	Serum	NR	NR	(71d) Total Ig: 1274 U/mL	Negative < 0.8 U/mL
Pinilla et al. 2021, Germany	Saliva	NR	(1m) IgG: 626 ng/mL Proportions of detection (1y after infection) IgG: 72% (15m after infection) IgG: 80%	NR	NR
	Serum	NR	(1m) IgG: 1458 µg/mL Proportions of detection (4m after infection) IgG: 89% (12m after infection) IgG: 89% (15m after infection) IgG: 98%	NR	NR
Robinson et al. 2022, Canada	Saliva	Total Ig: <0.4 U/mL	Total Ig <0.4 U/mL	Total Ig (56d) <0.4 U/mL (70d) 14.3 U/mL (86d) 11.2 U/mL (>6m) 2.6U/mL	<0.4 U/mL Negative
	Serum	NR	NR	Total Ig (56d) 79 U/mL	NR

				(>6m) 1558 U/mL (70d) >2500 U/mL (86d) >2500 U/mL (>6m) 1558 U/mL	
Sheikh-Mohamed et al. 2022, Canada	Saliva	NR	Proportion of detection (2w) Anti-S-IgG: 97% (2w) Anti-RBD- IgG: 52% (2w) Anti-S-IgA: 93% (2w) Anti-RBD-IgA: 41%	Proportion of detection (NR) Anti-S-IgG: 94% (NR) Anti-RBD-IgG: 93% (NR) Anti-S-IgA: 41% (NR) Anti-RBD-IgA: 20%	NR
	Serum	NR	NR	NR	NR

Abbreviations: BAU, Binding Antibody Units; BNT, BioNTech 162b2 mRNA/Pfizer; CLIA - Chemiluminescence Immunoassay; COI, Cut off Index; d - Days; ELISA, Enzyme-linked Immunosorbent Assay; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; mL, milliliter; m - Months; MOD, Spikevax mRNA-1273/Moderna; ng, nanogram; NR, Not Reported; RBD, Receptor Binding Domain; S, Spike protein; U, Units; µg, microgram; WD, Wild type; y - years: *Data calculated by authors based on reported information. In parentheses is the time between collection and the first dose, second dose, or infection, when it is reported in the study.

Discussion

SARS-CoV-2 is an enveloped single-stranded RNA virus, of which surface glycoprotein spike mediates viral entrance into host cells, especially through angiotensin-converting enzyme 2 (ACE2) (38). Against it, antibodies modulate cell infection by neutralizing viral antigen binding (39). Potent neutralizing antibodies were readily isolated from convalescent individuals, suggesting that SARS-CoV-2 is a neutralization-sensitive virus. Those neutralizing antibodies are targeted against the RBD motif of the spike protein, which is a relevant antigen to vaccines goals (40, 41). In this context, vaccine based on mRNA anti-SARS-CoV-2 was the most reported in this review. It demonstrated the capability of inducing antibodies in both previously infected and not infected individuals, increasing humoral and cellular immunity after the second vaccination dose (42). Furthermore, mRNA vaccines encode trimerized RBD which is modified by adding a “foldon” trimerization domain to increase immunogenicity. The result is the induction of anti-RBD neutralizing antibodies specific for SARS-COV-2 in plasma, and a T cell response with Th1 cytokine and low-level CD8 T cell (43).

In this systematic review, the main outcome was the correlation between serum/plasma and saliva antibodies, with the purpose of comparing the two types of samples source. Among the techniques for detecting antibodies, ELISA is one of the most used for serological tests (44). For years, its use has been also reported for saliva samples in different disease responses, such as hepatitis A (45), leprosy (46), and autism spectrum disorder (47). Thus, ELISA is suggested as a method to detect anti-COVID-19 antibodies in saliva. In this view, as most SARS-CoV-2 vaccines in use or in advanced development are based on the viral spike protein subunits, the antigen used for antibody detection is the S protein or its RBD region. Protein S is present on virions as prefusion trimers, where RBD is arbitrarily open or closed (41). IgG, mainly IgG1, dominates S- and RBD-specific antibody responses, which are intended against structurally folded S and RBD and three distinct peptide epitopes in S2. Although immunity assessment assays may vary respecting antigen-antibodies reactions, the synthesis of results suggests that it does not impact antibody detection after vaccination.

From our results, the mean correlation coefficient between serum/plasma and saliva was stronger for IgG than IgA. Nevertheless, at mucosal sites secretory IgA act with an essential role in protection mucosal surfaces by preventing the binding of viruses to epithelial cells (48). Salivary IgG, as well as a very limited amount of monomeric IgA, are derived from plasma via gingival crevices and could participate in viral protection (28, 49). Some articles comparing the immune responses of vaccinated and previously infected individuals suggested that salivary IgA titers were higher in the saliva of the infected, whereas IgG presented a higher salivary titer in vaccinated individuals (25, 27, 28, 35). As the current anti-COVID-19 vaccines used a systemic injection, they predominantly induce circulatory IgG (20, 50) indicating that after a mRNA vaccination, the IgG are translocated into saliva in sufficient amounts to have a high predictive value of induced seroconversion. However, as the testing methods used only the S protein (or its RDB subunit) as antigens, it is difficult to compare the IgG titers obtained after vaccination or natural infection. Indeed, except for vaccines that used inactivated viruses, the vaccinal antigen is based on the S protein, and only anti-S antibodies were obtained after vaccination, whereas the natural infection induces various antibodies specific to the various proteins of the virus (39). Thus, the testing methods are built to evidence the performance of the vaccination rather than the complexity of the antibody response obtained after natural infection. For IgA, several reports concluded that SARS-CoV-2 infection was associated with a mucosal secretory IgA response (25, 48) whereas, in vaccinated individuals, the IgA present in saliva were from blood origin (28). Thus, salivary IgAs induced by vaccination seems to be mainly exuded from serum while in previous infected individuals it came from a local mucosal immunity response.

Neutralizing antibodies were also assessed in the included studies. Viral neutralization plays a key role in anti-viral immunity and assessing its capacity is an important strategy to measure protective immunity (43, 51). In serum, neutralizing activity seems to be similar in previously infected individuals, either vaccinated or not, and uninfected vaccinated ones. However, neutralizing activity in saliva was high in convalescents and scarcely detected after vaccination (31). This observation could be explained by the absence of mucosal immunity induction in vaccinated individuals, associated with a quantity of Ig issued from the crevicular fluid that was insufficient to be neutralizing. Furthermore, one dose of vaccine was able to boost an anti-SARS-

CoV-2 response in previously infected individuals, whilst the third dose with a different vaccine type led to a significantly stronger response than only two doses (31, 35). Although a neutralizing activity was detected in saliva, intramuscular vaccines are not proved to be effective in producing salivary effects.

Besides the protection against the severe form of COVID-19, it is also essential to understand whether and how vaccination can decrease SARS-CoV-2 transmission (52). According to included studies, available data provided a weak response of intramuscular vaccines to elicit readily detectable mucosal immunity, suggesting the importance of local induction. Considering the respiratory tropism of the SARS-CoV-2 virus, a vaccine delivered intranasally would be useful to induce mucosal immunity directly at the port of virus entrance, also preventing transmission (53). Furthermore, nasal immunization is better than parenteral routes when seeking to achieve mucosal immunity, since the capability to induce IgA specific for SARS-CoV-2 in the respiratory tract may avoid virus spreading to the lung and avert respiratory problems (54). In this field, orally and intranasally administered vaccines have already been approved for humans against various mucosal pathogens (55). Currently, at least 12 projects are presenting intranasal candidates anti-SARS-CoV-2 at pre-clinical or clinical phases (56). In addition to potentially inducing sterilizing immunity, intranasal alternatives for COVID-19 vaccines are predominantly focused on viral vectors and protein subunits, representing safer delivery platforms than the whole pathogens used in all the licensed mucosal vaccines (55, 56). However, the development of a new safe, and an efficient mucosal vaccine is a complex process and several factors including antigen doses, formulation, administration route and adjuvants should be considered (54, 57). Thus, the kinetics and durability of the mucosal responses are also key factors in vaccine development. As mucosal vaccines seem to be potential alternatives for decreasing SARS-CoV-2 transmission, many efforts may focus on those strategies development turning into a current field of interest.

There are some limitations to be highlighted. First, the used techniques for antibodies detection in saliva were validated for serum analysis, resulting in difficulties to establish reference values for saliva quantification in some studies. Second, the included studies are highly heterogeneous with respect to samples, collection intervals, and strategies for reporting data. Third, in some studies, the sample size was not well

defined for all reported results, varying during the collection and analysis period process. Lastly, as COVID-19 is an urgent field, especially on vaccination at this time, we also included pre-print studies and letters for the editor, which impact detailed collection and quality.

Conclusion

Saliva is a suitable biofluid alternative for anti-SARS-CoV-2 antibodies detection in vaccinated and in previously infected individuals. Although salivary antibody titers are lower than serum titers, the detection of anti-SARS-CoV-2 immunoglobulins in saliva are satisfactory. Concerning specific immunoglobulins in vaccinated individuals, saliva seems to frequently present IgG but not uniformly IgA. The mean correlations in serum/plasma and saliva were moderate for IgG and weak for IgA. Thus, the results also suggest and pointed out the possible lack of mucosal immunity induction after anti-SARS-CoV-2 vaccination. It highlights the importance of new vaccination strategies focused also on mucosal alternatives directly on primary routes of SARS-CoV-2 entrance.

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Author Contributions

ENSG, ACA and HC designed the study. ENSG, VTC and JAS wrote the first draft of the manuscript. JAS conducted the search strategy. VTC and JAS selected the

articles. ENSG was involved when required to make a final decision. VTC and JAS collected the required information from each selected article and ENSG cross-checked all data to confirm its accuracy. ACA and HC reviewed and revised the draft. All authors contributed to the writing of the final manuscript.

Conflict of interest

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3.2 MANUSCRITO 2

O artigo a seguir foi escrito de acordo com as normas da revista *The International Journal of Infectious Diseases*, ISNN 1201-9712, classificada como periódico A3 no Qualis-Cape, com fator de impacto 12,07, onde será posteriormente submetido para publicação. A escolha da revista foi influenciada pelo seu escopo, onde são publicadas investigações laboratoriais originais na área de imunologia.

Detection of anti-SARS-CoV-2 salivary antibodies in vaccinated adults

Running title: SARS-CoV-2 salivary antibodies

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ABSTRACT

Since the introduction of efficient anti-SARS-CoV-2 vaccines, the detection of antibodies becomes useful for immunological monitoring and COVID-19 control. Therefore, this longitudinal study aimed to evaluate the detection of SARS-CoV-2 antibodies in the serum and saliva of vaccinated adults. The study included 13 negative control and 35 vaccinated participants with two doses of the CoronaVac (Sinovac/Butantan) vaccine who subsequently received the BNT162b2 (Pfizer-BioNTech) vaccine as a third dose. The vaccinated participants were evaluated approximately two months after the second dose, one month after the third dose and five months after the third dose, with the total of 118 saliva samples. ELISA was used to detect neutralizing antibodies (NAb), IgA and IgG and ECLIA to detect total antibodies (TAb) in 20 samples. The serum NAb was detected in 34/35 participants after the second dose (mean concentration= 57.86% ±20.74) and 35/35 participants one month (mean concentration: 95.6% ±3.34) and five months (mean concentration= 95.03% ±1.17) after the third dose ($p<0.0001$). In saliva NAb was detected in 30/35 samples after the second dose (mean concentration: 6.54%±5.54), and in 35/35 samples one month (mean concentration: 29.51%±11.96) and five months (10.17%±4.99) after the third dose ($p= 0.0001$). IgA was detected in 19/34 saliva samples after the second dose (ratio= 1.46±1.01), 18/35 saliva samples one month after the third dose (1.71±1.65) and 30/ 35 five months after the third dose (2.69 ±1.72) ($p<0.0013$). IgG was detected in 1/34 saliva samples after the second dose (ratio= 0.38±0.21), 33/35 saliva samples one month after the third dose (3.08±1.63) and 20/ 35 saliva samples five months after the third dose (1.44±0.76) ($p<0.0001$). There was a positive moderate correlation between NAb and TAb in serum ($r=0.6634$), NAb in serum and IgG in saliva ($r=0.7896$), and NAb and IgG both in saliva ($r=0.6115$). There was excellent sensitivity for the salivary NAb test (95%) and salivary IgA test five months after the third dose (88.2%). The salivary IgG test showed excellent specificity (100%) after the second dose, one and five months after the third dose, excellent accuracy one month after the third dose (100%) and still good accuracy five months after the third dose (86.8%). NAb, IgA and IgG were found in saliva from vaccinateds. Despite the inconsistencies of the data referring to the values of antibodies and correlations among themselves, the heterologous vaccination contributed to increase

anti-SARS-CoV-2 antibodies in the Brazilian health context.

Keywords: COVID-19; SARS-CoV-2; saliva; antibodies; neutralizing antibodies; IgG; IgA; COVID-19 vaccines.

BACKGROUND

Prediction models for the coronavirus disease 2019 (COVID-19) are quickly entering the academic literature to support medical decision making at a time when they are urgently needed (Wynants et al., 2020). Since the outbreak, the development of efficient vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was crucial for controlling the COVID-19 pandemic (Ketas et al., 2021).

Several vaccines were tested and developed at an unprecedented pace, the approved vaccines have been extremely effective in preventing COVID-19, particularly severe disease (Tregoning et al., 2021). CoronaVac (Sinovac/Butantan) is a widely used vaccine authorized in 48 countries. This vaccine was developed by the Chinese company Sinovac Biotech, uses inactated whole vírus (β-propiolactone-inactivated) and aluminum hydroxide as an adjuvant. It was created from African green monkey kidney cells (vero cells) inoculated with SARS-CoV-2 virus and the elentary vaccination regimen include two vaccination doses with an interval of 2 to 4 weeks. The effictiveness agaisnt hospital admission is 85% and 80% against death (Jara et al., 2021; Jin et al., 2022; Pérez-Then et al., 2022).

Differently, Pfizer-BioNTech developed mRNA vaccines targeting the surface protein of SARS-CoV-2 (Patel et al., 2022). The BNT162b2 vaccine (Pfizer–BioNTech) was globally used and authorized in 117 countries, it contains a nucleoside-modified messenger RNA encoding the spike glycoprotein of SARS-CoV-2 (Moreira et al., 2022). This vaccine commit to prevent the transmission of disease and reduce morbidity and mortality associated with SARS-CoV-2 infection, by inducing Spike protein-specific immunoglobulin antibodies providing protective immunity (Han et al., 2022).

Therefore, in the current moment of mass vaccination, antibody quantification becomes increasingly useful to assess vaccine efficacy and population immunity (Casian et al., 2021). Furthermore, continuated monitoring and longitudinal studies are

required to assess the duration of protection after COVID-19 vaccines shots over longer time periods (Feikin et al., 2022). Also, a great comprehension of immune memory after SARS-CoV-2 vaccination might contribute for the COVID-19 public health emergency management. Screening for protective immunity against SARS-CoV-2 will be decisive to preventing future outbreaks, taking effective public health measures, avoiding new infections, deaths and economic problems (Adi et al., 2022). Thus, antibody response testing postvaccination is a substantial and possible instrument for monitoring people after vaccination and would help to decide the specific time point for each individual to receive a booster dose (Wheeler et al., 2021). A combination of heterologous COVID-19 vaccines are safe and effective against SARS-CoV-2 infections, and they can be used as a great strategy since heterologous prime-boost regimens may induce comparable or higher antibody (spike protein) titers than homologous prime-boost (Garg et al., 2022).

The identification of specific antibodies anti-SARS-CoV-2 supports the evaluation of the seroprevalence, the monitoring of herd immunity and the generation of risk prediction models. Anti-SARS-CoV-2 neutralizing antibodies (NAbs) play a significant role in immunity since they inhibit the binding between the protein spike receptor-binding domain and the human angiotensin-converting enzyme 2 (ACE2) receptor. The binding between the virus S protein and human ACE2 makes the virus capable of entering the cell, suggesting that inhibit this process may prevent infection (Favresse et al., 2021). Considering the strong evidence of a protective role for neutralizing serum antibodies, a defined correlation between antibody titers against SARS-CoV-2 infection and how changes in immunity will affect clinical symptoms, would permit more confidence in community protection (Khoury et al., 2021).

Serological testing was widely applied to identify exposure to the virus by detecting SARS-CoV-2 specific antibodies and is now either for vaccination follow-up (Derruau et al., 2021). However, the invasive process needed for blood collection can limit the use of serological tests. Therefore, introducing saliva samples, composed by both gingival fluid and salivary secretion, can represent huge advantages as saliva collection is easy, non-invasive, and can be self-administrated with lower risk of contamination (Pisanic et al., 2021; To et al., 2020). Although the quantity of immunoglobulin G (IgG) secreted by gingival fluid is limited compared to polymeric immunoglobulin A (IgA) from salivary glands, the advantages associated with saliva

sample may improve the efficiency of monitoring and assessing vaccine responsiveness (Heaney et al., 2021).

There is evidence that saliva is a valuable fluid in assessing immunity for various diseases, and some studies are already pointed to a positive analysis of SARS-CoV-2 antibodies in saliva (Z. Huang et al., 2022; Isho et al., 2020; Ketas et al., 2021; Varadhachary et al., 2020). Compared with blood antibodies, saliva appears to be a viable alternative for obtaining information about acquired immunity after and during infection and as a tool to evaluate the effectiveness of vaccines (Aita et al., 2020; Faustini et al., 2020; Isho et al., 2020). However, there is a lack of information about how antibody titers in saliva correlate with those measured using plasma serologic assays to identify SARS-CoV-2-specific immunoglobulin activity.

Although mucosal immune responses have a critical role in protection against viral infections, it has been largely underestimated in the context of COVID-19. In a previously systematic review regarding detection of anti-SARS-CoV-2 antibodies in saliva, none of the included studies were in Brazil and none of them analyzed participants immunized with the CoronaVac vaccine (Guerra et al., 2022).

The majority of clinical studies focus on antibodies and cellular immunity in peripheral blood while vaccine abilities to elicit mucosal immune is still under study (Fiorelli et al., 2023). Few studies have explored mucosal immunity in individuals immunized with inactivated virus vacines (Chan et al., 2021; Ortega et al., 2022). And none of them provided a longitudinal follow-up of the participants after the booster dose, with the view to monitoring salivary and serum antibodies and their time duration, as is the case of this study.

Thus, the aim of this longitudinal study is to detect serum and saliva SARS-CoV-2 antibodies in vaccinated adults with two doses of CoronaVac vaccine who subsequently received the Pfizer vaccine as a third dose.

METHODS

This study is in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki, and get ethical approval from The Ethics

Committee of the Faculty of Health Sciences, University of Brasília – UnB protocol number 48224221.2.0000.0030.

Participants

The inclusion criteria were being over 18 years of age; not test positive for the serological test for anti-SARS-CoV-2 neutralizing antibodies; not presenting symptoms compatible with COVID-19; and agreed to the informed consent form. The exclusion criterion was withdrawal of the participants.

Between July 2021 and July 2022 all individuals donated serum and saliva samples, with a maximum interval of 7 days between serum and blood collection. As a negative control, 13 individuals were included, they were not vaccinated and not previously infected by SARS-CoV-2. The negative control group donated saliva and serum once. As participants, 35 individuals vaccinated with two doses of CoronaVac vaccine (Sinovac/Butantan) and later with an extra dose of BNT162b2 (Pfizer/BioNTech) vaccine were included. All the 35 vaccinated individuals donated saliva and serum in three different time points: after the second dose, one month after the booster dose and five months after the booster dose. The vaccinated group were not previously infected by SARS-CoV-2 (Figure 1). Therefore:

Negative control group (n=13):

- Group not vaccinated and not previously infected by SARS-CoV-2
- Donated saliva and serum once.

Vaccinated group (n=35):

- Donated saliva and serum after two doses of CoronaVac vaccine (Sinovac/Butantan): **collection of saliva and serum 2.5 months after the second dose.**
- Donated saliva and serum after doses of CoronaVac vaccine (Sinovac/Butantan) and one booster dose of BNT162b2 (Pfizer/BioNTech) vaccine: **collection of saliva and serum one month after the booster dose**.
- Donated saliva and serum after 2 doses of CoronaVac vaccine

(Sinovac/Butantan) and one extra dose of BNT162b2 (Pfizer/BioNTech) vaccine: **collection of saliva and serum five months after the booster dose**.

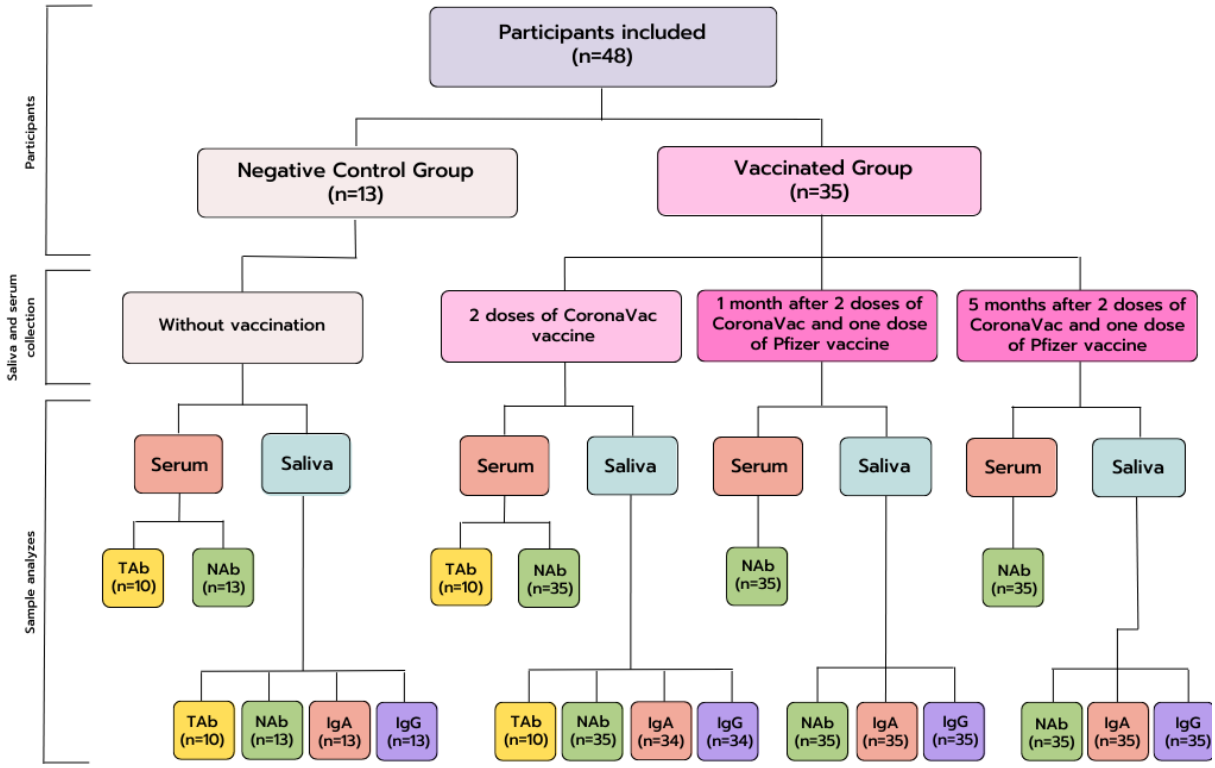

Figure 1. Flow diagram of participants included and sample analyzes. **Abbreviations:** IgA - Immunoglobulin A; IgG – Immunoglobulin G; NAb – Neutralizing Antibodies; TAb – Total Antibodies.

Demographic data collection

On the same day for serum and saliva collection, the participants answered a questionnaire about demographic data regarding being a health professional or not, age, gender, use of continuous medication, having systemic diseases (diabetes, hypertension, hypercholesterolemia, kidney disease, liver disease, depression, anxiety).

Collection and analysis of serum

Serum samples were obtained by venipuncture, perfomed after site asepsis. Serum collection was performed in a serum tube with clot activator and separator gel.

After collection, the tube is homogenized by inverting it 6 to 10 times and sent to sorting where the clot retraction is awaited (25 to 30 minutes on average) and the sample is centrifuged at 3,450 RPM for 15 minutes. The tube is sent to the technical area and stored in a specific transport box, with monitored temperature (-20ºC) until processed at the Sabin Laboratory (Brasília, Federal District, Brazil).

Collection, transport, and preparation of saliva

All participants were instructed not to use oral hygiene products, not to consume alcoholic beverages, cigarettes, and/or food for at least 1 hour before saliva collection. They were instructed to chew a cotton swab (Salivette® Cortisol, with roll of synthetic fibers, lid: blue, with paper label, code: 51.1534.500, SARSTEDT AG & Co, Germany) for 2 minutes to collection of stimulated saliva. Saliva was stored in a container with ice and transported to the laboratory within a maximum of 4 hours. Each swab containing saliva was centrifuged at 3,000 rpm for 5 minutes at 8ºC. Then, the samples were stored at -80°C until processing. No inhibitors were used in the samples.

Total Antibodies (TAb) analyses

The anti-Spike total specific antibodies (TAb) were quantified by the electrochemiluminescence assay (ECLIA) (Cobas e801, *Roche Diagnóstica BrasilLtda*) with RBD protein antigen, for 10 serum samples (after 2 doses of CoronaVac) and 20 saliva samples (10 from negative control and 10 after 2 doses of CoronaVac), at Sabin Laboratory (Brasilia, Federal District, Brazil). Total antibodies were measured in U/mL. Saliva was not diluted. The machine can not detect values lower than 0.4 U/mL. For serum, values higher than 0.8 were considered positive and values lower than 0.8 were considered negative. For saliva, values higher than 0.4 were considered positive and lower than 0.4 were considered negative.

Neutralizing Antibodies (NAb) analyses

For analyzing NAb, the enzyme-linked immunosorbent assay (ELISA) (GenScript, USA) was used with RBD protein antigen. NAb were analyzed in all the

samples of serum and saliva, using RBD as antigen. Saliva was not diluted. Neutralizing Antibodies were analyzed at Sabin Laboratory (Brasilia, Federal District, Brazil), measured in Optical Density (OD) and reported as a percentage using the following calculation: 1 minus the sample OD value divided by negative control of each plate multiplied by 100. Values higher or equal to 20 were considered positive and lower than 20 negative for serum. Values higher than 0 were considered positive and lower or equal to 0 negative for saliva.

$$X = \frac{1 - \text{Sample OD}}{\text{Negative Control}} \times 100 = \%$$

Anti-SARS-CoV-2 IgA and IgG analyses

Anti-SARS-CoV-2 IgA and IgG were both quantified in saliva samples by an ELISA test specific for IgA and an ELISA test specific for IgG, using a well plate coated with recombinant S protein antigen (Euroimmun Medizinische Laboradiagnostika, Luebeck, Germany, EI 2606-9601A EI 2606-9620A IgA, EI 2606-9601G EI 2606-9620G IgG), using a ELISA reader (EnSpire® Multimode Plate Reader, PerkinElmer, USA), at Laboratory of Oral Histopatology (University of Brasilia, Federal District, Brazil) and Laboratory of Natural Products (University of Brasilia, Federal District, Brazil). Saliva was tested in the dilutions 1:101 (Manufacturer's Instructions for blood), 1:50, 1:10, 1:5 and 1:2. The dilution 1:10 was chosen for IgA and 1:5 for IgG.

Sensitivity, specificity, and accuracy values for salivary test

A 2x2 table was performed regarding sensitivity, specificity, and accuracy of ELISA for Nab (values > 0% - positive; values ≤ 0% - negative), ECLIA for TAb (values > 0.4U/mL – positive; values ≤ 0.4U/mL – negative) and ELISA for IgA and IgG (Ratio ≥1.1 positive; < 0.8 negative, ≥ 0.8 to <1.1 borderline samples were excluded from this analysis). The parameter test used for comparition was NAb in serum. The sensitivy values were considered excellent when they were higher the 80%, 70-80% was considered good, 60-69% fair and < 60% poor. For specificity >90% was excellent, 80-90% good, 70-79% fair and < 70% poor. Regarding accuracy, > 90% was excellent, 30-90% moderate and < 30% poor (Appendix I).

Statistics Analyses

Descriptive and analytical statistics were performed using the Graphpad Prism software, version 9.3.0 (California, USA). The Shapiro-Wilk test was applied to assess data normality. Thus, Pearson's correlation test was performed for parametric data and Spearman's correlation test was performed for non-parametric. For analyzing NAb titers in both serum and saliva samples, the T-test was used for parametric data and the Mann-Whitney test for non-parametric data to compare two different time points. To compare the values for the four groups, Kruskal-Wallis test was used. A Linear Regression was performed using Jamovi (version 2.3.15). To the values obtained from each time point the Dunn's multiple comparisons test was perfomed.

RESULTS

The negative control group was composed by 7/13 (53.85%) health professionals and the vaccinated group composed by 19/35 (54.29%) health professionals. The average age for negative control was 29.23 years and for vaccinated group 30.31 years, regarding sex 7/13 (53.85%) were woman in negative control and 23/35 (65.71%) in vaccinated group. In the negative control group, a total of 5 participants (38.46%) had some systemic disease while in the vaccinated group, 6 participants (17.14%) had some systemic disease. As for the use of systemic medication, 5 persons (38.46%) used it in the negative control group and 9 participants (25.71%) in the vaccinated group. The groups composed of negative controls and vaccinated participants do not show statistically significant differences (Table 1).

Table 1. Description of demographic data based on participants records.

Characteristics	Variables	Groups		p-value
		Negative Control (n=13)	Vaccinated individuals (n=35)	
Health professional	No (%)	6 (46.15%)	16 (45.71%)	>0.999
	Yes (%)	7 (53.85%)	19 (54.29%)	

Sex	**Male (%)**	6 (46.15%)	12 (34.29%)	0.871
	Female (%)	7 (53.85%)	23 (65.71%)	
Age	**Median (Min-Max)**	25 (20-53)	25 (19-73)	0.842
	Mean (± SD)	29.23 (±10.22)	30.31 (±13.75)	
	95% CI	23.06-35.06	25.75-34.87	
	19 - 49 years	12 (92.30%)	30 (85.71%)	0.934
	50 - 79 years	1 (07.69%)	5 (14.29%)	
Systemic Disease	**No (%)**	8 (61.54%)	29 (82.86%)	0.344
	Yes (%)	5 (38.46%)	6 (17.14%)	
Use of medication	**No (%)**	8 (61.54%)	26 (74.29%)	0.815
	Yes (%)	5 (38.46%)	9 (25.71%)	
	Yes (%)	7 (53.85%)	19 (54.29%)	

Abbreviations: CI – Confidence Interval; Max – Maximum; Min – Minimum; SD – Standard deviation. Analytical statistics: Kruskal-Wallis test (*p<0.05).

Total Antibodies (TAb) (n=10)

Total antibodies (TAb) were analysed in saliva of negative control (n=10) and in serum and saliva of vaccinated individuals after two doses of CoronaVac (n=10), as a preliminary test.

After two doses of CoronaVac TAb was detected in 10/10 serum samples (158.13±88.1 U/mL) but only in 3/10 saliva samples (0.63±0.46 U/mL). TAb was not detected in saliva of any of 10 samples of negative control (<0.4±0 U/mL- limit of the machine) (Table 2). This preliminary result indicates that TAb could not be properly detected in saliva by the ECLIA assay.

Table 2. Total Antibodies titers in serum and saliva of negative control and vaccinated with 2 doses of CoronaVac.

Antibodies Titers	Variables	Negative control (n=10)	2 doses of CoronaVac (n=10)	Reference value	p-value

Total Ab Serum (U/mL)	**Median (Min-Max)**	NA	156 (31.40-250)	Non-reactive: <0.80 U/mL Reagent: ≥0.80 U/mL Values not detectable by the method: ≥0.40 U/mL	-
	Mean (± SD) 95% CI	NA	158.13 ±88.10 95.11-221.20		
Total Ab Saliva (U/mL)	**Median (Min-Max)**	<0.4 (0.4-0.4)	0.4 (0.4-1.5)	Non-reactive: <0.80 U/mL Reagent: ≥0.80 U/mL Values not detectable by the method: ≥0.40 U/mL	0.2105
	Mean (± SD) 95% CI	<0.4 (±0) 0.4-0.4	0.63 (±0.46) 0.3-0.96		

Abbreviations: CI – Confidence Interval; Max – Maximum; Min – Minimum; NR – Not reported; SD – Standard deviation. Analytical statistics: Mann Whitney test (*p<0.05). Vaccinated with 2 doses: 2 doses of CoronaVac (Sinovac/Butantan).

Correlation between NAb and TAb in Serum of CoronaVac vaccinated adults (n=10)

A Pearson's correlation was made between NAb (n=10) and TAb (n=10) values both in serum after two doses of CoronaVac. Notably, anti-SARS-CoV-2 NAb (by ELISA) concentrations in serum showed a positive and moderated correlation with TAb (by ECLIA) in adults who took two doses of CoronaVac (r= 0.6634; p=0.03565) (Figure 2, Table 3).

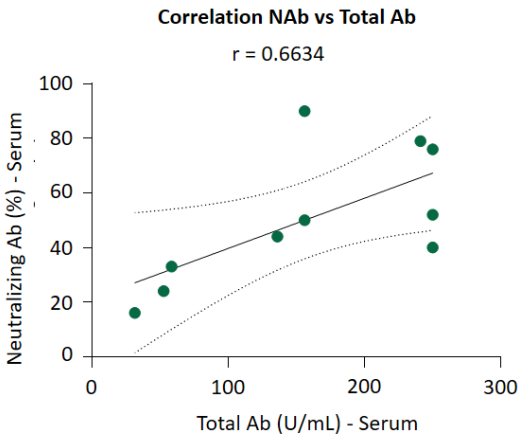

Figure 2. Correlation between Neutralizing Antibodies (n=10) and Total Antibodies (n=10) in serum of group 2 (vaccinated group with 2 doses of CoronoVac). Statistical analysis: Pearson's correlation for parametric data, p (two-tailed) = 0.03565. Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies; NAb - neutralizing antibodies; TAb - total antibodies.

Table 3. Correlations of diferente types of antibodies.

Antibodies	Groups	Time for collection (n of pairs)	Estatistic Correlation Analysis	R-value	95% confidence interval	P-value (two-tailed)
NAb versus TAb in serum	Vaccinated	2.5m after 2nd dose (n=10)	Pearson	0.6634	0.06 to 0.91	0.03565*
NAb in saliva versus NAb in serum	Negative Control	(n=13)	Pearson	-0.3357	-0.75 to 0.26	0.2621
	Vaccinated Individuals	2.5m after 2nd dose (n=35)	Spearman	-0.158	-0.47 to 0.19	0.3641
		1m after 3rd dose (n=35)	Spearman	0.223	-0.13 to 0.52	0.1977
		5m after 3rd dose (n=35)	Spearman	-0.021	-0.36 to 0.32	0.9011
	All samples	(n=118)	Spearman	0.7150	0.61 to 0.79	<0.0001*
IgA in saliva versus NAb in saliva	Negative Control	(n=13)	Spearman	-0.1637	-0.67 to 0.44	0.5908
	Vaccinated Individuals	2.5m after 2nd dose (n=34)	Spearman	-0.09204	-0.43 to 0.26	0.6047
		1m after 3rd dose (n=35)	Spearman	0.1623	-0.19 to 0.48	0.3516
		5m after 3rd dose (n=35)	Spearman	0.3233	-0.02 to 0.59	0.0582
	All samples	(n=117)	Spearman	0.04891	-0.14 to 0.23	0.6005
IgG in saliva versus NAb in saliva	Negative Control	(n=13)	Pearson	-0.3710	-0.76 to 0.23	0.2120
	Vaccinated Individuals	2.5m after 2nd dose (n=34)	Spearman	-0.1155	-0.44 to 0.24	0.5154
		1m after 3rd dose (n=35)	Spearman	0.3631	0.02 to 0.63	0.0321*

		5m after 3rd dose (n=35)	Spearman	0.5380	0.23 to 0.74	0.0009*
	All samples	(n=117)	Spearman	0.7203	0.62 to 0.79	<0.0001*
IgG in saliva versus NAb in serum	Negative Control	Negative Control (n=13)	Pearson	0.2582	-0.34 to 0.71	0.3944
	Vaccinated Individuals	2.5m after 2nd dose (n=34)	Spearman	0.3838	0.04 to 0.64	0.0250*
		1m after 3rd dose (n=35)	Spearman	0.09745	-0.25 to 0.43	0.5776
		5m after 3rd dose (n=35)	Spearman	-0.03893	-0.38 to 0.31	0.8243
	All samples	(n=117)	Spearman	0.8076	0.73 to 0.86	<0.0001*

Abbreviations: IgA - immunoglobulin A; IgG - immunoglobulin G; m – months; NAb - neutralizing antibodies.2 doses: vaccinated with two doses of CoronaVac (Sinovac/Butantan); 3 doses: vaccinated with two doses of CoronaVac (Sinovac/Butantan) vaccine + one booster dose of BNT162b2 (Pfizer-BioNTech).

Linear Regression to NAb and TAb in Serum of CoronaVac vaccinated adults (n=10)

A linear regression was performed (Jamovi, version 2.3.15) to estimate if neutralizing antibodies significantly predicted total antibodies (Table 4).

The fitted regression model was TAb = (-4.53) + 2.97x (x= value of neutralizing antibodies in percentage). The overall regression was statistically significant (R2= 0.465, F= 6.96, p= 0.03). It was found that NAb can significantly predicted TAb (β= 2.97, p= 0.03).

Table 4. Simple linear regression to test if Neutralizing Antibodies (NAb) significantly predicted Total Antibodies (TAb).

Normality Test (Shapiro-Wilk)		**Model Fit Measures**				**Model Coefficients – TAb**				
							Em	**SE**	**T**	**p**
Est	**p**	**R**	**R²**	**F**	**p**	**Ip**	-4.53	67.42	-0.07	0.94
0.95	0.66	0.68	0.46	6.10	0.03	**NAb**	2.97	1.13	2.64	0.03

Abbreviations: Est – Estatistic; Em – Estimate; Ip – Intercept; NAb - neutralizing antibodies; SE – Standard Error; TAb - total antibodies.

Neutralizing antibodies (NAb) in serum and saliva samples

The means values for serum and saliva antibodies titers are reported in percentage. NAbs were detected in saliva of 30/35 after two doses and 35/35 one month and five months after the booster dose (Table 5). Anti-SARS-CoV-2 NAb values were higher in vaccinated groups in all time points compared to the non-vaccinated group in both serum (negative control: 9.61% ±6.06; vaccinated with two doses: 57.86% ±20.74; one month after the booster dose: 95.6% ±3.34; five months after the booster dose: 95.03% ±1.17, p= 0.0001*) and saliva (negative control: 0% ±3.61; vaccinated after two doses: 6.54% ±5.54; one month after the booster dose: 29.51% ±11.96; five months after the booster dose: 10.17% ±4.99, p= 0.0001*) (Table 6, Figure 3). The values of neutralizing antibodies increased one month after the booster dose for serum and saliva when compared with 2 doses, and 5 months after the booster dose, the values remained the same for serum but dropped in saliva when compared to 1 month after the booster dose.

Table 5. Proportions of individuals positive for NAb, IgA and IgG antibodies.

Groups	**NAb**		**IgA**	**IgG**
	Serum	**Saliva**	**Saliva**	**Saliva**
Negative control	0/13 (0%)	3/13 (23.08%)	4/13 (30.77%)	0/13 (0%)
Vaccinated with 2 doses of CoronaVac (Sinovac/Butantan)	34/35 (97.14%)	30/35 (85.71%)	19/34 (55.88%)	1/34 (2.86%)
Vaccinated with 2 doses of CoronaVac (Sinovac/Butantan) and one booster dose of BNT162b2 (Pfizer-BioNTech) – one month after the last dose	35/35 (100%)	35/35 (100%)	18/35 (51.48%)	33/35 (94.29%)
Vaccinated with 2 doses of CoronaVac CoronaVac (Sinovac/Butantan) and one booster dose of BNT162b2 (Pfizer-	35/35 (100%)	35/35 (100%)	30/35 (85.71%)	20/35 (57.14%)

BioNTech) – five months after the last dose

Abbreviations: IgA - immunoglobulin A; IgG - imunoglobulin G; NAb - neutralizing antibodies.

Table 6. Median and mean concentration of neutralizing antibodies in serum and saliva.

		Groups				
	Variables	**Negative Control (n=13)**	**Vaccinated with 2 doses (n=35)**	**Vaccinated with 3 doses after 1 month (n=35)**	**Vaccinated with 3 doses after 5 months (n=35)**	**p-value**
NAb Serum (%)	**Median (Min-Max)**	9.00 (1-20)	56 (16-94)	96 (78-98)	95 (91-97)	<0.0001*
	Mean (± SD)	9.61 (±6.06)	57.86 (±20.74)	95.6 (±3.34)	95.03 (±1.17)	
	95% CI	5.95-13.28	50.73-64.98	94.45-96.75	94.62-95.43	
NAb Saliva (%)	**Median (Min-Max)**	0 (0-4)	8 (0-15)	26 (15-77)	9 (4-26)	<0.0001*
	Mean (± SD)	0 (±3.61)	6.54 (±5.54)	29.51 (±11.96)	10.17 (±4.99)	
	95% CI	-3.42-0.95	4.64-8.44	25.41-33.62	8.45-11.89	
Days between vaccines last dose and saliva donation	**Median (Min-Max)**	NA	74.00 (36-133)	36 (25-68)	163 (152-198)	0.0001*
	Mean (± SD)	NA	74.51 (±26.5)	38.54 (±9.99)	165.51 (±11.51)	
	95% CI		65.41-83.62	35.11-41.97	161.60-169.50	

Abbreviations: CI – Confidence Interval; Max – Maximum; Min – Minimum; NA – Not Available; NAb - neutralizing antibodies; SD – Standard deviation. Analytical statistics: Kruskal-Wallis test (*p<0.05). Vaccinated with 2 doses: two doses of CoronaVac (Sinovac/Butantan). Vaccinated with 3 doses: two doses of CoronaVac (Sinovac/Butantan) + one booster dose of BNT162b2 (Pfizer-BioNTech).

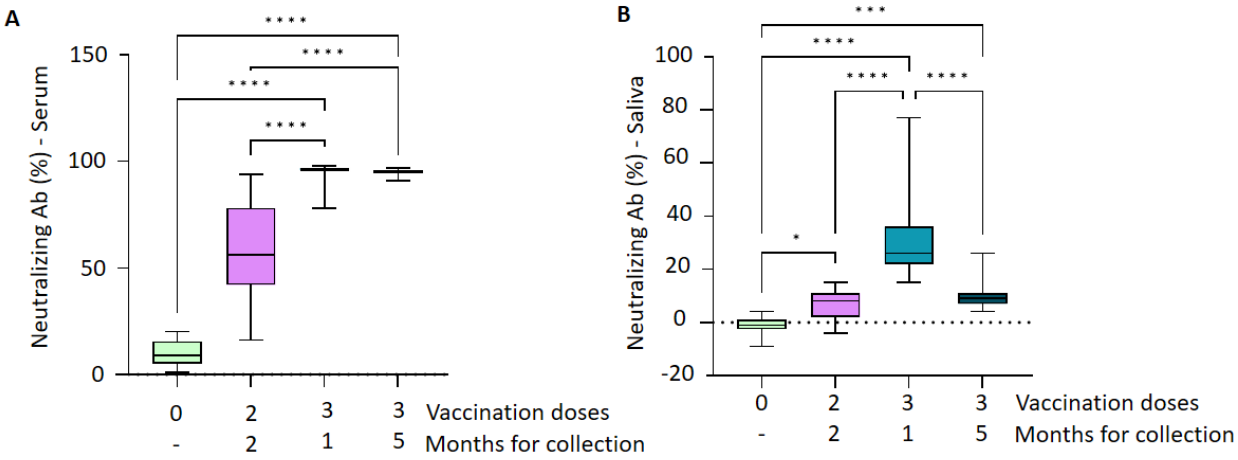

Figure 3. Median concentration of neutralizing antibodies in serum and saliva for each group. (A) Neutralizing antibodies in serum. (B) Neutralizing antibodies in saliva. Statistical analysis: Kruskal-Wallis test for non-parametric data, (*p<0.05). Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies.

Correlation NAb serum and NAb saliva

A correlation betweeen NAb in serum and NAb in saliva was performed to all three points of colletion of vaccinated group. NAb in serum vs NAb in saliva after 2 doses of CoronaVac had a week and negative Pearson's correlation (r= -0.158, p= 0.3641). One month after the booster dose the Spearman's correlation was also weak (r= 0.223, p= 0.1977). Five months after the booster dose the Spearman's correlation was contiunated as not significant (r= -0.02, p= 0.9011). The only significant correlation (r= 0.7150, p <0.0001) found between NAb in serum and NAb in saliva was when all the samples of all groups were compared including negative control and vaccinated group (n=118 pairs) (Table 3, Figure 4)

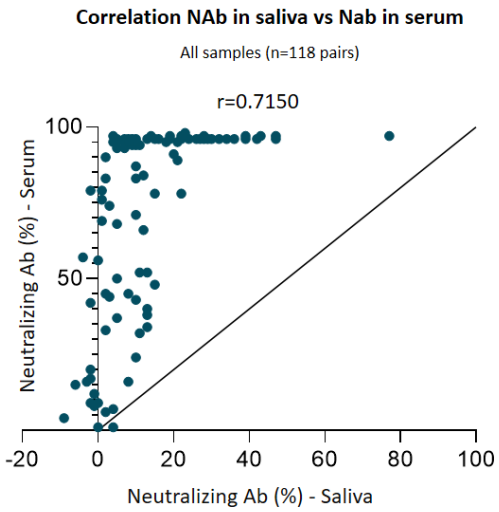

Figure 4. Correlation neutralizing antibodies in saliva versus neutralizing antibodies in serum for all the samples including negative control and vaccinated group (n=118 pairs). A significant and moderated correlation was found (r=0.7150). Statistical analysis: For non-parametric data Spearman's correlation, p (two-tailed) = <0.0001*. Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies; NAb - neutralizing antibodies.

IgA detection in saliva samples

IgA antibodies were detected in saliva of 4/13 in negative control, 19/34 after two doses of CoronaVac vaccine, 18/35 one month after the booster dose and 30/35 five months after the booster dose (Table 5). Negative control had the lowest median of IgA antibodies detected (ratio= 0.96), classified as "limit", by the manufacturer's instructions. While vaccinated in all time-points had a positive value, according to the manufacturer's instructions. After 2 doses of CoronaVac the IgA ratio median was 1.23; one month after the booster dose, the IgA ratio median was 1.16; and after five months from the booster dose, this value increase to 2.00 (p= 0.0013) (Table 7, Figure 4). To the values obtained from each time point the Dunn's multiple comparisons test was perfomed and the only significant difference was between group vaccinated with three doses five months after the last dose and all the other groups (Figure 5).

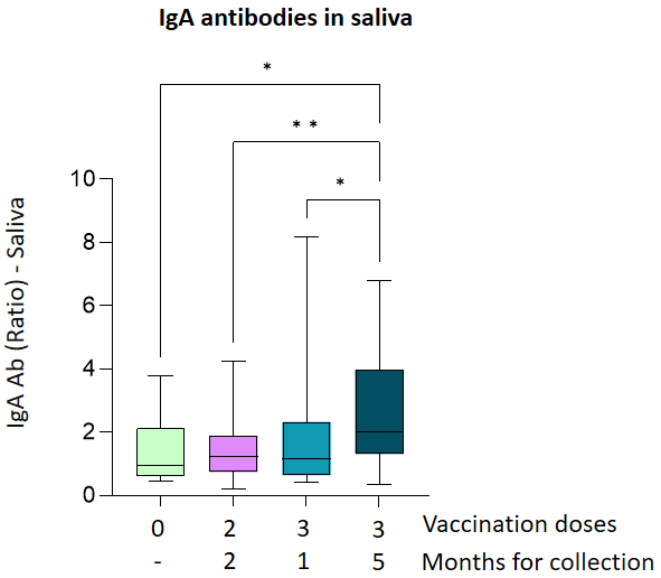

Figure 5. Median of IgA antibodies detected in saliva by ELISA method (Ratio). Statistical analysis: Kruskal-Wallis test (*p<0.05). p = 0.0013. Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies; IgA - Immunoglobulin A.

Table 7. Median and mean concentration of IgA and IgG antibodies in saliva using ELISA method.

		Groups				
	Variables	**Negative Control (n=13)**	**Vaccinated with 2 doses (n=34)**	**Vaccinated with 3 doses after 1 month (n=35)**	**Vaccinated with 3 doses after 5 months (n=35)**	**p-value**
IgA saliva (ratio)	**Median (Min-Max)**	0.96 (0.44-3.78)	1.23 (0.20-4.25)	1.16 (0.40-8.16)	2.00 (0.33-6.79)	<0.0013*
	Mean (± SD) 95% CI	1.39 (±1.15) 0.70-2.09	1.46 (±1.01) 1.11-1.81	1.71 (±1.65) 1.15-2.28	2.69 (±1.72) 2.10-3.28	
IgG saliva (ratio)	**Median (Min-Max)**	0.31 (0.19-0.43)	0.32 (0.19-1.40)	3.08 (0.88-9.11)	1.20 (0.53-3.86)	<0.0001*
	Mean (± SD) 95% CI	0.31 (±0.07) 0.27-0.35	0.38 (±0.21) 0.30-0.45	3.08 (±1.63) 2.52-3.64	1.44 (±0.76) 1.18-1.70	

Days between vaccines last dose and saliva donation	**Median (Min-Max)**	NA	74.00 (36-133)	36 (25-68)	163 (152-198)	
	Mean (± SD)	NA	74.51 (±26.5)	38.54 (±9.99)	165.51 (±11.51)	0.0001*
	95% CI		65.41-83.62	35.11-41.97	161.60-169.50	

Abbreviations: CI – Confidence Interval; IgA - immunoglobulin A; IgG - imunoglobulin G; Max – Maximum; Min – Minimum; NA – Not Available; SD – Standard deviation. Analytical statistics: Kruskal-Wallis test (*p<0.05). Vaccinated with 2 doses: two doses of CoronaVac (Sinovac/Butantan). Vaccinated with 3 doses: two doses of CoronaVac (Sinovac/Butantan) + one booster dose of BNT162b2 (Pfizer-BioNTech).

Correlation salivary IgA and NAb in serum and saliva

No significant correlation was found between IgA antibodies in saliva and neutralizing antibodies in saliva in any time of colletion (Table 3).

IgG detection in saliva samples

IgG antibodies were not detected in saliva of negative control group. After two doses of CoronaVacm, 1/34 individuals were positive for IgG; one month after the booster dose, 33/35 were positive for IgG and 20/35 individuals were positive for IgG five monoths after the booster dose (Table 5). The group non-vaccinated and the group vaccinated with two doses had similar medians (ratio=0.31 and ratio=0.32, respectively), this value increases for the group vaccinated with 3 doses after one month (ratio= 3.08) and decreases after 5 months (ratio= 1.20), although it remains higher than the values of the two initial groups (Table 7, Figure 6).

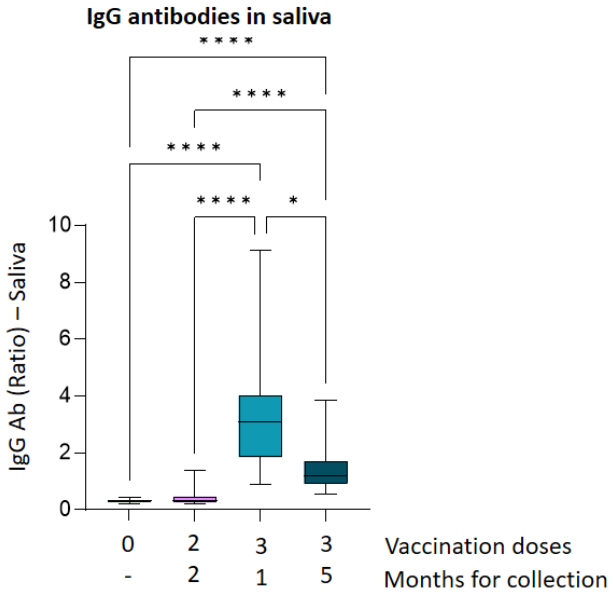

Figure 6. Median of IgG antibodies detected in saliva by ELISA method (Ratio). Statistical analysis: Kruskal-Wallis test (*p<0.05), p = <0.0001. Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies; IgG - Immunoglobin G.

Correlation IgG in saliva and NAb in saliva

A significant correlation was found between IgG antibodies in saliva and neutralizing antibodies in saliva one month after the third dose (r= 0.3631, p= 0.0321), five months after the third dose (r= 0.5380, p= 0.0009) and a moderate correlation was observed for IgG antibodies in saliva and neutralizing antibodies in saliva when all the samples were compared for all time of colletion (r= 0.7203, p= <0.0001) (Figure 7). All the correlations are presented in Table 3. After the third dose, all correlations were significant for IgG in saliva versus NAb in saliva.

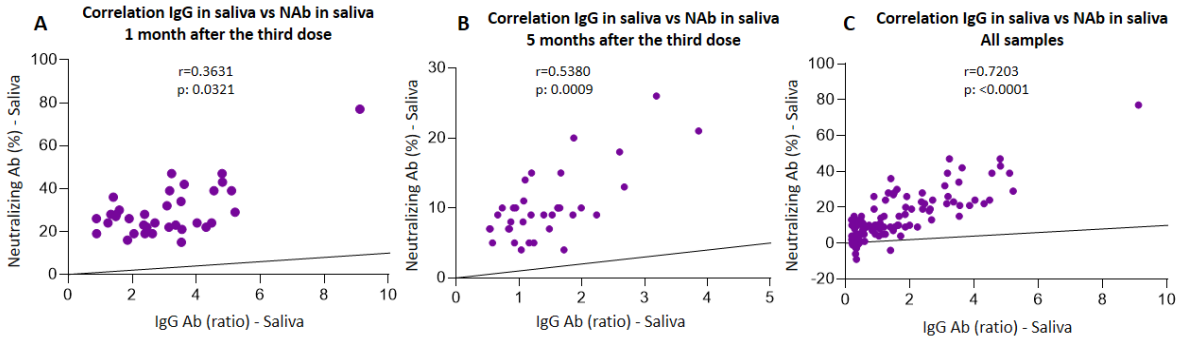

Figure 7. Significant correlations between IgG in saliva versus NAb in saliva. (A) Correlation IgG versus Nab in saliva one month after the third dose (r= 0.3631, p= 0.0321). (B) Correlation IgG versus Nab in saliva five months after the third dose (r= 0.5380, p=0.0009). (C) Correlation IgG versus Nab in saliva for all the samples (n=117 pairs) (r= 0.7203, p= <0.0001). Statistical analysis: For non-parametric data Spearman's correlation. Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies; IgG - immunoglobin G; NAb - neutralizing antibodies; r- correlation coefficient; vs - versus.

Correlation IgG in saliva and NAb in serum

A significant but weak correlation was found after two doses of CoronaVac vaccine (r= 0.3838, p= 0.0250) (Figure 8A) and a significant strong correlation was observed when all the samples are analyzed (r= 0.8076, p <0.0001) (Figure 8B, Table 3).

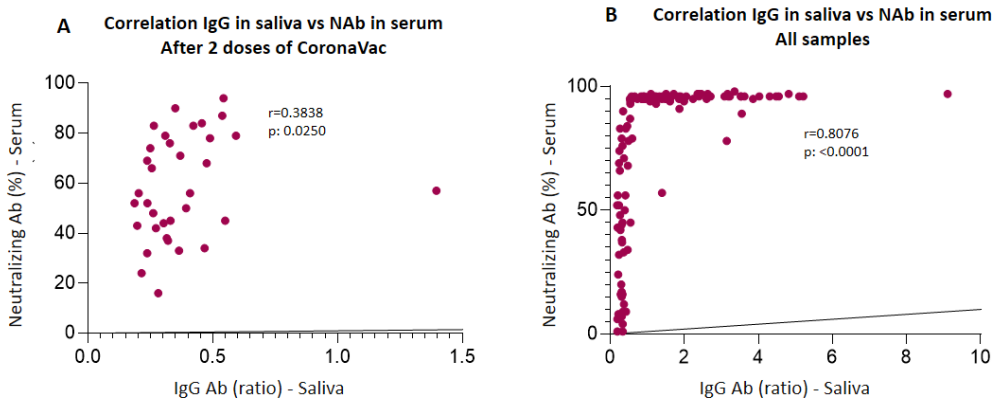

Figure 8. Significant correlations between IgG in saliva and NAb in serum. (A) Correlation IgG in saliva vs Nab in serum after 2 doses of CoronaVac vaccine. (B) Correlation IgG in saliva vs NAb in serum for all the samples (n=117 pairs). Statistical analysis: For non-parametric data Spearman's correlation. Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies; IgG - Immunoglobulin G; NAb - neutralizing antibodies;

Comparation between group infected by SARS-CoV-2 and not infected by SARS-CoV-2

Since this is a longitudinal study, some participants vaccinated were infected by SARS-CoV-2 during the follow-up. In total, 9/35 participants were infected by SARS-CoV-2 one month after the third dose. Then 13/35 were infected five months after the third dose. A comparison was used between the values of antibodies to check if there was a statistical difference between individuals infected and not infected by SARS-CoV-2 (Figure 9).

One month after the third dose, the values of neutralizing antibodies were similar for infected and not infected participants with no statistical difference for serum (infected: 96.22% ±0.44 versus not infected: 95.38 ±3.87, p= 0.885) or saliva (infected: 31.55% ±9.57 versus not infected: 28.81% ±12.78, p= 0.234). The same was observed after five months, in serum (infected: 95% ±0.82 versus not infected: 95.04% ±1.36, p= 0.727) and saliva (infected: 9.92% ±3.80 versus not infected: 10.32% ±5.66, p= 0.678) (Table 8).

The same analysis were performed for IgA and IgG antibodies in saliva comparing infected and not infected, however no statistical different were found to IgA or IgG titer in saliva (Table 8).

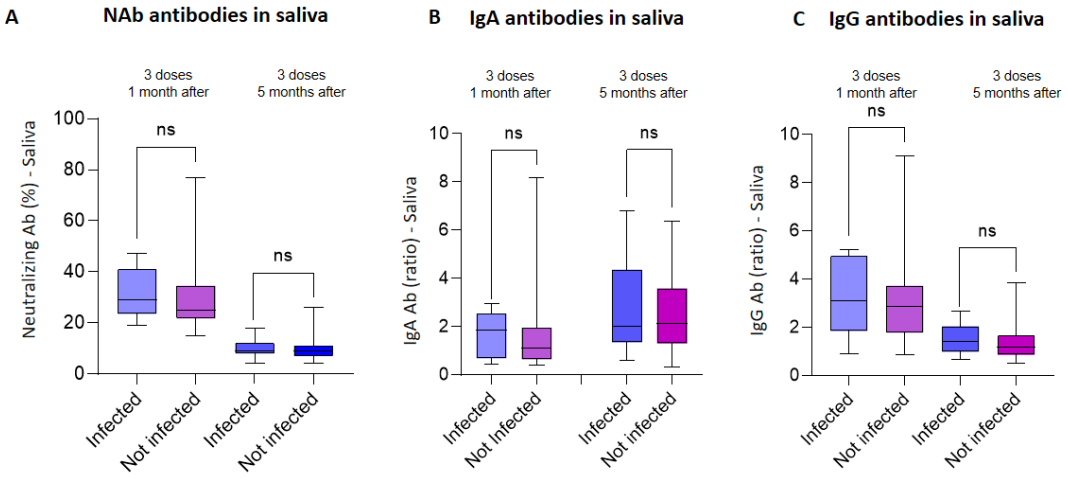

Figure 9. Comparation between salivary antibodies of infected and not infected participants. (A) Comparation between salivary antibodies of infected and not infected participantes for NAb concentration in saliva. (B) Comparation between salivar antibodies of infected and not infected participants for IgA concentration in saliva. (C) Comparation between infectted and not infected participants for IgG concentration in saliva. Statistical analysis: Mann-Whitney test for non-parametric data (*p<0.05). Graphpad Prism, version 9.5.0 (California, USA). Abreviations: Ab - antibodies; IgA - immunoglobulin A; IgG - immunoglobulin G; NAb - neutralizing antibodies; NS – Not significant.

Table 8. Comparation between group infected by SARS-CoV-2 and not infected by SARS-CoV-2.

	Variables	Vaccinated with 3 doses after 1 month (n=35)			Vaccinated with 3 doses after 5 months (n=35)		
		Infected by SARS-CoV-2 (n=9)	Not infected by SARS-CoV-2 (n=26)	p-value	Infected by SARS-CoV-2 (n=13)	Not infected by SARS-CoV-2 (n=22)	p-value
NAb Serum (%)	**Median (Min-Max)**	96 (96-97)	96 (78-98)		95 (93-96)	95 (91-97)	
	Mean (± SD)	96.22 (±0.44)	95.38 (±3.87)	0.885	95 (±0.82)	95.04 (±1.36)	0.678
	95% CI	95.93-96.51	93.89-96.87		94.56-95.44	96.47-95.61	
NAb Saliva (%)	**Median (Min-Max)**	29 (19-47)	25 (15-77)		9 (4-18)	9 (4-26)	
	Mean (± SD)	31.55 (±9.57)	28.81 (±12.78)	0.234	9.92 (±3.80)	10.32 (±5.66)	0.728
	95% CI	25.3-37.8	23.9-33.72		7.86-11.98	7.96-12.68	
IgA Saliva (Ratio)	**Median (Min-Max)**	1.86 (0.46-2.97)	1.10 (0.40-8.16)		2.00 (0.59-6.79)	2.13 (0.33-6.37)	
	Mean (± SD)	1.68 (±0.97)	1.72 (±1.85)	0.540	2.76 (±1.89)	2.65 (±1.65)	0.855
	95% CI	0.93-2.42	0.98-2.47		1.62-3.90	1.92-3.38	
IgG Saliva (Ratio)	**Median (Min-Max)**	3.08 (0.88- 5.21)	2.89 (0.88-9.11)		1.39 (0.66-2.68)	1.18 (0.53-3.86)	
	Mean (± SD)	3.19 (1.59)	3.04 (1.68)	0.697	1.51 (0.67)	1.40 (0.82)	0.468
	95% CI	1.97-4.41	2.36-3.72		1.10-1.91	1.04-1.76	

Abbreviations: CI – Confidence Interval; IgA – Immunoglobulin A; IgG – Immunoglobulin G; Max – Maximum; Min – Minimum; NA – Not Available; NAb – neutralizing antibodies; SD – Standard deviation. Analytical statistics: Kruskal-Wallis test (*p<0.05). Vaccinated with 3 doses: two doses of CoronaVac (Sinovac/Butantan) + one booster dose of BNT162b2 (Pfizer-BioNTech).

Sensitivity, specificity, and accuracy for salivary tests

The salivary test for detecting neutralizing antibodies using ELISA (n=118) method showed an excellent sensitivity (95%), fair specificity (77%), and excellent

accuracy (93%). ECLIA method for total antibodies had an excellent specificity (100%). however, the sensitivity was reduced to 30% and accuracy dropped to 65% (Appendix II). In view of the results, TAb could not be properly detected in saliva by the ECLIA assay. Nonetheless, it was possible to detect NAb in saliva of vaccinated adults by the ELISA method.

The sensitivity, specificity and accuracy for salivary NAb test using ELISA was performed also for the samples separately by groups, the calculation was made using matched (serum and saliva) samples collected exclusive after the second dose, then only samples collected one month of the third dose and also to the samples collected five months after the third dose. The specificity remains the same (76.9%) for all time points. The group vaccinated with 2 doses of CoronaVac present a great sensitivity (85.7%) and accuracy (83.3%), but lower than the other groups and groups vaccinated with 3 doses, which demonstrates an excellent sensitivity (100%) and accuracy (93.7%) (Appendix II). To calculate specificity, sensitivity and accuracy for salivary IgA and IgG test the samples considered borderline were excluded (Appendix II).

The salivary test for IgA detection had a sensitivity of 72%, specificity of 55.5% and accuracy of 70.5% when all the groups vaccinated were compared to negative control group. When the parameters were analyzed separately for each group, the salivary test for IgA presents a specificity of 55.5% to all the groups. The highest values to sensitivity and accuracy were after five months of the vaccine (sensitivity= 88.2%, accuracy= 81.3%), followed by the values after 2 doses of CoronaVac (sensitivity= 63.3%, accuracy= 61.5%) and the lowest values were one month after the third dose (sensitivity= 62%, accuracy= 60.5%).

The salivary test for IgG detection had a sensitivity of 58.6%, specificity of 100% and accuracy of 63.8% when all the groups vaccinated are compared to negative control group. For IgG detection to each group, the specificity was 100% to all the groups. The highest sensitivity and accuracy were observed one month after the third dose (sensitivity= 100%, accuracy= 100%), followed by five months after the third dose (sensitivity= 80%, accuracy=: 86.8%). The lowests values were after two doses of CoronaVac, because IgG were detected only in one of 34 samples (sensitivity: 2.9%, accuracy: 29.7%).

DISCUSSION

Total antibodies by ECLIA could not be properly detected in saliva after two doses of CoronaVac (Sinovac/Butantan). ECLIA anti-CoV-2 anti-S (Cobas e801, *Roche DiagnósticaBrasilLtda*) measures total antibodies (Iga, IgG, IgM) against SARS-CoV-2 S-glycoprotein validated to serum samples. Previsouly studies detected anti-SARS-CoV-2 salivary antibodies using ECLIA assay, however, the participants of the study were vaccinated with two doses of BNT162b2 (Pfizer-BioNTech), while on our study the participants were vaccinated with two doses of CoronaVac (Sinovac/Butantan) vaccine (Lapić et al., 2021). Working around it, this study had a greater focus on detecting antibodies using ELISA, as it was a better technique to saliva in our samples.

The positive detection of antibodies in saliva are in accordance with what is found in literature (Ketas et al., 2021; Klingler et al., 2021; Pinilla et al., 2021; Tu et al., 2022). In our study, antibodies could be detected even 5 months after the last vaccine dose. Our results are in agreement with the evidence of long-term and constant anti-SARS-CoV-2 antibodies levels for most COVID-19 convalescent and vaccinated individuals not only in plasma but also in saliva. Indeed, anti-SARS-CoV-2 salivary antibodies levels may serve as a proxy for monitoring immune response (Pinilla et al., 2021).

Some studies have proven the proper detection of antibodies in saliva of vaccinated individuals, confirming that salivary tests might be alternative methods to monitor the levels of population immunity against SARS-CoV-2 (Ketas et al., 2021; Pinilla et al., 2021). All these studies have accessed antibodies from mRNA-based vaccines, such as Pfizer-BioNTech and Moderna (Ketas et al., 2021; Klingler et al., 2021; Tu et al., 2022). On the other hand, our study evaluated salivary antibodies in adults vaccinated with the non-mRNA-based vaccine CoronaVac, and also monitored this population after receiving a booster dose from Pfizer vaccine in three longitudinal time points.

Neutralizing antibodies increased one month after booster dose in saliva and serum. Five months after the last vaccination, it remained the same for serum. Howerver, for saliva, the levels decreased even though it still detectable. The same behavior was observerd for IgG antibodies, what may explain the moredate correlation

between IgG and NAb antibodies in saliva. This result is in agreement with Darwich et al., (2022) in which salivary anti-SARS-CoV-2 specific antibodies IgG decreased in saliva three months after BNT162b2 (Pfizer/BioNTech) vaccination.

The IgA levels increased from one month after the booster dose to five months after the booster dose. This result may indicate that IgA antibodies increased with time. However, IgA antibodies was detected in 4/13 negative control samples. A previously study suggest that SIgA responses has an influence of pre-existing immunity since SIgA induction after vaccination was more efficient in patients with a history of COVID-19 (Sano et al., 2022). Ketas et al. (2021) detected IgA antibodies in an individual with no evidence of SARS-CoV-2 infection hypothesizing salivary IgA against the SARS-CoV-2 S-protein reflect virus exposure that did not lead to systemic infection but was sufficient to trigger a mucosal immune response. Ortega et al. (2022) also detected IgA in 38% of the negative control group, suggesting the presence of polyreactive sIgA, which can cross-react with several antigens. Also, Tsukinoki et al. (2021) detected SARS-CoV-2 cross-reactive sIgA antibodies to spike protein in the saliva of 46.7% of donors who were PCR- and IgM-negative for COVID-19 (Mestecky and Russell, 2009; Ortega et al., 2022; Tsukinoki et al., 2021). There are other reports of IgA detected in unvaccinated and infected individuals (Cervia et al., 2021b; Tosif et al., 2020). Thus, over time, individuals may have been exposed to the virus not leading to a systemic infection but sufficient for a mucosa immune response. One reason to explain this false-positive result may be the time of saliva collection. When our collections were perfomed, the pandemic was not under control in Brazil, and the virus was contaminating many people at that time point.

IgA antibodies were not well correlated with neutralizing antibodies in serum or in saliva, while IgG presented a significant moderate correlation with neutralizing antibodies in saliva and a high correlation with neutralizing antibodies in serum when all the samples are analyzed. Besides that, test for IgG salivary antibodies presented an excellent accuracy of 100% one month after the third dose. Thus, these higher correlations and specificity could indicate that salivary anti-SARS-CoV-2 antibodies are exudate from serum and vaccination are inducing higher levels of IgG (Brandtzaeg, 2013; Guerra et al., 2022; Guerrieri et al., 2021).

The low specificity of the IgG salivary test after 2 doses of CoronaVac vaccine may indicate that this test was not appropriate to analyzed antibodies against

CoronaVac. The IgG test uses the protein S1 as antigen, while the NAb test uses the RBD protein as antigen. A similar result was found in a previously study with nasal secretion, CoronaVac did not induced S1-specific IgA and IgG responses with neutralizing activity in the nasal mucosa (Chan et al., 2021). However, Ortega et al. (2022) found different results, indicating that CoronaVac was able to induce IgG antibodies in 77% of the saliva sample analyzed in the study. This divergence of results can perhaps be explained by the time of saliva collection, since the group vaccinated with two doses donated the saliva approximately 74 days after the last dose and the group vaccinated with three doses donated salivary samples around 36 days after the last dose.

There was no higher significant correlation between the levels of antibodies in saliva and serum when they were compared separately for each group, in accordance with reported literature (Azzi et al., 2022; Darwich et al., 2022; Garziano et al., 2022). All theses studies found a weak correlation. These inconsistencies may indicate a not completed information provided by SARS-CoV-2 serological tests on the protective feature of systemic or local immunity (Garziano et al., 2022). Intramuscular doses of vaccines anti-SARS-CoV-2 not confer sterilizing immunity and do not induce sterilizing immunity in the upper airway (Chavda et al., 2021; Hassan et al., 2020). This may explain why the correlation of neutralizing antibodies is not high and suggests more research about vaccines focused on increasing antibodies in the mucosa in order to reduce transmission levels.

Since it was a longitudinal study, 13 of 35 participants who were not previously dignosed with COVID-19, were eventually infected by SARS-CoV-2 during the follow-up. To assess whether this interfered with the results, antibody levels in the saliva and blood of infected and non-infected participants were compared and there was no significant difference between the two groups. A previously study reported that after one single dose of mRNA vaccine, individuals previously diagnosed with COVID-19 responded with high levels of anti-RBD IgG while it was not enough to individuals seronegative (Demonbreun et al., 2021). The literature also reports an earlier and more intense immune response for individuals only infected by the virus; however, antibody titers were significantly higher and more durable against COVID-19 for priming vaccination (Trougakos et al., 2021). Having the disease after two doses of vaccination does not seem to increase or change the antibodies concentration in saliva and serum

when comparing infected vaccinated people and uninfected vaccinated people, perhaps the levels of antibodies produced by vaccination were equal or more effective than the exposure to the virus.

Some methodological limitations of this study should be considered. First, the number of participants was small. Second, the tests for detecting anti-SARS-CoV-2 antibodies available on the market are made for blood to be used as a fluid. In this work, we attempted to adapt the use for saliva. Therefore, one of the difficulties was to define the cut-off values for salivary samples considered positive or negative. Third, tests were developed to evaluate de efficacy of RNA vaccines.

CONCLUSION

After two doses of CoronaVac, NAb could be detected in serum (34/35) and in saliva (30/35), IgA (19/34) also could be detected in saliva, but not IgG (1/34). One month after the bostter dose with Pfizer, NAb could be detected in both serum (35/35) and saliva (35/35). IgA (18/35) and IgG (33/35) could also be detected. Five months after the bostter dose, NAb could be detect in serum (35/35) and in saliva (35/35). IgA (30/35) and IgG (20/35) also were detected. After the bostter dose, NAb remains the same in serum. However, IgG and NAb decreased over time in saliva, IgA increased in dose and time dependent model.

The salivary NAb test presented excellent sensitivity (95%), fair specificity (77%) and excellent accuracy (93%). The salivary IgA test presented good sensitivity (72%), poor specificity (55.5%) and fair accuracy (70.05%). The salivary IgG test presented poor sensitivity (58.6%), excellent specificity (100%) and fair accuracy (63.8%).

Despite the inconsistencies of the data referring to the values of antibodies and correlations among themselves, the heterologous vaccination contributed to increase anti-SARS-CoV-2 antibodies in the Brazilian health context.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ETHICAL APPROVAL STATEMENT

This study is in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki, and get ethical approval from The Ethics Committee of the Faculty of Health

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APPENDIX I. Test indicators**.** Adpted from De Luca Canto G, Pachêco-Pereira C, Aydinoz S, Major PW, Flores-Mir C, Gozal D. Diagnostic capability of biological markers in assessment of obstructive sleep apnea: a systematic review and meta-analysis. J Clin Sleep Med. 2015;11(1):27-36. Published 2015 Jan 15. doi:10.5664/jcsm.4358

	Data analysis			
Test indicators	Excellent	Good	Fair	Poor
Sensitivity	> 80%	70-80%	60-69%	< 60%
Specificity	> 90%	80-90%	70-79%	< 70%
Accuracy	> 90%	80-90%	30-80%	< 30%

APPENDIX II. Table 2x2 used for the calculation of sensitivity, specificity and accuracy.

1. Calculation of sensitivity, specificity and accuracy for salivary antibodies detection using ECLIA after 2 doses of CoronaVac for detection of salivary TAb.

ECLIA for detection of TAb (n=20)		
	Vaccinated (n=10)	Negative Control (n=10)
Positive for TAb	3	0
Negative for TAb	7	10

Parameters for TAb	**ECLIA**
Sensitivity [a / (a + c)]	30%
Specificity [(d / (b + d)]	100%
Accuracy [(a+d) / (a + b + c + d)]	65%

2. Calculation of sensitivity, specificity and accuracy for salivary antibodies detection using ELISA for detection of salivary NAb.

ELISA for detection of salivary NAb	Vaccinated	Negative Control
Positive for NAb	30	3

Vaccinated with 2 doses of CoronaVac (n=48)	Negative for NAb	5	10
Vaccinated with 3 doses 1 month after the third dose (n=48)	Positive for NAb	35	3
	Negative for NAb	0	10
Vaccinated with 3 doses 5 months after the third dose (n=48)	Positive for NAb	35	3
	Negative for NAb	0	10
General (n=118)	Positive for NAb	100	3
	Negative for NAb	5	10

Parameters for NAb	**2 doses**	**3 doses after 1 month**	**3 doses after 5 months**	**General**
Sensitivity [a / (a + c)]	85.7%	100%	100%	95%
Specificity [(d / (b + d)]	76.9%	76.9%	76.9%	76.9%
Accuracy [(a+d) / (a + b + c + d)]	83.3%	93.7%	93.7%	93%

3. Calculation of sensitivity, specificity and accuracy for salivary antibodies detection using ELISA for detection of salivary IgA.

ELISA for detection of salivary IgA		Vaccinated	Negative Control
Vaccinated with 2 doses of CoronaVac (n=39)	Positive for IgA	19	4
	Negative for IgA	11	5
Vaccinated with 3 doses 1 month after the third dose (n= 38)	Positive for IgA	18	4
	Negative for IgA	11	5
Vaccinated with 3 doses 5 months after the third dose (n=43)	Positive for IgA	30	4
	Negative for IgA	4	5
General (n=102)	Positive for IgA	67	4
	Negative for IgA	26	5

Parameters for IgA	**2 doses**	**3 doses after 1 month**	**3 doses after 5 months**	**General**

Sensitivity [a / (a + c)]	63.3%	62%	88.2%	72%
Specificity [(d / (b + d)]	55.5%	55.5%	55.5%	55.5%
Accuracy [(a+d) / (a + b + c + d)]	61.5%	60.5%	81.3%	70.5%

4. Calculation of sensitivity, specificity and accuracy for salivary antibodies detection using ELISA for detection of salivary IgG.

ELISA for detection of salivary IgG		Vaccinated	Negative Control
Vaccinated with 2 doses of CoronaVac (n=47)	Positive for IgG	1	0
	Negative for IgG	33	13
Vaccinated with 3 doses 1 month after the third dose (n= 46)	Positive for IgG	33	0
	Negative for IgG	0	13
Vaccinated with 3 doses 5 months after the third dose (n= 38)	Positive for IgG	20	0
	Negative for IgG	5	13
General (n=105)	Positive for IgG	54	3
	Negative for IgG	38	13

Parameters for IgG	**2 doses**	**3 doses after 1 month**	**3 doses after 5 months**	**General**
Sensitivity [a / (a + c)]	2.9%	100%	80%	58.6%
Specificity [(d / (b + d)]	100%	100%	100%	100%
Accuracy [(a+d) / (a + b + c + d)]	29.7%	100%	86.8%	63.8%

Abbreviations: ECLIA – electrochemiluminescence; ELISA – enzyme-linked immunosorbent assay; IgA - Immunoglobulin A; IgG - Immunoglobulin G; NAb - Neutralizing antibodies; TAb – Total Antibodies.

4. CONSIDERAÇÕES GERAIS E PERSPECTIVAS

Os métodos disponíveis no mercado para avaliação de anticorpos anti-SARS-CoV-2 são aprovados para utilização em amostras sanguíneas. A concentração de IgG salivar total, por exemplo, é influenciada pelo dispositivo de coleta da amostra, técnica de coleta, duração da coleta da amostra e ainda pelo tipo de saliva que está sendo coletada (saliva em repouso ou estimulada, da parótida, sublingual ou fluido crevicular gengival). A IgG salivar é majoritariamente exsudada do sangue, ao contrário da IgA salivar (Brandtzaeg, 2013; Pisanic et al., 2023). O fluido crevicular gengival entra por filtração do epitélio crevicular gengival e é enriquecido com IgG derivado do soro. Portanto, contém concentrações totais de IgG mais altas do que outros tipos de fluido oral (Brandtzaeg, 2013, 2007; Pisanic et al., 2023). Os dispositivos de coleta que visam e estimulam o vazamento de fluido crevicular gengival geralmente resultam em fluido oral com concentrações totais de IgG mais altas em comparação com saliva total ou técnicas de coleta passiva de saliva. Idade, estado de hidratação, saúde gengival e horário de coleta da saliva, podem ser outros fatores que influenciam na concentração de anticorpos salivares (Pisanic et al., 2023). Portanto, ainda é necessária uma padronização do dispositivo de coleta, tipo de saliva, melhor horário para a saliva ser coletada, e ensaios imunológicos validados para uso de saliva como fluído, para que os anticorpos salivares sejam melhor detectados e para que os títulos de anticorpos encontrados em cada trabalho possam ser comparados de maneira ideal.

Após a validação dos testes também para saliva, seriam necessários estudos para determinação do *cut-off* negative e positivo. Um estudo anterior propos avaliar a confiabilidade e acurácia dos testes diagnósticos para saliva e secreções nasais pela análise da curva ROC, a fim de obter um valor de corte negativo adequado. A curva ROC desempenha papel central na avaliação da capacidade diagnóstica dos testes para discriminar o verdadeiro estado do sujeito e encontrar os valores de corte ideais, utilizando como parâmetro um grupo controle negativo e que nunca recebeu vacinação para COVID-19 (Fiorelli et al., 2023). A curva ROC pode ser uma alternativa para determinação do *cut-off* embora sejam necessários mais estudos com número maior de participantes.

Embora anticorpos salivares possam ser encontrados na saliva após a vacinação intramuscular e ser uma alternativa de monitoramento imunológico populacional, a concentração desses anticorpos é mais baixa do que no soro. Além disso, o surgimento contínuo de variantes mutantes do vírus SARS-CoV-2 são motivo de preocupação. Assim, a otimização das vacinas e dos métodos de administração continua sendo fundamental (J. Huang et al., 2022). Sendo assim, propostas para vacinas mucosas se destacam. As vacinas intranasais, por exemplo, apresentam vantagens pois a mucosa nasal costuma ser o local inicial da infecção, além de serem não invasivas e poderem ser autoadministradas (Chavda et al., 2021). Uma vacina oral ou nasal poderia acionar o sistema imunológico na mucosa para inibir o vírus, impedindo ou diminuindo a transmissão posterior por meio de uma forte resposta immune de mucosa (Kar et al., 2022).

Diante do exposto, as perspectivas incluem a validação de testes imunológicos utilizando saliva como fluido para detecção de anticorpos anti-SARS-CoV-2, determinação do *cut-off* negativo e positivo para uso da saliva, padronização dos métodos para coleta de saliva para que a saliva possa ser utilizada de forma ideal. Além disso, o desenvolvimento de uma vacina mucosa poderia aumentar a concentração de anticorpos mucosos, afim de evitar também transmissão, além da própria infecção por SARS-CoV-2.

5. CONCLUSÕES

A saliva é um biofluído alternativo adequado para a detecção de anticorpos anti-SARS-CoV-2 em vacinados e indivíduos previamente infectados. Embora os títulos de anticorpos salivares sejam inferiores aos títulos séricos, a detecção de imunoglobulinas anti-SARS-CoV-2 na saliva é satisfatória. Foram observadas correlações sangue e saliva fracas para IgA, e de moderada a forte para IgG.

No estudo experimental foi observado que após duas doses de CoronaVac, NAb pôde ser detectado no soro (34/35) e na saliva (30/35), IgA (19/34) também pôde ser detectado na saliva, mas não IgG (1/34). Um mês após a dose de reforço com Pfizer, NAb pôde ser detectado tanto no soro (35/35) quanto na saliva (35/35). IgA (18/35) e IgG (33/35) também podem ser detectados na saliva, cinco meses após a dose de

reforço, NAb pôde ser detectado no soro (35/35) e na saliva (35/35). IgA (30/35) e IgG (20/35) também foram detectados. Após a dose de reforço, NAb permanece o mesmo no soro. No entanto, IgG e NAb diminuíram ao longo do tempo na saliva, IgA aumentou no modelo dependente de dose e tempo. O teste de NAb salivar apresentou uma excelente sensibilidade (95%), especificidade justa (77%) e excelente acurácia (93%). O teste de IgA salivar apresentou boa sensibilidade (72%), baixa especificidade (55,5%) e justa acurácia (70,05%). O teste de IgG salivar apresentou baixa sensibilidade (58,6%), excelente especificidade (100%) e acurácia justa (63,8%).

Mais estudos são necessários para validação de um teste imunológico adequado para detecção de anticorpos utilizando a saliva como fluído. Ainda, faz se necessário uma padronização da técnica de coleta de saliva e determinação de um *cut-off* para deteção de anticorpos anti-SARS-CoV-2 na saliva. Porém, apesar das inconsistências dos dados referentes aos valores de anticorpos e correlações entre si, a vacinação heteróloga contribuiu para aumentar os anticorpos anti-SARS-CoV-2 no contexto da pandemia brasileira por COVID-19.

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ANEXOS

ANEXO A – APROVAÇÃO PELO COMITÊ DE ÉTICA EM PESQUISA, CAAE 48224221.2.0000.0030

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Detecção de anticorpos anti-SARS-CoV-2 na saliva
Pesquisador: Vitória Tavares de Castro
Área Temática:
Versão: 3
CAAE: 48224221.2.0000.0030
Instituição Proponente: DEPARTAMENTO DE ODONTOLOGIA DA UNIVERSIDADE DE BRASILIA
Patrocinador Principal: FUNDACAO UNIVERSIDADE DE BRASILIA
Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.975.449

Apresentação do Projeto:
Conforme o documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1771422.pdf" postado em 21/08/2021:
"Resumo:
Introdução: Considerando a importância de fortalecer o momento atual de controle e vacinação contra a COVID-19, traçar o perfil de anticorpos de indivíduos vacinados ou que tiveram a doença torna-se necessário para confirmação e acompanhamento do tempo de imunização populacional. Assim, propostas de novos métodos para detecção de anticorpos, utilizando técnicas menos invasivas, seguras e indolores, estão sob constante interesse. Diversos testes salivares já são utilizados para o diagnóstico de várias doenças, inclusive da COVID-19. Objetivos: Analisar a presença de anticorpos anti-SARS-CoV-2 na saliva em indivíduos diagnosticados com COVID-19 e em vacinados contra COVID-19 pelas vacinas disponíveis no Brasil. Além disso, pretende-se comparar a detecção de anticorpos salivares anti-SARS-Cov-2 por três métodos para eleição do método mais sensível e específico. Materiais e Métodos: As amostras de saliva serão coletadas a partir de doadores voluntários que já tiveram COVID-19 ou que foram vacinados contra a doença, no período de um mês, três meses, seis meses e um ano após a segunda dose. A saliva será coletada, centrifugada e congelada a -80 °C até serem processadas. Como controle positivo, o sangue do mesmo participante também será coletado e analisado para detecção de anticorpos séricos anti-SARS-CoV-2. Os métodos para avaliação dos anticorpos serão o ensaio de

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Conforme o documento 'PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1771422.pdf' postado em 21/08/2021:
"Resumo:
Introdução: Considerando a importância de fortalecer o momento atual de controle e vacinação contra a COVID-19, traçar o perfil de anticorpos de indivíduos vacinados ou que tiveram a doença torna-se necessário para confirmação e acompanhamento do tempo de imunização populacional. Assim, propostas de novos métodos para detecção de anticorpos, utilizando técnicas menos invasivas, seguras e indolores, estão sob constante interesse. Diversos testes salivares já são utilizados para o diagnóstico de várias doenças, inclusive da COVID-19. Objetivos: Analisar a presença de anticorpos anti-SARS-CoV-2 na saliva em indivíduos diagnosticados com COVID-19 e em vacinados contra COVID-19 pelas vacinas disponíveis no Brasil. Além disso, pretende-se comparar a detecção de anticorpos salivares anti-SARS-Cov-2 por três métodos para eleição do método mais sensível e específico. Materiais e Métodos: As amostras de saliva serão coletadas a partir de doadores voluntários que já tiveram COVID-19 ou que foram vacinados contra a doença, no período de um mês, três meses, seis meses e um ano após a segunda dose. A saliva será coletada, centrifugada e congelada a -80 °C até serem processadas. Como controle positivo, o sangue do mesmo participante também será coletado e analisado para detecção de anticorpos séricos anti-SARS-CoV-2. Os métodos para avaliação dos anticorpos serão o ensaio de

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científica tem buscado identificar métodos alternativos ao exame sorológico, que apresentem maior aceitação, fácil aplicabilidade e menor risco, com eficácia similar ou superior aos existentes, para detecção de respostas às vacinas anti-SARS-CoV-2 e controle da COVID-19. Com isso, destaca-se a possibilidade de utilização de testes salivares já que, por mais específicos e sensíveis que sejam os testes sorológicos usando sangue, o uso da saliva permite agregar mais conforto e segurança aos pacientes e aos profissionais da saúde, quando comparado à venopunção para coleta de sangue. (To et al., 2020; Ceron et al., 2020; Randad et al., 2020)A coleta salivar é realizada de forma fácil, pelo próprio paciente, dispensa treinamento e é indolor, o que facilita múltiplas coletas e propicia diminuição de custos (Ceron et al., 2020, Azzi et al., 2020). Sabe-se que a saliva é formada por produtos de secreção das glândulas salivares e contém células descamadas, microrganismos, uma gama variada de proteínas - como imunoglobulinas, mucinas e enzimas - além de metabólitos, hormônios e eletrólitos. Com isso, é propicia a identificação de patógenos e de biomarcadores que podem trazer informações importantes acerca da imunidade adquirida, da presença de doenças e de alterações fisiológicas, já sendo utilizada há décadas como instrumento de avaliação de saúde. (Assad et al., 2020; Randad, et al., 2020; Tvarijonaviciute et al., 2020). Além disso, as evidências disponíveis apontam uma análise positiva da presença de anticorpos anti-SARS-CoV-2 na saliva. Em comparação com a presença de anticorpos no sangue, a saliva mostra-se como uma alternativa viável na obtenção de informações acerca da imunidade adquirida após e durante a infecção e como ferramenta para avaliar a efetividade das vacinas (Isho et al., 2020; Aita et al., 2020; Faustini et al., 2020).Os estudos mostram que a saliva é um rico fluido na avaliação de imunidade para diversas doenças, sobretudo, nas que possuem a boca como principal via de infecção, uma vez que na própria mucosa há produção de anticorpos detectáveis para o vírus. Além disso, anticorpos do sangue podem ser secretados na cavidade oral por meio da saliva (Varadhachary et al., 2020; Ketas et al.,2021, Huang et al., 2021, Isho et al., 2020).Frente à importância de identificar métodos rápidos, seguros e confiáveis para acompanhar tanto as campanhas de controle, quanto as de imunização contra COVID-19, este trabalho objetiva verificar a utilização de saliva como alternativa para a detecção de anticorpos anti-SARS-CoV-2.Problema: A saliva é adequada para detecção de anticorpos (imunoglobulinas) anti-SARS-CoV-2?

Hipótese:

Apoiamos o conceito de que a análise da saliva em pacientes com COVID-19 poderia permitir a detecção de anticorpos produzidos contra o SARS- CoV-2, bem como a avaliação da resposta imune inata inespecífica. Além disso, formulamos a hipótese de que os anticorpos salivares anti-

SARS- CoV-2 poderiam servir como uma alternativa não invasiva aos testes sorológicos para monitoramento da infecção e resposta às vacinas em escala populacional."

"Metodologia Proposta:
Amostras de saliva: Os participantes serão orientados a não utilizarem produtos de higiene oral, não consumir bebida alcoólica, cigarro e/ou alimentos por pelo menos 1 hora antes da coleta de saliva, deverão concordar com o Termo de Consentimento Livre e Esclarecido (TCLE) específico para coleta de sangue e saliva (Anexo A). Serão coletados dados gerais dos participantes e eles serão instruídos a mastigar um swab de algodão (Salivette®, SARSTEDT AG & Co) por 2 minutos. Cada swab contendo saliva será devolvido para um recipiente plástico, embalado e armazenado em isopor com uma camada de gelo reciclável. Após 4 horas, as amostras deverão estar no laboratório para início das análises. As amostras de salivas serão centrifugadas a 3.600g por 5 minutos a 8oC. Após centrifugadas, elas serão transferidas para um eppendorf estéril e congeladas em freezer -80oC até o processamento. Para detecção de anticorpos anti -SARS-CoV-2, as amostras serão descongeladas à temperatura ambiente. Eletroquimioluminescência (ECLIA): A mensuração da quantidade de anticorpos anti-SARS-CoV-2 (IgA, IgG, IgM) encontrados na saliva por ECLIA será realizada em um analisador Roche Cobas 8000 automatizado com um Módulo e801 (Roche Diagnostics) e será dada em U/mL. Serão determinados os limites para o blank e para a detecção dos anticorpos, além da quantificação.Quimioluminescência (CLIA): O metodo sanduiche CLIA e um teste automatizado em que apos o tratamento das amostras, a deteccao de anticorpos anti-SARS-CoV-2 (IgA, IgG e IgM) se dará pelo sinal luminescente medido no luminometro. Ensaio de imunoabsorção enzimática (ELISA): O ELISA é um teste imunoenzimático que permite a detecção de anticorpos específicos, usado no diagnóstico de doenças que induzem produção de imunoglobulinas. Serão utilizados os kits da Euroimmun Anti-SARS-CoV-2 (IgA, IgG e IgM), em que proteínas estruturais do SARS-CoV-2 são empregadas como antígenos. Os métodos citados serão realizados segundo instruções do fabricante e como descritos por Assad et al (2020).Amostras de sangue: As amostras de sangue serão obtidas por venopunção e coletadas em tubos de soro com gel separador, no dia da primeira coleta de saliva. O sangue será centrifugado a 3500 -5000 rpm por 5 minutos e o volume total obtido será separado em 2 eppendorfs e congelado -20°C até análise das amostras. As análises serão realizadas pela técnica de eletroquimioluminescência (EQL), usando o equipamento COBAS® 8000 Roche. Questionário online: Será desenvolvido questionário online pelo aplicativo Google Forms (Google Search, Melon Park, Estados Unidos), que poderá ser respondido por aparelhos conectados à Internet. A

participação será voluntária e os participantes deverão concordar com o TCLE específico para o questionário online (Anexo B) para prosseguir com as respostas. No questionário (Anexo C) serão coletados dados gerais, relação como tabagismo e álcool, bem como estado de saúde geral e uso de medicamentos (presença ou ausência de doenças crônicas). Além disso, serão incluídas perguntas sobre o quadro de COVID-19, sobre a presença e a duração dos sinais e dos sintomas mais comuns apresentados e necessidade do uso de medicamentos durante o tratamento da doença. Haverá questões sobre o estado de higiene oral do paciente antes e durante a infecção viral e também sobre as manifestações orais do paciente enquanto estavam infectados com a COVID-19 (presença e frequência). Ademais, será avaliado o grau de xerostomia relatado antes e durante a infecção por COVID-19, em que o participante deverá responder segundo escala Likert. Por fim, haverá indagações sobre a permanência de alguns dos sinais e dos sintomas apresentados durante a infecção pelo SARS- CoV-2 após um período de 15 dias da resolução da doença, quando aplicável. O questionário foi baseado nos estudos de AbuBakret al., 2021, Thompson et al., 2011, Freni et al., 2020.

Critério de Inclusão:
Os critérios de inclusão serão: Grupo 1- indivíduos imunizados por vacina: serão incluídos indivíduos maiores de 18 anos que tenham sido vacinados pela segunda dose de uma das vacinas administradas no Brasil, que não tenham sido diagnosticados com COVID-19 antes ou após receberem as doses da vacinação e que concordem com TCLE. Grupo 2 - serão incluídos indivíduos maiores de 18 anos que tenham sido diagnosticados com COVID-19 por método PCR em tempo real, que não tenham participado da campanha de vacinação contra a COVID-19, e que concordem com TCLE. Grupo 3 – grupo controle: serão incluídos indivíduos maiores de 18 anos, que não foram vacinados por nenhuma das duas vacinas ministradas no Brasil, que não tiveram histórico de COVID-19, e que concordem com TCLE. Respondentes do questionário online: serão incluídos indivíduos com mais de 18 anos que tenham o diagnóstico de COVID -19 confirmado. Assim, serão incluídos os respondentes com confirmação da doença recente ou ainda aqueles que já se curaram da infecção a priori, mas também obtiveram um diagnóstico. Serão incluídos os indivíduos que assinarem o TCLE, confirmarem o diagnóstico de COVID-19 e responderem ao questionário por completo.

Critério de Exclusão:
Os critérios de exclusão serão: Grupo 1- indivíduos imunizados por vacina: Serão excluídos

indivíduos que sejam diagnosticados com COVID-19 após o início da pesquisa e desistentes da pesquisa. Grupo 2 - indivíduos com histórico de COVID-19: Serão excluídos indivíduos que sejam vacinados após o início da pesquisa, caso ainda estejam no período de doação de sangue e saliva, e desistentes da pesquisa. Grupo 3 – grupo controle: Serão excluídos indivíduos que testarem reagente para os anticorpos Anti-Sars-Cov-2 no exame de sangue e participantes que desistirem da pesquisa. Respondentes do questionário online: Serão excluídos indivíduos que relatarem disfunções olfativas, gustativas e salivares anteriormente à pandemia; indivíduos com diagnóstico baseado em aspectos clínicos e desistentes da pesquisa."

"Metodologia de Análise de Dados:
A análise das características clínicas e sociodemográficas da amostra será feita por meio do teste Exato de Fischer ou Qui-quadrado. Para comparar os grupos de pessoas vacinadas vs. grupo controle e de pessoas que foram diagnosticadas com COVID-19 vs. controle será utilizado teste T de Student para amostras independentes. Para verificar a correlação do pool de anticorpos a nível sérico e salivar será utilizada a Correlação de Pearson. Serão adotados os valores de $P<0,05$ (IC de 95%) e poder de 80% para significância estatística. Os softwares utilizados para as análises serão o Statistical Package for Social Science (IBM SPSS), versão 26 para Mac, e o R .

Desfecho Primário:
Espera-se que o desfecho primário seja abrir novas frentes de pesquisa e validar um novo método de análise de anticorpos para o vírus causador da COVID-19, utilizando a saliva como fluido biológico. Espera-se que os anticorpos salivares anti-SARS-CoV-2 possam servir como uma alternativa não invasiva aos testes sorológicos para o monitoramento da infecção e da imunização por SARS-CoV-2 em toda a população. Ainda, espera-se a possibilidade de um teste eficaz e indolor para a avaliação da eficácia das vacinas contra a COVID-19, comparação entre elas e detecção do tempo de imunização proporcionado.

Tamanho da Amostra no Brasil: 80

Haverá uso de fontes secundárias de dados (prontuários, dados demográficos, etc)?
Sim

Detalhamento:

Ao concordar com o TCLE, o participante receberá um formulário acerca de coleta de dados demográficos, no Laboratório de Histopatologia Oral da Faculdade de Ciências da Saúde. Este formulário deve ser respondido antes da primeira coleta de saliva. Ele contém perguntas sobre idade, sexo, nível de escolaridade, região administrativa onde o participante reside, profissão do participante, nacionalidade, se possui doenças sistêmicas, se faz uso de medicamentos, data de vacinação (caso o participante tenha sido vacinado), data do diagnóstico de COVID-19 (caso o participante tenha sido COVID-19 positivo), data das coletas de saliva e sangue, cor da pele, se é fumante ou se faz uso de bebidas alcoólicas. Será aplicado uma única vez, antes da primeira coleta de saliva. Não serão coletados dados demográficos no Laboratório Sabin. O formulário foi anexado na Plataforma Brasil após as considerações do CEP (Documento: "Formulario_Dados_Demograficos_2.docx"). Os participantes que concordarem em doar saliva e sangue deverão responder o formulário de dados demográficos, mas não obrigatoriamente devem responder o questionário online. Quanto ao questionário online, os dados coletados serão referentes ao próprio corpo de texto do questionário, que foi anexado na Plataforma Brasil (Documento: "Questionario_online_anexo.pdf). Os participantes que concordarem em responder o questionário online não obrigatoriamente deverão participar das coletas de saliva e sangue.

Informe o número de indivíduos abordados pessoalmente, recrutados, ou que sofrerão algum tipo de intervenção neste centro de pesquisa: 80"

Objetivo da Pesquisa:

Conforme o documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1771422.pdf" postado em 21/08/2021:

"Objetivo Primário:

Avaliar a presença de anticorpos salivares anti-SARS-CoV-2 em pacientes que foram diagnosticados com COVID-19 e indivíduos imunizados contra a COVID-19 pelas vacinas disponíveis no Brasil.

Objetivo Secundário:

- Verificar a resposta imunológica dos doadores voluntários à infecção pelo SARS-CoV-2 por meio da detecção de anticorpos salivares anti-SARS- CoV-2 ao longo do tempo;- Verificar a resposta imunológica dos doadores voluntários que foram imunizados contra a COVID-19, pelas vacinas disponíveis no Brasil, por meio da detecção de anticorpos salivares anti-SARS-CoV-2 ao longo do

tempo;- Comparar diferentes métodos para detecção de anticorpos salivares: ensaio de imunoabsorção enzimática (ELISA); o imunoensaio de eletroquimioluminescência (ECLIA); e o ensaio de quimioluminescência (CLIA);- Investigar, por meio de um questionário online, a prevalência das manifestações orais que podem ser encontradas em pacientes com COVID-19."

Avaliação dos Riscos e Benefícios:

Conforme o documento 'PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1771422.pdf' postado em 21/08/2021:

"Riscos:

Destacam-se como possíveis riscos o desconforto local ou ânsia de vômito decorrentes do contato do algodão com a boca. Para minimizar os riscos, o participante receberá orientações previamente ao procedimento para não ingerir alimentos ou líquidos uma hora antes da coleta da saliva. Além disso, a equipe de saúde estará prontamente disponível para auxiliar na coleta, esclarecer dúvidas e/ou prestar assistência caso ocorra qualquer tipo de reação adversa ou intercorrência. Outro possível risco é o desconforto local na coleta de sangue, entretanto, este teste será realizado por profissionais capacitados, evitando dificuldades de punção e a necessidade de repetição do teste.Pode haver constrangimento do voluntário ao responder os questionários feitos em relação aos sinais e sintomas apresentados durante o tempo da doença ou em relação a data de vacinação. Ressaltamos a liberdade do participante para desistir da pesquisa a qualquer momento. Ademais, asseguramos que não haverá identificação dos participantes, com manutenção do mais rigoroso sigilo a partir da omissão total de quaisquer informações que permitam a identificação do voluntário.Existe também o risco baixo de infecção ou reinfecção pela doença COVID-19, pois os voluntários estarão sujeitos ao atendimento presencial e precisarão retirar a máscara durante o momento da coleta de saliva. No entanto, para minimizar esse risco, os profissionais que realizarão a coleta do exame estarão devidamente paramentados com máscara n95, máscara faceshield e todos os cuidados em relação a biossegurança serão rigorosamente seguidos. Os atendimentos serão agendados para evitar filas de espera e aglomerações. Além disso, os participantes da pesquisa serão voluntários já vacinados, pessoas que já contraíram a doença, e participantes que possuam diagnósticos negativos confirmados contra a COVID-19, o que diminui ainda mais o risco de infecção/reinfecção. Ademais, o risco da partição envolve gasto de tempo durante a realização do exame e a resposta do questionário. No entanto, destacamos que o exame será agendado de acordo com a disponibilidade de horários dos participantes com intervalo padrão entre um indivíduo e outro para evitar filas de espera. Além disso, os profissionais estarão

capacitados e treinados para realizar os exames com qualidade no menor tempo possível.

Benefícios:
Como benefícios, os participantes terão acesso aos próprios exames, o que permitirá acompanhar o padrão de imunização durante o tempo, de acordo com a quantidade de anticorpos produzidos contra a COVID-19. E, ao final da pesquisa, almeja-se que consigam saber se a saliva é um fluido adequado para a detecção de anticorpos contra o SARS-CoV-2, podendo contribuir para a validação de um método não invasivo e indolor como alternativa ao exame sorológico."

Comentários e Considerações sobre a Pesquisa:
Trata-se de projeto de Mestrado Acadêmico do Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Ciências da Saúde a ser executado pela mestranda Vitória Tavares de Castro, que é a Pesquisadora Responsável, sob a orientação da Profa. Dra. Eliete Neves da Silva Guerra.

O orçamento, que recebeu aporte financeiro da Fundação Universidade de Brasília através do Edital COPEI-DPI/DEX No 01/2020: SEI 23106.059439/2020-18, prevê gastos no total de R$57.520,00 com pagamento de exames laboratoriais, informática e reagentes de laboratório.

O cronograma prevê o início da coleta de dados junto aos participantes para o período de 01/09/2021 a 06/06/2022.

Considerações sobre os Termos de apresentação obrigatória:
Documentos acrescentados ao processo e analisados para emissão deste parecer:

1. Informações Básicas do Projeto: "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1771422.pdf" postado em 21/08/2021.

2. Carta ao CEP/FS-UnB em resposta às pendências apresentadas: "CARTA_DE_RESPOSTAS_AS_PENDENCIAS_2.docx" postado em 19/08/2021.

3. Instrumento de coleta de pesquisa: "Formulario_Dados_Demograficos_2.docx" postado em 19/08/2021.

4. Modelo de TCLE ATUALIZADO: "TCLE_coleta_saliva_sangue_3.docx" postado em 19/08/2021.

5. Projeto Detalhado ATUALIZADO: "PROJETO_SALIVA_COVID_3.docx" postado em 19/08/2021.

6. Parecer Anterior: "PB_PARECER_CONSUBSTANCIADO_CEP_4904365.pdf" postado em 19/08/2021.

Recomendações:
Não se aplicam.

Conclusões ou Pendências e Lista de Inadequações:
Análise das respostas às pendências apontadas nos Pareceres Consubstanciados No. 4.822.809 e 4.904.365:

1. Solicita-se que o Laboratório Sabin seja adicionado como Instituição Co-participante no formulário online da Plataforma Brasil, com a adição do CNPJ da empresa.
RESPOSTA: O Laboratório Sabin foi adicionado como Instituição Co-partipante no formulário online da Plataforma Brasil, com adição do CNPJ da empresa.
ANÁLISE: PENDÊNCIA ATENDIDA.

2. Solicita-se que seja apresentado o Termo de Concordância da Instituição Co-participante para o Laboratório Sabin, conforme modelo disponível em http://fs.unb.br/documentos-modelos. RESPOSTA: Não foi possível a apresentação do Termo de Concordância da Instituição Co-participante para o Laboratório, pois o diretor do Sabin encontrava-se indisponível durante o mês de julho, foi enviado uma justificativa para falta desse documento, que será anexado posteriormente, o mais breve possível, assim que o diretor da Instituição retornar às suas funções.
ANÁLISE: A pesquisadora enviou o documento e foi anexado na PB. PENDÊNCIA ATENDIDA.

3. Solicita-se esclarecimentos sobre onde e como será feito o recrutamento de participantes para o estudo.
RESPOSTA: Serão recrutados profissionais de saúde, alunos, professores, funcionários, pacientes e prestadores de serviço que freqüentem a Universidade de Brasília e Hospital Universitário, que possuam mais de 18 anos de idade.
RESPOSTA: Serão recrutados profissionais de saúde, alunos, professores, funcionários, pacientes e

prestadores de serviço que freqüentem a Universidade de Brasília e Hospital Universitário, que possuam mais de 18 anos de idade.

Original (página 6, item 5 | POPULAÇÃO A SER ESTUDADA, "PROJETO_SALIVA_COVID_JUN_13_2021.pdf"): A saliva será coletada a partir de adultos (>18 anos) voluntários, conforme conveniência. Dessa forma, as pessoas serão recrutadas para doar saliva nas seguintes situações:

Atualização perante as orientações do CEP (página 6, item 5 | POPULAÇÃO A SER ESTUDADA, "PROJETO_SALIVA_COVID_2.pdf"): A saliva será coletada a partir de adultos (>18 anos) voluntários. Serão recrutados profissionais de saúde, alunos, professores, funcionários, pacientes e prestadores de serviço que freqüentem a Universidade de Brasília e Hospital Universitário, conforme conveniência. Dessa forma, as pessoas serão recrutadas para doar saliva nas seguintes situações"

ANÁLISE: PENDÊNCIA ATENDIDA.

4. Solicita-se que seja apresentada, de forma detalhada, como será a sequência de coleta de dados nos três espaços indicados no projeto de pesquisa: UnB, Laboratório Sabin e questionários online, esclarecendo, também, se todos os participantes participarão dos três momentos de coletas de dados.

RESPOSTA: Ao concordar com o TCLE, o participante receberá um formulário acerca de coleta de dados demográficos, no Laboratório de Histopatologia Oral da Faculdade de Ciências da Saúde. Este formulário deve ser respondido antes da primeira coleta de saliva. Contém perguntas sobre idade, sexo, nível de escolaridade, região administrativa onde o participante reside, profissão do participante, nacionalidade, se possui doenças sistêmicas, se faz uso de medicamentos, data de vacinação, data das coletas de saliva e sangue, cor da pele, se é fumante ou se faz uso de bebidas alcoólicas. Será aplicado uma única vez, antes da primeira coleta de saliva. Não serão coletados dados no Laboratório Sabin. O formulário foi anexado na Plataforma Brasil após as considerações do CEP (Documento: "Formulario_Dados_Demograficos"). Os participantes que concordarem em doar saliva e sangue deverão responder o formulário de dados demográficos, mas não obrigatoriamente devem responder o questionário online. Quanto ao questionário online, presente no documento "Questionario_online_anexo.pdf", os dados coletados serão referentes ao próprio corpo de texto do questionário, que foi anexado na Plataforma Brasil. Os participantes que concordarem em responder o questionário online não obrigatoriamente deverão participar das coletas de saliva e sangue.

Essa resposta foi alterada na Plataforma Brasil.

Original (página 5, Plataforma Brasil, "Haverá uso de fontes secundárias de dados (prontuários, dados demográficos, etc?"): Serão solicitados dados demográficos dos doadores voluntários de saliva, que incluirão: idade, sexo, etnia, data de vacinação (caso tenha sido vacinado), data dos primeiros sintomas de COVID-19 (caso tenha sido infectado pela doença).

Atualização perante considerações do CEP (página 5, Plataforma Brasil, "Haverá uso de fontes secundárias de dados (prontuários, dados demográficos, etc?"): Ao concordar com o TCLE, o participante receberá um questionário acerca de coleta de dados demográficos, no Laboratório de Histopatologia Oral da Faculdade de Ciências da Saúde. Este questionário deve ser respondido antes da primeira coleta de saliva. Ele contém perguntas sobre idade, sexo, nível de escolaridade, região administrativa onde o participante reside, profissão do participante, nacionalidade, se possui doenças sistêmicas, se faz uso de medicamentos, data de vacinação, data das coletas de saliva e sangue, uso de medicamentos, cor da pele, se é fumante ou se faz uso de bebidas alcoólicas. Será aplicado uma única vez, antes da primeira coleta de saliva. Não serão coletados dados no Laboratório Sabin. O questionário foi anexado na Plataforma Brasil após as considerações do CEP (Documento: "Formulario_Dados_Demograficos"). Os participantes que concordarem em doar saliva e sangue deverão responder o questionário de dados demográficos, mas não obrigatoriamente devem responder o questionário online. Quanto ao questionário online, os dados coletados serão referentes ao próprio corpo de texto do questionário, que foi anexado na Plataforma Brasil. Os participantes que concordarem em responder o questionário online não obrigatoriamente deverão participar das coletas de saliva e sangue.

ANÁLISE: No 'PROJETO_SALIVA_COVID_2.docx' postado em 31/07/2021, na página 6, lê-se: "4. LOCAL DE REALIZAÇÃO DA PESQUISA

As coletas de saliva serão realizadas no Laboratório de Histopatologia Oral da Faculdade de Ciências da Saúde. O laboratório dispõe de infraestrutura necessária para desenvolver estudos de biologia celular e molecular relacionados à detecção de anticorpos na saliva dos participantes avaliados. Já a coleta de sangue e a análise laboratorial das amostras, tanto as de sangue quanto as de saliva, serão realizadas no Laboratório SABIN." Solicita-se esclarecimentos sobre onde e como será feita a coleta de sangue. Se houver diferença na sequência da coleta de dados entre os 3 grupos (controle, imunizados e positivos para COVID), essa diferença também deve ser detalhada. Essa modificação deve constar no Projeto Detalhado, na Plataforma Brasil e no modelo de TCLE.

PENDÊNCIA PARCIALMENTE ATENDIDA.

RESPOSTA: O participante voluntário será convidado a doar sua saliva, na Universidade de Brasília,

no laboratório de Histopatologia Oral da Faculdade de Ciências da Saúde. Antes da coleta de saliva, e após concordar com o TCLE, o participante deverá responder um formulário acerca de dados demográficos, esse formulário foi atualizado de forma que os três grupos possam responder o mesmo formulário. O documento foi anexado à Plataforma Brasil, "Formulario_Dados_Demograficos_2.docx". Após o participante responder o formulário de dados demográficos, a saliva será coletada no próprio laboratório de Histopatologia Oral, pela pesquisadora responsável. Após a coleta de saliva, será solicitado que o participante se dirija a qualquer unidade do Laboratório Sabin, assim, o participante poderá escolher a unidade que seja mais conveniente. Na unidade do Laboratório SABIN, o participante deve realizar um exame de sangue, por venopunção, que avaliará os anticorpos anti-SARS-CoV-2 presentes no sangue do participante. Os custos de transporte entre os locais das coletas serão ressarcidos pelo pesquisador, conforme consta do TCLE. Se houver qualquer custo com a realização de exames de sangue, os custos também serão ressarcido pelo pesquisador responsável. Não haverá nenhuma coleta de dados demográficos no laboratório SABIN.

O participante que aceitar ser doador voluntário de saliva e sangue deverá responder o formulário de dados demográficos, mas não obrigatoriamente responderá ao questionário online. Os participantes que concordarem em responder o questionário online não obrigatoriamente deverão participar das coletas de saliva e sangue. Quanto ao questionário online, presente no documento "Questionario_online_anexo.pdf", os dados coletados serão referentes ao próprio corpo de texto do questionário, que foi anexado à Plataforma Brasil.

Portanto:

1. UnB: Serão coletados dados demográficos, referentes ao formulário de dados demográficos (Documento: "Formulario_Dados_Demograficos_2.docx"), antes da primeira coleta de saliva.
2. Laboratório Sabin: Não serão coletados dados demográficos no Laboratório Sabin.
3. Questionário online: Será desenvolvido um questionário online pelo aplicativo Google Forms, no qual as perguntas estão listadas no documento, "Questionario_online_anexo.pdf". Os participantes que responderem a este questionário não necessariamente serão doadores de saliva e sangue.

A atualização do texto foi feita no Projeto Detalhado, no modelo de TCLE e na Plataforma Brasil.

ANÁLISE: PENDÊNCIA ATENDIDA.

5. Solicita-se que no orçamento do projeto esteja contemplado o ressarcimento com gastos para o transporte dos participantes entre os locais de coleta, e que a previsão deste ressarcimento conste no TCLE.

RESPOSTA: A descrição "Transporte dos participantes entre os locais de coleta" foi adicionada ao orçamento do projeto, no item "visitas clínicas", e encontra-se em vermelho no Anexo "PLANILHA_DE_ORCAMENTO_2". E a previsão deste ressarcimento foi adicionada ao TCLE. Original (página 1, parágrafo 3, TCLE, "TCLE_coleta_saliva_sangue_JUN_13_2021): A sua participação se dará por meio de fornecimento de amostra da sua saliva para o estudo dos anticorpos salivares e coleta de sangue para controle de pesquisa. A coleta da saliva será com um algodão específico que será mastigado por 2 minutos e depois armazenado, essa coleta será realizada no laboratório de Histopatologia Oral da Universidade de Brasília. Serão realizadas quatro coletas da saliva, em horários que serão agendados e combinados previamente com o pesquisador. A saliva coletada será armazenada em tubos que serão identificados por números, dessa forma será garantida sua privacidade e sigilo. A coleta de sangue será realizada no laboratório Sabin, as amostras coletadas e seus fracos serão coletadas por números para garantir o sigilo dos pacientes. Além disso, será solicitado ao participante, que responda um questionário acerca dos sinais e sintomas apresentados durante a infecção por Covid-19, caso o mesmo já tenha adquirido a doença. Atualização perante as orientações do CEP (página 1, parágrafo 3, TCLE, "TCLE_coleta_saliva_sangue_2"): A sua participação se dará por meio de fornecimento de amostra da sua saliva para o estudo dos anticorpos salivares e coleta de sangue para controle de pesquisa. A coleta da saliva será com um algodão específico que será mastigado por 2 minutos e depois armazenado, essa coleta será realizada no laboratório de Histopatologia Oral da Universidade de Brasília. Serão realizadas quatro coletas da saliva, em horários que serão agendados e combinados previamente com o pesquisador. A saliva coletada será armazenada em tubos que serão identificados por números, dessa forma será garantida sua privacidade e sigilo. A coleta de sangue será realizada no laboratório Sabin, as amostras coletadas e seus fracos serão coletados por números para garantir o sigilo dos pacientes. Além disso, será solicitado ao participante, que responda um questionário acerca dos sinais e sintomas apresentados durante a infecção por Covid-19, caso o mesmo já tenha adquirido a doença. Caso o(a) senhor(a) tenha qualquer gasto em relação ao transporte entre os locais das coletas de amostras, o valor será ressarcido pelo pesquisador em até 48h via transferência bancária e/ou mediante acordo entre vossa senhoria e o pesquisador.
ANÁLISE: PENDÊNCIA ATENDIDA.

6. Solicita-se que seja esclarecido nos objetivos secundários do projeto, na PB e no Projeto Detalhado, explicações sobre a justificativa e os objetivos dos questionários online.

RESPOSTA: Foram esclarecidos nos objetivos secundários do projeto, na PB e no Projeto Detalhado, explicações sobre a justificativa e objetivos do questionário. O objetivo é investigar por meio de um questionário online, a prevalência das manifestações orais que podem ser encontradas em pacientes com COVID-19, visto que uma das linhas de pesquisa do trabalho é a COVID-19 no contexto da saúde oral.
Original (página 5, item 2.2 | OBJETIVOS SECUNDÁRIOS, "PROJETO_SALIVA_COVID_JUN_13_2021.pdf"):
- Verificar a resposta imunológica dos doadores voluntários à infecção pelo SARS-CoV-2 por meio da detecção de anticorpos salivares anti-SARS-CoV-2 ao longo do tempo; - Verificar a resposta imunológica dos doadores voluntários que foram imunizados contra a COVID-19, pelas vacinas disponíveis no Brasil, por meio da detecção de anticorpos salivares anti-SARS-CoV-2 ao longo do tempo;
- Comparar diferentes métodos para detecção de anticorpos salivares: ensaio de imunoabsorção enzimática (ELISA); o imunoensaio de eletroquimioluminescência (ECLIA); e o ensaio de quimioluminescência (CLIA);
Atualização perante as orientações do CEP (página 5, item 2.2 | OBJETIVOS SECUNDÁRIOS, "PROJETO_SALIVA_COVID_2.pdf"):
- Verificar a resposta imunológica dos doadores voluntários à infecção pelo SARS-CoV-2 por meio da detecção de anticorpos salivares anti-SARS-CoV-2 ao longo do tempo;
- Verificar a resposta imunológica dos doadores voluntários que foram imunizados contra a COVID-19, pelas vacinas disponíveis no Brasil, por meio da detecção de anticorpos salivares anti-SARS-CoV-2 ao longo do tempo;
- Comparar diferentes métodos para detecção de anticorpos salivares: ensaio de imunoabsorção enzimática (ELISA); o imunoensaio de eletroquimioluminescência (ECLIA); e o ensaio de quimioluminescência (CLIA);
- Investigar, por meio de um questionário online, a prevalência das manifestações orais que podem ser encontradas em pacientes com COVID-19.
ANÁLISE: PENDÊNCIA ATENDIDA.

7. Solicita-se que, caso necessário, o cronograma do projeto de pesquisa seja ajustado a fim de permitir tempo hábil para o trâmite no CEP-FS/UnB, que é de pelo menos 7 dias úteis.
RESPOSTA: O cronograma do projeto de pesquisa foi ajustado, com início para agosto de 2021. As datas também foram alteradas na Plataforma Brasil.
ANÁLISE: PENDÊNCIA ATENDIDA.

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

Todas as Pendências foram atendidas. Não foram observados óbices éticos.
Protocolo de pesquisa em conformidade com as Resolução CNS 466/2012, 510/2016 e complementares.

Considerações Finais a critério do CEP:
Conforme a Resolução CNS 466/2012, itens X.1.- 3.b. e XI.2.d, os pesquisadores responsáveis devem apresentar relatórios parciais semestrais, contados a partir da data de aprovação do protocolo de pesquisa; e um relatório final do projeto de pesquisa, após a conclusão da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1771422.pdf	21/08/2021 17:11:20		Aceito
Outros	CARTA_DE_RESPOSTAS_AS_PENDENCIAS_2.docx	19/08/2021 20:19:02	Vitória Tavares de Castro	Aceito
Outros	Formulario_Dados_Demograficos_2.docx	19/08/2021 20:17:53	Vitória Tavares de Castro	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_coleta_saliva_sangue_3.docx	19/08/2021 20:16:21	Vitória Tavares de Castro	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_SALIVA_COVID_3.docx	19/08/2021 20:14:26	Vitória Tavares de Castro	Aceito
Parecer Anterior	PB_PARECER_CONSUBSTANCIADO_CEP_4904365.pdf	19/08/2021 20:13:40	Vitória Tavares de Castro	Aceito
Outros	Termo_instituicao_coparticipante.pdf	06/08/2021 09:43:45	Fabio Viegas Caixeta	Aceito
Orçamento	PLANILHA_DE_ORCAMENTO_2.pdf	31/07/2021 19:10:44	Vitória Tavares de Castro	Aceito
Orçamento	PLANILHA_DE_ORCAMENTO_2.docx	31/07/2021 19:10:24	Vitória Tavares de Castro	Aceito
Cronograma	Cronograma_2.docx	31/07/2021 19:07:52	Vitória Tavares de Castro	Aceito
Outros	Carta_de_resposta_as_pendencias.pdf	31/07/2021 18:51:59	Vitória Tavares de Castro	Aceito
Cronograma	Cronograma_2.pdf	31/07/2021 18:49:31	Vitória Tavares de Castro	Aceito
Parecer Anterior	PB_PARECER_CONSUBSTANCIADO_	31/07/2021	Vitória Tavares de	Aceito

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

Continuação do Parecer: 4.975.449

Parecer Anterior	P_4822809.pdf	18:49:04	Castro	Aceito
Outros	Lattes_Vitoria_Tavares_de_Castro.pdf	13/06/2021 20:53:53	Vitória Tavares de Castro	Aceito
Outros	Lattes_Larissa_Di_Carvalho_Melo_e_Silva.pdf	13/06/2021 20:53:40	Vitória Tavares de Castro	Aceito
Outros	Lattes_Juliana_Amorim_dos_Santos.pdf	13/06/2021 20:53:25	Vitória Tavares de Castro	Aceito
Outros	Lattes_Gustavo_Barcelos_Barra.pdf	13/06/2021 20:53:09	Vitória Tavares de Castro	Aceito
Outros	Lattes_Eliete_Neves_da_Silva_Guerra.pdf	13/06/2021 20:52:52	Vitória Tavares de Castro	Aceito
Outros	Lattes_Bruna_Bastos_Silveira_da_Silva.pdf	13/06/2021 20:52:29	Vitória Tavares de Castro	Aceito
Outros	Lattes_Ana_Carolina_Acevedo.pdf	13/06/2021 20:52:12	Vitória Tavares de Castro	Aceito
Outros	Termo_de_Responsabilidade_e_Compromisso.docx	13/06/2021 20:51:29	Vitória Tavares de Castro	Aceito
Outros	Carta_Encaminhamento.docx	13/06/2021 20:50:57	Vitória Tavares de Castro	Aceito
Declaração de Instituição e Infraestrutura	Termo_Instituicao_Proponente.docx	13/06/2021 20:50:31	Vitória Tavares de Castro	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_questionario_manifestacoes_orais_JUN_13_2021.pdf	13/06/2021 20:45:40	Vitória Tavares de Castro	Aceito
Outros	Questionario_online_anexo.pdf	13/06/2021 20:44:21	Vitória Tavares de Castro	Aceito
Declaração de Instituição e Infraestrutura	Termo_Instituicao_Proponente.pdf	10/06/2021 22:16:21	Vitória Tavares de Castro	Aceito
Outros	Termo_de_responsabilidade.pdf	10/06/2021 22:15:04	Vitória Tavares de Castro	Aceito
Outros	Carta_Encaminhamento.pdf	10/06/2021 22:13:05	Vitória Tavares de Castro	Aceito
Folha de Rosto	Folha_de_Rosto.pdf	10/06/2021 22:08:40	Vitória Tavares de Castro	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Endereço: Faculdade de Ciências da Saúde, Universidade de Brasília - Campus Darcy Ribeiro
Bairro: Asa Norte **CEP:** 70.910-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-1947 **E-mail:** cepfsunb@gmail.com

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

BRASILIA, 15 de Setembro de 2021

Assinado por:
Fabio Viegas Caixeta
(Coordenador(a))

Endereço: Faculdade de Ciências da Saúde, Universidade de Brasília - Campus Darcy Ribeiro
Bairro: Asa Norte **CEP:** 70.910-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-1947 **E-mail:** cepfsunb@gmail.com

APÊNDICES

APÊNDICE A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - TCLE

Universidade de Brasília

Pós-Graduação em Ciências da Saúde

Termo de Consentimento Livre e Esclarecido - TCLE

Convidamos o(a) Senhor(a) a participar voluntariamente do projeto de pesquisa "**Detecção de anticorpos anti-SARS-CoV-2 na saliva**", sob a responsabilidade da pesquisadora Vitória Tavares de Castro. Trata-se de um estudo transversal realizado na Faculdade de Ciências da Saúde da Universidade de Brasília, e tem como objetivo principal avaliar a presença de anticorpos anti-SARS-CoV-2 na saliva de indivíduos diagnosticados com COVID-19 e em vacinados pelas vacinas disponíveis no Brasil.

O(a) senhor(a) receberá todos os esclarecimentos necessários antes e no decorrer da pesquisa e lhe asseguramos que seu nome não aparecerá sendo mantido o mais rigoroso sigilo pela omissão total de quaisquer informações que permitam identificá-lo(a).

A sua participação se dará por meio de fornecimento de amostra da sua saliva para o estudo dos anticorpos salivares e coleta de sangue para controle de pesquisa. A coleta da saliva será com um algodão específico que será mastigado por 2 minutos e depois armazenado, essa coleta será realizada no laboratório de Histopatologia Oral da Universidade de Brasília. Serão realizadas quatro coletas da saliva, em horários que serão agendados e combinados previamente com o pesquisador. A saliva coletada será armazenada em tubos que serão identificados por números, dessa forma será garantida sua privacidade e sigilo. A coleta de sangue será realizada no laboratório Sabin, as amostras coletadas e seus fracos serão coletados por números para garantir o sigilo dos pacientes. Caso o(a) senhor(a) tenha qualquer gasto em relação ao transporte entre os locais das coletas de amostras, ou na realização do exame de sangue, o valor será ressarcido pelo pesquisador em até 48h via transferência bancária e/ou mediante acordo entre vossa senhoria e o pesquisador. Também será solicitado que o participante, antes da primeira coleta de saliva, responda a um formulário acerca de dados demográficos, que contém perguntas sobre idade, sexo, nível de escolaridade, região administrativa onde o participante reside, profissão do participante, nacionalidade, se possui doenças sistêmicas, se faz uso de medicamentos, data de vacinação (caso o participante tenha sido vacinado), data e método do diagnóstico de COVID-19 (caso o participante tenha sido COVID positivo), data das coletas de saliva e sangue, cor da pele, se é fumante ou se faz uso de bebidas alcoólicas.

Todos os cuidados necessários para diminuir os riscos dos participantes serão observados no presente estudo, garantindo, dessa forma, a manutenção de sua dignidade e privacidade, como a realização de procedimentos não invasivos que são mais confortáveis, sendo que os benefícios possíveis do estudo explicam os benefícios de fazer os testes.

Os riscos de participar da pesquisa envolvem desconforto local ou ânsia de vômito decorrentes do contato do algodão com a boca e para minimizar os riscos, o senhor(a) será orientado(a) a não ingerir alimentos ou líquidos uma hora antes da coleta da saliva. Caso ocorra qualquer tipo de reação, a equipe médica do hospital sempre dará assistência, para que esses riscos sejam evitados ou

diminuídos. Se você aceitar participar da pesquisa, contribuirá para a avaliação de novas formas de diagnóstico e controle da Covid-19.

Outro risco é desconforto local na coleta de sangue, para minimizar esse risco, essa coleta será realizada uma única vez, por um profissional adequadamente treinado. Existe também o risco de constrangimento ao responder a perguntas do questionário relacionadas aos sintomas apresentados durante a infecção por Covid-19, ressaltamos que a identidade de cada participante será preservada e que ele pode desistir a qualquer momento de participar da pesquisa. E por fim existe o risco baixo de infecção ou reinfecção por Covid-19, pois será realizado atendimento presencial para realização das coletas de sangue e saliva, ressaltamos que os profissionais farão uso de Equipamentos de Proteção Individual, todos os cuidados serão tomados em relação à biossegurança, e os procedimentos serão agendados para evitar qualquer tipo de aglomeração.

O(a) Senhor(a) pode se recusar a responder (ou participar de qualquer procedimento) qualquer questão que lhe traga constrangimento, podendo desistir de participar da pesquisa em qualquer momento sem nenhum prejuízo para o(a) senhor(a). Sua participação é voluntária, isto é, não há pagamento por sua colaboração.

Caso o(a) senhor(a) tenha gastos diretamente relacionados à pesquisa, como alimentação, transporte e exames, estas despesas serão pagas pelo pesquisador responsável. Se for necessária a presença de acompanhante, as despesas dele relacionadas à pesquisa também serão pagas pelo pesquisador.

Caso haja algum dano direto ou indireto decorrente de sua participação na pesquisa, o(a) senhor(a) deverá buscar ser indenizado, obedecendo-se as disposições legais vigentes no Brasil.

Os resultados da pesquisa serão divulgados na Universidade de Brasília podendo ser publicados posteriormente. Os dados e materiais serão utilizados somente para esta pesquisa e ficarão sob a guarda do pesquisador por um período de cinco anos, após isso serão destruídos.

Se o(a) Senhor(a) tiver qualquer dúvida em relação à pesquisa, por favor telefone para: Vitória Tavares de Castro,no telefone (61) 99927-5825, disponível inclusive para ligação a cobrar ou entre em contato por e-mail (vitoriatavarescastro@gmail.com).

Este projeto foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Ciências da Saúde (CEP/FS) da Universidade de Brasília. O CEP é composto por profissionais de diferentes áreas cuja função é defender os interesses dos participantes da pesquisa em sua integridade e dignidade e contribuir no desenvolvimento da pesquisa dentro de padrões éticos. As dúvidas com relação à assinatura do TCLE ou os direitos do participante da pesquisa podem ser esclarecidas pelo telefone (61) 3107-1947 ou do e-mail cepfs@unb.br ou cepfsunb@gmail.com, horário de atendimento de 10:00hs às 12:00hs e de 13:30hs às 15:30hs, de segunda a sexta-feira. O CEP/FS se localiza na Faculdade de Ciências da Saúde, Campus Universitário Darcy Ribeiro, Universidade de Brasília, Asa Norte.

Caso concorde em participar, pedimos que assine este documento que foi elaborado em duas vias, uma ficará com o pesquisador responsável e a outra com o(a) Senhor(a).

_____

Nome e assinatura do Participante de Pesquisa

_____

Nome e assinatura do Pesquisador Responsável

Brasília, ___ de _____de _____.