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DESENVOLVIMENTO DE BIOSSENSORES ELETROQUÍMICOS PARA DETECÇÃO PRECOCE E TRATAMENTO OPORTUNO DE LEISHMANIOSE VISCERAL HUMANA EM PACIENTES ASSINTOMÁTICOS: NOVAS FRONTEIRAS NO COMBATE À ENFERMIDADE

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BEATRIZ RODRIGUES MARTINS

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DEDICATÓRIA

Minha jornada até aqui não seria possível sem mencionar os pilares que me sustentame me guiam. Meus pais, alicerces inabaláveis, sempre me ofereceram apoio e sabedoria, especialmente nos momentos em que a bússola interna parecia falhar. Minha mãe, Ex-diretora de Escola em Uberlândia, agora aposentada, me inspirou com sua paixão pelo trabalho e seu apurado senso crítico. Meu pai, apesar de não ter graduação formal, sempre foi um exemplode força de vontade e determinação, representando as raízes firmes que nutrem nossa família. Minha irmã, um furacão de energia e convicção, que, mesmo com suas intensidades, me ensinou a perseguir meus sonhos com total convicção. Meu namorado, Guilherme, ou simplesmente Gui, dedicado e resiliente, certo do que quer e acredita, é um exemplo de que estou no caminho certo e de que devo todos os dias me levantar para lutar pelo que acredito. E eu não poderia deixar de citar aqui, minha Maiazinha, um dos meus melhores presentes, uma gatinha sem raça definida, com características de uma "frajolinha", que recebeu o nome de Maia devido à minha profunda admiração pela cultura Maia, ela representa um voltar seguro e cheio de ternura para casa, mesmo nos dias mais difíceis.

Dedico à minha tese, primeiramente, à essas figuras extraordinárias que moldaram meu caráter, meus valores e meu senso de responsabilidade. Elas me ensinaram a ser resiliente, a buscar o conhecimento incessantemente e a lutar pelos meus objetivos com garrae determinação. São a fonte da minha força interior, a inspiração que me impulsiona a voar cada vez mais alto. Sou eternamente grata a cada um deles por me proporcionarem um lar cheio de amor, apoio e ensinamentos valiosos. Eles são meus maiores tesouros, a força motriz que me impulsiona a conquistar meus sonhos e a contribuir para um mundo melhor. Minha jornada na faculdade foi marcada por uma busca incessante pelo sentido aristotélico de ética, eu precisava encontrar um propósito que me trouxesse benefícios também, eu precisava trilhar por um caminho que me fizesse vibrar e me conduzisse ao equilíbrio. Atravessei diversos campos de pesquisa, inclusive laboratórios de Fisiologia e Bioquímica, mas o fascínio pelos experimentos não se concretizava. Sentia-me como um navio à deriva em um mar de incertezas. Em meio a essa busca, quase no fim da minha graduação, o destino me presenteou com a Professora Dra. Renata Pereira Alves, minha orientadora. Confesso que, no início, suas palavras me soavam como um idioma estrangeiro, e meu interesse era nulo. Mas a persistência da professora, aliada à minha própria vontade de

encontrar um norte, me impulsionou a mergulhar de cabeça em seu universo. Foi como entrar em uma nova dimensão, onde a beleza dos sensores eletroquímicos se revelava diante dos meus olhos. A cada dia que passava, a paixão pela pesquisa se intensificava, e a crença no propósito se tornava inabalável. O equilíbrio que tanto buscava finalmente foi encontrado, e trilhei o caminho dos biossensores eletroquímicos durante o final da minha graduação, por todo o meu mestrado e doutorado e pretendo segui-lo durante o meu pós-doutorado e por toda a minha caminhada de vida queainda virá.

Para mim, a pesquisa com sensores eletroquímicos se tornou mais do que um trabalho, mas sim uma missão com potencial para impactar a vida das pessoas, com o poder transformador da pesquisa científica consigo trilhar meu caminho dentro das minhas possibilidades e vislumbrar o poder transformador e prático da ciência em uma sociedade. Acredito, acima de tudo que, somos um mosaico composto por diversas peças, cada uma representando um momento, uma escolha, uma influência. Nossos trejeitos, refletem de alguma forma, nossa família, orientadores, colegas de trabalho, amigos, das batalhas que travamos e das vitórias que conquistamos. E assim, nessa jornada, encontramos exemplos que inspiram e nos guiam, iluminando o caminho que queremos percorrer. Por isso, dedico aqui, essa tese à todas as pessoas especiais e aos aprendizados que tive nesta minha jornada.

A pesquisa científica não é um mero jogo ou um atalho para títulos. É uma jornada árdua, permeada por desafios e frustrações, mas também por recompensas imensuráveis. Para nós, pesquisadores, a pesquisa é a chave para desvendar o potencial do conhecimento e transformá-lo em algo tangível, capaz de mudar o mundo. No meu caso, a pesquisa é um compromisso com a vida, um chamado para aliviar o sofrimento daqueles que mais precisam, como os pacientes nos corredores dos hospitais públicos, muitas vezes privados de recursos e atenção adequada. O ambiente de pesquisa, por vezes hostil e desafiador, nãome intimida. Pelo contrário, me impulsiona a superar obstáculos e buscar novas soluções.

Mais do que muitos, compreendo a realidade dos pacientes e da família dos pacientes. Já vivenciei em carne própria a agonia da espera, a angústia do diagnóstico e o medo do desconhecido. Sei o que significa sentir todas as emoções humanas em um único instante, seio que é ver a família sofrer junto, em um mar de incertezas. Em 2019, um acidente me fez revisitar minhas escolhas e reafirmar meus propósitos com ainda mais convicção. Em 2021, a perda em meu ciclo familiar, decorrente do SARS-Cov-2,

me agoniou. No seu leito tio, eu disse que te dedicaria um artigo científico, mais do que isso, dedico a minha tese, a nossa família, por me incentivar a continuar, perseverar e inspirar outros. Hoje, sei que a pesquisa se tornou mais do que uma paixão, mas sim uma missão de vida, por isso dedico a todos os pesquisadores e aos futuros, que ainda trilharão essa jornada, espero conseguir um dia transmitir um pouco do que me transmitiram, e assim, contribuir para uma sociedade melhor.

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unida, onde o amor e o respeito prevalecem, e onde cada um tem aliberdade de ser quem realmente é. Agradeço a Deus por essa benção, por me ter presenteado com essa família tão especial.

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“Educação não transforma o mundo. Educação muda as pessoas. Pessoas transformam o mundo.”

Paulo Freire

RESUMO

Este trabalho, descreve a importância dos biossensores eletroquímicos para o diagnóstico de doenças. O tema central da tese de doutorado apresentada, baseia-se na descrição de uma metodologia diagnóstica que se refere ao desenvolvimento de um biossensor eletroquímico para a detecção de pacientes sintomáticos e assintomáticos de leishmaniose (LSH), que suprime a reação cruzada com a doença de Chagas (DC). Trata-se de uma doença parasitária transmitida por mosquitos infectados. Seus sintomas, muitas vezes confundidos com outras enfermidades, dificultam o diagnóstico preciso e atrasam o início do tratamento adequado. Desta forma, o desenvolvimento de um biossensor eletroquímico que detecte adequadamente até mesmo os pacientes assintomáticos, e mantenha as características prevalentes nessa técnica, como excelente custo benefício, rápida execução e livre de interferências, faz-se necessário. Assim, eletrodos *screen-printed* de grafite modificados com óxido de grafeno e foram selecionados como plataformas para a imobilização de antígenos totais brutos de *Leishmania infantum*. As propriedades eletroquímicas foram avaliadas por voltametria cíclica (0,1 $V.s^{-1}$). O par *redox* ferri/ferrocianeto de potássio, possibilitaram a análise dos picos de corrente anódicas e catódicas. As análises comparativas com Enzyme-Linked Immunosorbent Assay (ELISA) indireto foram realizadas com os mesmos grupos de pacientes, em condições semelhantes às desenvolvidas no biossensor eletroquímico. Quanto a curva de calibração, nossos resultados apresentam um limite de detecção (LOD) em 5.58 $mg.mL^{-1}$. Portanto, este sensor pode ser apresentado como um dispositivo promissor para um diagnóstico eficiente e eficaz dos anticorpos de Leishmaniose, presentes nos soros de pacientes sintomáticos e possivelmente assintomáticos.

Palavras-chaves: Grafeno. Imunossensor. Eletroquímica. Leishmaniose. Assintomáticos. Sintomáticos.

ABSTRACT

This work describes the importance of electrochemical biosensors for the diagnosis of diseases. The central theme of the doctoral thesis presented is based on the description of a diagnostic methodology that refers to the development of an electrochemical biosensor for the detection of symptomatic and asymptomatic patients with leishmaniasis (LSH), which suppresses the cross-reaction with the disease leishmaniasis. Chagas (DC). It is a parasitic disease transmitted by infected mosquitoes. Its symptoms, often confused with other diseases, make accurate diagnosis difficult and delay the initiation of appropriate treatment. Therefore, it is necessary to develop an electrochemical biosensor that adequately detects even asymptomatic patients, and that maintains the predominant characteristics of this technique, such as excellent cost-benefit, fast execution and free from interference. Therefore, screen-printed graphite electrodes modified with graphene oxide were selected as platforms for the immobilization of raw *Leishmania infantum* total antigens. The electrochemical properties were evaluated by cyclic voltammetry (0.1 $V.s^{-1}$). The ferri/potassium ferrocyanide redox couple made it possible to analyze the anodic and cathodic current peaks. Comparative analyzes with Indirect Enzyme Immunosorbent Assay (ELISA) were carried out with the same groups of patients, under conditions similar to those developed in the electrochemical biosensor. As for the calibration curve, our results present a limit of detection (LOD) of 5.58 $mg.mL^{-1}$. Therefore, this sensor may present itself as a promising device for an efficient and effective diagnosis of antibodies against Leishmaniasis, present in sera from symptomatic and possibly asymptomatic patients.

Key words: Graphene. Immunosensor. Electrochemistry. Leishmaniasis. Asymptomatic. Symptomatic.

LISTA DE ILUSTRAÇÕES

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LISTA DE ABREVIATURAS E SIGLAS

Ac – Anticorpo

Ag – Antígeno

Au – Ouro

BSA – Albumina sérica bovina

CDC – Centros de prevenção e controle de doenças dos Estados Unidos

ELISA – *Enzyme-linked immunosorbent assay*

$K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$. $3H_2O$ – Ferri/Ferrocianeto de potássio

KCl – Cloreto de potássio

LOD – Limite de detecção

LV – Leishmaniose visceral

LVH – Leishmaniose visceral humana

OMS – Organização mundial da saúde

OPAS – Organização pan-americana da saúde

RIDT - Reação de imunodifusão dupla

VC – Voltametria cíclica

Screen-printed – Eletrodo impresso

SUMÁRIO

1. REVISÃO DE LITERATURA

Este capítulo apresenta uma abrangente revisão da literatura sobre leishmaniose e biossensores eletroquímicos, com foco nas aplicações no diagnóstico da doença. A leishmaniose é uma parasitose transmitida por flebotomíneos, com diversas formas clínicas e alta prevalência em regiões tropicais e subtropicais. O diagnóstico preciso e precoce da doença é crucial para o sucesso do tratamento e controle da leishmaniose, especialmente em pacientes assintomáticos e em áreas endêmicas para a doença de Chagas, onde o diagnóstico diferencial é um desafio.

2. INTRODUÇÃO

A eletroquímica, como um campo científico, teve seu início marcado por diversas descobertas e experimentos inovadores de químicos, físicos e cientistas naturais que moldaram a nossa compreensão da relação entre eletricidade e reações químicas. As observações e os experimentos realizados por William Gilbert, Benjamin Franklin, Luigi Galvani, Michael Faraday, Alessandro Volta, William Nicholson, Anthony Carlisle, Svante Arrhenius, John Frederic Daniell, entre outros estudiosos, marcaram as descobertas dos princípios fundamentais para o desenvolvimento da eletroquímica (ALVAREZ, 2007; BOULABIAR *et al*., 2004; BRESADOLA, 1998; BURNS *et al*., 1993; COTTI, 1995; FARA, 2009; FERREIRA, 1978; GUEDES, 1999; KNIGHT, 2000; PICCOLINO, 2006, 1997; ROSS, 2002).

Deste modo, as observações de contração muscular em resposta à estímulos de eletricidade, fizeram com que Galvani pudesse observar a eletricidade animal (CAJAVILCA *et al*., 2009; PICCOLINO, 1998). Enquanto isso, Volta, começava a propor o desenvolvimento da pilha voltaica, trabalho esse que geraria grande inovação nos aspectos que envolviam a geração de corrente elétrica por meio de reações químicas (PICCOLINO, 2000; SCROSATI, 2003). Assim, a eletroquímica começava a se apresentar como área da ciência capaz de investigar as relações existentes entre os fenômenos elétricos e as reações químicas.

Os estudos do século XIX, apresentariam Michael Faraday como um dos grandes nomes relacionados à essa ciência, de modo que os seus experimentos fundamentais, dentre eles, as leis da eletrólise, estabeleceram base teórica para a continuação de estudos relacionados à área

(ROWE, 2001). No final do mesmo século, o estudioso Svante Arrhenius introduziria o termo íon, o que acarretaria em benefícios para os estudos de reações eletroquímicas (PEREIRA *et al*., 2021).

Já no século XX, Jaroslav Heyrovsky; William Grove e outros cientistas, conseguiram aprimorar os estudos eletroquímicos, e estabelecer o desenvolvimento de técnicas analíticas relevantes para a área, como à exemplo, a voltametria cíclica, avanços das células a combustível, entre outras (PETROVIC, 2021). A partir desse momento, a eletroquímica, ganhou relevância mundial com os estudos de pesquisadores empenhados em resolver os problemas da área, como, Ludwig Eduard Boltzmann, Max Born, Leland Charles Clark, Charles Augustin de Coulomb, Carl Friedrich Gauss, Stephen Gray, Ronald Wilfrid Gurney, Joseph Henry, William Thomson, Baron Kelvin of Largs (Kelvin), Nevill Francis Mott, John Randles, Ernst Werner von Siemens, Nikola Tesla, William Whewell, Ludwig Ferdinand Wilhelmy, William Hyde Wollaston, entre outros (FLETCHER, 2008; GULABOSKI; MIRCESKI, 2024).

Desse modo, a "enciclopédia" científica desenvolvida por esses pesquisadores, foi responsável por estabelecer o início dos avanços que culminariam nas aplicações visualizadas das mais diversas áreas que envolvem a eletroquímica, como o aprimoramento e desenvolvimento de baterias recarregáveis, sensores eletroquímicos e desenvolvimento de dispositivos médicos para a detecção de anomalias biológicas (BREITKOPF; SWIDER-LYONS, 2017; LUBERT; KALCHER, 2010).

Os avanços significativos alcançados no desenvolvimento e fabricação de eletrodos permitiu o desenvolvimento de métodos relacionados às medições que se tornaram mais precisas e confiáveis (SHUKLA; KUMAR, 2008). Neste período, a criação e inserção dos eletrodos de vidro, inventado por Max Cremer, e eletrodos de disco de platina, contribuíram de maneira significativa para a evolução dos sensores eletroquímicos (KORYTA, 1990). Com esses avanços, em 1930, houve o desenvolvimento dos primeiros sensores de pH baseados em medições eletroquímicas, a partir dos eletrodos de vidro (SZABADVÁRY; BELCHER; GORDON, 2016). Em 1940, as técnicas voltamétricas, em especial a polarografia desenvolvida por J. Heyrovsky, começaram a dar indícios dos novos avanços conquistados pela eletroquímica, a detecção e quantificação de espécies eletroativas em solução começa ser possível (HEYROVSKÝ, 1960).

A partir dos anos 1960, os pesquisadores começaram a incorporar a leitura de substâncias biológicas como incumbência dos sensores eletroquímicos, neste sentido, o

caminho dos biossensores eletroquímicos estavam começando e se integrando a área eletroquímica (GUERRA, 2018). O grande marco desse período foi a descoberta da enzima glucose oxidase, bem como a sua aplicação como molécula alvo para detecção de anomalias biológicas. Deste modo, ambas as ciências foram integradas e a aplicação dessa biomolécula nos sensores eletroquímicos deu origem aos sensores de glicose, sendo um marco importante desse campo de pesquisa (CLARK; CLARK, 1973). Doravante, os avanços nos biossensores eletroquímicos se tornaram mais evidentes.

Em suma, a evolução dos biossensores eletroquímicos remonta a uma ciência diagnóstica, que apesar das várias décadas de estudos e aprimoramentos, é relativamente nova quando comparada com outras metodologias diagnósticas (KOHLES *et al*., 2011; ZHANG *et al*., 2020). Desde as primeiras tentativas de desenvolvimento de sensores eletroquímicos para detecção de compostos químicos, até a integração de componentes biológicos, a vasta aplicação dos biossensores eletroquímicos têm tornado esses dispositivos cada vez mais relevantes, em especial no âmbito dos diagnósticos biológicos (WANG *et al*., 2023). Assim, a capacidade desses dispositivos de detectar espécimes biológicas e químicas com alta sensibilidade e seletividade, mesmo quando diante de amostras complexas, têm ampliado os interesses de pesquisa voltados para o desenvolvimento dessa área no que tange a monitoramento e diagnóstico de doenças complexas, controle de qualidade alimentar, vigilância ambiental, controle de qualidade da produção industrial, dentre outros.

Os últimos anos são marcados por tentativas de integrar os sensores eletroquímicos às novas tecnologias. Assim, a otimização, a possibilidade de torná-los vestíveis e miniaturização desses dispositivos tem se tornado possível e uma crescente, uma vez que, essas pesquisas são responsáveis por tornar esses métodos aplicáveis no vasto campo de atuação que estes podem ser inseridos (GU *et al*., 2021; XU *et al*., 2021; ZENG *et al*., 2022; ZHANG *et al*., 2020). As microtecnologias e nanotecnologias tem contribuído com algumas características importantes desta técnica, assim, torna-se possível a fabricação de sensores portáteis, baratos e com alta sensibilidade e especificidade para as diversas aplicações possíveis (DE LA ESCOSURA-MUÑIZ; PAROLO; MERKOI, 2010; FAUSTINO *et al*., 2022).

Tendo em vista a relevância da eletroquímica em um mundo cada vez mais complexo e interconectado, a busca por soluções inovadoras para os desafios da sociedade moderna se torna cada vez mais urgente. Nesse contexto, o trabalho desenvolvido aborda os sensores eletroquímicos como ferramentas protagonistas e indispensáveis para a detecção de LVH. A

partir de princípios eletroquímicos, os sensores desenvolvidos transformam a presença de um analito em um sinal elétrico mensurável, permitindo a quantificação precisa e confiável de substâncias nocivas à saúde humana.

3. LEISHMANIOSE HUMANA

As leishmanioses constituem um conjunto de doenças parasitárias complexas, caracterizadas por um amplo espectro de manifestações clínicas, desde formas cutâneas até viscerais potencialmente fatais (VAN GRIENSVEN; DIRO, 2019). Os protozoários do gênero *Leishmania*, são transmitidos pela fêmea do flebotomíneo, popularmente conhecido como tatuquira, asa branca, birigui, mosquito-palha ou cangalhinha. Esses mosquitos, pertencentes à ordem *Diptera*, família *Psychodidae* e subfamília *Phlebotominae* e, apresentam alta prevalência em regiões tropicais e subtropicais, onde as condições climáticas favorecem sua proliferação (CAMARGO; LANGONI, 2006; PIMENTA; FREITAS; SECUNDINO, 2012; TAVARES *et al*., 2022).

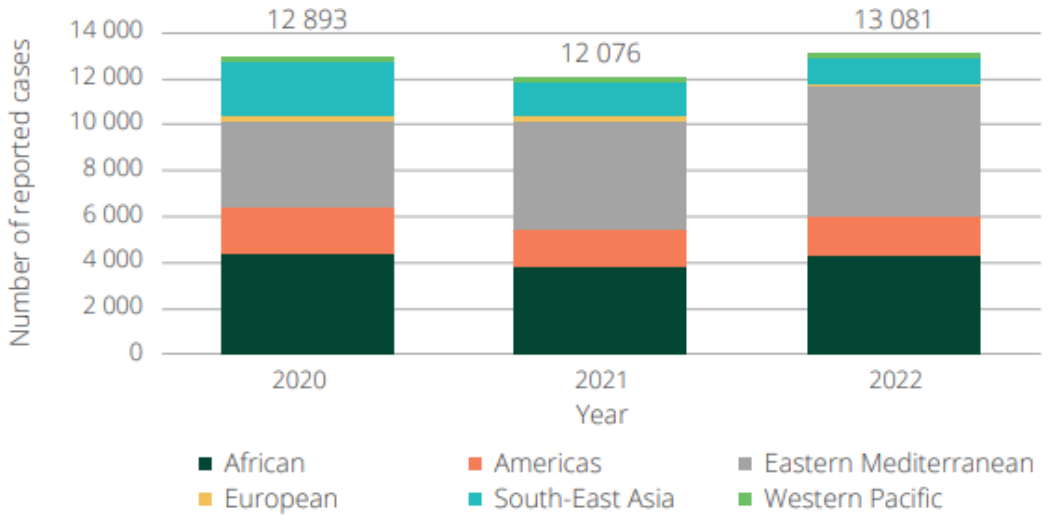
O pioneirismo de descoberta da Leishmaniose decorreu da observação do parasita do gênero *Leishmania* por Cunningham (1885) e William Boog Leishman (1903) na Índia, em casos de Leishmaniose Visceral (LV) (STEVERDING, 2017; VINCENT, 2017). Atualmente, segundo dados recentes da Organização pan-americana da saúde (OPAS), a LV concentra sua prevalência em quatro países, Índia, Sudão, Brasil e Quênia, respondendo por cerca de 70% dos casos globais (RUIZ-POSTIGO *et al*., 2022). A LV afeta o sistema imunológico e órgãos internos, podendo levar à morte se não for diagnosticada e tratada adequadamente. Dessa maneira, métodos eficientes de combate devem incluir medidas de prevenção, como controle do vetor e educação em saúde, além de acesso universal a diagnóstico e tratamento adequados.

A organização mundial da saúde (OMS) desempenha um papel crucial na identificação e classificação de doenças negligenciadas, doenças que afetam desproporcionalmente populações marginalizadas e recebem atenção e recursos limitados em pesquisa, desenvolvimento e controle (CAMARGO, 2008; MOREL, 2006; VASCONCELOS *et al*., 2016). Apesar de representarem um problema de saúde pública significativo, as leishmanioses são classificadas como doenças tropicais negligenciadas, recebendo atenção limitada de grandes empresas farmacêuticas e órgãos de financiamento. Esse cenário preocupante se deve, em grande parte, ao perfil socioeconômico da população mais afetada, majoritariamente, indivíduos com baixo poder aquisitivo, residentes em áreas com infraestrutura precária e acesso limitado a serviços de saúde (VALERO; PRIST; URIARTE,

2021).

A negligência no investimento em pesquisa e desenvolvimento de ferramentas diagnósticas e terapêuticas para as Leishmanioses representa um enorme desafio para o controle e a erradicação dessas doenças (ARAUJO-JORGE *et al*., 2014). No entanto, iniciativas recentes, como o Plano Global de Controle das Leishmanioses 2020-2030, demonstram um compromisso crescente em abordar essa problemática (BAMOROVAT *et al*., 2024; RUIZ-POSTIGO *et al*., 2022).

Esse conjunto de doenças parasitárias complexas, apresentam distribuição global, com endemicidade em regiões tropicais e subtropicais de aproximadamente 98 países (MICHEL *et al*., 2011). A prevalência da LVH se concentra em áreas da África, América Latina e Ásia (Figura 1), com estimativas que indicam cerca de 12 milhões de casos em todo o mundo. Anualmente, observa-se a ocorrência de 1,5 a 2 milhões de novos casos, evidenciando a persistência e o impacto significativo dessa doença na saúde pública global (LESSA *et al*., 2007) (WORLD HEALTH ORGANIZATION, 2024).



Fonte: OMS, 2024.
Figura 1: Prevalência global de Leishmaniose Visceral de acordo com a Organização Mundial da Saúde.

Essas enfermidades não se limitam a um problema de saúde, mas também representam um desafio socioeconômico significativo. Em virtude de afetar desproporcionalmente populações de baixa renda, residentes em áreas com infraestrutura precária e acesso limitado a serviços de saúde, por vezes, essa patologia é associada à incapacidade física e à disfunção social, impactando negativamente na qualidade de vida dos indivíduos e das comunidades afetadas (CARVALHO FILHO *et al*., 2022).

O combate à LV se configura como um desafio crucial para a saúde pública, necessitando de uma abordagem multifacetada que englobe a prevenção, o diagnóstico precoce, o tratamento adequado e o controle do vetor transmissor (GRAEFF-TEIXEIRA, 2013).

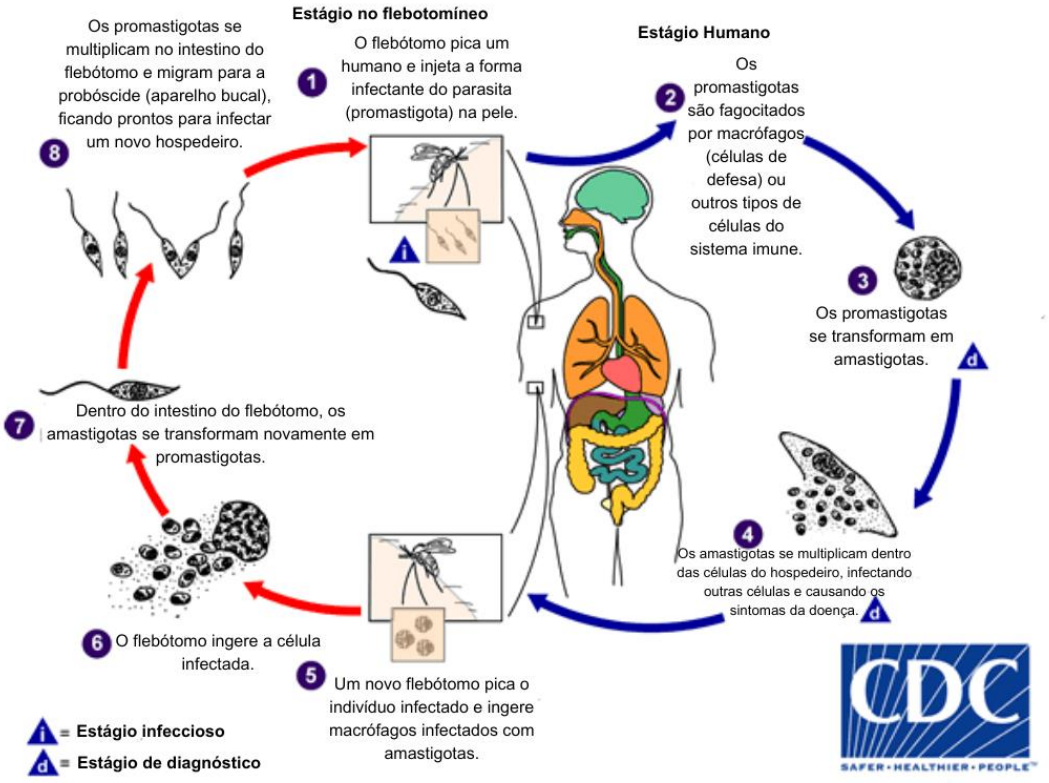
Diante disso, o investimento em pesquisa e desenvolvimento, a capacitação de profissionais de saúde, a educação em saúde e a colaboração intersetorial são elementos essenciais para alcançar o controle efetivo dessa doença devastadora e promover a saúde pública global.

3.1 Ciclo de vida de *Leishmania* spp.

A leishmaniose é uma doença parasitária complexa e multifacetada. Apresenta como principal via de transmissão a picada de fêmeas infectadas de mosquitos flebotomíneos (Figura 2), responsáveis pela propagação da doença, atuando como vetores biológicos do parasita *Leishmania* (SILVA; STEBUT, 2021).

Em dados publicados pelos centros de prevenção e controle de doenças dos Estados Unidos (CDC), o ciclo de vida de *Leishmania*, protozoário parasita causador da leishmaniose, inicia-se com um mosquito flebotomíneo fêmea infectado, que durante o seu período de alimentação, transmite a saliva infectada com promastigotas metacíclicos, a forma infectante do parasito, para um indivíduo. Assim, os promastigotas metacíclicos migram da saliva do mosquito para a corrente sanguínea e linfática do indivíduo, e em seguida, invadem os macrófagos, células do sistema imunológico presentes na pele, tecidos e órgãos internos.

Dentro dos macrófagos, os promastigotas se transformam em amastigotas, a forma replicativa da *Leishmania*, e se multiplicam intensamente dentro dos macrófagos, invadindo novas células e disseminando a infecção pelos tecidos do indivíduo, local onde os amastigotas se transformam em promastigotas metacíclicos. Quando outro mosquito flebotomíneo fêmea se alimenta de um indivíduo infectado com Leishmaniose ela ingere os promastigotas metacíclicos. No intestino do mosquito flebotomíneo, os promastigotas metacíclicos se transformam em amastigotas e se multiplicam. Esses amastigotas migram para as glândulas salivares do mosquito, prontos para serem inoculados em um novo hospedeiro durante a próxima picada, reiniciando o ciclo de vida da *Leishmania*.



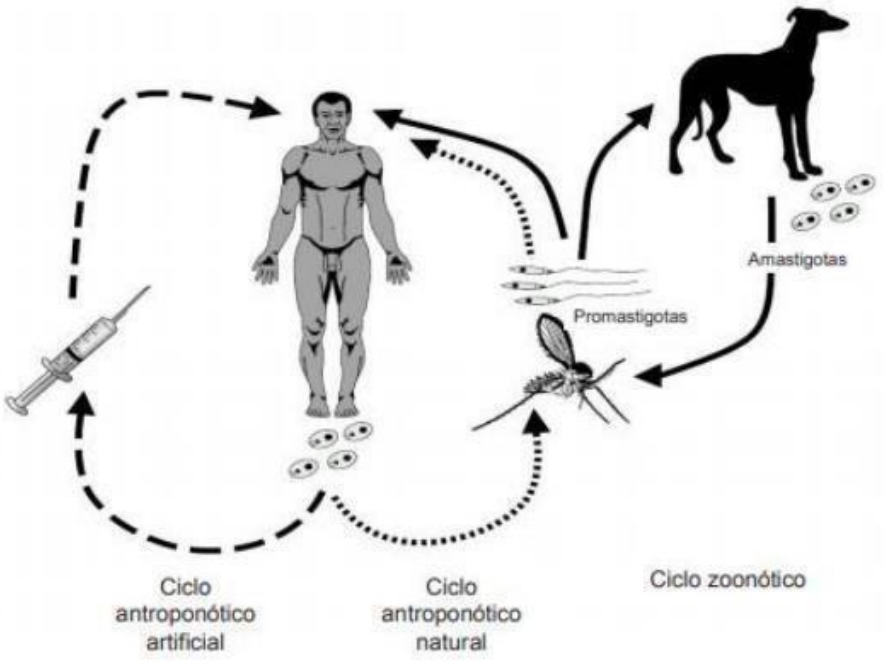
Fonte: CDC, 2017 (traduzida).
Figura 2: Representação esquemática do ciclo de infecção antroponótico natural de casos de *Leishmania* spp. Onde são simbolizados os estágios de infecção e de diagnóstico da doença.

A depender da espécie de *Leishmania* e da resposta imunológica do indivíduo, a infecção pode apresentar diferentes manifestações clínicas (SOUSA *et al*., 2021). A LV, a forma mais grave da doença, afeta órgãos internos como fígado, baçoe medula óssea. Já a leishmaniose cutânea se manifesta por lesões na pele, enquanto a leishmaniose mucocutânea causa lesões na pele, mucosa nasal e boca. Em alguns casos, a infecção pode persistir por meses ou anos, mesmo após o desaparecimento dos sintomas. Essa persistência, conhecida como infecção latente, pode levar à reativação da doença no futuro. Em indivíduos com leishmaniose cutânea ou mucocutânea, os amastigotas podem infectar outros tipos de células, como células dendríticas. Essas células migram para a pele, onde os amastigotas se transformam em promastigotas metacíclicos, a forma infectante para o mosquito flebotomíneo (NETO *et al*., 2021).

Além do ciclo antroponótico natural, acima citado, a Leishmaniose ainda pode ser transmitida por meio do ciclo antroponótico artificial e do ciclo zoonótico (Figura 3) (MARQUES *et al*., 2007). No ciclo antroponótico artificial, o parasito, na forma de amastigotas, encontra um novo meio de transporte, principalmente realizado por objetos perfurantes ou cortantes contaminados com sangue ou secreções de indivíduos infectados.

Esse mecanismo de transmissão torna-se ainda mais alarmante, em zonas endêmicas para a doença, para a qual a transfusão de sangue pode representar uma via de propagação a partir de indivíduos oligossintomáticos e/ou assintomáticos que representam cerca de 85% dos reais casos, ressaltando assim, a necessidade de implementação de diagnósticos mais eficientes para essas localidades (BURZA; CROFT; BOELAERT, 2019; MICHEL *et al*., 2011).

Apesar de raro, esse ciclo de transmissão direto, que acontece sem a participação do mosquito flebotomíneo, por meio do contato direto com lesões cutâneas de indivíduos infectados, da placenta de mãe para filho ou por transfusão de sangue contaminado é uma realidade observada por pesquisadores (AMATO NETO; BLANCO FILHO, 1981; EVANGELISTA *et al*., 2022; FIGUEIRÓ FILHO *et al*., 2005; URIAS *et al*., 2009). Já no ciclo zoonótico,o mosquito flebotomíneo se alimenta de um animal infectado, como um cão ou roedor, e adquire os amastigotas, e, ao picar um humano, o mosquito transmite os promastigotas, iniciando a infecção (MARQUES *et al*., 2007). Assim, a compreensão aprofundada desses ciclos, aliada a medidas de prevenção e controle adequadas, é essencial para minimizar o impacto da Leishmaniose na saúde pública e promover o bem-estar das populações afetadas.



Fonte: Marques et. al., 2007.
Figura 3: Representação esquemática dos diferentes ciclos de infecção de *Leishmania* spp.

3.2 Diagnósticos de leishmaniose

O diagnóstico da leishmaniose, envolve a combinação de diferentes metodologias que possibilitem o aumento da precisão e o direcionamento correto do tratamento. Nesse sentido, comumente, o diagnóstico é baseado em avaliação clínica, associada à testes parasitológicos, sorológicos ou moleculares (ANVERSA *et al*., 2018). Os indivíduos portadores de *Leishmania* podem apresentar sintomas clínicos clássicos, podem ser oligossintomáticos e podem ser assintomáticos, o que justifica a necessidade da incorporação de outros testes diagnósticos (BURZA; CROFT; BOELAERT, 2019).

Decorrido o tempo de incubação da infecção, indivíduos infectados, independentemente da faixa etária, podem começar a apresentar como sintomas clínicos febre recorrente, palidez cutâneo-mucosa, diarreia, tosse, presença de feridas, aumento do volume abdominal, e parâmetros laboratoriais alterados, indicando anemia, leucopenia, trombocitopenia, elevação dos níveis de ureia, creatinina e bilirrubinas. Esses sintomas, que por vezes podem ser considerados inespecíficos, retardam o início do tratamento (RUIZ-POSTIGO *et al*., 2022; ZIJLSTRA *et al*., 1991).

Constatada a possibilidade diagnóstica de leishmaniose, outros métodos diagnósticos podem ser associados à clínica (GUERRA *et al*., 2016). Os exames parasitológicos comumente são utilizados para diagnosticar indivíduos com Leishmaniose (BARBOSA *et al*., 2012; TASCA *et al*., 2009). No entanto, o procedimento muitas vezes invasivo, exige técnicas específicas e apresenta sensibilidade e especificidade variáveis, dependendo do tecido analisado. A medula óssea, apesar de ser um local importante para a replicação do parasita, apresenta baixa sensibilidade para a detecção de *Leishmania* spp (COSTA *et al*., 2020). Esses fatores aumentam a necessidade de alternativas complementares que confirmem o diagnóstico e possibilitem a escolha do tratamento mais adequado.

Os métodos imunológicos apresentam uma alternativa plausível para o diagnóstico de leishmanioses. Dentre os testes mais utilizados, destacam-se a Reação de Imunodifusão Dupla (RIDT), Testes imunocromatográficos, Teste de Aglutinação Direta e o ELISA (*enzyme-linked immunosorbent assay*) (MICHEL *et al*., 2011). O ELISA, um teste mais específico e sensível, é útil para confirmar o diagnóstico e acompanhar a resposta ao tratamento (RIERA *et al*., 2004; ROMERO *et al*., 2009). Embora os testes imunológicos comerciais apresentem vantagens significativas, é importante considerar suas limitações. A principal delas reside na baixa especificidade, que pode gerar resultados falso-positivos em indivíduos com outras doenças parasitárias (PAIVA-CAVALCANTI *et al*., 2015). Além disso, a sensibilidade

dos testes pode variar de acordo com a espécie de *Leishmania* e o estágio da doença.

Como alternativa de proposição diagnóstica, os testes moleculares emergem como ferramentas promissoras para o diagnóstico preciso e eficaz da doença (SUDARSHAN *et al*., 2011). Essa tecnologia, baseada na detecção do DNA do parasita *Leishmania* em amostras de tecidos, sangue ou secreções, destaca-se pela alta sensibilidade e especificidade, que superam as limitações dos métodos diagnósticos tradicionais, como os exames parasitológicos e imunológicos (FARIA; ANDRADE, 2012).

Essa característica permite a detecção do parasita mesmo em casos assintomáticos ou com baixa carga parasitária, aumentando as chances de um diagnóstico precoce e eficaz. A tecnologia também pode ser utilizada para monitorar o sucesso do tratamento, avaliar a resposta do paciente à terapia e identificar casos de recidiva da doença (CHAGAS, 2019). Essa capacidade de acompanhamento contínuo é essencial para garantir a cura completa e prevenir complicações. Apesar dos benefícios promissores, os testes moleculares ainda apresentam alguns desafios. O alto custo da tecnologia e a necessidade de infraestrutura laboratorial especializada podem limitar o acesso a essa ferramenta em áreas com recursos limitados.

Diante das limitações apresentadas por testes diagnósticos comerciais, o desenvolvimento de novos testes diagnósticos se torna crucial para o avanço no combate à doença, principalmente quando avaliado a preocupação com pacientes possivelmente assintomáticos (SILVA *et al*., 2011; WEIRATHER *et al*., 2011). Com o avanço da pesquisa e o desenvolvimento de biossensores eletroquímicos robustos e acessíveis, essa tecnologia tem o potencial de revolucionar o diagnóstico da Leishmaniose, contribuindo para o controle da doença, o direcionamento do tratamento adequado e a melhoria da qualidade de vida das populações afetadas.

4. ELETROQUÍMICA NO MUNDO CONTEMPORÂNEO

A eletroquímica, muitas vezes associada à um determinado nicho científico, se revela como uma área multifacetada, utilizada para o desenvolvimento de diversos diagnósticos contemporâneos (PEREIRA *et al*., 2006). Essa subárea da química, permite explorar a relação entre eletricidade e reações químicas, possibilitando o desenvolvimento de tecnologias inovadoras que impactam diversos setores da sociedade, com o desenvolvimento de baterias que alimentam nossos dispositivos eletrônicos, desenvolvimento de diagnósticos para vários campos do conhecimento e aplicabilidade nos processos industriais essenciais (BURNS *et al*.,

1993).

Nesse campo de estudo o conhecimento das estruturas dos elétrons, que são partículas carregadas negativamente que orbitam os átomos, são fundamentais para o desenvolvimento de pesquisas e produtos (GUERRA, 2018). As reações eletroquímicas envolvem a transferência de elétrons entre espécies químicas, gerando corrente elétrica ou consumindo-a. Essa transferência de elétrons é conhecida como processo *redox* (oxirredução), para o qual uma espécie química perde elétrons (oxidação) enquanto outra os ganha (redução) (LIMA *et al*., 2018).

Para que as reações eletroquímicas ocorram, é necessário um impulso que oriente o fluxo de elétrons. Essa força motriz é representada pelo potencial eletroquímico, uma grandeza termodinâmica que mede a tendência de uma espécie química de doar ou receber elétrons. A diferença de potencial eletroquímico entre duas espécies determina a viabilidade e a espontaneidade da reação eletroquímica. Essas reações ocorrem nas células eletroquímicas, fundamentalmente compostas por dois eletrodos (cátodo e ânodo) imersos em um eletrólito. As células eletroquímicas fornecem o ambiente ideal para a transferência de elétrons e a geração de corrente elétrica.

Em suma, os eletrodos, componentes essenciais das células eletroquímicas, servem como portas de entrada e saída para os elétrons. O cátodo, local da redução, recebe os elétrons do eletrólito, enquanto o ânodo, local da oxidação, doa os elétrons para o eletrólito. Assim, a natureza do material do eletrodo, sua superfície e seu potencial influenciam diretamente a cinética e a eficiência da reação eletroquímica.

A fim de quantificar e qualificar as substâncias de análise em solução, a técnica de eletroanálise, baseada em princípios eletroquímicos, é aplicada a partir de técnicas como voltametria, amperometria e potenciometria, nas quais os eletrodos são empregados para medir correntes ou potenciais eletroquímicos, fornecendo dessa forma, informações valiosas sobre a composição e propriedades das soluções (PACHECO *et al*., 2013).

Assim, em um mundo em constante transformação, a eletroquímica apresenta-se como um campo dinâmico e em constante evolução que contribui para o enfrentamento de diversos desafios do século XXI. Compreendendo seus princípios fundamentais, aliados à sua alta sensibilidade e versatilidade, podemos solucionar problemas globais e impulsionar o desenvolvimento tecnológico em diversos setores.

4.1 Sensores Eletroquímicos

Na química analítica, os sensores eletroquímicos (Figura 4) se destacam como ferramentas úteis para desvendar as composições químicas de diversos materiais. Mais doque simples dispositivos, eles representam uma associação entre o mundo molecular e o macroscópico, convertendo informações químicas em sinais elétricos mensuráveis. Uma resposta eletroquímica é desencadeada pela interação entre uma espécie química alvo e a superfície do sensor (FREIRE; PESSOA; KUBOTA, 2003). Essa resposta, na forma de corrente ou potencial elétrico, é proporcional à concentração da espécie alvo, permitindo a quantificação e qualificação precisa da substância em questão.

Fonte: Lowinsohn, Bertotti, 2006.

Figura 4: Representação esquemática dos componentes de um sensor.

Um sensor, por meio do transdutor, é capaz de captar informações e traduzi-las em uma linguagem compreensível (RICARDO *et al*., 2005). Assim como demonstrado na figura, esses dispositivos apresentam como unidades básicas o receptor ou transdutor, o comunicador e o processador de dados (TREVISAN; POPPI, 2006). Dessa forma, a substância alvo da investigação, também denominada analito, irá interagir com a camada receptora, gerando um sinal químico (ROSINI; ANTONA; POLLEGIONI, 2020). Esse sinal precisa ser traduzido pelo transdutor em um sinal compreensível que pode ser elétrico, óptico ou mecânico.

Nos transdutores eletroquímicos, a interação analito-receptor, pode gerar uma diferença de potencial ou reações de oxirredução, que serão compreendidas por meio dos sinais eletroquímicos potenciométricos, assim, na ausência de corrente a diferença de potencial na interface eletrodo-solução é verificada. Já nos amperométricos a corrente gerada por processos de oxirredução de espécies eletroativas é verificada por meio de um potencial fixo. E nos condutométricos, o transdutor mede a condutividade da solução na presença de corrente

elétrica. (PORFÍRIO; GIAROLA; PEREIRA, 2016).

Dentre os benefícios desses dispositivos encontram-se a alta sensibilidade capaz de gerar análises precisas de compostos em diferentes matrizes; a seletividade que proporciona a distinção entre as diferentes espécies químicas presentes na amostra, garantindo resultados confiáveis e livres de interferências; a agilidade diagnóstica ao apresentar a capacidade de fornecer resultados em tempo real ou em um curto intervalo de tempo, otimizando o tempo de análise e a tomada de decisões; a facilidade de uso por meio de operação simples e intuitiva, permitindo que pessoas com diferentes níveis de experiência os utilizem com eficiência; a versatilidade, uma vez que esses dispositivos são adaptáveis a diversos tipos de análises tornando-os ferramentas universais; o baixo custo o que democratiza o acesso à tecnologia e viabilizando seu uso em larga escala; a possibilidade de portabilidade, uma vez que os equipamentos podem ser compactos e leves, facilitando o transporte e a realização de análises em campo; e a possibilidade de miniaturização que abre caminho para aplicações em dispositivos vestíveis e miniaturizados (CAMPOS *et al*., 2023).

Nos sensores eletroquímicos, são componentes essenciais da técnica, os eletrodos utilizados como plataformas em diversas áreas, compreendendo assim, desde análises químicas até biossensores e dispositivos vestíveis. Dessa maneira, cada tipo de eletrodo apresenta características e funcionalidades específicas, impactando diretamente na aplicabilidade e no desempenho do sistema.

Os eletrodos convencionais, como platina, ouro e grafite, são os mais tradicionais e amplamente utilizados em eletroquímica (SOUZA, 1997). A platina é conhecida por sua alta atividade catalítica, baixo sobrepotencial e excelente resistência à corrosão. Por isso, é frequentemente utilizada em eletrodos para reações de oxirredução (NASCIMENTO; ANGNES, 1998). O ouro apresenta alta condutividade elétrica, biocompatibilidade e resistência à corrosão (FOGUEL *et al*., 2009). O carbono é um material de baixo custo com alta condutividade elétrica e boa biocompatibilidade (MONTEIRO; RIBEIRO; FONSECA, 2014).

Dessa forma, a estabilidade química e eletroquímica, alta condutividade elétrica e boa reprodutibilidade de medidas tornam os eletrodos convencionais a escolha ideal para diversos estudos e aplicações. No entanto, seus formatos rígidos e volumosos limitam a miniaturização e a integração em dispositivos vestíveis. Além disso, a fabricação individual de cada eletrodo pode ser complexa e trabalhosa, impactando no custo final do sistema.

Nesse sentido, os eletrodos *screen-printed* surgem como alternativa aos eletrodos convencionais, e se destacam pela simplicidade e baixo custo de fabricação (BERGAMINI;

ZANONI, 2005). Tintas condutoras à base de carbono, como grafeno e nanotubos de carbono, oferecem alta condutividade elétrica e boa processabilidade para impressão serigráfica (MONTEIRO; RIBEIRO; FONSECA, 2014). A partir de técnicas de impressão serigráfica, é possível produzir eletrodos em massa com geometrias e *designs* personalizados, facilitando a miniaturização e a integração em diversos dispositivos. Essa característica os torna uma alternativa promissora para sensores descartáveis e dispositivos de diagnóstico de ponto de atendimento. No entanto, como pontos a melhorar, destaca-se o fato de a reprodutibilidade de medidas e a estabilidade a longo prazo tolerarem condições inferiores às suportadas por eletrodos convencionais.

Na busca por biossensores e dispositivos de monitoramento de saúde mais eficientes, os eletrodos vestíveis surgem como uma inovação promissora da área. A flexibilidade, conformabilidade e capacidade de integração à pele permitem o monitoramento contínuo de parâmetros fisiológicos relevantes para o quadro do paciente monitorado, como frequência cardíaca, temperatura corporal e biomarcadores (MATTIONI; WURZEL; EVALD, 2020). Para isso, polímeros condutores, como PEDOT:PSS (poli(3,4-etilenodioxitiofeno):poli(estirenossulfonato)) e PANI (polianilina), podem ser utilizados, pois oferecem flexibilidade, biocompatibilidade e condutividade elétrica ajustável (JULIANE FAVERO *et al*., 2016). Essa característica os torna ferramentas valiosas para o diagnóstico precoce de doenças, acompanhamento de pacientes e promoção da saúde preventiva. No entanto, a integração à pele pode apresentar desafios como a biocompatibilidade, desconforto ao paciente, a estabilidade a longo prazo e a transferência de sinais elétricos.

Sendo assim, a escolha do tipo de eletrodo eletroquímico ideal depende das necessidades específicas da aplicação. Os eletrodos convencionais oferecem alta performance e reprodutibilidade, enquanto os eletrodos vestíveis possibilitam o monitoramento contínuo e a integração à pele. Já os eletrodos *screen-printed* se destacam pela simplicidade e baixo custo de fabricação. Portanto, o conhecimento e a análise comparativa detalhada contribuem para a seleção consciente do tipo de eletrodo mais adequado, otimizando o desempenho e a aplicabilidade do sistema final.

Além da escolha do tipo de eletrodo de trabalho, uma etapa crucial de avaliação é a análise da necessidade da modificação de superfície dos eletrodos eletroquímicos com nanomateriais, em busca de aprimorar suas propriedades e ampliar suas aplicações (ZHU *et al*., 2015). Os nanomateriais oferecem características únicas que podem melhorar a sensibilidade, seletividade, atividade catalítica e estabilidade dos eletrodos. Em biossensores eletroquímicos, além das características químicas dos materiais, a biocompatibilidade e

bioquímica devem ser avaliadas em busca de um diagnóstico mais eficiente.

A incorporação de micromateriais e nanomateriais amplia a área superficial, uma vez que a elevada área superficial permite maior adsorção de moléculas reagentes na superfície do eletrodo, aumentando a sensibilidade e a corrente de resposta, maior flexibilidade e conformabilidade, biocompatibilidade aprimorada, sensibilidade aprimorada para biomarcadores (CASTRO *et al*., 2019; MARTINS *et al*., 2020).

Sendo assim, diversas técnicas de modificação da superfície de eletrodos podem ser utilizadas, uma vez avaliados os benefícios e desafios associados à técnica. Logo, a eletroquímica se transforma com a introdução dos nanomateriais, proporcionando o desenvolvimento de eletrodos com propriedades aprimoradas e aplicações inovadoras. As modificações com materiais permitem controlar a estrutura, a composição e a funcionalidade da superfície do eletrodo em escala nanométrica, resultando em eletrodos com alta sensibilidade, seletividade, atividade catalítica e biocompatibilidade.

4.2 Biossensores eletroquímicos

A introdução de novos materiais, à exemplo os polímeros condutores e nanomateriais, e o aperfeiçoamento das técnicas de imobilização biomolecular, contribuíram para impulsionar o desempenho e proporcionar de forma mais efetiva a aplicação desses dispositivos nos diversos ramos dos diagnósticos biológicos (AMADOR SALOMÃO, 2018; D'ORAZIO, 2003; NARESH; LEE, 2021) . Dessa forma, os biossensores se consolidam como ferramentas essenciais na busca por um futuro mais saudável, seguro e sustentável.

Nos biossensores eletroquímicos, diversos componentes biológicos podem ser integrados aos materiais eletroquímicos, à exemplo, enzimas, anticorpos, antígenos e materiais genéticos, desses dispositivos (KAUR; BHOSALE; SHRIVASTAV, 2018). Dessa maneira, a interação entre as ciências biológicas, ciências médicas e ciências dos materiais fornecem um conjunto de conhecimentos essenciais quanto à interação dos componentes biológicos e não biológicos. Sendo assim, representam ferramentas essenciais para a seletividade, reconhecimento do analito de interesse, previsão de aplicação clínica e monitoramento de biocompostos e condições de saúde, desenvolvimento de materiais eletroquímicos avançados, estudo de técnicas de imobilização e interface dosmateriais com os componentes biológicos (URBAN, 2018; ZHANG *et al*., 2020).

Os biossensores eletroquímicos têm sido amplamente utilizados em aplicações

diagnósticas, proporcionando resultados rápidos, sensíveis e precisos (ROCCHITTA *et al*., 2016). Em vista disso, comercialmente podemos observar esses dispositivos aplicados na detecção de biomarcadores de doenças, como glicose para monitoramento de diabetes (CAMPOS *et al*., 2023; JUSKA; PEMBLE, 2020), monitoramento de troponina para detecção de infarto do miocárdio (FONSECA *et al*., 2011), marcadores tumorais para o diagnóstico de câncer (BOHUNICKY; MOUSA, 2011), monitoramento de amilase salivar (MARTINS *et al*., 2021). Além disso, biossensores têm sido aplicados para detecção de patógenos circulantes, comoos desenvolvidos para diagnósticos de bactérias (GU *et al*., 2023; JONES; PADILLA- PARRA, 2016), biossensores para a detecção de vírus (BALVEDI *et al*., 2014; MIRANDA *et al*., 2019), protozoários como *Leishmania infantum* (MARTINS *et al*., 2020), permitindo assim, a rápida identificação de infecções.

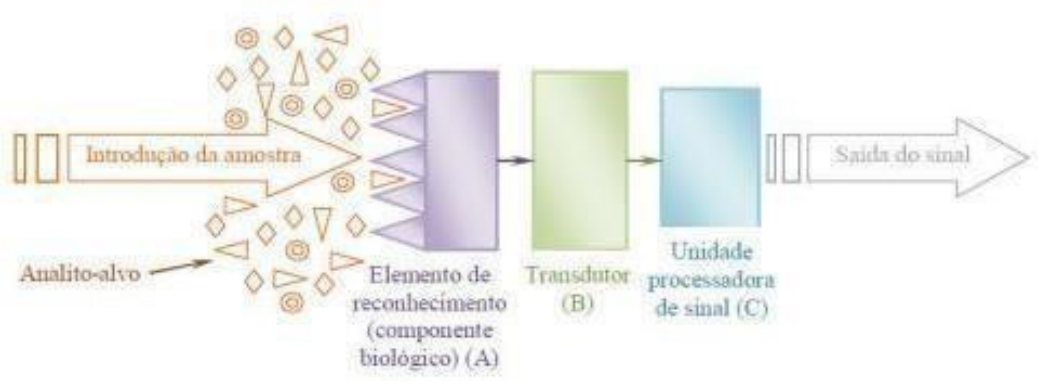
O desenvolvimento e evolução dos biossensores eletroquímicos nos mostra que esses dispositivos estão, a cada vez mais, avançando e desempenhando um papel importante na área diagnóstica de enfermidades (GRIESHABER *et al*., 2008). No que tange os processos de desenvolvimento de plataformas diagnósticas, esses dispositivos apresentam diversas vantagens, quando comparadas às metodologias diagnósticas inseridas no mercado e consideradas “padrão ouro” em processos que envolvem a determinação e diagnóstico de processos biológicos (SILVA, 2021). Assim, dentre as vantagens oferecidas pelos biossensores eletroquímicos, podem ser evidenciadas, a seletividade, especificidade, bom custo-benefício e facilidade de automação.

Destarte, os dispositivos eletroquímicos, desempenham papeis significativos no que tange a sua inserção como alternativa viável para as análises diagnósticas, complementando as necessidades do mundo cotidiano, e em especial, de áreas negligenciadas que possuem baixos recursos para a aplicação e desenvolvimento de sistemas diagnósticos precisos. Ainda assim, apesar das inúmeras vantagens que favorecem a implementação dessa técnica no cotidiano diagnóstico em centros de referências e pesquisas biológicas, esses, ainda hoje, não podem substituir completamente as técnicas analíticas clássicas (MARTINS *et al*., 2020; SARAF *et al*., 2017). Consideradas as suas limitações, os dispositivos eletroquímicos devem ser usados em conjuntos com outras técnicas analíticas que garantam confiabilidade e precisão de resultados, ao menos nas etapas de construção e estudos iniciais da aplicação dessas técnicas nos grupos alvo (ROSATTO *et al*., 2001). Dentre os fatores que contribuem para essas recomendações, estão algumas instabilidades que podem limitar a aplicação dessa metodologia no cotidiano. Dessa maneira, recomenda-se, atualmente, a inclusão dessas

metodologias como ferramentas complementares aos testes diagnósticos já existentes.

Suscintamente, o funcionamento dos biossensores eletroquímicos como sistemas analíticos de detecção de doenças podem ser explicados por meio da avaliação da interação entre os componentes biológicos finais da avaliação (GRAÇA; FERREIRA, 2015). Assim, essas substâncias biológicas são ligadas às superfícies transdutoras disponíveis no sistema eletroquímico desenvolvido para esse fim, de modo que, ao serem conectados ao transdutor, os sinais biológicos do biossensor são convertidos em sinal elétrico, e esse sinal elétrico será quantificado e analisado. Essa conversão permite a detecção e análise das amostras biológicas com base nas respostas elétricas geradas pelo dispositivo criado.

Nesse sentido, os biossensores eletroquímicos podem desempenhar papeis cruciais na obtenção de informações quantitativas, no que diz respeito à presença e concentração de analitos em uma amostra biológica (BARBIERI, 2017). Com tal característica, essas ferramentas são capazes de proporcionar uma abordagem de reconhecimento biológico mais sensível e precisa para a detecção e monitoramento das diversas substâncias de interesse do pesquisador. Em suma, o esquema representando o funcionamento dos biossensores eletroquímicos pode ser observado na Figura 5, a seguir.



Fonte: Vitoreti , 2014.

Figura 5: Representação esquemática dos componentes de um biossensor eletroquímico.

No âmbito dos diversos métodos diagnósticos disponíveis nos sistemas de saúde, os biossensores destacam-se como destaque como sistemas analíticos, principalmente em enfermidades em que a sua inserção é possível e comercializável (FURTADO *et al.*, 2008). Dentre os dispositivos já desenvolvidos e utilizados no cotidiano humano, o diagnóstico

realizado por glicosímetros, que são biossensores enzimáticos, destaca-se e serve de inspirações para os pesquisadores dessa área.

De modo geral, as inúmeras características relevantes dos biossensores, tornam seu uso essencial no que tange ao desenvolvimento de um diagnóstico efetivo até mesmo para as regiões mais remotas do globo.

No que se refere ao diagnóstico de doenças, um dispositivo que possua a capacidade de identificar doenças antecipadamente e de modo realista com o quadro do paciente é fundamental para garantir um tratamento adequado e que busque melhorar a qualidade de vida deste (BERNARDES *et al*., 2019). Atualmente, diversas técnicas diagnósticas estão disponíveis e contribuem para a detecção de enfermidades, mas, por vezes, várias dessas técnicas apresentam características que contribuem para a dificuldade de acesso à diagnósticos efetivos para os vários grupos populacionais.

As dificuldades diagnósticas podem ser observadas, à exemplo, nos diagnósticos sorológicos de doenças parasitárias como a leishmaniose e a doença de Chagas, que apesar de possuírem vários métodos diagnósticos já desenvolvidos, tal como, o método de hemaglutinação indireta, a imunofluorescência e o ELISA, por vezes apresentam como limitações o grande número de reações cruzadas, obstáculos para a detecção dos pacientes assintomáticos e resultados que refletem a realidade falso-positivos e falso-negativos (MATOS *et al*., 2015; ROMERO *et al*., 2009). Assim, somadas, essas limitações reforçam a necessidade de pesquisadores investirem em análises e estudos adicionais para corrigir esses problemas, buscando também aprimorar a precisão e confiabilidade dos testes diagnósticos para essas doenças propícias a gerar confusões tais como observados em estudos para as doenças parasitárias à exemplo da leishmaniose e a doença de Chagas.

Ademais, salienta-se a importância de superar essas limitações, com a criação de diagnósticos de doenças que se apresente mais preciso e confiável, e que, identifique corretamente os pacientes afetados com essas enfermidades, para que então, estes, possam receber um tratamento adequado à sua realidade patológica. Assim, buscando contribuir como aprimoramento na detecção de doenças, alguns biossensores eletroquímicos estão sendo criados e sendo empreendidos por diversos pesquisadores como linhas de pesquisa, quevisam obter um diagnóstico capaz de possuir um desempenho desejado em quesitos como, sensibilidade, alcance dinâmico e reprodutibilidade (SILVA *et al*., 2011).

Esses fatores, somados, são essenciais para garantir a eficácia e precisão diagnóstica de

dispositivos sensoriais e suas aplicações. Os avanços nas áreas dos biossensores eletroquímicos, têm, cada vez mais, sido impulsionado pela necessidade crescente de criação de sensores mais sensíveis, mais tecnológicos e mais sustentáveis, que ao mesmo tempo, mantenham a capacidade de detectar com precisão uma ampla faixa de concentrações do analito de interesse. Nesse mesmo contexto, a busca pela reprodutibilidade é um aspecto crucial para o desenvolvimento desses novos dispositivos, já que os resultados devem ser apresentados de maneira consistente e replicável para os diversos grupos de pacientes dos quais ele possa se deparar no cotidiano clínico.

Em especial, a capacidade de realizar testes de doenças complexas de forma rápida e precisa é fundamental, especialmente nas situações onde o tempo é essencial para a tomada de decisões clínicas precisas. Além do mais, a utilização de pequenas quantidades de amostras é interessante na área clínica, uma vez que há redução do quantitativo de desconforto do paciente frente às coletas de amostras invasivas. A portabilidade dos biossensores eletroquímicos proporciona para esses diagnósticos a flexibilidade e praticidade frente a ambientes remotos ou a pontos de atendimento fora de um ambiente laboratorial tradicional.

Em síntese, os biossensores eletroquímicos possuem características ímpares e são reconhecidos pela capacidade de execução rápida, baixo consumo de amostras, portabilidade, especificidade, tornando-os promissores para o diagnóstico eficaz em áreas da saúde. Dessa forma, o contínuo desenvolvimento de instrumentos que possam otimizar essas plataformas portáteis é fundamental para progredir e atingir os máximos benefícios oferecidos por essa tecnologia.

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6. APÊNDICE A – ARTIGO REFERENTE AO TEMA DA TESE

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A comparative study of graphene-based electrodes for electrochemical detection of visceral leishmaniasis in symptomatic and asymptomatic patients

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ABSTRACT

Background: Visceral Leishmaniasis is a neglected tropical disease with a high rate of infection and mortality in affected areas. Around 50,000 to 90,000 new cases of visceral leishmaniasis (VL) are estimated every year. Individuals asymptomatic for the disease should also be considered in epidemiological surveillance of the disease, as they can help spread the parasite. Thus, the development of low-cost diagnosis methods that allow the identification of infected and asymptomatic individuals is required, especially in developing countries where this disease is endemic.

Results: In this work, we developed an immunosensor for recognizing anti-*Leishmania* antibodies in asymptomatic individuals and avoiding cross-reaction with Chagas disease (CD). For that, we used carbon-based screen-printed electrodes, modified with graphene oxide and gold. Reproducibility was assessed by calculating the relative standard deviation (RSD < 5 %) from cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ using three different electrodes, screen-printed carbon electrodes (DPR-110) and graphene modified screen-printed electrodes (DPR-110 GPH) were purchased from DropSens (Oviedo, Asturias, Spain).

Significance: As an electrochemical methodology, we use cyclic voltammetry. After the tests were carried out, we considered that carbon electrodes adsorbed with reduced graphene oxide and modified with gold nanoparticles were the best platforms for detecting anti-*Leishmania* antibodies. In the study carried out, the limit of quantification (LOQ) for anti-*Leishmania* antibodies was established at 16.75 mg/mL, while the limit of detection (LOD) was 5.58 mg/mL. These limits indicate the minimum antibody concentration values that can be quantified and detected accurately and reliably in the analyzed sera.

1. Introduction

Visceral leishmaniasis (VL) is a neglected tropical disease that can be potentially mortal if left untreated. The causative parasite, *Leishmania infantum*, is transmitted by the bite of infected female phlebotomine sandflies. It is estimated that around 50,000 to 90,000 new cases of VL occur every year ([6]; WHO, 2023). According to the World Health Organization (WHO, 2023), the disease is primarily prevalent in Brazil, India, and countries in East Africa [35].

Symptoms can vary, ranging from severe to mild, such as diarrhea, fever, hepatomegaly, and mild splenomegaly, among others. Some individuals may be infected and asymptomatic for long periods. Although they do not always develop symptoms, such individuals should be the focus of attention, as they can assist in the spread of the parasite; they

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can serve as a possible reservoir or even contribute to the transmission through blood donations [2,25]. Studies have shown that parasites of the *Leishmania* genus can survive and remain infectious in stored blood bags. In Brazil, among healthy blood donors, some individuals exhibited immunoglobulin G (IgG) antibodies against *Leishmania*, suggesting prior contact with *Leishmania infantum*. This highlights the need for testing for leishmaniasis in asymptomatic individuals, especially blood donors in endemic areas [13,27].

Although there are some commercial tests available for the detection of the disease, such as Biolisa (BIOLISA LEISHMANIOSE VISCERAL®-Bioclin, Belo Horizonte, Brazil), rk39 protein (most present in *Leishmania donovani*), offer valuable diagnostic tools for leishmaniasis, in addition to recent academic studies involving the topic, they are sometimes insufficient in detecting asymptomatic infections and/or present a high financial cost [18,28]. Therefore, it is important to develop an efficient, low-cost, easily accessible test that guarantees sensitivity and specificity.

In this context, electrochemical biosensors are platforms that possess these characteristics and could serve as an option for the diagnosis of asymptomatic patients.

There are some studies showing the development of biosensors aimed at detecting leishmaniasis, such as the study by Barraza and collaborators, who developed a paper platform for detecting anti-American cutaneous leishmaniasis antibodies [5] and the study by Perk and collaborators, who made an electrochemical immunosensor for detecting antibodies against the surface protease (Gp63) of *Leishmania major* [29], both in human sera; and the study of an impedimetric immunosensor for the detection of *Leishmania infantum* [8], the latter in canine serum. However, there are still few biosensors currently reported in the literature focusing on the diagnosis of visceral Leishmaniasis in humans, mainly those that identify the detection of antibodies anti--*Leishmania infantum* in possibly asymptomatic and oligosymptomatic individuals.

Previously, our research group developed an immunosensor (graphite electrodes with the incorporation of gold nanoparticles) capable of detecting anti-*Leishmania* antibodies in sera from infected symptomatic individuals without showing significant cross-reactivity with Chagas Disease (CD) [22]. Leishmaniasis and Chagas Disease represent important public health problems, with high prevalence in several regions of the world. Accurate and early diagnosis of these diseases is crucial for successful treatment and control of transmission. However, the cross-occurrence between the disease-causing agents, *Trypanosoma cruzi* and *Leishmania spp.*, represents a significant challenge for traditional diagnostic methods, such as serological and molecular tests [4]. This cross-reactivity occurs due to the antigenic similarity between the parasites, which can lead to cross-recognition of antigens by the patient's immune system [21]. Consequently, diagnostic tests can present false-positive results, making it difficult to correctly identify the disease and appropriately direct treatment [23].

This problem is especially critical in areas endemic for both diseases, where the coexistence of cases of Chagas and leishmaniasis makes the diagnosis even more complex. Interpreting test results becomes a challenge and cross-reactivity can lead to delays in diagnosis, prescription of inappropriate treatment and, in serious cases, complications for the patient's health. The development of more specific and sensitive diagnostic methods is crucial to overcome these obstacles. New techniques, such as tests based on specific antigens and biomarkers, can offer greater diagnostic accuracy and help differentiate between Chagas and Leishmaniasis. Continuous studies and investments in research are essential to improve diagnostic tools and ensure accurate and timely diagnosis of these neglected diseases.

Despite advances in the diagnosis of human visceral leishmaniasis (HVL), there are still challenges to be overcome. Current tests, although effective, have limitations in detecting asymptomatic cases, which may represent an important reservoir of the disease and contribute to its spread, making early diagnosis and timely intervention difficult. Faced with this gap, our research group set out to develop new diagnostic tests using electrochemical biosensors, based on research into efficient nanomaterials for this purpose, as early diagnosis is crucial for the successful treatment of HVL, reducing mortality and morbidity. associated with the disease.

Graphene, one of the most explored carbon-based nanomaterials, has emerged as a promising tool for enhancing detection capabilities. Since its discovery in 2004, it has garnered significant interest from the research community, particularly for its potential applications in manufacturing electrochemical biosensors [11,24]. This study aims to evaluate a graphene-based immunosensor for the diagnosis of leishmaniasis. The developed immunosensor is capable of identifying asymptomatic individuals without cross-reaction with CD and allows the construction of analytical curve to evaluate the analytical performance of the modified electrodes.

2. Materials and methods

2.1. Sample acquisition

Serum samples from 4 patients diagnosed with VL (2 symptomatic and 2 asymptomatic patients), 2 patients diagnosed with cutaneous leishmaniasis, and 2 patients diagnosed with CD were obtained at the Immunology Research Laboratory and Hematology Research Laboratory of the Federal University of Triangulo Mineiro (Uberaba, State of Minas Gerais, Brazil).

The diagnosis of symptomatic individuals for VL was made through anamnesis, physical examination, and confirmation by laboratory tests, while the diagnosis of CD was made through laboratory tests. The collection and use of samples from patients who tested positive for infection by *Leishmania infantum* for these experiments were authorized by the Research Ethics Committee (CEP) under protocol number 1 846 584 (CAAE 58,301,516.8.0000.5154), while the collection and use of samples from patients infected with *Trypanosoma cruzi* were authorized by the CEP under protocol number 2 163 043 (CAAE 64,048,117.3.0000.5154).

Samples were collected from individuals who had received at least 4 blood transfusions and from individuals residing in the same households as the polytransfused patients in endemic areas for VL. Asymptomatic infection was determined by enzyme immunoassay to confirm the presence of anti-*Leishmania* spp. antibodies and Polymerase Chain Reaction (PCR) to detect parasitic DNA. These samples were authorized by CAAE number 29,950,120.4.0000.5154.

2.2. Techniques used to confirm positive samples for Leishmania infantum

2.2.1. qPCR protocol used for selection and confirmation of positive samples from asymptomatic individuals selected for standardization of tests in the developed immunosensor

For molecular biology analyses, DNA extraction from the blood samples was performed using Qiagen's DNA Mini Kit from Qiagen (Hilden, Germany), following the manufacturer's recommended method. The samples were subjected to qPCR to identify *Leishmania spp*. With primers described by Nicolas et al. [26]. The reaction consisted of 1XSYBR Green PCR Master Mix (Life Technologies, Carlsbad, CA, USA), 10pM of each primer, 50 ng of DNA, and ultrapure water for a final volume of 20 μL. Amplification was performed at an initial holding temperature of 95 °C for 5 min, followed by 40 cycles at 95 °C for 1 min, 60 °C for 1 min, and melting curve analysis in 1 °C increments from 60 °C to 95 °C. Each assay included an internal control of human beta-actin in each sample. The database was analyzed using Applied Biosystems 7500 Real-Time PCR Software v2.3 (Thermo Fisher Scientific, USA). DNA from *Trypanosoma cruzi* was used to demonstrate the absence of cross-reactivity of the selected target, as described by Nicolas et al. [26].

2.2.2. Commercial enzyme-linked immunosorbent assay (ELISA) protocol used to evaluate the positivity of the selected sample from asymptomatic individuals for the standardization of immunosensor tests

The qualitative detection of IgG antibodies against *Leishmania infantum* was performed from the serum of the samples collected through an enzyme immunoassay (ELISA) with a commercially available kit (BIOLISA LEISHMANIOSE VISCERAL® - Bioclin, Belo Horizonte, Brazil) according to the manufacturer's recommendations. Samples whose Elisa Index (EI) was ≥ 1.2 were considered positive.

2.2.3. Indirect ELISA

The indirect ELISA for the detection of IgG antibodies was performed as described in Martins et al. [22] (Figure S1).

The indirect ELISA method was used to detect immunoglobulin G (IgG) antibodies against *Leishmania infantum*. High-affinity plates (Thermo Scientific™ Nunc™, Waltham, MA, USA) were sensitized with antigens (1 μg/mL) diluted in 0.06 mol/L carbonate-bicarbonate buffer (pH 9.6) and incubated for 18 h at 4 °C. The plates were then washed six times with PBS containing 0.05 % Tween 20 (PBS-T) and blocked with PBS containing 5 % skimmed milk powder (Molico®, Nestlé®, São Paulo, Brazil - PBS-M5 %) for 4 h at room temperature. After washing again, serum samples diluted 1:40 in PBS-M5 % were incubated for 2 h at room temperature. Six subsequent washes were performed before adding peroxidase-conjugated anti-human IgG antibody (IgG/horseradish peroxidase (HRP), Dako), diluted 1:2000, for incubation for 2 h at room temperature.

After washing again, the reaction was revealed with the addition of the enzyme substrate 1,2-orthophenylenediamine (OPD, Dako) with 0.05 % H_2O_2, subsequently stopped with H_3PO_4. Positive and negative controls were included on each plate. Quantification of antibody levels was performed using the ratio between the optical absorbance (Abs) of the sample and the cut-off value. The cut-off was calculated as the mean Abs of the negative control serum plus three standard deviations. Samples with an enzyme index (IE) greater than 1.4 were considered positive.

2.3. Maintenance of L. infantum parasite cultures

The promastigote form of *Leishmania infantum* was cultured in the Schneider medium, supplemented with 10 % fetal bovine serum. When the exponential phase was reached, the parasites were quickly centrifuged at 200x g for 10 min at 25 °C to eliminate the dead ones. Then, the supernatant containing the live parasites was centrifuged at 3500 rpm for 25 min at 25 °C and then washed twice at 3500 rpm for 20 min at 25 °C using incomplete Roswell Park Memorial 17 Institute (RPMI, Gibco, USA) medium. The supernatant was discarded, and the pellet containing the parasites was frozen at −80 °C until antigen extraction.

2.3.1. Production of promastigote antigens from parasites

For extraction of soluble crude antigens, the pellet was resuspended in phosphate-bufferred saline (PBS) containing 0.05 % NP40 (Nonidet P-40 Substitute, Roche - Switzerland) with the COMPLETE protease inhibitor (COMPLETE™ ULTRA Tablets, Mini, EASYpack, Roche 2 Applied Science, Switzerland), and the antigen was obtained by the method of freezing in liquid nitrogen and thawing in a 37 °C water bath, followed by centrifugation at 10,000x g for 30 min at 4 °C. After that, the supernatant containing the crude soluble antigen was aliquoted and stored at −80 °C. The protein concentration of the antigen was determined by the NanoDrop™ 2000 spectrophotometers (Thermo Fisher Scientific, USA). The technique was performed and adapted based on Scott et al. [32]. A polyacrylamide gel electrophoresis was also performed to € the presence of soluble proteins of *Leishmania infantum* (Figure S2).

2.4. Construction of Immunosensor

2.4.1. Electrode selection

For the construction of electrochemical immunosensors, the choice of specialist platforms for the target analytes is a crucial step. Thus, in this work, screen-printed carbon electrodes (DPR-110, Metrohm DropSens, Spain) modified with electrodeposited gold were used, a protocol previously developed by Martins et al. [22] [22]. Furthermore, printed graphene electrodes (DPR-110 GPH, Metrohm DropSens, Spain), printed carbon electrodes (DPR-110) modified with graphene oxide (DPR-110-GO), and printed carbon electrodes (DPR-110) modified with graphene oxide with electrodeposited gold (DPR110-GO-Au) were evaluated to obtain a diagnostic platform capable of detecting asymptomatic patients.

2.4.2. Equipment selection

The potentiostat Em Stat 1 (PalmSens BV, The Netherlands) connected to a notebook (Vaio, Intel Core i5 (3rd Gen) 3210 M / 2.5 GHz) was used to take the readings.

2.4.3. Electrochemical experiments

Electrochemical analyses were performed by cyclic voltammetry (CV). The changes in the electrochemical signals of Ferri-Ferro Potassium Cyanide $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ (5×10^{-3} mol/L) containing KCl (0.10 mol/L) were evaluated (scan rate of 100 mV/s, at room temperature (25 ± 1 °C)). All solutions used were prepared using ultrapure water (MilliQ, resistivity value greater than 18.2 MΩ, Millipore Corporation, Burlington, MA, USA).

2.4.3.1. Devices. For the experiments, carbon screen-printed electrodes (DRP-110) and screen-printed electrodes modified with graphene (DRP-110 GPH) were used both purchased from DropSens (Oviedo, Asturias, Spain). These electrodes consist of a ceramic strip with a three-electrode system: working, reference and counter-reference, ideal for analyzing single drops. The reference is made of silver ink (known as silver pseudoreference electrode), while the counter reference and working electrode are made of carbon ink (DRP-110) or graphene ink (DRP-110 GPH), respectively.

In addition to the commercial electrodes mentioned, in this work we compared tests with carbon electrodes modified with graphene oxide. In graphene oxide-modified electrodes (DRP-110-GO), functionalized graphene oxide was added (15 μL) by adsorption onto carbon electrodes (DRP-110) at a concentration of 0.5 mg/dL and then electrochemically reduced.

Subsequently, in another group of carbon electrodes (DPR-110), in addition to the adsorption of graphene oxide, there was also performed the deposition of a gold film through CV in a gold chloride solution ($HAuCl_3$, 1 g/L) prepared in 1 mol/L H_2SO_4 [22].

2.4.4. Immunosensor

For the construction of the immunosensor, 4 μL of the solution containing the total soluble antigens of *Leishmania infantum* was pipetted onto the working electrode and immobilized by adsorption. After drying the solution, blocking was performed to prevent non-specific binding by pipetting 4 μL of 1 % bovine serum albumin (BSA). Then, 4 μL of the patient's serum was pipetted. After all these steps, the electrodes were washed and dried. For the electrochemical evaluation of the antigen-antibody interaction, a solution of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ (5 mM) was used, and the reading was performed using the CV technique (Fig. 1). All readings were performed at room temperature (25ºC ± 1ºC), and all tests were performed in triplicate.

2.4.5. Raman spectrophotometry

Electrode surface analyses were performed using the LabRAM HR Evolution, HORIBA equipment with HORIBA Scientific's LabSpec

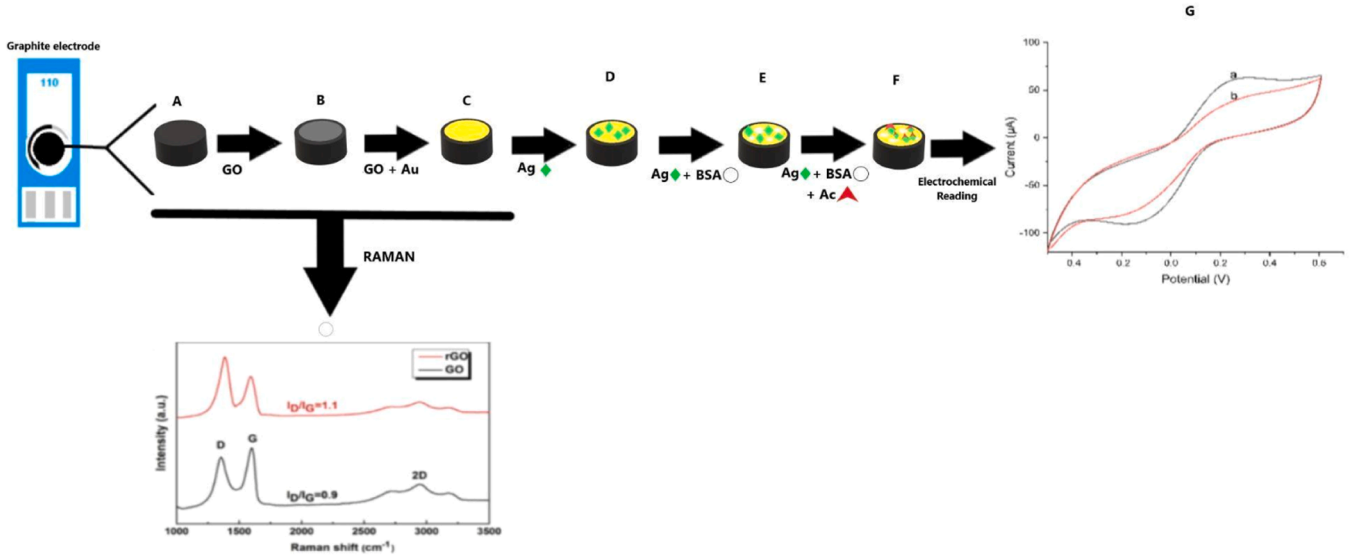

Fig. 1. Representative image of the electrochemical immunosensor developed using commercial carbon electrodes modified with graphene oxide and gold nanoparticles (DPR-110-GO-Au). The preparation steps of the immunosensors are as follows: (a) the commercial carbon electrode was used as the base platform; (B) carbon was modified with graphene oxide nanoparticles; (C) the electrodes already modified with graphene oxide were electroplated with gold nanoparticles; (D) after material modifications, the gross antigen of *Leishmania infantum* was immobilized in modified surface of the electrodes; (E) for blocking nonspecific interactions, a 1 % bovine serum albumin (BSA) blocking solution was coupled to the platform; (F) after preparations, the antibodies were coupled (real sample/serum); and (G) finally, the electroanalytic solution was inserted, and the process of transduction was started; thus, the surface with the antigen probe was autonomized as presented in a cyclic voltammetry (CV). For the evaluation of chemical modifications performed on the surface of the electrodes, Raman spectrophotometry was used as a means of verification.

software (LabSpec 6 Spectroscopy Suite) and optical microscope (Olympus BX41) in x10Vis, x40UV, and x100Vis objectives. The 10x (x10Vis) and 100x (x100Vis) objectives are used for wavelengths in the visible region (vis), and the 40x (x40UV) objective is used for the ultraviolet region. The images were generated at the Laboratory of New Insulating and Semiconductor Materials as well as the Multiuser Microscopy Laboratory at the Federal University of Uberlândia, Minas Gerais (MG), Brazil.

2.4.6. Specificity and selectivity

Sera containing anti-*Trypanosoma cruzi* antibodies from individuals with CD were also tested to confirm that the immunosensor would be able to eliminate possible cross-reactions.

2.4.7. Statistical analysis

With the aim of ensuring uniformity and clarity in the analyses, and following protocols described by [22], we constructed column charts using peak current data obtained using cyclic voltammetry (CV). The current percentages, which represent the oxidation and reduction currents of the redox probe, were calculated in relation to the initial CV (without biomolecule), considered as a reference value (100 %). Consequently, a low percentage of current indicates a high coverage of the electrode surface by biomolecules, either through proportional immobilization or specific molecular recognition. In summary, column plots and analysis of current percentages provide valuable information about the interaction between the biomolecules and the redox probe. The decrease in current reflects the extent of electrode surface coverage, revealing details about the efficiency of immobilization and the specificity of molecular recognition. The analyses are descriptive and are based on the comparative study of the voltammograms and their reinterpretations in bar charts and linear graphs (calibration).

3. Results and discussion

3.1. Samples

The asymptomatic individuals used in our tests come from an endemic area for VL, and the confirmation of positivity in these patients was confirmed through positive qPCR for the detection of parasitic DNA or positive commercial ELISA tests for the detection of antibodies. Table 1 shows that the samples of selected asymptomatic individuals were identified as follows: SBP-14 and FO 01P Patients diagnosed with leishmaniasis were identified as follows: VAL and LV1. Those diagnosed with CD were denoted as CH1 and CH2.

3.2. Graphene and modification

Fig. 2 shows the activation of screen-printed electrodes sold with graphene (110-GPH) and screen-printed graphite electrodes with graphene oxide adsorbed (before and after the reduction). The choice of KCl as electrolyte and the use of cyclic voltammetry were based on the ionic properties of the KCL electrolyte, as it dissociates into $K+$ and Cl- ions, improving the transfer of electrons between the electrode surface and the biochemical material, optimizing the biosensor performance. Furthermore, its ionic strength and neutrality are responsible for improvements in the adsorption of graphene oxide, promoting a stable and

Table 1
Identification of selected samples and description of confirmatory tests used.

	Identification	Bioclin ELISA	Indirect ELISA	qPCR
Asymptomatic	SBP 14	7.45	2.78	Negative
	FO 01P	2.14	1.98	Positive
Symptomatic	VAL	NP	4.53	NP
	LV1	NP	4.24	NP
Chagas disease	CH1	NP	5.94	NP
	CH2	NP	8.89	NP
Cutaneous	LA 01	NP	2.48	NP
	LA 05	NP	2.64	NP

*NP = Test not performed. Bioclin ELISA (EI > 1.2); Indirect ELISA (EI > 1.4).

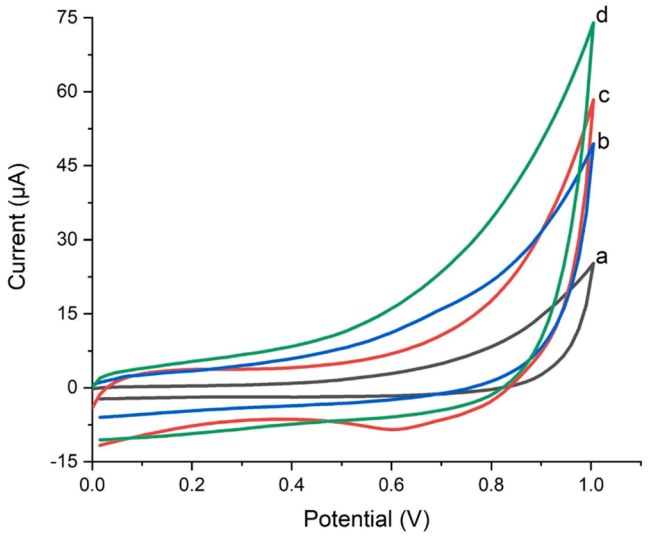

Fig. 2. Cross-platform comparison cyclic voltammogram in electroanalytical substance KCl 0,1 mol/L; a: commercial graphene electrode, area in value 4.9780; b: commercial graphene electrode after reduction, area in value 13.0393; c: graphite electrode with adsorbed graphene oxide, area in value 14.7234; d: graphite electrode with graphene oxide adsorbed after reduction, area in value 21.8200.

efficient interface for the immobilization of the biomaterial, and not influencing undesirable electrostatic effects, contributing to the reproducibility of the process.

In this work, the OriginLab Corporation 2019 software, a widely used tool for scientific data analysis, was used to study cyclic voltammograms (VCs). The area under the VC curve, a crucial parameter for understanding the electrochemical system, was calculated using the specific function for area analysis. Through this analysis, we obtained valuable information about the electrochemical behavior of the system under study. The type (Area Type) parameter was used to based on results described in Fig. 2 determine the absolute area of the VC, considering all current values, both positive and negative. This area under the curve represents the quantification of charge transferred during the redox process, providing information about the efficiency of electrode modification and the interactions between the analyte and the electrochemical surface. The joint analysis of the area under the VC curve with the physicochemical characterization of the analyte and the developed electrochemical system is fundamental for a complete understanding of the redox process and the efficiency of electrode modification. This approach allows optimization of the electrochemical system for specific applications, such as electrochemical sensors and biosensors.

The results show a higher current in graphite with adsorbed graphene oxide. On comparing the percentage areas in value, we observed the following: A-B: 161.936349 % gain; C-D: 48.199199 % gain; A-C: 195.7666787 % graphite gain with graphene adsorbed on initial activation; and B-D: 67.339893 % graphite gain with adsorbed graphene in the final activation.

Previous research has reported that the use of graphene oxide compared to graphene is more advantageous in aqueous solutions due to the ability to increase the surface area of the electrodes, improve electron transfer, and increase the conductivity [7,30].

Graphene-based electrochemical detection platforms are responsible for increasing the surface area, electron transfer, sensitivity, and specificity of these devices [3].

Graphene oxide interacts well with biomolecules, showing good biocompatibility. It has several applications in the health sector and can be used both in diagnoses and disease treatments. It has antimicrobial, antitumor, antioxidant, and antiparasitic actions; it can also be used to identify various biomolecules [17,20,34]. However, graphene oxide presents better results when it is reduced [1,14].

Based on results described in Fig. 2, we initially proceeded to develop an immunosensor, comparing the performance of commercial graphene (DPR-110 GPH) and graphite with adsorbed graphene oxide (DPR-110-GO). The results showed that graphene was able to increase the surface area, but graphene oxide provided a better reading area, enabling improved detection of anti-*Leishmania* antibodies in sera from asymptomatic individuals (Fig. 3).

The graphene oxide (GO) modified electrode showed a higher percentage current response compared to the commercial graphene electrode. This difference can be attributed to the structural characteristics of the GO-modified electrode, such as its larger surface area, which facilitates the adsorption of the analyte and, consequently, increases the sensitivity of the platform for the detection of anti-*Leishmania* antibodies. In summary, the search for a greater percentage difference in the current response aims to optimize the detection of anti-*Leishmania* antibodies, allowing a more precise differentiation between patients with the disease and those with other conditions that may present crossover results. The structural characteristics of the GO-modified electrode, such as its greater surface area, contribute to this optimization of the platform.

Based on these results (Fig. 3), we chose to follow the tests with graphite electrodes with adsorbed graphene oxide since this modification presented a better potential for the construction of this electrochemical immunosensor. This platform makes it possible to enhance the properties of graphene oxide and provides a better financial cost-benefit compared to the diagnostic proposal presented. Furthermore, in electrochemical tests evaluated by CV, the results can follow the analysis based on oxidation or reduction; in this work, we chose to follow our analysis evaluating the oxidation peaks, as these exhibited better performance on the target platform.

The article by Martins et al. [22] [22] showed the need to incorporate gold electrodeposition to improve the surface area and consequent increase in the detection window. Thus, we tested whether gold electrodeposition would be the best option for the graphite electrode adsorbed with graphene oxide as well. We also decided to test if the graphite electrodeposited with gold platform developed by Martins et al. [22] could detect antibodies anti-*Leishmania infantum* in asymptomatic as well as symptomatic individuals (fig. 4a-b).

In fact, the electrodeposition of gold nanoparticles is necessary to increase the surface area of electrochemical platforms since it is capable of generating a decisive current gain to increase the electrochemical precision. In addition, VL antigens have specificities that favor the electrodeposition of gold [8,22].

The graphite electrode modified with gold demonstrated recognition of anti-*Leishmania* antibodies in the serum of the asymptomatic individual (Fig. 4a), but the detection process was less efficient than the graphite electrode adsorbed with graphene oxide and gold, which showed a significant drop in current (Fig. 4b).

Finally, in addition to demonstrating the ability to detect both symptomatic and asymptomatic individuals, we tested serum samples from chagasic individuals to prove that the differentiation between anti-*Leishmania infantum* and anti-*Trypanosoma cruzi* antibodies also occurred in this electroanalytical platform. As shown in Fig. 5 and the correlation chart in Fig. 6, the detection of individuals positive for CD showed only a slight drop in current, which was considered negative. Fig. 6 also shows an advanced analysis of the performance of the developed electrochemical biosensor, demonstrating its ability to detect anti-*Leishmania* antibodies with precision and efficiency, while in the ELISA method the anti-*Trypanosoma cruzi* antibodies present in samples from chagasic patients were "confused" with antibodies anti-*Leishmania infantum*. This is important because the parasites of the *Leishmania* present structures and proteins similar to *Trypanosoma cruzi*, which causes CD, as both are trypanosomatids. Thus, serological tests for the detection of anti-*Leishmania* antibodies may present false-positive results in individuals with CD and even other diseases [23,36].

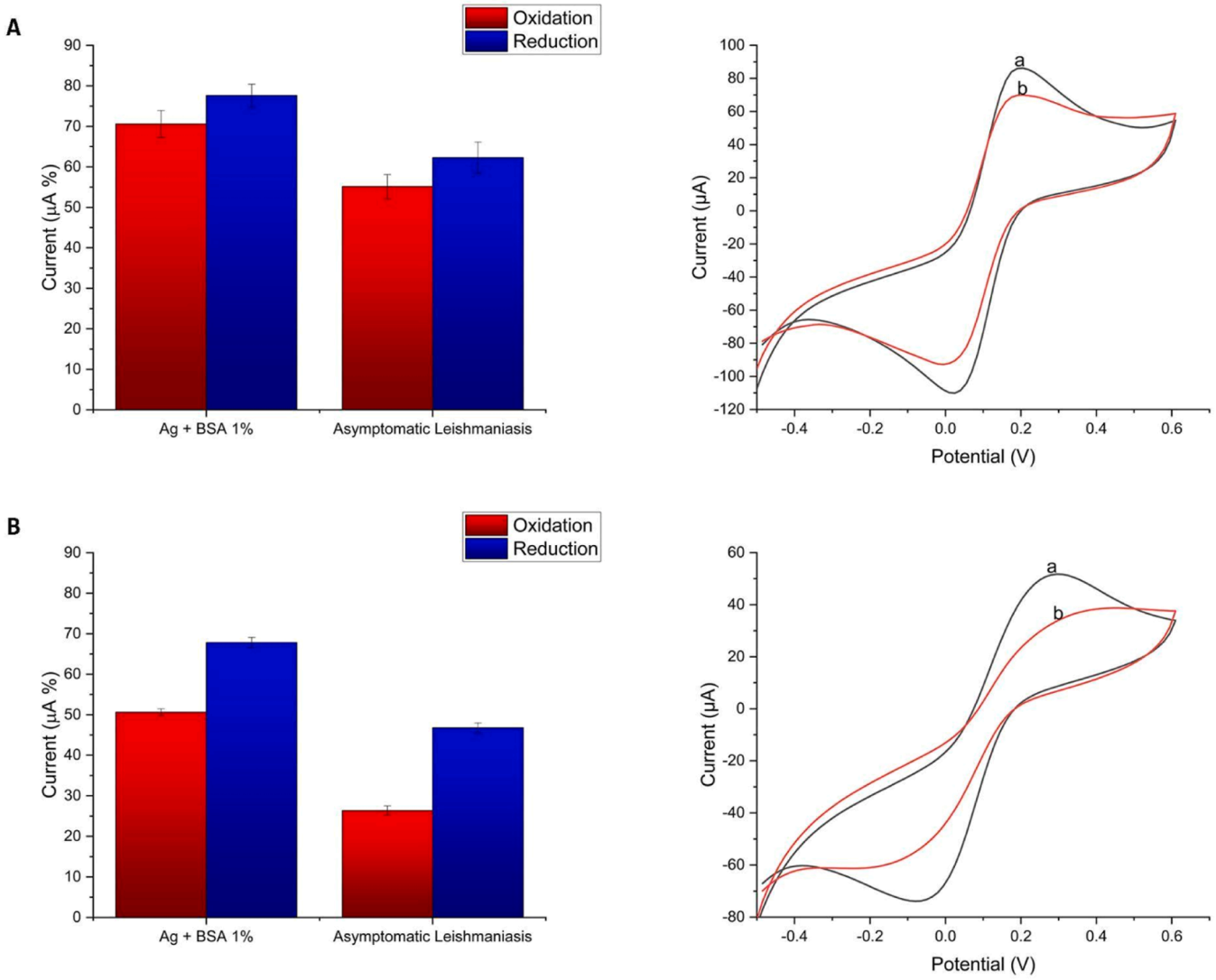

Fig. 3. Cyclic voltammograms for the redox probe and bar plots representing data referring to the detection of anti-*Leishmania* antibodies in an asymptomatic individual for leishmaniasis infection from an endemic area on a commercial graphene platform (A) and adsorbed graphene oxide (B). We emphasize that in "a" we represent the electrochemical platform consisting of a surface modified with biomolecules, such as BSA (bovine serum albumin) and specific antigen, in this case BSA acts as a blocking layer, while the antigen serves as a recognition site for the analyte. In "b" we represent the analyte, in this case, the antibody, which binds to the antigen on the surface of the electrochemical platform, triggering a measurable electrochemical response, this response is used to detect and quantify the presence of the antibody in the tested sample. Error bars represent the mean and standard deviation (SD) of triplicate measurements for each experimental condition.

Sera from individuals positive for cutaneous leishmaniasis were also tested. Although the antigen used is from *Leishmania infantum*, it was expected that there would be some recognition of cutaneous anti-*Leishmania* antibodies since both parasites belong to the same genus and therefore have very similar structures (antigens). A study by Viana et al. [33] even showed that the immunotherapeutic treatment of dogs naturally infected with *Leishmania infantum*, using *Leishmania amazonensis* antigens (etiological agent of cutaneous leishmaniasis) as a basis, resulted in clinical improvement in the animals, with a reduction in the parasite burden, at least initially [33]. Leal et al. (2015) also observed partial protection in mice challenged with *Leishmania infantum* and immunized with a *Leishmania amazonensis* antigen vaccine associated with an adjuvant [16].

The identification of *Leishmania* proteins using bioinformatics tools showed the existence of proteins shared between species as well as the existence of exclusive proteins [19]. The immunosensor developed here was able to differentiate the antibody detection of patients with visceral and cutaneous leishmaniasis, although not as well as the exclusion of patients with CD. However, a form of future improvement would be the purification of antigens from both species to further increase the specificity [10].

In our study, we also performed an immunoenzymatic assay on the same samples used in the immunosensor, and as expected, there was a cross-reaction with CD (Figure S1). These results corroborate data in the literature that indicate high phylogenetic compatibility between the parasites *Leishmania* sp. and *T. cruzi*, which influences the creation of effective diagnoses for the detection and differentiation of diseases caused by these pathogens. In line with this ELISA finding, currently, there are tests available that may have low sensitivity and/or specificity [31]. When analyzing the literature in search of devices aimed at detecting VL (Table 2), it is possible to observe a better rate of sensitivity and specificity of electrochemical techniques compared to the other techniques studied.

Fig. 7 shows the analytical curve of anti-*Leishmania* antibodies. In this redox probe, the current value is inversely proportional to the consumption of antibodies. Sera at different dilutions were analyzed to validate sensor sensitivity analyses. The sera were diluted in the following ratios: 1, 1:3.5, 1:50, 1:1.000, and 1:100.000. This plot shows

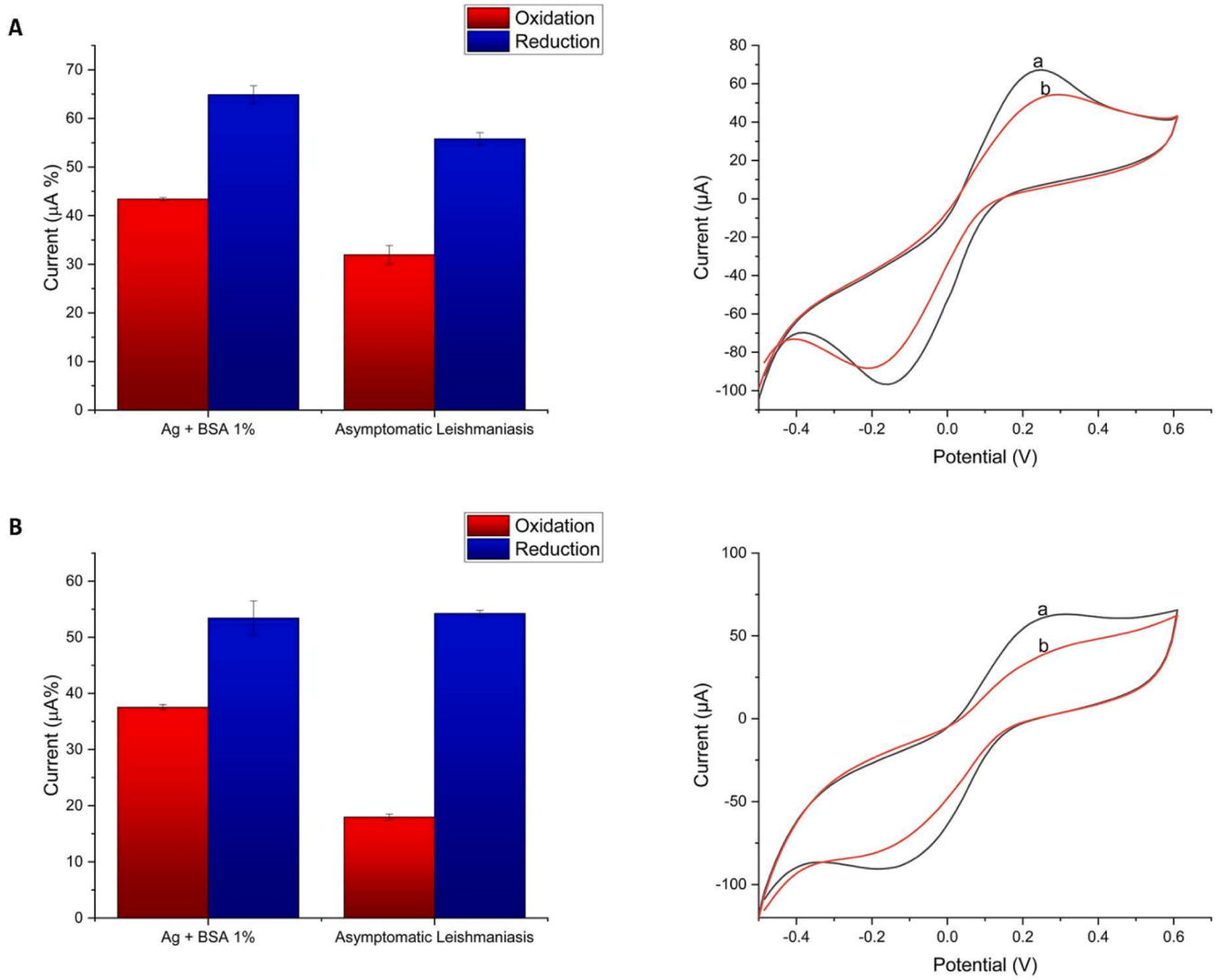

Fig. 4. - Cyclic voltammograms for the redox probe and bar plots representing data referring to the detection of anti-*Leishmania* antibodies in an asymptomatic individual for leishmaniasis infection from an endemic area on a commercial graphite electrode modified with gold (A) and commercial graphite adsorbed with graphene oxide and modified with gold (B). We emphasize that in "a" we represent the electrochemical platform consisting of a surface modified with biomolecules, such as BSA (bovine serum albumin) and specific antigen, in this case BSA acts as a blocking layer, while the antigen serves as a recognition site for the analyte. In "b" we represent the analyte, in this case, the antibody, which binds to the antigen on the surface of the electrochemical platform, triggering a measurable electrochemical response, this response is used to detect and quantify the presence of the antibody in the tested sample. Error bars represent the mean and standard deviation (SD) of triplicate measurements for each experimental condition.

the correlation coefficient of 0.99889 (for the equation: i(%) = 49.6972× 7.69631[serum dilution ratio]), revealing a quantification limit of 17.25 % and detection limit 5.75 % (in current percentage data) or revealing a quantification limit of 16.75 mg/mL and detection limit 5.58 mg/mL (SBP-14). Inset shows tolerance from linear regression of a peak current (%) vs. concentration of antibodies of leishmaniasis.

Thus, given our results, we carried out an analysis of the surface of the electrodes using the Raman spectrophotometry technique. This tool is very useful in the characterization of carbonaceous materials, such as graphene and its derivatives. These materials have about 3 absorption bands and vibrational modes that are active in Raman spectroscopy, giving rise to the D and G bands. The D band (appearing around 1330 cm-1) is the result of the breathing mode of the six-membered ring formed by sp2 carbon.

In a perfect crystalline lattice, the vibrational mode is prohibited. So in graphene, this band appears with low intensity. However, if there are defects in the crystalline lattice — the result of oxidation, for example, in the preparation of graphene oxide (GO) — the intensity of this band increases. The G band, on the other hand, arises from two vibrational modes (also in the plane, originating from E2g and E1u) around 1590 cm-1 [12]. These bands can be used as an indication of material change. Generally, in GO, the intensities of these bands are very close, with the G band exhibiting slightly higher intensity. The ratio of the intensities between these bands, known as ID/IG bands, is typically below 1.

The production of reduced graphene oxide (rGO) via GO reduction results in a small shift in the number of waves (cm-1) of absorption in the D, G, and 2D bands, with a more significant increase in the intensity of the D band resulting in a larger ID/IG than the GO [9,15]. In the region of approximately 2700 cm-1, the 2D band appears. This band is a D harmonic band, which occurs in a vibrational spectrum of a molecule when the molecule makes a transition from the ground state ($v = 0$) to the second excited state ($v = 2$) [12].

When analyzing the results, we observed that the Raman spectrum of the commercial graphene electrode (Fig. 8a- 1) presents a characteristic profile of a material composed of graphene, as the D band presents low intensity, as mentioned before. The spectra of the initial samples of the

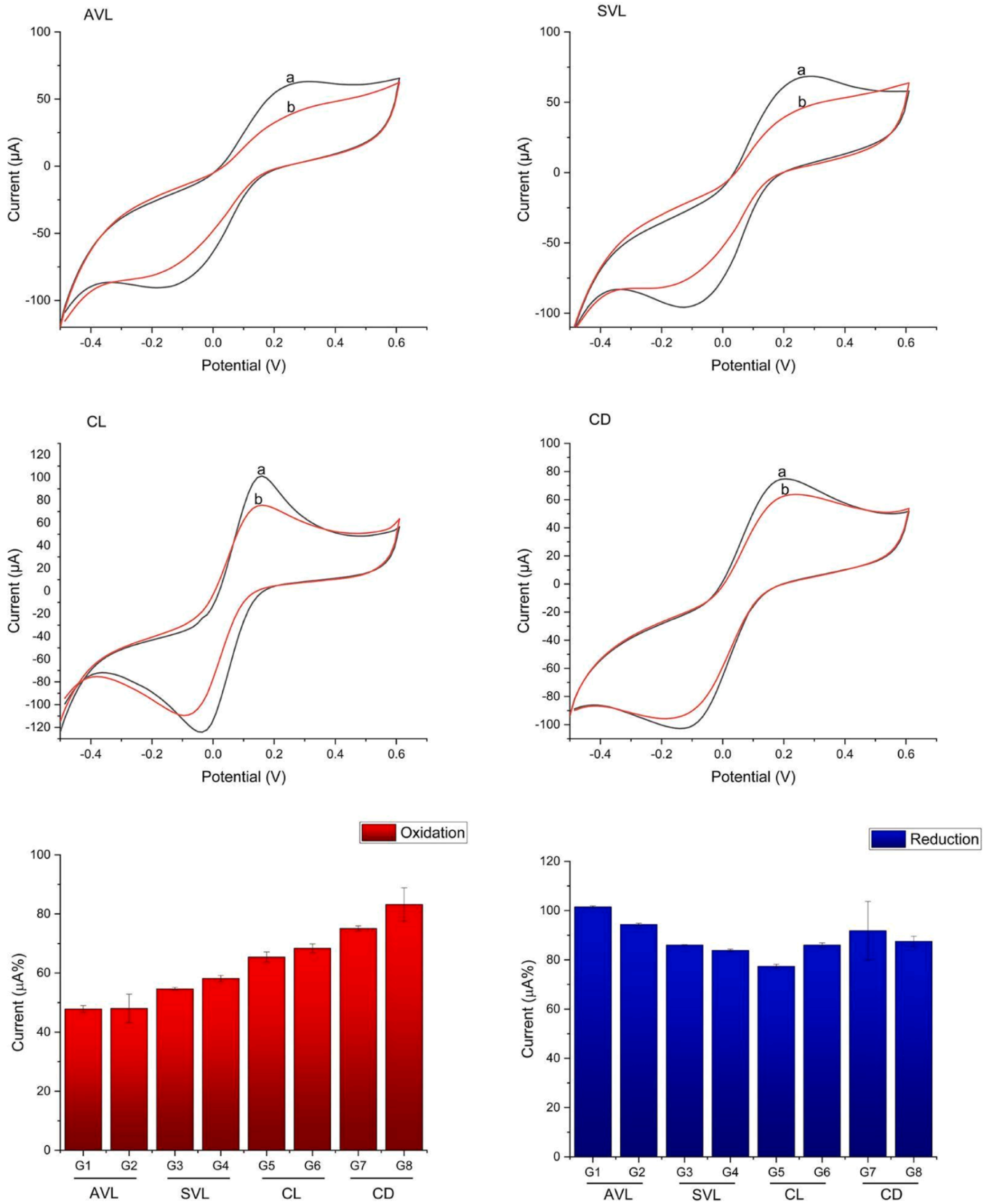

Fig. 5. Graphite electrode with adsorbed graphene and electrodeposited gold, where we can observe asymptomatic visceral leishmaniasis (AVL), symptomatic visceral leishmaniasis (SVL), cutaneous leishmaniasis (CL), and Chagas disease (CD). We emphasize that in "a" we represent the electrochemical platform consisting of a surface modified with biomolecules, such as BSA (bovine serum albumin) and specific antigen, in this case BSA acts as a blocking layer, while the antigen serves as a recognition site for the analyte. In "b" we represent the analyte, in this case, the antibody, which binds to the antigen on the surface of the electrochemical platform, triggering a measurable electrochemical response, this response is used to detect and quantify the presence of the antibody in the tested sample. Error bars represent the mean and standard deviation (SD) of triplicate measurements for each experimental condition.

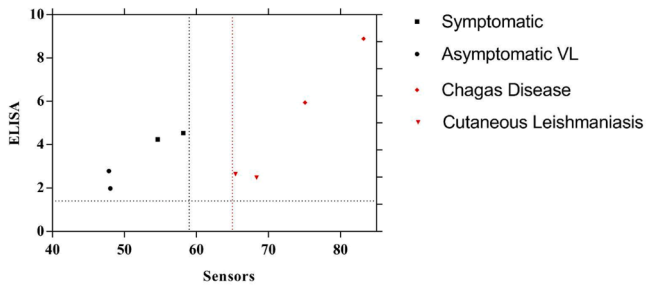

Fig. 6. Correlation graph between results from electrochemical sensors (x-axis) and ELISA results (y-axis), where we can observe asymptomatic visceral leishmaniasis, symptomatic visceral leishmaniasis, cutaneous leishmaniasis, and Chagas disease.

Table 2
Comparison of Visceral Leishmaniasis detection methods.

Method	Material	Sensitivity/ Specificity	References
ELISA	DNA/blood	91.47–97.5 %/100 %	[1,6]
PCR	Urine	93.8 %–95 %/100 %	[1,4]
PCR	Serum	80.7–93.9 %/95.7–100 %	[7]
Electrochemical	Serum	100 %/100 %	[2,5]
Immunochromatographic	Serum/total blood*	92.7–96.3 %/100 %	[3*,6]

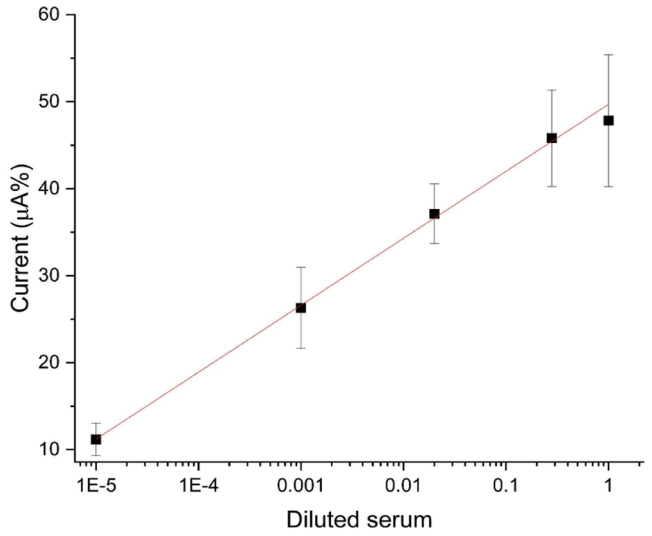

Fig. 7. Analytical curve obtained from current peak percentages for measurements of the biosensors in the presence of diluted serum (1; 1:3.5; 1:50; 1:1000; and 1:100,000) containing antibodies. The percentages were calculated from the initial CV (without biomolecule), totalling 100 %.Error bars represent the mean and standard deviation (SD) of triplicate measurements for each experimental condition. To carry out these tests, graphite modified with graphene oxide and gold was used. The analytical curve of the device developed for leishmaniasis has the "Y" axis representing the current in micro Ampere (%) and the "X" axis representing the dilution of the serum from the positive asymptomatic patient, this provides valuable information about the sensitivity and specificity of the biosensor for disease detection. Thus, the analytical curve shows a gradual increase in current as serum dilution decreases.

pre-treatment graphene electrode (Fig. 8a- 4); 1 cycle between 0.0 and 1.0 V), post-treatment graphene electrode (Fig. 8a- 3); 1 cycle between 0.0 and 1.0 V), graphene electrode in electrochemical reduction treatment with KCl (Fig. 8A- 2); 10 cycles between 0.015 and −1.5 V) showed a small increase in I_D/I_G ratio, as shown in Fig. 8, due to the increase in

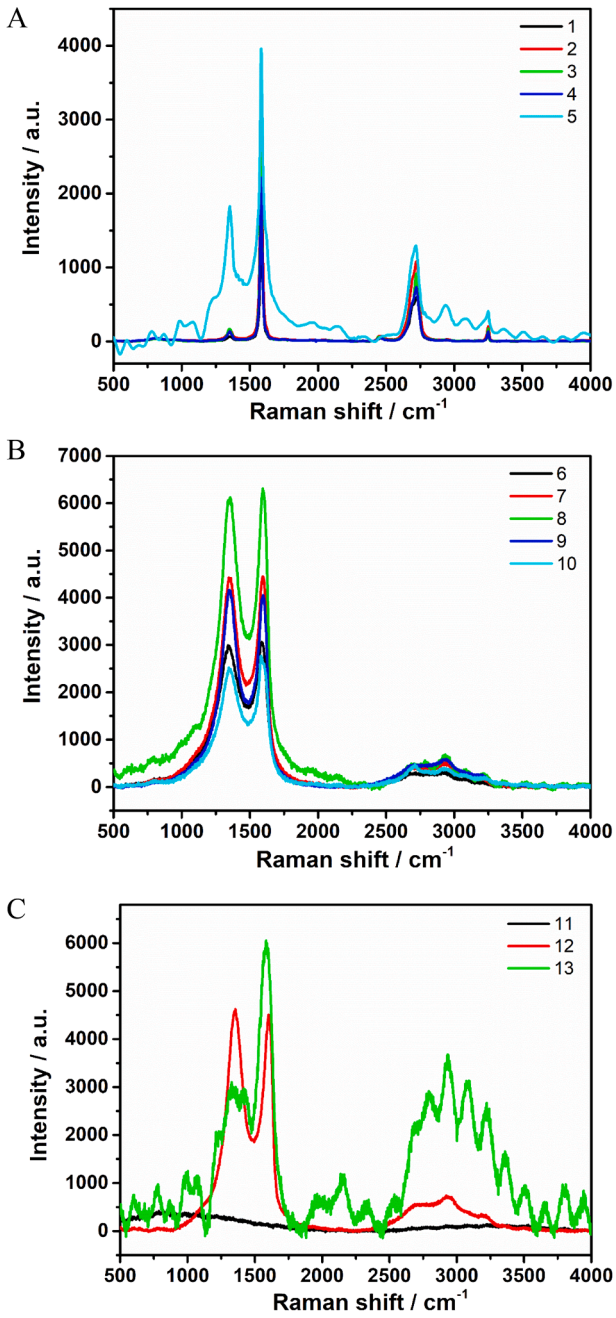

Fig. 8. In (A), representative figure of analysis by Raman spectrophotometry of commercial graphene electrodes. In (1), the commercial graphene data - laser 532 BL-SD are represented, in (2) the reduced commercial graphene data - laser 532 BL-SD are represented, in (3) the pre-reduced activated commercial graphene data - laser 532 BL-SD are represented, in (4) the post-reduced ativated commercial graphene data - laser 532 BL-SD are represented, in (5) the gold-modificed commercial graphene data - laser 532 BL-SD are represented. In (B) representative figure of analysis of commercial carbon electrodes modified with graphene oxide by Raman spectrophotometry. In (6), we observe the graphite electrode laser 532 BL-SD, in (7) we observe the graphite electrode the reduced graphene data - laser 532 BL-SD are represented, in (8) we observe the gold-modificed graphite electrode the reduced graphene data - laser 532 BL-SD are represented, in (9) the post-reduced activated commercial graphite electrode data - laser 532 BL-SD are represented, in (10) the pre-reduced ativated commercial graphite electrode data - laser 532 BL-SD are represented. In (C) representaitive figure of analysis by Raman spectrophotometry of the chemical elements used. In (11) are represented the KCL, in (12) the Grafeno and in (13) the gold - laser 532 BL.

the defect in the crystalline graphene network resulting from oxidation caused by the electrochemical treatment. On the other hand, the Au deposition (20 cycles between −0.4 and 1.2 V and sulfuric acid H_2SO_4), sample commercial graphene electrode and Au, resulted in a greater introduction of defects in the graphene crystal lattice ($I_D/I_G = 0.46$) given the greater potential and greater number of electrochemical cycles in H_2SO_4 used in the preparation of this sample.

The addition of GO to the graphite electrode produced samples with a Raman spectrum characteristic of GO i.e., the intensity of the D and G bands was very close, as can be seen in Fig. 8. The samples commercial graphite electrode and graphene (Fig. 8b– 10) (1 cycle between 0.0 and 1.0 V) resulted in similar I_D/I_G ratios, showing that this type of treatment did not significantly change the material. On the other hand, the samples commercial graphite electrode and graphene (Fig. 8b– 9) (1 cycle between 0.0 and 1.0 V after the electrochemical reduction of GO) and commercial graphite electrode and reduced graphene (Fig. 8B– 7) (10 cycles between 0.015 and −1 0.5 V) resulted in an increase in the I_D/I_G ratio, indicating the conversion of GO to rGO via electroreduction.

Fig. 8 show the spectra of the graphene and gold samples, respectively, while the I_D/I_G ratios for these samples are shown. The difference between is that a baseline correction was performed in Fig. 8a. Upon analyzing Fig. 8b, it is observed that in the region around 1300 cm-1, there does not seem to be an absorption band but only noise. Therefore, even after correcting the baseline, as shown in Fig. 8a, it is not possible to determine the true intensity of the D band for these samples, which in turn affects the obtained I_D/I_G value.

4. Conclusion

The research developed in this work expands the diagnosis of human visceral leishmaniasis, here we highlight the early detection of asymptomatic patients. Through the electrodeposition of gold nanoparticles on carbon electrodes, we developed electrochemical immunosensors with high sensitivity and specificity, overcoming the challenges of traditional methods.

The results demonstrated that the graphite electrode modified with graphene oxide and gold was efficient in detecting anti-*Leishmania* antibodies in symptomatic and asymptomatic individuals, without risk of cross-reactivity with CD. Gold electrodeposition on carbon electrodes modified with graphene oxide provided an ideal platform for the immobilization of specific antigens, allowing the precise recognition of epitopes present in the serum of patients with visceral leishmaniasis.

This innovative approach solves a crucial problem with commercially available screening tests, which often fail to identify asymptomatic patients. Due to the test's high sensitivity and specificity and low cost, it can be a valuable diagnostic tool, especially in regions with limited infrastructure. The results showed that surfaces functionalized with GO and electrodeposited with gold are interesting platforms for the development of an immunosensor.

The biosensor produced presents interesting properties, such as good selectivity and sensitivity. This is a promising technique for molecular analysis of a VL-specific biomarker. Future studies will expand the system to determine VL in plasma and saliva samples, in addition to adding stability and repeatability tests. Our research is not limited to sensor development. We intend to improve and adapt them for public health applications, enabling large-scale, high-performance tests. Future studies will focus on the selection of purified antigens for the diagnosis of asymptomatic patients and the development of sensors with long stability periods.

We believe that electrochemical immunosensors modified with graphene oxide and gold electrodeposition represent a promising tool for the specific and selective diagnosis of visceral leishmaniasis. With a reduced cost compared to Western blot and superior performance to ELISA, this technology paves the way for more effective detection of the disease, especially in its early stages, when treatment is most crucial and the chances of a cure are greater.

Our research contributes significantly to the advancement of the area of biosensors and the fight against visceral leishmaniasis, with a main focus on the early detection of asymptomatic patients. Through the optimization and validation of electrochemical immunosensors, we hope to contribute to future research that aims to transform this technology into a practical and accessible tool for the accurate and effective diagnosis of disease, saving lives and promoting public health.

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CRediT authorship contribution statement

Beatriz R. Martins: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cristhianne Molinero R. Andrade:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Guilherme F. Simão:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Rhéltheer de Paula Martins:** Writing – review & editing, Investigation. **Luana Barbosa Severino:** Writing – review & editing, Investigation. **Sarah Cristina Sato Vaz Tanaka:** Writing – review & editing. **Loren Q. Pereira:** Writing – review & editing. **Marcos Vinicius da Silva:** Writing – review & editing. **Fernanda Bernadelli de Vito:** Writing – review & editing. **Carlo José Freire de Oliveira:** Writing – review & editing. **Helio Moraes de Souza:** Writing – review & editing. **Anderson Barbosa Lima:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Virmondes Rodrigues Júnior:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation. **José Roberto Siqueira Junior:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation. **Renata Pereira Alves:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

The data that has been used is confidential.

Acknowledgments

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7. APÊNDICE B – Lista de participação de co-autores do artigo "A Comparative Study of Graphene-Based Electrodes for Electrochemical Detection of Visceral Leishmaniasis in Symptomatic and Asymptomatic Patients"

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8. APÊNDICE C – Normas da Revista Científica

Disponível em: <https://www.sciencedirect.com/journal/talanta/publish/guide-for-authors>

9. ANEXO A – Demais trabalhos publicados durante o doutorado

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Diagnostic efficacy of electrochemical biosensor compared to RT-PCR for diagnosis of Covid-19: a systematic review

Eficácia diagnóstica do biossensor eletroquímico em comparação com o RT-PCR para o diagnóstico da Covid-19: uma revisão sistemática

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ABSTRACT

In December 2019, the new coronavirus that was named SARS-CoV-2 was identified in China. The virus spreaded quickly and in March of 2020 it was declared a pandemic by the World Health Organization. Due to the initial lack of rapid diagnoses and effective treatments, the health systems were overloaded as a consequence of the high number of infected and severe cases. The diagnostic techniques currently available have limitations, for this reason, the search for new methods with sensitive, fast, cheap and locally used techniques, such as electrochemical biosensors, has been widely explored. Given the advantages of developing electrochemical biosensors for sensitive and selective diagnosis, this systematic review aimed to search for publications on the subject and answer the

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following question: The diagnosis of COVID-19 performed through an electrochemical biosensor, in individuals with suspected disease is it as efficient as that performed by RT-PCR? A study protocol was developed following the PRISMA-DTA guidelines and registered with PROSPERO under the approval code CRD42021282561. Searches were carried out in six electronic databases, inclusion and exclusion criteria were applied and seventeen publications were selected for this review. Based on the data, the analyse of risk was done using QUADAS-2. The results were presented in a descriptive qualitative manner it was not possible to carry out a meta-analysis.

Keywords: Covid 19, electrochemical biosensor, diagnostic.

RESUMO

Em dezembro de 2019, o novo coronavírus, denominado SARS-CoV-2, foi identificado na China. O vírus se espalhou rapidamente e, em março de 2020, foi declarado uma pandemia pela Organização Mundial da Saúde. Devido à falta inicial de diagnósticos rápidos e tratamentos eficazes, os sistemas de saúde ficaram sobrecarregados em consequência do alto número de casos infectados e graves. As técnicas de diagnóstico atualmente disponíveis têm limitações, por isso, a busca por novos métodos com técnicas sensíveis, rápidas, baratas e de uso local, como os biossensores eletroquímicos, tem sido amplamente explorada. Dadas as vantagens do desenvolvimento de biossensores eletroquímicos para o diagnóstico sensível e seletivo, esta revisão sistemática teve como objetivo buscar publicações sobre o assunto e responder à seguinte pergunta: O diagnóstico da COVID-19 realizado por meio de um biossensor eletroquímico, em indivíduos com suspeita da doença, é tão eficiente quanto o realizado por RT-PCR? Um protocolo de estudo foi desenvolvido de acordo com as diretrizes PRISMA-DTA e registrado no PROSPERO com o código de aprovação CRD42021282561. Foram realizadas buscas em seis bancos de dados eletrônicos, critérios de inclusão e exclusão foram aplicados e dezessete publicações foram selecionadas para esta revisão. Com base nos dados, a análise de risco foi feita usando o QUADAS-2. Os resultados foram apresentados de forma descritiva e qualitativa e não foi possível realizar uma meta-análise.

Palavras-chave: Covid 19, biossensor eletroquímico, diagnóstico.

1 INTRODUCTION

In December 2019, the new coronavirus was identified in China, which was named SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). The virus is responsible for causing severe acute respiratory syndrome, with a high rate of transmission and contagion. The disease caused by SARS-CoV-2, called COVID-19, spread quickly. Before the end of January 2020, there were already

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reports of contamination in different locations around the world (RAHMAN et al., 2021; SHAHRIAR et al., 2021).

The World Health Organization (WHO) declared COVID-19 a global pandemic in March 2020 and the world has suffered the consequences of this quick spread. The initial lack of rapid diagnoses, effective treatments and containment of those infected, generated a high number of hospitalizations, which increased expenses and overloaded the health systems of several countries (BÖRGUER et al., 2021).

The first case of genome SARS-CoV-2 was discovered in January 2020 and since then, a lot of research has been done to diagnose the pathology caused by the virus (LAI and LAM, 2021). The best way to control the spread is identifying the disease, therefore the search for effective and fast methods is a constant routine for researchers. The tools most used in the identification of SARS-CoV-2 are molecular tests, serological tests and lateral flow immunoassays (SHARMA et al., 2021).

The thehnique of lateral flow, called as “rapid tests”, are considered to have low sensitivity and may cross-react due to the similarity of SARS-CoV-2 to other coronaviruses. (LAI and LAM, 2021).

The RT-PCR (reverse transcriptase polymerase chain reaction) is a molecular test, considered the gold standard for the diagnosis of COVID-19, however, it has limitations such as high cost, it is required a qualified person to collect and proceed the test, the test take a long period of time to be completed, and also, the results can take long to be released.(SHARMA et al., 2021).

A very good option is the serological tests, however, this type of test depends on the production of antibodies by the body, usually they are produced by our organism only after two weeks of contamination, contraindicating its use in the first days of suspicion of infection (SHARMA et al., 2021). They may also have low specificity and imprecision, due to cross-reaction between SARS-CoV-2 and other types of viruses with a similar structure (TALEGHANI and TAGHIPOUR, 2021).

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Thus, the search for new diagnostic methods with sensitive, fast, cheap and locally used techniques has been explored by scientists around the world. Electrochemical biosensors have these characteristics and can allow accurate and efficient diagnosis. They are devices that measure biological or chemical reactions, they have an integrated receptor and transducer capable of converting a biological response into an electrical signal and measuring it (VARNAKAVI and NOHYUN, 2021).

The Electrochemical biosensors are the most used and studied because they are sensitive and selective devices. Its operation is based on the electrochemical reaction between the bioreceptor and the sample to be analyzed (SALOMÃO, 2018).

Due to the advantages of developing electrochemical biosensors for diagnosis, this systematic review aimed to answer the following question: The diagnosis of COVID-19 performed through an electrochemical biosensor, in individuals with suspected disease, is as efficient as that performed by RT- PCR?

2 MATERIALS AND METHODS

2.1 PROTOCOL AND REGISTRATIO

The question in this review was based on the acronym PIRO (P= Population, I= *Index test*, R= *Reference Standard*, O = *Outcome).*

P = Individuals with suspected COVID-19;

I= Electrochemical Biosensor;

R=RT-PCR;

O = Sensitivity and specificity of the index test;

A protocol was developed following the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies 2020 (PRISMA-DTA) guidelines and registered in the Prospective Register of Systematic Reviews (PROSPERO) under approval code CRD42021282561.

2.2 ELIGIBILITY CRITERIA

Studies that met the following inclusion criteria were included: articles

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published in the last 4 years (2019 to 2022), which compared the diagnosis of COVID-19 using an electrochemical biosensor with the RT-PCR test in samples from individuals with suspected of the disease. The period of search for publications was between 2019 and 2022 due to the emergence of COVID-19 in 2019. The first search in the databases was carried out on 09/26/2021 and the last on March 17, 2022.

Exclusion criteria for articles: 1) They did not use samples of individuals; 2) They did not compare the index test to RT-PCR; 3) Lack of comparator method; 4) Matters unrelated to the topic of the review (diagnosis of other diseases, other matters related to COVID-19 other than diagnosis); 5) Review studies; 6) They used a technique other than electrochemistry; 7) Full-text articles and author contact available in Chinese only; 8) Full text not available.

2.3 DATABASE AND SEARCH STRATEGY

The searches were performed in six electronic databases (PUBMED, SCOPUS, WEB OF SCIENCE, LILACS, EMBASE and LIVIVO). For each database was structured a strategy of searching (supplementary material 1). A gray literature search was also performed on Google Scholar and Open Gray. Endnote Web software was used to manage references and check for duplication, and for organization and selection of publications, we used Rayyan (Intelligent Systematic Review).

2.4 SELECTION OF STUDIES

The selection was performed blindly and independently in two phases. In the first one, two reviewers R1 and R2 (R.F.B and B.R.M) made the selection by reading the title and abstract and identified the studies that they considered to meet the inclusion criteria. The second stage of selection included the reading of the full texts selected in the first stage by the same pair of reviewers, applying the same eligibility criteria. In case of disagreements, a discussion was held between the two reviewers to define the inclusion of the publication. If the disagreement persisted, a third reviewer (R3 – R.P.A.) was invited for the final definition.

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2.5 DATA EXTRACTION

The data extraction was performed by R1, through a spreadsheet, where the following information from the included articles was described: number of clinical samples tested, type of biosensor, sample used, test execution time, sensitivity, specificity, detection limit and cost. Then, after the R1 has completed all the data, the R2 has checked the information, and if found a disagreement between the information collected by R1 and R2, a third reviewer would be required again. In addition, the data on authorship, the year of publication, the objective and then conclusion of the articles were also collected.

2.6 RISK OF BIAS ANALYSIS OF THE INCLUDED ARTICLES

The risk of bias was assessed using the QUADAS-2 tool. The analysis was performed by R1 and R2 independently and the results were evaluated, in the case of different answers, R3 was activated to solve the question.

2.7 SYNTHESIS AND ANALYSIS OF RESULTS

The results were presented in table format and qualitative description. Due to the variability of publications, it was not possible to perform a meta-analysis.

3 RESULTS

3.1 SELECTION OF STUDIES

The Figure 1 shows the steps of slection that was included on the studies, for this revision were included 17 publications in total. Initially, 383 publications were found, of which 143 were excluded due to duplicity and 240 entered the evaluation by reading the title and abstract, with 209 articles excluded and 31 selected for reading the full text. After completing the reading, 15 articles were included in the systematic review.

It was found 3920 citations searching on gray literature, with the first 100 being evaluated. Of these, 31 were excluded due to duplicity and 69 were included for evaluation, 67 of which met exclusion criteria and 2 were included. The appendix 2 of the supplementary material presents the author's name and

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the reason for excluding articles selected for full reading.

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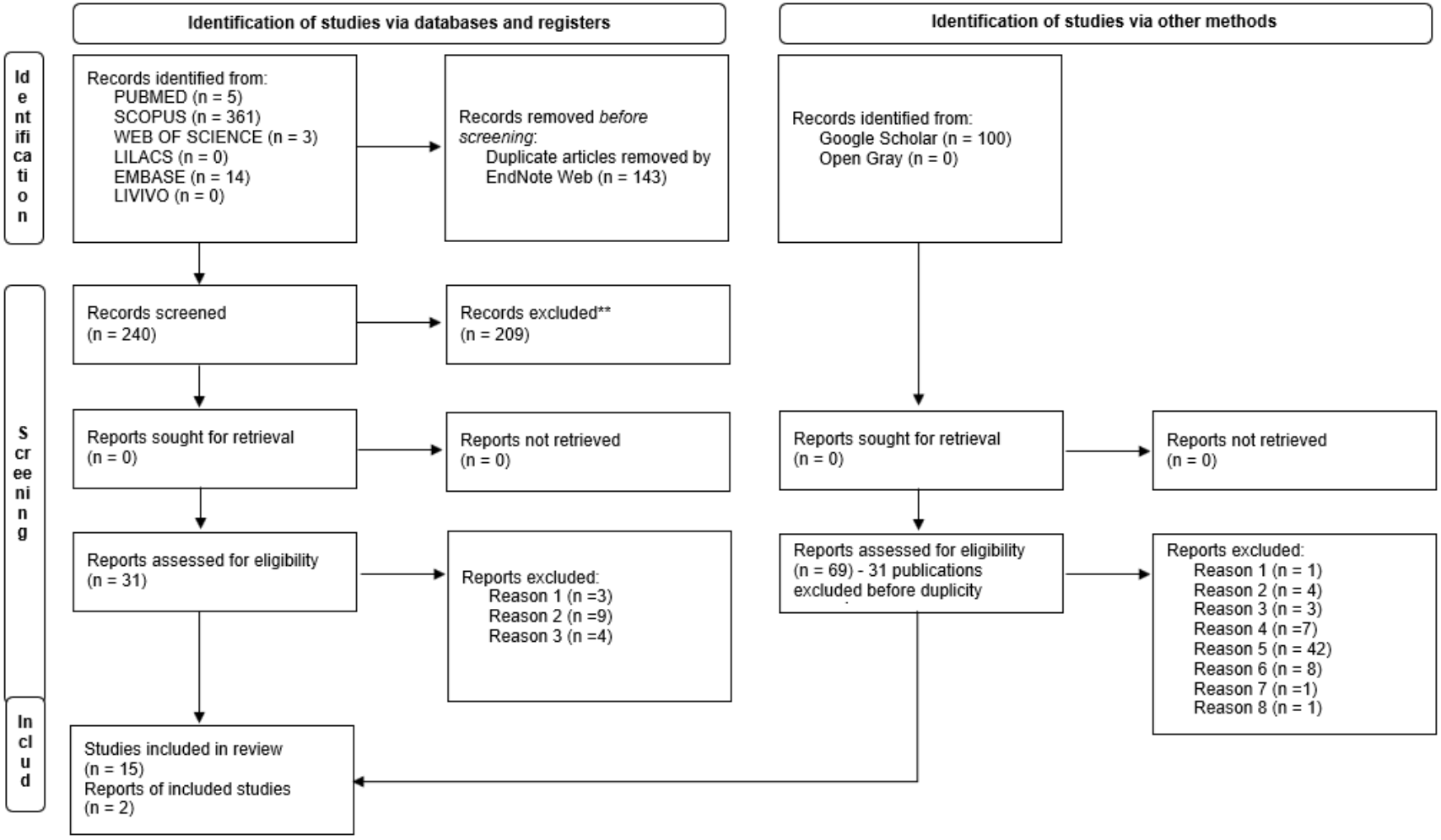
Figure 1 - Flowchart of identification and selection of studies (PRISMA, 2020).



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3.2 CHARACTERISTICS OF THE INCLUDED STUDIES

The articles did not describe the characteristics of the participants and most of the samples used were provided by health institutions. All of them presented the development of the sensor and the technique used. The articles included showed variability in the data. The types and number of samples tested were heterogeneous, in addition to the different measurement units to present the detection limit of each sensor. Not all the publications included were able to describe the sensitivity information, specificity information and detection of time. All the variables extracted from the studies included are described in table 1.

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Table 1 - Characteristics of included studies.

IDENTIFICATION			METHODS				RESULTS				
AUTHOR	**PLACE/ YEAR**	**OBJECTIVE**	**BIOSENSOR TYPE**	**SAMPLE USED**	**TEST EXECUTION TIME**	**Nº TESTED SAMPLES**	**SENSITIVITY**	**SPECIFICITY**	**LIMIT OF DETECTION**	**COST**	**CONCLUSION**
Alafeef et al	United States 2020	To develop a rapid, low-cost, easy-toimplement, and quantitative paper-based electrochemical sensor chip to enable the digital detection of SARS-CoV-2 genetic material.	Genosensor	Nasopharyngeal swab	Not available	48	Almost 100% 231 (copies/µL) -1	Almost 100%	6.9 copies/µL	Not available	Was developed herein an electrochemical platform made up of graphene and gold nanoparticles conjugated with suitably designed antisense oligonucleotides for the rapid, accurate, selective, and ultrasensitive detection of SARS-CoV-2 viral RNA within a time period of less than 5 min. The developed test can detect the early stage of infection. The sensor chip showed a significant change as a response to COVID-19 positive samples, whereas an insignificant change has been observed as a response to the healthy subjects' samples, with a classification accuracy of nearly 100%.
Ayankojo et al	Estonia 2022		Genosensor	Nasopharyngeal swab	20 minutes	8	Not available	Not available	64fM (4.8pg/ml)	Not available	The work demonstrated the electrochemical ncovS1 sensor armed with a molecularly imprinted polymer synthetic receptor (ncovS1-MIP). The sensor has show a possibility rapid diagnostic. The

		Develop electrochemical sensor for rapid detection of SARS-CoV-2 S protein, where the disposable thin-film metal electrodes (Au-TFME) chip was modified with a molecularly imprinted polymer (MIP) film endowed with the selectivity for S protein subunit S1 (ncovS1) and used as a recognition element.									electrochemical characteristics of the sensor could be readily handled by a portable potentiostat allowing on-site measurements thus holding a great potential as a point-of-care testing platform for rapid and early diagnosis of COVID-19 patients.
Beduk et al	Turkey 2021	Was describe a method for the quantification of SARSCoV-2 levels in blood serum by recognizing its protein host cell receptor domain. Was made use of laser-scribed graphene (LSG) and	Immunosensor	Blood Serum	Not available	23	Not available	Not available	2.9 ng/mL	Not available	Was describe a smart antibody sensor based on electrodeposition of electrodeposited gold nanostructures (AuNS) on LSG electrodes for SARS-CoV-2. Was tested the correlation between our proposed test and commercially available test results, and our test yielded the best agreement with the RT-PCR test. The results show that the proposed sensor has the possibility to be an alternative detection method with a convenient detection time. The

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		electrodeposited gold nanostructures (AuNSs) in a disposable electrochemical immunoassay.									authors concluded though further improvements are required for LSG/AuNS electrodes to develop fully optimized POC diagnostic tools, the proposed sensing system offers a good and stable alternative platform for future applications.
Büyüksünetçi, Yudum; Çitil, Burak Ekrem and Anik, Ülkü	Turkey 2021	Develop a diagnostic technique for SARS-COV-2, immobilizing angiotensin-converting enzyme 2 (ACE2) and CD147 on different electrodes and monitoring their electrochemical interactions with protein S.	Immunossensor	Nasopharyngeal swab and Oropharyngeal swab	Not available	82	Not available	Not available	Receptor CD147: 38.99ng mL−1. Receptor ACE2: 299.30 ng mL-1	Not available	An electrochemical approach based on the mechanism of SARS CoV-2 infection was developed. This system has been shown to work for ACE2 and CD147 receptors. Very accurate and effective results were also obtained for analyzes of real samples that were validated with RT-PCR. The authors believe that this system would be effective in detecting existing and new mutations.
Chaibun et al	Thailand 2021	In this study, we describe an electrochemical biosensor based on multiplex isothermal	Genosensor	Nasal swab	< 2 hours	106	99%	Not available	1 copy/µL para genes N e S	Not available	Was developed an electrochemical biosensor coupled with RCA for the highly sensitive and specific detection of SARS-CoV-2. The high amplification capability of RCA and sensitivity of the electrochemical detection method enabled the detection of the viral N and S genes in synthetic linear targets as well as clinical samples. The whole assay took under 2 h to complete, from RNA extraction to the detection step, and does not require the use of a thermal

		rolling circle amplification (RCA) for the rapid detection of the N and S genes of SARS-CoV-2 from clinical samples.									cycler. The performance of the assay with clinical samples was comparable to that of RT-qPCR, which is currently the standard for SARS-CoV-2 detection, whereas also demonstrating zero false-positive results. This approach may have a significant impact in places where rapid detection is required to minimize emerging SARS-CoV-2 outbreaks.
Daniels et al	France 2021	Propose a face mask o collect exhaled breath condensate (EBC); Show the utility of an aptamer-based electrochemical biosensor and to report electrochemical sensing format targeting the spike protein (S) which is embedded in a lipidic membrane forming the SARS-CoV-2 viral outer wall.	Genosensor	Exhaled breath condensate (EBC)	Not available	14	Not available	Not available	3 pfu mL- 1	Not available	The sensor selectively detected SARS-CoV-2. Additionally, was shown that converting exhaled breath vapor into EBC provides a convenient and accessible sample source for SARSCoV-2 viral particles. The work highlighted that EBC can identify SARSCoV-2 by RT-PCR in patients identified as SARS-CoV-2 positive using nasopharyngeal swab samples. Some optimization would need to be implemented to make the sampling step more robust to overcome some false negative issues. Eventual integration of the sensor into the mask itself would likely make the method even more robust and user-friendly. Nevertheless, the results validate the concept that the detection of SARS-CoV-2 in the breath of COVID-19 patients using a rapid aptasensor is feasible.

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Ehsan, M.A; Khan, S.A.; Rehman, A	Saudi Arabia 2021	Present an impedance biosensor for the detection of the SARS-CoV-2 spike protein utilizing the IgG anti-SARS-CoV-2 spike antibody.	Immunosensor	Nasopharyngeal swab	Not available	5	Not available	Not available	0.25 fg/mL	Not available	The fabricated sensors show promised in direct, rapid, and low-cost diagnosis without sample pretreatments. Moreover, the sensor fabrication process could be automated, and such developments were underway in the authors lab.
Eissa, S.; Zourob, M.	Saudi Arabia 2021	Develop a cotton-tipped electrochemical immunosensor that could perform both collection and detection. To report for the first time the combination of cotton fibers and electrochemical assays for the detection of the	Immunosensor	Nasopharyngeal swab	Not available	3	Not available	Not available	0.8 pg/mL	Not available	The cotton-tipped electrochemical immunosensor integrated the sample collection and detection tools into a single platform by coating screen-printed electrodes with absorbing cotton padding. The biosensor did not show cross-reactivity with antigens from other viruses, implying high selectivity of the method. Moreover, the biosensor was successfully applied for the detection of the virus antigen in spiked nasal samples. The signal measurements can be realized using a handheld potentiostat and easily monitored using a smartphone device. The developed cotton based electrochemical immunosensor is a promising diagnostic tool for the

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		SARS-CoV-2 antigen.									direct, low cost, and rapid detection of the COVID-19 virus which requires no sample transfer or pretreatment.
Eissa et al	Saudi Arabia 2021	Develop of a label-free voltammetric-based immunosensor for the determination of SARS-CoV-2 N antigen using gold nanoparticles-modified screen-printed carbon electrodes.	Immunosensor	Nasopharyngeal swab	Not available	Not available	Not available	Not available	0.4 pg.mL −1	Not available	The immunosensor was considered a rapid, low-cost, selective and sensitive diagnostic method capable of being integrated into a portable potentiostat and controlled via a common cell phone for point-of-care testing. Work has focused on detection of the nucleocapsid protein, but future work should focus on comparing sensitivities using other target antigens.
		Develop a sensitive electrochemical biosensor for the detection of SARS-CoV-2 in saliva using an electrochemical assay based on magnetic beads (MBs) and screen-printed electrodes (SPEs) based on carbon black as a sensor									Was developed a smart immunosensor for SARS-CoV-2 detection in saliva by combining the use of MBs as support for immunological chain and carbon black-based SPEs for sensitive and reliable detection. This sensor configuration demonstrated the capability to detect S and N proteins in untreated saliva, without any crossreactivity when tested with others virus. The satisfactory analytical features found in terms of sensitivity, accuracy, and selectivity with the time of analysis, easiness to use, and the requirement of portable instrumentation boost this

Fabiani et al	Italy 2021	combined with a PALM SENS portable potentiostat as a reader. Both SARS-CoV-2 proteins, namely protein S and protein N, were used as a target analyte by developing a sandwich assay with immobilized antibodies to proteins S or N on MBs.	Immunossensor	Saliva	Not available	24	Not available	Not available	Protein S: 19 ng/mL - ProteinN: 8 ng/mL	Not available	biosensor to acquire a relevant position in SARS-CoV-2 device scenario, taking into account also the easy sampling of saliva.
Jo et al	South Korea 2021	To evaluate the clinical performance of the MARK-B test (intended for the qualitative and semi-	Immunossensor	Nasopharyngeal swab	15 minutes		90%	99%	1 x 10^2 pfu/mL	Not available	The MARK-B test, a MESIA-based rapid Ag test, showed higher sensitivity compared to commercial rapid Ag tests for the detection of SARS-CoV-2. Furthermore, the MESIA technique and automated portable device provided results with improved clarity in 15 min as well as reliable semi-quantitative measurement. These results indicate that these rapid Ag tests can be useful for preventing the spread of COVID-19 via timely diagnosis and subsequent containment measures.

		quantitative detection of the SARS-CoV-2 nucleocapsid antigens) based on magnetic force-assisted electrochemical immunoassay (MESIA), compared with RT-PCR and a commercially available rapid antigen (Ag) test.				170					
Kashefi-Kheyrabadi et al	South Korea2021	Develop a nucleic acid amplification-free electrochemical biosensor based on four-way junction (4-WJ) hybridization is developed, which can simultaneously	Genosensor	RNA samples isolated from nasopharyngeal swab samples	Not available		Not available	Not available	For the S and Orf1ab genes, 5.0 and 6.8 ag/μL were	Not available	The S and Orf1ab genes were detected in both synthetic and clinical samples thanks to signal amplification capability provided by nanotextured electrodes and high sensitivity of the 4-WJ based electrochemical detection method. This approach has the following advantages: (i) multiplexed detection that avoids the generation of falsenegative results; (ii) high specificity and ability to differentiate between

		detect SARS-CoV-2 spike (S) and open reading frame (Orf1ab) genes within 1 hour.				21			determined, respectively.		closely related RNA target sequences down to single nucleotide substitution; (iii) a single step procedure and short assay period; (iv) low LOD that satisfies sensitivity requirement and could potentially be used to detect SARS-CoV-2 RNA targets in the early stages of the disease while the viral genes load is low.
Lasserre et al	United Kingdom 2021	To present an impedimetric SARS-CoV-2 biosensor using SARS-CoV-2 truncated aptamers, compatible with lowcost electrode systems	Immunossensor	Nasopharyngeal swab	Not available	Not available	Not available	Not available	Not available	Not available	Showed the development of a specific aptamer sequence for the SARS-CoV-2 spike protein and its subsequent use for the detection of the virus from complex clinical samples. The detection system as presented has several key advantages including a simple impedance measurement to determine target binding, a high stability aptamer receptor to detect the spike protein, and the use of low-cost gold electrodes, similar to blood glucose sensors.
Liv et al	Turkey 2021	To develop a newly designed, easy-to-prepare, and more sensitive graphene oxide-modified glass carbon electrode sensor to	Immunosensor	Gargle and mouthwash samples	Not available	110	93.30%	92.50%	Not available	Not available	The developed method showed excellent reliability and precision for the diagnosis of COVID-19 in real samples and a perfect agreement with the RT-PCR results. The sensor could be used even long after preparation and a sensor could be used three times on positive samples which would minimize the cost of testing. Easily

		voltammetrically determine SARS-CoV-2 spike antigen protein.									manufactured and supplied as a ready-to-use kit on a commercial scale.
Rahmati et al	Iran 2021	To suggest a new ultrasensitive electroanalytical nanobiodevice made using Screen-printed carbon electrode (SPCE) modified by Staphylococcal Protein A and Cu_2O nanotubes as a substrate for the orderly orientation of IgG antibodies as a specific receptor.	Immunosensor	Nasopharyngeal swab	Less than 20 minutes	8	Not available	Not available	0.04 fg mL−1	Not available	Was developed an electrochemical nanobiodevice which was applied for rapid screening of people suspicious to SARS-CoV-2 with the aim of facilitating the point-of-care diagnosis. In this sense, the disposable SPCEs were modified with Cu2O NCs, and, then, the ProtA layer was used to immobilize the IgG antibody, as a receptor element, in the regular direction. This sensor was able to be used in clinical samples to detect the SARS-CoV-2 virus in less than 20 min, without any cross-reactivity when tested with influenza viruses 1 and 2.
Raziq et al	Estonia 2021	Report development of a molecularly imprinted polymers (MIP) based electrochemical	Immunosensor	Nasopharyngeal swab	Not available		Not available	Not available	0.7 pg/mL	Not available	Was developed first time a portable electrochemical sensor integrated with a molecular imprinted polymer (ncovNP-MIP) as a synthetic recognition element

		sensor for detection of SARS-CoV2 nucleoprotein (ncovNP).				8					capable of selective detection of SARS-CoV-2 antigen (ncovNP). The results of the sensor performance validation in the clinical samples of the nasopharynx swabs of patients were promising, confirming the capability of the sensor to detect ncovNP in the complex biological media.
Torres et al	Estados Unidos 2020	To describe a simple, inexpensive, and rapid test for the detection of SARS-CoV2. The RAPID 1.0 that transforms biochemical information from a specific molecular binding event between the SARS-CoV2 spike protein (SP) and angiotensin-converting enzyme-2 (ACE2) into an electrical signal that can be easily detected.	Genosensor	Saliva and Nasopharyngeal/oropharyngeal swab	4 minutos	Saliva: 50 Nasopharyngeal/oropharyngeal swab: 139	Saliva: 100% Nasopharyngeal/oropharyngeal swab: 83.5%	Saliva: 100% Nasopharyngeal/oropharyngeal swab: 100%	1.16 PFU mL−1	$4.67	The biosensor that has been described is inexpensive and portable, allowing for decentralized diagnosis at the point of care. The detection time (4 min) was significantly shorter than existing diagnostic tests and could be further reduced using engineered versions of human ACE2 with improved selective binding to SARS-CoV-2 SP. The use of these ACE2 variants would also help to reduce the false positive rate in complex biofluids such a saliva.

Source: Authors

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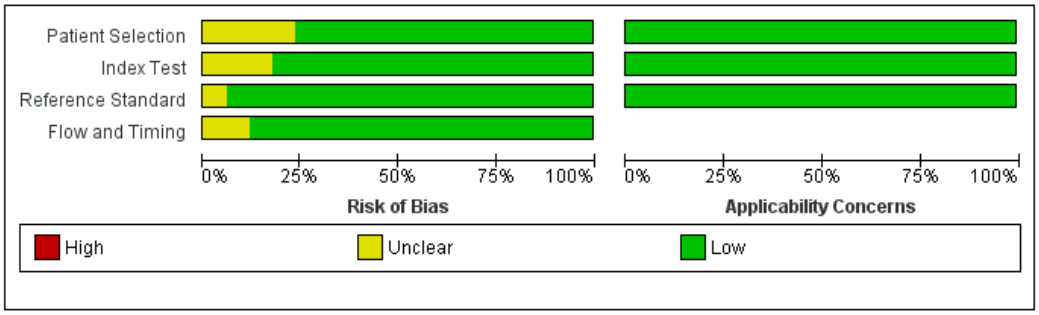
3.3 RISK OF BIAS ANALYSIS

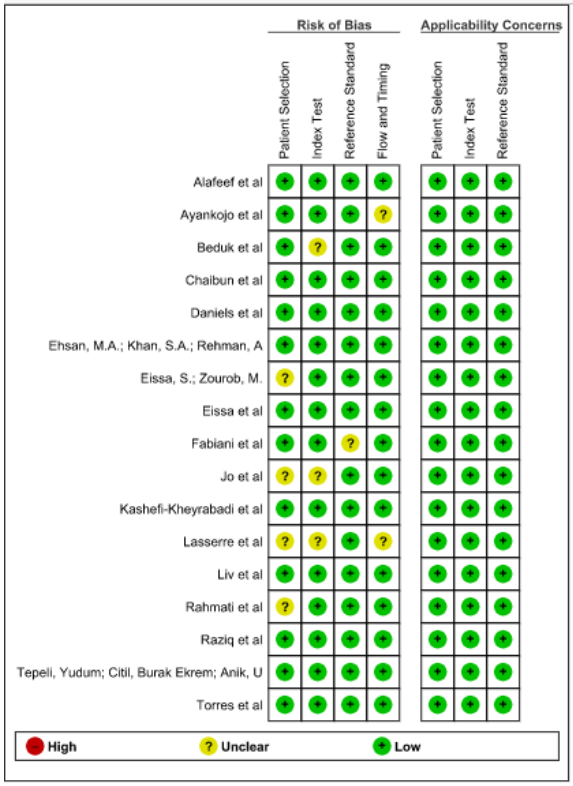
The assessment of the methodological quality of the included studies was performed according to the QUADAS-2 tool.

Figure 2 - Summary of the QUADAS-2 methodological quality assessment



Source: The author - through the Review Manager 5.4 program.

Figure 3 - Methodological quality of studies included in QUADAS-2 (individual assessment).



Source: The author - through the Review Manager 5.4 program.

The QUADAS-2 was adapted for the evaluation of studies included in this review, as it is generally used to evaluate methods already used in patients, which is not the case with the biosensors described here. All the devices presented are still in the experimental phase and they were tested only on samples provided. We realized that this may have interfered with our analysis, as some QUADAS-2 questions had no answers available in the articles. It is noted that 58.8% of the studies had a low risk of bias in all domains. Approximately 41.2% of the articles present uncertain risk in one or more of the one method`s domains.

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3.4 INDIVIDUAL STUDY RESULTS

Alafeef et al (2020) aimed to develop a graphene-based electrochemical biosensor. The device's performance was tested on nasopharyngeal swab and saliva samples and the authors concluded that the device outperformed serological tests, had an excellent detection limit, quick response, good shelf life, and reasonable cost.

Ayankojo et al (2022) reported the development of an electrochemical sensor modified with molecular imprint polymer with selectivity for one of the Spike protein subunits. The results coincided with those of RT-PCR, however, there was a difference in the intensity of the positive response and the authors discussed the possibility of dissimilarity in viral load and disease stages at the time of sample collection. The sensor was considered possible for rapid diagnosis, compared to available antigen tests.

Beduk et al (2021) used blood serum as a sample. The objective of this work was to describe a graphene immunosensor enriched with gold nanostructures. The samples tested showed agreement with RT-PCR. The sensor was considered a good alternative for detection, however, it was mentioned that modifications and improvements are needed.

Büyüksunetçi, Y.; Çitil, B.E. and Anik, U. (2021), developed an immunosensor based on the electrochemical interactions of angiotensin II converting enzyme and CD147 with the Spike protein of SARS-CoV-2. The device was tested on real oropharyngeal and nasopharyngeal swab samples and compared to RT-PCR results. The authors concluded that the response was accurate and effective.

Electrochemical biosensor for rapid isothermal rolling-circle amplification (RCA) based detection of N and S genes through nasopharyngeal swab was described by Chaibun et al. (2021). The tested samples showed results in agreement with the RT-PCR tests, without false-positive results.

Daniels et al (2021) developed an aptamer-based biosensor using a face mask to collect exhaled breath condensate as a sample for Spike protein detection. The results showed that the detection of SARS-CoV in the breath of

infected patients is feasible, however, they reported that some adjustments should be made to avoid false-negative problems.

The publication by Eissa, S. and Zourob M. (2021), integrated the collection method with the detection method. The development of an immunosensor for identification of the SARS-CoV-2 nucleocapsid virus antigen on cotton tip was described. Test results on clinical specimens showed compliance with RT-PCR tests.

For detection of Spike protein, Ehsan, M.A., Khan, S.A. and Rehman, A. (2021), reported an impedance immunosensor using the anti-SARS-CoV-2 IgG antibody. The sensor was tested on real samples, showing agreement with the RT-PCR tests. The results showed high selectivity and good reproducibility.

Eissa et al (2021) described an immunosensor for detection of the nucleuscapsid antigen, using carbon electrodes enriched with gold nanoparticles. Nasopharyngeal swabs were used as clinical samples. The research shows a strong correlation between test and RT-PCR results.

Fabiani et al (2021) developed an immunoassay to detect Spike protein or nucleocapsid protein in saliva. The test was compared to RT-PCR, showing high agreement.

Jo et al (2021) developed an immunosensor for SARS-CoV-2 antigen detection. The sensor was tested on nasopharyngeal swabs from patients with symptoms of COVID-19. ROC curve analysis was performed to assess device sensitivity and specificity. The authors confirmed that the test results had high agreement with RT-PCR and concluded that the sensor can be useful in rapid diagnosis.

Kashefi-Kheyrabadi et al (2022) described a biosensor for detecting spike and open reading frame genes (Orflab). To test the detection, the RNA samples were isolated from nasopharyngeal swabs obtained from medical laboratories. The sensor was able to identify positive and negative samples according to RT-PCR.

An antigen-based aptamer sensor was developed by Lassere et al (2022). Oropharyngeal and nasal swab samples were used to test the sensor that

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distinguished positive and negative samples.

Liv et al (2021) presented the detection of Spike SARS-Cov-2 protein antigen in gargle and mouthwash samples. Compared to RT-PCR, the sensor showed 91.7% of correct results. The authors concluded that the method described had good reliability, accuracy and would be an option for point-of-care diagnosis.

Rahmati et al (2021) developed an immunosensor to detect Spike protein. The device was tested on real nasopharyngeal swab samples and the results proved to be reliable.

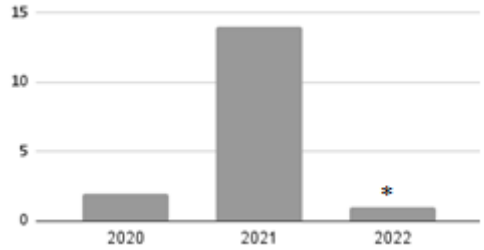
Raziq et al (2021) reported a sensor based on molecularly imprinted polymers for SARS-CoV-2 nucleoprotein antigen detection. Nasopharyngeal swabs from patients were previously tested by RT-PCR and then by the developed device, which showed promising results.

Torres et al (2021) described the development of a genosensor for the detection of SARS-CoV-2. The device was tested on real naso/oropharyngeal swab and saliva samples. Compared to RT-PCR, the sensor demonstrated high sensitivity, specificity and accuracy for both types of samples.

3.5 SUMMARY OF RESULTS

In this review, the data were summarized in a qualitative and descriptive manner.

Graph 1 - Number of publications per year



*Searches were performed until March 17, 2022.
Source: The author

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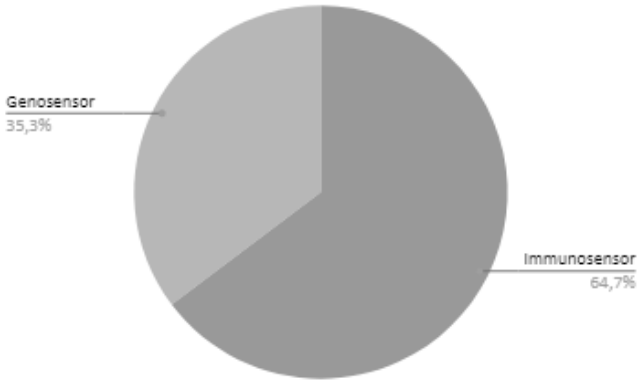
Graph 2 - Percentage of immunosensors and genosensors



Source: The author

Table 2 - Detection target of devices described in publications included in the review

Author	Detection target
Alafeef et al	Viral RNA
Ayankojo et al	Spike proteinsubunit S1
Beduk et al	Spike Protein
Büyüksünetçi, Y.; Citil, B. e. and Anik, U.	Spike Protein
Chaibun et al	N and S genes
Daniels et al	Spike Protein
Ehsan, M.A.; Khan, S.A.; Rehman, A.	Spike Protein
Eissa, S.; Zourob, M.	N Protein(nucleocapsid)
Eissa et al	Nucleocapsid Antigen
Fabiani et al	Spike Proteinand N Protein
Jo et al	Nucleocapsid Antigen
Kashefi-Kheyrabadi et al	S Gene and Orflab Gene
Lasserre et al	Spike proteinsubunit S1
Liv et al	Spike Protein
Rahmati et al	Spike Protein
Raziq et al	Nucleoprotein (ncovNP)
Torres et al	Spike Protein

Source: Authors

4 DISCUSSION

As mentioned and described, there was heterogeneity in the publications included in this review, from the sensor development methodology, to the number of samples tested and description of sensitivity and specificity. According to Cronin et al, 2018, when there is heterogeneity, it is appropriate to perform only the systematic review and not perform the meta-analysis.

The first descriptive analysis of the results was performed in relation to the year of publications included. The COVID-19 pandemic was decreed in March 2020 (PAHO, 2020) and due to uncertainties regarding the transmission,

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diagnosis and treatment of the disease, social isolation was the first measure to try to contain the spread of the virus. (BANERJEE and MAYANK, 2020).

According to Banerjee et al, 2020, at the beginning of the pandemic, health and educational institutions were greatly affected. Most universities stopped their activities, including research, and many professionals turned their occupations to assistance on the front lines of fighting the disease, which explains the small number of publications found by our search this year. In 2021, restrictions were relaxed and activities resumed, significantly increasing the number of studies.

Another point to be discussed in our review is the types of biosensors presented in the included studies. Rahman et al 2021 report that since the beginning of the pandemic, rapid and effective detection of the virus has become essential in order to identify the disease and contain its spread.

According to Lai and Lam, 2021 after the sequencing of the first genome of SARS-CoV-2, several tests were developed for the diagnosis of COVID-19, including rapid tests. These diagnostic methods varied in technology and detection target. As per our research we noticed with the use of sensonr the outcome was the same. Separating the tests only by the type of sensor developed, we observed 35.3% of the publications describing a genosensor and 64.7% presenting the description of an immunosensor.

Genosensors are biosensors that use DNA or RNA as recognition elements, based on the hybridization of the chains that occur in the electrode (YANG, MCGOVERNAN and THOMPSON, 1997; LIU et al., 2012). On the other hand, immunosensors are based on the binding between antigen and antibody, which form a stable complex (BAHADIR and SEZGINTÜRK, 2015).

Although genosensors have advantages over immunosensors such as lower cost and the need for less biomolecule in detections, immunosensors were predominant in the studies included in this review. The smaller number of genosensors reflects some difficulties in working with nucleic acids. According to Wang et al, 2021, techniques based on DNA/RNA samples have challenges such as the difficulty of maintaining structural integrity and the need to work with controlled and elevated temperatures.

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We also found a lot of diversity in the detection targets of the developed biosensors. This variability is not just a characteristic of sensor development methods. The range of different diagnostic tests and targets provides the option of choosing the best protocol, evaluating the patient's clinical conditions and the advantages and disadvantages of each technique (MOHAMADIAN et al., 2021).

We note that most studies chose the spike protein as a detection target, an external protein that mediates between the virus and the host cell receptors, playing an essential role in the entry and replication of the same, in addition to the junction of the virus with the membranes of the cells. (HARVEY et al., 2021).

We also observed the use of different clinical samples (nasopharyngeal, oropharyngeal and nasal swab, saliva, blood serum, exhaled breath condensate, gargle and mouthwash samples and isolated DNA). Borger et al. 2021 reported in a systematic review of the diagnostic accuracy of COVID-19 tests that the choice of sample type is important for successful diagnosis and concluded in their meta-analysis that respiratory samples were the ones that achieved the best sensitivity rates, despite of not being the most easily collected samples. Even so, these results explain the greater use of respiratory samples in the sensors presented in our studies.

We noticed the use of nanomaterials and nanoparticles in the presented sensors. Graphene, a type of nanomaterial, has been used in several devices, in agreement with the data available in the literature, which report that the use of the material has been widely diffused for improving the sensor surface, having high electrical conductivity, facilitating the transfer of electrons and excellent biocompatibility with different biomolecules (SZUNERITS and BOUKHERRROUB, 2018; BAHAMONDE et al., 2018).

The use of nanoparticles in biosensors, mainly made up of noble metals, is widespread in the literature. We verified the use of these nanostructures in some of the studies included in the review in order to amplify the detection signal, conductivity in electron transfer, consequently improving the performance and sensitivity of the device (TAN et al., 2020; IBRAHIN et al., 2021). In addition, the use of aptamers was also observed in some of the works included in this review.

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Aptamers are single-stranded RNA or DNA molecules designed to bind to specific substrates, having high affinity. They can be composed of proteins, nucleotides and oligonucleotides and, like nanoparticles, their use aims to improve sensor performance due to their specificity, low cost and great compatibility (MACKAY et al., 2014; XIANG et al., 2020; MO et al., 2021).

Data regarding the detection limits of the tests presented were also collected, which made it difficult to compare the limits presented. However, there was variability in the values and measurement units described. The measurement units are all sensitive and vary between plaque forming units (pfu/mL), copies/µL, nanogram (ng/ml), picogram (pg/mL), phentogram (fg/mL), attogram (ag/mL).

The sensitivity and specificity values of the publications included in the review were presented by only 29.41% and 23.53%, respectively, hindering the comparison of information and the performance of statistical analysis.

5 CONCLUSION

The diagnosis of COVID-19 performed through an electrochemical biosensor is on its way to being as efficient as that performed by RT-PCR. The articles found in this review show that electrochemical biosensors are effective in the diagnosis and most of the results were convergent with RT-PCR. However, there are some limitations for the proper comparison, among them: variability in the units of detection limits, lack of description of sensitivity and/or specificity and low number of clinical samples tested in most articles. Tests are required standardizing the methodology and using more samples for a safe statement.

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Biomolecules present in tick saliva with pharmacological potential: a systematic review

Biomoléculas presentes na saliva do carrapato com potencial farmacológico: uma revisão sistemática

Biomoléculas presentes en la saliva de garrapatas con potencial farmacológico: una revisión sistemática

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Abstract
Knowing that ticks have bioactive molecules in their saliva which modulate hemostatic and immunomodulatory activities in humans, we carried out a systematic search for biomolecules present in tick saliva with great pharmacological potential. We evaluated studies published in the last ten years. Following the recommendations of the Prisma tool, primary and secondary studies of a systematic nature were selected, with no language or country restriction. Studies that included arthropods other than ticks and studies in which the use of saliva had no pharmacological application were excluded. For searches, we used the following databases: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane and SCOPUS. The methodological quality was performed using the tools available in Joanna Briggs, always with two or more independent evaluators. The generated data were tabulated and summarized through qualitative narrative analysis. The methodology selected 19 articles that met the eligibility criteria. The saliva of hard ticks, found in the Americas, is more promising when used in experimental studies with human cells. The elucidation of the biomolecules was possible, with evasin and serpine being the biomolecules with the most evident pharmacological potential for anti-inflammatory action. In the selected studies, we found only experimental studies, with no pre-clinical or clinical studies, making methodological qualification difficult; in some studies, with the biomolecule Evasin and Serpin, the need for elucidation of these biomolecules in question was suggested. Thus, we found evidence that the saliva of American hard ticks is the most studied for pharmacological applications of anti-inflammatory and immunomodulatory action.
Keywords: Saliva; Tick; Pharmacological potential; Salivary biomolecules.

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Resumo

Sabendo que os carrapatos possuem moléculas bioativas em sua saliva as quais modulam atividades hemostáticas e imunomoduladoras em humanos, realizamos uma busca sistemática de biomoléculas presentes na saliva do carrapato com grande potencial farmacológico. Avaliamos estudos publicados nos últimos dez anos. Seguindo as recomendações da ferramenta Prisma, foram selecionados estudos primários e secundários de caráter sistemático, não havendo restrição de idioma ou país. Foram excluídos estudos que incluíam artrópodes diferentes de carrapatos e estudos em que o uso de saliva não tinha aplicação farmacológica. Para as buscas, utilizamos as seguintes bases de dados: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane e SCOPUS. A qualidade metodológica foi realizada com as ferramentas disponíveis no Joanna Briggs, sempre com dois ou mais avaliadores independentes. Os dados gerados foram tabulados e resumidos por meio de análise narrativa qualitativa. A metodologia selecionou 19 artigos que atenderam aos critérios de elegibilidade. As salivas de carrapatos duros, encontrados nas Américas, são mais promissoras quando utilizadas em estudos experimentais com células humanas. A elucidação das biomoléculas foi possível, sendo a evasina e a serpina as biomoléculas com os potenciais farmacológicos mais evidentes para ação anti-inflamatória. Nos estudos selecionados encontramos apenas estudos experimentais, não havendo estudos pré-clínicos ou clínicos, dificultando a qualificação metodológica; em alguns estudos, com a biomolécula Evasin e Serpin, sugeriu-se a necessidade de elucidação dessas biomoléculas em questão. Assim, localizamos evidências que a saliva de carrapatos duros americanos é a mais estudada para aplicações farmacológicas de ação anti-inflamatória e imunomoduladora.

Palavras-chave: Saliva; Carrapato; Potencial farmacológico; Biomoléculas salivares.

Resumen

Sabiendo que las garrapatas tienen moléculas bioactivas en su saliva que modulan las actividades hemostáticas e inmunomoduladoras en humanos, llevamos a cabo una búsqueda sistemática de biomoléculas presentes en la saliva de las garrapatas con gran potencial farmacológico. Los estudios publicados en los últimos diez años. Siguiendo las recomendaciones de la herramienta Prisma, se seleccionaron estudios primarios y secundarios de carácter sistemático, sin restricción de idioma o país. Se excluyeron los estudios que incluyeron artrópodos distintos a las garrapatas y los estudios en los que el uso de saliva no tuvo aplicación farmacológica. Para las búsquedas utilizamos las bases de datos: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane y SCOPUS. La calidad metodológica se realizó utilizando las herramientas disponibles en Joanna Briggs. Los datos generados fueron tabulados y resumidos mediante análisis narrativo cualitativo. La metodología seleccionó 19 artículos que cumplían con los criterios de elegibilidad. La saliva de las garrapatas duras, es más prometedora cuando se usa en estudios experimentales con células humanas. La elucidación de las biomoléculas fue posible, siendo la evasina y la serpina las biomoléculas con el potencial farmacológico más evidente para la acción antiinflamatoria. En los estudios seleccionados, encontramos solo estudios experimentales, sin estudios preclínicos ni clínicos, lo que dificulta la calificación metodológica; en algunos estudios, con las biomoléculas Evasin y Serpin, se sugirió la necesidad de dilucidar estas biomoléculas en cuestión. Así, encontramos evidencia de que la saliva de la garrapata dura americana es la más estudiada para aplicaciones farmacológicas de acción antiinflamatoria e inmunomoduladora.

Palabras clave: Saliva, Garrapata; Potencial farmacológico; Biomoléculas salivales.

1. Introduction

Ticks are distributed in the Arachnida class, Acari order, of the suborder Ixodida and 955 species have already been listed. The tick species are distributed into three families: Nuttalliellidae, which has only one species, Argasidae (soft tick) which is composed of 218 species and Ixodidae (hard tick) with approximately 736 species (Dantas, et al.; 2019), they are obligatory hematophagous arthropods that feed repeatedly by minutes, hours, days, or weeks on their hosts (Francischetti, et al., 2009). Due to this, to successfully obtain blood from their host, these invertebrate ectoparasites have developed a series of mechanisms that bypass vertebrate defenses. Among these mechanisms, we can highlight the production of saliva, which is a secretion rich in components that favor the success of blood acquisition and the perpetuation of its host's tick. In the vertebrate host, the insertion of the oral tract triggers the recruitment of defense cells and the production of chemokines, lipid inflammatory mediators and cytokines (Francischetti, et al., 2009). The presence of secreted saliva, exactly where the tick's mouthparts are fixed, is the main reason for its permanence, since it is where the cells and molecules of the host act precisely (Tatchell, 1967).

Just as researchers and pharmaceutical companies seek to discover synthetic or plant-derived bioactive molecules, they also seek to find molecules derived from vertebrate and invertebrate animals. In the case of hematophagous arthropods, such as ticks, mosquitoes, sandflies and triatomines, it is known that they are capable of producing and secreting potent bioactive

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molecules. Among these, there`s no doubt that ticks are the species with the best-known molecules and with a greater number of activities that have already been previously determined. However, there still hasn't been any careful evaluation of how many molecules that have already been identified to show a potential effect on the biology of human cells and molecules. For this reason, our proposal is to identify the main biomolecules existing in the saliva of ticks studied in the last decade in order to evaluate their interaction routes and potential pharmacological actions in human cells and molecules.

2. Methodology

This is a secondary study developed through a systematic review, following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Haddaway & McGuinness, 2020).

The study used a structure question with the aid of the acronym POT (P = population, O = outcome, T = type of study), being: P = Biomolecules with pharmacological activity present in tick saliva; O = type of activity; T = primary and secondary studies (only systematic review with or without meta-analysis), assisting in the stages of development of the methodological protocol.

2.1 Eligibility criteria

The criteria for inclusions were: types of original primary studies, in addition to secondary studies with a systematic character with or without meta-analysis, which covered the period of the last decade (2010 to 2023). For the present study, there were no language or country restrictions for the selections.

On the other hand, studies that were duplicated or that included only arthropods other than ticks, studies in which the use of tick saliva did not have pharmacological applications and, finally, in silico studies (bioinformatics) were excluded.

2.2 Information sources

The searches took place from 01/01/2010 to 06/02/2023, defining that for conventional publications the following 05 databases would be used: Medical Literature Analysis and Retrieval System Online (MEDLINE/PubMed), Web of Science, Latin American and Caribbean Health Sciences Literature (LILACS), Excerpta Medica Abstract Journal (EMBASE), Cochrane Library (Cochrane) and SciVerse Scopus.

For evaluation and the search in gray literature, unconventional publications, the following were investigated: Theses and dissertations cataloged by the Coordination for the Improvement of Higher Education Personnel (CAPES) and a detailed search on the topics indicated and suggested by the Gray's Matter manual (Grey Matters, 2020). In the theses and dissertations cataloged in the 04 most referenced universities in the study of ticks: Federal University of Triângulo Mineiro (UFTM), Federal University of Uberlândia (UFU), University of São Paulo (USP) and Osvaldo Cruz Foundation (FIOCRUZ). In addition, a consultation with an expert (CJFO) was carried out to evaluate the results, and to adjust the search strategy. A consultation was also carried out with the librarian on 08/26/2021, to confirm the searches and results, we closed the date of analysis of the articles 2010 on 02/06/2023.

2.3 Search strategy

To ensure accuracy in the search, descriptors and synonyms were established after searching the Medical Subject Headings (MeSH®).

Based on the structure question "Are there biomolecules in tick saliva that have pharmacological applications in humans?" descriptors and synonyms/alternative terms were selected come from DeCS. The descriptors were "Arthropod Proteins", "Tick", "Saliva", "Biological Products" and the synonyms were Tick Proteins, Tick, Salivas, Biologic Drugs, Biologic

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Medicines, Biologic Pharmaceuticals, Biologic Products, Biological Drugs, Biological Medicines, Biological (s), Biologic(s), Biopharmaceuticals, Drugs, Biologic, Drugs, Natural Products, Pharmaceuticals, Products.

The search strategy was adapted for each type of base evaluated. The Boolean operators “or” and “and” were used to guarantee the proper associations. The search key was generated automatically and below is an example used in the Medline/Pubmed database: "Ticks"[MeSH] OR Ticks OR Tick OR Ixodida OR Ixodidas AND "Saliva"[MeSH] OR Saliva OR salivas AND "Arthropod Proteins"[MeSH] OR (Arthropod Proteins) OR (Tick Proteins) AND "Biological Products"[MeSH] OR (Biological Products) OR (Products, Biological) OR (Biological Product) OR (Product, Biological) OR (Biological Product) OR (Product, Biologic) OR (Biologic Products) OR Biopharmaceutical OR Biopharmaceutical OR Biological OR Biological OR (Biological Drug) OR (Drug, Biological) OR (Biologic Drugs) OR (Drugs, Biologic) OR (Biological Medicine) OR (Medicine, Biological) OR (Biological Medicines) OR (Medicines, Biological) OR Biologicals OR (Biologic Medicines) OR (Medicines, Biologic) OR (Biologic Pharmaceuticals) OR (Pharmaceuticals, Biologic) OR Biologics OR (Biologic Drug) OR (Drug, Biologic) OR (Biological Drugs) OR (Drugs, Biological) OR (Natural Products) OR (Natural Product) OR (Product, Natural); filter=years: 2010 – 2023.

2.4 Study selection

The study selections were carried out independently by two researchers (Y.O.B. and C.M.A.R) and the disagreements were resolved by consensus and, when necessary, a third evaluator with broad experience for decision-making (R.P.A.) was included. The kappa coefficient for agreements was used to determine possible significant variations between the evaluators in different stages.

Articles were first selected based on their titles and abstracts, and those that were duplicates were excluded. Then, the complete essays were independently evaluated by the evaluators, and those that met the eligibility criteria were selected for this study.

2.5 Extraction of the data

Data from the selected studies were entered into a previously standardized Excel spreadsheet (Microsoft®), following the selection of the independent evaluators (Y.O.B. and C.M.A.R) and checked, when necessary, by a third evaluator (R.P.A.).

The analyzed data to be extracted from the eligible studies were study type, tick taxonomy, the place of origin of the tick, techniques used, anatomical parts that were extracted from the tick, molecules involved with pharmacological activity, promoted action and the methodology used.

2.6 Methodological quality assessment

Parameters linked to the methodological quality of the selected studies were carefully evaluated for all the selected studies. The recommendations from the Joanna Briggs; 2020 tools and the Checklist for Analytical Cross Sectional Studies form were followed.

For each study, a percentage of achievement was assigned in the topics that were suggested by the tool that was used, so that studies that met all quality topics were assigned 100% achievement, and reductions were associated with absences in the description and/or non-clear descriptions.

2.7 Data analysis

The data was charted in Microsoft® Excel and for the analysis and visual display of the data, the “Prism” program from Graphpad version 8.0 was also used. A qualitative narrative synthesis was carried out with the exposure of absolute (number) and relative (percentage) frequencies. Associations were assessed using the Chi-square test. For the temporal correlation of the

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frequencies of scientific production, the Person correlation test was used, after the notice of normality by the D'Agostino & Pearson test. The significance level used for all assessments was of 5% (Arango, 2001).

2.8 Records

The results pointed out in this systematic review are recorded at https://osf.io/yjuar/

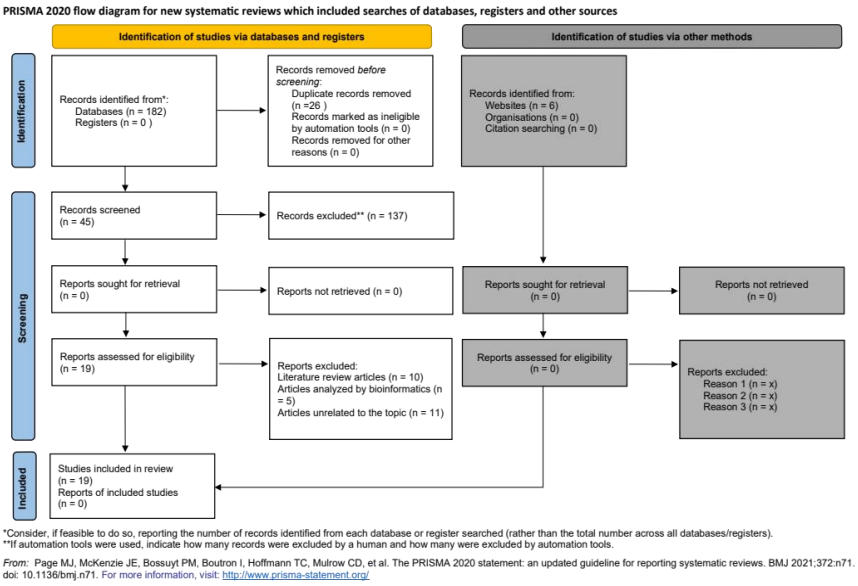
3. Results

3.1 Study Selection

The databases initially presented 182 articles and no records. With individual analysis, we observed that there were 26 duplicated articles. Among the 166, all titles and abstracts were read and the inclusion and exclusion criteria was applied. 137 articles were excluded for not being related to the topic, and none of them could have been used.

After completely reading the 45 studies, 26 were excluded for the following reasons: narrative reviews (n = 10), (n = 5) articles were analyzed exclusively by bioinformatics and did not relate to the topic (n = 11). Finally, the systematic search resulted in the selection of 19 articles according to the criteria established in the methodology and presented in detail in Figure 1 – flowchart.

Figure 1 - Flowchart belonging to the identification of the studies collected in database/records (in yellow) and Identification of studies collected by other methodologies (in gray).



Source: Authors.

3.2 Characteristics of the studies

After selecting the eligible studies, data such as: author and year, title, database, journal of publication, type of study and methodological quality were set in place in Table 1.

Among the 05 databases evaluated, it was possible to identify that those eligible studies are in 02: Medline/Pubmed (n = 15 - 79%) and Web of Science (n = 4 - 21%). In the evaluated articles, no preclinical or clinical studies were found. On the other hand, 79% of the studies used 2 or more study designs and 21% only used one. Assessing the study models separately these strategies were identified: *in vitro* (n = 16, 43%), *in vivo* (n = 13, 35%) and *in silico* (n = 8, 22%).

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All selected studies were evaluated for methodological quality. The data showed a variation between "moderate" (minimum value found = 62.50% of achievement) to "high" (100% of achievement). In the assessment of methodological quality, there was an average and standard deviation of quality achievement of 95 ± 10.18 (%). Of the 19 selected studies, 13 (68.42%) had the maximum achievement (100%) and only one study had the lowest achievement (62.5%).

Table 1 - Characteristic of studies and methodological quality. *PM: Pubmed/ WS: Web of Science.

Author / Year	Study	Data base	Journal/periodical	Type of study	Methodological quality
28 (2010)	A new Factor Xa inhibitor from *Amblyomma cajennense* with a unique domain composition.	PM	Elsevier - Archives of Biochemistry and Biophysics	In vitro, in vivo and in silico experimental study	93,75%
18 (2011)	Ixodid tick salivary gland products target host wound healing growth factors	PM	Elsevier - International Journal for Parasitology	In vitro, in vivo and in silico experimental study	62,50%
24 (2011)	The action of *Amblyomma cajennense* tick saliva in compounds of the hemostatic system and cytotoxicity in tumor cell lines	PM	Elvesier - Biomedicine e Pharmacotherapy	In vitro experimental study	100%
43 (2011)	Deconstructing tick saliva: non-protein molecules with potent immunomodulatory properties	WS	Jounal of Biological Chemisty	In vivo and in vitro experimental study	100%
12 (2013)	Evasin-4, a tick-derived chemokine-binding protein with broad selectivity can be modified for use in preclinical disease models	PM	The Febs Journal	In vivo and in vitro experimental study	90%
39 (2013)	Characterization of Ixophilin, A Thrombin Inhibitor from the Gut of *Ixodes scapularis*	PM	Plos One	In vivo and in vitro experimental study	100%
33 (2014)	Antihistamine response: a dynamically refined function at the host-tick interface	PM	Parasites and Vectors	In silico observational study	75%
41 (2014)	Longistatin in tick saliva blocks advanced glycation end-product receptor activation	PM	JCI - The Journal of Clinical Investigation	In vivo and in vitro experimental study	100%
20 (2015)	Effective inhibition of thrombin by *Rhipicephalus microplus* serpin-15 (RmS-15) obtained in the yeast *Pichia pastoris*	WS	Ticks and Tick-borne Diseases	In vivo experimental study	87,50%
21 (2015)	*Rhipicephalus microplus* serine protease inhibitor family: annotation, expression and functional characterisation assessment	WS	Parasites and vectors	In vivo and in vitro experimental study	100%
16 (2017)	Yeast surface display identifies a family of evasins from ticks with novel polyvalent CC chemokine-binding activities	PM	Nature – scientific reports	In vivo and in silico experimental study	100%
34 (2017)	Avathrin: a novel thrombin inhibitor derived from a multicopy precursor in the salivary glands of the ixodid tick, *Amblyomma variegatum*	PM	The Faseb	In vitro, in vivo and in silico experimental study	100%
40 (2018)	Ixonnexin from Tick Saliva Promotes Fibrinolysis by Interacting with Plasminogen and Tissue-Type Plasminogen Activator, and Prevents Arterial Thrombosis	PM	Nature Scientific Reports	In vivo and in vitro experimental study	100%
23 (2019)	Antitumoral effects of *Amblyomma sculptum Berlese* saliva in neuroblastoma cell lines involve cytoskeletal deconstruction and cell cycle arrest	PM	Brazilian Journal of Veterinary Parasitology	In vitro experimental study	100%
30 (2019)	The immunosuppressive functions of two novel tick serpins, HlSerpin-a and HlSerpin-b, from *Haemaphysalis longicornis*	PM	Immunology	In vitro, in vivo and in silico experimental study	100%
36 (2019)	Immunosuppressive effects of sialostatin L1 and L2 isolated from the taiga tick *Ixodes persulcatus Schulze*	PM	Elsevier - Ticks and Tick-borne Diseases	In vivo and in vitro experimental study	100%

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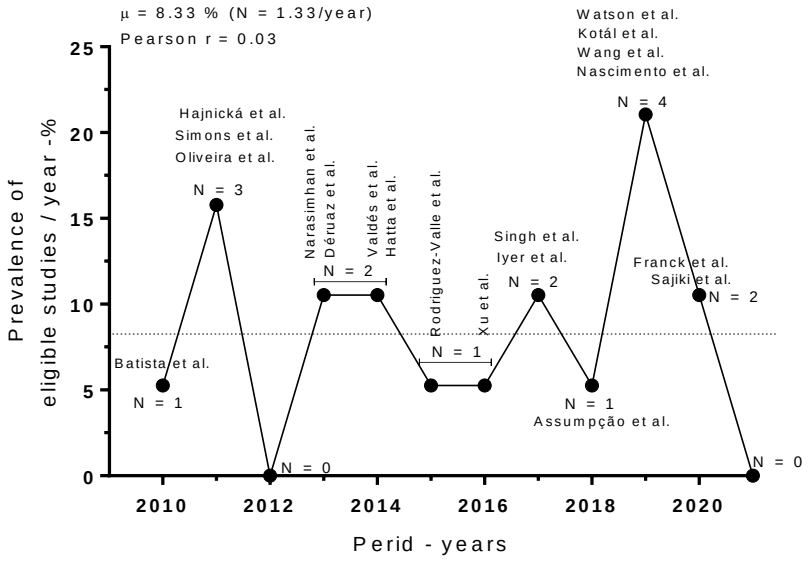
37 (2019)	The structure and function of Iristatin, a novel immunosuppressive tick salivary cystatin	PM	Cellular and Molecular Life Sciences	In vivo, in vitro and in silico experimental study	100%
42 (2019)	Rapid assembly and profiling of an anticoagulant sulfoprotein library	WS	PNAS - Proceedings of the National Academy of Sciences of the United States of America	In vitro and in silico experimental study	93,75%
17 (2020)	Semisynthesis of an evasin from tick saliva reveals a critical role of tyrosine sulfation for chemokine binding and inhibition	PM	PNAS - Proceedings of the National Academy of Sciences of the United States of America	In vitro and in silico experimental study	100%

Source: Authors.

Figure 2 shows the distribution of studies that evaluated bioactive molecules for pharmacological application between the periods of 2010 to 2023, and it was observed that annually it had an average percentage of 8.33 with a frequency of 1.33 studies. With these findings, it was not possible to identify a significant time correlation in the frequency of studies with the theme ($p>0.05$).

Figure 2 - Time correlation of studies that evaluated the pharmacological action of biomolecules extracted from tick saliva.



Source: Authors.

3.3 Report biasing

It was possible to qualify the distribution of tick genera and species evaluated in experimental studies for the application of saliva as a therapeutic form, as shown in Table 2.

The selected articles allowed the extraction of the following data: there are three genera of ticks, all of the Ixodidae family (hard tick) whose saliva are promising in pharmacological actions, since they contain bioactive compounds.

The prevalence of the genera and respective species with the highest prevalence can be observed: *Amblyoma sp* in 31.03% (Amblyomma cajennense 13.79% cited), *Rhipicephalus sp* 27.59% (*Rhipicephalus sanguineus* 10.34% cited) and *Ixodes sp* 20, 69% (Ixodes scapularis 10.34% cited), *Haemaphysalis sp* 10.34% (*Haemaphysalis longicornis* 10.34%), *Dermacentor sp* 6.90% (*Dermacentor andersoni* and *Dermacentorreticulatuse* 3.45% each) and *Hyalomma sp* 3.45% (Hyalomma marginatum rufipes with 3.45%).

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Table 2 - Prevalence of species and regions of ticks selected in the selected articles.

Genus	Prevalence % (n)	Species	Prevalence % (n)	Region	Study
Amblyomma sp.	31.03 (9)	*Amblyomma cajennense*	13.79 (4)	Brazil and Mexico	Batista, et al., 2010; Simons, et al., 2011; Franck, et al., 2020; Singh, et al., 2017
		Amblyomma maculatum	3.45 (1)	Brazil and Mexico	Singh, et al., 2017
		Amblyomma parvum	3.45 (1)	Brazil and Mexico	Singh, et al., 2017
		Amblyomma sculptum	3.45 (1)	Brazil	Nascimento, et al.,2019
		Amblyomma variegatum	6.90 (2)	Africa and Slovakia	Hajnická, et al.,2011; Iyer, et al., 2017
Dermacentor sp	6.90 (2)	*Dermacentor andersoni*	3.45 (1)	United States	Watson, et al., 2019
		Dermacentor reticulatus	3.45 (1)	Western Asia and Europe	Hajnická, et al., 2011
Haemaphysalis sp.	10.34 (3)	*Haemaphysalis longicornis*	10.34 (3)	Australia and Asia	Wang, et al.,2016, Anisuzzaman, et al.,2014, Watson, et al.,2019
Hyalomma sp.	3.45 (1)	*Hyalomma marginatum rufipes*	3.45 (1)	Africa, Asia and Europe	Watson, et al.,2019
Ixades sp.	20.69 (6)	*Ixodes persulcatus*	3.45 (1)	Europe, China and Japan	Sajiki, et al.,2020
		Ixodes ricinus	6.90 (2)	Europe and Asia	Hajnická, et al.,2011; Kotál, et al., 2019
		Ixodes scapularis	10.34 (3)	United States, Canada and Slovakia	Hajnická, et al., 2011; Narasimhan, et al., 2013; Assumpção, et al., 2018
Rhipicephalus sp.	27.59 (8)	*Rhipicephalus microplus*	6.90 (2)	Australia, South Africa and South America	Tao, et al., 2016; Rodriguez-Valle, et al., 2015
		Rhipicephalus appendiculatus	6.90 (2)	Africa	Hajnická, et al., 2011; Valdés, 2014
		Rhipicephalus pulchellus	3.45 (1)	Africa	Singh, et al., 2014
		Rhipicephalus sanguineus	10.34 (3)	Brazil	Déruaz, et al., 2013; Singh et al.,2017, Oliveira et al.,2011
Total	100 (29)		100 (29)		

Source: Authors.

It can also be observed that studies involving species with a prevalence higher than 10% converge to the statement that used ticks from the regions of Brazil, Mexico, United States, Canada, Slovakia, Asia, Africa and Australia.

To better assess these regional data, Table 3 shows the continental prevalence of ticks, whose saliva has possible pharmacological usage. It is interesting to note the predominance and frequency of ticks found in America, followed closely tied by Europe and Asia; followed by Africa and with lower prevalence in Oceania.

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Table 3 - Continental prevalence that assesses possible pharmacological applications for tick saliva.

Genus	Continent					Total
	America	Europe	Asia	Africa	Oceania	
Amblyomma sp.	4 (66.67)	1 (16.67)	0 (0.00)	1 (16.66)	0 (0.00)	6 (100)
Dermacentor sp.	1 (33.33)	1 (33.33)	1 (33.34)	0 (0.00)	0 (0.00)	3 (100)
Haemaphysalis sp.	0 (0.00)	0 (0.00)	1 (50.00)	0 (0.00)	1 (50.00)	2 (100)
Hyalomma sp.	0 (0.00)	1 (33.33)	1 (33.33)	1 (33.34)	0 (0.00)	3 (100)
Ixades sp.	2 (25.00)	3 (37.50)	3 (37.50)	0 (0.00)	0 (0.00)	8 (100)
Rhipicephalus sp.	2 (33.33)	0 (0.00)	0 (0.00)	3 (50.00)	1 (16.67)	6 (100)
Total	9 (158.33)	6 (120.83)	6 (154.17)	5 (100.00)	2 (66.67)	28

P-value <0.0001. Source: Authors.

Corresponding to the characteristics of the samples used through Table 4. We can stratify that 78.95% of the studies used human material, 68.42% used animals (non-humans) and 47.37% used unicellular organisms and other types of cells. It is noteworthy that the interaction of human and non-human materials (a+b) occupied third place in this prevalence, 57.89%, followed by the interaction of human materials and unicellular organisms (a+c) and non-human materials and unicellular organisms (b+c) with 31.58%.

Table 4 - Stratification of the characteristics of the samples used.

Sample characteristic	**Events (N)**	**Prevalence - % (N = 19)**
Human - (a)	15	78.95
Non-human animal - (b)	13	68.42
Unicellular organism - (c)	9	47.37
"In silico" - (d)	4	21.05
(a+b)	11	57.89
(a+c)/(b+c)	6/6	31.58/31.58
(a+d)	2	10.53
(b+d)	2	10.53
(c+d)	3	15.79
(a+b+c)	4	21.05
(a+b+d)	2	10.53
(a+c+d)	2	10.53
(b+c+d)	2	10.53
(a+b+c+d)	2	10.53

Source: Authors.

Table 5 summarizes the molecules shown in the studies as present in the saliva of ticks capable of interacting and acting on various molecules in the human body. Molecules listed in the studies include enzymes, cytokines, components of the complement system, antibodies, cell signaling molecules, and immune cell receptors. In selected studies and charted data, we can observe the prevalence of biomolecules and their respective pharmacological actions: Evasine (21.05%) that binds to host

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chemokines as an anti-inflammatory strategy; Serpin (15.79%) that acts as a protease inhibitor, also relating them to anti-inflammatory action. Assessing gross saliva (10.53%), the studies point to a possible antitumor and anticoagulant pharmacological action. The other biomolecules presented at lower frequencies (5.26%) had other specific actions such as: Amblyomin-X indicating anticoagulant and protease inhibitor action; Lipocalin with antihistamine action; Avatrin with anti-hemostatic activity; Ixophyllin: anticoagulant/thrombin inhibitor; Sialostatin with immunosuppressive and anti-inflammatory action; Iristatin: immunosuppressive; Ixonexin: possible anticoagulant; Longistatin: suppressor of adhesion molecule expression, cytokine secretion, prevention of NF-κB translocation, and reduction of cellular oxidative stress; Sulfoproteins: anticoagulant; Ado and PGE2 with important immunomodulating and inflammatory response modulation action.

Table 5 - Prevalence of biomolecules.

Biomolecule	Pharmacological Action	Prevalence %
Evasin	Anti-inflammatory (inhibition of chemokine), Action on inflammatory diseases (ID).	21.050%
Serpin	Action on ID, Protease inhibition	15.790%
Crude saliva	Antitumor action, Cytotoxic effects; Induction of cell death in cancer cell lines; Coagulation systems;Fibrinolysis and action on platelet aggregation; Factor Xa and inhibition of thrombin.	10.530%
Amblyomin-X	Anti-coagulant; Protease inhibitor.	5.263%
Lipocalin	Antihistamine	5.263%
Avatrina	Anticoagulant (thrombin inhibitor); Anti-hemostatic compounds	5.263%
Sialostatin	Immunosuppressant; Anti-inflammatory	5.263%
Ixophylline	Anticoagulant action (thrombin inhibitor)	5.263%
Ixonnexin	Anticoagulant	5.263%
Iristatin	Immunosuppressant	5.263%
Longistatin	Suppression of the expression of adhesion molecules Cytokine secretion; Prevention of NF-κB translocation; Reduction of oxidative stress.	5.263%
Sulfoproteins	Anticoagulant	5.263%
Ado e PGE2	Modulation - immune and inflammatory responses	5.263%
Total		100%

Source: Authors.

Table 6 shows the distribution focused on the potential pharmacological activities of tick saliva in different types of pathologies (target diseases). You can observe the prevalence of 42.11% on the selected studies, identifying molecules with anticoagulant activity (thrombin blockers). This pharmacological action is important in pathologies such as thrombosis, thromboembolism and stroke. Bioactive molecules with anti-inflammatory action were identified in the same percentage, which can help in therapies for pro-inflammatory pathologies such as arthritis. Anti-tumor and immunosuppressive pharmacological actions prevailed in 10.53% of the articles and in 5.26% anti-platelet aggregation, antihistamine and protease inhibitor actions. In combined/accumulated evaluation, we observed a greater tendency of studies to point out the use of tick saliva in pharmacological actions in inflammatory diseases (63.16%), followed by hemostatic activity (47.37%).

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Table 6 - Potential pharmacological activities for tick saliva in different types of pathologies.

Pharmacological action	**Target diseases**	**Prevalence - %**
Anticoagulant (blocks thrombin)	Thrombosis, thromboembolism, Brain stroke	42.11
Platelet anti-aggregation	Essential thrombocythemia, Polycythemia Vera	5.26
Antihistamine	Hypersensitivity (Allergies, Asthma)	5.26
Anti-inflammatory	Pro-inflammatory diseases (Arthritis, etc.)	42.11
Antitumor activity	Cancer	10.53
Imunossupressors	Autoimmune diseases	10.53
Protease inhibition	Acute pancreatitis	5.26
		Accumulated prevalence - %
Hemostatic activity	Thrombosis, thromboembolism, Brain stroke Essential thrombocythemia, Polycythemia Vera Hypersensitivity (Allergies, Asthma)	47.37
Inflammatory diseases	Pro-inflammatory diseases (Arthritis, etc.) Autoimmune diseases, Acute pancreatitis	63.16

Source: Authors.

4. Discussion

Pubmed is a search engine with open access to the MEDLINE database that contains publications of research articles in biomedicine. MEDLINE has around 4,800 magazines published in the United States and in more than 70 countries around the world and has been active from 1966 to the present day. Web of Science is a website that provides subscription-based access to 6 online databases that provide comprehensive information and citations for many different academic disciplines. LILACS is a health-specific database that provides articles published in 26 countries in Latin America and the Caribbean, important in our region. COCHRANE is a collection of databases focused on the health area with high quality evidence and also has a specific database for systematic reviews. EMBASE a database for pharmacological and biomedical articles covers a range of international publications. SCOPUS is a database where you can find a vast literature of publications such as articles, books, scientific journals and events in the most diverse areas such as: health, arts, medicine and others.

Of these, at least five are mandatory for the study to be carried out in a systematic review and this has assigned six bases to compose the search strategies. For this reason, the journals found in these databases were chosen because they relate to the theme, research profile and methodology determined in this systematic review. The systematic review, by limiting the investigation time in the last 10 years, allowed us to identify possible pharmacological applications with potential in humans. We observed a lack of scientific records on preclinical and clinical studies as shown in Table 7.

Finding only experimental studies (in vitro, in vivo, in silico) made the methodological classification difficult and there is still a lack of notes that need to be investigated to demonstrate the real action in human beings.

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Table 7 - Pharmacological applications in humans; *Articles selected in this systematic review.

Biomolecule	Possible human applications	In vivo	In vitro	Clinic	In Silico	Reference*	Reference literature
Evasin	Wound healing, inflammatory diseases	Yes	Yes	No	No	Déruaz, et al., 2013; Singh, et al., 2017; Frank, et al.,2020; Hajnická, et al., 2020	Chmelař. et al., 2019; Bonvin, et al; 2016; Déruaz. et al; 2019; Frauenschuh. et al., 2007; Vieira. et al., 2009
Serpin	Coagulation diseases, digestive system, pancreatic insufficiency and Alleviate Joint Swelling and Inflammatory Response in Arthritis Models	Yes	Yes	No	Yes	Tao, et al., 2016; Rodriguez-Valle, et al., 2015; Wang, et al., 2020	Chmelař, et al., 2019
Crude saliva	Neuroblastoma cell lines, effect on blood clotting, fibrinolysis and platelet aggregation, Cell death induction in cancer cell lines	No	Yes	No	No	Nascimento, et al., 2019; Simons, et al., 2011	-
Amblyomin-X	Coagulation diseases	Yes	Yes	No	Yes	Batista, et al., 2010	Chmelař, et al., 2019; Branco, et al.,2016; Decrem, et al., 2009; Corral, et al., 2016; Chudzinski-Tavassi, et al., 2010
Lipocalin	Allergy and asthma	No	No	No	Yes	Valdés, 2014	Wang, et al., 2016; Paesen, et al., 2000; Sangamnatdej, et al., 2002
Avatrina	Thrombosis	Yes	Yes	No	Yes	Iyer, et al., 2017	Kotsyfakis, et al., 2006
Sialostatin	Immunopathies and inflammatory diseases	Yes	Yes	No	No	Sajiki, et al., 2020	-
Ixophylline	Coagulation diseases	Yes	Yes	No	No	Narasimhan, et al., 2013	Kotsyfakis, et al., 2006,; Aounallah, et al., 2020
Ixonnexin	Thrombosis	Yes	Yes	No	No	Assumpção et al., 2018	-
Iristatin	Immunopathies and inflammatory diseases	Yes	Yes	No	Yes	Kotál et al., 2018	Narasimhan et al., 2013
Longistatin	Inflammatory diseases	Yes	Yes	No	No	Anisuzzaman, et al., 2014	-
Sulfoproteins	Coagulation diseases	No	Yes	No	Yes	Watson, et al., 2019	-
Ado e PGE2	Immunopathies and inflammatory diseases	Yes	Yes	No	No	Oliveira, et al., 2011	-
Total						19	

Source: Authors.

Evaluations of this same search in periods prior to 2010 will bring other results and discussions since 2000 new experimental techniques have been developed since then, as well as bioinformatics. However, to evaluate the scientific evidence that is closer to the discoveries and potential applicability, this temporal limitation was preferably chosen. Since the results of the last ten years did not show clinical studies, our review points to results with potential signaling of possible studies more focused on these biomolecules for the next ten years.

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The ticks listed are all from the Ixodidae (hard tick) family. Ticks successfully perform the blood meal, through the bite on the host's skin, spend days and even weeks to complete their feeding. For this to be successful, through their saliva, they trigger a series of immunomodulatory and homeostatic responses in the host, in addition to presenting major interferences in wound healing and suppressing the inflammatory response, and due to this their saliva are studied in search for possible actions and interesting pharmacological contributions (Nutall, 2019; Bowman, et al., 2008).

Considering the question on this systematic review and the data found, we converged information on some molecules present in tick saliva (regardless of genus and species) that are known for their specific pharmacological actions and functions in human target molecules and cells. We confirm the quantified results, highlighting the molecules that have been listed, favoring a greater elucidation of this pharmacological role and using as the base structure the associations found in the results with other scientific literature.

As evaluated, molecules have applications defined by their actions. Initial tests will guide the isolated or combined effects of these biomolecules so that in the next 10 years they can scientifically act in human therapeutics.

Evasin and Serpin prevailed in the results and the need for better elucidation of studies with crude or isolated saliva are still made noticed, since in this analysis there are interesting results with a promising percentage in antitumor action, as well as anticoagulant and anti-inflammatory actions.

5. Conclusion

It is observed that tick saliva is a promising universe to be explored. The prevalence of studies is not correlated with the greater effectiveness of the pharmacological action, but with the great number of studies identified in the systematic review.

We summarized, for futures research, the available evidence that the saliva of American hard ticks is the one with the most studies for pharmacological applications referring to anti-inflammatory and immunomodulatory action.

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PROPPG/UFTM, PPGCF, PPGCS, PPGMTI.

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