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Prominent events in the development of a simultaneous multidiagnostic system with synthetic peptides

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Abstract

The tropics are abundant in both animals and plants, but also in pathogenic agents. There, the world's greatest burden of diseases and mortality is concentrated. Co-infections are the rule, making laboratory diagnosis complex. Simultaneous multidiagnostic methods are desirable; however, they are mostly expensive and inaccessible to the populations of the region. The aim of our research was to produce synthetic peptides of the most important pathogens that can be used in a simultaneous multidiagnostic technique. Thus, we designed a low-cost method to detect antibodies, the multiple antigen blot assay (MABA), using synthetic peptides as the main source of antigens from endemic tropical diseases. This method allows the simultaneous detection of antibodies against 26 different agents with only a few microliters of sera, plasma, or saliva. The development of this system is the result of a long process, and the pipeline of our approach from then to nowadays is presented. Specific epitopes with the greatest antigenic potential using immunoinformatic algorithms have been selected from worldwide and tropical pathogens and then assayed by a successive chain of immunological techniques [PEPSCAN®, enzyme-linked immunosorbent assay (ELISA), and MABA] to evaluate the sensitivity and specificity of those synthetic peptides for their usefulness in diagnosis. Years of work have been required for this complex process, with the recent incorporation of new immunoinformatic predictive tools, methodologies, and cost advantages. It

can be concluded that synthetic peptides are a promising approach for diagnostic processes based either on the detection of antigens or antibodies.

Keywords

Multidiagnosis, synthetic peptides, tropical diseases, immunoinformatics, PEPSCAN®, synthesis of peptides on cellulose membrane (SPOT®), multiple antigen blot assay

Introduction

Laboratory diagnosis determines the definitive diagnosis of human and animal diseases beyond the clinical and epidemiological diagnosis. The improvement and development of diagnostic systems are currently more important due to the risk of new infectious agents, such as SARS-CoV-2, in which the rapid dispersion and severity of the pandemic forced experts to develop innovative diagnostic tools to guarantee the infection is detected.

It is clear that in recent decades there has been an evolution from diagnostic techniques from a single causal agent (with late reading and large volumes of both antigens and samples, conjugates and substrates) to rapid techniques of simultaneous multidiagnosis, with fast reading and very small quantities (nano) of both samples and reagents and novel approaches (multiplex, microarrays, etc.). Additionally, the use of fractions of these antigens (epitopes) instead of complete molecules makes the diagnosis more specific. These fractions can be produced as recombinant molecules or synthetic peptides.

The use of small molecules, such as synthetic peptides [usually less than 50 amino acids (AAs)], although more specific, is sometimes not sufficiently sensitive due to a genetically conditioned immunological blindness or tolerance (whereby the immune system fails to recognize or respond to a specific antigen, effectively rendering it "invisible" to the immune system) [1]. Therefore, in some cases, the use of synthetic peptide cocktails is required to guarantee higher sensitivity. Their thermostability, low cost, easy scalability, rapid modification, and purification make them attractive for use in immunodiagnostic techniques. As an example of the scalability of the strategy of chemically synthesized peptides, we used manual synthesis to obtain 4.5 g of a 45-AA peptide in just one month of work, and for multiple antigen blot assay (MABA), only 0.046 μg of each peptide is needed per patient per disease.

Considering the virtues of using synthetic peptides as a source of antigens, working in a tropical environment where the burden of infectious diseases is high and co-infections are frequent, we developed the MABA, a technique for the simultaneous diagnosis of infectious and parasitic diseases, as explained below [2]. A single MABA assay allows the simultaneous evaluation of at least 28 different sera against 26 different antigens with a few microliters of body fluids. This is equivalent to 13–14 conventional enzyme-linked immunosorbent assay (ELISA) plates. The cost of evaluation of each antigen per patient is $0.006 using MABA versus $0.129 using conventional ELISA [3].

To meet our needs, we first selected those infectious pathologies with high prevalence or health impact in our environment and searched for the sequences of the proteins recognized as most antigenic in the available databases. Once the complete sequences were loaded, we subjected them to analysis with predictive algorithms to select those that were potentially the most reactive sequences. Peptides were evaluated using immunological methods to determine sensitivity by previously well-characterized positive samples (serum or saliva) and specificity by negative ones.

The aim of our research was to produce synthetic peptides of the most important pathogens that can be used in a simultaneous multidiagnostic technique, namely the MABA technique, which is specially designed for the diagnostic demand in blood banks, obstetric units for monitoring pregnant women, infectious disease units, etc.

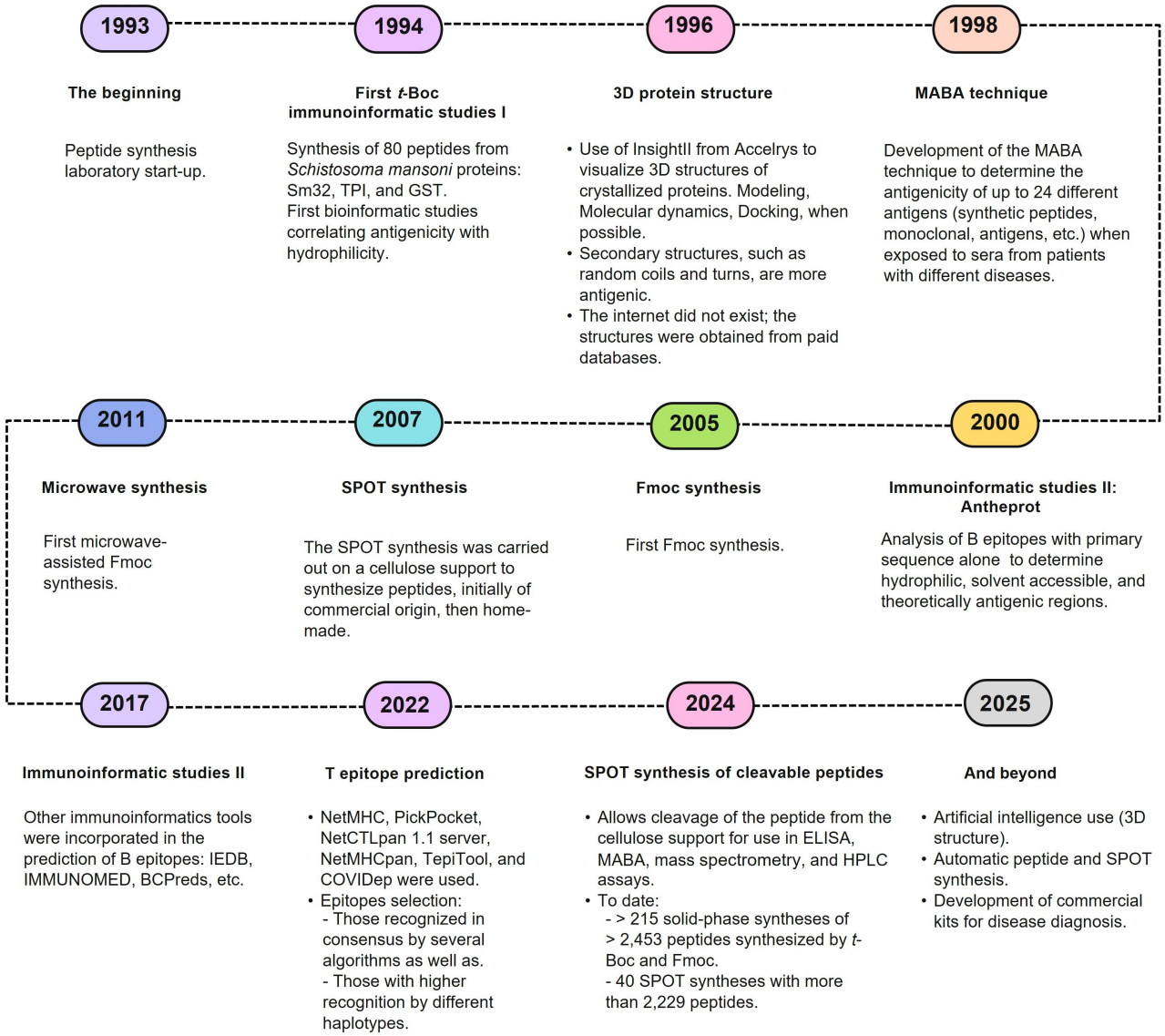

Figure 1. A pipeline representing the evolution of technologies used progressively between 1993 and 2025 to optimize the synthesis of the most reactive peptides to be used in different diagnostic methods (ELISA, rapid immunochromatographic tests, and MABA). ELISA: enzyme-linked immunosorbent assay; Fmoc: 9-fluorenylmethyloxycarbonyl; GST: glutathione-S-transferase; IEDB: immune epitope database; MABA: multiple antigen blot assay; SPOT: synthesis of peptides on cellulose membrane; *t*-Boc: *tert*-Butyloxycarbonyl; TPI: triosephosphate isomerase

Roadmap of the evolutionary process of peptide synthesis useful in multidiagnosis

The synthetic peptides of this research project are for use in diagnosis and particularly for use in multidiagnosis for the detection of co-morbidities. The authors view this subject as a permanent discussion for the improvement and innovation of the processes, adapting them to the available equipment and reagents. Analyzing the beaten track, we realize that this experience can help others, and it consolidates the successes achieved with many hours of work.

The sequence followed by our trajectory is shown in Figure 1. The peptide synthesis laboratory began its operations in 1993, and the journey of predicting T epitopes started in 1994 at the Instituto de Medicina Tropical (IMT), Universidad Central de Venezuela. Whole proteins were synthesized using the primary sequence by means of *tert*-Butyloxycarbonyl (*t*-Boc) methodology [4, 5] in windows of 20 AAs. Afterward, we initiated the selection of the most antigenic sequences with immunoinformatic studies (antigenicity and hydrophilicity using Antheprot software [6] and 3D structures visualized by InsightII®). Monomeric and polymerizable peptides were prepared with the addition or not of two AAs [cysteine-glycine (CG)] in order to favor polymerization, as previously described [6]. Later, crystalized 3D structures were available, and

secondary structures such as random coils and turns were added to the predictions. With the arrival of the internet, docking, modeling, and molecular dynamics [7] were incorporated, and free databases became available. In 2005, 9-fluorenylmethyloxycarbonyl (Fmoc) synthesis [8] was adopted because of its simpler technique and to avoid the use of hydrogen fluoride (HF) for cleavage. Some of the predicted epitopes from this strategy were then synthesized on paper [synthesis of peptides on cellulose membrane (SPOT) synthesis] [9] to be exposed to sera from patients with the disease to be investigated (PEPSCAN) [10]. Those peptides that were most frequently recognized by the patients' sera were subsequently synthesized in a solid-phase for further evaluation by MABA [3] and ELISA [11]. Dimers, trimers, tetramers, and multiple antigenic peptides (MAPs) [12] were prepared in order to increase recognition by infected patients. In 2011, microwave energy was used for Fmoc synthesis [13], allowing the process to be much faster. Later, other immunoinformatic tools, including immune epitope database (IEDB), IMMUNOMED, and ABCpreds [14–18], were used for the prediction of B epitopes. In 2022, we started working on the prediction of T-cell epitopes to evaluate the role of cellular immune response in diagnosis. NetMHC, PickPocket, and other online servers were used [19–25]. Those T-cell epitopes with higher recognition by different haplotypes and consensus were selected. Recently, the biotinylation [26] of peptides was assayed to look for a better binding to the membrane already impregnated with streptavidin.

Synthetic peptides have also been used as immunogens in laboratory animals to produce polyclonal antibodies, ideal for antigen-capture assays. Subsequently, it was determined whether they induced recognition of the parent molecule using Western blotting (WB). In order to confirm the specificity of the recognition, inhibition assays were performed by preincubating the immune serum with the peptide(s) used in the immunization [27, 28].

Between 1994 and 2025, 2,453 peptides were synthesized by solid-phase procedures and 2,229 peptides by SPOT synthesis according to the etiological agent (Figure 2).

Immunoinformatic studies

At the beginning of this research program, we observed a direct correspondence between the most hydrophilic regions of a protein [29] and its antigenicity [30]. The systematic use of computer algorithms selects more efficiently the regions of a protein that are potentially the most antigenic. This saves time and reagents and leads to the reduction of experimental animals, among other factors.

In general, there are three basic important requirements in the selection of B-cell epitopes: hydrophilicity, flexibility, and accessibility or exposure for interaction with antibodies, which allow the selection of exposed, mobile, and hydrophilic regions so that antibodies can bind to them effectively and generate an immune response. Incorporation of programs (InsightII®) allowed us to observe the 3D structure and relate the antigenicity observed in certain synthesized peptides with the topological location in the respective protein.

Exposed regions are those that interact more with the environment that surrounds the protein (rather than what is inside the protein structure). Those very flexible regions form secondary structures called "random arrangements" and "turns". They are generally more antigenic [31], unlike structures such as alpha helices or beta sheets, dominated by van der Waals-type forces and hydrogen or disulfide bonds that make them more rigid. The amino and carboxy-terminal regions of a protein generally meet these requirements, too.

Prediction programs were replaced with free access online programs that can be read on personal computers with internet access. Several algorithms have been developed in the last decades, and with their incorporation into online web portals, they have become more efficient and complex. Other physicochemical characteristics (many derived from experimental data) were added. Some of the most frequently used algorithms by our research group are listed in Table 1.

The arsenal of immunoinformatic algorithms was enhanced by the availability of crystallized structures [X-ray or nuclear magnetic resonance (NMR)] extracted from the Protein Data Bank (PDB) (https://www.rcsb.org).

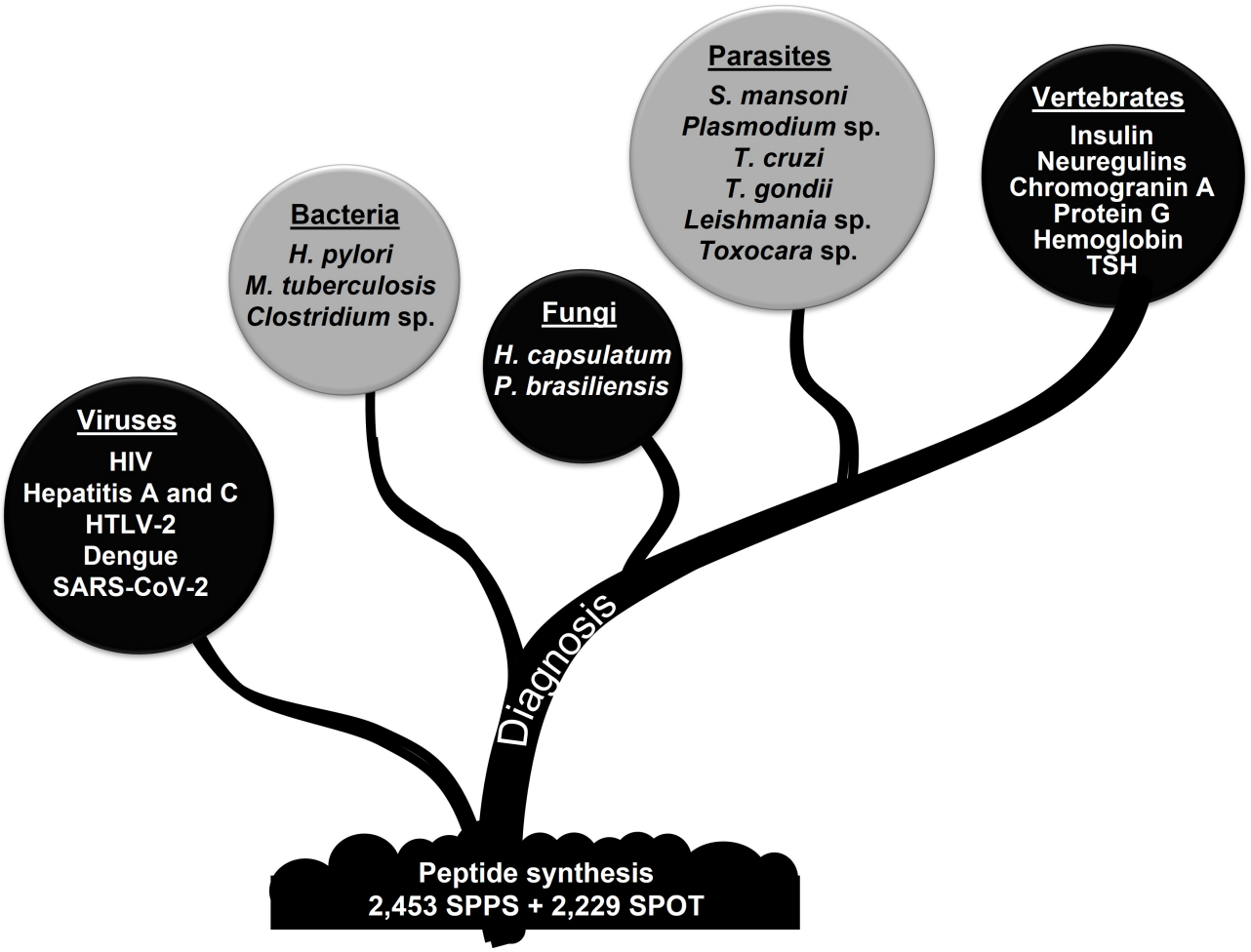

Figure 2. Peptides synthesized at the Instituto de Medicina Tropical, Universidad Central de Venezuela from 1994–2025. The total number of peptides made is 4,682. The size of the circles represents the proportion of peptides synthesized in each biological group. HIV: human immunodeficiency virus; HTLV-2: human T-lymphotropic virus 2; SPOT: synthesis of peptides on cellulose membrane; SPPS: solid-phase peptide synthesis; TSH: thyroid-stimulating hormone

Table 1. Algorithms used for the preselection of B-cell epitopes

Algorithm	Web address
Antheprot [6]	https://www.gdeleage.fr/antheprot/
Immune epitope database (IEDB) [14]	http://tools.iedb.org/bcell/
ABCpred [15]	https://webs.iiitd.edu.in/raghava/abcpred/ABC_submission.html
BCPREDS [16]	http://ailab-projects2.ist.psu.edu/bcpred/predict.html
Immnunomedicine group [17]	http://imed.med.ucm.es/Tools/antigenic.pl
SVMTriP [18]	http://sysbio.unl.edu/SVMTriP/prediction.php

In a similar approach but looking for Th1-type T-cell epitopes, immunoinformatics studies have been carried out. Up to six immunoinformatics algorithms were used and listed in Table 2.

Artificial intelligence (AI), which is acquiring increasing relevance, has recently been introduced and used in predictive studies of 3D structures of proteins not yet crystallized. It is a great tool for the visualization and selection of epitopic regions [32].

Peptide synthesis and evaluation

Synthetic peptides have won notoriety thanks to developments in biotechnology and are currently a key component of nanotechnological applications. Their chemistry is an area of research with many approaches, from vaccine design, drug development, drug delivery systems, and antimicrobial peptides to diagnostic methods and disease treatment. The peptides are very useful for the detection of various human and veterinary pathologies [33–38].

Table 2. Algorithms used for the preselection of T-cell epitopes

Algorithm	Web address
NetMHC-4.0 [19, 20]	https://services.healthtech.dtu.dk/services/NetMHC-4.0/
PicPocket [21]	https://services.healthtech.dtu.dk/service.php?PickPocket-1.1
NetCTLpan-1.1 server [22]	https://services.healthtech.dtu.dk/services/NetCTLpan-1.1/ (discontinued)
NetMHCpan-4.1 [23]	https://services.healthtech.dtu.dk/services/NetMHCpan-4.1/
TepiTool [24]	http://tools.iedb.org/tepitool/
COVIDep [25]	https://covidep.ust.hk/

The design of peptide libraries and their subsequent evaluation by immunoenzymatic methods permits the rapid screening of sequences of interest, considerably reducing the cost of synthesis per peptide compared to its conventional synthesis on polymer resins. Such is the case of the SPOT technique, which is easily integrated into research laboratories manually or semi-automatically, allowing the quantity of synthesized peptides to be varied. It can even integrate modifications, in addition to sieving or screening the peptide library in matrix form, which is widely used for the detection of peptide-ligand interactions [39, 40].

Bioinformatics tools, the SPOT, and their evaluation with immunoglobulins IgG, IgM, and IgA through the PEPSCAN immunoassay of structural and non-structural proteins were combined to identify potential candidate antigenic peptides to determine possible biomarkers of infection [41].

Our aim was to use this strategy to develop a commercial kit for the simultaneous and multiple diagnosis of endemic diseases with the appropriate mimicking of the antigenic proteins. Figure 3 describes the strategy we are currently using for the identification of antigenic peptides as candidates for diagnosis. These general steps are usually cyclical, and their number of repetitions is subject to the results obtained during the process. Optimizing the most antigenic peptides is possible by carrying out a series of structural modifications, such as: the design and synthesis of MAPs [12], site-directed mutagenesis by alanine scanning for the identification of critical residues [42], and obtaining 3D models of the synthesized peptides using PEP-FOLD [43] and its structural analysis.

For the immunological evaluation, first, SPOT synthesis was performed with peptides usually having a 16–20 AA length. Peptides were constructed on a gridded 15 × 15 cm cellulose filter paper (Whatman 50 or 540), and each spot was numbered using a graphite pencil. The membrane was exposed to a β-alanine solution, then deprotected with 20% 4-methylpiperidine/DMF (dimethylformamide) or NMP (*N*-methyl pyrrolidone) with acetic anhydride/diisopropyl ethylamine.

Afterward, it was stained with bromophenol blue (BPB), at which point the entire membrane should turn blue. Between 0.5 and 1.0 µL of the first AA, derived from the respective peptide sequence, was dissolved in DMF or NMP and previously activated. Next, the AA was coupled on each numbered spot, allowing it to react for 20 min, then this step was repeated. The membrane was acetylated using a 2% acetic anhydride/diisopropylamine mixture and stained with BPB. Finally, the Fmoc group on each spot was removed by subjecting it to a 20% 4-methylpiperidine solution for 5 min twice. After washing with NMP and methanol and subsequent staining with BPB, each spot should be colored blue.

The coupling, acetylation, and deprotection steps were repeated for each AA of the respective peptide sequence of each SPOT until the desired peptides were obtained. Finally, these were deprotected in their side chains using a mixture of 95% trifluoroacetic acid, 2.5% water, and 2.5% triisopropyl amine [44].

For the immunological evaluation, the membrane with the peptides to be evaluated was blocked with 5% skimmed milk in phosphate buffer saline with 0.05%-Tween 20 (PBST). After successive washes with PBST, it was exposed to a pool of serum dissolved in a 1/200 ratio for 90 min, subsequently incubated in an anti-antibody-enzyme conjugate solution (IgG, IgM, IgA, or IgE) overnight, and then a chemiluminescent or colorimetric substrate was added. Positive peptides were observed as spots in different shades of gray or

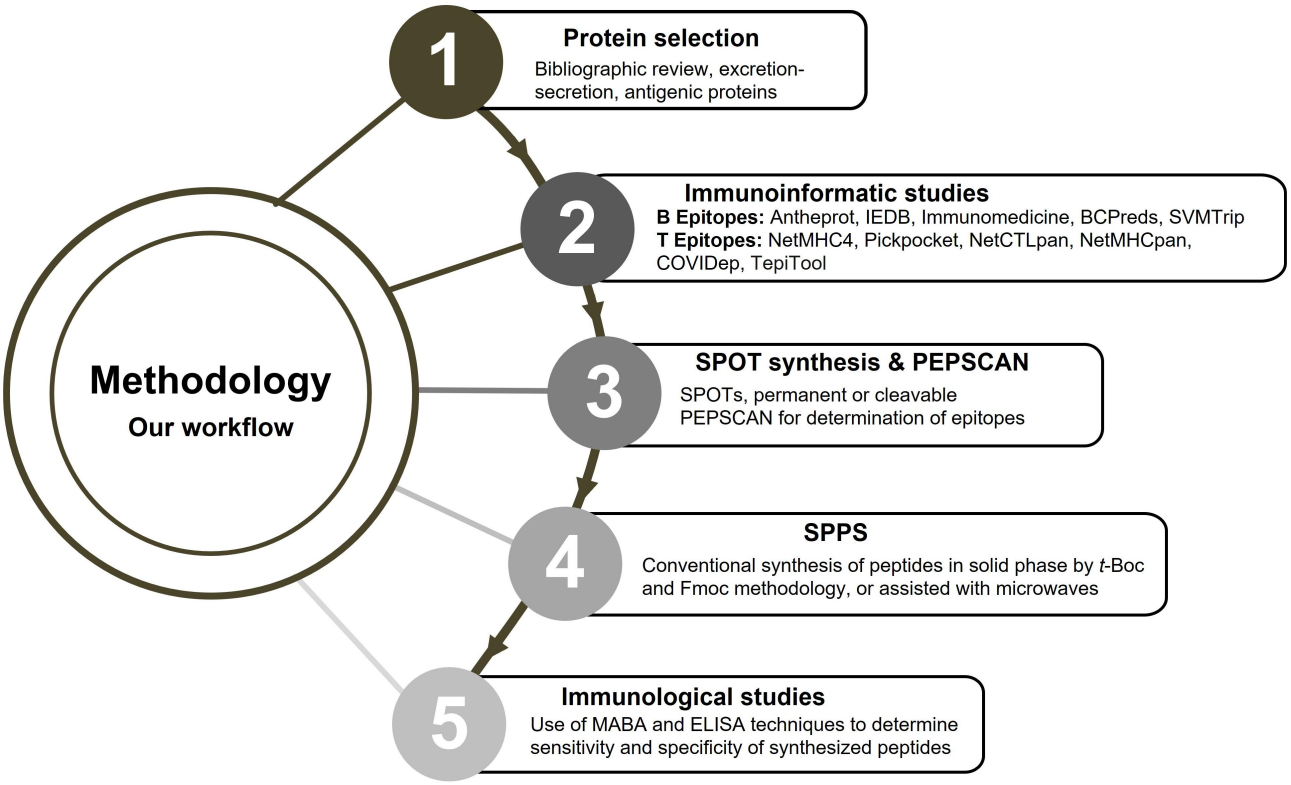

Figure 3. Methodological strategy followed for the development of new synthetic peptides. 1) Protein selection: the proteins of each pathogenic agent were selected based on the information collected from the literature; **2**) immunoinformatic studies: Web-based tools and databases were used to get possible B- and T-cell epitopes; **3**) SPOT/PEPSCAN: possible B epitopes were synthesized using the SPOT method (cleavable and not cleavable) and immunologically evaluated using the PEPSCAN technique; **4**) SPPS: synthesis of peptides identified in greater quantities using solid-phase synthesis on polymeric support (resin); **5**) peptides were evaluated for antigenicity using immunoenzymatic techniques such as ELISA, MABA, and/or WB. ELISA: enzyme-linked immunosorbent assay; Fmoc: 9-fluorenylmethyloxycarbonyl; IEDB: immune epitope database; MABA: multiple antigen blot assay; SPOT: synthesis of peptides on cellulose membrane; SPPS: solid-phase peptide synthesis; *t*-Boc: *tert*-Butyloxycarbonyl. WB: Western blotting

colored if a colorimetric substrate was used. These positive peptides were then produced in large quantities and used for evaluation using ELISA and/or MABA.

Conventional ELISAs are made by coating the plates with a 2–5 µg/mL dilution of the peptides, 1:100 dilution of sera, and using *p*-nitrophenylphosphate (*p*-NPP) as substrate, as previously described [11].

MABA is a dot-blot assay in which different antigens are adsorbed onto nitrocellulose paper, and 0.45 µm is inserted into a commercial or home-made acrylic chamber with 28 channels in carbonate bicarbonate buffer (pH 9.6) for 60 min [3]. Each strip can adhere up to 26 different antigens and two positive controls.

In our experiment, normal human serum (HS) was used as a source of immunoglobulins for positive controls. The nitrocellulose membrane was blocked with skimmed milk (5%) in PBST and cut perpendicularly to allow the 28 adsorbed antigens to be simultaneously exposed to the diluted body fluid (1:100 in blocking buffer). Secondary antibodies with enzymes were used to reveal positive signals with the corresponding substrates and were exposed to patient sera for antibody detection. Anti-antibodies conjugated to enzymes showed positive reactions as small colored boxes.

Highlighted case studies for the diagnosis of infectious and parasitic diseases

Using the previously described methodology, peptides of great diagnostic value from the following infectious and parasitic diseases were selected: human immunodeficiency virus (HIV), hepatitis A and C, schistosomiasis, Chagas disease, malaria, COVID-19, and *Helicobacter pylori* (*H. pylori*) infection. The results obtained with diseases caused by viral, bacterial, and parasitic agents are included. Other agents, such as *Toxoplasma gondii*, *Leishmania* sp., *Toxocara canis*, hepatitis delta virus (HDV), *Mycobacterium tuberculosis*,

Histoplasma capsulatum, *Paracoccidioides brasiliensis*, *Treponema pallidum*, and human T-lymphotropic virus 2 (HTLV-2), have been studied too.

For the analysis of peptides, the general concepts of comparing a diagnostic test with a standard reference test were used [45]; the results were quantified in terms of the percentages of sensitivity and specificity.

Sensitivity is the ability of a test to detect the highest number of positives in a sample of positives previously characterized by another reference test; it was calculated as (number of true positive samples/number of total positives) × 100, using a significant number of well-characterized human samples. While specificity is the ability to diagnose negatives from a pool of previously characterized negatives, it was calculated as (number of true negative samples/number of total negatives) × 100, this number of total sera can vary depending on the disease and its availability at the time. In some cases, saliva was also used.

The results are shown in Table 3, where a number of publications were gathered to display the evolution of our strategy and how these peptides may be used in a kit for multidiagnosis in the near future. These were preliminary studies with a limited number of samples. Larger numbers of sera and saliva are required to validate its use in a prototype. The Warao, mentioned in the table, are an indigenous population living in Venezuela.

HIV

Manually-synthesized peptides derived from conserved regions of HIV-1 glycoproteins 41 and 120 (gp41, gp120) were designed. The gp41 protein is a surface protein of HIV, and antibodies to the gp41 protein can be used to diagnose HIV infection [58]. The gp41 protein is a candidate for HIV-1 diagnosis; it has a high reactivity with HIV-1-positive sera and, therefore, a high antigenicity. Three pairs of synthetic peptides, 2 pairs derived from the HIV-1 gp41 and one from the gp120, were tested with HIV sera using MABA. Each pair consists of the same peptide, with some modifications. IMT-1954 is a 19-AA sequence derived from the gp41 protein that contains CG in the N-terminal and glycine-cysteine (GC) in the C-terminal. The addition of CG and GC AAs favors its polymerization and yields a higher reactivity by increasing the number of binding sites to patient antibodies [59]. We have observed in previous works carried out in our laboratory that polymeric peptides are more antigenic and immunogenic.

The peptide IMT-2197 is a modification of the p41 IMT-1954 with CG only in the N-terminal, allowing the formation of dimers. The peptide IMT-1961 is derived from the same protein (gp41) but contains part of the peptides IMT-1954 and IMT-2197 as a dimer. Peptides IMT-1965 (dimer) and IMT-1968 (polymer) were derived from the HIV-1 gp120 sequence. Different degrees of reactivity to these peptides were found in the HIV serum samples. In our studies, 146 sera [108 with positive polymerase chain reaction (PCR) results] were tested with a recognition frequency of 86.6% for the peptide IMT-1954 (non-published data, Figure 4, Table 3). The peptide IMT-2197 showed a recognition frequency of 82.8% in 105 patients. With the mixture of peptides IMT-1954 + 1961 + 2197, the recognition was 100% in 41 PCR-positive patients. Using MAPs from different proteins guarantees maximum recognition and avoids the possibility of a lack of response due to genetic background. In previous synthesis processes, these peptides were evaluated with 37 HIV-positive sera and were recognized by 86.5–91.9% of those patients. On the other hand, comparing patients with or without treatment, each of the peptides IMT-1954 and 2197 had a recognition frequency of 100% in 11 untreated patients, which decreased to 86.6% in 15 patients after treatment (Figure 5). Specificity was always 100%; this means there was no cross-reaction, and all negative sera evaluated were negative for this test. These peptides could be used for follow-up during antiretroviral therapy since it is known that patients under treatment exhibit attenuated responses, a phenomenon also reflected in rapid tests.

Our team, when working as Durango et al. [46], aimed to analyze the immune response to HIV synthetic peptides in different ethnic groups from Venezuela, with a particular interest in the indigenous Warao population, facing a devastating HIV-1 epidemic. The Warao displayed higher frequencies of recognition for all peptides. This distinctive pattern suggests a unique immune response profile within the

Table 3. Characteristics of the peptides produced for each biological agent from 1993 to 2024 in the Instituto de Medicina Tropical, Universidad Central de Venezuela

Agent	IMT code	Protein	Characteristics	Method	No. of sera tested	Sensitivity (%)	Specificity (%)	Ref.
HIV	957	gp41	CG-GC (polymeric)	MABA	37 HIV+	91.9	100	Not published
	953	gp41	CG-GC (polymeric)	MABA	37 HIV+	86.5	100	
	956	gp41	CG-GC (polymeric)	MABA	37 HIV+	86.5	100	
	959	gp41	CG-GC (polymeric)	MABA	37 HIV+	86.5	100	
	1954	gp41	CG-GC (polymeric)	MABA	146 HIV+	86.6	100	
					20 Warao HIV+	100	100	[46]
					15 with treatment	86.6	100	Not published
					11 without treatment	100	100	
	1961	gp41	Contains parts of 1954 + 2197, GC on C-terminal (dimeric)	MABA	59 HIV+	61	100	
	2197	gp41	Modification of 1954, CG on N-terminal (dimeric)	MABA	105 HIV+	82.8	100	
					15 with treatment	86.6	100	
					11 without treatment	100	100	
	1954 + 1961 + 2197	gp41	-	MABA	41 HIV+	100	100	
	1965	gp120	GC on C-terminal (dimeric)	MABA	59 HIV+	37.3	100	
					20 Warao HIV+	65	100	[46]
					15 with treatment	60	100	Not published
					11 without treatment	63.6	100	
	1968	gp120	CG-GC (polymeric)	MABA	59 HIV+	4.67	100	
					20 Warao HIV+	65	100	[46]
					15 with treatment	60	100	Not published
					11 without treatment	63.6	100	
	1954 + 1968	gp41 + gp120	CG-GC (polymeric)	MABA	28 HIV+	57.1	100	
					20 Warao HIV+	65	100	[46]
					15 with treatment	60	100	Not published
					11 without treatment	72.7	100	
HCV	286 + 59 + 290	Core + NS4 + NS5	Monomeric	ELISA	59 immunocompetent	100	100	[47]
					129 immunocompromised	91	100	
	59	NS4	Monomeric	ELISA	39 urban, rural, and Indigenous people	72	-	[48]
	286	Core				67		
	290	NS5				46		
	2052	Core	Monomeric	MABA	22 HCV+	100	100	[49]

Table 3. Characteristics of the peptides produced for each biological agent from 1993 to 2024 in the Instituto de Medicina Tropical, Universidad Central de Venezuela (*continued*)

Agent	IMT code	Protein	Characteristics	Method	No. of sera tested	Sensitivity (%)	Specificity (%)	Ref.
HAV	1996	VP1	Dimeric	PEPSCAN/ELISA/MABA	12–14 HAV+	87–100 IgG; 100 IgM	100	[50]
T. cruzi	9, 12, 14, 47	Hsp70	Polymeric, highly conserved	ELISA	9 chagasic	77.8	Low Inespecificity	[51]
	3972, 6303, 3973	TcCA-2 membrane protein	Monomeric	ELISA	133 chronic/non-acute, indeterminate symptomatic/non symptomatic	90	98	[52]
P. falciparum	94A, 154, 200	GLURP	Polymeric	ELISA/MABA	Rabbit hyperimmune sera	-	-	[27]
S. mansoni	164	Sm31	Polymeric	MABA	51	49	100	[53, 54]
	180	Sm31	Polymeric	MABA		86	100	
	178	Sm31	Polymeric	MABA		49	100	
	172	Sm31	Polymeric	MABA		35	100	
	164 + 180	Sm31	Polymeric	MABA		96	100	
	490, 491, 492, 487	Sm31	Monomeric/polymeric	ELISA/MABA/WB ELISA rabbit immunization for polyclonal Ab: capture assay	40 patient schistosomiasis+	22.5–67.5	100	[55]
	487, 488, 489, 493				40 rabbit hyperimmune sera	62.5	85.3	
	172, 180	Sm31	Polymeric	ELISA rabbit immunization for polyclonal Ab: capture assay	-	70.8	80.5	[56]
	12, 14, 64	Sm32	Polymeric	Rabbit immunization for polyclonal Ab: capture assay	-	-	-	-
SARS-CoV-2	Multiple peptides	S (S1, S2, RBD)	Monomeric	PEPSCAN	10 acute sera in 2 pools; IgM, IgG, IgA	High	-	[44]
		M				High		
		N				High		
		ORFs				0		
H. pylori	881	CagA	Monomeric	MABA/ELISA	349 sera (IgG); 390 saliva (sIgA)	92.6–96.3	100	-
	869			IgG, sIgA		62–87.6	100	
	866					67.2–100	99–100	
	1436					60.6–100	67–100	[57]
	1440					80.8–100	66–100	

Table 3. Characteristics of the peptides produced for each biological agent from 1993 to 2024 in the Instituto de Medicina Tropical, Universidad Central de Venezuela (*continued*)

Agent	IMT code	Protein	Characteristics	Method	No. of sera tested	Sensitivity (%)	Specificity (%)	Ref.
	1441					100	86–100	Data not
	1442					84.2–100	88.4–100	published
	1444					100	66.6–100	
	1445					75–80	96–100	
	Pools					99–100	100	

-: no data. Ab: antibody; CagA: protein from *Helicobacter pylori*; CG-GC: cysteine-glycine-glycine-cysteine; ELISA: enzyme-linked immunosorbent assay; GLURP: glutamate-rich protein; HAV: hepatitis A virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; IMT: Instituto de Medicina Tropical; M: membrane; MABA: multiple antigen blot assay; N: nucleocapsid; ORFs: open reading frames; RBD: receptor binding domain; S: spike; WB: Western blotting

Warao group. The antibody response in this group was notably robust, contrary to our expectations, considering their rapid evolution to acquired immunodeficiency syndrome (AIDS). This group revealed a surprisingly resilient antibody response, challenging prevailing notions regarding immune responses in unique subpopulations and highlighting the importance of considering population-specific factors in HIV research.

Our peptides detected HIV-1-positive samples in the general Venezuelan population and in an indigenous Warao population from Venezuela. Specificity was 100% since no reactivity was observed with negative sera. All of the peptides were recognized by three sera from HIV-1-positive samples infected with subtype A1. HIV-1 patients under antiretroviral therapy exhibited a lower frequency and intensity of peptide recognition.

Our study revealed that there was a significant difference in the antibody reactivity pattern between samples from patients with antiretroviral therapy and those from the Warao population for almost all of the peptides. The Warao group recognized the peptides with higher intensity than other ethnic groups. The application of MABA demonstrated its efficacy in detecting antibody reactivity patterns among different cohorts. This technique effectively identified specific peptides that were particularly recognized, showcasing its potential for analyzing the antibody response in depth [2]. The use of synthetic peptides, coupled with the robust performance of MABA, enriches our understanding of antibody responses in different HIV-1-infected populations.

Hepatitis C virus

Synthetic peptides have also proven to be useful tools for the immunodiagnosis of hepatitis C virus (HCV) infection. Synthetic peptides (monomers) from the subtype 1a polyprotein from the HCV core (40-AA IMT-286 N-terminal and 20-AA 716 derived from a region located inside 286), NS4 (20-AA IMT-59 that contains a highly conserved sequence among different HCV isolates), and NS5 (35-AA IMT-290) regions were evaluated using ELISA. The mixture of peptides IMT-286, -59, and -290 detected antibodies in 100% of the immunocompetent infected patients and in 91% of the immunocompromised patients. Therefore, this peptide cocktail seems to be useful for epidemiological studies in the general population [47].

Thereafter, these peptides were used to diagnose the hepatitis C seroprevalence in Venezuela [48]. A total of 2,592 sera from urban, rural, and indigenous populations were tested with an in-house-developed immunoassay based on the previously mentioned synthetic peptides. In summary, 39 of the samples were found to be positive for HCV peptides. This in-house, synthetic-peptide-based immunoassay seems to be a valuable tool for epidemiological studies. To improve

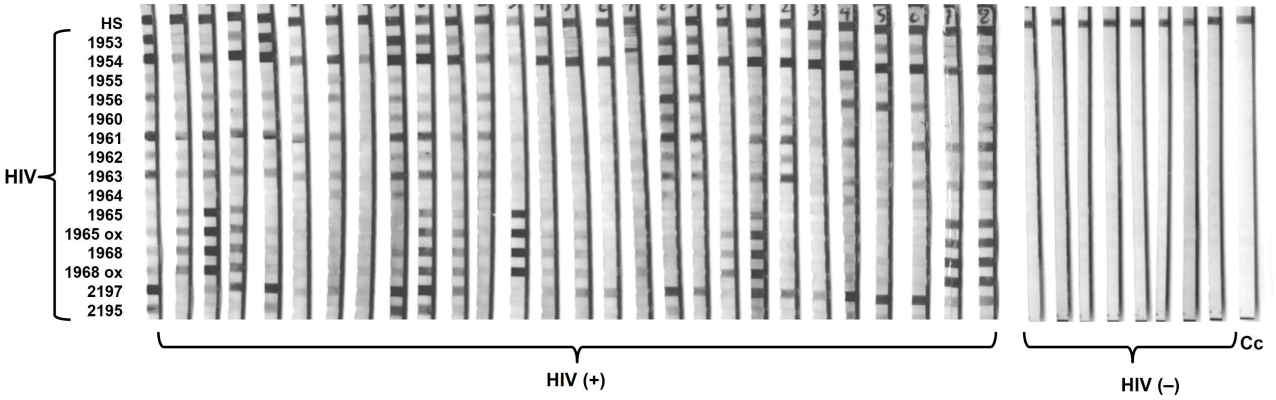

Figure 4. HIV-derived peptides were exposed to 28 HIV-positive sera [HIV (+)] and eight negative [HIV (–)] controls. Cc: conjugate control without sera; HS: human serum as a source of immunoglobulins for positive controls. HIV: human immunodeficiency virus; ox: oxidized form

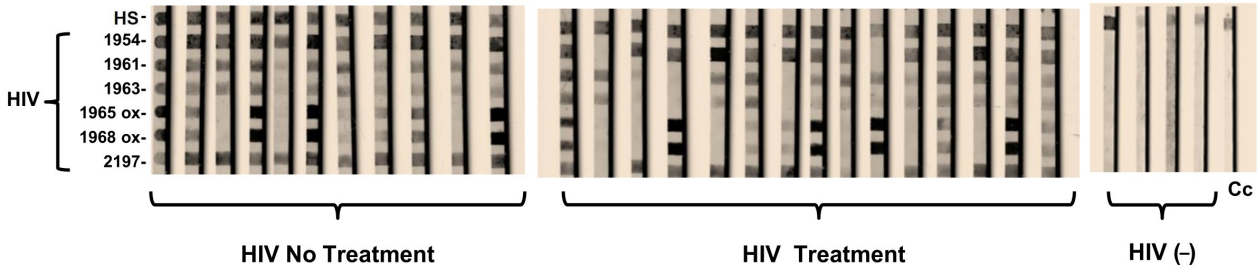

Figure 5. HIV peptides were exposed to HIV patients' sera with and without treatment. HIV (–): HIV negative sera; Cc: conjugate control without sera; HS: human serum as a source of immunoglobulins for positive controls. HIV: human immunodeficiency virus; ox: oxidized form

previous results and to decrease the size of one of the previous reactive peptides of 40-AA, we reanalyzed the core, E2, NS3, NS5A, structural, and non-structural viral proteins using immunoinformatics to select other antigenic peptides that were tested initially with the PEPSCAN technique [49]. Next, we performed the immunodiagnosis of HCV infection using MABA with modifications of these peptides with and without stearic acid at the N-terminal. A 20-AA linear peptide (IMT-2052) belonging to the N-terminal of the worldwide conserved core protein without the addition of stearic acid, showed 100% sensitivity and specificity in 22 confirmed patients [49]. This strategy (PEPSCAN plus MABA) allowed the selection of this peptide sequence with just 20 residues from the very conserved core protein, which leads to a low-cost alternative for prevalence studies and early diagnosis of HCV infection and highlights its relevance in the immunodiagnosis of viral hepatitis C.

Hepatitis A virus

A group of antigens from the exposed capsid proteins VP1, VP2, and VP3 that were previously known for their antigenicity was selected [50]. After its synthesis and the corresponding reactivity analysis by PEPSCAN using a pool of sera from hepatitis A virus (HAV) patients, a 15-AA peptide (IMT-1996) was selected for greater-scale synthesis on resin, one of which was derived from the C-terminal of the VP1 protein. The peptide was then evaluated using MABA and ELISA in a dimeric form, with a sensitivity of 87–100% with anti-IgG antibodies and 100% with anti-IgM. The HAV infection is usually an acute disease, in contrast with hepatitis B virus and HCV. Therefore, as shown in our assays and previously expected, the diagnosis is more sensitive in detecting IgM. The C-terminal region of the protein VP1 has been previously recognized as antigenic by others [60–63]. This recombinant antigen has been used successfully in epidemiologic studies [64–66].

However, synthetic peptides are easier to produce, more chemically stable, and less expensive than recombinant molecules, especially with sequences shorter than 20 residues. The evaluation of this peptide using saliva in addition to sera and IgA as immunoglobulin is ongoing.

Trypanosoma cruzi

We have also obtained good results using synthetic peptides derived from parasitic agents, such as *Trypanosoma cruzi* (*T. cruzi*), the pathogen responsible for Chagas disease. The reactivity of chagasic sera against a group of peptides covering most of the heat-shock protein Hsp70 has shown that > 50% of the peptides had a positive response, but a high reactivity was observed only against a few peptides (9, 12, 14, and 37) in 9 positive sera [51]. The general pattern of reactivity against the peptides is different in chagasic and healthy sera.

For the discrimination of the clinical stages of Chagas disease, we described those three out of five different repeats contained in the *T. cruzi* TcCA-2 membrane protein (3972, 6303, and 3973) that are recognized by > 90% of chronic Chagas disease patients but not by patients in the acute phase of the disease [52]. The 3973 peptide shows a specificity of > 98% since it is not recognized by patients with autoimmune and inflammatory diseases, nor by patients with a non-chagasic cardiomyopathy. It is noteworthy that the antibody levels against the 3973 epitope detected by ELISA with the sera from Chagas patients in the symptomatic chronic phase, involving cardiac or digestive alterations, are higher than those detected by the sera from Chagas patients in the indeterminate phase of the disease. We suggest that this diagnostic technique could also be used as a biomarker of pathology. We determined that certain AAs [F, Q, and diketopiperazine (DKP), located in the peptide at positions 1, 3, and 8 to 10, respectively] are essential to the conformation of the immunodominant antigenic epitope.

Plasmodium falciparum

Malaria is still a serious public health problem across the world, affecting hundreds of thousands of people annually, mainly in tropical countries. That is why it is crucial to make an accurate diagnosis in order to apply the appropriate treatment. Derived from *Plasmodium falciparum* (*P. falciparum*) excretory-secretory proteins {the glutamate-rich protein (GLURP), the histidine-rich protein 2 (HRP2), the HRP3 [the falciparum interspersed repeat antigen (FIRA)], and the serine-rich antigen homologous *P. falciparum* (SERPH)}, peptides were designed to be used in an antigen-capture assay [27]. Conformational and antigenic predictors were used. Polyclonal antibodies in rabbits were prepared using the peptides as antigens. Eight out of 14 constructs by ELISA and six by MABA elicited antibodies, corresponding to the predictive study and the immunogenicity obtained in rabbits. Western blots were performed with *P. falciparum* cultures to determine the binding activity and specificity of the anti-peptide antibodies, reacting against five molecules from 73 to 128 kDa. An inhibition assay was induced by preincubation with the homologous peptides.

Interestingly, the antibodies anti-HRP2 recognized the recombinant protein included in the ParaSight-F rapid test. In the same way, synthetic peptides from the HRP2 molecule were recognized by the monoclonal antibody present in the same rapid test. This confirms the potential value of synthetic peptides to induce monospecific polyclonal antibodies for developing antigen-capture assays.

Attempts to develop antigen-capture assays in blood and saliva have been performed. These peptides from the repetitive region of the GLURP, previously identified as IMT-94A, -154, and -200, and the peptide IMT-192 of HRP2 [27] were exposed to malaria patients' sera and saliva in PEPSCAN and were recognized with frequencies up to 47% in sera only, not in saliva (data non published). Preliminary results have been obtained with a group of peptides: IMT-2079 and -2082 (RAP-3), -2087 (MSP-9), and -2089 (FIRA), that were used to immunize hens in order to obtain specific IgY antibodies from the eggs. IgY against peptides IMT-2087 and -2089 were successfully produced by the hens, and now we are in the process of evaluating if it is possible to detect these antigens in a capture assay.

Schistosoma mansoni

Extensive studies have been developed on the diagnosis of schistosomiasis since the active search for potentially infected communities requires massive, sensitive diagnosis to detect asymptomatic cases of very low parasitic load [53–55]. Antigenic peptides derived from the proteins Sm31 (cathepsin B) and Sm32 (asparaginyl endopeptidase) were evaluated using 51 human sera infected with *Schistosoma mansoni*

(*S. mansoni*) [53]. Their immunogenicity in rabbits (in order to produce monospecific polyclonal antibodies) was measured using MABA [54]. Peptides IMT-164 and -180 from Sm31, when combined, showed sensitivities up to 96% with 100% specificity. Peptides from Sm31 were evaluated for antigenicity (in humans) and immunogenicity (in rabbits). The individual sensitivities ranged between 22.5 and 67.5%. Antigen-capture assays developed from rabbits immunized with synthetic peptides 487, 488, 489, and 493 recognized the native antigen in 62.5% of the patients' sera [55].

This is of particular relevance in areas of low and moderate intensities of infection (as low as 24 eggs/g feces), together with the evaluation of the efficacy of chemotherapy. With the same approach, 20-AA long peptides comprising the entire Sm31 molecule of the adult worm were synthesized, and their immunogenicity was evaluated in rabbits [56]. Peptides IMT-172 and -180 induced antibodies that adhered to the adult worm gut, where the Sm31 is present. They were also responsible for the recognition of the native Sm31 molecule via WB and by immunocytochemistry of cross-sections of adult worms [30]. Twenty-two polymeric peptides derived from Sm32 were synthesized, and rabbits were immunized [28]. Seventeen peptides were found to be immunogenic, and sera from immunized rabbits corresponding to the molecule from the first 335 AAs recognized the 32 kDa native protein from the adult worm antigen via WB. Sm32 is one of the excretory-secretory molecules released with the vomitus of the adult worm and is one of the target antigens for detection in the plasma of infected individuals. Sm32 was also studied thoroughly using "in silico" modeling and structural analysis [67] to assess its immunological and drug target implications. Sm32 can be exploited as a potential target for the development of biomarkers used in diagnosis.

Synthetic peptides are useful in studying the phylogenetic relationship between different schistosome species and also for application in diagnosis (pan-diagnosis) and/or cross-protective vaccines [68].

SARS-CoV-2 virus

RT PCR (real-time PCR) and antigen-detection tests are more useful for diagnosing acute COVID-19, while antibody tests measure the population's exposure. There is an urgent need for the development of diagnostic tests that can also be used for large-scale epidemiological screening, immunological screening, and the coverage of vaccine programs. A detailed analysis of the three structural proteins [spike (S), nucleocapsid (N), and membrane (M)] and the non-structural proteins [open reading frames (ORFs)] present in the SARS-CoV-2 virus was conducted by combining immunoinformatic methods, peptide SPOT synthesis, and the PEPSCAN immunoassay [44]. Sera from patients' pools in PEPSCAN showed a strong signal for peptides corresponding to the S, N, and M proteins of the virus, but not for the ORFs. The peptides exhibiting higher signal intensity were found in the C-terminal region of the N protein, showing different levels of recognition with IgM, IgG, and IgA isotypes. This difference in reactivities can ensure accurate diagnosis of infected patients, including their infection exposure times. A validation in the general population using an ELISA in-house assay is being performed to determine the seroprevalence of SARS-CoV-2/COVID-19 in Venezuela.

In a subsequent investigation, using immunoinformatic tools, continuous and discontinuous linear B-cell epitopes were identified for the N and M proteins of the SARS-CoV-2 virus. Peptides were synthesized using the SPOT method, and their antigenicity was evaluated using PEPSCAN with groups of sera for three immunoglobulins (IgG, IgM, and IgA). The prediction of the secondary structure of these sequences was carried out using PEP-FOLD, and the secondary structure obtained was compared with the corresponding region of the crystallized M protein. These were synthesized in solid-phase on resin and evaluated using the ELISA immunoenzymatic technique to determine their antigenicity, obtaining sequences that proved to be the most antigenic. These studies are still in progress.

A subsequent screening allowed us to identify sequences that showed the highest percentages of sensitivity and specificity. Individual peptides and mixtures were evaluated in ELISA to determine interactions that increase the sensitivity of the assay, showing in some cases an increase in sensitivity and specificity when mixtures were used to compare with individual peptides (results not yet published). This research allowed us to obtain antigenic peptides from the N and M proteins that can be used for the development of methods for diagnosing and monitoring COVID-19.

The methodology employed by our research group for the identification of B-cell epitopes has also been successfully applied in the identification of potential T-cell epitopes, applying each of the above-mentioned algorithms to the selected COVID-19 proteins [S, receptor binding domain (RBD), N, and M]. The best epitopes are being evaluated by measuring the release of interferon gamma by blood cells in patients with COVID-19 exposed to peptides [69].

H. pylori

We also developed valuable synthetic peptides for the diagnosis of the gram-negative bacteria, *H. pylori*, which colonizes the human gastric mucosa. Its role in the pathogenesis of gastritis, peptic ulcer disease, and gastric cancer has been demonstrated, where the protein from *H. pylori* (CagA) has an important role in the development of gastric carcinogenesis [70, 71].

The effector protein CagA is expressed by the highly virulent *H. pylori* strains possessing *cagPAI*, and it is absent from less virulent *cagPAI*-negative strains. The biological activity of CagA is determined by the number of repeats of the sequence glutamic acid-proline-isoleucine-tyrosine-alanine (EPIYA), present in the C-terminal region.

The diagnosis of this infection is complex, mainly due to the inconsistency that may exist between the available diagnostic tests since few tests detect its virulence factors [72]. The diagnosis of strains that produce the oncogenic protein CagA contributes to evaluating the risk of serious gastric diseases, guiding treatment, and monitoring infection. In this context, various sequences of the CagA were analyzed according to the methodology previously described, and different peptides were synthesized, obtaining 11 peptides of high sensitivity and specificity when determining IgG and secretory IgA in 349 sera and 390 saliva from infected individuals evaluated in Venezuela, both by MABA (Figure 6). A moderate level of agreement (Cohen's kappa = 0.58) was observed when comparing these results with the detection of gene from *H. pylori* (*cagA*)-positive strains in patient biopsies using PCR.

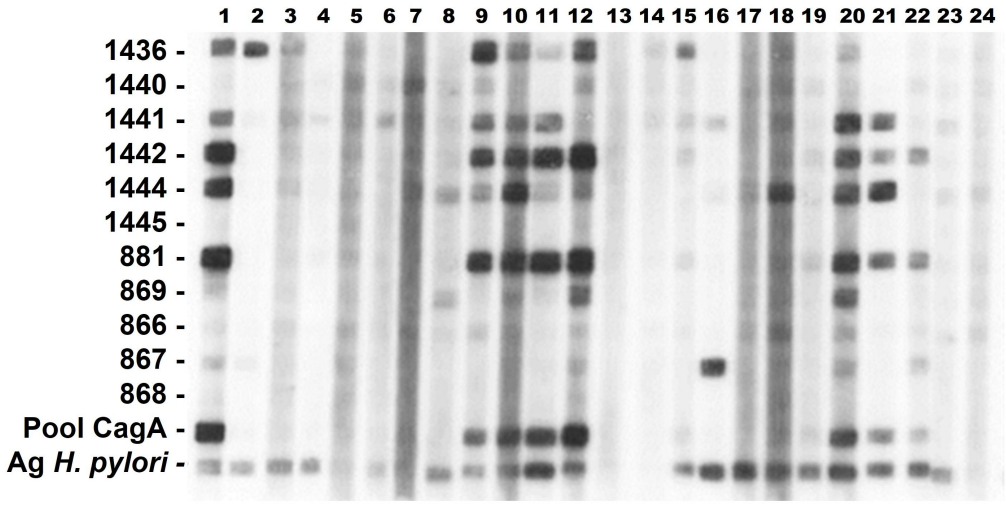

Figure 6. Reactivity of *H. pylori* CagA peptides in the sera (IgG) of *H. pylori*-infected and uninfected patients evaluated by MABA. *H. pylori*-negative: 5, 13, 14, 24; *H. pylori*-positive, CagA-negative: 2, 4, 15, 17, 19, 23; CagA-positive: 7; *H. pylori*-positive, CagA-positive: 1, 3, 6, 8, 9, 10, 11, 12, 16, 18, 20, 21, 22. CagA: protein from *Helicobacter pylori*; MABA: multiple antigen blot assay

Moreover, an ELISA assay was standardized, and six peptides from the CagA were selected based on their previously demonstrated good reactivity and sensitivity as determined by MABA (IMT-1436, -1440, -1441, -1442, -1444, and -1445), as well as a pool of these peptides (CagA pool). Peptides 1442, 1444, and 1445, which showed the highest antigenicity, were selected, and secretory IgA and serum IgG antibodies were determined in samples from patients positive and negative for the *cagA* detected by PCR. Statistically significant differences were observed in the median values of secretory IgA and serum IgG antibodies between patients with the *cagA* compared to those without the *cagA*, and these in turn were compared with

uninfected control patients. It should be noted that the studies have been carried out in symptomatic patients who, although negative at the time of evaluation, may have had a previous infection, so they may have specific memory antibodies.

Furthermore, the results demonstrate that secretory IgA had the highest recognition for all peptides, including the pool, compared to IgG. Some peptides were able to induce a stronger secretory IgA response (in saliva) than IgG (in serum), suggesting that these two types of antibodies recognize distinct epitopes in the peptides, reflecting the differences between the systemic IgG response and the mucosal immune response determined by secretory IgA, possibly related to active infection.

The development of synthetic peptides and the identification of *H. pylori*-specific epitopes, particularly CagA, and their use in immunodiagnosis, could be a significant contribution to the non-invasive prevention of gastric cancer, first-line diagnosis, and epidemiological studies, which are fundamental in high-risk populations with a high prevalence of infection.

Conclusions

The greatest burden of disease and mortality is found in the tropics, given that humans as well as wild and veterinary species are exposed to a large number of pathogens in tropical regions. This fact is aggravated by the possibility of being simultaneously co-infected with different pathogens, which should be detected by both epidemiological surveillance programs and the health facilities that receive those patients. Furthermore, since human populations are the most vulnerable, it is necessary to have simultaneous multidiagnostic tests accessible to local or regional health centers, which can be performed and interpreted by mid-level technical personnel due to their low cost and technical simplicity.

In this regard, the MABA test is a valid approach for developing countries, as it brings together the aforementioned qualities with the use of synthetic peptides as a source of antigens, allowing a low-cost system, plus offering thermostability and scaling up. Modular adaptability for different circumstances is a strength of the MABA. Therefore, the priority in its design should be directed towards programs such as blood banks, pregnancy monitoring, pre-surgery testing, immunocompromised patients, etc.

Developing a simultaneous multidiagnostic method is a complex challenge, as it is necessary to select not only the priority pathogens but also the best target antigens and, in this particular case, the best epitopes that guarantee high sensitivity with high specificity. This needs complex bioinformatic analyses and successive screening tests to identify the most reactive epitopes. It also requires harmonizing the technical conditions for binding and analyzing the different antigens on each MABA strip. Fortunately, only nanomolar quantities of the antigens in the form of peptides and a very small volume of blood or saliva are required. The MABA technique can also be designed for the capture of antigens.

This entire complex process, illustrated in Figure 3, faces difficulties inherent to the synthesis of certain peptides, in which it is hard to achieve the binding of some AAs. It is more likely that they can cyclize. What is more, redundant couplings may occur or may be truncated, interrupting the synthesis. There is also the possibility that the spatial conformation of the epitope cannot be imitated or that the epitopes are discontinuous sequences. Most of the peptides corresponding to B-cell epitopes have approximately 20 AAs or more, requiring in some cases a spatial conformation. In contrast, T-cell epitopes do not have more than 12 AAs, and only the primary structure is essential.

Regarding the type of synthesis, there are highly versatile and efficient automated synthesis equipment currently available. Nevertheless, we have maintained the manual chemical synthesis to obtain larger amounts of peptides and also for budgetary reasons.

On the other hand, we changed from *t*-Boc synthesis to Fmoc synthesis, avoiding the continued use of HF, an extremely dangerous and polluting acid, without any other benefits in terms of productivity and the quality of the peptides.

Throughout the process described in the pipeline in Figure 1, the most important changes in these 32 years have been the incorporation of new predictive algorithms developed with complex computer programs, as well as the incorporation of the SPOT synthesis technique and its subsequent development by PEPSCAN.

In a universe of 2,453 peptides synthesized by *t*-Boc and Fmoc methodologies plus 2,229 by SPOT synthesis, the success of the prediction after their immunological evaluation was 65% on average. However, recently we have observed that the use of immunoinformatics algorithms followed by SPOT synthesis (unpublished results) has shown a drop in sensitivity. This low percentage of recognition may be due to conformational rigidity when constructing the peptide attached to the cellulose paper; the peptide is built through the terminal carboxy group, which limits and, in many cases, prevents the peptide-patient antibody interaction, particularly by the carboxy-terminal region, which is relevant in the interaction. This can be prevented by constructing the peptide at the other end (amino-terminal group).

In general, 4 to 6 matching epitope regions per protein were determined across all the algorithms used, with sizes varying between 16 and 38 AAs. This suggests that when using different immunoinformatics algorithms, it is appropriate to search for consensus regions among these algorithms and then scan by synthesizing peptides of 12–15 contiguous AAs and accurately locate the epitope or epitopes.

One limitation of peptide synthesis is related to the difficulty of synthesizing conformational epitopes [73]. In our particular case, at least 95% are continuous epitopes that can be predicted and identified using the mentioned algorithms, including the SPOT synthesis technique. In this regard, there are two important aspects: first, the identification of discontinuous epitopes, which requires knowledge of the tertiary structure, either crystallized by NMR or through in silico models of the protein. The respective tertiary structure is often unavailable. Several platforms facilitate this prediction, such as PEPITO, ELLIPRO, DISCOTOPE, and CBTOPE. The second aspect is that the synthesis of these epitopes [74, 75] can be very complex. Computational predictions are often required, followed by synthesis with techniques that mimic the tertiary structure, for example, the use of pseudopeptides or mimetic peptides [76, 77].

Not all antigens are proteins; carbohydrates are the secondary targets to identify in any pathogen or tumor antigens, proinflammatory molecules, allergens, etc. In our experience, we have worked on two parasitic models in which carbohydrates are the ideal targets. This is the case for the blood parasite *S. mansoni*, the causal agent of schistosomiasis, one of the main parasitic diseases worldwide. It is also the case for Chagas disease (American trypanosomiasis), in which the protozoan *T. cruzi* exhibits a dense glycocalyx formed by a group of complex carbohydrates that are also highly antigenic [78]. In both the *S. mansoni* and *T. cruzi* models, short-lived antibodies are induced, which not only serve to indicate the presence of the causative agent but also whether the infection is active and are the best biomarkers of cure in treated patients. In contrast, in both cases, protein antigens induce long-lived antibodies by the activation of memory B lymphocytes, which persist for years after the patient is cured [79].

The results shown by our group and other groups worldwide demonstrate the great potential of synthetic peptides due to their multiple qualities: low production cost, easy modifiability, easy adaptability to any diagnostic format, rapid production, the feasibility of automating their synthesis, easy scalability, and thermostability (a fundamental property for tropical countries). But, above all, the development of algorithms that facilitate the selection of the most antigenic and immunogenic regions has developed at great speed, making the production process cheaper and faster. Likewise, the possibility of confirming the quality of the peptide with techniques such as paper synthesis (SPOT) and subsequently confirming reactivity by PEPSCAN reinforces the power of the technology developed so far, which is automatable in all its parts. For all these reasons, this strategy has allowed us to put together a simultaneous multidiagnostic test, named MABA, which can compete with other currently available techniques due to its low cost, especially in developing countries.

Among the drawbacks of MABA, it must be noted that not all peptides adhere to the nitrocellulose paper in a controlled and reproducible way. We have tried monomers, dimers, and polymers in order to evaluate if the size of the molecule affects the adsorption to the nitrocellulose membrane. It is estimated

that a larger surface increases the availability of the AA residues involved in the antigenic recognition by the corresponding antibodies [80]. As mentioned before, the streptavidin coating of the membrane was assayed for biotinylation in order to increase adsorption in a controlled manner [26].

We are looking forward to progressively incorporating synthetic peptides from additional infectious agents for simultaneous screening by using the MABA technique. This does not exclude the possibility that some of these peptides may also be used in the development of rapid tests for single infections.

Finally, we can conclude that synthetic peptides are a promising approach in the search for sensitive and specific diagnostic assays, based either on the detection of antigens or antibodies.

Abbreviations

AAs: amino acids

BPB: bromophenol blue

cagA: gene from *Helicobacter pylori*

CagA: protein from *Helicobacter pylori*

CG: cysteine-glycine

DMF: dimethylformamide

ELISA: enzyme-linked immunosorbent assay

FIRA: falciparum interspersed repeat antigen

Fmoc: 9-fluorenylmethyloxycarbonyl

GC: glycine-cysteine

GLURP: glutamate-rich protein

HAV: hepatitis A virus

HCV: hepatitis C virus

HF: hydrogen fluoride

HIV: human immunodeficiency virus

HRP2: histidine-rich protein 2

IMT: Instituto de Medicina Tropical

MABA: multiple antigen blot assay

MAPs: multiple antigenic peptides

N: nucleocapsid

NMP: *N*-methyl pyrrolidone

NMR: nuclear magnetic resonance

ORFs: open reading frames

PBST: phosphate buffer saline with 0.05%-Tween 20

PCR: polymerase chain reaction

SPOT: synthesis of peptides on cellulose membrane

t-Boc: *tert*-Butyloxycarbonyl

WB: Western blotting

Declarations

Acknowledgments

We dedicate this publication to Dr. Manuel Elkin Patarroyo Murillo, who passed away on January 9th, 2025. He was a fundamental factor in the creation of our research group and supported training in the immunochemical area of the team, particularly in everything related to the chemical synthesis of peptides. He was also a great role model as a researcher who dedicated his life to generating well-being for the most vulnerable populations on the planet as a pioneer in the development of synthetic vaccines and the training of a very large group of scientists at the Colombian Institute of Immunology in Bogotá.

Author contributions

ON: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing. HB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Resources, Validation, Visualization, Writing—original draft, Writing—review & editing. DP: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing—original draft, Writing—review & editing. BAdN: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing—original draft, Writing—review & editing. DOP: Data curation, Investigation, Methodology, Validation, Visualization, Writing—original draft, Writing—review & editing. FHP: Data curation, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing—original draft, Writing—review & editing. SL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

This article summarizes the research produced in the area of synthetic peptides with diagnostic value for infectious diseases, generated by several research projects that were approved by different bioethics committees that comply with international regulations to respect ethical standards related to studies with human beings. Regarding the non-published results for patients with HIV/AIDS, the Bioethics Committee of the Instituto Venezolano de Investigaciones Científicas (IVIC) approved the use of sera from patients in the project titled: "Viral hepatitis and AIDS: molecular characteristics of infections in Venezuela (G-2005000394)" at Meeting No. 1323 on May 20th, 2009. Similarly, the sera of patients with *Helicobacter pylori* infection were used with the consent of the Ethical Committee of the Instituto de Biomedicina on November 14th, 2006, with the project titled: "Immunological profile, genotype and pathologies associated with *Helicobacter pylori* in Venezuelan individuals". This study complies with the 2024 Declaration of Helsinki.

Consent to participate

Informed consent to participate in the study was obtained from all participants.

Consent to publication

Not applicable.

Availability of data and materials

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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Publisher's note

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