Send Orders of Reprints at reprints@benthamscience.org

Protein & Peptide Letters, **2013,** *20,* **0000-0000**

Evaluation and Comparison of the Ability of Online Available Prediction Programs to Predict True Linear B-cell Epitopes

Juan G. Costa[1], Pablo L. Faccendini[2], Silvano J. Sferco[3], Claudia M. Lagier[2] and Iván S. Marcipar[1,*]

[1]*Laboratorio de Tecnología Inmunológica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. Paraje El Pozo -3000- Santa Fe, Argentina;* [2]*IQUIR, CONICET, Dpto. de Química Analítica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. Suipacha 531 -2000- Rosario, Argentina;* [3]*Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Paraje El Pozo -3000- Santa Fe, Argentina, and INTEC (CONICET-UNL), Güemes 3450, 3000-Santa Fe, Argentin*

Abstract: This work deals with the use of predictors to identify useful B-cell linear epitopes to develop immunoassays. Experimental techniques to meet this goal are quite expensive and time consuming. Therefore, we tested 5 free, online prediction methods (AAPPred, ABCpred, BcePred, BepiPred and Antigenic) widely used for predicting linear epitopes, using the primary structure of the protein as the only input. We chose a set of 65 experimentally well documented epitopes obtained by the most reliable experimental techniques as our true positive set. To compare the quality of the predictor methods we used their positive predictive value (*PPV*), i.e. the proportion of the predicted epitopes that are true, experimentally confirmed epitopes, in relation to all the epitopes predicted. We conclude that AAPPred and ABCpred yield the best results, as compared with the other programs and with a random prediction procedure. Our results also indicate that considering the consensual epitopes predicted by several programs does not improve the *PPV*.

Keywords: Diagnostic, epitope, immunochemical, linear B-cell epitopes, prediction, program, reagents.

1. INTRODUCTION

Nowadays, defined antigenic regions are incresingly being used for immunoassays either as unique molecules, as a mixture of several components, or as single multiepitope molecules obtained by antigen fusion [1-2]. Experimental methods to identify B epitopes from infectious microorganisms are quite expensive and require long-term trials, preventing their use in the study of antigenic regions of complete proteomes from microorganisms [3-4]. Therefore, the development of *in silico* tools to reliably predict antigenicity can reduce the experimental work required to identify the regions of interest [4]. In particular, when researchers undertake the identification of diagnostic epitope candidates using sequence databases information, usually the tertiary structure of proteins is unknown, their primary structure being the only one that can be used. Accordingly, when the aminoacid (AA) sequence is available, linear epitope prediction is the most reliable approach currently used to select putative epitopes [5]. Hence, it is desirable to gain knowledge about the performance of programs focusing on linear epitopes.

The literature addressing applicability of B-cell epitope predictors is mainly oriented to identify vaccine candidates [4]; hence, some criteria important for the development of clinical diagnosis reagents are lacking in these assessments. Thus, sensitivity and specificity are indicators commonly used to assess the quality of prediction programs, and the program ability to find the real epitopes of a protein, described by the positive predictive value (*PPV*) is generally omitted. Indeed, in epitope-based vaccine research, a single epitope may be crucial to achieve an immunoprotective response [6-7]; hence, good sensitivity of predictors is a highly desired feature. However, to reduce the number of experimental procedures to confirm the epitope diagnostic utility, the reliability of the predicted epitope is more important than its sensitivity because any effective antibody-epitope interaction within the antigen could be useful to detect specific antibodies against the infection agent, the combination of many epitopes being necessary to ensure sensitivity [8-9]. It is important to differentiate both parameters because prediction programs may produce a low sensitivity indicator but, at the same time, if real epitopes are predicted, programs may render a high PPV.

Since the early report of Hopp et al [10] in 1981, several procedures have become popular to detect linear epitopes against B Lymphocytes (BL) starting from the primary structure of a protein. Then, since the advent of computing technologies a wide repertory of methods have been developed based on different algorithms that can be grouped by their similarity. For example: BcePred [11], Antigenic [12] and LEPDs [13] employ a simple combination of propensity scales even using the same scales. AAPpred [14], BCpreds [15], FBCpred [16], LEPs [17] and COBEpro [18] use the support vector method using propensity scales and antigenicity of AA pairs. Other programs, such as ABCpred [19] and

*Address correspondence to this author at the Laboratorio de Tecnología Inmunológica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. Paraje El Pozo -3000- Santa Fe, Argentina; Tel: +54-0342-4575206; Ext: 125.; E-mail imarcipr@fbcb.unl.edu.ar.

0929-8665/13 $58.00+.00

Bepipred [20], are supported by a neural network system and the Hidden Markov Model, respectively.

The performance of linear epitope prediction programs, unfortunately, has not still achieved optimal efficiency, as stated by the authors of the different programs [11-12-14-19-20]. In general, these authors have assessed their respective program by comparing it with simple algorithms of propensity scales (which evaluate, e.g., hydrophobicity of AA-windows, as compared with that of the whole protein), without comparing the performance of the new predictor algorithm with those previously proposed by other authors [11-12-14-19-20]. The lack of published literature comparing prediction programs prevents users from making the best choice. In the present work, we performed this comparison with the aim of evaluating the usefulness of prediction programs in selecting epitopes from database sites for the development of reliable diagnostic reagents. We evaluated and compared the five high popular, online available programs that are useful to identify linear epitopes from the protein primary structure available. We selected programs that are representative of each kind of algorithms employed.

To meet our objective, we investigated the quality of programs, mostly focusing on the rate of true positively predicted vs. the total number of predicted epitopes. Then, instead of evaluating sensitivity and specificity, we determined the *PPV*. We manually curated an epitope database to enhance the accuracy of the results. We studied 11 proteins that had been whole mapped experimentally using highly reliable techniques for epitope detection [21]. Each program was run and the predicted epitopes were compared with the 65 true epitopes displayed in these proteins. We analyzed if the epitopes consensually predicted by several programs were more antigenic than those that had been predicted by only one of the programs studied. Finally, to monitor the program prediction efficiency, we compared the *PPV* with that obtained when using randomly selected regions as possible epitopes of the molecule under study.

2. MATERIALS AND METHODS

2.1. Proteins Studied

The criterion to select the linear epitopes to be included in our dataset was previous experimental confirmation of epitopes by Enzyme-Linked Immunosorbent Spot (ELISpot) assays [21] or by using multispecific panels of monoclonal antibodies that recognized linear epitopes. Considering that our goal was to assess the true linear epitopes recognized by prediction programs, we constructed our own dataset instead of using an extended dataset available because the latter contain experimentally heterogeneous data. As a result, we manually selected 11 proteins for which a total number of 65 BL epitope data were obtained. We selected the proteins from BciPep Database [22], HIV Molecular Immunology database (http://www.hiv.lanl.gov/content/immunology/index.html) and from the literature [23-25] (see database or author sources for each protein in Table **1**). BciPep is a B epitope database focused on vaccine research projects and HIV Molecular Immunology Database is an annotated, searchable collection of HIV-1 T-cell epitopes and antibody binding sites. Minimal, experimentally determined linear AA sequences responsible for antibody recognition from the proteins are listed in Table **1**. Furthermore, unlike other authors who benchmarked predictor programs, we worked with the whole proteins instead of separated peptides to detect real antigenic zones. Thus, our results are closer to the real scenario because program users generally input the whole protein. Additionally, we selected proteins that do not participate in autoimmune response to restrict the assessment of the programs to epitopes recognized by antibodies developed against pathogens.

2.2. Theory and Setting up of the Programs

Some representative, free, online available programs were used to predict the linear epitopes from the protein AA sequences. All these programs use mathematical algorithms together with propensity scales and/or experimental antigenicity data. Unless otherwise stated, each program was run using the default parameters. The programs assessed in this study were *AAPPred* [14], *ABCpred* [19], *BcePred* [11], *BepiPred* [20] *and Antigenic* [12].

AAPPred [http://www.bioinf.ru/aappred/] determines the antigenicity of individual AAs by support vector method. It evaluates the frequency with which AA pairs occur, together with the propensity scales of hydrophobicity [26], flexibility [27], accessibility in a protein [28], turn location [29], antigenicity [30] and polarity [31]. The program allows us to perform the analysis using AA pairs together with propensity scales (SVM1) or alternatively, using AA pairs alone (SVM2). We used the SMV1 mode.

ABCpred [http://www.imtech.res.in/raghava/abcpred/] allows for prediction of BL epitopes of a defined length 10, 12, 14, 16 or 20 AAs. The analysis is performed using a neural network system trained with a database of known epitopes [19]. In the present work, the program was set up to obtain epitopes of 20 AAs in length.

BcePred [http://www.imtech.res.in/raghava/BcePred/] uses methodologies developed by other authors to assign antigenicity values to each individual AA, such as propensity scales of hydrophobicity [26], flexibility [27], accessibility in a protein [28], turn location [29], surface exposure [32], polarity [31] and antigenicity [30]. We performed the analysis taking into account all the mentioned features together.

BepiPred [http://www.cbs.dtu.dk/services/BepiPred/] defines antigenicity values for each AA applying the Hidden Markov Model to a hydrophobicity scale [26].

Antigenic [http://emboss.bioinformatics.nl/cgi-bin/emboss/antigenic]. This program determines variable length epitopes using the Kolaskar algorithm [12]. This program combines information of known antigenic determinants with values of flexibility, hydrophobicity and accessibility [26].

Prediction programs define the antigenicity degree by means of a score, provided for each epitope in some programs or, alternatively, for each individual AA in others. In the latter case, an epitope or antigenic region can be defined in the primary structure when several AAs with high score are adjacent. Moreover, the number of epitopes or antigenic regions that prediction programs yield depends on a threshold that is set by the user. All the prediction programs are set

Table 1. Experimental database: Proteins selected for this study. Protein NCBI accession code, total length, number of actual epitopes experimentally determined and location of epitopes or antigenic regions are indicated. * Proteins are from BciPep Database (A), HIV Molecular Immunology Database (B) or taken from the literature (see reference number 17 for VP1, 16 for protein N, and 18 for Gliadin).

Protein (*)	NCBI Sequence Code	Length (in number of AA)	Quantity of Epitopes	Location of Epitopes or Antigenic Regions
MPT70 (A)	NP_217391	193	2	31-70, 100-120
MSP-1 (A)	BAF62280.1	1693	3	29-39, 1594-1611, 1644-1662
Exotoxin A (A)	NP_249839	638	8	297-313, 324-333, 354-387, 412-421, 510-522, 528-539, 557-593, 596-638
GAG1 (A)	P20873	504	10	11-25, 113-122, 142-156, 176-214, 216-268, 280-308, 312-321, 330-367, 406-416, 428-448
Gp160 (A)	NP_057856	856	13	30-141, 161-191, 211-231, 252-281, 294-344, 346-413, 424-511, 525-558, 561-615, 639-701, 724-747, 761-778, 822-855
Pr55 (B)	NP_579876	132	2	11-38, 51-78
Rev (B)	NP_057854	116	4	5-15, 32-51, 70-91, 96-116
VP1 (17)	ABC87248	781	12	31-41, 55-65, 85-95, 151-161, 292-392, 316-326, 493-500, 529-539, 547-554, 571-578, 667-707, 712-722
Gliadin (18)	A27319	296	3	31-72, 165-176, 237-264
Protein N (16)	AAP13445	422	6	1-6, 45-61, 153-192, 211-223, 225-235, 354-412
Protease (B)	CAB66012	99	2	1-7, 36-47

at the optimum threshold (defined by the respective authors), by default. However, the results obtained for several proteins when using this setting predicted the whole protein sequences as being antigenic. This prompted us to input the protein sequences, run each program using its default threshold but considering as epitopes only top scored n/60 AA (where n is the protein length). Actually, one epitope in approximately 60 AAs is the real epitope frequency in the proteins studied.

The minimal AA length that a sequence should have to be recognized by antibodies is still under discussion [33-35]. Each program commonly predicts antigenic determinants of different lengths in the range of 1-30 AAs. In this work, we consider that a sequence is a predicted epitope if it has, al least, 6 AA in length. This has been proposed as the minimal AA length adequate to be bound by an antibody [36] in agreement with the criterion adopted by several authors [12-27-28-37]. Although prediction programs normally display separate epitopes, when results showed overlapped sequences, we considered all of them to be single antigenic region.

Sequences of 6 or more AAs, which were identified as antigenic by several programs, were evaluated and classified into two groups: weak and strong consensus groups. Epitopes were considered as belonging to the weak consensus group when they were predicted by three programs as antigenic with a low score, or they were graded as very antigenic displaying a high score by two programs. The strong consensus group contained epitopes that were predicted by a minimum of three different programs and simultaneously, at least one of them assigned it a high score or at least four programs marked them with low scores. We considered a score high when it was in the upper third of the rank of values of the method. Otherwise, we considered it a low score.

2.3. Program Comparison

The parameter we used to compare the programs was the *PPV*, i.e., the proportion between true and predicted epitopes, defined as:

$$PPV = \frac{C_{ep}}{T_{ep}} \; . 100\ \%$$

where C_{ep} is the number of correctly predicted epitopes which agrees, at least by 6 AAs with actual, experimentally found epitopes, and T_{ep} is the total number of epitopes, predicted or produced by programs or by the consensus. *PPV* was calculated for each program and each protein studied. We also evaluated whether the results produced by the programs were more truthful than epitopes obtained by a random prediction procedure. For this purpose, we calculated the average length of the epitopes predicted by the best program studied (AAPPred), and divided each protein into fragments of the calculated average length. Then, we evaluated how many of those fragments matched with real epitopes, and finally calculated *PPV* considering T_{ep}, the total number of the fragments obtained for the protein under study.

2.5. Statistical Analysis

Firstly, we verified the normal distribution of probability of the results obtained with the prediction programs and by random prediction. For this purpose, we calculated the asymmetry and Curtois indices, these belonging to (-1.174; 1.333) and (-0.540; 0.612) ranges, respectively. Results were graphed as a function of their cumulative frequency and, in all cases they turned out to be straight lines. Secondly, and once the Gaussian distribution of the probability was verified, the confidence intervals of *PPVs* were set at 90% level of significance for each different prediction procedure. As the same sample space was used to run all the programs, to compare the performance between them, we were able to calculate the difference among the *PPV*s using paired comparisons. Results were expressed as the 90% confidence intervals for each couplet of programs compared showing, with 90% of confidence, which program produced a *PPV* value different from that calculated for another program.

3. RESULTS

AAPPred, BcePred, ABCpred, BepiPred and Antigenic programs were run online to predict linear epitopes of all the proteins listed in Table **1**. As an example, we present the comparison of the results obtained for MPT70 protein using the different prediction programs and procedures (Fig. **1**).

The assessment of programs with the full panel of epitopes used in this work allowed us to determine the *PPVs* and the confidence intervals, set at 90% significance level (Fig. **2**). As an example, for the AAPPred predictor, a mean *PPV* value of 69.1 % means that of 100% of predicted epitopes (by this program itself, or by the weak- or by the strong-consensus), 69.1% are indeed experimentally found epitopes.

The differences of the means of the *PPV* between programs were calculated using the 65 experimental epitopes (Table **2**). When the confidence intervals contained the zero (0) value, then *PPV* means were considered to be equal (E), stated with 90 % significance; otherwise, *PPV* means were considered to be non-equal (NE).

4. DISCUSSION

The selection of data to evaluate program performance is always a quite difficult task; indeed, no database is to be considered gold standard because databases are incomplete

Figure 1. Linear epitopes predicted for MPT70 protein. The first line displays the real, experimentally found epitopes. In addition, there are results predicted by AAPPred, ABCpred, BcePred, BepiPred, Antigenic, weak consensus procedure and strong consensus procedure. Dark gray zones correspond to high score epitopes.

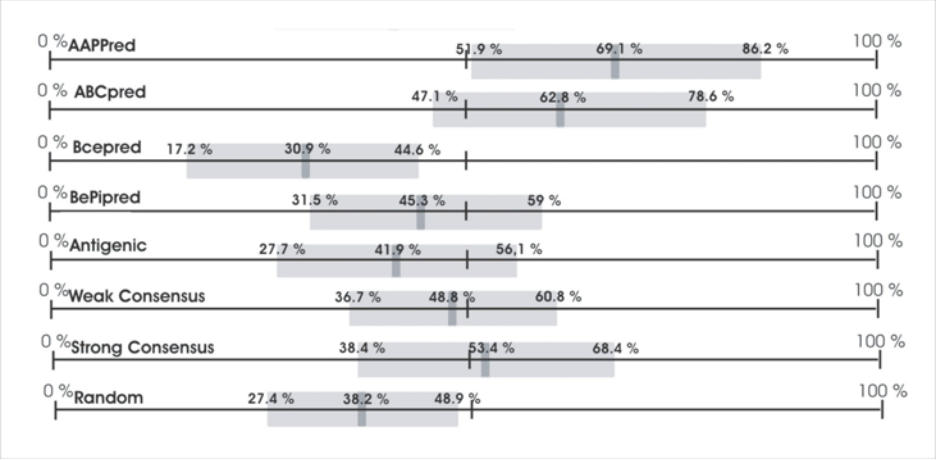

Figure 2. Comparison of the confidence intervals of the positive predictive value obtained for AAPPred, ABCpred, BcePred, BepiPred, Antigenic, weak and strong consensus, and the random prediction procedures, using all the experimental epitopes from the 11 proteins considered in this work (Table **1**).

Table 2. Program Comparison: Results of the Paired Comparison Between Each Couplet of Programs Studied, the Weak and Strong Consensus and Random Procedure, Having Set the Level of Significance at 90%. E=equal. NE=non-equal. See text.

	AAPPred	ABCpred	BepiPred	Antigenic	Bcepred	Weak consensus	Strong consensus
ABCpred	E						
BepiPred	NE	NE					
Antigenic	NE	NE	E				
Bcepred	NE	NE	NE	E			
Weak consensus	NE	NE	E	E	NE		
Strong consensus	E	E	E	E	NE	E	
Random	NE	NE	E	E	E	E	E

and based on heterogeneous experimental data [38]. Therefore, the automatic assessment of linear epitope prediction programs, using the whole database available is not reliable enough. Considering this difficulty, we used a moderate sample space, which in turn was very controlled. All the proteins were manually and carefully selected on the basis of the experimentally determined real epitopes. Furthermore, it should be noted that we did not use experimental negative epitopes simply because they are not necessary to determine the performance of programs when indicator *PPV*s are calculated. Hence, we consider that our results are highly reliable because negative epitopes, used to estimate specificity, are much less robustly defined in the experimental data available than positives epitopes [38], and because we made a meticulous selection of positive epitopes based on homogeneous experimental data. In addition, we have determined a confidence interval for our sample space that takes into account the number of epitopes analysed and the scatter of the *PPV* obtained; hence our comparison is of statistical significance.

In this work, we studied the programs AAPPred, ABCpred, BcePred, BepiPred and Antigenic. With all of these programs, the protein primary structure can be input. We compared their respective aptitude to predict linear epitopes that were already identified by experimental methods. Other comparative evaluations of prediction programs have also been published by Blythe & Flower [39] and Reimer [40], respectively. The former work evaluates propensity scales rather than online available programs. In that work, 484 propensity scales were used to predict AA antigenicity by using different, rather simple algorithms. Most of these algorithms compared the averages of the values of the propensity scales. The authors calculated Matthew's correlation and the mutual information coefficients, and concluded that the use of propensity scales only allowed for slightly better epitope prediction than random prediction [39]. It is noteworthy that, except for the algorithm used by BcePred program, which is comparable to those used by Blythe and Flower, the algorithms used by the programs studied in our work are very different and more complex. In fact, most of them use Markov chain or neural network models to perform calculations. Reimer's work, instead, evaluated two of the five prediction programs we studied, ABCpred and BepiPred [40]. The parameters assessed were program sensitivity, defined as the ratio between the number of epitopes correctly predicted over the total number of experimentally found epitopes, and program specificity, defined as the ratio between the number of the correctly predicted non-epitopes over the total number of non-epitopes experimentally found for different proteins. The author concludes that these programs predict slightly better than random prediction. However, these values (sensitivity and specificity) do not provide information on the reliability with which the program predicts, *i.e*., if the expected degree of confidence about the predicted epitope region is consistent with real epitope regions. Conversely, the analysis presented in our work allows us to evaluate reliability by calculating the program 90% confidence interval of *PPV*, as defined in the "Program comparison" subsection.

In the case of BcePred and ABCpred programs, the *PPV* was determined by their authors using their own database but, conversely to our analysis, confidence intervals have not been determined [11-19]. The *PPV*s reported were 58.7% and 65.6% for BcePred and ABCpred, respectively. As shown in Fig. 2, using our well controlled experimental epitope dataset, the *PPV* confidence interval obtained with BcePred ranged between 17.2-44.6 %, which is lower than the *PPV* reported by the authors, whereas with ABCpred, *PPV* ranged between 47.1-78.6%, with some values greater than those reported by the authors. Then, ABCpred was one of the prediction programs that best performed, as shown by the 90% confidence, whereas BcePred together with Antigenic did not perform better than random prediction (see Table **2**).

Regarding the program AAPPred, the authors did not evaluate the PPV [14]. For this predictor, our results show that *PPV* ranges between 51.9-86.2%, with 90% confidence (Fig. 2), indicating that AAPPred is one of the best performing programs together with ABCpred for the evaluated proteins (see Fig. 2). Interestingly, AAPPred uses the support vector method, also employed by other current programs, i.e.: BCpreds [15], FBCpred [16], LEPs [17] and COBEpro [18].

One putative reason to explain the difference among results produced by the programs studied is that the algorithms used by the programs are quite different. When comparing the results produced by AAPPred, which shares six of the seven propensity scales used by BcePred, except the one relying on surface exposure, it is apparent that programs lead to very different results (see Fig. 2 and Table **2**), therefore suggesting that the algorithm itself is the factor that best contributes to the differential prediction ability of a program. At the same time, even when quite simple algorithms appropriately weigh the physicochemical properties evaluated by several propensity scales, improved *PPVs* can be obtained [11-37].

Fig. 2 and Table **2** show that if we consider true epitopes only the regions that overlap those proposed as antigenic by several programs, this procedure by itself does not improve the *PPV* value, as compared with results produced by each program individually.

CONCLUSIONS

Having set the significance level at 90%, we obtained that the mean value of the positive predictive value, *PPV*, varied between 31% and 69%, BcePred resulting the only program displaying a mean *PPV* lower than *PPV* obtained with the random procedure (see Fig. **2**). Our results also indicate that only two of the programs studied, AAPPred and ABCpred, predicted epitopes with a statistically significantly higher *PPV* than a random procedure (see Table **2**). These two programs produced predictions with up to 86.2% and 78.6% accuracy, respectively, stated with 90% confidence. We also determined that the quality of the results obtained with programs that employ propensity scales are mainly determined by the algorithm used by the program rather than by the scale itself.

ABBREVIATIONS

AA	=	amino-acids
BL	=	B lymphocytes
NCBI	=	National Center for Biotechnology Information
PPV	=	positive predictive value
ELISpot	=	Enzyme-Linked Immunosorbent Spot
E	=	equal; NE, not equal

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGMENTS

This work was funded by the CONICET (Argentinean Research Council) and CAI+D PI 11-55/2009, from Universidad Nacional del Litoral, Argentina. J.G.C. is a fellow researcher of CONICET. I.S.M, and C.L are researchers of CONICET.

[1] Aguirre, S.; Silver, A.M.; Brito, M.E.F.; Ribone, M.E.; Lagier, C.M.; Marcipar, I.S. Design, Construction and Evaluation of a Specific Chimeric Antigen to Diagnose Chagasic Infection. *J. Clin. Microbiol*., 2006, *44*, 1043-46.

[2] Camussone, C.; Gonzalez, V.; Belluzo, M.S.; Pujato, N.; Ribone, M.E.; Lagier, C.M.; Marcipar, I.S. Comparison of recombinant Trypanosoma cruzi peptide mixtures versus multiepitope chimeric proteins as sensitizing antigens for immunodiagnosis. *Clin.Vaccine Immunol*., 2009, *16*, 899-905.

[3] Sobolev, B.N.; Olenina, L.V.; Kolesarova, E.F.; Poroikov, V.V.; Archakov, A. I. Computer design of vaccines: approaches, software tools and informational resources. *Curr. Comput. Aided Drug Des.*, 2005, *1*,207-222.

[4] Namrata, T.; Rajat, K.D. Immunoinformatics: an integrated scenario. *Immunology*, 2010, *131*, 153-168.

[5] Van Regenmortel, M.H.V. In: *Methods in Molecular Biology, Epitope Mapping Protocol;* Reineke, U.; Schutkowski, M., Eds.; Humana Press, Springer Science + Business Media: Berlin, 2009; Vol. 524, pp. 3-23.

[6] Wang, T.T.; Tan, G.S.; Hai, R.; Pica, N.; Ngai, L.; Ekiert, D.C.; Wilson, I.A.; García-Sastre, A.; Moran, T.M.; Palese, P. Vaccination with a synthetic peptide from the influenza virus hemagglutinin provides protection against distinct viral subtypes. *Proc. Natl. Acad. Sci. USA*, 2010, *107*, 18979-84

[7] Sutherland, J. N.; Maynard, J.A. Characterization of a key neutralizing epitope on pertussis toxin recognized by monoclonal antibody 1B7. *Biochemistry*, 2009, *48*, 11982-93.

[8] Noya, O.; Patarroyo, M.E.; Guzmán, F.; Alarcón de Noya, B. Immunodiagnosis of parasitic diseases with synthetic peptides. *Curr. Protein Pept. Sci*., 2003, *4*, 299-308.

[9] Alcaro, M.C.; Peroni, E.; Rovero, P.; Papini, A.M. Synthetic peptides in the diagnosis of HIV infection. *Curr. Protein Pept. Sci.*, 2003, *4*, 285-90.

[10] Hopp, T.P.; Woods, K.R. Prediction of protein antigenic determinants from amino acid sequences. *Proc. Natl. Acad. Sci.USA*, 1981, *78*, 3824-28.

[11] Saha, S.; Raghava, G.P.S. BcePred: prediction of continuous B-cell epitopes in antigenic sequences using physico-chemical properties, ICARIS 2004, LNCS 3239; Nicosia, G.; Cutello, V.; Bentley, P. J.Timis, J., Eds.; Springer: Berlin, 2004.

[12] Kolaskar, A.S.; Tongaonkar, P.C. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett.*, 1990, *276*, 172-74.

[13] Chang, H.T.; Pai, T.W.; Fan, T.C.; Su, B.H.; Wu, P.C.; Tang, C.Y.; Chang, C.T.; Liu, S.H.; Chang, M.D. A reinforced emerging methodology for mapping unique peptide motifs in members of protein families. *BMC Bioinformatics*, 2006, *7*, 38.

[14] Davydov, I.I.; Tonevitskii, A.G. Prediction of Linear B-Cell Epitopes. *Mol.Biol.(Mosk)*, 2009, *43*, 166-74.

[15] Chen, J.; Liu, H.; Yang, J.; Chou, K.C. Prediction of linear B-cell epitopes using amino acid pair antigenicity scale. *Amino Acids*, 2007, *33*, 423-28.

[16] El-Manzalawy, Y.; Dobbs, D.; Honavar, V. Predicting linear B-cell epitopes using string kernels. *J. Mol. Recognit.*, 2008, *21*, 243-55.

[17] Wang, H.W.; Lin, Y.C.; Pai, T.W.; Chang, H.T. Prediction of B-cell linear epitopes with a combination of support vector machine classification and amino acid propensity identification. *J. Biomed. Biotechnol.*, 2011, *2011*, 432830.

[18] Sweredoski, M.J.; Baldi, P. COBEpro: a novel system for predicting continuous B-cell epitopes. *Protein Eng. Des. Sel.*, 2009, *22*, 113-20.

[19] Saha, S.; Raghava, G.P. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*, 2006, *65*, 40-48.

[20] Larsen, J.E.; Lund, O.; Nielsen, M. Improved method for predicting linear B-cell epitopes. *Immunome.Res.*, 2006, *2*, 2.

[21] Shin, D.S.; Kim, D.H.; Chung, W.J.; Lee, Y.S. Combinatorial solid phase peptide synthesis and bioassays. *J. Biochem. Mol. Biol*., 2005, *38*, 517-25.

[22] Saha, S.; Bhasin, M.; Raghava, G.P. Bcipep: A database of B-cell epitopes. *BMC. Genomics*, 2005, *6*, 79.

[23] He, Y.; Zhou, Y.; Wu, H.; Kou, Z.; Liu, S.; Jiang, S. Mapping of Antigenic Sites on the Nucleocapsid Protein of the Severe Acute Respiratory Syndrome Coronavirus. *J. Clin. Microbiol*., 2004, *42*, 5309-14.

[24] Kaikkonen, L.; Lankinen, H.; Harjunpaa, I.; Hokynar, K.; Soderlund-Venermo, M.; Oker-Blom, C.; Hedman, L.; Hedman, K.

Acute-Phase-Specific Heptapeptide Epitope for Diagnosis of Parvovirus B19 Infection. *J. Clin. Microbiol*., 1999, *37*, 3952-56.

[25] Osman, A.A.; Gunnel, T.; Dietl, A.; Uhlig, H.H.; Amin, M.; Fleckenstein, B.; Richter, T.; Mothes, T. B cell epitopes of gliadin. *Clin. Exp. Immunol.*, 2000, *121*, 248-54.

[26] Parker, J.M.; Guo, D.; Hodges, R.S. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. *Biochemistry*, 1986, *25*, 5425-32.

[27] Karplus, P.A.; Schulz, G.E. Prediction of chain flexibility in proteins. *Naturwissenschaften*, 1985, *72*, 212-13.

[28] Emini, E.A.; Hughes, J.V.; Perlow, D.S.; Boger, J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. *J.Virol*., 1985, *55*, 836-39.

[29] Pellequer, J.L.; Westhof, E.; Van Regenmortel, M.H. Correlation between the location of antigenic sites and the prediction of turns in proteins. *Immunol. Lett*., 1993, *36*, 83-99.

[30] Lundegaard, C.; Lund, O.; Kesmir, C.; Brunak, S.; Nielsen, M. Modeling the adaptive immune system: predictions and simulations. *Bioinformatics*, 2007, *23*, 3265-75.

[31] Ponnuswamy, P.K.; Prabhakaran, M.; Manavalan, P. Hydrophobic packing and spatial arrangement of amino acid residues in globular proteins. *Biochim. Biophys. Acta*, 1980, *623*, 301-16.

[32] Janin, J.; Wodak, S. Conformation of amino acid side-chains in proteins. *J.Mol.Biol*., 1978, *125*, 357-86.

[33] Morris, G.E. In: Molecular biomethods handbook; Walker, M. J.; Rapley, R., Eds.; 2nd ed.; Humana Press, Springer Science+Business Media: New York, 2008; pp. 619-630.

[34] Davidson, H.W.; Watts, C. Epitope-directed processing of specific antigen by B lymphocytes. *J.Cell Biol*., 1989, *109*, 85-92.

[35] Moutsopoulos, N.M.; Routsias, J.G.; Vlachoyiannopoulos, P.G.; Tzioufas, A.G.; Moutsopoulos, H.M. B-cell epitopes of intracellular autoantigens: myth and reality. *Mol. Med*., 2000, *6*, 141-51.

[36] Alvarez, P.; Leguizamon, M.S.; Buscaglia, C.A.; Pitcovsky, T.A.; Campetella, O. Multiple overlapping epitopes in the repetitive unit of the shed acute-phase antigen from Trypanosoma cruzi enhance its immunogenic properties. *Infect. Immun*., 2001, *69*, 7946-49.

[37] Sollner, J.; Mayer, B. Machine learning approaches for prediction of linear B-cell epitopes on proteins. *J. Mol. Recognit*., 2006, *19*, 200-08.

[38] Caoili, S.E. Benchmarking B-Cell Epitope Prediction for the Design of Peptide-Based Vaccines: Problems and Prospects. *J. Biomed. Biotechnol*., 2010, *2010* (910), 524, 1-14.

[39] Blythe, M.J.; Flower, D.R. Benchmarking B cell epitope prediction: underperformance of existing methods. *Protein Sci*., 2005, *14*, 246-48.

[40] Reimer, U. In: *Methods in Molecular Biology, Epitope Mapping Protocols*; Reineke, U.; Schutkowski, M., Eds.; Humana Press, Springer Science + Business Media: Berlin, 2009; Vol. 524, pp. 335-346.

Received: ??? ??, 201? Revised: ????? 0?, 201? Accepted: ????? ??, 201?