© Український журнал клінічної та лабораторної медицини, 2010
УДК 577.27 + 577.152.193

Approaches to the synthesis of conjugates for enzyme immunoassay test-systems and evaluation of their use for diagnostics of infectious diseases

O.Yu.Galkin

National technical university of Ukraine «Kyiv polytechnic institute», Department of industrial biotechnology
Kyiv, Ukraine

This review sums up the available published data concerning the approaches aimed to obtain enzyme-antibody conjugates (including also conjugates containing monoclonal antibodies). The authors have analyzed the significance and role of bioconjugation in elaboration and production of up-to-date immunoenzyme test-systems for diagnostics of infectious diseases, detection of different substances etc. The review contains also the analysis of criteria determining the quantity of antibody-enzyme conjugates obtained and discusses different approaches for determination of their diagnostic value.

Key words: antibody, enzymes, conjugating reagents, conjugates, immunoenzyme test-systems, standard sera panels, specificity and sensitivity of analysis.

INTRODUCTION

The most of up-to date immunoenzyme test-systems are based on three detection schemas [11, 13, 16], indirect and sandwich ELISA modifications being the most widely used. Competitive ELISA variants are less preferable.

Diagnostic kits based on the ELISA approach permit to detect antibodies to different infectious agents of viral origin (HIV, human cytomegalovirus, herpes simplex viruses, rubella virus, hepatitis B and hepatitis C viruses) as well as to *Toxoplasma gondii, Treponema pallidum, Chlamydia trachomatis, Brucella abortus, Mycobacterium bovis* etc [5, 14, 20, 21, 33-36, 40]. Such kits include antispecies conjugates containing monoclonal antibodies to certain classes of human or animal immunoglobulin labeled by an enzyme — horseradish peroxodase.

As a rule, for the detection of some markers of infectious diseases (HBs-antigen, p24-antigen of the HIV) as well as for hormone detection (e.g. somatotrophic or thyreotropic hormone), test-systems based on the sandwich ELISA modification are used [1, 4, 17, 29, 38, 39]. Polyclonal or monoclonal antibodies to antigens of interest are included into such kits.

Such ELISA modification is also used to detect specific antibodies (e.g. in a test-system aimed for the detect of total anti-HIVS antibodies) [11, 13], the conjugate containing recombinant anti-HIVS antibodies and horseradish peroxidase being here present.

Some immunoenzyme test kits are based on the competitive ELISA approach. Such is a test detecting antibodies to the *Brucella abortus*; specific antibodies compete with a conjugate causing the decrease of signal intensity for positive samples [31]. The hormone detection (e.g. thyroxin, 3-iodine thyronine) carries also out by modifications of competitive ELISA protocols [4, 17, 22]. In this case, hormone molecules from samples to be investigated compete with its molecules in conjugated compounds.

It is evident that all nowadays test-systems include labeled antibodies/antigens without any dependence on the antigens/antibodies detection principle. That is why the obtaining of immunoenzyme conjugate belongs to the most important steps to be realized during any ELISA-based test-kit elaboration.

Our aim was to sum up the available data of scientific literature concerning the obtaining of conjugates containing enzymes and antibodies as well as approaches permitting to understand their diagnostic value.

MAIN APPROACHES FOR THE OBTAINING OF ANTIBODY-ENZYME CONJUGATES

The current publications describe a lot of approaches for antibody-enzyme conjugation permit-

ting to obtain conjugates of high antigen-binding and enzyme activities.

To obtain different enzyme-containing conjugates, different functional groups must be used present in antigen and enzyme molecules: amino- and sulfhydryl groups and aldehyde ones [16, 27]. If any molecule to be conjugated contains no active functional groups it should by modified by special chemical methods [24]. For example, free HS-groups in antibody molecules may be obtained following their disulphide bond reduction joining heavy Ig chains; free aldehyde groups may be obtained after the oxidation of carbon atoms in hydroxide groups of polysaccharide chains contained by Igs and some enzymes.

The conjugation approaches may be divided into two groups. The first group of approaches includes the conjugation obtaining by the aid of covalent antibody and enzyme binding. Another approach uses non-covalent interprotein bonds of high affinity including the binding between antigen and antibody.

The data already published [16, 27, 23, 25] demonstrate covalent conjugation approaches to be more popular; in their turn, these approaches may be classified according to the conjugating reagent type for zero-length, homo- and heterobifunctional conjugations. Zero-length sewing mediates the conjugation of two molecules due to the formation of bonds having no additional atoms. Such conjugating reagents are peculiar mediators absent in final reaction product; they remain free following conjugation. Similar reactions carry out with the participation of carbodiimids and N,N'-carbonyldiimidazole. This conjugation variant may be also reached during interactions of molecules with free aldehyde groups and amino groups leading to the formation of Schiff bases and their further reduction [16].

Homobifunctional agents contain two identical reactive groups and a so-called spacer — a region of the molecule taling no direct part in the conjugation. Such agents group includes homobifunctional N-hydroxysuccinimid ethers (NHS-ethers), imidoethers, sulfhydryl-reactive sewing, aldehydes etc [24].

Heterobifunctional agents contain two different reacting groups separated by a spacer. There are different sewing types described with different active group combinations — amino, carbonyl-, sulfhydryl-, carboxylate-, arginin-reactive as well as photoreactive [16, 27].

The variability of methodical approaches is due, first of all, to wide assortment of sewing agents. These agents are divided into two types — homobifunctional and heterobifunctional.

Our knowledge in the field of immunoglobulin and enzyme structure permits to obtain conjugates using different functional groups of these molecules.

To obtain immunoenzyme conjugates, the following enzymes are the most widely used: horseradish peroxidase (HRP), alkaline phosphatase, -galactosidase and glucosooxidase [24]. The HRP being a glycoprotein, it is usually activated by periodate and sewed with immunoglobulin amino groups in the process of reducing amination. The enzyme β-galactosidase contains a lot of free sulfhydryl groups participating in conjugation with sulfhydryl-reactive termini of heterobifunctional sewing agents, e.g. succineimidyl-4-(N-malein midomethyl)cyclohexan-1-carboxylate (SMCC). Any enzyme may be conjugated through its amino groupsusing the glutaraldehyde or different heterobifunctional reagents.

Below we shall discuss the most widely used chemical reactions enabling to synthesize immunoenzyme conjugates.

NHS ether-maleimid-mediated conjugation. Different heterobifunctional reagents containing amino-reactive N-hydroxysuccinimid (NHS) ether and sulfhydryl-reactive maleimid group are successfully used for conjugate synthesis. Such sewing substances assure well-controlled multi-step conjugation followed by the obtaining of highly active products of predetermined composition. The most widely used NHS ether maleimid sewing agents are succinimidyl-4-(N-maleimidomethyl)cyclohexan-1-carboxylate (SMCC), *m*-maleimidobenzoyl-N-hydroxysuccinimid ether (MBS), N-(γ-aleimido-butiryl-oxy) succinimid ether (GMBS) as well as their sulphoderivatibes [24, 27].

Immediately before conjugation with antibodies, the enzyme activated previously is to be purified from side products and sewing agents having not participated in the reaction. Such stepwise process dynamics enables the control of reaction course and direction as well as the prevention of molecule polymerization during this process [16, 25].

The first step of the NHS ether-maleimid-mediated conjugation is the enzyme activation accom-

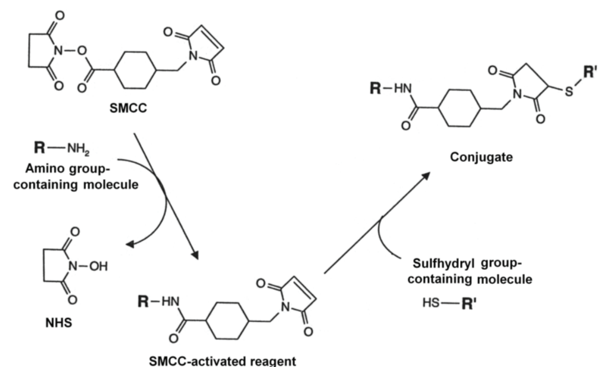

Fig. 1. Scheme of conjugate obtaining by the aid of the SMCC.

panied by the formation of a maleimid-activated derivative (fig. 1). According to the G.Hermanson's data, it is better to use the sulfo-SMCC, this water-soluble reagent possessing the most stabile maleimide groups. The antibody conjugation with the β-galactosidase has an important property: first the immunoglobulin molecules become activated by the aid of the sulfo-SMCC and then sewn with enzyme due to its free sulfhydryl groups.

The second conjugation step demonstrates the interaction between activated enzymes and antibody HS-residues. The immunoglobulin molecules are known to contain no free sulfhydryl groups, the chemical Ig molecule modifications being necessary. There are publications describing two approaches enabling the free HS-residue formation on the Ig molecules.

First, disulphide bonds between heavy immunoglobulin chains may be reduced, e.g. by 2-mercaptoethanolamine, and then halves of antibodies with free HS-residues interacts with activated enzyme [9, 26].

Second, the incorporation of free HS-residues to immunoglobulin molecules is possible by the aid of different thiolating reagents; the most widely used among them are 2-iminothiolane та N-succine-imidil-S-acetylthioacetate (SATA) [24].

It should be noted the thiolating reagents to be able to interact with any ε-aminogroups or free NH_2-residues of other amino acids. That is why a certain part of conjugated antibodies looses its activity because of screening effects or due to the blocking of antigen-binding center by an enzyme molecule. However, according to G.Hermanson's data, a part of non-active molecules contained in the conjugate obtained does not prevent its effective use in ELISA. We would also like to emphasize the use of the SATA to possess an important advantage: the sulfhydryl groups of SATA-modifies antibodies are stabile, so they may be conserved without inactivation during long time periods.

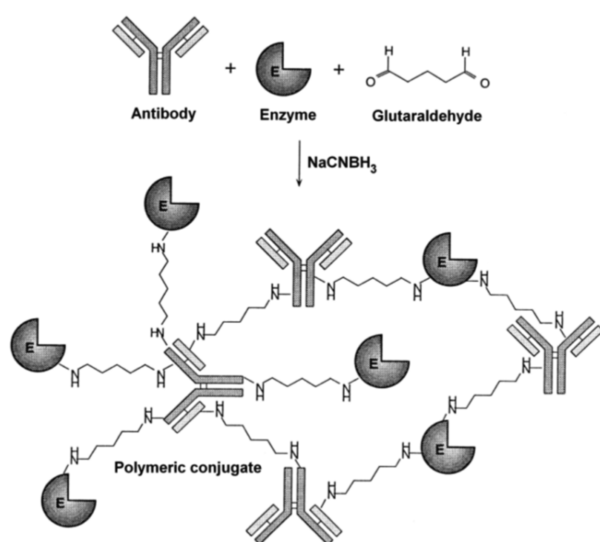

Fig. 2. Schematic description of the glutaraldehyde conjugation approach.

Glutaraldehyde approach. The glutaraldehyde belongs to the first reagents used for conjugation and taken often for this aim nowadays. It interacts with ε-aminogroups of antibody and enzyme lysine residues; of interest, in this case several reactions occur at the same time leading to the formation of a product containing more stabile chemical bonds comparing to simple Schiff bases (fig. 2) [19].

The main problems of the glutaraldehyde approach are formation of non-wanted side products and non-controlled polymerization of proteins to be conjugated [16, 27].

In aqueous solutions with pH values below 7.0, the glutaraldehyde becomes exposed to aldolic condensation accompanied by the formation of polymer structures containing α, β-non-saturated aldehydes. Another difficulty is the formation of conjugates of high molecular mass; such compounds may even loose their solubility. The enzymatic activity of high molecular mass conjugates may become significantly decreased.

All these difficulties are especially important for the single-step method requiring the glutaraldehyde addition to the solution containing both enzyme and antibodies [16]. This approach difficulties may be partly overcome during two-step conjugation; at the first step of this process, only the enzyme preparation becomes treated by the glutaraldehyde; following the removal of superfluous aldehyde the immunoglobulin preparation is added to the enzyme already modified [23]. The conjugate synthesis using the glutaraldehyde is mostly elaborated for horseradish peroxidase, alkaline phosphatase, and glucoamilase.

According to G.Hermanson's data [24], the conjugate of many commercial test-kits were obtained namely by the aid of the glutaraldehyde; this approach is so widely used due to its simplicity, first of all [18, 19].

Periodate method. This approach was first proposed by P. Nakane [32] in 1974; from this date it has become the most widely used to obtain antibody-containing conjugates with enzymes including carbohydrate residues. This method is shown to be of high effectiveness for the preparation of the HRP conjugates, the mass party of carbohydrates in this enzyme reaching 10-15 % [3].

The essence of the approach is the HRP modification accompanied by the formation of active aldehyde groups which then react with immunoglobulin amino

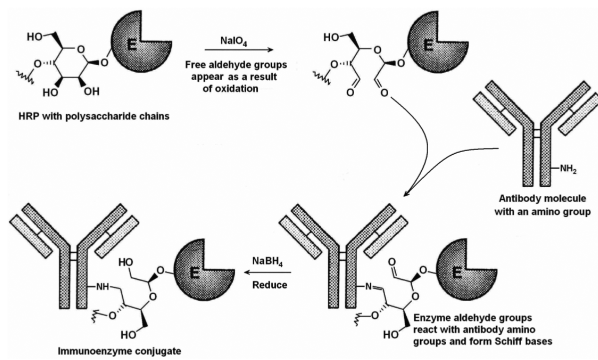

Fig. 3. Periodate approach of conjugate obtaining.

groups and form Schiff bases (fig.3). Aldehyde groups appear in the HRP due to oxidation of the enzyme carbohydrate components by the sodium periodate; the HRP amino groups must be previously blocked by the 1-fluoro-2,4-dinitrobenzene or protonated. This approach permits to prevent the enzyme autoconjugation during the reducing amination [24].

Sodium borhydride or sodium cyanoborhydride are used as reagents favoring the reducing amination [16]. To block aldehyde groups which have not participated in the reaction, a lot of reagents may be used including lysine, glycine, ethanolamine, Tris.

Carrying out the reducing amination process it is important to control the pH value of the medium: at neutral pH values the formation of low molecular mass conjugates occurs; at higher (alkaline) pH values (pH 9-10) a single Ig molecule interacts with a lot of enzyme molecules, the conjugates obtained being of considerable molecular mass [3, 16, 25].

The conjugate molecular mass is of great importance from the point of view of this compound use following its synthesis, the control of this parameter being of exclusive value. Carrying out histochemical researches, conjugates of low molecular mass are mostly used because of their ability to enter into the cell through its membranes. Simultaneously, different ELISA modifications prefer the conjugates of high molecular mass conjugates to reach perfect sensitivity values of analysis [24].

Non-covalent protocols of conjugate synthesis. All approaches mentioned above are based on the covalent bond formation between antibody and enzyme during the synthesis of their conjugates. However, there is an also promising conjugate-forming method based on the use of highly specific immune interactions or intermolecular «protein-protein» interactions.

To obtain HRP-containing conjugates, anti-HRP antibodies may be used forming a so-called «HRP-anti-HRP complex». A similar complex may be also prepared for alkaline phosphatase [2, 9, 16].

There is also an approach based on the formation of immune binding — «hybride antibody method». A mixture including F(ab’)$_2$-fragments to a given antigen and to antibody molecules is to be treated by β-mercaptoethanol. In this case the F(ab’)$_2$-fragments dissociate back to Fab-ones following the removal of reducing agent, and we obtain hybrid Ig molecules specific both to the enzyme and the antigen of interest. When added to enzyme, these hybrid molecules form “antibody-enzyme” conjugate [15].

The ability of A-protein isolated from Staphylococcus to form a stabile complex with Fc-fragment of antibody is also used to obtain conjugates; the enzyme must be previously sewed with A-protein by any chemical approach and bound then to the Fc-fragment through a protein “bridge”.

A promising approach enabling the conjugate obtaining is the use of avidin-biotin complex, its affinity constant being about 10^{15} M^{-1} [3, 28]. To realize such protocol, antibody conjugates with biotin and enzyme conjugates with avidin must be previously obtained; a more complex conjugate is formed following their mixture preparation.

All these non-covalent protocols for conjugate synthesis are easy to obtain, the conjugates keeping completely their enzyme and immune activities.

APPROACHES FOR EVALUATION OF CONJUGATE DIAGNOSTIC PROPERTIES

In ELISA-based test-systems MAbs are used as components of immune sorbents or enzyme-containing conjugate components (HPR-containing conjugates are the most widely used). The most of up-to-date diagnostic test-kits include MABs-based conjugates specific to different infectious and other antigens [5, 10, 11, 13, 14].

The immunoenzyme test-system quality depends mostly from two components — immune sorbent and immunoenzyme conjugate. Therefore, a promising way leading to the perfection of test-kit indices is the perfection of immunoenzyme conjugates. Taking this circumstance into consideration, it should describe the criteria predetermining diagnostic value of conjugates.

First, the quality of any conjugate is due to its components [3]. If conjugates include MAbs, their quality indices depend on specificity, affinity, stability, and purity of antibodies; on the other hand, these indices are also dependent on enzyme activity, purity, and stability.

Second, the conjugate quality is influenced directly by the method used for its synthesis. From this point of view, the next “critical points” should been taken into account. All reagents used for conjuga-

tion must influence as minimally as possible on the activity of enzyme molecules. It is also important to control the presence of non-bound Ig molecules in conjugate preparations, their activity leading inevitably to decreased conjugate sensitivity. Non-bound enzyme molecules in conjugate preparations may be a cause of increased background signal [30, 37].

Third, an extremely important factor is the conjugate's enzyme/antibody ratio. In different test-kits conjugates of different molar enzyme/antibody ratios are used [16, 24]. To overcome the effect of high serum HBsAg levels during the performance of sandwich ELISA modification it should use MAbs-containing conjugates with polymeric HRP, an Ig molecule having bound up to 10 HRP molecules [8]. At the same time, to obtain antispecies conjugates aimed for the detection of antibodies against different agents in indirect ELISA, it is useful to reach molar enzyme/antibody ratios about 1:2 [16].

Fourth, the diagnostic value of conjugates is indissolubly interconnected with their stability. Conjugates are the sole test-system components whose instability leads to limited test-system shelf-life [6]. Because of such situation, multi-component stabilizing substances are taken for conjugate storage including albumin, oligosaccharides, phenol derivatives, inorganic salts etc.

It is seen the quality of immunoenzyme conjugate is influenced by a lot of different factors. Even the same conditions of the conjugate synthesis using different MAbs lead to different indices of products obtained while using them in different test-systems. That is why it is necessary to possess a wide choice of antibodies against a given antigen: sometimes (and very often) only single or several MAbs of the assortment available give satisfactory results in a given test-system.

The evaluation of immunoenzyme conjugates as components test-kit is usually carried out during several steps [6].

Initial conjugate characterization is obtained using a special sera panel prepared for such aim; it is usually an intra-manufactory sera panel of a given producer. This panel includes the sera of both infected persons (citizens of a given country) and healthy (non-infected) donors [12].

The results of such investigations are usually evaluated according to the test-system positivity coefficient — ratio between positive sera optical density (OD^+) and limit value, or cut off (CO). In its turn, the CO is determined according to the formula: $CO = OD^- + 3\sigma$, where OD^- is the value of mean OD for negative samples, and σ is the standard deviation of negative sera OD values [7].

Further characterization (evaluation of their sensitivity and specificity indices) must be carried out for conjugates with the best results obtained during their control using different sera panels.

It is known the sensitivity is the index describing the test-system ability to catch maximally possible quantity of truly positive sera. This index reflects the percent of infected persons/animals which may be detected due to the use of a given test-kit [7].

The evaluation of test-system sensitivity is usually carried out according to the recommendation of the WHO, CDC (USA Centers for Disease Control and Prevention), FDA (USA Food and Drug Administration); the investigations are aimed to determine the test-system ability to detect positive sera in standard control panels [12].

Leading manufacturers produce two different the most important panel sera types to verify the test-kit quality — low titer sera and seroconversial sera panels.

Seroconversional sera panels are sera assortments taken from the same donor at different intervals from the moment of infecting assumed. Such panels enable to determine a possible term post infection following which it the test-system studied may catch antibodies to the agent of interest [7]. Using such panels it is easier to select an immunoenzyme conjugate assuring the shortest "seroconversial window" with a test-system being elaborated.

Low titer sera panels are sera assortments containing low antibody levels to a given agent [12]. The most sensitive conjugates are to be selected using such panels; the test-systems give in such cases the least quantities of false-positive results.

Another index of a test-kit diagnostic value is its specificity, i.e. its ability to determine exclusively the compound for which the test-system has been developed; this index describes the test-kit ability to register false-positive responses; their part is minimal for good tests [12].

The specificity index should be determined using a wide assortment of negative sera which had been previously characterized in other test-systems or with other conjugate preparations.

According to the recommendations of the WHO experts, the sensitivity and specificity indices of preparations must meet the minimal requirement and not be lower than 99 % and 95 %, respectively [12].

Therefore, to evaluate the suitability of antibody-enzyme conjugates for ELISA test-systems, the researches should be carried out determining the sensitivity and specificity indices as well as investigations permitting to ascertain the conjugate stability during prolonged storage.

CONCLUSIONS

1. This review sums up the available data of scientific literature on the current methods of obtaining antibody-enzyme conjugates.

2. The authors discuss different approaches permitting to describe diagnostic value of antibody-enzyme containing conjugates.

REFERENCES

1. Антипкін Ю.Г., Арабська Л.П., Толкач С.І. та ін. Соматотропна активність аденогіпофіза та фізичний розвиток дітей, народжених після аварії на ЧАЕС // Укр. мед. часопис. — 2001. — №5 (25). — С. 130-134.
2. Антитела. Методы: Кн. 1; пер. с англ. / Под ред. Д.Кэтти. — М.: Мир, 1991. — 287 с.
3. Антитела. Методы. Кн. 2; пер. с англ. / Под ред. Д.Кэтти. — М.: Мир, 1991. — 312 с.
4. Велданова М.В. Эутиреоидный зоб: диагностика, лечение и профилактика // Пробл. эндокринол. — 2001. — №3. — С. 1-12.
5. Вирусный гепатит С. Информационные материалы / Сост.: Обрядина А.П., Чепурченко Н.В., Фриго Н.В., Никулин Н.К., Комарова В.Д. — Нижний Новгород: НПО «Диагностические системы», 2003. — 46 с.
6. ВІЛ-інфекція/СНІД: проблеми етіології, епідеміології та діагностики / За ред. В.П.Семиноженка, В.Ф.Москаленка, А.Л.Гураля. — К.: Поліграф-Експрес, 2004. — 160 с.
7. Гураль А.Л., Сергеева Т.А., Кислых Е.Н. и др. К проблеме оценки диагностических характеристик тест-систем для выявления антител к вирусу иммунодефицита человека (ВИЧ) // Лабораторная диагностика. — 1999. — №1. — С. 50-54.
8. Донская Е.С., Раевская Г.Е., Николаенко И.В. и др. Применение конъюгата моноклональных антител с полимерной пероксидазой как способ повышения чувствительности и снижения hook-эффекта высоких доз в ИФА для определения HBsAg // Вирусный гепатит В — диагностика, лечение и профилактика (к 40-летию открытия HBsAg): Тез. докл. Рос. науч.-практ. конф. с междунар. уч. (19-20 мая 2004 г., г. Москва). — М.: Гепатитинфо, 2004. — С. 49-50.
9. Иммунологические методы / Под ред. Х.Фримеля. — М.: Мир, 1979. — 520 с.
10. Імунобіологічні препарати. Довідник / В.В.Смірнов, О.П.Сельнікова, В.Д.Думанський [та ін.]. — К.: Моріон, 2001. — 192 с.
11. Материалы I международной научно-практической конференции «Специфическая диагностика инфекционных болезней» (20-21 января 2004 г., г. Киев) / Под ред. Е.Г.Бочкарева, Л.Л.Громашевской, А.Л.Гураля. — К., 2004. — 120 с.
12. Навчальний посібник з лабораторної діагностики ВІЛ-інфекції/СНІДу / В.М.Гирін, І.В.Дзюблик, В.Г.Порохницький, А.Л.Гураль [та ін.]. — К.: Медпромінфо, 1999. — 140 с.
13. Ніколаєнко І.В., Шинкаренко Л.М., Галкін О.Ю. та ін. Принципи, особливості та застосування гібридомної технології // Імунологія та алергологія. — 2003. — №4. — С. 7-17.
14. Сифилис: лабораторная диагностика. Информационные материалы / Сост.: Маянский А.Н., Обрядина А.П., Уланова Т.И., Блинова Т.В., Бочкова Г.А., Бурков А.Н. — Нижний Новгород: НПО «Диагностические системы», 2003. — 42 с.
15. Структура и функции антител: пер. с англ. / Под ред. Л.Глин, М.Стьюард. — М.: Мир, 1983. — 380 с.
16. Теория и практика иммуноферментного анализа / А.М.Егоров, А.П.Осипов, Б.Б.Дзантиев, Е.М.Гаврилова. — М.: Высш. шк., 1991. — 288с.
17. Трошина Е.А., Мазурина Н.В. L-тироксин и йодид калия в лечении узлового коллоидного зоба // Пробл. эндокринол. — 2002. — Т. 48, №2. — С. 27-33.
18. Avrameas S. Coupling of enzymes to proteins with glutaraldehyde use of the conjugates for the detection of antigens and antibodies // Immunochem. — 1969. — Vol. 6, 1. — P. 43-48.
19. Avrameas S., Ternynck T. The cross-linking of proteins with glutaraldehyde and its use for the preaparation of immunoadsorbents // Immunochem. — 1969. — Vol. 6, 1. — P. 53-66.
20. Chand P., Rajpurohit B.S., Malhotra A.K. et al. Comparison of milk-ELISA and serum-ELISA for the diagnosis of Brucella melitensis infection in sheep // Vet. Microbiol. — 2005. — Vol. 108, 3-4. — P. 305-311.
21. Erensoy S. Diagnosis of hepatitis C virus (HCV) infection and laboratory monitoring of its therapy // J.Clin. Virol. — 2001. — Vol. 21, 3. — P. 271-281.
22. French S.W. The role of hypoxia in the pathogenesis of alcoholic liver disease // Hepatol.Res. — 2004. — Vol. 29, 2. — P. 69-74.
23. Harlow E., Lane D. Antibodies. A Laboratory Manual. — N.-Y.: Cold Spring Harbor, 1988. — 726 p.
24. Hermanson G.T. Bioconjugate Techniques. — San Diego: Academic Press, 2000. — 760 p.
25. Hudson L., Hay F. C. Practical Immunology. — Oxford: Blackwell, 1976. — 344 p.
26. Johnstone A. Immunochemistry 2: A Practical Approach. — Oxford: IRL Press, 1997. — 270 p.
27. Johnstone A., Thorpe R. Immunochemistry in Practice. — Oxford — : Blackwell, 1982. — 380 p.
28. Jonstone A. Analytical techniques in immunochemistry. — New York: Phillips, T.M. — 1992. — 343 p.
29. Klein S.A., Karstena S., Rьster B. et al. Comparison of TaqMan™ real-time PCR and p24 ELISA for quantification of in vitro HIV-1 replication // J. Virol. Meth. — 2003. — Vol. 107, 2. — P. 169-175.
30. Lombardi V.C., Schooley D.A. A method for selective conjugation of an analyte to enzymes without unwanted enzyme-enzyme cross-linking // Analyt. Biochem. — 2004. — Vol. 331, 1. — P. 40-45.
31. McGiven J. A., Tucker J. D., Perrett L. L. et al. Validation of FPA and cELISA for the detection of antibodies to Brucella abortus in cattle sera and comparison to SAT, CFT, and iELISA // J. Immunol. Meth. — Vol. 278, 1-2. — P. 171-178.
32. Nakane P., Kawaio A. Peroxidase-labeled antibody a new method of conjugation // J. Histochem. Cytochem. — 1974. — Vol. 22, 12. — P. 1084-1091.
33. Ory F., Guisasola E.M., Casas I. et al. Evaluation of new reagents for typing IgG to HSV-1 and HSV-2 // Opportunistic Pathogens. — 1997. — Vol. 9, 1. — P. 39-41.

34. Poon S., Rosenberg E. Clinical and laboratory implications of acute HIV-1 infection // Clin.Microbiol. Newsletter. — 2000. — Vol. 22, 5. — P. 33-37.
35. Princea H.E., Wilson M. A simplified assay for measuring Toxoplasma gondii IgG avidity // Clin.Appl.Immunol.Revs. — 2002. — Vol. 3, 1-2. — P. 7.
36. Ross S.A., Boppana S.B. Congenital cytomegalovirus infection: Outcome and diagnosis // Seminars in Pediatric Infectious Diseases. — 2005. — Vol. 16, 1. — P. 44-49.
37. Saegerman C., De Waele L., Gilson D. et al. Evaluation of three serum i-ELISAs using monoclonal antibodies and protein G as peroxidase conjugate for the diagnosis of bovine brucellosis // Vet. Microbiol. — 2004. — Vol. 100, 1-2. — P. 91-105.
38. Schьpbach J., Tomasika Z., Nadalb D. Use of HIV-1 p24 as a sensitive, precise and inexpensive marker for infection, disease progression and treatment failure // Int.J.Antimicrob.Agents. — 2000. — Vol. 16, 4. — P. 441-445.
39. Tsitsilonis O., Thrasyvoulides A., Balafas A. Serological detection of hepatitis B viral infection by a panel of solid-phase enzyme-linked immunosorbent assays (ELISA) // J.Pharmaceut.Biomed.Analysis — 2004. — Vol. 34, 4. — P. 811-822.
40. Vernet G. Molecular diagnostics in virology // J.Clin. Virol. — 2004. — Vol. 31, 4. — P. 239-247.

О.Ю.Галкін. Підходи до синтезу імуноферментних кон'югатів та оцінки їх використання для діагностики інфекційних захворювань. Київ, Україна.

Ключові слова: *антитіла, ферменти, зшивальні реагенти, імуноферментні кон'югати, імуноферментні тест-системи, стандарті панелі сироваток, специфічність та чутливість аналізу.*

В огляді літератури узагальнено літературні дані щодо підходів до одержання кон'югатів антитіл (у тому числі і моноклональних) з ферментами. Проаналізовано місце і роль техніки біокон'югації в розробці та виробництві сучасних імуноферментних тест-систем для діагностики інфекційних захворювань, визначення речовин тощо. Проаналізовано критерії, від яких залежить якість одержуваних імуноферментних кон'югатів, та розглянуто підходи до оцінки їх діагностичної якості.

А.Ю.Галкин. Подходы к синтезу иммуноферментных коньюгатов и оценки их использования для диагностики инфекционных заболеваний. Киев, Украина.

Ключевые слова: *антитела, ферменты, сшивающие реагенты, иммуноферментные коньюгаты, иммуноферментные тест-системы, стандартные панели сывороток, специфичность и чувствительность анализа.*

В обзоре литературы обобщены литературные данные о подходах к получению коньюгатов антител (в том числе и моноклональных) с ферментами. Проанализированы место и роль техники биоконьюгации в разработке и производстве современных иммуноферментных тест-систем для диагностики инфекционных заболеваний, определения веществ и т.д. Проанализированы критерии, от которых зависит качество получаемых иммуноферментных коньюгатов, и рассмотрены подходы к оценке их диагностического качества.

Надійшла до редакції 12.10.2010 р.