Медицинская иммунология
2023, Т. 25, № 5,
стр. 1197-1204

Краткие сообщения
Short communications

Medical Immunology (Russia)/
Meditsinskaya Immunologiya
2023, Vol. 25, №5, pp. 1197-1204

КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ С1-ЭСТЕРАЗНОГО ИНГИБИТОРА В СЫВОРОТКЕ КРОВИ ЧЕЛОВЕКА МЕТОДОМ ИММУНОФЕРМЕНТНОГО АНАЛИЗА: КОРРЕЛЯЦИЯ С ТУРБИДИМЕТРИЧЕСКИМ МЕТОДОМ

Горбунов Н.П.[1], Жахов А.В.[1], Горбунова И.Н.[1], Миличкина А.М.[1], Дрозд И.В.[1], Губанова А.В.[1], Данилова Е.М.[1], Кузнецова Р.Н.[1], Савин Т.В.[1], Бурцева А.Г.[1], Пигарева Н.В.[1], Ищенко А.М.[1], Тотолян Арег А.[1, 2]

[1]ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия
[2]ФГБОУ ВО «Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова» Министерства здравоохранения РФ, Санкт-Петербург, Россия

Резюме. С1-ингибитор сериновых протеаз (С1-INH) выполняет регуляторную функцию в системе комплемента и проницаемости сосудов. Дефицит С1-INH приводит к различным формам ангиоотеков, в том числе наследственному ангионевротическому отеку (НАО). Причиной НАО является генетически обусловленное нарушение синтеза С1-INH. Снижение уровня С1-INH до 50% относительно нормы приводит к увеличению продукции брадикинина, что является основанием для постановки диагноза «НАО». Разработка доступных иммуноферментных тест-систем (ИФА) для количественного определения С1-INH является востребованным направлением для клиницистов. В процессе разработки нового набора для количественного определения С1-INH были получены два моноклональных антитела мыши (МАТ), обладающих разной эпитопной специфичностью. На их основе была разработана ИФА тест-система по типу сэндвич. Специфичность полученных МАТ была подтверждена с использованием коммерческого препарата «Беринерт». Для приготовления калибраторов С1-INH был аффинноочищен из плазмы крови человека с использованием сорбента с иммбилизованными МАТ. Идентичность белку С1-INH подтверждали при помощи электрофореза в ПААГ, иммуноблоттинга и масс-спектрометрии на MALDI-TOF/TOF масс-спектрометре UltrafleXtreme. Для оценки показате-

Адрес для переписки:
Горбунов Николай Петрович
ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера»
197101, Россия, Санкт-Петербург, ул. Мира, 14.
Тел.: 8 (921) 786-38-16.
E-mail: niko_laygo@mail.ru

Address for correspondence:
Nikolay P. Gorbunov
Saint Petersburg Pasteur Institute
14 Mira St
St. Petersburg
197101 Russian Federation
Phone: +7 (921) 786-38-16.
E-mail: niko_laygo@mail.ru

Образец цитирования:
Н.П. Горбунов, А.В. Жахов, И.Н. Горбунова, А.М. Миличкина, И.В. Дрозд, А.В. Губанова, Е.М. Данилова, Р.Н. Кузнецов, Т.В. Савин, А.Г. Бурцева, Н.В. Пигарева, А.М. Ищенко, Арег А. Тотолян «Количественное определение С1-эстеразного ингибитора в сыворотке крови человека методом иммуноферментного анализа: корреляция с турбидиметрическим методом» // Медицинская иммунология, 2023. Т. 25, № 5. С. 1197-1204.
doi: 10.15789/1563-0625-QOC-2794

For citation:
N.P. Gorbunov, A.V. Zhakhov, I.N. Gorbunova, A.M. Milichkina, I.V. Drozd, A.V. Gubanova, E.M. Danilova, R.N. Keznecova, T.V. Savin, A.G. Burtseva, N.V. Pigareva, A.M. Ischenko, Areg A. Totolian "Quantification of C1 esterase inhibitor in human serum by enzyme-linked immunosorbent assay: Correlation with turbidimetric immunoassay", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 5, pp. 1197-1204.
doi: 10.15789/1563-0625-QOC-2794

DOI: 10.15789/1563-0625-QOC-2794

лей качества разработанного набора реагентов были проведены исследования в соответствии с ГОСТ Р 51352-2013 и ТУ 21.20.23-041-01967164-2022. Значения показателей качества: точность (правильность) – 93,53%; интервал линейности измерений – 22,00-176,07 нг/мл. Присутствие интерферирующих агентов (гемоглобина, билирубина, триглицеридов) не влияло на эффективность измерений. С помощью разработанной ИФА тест-системы нами были исследованы 28 сывороток крови здоровых доноров и 7 сывороток крови пациентов с подтвержденным диагнозом «НАО». В этих же образцах определяли содержание С1-INH при помощи турбидиметрического метода, используя медицинское изделие «Реагенты диагностические для иммунохимических исследований *in vitro* специфических белков крови. Модель: С1-ингибитор эстеразы (C1 EsteraseInhibitor)» (Aptec, Бельгия). Коэффициент корреляция составил 0,94 ($p < 0,05$). Было установлено, что диагностическая чувствительность и специфичность разработанной тест-системы составляет 100%. В результате проведенного исследования разработана оригинальная ИФА тест-система для количественного определения С1-INH «Набор реагентов для иммуноферментного количественного определения С1-ингибитора человека (C1-inh PS)».

Ключевые слова: С1-ингибитор, моноклональное антитело, иммуноферментный анализ, турбидиметрия, калибратор, корелляционный анализ

QUANTIFICATION OF C1 ESTERASE INHIBITOR IN HUMAN SERUM BY ENZYME-LINKED IMMUNOSORBENT ASSAY: CORRELATION WITH TURBIDIMETRIC IMMUNOASSAY

Gorbunov N.P.[a], Zhakhov A.V.[a], Gorbunova I.N.[a], Milichkina A.M.[a], Drozd I.V.[a], Gubanova A.V.[a], Danilova E.M.[a], Kuznecova R.N.[a], Savin T.V.[a], Burtseva A.G.[a], Pigareva N.V.[a], Ischenko A.M.[a], Totolian Areg A.[a, b]

[a] *Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*
[b] *First St. Petersburg State I. Pavlov Medical University, St. Petersburg, Russian Federation*

Abstract. C1 inhibitor of serine proteases (C1-INH) performs a regulatory function in the complement system and vascular permeability. Deficiency of C1-INH leads to various forms of angioedema, including hereditary angioedema (HAE). The cause of HAE is a genetically determined violation of the synthesis of C1-INH. A decrease in the level of C1-INH to 50% relative to the norm leads to an increase in the production of bradykinin, which is the basis for the diagnosis of HAE. The development of affordable ELISA for the quantitative determination of C1-INH is a popular direction for clinicians. During the development of a new kit for quantitative determination of C1-INH, two mouse monoclonal antibodies (mAb) with different epitope specificities were obtained. On their basis, a sandwich-type ELISA was developed. The specificity of the obtained mAb's was confirmed using the medical device "Berinert". To prepare calibrators, C1-INH was affinity purified from human blood plasma using a sorbent with immobilized mAbs. The identity of the C1-INH protein was confirmed by PAGE electrophoresis, immunoblotting, and mass spectrometry on MALDI-TOF/TOF UltrafleXtreme mass spectrometer. To assess the quality indicators of developed reagents kit, studies were carried out in accordance with GOST R 51352-2013 and TU 21.20.23-041-01967164-2022. Values of quality indicators: accuracy – 93.53%; measurement linearity interval – 22.00-176.07 ng/mL. Using the developed ELISA test system, we examined 28 blood sera from healthy donors and 7 blood sera from patients with confirmed HAE. In the same samples, the content of C1-INH was determined by turbidimetric method, using the "Diagnostic reagents for *in vitro* immunochemical studies of specific blood proteins. Model: C1-esterase inhibitor (C1 EsteraseInhibitor)" (Aptec, Belgium). The correlation coefficient was 0.94 ($p < 0.05$). It was found that the diagnostic sensitivity and specificity of the developed ELISA is 100%. As a result of the study, an original ELISA test system for the quantitative determination of C1-INH was developed "Reagent kit for enzyme-linked immunosorbent assay of human C1-inhibitor (C1-inh PS)".

Keywords: C1 inhibitor, monoclonal antibody, enzyme immunoassay, turbidimetry, calibrator, correlation analysis

Introduction

C1 esterase inhibitor (C1-INH) belongs to the superfamily of serine protease inhibitors (serpins) and is a single-chain highly glycosylated protein. The main function of C1-INH is to control the inflammatory response by inhibiting spontaneous complement activation via the classical and lectin pathways, as well as regulate vascular permeability by inactivating proteases of the fibrinolytic, coagulation, and kinin pathways, preventing unregulated production of bradykinin [2, 3].

Deficiency of the C1-INH inhibitor leads to increased generation of complement anaphylatoxins, which stimulate the release of vasoactive amines by mast cells and eosinophils and cause a strong inflammatory response. The weakening of control over the activation of contact systems leads to enhanced production of the powerful vasodilator bradykinin. In both cases, this leads to systemic inflammation, which manifests in the form of angioedema (AE) [2, 4].

The most severe manifestation of AE is hereditary angioedema (HAE), which can occur in presence or absence of urticaria, accompanied by repeated, self-limiting relapses and not reversed by antihistamines [1].

The cause of HAE disease is a rare dominant mutation in the human autosome, which leads to genetically determined defects in the genes encoding C1-INH synthesis. The incidence of HAE ranges from 1:20,000 to 1:100,000 [1, 6].

The symptoms of AE are similar to those of other diseases (allergies, asthma, inflammatory processes, etc.), which causes difficulties in determining of its cause, diagnostics and choosing a therapy strategy by clinicians. A decrease in the level of C1-INH to a value below 50% of normal (20-40 mg/L) is strictly associated with the diagnosis of HAE, which makes it relevant to determine its concentration in the blood of patients [5, 6].

In this regard, **the aim of the work** was to develop an available ELISA test system for the quantitative determination of C1-INH, which can compete with other registered more expensive test systems.

Materials and methods

Human serum samples

The panel of serum samples from healthy donors and confirmed type I HAE patients was provided by the Pasteur Institute Medical Center (St. Petersburg, Russia).

Obtaining mouse monoclonal antibodies (mAbs) against human C1-INH

Monoclonal Abs against human C1-INH used to develop human C1-INH enzyme-linked immunosorbent assay (ELISA) test system were obtained according to Köhler and Milstein hybridoma technology. To immunize animals (Balb/c mice), commercial intravenous human C1-INH «Berinert» (Aptec) was used. Immunization was carried out in three stages with 31 day interval. At the first stage, 20 μg of protein together with Freund's complete adjuvant (FCA) was administered subcutaneously by footpad injection. Re-immunization was carried out with the same dose of antigen with Incomplete Freund's Adjuvant (IFA), followed by booster dose of 100 mg protein in saline.

Six days after boosting, lymphocytes were harvested and fused with SP 2/0 mouse myeloma cells. The cells were cultured in RPMI-1640 (Sigma) supplemented with 10% fetal bovine serum (Sigma) and HAT selection agent (Sigma) containing hypoxanthine, aminopterin, thymidine. Thus obtained hybridoma clones were screened by ELISA. The antigen was immobilized on the plate well surface, then the supernatants from hybridoma cells were added to the wells, and the resulting immune complexes were detected using commercial goat anti-mouse IgG conjugated with horseradish peroxidase (A4416, Sigma). mAbs were produced by intraperitoneal injection of hybridoma cells into experimental animals, followed by collecting of ascitic fluid once ascites was developed. To purify the mAbs, ascitic fluid was preliminarily precipitated with ammonium sulfate and applied to a column filled with a protein A resin (MabSelectXtra, GE), elution was carried out according to the manufacturer's instructions, having regard to the antibody isotype, previously determined using Mouse Monoclonal Antibody Isotyping Reagents (ISO 2, Sigma).

Monoclonal antibodies labeling with horseradish peroxidase

Monoclonal Ab conjugates with horseradish peroxidase were obtained by the periodate-oxidation method.

Development of an enzyme-linked immunosorbent assay test system for determination of human C1-INH

To create a sandwich-type ELISA test system for determination of human C1-INH, a selection of mAbs with different epitope specificity was made. For this purpose, each of the obtained mAbs was tested as both a capturing (immobilized on the wells of the plate) and detecting (conjugated with horseradish peroxidase) antibody with respect to other mAbs against C1-INH. Concentration of C1-INH in the calibration samples was selected by successive dilutions of the initial sample. The sample was diluted until no saturation of capture antibodies with C1-INH was observed. The titer of capturing antibodies was

selected in order to ensure optimal characteristics of the ELISA test system, i.e., the consistency of the following parameters: low background values, the calibration sample signal level providing a relatively linear relationship between C1-INH concentration and optical density values on the calibration curve. The titer of the conjugate was determined by ELISA. The antigen was immobilized on the plate well surface, then serial dilutions of the tested mAb HRP conjugate were added into the wells, and the first dilution after which a decrease in the signal level was observed relative to the previous dilution was determined.

Preparation and characterization of human C1-INH for calibration samples

Human C1-INH was obtained by chromatography purification from blood plasma using an affinity sorbent with immobilized monoclonal antibodies. Phosphate-buffered saline, pH 7.5 was used as an equilibration buffer, and 3M $MgCl_2$ solution was used as an eluent. The matrix for affinity sorbent was BrCN-activated Sepharose 6B (GE), antibodies were immobilized according to the manufacturer's instructions.

The physicochemical and immunological properties of the obtained human C1-INH were compared with human C1-INH from "Berinert" drug (Aptec) using such methods as SDS-PAGE, immunoblotting, ELISA, and mass spectrometry on UltrafleXtreme MALDI-TOF/TOF mass spectrometer.

Study of the analytical characteristics of an experimental test system for the quantitative determination of human C1-inhibitor

Such parameters as the accuracy (correctness) of measurements, assessment of control samples $K1^+$ and $K2^+$, precision under reproducibility conditions; sensitivity, detection limit, linearity, analytical measurement range; possible influence of interfering substances in blood serum samples; concordance with the reference medical device (MD), diagnostic significance were determined.

To evaluate these parameters, the components of the developed ELISA test system (calibration and control samples) were used, as well as 2 panels of biological samples: the first comprising 24 sera from healthy donors, and 11 sera from patients with confirmed HAE; the second comprising 28 sera from healthy donors, and 7 sera from patients with confirmed type I HAE. As a reference MD, a commercial C1 Esterase Inhibitor assay kit (Aptec) was used, designed for the analysis of C1-INH by the turbidimetric method on a FURONO 270 Clinical Chemistry Analyzer.

To assess the possible influence of interfering substances in blood serum samples, solutions of hemoglobin 1000 mg/dL, bilirubin 60 mg/dL, triglycerides 50 mg/dL were used.

Results and discussion

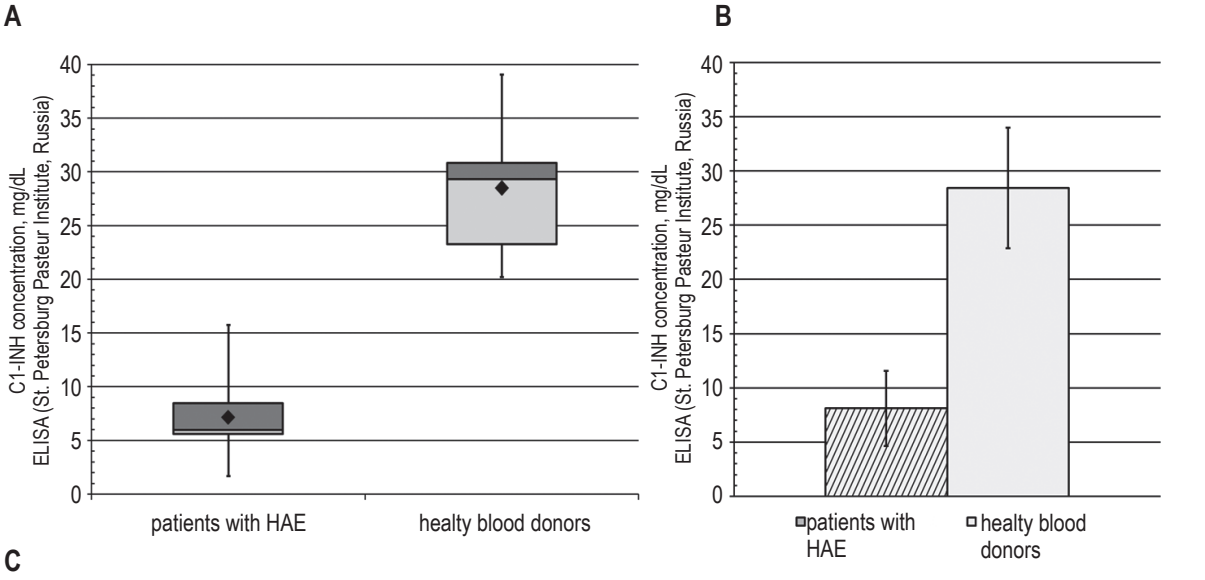
As a result, two mouse monoclonal antibodies, C1-1i and C1-2i, against human C1-INH were obtained.

The antibodies were characterized by ELISA and immunoblotting. The specificity of the obtained antibodies was confirmed by detection in the above assay of the immune complex of target antibodies with the C1-INH protein contained in the commercial "Berinert" drug (control protein), a similar complex was also detected in the presence of human blood serum. One band was stained on the nitrocellulose membrane, corresponding to the molecular weight of C1-INH, 105 kDa, when analyzing a sample loaded to the gel without addition of β-mercaptoethanol. It was determined that the mAbs have different epitope specificity, which made it possible to implement the developed ELISA test system as a sandwich-type assay, where one of the antibodies is capturing and immobilized in the wells of the plate, and the second one is detecting and conjugated with horseradish peroxidase.

A combination of mAbs was determined for the developed ELISA test system, where mAb C1-2i is capturing, and mAb C1-1i is detecting.

A sample of human C1-INH was obtained and characterized, intended for the preparation of calibration samples. This sample was analyzed using various analytical methods. According to SDS-PAGE, the molecular weight of the protein was 105 kDa, which corresponded to the molecular weight of the control protein. According to the results of immunoblotting using a conjugate of the obtained specific mAb against C1-INH with horseradish peroxidase (C1-1i-HRP), one band was observed on the nitrocellulose membrane, corresponding to the molecular weight of the control protein. MALDI-TOF/TOF mass spectrometric analysis on UltrafleXtreme mass spectrometer was also carried out. The processed spectra were used to identify proteins by accessing the NCBI or SwissProt databases using MASCOT. The mass determination error was limited to 20 ppm. According to the analysis, the studied protein was reliably identified as "complement C1-inh [Homo sapiens]" (NP_000055.2); the Score value was 491 with a threshold value of 68.

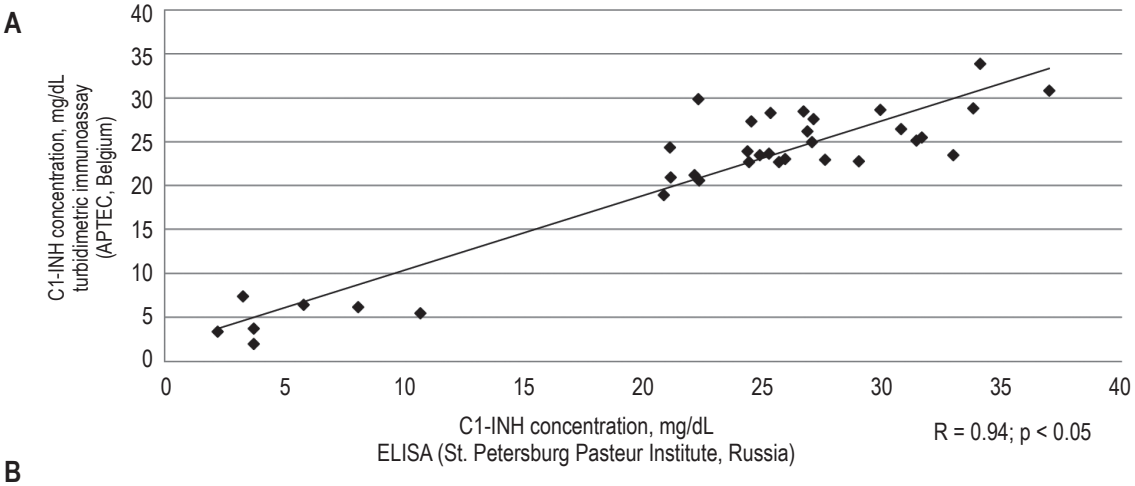
Studies of the developed original ELISA test system for the quantitative determination of C1-INH were carried out. In the study of the diagnostic significance, sera from the first panel of biological samples were



C

seprum samples	sample quantity	average C1-INH concentration, mg/dL	standard deviation	min. C1-INH concentration, mg/dL	max. C1-INH concentration, mg/dL
patients with HAE	11	7.19	3.63	1.72	15.76
healthy blood donors	24	28.51	5.57	20.26	39.09

Figure 1. Results obtained when studying the diagnostic significance for the developed original ELISA test system for the quantitative determination of C1-INH



B

seprum samples	sample quantity	analysis type	average C1-INH concentration, mg/dL	standard deviation	min. C1-INH concentration, mg/dL	max. C1-INH concentration, mg/dL
patients with HAE	7	ELISA (St. Petersburg Pasteur Institute, Russia)	27.02	4.33	36.98	20.86
		turbidimetric immunoassay (APTEC, Belgium)	25.25	3.43	33.89	19.00
healthy blood donors	28	ELISA (St. Petersburg Pasteur Institute, Russia)	5.38	3.04	2.23	10.70
		turbidimetric immunoassay (APTEC, Belgium)	4.98	1.96	2.00	7.47

Figure 2. Results obtained when studying concordance with reference MD for the developed original ELISA test system for the quantitative determination of C1-INH

TABLE 1. CHARACTERISTICS OF THE DEVELOPED ORIGINAL ELISA TEST SYSTEM FOR THE QUANTITATIVE DETERMINATION OF C1-INH, OBTAINED DURING THE STUDY

Parameter	Value
Accuracy	within 90-110%
Limit of detection	2.00±0.60 ng/mL
Analytical measuring range	22.00 to 175.00 ng/mL
Linearity	within 90% to 110% in the range from 22.00 to 175.00 ng/mL
Repeatability and reproducibility	the coefficient of variation (CV) does not exceed 15%
Concordance with reference MD	corresponds
Diagnostic specificity	100%
Diagnostic sensitivity	100%

analyzed. The value of diagnostic sensitivity and diagnostic specificity was determined to be at the level of 100%, which indicates the absence of both false negative and false positive results. Based on the data, a graph (Figure 1A) and a histogram (Figure 1B) were plotted, reflecting statistically significant differences between the studied groups of sera, and Figure 1C shows the data used to plot the above graphs.

In the study of concordance with the reference MD, a correlation graph was plotted reflecting the relationship between the values of C1-INH, mg/dL, determined using the developed test system, and the values of C1-INH, mg/dL, determined using the reference MD (Figure 2). For this study, a second panel of biological samples was used. The coefficient R was determined to be 0.940, $p < 0.05$. The value of this coefficient indicates a strong positive correlation of data on the level of C1-INH in the studied samples analyzed using the two test systems. The graph of the correlation is presented in Figure 2A, Figure 2B shows the data used to plot the graph.

Also, such parameters as the accuracy of measurements, assessment of control samples $K1^+$ and $K2^+$, precision under reproducibility conditions; sensitivity, detection limit, linearity, analytical measurement range; possible influence of interfering substances in blood serum samples were determined. All the studied parameters corresponded to the declared ones. The results of the study of these parameters are shown in Table 1.

Conclusion

As a result of the study, an original ELISA test system for the quantitative determination of C1-INH was developed. "Reagent kit for enzyme-linked immunosorbent assay of human C1-inhibitor (C1-inh PS)".

References

1. Borisova N.V., Sokurenko S.I. Classification, diagnosis, and approach, to treatment for angioedema. *Clinical Practice, 2014, Vol. 10, no. 3, pp. 71-79.* (In Russ.)

2. Davis A.E., III, Mejia P., Lu F. Biological activities of C1 inhibitor. *Mol. Immunol., 2008, Vol. 45, no. 16, pp. 4057-4063.*

3. Drouet C., López-Lera A., Ghannam A., López-Trascasa M., Cichon S., Ponard D., Parsopoulou F., Grombirikova H., Freiberger T., Rijavec M., Veronez C.L., Pesquero J.B., Germenis A.E. SERPING1 variants and C1-INH biological function: a close relationship with C1-INH-HAE. *Front. Allergy, 2022, Vol. 3, 835503.* doi: 10.3389/falgy.2022.835503

4. Karnaukhova E. C1-Inhibitor: structure, functional diversity and therapeutic development. *Curr. Med. Chem., 2022, Vol. 29, no. 3, pp. 467-488.*

5. Tange C.E., Kaur A., Verma N., Hickey A., Grigoriadou S., Scott C., Kiani S., Steven R., Ponsford M., El-Shanawany T., Jolles S., Harding S., Parker A.R. Quantification of human C1 esterase inhibitor protein using an automated turbidimetric immunoassay, *J. Clin. Lab. Anal., 2019, Vol. 33, no. 1, e22627.* doi: 10.1002/jcla.22627.

6. Wagenaar-Bos I.G.A., Drouet C., Aygören-Pursun E., et al. Functional C1-inhibitor diagnostics in hereditary angioedema: assay evaluation and recommendations. *J. Immunol. Methods, 2008, Vol. 338, no. 1-2, pp. 14-20.*

Авторы:

Горбунов Н.П. *— младший научный сотрудник лаборатории гибридомных технологий ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Жахов А.В. *— научный сотрудник лаборатории гибридомных технологий ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Горбунова И.Н. *— научный сотрудник лаборатории гибридомных технологий ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Миличкина А.М. *— к.м.н., главный врач, ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Дрозд И.В. *— к.б.н., заведующая центральной клинико-диагностической лабораторией ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Губанова А.В. *— врач клинической лабораторной диагностики центральной клинико-диагностической лаборатории ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Данилова Е.М. *— к.м.н., заведующая диспансерно-поликлиническим отделением ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Кузнецова Р.Н. *— к.м.н., врач — аллерголог-иммунолог диспансерно-поликлинического отделения ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Савин Т.В. *— врач — аллерголог-иммунолог диспансерно-поликлинического отделения ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Бурцева А.Г. *— младший научный сотрудник лаборатории гибридомных технологий ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Authors:

Gorbunov N.P., *Junior Research Associate, Laboratory of the Hybridoma Technology, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Zhakhov A.V., *Research Associate, Laboratory of the Hybridoma Technology, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Gorbunova I.N., *Research Associate, Laboratory of the Hybridoma Technology, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Milichkina A.M.*, PhD (Medicine), Chief Physician, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Drozd I.V.*, PhD (Biology), Head, Central Clinical Diagnostic Laboratory, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Gubanova A.V.*, Doctor, Clinical Laboratory Diagnostics, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Danilova E.M.*, PhD (Medicine), Head, Dispensary and Polyclinic Department, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Kuznecova R.N.*, PhD (Medicine), Allergist-Immunologist, Dispensary and Polyclinic Department, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Savin T.V.*, Allergist-Immunologist, Dispensary-Polyclinic Department, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Burtseva A.G., *Junior Research Associate, Laboratory of the Hybridoma Technology, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Пигарева Н.В. *— к.м.н., старший научный сотрудник лаборатории гибридомных технологий ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Ищенко А.М. *— к.б.н., заведующий лабораторией гибридомных технологий ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера», Санкт-Петербург, Россия*

Тотолян Арег А. *— д.м.н., профессор, академик РАН, заведующий лабораторией молекулярной иммунологии, директор ФБУН «Санкт-Петербургский научно-исследовательский институт эпидемиологии и микробиологии имени Пастера»; заведующий кафедрой иммунологии ФГБОУ ВО «Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова» Министерства здравоохранения РФ, Санкт-Петербург, Россия*

Pigareva N.V.*, PhD (Medicine), Senior Research Associate, Laboratory of the Hybridoma Technology, Saint Petersburg Pasteur Institute, St. Petersburg, Russian Federation*

Ischenko A.M., *PhD (Biology), Head, Laboratory of the Hybridoma Technology, Saint Petersburg Pasteur Institute, St. etersburg, Russian Federation*

Totolian Areg A., *PhD, MD (Medicine), Professor, Full Member, Russian Academy of Sciences, Head, Laboratory of Molecular Immunology, Director, Saint Petersburg Pasteur Institute; Head, Department of Immunology, First St. Petersburg State I. Pavlov Medical University, St. Petersburg, Russian Federation*

Поступила 14.04.2023
Отправлена на доработку 20.04.2023
Принята к печати 24.04.2023

Received 14.04.2023
Revision received 20.04.2023
Accepted 24.04.2023