Lithuanian Journal of Physics
Vol. **66**, No. 2, pp. 75–81 (2026)
ISSN 1648-8504 eISSN 2424-3647
DOI: https://doi.org/10.3952/physics.2026.66.2.2

INFRARED SPECTROSCOPIC DETERMINATION OF DRY INFECTIOUS AND NORMAL CEREBROSPINAL FLUIDS

R. Bandzevičiūtė [a], V. Šablinskas [a], G. Steiner [b, c], J. Kasper [b], K. Sulin [b], R. Brilmayer [b], Ž. Chomanskis [d], G. Lukšys [d], S. Ročka [d], J. Jakučionytė [a], and M. Kirsch [b]

[a] *Institute of Chemical Physics, Faculty of Physics, Vilnius University, Saulėtekio 3, 10257 Vilnius, Lithuania*
[b] *Clinic and Polyclinic for Neurosurgery, Klinikum Chemnitz, Flemmingstraße 2, 09116 Chemnitz, Germany*
[c] *Department of Anesthesia and Intensive Care, Clinical Sensoring and Monitoring, University Hospital and Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Germany*
[d] *Clinic of Neurology and Neurosurgery, Faculty of Medicine, Vilnius University, M. K. Čiurlionio 21, 03101 Vilnius, Lithuania*
Email: rimante.bandzeviciute@ff.vu.lt; valdas.sablinskas@ff.vu.lt

Received 2 December 2025; accepted 21 January 2026

Cerebrospinal fluid (CSF) reflects the pathological processes in neural tissues, therefore, by analyzing it, pathologies can be detected. In our study, we applied infrared (IR) spectroscopy for the analysis of dried infectious and normal CSF films of 30 patients. The presence of glucose, bicarbonates, amino acids and lactate was established from the attenuated total reflectance (ATR) IR spectra. The differences between infectious and normal CSF samples are related to lactate and amino acids and allow one to distinguish these two sample groups. The applied method allows one to analyze a small sample amount that is an advantage compared to the routine clinical evaluation of the CSF where significantly larger amounts of the fluid are required.

Keywords: cerebrospinal fluid, CSF, infrared spectroscopy

1. Introduction

Cerebrospinal fluid (CSF) is a colourless biological fluid that surrounds the brain and spinal cord, providing mechanical protection, nutrient transport, and metabolic waste removal [1]. Because CSF is continuously exchanged with neural tissue, even subtle changes in its biochemical composition can signal underlying pathological neural tissue processes, including neoplastic lesions, intracranial hemorrhages, infectious and demyelinating diseases of the central nervous system [2].

Routine clinical evaluation of CSF typically involves cytological examination, biochemical testing, microbiological assays, immunological profiling, and more recent molecular diagnostic approaches [2–4]. These well-established conventional CSF analysis methods still exhibit several important constraints [3, 4]. Many of these procedures are labour-intensive, require multiple specialized reagents, and involve manually performed steps that introduce user-dependent variability and extend the overall time required to obtain diagnostic results. Furthermore, the need to allocate CSF into separate tubes for different assays increases sample consumption – a particular challenge in patients with elevated intracranial pressure, where limiting CSF withdrawal is essential to avoid exacerbating the risk of herniation. Most importantly, conventional CSF analyses focus on predefined biomarkers and therefore provide only a narrow window into the molecular landscape of neurological disease. Consequently, early and subtle biochemical perturbations may remain undetected.

Advances in molecular spectroscopy have underscored its potential as a powerful analytical method for disease diagnostics, as spectroscopic signatures encode rich molecular information about

biological systems [5]. Infrared (IR) spectroscopy, in particular, presents several advantages when applied to CSF [6, 7]. Being inherently label-free, it eliminates the need for chemical reagents, antibodies, or enzymatic substrates, reducing experimental complexity and enhancing reproducibility. Spectra can be acquired within minutes and require a minimal sample preparation, especially when using attenuated total reflection (ATR) configurations, which enable the direct measurement of microlitre-scale volumes. IR spectroscopy also yields an overall biochemical information of the sample, capturing shifts in proteins, lipids, nucleic acids, and other macromolecules that may precede clinically detectable abnormalities. In addition, with the rapid development of machine-learning and AI-based analytical frameworks, IR CSF analysis could be integrated into automated or robotic platforms, providing rapid, standardized, and operator-independent assessments for clinical decision support.

A number of exploratory studies have already evaluated IR-based CSF profiling across several neurological disorders. The research employing FTIR (Fourier transform IR) spectroscopy has identified distinct spectral patterns associated with amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease, and tumours of central nervous system [8–11]. Those investigations reported disease-related perturbations in the protein secondary structure, lipid composition, and nucleic-acid-associated vibrational modes, illustrating the capacity of IR spectroscopy to detect biochemical features that extend beyond the reach of routine diagnostic assays. Nevertheless, these early studies are limited by small cohort sizes, diverse acquisition protocols, and non-uniform data-processing strategies. Consequently, the broader diagnostic utility of IR spectroscopy for CSF evaluation – particularly for the early, rapid, and reagent-free disease characterization – remains insufficiently established.

In this manuscript, we describe the determination of infectious and normal dry cerebrospinal fluid by means of IR spectroscopy.

2. Materials and methods

Samples were prepared following lumbar puncture by placing a drop (approximately 1 μL) of the CSF onto an optical slide covered with aluminum foil and allowing it to air dry. This sample preparation method was chosen since it allows 1) an easy transportation and storage of the samples, 2) an effective IR spectroscopic analysis by pressing the aluminum foil against the ATR element during the measurements. For the measurements, a small section of the foil containing the dried CSF sample was cut out and pressed against the diamond ATR element (Fig. 1).

Infrared (IR) spectra were recorded using a FTIR spectrometer Alpha (*Bruker Optik GmbH*, Ettlingen, Germany) with a single-reflection diamond ATR module attached.

Spectra were acquired at a resolution of 4 cm^{-1}, averaging 128 scans per measurement in the spectral range between 400 and 4000 cm^{-1}. The three-term Blackman–Harris apodization function and zero filling factor of 2 were applied for Fourier transformation. Before each measurement, the ATR element was cleaned with distilled water and 2-propanol following background measurement.

For the subsequent data analysis, two groups were formed in accordance with the gold standard of laboratory analysis: spectra of normal CSF samples and spectra of infectious CSF samples.

Data preprocessing and statistical data analysis were performed using the Matlab package (*Mathworks Inc.*, Natric, MA, USA). In the first step, the spectra were subjected to a linear baseline correction using the msbackadj function. For further

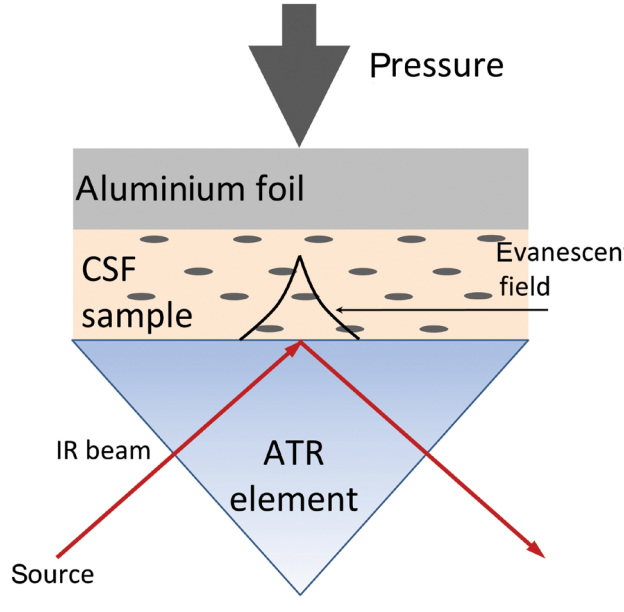

Fig. 1. The experimental setup for ATR FTIR measurements of CSF.

analysis, the spectra were area normalized in the spectral ranges under consideration. The mean values and standard deviations were calculated using the mean and std functions. The statistical two-sample t-test was performed using the ttest2 function. This function performs the t-test of the hypothesis that two independent extinction values of the 'normal' and 'infectious' groups originate from distributions with equal mean values. The significance level was set at 5%. The function outputs both the test result and the significance underlying the test result.

In total, 30 patient cases were included into the study, 8 with infection and 22 cases without pathology, hereinafter referred to as normal. Table 1 summarizes the number and the age ranges of the patients.

Table 1. CSF sample types included to the study based on patient diagnosis.

Pathology	Number of samples	Age range
Infection	8	26–83; median: 53
Normal	22	19–87; median: 42

3. Results and discussion

The calculated mean ATR IR spectrum and the standard deviation of all the measured CSF samples are presented in Fig. 2.

In general, the composition of the CSF includes several components such as various proteins, glucose, ions, vitamins and neurotransmitters [1]. Together with other electrolytes, CSF contains bicarbonate ions (HCO_3^-) involved in the acid-base (pH) balance process [12]. HCO_3^- together with PO_4^{3-} are rather different from conventional bodily electrolytes such as Na^+, K^+, Cl^-, Ca^{2+} and Mg^{2+} from the point of view of IR spectroscopy. They have a molecular origin with chemical bonds causing strong spectral bands in IR absorption spectra, and this makes them detectable by means of IR spectroscopy. Despite the low concentration of HCO_3^- in CSF (21.8 mM/l) [13], it is observable by IR spectroscopy – in the presented CSF spectrum (Fig. 2), the spectral bands located at 699 and 834 cm^{-1} are attributed to HCO_3^- (experimental spectra of pure constituents are presented in Fig. 3).

The spectral range 685–840 cm^{-1} besides the bicarbonates can be associated with the presence of lactate. The spectral region 953–1177 cm^{-1} is predominantly assigned to glucose and lactate (Fig. 3). Particularly strong signals occur in the range from 1050 to 1150 cm^{-1}. Here, various vibration modes of glucose, lactate, and vibrations of carbonyl groups and their derivates overlap. The spectral range 1220–1500 cm^{-1} can be attributed to free amino acids, while the narrower spectral range 1477–1497 cm^{-1} can additionally reflect

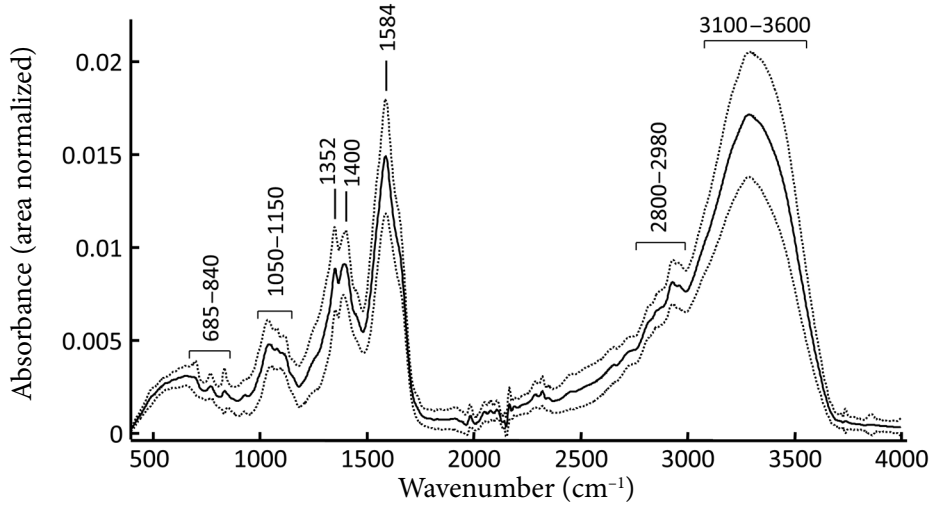

Fig. 2. Mean ATR IR spectrum (bold line) and the standard deviation band (dotted line) of all measured spectra of the 22 normal and 8 infectious CSF samples. The positions of particularly distinctive spectral bands are marked and explained in the text.

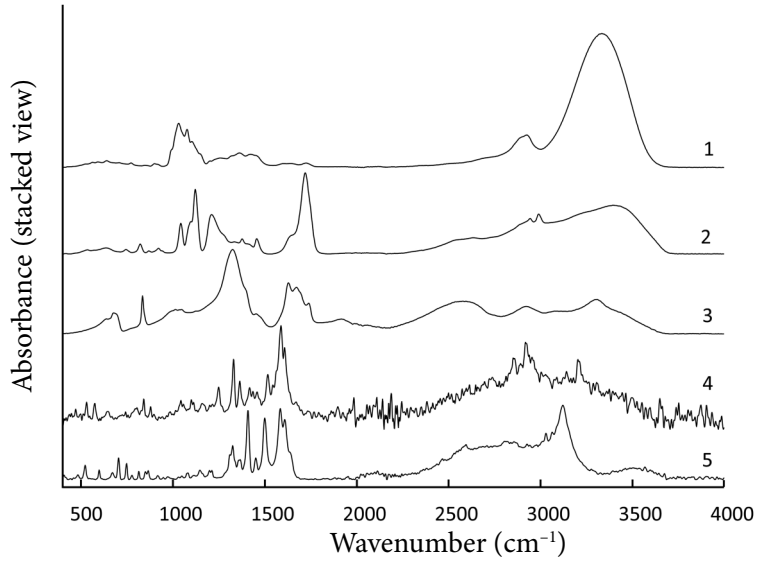

Fig. 3. Experimental ATR IR spectra of pure constituents ((1, glucose; 2, lactic acid; 3, $NaHCO_3$; 4, L-tyrosine; 5, L-phenylalanine).

contributions of lactate and also of bicarbonates. The clearly prominent bands at 1352 and 1400 cm^{-1} can be attributed primarily to the C–H and N–H deformational vibrations [14] of amino acids. Another strong and broad signal appears between 1500 and 1700 cm^{-1}. In this range, the amide-I and amide-II spectral bands of proteins as well as phenyl ring vibrational modes and stretching modes of amino groups and amines are visible. The comparatively strong signal at 1584 cm^{-1} can be attributed to the ring C–C stretching vibration of phenyl groups [14]. The spectral range between 2700–4000 cm^{-1} is less specific, it is related to the C–H, N–H and O–H stretching vibrations as well as the amide-A vibrational mode [14]. Between 2800–2980 cm^{-1} occur the antisymmetric and symmetric stretching modes of CH_2 and CH_3 groups [14]. The broad and strong spectral signal between 3100 and 3600 cm^{-1} originates from the OH and NH stretching modes [14].

When comparing the spectra of normal CSF samples to those of infection samples, the spectral range from 950 to 1800 cm^{-1} will be considered. This range is also referred to as the fingerprint region due to its high and specific information content.

Figure 4 shows the calculated mean spectra and the range of the standard deviation of normal and infectious CSF samples.

Clear spectral differences occur around 1400 cm^{-1} and in the region from 1050 to 1150 cm^{-1}. As mentioned above, signals around 1400 cm^{-1} can be attributed primarily to the C–H and N–H deformation vibrational modes of amino acids. Infectious samples have obviously a lower level of free amino acids than normal CSF samples. In the region from 1050 to 1150 cm^{-1}, vibrations of carbohydrates and lactate occur in particular. Infectious samples show an elevated level, which can be explained by inflammatory reactions and their associated degradation products. However, the plot of standard deviations in Fig. 4 also reveals that the spectra of the CSF samples exhibit a comparatively large variation, and thus the classification of the spectral profiles of normal and infectious CSF samples is questionable.

To investigate this more closely, all spectra of the two classes were subjected to a t-test. Using a t-test, the hypothesis that the absorbance values of the two classes differ significantly is tested for each wavenumber data point. The result of the t-test and the calculated significance values are plotted in Fig. 5.

The t-test shows three regions with a test result of 1. This means the hypothesis that the absorbance values of these spectral regions are different cannot be rejected. In other words, the spectral profiles of the respective groups are significantly different from each other. Accordingly, 0 means that the absorbance values of the respective groups are not significantly different from each other. The three regions in which the absorbance values of the two groups are significantly different from each other are interpreted as follows. Region ① is from 1106 to 1122 cm^{-1} and corresponds to the C–O–C

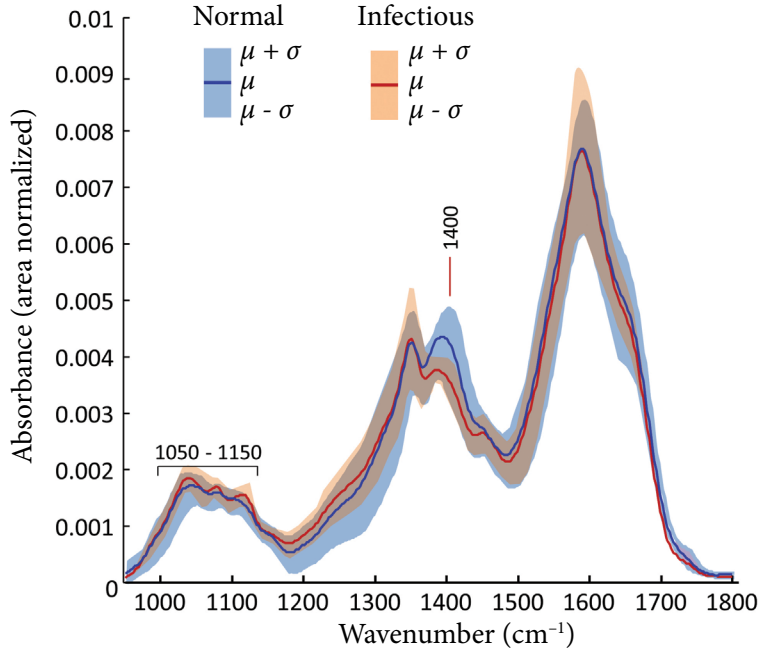

Fig. 4. Mean ATR IR spectra (bold line; μ) and the range of standard deviation (σ) of normal CSF samples (blue) and infectious CSF samples (red).

asymmetric stretching vibration of lactate. The next region of significant differences ② is between 1380 and 1400 cm^{-1} and corresponds to free amino acids. The third region ③ between 1690 and 1720 cm^{-1} represents the C=O stretching mode of lactate.

The t-test proves that the spectra of normal CSF samples can be significantly distinguished from infectious CSF samples based on lactate and free amino acids, i.e. ATR-IR spectroscopy can be used to perform a quick on-site evaluation of CSF.

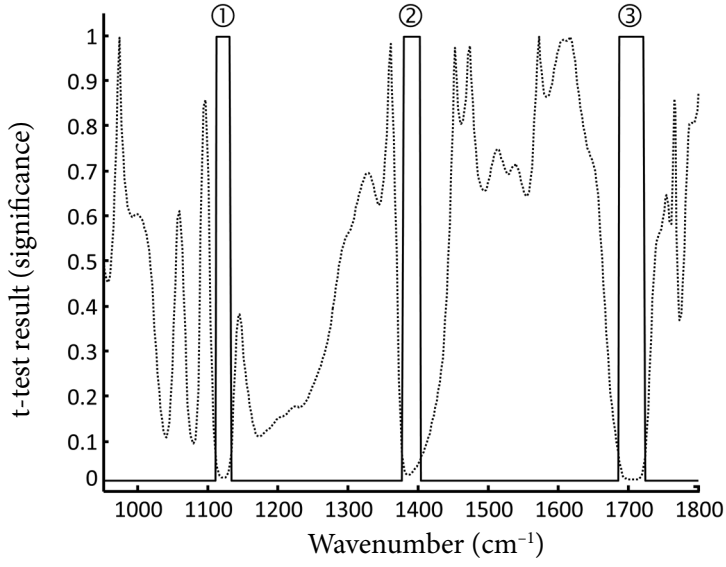

Fig. 5. Plot of the results of the t-test of the hypothesis that the differences between the spectra of the infectious samples and those of the normal samples are different. Bold line: result of the t-test. The dotted curve shows the significance that the spectra of both groups are the same.

4. Conclusions

In this study, approximately 1 μL of CSF was transferred onto an aluminum foil and, after quick drying, ATR-IR spectra were recorded. Normal CSF samples, i.e. those without known pathology, and CSF samples under infection were examined. Although the spectra show a high variability, which is obviously due to the respective highly varying biochemical compositions, the spectra of both classes can be reliably distinguished. Using a statistical t-test, three spectral regions that allow separation were identified. These are associated with lactate and amino acids. The study demonstrates that IR spectroscopy can be used for the rapid analysis and classification of native CSF.

Acknowledgement

This study has been funded by the Baltic-German University Liaison Office (Project Agreement No. 2025/8).

References

[1] N. Czarniak, J. Kamińska, J. Matowicka-Karna, and O. Koper-Lenkiewicz, Cerebrospinal fluid–basic concepts review, Biomedicines **11**(5), 1461 (2023).

[2] R. Spector, S.R. Snodgrass, and C.E. Johanson, A balanced view of the cerebrospinal fluid composition and functions: Focus on adult humans, Exp. Neurol. **273**, 57–68 (2015).

[3] F. Deisenhammer, A. Bartos, R. Egg, N.E. Gilhus, G. Giovannoni, S. Rauer, F. Sellebjerg, and EFNS Task Force, Guidelines on routine cerebrospinal fluid analysis. Report from an EFNS task force, Eur. J. Neurol. **13**(9), 913–922 (2006).

[4] E.S. Theel, M.E. Aguero-Rosenfeld, B. Pritt, P.V. Adem, and G.P. Wormser, Limitations and confusing aspects of diagnostic testing for neurologic Lyme disease in the United States, J. Clin. Microbiol. **57**(1), e01406-18 (2019).

[5] J.M. Cameron, C. Rinaldi, S.H. Rutherford, A. Sala, A.G. Theakstone, and M.J. Baker, Clinical spectroscopy: Lost in translation?, Appl. Spectrosc. **76**(4), 393–415 (2021).

[6] M.J. Baker, J. Trevisan, P. Bassan, R. Bhargava, H.J. Butler, K.M. Dorling, P.R. Fielden, S.W. Fogarty, N.J. Fullwood, K.A. Heys, et al., Using Fourier transform IR spectroscopy to analyze biological materials, Nat. Protoc. **9**, 1771–1791 (2014).

[7] S.G. Kazarian and K.L.A. Chan, ATR-FTIR spectroscopic imaging: Recent advances and applications to biological systems, Analyst **138**, 1940–1951 (2013).

[8] T. Dučić and J.C. Koch, Synchrotron-based Fourier-transform infrared micro-spectroscopy of cerebrospinal fluid from amyotrophic lateral sclerosis patients reveals a unique biomolecular profile, Cells **12**(11), 1451 (2023).

[9] D. Yonar, L. Ocek, B.I. Tiftikcioglu, Y. Zorlu, and F. Severcan, Relapsing-remitting multiple sclerosis diagnosis from cerebrospinal fluids via Fourier transform infrared spectroscopy coupled with multivariate analysis, Sci. Rep. **8**, 1025 (2018).

[10] J. Depciuch, I. Zawlik, M. Skrzypa, J. Pająk, N. Potocka, K. Łach, H. Bartosik-Psujek, A. Koziorowska, E. Kaznowska, and J. Cebulski, FTIR spectroscopy of cerebrospinal fluid reveals variations in the lipid: Protein ratio at different stages of Alzheimer's disease, J. Alzheimer's Dis. **68**(1), 281–293 (2019).

[11] M. Griebe, M. Daffertshofer, M. Stroick, M. Syren, P. Ahmad-Nejad, M. Neumaier, J. Backhaus, M.G. Hennerici, and M. Fatar, Infrared spectroscopy: A new diagnostic tool in Alzheimer disease, Neurosci. Lett. **420**(1), 29–33 (2007).

[12] H.H. Damkier and J. Praetorius, Cerebrospinal fluid pH regulation, Pflugers Arch. – Eur. J. Physiol. **476**, 467–478 (2024).

[13] G. Schnaberth, H. Schubert, and K. Summer, Das Säure-Basengleichgewicht im normalen Liquor Cerebrospinalis [Acid-base balance in the cerebrospinal fluid of normal subjects. Comparison of cisternal and lumbar CSF (author's transl.)], Wien. Klin. Wochenschr. **87**(9), 294–297 (1975).

[14] A.C.S. Talari, M.A.G. Martinez, Z. Movasaghi, S. Rehman, and I.U. Rehman, Advances in Fourier transform infrared (FTIR) spectroscopy of biological tissues, Appl. Spectrosc. Rev. **52**(5), 456–506 (2017).

IŠDŽIOVINTO INFEKCINIO IR NORMALAUS SMEGENŲ SKYSČIO ANALIZĖ INFRARAUDONOSIOS SPEKTROSKOPIJOS METODU

R. Bandzevičiūtė [a], V. Šablinskas [a], G. Steiner [b, c], J. Kasper [b], K. Sulin [b], R. Brilmayer [b], Ž. Chomanskis [d], G. Lukšys [d], S. Ročka [d], J. Jakučionytė [a], M. Kirsch [b]

[a] *Vilniaus universiteto Fizikos fakulteto Cheminės fizikos institutas, Vilnius, Lietuva*

[b] *Kemnico klinikų Neurochirurgijos klinika ir poliklinika, Kemnicas, Vokietija*

[c] *Karlo Gustavo Karuso Medicinos fakultetas ir universitetinė ligoninė Technikos universitete, Drezdenas, Vokietija*

[d] *Vilniaus universiteto Medicinos fakulteto Neurologijos ir neurochirurgijos klinika, Vilnius, Lietuva*

Santrauka

Smegenų skysčio (CSF) sudėtis yra tiesiogiai susijusi su patologiniais procesais nerviniuose audiniuose, todėl jo analizė leidžia aptikti šias patologijas. Mūsų tyrime išdžiovintų infekcinio ir normalaus CSF plėvelių analizei buvo pritaikyta infraraudonoji (IR) spektroskopija 30 pacientų atveju. Iš ATR IR spektrų nustatytas gliukozės, bikarbonatų, aminorūgščių ir laktato buvimas. Laktato ir aminorūgščių skirtumai tarp infekcinio ir normalaus CSF mėginių leidžia patikimai atskirti šias dvi grupes. Taikytas metodas tinkamas mažų mėginio kiekių analizei, todėl yra pranašesnis už įprastinį CSF tyrimą, kuriam būtinas žymiai didesnis skysčio kiekis.