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Maintaining the collections of microorganisms requires the long-term conservation of strains in viable state without changes in their biological properties. Cryopreservation is considered as one of the most effective means of long-term storage of microorganisms. The use of cryoprotective compounds ensures new possibilities to protect microbial cells from cold shock and alterations at freezing procedure. Specification of the preservation methods adapted for a specific type of microorganisms, that would provide high viability and stability of biological properties, is an actual task of modern microbiology. **Objective**. This study aimed to investigate the effect of cooling regiments, composition of conservation medium, and low temperature storage on the viability of different types of microorganisms. **Methods**. The study was conducted on 5 strains of E.coli and 5 strains of Staphylococcus spp. Cryoprotective mediums comprising 1% glucose or 10 % glycerol were used for deep freezing. Two cooling modes were used: mode 1 - direct immersion of the samples in liquid nitrogen; mode 2 - cooling with programmable freezer from 20 to −70 ° C with a speed of 10 ° C / min, followed by a temperature stop at −70 ° For 10 min and further immersion. The samples were thawed in a water bath at a temperature of +37 ° C for 120 seconds. Viability of tested strains was tested by the Koch method after 3 and 6 month of storage in liquid nitrogen. Statistical analysis was performed with nonparametric methods using MX Excel 2007 and STATISTICA 6.0 software. Results. Significant strain-specific differences in survival rates were established. At both cooling modes adding of the glycerol as a cryoprotectant provided significantly higher viability of bacterial cells then adding of glucose. The average number of viable cells after freezing was 8.1 × 108CFU / ml vs 1.3 × 108CFU / ml for glycerol and glucose respectively ($p < 0,05$). There was no significant difference in viability of strains after deep frozen storage for 3 and 6 month. All strains had similar viability rates when glycerol was used as a cryoprotectant. Staphylococci were less vulnerable to freezing stress at both cooling modes when medium with glucose was used. **Conclusion**. High efficiency of one step deep freezing with 10 % glycerol as a method for preserving of Staphylococcus and Escherichia collection strains has been demonstrated. The viability of bacteria during cryopreservation was influenced by the composition of the preserving medium, the cooling mode and the species and stain-specific morpho-functional features. Glycerine was found to be the optimal cryoprotectant at both single-stage and two-stage cooling mode. The one stage freezing was much more preferable when a cryoprotective medium with glucose was used. Duration of the sample storage in liquid nitrogen did not affect the number of viable cells.
Keywords: cryopreservation; freezing; cryopreserving formulation; staphylococci; Escherichia.

Justification of the gel formers selection in the development of oromucosal drug in the form of troches 42-44

Semchenko K. V., Vyshnevska L. I.

Introduction. Troches deserve special attention among the existing oromucosal dosage forms. They have good consumer characteristics and an adequate level of bioavailability, which makes it expedient to expand the range of medicines to be used in the form of troches, especially for pediatric use. The purpose of this work is to justify the optimal composition of troches by selecting the most rational gel-forming agent and its concentration. **Materials & methods.** Gel formers gelatine (250 g/cm^2, according to Bloom), agar (800 g/cm^2, according to Bloom) and apple pectin were used as objects of the study. The ratio of the ingredients was investigated in 6 samples. Samples with gelatine and agar were prepared as follows: the calculated amount of gelatine or agar was poured with the calculated amount of purified water and left for swelling. When gelatine/agar swelled, it was melted in a water bath and mixed with concentrate of citric acid, glucose syrup, glycerol, fruit flavouring and food colouring. Samples with apple pectin were prepared as follows: the calculated amount of apple pectin was mixed with half the sugar and heated to (50.0 ± 2.0) °C with water purified with vigorous stirring. After swelling, it was mixed with concentrate of citric acid, glucose syrup, glycerol, fruit flavouring and food colouring. The resulting mass was poured into silicone form and placed in a refrigerator for hardening. **Results & discussion.** The obtained troches were evaluated by such quality indicators as organoleptic control, uniformity of dosage units, dissolution time. It was found that sample No. 5 shows the best consumer characteristics, while the other samples do not meet the requirements of the studied quality indicators. For example, sample No. 1 was not formed, the texture remained semi-solid; samples No. 2 and No. 3 were formed, but were opaque and easily destroyed when pressed; samples No. 4 and No. 6 contained too much gelatine, some of which remained undissolved, resulting in deterioration of taste characteristics (less pleasant taste, "crunch", sticking during chewing). The critical parameters of the technological process of troches preparation are time of gelatine swelling, temperature of gelatine melting, time of troches hardening. **Conclusion.** The findings showed that samples of gelatine-based troches with its content of 8.65 mass. % have the best consumer characteristics. The resulting troches are homogenous, with a pleasant fruity aroma and sweet taste. They meet the requirements of SPhU by the quality indicators organoleptic indicators, the uniformity of the dosage units and the dissolution time. The established critical parameters of the technological process and their values were established.
Keywords: gel; oromucosal; troches; gel formers

Study of antibacterial properties of the emulgel with *Scutellaria baicalensis* extract 45-50

Slipchenko G.D., Osolodchenko T.P., Ruban O.A.

Introduction. One of the important problems of medicine for many years remains the search for effective treatments for infected wound processes. Semisolids remain an indispensable dosage form in the treatment of wound injuries, the interest in which has increased recently due to the new tend of including phytopreparations, providing soft action and low toxicity against the background of high efficiency, and the complex of biologically active substances has a versatile and complementary effect. This makes it possible to use them for a long time with a minimal number of side effects. Particular attention is paid to the properties of the Scutellaria baicalensis among the plants. Considering the variety of compounds and the action inherent in the raw material, we have developed an emulgel based on the dry extract of Scutellaria root and rhizomes. The purpose of our work is to study the antimicrobial activity of the created emulgel and to choose a preservative. **Materials and methods.** For the study of microbiological purity, three samples of emulgel with extract content of 1%, 2% and 2.5% were taken. The following test strains were used to evaluate the activity of the drug samples: *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633, Proteus vulgaris ATCC 4636, Candida albicans ATCC 885/653.* Determination of antibacterial activity of the study drug was carried out by the method of diffusion into agar (method of "wells") on two layers of agarised nutrient medium on Petri dishes. Cultivation media were used according to the type of microorganisms in accordance with existing methodological developments and recommendations. Antimicrobial and anticancer effects of new drugs were determined by the standard method of two-fold serial dilutions in nutrient broth (macromethod). Microbiological purity tests were performed by direct cropping on liquid nutrient media. The soybean-casein broth, thioglycol medium and Sabouraud liquid medium were sterile poured into tubes of 10.0 ml. The most common substances that are authorized for use in pharmacy in Ukraine were used to select a preservative. Parabens derivatives (Nipagin, Nipasol, germaben) and glycol (euxyl) were selected for the research. **Results and discussion.** As a result of the study, it was found that the samples of the emulsifier based on the dry extract of Scutellaria root (1%, 2% and 2.5%) possess antibacterial action against the test strains. The diameters of growth retardation zones were 20-23 mm relative to *Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633, 18-20* mm - to *Escherichia coli ATCC 25922,* to *Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853 - the diameter was 16* - 19 mm, relative to the fungus *Candida albicans ATCC 653/885* the zone was 16-17 mm. In comparison drug, the solution of chlorophyllin the diameter of the growth retardation zone was smaller, and with respect to Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853 antibacterial properties were not registered. It was also found that the samples had antibacterial activity against clinical strains. The diameters of growth inhibition zones were 15–20 mm relative to *Pseudomonas aeruginosa 46*, *Proteus vulgaris* 18, *Staphylococcus aureus 25, Escherichia coli 7.* In the study of microbiological purity by direct sowing on the dishes it has been found that the growth of fungi was absent in the study of all samples. The number of microorganisms that grew on 0.1 g samples of the preparation did not exceed 10^3 CFU / ml, which meets the requirements of the State Pharmacopoeia of Ukraine. Microscopy showed the presence of a gram-positive vegetative spore bacillus in the 2.0% emulgel samples based on the dry extract of Scutellaria. Confirmation was obtained by cropping on differential nutrient media. Obtained data showed that in morphology of the colonies and some biological properties

the isolated microorganisms found in the study belong to the genus *Bacillus sp*. On differential media (Chistovich medium and Endo medium) for the isolation of intestinal group and pathogenic staphylococci the growth among other microorganisms was not observed. The next step was to investigate the effectiveness of the antimicrobial preservatives (Nipagin + Nipasol, germaben and euxyl). Based on the studies and according to the literature, euxyl has been chosen as a preservative, as it has the widest spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, yeast and fungi in the pH range of 3.0-12.0 and is a safer and economically reasonable option. **Conclusions.** According to the results of the study of the antimicrobial activity of samples of emulgel with Scutellaria baikalensis extract, it has been found that the optimal content of dry extract in the developed drug is 2%. The microbiological purity of the freshly made specimen was investigated and antibacterial activity against a wide range of microorganisms of different taxonomic groups has been proven. Studies on the preserving ability of antimicrobial preservatives (Nipagin + Nipasol, germaben and euxyl) have established the effectiveness of all selected substances. All samples met the requirements of SPU (criterion "A"). As the most promising one Euxyl preservative has been selected, which is the safest and most economically reasonable with a wider pH range.
Keywords: antibacterial; emulgel; *Scutellaria baicalensis*; extract

Lethal activity of the museum and clinical strains of *C. Difficile* 51-54

Voronkina I. A., Kheder S. S., Biryukova S. V., Maryuschenko A. M., Dyachenko V. F., Panchenko L.O.

Introduction. At present C. *difficile* infection (CDI) is considered to be nosocomial worldwide, this problem is studied in depth and currently there are large-scale monitoring studies being carried out. There is a considerable quantity of different tests for CDI diagnosis. These tests include the cytopathogenic test and the toxin neutralization reaction (determination of toxin B), latex agglutination (determination of the glutamatedehydrogenase), ELISA test (determination of the A or B toxins, or determination of the A and B toxins simultaneously), PCR. At the same time, it is known that the isolation of the agents and its identification without determination of the ability of the latter to produce toxins is insufficient, as the non-toxicogenic strains do not play any role in the human pathology. The modern diagnostic methods present in Ukraine (PCR, ELISA, etc.) have not become widespread in the laboratories of the state health establishments because of their complexity and cost, moreover, they cannot provide for all aspects required for the solution of this problem, such as the study of the properties and variability of the agent itself. The toxins A and B have powerful cytotoxic and pro-inflammatory properties. These toxins are glycosyltransferase enzymes that catalyze the inactivation of Rho proteins responsible for actin cytoskeleton organization and epithelial barrier function. The destruction of the epithelial cells leads to the disruption of the water-electrolyte exchange, which facilitates the secretion of the fluid into the intestinal space. The modern studies confirm the important role of both toxins A and B, and the distribution of the clinical symptoms beyond the intestine to other organs (heart, kidneys, brain), which points to the presence of the systemic toxemia and plays the main role in the prognosis of the CDI development. In the laboratory of the anaerobic infections of the SI "IMI NAMS" in the 2017-2019 years, studies devoted to the identification and study of the circulating clinical *C. difficile* strains were carried out. **Aim of the study.** To verify the toxin-producing properties of the clinical and museum strains of *C. difficile* in different nutrient media. **Materials&methods.** The toxin producing properties were verified in 7 isolated clinical strains and 2 museum strains (*C. difficile* №258, №281 A), obtained from the L. V. Tarasevich SISK in 1988. In order to isolate the toxin, the microorganisms were cultivated on the liver and heart-brain broth (HBB) in anaerobic conditions. After 72 hours of cultivation, bacterial cells were isolated with the help of centrifugation for 30 minutes at 8 000 rpm with the subsequent filtration of the supernatant through 0, 45 μm membranous filters (Sartorius) [2, 18]. Lethal activity was determined in the experiments on white mice with the help of the LD_{50}/ml determination method [18, 19]. The animal experiments were carried out according to the requirements of the European Convention of the Protection of Vertebrate animals that are used for research and other scientific purposes and practical scientific recommendations [20-21]. **Results&discussion.** The carried out experimental studies have shown tha the museum strains of *C. difficile* produced toxins with different lethal activity. In the cultural filtrate of the museum strain № 258, obtained on the liver broth, the lethal activity was 7,9 LD_{50}/ml ($LD_{50\ max}$ = 1:5,4 ml; $LD_{50\ min}$ = 1:9,5 ml, p = 0,05), on the heart-brain broth – 10,0 LD_{50}/ml ($LD_{50\ max}$ = 1:8,3 ml; $LD_{50\ min}$ = 1:12,5 ml, p = 0,05). Museum strain № 281 A produced a more active toxin. Its lethal activity was correspondingly 12,5 LD_{50}/ml ($LD_{50\ max}$ = 1:10,0 ml; $LD_{50\ min}$ = 1:14,8 ml, p = 0,05), and – 19,9 LD_{50}/ml (LD_{50max} = 1:17,8 ml; $LD_{50\ min}$ = 1:20,5 ml, p = 0,05). **Conclusion.** Filtrates obtained from clinical strains of *C. difficile* have not demonstrated lethal activity after an intraperitoneal injection into mice, which allows us to consider them non-toxicogenic in advance and as such as those that do not have epidemic significance. The studies museum strains of *C. difficile* № 258 and 281 A produced lethal toxins of different activity. The toxin-producing activity of the *C. difficile* 281 A strain was reliably ($p \leq 0,05$) higher than that of the *C. difficile* № 258 strain. The toxin-producing activity of both strains was reliably higher on the heart-brain broth compared to the data obtain on the liver broth.
Key words. *C. difficile,* toxin-producing activity, nutrient media.

Study of the antimicrobial activity of phytoointment for treatment of mechanical damages of dairy tissue 55-61

Zuikina S. S., Vyshnevska L. I., Silaeva L. F.

Introduction. One of the common pathologies found in gynaecological practice is cracking nipples, which represent the rupture of an external sensitive epidermis due to its mechanical damage. Cracks have different shapes (straight and stellar), as well as different depths (superficial and deep). The nature of cracks is quite diverse: poor quality personal care products or non-compliance with personal hygiene rules, low-quality gels and cosmetics, improper breastfeeding, vitamin A and E deficiency, early use of hormonal contraceptives and other medicines, tanning bed, improperly selected linen and other. Diagnosis is performed only when there are complaints, namely: when the nipple swells, blushing, pain and heaviness in the chest are felt. The treatment is prescribed by the doctor depending on the duration of the disease and its severity. If the disease lasts up to three days, then wound healing, antiseptic and palliative drugs are prescribed. If the disease lasts more than three days, then antibiotic therapy is added to these agents, as there is a secondary infection. In such cases, the breastfeeding of the baby stops.Taking into account the above, the composition of the soft dosage form for use in gynaecology, which has reparative, wound healing action, was developed. Ointment under the conventional name "Phytolan" contains in its composition vegetable oils: amaranth, sea buckthorn, parsley leaves, melaleuca and lanolin anhydrous as the basis. The aim of the research was comparative study of the spectrum and level of antimicrobial activity in vitro ointment "Phytolan", the comparator preparation - the analogue by the action of the cream "Mama care" and specimens containing ointment base - anhydrous lanolin and a separate phytooil in a concentration of 10%. **Material&methods.** As test strains we took standard strains from the American standard collection of microorganisms: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Bacillus subtilis ATCC 6633, Candida albicans ATCC 885-653. Cultivation of cultures was carried out by traditional methods in nutrient media with subsequent confirmation of morphological, cultural and biochemical properties (3). In the course of research, appropriate nutrient media indicated in the national part of the SPHU were used: medium # 1 - in the study of antibacterial activity and medium # 2 - in the study of antifungal activity of the samples. The antimicrobial activity of the drugs was studied in vitro in commonly accepted in microbiological practice method of diffusion in agar in the modification of wells. This method is based on the ability of active substances to diffuse into agar medium, which is pre-sown with the test culture. The molten agar nutrient medium was cooled to 45° C, poured into the bottom layer into Petri dishes in a volume of 10 ml. After hardening of the agar on it were placed six sterile cylinders of stainless steel 10 mm in height and an internal diameter of 8 mm, around which the second layer of medium was poured into a volume of 15 ml, seeded with appropriate cultures of microorganisms. The microbial load was 0,5 units. Mc Farland turbidity per 1 ml of medium. After clamping the upper layer of agar, the cylinders were removed with sterile tweezers and in the formed wells, the test specimens were administered. Petri dishes were kept for one hour at room temperature, after which they were placed in a thermostat and incubated for 24 hours at a temperature of 37° C with Muller - Hinton agar and 25° C with Sabur agar. The level of antimicrobial activity of the developed ointment, experimental specimens and the comparison preparation was evidenced by the

diameter of zones of growth retardation of microorganisms around the well. The research was conducted in six-time repetitions for each test culture. Statistical processing of the results of the study was carried out according to Student's criterion (P <0.5). **Results&discussion.** According to the results of the research, ointment samples exhibited a moderate level of antimicrobial activity against Staphylococcus aureus culture and practically no activity compared to other cultures. Thus, the growth retardation zone of S. aureus culture around the ointment samples was 13,6 mm, around other cultures 12,1 – 12,2 mm. Other study samples: the comparison drug "Mama care" (analogue by effect) and individually samples with lanolin and amaranth, sea buckthorn, parsley, tea tree almost did not exhibit antimicrobial activity to used test cultures.Despite the known antimicrobial properties of essential oils, in particular tea tree (6), the absence of a pronounced effect in vitro may be due to the technological difficulties of creating an effective antimicrobial concentration in the ointment. The tendency towards the manifestation of antistaphylococcal activity of the ointment may be related to the effect of synergy of essential oils in the composition, in particular the influence of tea tree oil and requires further study of the mechanism of this phenomenon. **Conclusion.** 1. The use of essential oils in the treatment of mastopathy is substantiated. 2. Antimicrobial activity of the developed ointment in relation to S. aureus culture was established and the synergistic effect of essential oils in the composition of the designed drug was revealed. 3. The promise of the application of the developed preparation for the treatment of mechanical damage to the tissues of the mammary gland, complicated by bacterial infections, and the prevention of mastopathy has been proven.

Key words: mastopathy, ointment, antimicrobial activity, synergism, phytooil.

Spectrum and levels of antimicrobial activity of modified amino acids 62-65

Osolodchenko T. P., Andreieva I. D., Zavada N. P., Ryabova I. S., Volyansky A. Yu.

Introduction. Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections and is rising to dangerously high levels in all parts of the world. The search among the natural and synthetic biologically active compounds that influence the development of resistance in against antibacterial agents in the clinically relevant strains acquires increasingly greater significance. **Material & methods.** Were studied the spectrum and levels of antimicrobial activity of 10 synthetic amino acid derivatives, the most active according to the results of the primary screening study. Investigations of the degree and spectrum of antimicrobial activity selected on the basis of screening of promising substances were performed on 40 museum and clinical strains of microorganisms, among them 17 gram-positive, 13 strains of gram-negative bacteria and 10 strains of fungi of the genus *Candida*. Determination of antimicrobial and anticandidisis effects of new compounds was carried out using the standard method of double serial dilutions. **Results & discussion.** It has been established that synthesized derivatives of lysine and arginine possess high in vitro activity against gram-positive microorganisms with MIC values in the range of 3.9-31.25 μg / ml. Among the investigated substances, the most active compounds, namely the derivatives of lysine 6.1 and arginine 7.1.5 (MIC and MBCB in the range 3.9-15.6 μg / ml), were determined according to their degree of influence on gram-positive microorganisms. The effect of modified amino acids relative to *P. aeruginosa* ATCC 27853 was demonstrated at concentrations of 15.6-62.5 μg / ml. The MIC synthetic derivatives of amino acids relative to *E.coli ATCC 25922* were within the range of 7.8-15.6 μg / ml. Indicators of MIC of synthesized substances for strains *S. enteritidis* gr. P, Y / ratin No. 27, *S. flexneri* DSIK 170 and *S. sonnei* DISK 5772 were in the range of 15.6 - 31.25 μg / ml., The most active for strain *P. aeruginosa* ATCC 27853 were derivative of lysine 6.6 and derivative of arginine 7.1.6. For representatives of the *Enterobacteriaceae* family were the most active derivative of lysine 6.3 and derivative of arginine 7.1.5. The activity of modified amino acids relative to fungi of the genus Candida was not high. The inhibitory growth of the concentrations of the tested substances was in the range of 62.5-125.0 μg / ml. According to the degree of antifungal activity, none of the studied substances did not show better than others. **Conclusion.** The obtained results confirmed the promising research of modified amino acids with the ultimate goal of creating new antimicrobial agents on their basis.

Keywords: modified amino acids; microorganisms; antimicrobial activity

Clinical case of multiple sclerosis associating with persistent herpes virus infection: dynamics on the background of antiviral and immunocorrective treatment 66-70

Davydova T.

Rationale: Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system. Infectious triggers of MS are being actively investigated. Substantial evidence supports the involvement of the Epstein-Barr virus (EBV), though other viruses, bacteria, protists, and fungi are also being considered. For many years, researchers have discussed the relationship of demyelinating processes and development of multiple sclerosis (MS) associated with the activation and persistence of herpes viruses. In recent years, studies have increasingly proved the pathogenetic role herpes viruses in the development of this disease, but this requires further study. There is growing evidence that viruses can play a role by acting as external triggers. However, it is not known, one virus is the cause MS or several viruses can act as an impulse to the development the disease. **Clinical case:** Patient N., female, 35 years old, was diagnosed with multiple sclerosis, disseminated, relapsing remitting, exacerbation stage with moderately expressed right-sided paraparesis, with motor and sphincter disorders, pronounced vestibule-ataxic (vestibulocerebellar) syndrome, cognitive impairment, EDSS 4.5–5.0 in 2013. A brain and spine MRI showed numerous bilateral hyperintense (17) T1, and several (5) T2 lesions and FLAIR contrast-enhancing over the hemispheres and cerebellum and several (6) T1, (4) T2 lesion in her cervical spinal cord sizes from 0.3 cm to 1.1x0.6x1.1 cm individual foci with signs of perifocal edema. At the time of diagnosis complaints of dizziness, shakiness when walking, weakness and a feeling of numbness in the limbs, discoordination of movements, impaired urination by the type of delay with frequent urges, decreased performance, fatigue, unstable gait, muscle cramps, decreased muscle strength (4), slight speech disturbances, depression and anxiety. She treated with pulses of corticosteroids (1.5 g methylprednisolone) and plasmapheresis with gradual tapering of the steroids over a period of 4 weeks according to standard treatment protocols in the neurological department Kharkiv Regional Clinical Hospital. The first symptoms appeared 6 months before the diagnosis was established and gradually increased. Complaints were preceded by an episode of acute viral infection and prolonged low-grade fever for 3 months. We consider the clinical case patient with MS, it was detection the abnormalities in the immune status and viral load (herpes type 4 – Epstein-Barr virus, EBV and human herpes virus 6 – HH6), and positive dynamics was observed in condition of patient and MRI data after antiviral and immunocorrective therapy. **Interventions:** We administered valacyclovirum as the first therapy in combination with recombinant interferons α2b and cridanimodum. Additionally was recommended high doses of vitamins. **Outcomes:** The patient's condition improved after treatment: EDSS 3.0–3.5. MRI also showed positive dynamics: several small lesions in the brain disappeared, large MS lesions became smaller in size. (11) T1, and several (5) T2 over the hemispheres and cerebellum and FLAIR contrast-enhancing and several (4) T1, (3) T2 lesion in her cervical spinal cord sizes from 0.3 cm to 0.7x0.4x0.8 cm individual foci without signs of perifocal edema. **Conclusion:** Early diagnosis and active antiviral and immunocorrective therapy is important when herpes are detecting in MS patients for treating and preventing further development of the disease, so we would like to highlight some aspects of the therapy carried out in this case for the perspective planning relevant clinical studies in the similar direction.

Keywords: Circulating immune complexes (CIC); Central nervous system (CNS); Epstein – Barr virus (EBV); Human herpesvirus 6 (HH6); Herpes simplex virus 1 (HS1) ; Immunofluorescence coefficient (Ic); Magnetic resonance imaging (MRI); Multiple sclerosis (MS); Optical density units (Op.d.u); Peripheral blood mononuclear cell (PBMCs); Varicella – Zoster virus (VZV)