

CLINICAL TRIAL PROTOCOL

Randomized, prospective, sham-controlled, blinded, cross-over clinical study of the effect of the virtual reality (VR) technology on recovery of the indicators of the autonomic nervous system in healthy volunteers affected by moderate stress

Protocol code:	PurePurr-001
Protocol version:	No. 2.6
Date:	January 29, 2018
Study type:	Interventional
Regulatory approval:	Not required
Study phase:	N/A

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PROTOCOL AGREEMENT

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Protocol Consent Form

I, physician _____,
have reviewed the PurePurr-001 Clinical Study Protocol *Randomized, prospective, sham-controlled, blinded, cross-over clinical study of the effect of the virtual reality (VR) technology on recovery of the indicators of the autonomic nervous system in healthy volunteers affected by moderate stress.*

Position: _____

Name of the Healthcare Facility: _____

Address: _____

Tel.: _____

E-mail: _____

I have discussed the objectives of the study and the content of the Protocol with the Authorized Representative of Pharmaxi-Ukraine LLC in detail.

I undertake to conduct the study in accordance with the requirements of the current legislation of Ukraine, the principles of the Good Clinical Practice and this Protocol, and agree to comply with all its requirements, taking into account all ethical considerations and reasons.

I agree to observe the privacy of this Protocol, not to disclose it to third parties, and to use it solely for the purposes of this study.

I understand that should the Company decide to suspend or terminate the study early at any time and for any reason, I would be notified of such decisions in writing. And vice versa, should I decide to refuse to conduct the study, I would immediately notify the Company of my decision in writing.

I confirm the absence of my interest in certain findings and outcomes of this study and any other conflict of interest in the performance of my duties as the investigator.

Sponsor's Representative	Physician taking part in the study
Last name: Yu. V. Lebid	Last name: _____
Signature: _____	Signature: _____
Date: _____	Date: _____

LIST OF ABBREVIATIONS

BP	Blood pressure
DBP	Diastolic blood pressure
BMI	Body mass index
CRF	Case Report Form
CT	Clinical trial
CRO	Contract Research Organisation
HCF	Healthcare Facility
SS	Study site
AE	Adverse event
AR/ADE	Adverse reaction/Adverse device effect
SOP	Standard operating procedure
SBP	Systolic blood pressure
HR	Heart rate
RR	Respiratory rate
CEB	Central executive body
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
t_{body}	Body temperature
TP	Total power
SDNN	Standard deviation of the NN interval
RMSSD	Square root of the mean squared differences of successive NN intervals
HF	High frequency
LF	Low frequency
LF/HF	Ratio LF [ms ²]/HF [ms ²]
HRV	Heart rate variability
VR	Virtual reality
MC	Mental calculation
STAI	Spielberger State-Trait Anxiety Inventory
ASMR	Autonomic Sensory Meridian Response

PROTOCOL SYNOPSIS

Study technology	VR (Pure Purr)
Study title	Randomized, prospective, sham-controlled, blinded, cross-over clinical study of the effect of the virtual reality (VR) technology on recovery of the indicators of the autonomic nervous system in healthy volunteers affected by moderate stress.
Study code	PurePurr-001
Protocol version	No. 2.6 of January 29, 2018
Study phase	N/A
Study objective	<i>Primary objective:</i> To assess the effect of the Pure Purr technology on the recovery of the autonomic nervous system in healthy volunteers affected by moderate stress. <i>Secondary objective:</i> To assess the effect of 5-minute VR exposure on the situational anxiety parameters before and after exposure to moderate stress.
Design	Randomized, prospective, sham-controlled, blinded, cross-over clinical study.
Number of volunteers:	Approx. 100 randomized volunteers.
SS:	1 centre
Design	<p>Randomized, prospective, sham-controlled, blinded, cross-over clinical study. The study will include healthy volunteers 20 to 60 years of age, who have applied to the study site. After signing the Informed Consent Form for participation in the clinical study, an assessment of healthy volunteers' eligibility with the inclusion/non-inclusion criteria will be carried out. Eligible volunteers will be randomized at the 1:1 ratio to one of the following 2 groups:</p> <p><u>Group 1:</u> the volunteers will first be tested with the VR headset A, and during the next visit – with the VR headset B.</p> <p><u>Group 2:</u> the volunteers will first be tested with the VR headset B, and during the next visit – with the VR headset A.</p> <p>The study includes two visits:</p> <p><u>Visit 1 (Day 1):</u> verification of inclusion/non-inclusion criteria, randomization, and testing.</p> <p><u>Visit 2 (Day 2):</u> verification of exclusion criteria, testing.</p> <p>The minimum interval between the two visits is 24 hours and nocturnal sleep is mandatory. The next visit should take place at the same time, taking into account the circadian rhythms. The total duration of the study per volunteer is 2 days (+7 days). Visit 2 may be postponed for the maximum period of 7 days, if necessary.</p> <p>Volunteers' tests during each visit consist of the following stages:</p> <p><u>Stage 1:</u> a volunteer lies down on the bed and 5-minute ECG is recorded at rest.</p> <p><u>Stage 2:</u> a volunteer undergoes the software-based "mental calculation" test while lying on the bed for 5 minutes. ECG is recorded simultaneously.</p> <p><u>Stage 3:</u> a volunteer lies down on the bed, puts on the VR headset, the investigator turns the program on for 5-minute exposure, ECG is recorded simultaneously.</p> <p><u>Stage 4:</u> a volunteer takes off the VR headset, lays on the bed for 5 minutes during which ECG is recorded, then the volunteer takes the Spielberger State-Trait Anxiety Inventory (STAI).</p> <p>Thus, for each visit, 4 ECG will be registered for each volunteer: before VR</p>

	exposure, during exposure to moderate stress, during VR exposure, and after VR exposure.
Duration of monitoring over volunteers	The duration of the study per volunteer is 2 days (+7 days). Volunteers will be enrolled for 2 months.
Administration of medicinal products and food intake.	Volunteers are allowed to take medicinal products upon prior agreement with the investigator. Administration of medicinal products known to have a pronounced effect on the central nervous system and the cardiovascular system that may cause erroneous study results is prohibited. Tonics and energy drinks and foods are prohibited for at least 2 hours prior to participation in the study.
Inclusion criteria:	The study will enrol healthy volunteers meeting all inclusion criteria: <ol style="list-style-type: none"> 1. Females and males 20-60 years old; 2. Volunteers have no history of cardiovascular diseases; 3. Volunteers have no essential abnormalities on the ECG; 4. Normal or moderate level of situational anxiety based on the Spielberger State-Trait Anxiety Inventory (STAI); 5. Signed Informed Consent Form for participation in the study.
Exclusion criteria:	The study will not enrol volunteers meeting one or more non -inclusion criteria: <ol style="list-style-type: none"> 1. Intolerance to the VR technology; 2. HR 110 bpm or below 50 bpm; 3. RR above 23 breaths per minute; 4. Abuse of alcohol, psychotropic substances and narcotic agents; 5. Smoking more than 100 cigarettes a week; 6. Administration of medicinal products for the treatment of cardiovascular and nervous system diseases 3 days prior to participation in the study; 7. Drinking tonics and energy drinks for at least 2 hours prior to participation in the study; 8. Severe or acute forms of respiratory, urogenital, gastrointestinal, haematological, metabolic, endocrine, or neurological diseases; 9. Mental disorders, which, in the opinion of the investigator, may distort the results of the study; 10. Participation in any clinical study over the last 3 months prior to enrolment to the study; 11. Parkinson's disease, Parkinson's symptoms, tremor, restless leg syndrome, and other motility disorders; 12. Pregnancy; 13. Any disease of the thyroid gland.
Efficacy evaluation	<p><i>Primary variable:</i></p> <ul style="list-style-type: none"> • Change in HF component after 5-minute exposure to the VR audio-visual sequence; <p><i>Secondary variables:</i></p> <ul style="list-style-type: none"> • rMSSD variable after 5-minute exposure to the VR audio-visual sequence; • LF/HF ratio after 5-minute exposure to the VR audio-visual sequence; • SDNN variable after 5-minute exposure to the VR audio-visual sequence; • Total power (TP) variable after 5-minute exposure to the VR audio-visual sequence; • HR variable after 5-minute exposure to the VR audio-visual sequence; • BP variable after 5-minute exposure to the VR audio-visual sequence; • Spielberger State-Trait Anxiety Inventory (STAI) variable after 5-minute exposure to the VR audio-visual sequence.

Safety and tolerability evaluation	<p>For the purposes of safety evaluation, security measures will be taken and the following data will be evaluated:</p> <ul style="list-style-type: none"> • the number of AE/ADE occurring during the participation in the study; • the results of measurement of vital signs; • the overall tolerability evaluation of the VR technology provided by the investigator.
Examination methods	<p>The following examination methods will be used during the study:</p> <ul style="list-style-type: none"> • Collection of medical history; • Anthropometric examination (weight, height); • Measuring BP, HR, RR, and body t°; • Spielberger State-Trait Anxiety Inventory (STAI); • Electrocardiography.

1. BACKGROUND INFORMATION

Information about the protocol

Randomized, prospective, sham-controlled, blinded, cross-over clinical study of the effect of the virtual reality (VR) technology on recovery of the indicators of the autonomic nervous system in healthy volunteers affected by moderate stress

Protocol code: PurePurr-001,

Protocol version: No. 2.6, Date: January 29, 2018

Study type: interventional, Study phase: N/A

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2. STUDY JUSTIFICATION

Stress is a non-specific response of the body to any external stimulus. There is evidence that stress causes many diseases, in particular, diseases of the cardiovascular and nervous systems (depression, erectile dysfunction, anxiety disorder), etc. These diseases can be caused and aggravated by stress. Also, stress affects the emotional sphere by lowering the quality of life. In everyday life, people interact with large numbers of stressors – the factors that cause stress. The neurohumoral basis of the stress response is the activation of the sympathoadrenal system with a corresponding increase in the activity of the sympathetic nervous system and suppression in the activity of the parasympathetic nervous system [12].

Stress can be related to physiological response, as well as cognitive load [13]. Mental calculation (MC) is one of the most common psychological stressors, which is widely used for stress modelling. In addition, it has been proven that mental calculation is accompanied by an increase in HR and a change in the HRV [14].

The anti-stress effect of relaxation, meditation, listening to musical pieces and watching visual stimuli (landscape therapy, review of paintings, drawings, etc.) is well-established. A certain set of visual and auditory stimuli, which are subjectively linked to calmness, relaxation and safety, contributes to the loss in the tone of the sympathetic nervous system, and activation of the parasympathetic nervous system. The activation of the sympathoadrenal system is aimed at mobilization of the body organs and systems, triggering catabolic processes, spasm of the peripheral arteries (centralization of blood circulation), providing breakdown of the glycogen and supplying glucose to the organs and systems, suppressing the digestive processes. Activation of the parasympathetic nervous system leads to the activation of anabolic processes in the body and promotes recovery, growth, and regeneration.

In order to maintain homeostasis, the central nervous system responds to environmental factors through nerve feedback. Due to the fact that stress affects the rhythmic structure of autonomous conditions and human behaviour, and nervusvagus plays an integral role in the function of the parasympathetic system with the help of cardiac contractions. Thus, the heart rate variability (HRV) is the sensitive method for evaluation of the integral degree of activation of the sympathetic and parasympathetic nervous systems [11]. The HRV parameters make it possible to assess the level of activation of the sympathetic nervous system and, accordingly, estimate the level of stress [1].

The Pure Purr technology combines several audio and visual stimuli aimed at activation of the parasympathetic nervous system. Therefore, this study will investigate the effect of this technology on the recovery of autonomic nervous system performance after moderate stress.

Existing polyvagal theory specifies two functionally distinct branches of the vagus, or tenth cranial nerve. A vagus nerve is a major component of the autonomic nervous system. The theory links the evolution of the autonomic nervous system to affective experience, emotional expression, facial gestures, vocal communication, and contingent social behavior. In this way, the theory provides a plausible explanation for the reported covariation between atypical autonomic regulation (eg, reduced vagal and increased sympathetic influences to the heart) and psychiatric and behavioral disorders that involve difficulties in regulating appropriate social, emotional, and communication behaviors. In addition polyvagal theory offers strategies that help create the sense of security and among them solitude in a calm, quiet place, calm, silent conversation, or even listening to music. This interlaced with VR technology using. [11]

The HF component has been selected as the primary variables for efficacy evaluation because HF peak indicates parasympathetic nervous system activity when LF indicate sympathetic branch of the nervous system [22].

Efferent vagal activity is considered to be an important component of HF and its power is justified by the power of parasympathetic influences. The HF power is significantly influenced by the respiratory center (cardiorespiratory arrhythmia). The respiratory nuclei and nuclei of the vagus nerves are located near the brain stem, which is the cause of the modulating effect of the first on the latter. The direct subordination of the respiratory center to the cortical functions mediates direct central effects on the cardiac spectrum. Mental stress is accompanied by a decrease in the total power of HRV (TP) due to the predominant reduction of HF not only in basal conditions, but even with modulated breathing.

HF, however, reflects direct extracardiac effects on the heart rhythm and is determined by the state of the nervous regulation. Under the influence of hypnosis, the power of the high-frequency component (HF) increases, and the mid-frequency component decreases, and these changes correlate with the degree of hypnability. [21]

Thus, we expected that investigational virtual reality exposure will reflect in HF component and show the effect of relaxation.

2.1. Name and description of the VR technology

Studied VR technology

The **Pure Purr** technology consists of a virtual reality headset with pre-installed software. The virtual reality headset reproduces a dynamic video content that is visually perceived with the help of the high-resolution screen. Also, the headset is able to play audio and transmit it via the headphones. During the Pure Purr study, the volunteer puts on the BoboVR X1 headset, which creates the illusion of a gazebo located by the ocean. The visual sequence also includes a horizon created by the surface of the ocean and blue sky. The dynamics is achieved with the help of the ocean waves moving towards the observer. Curtains enclosing the gazebo from two opposite sides are also moving as if fluttering with fresh wind, and in front of the eyes of the observer a cat is walking in time with the calm breath of a person. The audio support was created on the basis of a slow musical composition and modified by adding a binaural effect, and a background cat purring that was slowed down and supplemented with a binaural effect.

The total length of the audio-video sequence is 6 minutes 11 seconds, of which 1 minute 11 seconds is the Pure Purr promotional video, and the duration of the investigational audio-visual sequence itself is 5 minutes.

Sham VR technology

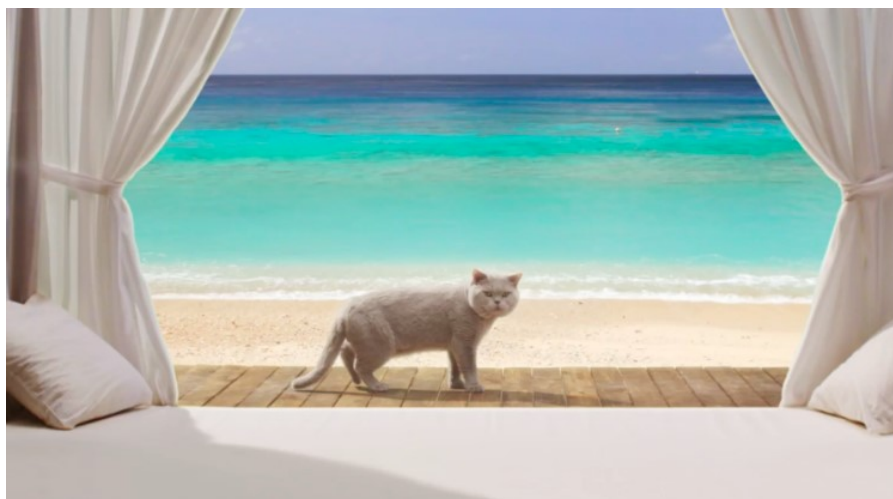
The sham technology includes an identical BoboVR X1 virtual reality headset with pre-installed software. The audio-visual sequence is similar to the one in the investigational version of the software. The key difference is that the audio sequence has not been modified with the binaural effect and has not been synchronized with the tact of respiratory movements and the frequency of heart rate.

The total length of the audio-video sequence is 6 minutes 11 seconds, of which 1 minute 11 seconds is the Pure Purr promotional video, and the duration of the investigational audio-visual sequence itself is 5 minutes.

Figure 1. Still frame of the visual sequence seen by the volunteers during the study of the effect of the VR technology (sham).



Figure 2. Still frame of the visual sequence seen by the volunteers during the study of the effect of the VR technology (studied technology).



2.2. Brief description of known and foreseen risks and benefits for study subjects

Virtual reality technologies are widely used in entertainment, and clinical studies of its medical applications confirms the safety and possible clinical efficacy of the VR. Literature data shows that the risks of serious adverse reactions are insignificant.

The planned clinical study will examine the effects of the use of the VR technology, as well as collect additional data on the safety and tolerability of the VR technology.

Based on the foregoing, the assessment of the risk/benefit ratio allows us to conclude that this study is possible.

The foreseen adverse effects include the symptoms of VR/cyber sickness or kinetosis (ICD-10, T75.3), in particular:

- general discomfort;
- headache;
- feeling of own stomach;
- nausea;
- vomiting;
- pallor;
- hyperhidrosis;
- fatigue;
- somnolence;
- disorientation;
- apathy.

2.3. Description and justification for the method of application of the VR technology

Pure Purr virtual reality technology

Pure Purr technology is based on discoveries in neurophysiology, medicine, acoustics, and computer engineering, which are used to initiate the physiological process of recreation, i.e. allostasis. Literally this term means “achieving stability through change”. This is an active mechanism playing a part in normalization of the tone of the autonomic nervous system and maintaining stability of the body internal environment. The condition in which all our functions are implemented fully and sparingly in response to the load, and the level of adaptation remains high for a long time. Pure Purr is a software and hardware complex for relaxation and recreation based on a virtual reality headset with a display with sufficient resolution, built-in processor, memory, and extended-range stereo speakers. Positive effect on the cognitive function of the brain and the system of neurohumoral regulation is achieved due to the synchronous actuation of a number of stimuli, such as: visual sequence enhanced by the effect of virtual reality, harmonious music with the binaural rhythm technology, and modified cat purrs. Currently, the use of each technology is a scientifically proven trigger for the activation of individual parts of the human nervous system. And combining them in one software complex enhances the overall effect.

Virtual reality technology

The virtual reality technology has been introduced at the end of the last century, and is now rapidly developing. While in the VR, the user interacts with the virtual environment created by the computer, using a special headset or glasses that provide video and audio information. The headset consists of a display and a system of lenses for adjustment of the image geometry, as well as a tracking system that tracks spacial orientation of the device.

Besides various forms of interactive entertainment, virtual reality systems are also widely used in education and medicine. One aspect that influences the efficiency of diving into the VR is the feeling of “presence” in the virtual environment compared to the real environment. That is, when viewing the pieces of virtual content, it is easier for us to unwittingly focus on the image in the headset than on the thoughts and events in the surrounding world. This effect is successfully used by scientists and healthcare professionals for pain relief in dentistry [2], treatment of burn wounds [3], and other cases.

Reduced anxiety, positive emotional background, satisfaction with the presence in the VR are the key factors associated with subjective pain relief when using the VR technology.

It has been established that the modulation of attention via the ascending nociceptive pathways due to the attention to the interactive environment leads to a decrease in impulses through the activation of the inhibitory cortical and subcortical paths [4]. Also, the connection between emotional conditions and cognitive interpretations of sensations obtained through interaction with a virtual video sequence [4] has been confirmed.

Thus, the VR triggers emotions and, as a result, the feelings associated with them – this effect is implemented in Pure Purr.

Harmonious sound sequence

The Pure Purr technology utilizes the virtual reality sound with a harmonious tune subject to the laws of classical harmony. The use of harmonious tune is due to the fact that it activates basal ganglia, motor zones, cerebellum, and limbic-reticular formation. As a result, neuromediator patterns of synaptic transmission change, as well as the levels of such important regulatory hormones as adrenocorticotrophic hormone, cortisol and beta-endorphin.

The processes of perception of sound and its effect on the body are studied using audiometry, electroencephalography, auditory induced potentials techniques, functional magnetic resonance therapy. Harmonious sound is used to treat depression [5], to improve the therapeutic effect in severe neurodegenerative processes [6,7], for preoperative relaxation [17], and in other diseases.

Thus, the properly chosen sound stimulus is a physiological method of influence on the nervous system.

Binaural rhythm

The phenomenon of beating occurs when two sound vibrations of a slightly different frequency overlap. The beating also occurs when the brain receives two signals of a slightly different frequency for different ears [15]. Binaural rhythm is a brain function artefact, when the brain mistakenly perceives certain sounds, although there are no sounds on this frequency. Binaural rhythms are generated when sinusoidal waves in the near range reach each ear separately. The perception of the binaural rhythm is the result of a central alignment in the upper nuclei of the olivary body, neurons of which are sensitive to phase interactions.

There is evidence that binaural rhythms greatly increase the HF power – a known parasympathetic activation marker, stipulated by the activity in the areas of the anterior lumbar gyrus and the medial prefrontal cortex. The binaural effect also reduced the power of LF and the LF/HF ratio [8].

Numerous studies reported a positive effect of listening to the binaural rhythm for achievement of clinically significant results, including: slowing down the heart rate, lowering blood pressure [1], increasing productivity and improvement of mood, responsiveness to hypnosis, mental and physical relaxation, improvement of attention and memory, relief of depression, general anxiety [8,9]. The technology of binaural rhythms is also used in Pure Purr to relief stress.

Autonomic Sensory Meridian Response (ASMR)

An autonomous sensory meridian response is a phenomenon, which makes people experience various pleasant tactile sensations in the scalp, the back of the neck, back, and sometimes in other areas in response to specific audio and visual stimuli.

Usually these feelings are accompanied by a feeling of relaxation and comfort. The most common triggers, i.e., the triggers for the emergence of ASMR, are whispers, cat purrs, crackling sounds, slow movements.

There is evidence that such sensory experience reduces stress, temporarily improves well-being in cases of depression and chronic pain syndrome [10].

Also, preliminary data suggest that sounds used in Pure Purr are potential ASMR triggers.

Cat purrs

Domestic cats are one of the most popular animals, virtually any individual is familiar with the sound of cat purrs. Yet, currently there is no reliable data on the source of purring [16]. Also, the data that describes the purring and its impact on humans is scarce. Purring is a vocalization form unique to cats. Purring often occurs during social contacts between cats and humans, and is usually perceived as an expression of cats' satisfaction. In turn, for humans, the sounds of purring can have a positive psycho-emotional effect.

The Pure Purr uses modified cat purring, which is likely to activate the parasympathetic link in the autonomic nervous system.

2.4. Study conditions

This clinical study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, observation of the approved PurePurr-001 Clinical Trial Protocol and taking into account the requirements of the ICH GCP.

2.5. Characteristics of the study population

The study will include healthy volunteers aged 20 to 60 years, who have applied to the study site. The investigator will suggest taking part in the study to all visitors of the SS, who turned to the SS for any reason. Information about the study and the invitation to take part in the study will also be published on the Internet.

After signing the Informed Consent Form for participation in the clinical study, an assessment of healthy volunteers' eligibility with the inclusion/non-inclusion criteria will be carried out.

Approximately 100 randomized healthy volunteers will be enrolled to the study.

2.6. References to publications and other sources of information used for planning and justification of the clinical trial

For planning and justification of this clinical trial, the literary sources specified in the List of Regulatory Documents and References section were used.

3. CLINICAL TRIAL OBJECTIVE

Primary objective: To assess the effect of the Pure Purr technology on the recovery of the autonomic nervous system in healthy volunteers affected by moderate stress.

Secondary objective: To assess the effect of 5-minute VR exposure on the situational anxiety parameters before and after moderate stress.

4. TRIAL DESIGN

4.1. Primary and secondary parameters to be determined during the study

Primary variable:

- Change in HF component after 5-minute exposure to the VR audio-visual sequence;

Secondary variables:

- rMSSD variable after 5-minute exposure to the VR audio-visual sequence;

- LF/HF ratio after 5-minute exposure to the VR audio-visual sequence;
- SDNN variable after 5-minute exposure to the VR audio-visual sequence;
- Total power (TP) variable after 5-minute exposure to the VR audio-visual sequence;
- HR variable after 5-minute exposure to the VR audio-visual sequence;
- BP variable after 5-minute exposure to the VR audio-visual sequence;
- Spielberger State-Trait Anxiety Inventory (STAI) variable after 5-minute exposure to the VR audio-visual sequence.

The criterion for the prevailing efficiency for each variable is the presence of statistically significant differences between groups in favour of any of the groups with a directivity, which indicates the effectiveness of the impact of the studied VR technology.

Tolerability evaluation

Tolerability of the VR technology will be evaluated based on: vital signs, assessment of AR/ADE.

Upon completion of the study (upon completion of Visit 2), the Investigator should provide an assessment of the tolerability of the studied VR technology for each healthy volunteer based on the 5-point verbal analogue scale given in Table 1.

Table 1. Tolerability evaluation

Points	Category	Category description
5 points	“very good ”	No adverse reactions associated with the VR technology
4 points	“good”	There is a minor adverse effect that does not cause serious problems in a healthy volunteer and does not require discontinuation of the use of the VR technology.
3 points	“satisfactory”	There is a minor adverse effect that causes discomfort in a healthy volunteer, but does not require discontinuation of the use of the VR technology.
2 points	“unsatisfactory”	There is an adverse reaction that causes a significant negative effect on the condition of a healthy volunteer, it requires discontinuation of the use of the VR technology, but does not require additional medical care
1 point	“extremely unsatisfactory”	There is an adverse reaction that causes a significant negative effect on the condition of a healthy volunteer, it requires discontinuation of the use of the VR technology and additional medical care

4.2. Description of type/design of the trial/randomization/intended purpose of the VR technology

PurePurr-001 is a randomized, prospective, sham-controlled, blinded, cross-over clinical study. The study will include healthy volunteers aged 20 to 60 years, who have applied to the study site for other medical reasons or those who read the advertisement on the Internet. After signing the Informed Consent Form for participation in the clinical study, an assessment of healthy volunteers' eligibility with the inclusion/non-inclusion criteria will be carried out.

Study groups

Eligible volunteers will be randomized at the 1:1 ratio to one of the following study groups (1 or 2):

Group 1: the volunteers will first be tested with the VR headset A, and during the next visit – with the VR headset B.

Group 2: the volunteers will first be tested with the VR headset B, and during the next visit – with the VR headset A.

Visits

The study includes two visits:

Visit 1 (Day 1): verification of inclusion/non-inclusion criteria, randomization, and testing.

Visit 2 (Day 2): verification of exclusion criteria, testing.

The minimum interval between the two visits is 24 hours and nocturnal sleep is mandatory. The next visit should take place at the same time, taking into account the circadian rhythms. The overall

duration of the study for each volunteer is 2 days (+7 days), if necessary, Visit 2 may be postponed for a maximum period of 7 days.

Stages of each visit are described in details in Section 6.1.

Layout 1. General study layout

Group 1

Headset A, Visit 1

Stage 1	Stage 2	Stage 3	Stage 4
---------	---------	---------	---------

Headset B, Visit 2

Stage 1	Stage 2	Stage 3	Stage 4
---------	---------	---------	---------

Group 2

Headset B, Visit 1

Stage 1	Stage 2	Stage 3	Stage 4
---------	---------	---------	---------

Headset A, Visit 2

Stage 1	Stage 2	Stage 4	Stage 4
---------	---------	---------	---------

4.3. Description of randomization and blinding

In order to observe the “blindness”, the study group of each volunteer will be determined in accordance with their randomization group.

The blind study suggests volunteers’ lack of information about used VR technology. VR headsets will be labelled as A and B, and the volunteer will not be aware of the corresponding technology. Unblinding will take place upon completion of the study.

With a view to a more even distribution of the volunteers, a block randomization design (fixed-length blocks of 10 volunteers balanced by groups) will be used.

Randomization is carried out only after the signing of the Informed Consent Forms and verification of the volunteers’ compliance with all inclusion/non-inclusion criteria (the volunteers are assigned with randomization numbers).

In practice, the implementation of the developed randomization scheme will be carried out using numbered envelopes provided by the Sponsor to the site. Each envelope will contain 10 randomization cards. The investigator will open an envelope and randomly take out randomization cards, which will say “Group 1” or “Group 2”, according to which the volunteers will take part in the study.

The next envelope will be opened as the investigator runs out of randomization cards in the previous envelope in ascending order of the numbers on envelopes.

The distribution by groups is given in Table 2.

Table 2. Distribution of healthy volunteers

	Group 1	Group 2
Number of volunteers	50 healthy volunteers	50 healthy volunteers

4.4. Information about the VR headset

The study will use two identical BoboVR X1 virtual reality headsets. Pure Purrr software will be installed on one of the headsets, and another headset will run the sham software. Each of the VR headsets will have the following labelling:

- A or B marking;
- clinical trial protocol code (PurePurr-001).

4.5. Information about ECG recorder

The study will use the same properly calibrated 12-channel ECG recorder CONTEC8000G ECG Workstation manufactured by CONTEC™ (China).

4.6. Estimated duration of participation of subjects, description of the sequence and duration of all stages of the study

The duration of the study for each volunteer is 2 (+7) days.

4.7. Description of conditions for termination or interruption of the entire study, its parts, or the participation of individual subjects

The study may be terminated in the event of severe adverse reactions, as well as inability to comply with the requirements of the Protocol or upon the decision of the study Sponsor. The investigator is required to inform the study Sponsor and the Ethics Committee at the HCF in writing about termination of the study.

For each volunteer, the reason for withdrawal from the study is the onset of one of the following **exclusion criteria**:

- Emergence of any medical condition, an adverse event or circumstances when, in the opinion of the investigator, it is not in the best interests of the volunteer to proceed with the participation;
- Failure to comply with the protocol requirements;
- Onset of one of the non-inclusion criteria.

The reasons for the early withdrawal of the volunteer from the study are specified in the CRF.

4.8. Accounting procedures for virtual reality headsets and volunteers

Storage of virtual reality headsets

Virtual reality headsets should be stored in original packaging, in a safe, dry place, in a closed room or a closet, accessed only by the responsible Investigator and authorized persons.

Terms of distribution, accounting and return of virtual reality headsets

For the purposes of the study, the HCF will receive 2BoboVR X1 virtual reality headsets with pre-installed software (sham and Pure Purr). Each virtual reality headset will be labelled as A or B.

Having received the virtual reality headsets, the responsible Investigator provides signing of the Note of Delivery to the Study Site (2 copies) by the administration of the HCF. All received virtual reality headsets should be registered on the off-balance sheet of the HCF.

Under no circumstances can the investigator allow the use of virtual reality headsets for any purposes other than that specified in the Clinical Study Protocol. The virtual reality headsets should be delivered to the SS in intact packaging.

Upon completion of the study the virtual reality headsets are returned to the Sponsor, as evidenced by the Note of Return from the Study Site (2 copies).

The Investigator conducting the study should keep the Virtual Reality Headset Use Form, stating the following

- 1) Date, time and duration of use of the virtual reality headset
- 2) Type of headset, A or B
- 3) Volunteer's ID

The virtual reality headset will be used by the volunteers in accordance with the regimen corresponding to the study group

Record form for enrolled/screened/randomized volunteers

Volunteers who have signed the Informed Consent Form are registered in the Record Form for Enrolled/Screened/Randomized Volunteers, which states:

- 1) Volunteer's full name
- 2) Volunteer's ID (five digits)
- 3) Date of signing of the Informed Consent Form
- 4) Did the volunteer pass the screening (yes/no)?

- 5) Did the volunteer pass the randomization (yes/no)?
- 6) Date of randomization
- 7) The group to which the volunteer was assigned (1 or 2)
- 8) In case of volunteer's non-compliance based on the results of the screening or his/her exclusion, the reason should be specified in the Volunteer Exclusion column

4.9. Disclosure of randomization code

Information related to decoding of randomization codes will be retained by the Sponsor throughout the study. Disclosure of randomization codes will be carried out after the Sponsor receives statistical data on the findings and outcomes of the study.

An Authorized Representative of the Sponsor retains confidentiality of randomization codes and cannot disclose them directly or indirectly to volunteers or other parties/individuals/entities.

4.10. Working with Case Report Forms

All data entered in the Case Report Forms are considered to be source documentation.

Data on each volunteer enrolled in the study should be collected by the Investigator 2 times (Visit 1 and 2). Only the person to whom this right has been delegated in accordance with the Distribution of Duties and Signature Samples Log is allowed to make entries and corrections in the CRF.

4.11. Description of the sequence and duration of the stages of the CS before the use of the virtual reality technology

In order to enrol a volunteer in the study, the investigator has to take a number of consecutive steps:

1. Fill in and sign the Informed Consent Form with the volunteer. The terms and conditions of the study should be explained to potential study subjects and suggested to familiarize themselves with the Study Subject Information Sheet. A potential study participant should have enough time to familiarize himself/herself with the information, he/she has the right to ask any study-related questions, he/she should receive comprehensive information regarding his/her inquiries. Participation in the study is absolutely voluntary. In case of consent to participate in the study, the volunteer gives his/her written consent to participate in the study by signing the Informed Consent Form. 2 copies of the Informed Consent Form should be signed and dated by the participant in person, as well as the person, who informed study subject (the investigator).

2. The investigator should check the volunteer's compliance with the eligibility criteria for inclusion/non-inclusion. For the purposes of testing, the investigator adds the information about verification of the availability of all inclusion criteria and the absence of all non-inclusion criteria to the CRF.

3. Randomization will be carried out only after the signing of the Informed Consent Form and verification of the volunteers' compliance with all inclusion/non-inclusion criteria.

4. Once the volunteer has been successfully randomized, the investigator should indicate the volunteer's (five-digit) identification number:

- in the CRF;
- in the Virtual Reality Headset Use Form;
- in the Record Form for Enrolled/Screened/Randomized Volunteers.

4.12. Time and events schedule

The frequency of examination in volunteers and registration of the data obtained will be carried out in accordance with the schedule shown in Table 4.

Table 3. Visit matrix (time and events schedule)

Study procedures		
Visits ¹	Visit 1	Visit 2
Study days	Day 1	Day 2 (+7)
Basic data		

Study procedures		
Visits ¹	Visit 1	Visit 2
Study days	Day 1	Day 2 (+7)
Obtaining informed consent	X	
Evaluation of volunteer's compliance with the inclusion/non-inclusion criteria	X	
Randomization	X	
Evaluation of availability of exclusion criteria		X
Registration of demographic data	X	
Collection of medical history	X	
Physical examination	X	X
Measuring BP, HR, RR, and body t°	X	X
Registration of concomitant treatment and comorbidities	X	
Investigation of the VR technology		
Stress modulation and ECG recording before/during/after VR exposure	X	X
Evaluation of volunteers based on the Spielberger State-Trait Anxiety Inventory (STAI)	X	X
Safety and tolerability evaluation		
Registration of AR/ADE	X	X
Tolerability evaluation	X	X

4.13. Study procedures and methods used throughout the study

Obtaining informed consent

All volunteers must provide the written informed consent to participate in the study before study initiation.

Volunteers, who are potential participants in the study, should be informed by the investigator in a clear form about the nature of the clinical trial, the technology under investigations, and the possible risks associated with administration of the medicinal product. All information and the Informed Consent Form are given to the volunteers at their request in Russian or in Ukrainian.

The volunteers should be informed that at any given moment they may refuse to participate in the study. The volunteers should know that the fact of their participation in the study is strictly confidential.

The volunteers also have to be explained that their personal data obtained during the study will be used for statistical processing of the study results and drawing of a report, and can also be discussed by the individuals conducting the study.

A volunteer should be given enough time to make a decision regarding participation in the study and have the opportunity to discuss all questions with his or her investigator. The investigator should not put pressure on the volunteer to have an effect on his/her decision.

2 copies of the Informed Consent Form should be signed and dated by the volunteer in person, as well as the person, who informed the volunteer (the investigator). One copy of the signed and dated Informed Consent Form is given to the volunteer, the second copy will be attached to the Investigator's File and the SS archive for at least 15 years upon the completion of the trial.

In the event that new safety data are obtained that will lead to a significant change in the risk/benefit ratio, it is necessary to analyse the Informed Consent Form and introduce the necessary changes. In this case, the volunteers, who continue to participate in the study, should be informed of the emergence of new information, provide them with a new version of the Informed Consent Form, and give them the opportunity to reconsider their decision to participate in this study, to sign and to date 2 copies of the Informed Consent Form for participation in the clinical trial.

The fact of obtaining informed consent must be recorded in the CRF with indication of the date of signing the Informed Consent Form.

Screening procedures/evaluation for collection of data on the volunteers' baseline condition/volunteers' inclusion to the study

Upon obtaining of the informed consent, it is necessary to confirm the possibility of the volunteer's participation in the study based on the inclusion/non-inclusion criteria. For this purpose, it is necessary to conduct an examination, which includes:

- registration of demographic data (sex, age) and anthropometric data (height, body weight, BMI calculation);
- objective examination;
- measurement of BP, HR, and body t°;
- collection of medical history;
- data on current treatment;
- evaluation of volunteer's compliance with the inclusion/non-inclusion criteria.

Confirmation of compliance with each of the inclusion/non-inclusion criteria should be reflected in the relevant sections of the CRF.

Simulation of stress using calculations in head

During each visit, prior to the investigation of the effects of the audio-visual sequence in the VR, volunteers will undergo a 5-minute mental calculation test. The investigator or his assistant will read for the volunteers two three-digit numbers, which the volunteers have to add and state their sum, which they will have to decompose and sum up to obtain a one-digit number. In the case the volunteer gives a wrong answer, the investigator clearly tells him/her about it and says "that is not correct". Volunteers will hear the same sets of three-digit numbers.

Measurement of BP and HR

Measurement of HR and BP will be performed with the help of properly calibrated equipment at the study site in a sitting position after at least a 5-minute rest.

Measurement of body temperature

Measurement of body temperature will be done for 10 minutes in the armpit using a mercury thermometer once during each visit.

Physical examination

It includes: auscultation of the heart and lungs, examination of skin, organ systems, and visible mucous membranes. Objective examination is carried out during each visit in full.

Anthropometric data

Collection of anthropometric data (height and weight) will be carried out using equipment available at study sites during visits to the SS at visit 1.

Electrocardiography

Electrocardiograms will be taken by the properly qualified investigator, the study will use the same properly calibrated 12-channel ECG recorder CONTEC8000G ECG Workstation manufactured by CONTEC™ (China).

Spielberger State-Trait Anxiety Inventory

Testing the volunteers using the Spielberger STAI will be performed in the process of assessment of compliance with the inclusion/non-inclusion criteria, and upon completion of investigation of the virtual reality technology.

4.14. Study data collection procedure

The study will be collected according to the following plan:

Visit 1.

The visit takes place at the SS and is considered to be Day 1 of the study.

Having received the informed consent for participation in the study, the investigator evaluates the compliance of the volunteer with the inclusion criteria and verifies that he/she has no non-inclusion criteria.

During the visit, the investigator:

- Obtains the Informed Consent Form from the volunteers;
- Checks the inclusion/non-inclusion criteria, evaluates the volunteers with the help the Spielberger STAI. In case a volunteer has all the inclusion criteria and no signs that meet the non-inclusion criteria, the investigator performs further data collection and randomization;
 - Anthropometric data (weight, height and body mass index);
 - Evaluates vital signs (BP, HR, RR, and body t°);
 - Physical examination;
 - Collects demographic data (date of birth, gender);
 - Collects clinical data on concomitant diseases, therapy and records them;
 - Investigates the technology of virtual reality (Stages 1, 2, 3, and 4);
 - Evaluates the volunteers' complaints in terms of the development of adverse events/adverse device effect (AE/ADE);
- Evaluates tolerability;
- The clinician records all indicators collected at Visit 1 and transfers them to the CRF.

Visit 2.

The visit takes place at the SS.

During the visit, the Investigator:

- Checks the inclusion criteria. In case a volunteer has no signs that meet the exclusion criteria, the Investigator performs further data collection;
 - Evaluates vital signs (BP, HR, RR, and body t°);
 - Evaluated physical signs;
 - Investigates the technology of virtual reality (Stages 1, 2, 3, and 4);
 - Evaluates volunteers based on the Spielberger State-Trait Anxiety Inventory (STAI);
 - The investigator records all indicators collected at Visit 2 and transfers them to the CRF;
 - Evaluates the volunteers' complaints in terms of the development of adverse events/adverse device effect (AE/ADE);
- Evaluates tolerability;
- Informs volunteers on study completion.

4.15. Data management

Data collection and validation

Data will be collected using the CRF. Upon completion of the study, all filled CRFswill be provided to the CRO for data transfer into a spreadsheet and further statistical analysis. Data collection procedures and their verification will be described in detail in the operating procedures.

The investigator should submit information to the CRF on the day of the visit, but notlater than within 12 hours.

5. INCLUSION AND EXCLUSION OF THE SUBJECTS

It is planned to enrol approximately 160 healthy volunteers to the study for further randomization of 100 volunteers.

For the purposes of randomization the volunteers should meet the inclusion criteria and have no non-inclusion criteria, be familiar with the terms of participation in the study, and sign the Informed Consent Form for participation in the study.

5.1. Inclusion criteria for the study subjects:

The study will enrol healthy volunteers meeting all the following inclusion criteria:

1. Females and males 20-60 years old;
2. Volunteers have no history of cardiovascular diseases;
3. Volunteers have no essential abnormalities of the ECG;
4. Normal or moderate level of situational anxiety based on the Spielberger State-Trait Anxiety Inventory (STAI);
5. Signed Informed Consent Form for participation in the study.

5.2. Non-inclusion criteria for the study subjects:

The study will not enrol volunteers meeting one or more non-inclusion criteria:

1. Intolerance to the VR technology;
2. HR above 110 bpm or below 50 bpm;
3. RR above 23 breaths per minute;
4. Abuse of alcohol, psychotropic substances and narcotic agents;
5. Smoking more than 100 cigarettes a week;
6. Administration of medicinal products for the treatment of cardiovascular and nervous system diseases 3 days prior to participation in the study;
7. Drinking tonics and energy drinks for at least 2 hours prior to participation in the study;
8. Severe or acute forms of respiratory, urogenital, gastrointestinal, haematological, metabolic, endocrine, or neurological diseases;
9. Mental disorders, which, in the opinion of the investigator, may distort the results of the study;
10. Participation in any clinical study over the last 3 months prior to enrolment to the study;
11. Parkinson's disease, Parkinson's symptoms, tremor, restless leg syndrome, and other motility disorders;
12. Pregnancy;
13. Any disease of the thyroid gland.

5.3. Exclusion criteria for the subjects:

The study will not enrol volunteers meeting one or more exclusion criteria:

1. Emergence of any medical condition, an adverse event or circumstances when, in the opinion of the investigator, it is not in the best interests of the volunteer to proceed with the participation;
2. Failure to comply with the requirements of the protocol;
3. Onset of one of the non-inclusion criteria.

The reasons for the early withdrawal of the volunteer from the study are specified in the CRF. The discontinued patients will not be replaced.

5.4. Procedure of early withdrawal from the study

At any given time and for any reason, the Sponsor may decide to terminate or suspend the study early. Such decision will be brought to the attention of the investigator and the LEC in writing. Accordingly, in case the investigator decides to discontinue his/her participation in the study, he/she must promptly notify the Sponsor in writing. The authorized representative of the Sponsor or the CRO will inform the local Ethics Committee at the HCF.

6. STUDY SUBJECT INVESTIGATION

6.1. Procedure for use of the virtual reality technology

Depending on the results of randomization, the volunteers are assigned to Group 1 or Group 2:

Group 1: the volunteers will first be tested with the VR headset A, and during the next visit – with the VR headset B.

Group 2: the volunteers will first be tested with the VR headset B, and during the next visit – with the VR headset A.

Stages for each visit

Volunteers' tests during each visit consist of the following stages:

Stage 1, a volunteer lies down on the bed and 5-minute ECG is recorded at rest.

Stage 2, a volunteer undergoes the software-based “mental calculation” test while lying on the bed for 5 minutes. ECG is recorded simultaneously.

Stage 3, a volunteer lies down on the bed, puts on the VR headset, the investigator turns the program on for 5-minute exposure, ECG is recorded simultaneously.

Stage 4, a volunteer takes off the VR headset, lays on the bed for 5 minutes during which ECG is recorded, then the volunteer takes the Spielberger State-Trait Anxiety Inventory (STAI).

Thus, for each visit, 4 ECG will be registered for each volunteer: before the test, during the test, and after the test. Two assessments based on the Spielberger State-Trait Anxiety Inventory (STAI) will be carried out, as well: before the use of the virtual reality technology and after the use of the virtual reality technology.

6.2. Medicinal products allowed for use in the course of the study

Volunteers participating in the study may proceed with administration of medicinal products that are permanently used to treat concomitant diseases, having previously agreed their list with the investigator and provided they are not forbidden to use.

6.3 Medicinal products and food products prohibited for use in the course of the study

When a volunteer participates in the clinical study, it is prohibited to use the following groups of medicinal products. After Visit 2, the volunteers can take them in the event of prescription from a physician.

- medicinal product for the treatment of diseases of the nervous system (Code ATC-N);
- medicinal products for the treatment of diseases of the cardiovascular system (Code ATC-C).

In case a volunteer takes a prohibited medicinal product, he/she should be excluded from the study.

Volunteers are forbidden to consume tonics (tea, coffee, etc.) and energy drinks (Red Bull, etc.), and foods possessing a pronounced effect on the nervous and cardiovascular system. The minimal period after consumption of these foods to participation in the study is 2 hours.

6.4. Quality control and clinical monitoring

The monitoring procedures are in place to ensure that the rights of the volunteers are respected and the investigator conducts the study in accordance with the Protocol. Within the framework of this study, an on-site monitoring is planned.

During the study, a pre-scheduled visit of the monitor to the SS can take place. Its objective is to check data, monitor the correctness of obtaining volunteer consent, control over protocol guidelines and clinical study standards. During this visit, a check of the completeness of documents in the Investigator's File, verification of the availability of the signed Informed Consent Forms, verification of CRF will be performed.

Quality control will be implemented by skilled and trained personnel purposefully assigned for these tasks.

7. EFFICACY EVALUATION

7.1. Efficacy parameters

Principal and secondary parameters to be determined during the study:

Primary variable:

- Change in HF component after 5-minute exposure to the VR audio-visual sequence;

Secondary variables:

- Change in rMSSD after 5-minute exposure to the VR audio-visual sequence;
- Change in LF/HF ratio after 5-minute exposure to the VR audio-visual sequence;
- Change in SDNN after 5-minute exposure to the VR audio-visual sequence;
- Change in TP after 5-minute exposure to the VR audio-visual sequence;
- Change in HR after 5-minute exposure to the VR audio-visual sequence;
- Change in BP after 5-minute exposure to the VR audio-visual sequence;
- Change in Spielberger State-Trait Anxiety Inventory (STAI) score after 5-minute exposure to the VR audio-visual sequence.

The criterion for the prevailing efficiency for each variable is the presence of statistically significant differences between groups in favour of any of the groups with a directivity, which indicates the effectiveness of the technology. Procedures and methods required for efficacy evaluation are described in Section 4.13. The time and events schedule is shown in Section 4.12.

8. SAFETY AND TOLERABILITY EVALUATION

Detection and registration of AR/ADE

In the course of the study, beginning with the moment of putting the virtual reality headset on the volunteers, registration of AR/ADE will be carried out by carefully monitoring the health of each of them.

To this end, during each visit, the investigator asks the volunteers questions related to their well-being, conducts physical examination, evaluates vital signs (blood pressure, heart rate, and body temperature).

Diseases, signs or symptoms, results of electrocardiography that are not compliant with the normal values and were observed in the volunteer prior to the beginning of the study are not considered to be adverse reactions if they were detected during the trial (except for cases when there was a deterioration in the severity or frequency of manifestations).

Adverse reactions occurring when the virtual reality technology is applied are described in Section 2.2. of this Protocol.

In the event of any symptoms of AR/ADE, the volunteers should immediately report this to the investigator. Volunteers should report any undesirable events, regardless of whether they are related to the virtual reality technology (from the perspective of the volunteer).

The following should be classified as AR/ADE:

- exacerbation of concomitant diseases;
- an increase in the frequency or severity of previous diseases, or episodic events;
- the disease detected or diagnosed after the introduction of the virtual reality technology, even if it is possible that the disease was pre-existing prior to the volunteer's participation in the study;
- prolonged, persistent diseases or symptoms that were detected at the initial stage and the severity of which increased after the volunteer started taking part in the study;
- clinically significant disorders detected by the results of electrocardiography or other surveys after the introduction of the virtual reality technology, or those that were observed at the initial stage, and the severity of which increased against the background of the use of the virtual reality technology.

The following should not be classified as AR/ADE:

- diseases or conditions detected or diagnosed before the beginning of the use of the virtual reality technology, the severity of which has not increased;
- situations in which there is no adverse event, from a medical point of view (e.g., hospitalization due to scheduled surgery);
- any clinically significant laboratory deviations that was detected before the first application of the virtual reality technology.

All cases of AR/ADE observed by the volunteers and/or the clinician during the study, including events not directly related to the virtual reality technology, should be recorded in the appropriate section of the CRF.

Information about AR/ADE should include: date and time of beginning and ending, nature of manifestations, severity, likelihood of connection with the virtual reality technology, measures taken to eliminate AR/ADE, seriousness, resolution of the event/reaction. In addition, these data should be provided in the final Clinical Study Report.

The investigator should notify the Sponsor of the study of all AR/ADE. The Sponsor is required to provide complete information about all AR/ADE registered during the clinical study in the Clinical Study Report.

The relationship between AR/ADE and the investigational virtual reality technology

In this study, the following definitions are used in accordance with the Guidelines on medical devices. Clinical Investigations: Serious adverse event reporting. Under Directives 90/385/EEC AND 93/42/EEC

Adverse reaction (AR) is any adverse manifestation, unpredictable disease or injury, or any undesirable clinical signs (including laboratory abnormalities) in the subject, users or other individuals, regardless of their association with the investigational medical device. [20]

Adverse device effect(ADE) – side effect associated with the use of the investigational medical device. [20]

AR/ADE are classified as serious and non-serious.

A serious adverse device effect (SADE) or a serious adverse event is any adverse medical event that results in:

- a) led to a death, injury or permanent impairment to a body structure or a body function.
- b) led to a serious deterioration in health of the subject, that either resulted in:
 - a life-threatening illness or injury, or
 - a permanent impairment of a body structure or a body function, or
 - in-patient hospitalization or prolongation of existing hospitalization, or
 - in medical or surgical intervention to prevent life threatening illness
- c) led to foetal distress, foetal death or a congenital abnormality or birth defect

Planned hospitalization for pre-existing condition, or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered a serious adverse event.

Non-serious AR/ADE – all undesirable reactions that do not meet the above criteria.

ADE are also classified as unanticipated and anticipated.

Unanticipated Serious Adverse Device Effect (USADE) Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

Anticipated SADE (ASADE): an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report

Causality assessment

The relationship between the use of the medical device and the occurrence of each adverse event shall be assessed and categorized. During causality assessment activity. The presence of confounding factors, such as concomitant medication/treatment, the natural history of the underlying disease, other concurrent illness or risk factors shall also be considered. The above considerations apply also to the serious adverse events occurring in the comparison group For the purpose of harmonising reports, each SAE will be classified according to five different levels of causality. The sponsor and the investigators will

use the following definitions to assess the relationship of the serious adverse event to the investigational medical device or procedures.

Table 4. Categories and criteria for determination of the causal relationship

Degree of probability	Manifestation
Not related:	Relationship to the virtual reality technology is excluded
Unlikely	The relationship with the use of the virtual reality technology seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.
Possible	The relationship with the use of the virtual reality technology is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases were relatedness cannot be assessed or no information has been obtained should also be classified as possible.
Probable	The relationship with the use of the virtual reality technology seems relevant and/or the event cannot reasonably explained by another cause, but additional information may be obtained.
Causal relationship:	The serious event is associated with the virtual reality technology beyond reasonable doubt.

Determination of the severity of AR/ADE

The severity of adverse reactions is evaluated on the basis of the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Table 5. AR/ADE severity evaluation criteria

Degree	Evaluation criterion
1	Low-severity AR/ADE (insignificant, do not require medical intervention, asymptomatic disorders, which are determined by the results of laboratory diagnostics and have no substantial clinical significance)
2	Moderate AR/ADE (require minimal intervention from a physician)
3	Severe AR/ADE (significant symptoms requiring hospitalization or invasive intervention)
4	Life-threatening or disabling AR/ADE (complicated by acute, life-threatening conditions, require intensive care or invasive procedures)
5	AR/ADE with a fatal outcome

Categories of AR/ADE outcomes and their definition

- **The event has resolved without consequences** – there are no symptoms and the volunteer is not treated to eliminate **AR/ADE**.
- **Stabilization of condition** – the outcome of AR/ADE is classified as stabilization of condition, according to the investigator's assessment.
- **The event has resolved with consequences** – the event was reversed, but its consequences remained: as a result of AE/AR the volunteer experienced a temporary or permanent disability/inability to work. Any AE/AR that have resolved with consequences are classified as serious AE/AR.
- **The event has not resolved** – symptoms persist.
- **Death of a volunteer.**

Actions to be taken after development of AR/ADE

The investigator should keep records of measures taken and consequences for each case of AR/ADE based on the following criteria:

- no actions were performed;
- treatment prescription;

- hospitalization;
- termination of application of the virtual reality technology;
- cancellation of application of the virtual reality technology;
- resuscitation measures (in cases of development of life threatening AR/ADE).

The investigator should monitor changes in the development of clinical manifestations over time, as well as provide information to the Sponsor in accordance with the requirements of the Protocol.

In case of serious unpredicted AR/ADE, provide information to the study Sponsor and the Ethics Committee at the SS.

Measures taken in cases of development of AR/ADE

In case of AR/ADE, the investigator should take medical measures and actions aimed at their reversal. In the event of the occurrence of AR/ADE health-threatening and/or life-threatening for the volunteer, the investigator is allowed to unblind the randomization code making a telephone call to the study monitor or to another employee of the Sponsor or the CRO (tel.: +38 (095) 735-28-75). The reason for disclosure of the code should be described in detail in the CRF.

All volunteers, who had registered AR/ADE during the study period, should be monitored until their resolution or achievement of clinical stability.

Observation of the volunteers who have suffered from AR/ADE, should continue until complete recovery or determination of the causes of its occurrence. All attempts to obtain additional information should be documented.

The investigator is responsible for all the therapeutic measures and final procedures.

Reporting about AR/ADE

All cases of AR/ADE observed by the investigator or spontaneously reported by the volunteers during the study, including events not directly related to the virtual reality technology, should be recorded in the appropriate section of the CRF.

The Investigator should provide full description of AR/ADE, the degree of severity, the date and time of the beginning and end of the event, the pharmacotherapy, study outcomes, describe the measures for the elimination of AR/ADE, and their consequences. In addition, these data should be provided in the final Clinical Study Report.

The investigator should notify the Sponsor of the study of all RE/ADE with respect to the confidentiality of data: in the primary and subsequent messages, the volunteers are identified by their identification (randomization) numbers, initials and date of birth.

9. STATISTICS

9.1 Justification of the sample size

The required sample size is the minimum sufficient number of subjects to obtain clinically and statistically significant outcomes of the clinical trial. Depending on the type of clinical trial and its design, as well as the types of variables used to evaluate the treatment effect, the sample size may vary.

The sample size depends on the following parameters:

- The size of the clinically significant differences (δ);
- Limiting probability of type I error α (usually for the tests of the prevailing efficiency is equal to 0.05 or 5% (two-tail));
- Limiting probability of type II error β (usually equal to 0.2) and, respectively, the power (80 %);
- Estimated on the basis of previous studies of intrasubject variability.

This study is designed to prove the superiority of the use of VR headset A vs. the use of the VR headset B for the main variable — “Change in HFcomponent after 5-minute exposure to the audio and visual VR sequence”.

To estimate the sample size, it is assumed that the following statistical hypotheses are verified for the demonstration of superiority under conditions of the single-group design:

$$H_0: \varepsilon \leq \delta \text{ vs. } H_a: \varepsilon > \delta, \quad (1)$$

where δ — the limit of superiority — the marginal value of the clinically significant differences in which it can be assumed that the investigational device (VR headset A) is superior to the comparable device (VR headset B);

$$\varepsilon = \mu_A - \mu_B \quad (2)$$

true difference between the arithmetic mean of the results obtained using the VR headset A and the VR headset B.

According to Chow et al [18], the sample size can be estimated using the following expression:

$$n = \frac{(z_{\alpha} + z_{\beta})^2 \sigma_m^2}{2(\varepsilon - \delta)^2} \quad (3)$$

where d — the size of clinically important differences as determined by experts; a — limiting probability of type I error (significance level), which in most cases is equal to 0.05 (5%); b — limiting probability of type II error; s_m — coefficient of variation, which is evaluated based on the results of previous studies.

In this study, the size of the clinically important differences d is 0.1 (10%), a = 0.05 (two-tailed), b = 0.05 (study power of 95 %). It is assumed that the difference e will be 0.25 (25 %), and s_m will be 0.35 (35 %). The results of calculations are given in the table below:

Statistical parameter	Value
a — limiting probability of type I error	0.05
b — limiting probability of type II error	0.05
Percentage point of the standardized normal distribution for a	1.96
Percentage point of the standardized normal distribution for b	1.64
Difference in means e	0.25
The value of clinically significant differences d	0.1
Intrasubject variability s_m	0.35
Estimated sample size	36

Thus, it is necessary to enrol 36 volunteers to this study, according to the calculations. Yet, given that a large proportion of volunteers may not meet the inclusion criteria or non-inclusion criteria, and are the non-responders, at least 80 volunteers should be enrolled to the study. Yet, since it is planned to conduct the study of the virtual reality effect separately for different groups of volunteers, it is recommended that approximately 160 volunteers are included to get 100 randomized volunteers.

9.2 Statistical analysis plan

9.2.1 General principles of the statistical analysis

Statistical analysis will be conducted by a qualified statistician.

It should include:

- description of the volunteers enrolled to the study;
- number of volunteers who discontinued the study early;
- number of adverse events;
- efficacy analysis in groups;
- efficacy analysis in subgroups of volunteers;
- tolerability and safety analysis;
- statistical conclusions.

9.2.2 Description of volunteers at baseline

Give a description of the groups by age, gender, features of the diagnosis, medical history and efficacy and safety parameters.

To do this, one should use the descriptive statistics methods (for quantitative variables: n, arithmetic mean, median, standard deviation, minimum and maximum value; for categorical variables – frequency and proportion in %).

Assess 95 % confidence intervals for all parameters.

9.2.3 Efficacy analysis

Efficacy analysis based on quantitative variables

For each quantitative parameter, estimate its relative change after the exposure of the headset in comparison with the values before its exposure in % compared with the baseline using the following formula:

$$X(\%) = \frac{T_{\text{нисл}} - T_{\text{до}}}{T_{\text{до}}} \times 100\% \quad (3).$$

Give descriptive statistics values (n, arithmetic mean, median, standard deviation, minimum and maximum value) for each time point, grouping the data separately for each headset, and, also, show the difference between these indicators for each headset (“after” – “before”), and between the headsets (“VR headset A” – “VR headset B”). Present changes of the parameters over time graphically.

To analyse the efficacy of the four-factor dispersion analysis (ANOVA) based on the mixed model: the “exposure sequence” factors (levels “A – B” and “B – A”, “exposure period” (levels: “Period 1” and “Period 2” and “headset” (levels: “VR headset A” and “VR headset B”) are fixed, the “subjects” factor is random, distributed in the “exposure sequence” factor followed by the use of contrast analysis between the levels of the “headset” factor (level “VR headset B” – reference).

Check the normal distribution of the ANOVA residues using the Shapiro-Wilk test. In case the residues are not distributed normally, perform the ANOVA on ranks [19].

Efficacy analysis based on categorical variables

Calculate the descriptive statistics parameters (frequency and proportion in percentage).

Present results graphically.

To compare the proportions for the “VR headset A” and the “VR headset B” McNemar’s test should be used. In case the categorical variable has more than two categories, the marginal frequency test should be used.

9.2.4 Safety and tolerability analysis

Provide parameters of descriptive statistics used to assess the safety and tolerability of each individual headset.

Since the safety of the product will be evaluated based on the development (or lack thereof) of negative (undesired) adverse events and reactions, the parameters of descriptive statistics (frequency and rate as a percentage) should be provided. Evaluate serious AR/ADE and non-serious AR/ADE both together and separately.

9.2.5 Significance levels

The level of significance is equal to 0.01 for the Shapiro-Wilk test, and equal to 0.05 for other tests.

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

10.1. Data management

The CRF should be filled by 100 % and contain valid information. In case of availability of data, the validity of which is reasonably doubted, and it is impossible to check such data, they should be excluded from statistical processing.

All documentation related to the study, as well as information regarding the volunteers participating in it, is confidential. If necessary, the report and study documents will use only the identification numbers of the volunteers participating in the study.

10.2. CRF

Case Report Forms will be used in the study to collect information. Data from the paper CRF will be introduced by several CRO employees into an electronic data base, after which a comparison of data will be carried out to detect physical errors.

10.3. Source documentation

In this study, the CRF is considered to be the source documentation and is a confirmation of the reality of existence of a particular volunteer, and confirms the reliability of the collected/obtained data. The source documentation is filled in at the study site.

10.4. Corrections in source documentation

All entries in the source documentation (CRF) must be legible. In case of corrections, it is necessary to leave the previous values so that they are eligible. Each correction must be dated, the reasons for the correction explained, and must be verified with the signature of the person who made the correction.

10.5. Direct access to source documentation

For the purposes of ongoing inspections, the documentation for this study should be provided by the investigator and the Healthcare Facility (HCF) to the Ethics Committee at the Healthcare Facility, as well as persons authorized to carry out the audit and monitoring of the study.

11. QUALITY CONTROL AND ASSURANCE

The control over quality and completeness of data entry will be performed by the authorized representative of the CRO or the Sponsor during the monitoring visit.

A 100 % verification of signed Informed Consent Forms will be conducted. The quality control will be carried out by the qualified personnel, who will monitor the study on behalf of the CRO or the Sponsor.

The responsible investigator should provide free access to the CRF, virtual reality headsets, and study documentation (Investigator's personal file) for representatives of the Sponsor and the CRO. Also, the responsible investigator provides free access to all study documentation for monitoring and auditing.

The investigator is obligated to notify the participants of the study and to obtain their consent, according to which all personal information may be studied in the process of data verification within the framework of monitoring/audit conducted by the Sponsor, its authorized representatives, or may be subject to verification by the regulatory authorities. In this case, participation in the study and personal information will be considered as confidential, as it is established by the law, and not subject to disclosure. Audits and inspections are conducted to assess the compliance with the principles of the Good Clinical Practice, in accordance with the legislation of Ukraine in force and the Study Protocol.

Audits may be conducted by the Sponsor or a designated agent in accordance with its Standard Operating Procedures (SOPs) or according to the SOP of the Authorized Agent.

In the process of monitoring and clinical audit, the availability of the set of documents in the Investigator's File, the execution of the study in accordance with the Protocol, the filling of the CRF will be inspected. An audit or verification may include, for example, examination of all source documentation, distribution logs for virtual reality headsets, the CRF, some or all equipment used in the study.

Representatives of the Sponsor can conduct a number of visits to the SS. Prior to study initiation, monitors acting on behalf of the Sponsor may visit the SS to verify the suitability of the institution for conduction of the study, as well as to discuss responsibilities of investigator in the study. After successful procurement of all the necessary documentation (including autobiographies and supporting documents, signed agreements on conduction of clinical study, approval of the Protocol by the Ethics Committee at the HCF), the Sponsor will arrange for delivery of virtual reality headsets and other equipment to the study site.

The initial visit to the study site will be scheduled for a date agreed by both parties. The enrolment of volunteers is not allowed prior to the initiating visit to the study site, as well as before the staff of the study site obtains a written approval for the beginning of enrolment of volunteers. During the initiating visit to the study site, all employees who are likely to participate in the study should receive introductory information including, but not limited to (the list is not exhaustive), a review of the study protocol and the processes for obtaining informed consent, the general responsibilities of the study site staff, the questions for testing. Information about this visit will be documented in the report.

Throughout the study, the Sponsor will contact the study site staff on a regular basis. Study monitors will periodically visit the study site during the study to perform routine monitoring visits. The monitor will carry out audits to assess the accuracy, completeness, and relevance of the clinical and source documentation. The monitor will also check the proper protection of the rights of the study subjects, as well as provide an assessment of the compliance of the study procedures with the requirements of the Protocol, the requirements of the Good Clinical Practice, and all regulations.

As part of the data verification, it is assumed that all source documentation will be provided for verification to the Sponsor or its representatives/monitors.

In accordance with the current standard operating procedures (Sponsor's or its representative's) at the SS, the site closing visit will be conducted, during which the Sponsor or its representative will analyse all Informed Consent Forms, the CRF, and other documentation.

The Sponsor or its representative will meet the investigator or the study site staff during the closing visit to explain the documentation retention procedures, publication policies, the possibility of audits at

the site, and the disclosure of financial information. The investigator is obliged to inform the Ethics Committee at the HCF of the completion of the study.

The investigator must keep all documentation pertaining to this study in the archives of the institution for 15 years. Documentation is submitted to the archive once the study is over and the site is closed. The fact of the submission of documents to the archive by the Investigator should be documented by an appropriate act submitted to the Sponsor or the CRO.

12. REGULATORY, ETHICAL AND ADMINISTRATIVE RULES

12.1. Ethical principles

This study is conducted in accordance with the principles proclaimed at the 18th World Medical Association (Helsinki, 1964), last edited in 2013, the Guideline for Good Clinical Practice (GCP), as well as the legislative acts of Ukraine.

A copy of the letter of the Ethics Committee at the HCF with the approval of the Protocol should be given to the Sponsor to obtain consent to participation in this study from any volunteers.

This study will start only upon approval of the Clinical Study Protocol by the Ethics Committee at the HCF, where the study will take place.

In the course of the study, the Investigator should submit timely reports on the progress of the study to the Ethics Committee at the HCF. Copies of all communications and correspondence with the Ethics Committee at the HCF should be provided to the Sponsor. In addition, upon termination or early termination of the study, the investigator should submit a final report to the Ethics Committee at the HCF. A copy of the report will be provided to the Sponsor.

12.2. Protection of volunteer's personal information

All documents and information related to this study, which will be provided by the investigator to the Sponsor, are strictly confidential. The investigator and other participants in the study will be obliged to use this information solely within the framework of this study to implement the Protocol.

The investigator must fill in and keep the Record of Enrolled/Screened/Randomized Volunteers, as well as other accounting records.

The investigator and the Sponsor should ensure protection of the personal data of the volunteers involved in the study. The required personal data of the study subjects (for example, social and demographic parameters) will be collected solely for the purpose of achievement of the study objectives and to a minimum extent.

No documentation identifying volunteers will be disclosed. Names of volunteers will not be disclosed to the third parties.

Before enrolment to the study, the volunteers will be introduced to the confidentiality provisions and use of their personal data, including the need for access by the monitor and other authorized persons (in cases of audits, inspections, etc.). These conditions will be reflected in the information for the volunteers.

12.3. Amendments to the Protocol

Any changes to the study plan should be agreed with the Sponsor, the State Expert Centre of the Ministry of Health of Ukraine and the Ethics Committee at the Healthcare Facility where the study will be conducted. Additions or amendments should be provided in writing, signed by all parties, dated and attached to the Protocol.

12.4. Agreement to the terms and conditions of the Protocol

The investigator conducting this study should not be entitled to deviate from the requirements of the Protocol and must confirm the agreement to work in accordance with this Protocol in writing. The contents of the Protocol cannot be disclosed to other individuals or entities without the written consent of the Sponsor.

12.5. Laws and regulations

The study will be conducted in compliance with the:

- 1) ICH Good Clinical Practice E6 (R2)
- 2) WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects

13. DATA PROCESSING AND RECORD MAINTENANCE

13.1. Investigator File

The Sponsor of the study or its authorized representative is required to provide the Investigators with the documentation required for the clinical trial.

13.2. Data processing

The data that will be obtained by the investigator during the study will be recorded and kept in the CRF. After the verification procedures (monitoring and remote monitoring), the data will be processed for the statistical report.

13.3. Record maintenance and data protection

The investigator should ensure that the study documentation is retained until completion of the study. According to the contractual obligations, the investigator should keep the study documentation for at least 15 years.

Handling personal data of the volunteer and personal data of the investigator, which may be kept in the Sponsor's database, will be carried out in accordance with the Law of Ukraine On Personal Data Protection, as amended. When storing or processing personal data relating to the investigator and/or a volunteer, the Sponsor undertakes to take all necessary measures and precautions to ensure that the data is retained, and to prevent third parties from accessing them.

13.4. Amendments to the Protocol

The investigator should confirm his/her consent to work in accordance with the Protocol in writing. The investigator has no right to deviate from its requirements. During the study, corrections and additions that are considered as amendments can be made.

Amendments to the Protocol can be made after their approval by the local Ethics Committee at the SS. The history of all amendments to the Protocol should be kept on record.

14. FUNDING

Funding of activities related to the implementation of the provisions of this Protocol will be carried out by the Sponsor of the clinical trial on the basis of the concluded agreements between the Sponsor and the HCF, as well as between the Sponsor and the investigators.

15. PUBLICATION POLICY

The ownership of the data, outcomes, reports, findings, discoveries relating to this study belongs to the Sponsor. Therefore, the Sponsor has the right to use the data obtained during this study for any purpose, including filing for examination to the competent authorities in any other country.

The Sponsor's policy is aimed at promotion of presentation and/or publication of the findings of own studies using validated data, which will guarantee the reliability of the findings. The Sponsor approves the release of publications and may delay publication or communication for a limited period of time to protect the privacy or ownership of the information contained therein.

The ownership of the data, outcomes, reports, findings, discoveries relating to this study belongs to the Sponsor. Therefore, the Sponsor has the right to use the data obtained during this study for any purpose, including filing for examination to the competent authorities in any other country.

16. CONFIDENTIALITY OF INFORMATION

All materials, information (oral or written), and unpublished documents provided to the investigator (or any actions taken by the Company on its own behalf), in particular, this Protocol and the ICF, are the exclusive property of the Sponsor. The Investigator should treat all information provided to him/her or obtained during the investigation as confidential, and take all necessary measures to ensure that its confidentiality is preserved, except for the information to be disclosed in accordance with the legislation in force.

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18. ATTACHMENTS

Attachment No. 1. Spielberger State-Trait Anxiety Inventory (STAI)

Attachment No. 2. Numeric character sets for mental calculation

Attachment No. 1. Spielberger State-Trait Anxiety Inventory (STAI)

Spielberger State-Trait Anxiety Inventory (STAI)

Examination of the level of situational anxiety

Volunteer's ID: _____

Test time: _____:_____, _____/_____/_____
hours minutes day month year

Instructions: read each of the following sentences/statements carefully and cross out the number in the corresponding box on the right, depending on the way you feel at this time. Do not reflect on the questions for long, because there are no right or wrong answers.

No.	Statement	Never	Sometimes	Frequently	Almost always
1	Calm down	1	2	3	4
2	Safe	1	2	3	4
3	Tense	1	2	3	4
4	Annoyed	1	2	3	4
5	Comfortable	1	2	3	4
6	Upset	1	2	3	4
7	Concerned with future misfortunes	1	2	3	4
8	Relaxed	1	2	3	4
9	Anguished	1	2	3	4
10	At ease	1	2	3	4
11	Self-confidence	1	2	3	4
12	Nervous	1	2	3	4
13	Restless	1	2	3	4
14	Downhearted	1	2	3	4
15	Rested	1	2	3	4
16	Satisfied	1	2	3	4
17	Concerned	1	2	3	4
18	Stunned	1	2	3	4
19	Happy	1	2	3	4
20	I felt good	1	2	3	4

Volunteer's ID: _____

Processing of results

Calculate the total score based on answers given by volunteers.

While interpreting the results, use the following levels of anxiety:

- up to 30 points — low,
- 31 – 44 points — moderate;
- 45 and more — high.

Total score: _____

Key

Question No.	Answers			
	Never	Sometimes	Frequently	Almost always
1	4 points	1 point	3 points	2 points
2	4 points	1 point	3 points	2 points
3	1 points	4 points	2 points	3 points
4	1 point	4 points	2 points	3 points
5	4 points	1 point	3 points	2 points
6	1 point	4 points	2 points	3 points
7	1 point	4 points	2 points	3 points
8	4 points	1 point	3 points	2 points
9	1 point	4 points	2 points	3 points
10	4 points	1 point	3 points	2 points
11	4 points	1 point	3 points	2 points
12	1 point	4 points	2 points	3 points
13	1 point	4 points	2 points	3 points
14	1 point	4 points	2 points	3 points
15	4 points	1 point	3 points	2 points
16	4 points	1 point	3 points	2 points
17	1 point	4 points	2 points	3 points
18	1 point	4 points	2 points	3 points
19	4 points	1 point	3 points	2 points
20	4 points	1 point	3 points	2 points

Attachment No. 2. Numeric character sets for mental calculation

Volunteer's ID: _____

1)	854	+	746	=	1600	=7	
2)	191	+	733	=	924	=14	=5
3)	793	+	966	=	1759	=23	=5
4)	555	+	419	=	974	=20	=2
5)	271	+	955	=	1226	=11	=2
6)	591	+	862	=	1453	=13	=4
7)	688	+	531	=	1219	=13	=4
8)	358	+	696	=	1054	=10	=1
9)	422	+	685	=	1107	=9	
10)	772	+	969	=	1741	=13	=4
11)	493	+	650	=	1143	=9	
12)	128	+	822	=	950	=14	=5
13)	752	+	383	=	1135	=10	=1
14)	764	+	550	=	1314	=9	
15)	408	+	807	=	1215	=9	
16)	194	+	467	=	661	=13	=4
17)	299	+	959	=	1258	=16	=7
18)	502	+	325	=	827	=17	=8
19)	302	+	552	=	854	=17	=8
20)	562	+	891	=	1453	=13	=4
21)	339	+	930	=	1269	=18	=9
22)	826	+	397	=	1223	=8	
23)	196	+	427	=	623	=11	=2
24)	576	+	252	=	828	=18	=9
25)	120	+	788	=	908	=17	=8
26)	323	+	735	=	1058	=14	=5
27)	759	+	132	=	891	=18	=9
28)	839	+	723	=	1562	=14	=5
29)	493	+	685	=	1178	=17	=8
30)	520	+	685	=	1205	=8	
31)	220	+	560	=	780	=15	=6
32)	222	+	921	=	1143	=9	
33)	457	+	734	=	1191	=12	=3
34)	549	+	528	=	1077	=15	=6
35)	392	+	838	=	1230	=6	
36)	886	+	560	=	1446	=15	=6
37)	294	+	460	=	754	=16	=7
38)	805	+	382	=	1187	=17	=8
39)	413	+	895	=	1308	=12	=2
40)	151	+	461	=	612	=9	
41)	768	+	861	=	1629	=18	=9
42)	516	+	272	=	788	=23	=5
43)	172	+	373	=	545	=14	=5
45)	371	+	103	=	474	=15	=6
46)	220	+	931	=	1151	=8	
47)	118	+	219	=	337	=13	=4
48)	371	+	993	=	1364	=16	=7
49)	596	+	620	=	1216	=10	=1
50)	329	+	486	=	815	=14	=5

State the number of successful calculations: _____

Comment:
