

GUIDELINES FOR STANDARDIZATION OF BED REST STUDIES IN THE SPACEFLIGHT CONTEXT



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International Academy of Astronautics



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Guidelines for Standardization of Bed Rest Studies in the Spaceflight Context

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1. Introduction

Bed rest studies, in which healthy volunteers are confined to bed in a 6° head-down tilt position, are a well-established model for some of the adaptations experienced by astronauts during spaceflight. They are therefore a very valuable tool both for investigating possible mechanisms and for testing measures to counter these adaptations. Further, the results obtained in these studies have obvious relevance and applications in terrestrial clinical contexts, which make them even more useful.

Many space agencies (and in some cases even individual investigator teams) around the world are involved in organizing bed rest studies. However the conditions in which these studies are performed are quite diverse. Differences lie, for example, in the duration of studies, angle (6° tilt or horizontal), sunlight exposure, sleep/wake cycles, nutritional standards and control. This complicates drawing overall conclusions and comparing results on countermeasure effectiveness between different studies.

In order to achieve better standardization of bed rest studies in the spaceflight context, an International Academy of Astronauts (IAA) study group was initiated, including members from most of the entities that are actively pursuing this type of activity. The following document represents the outcome of the work of this study group.

Standardization can be differentiated to two different aspects: standardization of the conditions of bed rest studies and standardization of a core set of measurements to ensure that a minimum of outcome data are available from every study. Consequently this report contains description of these two aspects.

While bed rest conditions were elaborated within the study group, for the standardized measurements valuable input was collected from discipline specific experts before refinement by the study group. The following table (Table 1-1) lists these contributors who deserve our gratitude.

Table 1-1: Discipline Experts

Discipline	ESA	IBMP	NASA
Bone	Joern Rittweger	Victor Oganov	Jean Sibonga
Cardiovascular	Richard Hughson	Olga Vinogradova	Steve Platts
Hematology	Alexander Chouker	Marina Rykova	Scott Smith
Immunology	Alexander Chouker	Marina Rykova	Brian Crucian
Muscle	Joern Rittweger	Boris Shenkman	Lori Ploutz-Snyder
Nutrition	Petra Frings-Meuthens	Irina Larina	Scott Smith
Psychology	Elisabeth Rosnet	Vadim Gushin	Walt Sipes, Kim Seaton, Camille Shea
Sensorimotor	Gilles Clement	Inessa Kozlovskaya	Jacob Bloomberg, Millard Reschke, Scott Wood

Following the guidelines laid out in this document should ensure high quality and basic comparability between the studies performed in the future. This will make the collective investments even more efficient and valuable, enable still better scientific progress, and also fulfill the ethical obligation towards our volunteers to maximize the benefit derived from their efforts.

2. Study management and project communication

2.1. Study Management

The detailed study management setup may differ considerably for a given bed rest study depending on complexity, national/local legislature, organizational frame, and so on. However the following key roles for a study management team are typically present:

- Project Scientist: Person in charge of all scientific aspects of the study and the representative of all investigators in the study.
- Project Manager: Person in charge of the organization and management of the study.
- Head Medical Doctor: Independent medical doctor with no scientific interest in the study, representing the medical interest of the test subjects.

Depending on the dimensions of a study, the tasks of the Project Manager and the Project Scientist could be conducted by one person. However it is strongly recommended that the function of the Head Medical Doctor should always be a separate person.

The management team shall be available as much as possible during a study to ensure consistency in the performance of the study. However, for each position a backup person should be declared. The backup positions shall be well communicated between the management team and all staff working in the study. To ensure availability a list with all the phone numbers (private and office) of the management team and the medical doctors should be posted at a central position in the study facility. All staff shall be familiar with the phone list and they are asked to call a member of the management team for important questions rather than making arbitrary decisions.

2.2. Project Communication

Implementing a well-organized communication plan is the key factor in successfully organizing a study. There are different levels of communication that shall be considered. Internal communication includes the management team and all other staff at the study site. External communication includes the Principal Investigator (PI) teams and possible cooperating facilities and laboratories. The following paragraphs will give an overview about how to ensure proper communication between all scientists and staff that are involved in a study.

Generally, it is recommended to post the study protocol electronically, for instance on a webpage with access codes for all involved staff and scientists in a study.

2.2.1. Internal Communication

2.2.1.1. General Internal Communication

With kick off of a study the management team should perform regular meetings to exchange the status of the ongoing processes such as the progress of subject and staff recruitment, progress of study preparation, implementation of PI experiments, and so on. In case the members of the management team are not located at the same site, telephone conferences may be used. However, face-to-face meetings are the preferred method for communicating. Progress in study planning shall be documented on a regular basis.

One week before the start of a study campaign a general meeting should be organized by the Project Manager where all staff working in the study should participate to enhance communication during a study campaign. This enables the team members to get to know each other and learn about the responsibilities of each staff member.

2.2.1.2. Internal Communication during a Study

During a study internal communication is more complicated as people work in different shifts or different locations. Therefore communication tools should be established that ensure optimal work flow independently from personal reachability of the staff members.

Recommended tools are for instance the 'handover book' or electronic case report forms (e-CRF). The Head Medical Doctor and his staff should document their daily ward round separately.

All staff is obliged to read the documentation when they start their new shift. Thus it is possible to follow the internal communication from the last shift they worked until the present.

Regular meetings are recommended during the actual performance of a study to report the progress of the study and to exchange problems or news.

2.2.1.3. Documentation and Tracking of Adverse or Unexpected Events

To document that subject rights, safety, and well-being are protected and to facilitate and support the interpretation of data from each campaign, all adverse and unexpected events shall be documented.

2.2.2. External Communication – Investigators

2.2.2.1. Prior to the Study

2.2.2.1.1. Experiment Data Sheet (EDS)

The experiment data sheet contains relevant information about the PI experiments including their specific needs. A draft version of the EDS should be sent to the Project Scientist of the specific study at least 6 weeks prior to the first investigators working group meeting.

2.2.2.1.2. First Investigator Information Package

The investigator information package is created by the Project Scientist for the rest of the investigator team. It is the compilation of relevant information covering all aspects of the study necessary for the joint creation of experiment proposals and research protocols by the whole investigator team. The purpose of the investigator information package is to ensure investigator team members' understanding of the rationale for and compliance with the key features of the specific bed rest campaign. The investigator information package will include

- General background of the study
- Organization of the study (key functions)
- Equipment and hardware
- Data rights, access and publication
- Investigator rules:

- ◇ Obligations (such as dry runs)
- ◇ Responsibilities of Agencies and PIs
- ◇ Confidentiality

- Preliminary study protocol
- Capabilities available at the bed rest facility

All recipients shall treat the investigator information package as a confidential document.

2.2.2.1.3. Second Investigator Information Package

The second investigator information package includes the final versions of the EDS, the data sharing agreement, and the study protocol.

2.2.2.1.4. Investigator Working Group (IWG) Meetings

Approximately 6 weeks after receiving the preliminary EDS, the study management team shall organize the first Investigator Working Group (IWG) meeting. The meeting should take place at the bed rest facility where the study will be conducted. This will give the PIs and the study management team the opportunity to get to know the other PI groups and to get more insight about the different experiments and the facility. It is mandatory to schedule the meeting before the preparation of the ethics file. During the meeting the PIs will be provided with the first investigator information package.

Such a meeting also simplifies the discussion about the study protocol that shall be part of the meeting.

Mandatory topics shall be:

- Introduction and signed consent of the investigator rules by the PI's before any other action in the scope of the meeting and the study
- Introduction of the different investigator groups and their experiments
- Discussion about possible interferences between individual experiments
- Introduction of the first draft of the general plan by the Project Scientist
- Discussion of the general plan
- Introduction and discussion of a data sharing plan
- Harmonization and cooperation between different experiments

Besides the personal communication between each investigator and the management team during the planning of the study there should also be a monthly email distributed about the progress of the study planning.

A final IWG meeting will be scheduled at least 2 weeks prior to the start of the first study campaign. Mandatory topics shall be:

- Introduction of the final version of the EDS
- Introduction of the final version of the study protocol
- Introduction and signed consent on the final data sharing agreement

The two described IWGs are the minimum amount of meetings. If necessary, additional meetings can be scheduled in between.

2.2.2.2. During the Study

When the investigator groups arrive to perform their experiment, a mandatory face to face meeting between the Project Scientist and each respective investigator group should be scheduled the day before they actually perform their experiment in the running study. This will vary for the different PIs as it depends on the study day when their first experiment is scheduled. In this meeting the respective investigator group shall be informed about special events during the study so far. They will be instructed on the processes related to the study and also be introduced to the staff and the subjects on site of the study. A mandatory topic of the meeting shall also be to check whether the management team on site and the investigator groups have fulfilled their obligations.

2.2.2.3. After the Study

It is the responsibility of the study management team to schedule a debriefing for all of the PIs 6 months after the end of the laboratory phase of the study. In this meeting the PIs should present preliminary results of their gained data in the specific study. It should give everyone the opportunity to discuss the results among all the researchers involved and to maybe discover any parallel findings in the results.

2.2.3. Communication – Press / Media

All communication with media outlets such as newspapers, television, and radio stations should be coordinated by the Project Scientist possibly in close coordination with other involved parties. For the process of subject recruitment, the Project Manager is in charge to get in contact with different media outlets. However, only the Project Scientist shall communicate with the media about study-related issues or he/she shall decide who would also be appropriate to give interviews. No other team members should act on their own authority regarding this topic. Interview requests regarding the specific experiments should be transferred to the respective PIs by the Project Scientist.

To avoid any interference with any experiment session, all media contact especially in short and medium-term bed rest studies should be postponed until after the entire study campaign.

No media will be allowed to get in touch with the subjects without prior notice and written consent obtained from the subjects.

To avoid direct contact with media, it is suggested to request the respective public relations department of the bed rest facility or the agency to delegate the documentation of the study to a professional cameraman and photographer. The graphic material and film footage can then be distributed to the interested media by the public relations office without disturbing the accomplishment of the study.

A press conference might be scheduled by the Project Scientist at each local site after completion of the study campaign. In case any media agency would like to broadcast news from the study, a B-roll (pre-prepared film of the experiments) from experiment performance will be recorded some time in advance at each experiment site. This B-roll can be provided to the media during the study.

3. Development of the integrated study protocol

3.1. General

Development of the study protocol consists of two plans: a general plan and a daily schedule plan. During the planning phase of the study, it is the responsibility of the Project Scientist to design the study protocol in close collaboration with the Project Manager, individual PIs, and Head Medical Doctor. The following information about the study shall be provided by the PIs to the Project Scientist to facilitate integration of the studies. This information may be provided in the form of an individual protocol or other site specific documentation.

- Study description
- Specific aims and hypotheses
- Methodology to include:
 - ✧ Experimental design
 - ✧ Number of subjects
 - ✧ Inclusion/exclusion criteria
 - ✧ Screening requirements
 - ✧ Test descriptions and requirements
 - ✧ Study location and location of individual testing sessions (Will subjects leave the bed rest facility for testing?)
 - ✧ Study duration including timing of pre-, in and post bed rest phases
 - ✧ Study schedule (optimal time for each testing session, last day of the adaptation, first day of the recovery, and optimal point of time during one study day)
 - ✧ Experimental constraints (for example, specialized diet, fasting, special food, body position during the experiment)
 - ✧ Resources needed (nursing support, data/specimen collection)
- Study budget

Dry runs or readiness reviews of study procedures are needed to understand experimental procedures and operation prior to the beginning of the study.

Length of bed rest will be categorized into 3 different durations with associated pre- and post-bed rest phases. The table (Table 3-1) below describes these categories.

Table 3-1: Categories for Bed Rest Study Duration

Category	Pre-Bed Rest Baseline Data Collection (BDC)	Head-Down Tilt Bed Rest (HDT)	Post-Bed Rest Recovery (R)
Short-Duration Bed Rest	5 to 7 days	5 to 14 days	3 to 6 days
Medium-Duration Bed Rest	7 to 14 days	15 to 59 days	7 to 14 days
Long-Duration Bed Rest	14 days (or more)	60 days (or more)	14 days (or more)

The suggested naming convention used above indicates pre-bed rest days as baseline data collection (BDC) beginning at BDC-X and ending on BDC-1. In-phase bed rest days begin on HDT1 and continue to HDTX. Post-bed rest days for recovery (R) begin on R+0 and subjects are released on R+X.

3.2. General Plan

To develop daily schedules for study operations, a general plan for the entire study will be drafted. This general plan shall incorporate to the greatest degree possible study requirements of all participating PIs. Information about each individual experiment as collected from the PIs (described above) will be used to create this draft study schedule. The draft study schedule will be distributed to all PIs in an effort to identify possible interferences between their experiment and others. This may be an iterative process. An IWG may be necessary to optimize the final accepted plan. In addition to the general plan, development of a corresponding document that describes the amount of blood drawn from subjects for each test that requires blood samples. This blood volumes document will help to ensure compliance with any site-specific volume requirements for removal of blood from subjects. The general plan and blood volumes document is usually submitted with the proposal to the ethics committee at the respective study site. An example of a general plan is provided below.

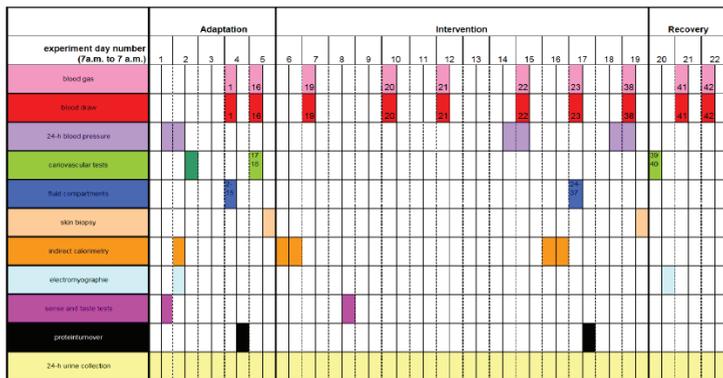


Figure 3-1: Example of a general plan.

Once the general plan is finalized, a decision is made regarding the timing with which subjects will enter the study. In other words, subjects may enter all at once to fill all available beds on the same day or their admission will be staggered. This decision is based upon factors such as the availability of subjects, beds, equipment, and study personnel. For example, the first two subjects may start 1 day earlier than subjects three and four, and so on (1-day subject shift). Other combinations or time shifts are possible to suit the needs of the study.

3.3. Daily Schedule

Using the general plan, a detailed daily schedule is created for each test subject for every day that the subject participates in the study. The daily schedule documents all testing and activities (such as, showering and meals) that are planned for each individual subject.

Depending on the study design and the experiments involved, a significant amount of information is required to create the daily schedule. From the perspective of study operations, the following points shall be considered while designing the daily schedule:

Time necessary for transportation to experiment sessions. For experiments done outside of the bed rest facility, time spent in transportation with taxis or patient transport ambulances should be considered.

Time allotted for appointments for physiotherapy or other routine activities.

Time needed for personal hygiene (showering) of the test subjects

Time necessary for setting up and calibrating specific experiment hardware

An example of a daily schedule is provided in Figure 3-2.

Study day	day#	subject A	subject B	subject C	subject D	subject E	time (a.m.)
		0.00-01.00 a.m.					0.00-01.00 a.m.
		01.00-02.00 a.m.					01.00-02.00 a.m.
		02.00-03.00 a.m.					02.00-03.00 a.m.
		03.00-04.00 a.m.					03.00-04.00 a.m.
		04.00-05.00 a.m.					04.00-05.00 a.m.
		05.00-06.00 a.m.					05.00-06.00 a.m.
		06.00-07.00 a.m.					06.00-07.00 a.m.
		07.00-08.00 a.m.					07.00-08.00 a.m.
		08.00-09.00 a.m.					08.00-09.00 a.m.
		09.00-10.00 a.m.					09.00-10.00 a.m.
		10.00-11.00 a.m.					10.00-11.00 a.m.
		11.00-12.00 p.m.					11.00-12.00 p.m.
		12.00-01.00 p.m.					12.00-01.00 p.m.
		01.00-02.00 p.m.					01.00-02.00 p.m.
		02.00-03.00 p.m.					02.00-03.00 p.m.
		03.00-04.00 p.m.					03.00-04.00 p.m.
		04.00-05.00 p.m.					04.00-05.00 p.m.
		05.00-06.00 p.m.					05.00-06.00 p.m.
		06.00-07.00 p.m.					06.00-07.00 p.m.
		07.00-08.00 p.m.					07.00-08.00 p.m.
		08.00-09.00 p.m.					08.00-09.00 p.m.
		09.00-10.00 p.m.					09.00-10.00 p.m.
		10.00-11.00 p.m.					10.00-11.00 p.m.
		11.00-12.00 a.m.					11.00-12.00 a.m.

Figure 3-2: Daily schedule example.

All study personnel should have access to copies of the general plan and the daily schedules. Each site should have processes in place to address changes to the general plan, daily schedule, and study protocol. For example, changes to these documents can only be made by the Project Scientist with concurrence of the Project Manager. Once changes are made, updated documents are distributed to all PIs and study personnel.

3.4. Ethics Approval Application

Studies shall be conducted in accordance with the Declaration of Helsinki (Version of Seoul 2008; <http://www.wma.net/en/30publications/10policies/b3/17c.pdf>) and its subsequent amendments. Subjects shall voluntarily confirm their willingness to participate in the study by providing a fully informed consent. Documentation by a written and dated consent form with signatures of the subject and responsible study personnel shall be obtained. A subject may not participate without signing the study consent form.

Ethics approval of the study is mandatory before starting subject recruitment. The Project Scientist is responsible for preparing the documentation and ensuring compliance with all necessary regulations. After development of the general plan, the Project Scientist in cooperation with the PIs prepares the ethics documentation for review by the appropriate ethics committee. Documentation is prepared or translated to the language preferred by the ethics committee. While each site-specific ethics committee will have their own unique requirements, the following is a list of typical requirements included in documentation submitted to ethics committees.

3.4.1. Scientific Background

This section contains items such as a discussion of related scientific literature, hypotheses, specific aims and objectives of the study.

3.4.2. Procedures

This section provides information on study design, subject selection, and sample size. Full descriptions of screening procedures and experimental measures along with appropriate analyses are also included. The general plan is typically included to provide information on the study schedule.

3.4.3. Sample Collection

This section covers requirements for collection of biological samples such as blood, urine, saliva, and feces. Blood volume requirements unique to the site-specific ethics committee shall be observed. The blood volumes document is typically included.

3.4.4. Risks and Benefits

This section describes potential risks and benefits to the subject and to society. Discussion of risk mitigation is also included. All potential hazards shall be described in detail. The Investigator Team is responsible for providing all necessary information related to their specific experiments, and the Project Scientists are responsible for describing the potential hazards of participation in bed rest studies.

3.4.5. Informed Consent

The subject shall voluntarily confirm his or her willingness to participate in the study by providing a fully informed consent. The Project Scientists or their authorized representatives are responsible for providing general and site-specific detailed information to the test subjects in layman's terms. The oral and written information shall use non-technical, understandable language. During the consent process subjects will be provided with a detailed description of

the study, risks and benefits of participation, study schedule, participating researchers and all other items required by the site-specific ethics committee. The following basic elements of informed consent shall be provided to each subject and included in the written consent form.

Statement that explains that the study involves research. An explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental.

Description of foreseeable risks or discomfort to the subjects is explained.

Description of any benefits to the subject or to others which may reasonably be expected from the research or a statement that the research is of no benefit to the subject.

Disclosure of the appropriate alternate procedures or courses of action or treatment that could be advantageous.

Statement to the effect that all identifiable subject records shall be confidential.

Explanation as to whether any compensation and medical assistance are available if injury or illness occurs and, if so, any relevant details relating to these items.

Identification of contacts for answers to pertinent questions concerning specifics of the research and the research subject's rights. Contact information in the event of a research related injury or illness to the subject also should be identified.

Statement that participation is voluntary and that the subjects have a right to participate and to discontinue participation in the research at any time and that they may do so without penalty or loss of benefits to which they would otherwise be entitled. If the subject, in fact, cannot withdraw at any given time, the circumstances shall be explained to the subject in writing as part of the informed consent document.

Statement that the particular treatment or procedure may involve risks to the subject which are currently unforeseeable.

Statement describing the anticipated circumstances under which the Investigator Team, without regard to the subject's consent, may terminate the subject's participation.

Statement that the subject shall be informed of significant new findings developed during the course of the research, including adverse reactions of other subjects participating in this research that may affect the subject's willingness to continue participation.

Statement identifying the approximate number of subjects in the study.

Statement of any collective impact of the multiple protocols, if applicable.

Statement regarding compensation for time and/or study related expenses.

Any appreciable changes to the study protocol shall be reported to the ethics committee. It is the responsibility of the Project Scientist to communicate changes to the ethics committee and to the PIs and study personnel.

4. Volunteer selection

4.1. Process

Subjects shall be assured that their rights, safety, and well-being are protected and consistent with the ethical principles of the latest version of the Declaration of Helsinki (current latest version: Seoul 2008 <http://www.wma.net/en/30publications/10policies/b3/17c.pdf>) and its subsequent amendments (see Section 3.4 – Ethics Approval Application). Therefore all subjects recruited shall be volunteers and can withdraw their consent at any time without any questions or punishment. Consistency with the principles of the Declaration of Helsinki should be mandatory for the entire test-subject recruitment process.

In addition to the Declaration of Helsinki the test-subject selection process needs to include:

- Experiment specific screening processes
- Detailed information for potential test subjects about the study schedule and potential risks of the experiments
- Medical screening
- Psychological screening

4.1.1. Test Subject Recruitment

After receiving approval of the relevant ethics committee, test-subject recruitment will start at the site of the bed rest facility where the study will be conducted. Depending on the aim of the study, both female and male test subjects can be recruited. It should be confirmed however, that the number of subjects in any group, male or female, is big enough for statistical analyses (statistical power) should the issue of gender be an aim of the study. When female subjects are included, the impact of menstrual cycle timing on the experimental design should be considered. This is of particular importance when bone metabolism or training countermeasures are part of a study. Synchronization of the menstruation cycle could be one potential measure that may be considered. Unless a compelling scientific rationale exists, composition of the subject sample should include subjects from a variety of ethnic and racial backgrounds. The Project Scientist and PIs will decide jointly on the gender, racial, and ethnic distribution for a particular study campaign based on the specific campaign science requirements.

4.1.2. Recruitment Methods

The Project Manager or a staff member under his/her supervision is responsible for the organization of the recruitment process. This team member organizes the contact to media outlets and is also the contact person for the potential test subjects. Recruitment of test subjects will include methods, such as use of test subject archives, announcements in print and electronic media in local and nationwide newspapers, radio and television, and the internet, to solicit as many interested volunteers as possible. The basic study information included in advertising materials comprises: title and purpose of the study, eligibility criteria, location, study duration, and contacts for further information. Candidates will be contacted only after they have shown interest in participating in the studies.

Interested subjects that respond to advertisements will receive initial screening via telephone, website questionnaire, or other similar communication. Screening should include questions about their personal data such as contact information, date of birth, weight, and height. Even more importantly it should contain questions that are tailored to the subject and study requirements according to the inclusion and exclusion criteria. Based on information gained from this screening, a first decision can be made whether the interested person would be a potential candidate. The questionnaire should be designed so that interested people who pass that screening would fit the basic subject requirements. Once subjects pass this part of the screening process, they will be eligible to proceed with the medical and psychological screening.

4.1.3. Information Provided to Test Subjects Prior to Screening Procedures

Potential test subjects will be provided with appropriate information about all aspects that are relevant to the subject's decision to participate in the screening procedures, including a description of all potential hazards. In addition to this information, the Project Scientists or their authorized representatives, will jointly develop material for the test subjects describing issues that might interest them regarding study participation. An involved qualified physician shall provide information for subjects regarding medical issues. Once qualified, the subject will be consented and enrolled in the study. A written and dated consent form with the signatures of the subjects and responsible study personnel will document the process of providing this information to the subjects.

4.2. Inclusion and Exclusion Criteria

In general only healthy test subjects should be recruited. The age range as well as gender shall be decided by the agency/sponsor in sufficient time. All inclusion and exclusion criteria should at least be named 10 months prior to the respective study by the agency / sponsor to ensure an adequate timeframe for subject recruitment.

The following inclusion and exclusion criteria only reflect the general requirements. More specific inclusion and exclusion criteria shall be discussed and appointed prior to each study specifically in dependence of the respective intervention and experiments.

4.2.1. Inclusion Criteria

- Physically and mentally healthy subjects (Successfully pass the psychological and medical screening)
- General age range 20 to 55 years, smaller ranges should be defined prior to each study
- Body mass index 20 to 30 kg/m²
- 158 to 190 cm (62 to 75 in.)
- Willing to be assigned randomly either to the treatment or the control group
- Signed informed consent

4.2.2. Exclusion Criteria

- Condition and/or history of: thyroid dysfunction, diabetes, considerable allergies, hypocalcaemia, uric acidemia, lipidaemia or hyperhomocystinaemia, hypertension, orthostatic intolerance, other cardiovascular diseases, vestibular disorders,

- considerable musculoskeletal issues, chronic back pain, head trauma, seizures, ulcers, renal stones, gastro-esophageal reflux disease or renal function disorder, Hiatus hernia, migraines, claustrophobia, mental illness
- Electrocardiogram abnormalities
 - Tuberculosis, hepatitis, HIV
 - Ferritin range outside 10 to 154 ng/ml (F); 20 to 245 ng/ml (M)
 - Family history of thrombosis or positive response in thrombosis screening
 - Bone mineral density (measured by DEXA) more than 2.0 standard deviation \leq t-score
 - Females: Using hormonal contraceptives (less than 6 months before), use of intrauterine device, Pregnancy, 1 year after pregnancy, breast feeding, abortion, menopause
 - Medication required that may interfere with the interpretation of the results
 - Recent sub-standard nutritional status
 - Special dietary requests (e.g. vegetarian, vegan or some other diet)
 - Use of metallic implants, osteosynthesis material
 - Blood donors in the past 3 months before the onset of the experiment
 - Smoker within 6 months prior to the start of the study
 - Abuse of drugs, medicine or alcohol
 - Participation in another study within the site-specific legally required time period 2 months before study onset
 - Cannot clear a criminal background check
 - No signed consent form before the onset of the experiment
 - Incarcerated persons

It is the decision of the responsible Project Scientist, Head Medical Doctor, and psychologist of the study to exclude a test subject from the study for any other reason.

The mentioned inclusion and exclusion criteria are the basic criteria. In case any specific experiment requires further consideration, the Project Scientists shall take care to add further inclusion/exclusion criteria into the above mentioned list.

In general, it should be defined which activity level / fitness level the subjects should have or should not have. There is a high variability of fitness levels between different individuals.

4.3. Medical and Other Screening Tests

Potential candidates are required to pass a medical screening within 3 to 6 months prior to starting the study, and each shall be capable of giving informed consent.

If more than 60 days has elapsed since initial testing, a hematology profile, fasting glucose, urea, creatinine, test of liver function, and electrocardiogram are repeated before study enrollment.

A medical examination prior to the acceptance of the candidates should include the following:

- Anamnesis / Full physical exam according to Section 4.3.1
- Collection of a blood sample and analysis according to Section 4.3.2
- Collection of urine samples and analysis according to Section 4.3.3
- DEXA scans according to Section 4.3.4

- Stand test according to Section 4.3.5
- 12-lead ECG
- Vision and hearing tests according to Section 4.3.6
- Chest X-ray
- Illegal drug, alcohol and nicotine screening according to Section 4.3.7
- Psychological screening according to Section 4.3.8
- Initial fitness level screening according to Section 4.3.9

Additional tests and/or analysis might be foreseen for specific experiments. These shall be defined before the subject recruitment in order to include those in the overall subject screening process.

4.3.1. Anamnesis / Physical Examination

The medical examination has to be performed by an independent medical doctor.

It should include the following:

- Contact data of the subject
- Questionnaire (the following only serves as an example, additional questions could be added):
 - ◇ Headaches at regular intervals
 - ◇ Dizziness or fainting
 - ◇ Head accident or other
 - ◇ Fainting
 - ◇ Eye problems
 - ◇ Ear problems
 - ◇ Allergies / hay fever
 - ◇ Asthma or bronchitis
 - ◇ Heart problems
 - ◇ High / low blood pressure
 - ◇ Stomach, intestine or liver problems
 - ◇ Blood or saccharide in urine
 - ◇ Epilepsy
 - ◇ Neurological problems
 - ◇ Suicide attempt
 - ◇ Alcohol or drug abuse (pharmaceutical or illegal)
 - ◇ Joint / spine disorders
 - ◇ Frequent skin rash
 - ◇ For women: gynecological history
- History of medical treatments
- History of pharmaceutical usage (including hormonal contraceptives for women)
- Full physical exam
- Resting heart rate / blood pressure

4.3.2. Blood Analysis

For blood analysis during the selection process the following parameters are currently analyzed are shown in Table 4-1:

Table 4-1. Blood Analysis Parameters

Parameters	Between 3 and 6 months prior to starting the study	To be repeated if more than 6 months
Blood chemistry		
Fasting glucose	x	x
Urea (BUN)	x	x
Uric acid	x	
Creatinine	x	x
Total bilirubin	x	x
Aspartate Aminotransferase (AST = GOT)	x	x
Alanine Aminotransferase (ALT = GPT)	x	x
Alkaline Phosphatase (AP)	x	x
Glutamyl transferase (GGT)	x	x
Sodium	x	x
Potassium	x	x
Chloride	x	x
Calcium		x
Phosphorous	x	
25-OHD (Vitamin D)	x	
TSH	x	
T4 (free)	x	
Total Protein	x	
Cholesterol	x	
Triglyceride	x	
High density Lipoprotein	x	
Low Density Lipoprotein	x	
C reactive protein (CRP)	x	
Hematology		
Red blood cells	x	x
White blood cells (Eosino, Neutro, Lympho, Mono, Baso)	x	x
Hemoglobin	x	x
Hematocrit	x	x
Ferritin	x	x

Mean corpuscular volume (MCV)	x	x
Mean corpuscular hemoglobin concentration (MCHC)	x	x
Platelet count	x	x
Red cell distribution width	x	x
PT	x	
PTT	x	
Fibrinogen	x	
Antithrombin III	x	
Protein S	x	
Protein C	x	
Factor V Leiden	x	
Factor II	x	
Lupus-like anticoagulant	x	

Table 4-2. Other Blood Tests

Other blood tests*	Around 4 weeks prior to starting the study
HIV screen,	x
Hepatitis B and C screen	x

*These tests should not be conducted earlier than approximately 4 weeks before the start of the study and could also be performed after the end of the study (period to be defined according to the technique).

Table 4-3. Vitamins and Minerals Status

Vitamins and Minerals Status
Retinol
Retinyl-palmitate
Beta-carotene / alpha-carotene
Serum phyloquinone (Vitamin K)
Alpha-tocopherol / gamma-totocopherol (Vitamin E)
Erythrocyte glutathione reductase (Vitamin B1)
Vitamin B6
Folate
Vitamin C

4.3.3. Urine Analysis

For urine analysis during the selection process, the following parameters are analyzed:

Table 4-4. Urine Parameters

Urine Parameters	
pH	Blood
Specific Gravity	Bilirubin
Appearance	Urobilinogen
Ketones	Nitrite
Protein	Leukocyte
Glucose	

For women, a pregnancy test has to be performed during the selection process and should be done again at the entry into the Clinical Research Facility. A pregnancy blood test could also be added during the pre-bed rest period (first blood collection).

4.3.4. DEXA Scans

DEXA scans (lumbar spine and hip) should be included in the medical screening process of all bed rest studies to evaluate the bone mineral density of the potential candidates. The bone mineral density for each subject should not exceed the mean value of the specific t-score ≤ 2.0 standard deviation.

4.3.5. Stand Test

This test will check the absence of orthostatic hypotension. The protocol for performing the test shall look like: measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) in both supine position (after the subject has rested comfortably for at least 10 minutes) and standing position after 3 and 10 minutes.

4.3.6. Vision and Hearing Tests

Vision tests:

- Visual acuity tests
- Fundoscopic examination
- Tonometry

Hearing tests:

- Physical examination
- Audiometry

4.3.7. Drug, Alcohol, and Nicotine Screening

Test subjects shall be screened for the use of drugs, alcohol, and nicotine during the selection procedure. In case they are selected for participation in the study, subjects shall be screened again when entering the Clinical Research Facility. In case of multiple study campaigns, the tests subjects shall be screened for illegal drug, alcohol, and nicotine use the first day of the respective study campaign.

The test for drug use (TCH, cocaine, opiates, amphetamines/metamphetamines, benzodiazepines, and barbiturates) as well as nicotine analyses shall be performed on urine samples by using commercially available test kits. Alcohol use shall be monitored by a breath analysis test.

4.3.8. Psychological Screening

In general, all subjects shall be tested and interviewed by an experienced psychologist to ensure that they have the mental fitness necessary to complete all aspects of the respective bed rest study. The psychological screening should comprise a paper and pencil test on biography and personal history of the subjects and a standardized test on personality. The final selection of the tests that will be used in the screening process shall be done by the psychologist who will perform the psychological screening. These tests shall be followed by a face to face interview.

The final selection of the subjects should be a joint decision of the psychologist or psychiatrist, the Project Scientist, and the Head Medical Doctor. We recommend performing the psychological interview after candidates have passed the medical screening.

4.3.9. Fitness Level Assessment

For each study it shall be discussed in advance by the study management team together with an exercise peer-group what fitness level subjects are allowed / not allowed to have. This decision may be more or less important depending on the main research question for each study. However, it should be taken into account, that the performance level itself prior to the study as well as the ability to adapt to training / detraining may have a significant impact on the results of a study, especially in the context of bed rest. It is therefore mandatory, beside the decision whether trained or untrained subjects will be needed for the study, to select a group of test subjects that is as homogeneous as possible regarding their fitness level.

For getting an estimate of the fitness level of a potential subject, we suggest measuring the VO_{2peak} during the screening procedures, using a bicycle step test with spirometry. Termination criteria have to be defined before, which can be for instance, subjective exhaustion or heart rate (220 beat per / minute minus age). However, homogeneity in the group of subjects should be favored over the absolute VO_{2peak} values.

The range of VO_{2max} values according to the age of the volunteers should be:

- if age < 35 years: 35 ml/min/kg < VO_{2max} < 60 ml/min/kg
- if age > 35 years: 30 ml/min/kg < VO_{2max} < 60 ml/min/kg

Additionally, a health related fitness assessment protocol to assess musculoskeletal strength and endurance shall be performed or or an ‘activity diary’, wherein subjects record physical

activity for 2 ‘normal weeks’ in their daily life, shall be performed to estimate the real fitness level of the candidates. This can be complemented by measuring the total number of steps a candidate performs per day with an activity monitor.

4.3.10. Screening Data Handling

The raw data from the test-subject screening process, meaning the subjects blood and urine analyses as well as those from the laboratory reference samples, shall be filed and stored according to the national regulations for medical data archiving. Storage shall be done in a locked filing cabinet according to standards of clinical practice accepted in the country where the study is being conducted. Good Clinical Practice (GCP) (<http://www.emea.europa.eu/pdfs/human/ich/013595en.pdf>) is an example of such practices used in Europe.

5. Volunteer rules/general conditions of bed rest

When designing the ethics application the national regulations for volunteer rules have to be checked. For the following activities and areas rules have to be defined by the study management team and agreed upon with the subjects, before they are selected as candidates.

- Activity during the ambulatory study phases
- Directive for bed rest (before, during and after)
- Activity monitoring of subjects
- Physiotherapy
- Subjects privacy
- Day-night-cycle
- Subjects motivation
- Telephone and internet access.

This checklist only serves as an example and can be amended if needed.

5.1. General Rules

Rules shall be defined and are mandatory during the studies to guarantee identical study performance during each study campaign and over a couple of studies. In some countries they may have to be validated by the ethics committee before they can be proposed to the subjects. Thus national regulations have to be checked and followed.

Once the study has started, subjects shall follow the rules for the study and the instructions of the personnel.

Generally, the subjects should not be allowed to leave the research facility after the beginning of the study campaign without an escort of the study personnel. During short and mid-term bed rest, leaving the research facility should be limited to external experiments. However, for long-term bed rest additional times can be discussed when subjects are allowed to leave the facility in the company of an assistant. This should then be the same length and frequency for all subjects.

5.2. Activity during the Ambulatory Study Phases

5.2.1. General

The activity level in the medical facility is usually lower than before the study starts. Thus, one shall allow for adaptation to the experimental conditions in the bed rest facilities. However to still have a good distinction between the ambulatory phase and the bed rest phase, subjects should not be allowed to lie on the couch or in their bed during the day in the ambulatory phases. All daily activities such as reading, eating, and watching TV shall be performed in a seated position with the feet on the ground rather than outstretched on the couch or chair. If this is for any reason not the case, times in other body positions than seated should be monitored and documented. Additionally, subjects should spend most of their ‘free time’ in the group, rather than by themselves in their bedrooms. If subjects prefer to spend time for studying in their own rooms the personnel shall ensure that the subjects keep the specified body position.

5.2.2. Exercise Interventions to Adapt Activity Level

Changing the activity level from before the study to the rather lower activity level in the bed rest facility during the ambulatory study phases may already have an impact on some parameters of interest (such as bone markers, muscle parameters, and cardiovascular system). To standardize the activity on each site, an individually tailored exercise program shall be developed and applied in the ambulatory study phases. An exercise peer-group shall be available to discuss and design exercise / activity interventions during the ambulatory phase for each study individually.

The aim of the exercise program should be to keep the activity level during the ambulatory study phases as close as possible to the activity level before the study. Thus, the activity level in the ambulatory phases of the study, including all experiments and other study related activities, should be assessed and compared with the activity diary and the outcome of the fitness level assessment. This comparison should be performed for each of the study days individually, as they usually are very different in the amount of experiments, especially in the recovery period after bed rest. It should result in an exercise program that is tailored for each subject according to their usual activity habits.

Simulation of daily activity with walking on a treadmill or using a step ergometer may be possible methods. However, this activity should not have exercise character! A well-trained person shall supervise the activity measures. Loss of body fluid and energy due to exercise interventions has to be adjusted with dietary intake.

5.3. Directives for Bed Rest

5.3.1. General

During bed rest, subjects spend 24 hours a day in bed. In addition for simulation of physiological effects of microgravity the bed is set in a 6° head-down tilt position (HDT position). They 'live' in bed during that time, meaning all activities such as hygienic procedures, eating, reading, and 'going' to the bathroom' (use of bed-pans and urine bottles) take place in bed continuously for the duration of the study. Thus, it is mandatory to provide the beds with high quality mattresses to avoid discomfort. The subjects are allowed to move slowly without pushing from supine to ventral or lateral positions but are not allowed to get up, sit, or stand at any time and shall maintain the HDT position.

The use of one small size flat pillow is allowed as long as the shoulders touch the mattress.

5.3.2. Start of Bed Rest

On 'day 1' of the bed rest phase of a study campaign, subjects shall follow the same morning procedures as on all other study days in the ambulatory phase. They shall get up at the predefined time, perform the normal morning duties such as urine sampling, blood draw, weighing, showering, and having breakfast in an upright position. Then they shall start the 6°-HDT bed rest at approximately 9:00 am. Day 1 (HDT 1) of bed rest ends approximately 24 hours with the morning blood draw and urine sampling in the morning of day 2 (HDT 2), which can be earlier than 9:00 am.

5.3.3. End of Bed Rest

The end of the bed rest is in the morning of R+0. Subjects get up after the daily morning procedures such as urine sampling, weighing procedure, and blood sampling. The difference in 'getting up' between subjects should not exceed 2 hours. This has to be considered when designing the study protocol.

5.3.4. Gurney Use

All activities that require to subjects leave their rooms should be done with a gurney tilted 6° head down. This includes weighing procedures, showering, or transport to other experiment facilities.

5.3.5. Accepted Activities during Bed Rest

All activities shall be carried out in 6° HDT. This includes:

- Eating: subjects shall maintain the correct body position. Food shall be served on a low desk at the bedside. The subjects consume beverages (with a straw) and eat all meals in a HDT position leaning out of the bed.
- Hygienic procedures: teeth brushing, shaving, washing with a washbowl in bed or showering shall be performed in the above-mentioned position.
- Times free of experiments: they are allowed to read or write, watch TV, use the computer or listen to music, use a telephone or talk to each other as long as the correct body position is maintained.

5.4. Activity Monitoring of Test Subjects

To document compliance of the subjects with the requirements, their activity shall be monitored. This should be done by redundant methods such as video control, pressure sensors, by subject monitors in person, or activity measurements by an actimeter. Activity during the bed rest period can also be recorded with telemetric electromyography on randomized study days.

For video control the rooms of the subjects are equipped with video cameras. Video recording shall be continuous. To respect the subject privacy the cameras should point only to their upper body. The period of non-video control should be documented in a specific form.

5.5. Physiotherapy

The experience from bed rest experiments is that staying in bed for several weeks without any stretching leads to muscle tension. For tension release it would therefore be ideal to include a regime of massage or physiotherapy on a regular basis in the study. It is preferred that stretching is not applied as it may influence exercise outcomes in some studies. However, should stretching be needed to maintain joint range of motion, it should be completed in the head-down position.

To keep the standardized study conditions, the Project Scientist decides if this is at all applicable for the relevant study. It should be discussed with the PIs during the IWG meeting and before the study whether physiotherapy could have a significant impact on any of the experiments. Conclusion of the discussion should be a decision on what kind of physiotherapy

can be applied and in what frequency and duration. Before the onset of a study, the conclusion of the discussion on the application of physiotherapy shall be prepared in a standard operating procedure (SOP). The Project Manager ensures that only a physiotherapist or massage therapist graduated from an accredited physical therapy program shall perform the tension release measures. If subjects complain about back pain due to the lying position, massage can be a helpful treatment as long as it does not interfere with the experiment.

5.6. Subject's Privacy

5.6.1. Privacy Spaces

Well-being of the subjects and respecting cultural differences is most important for their compliance with the requirements therefore subjects should feel comfortable about the situation. Privacy to a certain extent shall be respected. For example, sleeping in single rooms is not crucial, as long as specific experimental conditions are not affected.

5.6.2. Clothing

To contribute to the subject's well-being the choice of clothing (such as, light clothing, short trousers) shall be the subject's preference, except for specific experimental requirements. The clothing shall be appropriate; sweating or freezing shall be avoided.

5.6.3. Day-Night Cycle

Subjects will keep a strict day-night cycle. That means they will be awake for 16 hours, and asleep for 7 to 8 hours. They should awake around 7:00 am (+/- 1 hour) and go to sleep around 11:00 pm (+/- 1 hour). The sleeping times that were decided at the beginning of the respective study shall be maintained continuously during the entire study campaign period. When planning the waking times it has to be considered that in the morning, blood drawing and weighing procedures take a large amount of time. Sleeping times have to be checked by the respective care givers. Volunteers are allowed to have a little afternoon nap (15 to 20 minutes -in HDT position during the HDT period and in seated position in the BDC/recovery phase of the study) if it does not interfere with the experiment.

5.6.4. Enhancing of Subject's Motivation

As long as the experiments are not disturbed, a maximum number of possibilities for entertainment (books, newspapers, magazines, radio, TV, DVD etc.) should be offered. Also a telephone should be provided if technically possible, internet access also provides a well recognized communication tool.

To support the well-being of the subjects that live in single rooms during the bed rest phase, a large living room should be available so the subjects can join the others while lying on a gurney and strictly maintaining the 6° HDT if technically feasible.

Other mood supporting activities will likely improve the subjects' motivation and support their compliance with the rules, especially during long-term bed rest studies. These activities may comprise, if required, personal talks with the subjects or supporting of the subject's efforts to reach a certain goal during their stay in the bed rest facility (such as writing a novel, learning a language, and so on).

The care givers shall discuss all such measures before they promise anything to the subjects with the responsible person / Project Scientist on site to ensure that any impact on experimental procedures is avoided and that the subject feels free to decide whether to agree or to decline the offer. Any mood supporting activities that are questionable due to scientific or psychological reasons shall be excluded.

5.6.5. Use of Telephone and Internet

Conversations via telephone or internet video phone shall be allowed as long as any interference with experimental operations is excluded. The total duration of phone calls should be restricted to 2 hours per day.

Use of private mobile phones can be restricted or not permitted during the entire study.

Internet connection for the test subjects shall be provided at each site if technically possible and can be used as long as this does not interfere with experiments. However, the test subjects have to sign a form wherein they confirm that they will agree to the regulations at each site in using the internet.

Internet access and phone calls are not allowed during the sleeping times from 11:00 pm (+/-1 hour) until 7:00 am (+/-1 hour) and during experiment sessions.

5.6.6. Visitors

It is preferred that visitors not be allowed. However, visits from family or friends can boost the morale of bed rest subjects. When allowed, visits should be scheduled so as not to interfere with the subject's testing and meal schedule. Visitors are not allowed to bring food or other items that could compromise the subject's participation in the study.

5.6.7. Media Events, Interviews, Tours

Public relations activity shall be coordinated by the project scientist. Before any media-activity occurs, national regulations have to be fulfilled. The test subjects shall be informed prior to the communication with media and their written consent shall be obtained. Subjects are not allowed to contact media on their own authority. To avoid interference with any experiment session, all media contact should be postponed to after the entire study campaign. For further details on the communication with media see Section 2.2.3 – Communication – Press / Media.

6. (Para-) MEDICAL Care

6.1. Qualification of Personnel

Qualified personnel shall be provided for 24-hour subject care. Because of safety and rules of good clinical practice, an independent (meaning not an associated PI or Co-Investigator) and qualified medical doctor shall be responsible for all subject-related general medical care but not experiment-specific medical decisions.

Curriculum vitae and/or other relevant documents, such as proof of specific training measures or experience, shall be provided for all staff involved in the care of subjects or support of subject-related activities to prove their qualifications, eligibility to conduct a study or medical supervision or care of subjects. The Project Manager is responsible to collect the relevant documents.

6.1.1. Training of Staff

Any person involved in the study shall be qualified to perform the respective experimental procedures. The qualification of the on-site personnel shall be ensured by respective certification of the responsible PI. If the PI is not on site during an experiment, an authorized person shall be trained who is responsible for the experiment performance on site. This person might be either a co-worker from the PI's laboratory (external staff) or be selected out of the staff on site (internal staff).

6.1.1.1. Internal Staff

Training of staff shall be provided to achieve consistent quality of operations and procedures. In SOPs, the relevant procedures and the handling of protocols shall be described in a step by step manner to be used both as checklists to be tracked during the organization and conduction of the bed rest studies and as educational material for staff training prior to the implementation. The Project Manager is responsible for assigning the training measures and for providing the staff trainers and supervisors with the relevant information to enable them to perform the training.

This training on procedures and the use of SOPs and protocols for the in-house staff shall be documented.

6.1.1.2. External Staff

During the course of the bed rest studies, external staff may take part in the study performance. These personnel might come partly from other PIs' laboratories or may be recruited on site to support study-related activities. However, they are most likely not familiar with the detailed procedures on site. Such personnel shall be trained in a detailed way not only regarding specific experiment-related procedures but also regarding general operations / rules during bed rest studies and how to deal with the specific physical and psychological situations of the test subjects. They shall learn about the roles and responsibilities and how to deal with critical situations. Persons shall perform this training without jeopardizing the success of the study.

This training process of personnel on procedures, the use of SOPs, and so on, does not need to be identical at the different bed rest facilities. However, it should include security rules, instructions for all devices, general goal of the study, and rules for subjects and staff. The

training process shall be well documented, for example, which procedures are included in the training process as well as who attended the training. The training shall be performed by well-experienced researchers, technicians, or other staff. Although training processes may appear time consuming it is important to repeat them for every new study that will be performed. Also the staff, who has attended in previous studies, shall take part in the trainings again for every new study.

6.2. Test Subjects Nursing / Support / Supervision

Sufficiently well-trained care givers – either internal or external – shall be in the bed rest facility and available for 24 hours for the test subjects to support the test subjects and also to supervise those regarding their adherence to the study rules. Their job is to conduct the measurements for medical monitoring (blood pressure, heart rate, temperature, body weight), provide bottles for urine collection and bed pans for feces collection, and store urine and feces samples prior to laboratory processing. The care givers shall be trained on the operating procedures for weighing in bed rest position, and urine and feces collection, as well as any other measurements according to the relevant SOPs.

They also shall make sure that these measurements as well as any other experiment-related activities are on time. To check the point of time of each experiment a spread sheet from each experiment day for each test subject is located in the supervisor's room as well as in the subjects' rooms for information of the test subjects.

6.3. Medical Care

6.3.1. Medical On-Call Duty

To ensure that rights, safety and well-being of subjects are protected, 24-hour medical on-call duty is the minimum that shall be provided. For some countries physical presence of a medical doctor may be mandatory. If the medical doctor can be 'on call' during the night it shall be ensured that he can be in the laboratory within 30 minutes. Phone and/or pager number is provided to the care giver on duty in the bed rest facility to ensure that the medical doctor can be called in case of an emergency. A portable emergency bag shall be available in the bed rest facility.

6.3.2. Daily Ward Round

Each day of the study an independent qualified medical doctor shall perform a ward round to monitor subjects including the documentation of health status. Any medical intervention, such as subscription of medication, massage, and so on, should be discussed prior to the administration (except in case of emergency) with the project scientist to minimize potential effects on the outcome of the study.

6.3.3. Medical Monitoring Routine

Several measurements will be performed daily at the same time with respect to the study protocol to routinely monitor the subjects. This medical routine comprises measurement of blood pressure, heart rate, body temperature, and body weight. For blood pressure and heart rate measurements, a joint protocol should be used. This describes how often the measurements are done to calculate the mean value, the period between measurements, and so on (Table 6-1).

Table 6-1: Safety Parameters during Different Lengths of Bed Rest: Clinical Parameters and Clinical Blood Tests

Bed Rest Studies Standardization – Clinical Parameters and Clinical Blood Tests		
Clinical Parameters	Timing	
Body mass	Daily	
Temperature	Daily	
Blood pressure / heart rate	Daily	
Clinical Blood Tests	STBR	MTBR
	BDC-5 (±1); R+4 (±1)	BDC-5 (±1)
		HDT10 (±1); R+4 (±1)
Hemoglobin	<input type="checkbox"/>	<input type="checkbox"/>
Hematocrit	<input type="checkbox"/>	<input type="checkbox"/>
RBC	<input type="checkbox"/>	<input type="checkbox"/>
RBC volume	<input type="checkbox"/>	<input type="checkbox"/>
MCH	<input type="checkbox"/>	<input type="checkbox"/>
MCHC	<input type="checkbox"/>	<input type="checkbox"/>
Platelets	<input type="checkbox"/>	<input type="checkbox"/>
White blood cells	<input type="checkbox"/>	<input type="checkbox"/>
Neutrophils	<input type="checkbox"/>	<input type="checkbox"/>
Eosinophils	<input type="checkbox"/>	<input type="checkbox"/>
Basophils	<input type="checkbox"/>	<input type="checkbox"/>
Lymphocytes	<input type="checkbox"/>	<input type="checkbox"/>
Monocytes	<input type="checkbox"/>	<input type="checkbox"/>
Reticulocytes	<input type="checkbox"/>	<input type="checkbox"/>
Sodium	<input type="checkbox"/>	<input type="checkbox"/>
Potassium	<input type="checkbox"/>	<input type="checkbox"/>
Chloride	<input type="checkbox"/>	<input type="checkbox"/>
Calcium	<input type="checkbox"/>	<input type="checkbox"/>
Phosphorus	<input type="checkbox"/>	<input type="checkbox"/>
Urea	<input type="checkbox"/>	<input type="checkbox"/>

Creatinine	<input type="checkbox"/>	<input type="checkbox"/>
Total Protein	<input type="checkbox"/>	<input type="checkbox"/>
Glucose	<input type="checkbox"/>	<input type="checkbox"/>
ALAT (alanine aminotransferase)	<input type="checkbox"/>	<input type="checkbox"/>
ASAT (asparagine aminotransferase)	<input type="checkbox"/>	<input type="checkbox"/>
Alkaline Phosphatase	<input type="checkbox"/>	<input type="checkbox"/>
Glutamyl transferase	<input type="checkbox"/>	<input type="checkbox"/>
Creatine Kinase	<input type="checkbox"/>	<input type="checkbox"/>
Lactate Dehydrogenase	<input type="checkbox"/>	<input type="checkbox"/>
Prothrombin Time (PT)	<input type="checkbox"/>	<input type="checkbox"/>
Activated Partial Thromboplastin Time (APTT)	<input type="checkbox"/>	<input type="checkbox"/>
Fibrinogen	<input type="checkbox"/>	<input type="checkbox"/>
Ferritin	<input type="checkbox"/>	<input type="checkbox"/>
25-OHD (Vitamin D)	<input type="checkbox"/>	<input type="checkbox"/>

Additionally clinical safety parameters will be measured as indicated in Table 6-2:

Table 6-2: Safety Parameters during Different Lengths of Bed Rest

Urinalysis (dipsticks)	BDC-5 (±1) ; R+4 (±1)	BDC-5 (±1) ; HDT10 (±1) ; R+4 (±1)
pH	<input type="checkbox"/>	<input type="checkbox"/>
Blood	<input type="checkbox"/>	<input type="checkbox"/>
Glucose	<input type="checkbox"/>	<input type="checkbox"/>
Protein	<input type="checkbox"/>	<input type="checkbox"/>
Ketones	<input type="checkbox"/>	<input type="checkbox"/>
Leukocyte	<input type="checkbox"/>	<input type="checkbox"/>
Bilirubin	<input type="checkbox"/>	<input type="checkbox"/>
Urobilinogen	<input type="checkbox"/>	<input type="checkbox"/>

6.3.4. Medical Monitoring during Experimental Sessions of PIs

It is the responsibility of the Head Medical Doctor to organize and provide the medical monitoring of the subjects' health status during especially demanding experiments. The independent medical doctor is allowed to stop any experiment if the well-being of a subject is jeopardized. The experiments for which this might be necessary are defined in advance by the Project Scientist and the Head Medical Doctor. Termination criteria shall be defined by the Project Scientist, the Head Medical Doctor, and the experiment responsible PIs. These criteria shall be taken as joint termination criteria for the specific experiment.

6.4. Psychological Support

Psychological support is mandatory for the monitoring of the subjects' well-being and mood and shall be performed during the studies. It shall be defined in advance, how often during a study campaign psychologists shall visit the subject. Every 2 weeks seems a reasonable interval. The interview should be developed and accomplished by the same psychologists that performed the psychological screening during the volunteer selection. In the case where experts decide that cultural differences require changes to the standardized interview, site-specific modifications can be added as long as the necessary information regarding the subjects' mood can be judged from the questions. The content of the interviews is considered confidential, unless it is the wish of the individual test subject to transfer anything to the Project Scientist.

Additionally, the medical doctors monitor the psychological well-being of the subject during the daily ward rounds and contact the psychologists if necessary.

6.5. Physiotherapy

Immobilization through bed rest can cause side effects, for example, neck pain, back pain or headaches. Physiotherapy can provide relief in some cases and should be considered by the medical doctors as treatment before using drugs. It is the decision of the Head Medical Doctor to schedule physiotherapy sessions as a treatment, additionally to the mandatory physiotherapy described in Section 5.5. However, the Head Medical Doctor should only decide on physiotherapy as a treatment in consultation with the project scientist.

6.6. Hygienic Procedures

6.6.1. Daily Washing

Subjects shall be provided with the opportunity to clean themselves twice daily. During the bed rest phases it is essential to provide them with the opportunity to clean themselves without standing.

The procedures of showering during HDT shall ensure that the subjects keep the HDT position and are safe.

6.6.2. Collection of Urine / Feces

For the collection of urine, the subjects are asked to void into pre-weighed single-void containers at particular times. The particular times will be defined according to the experiment requirements and can be mandatory each morning at 7:00 am for 24-hour urine or at specific

times for any experiment requirements. As a default, 24-hour urine shall be collected from 7:00 am one day to 7:00 am the next day. The exact times of additional voids shall be defined by the Project Scientists according to the experiment requirements. A protocol shall be developed in which subject code, date, and time of all voids, including tare weight of the urine bottles shall be noted. Times of feces collection are usually not predefined. However the exact date and time of a feces sample shall be noted. Handling and labeling of urine and feces samples are described in Section 8.2.2 and 8.2.3 under Biological Samples.

During the ambulatory phase, subjects can use the normal bathrooms for urine and feces collection. During the bed rest phase, collection shall be performed in the HDT position. Subjects' privacy shall be respected during these procedures and they are asked to call for the care giver in the bed rest facility to retrieve a bottle for the urine voids. As some parameters analyzed out of the urine samples are temperature or light sensitive and since the time of voiding or the feces sample shall be documented, no bottles or bed pans shall be stored in the subjects' room. The care givers shall provide those only in case the subjects ask for it.

6.7. Environmental Conditions

To achieve standardized study conditions it is very important to control the environmental conditions such as room temperature, humidity, and daylight exposure.

6.7.1. Room Temperature and Humidity

Room temperature and humidity should be kept at comfortable levels. Values should be in the range of 19°C to 22°C and 50% to 70% relative humidity for most of the day so that sweating or freezing shall be avoided as both would affect study results. If not possible, temperature and humidity should be at least monitored. Room temperature and humidity shall be documented throughout the whole study.

6.7.2. Sunlight Exposure

The exposure to daylight should be controlled as it elicits physiological reactions in the human body that may influence the results of bed rest studies. Especially if study campaigns take place during different seasons, different exposure to daylight may jeopardize the results of the study. The exclusive use of artificial light would be the easiest way to control daylight. However, then supplementation of vitamin D would be mandatory (see Section 7.2).

7. Nutrition standardization

7.1. Basic Nutrient Intake Level

7.1.1. Levels of Recommended Nutrient Intake

To avoid any impact of inadequate nutrient supply to the human organism, adequate nutrient intake levels are defined in Table 7-1. These recommended values should be regarded either as an adequate range, if a range is mentioned, or as a minimum intake level as a leading line for each study if no other nutritional constraints or nutritional countermeasures from specific proposals are required. For some of these nutrients (vitamins and elements) these recommended intakes should be achieved on an average per week. For all other nutrients the recommended intake should be achieved every day. In case of specific nutrient requirements from the PIs, the levels/ranges of the specific experiments shall replace the mentioned ones. All other nutrient intake levels shall optimally reach the recommended adequate intake level listed in Table 7-1.

Table 7-1. Recommended Adequate Nutrient Intake Levels to be Achieved

Nutrient	Adequate intake
Energy and Macronutrients	
Energy (total energy expenditure, TEE)	WHO equation for Resting Metabolic Rate (RMR) – ideally RMR should be measured via indirect calorimetry to get individual data × 1.1 (bed rest: HDT) or × 1.4 (ambulatory: BDC, Recovery) + 10% (of TEE) for thermo genesis
Total fat (%TEE)	30% to 35 %
Saturated fatty acids (%TEE)	≤ 10
Monounsaturated fatty acids (%TEE)	≥ 10
Polyunsaturated fatty acids (%TEE)	≥ 7
Protein g/kgBW/d	1.2
Carbohydrates (%TEE)	50 to 60
Total Fibre (g/d)	≥ 30
Electrolytes and Water	
Sodium (g/d)	3.5 to 4.5
Chloride (g/d)	6.0 to 7.5
Potassium (g/d)	3.5 to 5.0
Calcium (mg/d)	1000 to 1200
Water (ml/kgBW/d)	35 to 50
Vitamins	
Biotin (µg/d)	100
Pantothenic Acid (mg/d)	5

Folate (µg/d)	400
Niacin (mg/d)	20
Riboflavin (mg/d)	1.5
Thiamin (mg/d)	1.5
Vitamin B6 (mg/d)	2
Vitamin B12 (µg/d)	2
Vitamin K (µg/d)	80
Vitamin D (ug/d)	5
Vitamin A (µg/d)	1000
Vitamin C (mg/d)	100
Vitamin E (mg/d)	20
Elements	
Copper (µg/d)	1500-3000
Fluoride (mg/d)	1.5 – 4
Iodine (µg/d)	200
Iron (mg/d)	Male: 10
Magnesium (mg/d)	300
Phosphorus (mg/d)	700-1500
Zinc (mg/d)	12-15

7.1.2. Procedure in Case of Additional Experiment Specific Nutrient Requirements

In case specific nutrient requirements from experiment proposals need to be included, these have to be extracted from the respective proposals and included into an overall dietary requirements document.

7.1.3. Handling of Different Traditional Cuisines

It is obvious that food habits are different in different countries. Therefore the traditional cuisine of each country shall be kept and the level of nutritional standardization shall be on the nutrient intake level. Food habits of selected test subjects from other countries might also be included in menu planning.

7.2. Dietary Supplements

To avoid seasonal differences all subjects have to be supplemented with 1000 IU vitamin D3 from final selection to the end of the study. If a subject shows a deficiency in (25-OHD) vitamin D at the time point of the medical screening, depending on the level of deficiency it should be recommended individually how to deal with it (amount of supplementation, duration of supplementation). Deficits in other vitamins and minerals should be discussed with the Head Medical Doctor and the Project Scientist. They have to decide in individual cases if the subject can be supplemented or if he has to be excluded from the study.

7.3. Dietary Restrictions Other than the Basic Nutrient Requirement

Besides matching the nutrient intake levels, some other dietary restrictions are mandatory. These are:

- No methylxanthine derivatives are allowed (coffee, decaffeinated coffee, black and green tea, energy drinks, chocolate, cola)
- No alcohol intake
- No flavor enhancer
- No sweat inducing spices (such as chili, hot curry)

7.4. Teaching Test Subjects on Constant and Controlled Nutrient Intake

One of the most important prerequisites for high compliance on food intake during metabolic balance studies is the education of the test subject. To prepare the test subjects adequately, a very detailed section on dietary control and constant nutrient intake shall be part of the informed consent document.

7.5. Menu Preparation in the Metabolic Kitchen

A 'metabolic kitchen' is a kitchen where metabolic meals are prepared in accordance with specific requirements. There is no need that the metabolic kitchen is part of the research facility; a metabolic kitchen in a hospital with a delivery service is also imaginable.

The metabolic kitchen personnel shall observe and follow the country-specific law according to industrial hygiene.

The metabolic kitchen staff is responsible that the food items on the menu will be provided to the test subject in the defined amount. The weight of each food item/beverage to be offered to the test subject should either be almost exactly the weight foreseen on the menu or the actual provided weight of the respective ingredients shall be recalculated in the nutrition software to be sure that at the end of the day the nutrient requirements are met. In case of any leftovers on the test subjects' plate, the food items will be separated and weighed separately. As the food shall probably be mixed together, a very accurate measurement is impossible. However, based on the weighed food items an estimation of the nutrient content in the leftovers is done by the nutrition software and the missing part of the nutrients is provided to the test subjects with the next meal. This procedure results in standardized nutrient intake level for each day.

7.6. Documentation of Actual Daily Nutrient Intake

Documentation of amount and time of consumption of the meals will be documented first on the individual hardcopies of the daily menus and then evaluated by nutrition software regarding nutrient content.

7.7. Verification of Individual Nutrient Intake

Although a very thorough menu planning is foreseen and thereby the calculation of nutrient content and nutrient intake should be very accurate, the calculated nutrient intake with food chemical analyses should be verified.

8. Biological samples

This chapter deals with the biological samples of blood, urine and feces. All other biological samples such as tissue or saliva are seen as additional experiment specific samples. The collection and processing of these additional samples has to be defined by each investigator and shall be discussed and arranged with the Project Scientist.

8.1. Coding of Samples

Each biological sample shall be labeled with a sample code. Each bed rest facility should define a clearly understood sample code system on its own. Then a decoding list shall be developed by each lab. The sample code or the respective decoding list should contain information like study name, study campaign, study site, subject code, sample number, blood type (such as, serum and plasma) only on monovettes and the parameter code on tubes (taken from the aliquot scheme). Each PI guarantees that only this sample code is used for any procedure that follows the site specific sample processing.

8.2. Blood Drawing and Other Sample Collection

8.2.1. Blood Drawing

Body position, time of blood drawing, and so on, may influence blood concentrations of certain parameters. Therefore it is necessary to define the exact procedure of blood drawing including prerequisites and other circumstances according to the study design. The investigator shall define his/her requirements and arrange this with the Project Scientist.

Blood drawing systems do not need to be standardized. This means that the preferred blood drawing systems (vacuettes, monovettes, or others) can be used independently. But for the analysis of different parameters, specific additives are mandatory. Some shall be pre-cooled and then stored on ice until centrifugation. The Project Scientist shall define the specific requirements (such as blood volume, additives, freezing temperature, and so on) based on the information provided by the PIs, named in the EDS, for the parameters to be measured and to check if the necessary circumstances are available at all sites.

Blood drawing logistics shall be organized by the Project Scientist. The Project Scientist will determine such factors as how much blood is drawn and who shall process the blood.

8.2.2. Urine collection

Urine might be collected as 24-hour urine in one collecting box or pooled out of single samples. In case samples from a single void are needed for some experiments and 24-hour urine is necessary (on an identical day) for other experiments, a calculation shall be carried out to pool certain amounts of the single voids to a 24-hour urine and aliquot the 24-hour urine sample accordingly. It is important to have completed 24 hours. Therefore the exact 24-urine collection period (including maximal deviations) should be defined. The procedure for collection should be as the following:

- First day of urine collection: After having woken up (7:00 am), the test subject immediately is instructed to urinate, using the toilet and not the bottle. Thus, the morning urine (7:00 am) of the first collection day is not kept for analysis. The exact

time is documented in the protocol and is the starting point for the 24-hour urine collection. For the next 24 hours, the urine is collected. The last urine to be included in the analysis shall be collected 24 hours after the starting point (7:00 am of the next day). The time of this last urine collection of the 24-hour period is noted in the protocol and signifies the end of the first collection period.

- Further collection days: The time of the last urine collection of the preceding collection period is at the same time the beginning of the new collection period.
- Each time urine is collected, the exact time has to be documented in the protocol.
- The urine bottle has to be stored in the dark, refrigerated immediately after each voiding, until it is transferred to the laboratory.

8.2.3. Feces Collection

It may be mandatory to collect samples or the entire feces. Requirements for collection, processing and storage need to be defined by the investigators.

8.3. Processing of Blood and Urine Samples

All blood and urine samples shall be processed in the way defined by the investigators and should be always the same for the respective parameter to be measured.

8.4. Biochemical Sample Analysis and Cross Calibration Procedures

The ideal case would be to analyze the same parameters in the same lab. But it is also reasonable that the samples need to be analyzed in different labs. For biochemical assay analysis different manufacturers of commercial assays are available to analyze one parameter. To avoid any difference in sample analysis because of different assay manufacturers, the assays from the same manufacturer shall be used in the participating laboratories. To control any differences because of different batches, different hardware and laboratory differences and to check the quality of biochemical analysis, it is necessary to create serum, plasma, urine, and saliva pools in the different laboratories that will be taken as control samples. The responsible laboratory that creates the control samples shall be assigned randomly. At least six different samples of each pooled material shall be prepared. Out of these six different samples of each material only two of each will randomly be chosen and sent for analysis to the labs of the sites. The samples shall be chosen by a person who is not involved in the lab analysis. These two samples will be included in each of the assays running in the different labs. The data of the different sites shall be collected in one data sheet. The exact procedure and timeline for pooling the control samples shall be defined. At least two different control samples shall be included in the analysis at each participating lab. In case the data at one site is not in a certain range, the Project Scientists shall check with the respective lab and find the cause for the deviation.

Additional to cross calibration tests it is mandatory that each lab takes part in interlaboratory tests to control the quality of analyses that is important for getting valid results.

8.5. Sample Storage

All samples shall be stored according to the parameters' (to be analyzed) specific freezing temperature for at least 10 years. The freezer should be connected to an automatic temperature

monitoring system to guarantee that the temperature is always constant. This system shall be connected to an alarm system in case of dysfunction to avoid thawing of the samples.

8.6. Sample Transport (between labs)

The Project Scientist shall describe the accepted sample transport procedures such as hand carry or by a method that ensures for a correct transport regarding temperature and other safety issues. It is the PI's responsibility to guarantee safe transport. The main interest should be to receive the samples in an optimal, frozen condition in the respective PI's labs. To ensure that the samples could be received, the Project Scientist has to contact the PI before shipping.

9. Data Management

9.1. General

Data generated during bed rest studies shall be archived according to national regulations and study results shall be made available to the scientific community. To allow transparent processing, publication and comparison of data it is useful to consider a few different types of data that should be captured for a given bed rest study.

9.1.1. Administrative Data

In addition to scientific data a wide range of descriptive data ('meta data') shall be captured to allow for comparison between individual studies.

9.1.1.1. Facility Data

Information about each facility that might be of relevance for bed rest studies should be compiled. This information may include:

- Name of the bed rest facility
- Size of the bed rest facility
- Number of subjects that can be hosted at the same time
- Hardware and measuring devices on site
- Professional experience and scientific focus of the laboratory

9.1.1.2. Test Subject Data

A unique personal code shall be given to every test subject accepted for the participation in the study allowing for anonymous data storage during selection and during the study. Subjects participating in more than one study shall keep their personal code throughout all studies, thus allowing for association of test results to individuals across studies. The personal code could, for example, reflect a subject's gender, year of birth, and nationality followed by a unique number (for example, F1989GE0023 for female, born 1989, German, 0023). In countries where ethics committees prohibit identifiers in subject codes, a random numbering scheme can be developed prior to the study. Numbers can then be assigned to subjects as they are enrolled.

Although the personal code is mandatory for all data transmissions between participating facilities / agencies, facilities are free to use a different code for internal purposes (such as labeling of medical samples).

Two strictly separated databases are required for test subject data.

9.1.1.2.1. Personal Subject Data

This database contains the relationship between an applicant's subject code and personal information such as name, address, date of birth, studies participated in, and so on. Data of rejected applicants shall be kept as well to identify re-applications.

It is obvious that access to this database shall be restricted to personnel directly involved in the organization of applicant selection and there shall be no connection to any other subject or study data.

9.1.1.2.2. Medical Subject Data (medical screening, daily medical routine data)

This database holds all medical / psychological data acquired during the selection process along with the subject code and studies participated. Moreover it shall hold information about any medical / psychological interventions and off-nominal events occurring during the study. Data of applicants rejected during the selection process due to any reason shall not be kept.

These data shall be accessible for all involved scientists as long as the subject participates in the respective study.

9.1.1.3. Descriptive study data

The main study characteristics should be captured. This includes:

- Study name
- Scientific rationale and methods
- Study campaigns, date, time, and duration
- Facility code
- Participating scientists
- Participating subjects' codes
- Summary of results

9.1.1.4. Study schedule data

All study relevant activities and events (planned or unplanned) during the course of a study shall be recorded along with for example:

- Subject code
- Study campaign and day
- Date and time
- Location
- Personnel involved

9.1.2. Scientific study data

Here, data derived from standardized measurements and experiment / study specific data can be differentiated. Standardized measurements are implemented in all applicable bed rest studies in a pre-defined scheme. Experiment specific data is usually driven by a concrete research interest by one or more investigators. Both types of data may be shared, in which case it is recommended to establish data sharing agreements before the study / data collection starts.

10. Approach and schedule for standardized measurements

Measures selected as standard measures are presented by discipline and categorized as either required or recommended. Required standard measures are those measures collected for all bed rest studies. Recommended measures are those that can be used when desired for relevant studies. Detailed methodology is described for the required standard measures in each discipline. When applicable, equipment manufacturers are listed for each test. These manufacturers are provided only as suggestions for equipment that is available to complete a particular test. Comparable equipment from other manufacturers will also satisfy testing needs. A summary of the required standard measures and schedule for testing can be found in Table 10-1. Additional familiarization sessions may be added if needed.

Table 10-1. Schedule of Required Standard Measures

Standard Measure	Baseline Data Collection (BDC)	Head-Down Tilt (HDT)	Recovery (R)
Postural Equilibrium Control	BDC-1		R+0
Treadmill Test	BDC-2		R+0
Tilt test	BDC-5		R+0
Maximal Aerobic Capacity	BDC-4		R+0
Muscle strength	BDC-5		R+2
Vertical Jump	BDC-5		R+0
Bone mineral density	BDC-13		R+13
Bone markers	BDC-3		R+0
Nutrition/Hematology	BDC-3		R+0
Immunology	BDC-3		R+0
Positive and Negative Affect Scale	BDC-13, BDC-1	HDT14, HDT28, HDT42, HDT56	R+1, R+13
General Health Questionnaire	BDC-13, BDC-1	HDT14, HDT28, HDT42, HDT56	R+1, R+13

In addition to standard measures, a recommendation is made for archiving biological samples such as serum, plasma, urine, and saliva. These samples can be used for post hoc analyses of some of the recommended measures, for other related analyses, or to support future analyses that may occur at a later time. Laws and rules related to informed consent, sample storage, and allowed duration of storage may vary internationally and should be explored prior to creating the sample archive.

11. Sensorimotor Bed Rest Standard Measures

11.1. Required Measures

11.1.1. Postural Equilibrium Control

This test will characterize changes in postural control following bed rest (Reschke et al., 2009). This test utilizes computerized dynamic posturography (CDP) to quantitatively assess both sensory and motor components of postural control (Black 2001). The Sensory Organization Tests (SOTs) objectively evaluate one’s ability to make effective use of (or suppress inappropriate) visual, vestibular, and proprioceptive information for balance control. The Motor Control Tests (MCTs) evaluate one’s ability to automatically recover from unexpected support surface perturbations.

Decrements in postural stability following spaceflight reflect the adaptation to novel patterns of sensory cues experienced during motion on orbit, most notably changes in how inertial cues from the otolith system are integrated with other sensory information. As bed rest does not alter the interaction of otolith with other sensory input in the same manner as spaceflight, we hypothesize that changes in postural control following bed rest will not be comparable to those observed after spaceflight. Nevertheless, the bed rest analog does mimic aspects of the restricted visual environment of space craft and the reduced stimulation of proprioceptive reflexes of the lower limbs. We predict that decrements in postural stability following bed rest will reflect altered proprioceptive function, and may also be affected by musculoskeletal and/or orthostatic deconditioning.

Postural stability will be evaluated using a computerized dynamic posturography system (Equitest, NeuroCom International, Clackamas, OR). The SOT objectively assesses one’s ability to make effective use of (or suppress inappropriate) visual, vestibular, and proprioceptive information for balance control. The more challenging SOT conditions involve disrupting proprioceptive and visual feedback by rotating the support surface and visual surround in proportion to body sway, referred to as sway-referencing. The standard SOT protocol with head erect is comprised of six conditions involving two support surface conditions (fixed and sway-referenced) and three visual conditions (eyes open, eyes closed and sway-referenced surround). Two modified SOT conditions continue to be used to increase sensitivity and specificity by including dynamic head tilts with eyes closed on either fixed (2M) or sway-referenced support surface (5M). The dynamic tilts involve pitching the head at 0.33 Hz ($\pm 20^\circ$) paced by an audible tone. For each SOT trial, data are recorded for 20 seconds or until there is a fall. Table 11-1 describes the SOT tests.

Table 11-1. SOT Tests

Condition	Support Surface	Vision	Head
1	Fixed support	Eyes open, fixed surround	Erect
2	Fixed support	Eyes closed	Erect
3	Fixed support	Sway-referenced surround	Erect
4	Sway-referenced	Eyes open, fixed surround	Erect
5*	Sway-referenced	Eyes closed	Erect
2M	Fixed support	Eyes closed	Dynamic (0.33 Hz, $\pm 20^\circ$)
5M*	Sway-referenced	Eyes closed	Dynamic (0.33 Hz, $\pm 20^\circ$)

*SOTs 5 and 5M are required posturography tests. SOTs 1 to 4 and 2M are recommended.

The MCTs assess the patient’s ability to quickly and automatically recover from unexpected support surface perturbations. Large forward and backward platform translations (400 ms, amplitude scaled to the subject height, approximately 2.3 inches for a 6-foot-tall subject) are performed to elicit automatic postural responses. Throughout each SOT and MCT trial, subjects are instructed to maintain stable upright posture with arms folded across the chest. External auditory orientation cues are masked by white noise supplied through headphones. Center-of-mass sway angles are estimated from instantaneous anterior-posterior (AP) and medial-lateral (ML) center-of-force positions computed from force transducers mounted within the EquiTest force plates. The AP peak-to-peak sway angle (p-p sway) is used to compute the equilibrium score (EQ), where 12.5° is the maximum theoretical p-p sway.

No exercise is permitted prior to testing on the day of the scheduled test. A normal or light meal may be consumed 3 hours before testing. However, no food should be eaten within 30 minutes of testing. Medications that effect sensorimotor performance should not be taken 24 hours prior to testing. No other medications should be administered within 12 hours of testing.

This test is completed once in pre-bed rest on BCD-1 and on R+0. This test takes about 30 minutes to complete.

11.1.2. Treadmill Test

The level of conditioning and consequently, the effectiveness of the training performed by crewmembers are also determined in long-duration flight according to the parameters of the treadmill test with gradually increasing locomotor loads on the treadmill. A distinctive feature of the test is the standardization of the sequence and length of each of the five load levels with voluntary selection of work intensity within each level. The speed limits of slow, moderate, and fast running are self-selected by the subject. Parameters collected during each of the five load levels are running speed (m/s) and heart rate (bpm). The intensity of work performed is then expressed as the ratio of peak heart rate to peak running velocity (beats/60 m).

The treadmill test consists of several steps: warm-up walking for 3 minutes, slow running for 2 minutes, moderate running for 2 minutes, fast running for 1 minute, and walking for 3 minutes as the final step. The test is performed in idling regimen (passive mode) of treadmill. In this mode, the treadmill belt is driven by the force of the running subject. The length of the test is 11 minutes, and the energy expended during the experiment is approximately 100 kcal. Steps for this test are summarized in Table 11-2.

Data are collected once during pre-bed rest and once during post bed rest at R+0. No large meals should be eaten within 1.5 hours of testing. However, a light complex carbohydrate meal is recommended before testing and should be eaten within 1.5 hours to 30 minutes prior to testing. Subjects should not undergo maximum exercise within 18 hours before testing. Regular exercise (submaximal exercise) should not occur within 8 hours prior to testing. This test takes about 20 minutes to complete.

Table 11-2. Steps for Locomotor Test

Steps	Time (min)
Warm-up walking	3
Slow running	2
Moderate running	2
Fast running	1
Walking	3

11.2. Recommended Measures

11.2.1. Dynamic Gait Index (DGI)

The Dynamic Gait Index (DGI) was developed as a clinical tool to assess gait, balance and fall risk (Shumway-Cook et al., 1997). It evaluates not only usual steady-state walking, but also walking during more challenging tasks. Eight functional walking tests are performed by the subject. Performance of each test is rated on a scale of 0 to 3 where 3 indicates the best score. Twenty four is the total individual score possible. Scores of 19 or less have been related to increased incidence of falls (Shumway-Cook et al., 1997).

11.2.2. Seat Egress and Walk Test

The ability to ambulate following bed rest is assessed with the Seat Egress and Walk Test. This test was designed to measure the ability to rise from a seated position and walk while avoiding obstacles. For this test subjects unbuckle a harness while in a seat and stand up. Testing occurs twice. Once with the seat upright and once with the seat back positioned on the floor. Following egress from the seat, subjects will walk through the obstacle course as quickly and as safely as possible without touching any of the obstacles. Subjects negotiate a portal and pylons, and walk up and down a sloped surface inclined at 18°. Time to complete the course and number of obstacles hit are recorded.

Upon completion of the obstacle course subjects perform a simple line walk test. Subjects walk 10 steps with the eyes closed, arms and hands folded across the chest, while placing the feet in the traditional tandem heel-to-toe position for each step. The performance metric is the number of correct steps made during the trial.

11.2.3. Gaze Control

A battery of tests will be performed to assess changes in gaze control associated with bed rest. These tests include:

- Spontaneous nystagmus and gaze-holding
- Visual saccades to unpredictable targets ranging from ± 5° to ± 30° eccentric in both the horizontal and vertical planes

- Pursuit of visual targets with and without background distraction (in a range of 5 to 30°)
- Combined active eye-head tracking using the same parameters as for pursuit and the saccade test
- Dynamic visual acuity (DVA)

For this test subjects will identify stationary optotypes on a display panel during head movements. The optotypes are displayed only when head velocity exceeds a threshold, so that DVA can be assessed.

11.2.4. Force Control

To determine the effects of bed rest on proprioceptive feedback, subjects will perform a force control test. Force control will be assessed with the use of the BTE PrimusRS System (BTE Technologies, Hanover, MD). This system features numerous attachments that are used for rehabilitation and work/task simulations. A hand-grip attachment will be affixed to the PrimusRS. Seated subjects will be asked to perform a series of isometric hand flexions from a minimal force to a sub-maximal force with minimal differences in the intensity of subsequent movements.

11.2.5. T-Reflex Function

This test compares the effects of bed rest on the amplitude and latency of the stretch reflex (T-reflex) (Reschke et al., 2009). The T-reflex is a monosynaptic reflex (MSR) that uses feedback to control increasing muscle tension by causing muscle relaxation before tension force becomes so great it may damage the muscle. For data collections, subjects will lay in the prone position either 0° horizontally (pre- and post-bed rest), or 6° head down (in bed rest) with the ankle firmly attached to an 80 ft-lb DC-servo motor using a footplate. Electromyography (EMG) electrodes with a high impedance probe are then placed on the triceps surae and the anterior tibialis muscle groups. A dorsiflexion torque will then be applied by the motor, moving the toe forward. During these trials, subjects are instructed to provide no resistance to the torque, enabling collection of T-reflex data. EMG is analyzed for both latencies and amplitudes.

12. Cardiovascular Standard Measures

12.1. Required Measures

12.1.1. Tilt Test

The tilt test is used to assess orthostatic tolerance before and after bed rest (Stenger et al., 2012; Stenger et al., 2010; Platts et al., 2009). Subjects are instrumented while supine and this position is maintained while baseline data are collected for 5 minutes. The table is then tilted to 80° placing subjects in a head-up tilt position at a rate of approximately 7°/s. Subjects remain in this position for 15 minutes or until they exhibit symptoms of presyncope. Total time standing at 80° head-up tilt is recorded. Longer durations for the tilt test are acceptable provided data are collected at the 15-minute time point.

During the test, continuous measures are obtained for blood pressure, electrocardiography (ECG), and Doppler ultrasound of blood flow velocity at the suprasternal notch. Finger arterial blood pressure is sampled at 200Hz using a photoplethysmography device (Finometer Pro, Finapres Medical Systems, Netherlands). This device uses a hydrostatic adjustment routine to provide an accurate estimation of blood pressure independent of sensor location with respect to the heart. Oscillometric brachial artery pressure is also measured using a cuff placed around the upper arm every minute (Dinamap XL Vital Signs Monitor, GE Medical Systems Information Technologies, Milwaukee, WI). Systolic and diastolic blood pressure are recorded and mean arterial pressure (MAP) is calculated as $MAP = [(2 \times \text{diastolic}) + \text{systolic}] / 3$. ECG data are collected at 100Hz using a 5-lead system (Escort II, Medical Data Electronics, Arleta, CA). Heart rate measures are derived from the ECG and ECG collection is synchronized with the suprasternal notch Doppler ultrasound signals for stroke volume and cardiac output calculations. Two-dimensional echocardiography is used to obtain the aortic annulus diameter from the parasternal long axis during supine rest prior to data collection. The aortic blood velocity time integral is measured for each beat during supine rest and during the period of 80° head-up tilt. These Doppler measurements are made at the suprasternal notch using a 1.9 MHz pulsed wave Doppler probe (Biosound MyLab Gold, Indianapolis, IN). Images are stored digitally for subsequent analyses. To insure accuracy, images from at least three cardiac cycles during inspiration are independently analyzed by two experienced sonographers. Stroke volume (annulus diameter x velocity time integral) and cardiac output (stroke volume x heart rate) are calculated.

Maximal exercise and medications are not permitted for a 24-hour period prior to testing. Caffeine, nicotine, or alcohol shall not be consumed within 12 hours prior to testing. Heavy meals should be avoided 4 hours before testing. However, a light snack of complex carbohydrates 2 hours before testing is permitted.

This test is completed once in pre-bed rest on BDC-5 to ensure that subjects have stabilized with respect to their dietary intake. The tilt test is performed on R+0 and is usually the first test on that day that brings the subject upright. This test takes about 50 minutes to complete.

12.1.2. Maximal Aerobic Capacity (VO_2 max)

Decreased aerobic capacity as a result of spaceflight has also been documented in the bed rest analog (Convertino et al., 1986). Maximum aerobic capacity is assessed using a graded exercise protocol on an electronically-braked cycle ergometer (Lode Excalibur Sport; Lode B.V., Groningen, The Netherlands), and a metabolic cart for gas exchange determination (TrueOne® 2400, ParvoMedics, Sandy, UT). For accurate collection of gases, subjects wear a nose clip and breathe through a respiratory valve. The graded exercise protocol provides an individualized approach to achieve subjects' maximum aerobic capacity using small increments in workload. Subjects warm up cycling at a light workload (0 to 75 Watts) for 1 to 3 minutes. During testing, subjects maintain a pedaling cadence of 70 to 75 revolutions per minute (rpm). Workload begins at 50 Watts for 3 minutes and is then increased by 25 W every minute. Subjects who are small or have low initial aerobic fitness may use an alternative light protocol starting at 45 W for 3 minutes followed by 15 W increases each minute. Increasing workload in small increments with each minute of exercise allows for optimal evaluation of ventilatory threshold and VO_2 max (Amann et al., 2004). Using this protocol, maximal exercise is achieved in approximately 8 to 15 minutes. Testing is completed when subjects: 1) are no longer able to maintain a pedaling rate of 70 rpm, 2) reach a plateau in oxygen uptake (VO_2) despite further increases in workload, 3) achieve a heart rate greater than 90% of age predicted maximum accompanied by a respiratory exchange ratio (RER) (VCO_2/VO_2) greater than 1.10, or 4) indicate a desire to stop the test.

Heart rate is monitored continuously during testing (Q-Stress ECG monitor, Quinton Instruments, Seattle, WA). Blood pressure is measured during each of the first 3 stages, and every 2 minutes during the subsequent 1-minute stages, using a sphygmomanometer and stethoscope. Measures collected (or derived) through gas exchange data include VO_2 , carbon dioxide elimination (VCO_2), RER, minute ventilation (V_E), anaerobic threshold and ventilatory threshold. Ventilatory threshold is calculated by identifying the breakpoint of V_E from VO_2 as described by Amann et al., 2004. Workload is also recorded.

This test is performed once in pre-bed rest and once in post-bed rest. This test can be performed in the morning. However, on R+0 this test is performed in the afternoon following all other testing activities. A light complex carbohydrate meal is recommended before testing and should be eaten within 3 hours to 30 minutes prior to testing. Subjects should not undergo maximum exercise within 18 hours before testing. Regular exercise (submaximal exercise) should not occur within 8 hours prior to testing. This test takes about 60 minutes to complete.

12.2. Recommended Measures

12.2.1. Electrocardiogram (ECG)

A 5-lead ECG is collected as part of the tilt test. Additional resting ECG measures can be collected using a 12-lead system during pre- and post-bed rest.

12.2.2. Holter Monitor

The Holter monitor is a wireless portable device that continuously monitors cardiac activity. Using this device, ECG signals can be collected over long periods of time (usually 24 hours). A 5-lead system is typically used.

12.2.3. Plasma Volume

Plasma volume is used to assess the cephalad fluid shifts that occur with 6° HDT bed rest. The carbon monoxide rebreathing technique is used to assess plasma volume (Platts et al., 2009). Values are corrected for body surface area and reported as plasma volume index.

12.2.4. Echocardiography

Two-dimensional Doppler ultrasound is used to collect cardiac measures during the tilt test. Vascular measures of the jugular and femoral vein can also be assessed. Additionally, more sophisticated measures can be collected using 3-dimensional ultrasound techniques.

13. Muscle Standard Measure

13.1. Required Measures

13.1.1. Muscle Strength

Isometric and isokinetic testing provide an assessment of muscle strength and endurance. These assessments can be completed using any isokinetic dynamometer (such as the Biodex Dynamometer, Biodex Medical Systems, Inc., Shirley, NY).

Isometric maximum voluntary contractions will be completed for muscles of the knee and ankle. This test provides a very fast and reliable measurement of isometric strength of two important lower body muscle groups.

To test isometric knee strength, the seated subject is positioned in the dynamometer with the knee flexed at 60°. The subject then performs three sets of maximum contractions alternating between flexion and extension muscle contractions. Each muscle contraction should last 5 to 7 seconds with 30 seconds of rest between flexion/extension contractions.

For testing isometric ankle strength, the subject is placed in the prone position with the ankle positioned at 90°. Three sets of maximum contractions are performed alternating between plantar flexion and dorsiflexion muscle contractions. Muscle contractions should last 5 to 7 seconds with 30 seconds of rest between plantar and dorsiflexion contractions.

For all isometric muscle testing, the highest value of torque (Nm) obtained is considered the subject's maximum. If a subject continues to improve at the third contraction, proceed with testing until no further improvement is observed.

Isokinetic muscle testing is used to assess strength of knee, ankle and trunk musculature. In addition, endurance testing for muscles of the knee is also performed. As a warm-up, subjects complete 5 minutes of light exercise (50 Watts) on a cycle ergometer. For each isokinetic test, muscles are warmed up prior to testing by completing 5 contractions with increasing force levels. A 2 to 3 minute rest period is provided between testing of each muscle group. For standardization purposes, knee and ankle testing are performed on the right lower extremity.

For isokinetic testing of the knee, the subject is placed in a seated position. Knee range of motion on the dynamometer is set for 20° to 95°. The weight of the limb is assessed with the knee positioned at 30°. Isokinetic peak torque (Nm) is assessed at a speed of 60°/s. The subject performs 3 repetitions of continuous concentric extension and flexion motion at maximal effort.

Subjects are provided a 2-minute rest period prior to endurance testing. Endurance testing of the knee is assessed at a speed of 180°/s. The subject performs 20 repetitions of continuous flexion and extension motion at maximal effort. Total work in Nm is recorded. Table 13-1 summarizes the isokinetic protocol for the knee.

Table 13-1. Isokinetic Knee Protocol

Speed	Warm-up	Test Repetitions	Rest
Knee (Concentric)			
0°/s (Isometric Mode)	None	Practice Test (2 Sub, 1 Near Max)	60 seconds
0°/s (Isometric Mode)	None	3 Max	60 seconds
60°/s	2 Submax	3 Max	60 seconds
Knee (Endurance)			
180°/s	2 Submax	20 Max	Set-up for next test

Isokinetic testing of the ankle is done with the subject in the prone position. Range of motion of the dynamometer is adjusted to 5° less than the subject's maximum position of dorsiflexion and 5° less than the subject's maximum position of plantar flexion. Weight of the limb is assessed with the ankle positioned at 15°. Isokinetic peak torque (Nm) is assessed at a speed of 30°/s. Three repetitions of continuous concentric dorsi- and plantar flexion contractions are performed at maximal effort. A rest period of about 2 minutes is provided before testing is repeated to assess peak torque (Nm) for eccentric muscle contractions. Table 13-2 summarizes the isokinetic protocol for the ankle.

Table 13-2. Isokinetic Ankle Protocol

Speed	Warm-up	Test Repetitions	Rest
Ankle (Concentric)			
30°/s	2 Submax	3 Max	60 seconds
Ankle (Eccentric)			
30°/s (Passive Mode)	None	Practice Test (3 Submax)	60 seconds
30°/s (Passive Mode)	None	3 Max	60 seconds
30°/s (Passive Mode)	None	Practice Test (3 Submax)	60 seconds
30°/s (Passive Mode)	None	3 Max	During set-up for next test

Assessment of isokinetic trunk strength is done with the subject in a standing position. Range of motion on the dynamometer is set for 0° to 90° of motion. Weighing of the trunk is not required. Peak torque (Nm) is assessed at a speed of 60°/s. The subject performs 3 repetitions of continuous concentric flexion and extension motion at maximal effort. Table 13-3 summarizes the isokinetic protocol for the trunk.

Table 13-3. Isokinetic Trunk Protocol

Speed	Warm-up	Test Repetitions	Rest
Trunk (Semi-standing position)			
60°/s	2 Submax	3 Max	Test Complete

Data are collected once during pre-bed rest and once during post-bed rest at R+2. No large meals should be eaten within 1.5 hours of testing. However, a light complex carbohydrate meal is recommended before testing and should be eaten within 1.5 hours to 30 minutes prior to testing. Subjects should not undergo maximum exercise within 18 hours before testing. Regular exercise (submaximal exercise) should not occur within 8 hours prior to testing. This test takes about 50 minutes to complete.

13.1.2. Neuromuscular Power – Vertical Jump

Vertical jump is used as an assessment of whole-body-power output (Rittweger et al., 2007). It is a highly reliable measure that is free of any learning effect (Rittweger et al., 2004). A ground-reaction platform (Kistler Instrument Corp., Amherst, NY) is used for data collection. Data are collected with a custom LabVIEW software program (National Instruments Corp., Austin TX) and sampled at 1000 Hz using a National Instruments data acquisition system (National Instruments Corp., Austin TX). Prior to the jumping task the subject performs a warm-up session and is instructed to perform 3 warm-up squats. The subject is then instructed to perform 2 to 3 practice countermovement jumps at 50% of maximum effort to ensure that proper technique is understood. Countermovement jump begins by anchoring the hands on the waist while standing with erect posture. The movement is performed by the subject quickly dropping into a squat then reversing the direction by pressing into the platform with maximum force with the aim to jump as high as possible. This motion does not allow pause during the movement and requires the hands to be anchored to the hips at all times to remove moments of inertia contributed by the arms swinging. Once the subject warms up and proper technique is demonstrated, the operator allows the subject to attempt 3 maximum effort jumps. Prior to jumping, body mass is measured during quiet stance assuming acceleration due to Earth’s gravity to be 9.8 m/s², then the subject is instructed to jump and encouraged to elevate the head as high as possible. The subject rests 60 to 90 seconds in between each jump or longer if the subject desires more time between tests. The sum of the vertical ground reaction forces collected from the force plate are divided by body mass to determine the subject’s acceleration profile. To calculate the subject’s jump height a double integration of the acceleration profile is computed. Instantaneous power is calculated as the product of acceleration and velocity. Variables of peak acceleration in m/s² (A_{peak}), peak velocity in m/s (V_{peak}), jump height in cm (H_{peak}), and peak power in kW (P_{peak}) are then assessed as the maximum of these respective curves.

This test is completed once during pre-bed rest and once during post-bed rest at R+0. No large meals should be eaten within 1.5 hours of testing. However, a light complex carbohydrate meal is recommended before testing and should be eaten within 1.5 hours to 30 minutes prior to testing. Subjects should not undergo maximum exercise within 18 hours before testing.

Regular exercise (submaximal exercise) should not occur within 8 hours prior to testing. This test takes about 30 minutes to complete.

13.2. Recommended Measures

13.2.1. Muscle Size

Magnetic resonance imaging (MRI) is recommended to obtain upper and lower leg cross sectional scans along the limb for the evaluation of muscle cross sectional area and volume. Any commercially available MRI scanner can be used. MRI provides an objective, volitional independent measure of muscle size to evaluate atrophy.

13.2.2. Muscle Biopsy

The purpose of the muscle biopsy is to provide an unbiased, motivation independent measure of muscle fiber size and function. Muscle biopsy provides an evaluation of muscle function in the absence of neural activation, and an evaluation of metabolic enzymes in the muscle. Muscle biopsies are typically taken from the thigh (vastus lateralis) and/or calf (soleus) muscles depending on the relevance for the study. Parameters measured from biopsy tissue include single muscle fiber size and contractile function on slow- and fast-twitch muscle fibers, single muscle fiber type identification, and biomarkers for aerobic and glycolytic enzyme activity.

14. Bone Standard Measures

14.1. Required Measures

14.1.1. Bone Mineral Density

Typical changes observed in bone due to long duration bed rest are rapid, but highly variable, decreases in areal bone mineral density (BMD) (g/cm²). These changes occur in weight-bearing skeletal sites and are similar to changes in spaceflight induced deficits, but at lower magnitude (Spector et al., 2009). Measures of 2-dimensional bone mineral density (BMD) are obtained by dual-energy X-ray absorptiometry (DXA) using, e.g., a Hologic Discovery Unit whole body densitometer (Hologic, Inc., Bedford, MA). Calibration shall be completed to ensure accuracy of the data. Specifically, a phantom scan is performed daily for quality control, which involves a self calibration process utilizing Hologic’s Automatic Internal Reference System. It is highly desirable to use the same operator to collect all scans for a study to further ensure data accuracy. Prior to testing, the operator shall ensure that the test subject is free of external metal objects such as metal buttons, jewelry, zippers and belts. The operator shall also confirm that the subject is free of internal metal objects such as pacemaker leads, radioactive seeds, metal implants and surgical staples. The subject is positioned by the operator for each scan. The subject is instructed to lie still during the scan, but may move between scans.

Scans are obtained in triplicate from the following sites: whole body, lumbar spine, and hip. The forearm and calcaneus are recommended sites depending upon study specific requirements. These sites have demonstrated minimal evidence for change in bed rest (Spector et al., 2009), but may be critical for evaluation of specific countermeasure studies. Triplicate values for each scan type are averaged to improve measurement precision. As mentioned, all scans are performed and analyzed by a single operator to reduce inter-operator variation. For repeated measures such as pre- to post-bed rest, scans should be completed using the same densitometer to allow for accurate comparisons. Variables of BMD and bone mineral content (BMC) are derived from the scans and percent change from pre- to post bed rest is calculated at each site. The changes in BMD are considered biologically significant if absolute BMD or percentage change from baseline exceed the least significant change as determined by a previously conducted precision assessment (<http://www.iscd.org/Visitors/positions/OfficialPositionsText.cfm>).

Subjects shall not have any radioisotopes or radiopaque contrast agents within one week prior to testing. Females shall have a negative pregnancy test within 24 hours of testing. DXA scans are completed once in pre-bed rest (BDC-13) and once 14 days post-bed rest. The 14 days are recommended as the post-bed rest time point for all imaging technologies based on the detection of a reversal in bone mass loss at the 2-week time point (Rittweger et al., 2010).

14.1.2. Bone Markers

Bone turnover markers suggest that bed rest increases bone degradation but has minimal impact on bone formation (Zwart et al., 2009).

Circulating bone- and calcium-related factors (such as parathyroid hormone [PTH], bone-specific alkaline phosphatase [BSAP], and osteocalcin) are assessed in serum. PTH is assayed for the intact peptide by radioimmunoassay (RIA). Serum osteocalcin is also determined by RIA (Gundberg et al., 1998; Zwart et al., 2011a). Serum BSAP activity and N-terminal propeptide of type I procollagen (PINP) is determined by enzyme linked immunoassay (ELISA) (Spector et al., 2009; Smith et al., 2005a).

Urine samples are analyzed for collagen crosslinks, including N-telopeptide, C-telopeptide, and deoxypyridinoline, using commercially available kits (Osteomark® ELISA kit, Ostex International, Inc., Seattle, WA; Urinary CrossLaps ELISA, Nordic Bioscience Diagnostics, Herlev, Denmark; Pylinks-D, Metra Biosystems, Palo Alto, CA). These widely used markers of bone resorption are extremely reliable when evaluating within-subject interventions (for example, spaceflight, countermeasure use) (Smith et al., 2005a; Heer et al., 2005).

Calcium assessments are also conducted on blood and urine samples. Whole-blood ionized calcium (and pH) is determined electrochemically using a portable analyzer (Smith et al., 1997; Smith et al., 2004). Serum total calcium and urinary calcium are determined using atomic absorption spectrophotometry (Smith et al., 2005a; Smith et al., 2008).

Vitamin D status is clearly altered after long-duration spaceflight (about 4 to 6 months). The absence of ultraviolet light during spaceflight diminishes vitamin D stores in the body (Leach et al., 1977; Smith et al., 2005b; Smith et al., 2009). Serum 1, 25 dihydroxyvitamin D is measured by RIA after extraction of samples with acetonitrile and purification on C18OH cartridges, and serum 25 hydroxyvitamin D determined by RIA after acetonitrile extraction, as reported previously (Smith et al., 2005a; Smith et al., 2005b; Zwart et al., 2011b; Zwart et al., 2009).

Endocrine factors that regulate and affect bone are also determined, to provide a complete profile of bone and calcium regulation and understand the effects of the countermeasures under consideration. These include testosterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), cortisol, estradiol, thyroxine (free T4), and thyroid stimulating hormone (Smith et al., 2012; Smith et al., 2003). Liquid chromatography with tandem mass spectrometry, ELISA, or RIA procedures are used for these analyses. These required bone standard measures are summarized in Table 14-1.

Table 14-1. Required Bone Standard Measures

Measures of Bone Mineral Content (BMC) and Bone Mineral Density (BMD)	
DEXA	BMC, Areal BMD (whole body, regional lumbar spine and hip, calcaneus and forearm*), Body Composition
Blood/Serum Chemistry**	
Calcium Homeostasis	Total Calcium, Whole-blood Ionized Calcium
Gonadal Hormones	Testosterone, Estradiol
Calcitropic Hormones	25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, intact Parathyroid Hormone (PTH)

Endocrine Regulators	Thyroxine (Free T4), Thyroid Stimulating Hormone (hTSH III), Cortisol
Bone Turnover Markers (Bone Formation)	Osteocalcin, Bone Specific Alkaline Phosphatase (BSAP), N-terminal propeptide of type I procollagen (PINP)
Urinary Measures	
Bone Turnover Markers (Bone Resorption)	N-telopeptide, C-telopeptide, Deoxypyridinoline
Minerals	Calcium

*Whole body, regional lumbar spine and hip are required measures. Calcaneus and forearm are recommended.

**Measures may overlap with the Nutrition/Hematology Discipline. When this is the case, measures are shared between disciplines.

14.2. Recommended Measures

The imaging technology of peripheral quantitative computerized tomography (pQCT) (Stratec, Pforzheim, Germany) is a recommended measure. The pQCT provides a 3-dimensional assessment of bone structure for trabecular and cortical bone compartments. The pQCT and other recommended measures are listed in Table 14-2.

Table 14-2. Recommended Bone Standard Measures

Measures of Bone Mineral Content (BMC), Bone Mineral Density (BMD), and Bone Structure	
Peripheral QCT	BMC, BMD, cross-sectional distributions (sites of lower limb (tibia), stress-strain index (calculated))
Urine and Blood	
Urine	Total volume, Creatinine, Phosphorus, Magnesium and Pyridinoline
Blood (Cytokines and Cell Signaling/Mediators)	Osteoprotegerin (OPG), Osteoprotegerin ligand (receptor activator of nuclear factor-kB ligand or RANKL), Insulin-like Growth Factor-1, Leptin, Vitamin D binding protein, total alkaline phosphatase. Cytokines*: TNF alpha, IL-6
Renal Stone Risk*	Sodium, Potassium, Uric Acid, Citrate, Oxalate, Sulfate, Supersaturation of Brushite, Struvite, and Calcium Oxalate

*Measure is required for Nutrition / Hematology and can be shared with Bone Standard Measures.

15. Nutrition / Hematology Standard Measures

15.1. Required Measures

General chemistries provide a general overview of crew member and subject health. Serum chemistry and hematology analyses are conducted using standard clinical techniques. Serum electrolytes, total protein, total cholesterol, triglycerides, albumin, transferrin, liver enzymes (ALT, AST), urinary magnesium, and total alkaline phosphatase are assayed using an automated clinical chemistry system (Olympus AU480, Olympus America Inc., Center Valley, PA). Ionized calcium (iCa), pH, and the partial pressures of carbon dioxide and oxygen (pCO2, pO2) are analyzed in whole blood from venous or finger-stick blood samples (Smith et al., 2004). Fibrinogen is measured by nephelometry using a BN2 analyzer (Dade Behring/Siemens Medical Solutions, Diagnostics GmbH, Bad Nauheim, Germany). Urinary creatinine and phosphorus are measured spectrophotometrically using an ACE Alera® clinical chemistry system (Alfa Wassermann, West Caldwell, NJ, USA). Urine pH is determined using a pH meter. For the complete list of panels (chemistry, hematology, and finger-stick), see Table 15.1.

Changes in iron metabolism represent a significant concern for astronauts (Smith et al., 2009) Therefore, indices of iron status, storage, and transport are determined for bed rest studies. Hemoglobin, hematocrit, and mean corpuscular volume (MCV) are determined using a Coulter LH750 instrument (Beckman Coulter, Brea, CA, USA). Serum ferritin is analyzed using the Advia Centaur XP immunoassay system (Siemens Medical Solutions, Diagnostics GmbH, Bad Nauheim, Germany). Standard clinical techniques are used for determination of transferrin and total iron-binding capacity (TIBC). Transferrin receptors are determined using a commercially available ELISA (Ramco Laboratories, Houston, TX, USA). Iron and ferritin iron content and concentration are determined by inductively-coupled plasma mass spectrometry (ICP-MS) using a method previously described (Smith et al., 2005b).

Calcium status is assessed via analyses of blood and urine samples. Whole-blood ionized calcium (and pH) is determined by ion-specific electrometry performed with a portable analyzer (i-STAT, Princeton, NJ, USA). Serum total calcium and urinary calcium are determined using atomic absorption spectrophotometry (Smith et al., 2005a; Smith et al., 2008).

Endocrine factors that regulate and affect bone are also determined to provide a complete profile of bone and calcium regulation and to help understand the effects of countermeasures. These factors include testosterone, DHEA, DHEA-S, cortisol, estradiol, thyroxine (free T4), and thyroid stimulating hormone (Smith et al., 2012 ; Smith et al., 2003). Liquid chromatography with tandem mass spectrometry, ELISA, or RIA procedures are used for these analyses. Cytokines (including interleukin-6 and tumor necrosis factor- α) are measured using ELISAs (Cayman Chemical, Ann Arbor, MI).

Study of serum and urinary minerals is critical for bed rest studies. Minerals such as magnesium and phosphorus were less in astronauts after landing when compared with pre-flight values (Smith et al., 2005b). Other minerals also play key roles in health status and are therefore examined in bed rest subjects. Copper, zinc, iodine, selenium, iron, magnesium, phosphorus are determined using ICP-MS and colorimetric techniques.

Generally, renal stone risk increases during spaceflight. This increase is related in large part to insufficient water intake along with increased calcium excretion in some crew members (Whitson et al., 2001). The renal stone risk profile is a panel of measures including urinary pH, sodium, potassium, calcium, oxalate, citrate, total volume, phosphorus, magnesium, uric acid, and sulfate. Supersaturation values for calcium oxalate, brushite, struvite, uric acid, and sodium urate are calculated from the panel. Multistix 10SG reagent strips (Siemens Healthcare Diagnostics Inc., Tarrytown, NY) are run on a Clinitek analyzer for urinalysis of protein, pH, color, specific gravity, glucose, bilirubin, ketone, blood, urobilinogen, nitrite, and leukocyte esterase.

Determining protein status is critical for many reasons related to muscle loss, hydration status, and renal function (Smith et al., 2009). Serum ceruloplasmin, retinol-binding protein, and transthyretin are analyzed by nephelometry with a BN2 analyzer (Dade Behring/Siemens Medical Solutions, Diagnostics GmbH, Bad Nauheim, Germany). Assay of urinary 3-methylhistidine is performed by ion-exchange chromatography on a Hitachi L8800 amino acid analyzer (Hitachi High Technologies, San Jose, CA). Urinary nitrogen is analyzed by pyrochemiluminescence using an Antek 7000 analyzer (Antek Instruments Inc., Houston, TX).

Water-soluble vitamins are a primary concern during spaceflight, and any other time when individuals are on a closed food system. RBC folate decreased about 20% ($P < 0.01$) after 4 to 6 months of spaceflight. Before launch, the RBC folate of most crew members was at or near the upper limit of the normal range. After landing, the RBC folate of approached the lower limit of normal range. It is not known if this decrease in folate status would level off or continue to decrease with missions of even longer duration (Smith et al., 2009). Vitamin B6 is critical in muscle metabolism, and is affected by muscle loss in bed rest (Coburn et al., 1995). One-carbon metabolism, the status of which is affected by folate, vitamin B12, and vitamin B6 status, is assessed through analysis of a homocysteine metabolite panel. The RBC transaminase, glutathione reductase, and transketolase assays, which assess the in vitro activity of the enzymes, are functional indicators of vitamin B6, riboflavin, and thiamin status. These are analyzed spectrophotometrically on the ACE Alera® clinical chemistry system (Alfa Wassermann, West Caldwell, NJ). RBC folate and serum folate are measured using a commercially available radioreceptor assay (Siemens Medical Solutions, Diagnostics GmbH, Bad Nauheim, Germany). Plasma pyridoxal 5'-phosphate and 4-pyridoxic acid are measured by high-performance liquid chromatography (HPLC). Samples for analysis of plasma homocysteine are analyzed by Metabolite Laboratories (Denver, CO) for gas chromatography-mass spectrometry. Vitamin C is analyzed by HPLC.

Fat-soluble vitamins are determined, including status markers for vitamins A, E, D, and K. As described under Bone Standard Measures (Section 14.1.2), vitamin D status is clearly altered after long-duration spaceflight (about 4 to 6 months) (Smith et al., 2005a; Smith et al., 2009). The absence of ultraviolet light during spaceflight diminishes vitamin D stores in the body. Serum 1,25 dihydroxyvitamin D is measured by RIA after extraction of samples with acetonitrile and purification on C18OH cartridges, and serum 25 hydroxyvitamin D is determined by RIA after acetonitrile extraction. Vitamin D-binding protein and plasma heme are assayed as previously described using commercially available kits (Zwart et al., 2011b; Zwart et al., 2009).

Vitamin K has relevance for bone health, primarily related to its involvement in the formation of gamma-carboxyglutamic acid (GLA) residues in proteins, such as osteocalcin and matrix GLA protein. Early studies showed that vitamin K supplementation during spaceflight significantly elevated urinary GLA excretion and decreased the excretion rate of undercarboxylated osteocalcin, suggesting that vitamin K status was compromised during spaceflight (Caillot-Augusseau et al., 2000). More recent studies on the ISS have documented that the current food system provides adequate vitamin K (Zwart et al., 2011a). Vitamin E is a critical antioxidant nutrient, and of the two primary vitamers, gamma-tocopherol but not alpha-tocopherol was decreased after long-duration spaceflight (Smith et al., 2005b). For these reasons, fat-soluble vitamins A, E, and K are examined during bed rest studies. Vitamins A and E and their metabolites are analyzed using HPLC with electrochemical detection. Vitamin K is analyzed using HPLC with fluorimetric detection.

Serum clinical chemistry (carbon dioxide, blood urea nitrogen, phosphorus, magnesium, bilirubin, glutamyltransferase, lactate dehydrogenase, creatine kinase, uric acid, C-reactive protein, creatinine, glucose) are analyzed using standard methods with a clinical analyzer (Beckman AU4080). Whole-blood chemistry (white blood cell count and differential, red blood cell count, hemoglobin, mean corpuscular volume, platelet count, and reticulocyte count) is also analyzed using a clinical analyzer (Beckman Coulter LH750). All of the required standard measures are summarized in Table 15-1.

Table 15-1. Required Nutrition / Hematology Standard Measures

Required Blood Measures	
Serum Chemistry*	Carbon Dioxide, Blood Urea Nitrogen, Phosphorous, Magnesium, Bilirubin, Glutamyltransferase, Alkaline Phosphatase, Lactate Dehydrogenase, Creatine Kinase, Uric Acid, C Reactive Protein, Sodium, Potassium, Chloride, Creatinine, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Cholesterol, Triglyceride, Glucose, Calcium
Whole Blood Analysis (CBC/differential/Platelets)	White Blood Count and differential, Red Blood Count, Hemoglobin, Mean Corpuscular Volume (MCV), Platelet Count, Reticulocyte Count Calculated values: Relative (Red Cell) Distributive Width (RDW), Hematocrit, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC)
Coagulation Test	Fibrinogen
Finger-Stick Tests (whole blood)	iCa, pH, PCO ₂ , PO ₂ (optional tests, if included in analysis: Na, K, glucose, hematocrit) Calculated Values: TCO ₂ , HCO ₃ , BE, sO ₂ , Hgb
Hematologic and Iron Status Indicators	Transferrin Receptors, Transferrin, Ferritin, Ferritin Iron, RBC Folate, Iron, Total Iron Binding Capacity (TIBC) Calculated values: Ferritin Iron % Saturation, Transferrin Saturation

Ionized Calcium Profile†	Whole-blood Ionized Calcium, pH-Whole-blood Calculated value: Ionized Calcium at pH 7.40
Hormones	Thyroxine (Free T4), Thyroid Stimulating Hormone (hTSH III), Testosterone, Estradiol, Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone Sulfate (DHEA-S), Cortisol, Cytokines: TNF alpha, IL-6
Mineral Status	Zinc, Selenium, Iodine, Copper, Ceruloplasmin
Protein Status	Retinol Binding Protein, Transthyretin, Total Protein, Albumin
Nutritional Assessment (if blood volume allows)‡	
Water Soluble Vitamin Status‡	Erythrocyte Transketolase Stimulation, Erythrocyte Glutathione Reductase Activity, Erythrocyte nicotinamide adenosine dinucleotide and nicotinamide adenosine dinucleotide phosphate (NAD/NADP), Erythrocyte Transaminase Activity, Red Cell Folate, Folate, Homocysteine, Vitamin C, Pyridoxal 5-phosphate (PLP)
Fat Soluble Vitamin Status‡	Retinol, Retinyl palmitate, β-carotene, α-carotene, Serum Phylloquinone, α-tocopherol, γ-tocopherol, Tocopherol : lipid ratio, vitamin D binding protein and plasma heme, 25-hydroxyvitamin D
Required Urinary Measures	
Urinanalysis	Specific Gravity, pH, Color, Appearance, Protein, Glucose, Bilirubin, Urobilinogen, Ketone, Nitrite, Blood, Leukocyte Esterase, Total volume, pH, Creatinine
Minerals	Calcium, Phosphorus, Magnesium, Copper, Selenium, Zinc, Iodine
Protein Status	3-methyl histidine, Nitrogen
Renal Stone Risk	Sodium, Potassium, Uric Acid, Citrate, Oxalate, Sulfate, Supersaturation of Brushite, Struvite and Calcium Oxalate
Nutritional Assessment (if blood volume allows for full blood testing complement, then the following urine tests shall be included as well)‡	
Water Soluble Vitamins‡	N-methyl nicotinamide, 2-pyridone, 4-pyridoxic acid
Other Measures	
Body Mass	Daily
DXA for body composition	Before and after bed rest
Dietary Intake	Daily

*Measures may overlap with the Immunology Discipline. When this is the case, measures are shared between disciplines.

†Measure is also required for bone standard measures and can be shared between disciplines.

‡These measures are considered required if blood volume is not exceeded.

15.2. Recommended Measures

The recommended measures include experiment specific assessments. These measures would be included as standard measures in the event that countermeasure testing was expected to have an effect on the listed systems. These include antioxidants and oxidative damage markers, bone markers, cytokines, inflammatory markers, and evaluation of metabolic rates via indirect calorimetry. Recommended measures are listed in Table 15-2.

Table 15-2. Recommended Nutrition / Hematology Standard Measures

Blood Measures	
Antioxidants and Markers of Oxidative Damage	Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Malondialdehyde (MDA), 4-OH-alkenal, 4-hydroxynonenal, Glutathione, Protein Carbonyls, Prostaglandin F2-α (PG F2-α)
Bone Markers*	Intact Parathyroid Hormone (PTH), Calcium, 1,25-dihydroxyvitamin D, Osteocalcin, Bone Specific Alkaline Phosphatase (BSAP), C-telopeptide (CTX), Osteoprotegerin (OPG), Osteoprotegerin ligand (receptor activator of nuclear factor-κB ligand or RANKL), Insulin-like Growth Factor, Leptin
Cytokines	Cyto- and chemokine profile (including: CCL2/MCP-1, CCL3/MIP-1a, CCL4/MIP1B, CCL5/RANTES, CXCL8/ENA-78, CXCL8/IL-8, FGF, G-CSF, GM-CSF, IL-1 β, IL-10, IL-17, IL-1a, IL-1ra, IL-2, IL-4, IL-5, INF gamma, TPO (Thrombopoietin), VEGF-1)
Urinary Measures	
Antioxidants/Oxidative Damage	8-OH deoxyguanosine
Bone Markers*	N-telopeptide, C-telopeptide, deoxypyridinoline, pyridinoline, Helical Peptide, γ-carboxy glutamic acid
Other Measures	
Metabolic Rate	Resting Metabolic Rate (pre- and post-bed rest only)

*Measure is required for Bone Standard Measures and can be shared with Nutrition/ Hematology Standard Measures.

15.3. Blood Volume Requirements

The volume of blood drawn from bed rest subjects during any study is of concern and is typically regulated by an ethics committee. What follows are breakdowns of the blood requirements for the required and recommended measures.

- Total blood volume for all measures: 2 × 6 ml SST; 1 × 6 ml blue mineral tube; 2 × 4 ml EDTA; 1 × 2.7ml LiHep Monovette; 1 × 1.8ml Na Citrate tube= 30.5 ml

If done alone, the volumes for the analyses are shown below (note that there is savings of blood based on blood tube sizes when all are combined).

- Total blood volume for required measures with vitamins: 2 × 6 ml SST; 1 × 6 ml blue mineral tube; 2 × 4 ml EDTA; 1 × 1.2 ml LiHep Monovette; 1 × 1.8 ml Na Citrate tube = 29.0 ml
- Total blood volume for required measures without vitamins: 2 × 6 ml SST; 1 × 6 ml blue mineral tube; 1 × 4 ml EDTA; 1 × 1.2 ml LiHep Monovette; 1 × 1.8 ml Na Citrate tube = 25.0 ml
- Total blood volume for recommended measures: 1 × 6.0 ml SST; 1 × 3 ml EDTA; 1 × 1.2 ml LiHep Monovette = 10.2 ml

16. Immunology Standard Measures

Immune system dysregulation during spaceflight is well characterized (Gueguinou et al., 2009; Morukov et al., 2010) and may result in specific clinical risk to crewmembers participating in exploration class space missions (Crucian and Sams, 2009). Causal factors for this phenomenon may include microgravity, physiological stress, isolation, altered nutrition and disrupted circadian rhythms. The effects of stress on human physiology and during spaceflight were recently characterized (Chouker, 2012).

The appropriateness of the bed rest analog to demonstrate impacts on the immune system is inconclusive. The best analog for immune dysregulation includes factors such as mission physiological stress, isolation and disrupted circadian rhythms. These items are somewhat missing from bed rest, which may limit the analog utility for immune dysregulation. However, there are some isolation and fluid shifts associated with bed rest which could influence the immune system. In addition, there is a need and scope to investigate a countermeasure effect on every organ system, including the immune system, and its interaction with the latter, where there is very little known.

The standard measures identified for this discipline are a minimal set of measures with accepted clinical utility that are limited to assays readily available world-wide, and do not require access to specialized equipment or reagents. All immunological measures described below are required measures. For particular bed rest studies however, additional research assays could be applied to target specific research questions.

The following list describes the required immune standard measures for bed rest studies:

- Complete blood count (CBC)
- Basic leukocyte subsets
- Stress hormone levels (saliva and or plasma measures)
- Plasma immunoglobulin G (IgG) levels
- Viral antibody levels
- Alpha 1 globulin, alpha 2 globulin, beta globulin, gamma globulin

Specific clinical rationale is as follows. A complete blood count and basic leukocyte subsets indicate constitutive immune activation for an individual subject. Concurrent with any functional dysregulation, it is important to know if a subject's current immune status remains within baseline values. Stress hormone levels provide an important physiological indicator regarding subject adaptation to the space-analog condition. Also, the effect of physiological stress on human immunity is well-established. Globulin fractionation is also an indicator of dysregulation in plasma protein concentrations, which may correlate with constitutive immune activation or inflammatory processes. Plasma IgG levels are also an indicator of constitutive immune activation, specifically humoral immune responses. Viral antibody levels however, are an indicator of latent herpes virus reactivation. Latent viral reactivation is associated with immune suppression both terrestrially and during spaceflight.

CBC consists of white blood cell, differential, red blood cell parameters, and platelet count. This analysis is performed by a certified clinical laboratory using a commercially available and validated hematology analyzer.

Analysis of basic leukocyte subsets is performed by flow cytometry. Suggested cell subsets for analysis are T cells, B cells, NK cells, CD4+/CD8+ T cells, memory/naïve T cells, aconstitutively activated T cells. Whole blood sample should be stained using the appropriate fluorescent-labeled monoclonal antibodies relevant for the leukocyte subsets of interest. The specific antibody matrix will depend on the configuration and analysis capabilities of the flow cytometer. Following leukocyte staining, RBCs should be lysed using any of several accepted methods (acid, osmotic). The stained leukocytes should be fixed (1% paraformaldehyde) prior to analysis. Flow cytometry analysis should include bead-based validation of instrument optical precision, appropriate positive/negative controls, as well as controls to verify the absence of spectral overlap.

Specific stress hormones are left to the discretion of the investigator, but may include cortisol and catecholamines. There are several methods available to measure stress hormone levels in plasma or serum, including competitive binding enzyme-linked immunosorbent assay (ELISA). Any available assay may be employed, provided an appropriate standard curve is generated and control values are as expected.

Plasma concentration of IgG and antibodies for specific latent herpes viruses should be determined by ELISA. Commercially available kits may be used, or sample may be sent to a reference laboratory.

To analyze serum globulin levels, concentrations of alpha 1 globulin, alpha 2 globulin, beta globulin, and gamma globulin should be determined by serum globulin electrophoresis.

17. Psychology Standard Measures

17.1. Required Measures

17.1.1. Positive and Negative Affect Scale (PANAS)

The PANAS is a 20-item self-evaluation questionnaire that measures affects, or indicators of emotional states. Emotional states are important to study during situations of isolation and confinement like bed rest experiments because they reflect the general state of the person at one step of the experiment. The PANAS separately assesses positive and negative affects. A positive affect reflects the extent to which a person feels enthusiastic, active and alert. Therefore, a person displaying high-positive affect has high energy, full concentration, and pleasurable engagement. Low-positive affect is characterized by sadness and lethargy. The separate measure of negative affect reflects the degree to which a person feels subjective distress and unpleasant engagement. A high-negative affect is described by anger, contempt, disgust, guilt, fear and nervousness. A low-negative affect describes a state of calmness and serenity. The PANAS is a highly valid and reliable measure of positive and negative affect (Watson, 1988; Watson et al., 1988).

To administer the test, subjects are provided the PANAS form and a pen or pencil. Subjects are asked to indicate to what extent they have felt each positive affect item and each negative affect item in the past week. Items are rated on a scale of 1 to 5 where 1 indicates not at all, and 5 indicates extremely. Scores for each affect are totaled and recorded.

Positive and negative affect should be assessed once every 2 weeks throughout the study (pre-, in, and post-bed rest). Each assessment should be completed about the same time of day; within ± 2 hours of the initial baseline assessment. This test takes about 5 minutes to complete. The PANAS should not be administered immediately after awakening, or following maximal exercise.

17.1.2. General Health Questionnaire (GHQ)

GHQ is a self-evaluation questionnaire measuring the current mental health of the individual. GHQ was first developed as a 60-item tool to screen for non-specific psychiatric morbidity (Goldberg, 1972; Goldberg and Williams, 1988). For bed rest studies, it is used to measure minor mental health problems and can be useful to prevent more important mental disorders during isolation and confinement situations (Ishizaki et al., 2002). There are 4 versions: GHQ-12, GHQ-28, GHQ-30 and GHQ-60. GHQ-12 or GHQ-28 is recommended for bed rest studies. GHQ-12 provides an overall total score for mental health. GHQ-28 provides an overall total score and scores on 4 subscales of somatic symptoms, anxiety, insomnia, social dysfunction and severe depression. The advantage of having these 4 subscales makes the GHQ-28 a useful measure for bed rest studies. Each item on the GHQ is rated on a 4-point scale (0 to 3) indicating 'less than usual', 'no more than usual', 'rather more than usual', and 'much more than usual'. The GHQ is available in many languages and can be purchased at: <http://www.mapi-trust.org/services/questionnairelicensing/cataloguequestionnaires/52-GHQ>.

Subjects are provided with the GHQ and asked to respond to questions inquiring about their health. GHQ is administered every 2 weeks throughout the study (pre-, in, and post-bed rest). Each assessment should be completed about the same time of day; within ± 2 hours of the initial baseline assessment. The GHQ-28 takes 10 minutes to complete and the GHQ-12 takes 5 minutes to complete. GHQ should not be administered immediately after awakening, or following maximal exercise.

17.2. Recommended Measures

17.2.1. Log of Critical Incidents

The log of critical incidents is completed by the monitoring psychologist. Observations of significant events that affect subject's psychological state are described. Entries may require an interview with the subject by the psychologist. This log is useful to help explain issues that cannot be quantified.

17.2.2. Subject Diary

The subject diary provides the subject's perspective on their experiences in the study. The diary is a self-report by the subject and can be used in conjunction with the log of critical incidents to obtain both the subject and psychologist perspectives.

17.2.3. Personal Self-Perception and Attitudes (PSPA)

PSPA is used to examine the dynamics of interrelationships in a group setting (Gushin et al., 2000). The methodology used is based on an analysis of the subject's system of subjective attitudes toward him/herself and others. In the first stage of assessment, the subject selects 9 personalities that are most important to him/her based on a suggested form. These personalities include self-image in the present, past (childhood), and future (ideal). Next, the subject formulates 12 pairs of personality assessment criteria (constructs), using those traits that seem most important to him/her in describing people. The selected traits in a pair shall be antonyms for example, optimism and pessimism. The assessment criteria should make it possible to distinguish the majority of the people being evaluated. Hence, 12 pairs of bipolar rating scales are formulated from the traits proposed by the test subject. In the third stage, the subject completes a personality rating according to the bipolar rating scales formed by the 12 pairs of assessment criteria. In the final stage of the examination, the personality series is replaced by the members of the group that are being studied, while retaining the list of assessment criteria. The main benefit of the PSPA method is that the subject chooses the criteria for assessment; the individual's own behavior is used as a source of evidence for beliefs and attitudes. This makes the assessment more precise, reflecting the subject's actual attitudes toward people. Principle component analysis is then used to process the data.

17.2.4. Cognitive Test Battery

The cognitive test battery is a series of cognitive tests embedded in a virtual reality scenario. It is more difficult to administer than standard patient tests, but is an excellent test to use with healthy subjects. This test is useful for measuring cognitive fluctuations as severe declines are not expected in bed rest subjects. The Cognitive Test Battery was validated in Mars 500.

The Cognitive Test Battery utilizes the VIRTU software that simulates manned-Mars mission operations. Practicing the VIRTU software should contribute to the elaboration of practical professional skills, necessary for the astronaut on another planet: landing, take-off, driving rovers, collecting samples, drilling, working in off-nominal situations. It allows learning solo and in pairs. The three main tasks included in this simulated mission are: a) planet investigation with the 'big' transport rover, b) planet investigation with the 'small' transport rover, and c) inspection of the Martian surface during a sand storm.

18. References

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19. Appendices

19.1 IAA in Brief

International Academy of Astronautics

A Brief Description

Founded:

16 August 1960, Stockholm, Sweden, by Theodore Von Karman. Independent non-governmental organization recognized by the United Nations in 1996.

Aims:

Foster the development of astronautics for peaceful purposes;
Recognize individuals who have distinguished themselves in space science or technology;
Provide a program through which members may contribute to international endeavors;
Promote international cooperation in the advancement of aerospace science.

Structure:

Regular Meeting (every two years). Board of Trustees (meets twice a year), consisting of: President; four Vice-Presidents and twenty-eight Trustees, seven from each Section: Basic Sciences, Engineering Sciences, Life Sciences and Social Sciences. Current President: Dr Madhavan G. Nair, Past-President: Prof. Edward C. Stone, USA, Vice-Presidents: Mr. Yannick d'Escatha, France; Prof Liu Jiyuan, China ; Dr. Hiroki Matsuo, Japan; Prof. Anatoly Perminov, Russia, Secretary General Dr. Jean-Michel Contant, France.

Activities:

Encourage international scientific cooperation through scientific symposia and meetings in the area of: - Space Physical Sciences, - Space Life Sciences, - Space Technology and System Development, - Space Systems Operations and Utilization, - Space Policy Law and Economy, - Space and Society Culture and Education. A major initiative of the Academy is the development of a series of "Cosmic Studies" and "Position Papers" dealing with the many aspects of international cooperation endeavors in: - The exploration and habitation of the solar system and beyond; - The space debris, - The small satellites, - Declaration of Principles Concerning Activities Following the Detection of Extraterrestrial Intelligence, - EVA Safety and Space Suit Interoperability, - Inexpensive Scientific Satellite Missions, - Lunar and Martian Exploration, - Next Steps in Exploring Deep Space, - Space to promote Peace, - Space Traffic Management, - Knowledge Management in Space Activities, - Cost Effective Earth Observation Missions.

Events:

Establishment of cooperation with national academies: The Royal Swedish Academy of Sciences (1985), the Austrian Academy of Sciences (1986, 1993), the Academy of Sciences of the Institute of France (1988, 2001), The Academy of Finland (1988), Indian Academy of Sciences (1990, 2007), The Royal Spanish Academy of Sciences (1989), German Academy of Sciences (1990), The Kingdom of Netherlands (1990), RSC: The Academies of Arts, Humanities and Sciences of Canada (1991), the U.S. National Academy of Sciences (1992, 2002), the U.S. National Academy of Engineering (1992, 2002), the Israel Academy of Sciences and Humanities (1994), Norwegian Academy of Science and Letters (1995), Chinese Academy of Sciences (1996), the Academy of Sciences of Turin (1997), the Australian Academy of Sciences (1998), The Royal Netherlands Academy of Arts and Sciences (1999), the Brazilian Academy of Sciences (2000), the U.S. National Institute of Medicine (2002) the Academy of Sciences of South Africa (ASSAf) (2011), the Royal Society of South Africa (2011), the Pontificia Academia Scientiarum (2012).

Publications:

The journal of the Academy, *Acta Astronautica* (elevating from the 27th to 13th position for 5 year Impact Factor); IAA e-newsletter; Yearbook, Dictionaries and CD-ROM in 24 languages (last languages added Afrikaner and Swahili), Position Papers and Cosmic Studies (<https://shop.iaaweb.org/>), IAA Book Series on Small Satellite - Programs, Missions; IAA Book Series on Conference and Symposium Proceedings; IAA Book Series on Remote Sensing of the Earth System - Science, Technologies and Applications; Scientific Papers Data Base on the IAA Web site.

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- Oceania: Australia, New Zealand.

Headquarters in Bern, Switzerland, **Secretariat:** 6 rue Galilée, 75116 Paris, France; Branches of Secretariat in Bangalore (India) and IAA Study Center in Beijing (China); Regional offices in Abuja (Nigeria), Tunis (Tunisia), Buea (Cameroon) and Nairobi (Kenya).

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