Measures of Autonomic Nervous System Regulation

Defense Centers of Excellence for Psychological Health and Traumatic Brain Injury

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Introduction

Mind-body health practices aim to regulate activation of the sympathetic nervous system (SNS) to maintain homeostasis within the autonomic nervous system (ANS). The activation of the SNS is directly related to stress response, which, if persistent or prolonged, may lead to physiological and psychological damage. The ability to return to homeostasis after exposure to a stressor or to moderate SNS activation is a measure of resilience. In order to measure the effects of mind-body skills in facilitating resilience, tools may be used that are sensitive to the changes in activation and allow for feedback to guide practice. The most promising and most often used measures of ANS activation encompass non-invasive tools, which measure cardiac, skin conductance, respiratory, and vascular activity. Choice of tools is dependent upon a variety of factors, including setting, purpose, user friendliness and psychometric properties. In this companion paper to Mind-body Skills for Regulating the Autonomic Nervous System, a brief overview of ANS activity, as well as a review of the physiological measurement of stress, is presented. A description of tools which measure the nine ANS pathways is also presented. Finally, a decision tree is offered to aid in choosing appropriate tools for measurement of the effects of mind-body skills.

Overview of the ANS

The nervous system is divided into the central and peripheral nervous systems. The peripheral nervous system (PNS) connects the central nervous system (CNS) to the rest of the body. The CNS includes the brain and brain stem; the PNS is comprised of the ANS (or visceral nervous system) and the somatic nervous system (or voluntary nervous system). The ANS regulates the CNS and promotes adjustments to environmental changes. For example, the ANS can adjust or modify some functions of the body in response to a perceived threat i.e. a stress response. The ANS continuously controls vital functions, such as temperature regulation, osmotic balance, metabolism, digestion, excretion, and cardiac and respiratory activity.

The ANS consists of the sympathetic and parasympathetic divisions and acts through a balance of the two divisions. The ANS is predominately controlled by centers in the spinal cord, brain stem and hypothalamus. The sympathetic stimulation causes excitatory effects ("fight or flight") in some organs and inhibitory effects in others. Sympathetic ganglia are located in two sympathetic chains close to the spinal cord: the prevertebral and paravertebral chains. Most sympathetic ganglia are not located in close proximity to the target organ. There are two exceptions in which preganglionic sympathetic nerve fibers directly innervate the target organ: the sweat glands and the adrenal medulla. Parasympathetic ganglia, in contrast, are located in close proximity to the target organ. For example, the submandibular ganglia are close to salivary glands and pericardia ganglia. Parasympathetic preganglionic fibers pass all the way to the organ with the postganglionic neurons located in the wall of the organ. The sympathetic and parasympathetic divisions both complement and oppose each other. In some organs, such as the heart, the two systems act reciprocally. For example, sympathetic and parasympathetic influences constantly modulate heart rate, as a function of the respiratory cycles. Generally, these two systems should be seen as permanently modulating vital functions to achieve homeostasis. Since both systems are continuously
active, the interplay between the divisions can increase or decrease the activity of a targeted organ. However, most organs are dominantly controlled by one or the other.

**Measurement of Stress**

Psychology and the physiological measurement of ANS activities have been intertwined well before Sigmund Freud and Joseph Breuer traced physiological symptoms to psychological processes in Studies on Hysteria (1955, translation to English, 1980 reprint). Some of the earliest psychologists were physiologists (e.g., Wilhelm Wundt, Ivan Pavlov, and Edward Thorndike) who looked to the physiological process to understand behavior and the mind. These researchers used medical and physiological methodologies to attempt to correlate observable changes with internal experiences. Other researchers at the time were using a method called introspection, observing and reporting individual internal experiences and extrapolating their findings to all humans to explore psychological processes. In response to inadequacies in both early research methodologies, Clark L. Hull (1933) suggested that the mind is a black box that could not be objectively measured and recommended a more useful approach in focusing research on observable and definable behaviors. From Hull’s theory, behaviorism arose in contrast to the analytic perspective, which continued to focus on the mind. Despite the differences in focus, both behavioral and psychoanalytic theorists continued to map physiological processes to human experiences.

One bridge between the mind and body is the study of stress and its impact on human performance. Stress occurs in response to real or imagined emotional or physical threats. Understood today as an orchestration of physiological mechanisms, the physiological stress response is initiated by sub-cortical structures in the brain, principally the amygdala, which initiates a chain of events to prepare the individual for “fight or flight” in response to the perceived stressor. First, the paraventricular nucleus of the hypothalamus releases corticotropin-release factor (CRF). CRF activates the locus coeruleus, causing release of norepinephrine throughout the brain, and stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH, in turn, stimulates the adrenal cortex to secrete glucocorticoids, mineralocorticoids and androgens. Cortisol, also known as the stress hormone, is a glucocorticoid that causes immunosuppression and increases blood glucose concentrations through glycogenolysis and gluconeogenesis. This pathway is known as the hypothalamic-pituitary-adrenal (HPA) axis. The stress response also stimulates secretion of catecholamines, primarily epinephrine and norepinephrine, through the release of acetylcholine from sympathetic preganglionic nerve fibers; this mechanism is known as the sympaetho-adrenal pathway.

In addition to HPA and sympaetho-adrenal activation, target organs are influenced through the stress-induced activation of the sympathetic nervous systems (SNS) and the inhibition of the parasympathetic nervous system (pSNS). The repercussions of the stress response are manifested physiologically through the following: pupillary dilation, increased heart rate and myocardial contractility, increased respiratory rate, diverted blood flow to organs vital to the fight or flight response, and increased concentrations of cortisol, catecholamines and related metabolites.

The stress response may be adaptive in that it allows for the activation of the immune system, memory enhancement and energy immobilization. Allostasis is the adaptive process of quieting the SNS and returning the ANS back to homeostasis. Allostatic overload is the result of chronic or rapid and repeated exposure to stressors resulting in a persistent SNS hyper-responsiveness.
The intensity and duration of stress, or allostatic overload, has been implied in a range of medical and psychological conditions. For example, stress has been linked to mood and anxiety disorders such as depression and post-traumatic stress disorder (PTSD). In animal and human studies, prolonged stress was demonstrated to change neuro-pathways and hormone levels and is believed to cause structural changes in the brain. These changes in the brain have been hypothesized to be associated with chronic anxiety disorders such as PTSD. The Stress Response Timeline below (Table 1) provides a general overview of the immediate effects of chronic stress, the physiological measures to gauge physiological impact of the stressor and the tools that can be used to quantify these measures. Additionally, as some studies have linked genetic predispositions to the onset of PTSD, the Stress Response Timeline notes the specific hormones and proteins coded by the identified genes.
### Table 1: Stress Response Timeline

**Physiological Measures**

<table>
<thead>
<tr>
<th>Immediate Effects</th>
<th>Chronic Stress Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Heart Rate</td>
<td>↑ CRF Secretion</td>
</tr>
<tr>
<td>↓ Heart Rate Variability</td>
<td>↓ Growth Hormone</td>
</tr>
<tr>
<td>↑ Blood Pressure</td>
<td>↑ CRF Secretion</td>
</tr>
<tr>
<td>↑ Pupillary Dilation</td>
<td>↑ Glucocorticoid</td>
</tr>
<tr>
<td>↑ Respiration Rate</td>
<td>↓ Gonadotropins</td>
</tr>
<tr>
<td>↑ Electrical Skin Resistance</td>
<td>↑ Mortality</td>
</tr>
<tr>
<td>↑ Cortisol in Circulation</td>
<td>↑ Mortality</td>
</tr>
</tbody>
</table>

**Tools**

- ELISA
- Galvanic Skin Response Monitor
- Individual Monitoring
- Pupillometer
- Sphygmomanometer
- Respiratory Sinus Arrhythmia
- EKG
- HPLC - Mass Spectrometry
- ELISA

Resilient individuals not only return to homeostasis relatively quickly, but also appear to activate the SNS in a more conservative fashion; i.e. strong enough to respond effectively while avoiding the negative effects of allostatic overload. In an attempt to increase resiliency and to ameliorate or alleviate the damaging effects of allostatic overload, interventions have been developed, including a multitude of mind-body health practices and programs as described in Mind-body Skills for Regulating the Autonomic Nervous System. Many of these skills and programs have used a variety of measures to validate their effects and aid in skill acquisition.

Of particular utility are those tools which directly measure the activity of the ANS and which are sensitive enough to measure the power of the intervention to aid in the return to homeostasis. Below is a table that
Measures of Autonomic Nervous System Regulation

identifies a selection of mind-body skills, which were described in Mind-body Skills for Regulating the Autonomic Nervous System paper, and the ANS pathways which have been measured for each. The ANS pathways identified in the table are broad domains rather than the specific activity; for example the cardiac pathway includes both heart rate and heart rate variability. As can be seen in the table, the ANS pathways most often measured include cardiac, galvanic skin response (GSR), respiratory and vascular. However, several of the practices have not been correlated with allostasis or the evidence is just emerging. Some ANS pathways, such as cortisol, appear to have been underutilized as outcome measures. Due to a variety of factors, other ANS pathways, such as gastrointestinal and catecholamine, may not lend themselves readily to such research. For each ANS pathway, there is a wide range of tools available to measure the effects of SNS activation. These tools vary on a number of dimensions which impact their effectiveness as a measure. The table below demonstrates those pathways commonly measured, which may be useful when choosing a tool for clinical or training purposes, as well as those pathways that have not been commonly measured, which may be of interest for further research.
## Measures of Autonomic Nervous System Regulation

### Table 2: Mind-Body Skills and ANS Pathway Table

<table>
<thead>
<tr>
<th>Practice</th>
<th>Mind-Body Skills</th>
<th>ANS Pathways Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cardiac</td>
</tr>
<tr>
<td>Manipulative Body-Based/Tension-Release Practices</td>
<td>Trauma Resiliency Model (TRM)*</td>
<td>X*</td>
</tr>
<tr>
<td></td>
<td>Trauma and Tension Releasing Exercises (TRE)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yoga (Asana) Postures</td>
<td>X</td>
</tr>
<tr>
<td>Breathing Practices</td>
<td>Slow-Paced Breathing</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Fast-Paced Breathing</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Warrior Breath (SKY)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Diaphragmatic Breathing</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>iBreathe/ Diaphragmatic</td>
<td></td>
</tr>
<tr>
<td>Meditative Practices</td>
<td>Mindfulness Mind-Fitness Training (MMFT)</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Mindfulness Based Stress Reduction (MBSR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yoga Nidra (iRest)</td>
<td>X</td>
</tr>
</tbody>
</table>

*Study currently in progress utilizing these measures
Identification of Tools

ANS activity may be categorized into nine measurable physiological systems or pathways, activated through sympathetic and parasympathetic mechanisms. These pathways provide a framework for a comprehensive review of stress reactivity measures. The physiological pathways are listed in Table 3 below:

<table>
<thead>
<tr>
<th>ANS Physiological Activities</th>
<th>Cardiac</th>
<th>Pupillary Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catecholamines</td>
<td></td>
<td>Respiration</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td>Salivary Amylase</td>
</tr>
<tr>
<td>Galvanic Skin Response</td>
<td></td>
<td>Vascular</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The ANS Measures Table in Appendix A provides a summary of over fifty tools identified for measuring ANS activity. Tools were identified through a literature review to clarify mechanisms affecting each measure and delineate complexities to be considered. Upon forming a comprehensive list of measures and pertinent descriptions, the tools were further explored through Web reviews and interviews with leading researchers. Tools were selected based on several factors, including the ability to produce objective results and to demonstrate reliability and validity. Some developing tools had not been tested for reliability and validity; they were noted as promising tools needing future development. The search resulted in tools ranging from the established highest standard measure for the respective measure (e.g., electrocardiogram for myocardial electrical activity) to instruments in development.

Tools are described in the ANS Measures Table through the assessment of five specifications: reliability, validity, maturity, usability and potential military settings. Each of these specifications is operationally defined and presented in ascending order of rigor, as follows:

**Reliability** was gauged by the ability to produce consistent results and classified into the following tiers:
- ✓ No information on reliability
- ✓ Reliability not consistently demonstrated
- ✓ Trial-to-trial repeatable results reported
- ✓ Subject-to-subject repeatable results reported
- ✓ Cronbach alpha exceeding 0.70

**Validity** was assessed by the tool's ability to quantify the data to be measured and was determined by meeting one or more of the following criteria:
- ✓ No information on validity
- ✓ Validity not consistently demonstrated
- ✓ Evidence-based on test content (or face validity) - determines if the tool has the ability to quantify its intended measure
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- Evidence-based on relations to other variables or measures - divides into convergent and discriminate validity based on strength of correlation, direct and inverse, to other measures
- Evidence-based on consequences of testing (or predictive validity) - gauges the ability to predict an outcome on a separate test based on the result of the tool

**Maturity** refers to the magnitude of the tool's utility. Technological development was not incorporated into assessing tool maturity. Maturity was categorized by the following:
- In development
- Limited use
- Widely used and accepted

**Usability** describes the specifications of using the tool. Usability is categorized by the following:
- Portability (mass, size and power source requirements)
- Technicality of operation (skilled/trained technician required)
- Fragility (environmental sensitivities and material durability)
- User friendliness (easy to use, painless, non-invasive, non-stigmatizing operation)

**Applicability to Potential Military Settings** provides a list of ideal operating environments for the tool, described using one or more of the following:
- U.S. military base (field)
- Military treatment facility (office or hospital)
- Overseas military base (field or office)
- Combat deployment (forward operating base)
- Field combat deployment (limited to those that may be carried on a person)

The ANS Measures Table in Appendix A provides a brief snapshot of ANS activities affected by stress reactivity and over fifty tools for quantifying each. The above specifications are utilized to aid in describing each tool and facilitate comparisons between tools.

**Measuring ANS Activity**

Approximately fifty tools identified according to ANS pathway are listed in the ANS Measures Table in Appendix A. Some of these tools, such as the electrocardiogram (EKG), are established and widely used. Others, such as the Intelligent Garment (also known as the Smart Shirt), are still in development. The utility of each tool is dependent upon its purpose of use, target environment and the sensitivity of measurement needed. The three ANS pathways most often used to measure the impact of mind-body skills, as seen in Table 2 on page 9, are cardiac, vascular and respiratory. These are the ANS pathways that physicians monitor via the vital signs (blood pressure, heart rate, temperature and respiratory rate).

**Cardiac Measurement**

Cardiac activity, one of the most commonly assessed ANS pathways, is among the most sensitive areas to ANS changes (Davidson, personal communication, 2010). Cardiac activity is measured via heart rate, with increased rate signifying activation of the SNS, and via heart rate variability, with decreased
variability signifying activation of the SNS. Heart rate variability may decrease due to either acute or chronic stress and there is some indication that low heart rate variability may be associated with reactivity to stress. One widely used measure is manually taking the pulse; it offers a non-invasive, non-painful, relatively quick measure of heart rate. An EKG is a standard measure of the electrical activity, i.e. the depolarization of the heart during each beat over time. Sensors placed on the skin using two, three, five or twelve leads capture electrical activity. Three other novel tools also effectively measure heart rate changes. The Doppler radar cardiopulmonary remote sensing unit, the Intelligent Garment and the wearable reflectance pulse oximeter are still in development. Standard pulse oximeters have been in use for many years and are widely used for measuring oxygen saturation of blood. The Doppler radar cardiopulmonary remote sensing unit and the wearable reflectance pulse oximeter have the benefit of being more portable; they do not require a trained technician and are more durable than the traditional EKG. The Intelligent Garment is portable, but does require a trained technician and is less durable, much like the EKG. These tools may all be used to measure heart rate and heart rate variability which have been identified as two pivotal ANS activities for measuring the effects of mind-body skills (Davidson, personal communication, 2010).

Vascular Measurement

The vascular or circulatory system is measured via temperature and blood pressure. As part of the stress response when the SNS is activated, blood vessels in the limbs constrict causing a decrease in temperature in the extremities while increasing the blood flow to the muscles, brain, lungs and heart, increasing core temperature. While thermometers can accurately measure body temperature, it can be influenced by illness, hormonal balance, circadian rhythms, muscle activity, clothing, environmental conditions and medication. There are a number of tools which measure temperature, some of which would be sensitive to changes associated with a stress response. Tools which may measure the decreased temperature of the extremities, (i.e. stress) include auxiliary sensors such as those used on the intelligent garment and biodots. Other tools such as a biobelt and rectal thermometers offer means to measure increased core temperature.

Blood pressure, the measure of the pressure of the blood within the arteries, is a product of heart rate, intravascular volume status, cardiac muscle contractility, and systemic vascular resistance. As part of a response to a stressor arterial blood pressure increases. Blood pressure may be measured through the following three methods. The auscultatory method includes listening to the internal sounds of the body, usually through a stethoscope in combination with a sphygmomanometer. Another method is the oscillometric method, which detects the oscillations in blood flow via the sphygmomanometer and an electronic sensor. The third method of measuring blood pressure is the photoplethysmo-graphic method, which detects the change in blood volume by measuring the amount of light either transmitted or reflected to a photodiode. The pulse oximeter, described above, is an example of a photoplethysmographic device. Portable and operable without a skilled technician, it measures blood volume in the arteries of the fingers. While it can reliably measure pulse in most individuals, its use in accurately measuring blood pressure is still in development.

Respiratory Measurement

The respiratory system includes the inhalation of air, which is then channeled into the alveoli of the lungs to deliver oxygen to the circulatory system and the exhalation of carbon dioxide. When the SNS is
activated due to exposure to a stressor, breath rate increases and breaths become shallower. There are two methods of monitoring the respiratory system: direct and indirect. One form of direct measurement includes spirometers. Spirometers measure the amount and rate of airflow over a period of time by having the individual breathe into a device, which visually illustrates the volume of air displaced, much like a breathalyzer. Indirect measures of respiration include the use of a plethysmograph. A pulmonary plethysmograph measures the total lung capacity and functional residual capacity of the lungs. As the individual breathes into a mouthpiece, the pulmonary plethysmograph measures pressure differences during respiration. Using Boyle’s Law, which relates pressure and volume, the plethysmograph calculates the volume within the lungs. Another method used more frequently in health care settings is the standard counting of the rise and fall of the chest. Rate of breathing rather than lung capacity is sensitive to mental and emotional states. Typically, a higher breath rate is indicative of a higher experience of stress while a lower breath rate correlates with a lower experience of stress. Spirometers, pulmonary plethysmographs and individual monitoring are all widely used and accepted. The peak flow meter is a device that measures peak expiratory flow rate (PEFR), which is air flowing out of the lungs. The optimal level of the individual’s lung function is measured by using three color-coded peak flow zones. The individual monitoring and peak flow monitor are both portable and user friendly; however, the peak flow monitor does not demonstrate consistent reliability. Additionally, the spirometer, pulmonary plethysmograph and individual monitoring require skilled technicians to use accurately.

**Galvanic Skin Response (GSR) Measurement**

GSR, one of the earliest tools used in psychological research, offers a method of measuring the electrical resistance of the skin. In response to a stressor, GSR nonspecifics increase and basal resistance decreases. GSR is obtained by attaching two leads to the skin and acquiring a base measure. As an activity is performed, recordings are made from the leads. Active GSR is when a current is passed through the body and the resistance is thereby measured. GSR is sensitive to immediate emotional arousal as well as general mood or acute stress responses. The original psychogalvanometer, a widely used and accepted measure of GSR, has been used since the 1930s with little change. The e-meter and polygraph are also versions of a GSR which are widely used. While the psychogalvanometer, the e-meter and the polygraph are widely accepted for measuring GSR, there is controversy regarding the validity of the data interpretation. These tools remain popular and mainstream for measuring GSR, yet their applications continue to be less widely endorsed by the scientific community due to the concerns regarding validity.

**Catecholamine Measurement**

Catecholamines, such as norepinephrine, epinephrine, and cortisol, are hormones released during a stress response. In response to a stressor, an endocrine signal cascade is initiated primarily by the release of corticotropin-release factor (CRF) from the paraventricular nucleus of the hypothalamus, resulting in increased secretion of glucocorticoids (i.e., cortisol), mineralocorticoids, and androgens. As a result, catecholamine metabolites, specifically vanillylmandelic acid (VMA), can be used to gauge sympathetic activity. Concentrations of catecholamine metabolites can be determined by measuring the following: VMA concentration in urine samples; dihydroxyphenylglycol (DHPG), 3-Methoxy-4-hydroxyphenylglycol (MHPG), and VMA levels in serum; and catecholamine and related metabolites in saliva. Some studies have suggested a direct correlation between catecholamine levels and PTSD, as well as major depressive disorders. However, further research is needed to explore the measurement of catecholamine metabolite concentrations to detect PTSD with consideration to environmental influences. For example, synthesis and secretion of catecholamines are affected by the
use of drugs, such as amphetamines, alcohol and monoamine oxidase inhibitors, which may interfere with accurate measurements of catecholamine metabolites. Three tools for measuring catecholamine metabolites are widely used and accepted: Radioimmunoassay (RIA) with High Performance, Enzyme-Linked Immunosorbent Assay (ELISA), and Liquid Chromatography-Mass Spectrometry (LC-MS). RIA, ELISA and LC-MS have adequate reliability and validity, but not all of them are portable.  

Cortisol Measurement

Cortisol, commonly referred to as the stress hormone, is secreted by the adrenal glands and impacts the sympathetic and parasympathetic nervous systems. Cortisol is the most potent glucocorticoid produced and secreted by the adrenal cortex. Its release is activated through the hypothalamus pituitary adrenal (HPA) axis, also known as the limbic-hypothalamic-pituitary-adrenal axis (LHPA axis). In response to a perceived threat, CRF is released from the hypothalamus and binds to receptors on the anterior pituitary lobe. This activates release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. Cortisol is released when ACTH binds to receptors in the adrenal medulla. In addition to stress or trauma, many studies link cortisol levels to circadian rhythms, temperature, infection, exercise, and obesity. Cortisol levels peak after waking and subsequently decrease throughout the day. This pattern of diurnal variation varies if the individual has irregular sleep patterns which are common in individuals with PTSD and other psychological disorders. These confounding factors should be taken into account when using cortisol as a measure.

Several tests are available to monitor cortisol levels: 24-hour urine collection, blood testing, and saliva sampling. Saliva sampling is a portable test that can be performed outside of the lab. Although this method is less intrusive, it requires special care in obtaining the sample. The sample is often collected when cortisol is at its lowest, which is typically in the late evening. The 24-hour urine collection method requires a collection of all the urine produced during a day and night to establish an averaged, daily baseline. The 24-hour urine sample may be ordered to measure the amount of free (unbound) cortisol secreted in the urine, but it will not allow the provider to evaluate variations in cortisol secretion. Cortisol levels in saliva have been shown to strongly correlate with its concentration in serum. Though cortisol levels in the blood are easier to collect, blood collection may induce a stress response.  

Pupillary Response Measurement

The pupillary response may occur for several reasons, including exposure to light, sexual stimulation, before sleep, or in response to a stressor. Pupillary response varies the size of the pupil of the eye via the iris dilator muscle which will dilate in response to a stressor. Three widely accepted and used measures of pupillary response are the Colvard Pupillometer, Dynamic Binocular Infrared Pupillometer, and the NeurOptics VIP™-200 Pupillometer. The Dynamic Binocular Infrared Pupillometer measures activity of ciliary muscle in response to brief flashes of light to the left, right or both eyes. It has real-time processing with a display of fitted pupil during patient positioning and data acquisition. The Colvard Pupillometer accurately measures scotopic pupil size and the corneal diameter of cataract and refractive candidates. It utilizes light amplification technology to allow visualization of the pupils in a darkened room. The NeurOptics VIP 200 Pupillometer is widely used to diagnose narcotic influence, head injury, Parkinson’s disease, rheumatoid arthritis or lupus. It may also be used to assess degeneration of eye tissue in people with severe diabetes. All the tools have satisfactory reliability and validity. The Colvard and NeuroOptic Pupillary are portable and battery operated. While the Colvard is hand-held, the Dynamic Binocular Infrared Pupillometer requires attachment to a computer for data processing and display. All three tools require a skilled technician to use accurately.
**Salivary Amylase Measurement**

Most measures of salivary amylase, the enzyme which initiates the chemical breakdown in the mouth, and gastrointestinal activity have limited use. However, the hand-held biosensor monitor, designed to measure salivary amylase, is still in development, and early results are promising. The device is reported to be portable and durable and would not require a skilled technician to use accurately. Acinar cells, which produce salivary amylase, are innervated by sympathetic and parasympathetic pathways. Some studies have found sympathetic activity increases amylase synthesis, which increases amylase concentration in the saliva, and parasympathetic activity increases saliva flow rate with no or little effect on amylase synthesis. As these effects produce an overall increase in absolute salivary amylase output, they should be considered in relating salivary amylase to stress reactivity.

**Decision Tree**

The aforementioned tools for measuring ANS activity have potential for use in research, prevention, diagnostic and treatment settings. One consideration, when choosing metrics to investigate the effects of mind-body skills upon ANS regulation, is the impact of stress on the ANS and the power of the intervention to catalyze or aid in the return to homeostasis. In a sense, this is a measure of resilience. Any measure chosen must be able to reliably quantify this effect. While some areas of ANS activity may be measurable, they may not be as readily available with consideration to the repercussions of measurement. For example, blood draws and gastrointestinal measures may cause a stress reaction, and salivary amylase concentrations may not be sensitive enough to reliably demonstrate an effect. On the other hand, non-invasive cardiac, skin conductance, respiratory, vascular and cortisol (as measured through saliva) measures offer the most promise for this type of analysis. Therefore, the primary consideration when choosing a tool to measure the impact of an intervention upon the regulation of the ANS is whether it measures an activity which will be sensitive to the expected change.

Another consideration is the ability for the integrated mind-body health practice or program to affect allostatic and aid in avoiding allostatic overload. In order to measure these effects, the tool must be responsive to minute changes in activation and allow precise measurement. It is equally important that baseline measurements are stable in order to assess patterns and intensity of changes. ANS activity, which is the most susceptible to a variety of environmental and biological influences, such as temperature, blood pressure and papillary response, may not give clear information about the impact of the technique. The most promising and most frequently used measures of the effect of mind-body skills upon the ANS include non-invasive cardiac, skin conductance, respiratory, and vascular measures, as indicated in Table 2 on page 10. One method to aid in clearly correlating the technique with the effect is to employ multiple measures, thus controlling for confounding variables.

Other considerations include the use of the tool (application or investigation), phase (training, crisis or treatment), reliability and validity of the tool, setting in which the tool will be used, as well as usability of the tool. The Decision Tree below visually depicts the steps for the selection of tools in ascending order from left to right.
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Table 4: Decision Tree

Use
- Application
- Theoretical

Phase
- Training
- Crisis Intervention
- Treatment

Reliability
- No Information on Reliability
- Reliability Not Consistently Demonstrated
- Trial to Trial Repeatable Results
- Subject to Subject Repeatable Results
- Cronbach Alpha >0.70

Validity
- No Information on Validity
- Validity Not Consistently Demonstrated
- Face Validity
- Convergent Validity
- Predictive Validity

Setting
- U.S. Military Base - Field
- Military Treatment Facility
- Overseas Military Base - Field
- Overseas Military Base - Office
- Combat Deployment – Forward Operating Base

Usability
- Portability
- Technicality of Operation
- Fragility & Durability
- User Friendliness
Prolonged and repeated exposure to stressors, causing persistent re-activation of the SNS within the ANS, or allostatic overload, may cause physiological and psychological harm. The ability to return the ANS to homeostasis, quieting and moderating the SNS, is a measure of resilience and is a goal of mind-body health practices and programs. Physiological measures of the ANS are used as an aid in learning mind-body health practices, as well as for measuring the effectiveness of the techniques. Breathing and meditative practices have utilized such measures, while there is relatively little evidence of body-based and tension-release practices having similar support. A wide range of tools, which vary in their psychometric properties, maturity, usability and application, can be used to measure ANS activity. Consideration of which tool to use begins with whether the ANS activity is sensitive to the impact of the intervention, followed by the use, phase, psychometric properties, setting and usability of the tools.
## APPENDIX A: ANS MEASURES

<table>
<thead>
<tr>
<th>Autonomic Nervous System (ANS) Measures</th>
<th>Neurophysiological Process Examined: Nervous System (NS) controlled target organ activity</th>
<th>Measurement Method: Transducer that converts physiological process to measurable variable</th>
<th>Measure Reliability: Consistency of measureable results when repeat tests are performed on the same individuals under the same condition</th>
<th>Measure Validity: Expert consensus for ability of the test/intervention/ modality to measure what it is supposed to do</th>
<th>Maturity: Level of conceptual, technical, and functional development and sophistication</th>
<th>Usability: Characteristics of the tool/system that facilitate or impede ease of use</th>
<th>Potential Military Settings: Tool/system suited for use in the following military/operational environments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual Pulse&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Heart rate variability</td>
<td>Place 2 fingers on wrist or carotid artery; count the beats for 15 seconds, then multiply by 4</td>
<td>• No information on reliability</td>
<td>• Face validity</td>
<td>Widely used and accepted</td>
<td>• Portable</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Armband Pulse-meter&lt;sup&gt;22&lt;/sup&gt;</td>
<td>Heart rate variability</td>
<td>Worn around the arm to measure heart rate</td>
<td>• No information on reliability</td>
<td>• Face validity</td>
<td>Widely used and accepted</td>
<td>• Portable</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Pocket Heart Rate Monitor&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Heart rate variability</td>
<td>Measures pulse and heart rate variability</td>
<td>• No information on reliability</td>
<td>• Face validity</td>
<td>Widely used and accepted</td>
<td>• Portable</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>PDA Monitoring System&lt;sup&gt;24&lt;/sup&gt;</td>
<td>Heart rate</td>
<td>Electrocardiography and photoplethysmography</td>
<td>• No information on reliability</td>
<td>• Face validity, Convergent validity</td>
<td>Widely used and accepted</td>
<td>• Portable</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Pulse Oximeter&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Arterial oxygen saturation</td>
<td>Spectrophotometry</td>
<td>• No information on reliability</td>
<td>• Face validity</td>
<td>In development</td>
<td>• Portable</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Intelligent Garment&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Blood pressure, Heart rate</td>
<td>Plethysmographic optical sensor on finger or earlobe (EKG is lab standard, finger to pulse/time)</td>
<td>• Correlated with physical activity, conditioning, rest versus engagement</td>
<td>• Face validity</td>
<td>Widely used and accepted</td>
<td>• Portable</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Cardiopulmonary Remote Sensing Unit&lt;sup&gt;27&lt;/sup&gt;</td>
<td>Heart rate variability</td>
<td>Plethysmographic optical sensor on finger or earlobe (EKG is lab standard)</td>
<td>• Trial to trial repeatable results reported</td>
<td>• Face validity</td>
<td>Widely used and accepted</td>
<td>• Portable</td>
<td>U.S. military base – field</td>
</tr>
</tbody>
</table>
| Cardiac (continued) | Biobelt<sup>24</sup> | • Heart rate  
• Heart rate variability  
• Blood pressure  
• Temperature  
• Electrocardiography  
• Respiration rate  
| Electrocadiogram (EKG)<sup>25</sup> | • Myocardial electrical activity  
• Heart rate variability | Plentifulmographic optical sensor on chest with a pill that is swallowed which will capture core temperature  
• Trial to trial repeatable results reported  
• Subject to subject repeatable results reported | • Face validity  
• Convergent validity  
• Predictive validity | • Portable  
• Skilled technician required  
• Durable  
• User-friendly operation | • U.S. military base – field  
• Military treatment facility  
• Overseas military base – field  
• Combat deployment – forward operating base | • Combat deployment - field  
| Adrenal Stress Index<sup>30</sup> | • Catecholamine concentration  
| Chromatography and spectrometry | • No information on reliability | • Face validity  
• Convergent validity | • Portable  
• No skilled technician required  
• Durable  
• User-friendly operation | • U.S. military base – field  
• Military treatment facility  
• Overseas military base – field  
| Radioimmunoassay<sup>21</sup> | • Catecholamine concentration  
| Chromatography and spectrometry | • Subject to subject repeatable results reported | • Face validity  
• Convergent validity  
• Predictive validity | Widely used and accepted  
• Not portable  
• Skilled technician required  
• Fragile  
• Rigorous operation | • U.S. military base – field  
• Military treatment facility  
| Enzyme-Linked Immunosorbent Assay<sup>22</sup> | • Catecholamine concentration  
| Chromatography and spectrometry | • Subject to subject repeatable results reported | • Face validity  
• Convergent validity  
• Predictive validity | Widely used and accepted  
• Not portable  
• Skilled technician required  
• Fragile  
• Rigorous operation | • U.S. military base – field  
| Liquid Chromatography- Mass Spectrometry<sup>23</sup> | Increase in catecholamine metabolites concentration as a result of sympathetic activation  
| Chromatography and spectrometry | • Subject to subject repeatable results reported | • Face validity  
• Convergent validity  
• Predictive validity | Widely used and accepted  
• Not portable  
• Skilled technician required  
• Fragile  
• Rigorous operation | • US military base – office  
• Military Treatment Facility  
| ELISA Saliva Kit (Enzyme-linked immunosorbent assay)<sup>31</sup> | • Salivary Cortisol  
| Level of cortisol secreted by adrenal glands | • Subject to subject repeatable results reported | • Face validity  
• Convergent validity  
• Predictive validity | Widely used and accepted  
• Portable  
• No skilled technician required  
• Durable  
• User-friendly operation | • U.S. military base – field  
• Military treatment facility  
| Chromatography<sup>20</sup> | • Serum Cortisol  
| Level of cortisol secreted by adrenal glands | • Subject to subject repeatable results reported | • Face validity  
• Convergent validity  
• Predictive validity | Widely used and accepted  
• Portable  
• No skilled technician required  
• Durable  
• User-friendly operation | • U.S. military base – field  
| Liquid Chromatography-Tandem Mass Spectrometry<sup>26</sup> | • Urinary free cortisol (UFC) measurements  
| Level of cortisol secreted by adrenal glands | • Subject to subject repeatable results reported | • Face validity  
• Convergent validity  
• Predictive validity | Widely used and accepted  
• Not portable  
• Skilled technician required  
• Fragile  
• Rigorous operation | • US military base – office  
• Military Treatment Facility  
| GSR Relaxation Monitor<sup>24</sup> | • Sympathetic control of sweating  
| Electrical resistance of the skin | • Reliability not consistently demonstrated | • Face validity  
• Convergent validity | Limited use  
• Portable  
• No skilled technician required  
• Durable  
• User-friendly operation | • U.S. military base – field  
<p>| Galvanic Skin Response (GSR) |
|----------------------|---------------------------------|-----------------------------------|------------------------------------------|--------------|-------------------|-------------|-----------|----------------------------|---------|-----------------------------|--------------------------|-----------------------------|-----------------------------|----------------------------|-------------------------------|---------------------------------|
| Polygraph            | Sympathetic control of sweating | Electrical resistance of the skin | Reliability not consistently demonstrated | Inconsistent information on validity | Widely used and accepted | Portable | Skilled technician required | Durable | Rigorous operation | Military treatment facility | Overseas military base – office |
| Gastrointestinal     | Gastric motility controlled by enteric NS and ANS | Passive electrodes record cutaneous voltages generated by gastric muscle activity. | Trial to trial repeatable results reported | Evidence based on test content | Evidence based on consequences of testing | Limited use | Not portable | Skilled technician required | Fragile | Rigorous operation | Military treatment facility |
| E-Meter              | Sympathetic control of sweating | Electrical resistance of the skin | Reliability not consistently demonstrated | Inconsistent information on validity | Widely used and accepted | Portable | Skilled technician required | Durable | User-friendly operation | Military treatment facility | Overseas military base – office |
| Neuro-Optics Pupilimeter | Pupil dilation | Ciliary muscle activity in response to stimuli | Trial to trial repeatable results reported | Face validity | Convergent validity | Predictive validity | Widely used and accepted | Portable | Skilled technician required | Durable | User-friendly operation | Combat deployment - field |
| Advanced Communication Index | Pupil dilation | Ciliary muscle activity in response to stimuli | Trial to trial repeatable results reported | Face validity | Convergent validity | Predictive validity | Widely used and accepted | Portable | Skilled technician required | Durable | User-friendly operation | Combat deployment - field |
| Binocular Infrared Pupilimeter | Pupil dilation | Ciliary muscle activity in response to stimuli | Trial to trial repeatable results reported | Face validity | Convergent validity | Predictive validity | Widely used and accepted | Portable | Skilled technician required | Durable | User-friendly operation | Combat deployment - field |</p>
<table>
<thead>
<tr>
<th>Device</th>
<th>Monitored Parameter(s)</th>
<th>Methodology</th>
<th>Reliability</th>
<th>Validity</th>
<th>Durability</th>
<th>Skilled Technician Required</th>
<th>Portable</th>
<th>Secure Operation</th>
<th>User-friendly Operation</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory</strong></td>
<td></td>
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<tr>
<td>Individual Monitoring</td>
<td>Chest movement</td>
<td>Direct observation</td>
<td>Trial to trial repeatable results reported</td>
<td>Face validity, Predictive validity</td>
<td>Widely used and accepted</td>
<td>Skilled technician required, User-friendly operation</td>
<td></td>
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<td></td>
<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
</tr>
<tr>
<td>Peak Flow Meter</td>
<td>Lung capacity</td>
<td>Measures respiratory intake and outtake</td>
<td>Reliability not consistently demonstrated</td>
<td>Face validity, Predictive validity</td>
<td>Widely used and accepted</td>
<td>Portable, No skilled technician required, Durable, User-friendly operation</td>
<td></td>
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<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
</tr>
<tr>
<td>Spirometers</td>
<td>Lung capacity</td>
<td>Measures respiratory intake and outtake</td>
<td>Subject to subject repeatable results reported</td>
<td>Face validity, Predictive validity</td>
<td>Widely used and accepted</td>
<td>Portable, Skilled technician required, Durable, User-friendly operation</td>
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<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
</tr>
<tr>
<td>Pulmonary Plethysmographs</td>
<td>Inhalation and exhalation</td>
<td>Functional, residual lung capacity and total lung capacity</td>
<td>Subject to subject repeatable results reported</td>
<td>Face validity, Predictive validity</td>
<td>Widely used and accepted</td>
<td>Not portable, Skilled technician required, Fragile, Rigorous operation</td>
<td></td>
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<td>U.S. military base – field, Military treatment facility, Overseas military base – office</td>
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<tr>
<td><strong>Salivary Amylase</strong></td>
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<tr>
<td>Biosensors Hand-Held Monitor</td>
<td>Salivary amylase concentration</td>
<td>Chromatography and spectrometry</td>
<td>No information on reliability</td>
<td>Face validity, Convergent validity</td>
<td>In development</td>
<td>Portable, No skilled technician required, Durable, User-friendly operation</td>
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<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
</tr>
<tr>
<td>Dye-Based Microsphere Absorbance Test</td>
<td>Salivary amylase concentration</td>
<td>Chromatography and spectrometry</td>
<td>No information on reliability</td>
<td>Face validity, Convergent validity</td>
<td>Limited use</td>
<td>Portable, No skilled technician required, Durable, User-friendly operation</td>
<td></td>
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<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
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<tr>
<td><strong>Vascular</strong></td>
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<tr>
<td>Forehead Thermometer</td>
<td>Temperature</td>
<td>Thermometer</td>
<td>Reliability not consistently demonstrated</td>
<td>Inconsistent information on validity</td>
<td>Limited use</td>
<td>Portable, No skilled technician required, Durable, User-friendly operation</td>
<td></td>
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<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
</tr>
<tr>
<td>Tympanic Thermometer</td>
<td>Temperature</td>
<td>Thermometer</td>
<td>Trial to trial repeatable results reported</td>
<td>Face validity, Predictive validity</td>
<td>Widely used and accepted</td>
<td>Portable, No skilled technician required, Durable, User-friendly operation</td>
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<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
</tr>
<tr>
<td>Oral Thermometer</td>
<td>Temperature</td>
<td>Thermometer</td>
<td>Trial to trial repeatable results reported</td>
<td>Face validity, Predictive validity</td>
<td>Widely used and accepted</td>
<td>Portable, No skilled technician required, Durable, User-friendly operation</td>
<td></td>
<td></td>
<td></td>
<td>U.S. military base – field, Military treatment facility, Overseas military base – field, Combat deployment – forward operating base, Combat deployment – field</td>
</tr>
<tr>
<td>Intestinal Thermometer (Lee SM, Williams WJ, Schneider SM, oral communication, 2000)</td>
<td>Temperature</td>
<td>Thermometer</td>
<td>Cronbach alpha reported and is &gt;0.70</td>
<td>Face validity Predictive validity</td>
<td>In development</td>
<td>Portable</td>
<td>Skilled technician required</td>
<td>Durable</td>
<td>User-friendly operation</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Intelligent Garment</td>
<td>Blood pressure</td>
<td>Thermometer</td>
<td>Pletysmographic optical sensor on finger or earlobe (EKG is lab standard), (finger to pulse/time)</td>
<td>Correlated with physical activity, conditioning, rest versus engagement</td>
<td>Widely used and accepted</td>
<td>Portable</td>
<td>Skilled technician required</td>
<td>Fragile</td>
<td>User-friendly operation</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Automatic Sphygmomanometer</td>
<td>Blood pressure</td>
<td>Sphygmomanometer</td>
<td>Trial to trial repeatable results reported</td>
<td>Face validity Predictive validity</td>
<td>Widely used and accepted</td>
<td>Portable</td>
<td>No skilled technician required</td>
<td>Durable</td>
<td>User-friendly operation</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Manual Sphygmomanometer</td>
<td>Blood pressure</td>
<td>Sphygmomanometer</td>
<td>Trial to trial repeatable results reported</td>
<td>Face validity Predictive validity</td>
<td>Widely used and accepted</td>
<td>Portable</td>
<td>Skilled technician required</td>
<td>Durable</td>
<td>User-friendly operation</td>
<td>U.S. military base – field</td>
</tr>
<tr>
<td>Vascular (continued)</td>
<td>Core body temperature</td>
<td>Temperature variance</td>
<td>No information on reliability</td>
<td>Face validity Convergent validity</td>
<td>Limited use</td>
<td>Not portable</td>
<td>Skilled technician required</td>
<td>Fragile</td>
<td>Rigorous operation</td>
<td>Military treatment facility</td>
</tr>
<tr>
<td>Computed Tomography and Ultrasound</td>
<td>Endothelial function</td>
<td>Brachial artery flow mediated dilation</td>
<td>No information on reliability</td>
<td>Face validity</td>
<td>In development</td>
<td>Not portable</td>
<td>Skilled technician required</td>
<td>Durable</td>
<td>Rigorous operation</td>
<td>Military treatment facility</td>
</tr>
<tr>
<td>Neuroscope</td>
<td>Core body temperature</td>
<td>Temperature variance</td>
<td>No information on reliability</td>
<td>Face validity</td>
<td>Limited use</td>
<td>Not portable</td>
<td>Skilled technician required</td>
<td>Durable</td>
<td>User-friendly operation</td>
<td>Military treatment facility</td>
</tr>
<tr>
<td>Photoplethysmography (Non-invasive)</td>
<td>Tissue blood volume</td>
<td>Volume changes in all blood vessels</td>
<td>Trial to trial repeatable results reported</td>
<td>Face validity Predictive validity</td>
<td>Widely used and accepted</td>
<td>Portable</td>
<td>Skilled technician required</td>
<td>Durable</td>
<td>User-friendly operation</td>
<td>Military treatment facility</td>
</tr>
<tr>
<td>Plethysmograph</td>
<td>Change in peripheral blood volume</td>
<td>Subject to subject repeatable results reported</td>
<td>Face validity</td>
<td>Widely used and accepted</td>
<td>Portable</td>
<td>Skilled technician required</td>
<td>Durable</td>
<td>User-friendly operation</td>
<td>U.S. military base – field</td>
<td>Military treatment facility</td>
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</tbody>
</table>
**KEY**

**Reliability** is gauged by the ability to produce consistent results and classified into the following tiers:

- ✓ No information on reliability
- ✓ Reliability not consistently demonstrated
- ✓ Trial-to-trial repeatable results reported
- ✓ Subject-to-subject repeatable results reported
- ✓ Cronbach alpha exceeding 0.70

**Validity** is assessed by the tool’s ability to quantify the data to be measured and was determined by meeting one or more of the following criteria:

- ✓ No information on validity
- ✓ Validity not consistently demonstrated
- ✓ Evidence-based on test content (or face validity) - determines if the tool has the ability to quantify its intended measure
- ✓ Evidence-based on relations to other variables or measures - divides into convergent and discriminate validity based on strength of correlation, direct and inverse, to other measures
- ✓ Evidence-based on consequences of testing (or predictive validity) - gauges the ability to predict an outcome on a separate test based on the result of the tool

**Usability** describes the specifications of using the tool. Usability is categorized by the following:

- ✓ Portability (mass, size and power source requirements)
- ✓ Technicality of operation (skilled/trained technician required)
- ✓ Fragility (environmental sensitivities and material durability)
- ✓ User friendliness (easy to use, painless, non-invasive, non-stigmatizing operation)
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