

Ultrasound Checklist

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IACUC considerations for “Non-Standard” Ultrasound use

I. Purpose

This set of guidelines describes recommended points of consideration for IACUC review of protocols involving the use of ultrasound protocols that are “non-standard”. “Non-standard” includes the use of High Intensity focused ultrasound (HIFU) and any use of custom ultrasound equipment, as these approaches do not have safety measures built in to the instruments and are potentially harmful to the animals. It is meant to serve as a supplement to the “Checklist for IACUC Protocols”.

This document does not cover ultrasound with regards to imaging or any other “standard” ultrasound technique, such as, but not limited to;

1. Contrast imaging (linear or nonlinear)
1. Doppler imaging (Power or Tissue)
2. M-mode imaging (with or without 3D capabilities)
3. B-mode imaging (with or without 3D capabilities)

These techniques are inherently safe and require no additional consideration for IACUC approval.

“Non-standard” techniques that are covered by these recommendations include, but are not limited to:

1. All techniques using “High Intensity” ultrasound (such as the use of HIFU).
2. All techniques using custom ultrasound equipment

II. Responsibility

Principal investigator (PI) and their research staff, veterinary care staff.

III. Approval for use

For the use of a “non-standard” ultrasound techniques, a description of the calibration of the equipment must be included in the IACUC protocol submission, including detailed acoustic pressures measured and method of calibration over the intended experimental ranges of applied voltage(s). The equipment must be calibrated annually and recorded in a log which will need to be made available during IACUC inspections and/or Post Approval Monitoring (PAM) visits. Failure to provide this information in the submission will result in a “nonapproval” of the protocol.

To calibrate a non-standard US, a calibration curve (input electrical energy plotted versus pressure measured from a hydrophone) should be generated and then periodically tested to ensure the device is operating properly. This is important in non-standard US, as the potential to harm the animals is high given the energies used can reach destructive levels. Generally, it is difficult to ascertain what an acceptable deviation from the calibration curve is, as each use is different and sometimes non-linear in response. Therefore, acceptable deviations from the calibration curve, should be defined by the Principal Investigator and supported by literature or theory.

IV. Definitions

1. IACUC: Institutional Animal Care and Use Committee
1. JABSOM: John A Burns School of Medicine
2. EHSO: Environmental Health and Safety Office
3. AVS: Animal and Veterinary Services
4. Focused ultrasound. Focused ultrasound can be accomplished by mechanically focusing the transducer *via* a convex face which essentially “focuses” the energy into conical beam aimed at a specific target area. It can be used in high intensity applications or low intensity applications.
5. Unfocused ultrasound. Unfocused ultrasound utilizes a transducer that does not

have a convex face and the energy is transmitted in a cylindrical beam from the face of the transducer. Unfocused ultrasound is typically used only in low intensity cases.

6. HIFU: High Intensity Focused Ultrasound. All HIFU applications are focused in nature. The unique aspect of HIFU is that ultrasound is emitted from the transducer at a much higher intensity than that used for imaging or “standard” ultrasound sound procedures. This creates a potential for tissue heating or ablation.
7. UTMD (Ultrasound Targeted Microbubble Destruction). The use of ultrasound to cavitate (pop) microbubbles *in vivo* where the “popping” of the microbubbles delivers gene to cells close to the cavitation events. High or low intensity approaches may be used, as well as focused or unfocused techniques.
8. EKG: electrocardiogram
9. BPM: Beats Per Minute
10. PAM: Post Approval Monitoring

V. Specific considerations for each ultrasound approach

Focused Techniques

Low intensity: Focused low intensity ultrasound is normally safe, in that the mechanical index (a calibrated and calculated measurement of the ultrasound intensity of energy used) typically never exceeds medically safe levels.

High intensity (HIFU): There are more considerations when using HIFU with respect to animal safety. It is not possible to calibrate a HIFU instrument precisely, as it is still an experimental approach and in development, though there are ways to approximate the acoustic pressure outputs in order to evaluate safety and must be included in any submission proposing the use of HIFU. HIFU is used due to its precise focusing capability. The acoustic pressures that can be achieved may result in thermal ablation of the targeted area within milliseconds. Animals need to be monitored closely throughout the procedures for signs of distress *via* physiological monitoring (see section VI. Monitoring requirements). Euthanasia endpoints need to be considered should there be any signs of post-procedural morbidity (see section VII. Humane intervention points).

Unfocused techniques

Low intensity only: Unfocused low intensity ultrasound is regarded as safe, in that the mechanical index (a calibrated and calculated measurement of the ultrasound intensity of energy used) typically never exceeds medically safe levels.

VI. Monitoring requirements:

The following physiological signals must be monitored when using any “non-standard” ultrasound techniques;

1. Circulation: heart rate and/or EKG (an anesthetized animal’s heart rate is ~200-300 BPM)
1. Oxygenation: mucous membranes color monitored by tongue color
2. Respiratory rate: typical respiration is ~105 breath/min.

VII. Humane intervention points

If the general condition of the animal does not improve after 48 hours post-treatment, mice should be considered for euthanasia after consulting with AVS veterinary staff (Refer to the IACUC Policy on Euthanasia).

Euthanize animals with:

1. Weight loss: due to inappetence and diarrhea
1. Lethargy
2. Hunched posture
3. Weight loss exceeding 20% of pre-treatment weight
4. Body condition score of less than 2
5. No or weak response to external stimuli
6. Labored breathing

