Contains Nonbinding Recommendations

Draft – Not for Implementation

Utilizing Animal Studies to Evaluate Organ Preservation Devices

---

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

Document issued on September 15, 2017.

You should submit comments and suggestions regarding this draft document within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions about this document, contact the Division of Reproductive, Gastro-renal, and Urological Devices at 301-796-7030 or Andrew Fu at (301) 796-5881 or Andrew.fu@fda.hhs.gov.

FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR DEVICES & RADIOLOGICAL HEALTH

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Preface

Additional Copies

Additional copies are available from the Internet. You may also send an e-mail request to CDRH-Guidance@fda.hhs.gov to receive a copy of the guidance. Please use the document number 1500083 to identify the guidance you are requesting.

Table of Contents

Utilizing Animal Studies to Evaluate Organ Preservation Devices

Draft Guidance for Industry and Food and Drug Administration Staff

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. Introduction

While the national transplant waiting list continues to grow, donation and transplant rates remain stagnant. The shortage of organs available for transplants has propelled a new wave of innovation in organ preservation technologies. These technologies are evaluated in animal models to demonstrate that they are suitable for clinical experience.

The intent of this draft guidance is to provide recommendations regarding best practices for utilizing animal studies for the evaluation of organ preservation devices. For information regarding Good Laboratory Practice (GLP) requirements that may apply to such studies, you should refer to 21 CFR Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies. FDA recommends balancing the ethical principles of The Three Rs (replacement, reduction and refinement)[1] as well as regulatory least burdensome principles, with the goal of using the minimum number of animals necessary to generate data to demonstrate device safety. You should consider the best practices for the development, conduct and presentation of these animal studies while incorporating modern animal care and use strategies.

FDA recognizes that best practices for conducting animal studies to evaluate organ preservation devices are evolving with the rapid advancements in such technologies. This guidance is not intended to be comprehensive or prescriptive. Instead, it aims to highlight FDA's initial thoughts on how animal transplant models can be utilized to evaluate organ preservation technologies, with careful considerations of regulatory least burdensome principles. While FDA expects that at

[1] Russell WMS, Burch, RL. The Principles of Humane Experimental Technique. London: Methuen & Co.; 1959. Special edition published by Universities Federation for Animal Welfare, 1992. http://altweb.jhsph.edu/pubs/books/humane_exp/het-toc. Accessed April 26, 2017.

this time, most of these animal studies will be submitted to support investigational device exemption (IDE) applications, they may also be used to support premarket approval (PMA) applications, premarket notifications (510(k)), humanitarian device exemption (HDE) applications, or De Novo classification requests.

FDA encourages members of industry to engage CDRH via the Pre-Submission process to obtain feedback for specific animal study protocols to evaluate organ preservation devices. For more information on Pre-Submissions, you should refer to "Requests for Feedback on Medical Device Submissions: The Pre-Submission Program and Meetings with Food and Drug Administration Staff" (https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm311176.pdf).

In this document, the terms "you" and "your" refer to members of industry, also known as "sponsors" or "applicants." The terms "we," "us," and "our" refer to FDA.

FDA's guidance documents, including this draft guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA guidance means that something is suggested or recommended, but not required.

II. Scope

The recommendations in this draft guidance document are applicable to devices intended to preserve human vascularized organs via machine perfusion (hypothermic or normothermic) from the time of organ procurement until transplant. The Health Resources and Services Administration (HRSA), not FDA, oversees the donation and transplantation of human organs.

Most of the devices to which this guidance applies are currently not classified. This guidance document is also applicable to the product code KDN, System, Perfusion, Kidney (21 CFR 876.5880, Class II).

The recommendations in this guidance document do not apply to devices intended to preserve organs via cold static storage, including those associated with product codes KDK, PIN, KDL, and MSB, regulated as Class II devices under 21 CFR 876.5880, Isolated kidney perfusion and transport system and accessories. In addition, human cells, tissues and cellular and tissue-based products (HCT/P's) regulated under 21 CFR 1271.3(d)(1) and Section 351 & 361 of the Public Health Service Act and the devices utilized to preserve and transport them are also outside the scope of this guidance document.

III. Definitions

For purposes of this guidance document, the following definitions apply:

Cold Ischemia Time: The amount of time that an organ is cold (~4°C) and not receiving adequate blood supply.

Cold Static Storage: The current standard method to preserve most organs. Organs are submerged in a preservation solution in a closed container that maintains the temperature at ~4°C.

Extended Criteria Organs: Donor organs that are suboptimal for transplant (e.g., donation after cardiac death (DCD) donor organs). The criteria may differ depending on organ type.

Ischemia Reperfusion Injury: Inflammation and oxidative damage to the tissue caused by the restoration of blood supply after a period of ischemia.

Machine Perfusion: A dynamic method to preserve organs, utilizing a device with a pump that drives the movement of a perfusate. Devices performing machine perfusion of organs may also contain oxygenators, heat exchangers, sensors, disposable circuits, and computer units for processing and displaying hemodynamic and metabolic data. Machine perfusion can be performed at various temperatures, e.g., ~4°C (hypothermic), ~37°C (normothermic).

Perfusate: The solution that is pumped through the donor organ.

Reperfusion: The restoration of blood supply to an organ.

Warm Ischemia Time: The amount of time that an organ is at body temperature or room temperature and not receiving adequate blood supply.

IV. Overview and General Study Design Considerations

FDA recommends a risk-based approach for developing animal study protocols for evaluating organ preservation devices. In order to determine the specific risks to be evaluated in an animal study, you should consider the known risks of your device type as identified through literature review, bench testing, and exploratory animal studies, as well as the risks inherent to the indications for use. For example, machine perfusion in general may be associated with increased risks of injuries due to organ manipulation and contamination of the perfusion circuit. In another example, a device indicated to preserve extended criteria organs (e.g., ones with longer preservation time than the current standard of practice) may also subject the organs to additional risks.

After determining the specific risks and their corresponding failure modes, you should develop a protocol with focused objectives and *a priori* acceptance criteria. When appropriate, FDA recommends including the scientific rationales for the chosen acceptance criteria. In addition, FDA recommends that you provide a rationale for the selection of a particular animal model for

your study, with careful considerations of anatomical, physiological, and immunological similarities and differences between the animal model and humans.

A typical experimental setup for such animal studies will consist of three phases: organ procurement, organ preservation, and organ reperfusion (see Figure 1 below).

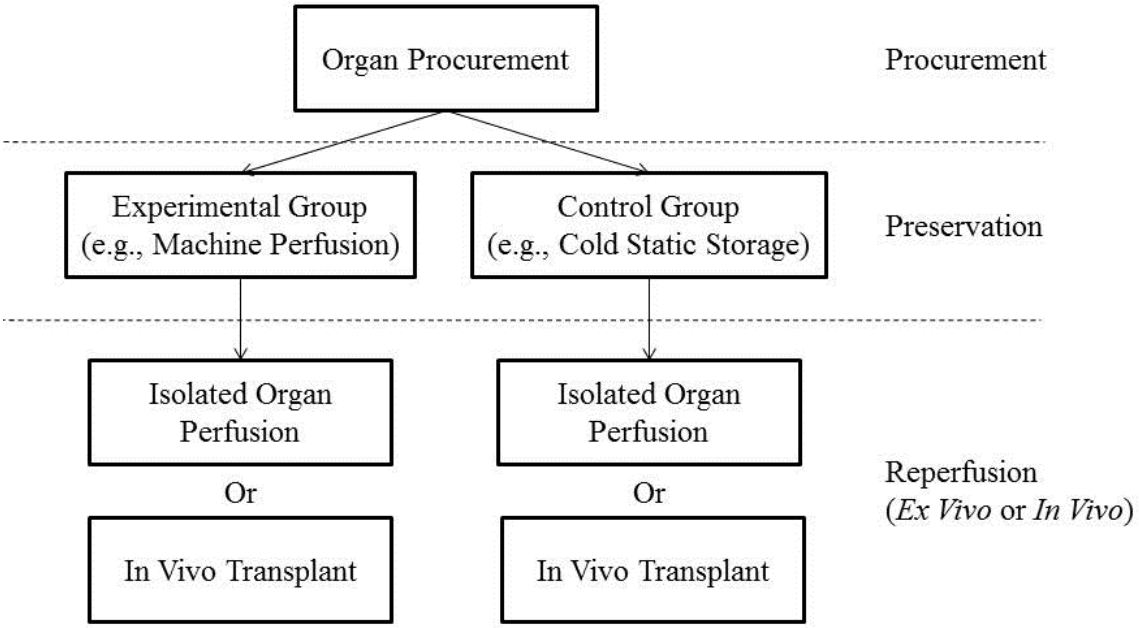

Figure 1: The Three Phases of a Typical Animal Study for Evaluating Organ Preservation Devices

To begin the safety assessment of the organ preservation device selected organs are procured from appropriate animal model donors. Then, these organs are preserved using either an experimental method (e.g., machine perfusion) or a control method (e.g., cold static storage). Organs from both groups are reperfused in either an *in vivo* or *ex vivo* model, to evaluate reperfusion injury. Due to its complexity, the reperfusion phase will be discussed in detail in Section V. In the section below, our recommendations focus on general study design considerations:

A. Procedure Duration

Procedure duration has a significant effect on the outcome of transplant studies. You should carefully consider the following recommendations regarding the duration of the experimental procedures:

- **Procurement Phase**: FDA recommends specifying warm ischemia time and cold ischemia time as part of the animal organ procurement protocol. The ischemia time should reflect the indications for use of the device. For instance, when evaluating a device indicated to preserve organs from non-heart-beating donors,

you should extend the period of warm ischemia by leaving the organ *in situ* after inducing cardiac arrest in the animal.

- **Preservation Phase**: Prior to initiating preservation, the time to successfully cannulate and connect the organ to the device should be evaluated based on *a priori* acceptance criteria. The total preservation time should be consistent with the indications for use of the device. Preservation time may vary based on organ type.
- **Reperfusion Phase**: At the end of the preservation phase, the organ should be cold-flushed per standard protocol and exposed to a realistic preparation period prior to the start of *ex vivo* reperfusion or *in vivo* transplant. The duration of reperfusion will be discussed in detail in Section V.

B. Contamination

Compared to cold static storage, machine perfusion has a higher risk of contamination due to the increased complexity of the perfusion circuit and manipulation of the organ. Therefore, FDA recommends performing bacterial cultures on perfusate samples taken at the end of a perfusion session to demonstrate contamination did not occur.

C. Transportability

If your organ preservation device is transportable, your animal protocol should assess whether the device and the organ can withstand the turbulence during transport (e.g., being driven in an ambulance). Normal handling, such as tilting the device, during transport may jeopardize the organ support system or cause transient changes in the perfusion parameters. FDA recommends developing and evaluating strategies that mitigate the risk of organ injury from mechanical trauma. For instance, if you plan to administer a vasodilator to regulate the spikes in hemodynamic parameters (e.g., vascular pressure) during transport, you should evaluate whether the amount of vasodilator administered achieves the intended effect.

V. Reperfusion Models

After an organ undergoes preservation, the clinical concern centers on the severity of the reperfusion injury. There are generally two models to assess reperfusion injury: an *in vivo* model in which the organ is transplanted into a recipient animal and an *ex vivo* model in which the organ is reperfused in an isolated setup. In order to establish a more focused animal study protocol, it is important to discuss the advantages and limitations of each model, in the context of recent technological advancements in organ preservation technologies.

A. *Ex Vivo* Models

The development of new organ preservation technologies (e.g., normothermic machine perfusion) has unlocked the potential to monitor and assess organs *ex vivo* prior to transplantation. Compared to the more traditional *ex vivo* models (e.g., Langendorff heart model), *ex vivo* models utilizing these new technologies are capable of continuously collecting more detailed hemodynamic, metabolic, and functional data under more relevant physiological conditions. In addition, compared to their *in vivo* counterparts, these *ex vivo* models typically offer a more controlled study environment with fewer potential confounders (i.e., non-device related factors that may affect the interpretation of study outcomes). Nevertheless, *ex vivo* models have two important limitations:

- The evaluation of ischemia reperfusion injury attributed to interactions between the coagulation and inflammatory cascades is hindered by 1) the use of anticoagulants (e.g., heparin) in the blood-based perfusates and 2) the lack of whole-body immune response.
- The association between organ viability and the hemodynamic, metabolic, and functional data collected in an *ex vivo* model has not yet been well-established. While the perfusate can be sampled during *ex vivo* reperfusion to measure levels of biomarkers for organ injury and function, some of these biomarkers are considered exploratory and are not well-accepted as surrogates for organ viability post-transplant.

While some of these limitations are inherent to the *ex vivo* model, other limitations can be mitigated through improved study design. FDA has the following recommendations for study designs in an *ex vivo* model:

- **Control group**: Due to the limitations discussed above, an *ex vivo* model cannot determine the absolute extent of ischemia reperfusion injury. Therefore, we recommend including a control group (e.g., cold static storage) in the study, so that the relative effects of the injury can be evaluated.
- **Near-physiological conditions**: In order to simulate *in vivo* conditions*, ex vivo* reperfusion should be performed under near-physiological conditions (e.g., temperature, pressure, flow, oxygenation). The performance of critical device components (e.g., pumps, sensors, oxygenators) should be validated using exploratory animal studies or studies using human organs not suitable for transplant.
- **Whole blood as perfusate**: FDA recommends that your perfusate consist primarily of whole blood collected from third party animals as blood donors. The use of blood from the organ donors should be avoided in order to 1) simulate the reperfusion conditions in clinical transplants and 2) limit the confounding effects of hypovolemia and catecholamine release on the donor organ.
- **Perfusate additives**: If you plan to supplement your perfusate with additives (e.g., sodium bicarbonate, vasodilators) through bolus or continuous infusions,

FDA recommends establishing pre-specified conditions for administering these additives to minimize bias.

- **Reperfusion duration**: You should specify the duration of *ex vivo* reperfusion to allow adequate assessment of organ function and viability. For instance, if you plan to assess the ability of a liver to synthesize a coagulation factor post-preservation, you should consider the half-life of the coagulation factor when specifying the duration of reperfusion.
- **Biomarkers**: Ischemia reperfusion injury may affect several distinct structures and functions of a single organ; therefore, FDA recommends evaluating a panel of biomarkers targeted to assess both organ injury and organ function. Due to the limitations of the *ex vivo* model, biomarkers for endothelial cell injury and activation of the inflammatory cascades should be evaluated. FDA recommends that you reference a sample panel of biomarkers developed for an *ex vivo* liver reperfusion model. See Appendix A for an example.
- **Edema**: FDA recommends weighing organs before and after reperfusion to assess the risk of machine perfusion-related edema. Machine perfusion parameters such as preservation duration, perfusate composition, temperature, pressure, and flow can contribute to edema, which in turn can adversely affect organ function. Extended hypothermic machine perfusion of the heart, for instance, is known to induce myocardial edema,[2,3] which is directly associated with increased ventricular stiffness and diastolic dysfunction.
- **Histopathology**: You should collect tissue biopsies from multiple representative regions of the organ before and after reperfusion. FDA recommends that a qualified independent pathologist evaluate the histopathology, with a focus on the integrity of endothelial cells using appropriate stains (e.g., CD31 immunohistochemistry stains for assessing sinusoidal endothelial cell integrity in the liver).

B. *In Vivo* Models

After an organ undergoes preservation, transplanting the organ in a survival model offers the most direct method for evaluating the preservation technology. Compared to *ex vivo* models, *in vivo* models rely on the most clinically relevant endpoint—graft survival, instead of biomarkers for organ injury and function. In addition, *in vivo* models allow for the whole-body immune response and the complex interplay between the coagulation and inflammatory cascades, so you can evaluate the full extent of ischemia reperfusion injury. Despite these advantages, *in vivo* models introduce many non-device related variables, which may affect transplant outcomes and hinder meaningful interpretation of data. To address these challenges, FDA recommends that you carefully consider the following:

[2] Van Caenegem O., et al., "Hypothermic continuous machine perfusion improves metabolic preservation and functional recovery in heart grafts." *Transpl Int* (2015) 28(2):224-231.

[3] Collins MJ, et al., "Preserving and evaluating hearts with ex vivo machine perfusion: an avenue to improve early graft performance and expand the donor pool." *Eur J Cardiothorac Surg* (2008) 34(2):318-325.

- **Confounders in the organ recipient**: FDA recommends that you collect baseline hemodynamic profiles in organ recipients and provide immunosuppressants to limit the effects of hemodynamic instability and immunologic heterogeneity, respectively, on transplant outcome.
- **Confounders in the transplant procedure**: The animal studies should be conducted in a highly controlled facility by qualified personnel with extensive experience in surgical transplants and post-operative care. Standard procedures, including antibiotic and immunosuppressant regimens and post-operative monitoring and care, should be applied to both the experimental and control groups. In order to reduce the risk of confounders such as rejections, FDA recommends a follow up period no longer than one week post-transplant, with endpoints that evaluate early injury patterns.

C. Conclusion

In the field of organ preservation, the outlook and utility of *ex vivo* and *in vivo* models will evolve with continued innovation in technology and our improved understanding of basic science. On one hand, as machine perfusion more closely mimics physiologic conditions and more biomarkers are accepted as surrogates for organ injury and function, the data collected in *ex vivo* models are expected to become increasingly predictive of transplant outcomes and subsequently reduce the number of animals used in the studies. On the other hand, *in vivo* models have the potential to utilize genetically-engineered animals with specific immunologic deficiencies or ischemic tolerance in order to simulate clinical scenarios.

While FDA understands that, the choice of the model may be restricted by many factors including utilizing animals and other available resources, your study should primarily be based on the study objectives and the risks of the device. For instance, *in vivo* models may be necessary to support an IDE application for a perfusion solution with multiple novel components or a first-of-its-kind device indicated to improve the quality of extended criteria donor organs. *Ex vivo* models may be sufficient to support, for example, a device modification or protocol modification of a previously approved IDE. Recognizing that each scenario is unique and that our understanding of these devices continues to evolve, FDA recommends that you engage us via the Pre-Submission process to obtain feedback on proposed animal studies.

Appendix A: Sample Panel of Biomarkers in Liver

Ischemia reperfusion injury may affect several distinct structures and functions of a single organ; therefore, FDA recommends evaluating a panel of biomarkers targeted to both organ injury and organ function. FDA recommends that you reference a sample panel of biomarkers developed for an *ex vivo* liver reperfusion model as a starting point. Our goal is not to prescribe any particular set of biomarkers for any given organ. Instead, this example illustrates the approach for identifying an appropriate panel of biomarkers.

Liver Injury	**Biomarkers**
Hepatocellular Injury	aspartate aminotransferase (ALT) alanine aminotransferase (AST)
Hepatobilliary Injury	γ-glutamyl transpeptidase (GGT) alkaline phosphatase (ALP)
Sinusoidal Endothelial Cell Injury	hyaluronic acid
Kupffer Cell Activation	β-galactosidase
Liver Function	**Biomarkers**
Synthetic	bile volume, Factor V or VII
Metabolic	pH, lactate

Table 1: Sample liver injury and function biomarkers.

A. Liver Injury Biomarkers

Ischemia reperfusion injuries in an *ex vivo* liver reperfusion model can affect both hepatocytes and cholangiocytes. As shown in Table 1, FDA suggests measuring the following injury biomarkers in the perfusate: ALT and AST for hepatocellular injury and GGT and ALP for hepatobiliary injury.

In the liver, sinusoidal endothelial cells are particularly sensitive to ischemia reperfusion injury. Adenosine triphosphate depletion during ischemia, followed by damages to the sinusoidal endothelial cells and activation of Kupffer cells, triggers a cascade of inflammatory responses, which can lead to microvascular thrombosis and graft dysfunction. In an *ex vivo* reperfusion model, the risk of microvascular thrombosis cannot be directly evaluated due to the use of anticoagulants in the perfusate. Therefore, as an alternative, FDA suggests measuring hyaluronic acid[4,5,6] and β-galactosidase[7,8] levels in

[4] Brockmann J, et al., "Normothermic perfusion – A new paradigm for organ preservation." *Ann Surg* (2009) 250(1):1-6.
[5] Schön MR, et al., "Liver transplantation after organ preservation with normothermic extracorporeal perfusion." *Ann Surg* (2001) 233(1):114-123.
[6] Spetzler VN, et al., "Subnormothermic ex vivo liver perfusion is a safe alternative to cold static storage for preserving standard criteria grafts." *Liver Transpl* (2016) 22(1):111–119.
[7] Reddy S, et al., "Non-heart-beating donor porcine livers: The adverse effect of cooling." *Liver Transpl* (2005) 11(1):35-38.

the perfusate as markers for sinusoidal endothelial cell injury and Kupffer cell activation, respectively.

B. Liver Function Biomarkers

In addition to injury biomarkers, maintenance of liver function is an important predictor of graft survival. FDA suggests measuring the following biomarkers: bile production and perfusate-level coagulation factor (e.g. Factor V, Factor VII) for hepatic synthetic function; and pH and lactate levels in the perfusate for hepatic metabolic function.

In *in vivo* models, prothrombin time or international normalized ratio is typically assessed as a marker for hepatic synthetic function. However, evaluation of prothrombin time is not feasible in *ex vivo* reperfusion models due to the use of anticoagulants in the perfusate. As an alternative, FDA suggests assessing perfusate-level coagulation factors[9,10] as biomarkers of hepatic synthetic function.

[8] Liu W, et al., "Glycohydrolases as markers of hepatic ischemia-reperfusion injury and recovery." *Hepatology* (1996) 24(1):157-162.
[9] Reddy S, et al., "Preservation of porcine non-heart-beating donor livers by sequential cold storage and warm perfusion." *Transpl* (2004) 77(9):1328-1332.
[10] Imber CJ, et al., "Advantages of normothermic perfusion over cold storage in liver preservation." *Transpl* (2002) 73(5):701-709.