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Utilizing Animal Studies to Evaluate Organ Preservation Devices

Draft Guidance for Industry and Food and Drug Administration Staff

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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.



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

Preface

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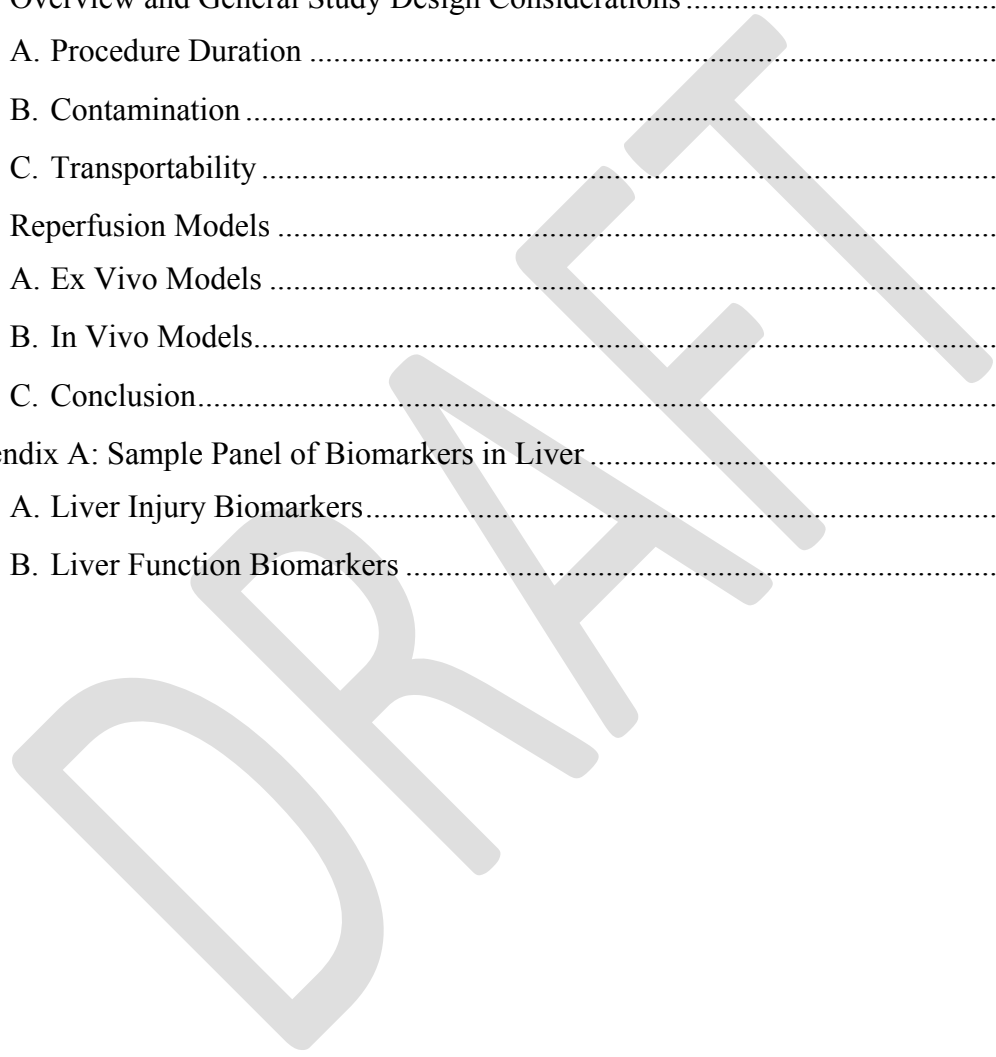
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This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative 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The intent of this draft guidance is to provide recommendations regarding best practices for utilizing animal studies for the evaluation of organ preservation devices.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.

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

100
101 FDA encourages members of industry to engage CDRH via the Pre-Submission process to obtain
102 feedback for specific animal study protocols to evaluate organ preservation devices. For more
103 information on Pre-Submissions, you should refer to “Requests for Feedback on Medical Device
104 Submissions: The Pre-Submission Program and Meetings with Food and Drug Administration
105 Staff”
106 (<https://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm311176.pdf>).
107

108
109 In this document, the terms “you” and “your” refer to members of industry, also known as
110 “sponsors” or “applicants.” The terms “we,” “us,” and “our” refer to FDA.

111
112 FDA's guidance documents, including this draft guidance, do not establish legally enforceable
113 responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be
114 viewed only as recommendations, unless specific regulatory or statutory requirements are cited.
115 The use of the word *should* in FDA guidance means that something is suggested or
116 recommended, but not required.

117 **II. Scope**

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

122
123 Most of the devices to which this guidance applies are currently not classified. This guidance
124 document is also applicable to the product code KDN, System, Perfusion, Kidney (21 CFR
125 876.5880, Class II).

126
127 The recommendations in this guidance document do not apply to devices intended to preserve
128 organs via cold static storage, including those associated with product codes KDK, PIN, KDL,
129 and MSB, regulated as Class II devices under 21 CFR 876.5880, Isolated kidney perfusion and
130 transport system and accessories. In addition, human cells, tissues and cellular and tissue-based
131 products (HCT/P's) regulated under 21 CFR 1271.3(d)(1) and Section 351 & 361 of the Public
132 Health Service Act and the devices utilized to preserve and transport them are also outside the
133 scope of this guidance document.

134 **III. Definitions**

135 For purposes of this guidance document, the following definitions apply:
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137 **Cold Ischemia Time:** The amount of time that an organ is cold (~4°C) and not receiving
138 adequate blood supply.

139
140 **Cold Static Storage:** The current standard method to preserve most organs. Organs are
141 submerged in a preservation solution in a closed container that maintains the temperature
142 at ~4°C.

143
144 **Extended Criteria Organs:** Donor organs that are suboptimal for transplant (e.g.,
145 donation after cardiac death (DCD) donor organs). The criteria may differ depending on
146 organ type.

147
148 **Ischemia Reperfusion Injury:** Inflammation and oxidative damage to the tissue caused
149 by the restoration of blood supply after a period of ischemia.

150
151 **Machine Perfusion:** A dynamic method to preserve organs, utilizing a device with a
152 pump that drives the movement of a perfusate. Devices performing machine perfusion of
153 organs may also contain oxygenators, heat exchangers, sensors, disposable circuits, and
154 computer units for processing and displaying hemodynamic and metabolic data. Machine
155 perfusion can be performed at various temperatures, e.g., ~4°C (hypothermic), ~37°C
156 (normothermic).

157
158 **Perfusate:** The solution that is pumped through the donor organ.

159
160 **Reperfusion:** The restoration of blood supply to an organ.

161
162 **Warm Ischemia Time:** The amount of time that an organ is at body temperature or room
163 temperature and not receiving adequate blood supply.

164 **IV. Overview and General Study Design Considerations**

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

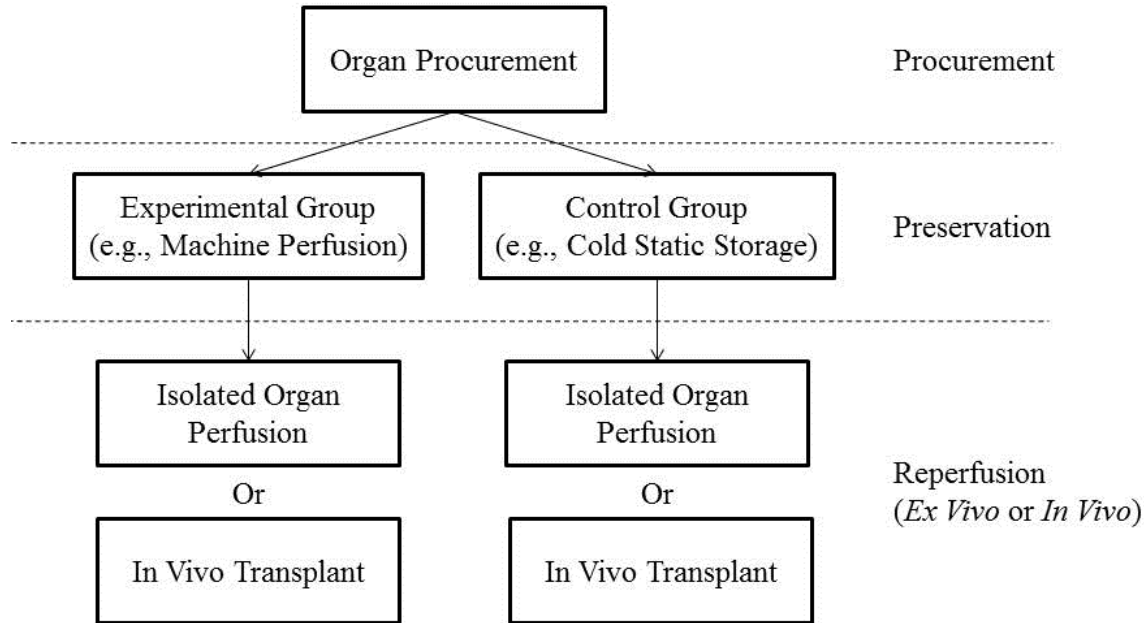
174
175 After determining the specific risks and their corresponding failure modes, you should develop a
176 protocol with focused objectives and *a priori* acceptance criteria. When appropriate, FDA
177 recommends including the scientific rationales for the chosen acceptance criteria. In addition,
178 FDA recommends that you provide a rationale for the selection of a particular animal model for

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

181
182 A typical experimental setup for such animal studies will consist of three phases: organ
183 procurement, organ preservation, and organ reperfusion (see Figure 1 below).
184



185
186 **Figure 1: The Three Phases of a Typical Animal Study for Evaluating Organ Preservation**
187 **Devices**

188
189 To begin the safety assessment of the organ preservation device selected organs are procured
190 from appropriate animal model donors. Then, these organs are preserved using either an
191 experimental method (e.g., machine perfusion) or a control method (e.g., cold static storage).
192 Organs from both groups are reperfused in either an *in vivo* or *ex vivo* model, to evaluate
193 reperfusion injury. Due to its complexity, the reperfusion phase will be discussed in detail in
194 Section V. In the section below, our recommendations focus on general study design
195 considerations:

196 **A. Procedure Duration**

197 Procedure duration has a significant effect on the outcome of transplant studies. You
198 should carefully consider the following recommendations regarding the duration of the
199 experimental procedures:
200

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

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

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

216 **B. Contamination**

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

221 **C. Transportability**

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

231 **V. Reperfusion Models**

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

238 **A. Ex Vivo Models**

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

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

259
260 While some of these limitations are inherent to the *ex vivo* model, other limitations can be
261 mitigated through improved study design. FDA has the following recommendations for
262 study designs in an *ex vivo* model:

- 263
264 • **Control group:** Due to the limitations discussed above, an *ex vivo* model cannot
265 determine the absolute extent of ischemia reperfusion injury. Therefore, we
266 recommend including a control group (e.g., cold static storage) in the study, so
267 that the relative effects of the injury can be evaluated.
- 268 • **Near-physiological conditions:** In order to simulate *in vivo* conditions, *ex vivo*
269 reperfusion should be performed under near-physiological conditions (e.g.,
270 temperature, pressure, flow, oxygenation). The performance of critical device
271 components (e.g., pumps, sensors, oxygenators) should be validated using
272 exploratory animal studies or studies using human organs not suitable for
273 transplant.
- 274 • **Whole blood as perfusate:** FDA recommends that your perfusate consist
275 primarily of whole blood collected from third party animals as blood donors. The
276 use of blood from the organ donors should be avoided in order to 1) simulate the
277 reperfusion conditions in clinical transplants and 2) limit the confounding effects
278 of hypovolemia and catecholamine release on the donor organ.
- 279 • **Perfusate additives:** If you plan to supplement your perfusate with additives
280 (e.g., sodium bicarbonate, vasodilators) through bolus or continuous infusions,

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- 281 FDA recommends establishing pre-specified conditions for administering these
282 additives to minimize bias.
- 283 • **Reperfusion duration:** You should specify the duration of *ex vivo* reperfusion to
284 allow adequate assessment of organ function and viability. For instance, if you
285 plan to assess the ability of a liver to synthesize a coagulation factor post-
286 preservation, you should consider the half-life of the coagulation factor when
287 specifying the duration of reperfusion.
 - 288 • **Biomarkers:** Ischemia reperfusion injury may affect several distinct structures
289 and functions of a single organ; therefore, FDA recommends evaluating a panel of
290 biomarkers targeted to assess both organ injury and organ function. Due to the
291 limitations of the *ex vivo* model, biomarkers for endothelial cell injury and
292 activation of the inflammatory cascades should be evaluated. FDA recommends
293 that you reference a sample panel of biomarkers developed for an *ex vivo* liver
294 reperfusion model. See Appendix A for an example.
 - 295 • **Edema:** FDA recommends weighing organs before and after reperfusion to assess
296 the risk of machine perfusion-related edema. Machine perfusion parameters such
297 as preservation duration, perfusate composition, temperature, pressure, and flow
298 can contribute to edema, which in turn can adversely affect organ function.
299 Extended hypothermic machine perfusion of the heart, for instance, is known to
300 induce myocardial edema,^{2,3} which is directly associated with increased
301 ventricular stiffness and diastolic dysfunction.
 - 302 • **Histopathology:** You should collect tissue biopsies from multiple representative
303 regions of the organ before and after reperfusion. FDA recommends that a
304 qualified independent pathologist evaluate the histopathology, with a focus on the
305 integrity of endothelial cells using appropriate stains (e.g., CD31
306 immunohistochemistry stains for assessing sinusoidal endothelial cell integrity in
307 the liver).

308 B. *In Vivo* Models

309 After an organ undergoes preservation, transplanting the organ in a survival model offers
310 the most direct method for evaluating the preservation technology. Compared to *ex vivo*
311 models, *in vivo* models rely on the most clinically relevant endpoint—graft survival,
312 instead of biomarkers for organ injury and function. In addition, *in vivo* models allow for
313 the whole-body immune response and the complex interplay between the coagulation and
314 inflammatory cascades, so you can evaluate the full extent of ischemia reperfusion injury.
315 Despite these advantages, *in vivo* models introduce many non-device related variables,
316 which may affect transplant outcomes and hinder meaningful interpretation of data. To
317 address these challenges, FDA recommends that you carefully consider the following:
318

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.

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

331 **C. Conclusion**

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

341
342 While FDA understands that, the choice of the model may be restricted by many factors
343 including utilizing animals and other available resources, your study should primarily be
344 based on the study objectives and the risks of the device. For instance, *in vivo* models
345 may be necessary to support an IDE application for a perfusion solution with multiple
346 novel components or a first-of-its-kind device indicated to improve the quality of
347 extended criteria donor organs. *Ex vivo* models may be sufficient to support, for example,
348 a device modification or protocol modification of a previously approved IDE.
349 Recognizing that each scenario is unique and that our understanding of these devices
350 continues to evolve, FDA recommends that you engage us via the Pre-Submission
351 process to obtain feedback on proposed animal studies.

352

353

354 **Appendix A: Sample Panel of Biomarkers in Liver**

355 Ischemia reperfusion injury may affect several distinct structures and functions of a single organ;
 356 therefore, FDA recommends evaluating a panel of biomarkers targeted to both organ injury and
 357 organ function. FDA recommends that you reference a sample panel of biomarkers developed for
 358 an *ex vivo* liver reperfusion model as a starting point. Our goal is not to prescribe any particular
 359 set of biomarkers for any given organ. Instead, this example illustrates the approach for
 360 identifying an appropriate panel of biomarkers.
 361

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

362 **Table 1: Sample liver injury and function biomarkers.**

363 **A. Liver Injury Biomarkers**

364 Ischemia reperfusion injuries in an *ex vivo* liver reperfusion model can affect both
 365 hepatocytes and cholangiocytes. As shown in Table 1, FDA suggests measuring the
 366 following injury biomarkers in the perfusate: ALT and AST for hepatocellular injury and
 367 GGT and ALP for hepatobiliary injury.
 368

369 In the liver, sinusoidal endothelial cells are particularly sensitive to ischemia reperfusion
 370 injury. Adenosine triphosphate depletion during ischemia, followed by damages to the
 371 sinusoidal endothelial cells and activation of Kupffer cells, triggers a cascade of
 372 inflammatory responses, which can lead to microvascular thrombosis and graft
 373 dysfunction. In an *ex vivo* reperfusion model, the risk of microvascular thrombosis cannot
 374 be directly evaluated due to the use of anticoagulants in the perfusate. Therefore, as an
 375 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.

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

378 **B. Liver Function Biomarkers**

379 In addition to injury biomarkers, maintenance of liver function is an important predictor
380 of graft survival. FDA suggests measuring the following biomarkers: bile production and
381 perfusate-level coagulation factor (e.g. Factor V, Factor VII) for hepatic synthetic
382 function; and pH and lactate levels in the perfusate for hepatic metabolic function.
383

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

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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.