Ensuring the quality of a test system using the principles of Good In Vitro Method Practices (GIVIMP): A case study of cryopreserved human precision-cut lung slices

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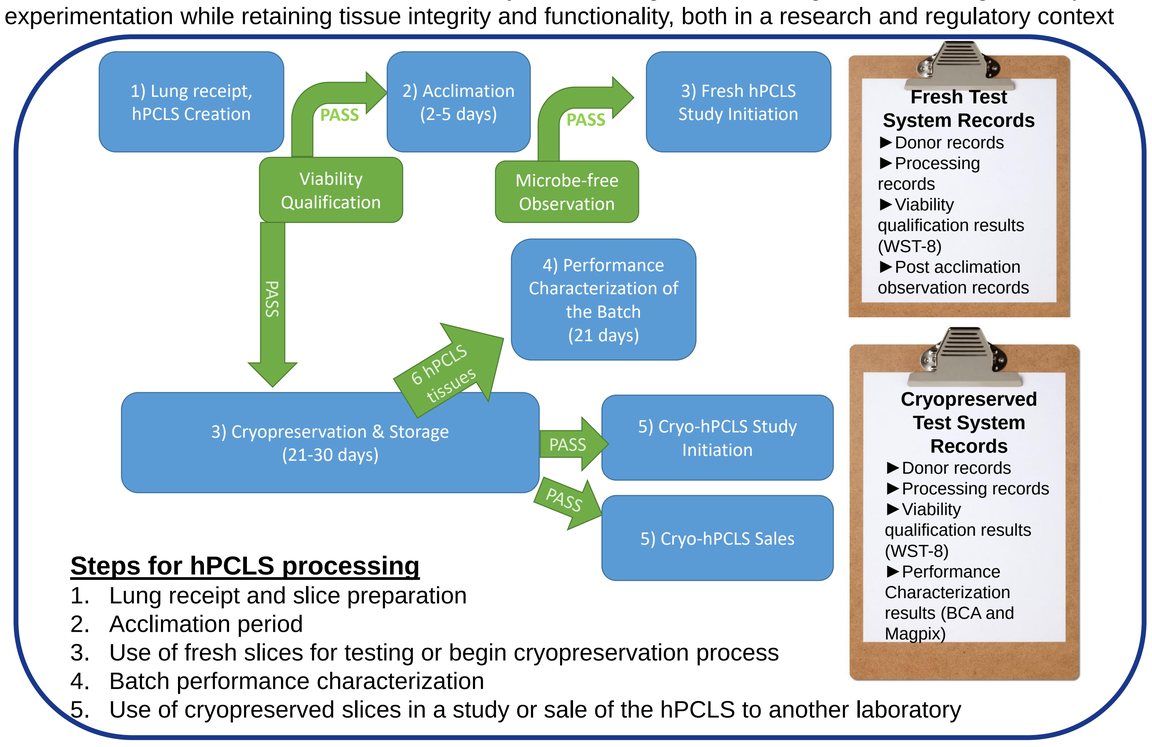
ABSTRACT

Human precision-cut lung slices (hPCLS) are a highly relevant 3-dimensional model of the lung. They offer native architecture and cells of the lung tissue including respiratory parenchyma, small airways, and immune competent cells involved in inflammatory and sensitization processes. The scarcity of human lung tissue available for research and the inability to conduct larger scale testing has limited the use of hPCLS as a test system for routine, high-throughput testing. To overcome this barrier, IIVS has refined the methodology behind the cryopreservation, storage, thaw and post-thaw maintenance of human lung slices. As primary tissues have varied quality and responsiveness, a standardized performance characterization (PC) is conducted on all donor batches. To establish credibility for the approach, the principles of the OECD guidance document (No. 286), "Good In Vitro Method Practices (GIVIMP)", were applied. Documentation for 1) Donor batches created (including blinded donor demographic/medical data), 2) hPCLS physical and functional characteristics (e.g., PC data) and storage conditions, 3) recommended thaw and culture protocol, and 4) chain of custody documents relevant to the distribution of hPCLS to other laboratories are maintained. The quality principles of GIVIMP are crucial to the hPCLS serving as a reliable test system for use in repeatable research and regulatory toxicology. With improvements in slice creation, storage, culture conditions, and the quality framework surrounding all of these efforts, the IIVS hPCLS can be confidently used for larger scale testing, tissue banking, and repeat donor experimentation while retaining tissue integrity and functionality, both in a research and regulatory context

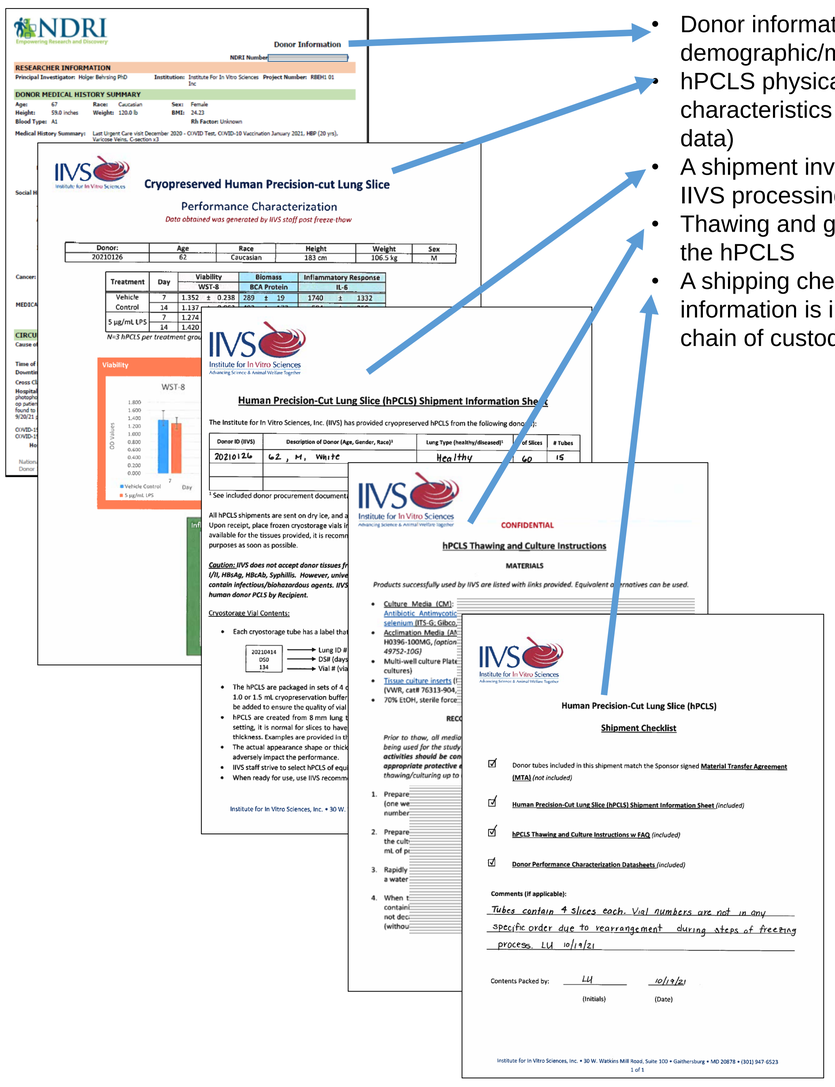


Steps for hPCLS processing

1. Lung receipt and slice preparation
2. Acclimation period
3. Use of fresh slices for testing or begin cryopreservation process
4. Batch performance characterization
5. Use of cryopreserved slices in a study or sale of the hPCLS to another laboratory

INFORMATION PROVIDED TO PURCHASERS

GIVIMP chapter 1 discusses the responsibility *in vitro* test system providers have to share information on test system characterization, authenticity, and safe transport, use, and disposal. IIVS provides a package of this information to test system purchasers to satisfy the GIVIMP requirements of test system providers.



- Donor information including blinded donor demographic/medical data
- hPCLS physical and functional characteristics of the bactch of PCLS (PC data)
- A shipment inventory sheet traceable to the IIVS processing batch records
- Thawing and general culture instructions for the hPCLS
- A shipping checklist to assure all required information is included and serving as a chain of custody document

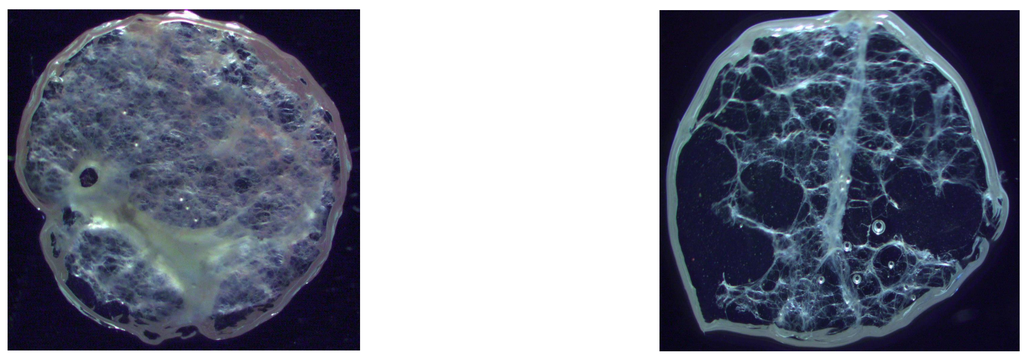
STANDARDIZATION AND DOCUMENTATION

In vitro test systems must reliably perform as expected when used in studies. GIVIMP recommends standardizing procedures Chapter 7), training to the standards (Chapter 2), and documenting that the standards have been followed (Chapter 10). GIVIMP also provides guidance for consideration when optimizing the procedures during the later stages of standardization. IIVS meets the recommendations in GIVIMP for cryopreservation of hPCLS activities.

- Step-by-step procedures from procurement of the lung, through preparation of slices and their assessment and cryopreservation, to shipment of the test system to the purchaser are documented in management approved Standard Operating Procedures (SOPs).
- Batch records are available for each lot of tissue that attribute each procedural step to trained individuals and provide traceability to media and equipment used. ALCOA + principles are followed with all paper and electronic based documentation.
- Personnel training is standardized and documented in training records.
- Several manuscripts and posters have been published describing the optimization of the precision cut lung slice procedure.

ETHICAL CONSIDERATIONS

Unlike other quality systems, GIVIMP addresses ethical concerns that may arise when using *in vitro* methods. GIVIMP stresses the need for donor consent when primary human tissues or cells are used. Chapter 4 of GIVIMP provides guidance for using alternatives to animal sources of serum in media components. The presence of Intellectual Property Rights (IPR), patents, and trademarks on test systems and/or media could be limiting factors in the ability of *in vitro* methods to be a viable testing option for many laboratories. These points have been assessed and addressed for hPCLS at IIVS.



Normal hPCLS **Emphysemic hPCLS**

The photographs above show a representative normal hPCLS and one from a lung with diagnosed emphysema. Organ Procurement Organizations assure donor consent for research for all donor characteristic requests.

- All media and components used in the processing and handling of hPCLS are free of animal products.
- No sera of any type are used.
- A laboratory does not need special equipment or a high level BSL facility to use the hPCLS for testing.
- There are no concerns about Intellectual Property Rights (IPR) related to the slices. Cryopreservation media is considered confidential, but instructions for all media needed for tissue handling, maintenance, and testing are shared with the purchaser.

TEST SYSTEM CHARACTERIZATION AND CONTROLS

Chapters 5 and 2 of GIVIMP discuss measures to improve the quality of the test systems used in *in vitro* testing. Some of these topic areas include: cell and tissue sourcing, handling and maintenance of the test system, cryopreservation and storage, contaminant screening, and quality control of test systems. The documentation and procedures in place at IIVS surrounding the preparation of hPCLS tissues was assessed against the applicable GIVIMP recommendations. Select points are discussed and examples given below.

- Lungs are ethically sourced through Organ Procurement Organizations. These groups assure donor consent in accordance with federal and state laws through the Organ Procurement Agencies.
- Specific characteristics for the batches of tissues are requested and documented prior to receive of the lungs for processing (see figure below for example).

LUNG DONOR ACCEPTANCE CRITERIA					
Lung Type:	**Normal**			**Clinical/Pre-Op Read**	
Special Conditions:			Chest tubes (X-ray)		
Donor Demographics			Lab Data (pCO2, pO2, etc.)		no restrictions
Donor Age	18-70		Atelectasis (X-ray)		
Gender	M or F		Pneumothorax (X-ray)		
Race	any		Effusions		
Donor Behavior History			Sarcoidosis		Ok if **NOT** diffuse
Tobacco use		1/2 PPD, chewing tobacco is Ok	Infiltrates		Ok if **NOT** diffuse
Electronic Cigs			Contusions (X-ray)		Ok, if some lung is undamaged
Illicit Drug use		opiates, IV	Aspiration (one/both Lungs)		recent ok, not extensive or multiple
Marijuana use			Edema		pulmonary edema due to heart ok
Alcohol use			Bacterial &Viral Meningitis		
Unacceptable Medicines		Inhalation based	MRSA in Sputum Culture		
Organ Specifics			Sepsis		
Warm ischemic time	2hr	Low-PMI	MRSA in BLOOD culture		
Cold ischemic time	36hr	Low-PMI	MRSA in NARES		
Downtime	1hr	Low-PMI	High Risk CDC donors		
Ventilator time	≤ 7 days		Fibrosis		
History/Current Disease States			Sepsis		
Diabetes		any type OK	Pneumontitis/Pneumonia (X-ray)		
Hypertension		systemic or pulmonary OK	Positive Sputum or BAL Cultures:		
History Chemotherapy		Ok if Lung not adversely impacted	Infection/Granuloma		
History Radiation		Ok if Lung not adversely impacted	Inflammation		
History Cancer		lung cancer IF we can isolate healthy	Foreign objects		
Asthma			Bleeding		
COPD			Pus-like secretions		
Emphysema			**Serologies**		
Cystic Fibrosis			Acceptable: Anti-CMV, EBV		
			Not acceptable: Anti-HCV, Anti-HIV I/II, HBsAg, HBcAb, Syphilis		
During the procurement process, IIVS is presented with specific donor information that will be limited to data obtained by the facility where the donor was provided medical care. IIVS will make the best effort to ensure the inclusion criteria are met and/or that the accepted donor lung meets the needs of the Sponsor's study objectives.					
Color Codes:	Accepatable	Not Acceptable	Conditional		

Example chart outlining screening characteristics necessary for acceptance of a "normal lung"

- Several quality control points are built into the process (see timeline to the left). The test system is viewed and assessed at each point in green.
- Lack of overt contamination is assured prior to cryopreservation of the processed lung slice.
- Tissue functionality and general characteristics are assessed for each batch and assured to be acceptable.

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