

Feb 04, 2026

Human Pancreas Processing

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v9oqwmv3e/v1>

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DOI: <https://dx.doi.org/10.17504/protocols.io.x54v9oqwmv3e/v1>

Protocol Citation: Anastasia Coldren 2026. Human Pancreas Processing. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.x54v9oqwmv3e/v1>

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Protocol status: Working

We use this protocol and it's working

Created: June 17, 2025

Last Modified: February 04, 2026

Protocol Integer ID: 220346

Abstract

Human Pancreas Processing

Guidelines

Human sample collection for this protocol requires prior approval by the users' Institutional Ethics Board or equivalent ethics committee.

Materials

Preparing Pancreatic Slices:

Supplies:

Sterile Sponge (Covidien, 8044)
Plastic Weigh Boat
Ruler with cm
Microsurgical tools

Equipment:

Dissection Tray
Analytical Balance
Ice-cooled Dissecting Platform
Digital camera

Human Pancreas Processing for Paraffin-embedding (PE)

Reagents:

- ⊗ Sodium Phosphate dibasic anhydrous (Na₂HPO₄) Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5011
- ⊗ KH₂PO₄ Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5655
- ⊗ NaCl Fisher Scientific Catalog #S640-10
- ⊗ KCl Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9333
- ⊗ PBS, no calcium, no magnesium Thermo Fisher Scientific Catalog #14190144
- ⊗ PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE EMS Catalog #15710
- ⊗ 70% ethanol VWR International (Avantor)

Supplies:

50 mL conical tubes
Fine scissors
10 mL Petri dishes

Equipment:

Equipment

Adjustable Tilt Rocker

NAME

LabNet

BRAND

S2035-DE

SKU

Human Pancreas Processing for Flash Frozen Sections (FF):

Supplies:

50 mL conical tubes
 Forceps
 Aluminum foil
 Zip-lock bags
 Dry ice

Reagents:

⊗ Liquid Nitrogen

⊗ Dry ice

Human Pancreas Processing for Fixed Cryosections (OCT):

Supplies:

50 mL conical tubes

⊗ Cryomold **VWR International (Avantor) Catalog #25608-916**

⊗ Andwin Scientific Tissue-Tek™ CRYO-OCT Compound **Fisher Scientific Catalog #14-373-65**

10 cm Petri dish
 Fine forceps
 Kimwipes
 Aluminum foil
 Zip-lock bags

Reagents:

⊗ PBS, no calcium, no magnesium **Thermo Fisher Scientific Catalog #14190144**

⊗ 16% Paraformaldehyde (PFA) **Fisher Scientific Catalog #50-980-487**

Equipment:

Equipment

Adjustable Tilt Rocker

NAME

LabNet

BRAND

S2035-DE

SKU

Human Pancreas Processing for Clarity (CLR):

Supplies:

50 mL conical tubes

Reagents:

 PBS, no calcium, no magnesium **Thermo Fisher Scientific Catalog #14190144**

 PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE **EMS Catalog #15710**

Equipment:

Equipment	
Adjustable Tilt Rocker	NAME
LabNet	BRAND
S2035-DE	SKU

Human Pancreas Processing for Transmission Electron Microscopy (EM):

Supplies:

50 mL conical tubes

Reagents:

 PBS, no calcium, no magnesium **Thermo Fisher Scientific Catalog #14190144**

 PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE **EMS Catalog #15710**

 8% Glutaraldehyde **Electron Microscopy Sciences Catalog #16020**

 Sodium Cacodylate Buffer **Electron Microscopy Sciences Catalog #11652**

Equipment:

Equipment	
Adjustable Tilt Rocker	NAME
LabNet	BRAND
S2035-DE	SKU



Protocol materials

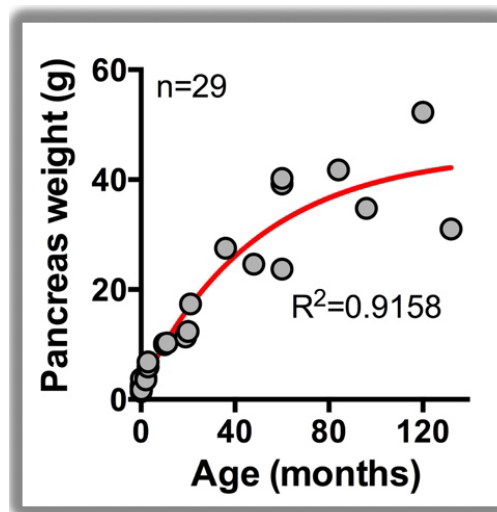
- ✕ 100 mM PBS **Electron Microscopy Sciences Catalog #19344-10**
- ✕ 70% ethanol **VWR International (Avantor)**
- ✕ Liquid Nitrogen
- ✕ Dry ice
- ✕ 16% Paraformaldehyde (PFA) **Fisher Scientific Catalog #50-980-487**
- ✕ D-Sucrose **Fisher Scientific Catalog #BP220-1**
- ✕ Andwin Scientific Tissue-Tek™ CRYO-OCT Compound **Fisher Scientific Catalog #14-373-65**
- ✕ Cryomold **VWR International (Avantor) Catalog #25608-916**
- ✕ PBS, no calcium, no magnesium **Thermo Fisher Scientific Catalog #14190144**
- ✕ 8% Glutaraldehyde **Electron Microscopy Sciences Catalog #16020**
- ✕ PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE **EMS Catalog #15710**
- ✕ Sodium Cacodylate Buffer **Electron Microscopy Sciences Catalog #11652**
- ✕ KH₂PO₄ **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5655**
- ✕ NaCl **Fisher Scientific Catalog #S640-10**
- ✕ KCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9333**
- ✕ Sodium Phosphate dibasic anhydrous (Na₂HPO₄) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5011**

Before start

Prepare all reagents needed before starting this protocol.

Preparing Pancreatic Slices

- 1 Upon arrival, transfer the pancreas and transport solution (UW or HTK) into a dissection tray placed on ice. Transfer enough of the the transport solution to keep pancreas submerged.
- 2 Dissect the pancreas by carefully removing duodenum, mesentery, fat, and spleen.
- 3 Once the pancreas is clean, blot it with a sterile sponge, and quickly take its wet weight by placing it into a pre-weighed clean plastic boat.



Range of pancreatic weights collected from neonatal and juvenile donors in the first 10 years of life.

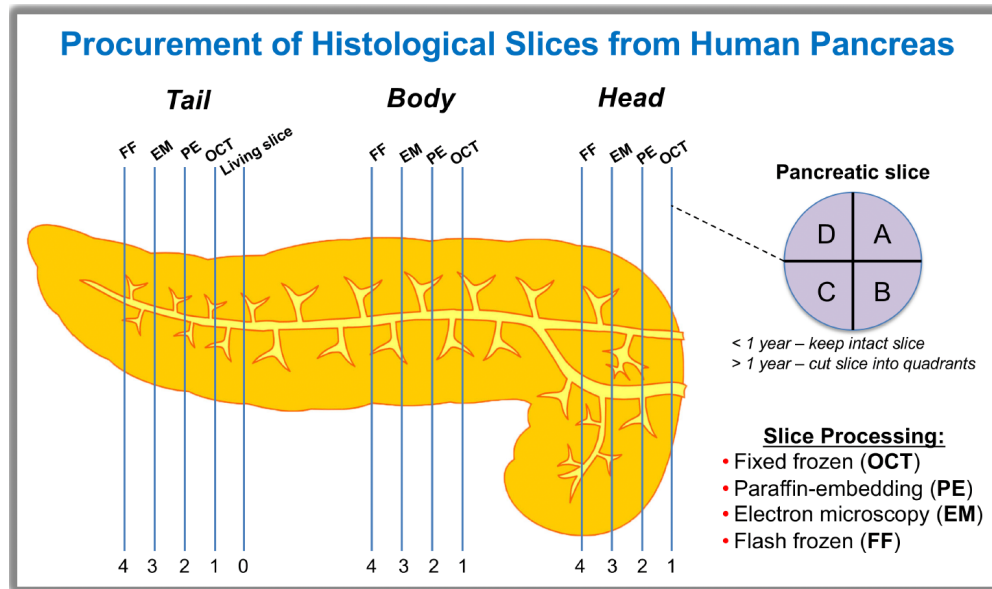
- 4 Transfer immediately to an ice-cooled dissecting platform and take a photograph with a ruler as shown below.





Example of pancreatic tissues from young donors of different age categories.

- 5 Immediately after photographing, start collecting approximately ± 2 mm thick slices as outlined below. Collect slices in head-to-toe order as shown below.



Map of pancreatic slices with reference to their anatomical location and processing procedure.

- 5.1 The number of slices and their processing will be determined on a case-by-case basis depending on tissue availability, age category, and requirements for downstream analyses.
- 6 Label pancreatic slices following the naming nomenclature and controlled vocabularies developed for Vanderbilt LIMS, which includes the position of each slice in a given anatomical location and the processing procedure.

LABELING EXAMPLES for a 10-year-old male donor without diabetes:

Slices collected from the pancreatic head:

- UNOS ID-ND-10y-M-PANC-OCT-H1
- UNOS ID-ND-10y-M-PANC-PE-H2
- UNOS ID-ND-10y-M-PANC-EM-H3
- UNOS ID-ND-10y-M-PANC-FF-H4

If the next set of slices is also collected from the pancreatic head:

- UNOS ID-ND-10y-M-PANC-OCT-H5
- UNOS ID-ND-10y-M-PANC-PE-H6
- UNOS ID-ND-10y-M-PANC-EM-H7
- UNOS ID-ND-10y-M-PANC-FF-H8

LABELING EXAMPLES for a male donor less than 1 year of age. Each slice would be divided into quadrants and labeled as follows:



- UNOS ID-ND-6mo-M-PANC-OCT-H1A
- UNOS ID-ND-6mo-M-PANC-PE-H1B
- UNOS ID-ND-6mo-M-PANC-EM-H1C
- UNOS ID-ND-6mo-M-PANC-FF-H1D

6.1 **LIVING SLICES:** Prior to pancreas processing, tissue from the Tail region will be removed to generate 100-140 living pancreatic slices following existing nPOD protocol.

Human Pancreas Processing for Paraffin-embedding (PE)

20h

7 Prepare  1 L  100 millimolar (mM) PBS:

Note

Steps that use this recipe:  [go to step #24](#)

7.1  12.07 g



Sodium Phosphate dibasic anhydrous (Na₂HPO₄) Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5011



2.04 g  KH₂PO₄ Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5655



8.0 g  NaCl Fisher Scientific Catalog #S640-10



2.0 g  KCl Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9333

7.2 Adjust to  7.5 .

7.3 Filter through a  0.22 μ m filter.

**Note**

[M] 100 millimolar (mM) PBS can be stored at 4 °C for up to 1 month.

- 8 Prepare 40 mL fixative for each slice: 4%
⊗ PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE EMS Catalog #15710 in
prepared [M] 100 millimolar (mM) PBS [go to step #7](#)
- 8.1 Transfer contents of 10 mL vial of
⊗ PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE EMS Catalog #15710 into a
50 mL conical tube.
- 8.2 Add 30 mL [M] 100 millimolar (mM) PBS. Mix and place the tube On ice .
- 9 Prepare and label one 50 mL conical tube with 40 mL of freshly-prepared fixative
for each slice.
- 10 Cut each cross-sectional slice from the Head, Body, and Tail into four quadrants (A, B, C,
D) and place all 4 tissue slices into the same 50 mL tube with fixative.

Note

For donors less than 1 year of age, keep the entire slice intact.

- 11 Place tube onto an adjustable tilt rocker at 4 °C , and set to mild agitation. Allow
tissue to fix at least 18 hours.
- 12 Decant the fixative and blot the tube onto a paper towel. Add 40 mL
⊗ 100 mM PBS Electron Microscopy Sciences Catalog #19344-10 and place back
onto the rocker. Wash for 00:30:00 .

18h



30m



- 13 Repeat **STEP 11** 3 times for another 01:30:00 . 1h 30m
- 14 After the final wash, add 40 mL 70% ethanol **VWR International (Avantor)** .
Place tubes at 4 °C .
- 15 Ship tissue tubes in sealed tubes to **Vanderbilt University Medical Center** with ice packs.

Human Pancreas Processing for Flash Frozen Sections (FF)

- 16 Fill one 50 mL conical tube with 2-5 mL Liquid Nitrogen for every tissue slice to be procured and place on Dry ice .
- 17 Cut a piece of aluminum foil (about 5 square inches) for each tissue slice, label, and place on Dry ice to cool down.
- 18 Collect about 2 mm thick cross-sectional slices from the Head, Body, and Tail (see figure in Step 5 [go to step #5](#)).
- 19 Cut each slice into 4 quadrants (A, B, C, D), and drop each piece of tissue, one at a time, into the same tube.
- Note

For donors less than 1 year of age, keep the entire slice intact and drop into the tube.
- 20 Allow the Liquid Nitrogen to evaporate on Dry ice .
- 21 Using forceps, transfer the frozen tissue to a pre-labeled and pre-cooled piece of aluminum foil. Wrap the tissue in the foil, and place in a zip-lock bag. Seal and store at -80 °C .

**Note**

Aluminum foil and zip-lock bag keep the tissue from drying out.

- 22 Ship tissue blocks to **Vanderbilt University Medical Center** on Dry ice .

Human Pancreas Processing for Fixed Cryosections (OCT)

9h

- 23 Label a Cryomold **VWR International (Avantor) Catalog #25608-916** and a piece of foil (about 5 square inches) for each tissue.
- 24 Prepare 100 millimolar (mM) PBS per go to step #7 .
- 25 Prepare 4% PFA solution:
- 25.1 Transfer contents of 10 mL 16% Paraformaldehyde (PFA) **Fisher Scientific Catalog #50-980-487** vial to a 50 mL conical tube.
- 25.2 Add 30 mL prepared 100 millimolar (mM) PBS.
- 25.3 Mix and place the tube On ice .
- 26 Prepare **30% sucrose solution**:
- 26.1 Weigh out 15 g D-Sucrose **Fisher Scientific Catalog #BP220-1** into a 50 mL conical tube.



26.2 Add  35 mL

 PBS, no calcium, no magnesium **Thermo Fisher Scientific Catalog #14190144** .

Place the tube on an adjustable tilt rocker and place  On ice .

Note

Larger quantities of 30% sucrose can be prepared ahead of time, filtered through a 0.22 μ M filter, and stored at  4 °C for up to 1 month.

27 Collect about  2 mm thick cross-sectional slices from the Head, Body, and Tail (see pancreas map).

28 Cut each slice into 4 quadrants (A, B, C, D), and drop each piece of tissue, one at a time, into the same tube.

Note

For donors less than 1 year of age, keep the entire slice intact and drop into the tube.

29 Transfer the tissue into a 50 mL conical tube containing  40 mL freshly prepared fixative and fix for  03:00:00  On ice under mild agitation using an adjustable tilt rocker.

3h



30 Wash the tissue four times in  40 mL

3h

 100 mM PBS **Electron Microscopy Sciences Catalog #19344-10** over

 03:00:00 . Blot the tube on a paper towel between washes.



31 Equilibrate the tissue in  40 mL **30% sucrose solution** at  4 °C  Overnight . The tissue will settle to the bottom of the tube.

3h



Note

If the pancreas is not cleaned sufficiently of fat, the tissue will not drop.



32 Prepare a flat piece of dry ice.

33 Fill a pre-labeled cryomold halfway with



Andwin Scientific Tissue-Tek™ CRYO-OCT Compound **Fisher**
Scientific Catalog #14-373-65

.

34 Pour the pancreas with some sucrose out into a 10 cm Petri dish. Pick the tissue up with fine forceps and blot with Kimwipes to remove any excess sucrose.

35 Place the tissue into the prepared cryomold, and gently push toward the bottom. Add more



Andwin Scientific Tissue-Tek™ CRYO-OCT Compound **Fisher**
Scientific Catalog #14-373-65

to completely fill the mold.

36 Place the mold on a flat piece of dry ice. As the OCT begins to freeze, push the tissue down into the bottom of the mold again.

37 Once OCT is completely frozen, wrap the mold in pre-labeled piece of foil, place it in a zip-lock bag, and store at -80 °C .

Note

Aluminum foil and zip-lock bag keep the tissue from drying out.

38 Ship tissue blocks to **Vanderbilt University Medical Center** on dry ice.

Human Pancreas Processing for Clarity (CLR)

3h

39 Collect about 2 mm thick cross-sectional slices from the Head, Body, and Tail (see figure in Step 5 [go to step #5](#)).



- 40 Cut each slice into 4 quadrants (A, B, C, D), and drop each piece of tissue, one at a time, into the same tube.

Note

For donors less than 1 year of age, keep the entire slice intact and drop into the tube.

- 41 Transfer the tissue into a 50 mL conical tube containing  40 mL freshly prepared fixative and fix for  03:00:00  On ice under mild agitation using an adjustable tilt rocker.

3h



- 42 Wash the tissue four times in  40 mL



 PBS, no calcium, no magnesium **Thermo Fisher Scientific Catalog #14190144**

over  03:00:00 . Blot the tube on a paper towel between washes.

- 43 After the last wash, add  40 mL

 PBS, no calcium, no magnesium **Thermo Fisher Scientific Catalog #14190144** and

store at  4 °C .

- 44 Ship tissue blocks to  **Vanderbilt University Medical Center** with ice packs.

Human Pancreas Processing for Transmission Electron Microscopy (EM)

1d

- 45 Prepare TEM fixative in a 50 mL conical tube:

Note

Prepare as many tubes as the number of tissue slices to be processed.

- 45.1 Add  5 mL

 PARAFORMALDEHYDE 16% Aqueous SOL. EM GRADE **EMS Catalog #15710** .



45.2 Add  10 mL

 8% Glutaraldehyde **Electron Microscopy Sciences Catalog #16020** .

45.3 Add  20 mL

 Sodium Cacodylate Buffer **Electron Microscopy Sciences Catalog #11652** .

45.4 Add  5 mL dH₂O.

46 Collect about  2 mm thick cross-sectional slices from the Head, Body, and Tail (see figure in Step 5  [go to step #5](#)).

47 Place each slice into a separate 10 cm Petri dish containing 10–15 mL EM fixative at  Room temperature .

Note

Cold depolymerizes cytoskeletal elements, so whenever possible fixative and sample should be at  Room temperature or  37 °C to initiate fixation.

48 Using fine scissors, mince the sample in the plate with fixative. Alternatively, the tissue can be minced by placing it into a drop of fixative on dental wax or parafilm and using fresh razor blades.

Note

Aldehyde fixatives penetrate tissues slowly, so samples must be minced very small (less than  0.5 mm per side.

49 Transfer the minced tissue into a 50 mL conical tube and fix in  40 mL fixative for  24:00:00 at  4 °C under mild agitation on an adjustable tilt rocker.

1d



- 50 Ship to  **Vanderbilt University Medical Center** within 3 days on ice packs.
- 51 The Vanderbilt group will submit the tissue in fixative to the VU EM Core for EM processing. Tissue will be embedded into Spurr resin within 7 days of fixation.

Reference Info

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LIMS RECORDS – NAMING NOMENCLATURE & CONTROLLED VOCABULARIES

Powers Laboratory / Core Informatics LIMS (core_informatics_specimen_nomenclature_r20 last saved on 2/8/2017 11:01 PM)

RECORD NAMING NOMENCLATURE

In order to maintain consistency in the identification of records in the LIMS, we have adopted a nomenclature. This nomenclature begins at the entity donor level and is passed down to LIMS samples and LIMS lots. These entity names are assembled as an ordered and separated list of allowed (controlled) tokens. The LIMS is configured to automatically generate these names, as long as the primary values exist.

Please note that the entity hierarchy within the LIMS: **Donor > Sample > Lot > Container**

General rule is to separate tokens with hyphens: **{TOKEN}-{TOKEN}**

DONOR LEVEL NAME	
Structure	{donorID}-{disease state-age-disease duration-gender}
Example	AALQ152-T1D-58y-31y-M
SAMPLE (SPECIMEN) LEVEL NAME	
Structure	{donorID}-{disease state-age-disease duration-gender}-{specimen type}
Example	AALQ152-T1D-58y-31y-M-PANC
LOT LEVEL NAME	
Structure	{donorID}-{disease state-age-disease duration-gender}-{specimen type}-{processing}-{specimen anatomical location}
Example	ABHQ115-T2D-49y-3y-F-PANC-PE-T
CONTAINER LEVEL NAME	
The container name cannot inherit our nomenclature. Its name is assigned a container-specific name identical to its barcode (eg. BLK72, for Block #72).	

Token Definitions

{Token}	Definition	Include for	Examples	Example names
donorID	UNOS ID	donor name, donor specimen name, donor lot name	AALQ152 ADBNO25	AALQ152-T1D-58y-31y-M ADBNO25-ND-19y-F
disease state	diabetic status (e.g. type 2 diabetic, non-diabetic)		T2D	
age	donor age (e.g. 49 years)	donor name, donor specimen name, donor lot name	49y	
duration	duration of diabetes in years (y)		3y	ABHQ115-T2D-49y-3y-F
gender	donor gender (e.g. M for male or F for female)		F	
specimen type	type of specimen (e.g. pancreas, blood, islet)	donor specimen name	PANC BLD ISL	AALQ152-T1D-58y-31y-M-PANC AALQ152-T1D-58y-31y-M-BLD
processing	type of processing (e.g. paraffin embedded, fixed frozen)	donor lot name	PE OCT	ABHQ115-T2D-49y-3y-F-PANC-PE
specimen anatomical location	section of the specimen, (e.g. head, body, tail - This only applies to pancreas.)	donor lot name	H B T	ABHQ115-T2D-49y-3y-F-PANC-PE-T



Notes

1. Entity names must be unique.
2. Spaces are not allowed.
3. Hyphens are used to separate tokens.
4. Each entity record name not controlled by this nomenclature will generated using a pre-configured 2-3 letter prefix, e.g. DON20, VAE31, etc.
5. Non-diseased donors (i.e. no disease or *ND*) will not have a **disease duration** in the donor name, e.g. ND-25y-F is a 25-year old non-diabetic female.
6. Donors whose **age** is less than one year require the label “d” or “w” or “m” for days/weeks/months of age, based on the selected “age unit” of the respective donor.
7. Any donor whose **age** is in weeks will have a “G” automatically inserted in front of the age, e.g. G39w would be gestational age 39 weeks.
8. In order to keep things consistent, a dash is used between each token, e.g. ABHQ115-T2D-49y-3y-F.



CONTROLLED VOCABULARIES

KEY		
Token	Term	Acronym
diabetic status	auto-antibody positive	AAB
	cystic fibrosis	CF
	cystic fibrosis related diabetes	CFRD
	non-diabetic	ND
	type 1 diabetic	T1D
	type 2 diabetic	T2D
age	<i>See AGE table below</i>	
gender	female	F
	male	M
specimen type	blood	BLD
	islets	ISL
	lymph nodes	LN
	pancreas	PANC
	spleen	SPL
	islet transplant	TX
	FACS sorted cells	FACS
processing	plasma	PLA
	serum	SER
	perifusion fractions	PERIF
	hormone extract	HOREXT
	cytopsin	CYTO
	RNA	RNA
	collagen I gel embedded islets	GEL
	frozen unpurified	FRZUNP
	clarity	CLR
	electron microscopy embedding	EM
	flash frozen	FF
	frozen fixed	OCT
	paraffin embedding	PE
	in vivo imaging	IMG
	fasting glucose plasma from mouse	GLU
specimen anatomical location	glucose/arginine plasma from mouse	GLUARG
	glucose tolerance test from mouse	GTT
	unknown	UNK
	head	H
	body	B
	tail	T
	unknown	U



AGE		
Unit	Abbreviation	Example
gestational (weeks)	G(<i>number</i>)w	G39.9w
days	d	1d
months	m	10m
years	y	33y-16y