UNIVERSITÀ
DEGLI STUDI DI TRIESTE

FREEZING OF SPECIMEN

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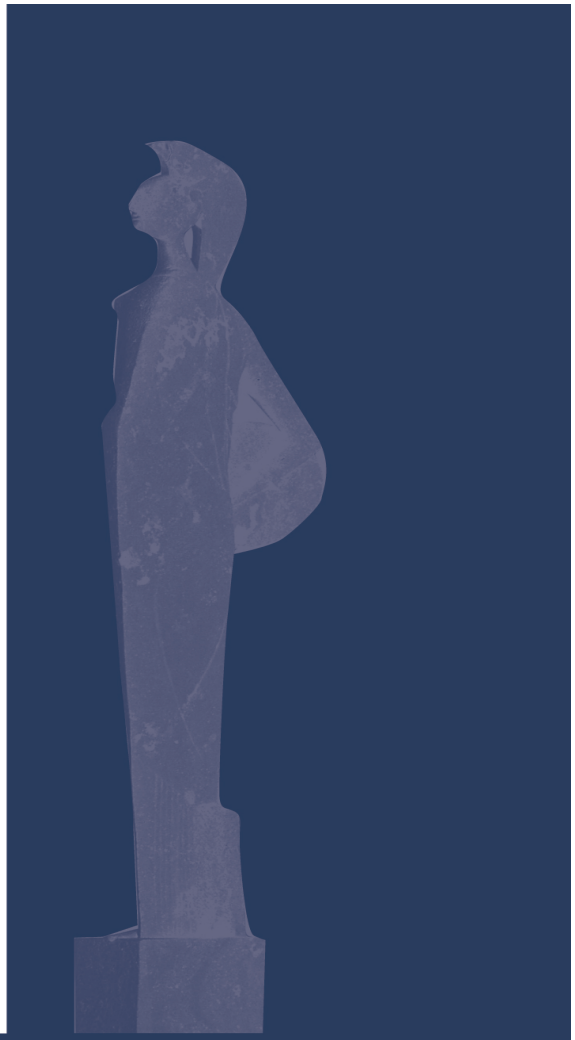
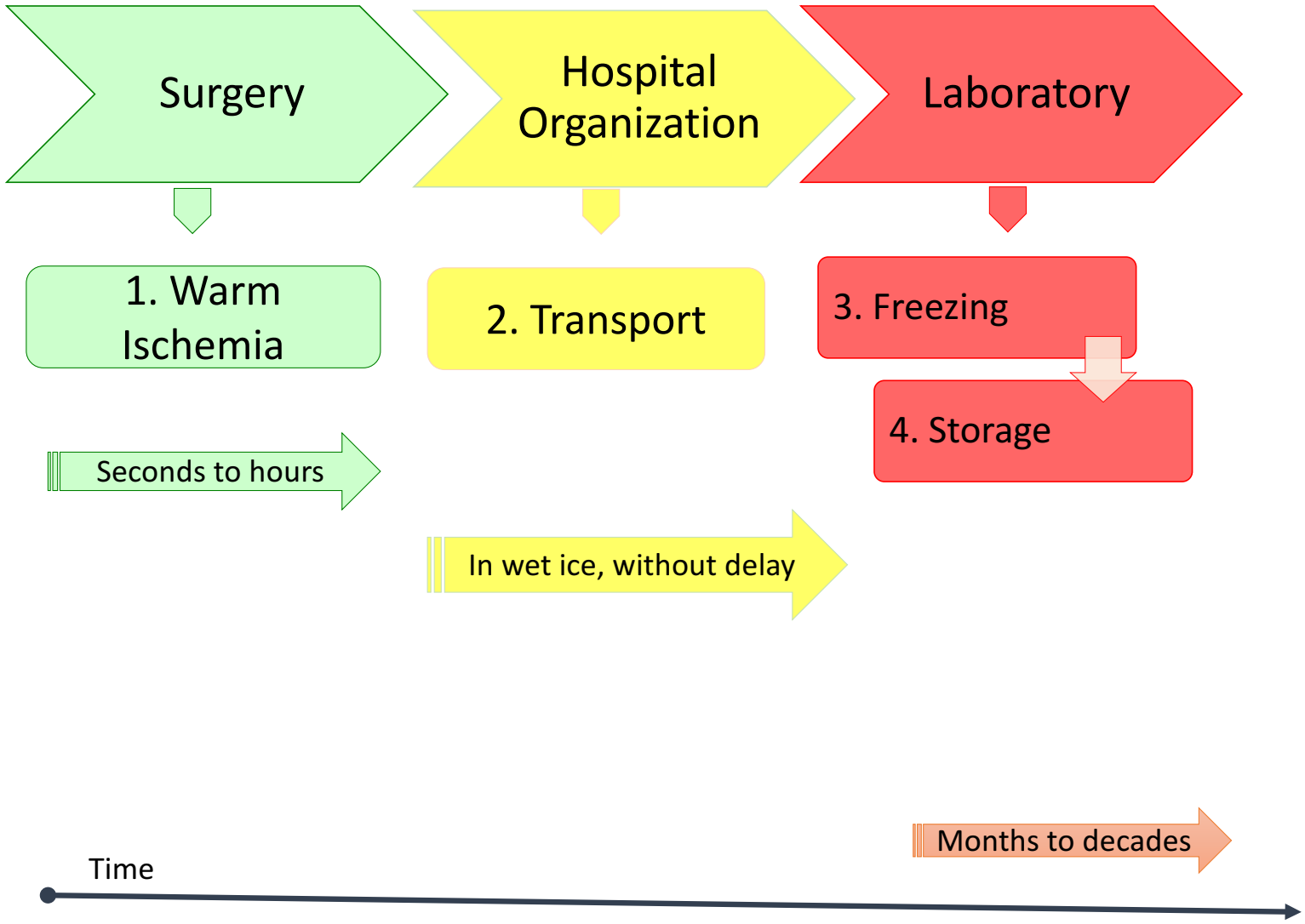
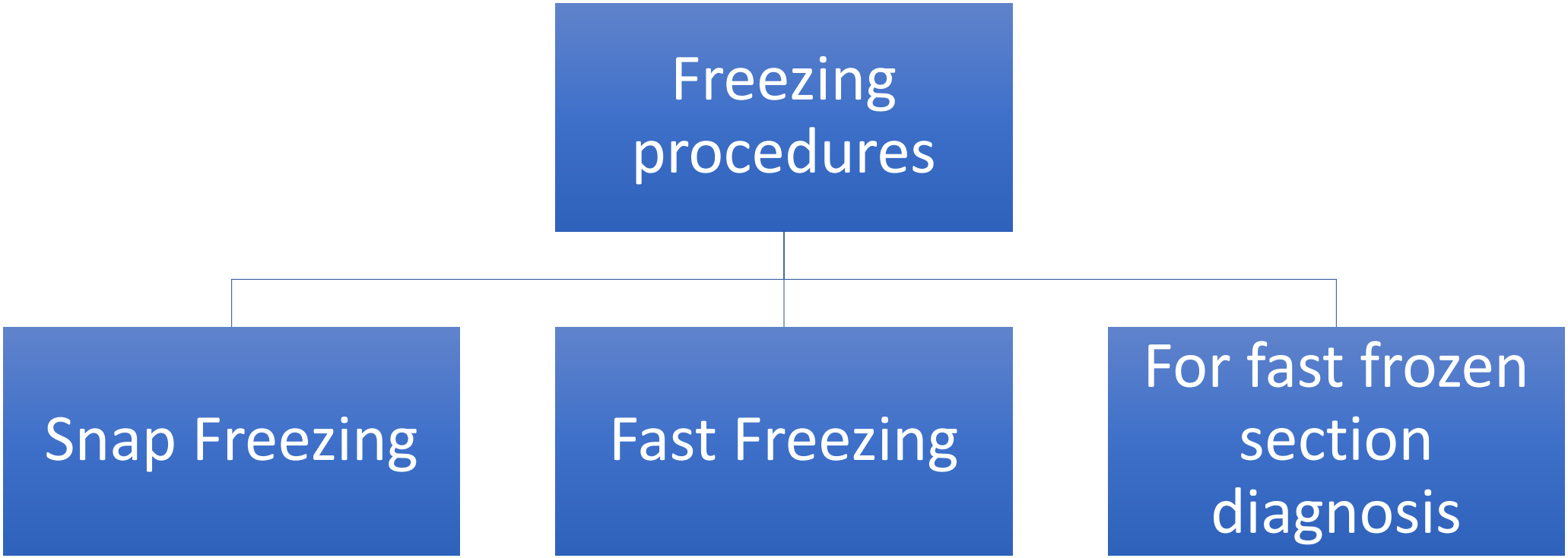



Surgery
Hospital Organization
Laboratory
1. Warm Ischemia
2. Transport
3. Freezing
4. Storage
Seconds to hours
In wet ice, without delay
Months to decades
Time


Freezing procedures
Snap Freezing
Fast Freezing
For fast frozen section diagnosis

SNAP FREEZING

- ***It gives the best preservation of morphology in frozen tissue samples.***
- ***Medium- Isopentane (C_5H_{12}, also called methylbutane or 2-methylbutane),***
- ***It shall be pre-cooled from ≤ –80 °C to > –160 °C***
- ***Pre-cooling: with liquid nitrogen (–196 °C), dry ice (–80 °C), –80 °C freezers or dedicated freezing appliances keeping the isopentane ≤ –80 °C.***
- ***The isopentane shall be cooled in a tube or other container (e.g. glass beaker) temperature shifts.***
- ***The volume of pre-cooled isopentane: at least 10x the volume of the specimen or sample.***
- ***Tissue sample shall be completely submerged into the pre-cooled medium.***
- ***After the tissue is frozen, it shall be transferred into a pre-cooled labelled cryo-vial.***
- ***Isopentane should be refreshed when debris is seen at the bottom of the tube.***
- ***Isopentane is extremely volatile and flammable at room temperature and pressure.***
- ***Laboratory should be well ventilated.***
- ***Isopentane in the tube should be cooled.***

FAST FREEZING PROCEDURES

- ***Samples are frozen on a pre-cooled metal plate, or metal basket***
- ***Those are placed on the surface of liquid N_2, or on dry ice.***
- ***Pre-cooling metal surface: from ≤ −80 °C to > −196 °C.***
- ***There is the possibility to fix the plate or the basket stand and clamp.***
- ***Sample can be frozen directly in liquid N_2 as it is or in the storage vial (labelled and closed) in liquid N_2 or in dry ice.***
- ***Slow freezing process ⟹ membrane disruption and crystal formation (can affect morphology)***
- ***To avoid cross-contamination, clean basket or plate between freezing samples.***
- ***NOTE Freezing liquid N_2 ⟹ Leidenfrost effect The Leidenfrost effect is a physical phenomenon in which a liquid, close to a mass that is significantly hotter than the liquid's boiling point, produces an insulating vapor layer that keeps the liquid from boiling rapidly. Because of this 'repulsive force', a droplet hovers over the surface rather than making physical contact with the hot surface.This reduces the heat conduct from the sample to the liquid nitrogen; this becomes worse when the sample is placed in the labelled vial.***

FREEZING FOR FAST FROZEN SECTION DIAGNOSIS

- ***Tissue transported freshly to the laboratory without delay.***
- ***The specimen is frozen onto a specific support device (e.g. metal grid, pedestal, disc) fitting onto the cryostat in an appropriate freezing medium.***
- ***The freezing medium used shall be documented.***
- ***The support device with tissue and freezing medium are frozen preferably in liquid nitrogen or dry ice for improved morphology.***
- ***Cut the frozen sections,***
- ***The remainder is removed from the support device without thawing and stored in a pre-cooled vial for long term storage.***

STORAGE

- ***The constant temperature: ≤ –70 °C.***
- ***Systems monitoring the temperature.***
- ***Freezers or liquid nitrogen tanks shall have a temperature alarm system.***
- ***Major temperature shifts during retrieval of the specimen(s) or sample(s).***
- ***Retrieval times as short as possible to avoid the thawing of samples.***
- ***Documentation of Temperature shifts with accidentally thawed the specimen(s) or sample(s) to be processed or to be further stored.***
- ***Back-up cryo-storage facilities.***
- ***Documentation of the storage position, storage temperature, time and date of the retrieval of any specimen or sample from the storage system.***

FURTHER INFORMATION

- ***All materials (excluding the lysis buffer and vial containing lysis buffer) and tools to manipulate frozen samples for cryo-sectioning or transferring to the lysis buffer shall be cooled to < 0 °C while kept in an environment of ≤ –20 °C before use.***