Cryo Bio System
Groupe I.M.V. Technologies

Straw-based specimen banking

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ABSTRACT

When archiving biological specimens, researchers have a choice of methodologies and technologies. This poster compares the most common methods from scientific and economic standpoints. The parameters of consideration are the storage temperature (e.g., liquid nitrogen vs. -80°C; LN2 liquid vs. vapor phase), the type of container (e.g., upright vs. chest freezer), as well as the packaging (e.g., vials vs. straws) used for the repository. Different configurations were assessed according to the following criteria: "Quality" preservation as measured by markers stability and cell survival rates, "Safety" assessed from the standpoint of bio-containment and cross-contamination risk, "Efficiency" quantified by the number of aliquots per container unit, "Traceability" of the samples throughout the chain of custody, as well as the level of "workflow automation" and general "ease-of-use". A comprehensive cost model was developed to evaluate the overall cost per sample for any storage configuration. The model includes capital equipment, maintenance, "fuel" (electricity or LN2), technician labor, and additional air conditioning required.
The poster presents the results and outlines the scientific and economic benefits of a straw-based, integrated and automated solution such as the Cryo Bio System.

QUALITY CONCERNS

Aliquot packaging considerations

Simple physics dictates that a larger radius impedes heat transfer so that the cooling rate achieved lags behind the desired cooling curve, and there is uneven heat exchange throughout the specimen (Morris, 2002). The issue was discussed at length by Mortimer (2004a), whose analysis showed this to be a marked disadvantage of cryovials, and likely to lead to impaired survival of specimens frozen in such large diameter packages.

The other side of this issue is the effective warming rate that can be achieved, impacting not only the effective thawing of specimens but also the risk of their warming during handling for brief periods outsize the cryogenic storage tank. Here the ability to achieve rapid warming rates in straws is a double-edged sword as it leads to an increased risk of damage: a 0.25 ml straw will warm to -80°C within 15 seconds in air at ambient temperature (Tyler et al, 1996), cf. **figure 1A**, **figure 1B** and **figure 2** below.

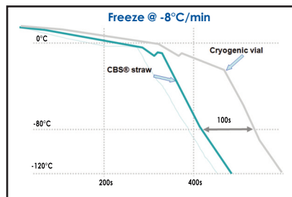


Fig 1A - Cooling curve measured by thermocouple inside CBS™ straw and cryovial

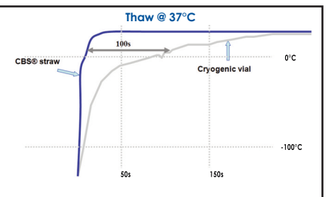


Fig 1B – Warming curve measured by thermocouple inside CBS™ straw and cryovial

	1.8ml cryovial	1ml cryovial	0.5ml straw
OD (mm)	12.5	12.5	3.1
L (mm)	48.0	42.0	133.0
S (cm²)	18.8	16.5	13.0
V (ml)	1.8	1.0	0.5
S/V (1/cm)	**10.5**	**16.5**	**25.9**

Fig 2 – Geometry and surface/volume ratios of cryovials and CBS™ straws

Freezing container & Storage Temperature implications

T. Moore et al. have conducted at BBI Research Laboratories a validation study of upright mechanical -80°C freezers under full specimen load. They found that temperatures measured within the freezer chamber (in 36 points) ranged from -19°C to -83°C (>60°C variation) depending on position within the unit and the racking configuration. Only by modifying the factory-installed shelving configuration so that to facilitate airflow, were the authors able in their experiment to uniformly maintain specimen temperatures below -70°C.

When dealing with specific biomarkers or with more sensitive materials than just plasma or serum (e.g. cells), in order to maintain biological integrity, specimens must be maintained below the glass transition temperature of water, i.e., below -132°C so that to stop all biological activity (Mazur, 1984). Because some biological activity might continue even at -80°C in an "ultracold" mechanical refrigerator, or at -79°C on dry ice, degradation of cryobanked biological material accumulates over time.

Of particular importance to the storage and handling of all cryobanked materials is what happens to water as it warms from cryogenic temperatures. The glass transition of a frozen aqueous solution is not a sudden event at exactly -132°C, glass transition occurs progressively between this temperature and about -90°C; so that by -80°C substantial change would have already occurred. During warming, energy is returned to the water molecules, allowing them to resume their natural orientation: because very small ice crystals are unstable , with a large surface/volume ratio, they tend to fuse together to reduce that ratio. It is therefore essential that cryopreserved material be kept below -132°C, and hence storage in liquid nitrogen vapor (-150°C) or in liquid nitrogen (-196°C) must be employed. The lower the temperature the greater the margin of safety, for example when a specimen is removed briefly to check its identity.

This margin of safety becomes crucial in a dynamic biobanking situation with respect to the occurrences of opening and closing the freezer container to input or retrieve samples, as shown on **figure 3** below (Pope, 2007). When considering storage in liquid nitrogen vapor or in mechanical cryogenic freezers (-140°C), a major concern is that liquid nitrogen vapor and super-cold air are poor conducts of heat and have very low thermal capacity, so they cool specimens poorly and heat up very quickly in the presence of a warm object, even ambient air. Because every second spent above -132°C (and especially above -80°C, see above) potentially causes accumulation of irreversible damage to the frozen materials, extreme care must be taken to ensure that specimens are kept below -132°C whenever they are manipulated, e.g. during transfer into the cryobank, during storage audits, and when they are being retrieved (Simione, 1999).

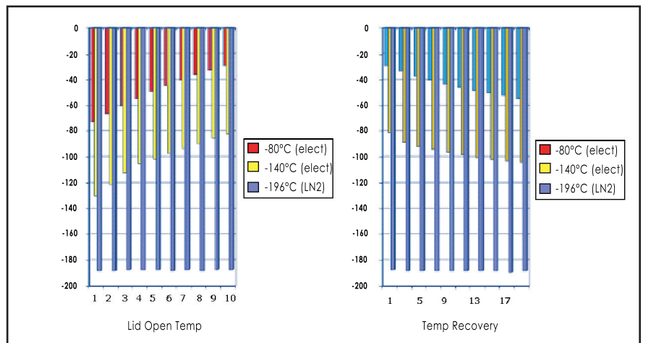


Fig 3 – Temperature changes when opening and closing common freezer containers

COST CONSIDERATIONS

When assessing the **true** costs of the bio-repository activity consisting of **aliquoting**, **packaging** and **storing** biological specimens, one needs to consider the following variables over the intended duration of storage (e.g, 10 years):

- **CONSUMABLES**: e.g., vials, straws, pipette tips, etc.
- **LABOR**: technician time to accomplish processing operations (e.g., aliquoting, vial labeling, etc.)
- **FREEZER EQUIPMENT**: capital expense of the freezer units
- **STORAGE ELEMENTS**: racking system to install inside the freezer
- **MAINTENANCE**: cost of maintenance contract + expected spare parts replacement
- **"FUEL"**: consumption of either LN2 or electricity (or both in case of -140°C mechanical freezers)
- **AIR CONDITIONING**: needed to evacuate additional head produced with electrical freezers
- **SPACE**: rent or opportunity cost of using the storage space – ***was not included in present cost model, due to high geographical variability***

Assuming the **same protocol of 12 aliquots per sample to be stored over a 10 year period**, we run the model for 2 types of packaging system CBS™ straws and cryovials, and across 3 types of freezer containers : i) a LN2 freezer (model Chart-MVE 1879P-190), ii) a -80°C mechanical freezer (model REVCO 24.4 cu ft), iii) a -140°C "cryogenic" mechanical freezer (model REVCO ULTIMA II – 10.3 cut ft), resulting in 6 configuration scenarios. The table in **figure 4** summarizes the results obtained for the overall cost per sample and **Figure 5** provides the cost breakdown for each considered storage configuration:

	Mechanical freezer		LN2 freezer
Temp	-80°C	-140°C	-196°C
Packaging			(-150°C vap)
Vial	$ 19.61 (-37%)	$ 53.18 (-40%)	$14.71 (-27%)
Straw	$ 12.32	$ 32.15	$ 10.71

Fig 4 – Cost of processing & storage for 1 sample (divided in 12 aliquots) over 10 years

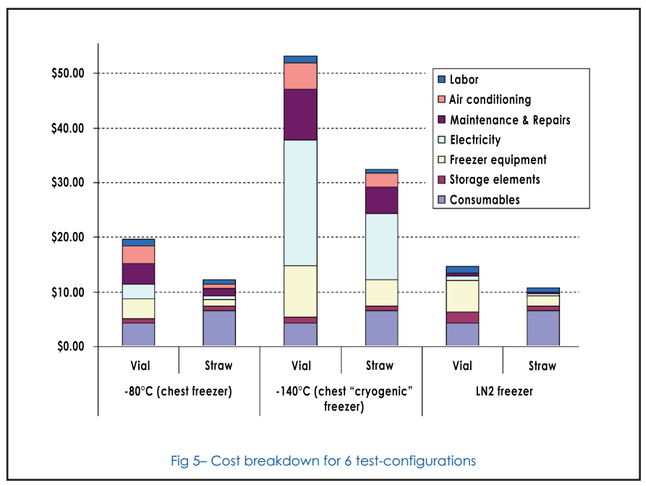


Fig 5– Cost breakdown for 6 test-configurations

STORAGE EFFICIENCY

When considering storage space efficiency, especially when at the repository planning stage, one may remind some rules of thumbs between capacity and floor space, cf. **figure 6** (Pope, 2007).

Floor space: 2 upright fr. = 1 chest fr. = 1 LN2 fr.

Storage capacity: 2 upright fr. = 2 chest fr. = 1 LN2 fr.

Capacity/Floor space: 1 upright fr. = 2 chest fr. = 1LN2 fr.

~1.1 cu ft / sq ft ~0.55 cu ft / sq ft ~1.1 cu ft / sq ft

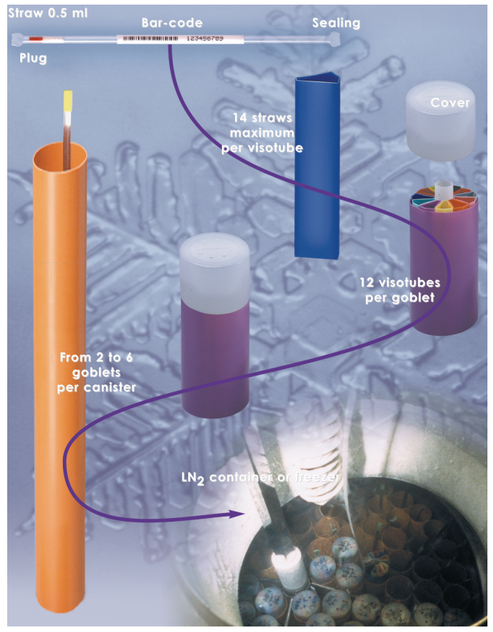
Fig 6 – Rule of thumbs on storage capacity and floor space

For any given storage container however, storage space can be optimized by using the appropriate aliquot packaging configuration. **Figure 7** below illustrates the space efficiency advantage of the straws in this respect for a number of standard units in LN2, at -80°C (chest & upright) and at -140°C. The average ratio between the number of straws and the number of cryovials that can be stored in a fully-organized fashion in any container is close to 4.

	Container	Container capacity (liter)	Straw Capacity	Cryovial (1.2ml) capacity	Ratio straws/vials
	LN2 FREEZERS				
Taylor Whart. MVE	MVE 815P-150	370	69,720	15,600	4.47
	MVE 1536P-150	756	157,248	36,400	4.32
	MVE 1879P-190	1672	321,552	79,950	4.02
	TW K Series 38K	590	146,160	38,350	3.81
	TW LABS Series 20K	407	66,360	19,500	3.40
	TW LABS Series 80K	1350	262,920	79,300	3.32
Air Liquide	AL Espace 330/331 - rotating drum	386	71,400	19,800	3.61
	AL Espace 660/661	786	132,720	38,400	3.46
	AL RCB 600 (Liquid phase)	575	113,904	29,400	3.87
	AL RCB 1001 (Liquid phase)	1110	209,664	52,000	4.03
	- 80C FREEZERS				
Type Forma	Hupright freezer 13 cu. ft.	368	72,576	19,440	3.73
	Hupright freezer 17.3 cu. ft.	490	96,768	25,920	3.73
	Hupright freezer 23 cu. ft.	651	133,056	32,400	4.11
	Hupright freezer 28 cu. ft.	793	157,248	38,880	4.04
	Chest freezer 12.7 cu. ft.	360	75,600	20,412	3.70
	Chest freezer 20 cu. ft.	566	120,960	32,076	3.77
	- 140C FREEZERS				
Revco	ULTIMA II - ULT 10140-9	292	48,384	21,600	2.24
				AVERAGE	3.74

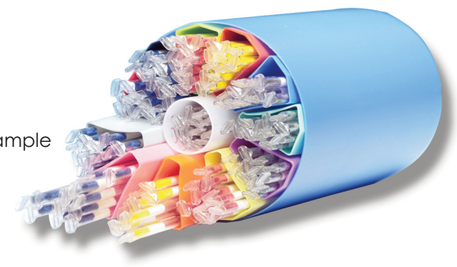
Fig 7 – Straw vs. Vial capacity of standard cryogenic freezers

STORAGE CONCEPT



CONCLUSION - Benefits of Straw-based specimen banking

- Storage EFFICIENCY
  4-fold improvement factor vs. cryovials
- Enhanced TRACEABILITY
  color-coding & barcoding of each aliquot
  storage management software
- Better QUALITY preservation
  biocompatibility of material
  higher surface/volume ratio
- Higher SAFETY conditions
  sealed container → no cross-contamination
  no infiltration of LN2 inside sample
- Increased ALIQUOTING possibilities
  aliquot size of 500µl, 300µl or 150µl
  fully-automated aliquoting platform
- Overal STORAGE cost savings > 35%



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