

An economical insulated shipping container providing a > 4 day lifetime for frozen biosamples without dry ice

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Abstract

Many research projects in the life sciences require the shipment of biosamples which *must* remain frozen. We describe the design, construction, testing, and use of an economical and reusable insulated “super-box” which, in conjunction with reusable phase-change-material “cold packs”, reliably maintains a volume of about 38 liters (sufficient to hold 34 standard $13.4 \times 13.4 \times 5.2$ cm cryoboxes) below -15°C for about 3.5 days, and below -5°C for about 4.5 days. The entire system is readily constructed from commercially-available materials at a cost of under US\$500. In contrast to systems using dry ice (frozen CO_2) during shipment, the super-box and cold packs do not require special handling during shipment and are not considered “dangerous cargo” by commercial shippers.

1 Introduction

Many research projects in the life sciences involve the collection and assaying of biosamples (e.g., blood, saliva, urine, or solid tissues). In some cases the biosamples can be preserved (e.g., with sodium azide) so as to be stable at ambient temperature, but in many cases the biosamples must be frozen to avoid degradation between collection

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and assaying. This requires the maintenance of a continuous “cold chain” to keep the samples frozen from soon after collection at a field site (possibly in a remote location), through storage and transport to an assay laboratory. Here we consider the problem of maintaining biosamples in a frozen state during long-distance transport.

Active refrigeration during transport can offer essentially any desired temperature with long cold lifetimes. But active refrigeration systems are often expensive and/or heavy, and they may require treatment as “dangerous cargo” in shipping (due to substantial batteries and/or fuel supplies to power the refrigerator during transport). Moreover, there’s a serious risk of sample loss if there’s any failure in the power or refrigeration system.

In contrast, passive systems relying solely on thermal insulation and heat absorption by phase-change materials can be economical, relatively lightweight, and highly reliable (there are no moving parts or active components to fail). Passive systems offer less precise temperature control and somewhat limited cold lifetimes, but in practice samples can still be maintained frozen for useful lifetimes.

In some cases a passive system can rely on thermal insulation alone, but adding suitable phase-change materials can greatly improve the cold lifetime. In such a system the phase-change material can either be expendable or reusable.

A common choice of expendable phase-change material in such a system is dry ice (frozen CO₂). This absorbs heat while sublimating at approximately -79°C ; the resulting CO₂ gas is vented to the surrounding atmosphere. Dry ice is economical, fairly readily available, and non-toxic, but (because of the very large volume of CO₂ gas produced as it sublimates) it’s treated as a dangerous good for air or water shipment.¹ Commercial shippers such as airlines, UPS^(TM), DHL^(TM), and FedEx^(TM) either forbid it entirely or strictly regulate the amount of dry ice allowed in a shipment.

A reusable alternative to dry ice is the use of phase-change materials formulated to have a chosen melting temperature. If initially frozen at a temperature below their melting temperature, these materials absorb heat in the process of melting. They are commercially available in convenient “cold packs” which are relatively economical, non-toxic, and are not treated as dangerous goods by commercial shippers. They can be thawed and re-frozen as many times as desired, allowing for a fully reusable cold-chain system.

Along with suitable cold packs, the other key ingredient of this type of cold-chain system is excellent thermal insulation. An inadequately-insulated shipping container filled with cold material (such as frozen biosamples) will leak a large amount of heat in from the ambient environment, leading to a relatively short cold lifetime before the samples warm to their melting point. To obtain a long cold lifetime the choice of insulating material, the thickness of the insulation, the overall size and shape of the container, and the quality of the container’s construction are all important.

¹See, e.g., https://en.wikipedia.org/wiki/Dry_ice#Safety.

In this report we describe a passive and fully reusable cold-chain system (comprising an insulated shipping container and a number of cold packs) which offers a cold lifetime sufficient for commercial air shipment almost anywhere in the world, without using dry ice. International air shipment normally promises roughly 24- to 48-hour delivery times, but occasionally shipments misconnect at a shipment hub or are even temporarily “lost”. For our system we have set a minimum requirement of a 72-hour (3-day) cold lifetime, with >96 hours (>4 days) preferred to give a comfortable safety margin in case of shipping delays. Given the range of melting temperatures of various biosamples, we measure the cold lifetime as the time for the biosamples to warm to either -15°C or -5°C .

The outside dimensions of our insulated “super-box” are approximately $64 \times 63.5 \times 62.9$ cm ($25.25 \times 25 \times 24.75$ inches). The super-box and cold packs together weigh approximately 23.2 kg (51.1 lbs). The interior dimensions (volume available for biosamples) are approximately $36.9 \times 36.2 \times 28.6$ cm ($14.5 \times 14.25 \times 11.25$ inches); this is a volume of approximately 38 liters, and can hold 34 standard $13.4 \times 13.4 \times 5.2$ cm ($5.25 \times 5.25 \times 2$ inch) cryoboxes. Starting with samples and cold packs frozen using dry ice or in a -80°C freezer, the samples are maintained below -15°C for about 3.5 days, and below -5° for about 4.5 days. The super-box can be built from readily-available commercial supplies for under US\$500, with less than one person-day of labor.

The remainder of this report is organized as follows: Section 2 describes the design and construction of the super-box and the choice of the cold packs. Section 3 describes how to pack biosamples and cold packs in the super-box. Section 4 describes the testing we performed to validate the cold lifetime of the “as-built” super-box in realistic environmental conditions. The appendix presents a simple 1-dimensional mathematical model of the super-box’s thermal performance; this is useful for estimating how the cold lifetime varies in different conditions.

2 Design and Construction

The basic ingredient of our “super-box” is a commercial insulated shipping box. The main way in which heat flows into such a box is by conduction through the walls of the box.² Wikipedia offers an excellent overview of thermal insulation and the basic concepts of heat conduction.³ Another excellent discussion, with specific applications to insulated shipping boxes, is the paper by Singh, Burgess, and Singh.⁴

²The leakage of warm air into and/or cold air out of the interior of the box is also a factor, particularly in air shipment where the ambient air pressure changes dramatically during transport. It’s not practical to perfectly seal a box (this would require impractically heavy and expensive “vacuum chamber” construction), but reducing air leakage by tightly taping box seams and lids is valuable.

³[https://en.wikipedia.org/wiki/R-value_\(insulation\)](https://en.wikipedia.org/wiki/R-value_(insulation)) .

⁴S. P. Singh, Gary Burgess, and Jay Singh, “Performance Comparison of Thermal Insulated Packaging Boxes, Bags and Refrigerants for Single-parcel Shipments”, *Packaging Technology and Science*

In comparing the available models of insulated shipping boxes, the most obvious criteria are the type of insulation material, the box’s wall (insulation) thickness, and the overall size and shape of the box.

2.1 Type of insulation material

Insulation materials are generally rated by their “R value” or “thermal resistance per unit thickness”. As a rough approximation, the cold lifetime of an insulated box will be proportional to the product of the insulation R value and the insulation thickness. Table 1 gives typical R values of some common insulation materials.

From inspecting table 1 it would appear that vacuum insulated panels (VIPs)⁵ would be an ideal insulation material for a shipping box. Unfortunately, we have found that commercially-available VIPs are not suitable for this purpose, because they’re mechanically somewhat fragile and vulnerable to having their vacuum seal punctured during shipping (which causes a catastrophic loss of insulation). Even if not punctured, air may gradually leak into their “vacuum” region, causing a gradual loss of insulation over time.

In practice, we have found commercial insulated shipping boxes with polyurethane foam walls to be an excellent choice. These are fairly economical, mechanically quite strong, and their insulation qualities do not degrade seriously with normal shipping wear-and-tear.

21, 25–35.

⁵See, e.g., https://en.wikipedia.org/wiki/Vacuum_insulated_panel.

Material	R value	
	(imperial)	(SI)
typical hardwood	0.7	5
typical softwood	1.4	10
polyethylene foam	3	20
cardboard	3.5	25
extruded polystyrene (“styrofoam” ^(TM))	5	35
polyisocyanurate foam (foil-faced)	7	50
polyurethane foam	7	50
vacuum insulated panel	40	275

Table 1: This table gives the typical R-values (thermal resistance per unit thickness) of some common insulation materials. The imperial units for R-values are $\text{foot}^2 \cdot \text{hour} \cdot ^\circ\text{F}/(\text{BTU} \cdot \text{inch})$; the SI units are $\text{meter} \cdot ^\circ\text{C}/\text{Watt}$. R-values in SI units are sometimes known as RSI values.

2.2 Box size and shape

Obviously a larger box will tend to be heavier, particularly when fully loaded with cold packs and biosamples. Many commercial shippers place an upper limit of about 32 kg (70 lb) on the weight of any one parcel in a shipment. When fully loaded our super-box is typically quite close to this limit, and the total parcel weight needs to be carefully monitored.

As noted above, the main way in which heat flows into an insulated box is by conduction through the walls of the box. This means that, all other factors being equal, this heat flow can be minimized by minimizing the box's wall (surface) area. In practice, this means that for a given box volume, near-cubical shapes are preferred, while low/flat or long/narrow shapes are undesirable.

2.3 Wall thickness and insulation

For a given insulation material and a given weight of cold packs, the thicker the walls of an insulated container the less heat will leak into the container and the longer the cold lifetime will be.

For our “super-box” we begin with a Thermosafe^(TM) E337UPS insulated shipping box.⁶ This consists of an outer cardboard box inside which is glued a 3 inch (7.6 cm) layer of polyurethane foam insulation,⁷ with an inner cardboard layer glued inside the insulation. The box has a removable 4 inch (10.2 cm) thick foam lid which sits flush in the top of the box; this can be seen in figure 7.

In tests we found that the E337UPS box alone was not sufficiently well-insulated for our needs. (That is, it did not give a sufficient cold lifetime when loaded with a reasonable number of cold packs; increasing the number of cold packs would have made the fully loaded super-box too heavy for standard commercial shipping.)

Therefore, we add an additional insulation layer inside the box, by lining the box's interior with 1 inch thick polystyrene (“styrofoam”^(TM)) sheets⁸ on the bottom and all 4 sides, and 2 or 2.5 inches (5 or 6.3 cm) of styrofoam under the foam lid. Styrofoam sheets are readily available at building supply stores in 2×8 or 4×8 foot sizes, and are easily cut with a box-cutter knife. Such sheets are often available in both open-cell and closed-cell forms; the open-cell forms are less desirable for a super-box because they tend to absorb water (e.g., from condensation on the cold packs), which degrades their

⁶<https://www.thermosafe.com/subcategory/pur+shippers> .

⁷This is the greatest wall thickness of polyurethane insulated shipping box we have found commercially available. If a 4 inch (10.2 cm) thickness were available, the styrofoam lining could be eliminated from our super-box design, and the resulting overall insulation performance and cold lifetime would still be slightly better than our design.

⁸“Styrofoam” is the trademarked name of one popular brand of polystyrene sheet, but any brand should work equally well for a super-box.

R-value.

If 1-inch thick styrofoam sheets are not available, 2 layers of 0.5 inch sheets (4 or 5 layers under the foam lid) would work equally well. A mixture of 1-inch and 0.5-inch styrofoam sheets may also be useful to best fill the available space under the foam lid.

If polyurethane sheets were available, they would give somewhat better insulation, but we have not found these readily purchasable. Polyisocyanurate sheets may be an excellent substitute.

2.4 Choice of Cold Packs

Cold packs are commercially available with various melting points from vendors such as Cold Chain Technologies⁹ and Cryopak¹⁰.

For keeping biosamples (which typically have a melting temperature a few °C below 0°C) solidly frozen, we have selected the Cryopak “20 Below”^(TM) cold packs,¹¹ which have a melting temperature of −20°C. These are available in several package sizes and shapes; we have found the model FGEL00055 “small panel” to be particularly convenient. This is a rigid plastic box,¹² 14 × 14 × 2.5 cm (5.5 × 5.5 × 1 inch) in size, weighing 385 grams (13.6 ounces), containing the phase-change material (a non-toxic water-starch solution which is liquid at room temperature). Because of the rigid plastic box, these cold packs don’t change size or shape appreciably when freezing/thawing.

It’s **essential** that the cold packs be solidly frozen (before packing the super-box) in order for the full cold lifetime to be attained.¹³ The cold packs can be frozen in a −80°C freezer or with dry ice; a standard household freezer usually does not get cold enough to fully freeze them. We find that starting from room temperature, solidly freezing the cold packs takes about 2 days in a −80°C freezer or dry-ice container. (To avoid overly warming other freezer contents, in practice it’s useful to space out the loading of the cold packs into the freezer over a 4–5 day period.)

An easy way to check whether or not a cold pack is frozen is to shake it and listen for the sound of liquid sloshing inside it; if this can be heard then it’s not frozen. To avoid cold burns, insulated gloves or mittens should be used when handling frozen cold packs.

⁹<https://www.coldchaintech.com/koolit-refrigerants/>

¹⁰<https://www.cryopak.com/packaging-and-refrigerants/phase-change-materials/>

¹¹<https://www.cryopak.com/packaging-and-refrigerants/phase-change-materials/20-below/>

¹²We have also experimented with phase-change materials packaged in a flexible plastic or metal-foil pouch. These proved to be less convenient both because of their vulnerability to punctures in handling, and because they tend to freeze in “pear shapes” which are wider at the bottom than at the top, which makes them inconvenient to place in and remove from the super-box.

¹³In terms of the 1-dimensional thermal model in the appendix, starting with non-frozen cold packs at a temperature just above −20°C would correspond to only having stage 3. From figure 9, this would cut the cold lifetime by about a factor of 3 to 4, i.e., to about 1 day to −5°C, and much less than that to −15°C.

2.5 Construction of the super-box

We have constructed several super-boxes to this design. We find that the E337UPS boxes differ slightly in size (by a few mm), even even for boxes ordered at the same time from the same supplier. This means that the styrofoam pieces must be cut specifically for each super-box in order to fit tightly (which is desirable to reduce air leakage, cf. footnote 2). If the research group has more than one super-box, the removable styrofoam lid pieces should be labelled as to which super-box they belong to.

The E337UPS box is not quite square in cross-section, so it's useful to label the 4 sides of the box and, correspondingly, the 4 sides of all the removable styrofoam and foam lid pieces, to ensure that all the components are correctly oriented during reassembly/packing. The removable pieces should also be labelled for orientation (e.g., "this side up"). Looking down on the E337UPS box from above, with its wider (64 cm, 25.25 inch outside) dimension oriented left-to-right and its narrower (63.5 cm, 25 inch outside) dimension oriented top-to-bottom, we number the 4 sides of the box ① at the top, ② on the left, ③ on the right, and ④ at the bottom. These labels can be seen in figure 1a. All the super-box photos and packing diagrams in this report use this same orientation.

Note that since the foam lid sits flush in the top of the polyurethane-foam insulation, the styrofoam pieces lining the inside box walls do not extend up to the top of the polyurethane-foam insulation; this can also be seen in figure 1a.

Since the styrofoam lid pieces should fit snugly, they need "handles" in order to be removable. We make these handles from 2-inch plastic strapping tape, running a single long piece of tape under the lid piece (held horizontally), up on each of 2 opposite sides to extend about 10 cm above the top surface of the lid piece, and then folded back on itself at each end to provide a pair of handles on opposite sides of the lid piece. Each lid piece then has 2 or 4 such pairs-of-handles, providing 1 or 2 handles on each side (thinner styrofoam is more fragile, and needs more handles to avoid cracking during insertion/removal). Figure 1b shows one of the lid pieces with its handles.

We find it convenient to first cut and fit a bottom piece of styrofoam whose size we trim to just fit snugly within the E337UPS inner cardboard liner. We then cut and fit the 4 side wall pieces, then finally cut and fit the lid pieces. Fitting the lid pieces is somewhat tricky, since it's easy for them to get stuck inside the styrofoam wall pieces¹⁴ and be difficult or impossible to (non-destructively) remove. We found it easiest to start by measuring the desired dimension of a lid piece and cutting it to be 1 to 2 mm too large. Then we could obtain a snug fit by carefully cutting down of the styrofoam one side at a time in a trial-and-error fashion, making repeated trial fits during the process. By making each trial fit on only one side of the lid at a time, we could avoid having the lid piece get stuck in the super-box. If a lid piece is mistakenly cut too small (so that

¹⁴At this stage the lid pieces won't yet have handles, because handles would be in the way when trimming the lid pieces for a correct fit.

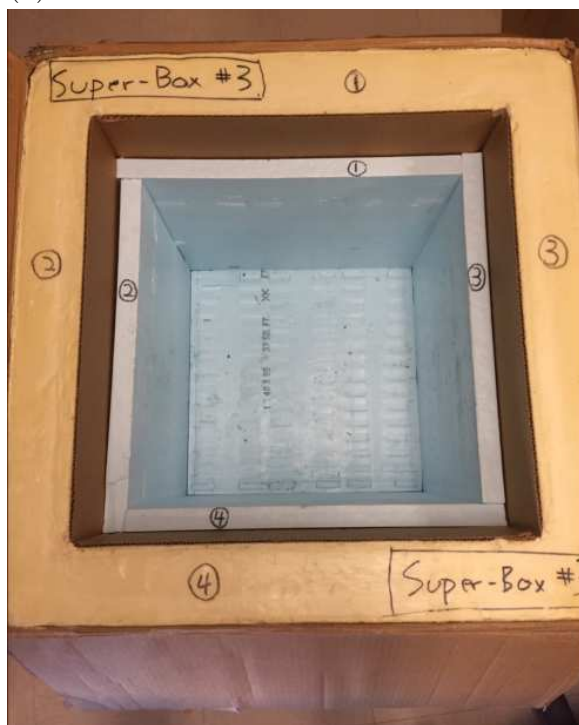
it's a loose fit), it should be discarded and a new piece cut, as the loose fit would allow air leakage and shorten the cold lifetime.

Cutting the styrofoam pieces tends to produce a mess of tiny styrofoam fragments and shavings. These are hard to clean up because they tend to adhere to tabletops, floors, and other nearby surfaces by static electricity. An easy way to collect these pieces is to take a strip of masking tape or plastic strapping tape and put it adhesive-side-down onto the surface. The tape can then be lifted away, and the styrofoam pieces will stick to the tape.

3 Packing a Super-Box

For use in a super-box, the cold packs are tightly packed lining the inside styrofoam walls of the super-box, so that the samples (in the middle) are surrounded by cold packs on the bottom, all 4 sides, and the top. The super-box interior temperature tends to be highest at the top of the box, so it's useful to have more and more-tightly-packed cold packs there. Cold air tends to sink, so these cold packs help keep the entire sample

(a)



(b)

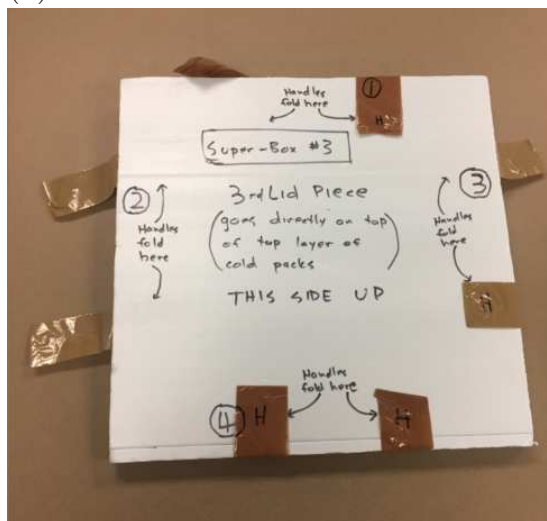


Figure 1: This figure shows (a) an empty super-box with the foam and styrofoam lid pieces removed, and (b) a styrofoam lid piece showing the tape “handles”.

volume cold.

To repeat, it's **essential** that the cold packs be solidly frozen before packing them into the super-box, and insulated gloves or mittens should be used to avoid cold burns when handling frozen cold packs.

It's important to plan the packing in advance (without the pressure of valuable samples thawing and a shipping-pickup deadline approaching) to ensure that the desired number of sample cryoboxes and cold packs all fit together within the super-box's styrofoam lining and lid, with minimum empty (wasted) space. We experimented with a number of different packings (using empty cryoboxes and cold packs at room temperature for ease of handling), and then tested selected configurations with frozen cold packs and simulated samples in order to finally converge on a configuration which met all our requirements for cold lifetime, number of cryoboxes per super-box, and overall (loaded) super-box weight.

Our final packing is quite tight, particularly if packing a super-box which is a few millimeters smaller than average. If you have multiple super-boxes of slightly different sizes, we recommend using the largest one for your first packing.

We suggest reviewing the following packing instructions, diagrams, and photographs *before* starting a packing with actual samples. If this the first time you've packed a super-box, it might be useful to do a preliminary "dry run" with room-temperature cold packs and cryoboxes.

If 0.5 inch styrofoam sheets are used for the lid pieces, be careful not to crack them when putting them into place or removing them. Fold the tape "handles" flat against each piece before fitting the next piece.

3.1 Sealing cryoboxes in plastic bags

Commercial shippers typically require triple-barrier protection (sealing) of biosamples. Taking an individual cryotube as the 1st protection layer and the superbox itself as the 3rd layer, this means that an additional protection layer is needed in between. We provide this by sealing each cryobox with an absorbent material (a paper towel) inside an individual zip-lock plastic bag (taped shut) before loading it into the super-box. While most of the super-box loading can easily be done by a single person, sealing the cryoboxes into plastic bags is much easier with two people.

Overall, the samples will stay colder if the cryoboxes are plastic-bagged one at a time, then returned immediately to a freezer, and the super-box packing isn't started until all the cryoboxes are plastic-bagged. But sometimes this isn't practical (e.g., because it requires opening the freezer door too many times), so plastic-bagging may be interleaved with packing into the super-box.

Figure 2 shows the the supplies for sealing a cryobox inside a plastic bag. 2 inch (5 cm)

wide plastic packing tape is easiest, but 1 inch (2.5 cm) wide plastic tape would also suffice. It doesn't matter if the plastic tape is transparent or opaque.

Here's how to seal up a cryobox:

1. Put the cryobox and a piece of absorbent paper into the zip-lock bag, **squeeze out as much air from the bag as you can (this is important because it's a tight fit to get all the bagged cryoboxes and cold packs into the super-box!)**, and press the zip-lock bag's seal so it clicks together (figure 3a).
2. Stick a large piece of tape onto one side of the zip-lock bag seal, so it looks like figure 3b. (This is where it's particularly useful to have two people: one can hold the bagged cryobox while the other attaches and cuts the tape.)
3. Fold the tape over to seal the zip-lock bag shut, so it looks like figure 3c.
4. Cut off the extra tape at the ends of the bag, so it looks like figure 3d.



Figure 2: Supplies for sealing a cryobox inside a plastic bag. (Each cryobox needs its own plastic bag and piece of absorbent paper.)

(a)



(b)



(c)



(d)



Figure 3: This figure shows the process of sealing a cryobox with absorbent paper into a plastic bag.

3.2 Packing the cold packs and cryoboxes in the super-box

Our standard packing has the super-box holding 34 cold packs and 34 cryoboxes. Inside the super-box, the cold packs and samples are each arranged in layers, which we number from layer #1 on the bottom up to layer #4 on the top:

- Layer #1 contains 5 cold packs.
- Layer #2 contains 10 cold packs and 17 cryoboxes.
- Layer #3 contains 10 cold packs and 17 cryoboxes.
- Layer #4 contains 9 cold packs.

Important: When you are packing the box, you need to insert the cold packs in a slightly different order, with the layer #2 cold packs coming **before** the layer #1 ones. This is explained in detail below.

The following diagrams all show the interior of the E337UPS box, looking down from above, with the 1 inch (25 mm) styrofoam lining the walls shown in blue, cold packs shown in gray, and cryoboxes shown in red. (Of course, the actual materials may be of any color.) The 4 sides of the super-box are labelled ①, ②, ③, and ④, with you (the person packing the super-box) on side ④, side ② at your left, side ③ at your right, and side ① opposite from you. The side labels can be seen in figure 1a. The wider (64 cm, 25.25 inch outside) dimension of the E337UPS box always runs left-to-right in the diagrams, between sides ③ and ④, while the narrower (63.5 cm, 25 inch outside) dimension runs top-to-bottom in the diagrams, between sides ① and ④.

The actual packing of the cryoboxes is considerably tighter than the diagrams here would suggest, because of the extra space occupied by the absorbent paper, zip-lock plastic bags, and tape.

Starting with sample cryoboxes and cold packs in a freezer, and all the auxiliary supplies ready at hand (zip-lock plastic bags, paper towels, plastic tape, scissors, and a pair of insulated gloves for handling the frozen cold packs), we find that two experienced people can plastic-bag the cryoboxes and pack a super-box in about an hour. Or, if the cryoboxes are already sealed in plastic bags, then one experienced person can pack a super-box in about 20 minutes, or slightly longer if s/he is also recording sample inventory/tracking information for each cryobox.

The actual packing process is as follows:

1. Start by putting in 10 cold packs for layer #2, as shown in figure 4a. These cold packs rest on their edges around the side walls on the styrofoam bottom of the super-box. Note that because the super-box isn't quite square, the cold packs will only (just barely!) fit in the orientation shown – they won't fit if you mistakenly rotate the configuration by 90 degrees.

The cold packs against sides ① and ④ are a tight fit (particularly in a super-box which is a few millimeters smaller than average). What we do to squeeze them in is to first put in the left and right cold packs at a slight angle, as shown in figure 4b, then put in the middle cold pack and push all 3 cold packs flat against the styrofoam inside wall of the super-box.

2. Now put in 5 cold packs for layer #1. These cold packs rest flat on the styrofoam bottom of the super-box, as shown in figure 4c. At this point the super-box should contain 15 cold packs, and look like the photograph in figure 4d.
3. Now put in 17 cryoboxes (each of which should already each be sealed in its own ziplock plastic bag as described earlier) for layer #2, as shown in figure 5(a). The cryoboxes sit on edge, resting on the layer #1 cold packs. At this point you've finished layers #1 and #2. The super-box should now contain 15 cold packs and 17 cryoboxes, as shown in figure 5(b).
4. Put in 10 cold packs for layer #3. These cold packs are arranged just like those in layer #2: they rest on their edges on top of the layer #2 cold packs inside the styrofoam side walls of the super-box, as shown in figure 6a.
5. Put in 17 cryoboxes (each of which should already each be sealed in its own ziplock plastic bag as described earlier) for layer #3. As shown in figure 6b, these cryoboxes are arranged just like those in layer #2.

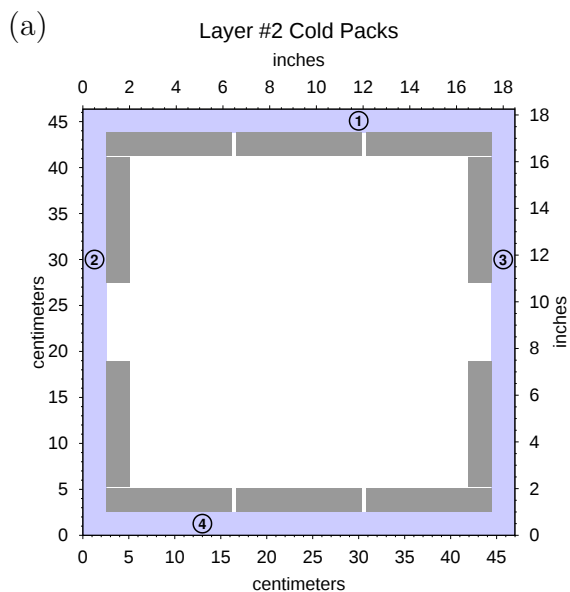
At this point you've finished layers #1, #2, and #3. The super-box should now contain 25 cold packs and 34 cryoboxes (there are still more cold packs to be packed, but that's all the cryoboxes that will go in this super-box).

6. Put in the last 9 cold packs (which are layer #4) in a 3×3 grid on top of the layer #3 cryoboxes, as shown in figure 6c.

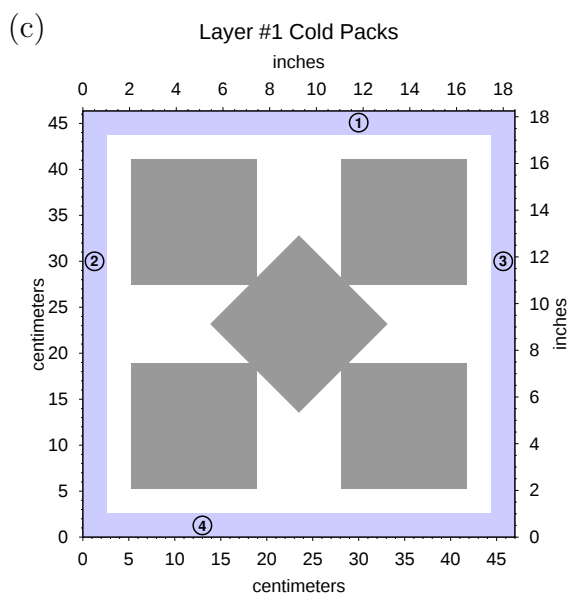
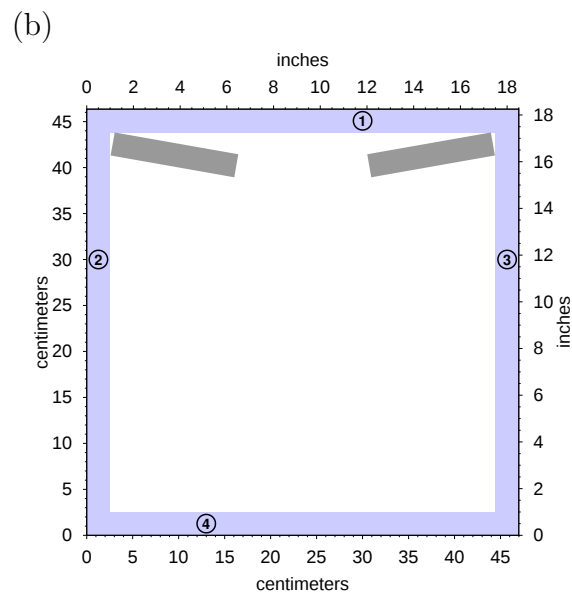
This layer of cold packs is a tight fit (particularly in a super-box which is a few millimeters smaller than average). It's easiest to put in the corner cold packs first, then put in the middle ones. We found that we usually couldn't get the whole layer of 9 cold packs to lay flat; the middle row of cold packs was about $\frac{1}{4}$ inch (5 mm) higher than the side-① and side-④ rows. That's ok – there should be enough height to accommodate this. You'll be able to check the height when you finish step 7 of these packing instructions; the instructions there also describe what to do if there's not enough height.

At this point you've finished layers #1, #2, #3, and #4. The super-box should now contain 34 cold packs and 34 cryoboxes (that's all the cold packs and cryoboxes that will go in this super-box), and look like the photograph in figure 6d.

7. Now put in the styrofoam lid pieces. To repeat, different super-boxes may be slightly different in size, so make sure to only use the lid pieces that are labelled for the super-box you're packing. Also, make sure each lid piece is oriented correctly



this diagram: 10 cold packs
 These cold packs stand on edge on styrofoam bottom of box.
 Note: the cold packs against sidewalls 1 and 4 are a tight fit;
 see the instructions for tips on how to squeeze them into place.



this diagram: 5 cold packs
 These cold packs rest flat on styrofoam bottom of box.
 The outer 4 cold packs are positioned just inside the layer #2 cold packs,
 which you should place in the box **before** you place this layer.

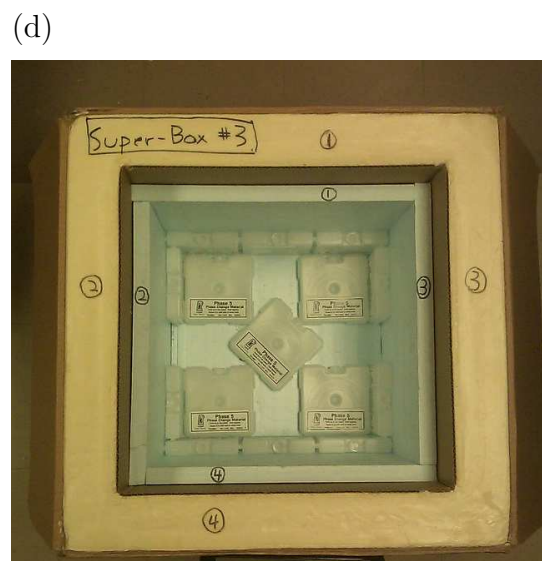


Figure 4: This figure shows (a) the packing of the 10 layer #2 cold packs, (b) a detail of part of this packing along side ① of the super-box, (c) the packing of the 5 layer #1 cold packs (which should be done *after* the packing of the 10 layer #2 cold packs), and (d) the appearance of the super-box with these cold packs (but not yet any cryoboxes) in place.

(“this side up” label facing up, and labels for sides ①, ②, ③, and ④ matching those on the side walls of the super-box).

The styrofoam lid pieces need to be inserted in the super-box in the correct order; if the pieces are numbered as we suggest (from top to bottom) then they need to be inserted in **decreasing** order of their numbers. That is, for example, if there are 3 styrofoam lid pieces, then when you are packing the super-box you put in the 3rd piece lid first (directly on top of the layer #4 cold packs), then the 2nd lid piece, and then finally the 1st (outermost) lid piece.

At this point the outermost styrofoam lid piece should be roughly level with (or no more than about $\frac{1}{4}$ inch (5 mm) above) the top of the styrofoam lining the walls of the super-box. If the lid pieces stick up farther than that, you should take them out and reinsert them, omitting a $\frac{1}{2}$ inch (12 mm)-thick lid piece (or if there is no such piece, replacing a 1 inch (25 mm)-thick lid piece with a $\frac{1}{2}$ inch (12 mm)-thick lid piece).

8. Next, put in the soft foam lid piece. Figure 7 shows the appearance of the super-box with the foam lid in place.

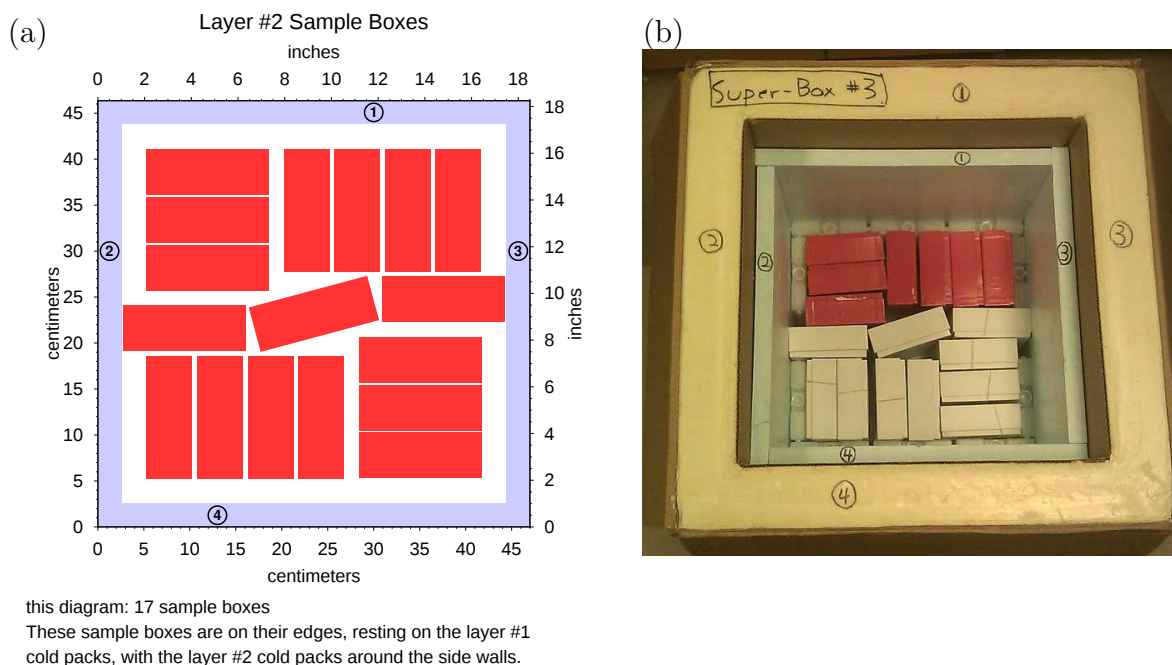
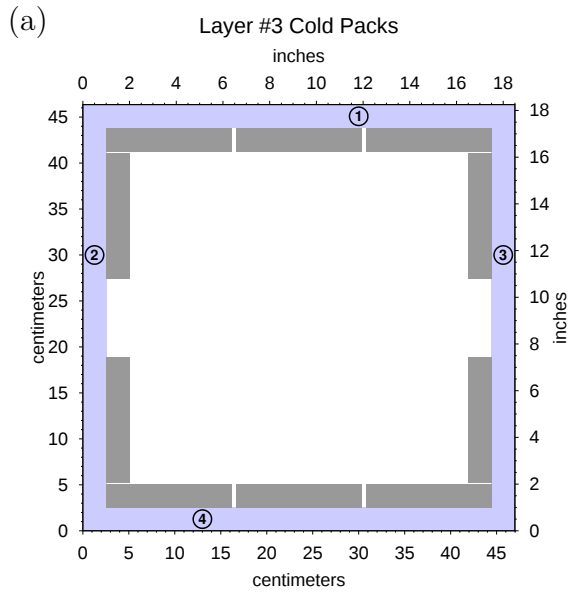
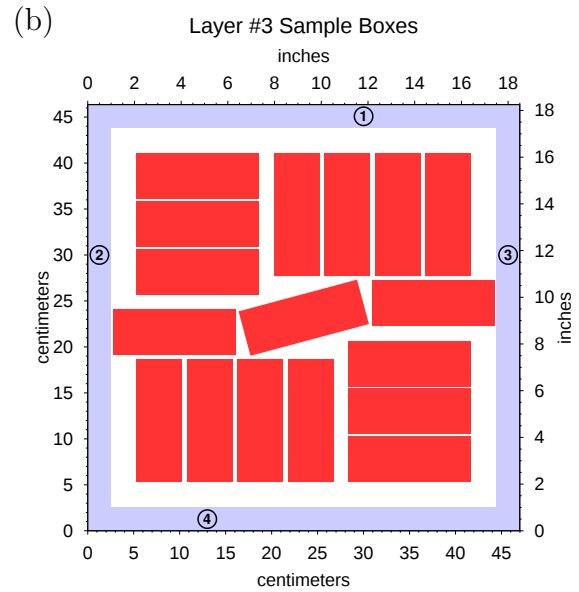


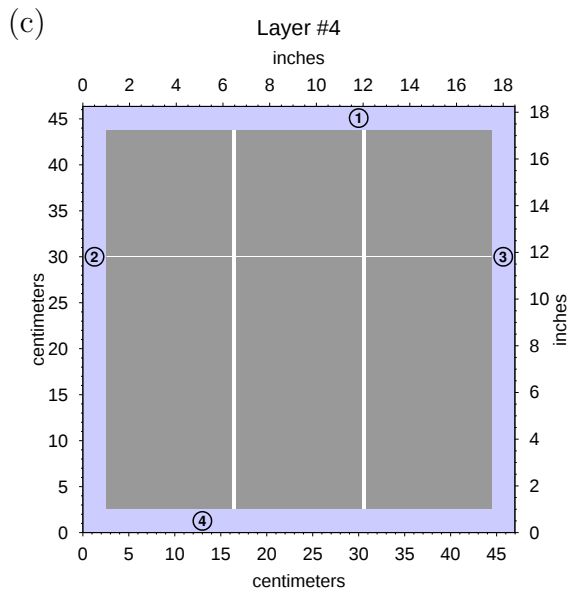
Figure 5: This figure shows (a) the packing of the 17 layer #2 cryoboxes, and (b) the appearance of the super-box when layers #1 and #2 are complete; for actual shipping the cryoboxes will likely be sealed in plastic bags.



this diagram: 10 cold packs
 These cold packs stand on edge on top of the layer #2 cold packs.
 Note: the cold packs against sidewalls 1 and 4 are a tight fit;
 squeeze them into place just like the layer #2 cold packs.



this diagram: 17 sample boxes
 These sample boxes are on their edges, resting on the layer #2 sample boxes, with the layer #3 cold packs around the side walls.



this layer: 9 cold packs
 cold packs rest flat on top of layer #3 sample boxes

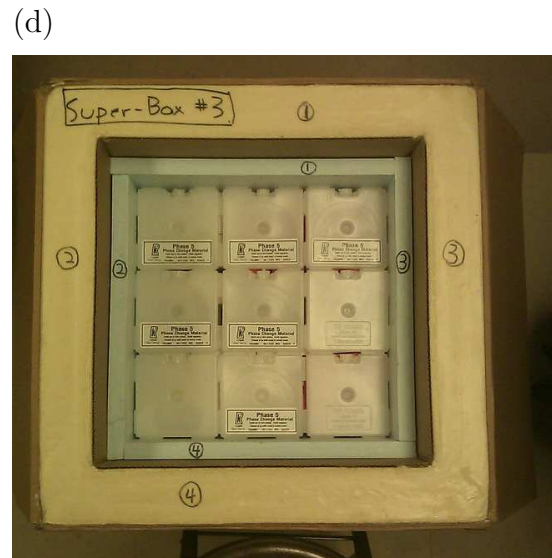


Figure 6: This figure shows the packing of (a) the 10 layer #3 cold packs, (b) the 17 layer #3 cryoboxes, (c) the 9 layer #4 cold packs placed in a 3 × 3 grid, and (d) the appearance of the super-box when layers #1, #2, #3 and #4 are complete.

9. On top of the foam lid enclose a plastic envelope containing copies of the samples' biosafety approval letters, the cold packs' materials safety data sheets (MSDSs), and the sender and recipient's contact information. Close the outside cardboard lid flaps over the foam lid and documentation envelope. Finally, since air leaking in or out will make the super-box contents warm up faster, seal the closed lid flaps as tightly as possible with plastic packing tape.

4 Testing a super-box

It's important to test a super-box to verify that it gives an adequate cold lifetime before using it with actual (precious) biosamples.

4.1 Thermometers

For such a test, it's very useful to take actual temperature readings at several places in the super-box's sample volume throughout the test. Inexpensive digital thermometers with remote temperature probes are readily available; we have used both the Fisher Sci-



Figure 7: This photograph shows the appearance of the top of the super-box with the foam lid in place. The black wires lead to thermometer probes.

entific #S95895 “Jumbo Refrigerator/Freezer Thermometer with Sealed Bottle Probe”¹⁵ (rated down to -50°C)¹⁶ and the Fisher Scientific #15-077-961 “Traceable^(TM) RTD Platinum Freezer Thermometer”¹⁷ (rated down to -100°C). Some thermometer models also allow multiple temperature probes to be read out through a single display unit. For the thermometers we used, the temperature probes are connected to the display unit by a long insulated wire; this wire is narrow enough to be passed through the corners of the styrofoam and foam lid pieces, so the super-box’s packing and cold lifetime should be only trivially affected by the temperature measurements. Two of these temperature-probe wires can be seen in figure 7.

Many thermometer models can also log the maximum and/or minimum temperature recorded during an extended period. It may be useful to include such a logging thermometer in an actual “biosamples” super-box shipment, for extra assurance that the package didn’t warm up excessively in transit. Comparison of a maximum-temperature reading when the super-box is received at its destination with the results of a cold test like the one shown in figure 8 can also help in assessing a super-box’s safety margin in real-world conditions.

4.2 Simulated samples

To test a super-box without endangering actual biosamples, some sort of simulated samples are necessary. Ideally these would take the same form as actual biosamples, e.g., some liquid (it could be water) in cryotubes stored in a grid in cryoboxes. However, preparing thousands of cryotubes this way would be labor-intensive, and the amount of wasted plastic and the cost of the cryotubes (which would no longer be sterile and thus couldn’t be used for actual biosamples thereafter) would be substantial. Instead, we have simulated the thermal inertia of biosamples by using the same total mass of water spread among a number of small plastic bottles. This arrangement isn’t a perfect simulacrum (e.g., the simulated samples don’t have the same air circulation or thermal conductivity as actual samples), but in practice these simulated samples give test temperature profiles and cold lifetimes similar to those we have observed in actual use of the super-box.

¹⁵<https://www.fishersci.com/shop/products/fisher-scientific-jumbo-refrigerator-freezer-thermometers-with-sealed-bottle-probe/s95895>

¹⁶Even though the cold packs start out near -80°C , using a thermometer rated down to only -50°C isn’t actually a serious problem: even when a super-box is loaded with cold packs frozen in dry ice or taken directly from a -80°C freezer, the super-box interior temperature is only below -50°C for a few hours. This can be seen in figure 8.

¹⁷<https://www.fishersci.com/shop/products/fisher-scientific-traceable-rtd-platinum-freezer-thermometer/15077961>

4.3 Cold Lifetime

We have tested a number of super-box configurations, but unfortunately not the exact (final) configuration described in this report. For a conservative estimate of the final configuration's cold lifetime, here we present test results for a configuration using the same insulated shipping box lined with styrofoam as is described in this report, loaded with a total of 7.35 kg of simulated samples (spread among 14 plastic bottles), but only 29 cold packs (rather than the 34 cold packs used in our final configuration). The simulated samples and cold packs were initially frozen in a -80°C freezer, then transported in an insulated (styrofoam) box for about an hour before beginning to load the super-box (which was initially at ambient temperature). The super-box was placed on a cement floor in an uninsulated garage; the super-box was not exposed to direct sunlight. The ambient (air) temperature during the trial varied from a high of 30°C at beginning of the trial to a low of 23°C near the end.

Figure 8 shows the ambient and super-box temperatures as a function of time, with temperature probes at 4 locations in the super-box. Within the first hour the super-box interior cooled down to between -45°C and -60°C , depending on the position of the thermometer probe. It then took about a day for the super-box to warm up to about -20°C . The temperatures then stayed almost constant for about 2 days while the frozen cold packs gradually thawed.¹⁸ At this point the warmest temperature (a top corner of the super-box, just under the foam lid) was at about -15°C . After all the cold packs had thawed it took about another day for the warmest part of the samples to warm up to -5°C . That is, in this trial the super-box and cold packs maintained the simulated samples below -15°C for about 3 days, and below -5° for about 4 days. This meets our design goals, and is long enough for commercial air shipment almost anywhere in the world with a comfortable safety margin.

For our final super-box configuration as described in this report (with 34 cold packs), we expect the cold lifetime to be somewhat longer. The simple 1-dimensional thermal model presented in the appendix shows cold lifetimes which are approximately proportional to the number of cold packs. This argues for linearly extrapolating the cold lifetime, giving about 3.5 days to -15°C and 4.5 days to -5°C .

In actual use air-shipping biosamples from Bolivia, Germany, and Iceland to the USA, the longest transit time we have experienced was about 4 days; the biosamples were all still solidly frozen on arrival.

¹⁸The key point here is that the cold packs *absorb heat* in the process of thawing; this is why the temperature stays almost constant during this period despite heat leaking in from the ambient environment.

5 Acknowledgements

We thank Indiana University's Office of the Vice Provost for Research for financial support.

A 1-dimensional thermal model

In this (slightly mathematical) appendix we present an approximate 1-dimensional thermal model to estimate the cold lifetime of a super-box.

The major approximations in this model are:

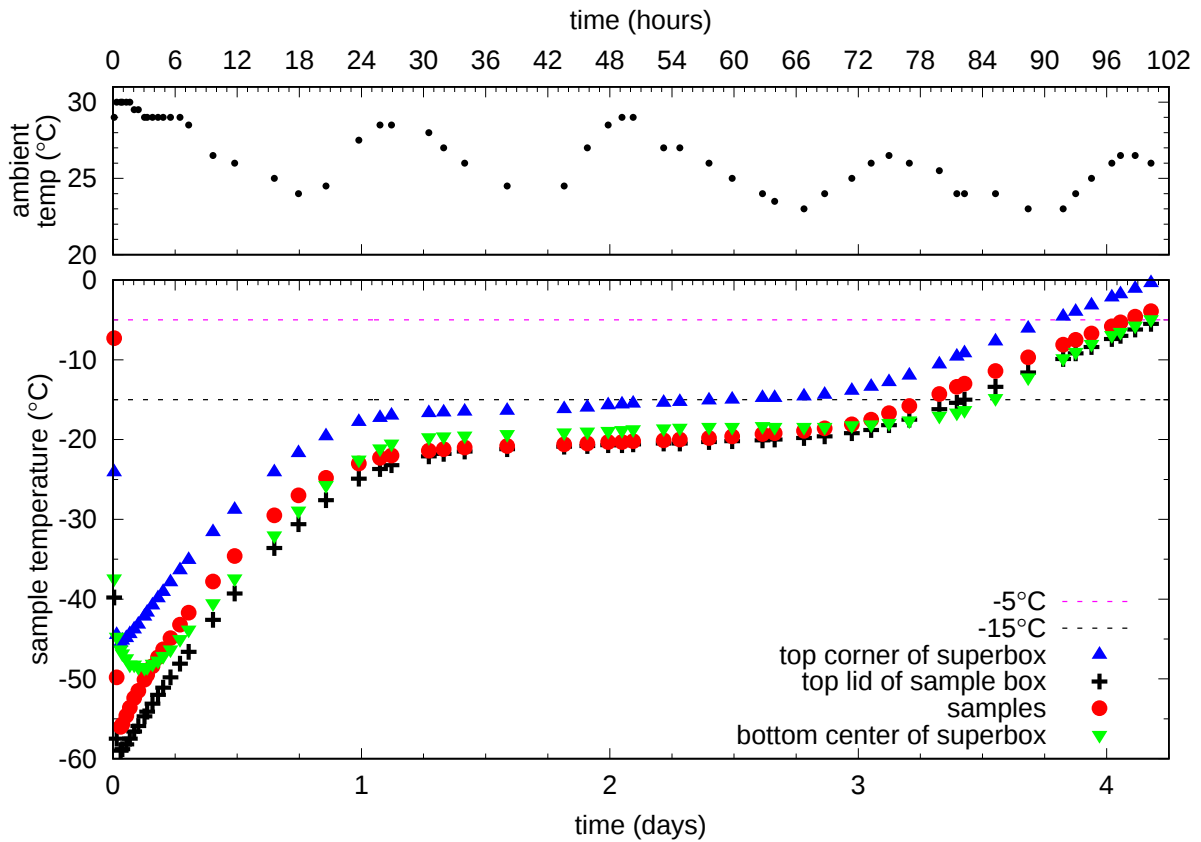


Figure 8: This plot shows a test of the super-box with only 29 cold packs (rather than the 34 of our final configuration). The upper subplot shows the ambient temperature during the trial. the temperature in the super-box as measured by thermometer probes at 4 different places within the box. The thin horizontal dashed lines show temperatures of -5°C (pink) and -15°C (black).

- It treats the ambient environment as an infinite heat sink at a constant temperature.
- It assumes the entire super-box interior is at a uniform (time-dependent) temperature, neglecting any spatial variation in this temperature.
- It assumes that conduction through the super-box walls is the only source of heat flow into the super-box; i.e., it neglects air leakage, convection, and thermal radiation.
- It treats all 6 faces of the super-box as identical (in reality the top lid probably has a lower R-value than the bottom and side walls).
- It assumes that the thermal resistances of the different layers of insulation can be added together to obtain the total thermal resistance of the super-box walls.
- It neglects 3-dimensional effects, i.e., it treats the super-box walls as being thin relative to the super-box size and it idealizes the heat flow into the super-box as being always perpendicular to each wall (the model neglects the non-perpendicular heat flows near the super-box edges and corners).
- It neglects the heat capacity of the super-box itself.
- It neglects the heat capacity of any super-box contents except the cold packs and the samples, i.e., it neglects the heat capacity of the cryotubes, cryoboxes, paper towels, plastic bags, and any other packaging material inside the super-box.
- It assumes Newton's law of cooling, i.e., it assumes that the heat flow into the super-box is proportional to the difference between the interior and ambient temperatures.

Despite these approximations, this model can still give useful information about the general dependence of the cold lifetime on the super-box configuration. For example, the model can be used to estimate how the super-box's cold lifetime would be changed if more or fewer cold packs were used, or if the ambient temperature were different.

By the definition of R-values (this also incorporates Newton's law of cooling), the (time-dependent) rate at which heat leaks into the package, Q ,¹⁹ is given by

$$Q = \frac{A \cdot \Delta T}{Rw} , \quad (1)$$

where A is the total surface area of the package,²⁰ $\Delta T = T_{\text{ambient}} - T_{\text{inside}}$ is the (time-dependent) temperature difference between the ambient temperature and the package interior temperature, R is the R-value (thermal resistance per unit thickness) of the

¹⁹In SI units, Q is measured in Watts.

²⁰Within the approximations of this model, it's not clear whether this should be the inside or exterior surface area. The area enters the model as a *multiplicative* factor in equation (1), so in practice we use the geometric mean of the inside and exterior areas.

package walls, and w is the wall thickness. If the package walls are composed of (say) 2 layers which are thermally “in series” (i.e., one layer is completely contained within the other layer, so that heat leaking into the package must flow first through one layer, then through the other layer), then Rw is replaced by $R_1w_1 + R_2w_2$. This latter case applies to our superbox, with the polyurethane foam as the 1st layer and the styrofoam as the 2nd layer.

If T_{inside} differs from the melting point of the cold packs, then the inflowing heat warms the cold-packs and samples according to

$$Q = (M_{\text{samples}}c_{\text{samples}} + M_{\text{cold pack}}c_{\text{cold pack}}) \frac{dT_{\text{inside}}}{dt} , \quad (2)$$

so that

$$\frac{dT_{\text{inside}}}{dt} = \frac{Q}{M_{\text{samples}}c_{\text{samples}} + M_{\text{cold pack}}c_{\text{cold pack}}} , \quad (3)$$

where M_{samples} and $M_{\text{cold pack}}$ are the total mass of the samples and the cold-packs respectively, and c_{samples} and $c_{\text{cold pack}}$ are the specific heat capacities²¹ of the samples and the cold-packs respectively. Note that $c_{\text{cold pack}}$ is typically different for frozen versus melted cold packs.

If T_{inside} equals the melting point of the cold packs and the cold packs have not yet completely melted, then the inflowing heat melts the cold-packs (at constant temperature) at a rate

$$Q = -\frac{dM_{\text{frozen}}}{dt}L , \quad (4)$$

so that

$$\frac{dM_{\text{frozen}}}{dt} = -\frac{Q}{L} , \quad (5)$$

where M_{frozen} is the mass of cold-pack phase-change-material that is still frozen, and L is the specific latent heat of fusion²² of the cold-packs.

These equations can be readily solved either analytically or numerically.

A.1 Analytical solution of the model

Given the approximations of the model, it's easy to see that starting with samples and cold packs frozen, the super-box passes sequentially through 3 stages before the samples thaw:

1. The (frozen) cold packs and samples warm from the initial temperature to the melting temperature of the phase-change material, $T_{\text{melt}} = -20^\circ\text{C}$.

²¹The specific heat capacity of a material is the amount of energy per unit mass per unit temperature required to warm or cool the material. In SI units, it's measured in Joules/(Kilogram $\cdot$ $^\circ\text{C}$).

²²The specific latent heat of fusion of a material is the amount of energy per unit mass required to melt the material. In SI units, it's measured in Joules/Kilogram.

2. The cold packs thaw at (within the approximations of the model) a constant temperature T_{melt} .
3. The (melted) cold packs and frozen samples warm from T_{melt} until the samples thaw.

We take the initial conditions to be time $t = 0$ and temperature $T_{\text{inside}} = T_{\text{initial}}$. We denote the time at which stage 2 starts as $t = t_{12}$, and the time at which stage 2 ends as $t = t_{23}$, and the time at which the samples reach their thawing temperature T_{thaw} as $t = t_{\text{thaw}}$.

For stages 1 and 3 we consider an exponential-decay ansatz (this is also implied by Newton's law of cooling),

$$\Delta T = K e^{-(t-t_0)/\tau} \quad (6a)$$

$$T_{\text{inside}} = T_{\text{ambient}} - K e^{-(t-t_0)/\tau} . \quad (6b)$$

Substituting equation (6) into equations (1) and (3) it follows that the exponential-decay time constant is given by

$$\tau = \frac{Rw(M_{\text{samples}}c_{\text{samples}} + M_{\text{cold pack}}c_{\text{cold pack}})}{A} , \quad (7)$$

while t_0 and K are determined by the initial conditions.

For stage 1 the initial conditions give $t_0 = 0$ and $K = T_{\text{ambient}} - T_{\text{initial}}$. Solving equation (6b) for $T_{\text{inside}} = T_{\text{melt}}$ then gives the duration of stage 1 as

$$t_{12} = \tau_{(\text{stage 1})} \ln \left(\frac{T_{\text{ambient}} - T_{\text{initial}}}{T_{\text{ambient}} - T_{\text{melt}}} \right) . \quad (8)$$

During stage 2 the cold packs melt at a constant rate given by equation (5), so the duration of stage 2 is

$$t_{23} - t_{12} = \frac{M_{\text{cold pack}}L}{Q} , \quad (9)$$

and hence the time at which stage 2 ends is

$$t_{23} = t_{12} + \frac{M_{\text{cold pack}}L}{Q} . \quad (10)$$

For stage 3 the initial conditions give $t_0 = t_{23}$ and $K = T_{\text{ambient}} - T_{\text{melt}}$. Solving equation (6b) for $T_{\text{inside}} = T_{\text{thaw}}$ then gives the duration of stage 3 as

$$t_{\text{thaw}} - t_{23} = \tau_{(\text{stage 3})} \ln \left(\frac{T_{\text{ambient}} - T_{\text{melt}}}{T_{\text{ambient}} - T_{\text{thaw}}} \right) . \quad (11)$$

The cold lifetime t_{thaw} is given by the sum of the durations of the 3 stages, equations (8), (9), and (11).

A.2 Parameters of our super-box

The parameters of our super-box are as follows:

The total surface area of a rectangular solid of dimensions $x \times y \times z$ is $2xy + 2xz + 2yz$. For our super-box the interior dimensions are approximately $x=36.9 \times y=36.2 \times z=28.6$ cm, for a surface area of 0.685 m^2 . The exterior dimensions are $x=64 \times y=63.5 \times z=62.9$ cm, for a surface area of 2.42 m^2 . The geometric mean of these areas is $A = 1.29 \text{ m}^2$.

The wall thicknesses are $w_1 = 7.6$ cm and $w_2 = 2.5$ cm. From table 1, the wall R-values (thermal resistances per unit thickness) are $R_1 = 50$ and $R_2 = 35 \text{ m} \cdot ^\circ\text{C}/\text{W}$.

Each cold pack contains 298 g of phase-change material, with specific latent of fusion $L = 270 \text{ kJ/kg}$ and heat capacity similar to that of water,²³ $c_{\text{cold pack}} = 2050 \text{ J}/(\text{kg} \cdot ^\circ\text{C})$ when frozen (solid) and $c_{\text{cold pack}} = 4181 \text{ J}/(\text{kg} \cdot ^\circ\text{C})$ when melted (liquid).

At 298 grams of phase-change material per cold pack, 29 cold packs contain 8.6 kg of phase-change material and 34 cold packs contain 10.1 kg of phase-change material. 34 cryoboxes each loaded with a 9×9 grid of 2 ml cryotubes, each containing about 1.7 g of biosample, gives a biosample mass of $M_{\text{samples}} = 4.7 \text{ kg}$. Assuming the biosamples to be frozen throughout the super-box's cold lifetime, we approximate their specific heat as similar to that of frozen water, $c_{\text{samples}} = 2050 \text{ J}/(\text{kg} \cdot ^\circ\text{C})$.

A.3 Model results

Figure 9 and table 2 show the model results for the super-box configuration used in figure 8 (29 cold packs, ambient temperature 26.5°C), as well as for our “final” super-box configuration (34 cold packs) at 3 different ambient temperatures (35°C , 26.5°C , and 20°C). The model results are surprisingly close to the test results: the predicted cold lifetimes to -15°C or -5°C are within about 10% of the test results.

²³https://en.wikipedia.org/wiki/Table_of_specific_heat_capacities

Number of cold packs	Ambient temperature ($^\circ\text{C}$)	Cold lifetime to ...		Notes
		-15°C (hr)	-5°C (hr)	
34	35	70	82	very high ambient temperature
29	26.5	71	84	fewer cold packs (matches figure 8)
34	26.5	82	97	higher ambient temperature
34	20	95	113	lower ambient temperature

Table 2: This table gives the calculated cold lifetime from our 1-dimensional thermal model for various super-box configurations and ambient temperatures. See figure 9 for plots of these same cases.

The model is particularly useful for seeing how the cold lifetime would vary for different system parameters. For example, comparing the model results for 34 versus 29 cold packs at the same ambient temperature, the modelled cold lifetimes are very close to proportional to the number of cold packs. (Going from 29 to 34 cold packs is an increase of 17%; the cold lifetimes to -15°C and -5°C increase by 16% and 15% respectively.) This argues for linearly extrapolating the 29-cold-pack test results in figure 8 to estimate the actual cold lifetime of our final 34-cold-pack super-box configuration. We have done this.

Another useful comparison is how the cold lifetime varies with the ambient temperature. Compared to the results for an ambient temperature of 26.5°C , the cold lifetime is approximately 14% shorter at an ambient temperature of 35°C , and it's approximately

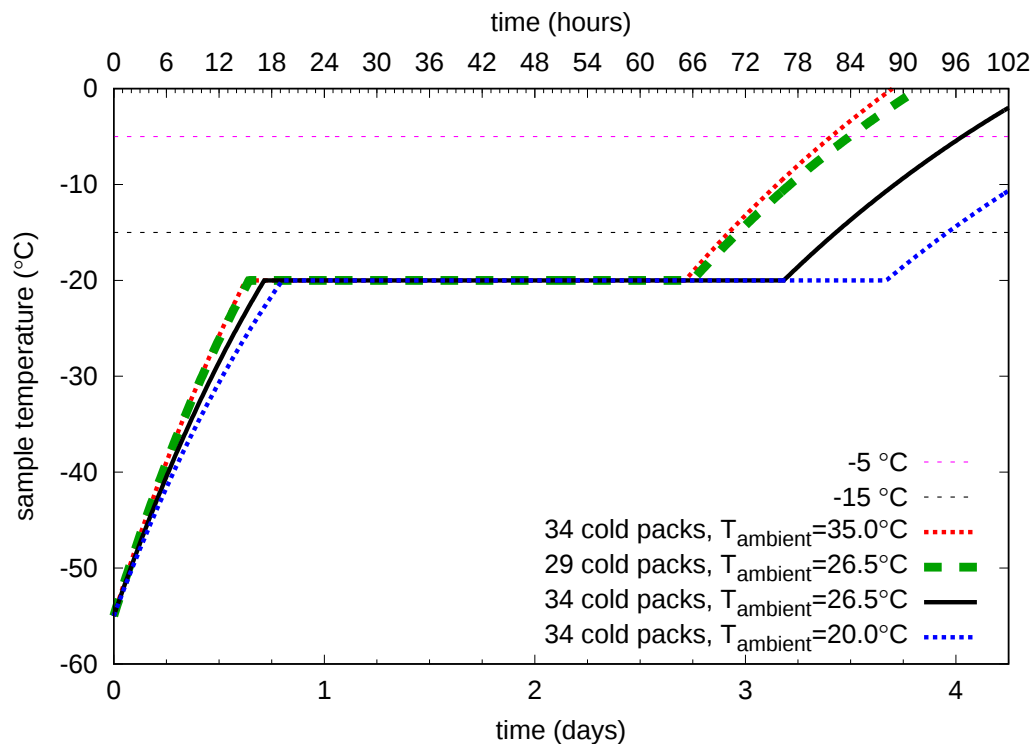


Figure 9: This figure shows the interior temperatures of the super-box calculated from our 1-dimensional thermal model, for various super-box configurations and ambient temperatures. The thick green dashed line shows the case matching figure 8. The thin horizontal dashed lines show temperatures of -5°C (pink) and -15°C (black). See table 2 for the calculated cold lifetimes for all these cases.

15% longer at an ambient temperature of 20°C. We expect these percentage changes to also apply fairly accurately to the actual super-box cold lifetime. These results can be summarized by the statement that the actual cold lifetime is (approximately) inversely proportional to the difference between the ambient temperature and the melting temperature of the cold packs (-20°C).

A.4 Discussion

Figure 8 shows that (over the range of parameters considered here) the changes in (modelled) cold lifetime are mainly due to changes in the duration of stage 2. Equations (1) and (9) show that this duration is directly proportional to the total mass of the cold packs (and hence to the number of cold packs), and inversely proportional to the difference between the ambient temperature and the melting temperature of the cold packs ($T_{\text{melt}} = -20^{\circ}\text{C}$).