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Logistics and Sample Transport: Overview, Recommendations, Validation Protocol

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Introduction

Logistics and sample transport can introduce significant preanalytical bias. The ISBER Best Practices include detailed guidelines on sample transport (<http://www.isber.org/Pubs/BestPractices2008.pdf>). The ISBER Biospecimen Science Working Group has published a standard Biospecimen research experimental protocol in order to study and reduce in vitro preanalytical variables related to sample processing (CEBP 2009, *in press*).

The purpose of this document is to further reduce pre-analytical variables once the optimum conditions for the sample and assay have been established.

Increasingly, biological specimens are transported domestically and internationally in support of research. Numerous factors can affect specimen integrity throughout this process.

The shipper has the responsibility to be compliant with International Air Transport Association (IATA) requirements if shipping by air, and must demonstrate training to meet the standards. While it is not in the scope of this document to address this training, www.iata.org can provide additional information. It is essential that prior to shipping, staff involved with shipping must be trained and certified in the handling of dangerous goods according to IATA Dangerous Goods Regulations and CFR 172.700.

Once optimum temperature for the sample and assay has been defined, the logistics process must be designed to maintain the sample at this temperature and reduce fluctuations during shipment while expediting transport to its destination.

Globally there are many products on the market for sample transport with additional products that offer thermal protection. The manufacturers of the products can provide validation data, and the shipper should ensure that the data supports their need, or if additional data is needed. The manufacturer should be encouraged to provide the data in order to reduce cost and time to the institution.

Variables

Many variables can influence the sample integrity during the logistics process, including: temperature, packaging, courier, sample type, import/export requirements, seasons, costs, and transit time/ship days. Each of these variables is described in more detail below.

Temperature

For the purposes of this document, the following temperature states are defined:

Ambient (20-25 degrees C)

Refrigerated (2-8 degrees C)

Frozen (<-20 degrees C) but generally at ultra-low temperatures (-80 degrees C)

Liquid Nitrogen (<-130 degrees C)

Packaging

Packaging material can vary widely.

Ambient shippers alone may not offer sufficient protection during flight. Air temperatures will drop to sub-zero temperatures. Depending on sample type, further thermal protection may be required in order to reduce variability, and this may be further affected by the season. Various products can offer thermal protection and may include gel materials, foam materials, or other products.

Refrigerated shipments can be protected by the use of materials that can be frozen or refrigerated prior to shipment, and this may vary according to the season as well.

When shipping frozen (dry ice) samples, the packaging container should be large enough to add sufficient dry ice for the duration of the shipment with a margin of safety (recommend 72 hours) and be evaluated in conjunction with a typical sample shipment size. Dry ice is available as pellets or blocks. Selection may be driven by local availability. Block ice will provide more longevity. If block ice is used, any vacant space can be filled with packing material or wadded paper to slow evaporation and cushion the samples. If pellet ice is used, place a layer of dry ice lining the bottom of the shipper, and then add samples, followed by additional dry ice to fill the chamber.

Liquid nitrogen (LN₂) dry shippers offer the longest protection during transport although may somewhat limit the number of samples that can be shipped. Availability and/or requirements governing the use of liquid nitrogen at the facility must also be taken into consideration.

Courier

Couriers can be divided into the common carriers (i.e. FedEx, TNT, etc.) who transport specimens on their own planes, or premium couriers who often transport on the commercial airlines. Premium couriers can provide dry ice to the shipper if procurement is difficult, and re-ice the shipment during transport. They can advise on customs regulations and assist with the process.

It is advisable to have proof of delivery when the shipment reaches its destination or other method of tracking the shipment.

Sample type

Sample type, anticoagulants, transport media, primary container (glass versus plastic versus cryotubes), and total shipment volume should also be considered during shipment. Again, IATA regulations should be reviewed for any limitations.

Import/Export Requirements

Increasingly research is conducted on a global basis and it is very challenging to ensure compliance with regulatory bodies. Regulations are country specific and can change rapidly. It is important to determine if any permits are required, who is the party that is required to hold the permit, if it is required per shipment or if a single permit covers the duration of the trial, and the lead time required to obtain the necessary permit. It is important to prevent any delay at customs as this can negatively impact the sample integrity rendering it useless for testing.

Seasonal differences

Summer versus winter variations can also negatively affect sample integrity. This is most often seen with ambient samples. During the transport process, samples may sit on a tarmac in extreme heat, or conversely be subjected to extremely cold temperatures.

Cost

Shipment costs are extremely important when considering the shipping – and shipping frequency – of samples. It is estimated that in a clinical trial, approximately one third of the cost can be in transportation.

Shipping domestically versus shipping internationally can heavily weigh the decision. While a premium courier will be significantly more expensive than a standard courier, assistance with customs and the ability to re-ice if needed are important considerations.

Transit time/Shipping days

Shipments should be planned to minimize transit times. It is advisable to avoid weekend shipments if using standard couriers. The courier can provide specific guidance for international shipments. Considerations include pick-up times (and documentation required), time zone differences, local and national holidays, optimum routes, transit time to destination, contingency plans, notification of delays, and proof of delivery. The length of time a flight is in the air will increase the time that cargo is subjected to sub-zero temperatures.

Additional recommendations

- Send the intended receiver a notification, including tracking ID, when samples are en route so that both shipping and receiving parties are able to track the shipment. As needed, an electronic copy of the packing slip may be included as an attachment.
- Have the recipient email the shipper as soon as the shipment is received. Any comments regarding the condition of the shipment should be noted at this time.

- Include a packing slip (manifest or roster) with the shipment in compliance with IATA requirements.
- Consider other instructions or labelling as part of the shipment, such as “Store @ __ degrees C immediately upon receipt”

Validation Protocol

After evaluation of the variables listed above, if it is determined that a test shipment is required, the following protocol can be used to determine if the components selected will meet the needs of expediting the shipment while reducing temperature variation.

Note: If shipping PBMCs, consider comparing viability and cell counts prior to shipping and after shipping. It must be noted if cells were shipped on dry ice or LN₂, and if the cells were sitting at -80 degrees C and not placed in LN₂ prior to shipping.

Materials

- IATA compliant shipping materials
- 2 pre-calibrated temperature recording devices to provide redundancy. Many products can monitor temperature during shipment. It is important to select a product that will measure temperature within the acceptable range for the shipment (i.e. below -40 degrees C, warmer than -40 degrees C, etc.). It may be as simple as a product that simply measures variance from the expected range. Or it may be a more sophisticated product where the user can set the temperature recording intervals. These products are purchased with software where the data can be downloaded and plotted on a graph of temperature (x-axis) versus time (y-axis). The products may be re-usable.
- Materials to simulate actual samples including size of containers and number of containers per shipment

An agreement should be made with the intended recipient to participate in the test run. The shipper should be packaged according to IATA and manufacturer's instructions.

Prior to sealing the shipper, the 2 temperature recording devices should be activated and inserted into the shipment per manufacturer's directions.

The shipment should be sent per expected process.

Attributes of the shipment should be recorded to later determine conformity/non-conformity, and if a failure is significant to warrant a repeat. Examples of attributes of the shipment that should be recorded include the following:

- Sample type
- Number of samples
- Primary container (cryotubes, etc)
- Secondary shipping container
- Outer shipper
- Expected temperature range
- Courier
- Pick up day

- Pick up time
- Weight of dry ice (if applicable)
- Ice bricks or other material (if applicable)
- Size of LN shipper (if applicable)
- Season
- Expected delivery day/date
- Actual delivery day/date

Temperature during shipment (x-axis) should be plotted against time (y-axis) to determine if the samples were maintained within acceptable limits.

Non-conformity can be evaluated using the following table:

Non-conformity	Critical	Non-Critical	Repeat test	Conclusion
Extended transit time				
Temperature range outside established limits				
Customs delays				
Insufficient documentation				

In the table above, a final conclusion should be entered stating whether the combination of shipping materials, courier, internal protection, and documentation procedures are robust enough in order to offer maximum protection and minimize risk to the samples in shipment.

Conclusion

The critical nature of extended transit time or temperature range outside established limits can be assessed by performing Quality Control assays on dedicated aliquots of the samples, so as not be destructive for the samples. Quality Control can be performed both prior and after shipment, or after shipment only. Testing both before and after shipment allows specific assessment of the preanalytical impact of shipment.

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