Logging Temperatures in Biological Cryo Transport

In 1999 experts from the Zoological Society of London (ZSL) returned from Russia with blood samples from the Amur leopard which, with only about forty animals left in the wild and a handful in zoos, was perhaps the world's most endangered member of the cat family. The expedition involved collecting blood and semen samples from several of these cats. With the strict prevailing quarantine laws, the semen had to remain in Russia under cryogenic conditions for six months. However, the blood samples could be brought back to the UK immediately for analysis.

With so few animals left in the world conservation was important and this team was part of a wider inter zoo collaboration to conserve the leopards' genetic diversity and improve the overall genetic makeup of the population. Semen from the pure-bred Amur leopards in Moscow was be banked and could in future be used for artificial insemination of the rest of the population, thus increasing the overall levels of these valuable founder genes. It is likely that further research will be needed before AI is successful, but, once the semen is stored it is there for posterity as a long-term insurance policy against the death or failure to breed of the male concerned.

In transporting and storing specimens cryogenically Dr. Bill Holt and his colleagues from London used freezing techniques and equipment that have been so widely accepted they are commonplace now throughout the world. However, in this case, the team was the first in the world to test a new system that monitored the vital parameters of the valuable cargo during its progress.

Over the last fifty years the preservation of biological material at liquid nitrogen temperatures has come from being an experimental technique to a cornerstone of much of medicine and biology. Early items preserved were animal sperm for artificial insemination; subsequently human sperm, human embryos, bone marrow, plant matter, cell lines, cornea, skin and many other biological specimens have all been preserved.

As a consequence of this it became more and more important to monitor the actual temperatures experienced in cryogenic vessels and refrigerators. A rise in temperature can easily damage sample viability and so temperature logging is necessary to check that the requisite protocol is available and that the sample has been, or continues to be, safely stored; traceability and Good Laboratory Practice demand this as well as scientific needs.

As the use of cryo-preservation techniques became widespread, the need for the transportation of samples from location to location increased. On the face of it, this was a theoretically simple task of transferring the frozen specimen to a container, also filled with liquid nitrogen and then shipping it. In practice it was not simple - from the physical handling and from the sample integrity point of view.

For example, the ZSL researchers used a lightweight 'dry shipper' to ferry their samples from Moscow to the UK. This is a metal container with an inorganic porous liner. Liquid nitrogen 'soaks' into the liner providing a 'cold sink'; this approach

obviously posed far less of a safety hazard for transportation than an equivalent liquid only container might. Transporting a valuable sample has potential risks for the specimen as well as the handlers. For example it is desirable that the temperature of the Amur Leopard samples should not go above -128 °C. The researchers utilized the Planer ShipsLog™, a type of data logger integral to the 'dry shipper' and which traveled with it.

After loading the 'dry shipper' and ShipsLog™, into the hold of a ‘plane from Moscow, the London team were able to download a temperature history after their sample had been collected from Heathrow. This showed that a temperature of no more than -140 °C had been kept for five days. Peaks could be seen when the container had been opened briefly, but these would not have been sufficient to damage cell integrity. Interestingly the researchers found that a 'negative peak' seemed to occur after takeoff - possibly due to pressure drop. Again the team was confident that this would not have affected sample viability.

The ShipsLog™ system is a data logger attached to the upper side of a 'dry shipper' container with a range of -200°C to 0°C. A platinum resistance thermometer senses the chamber temperature and is attached to a recorder through the 'dry shipper' lid. The subsequent recording is accurate to +/- 5 °C with a resolution of about 1°C. The data logger takes over 8,000 readings at user defined intervals of between 30 second and 99 minutes. An especially useful feature is the existence of three indicator lamps giving an audible or visual warning or alarm condition. Both high and low alarm levels can be pre set and a magnetic start/stop non contact system is applied via a special fob key. A standard RS232 port allows communication with, and downloading to, the users PC and a custom programme for interpretation is available. The internally fitted Lithium battery allows a long life of approximately five years before factory refitting.

In the transport of the leopard blood samples described, a liquid nitrogen system was used since very low temperatures were needed, but related equipments using solid carbon dioxide as a cold source are frequently used. Similar solutions can apply to pharmaceutical companies who need to ship product for clinical trials, where temperatures of -70°C need to be maintained for viability of product. The ShipsLog™ provided a record of temperature against time to check viability when the sample is out of view. Other data loggers will provide an alarm at a predetermined low level of liquid nitrogen or when a specified temperature is reached. These alarms can be audible or visual and can trigger a number of actions - automatic refill being a standard one. Planer offer a number of these loggers and alarms for both mechanical and liquid nitrogen storage vessels and containers.

The Amur leopard blood samples are valuable because of their scarcity. However any sample worth storing must be important - pharmaceutical cell lines, human or animal tissue. If the material is going to be out of sight for any time an investment for the reassurance of its integrity will always be worthwhile.