

Good Things Come in Threes



The Three Rs (Reduction, Refinement, Replacement)

May 2018 Newsletter



Introduction

“It is widely recognised that the humanist possible treatment of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments.”

W.M.S. Russell & R.L. Burch, *The Principles of Humane Experimental Technique*

The Principles of Humane Experimental Technique, published in 1959, was the foundation of what is now known as the Three Rs field of Reduction, Refinement and Replacement of animal use. In their 1959 book, William Russell and Rex Burch proposed a novel applied science, which aimed at ameliorating the treatment of research animals while improving the quality of scientific and medical research and testing. They suggested that implementation of the Three Rs yields more reliable data as experiments would be appropriately designed in a manner that decreases variation, provides standardized optimal conditions of animal care and minimizes unnecessary stress.

Current regulatory guidelines take the Three R Principles into account; for example the AAALAC's (Association for Assessment and Accreditation of Laboratory Animal Care International) and CCAC's (Canadian Council on Animal Care) ethical standards of animal experimentation is based on these three guiding principles of Reduction, Refinement and Replacement. ITR, being CCAC- and AAALAC-accredited, is strongly committed to adhering to the principles of the Three Rs in order to produce quality scientific data while meticulously caring for our research animals. Our mission is to promote animal welfare through incorporating these guiding principles in all of our practices, thereby enabling the propagation of Russell and Burch's legacy.

In this newsletter, we delve into the role ITR plays in implementing the Three Rs:

- Reduction through developing a single-handed jugular venipuncture blood collection technique
- Refinement through selecting European-style housing
- Replacement through using human cells for certain types of studies

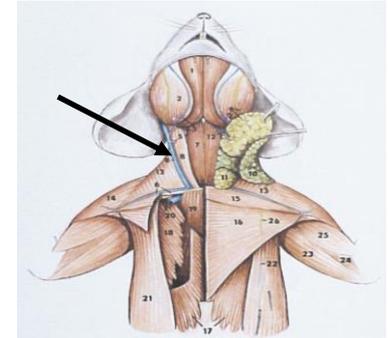


I.

A Single-Handed Jugular Venipuncture Blood Collection Technique

ITR is continuously striving towards refining techniques used in preclinical testing and conforming to the Three Rs guiding principles for the more ethical use of animals in safety testing. Our most recent improvement, the development of a single-handed jugular venipuncture blood collection technique, results in a considerable reduction in the total number of mice used in toxicology studies, specifically for toxicokinetic evaluation subsets. This technique consists of collecting blood from the jugular vein in unanesthetized mice. The blood samples are collected and subsequently assessed for quality (presence of clots). There has been no mortality associated with this technique and no clinical signs have been observed during the procedure. The lack of clinical signs and the fact that the proposed technique is quite similar to that used for oral gavage suggest that the procedure is well tolerated by the mice. The sample quality of the blood was not affected by the jugular collection technique; all samples were considered of acceptable quality, as noted by the absence of clots.

Up until recently, blood collection in mice consisted mainly of collecting terminal blood samples via the abdominal aorta or by cardiac puncture, a practice requiring the use of a new subset of animals for each blood sampling occasion. Collecting blood samples via the jugular vein from conscious animals allows for the same mice to be used on multiple blood sampling occasions.



Location of Jugular Vein in the Mouse

II. European-Style Housing

In order to meet the physical and behavioral needs of the animals, thereby improving the quality of the animal housing environment, animal welfare, animal health and consequently study data, ITR has installed European-style housing for nonhuman primates. This housing system allows animals to be in a socially enriched environment that permits them to express species-typical behavior. The Nonhuman Primate Group Housing Unit is designed to meet European-style open housing. This caging system creates more naturalistic group-housing scenarios that allow healthy, socially-housed animals to participate in studies while remaining housed in a social setting. In our efforts towards refinement, we installed a European-style housing system for most of our nonhuman primate studies. These are characterized by their size as they have double the space of regular cages. One animal can take up a whole stage or even the entire cage. Study animals are usually housed in groups of up to 3 per housing unit. European-style units allow the free movement of the animals vertically as well as horizontally. Multiple units could be joined together through opening the separators on the side thereby increasing the space available for the animals in each study. A mezzanine is attached on the front of the cages and allows monkeys to have more private space during their social interaction process. Swings are provided for all units to respect the monkeys' penchant for climbing and swinging.

In conclusion, our exemplary environmental enrichment and behavioral management programs exceed industry requirements and allow us to continue to successfully conduct studies involving different kinds of species while meeting their species-specific needs.



European-Style Housing for Nonhuman Primates

III. Migration and Proliferation Assays in Human Cells

Regulatory agencies are increasingly recommending the replacement of animals involved in testing and encouraging the use of alternatives whenever possible. To address this, ITR is developing new methods such as cellular migration and proliferation assays, which use *in vitro* human cell cultures. The migration assay involves the quantification of movement of a cell population, for example from the top side of a microporous membrane to its bottom side in a microplate assay format. Inhibition or increase of such movement can point to a Test Item effect on wound healing (e.g. epithelial or muscular cells) or on the immune response (e.g. activity of lymphocytes, macrophages). These cell-membrane experiments can also be used to study the transport of drugs through a cellular matrix (e.g. lung alveoli-blood interface). As for proliferation assays, there are many different protocols available. These *in vitro* proliferation tests are used to quantify the processes involved in the division of cells either as an effect of a Test Item or inversely from a stimulant, which allows the detection of an inhibitory effect from the Test Item. Once in place these protocols will be added to the growing list of *in vitro* assays available at ITR employing human blood/cells that are aimed at replacing laboratory animals. The current list of validated tests includes the Mammalian Chromosome Aberration Test (MCAT), the COMET assay, hemolytic potential and blood compatibility testing.



Cell Culture Lab

Conclusion

While there are still many areas where animal testing is necessary and non-animal testing is not yet a scientifically valid and available option, it is critical to acknowledge the fact that Russell and Burch's Three Rs provide a means to improve animal welfare and provide a legal and ethical framework for *in vivo* research and we, at ITR, conform to these guiding principles.

