

The Science Behind GentleSharp™ and the 3 Rs.

What is GentleSharp?

GentleSharp is a handheld device designed to reduce needle penetration force. It adds directed, low frequency, oscillatory motion to the needle as it is inserted. The resulting micro-vibrations help the needle puncture and glide through tissue more smoothly with less resistance.

What are the 3 Rs?

Russell and Burch first introduced the principles behind the 3 Rs in 1959.¹ To this day, these concepts continue to guide our conduct of experiments using animal subjects:

- > **Refinement** - Any method that allows for the reduction or elimination of pain and distress to animals during experimentation.²
- > **Reduction** - Any methods that allow for the use of fewer animals in an experimental protocol.²
- > **Replacement** - Any technique that does not require use of living animals (absolute replacement) or those that avoid or replace the use of protected animals (relative replacement).³

Blood sampling is a stressful procedure.

While blood sampling is a common practice in biomedical research, it's also inherently stressful to the subjects. Sampling stress, which is partially caused by repeated painful needle punctures, can increase stress hormone levels and variability in research subjects. Addressing the concept of Refinement can be a genuine challenge in this type of research.

To protect these animals, most institutions establish strict guidelines regarding the frequency of sampling and the amount of blood that can be collected from a single subject.⁴ To maintain compliance, researchers often employ cross-sectional study designs requiring large numbers of animals at different points in the species' lifespan. This allows them to obtain unaffected blood samples without violating frequency guidelines. Unfortunately, this approach is at odds with the concept of Reduction as it requires use of many animals for each time-point of interest.⁵

How did you determine GentleSharp reduces stress?

Research was conducted to evaluate the impact of the device with repeated blood tail samplings in rats and mice.

Experimental Design

In vivo serial blood sampling was completed using rats and mice.

| 3 sample collections on the same day | Sampling Week 1 | | | Sampling Week 2 | | | Sampling Week 3 | | |
|--------------------------------------|----------------------|---|---|-----------------|---|---|--------------------|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Treatments | OFF: GentleSharp Off | | | | | | ON: GentleSharp On | | |
| Rats | N=9 | | | | | | N=10 | | |
| Mice | N=23 | | | | | | N=24 | | |

Experimental Endpoints

| Target | How Target was Measured/Calculated |
|-----------------------|---|
| Plasma Corticosterone | Radioimmunoassay |
| Needle Insertions | Counts required for successful collection |
| Total Blood Mass | Total blood mass collected (g) |
| Success (%) | Successful/Attempted Collections |
| Movement/Vocalization | 4 independent blinded viewers of video footage of the blood sampling procedures |



Explain how GentleSharp addresses 2 of the 3 Rs.

GentleSharp improved blood-sampling outcomes with both rats and mice. The data suggests a strong trend in the device enabling the acquisition of blood samples while eliciting a reduced stress response. This can benefit studies conducted with either rats or mice.

Refinement

By multiple factors, the animals exhibited reduced pain and distress during collection:

- > *Reduced levels of plasma corticosterone in rats.*
- > *Trend for reduced levels in mice.*
- > *Rats also exhibited reduced behavioral indications of stress when the investigational device was ON vs. OFF.*

Reduction

GentleSharp reduced the stress hormone level, corticosterone, and additionally reduced the individual variance in corticosterone levels over the study span in the animals. This decrease in variations could:

- > *Allow for statistical significant data to be obtained with fewer animals in certain types of studies.*
- > *Reduced animal number could further lead to lowered animal costs, per diem and associated analysis costs.*
- > *Reduce the need for cross sectional study design by allowing individual animals to be repeatedly sampled over their life spans and/ or treatments.*

Data Analysis

Data from replicate experiments were analyzed by t-test or by one-way ANOVA. All tests were two-tailed and statistical significance was set at alpha = 0.05, depicted by *.

Rat Study Results

| Target | Gentle Sharp ON vs OFF |
|---|--|
| Insertion Force | *Reduced (67.5%) |
| Plasma Corticosterone | *Reduced 49% (Week 2); Reduced 65% (Week 3) |
| Plasma Corticosterone-Individual Variance | Reduced (71%) |
| Rat Movement | *Reduced 50% (Week 2); *Reduced 40% (Week 3) |
| Rat Vocalization | *Reduced 60% (Week 2); *Reduced 52% (Week 3) |

Mice Study Results

| Target | Gentle Sharp ON vs OFF |
|--|-------------------------|
| Success Rate | *Increased (10%) |
| Needle Insertions | *Reduced (12.4%) |
| Trials requiring single needle insertion | *51% (ON) vs. 38% (OFF) |
| Total Blood Mass | *Increased (26.7%) |
| Training Tool Capability: Novice Collector 1st Collection Success Rate | 100% (ON) vs. 45% (OFF) |

CITATIONS:

1. Russell, W.M.S. and Burch, R.L., (1959). *The Principles of Humane Experimental Technique*. Methuen, London. ISBN 0900767782
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3. [Internet]. 2014. NC3Rs - What are the 3Rs? Available at: <http://www.nc3rs.org.uk/page.asp?id=7>.
4. Guidelines for Survival Bleeding of Mice and Rats. National Institutes of Health.
5. Cano P, Cardinali DP, Spinedi E, Esquifino AI. 2008. Effect of aging on 24-hour pattern of stress hormones and leptin in rats. *Life Sci* 83:142-148.

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All procedures described herein were reviewed and approved by the Pennsylvania State University Institutional Animal Care and Use Committees and were performed in accordance with The Guiding Principles for the Care and Use of Laboratory Animals.



A More Humane Blood Sampling System

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