

## **Mouse bleeding times** (Shivdasani Lab - Dana-Farber Cancer Institute)

The widely accepted way to measure mouse bleeding times is as follows:

Pre-warm tubes of saline (PBS) to 37°C and maintain at that temperature during the measurements.

Use inhalation of isoflurane vapor or, alternatively, intraperitoneal injection of avertin to induce general anesthesia.

Using a sharp new razor or scalpel blade, briskly cut exactly 0.5 cm of the distal tip of the tail of an adult mouse and immediately insert into the pre-warmed tube of saline. Start a stopclock at this time.

Hold the tail gently, near its base, to avoid a "tourniquet effect." You will see venous blood flowing into the tube and can readily detect when this bleeding stops. The stopclock provides an accurate bleeding time.

Keeping the following three caveats in mind, you should be able to record highly reproducible bleeding times of 50-70 seconds in most inbred or outbred strains of laboratory mice.

(1) Mice that have previously been genotyped by tail biopsy are not appropriate for this measurement because the scar tissue from the tail biopsy interferes with the study. We get around this problem by genotyping AFTER the bleeding time measurement, which has the added advantage of the bleeding times being studied in blinded fashion.

(2) Anesthesia is critical. If the animal is moving around too much (or at all), motion of the tail interferes with measurements substantially and variably.

(3) Once the bleeding into pre-warmed saline has stopped for 5-10 seconds, we return the animals to their cage, where anesthesia reverses gradually. Bleeding may resume after that time, but its significance is unclear because factors other than stability of the initial platelet plug may come into play, especially if the animals move, sniff each others' wounds, etc. If you find that bleeding continues in anesthetized mice while their tails are held gently in the saline, that should legitimately be included in the bleeding time measurement.