



Version: 093109

SELECTION AND RANDOMIZATION OF ANIMAL SUBJECTS FOR PRECLINICAL STUDIES.

Purpose: This procedure is promulgated to assure an unbiased assignment of animal subjects for preclinical studies, as part of the CRL's commitment to the highest standards of scientific quality, integrity and clinical relevance.

Scope: This protocol is to be followed for all studies considered "translational" or "preclinical," and in particular for all studies designed specifically to evaluate the therapeutic potential of putative neuroprotective strategies.

Review: This protocol will be reviewed and updated annually or at the discretion of the Associate Director or Associate Chair for Research. Suggestions for improvement are to be forwarded to the Associate Director at jsulliva@med.wayne.edu.

OVERVIEW: This procedure removes the surgeon and PI from processes involving animal selection and randomization, and imposes anonymity on post-procedure animals for purposes of outcome measurement. The procedure is driven by coin-toss and involves the following stages: *Group Designation, Cage Randomization, Permanent Designation, Randomization to Injury, Randomization to Treatment, and Post-Surgical Blinding*. The procedure is described in great detail below, but is actually much simpler in practice than it sounds, as the attendant examples should demonstrate.

1. **Group Designation.** Upon arrival at the Lande DLAR facility, all animals will be assigned a *group designation* based on date.
 - a. **Example:** if rats arrive on 081109, all animals will carry 081109 as part of their permanent designation. The first animal to be removed from the cage for study (see below) will be designated 081109a, the second 081109b, etc. A good way to think about it is that all the rats get their "family" name (081109 in this example) when they arrive, and get their "given" name (a, b, c...) when they are taken out for study.
2. **Cage Randomization.** On the day of surgery, a cage will be selected randomly from the pool of all cages containing animals eligible for inclusion in the study, regardless of arrival date. *Primary randomization is conducted by an individual other than the surgeon* (the randomizing individual is hereafter referred to as the "registrar"). On the basis of their position on the rack, cages are given a *temporary numerical designation* for the purpose of primary randomization. Temporary numerical designations range from 1 (upper left) to x (lower right), where x = total number of eligible animal cages.
 - a. **Example:** if ten animal cages are on the cart, the cage in the upper left is number one, and the cage in the lower right is number 10.

The registrar will then produce a coin and perform a coin toss to progressively bisect the set of available cages until a selection is made. Tails will exclude animals in the cages half of the set, and heads will exclude cages in

the lower half. Odd numbered sets are bisected such that the remainder set is the next higher number (i.e., “rounded up”).

- b. **Example:** if there are ten eligible cages, with temporary numerical designations 1-10, and the first toss is heads, only cages numbered between 6 and 10 are now eligible. If the second toss is tails, only cages between 6 and 8 are eligible. (Again, odd-numbered sets are split to the next higher number). If the third toss is heads, only cages 7 and 8 are eligible. If the fourth toss is heads, cage 8 is then selected.

If multiple animals are to be used for surgery, subsequent cages are selected by the same method, after a repeat numerical designation.

- c. **Example:** In the first randomization, cage 8 is selected and removed. 9 cages remain, and are reassigned numerical designations 1-9. Randomization is repeated by coin toss to select the second cage. (In this case, the cages previously designated 9 and 10 would become 8 and 9, respectively.)
3. **Permanent Designation.** Animals are then removed from the cage for study and given their *permanent designation* (a, b, c...) by the registrar. The registrar notes on the cage card that animal (a, b, c...) has been removed, to prevent repeat designations.
 - a. **Example:** Two cages have been selected by randomization. One cage is for animals that arrived on 081109, one cage is for animals that arrived on 073009. All animals that arrived in cage 081109 are still present. One animal is removed at random and is given the *permanent designation* 081109a. The cage card is annotated accordingly and the cage is returned to the rack. The second cage has two animals remaining; 073009a has already been taken. The registrar removes one of the remaining animals at random and assigns permanent designation 073009b. The cage card is annotated and the cage is returned to the rack.
 4. **Randomization to Injury.** At surgery, all animals receive the same anesthesia, prep, sedation, and preliminary surgical procedures *up to the point at which injury may be induced* by either blood removal and clamping (2VOH), placement of a filament (MCAO), or TBI. *Only then may randomization to injury/no injury take place.* The registrar will flip a coin and the surgeon proceeds accordingly. For standardization purposes *heads = injury* and *tails = sham*.

The animal is treated accordingly, following standard procedures. At the appropriate time, the animal is then randomized to treatment by coin toss. For the purposes of standardization, *heads = treatment* and *tails = vehicle/sham treatment*.

- a. **Example:** Assume that we are conducting a study of combination therapy with treatments A and B. An animal selected by the methods above has been anesthetized, shaved, and instrumented to the point of ischemia. The registrar flips a coin and it is heads. The surgeon proceeds to stroke the animal per protocol. At the time point when a decision on therapy A must be made, the registrar flips a coin. It is tails, and therefore the subject receives vehicle/sham therapy instead of therapy A. At the time point for decision on therapy B, the registrar flips the coin again, and it is heads. Therapy B is administered. The animal has now been randomized to ischemia with therapy B only.

Prohibited groups are groups to which an animal may randomize, but which are not called for by the experimental protocol or are already filled by previous animal preparations (as indicated by the anonymous experimental registry, maintained by the registrar). If an animal randomizes to any such group, the registrar will repeat the randomization (Example a). Alternatively, the registrar may observe that randomization to an interim point only allows assignment to one permitted group, and make that assignment (Example b).

- b. **Example:** The surgeon has performed ischemia and reperfusion, and the animal has randomized to treatment A. On the final toss, the animal randomizes to treatment B as well. Referring to the registry for this study, the registrar notes that the set isch + txA + txB is already full, and is therefore a prohibited set. The registrar then repeats the randomization and the animal falls into the group isch + shA + shB. This

group is not part of the experiment, and is also prohibited. On the next randomization, the animal falls into the group isch + shA + txB, which is an incomplete experimental group and therefore permitted. The surgeon proceeds.

- c. **Example:** The surgeon has performed ischemia and reperfusion per secondary randomization, and on tertiary randomization the animal has assigned to treatment A (isch + txA +?). The registrar may at this point note that the group isch + txA + txB is already full (prohibited), while the group isch + txA + shB is not. *It is acceptable* at this juncture for the registrar to then declare the animal as having randomized to the isch + txA + shB group, the only remaining non-prohibited possibility, instead of starting the randomization over again.

5. **Post-Surgical Blinding.** The surgeon will complete the animal surgery/data documents and deliver them to the registrar. These forms will include information on ischemia/no ischemia/treatment/no treatment. These will be sealed into the animals packet by the registrar, who will then label the packet with the animal's permanent designation obtained in step 5 above (*to which the surgeon and PI have been blinded.*) The registrar will then record/check off the animal in the general experimental registry (egg, "8 min 2voh + txA+txB"). *The permanent designation is not recorded in the experimental registry.* This registry simply tells us which groups are planned for a particular experiment and how many animals have been completed for a particular group. Similarly, *the cage card and visible part of the animal's paperwork reveal no information on the animal's treatment/experimental group.*

In other words, looking at the rat's cage card or its sealed up note only reveals the animal's "name" (permanent designation, e.g. "101509c"), not what happened to it. Looking at the registry only reveals which animal preparations have been completed for a particular experiment, not the "names" of the animals that have been used for those preparations. *After surgery, the animal's permanent designation is the only available identifier until its record is unsealed for final data analysis. No information on what has happened to a particular animal is accessible until its surgical record has been unsealed.*

6. **Exceptions:** For preclinical studies, deviations from this protocol require prior approval of the Associate Director or Associate Chair for Research, and will be duly noted (with justification) in all publications, presentations and abstracts.
7. **References:** This protocol may be referenced by the following URL:

<http://www.brainischemia.net>

Approved:



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Associate Director
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