STANDARD OPERATING PROCEDURE

TITLE: **Elevated Plus Maze**

CATEGORY: **Behavioral Assay**

Introduction

Goal: This document aims to provide the reader information on how to conduct the Elevated Plus Maze. The test is used to evaluate anxiety levels, and parameters collected include time and frequency in each arm. As this is a general description of standard materials, test settings, and procedures, variations may be made to fit specific needs.

Materials
- *Subjects*: any strain of rats or mice. No prior training is required, though subjects should be acclimated to testing environment and experimenter before testing.
- *Apparatus*: Plus-shaped maze with two closed and two open arms (see Figure 1).
- *Overhead camera*: mounted to ceiling directly above apparatus.
- *Software*: automated tracking system (ex. EthoVision).
- *Privacy blinds*: placed around apparatus to eliminate external room cues.
- *Standing lamps with white light bulbs (4)*: placed at corners outside privacy blinds pointed away from apparatus.
- *Light meter*: used to measure illumination in arms of apparatus.
- *Virkon*: used between trials to eliminate visual and olfactory residue in arena.

Test Settings
- *Lighting*: consistent illumination throughout apparatus (approximately 200lux).
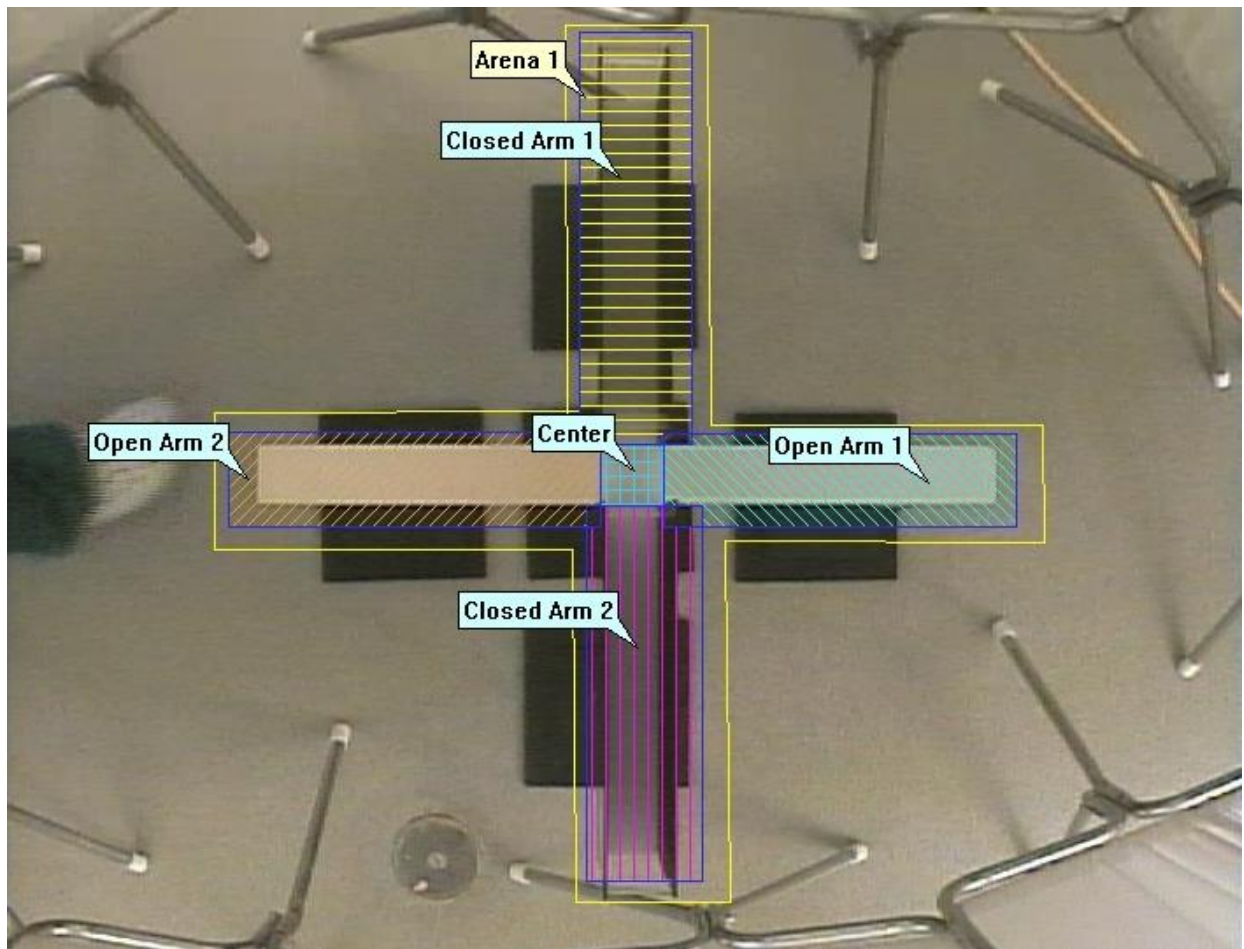
Figure 1. Elevated Plus Maze Zones

Detailed Standard Operating Procedure

Before testing:
- *Acclimation*: subjects in home cage are placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing.
- *Subject training*: none required.

Testing procedures:
- Animal is placed in center square region of apparatus facing away from experimenter and allowed to move through apparatus for 5min. Trial begins immediately and ends when defined duration has elapsed.
- Animal is returned to home cage and number of fecal pellets in each zone is recorded.
- Arena is cleaned with Virkon between trials.

Data Analysis
- The following parameters are collected for analysis:
    - Distance moved
    - Mean velocity
    - Time & frequency in each zone
    - Total & percent time in open arms
    - Number of entries into open arms