Standard Operating Procedure

Sudden Object Test (SUD) – Bedded Pack - Group

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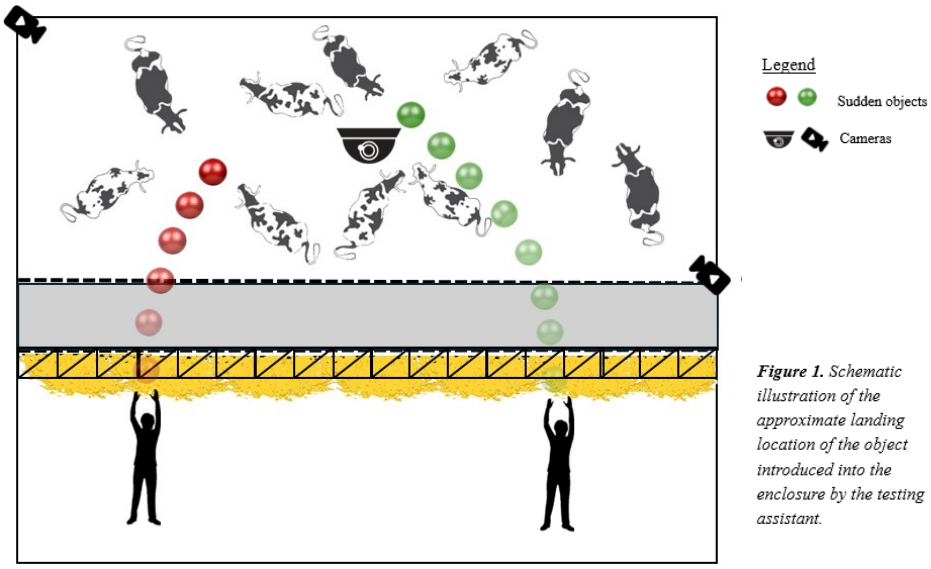
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Description/objective

The Sudden Object Test evaluates animal's behavioural reactions to a sudden and unexpected stimulus. It is designed to assess startle responses, approach–avoidance tendencies, and recovery following the sudden stimulus. This test is adaptable to various environments and all age classes and is designed for both individual and group testing. *The test procedure described in this SOP is performed in a bedded pack enclosure, using one object presentation methods on two or more animals simultaneously.*

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Terminology

Object presentation method:	
Object placement:	A system into which an object is introduced by being thrown from outside the enclosure.  ***Figure 1.*** *Schematic illustration of the approximate landing location of the object introduced into the enclosure by the testing assistant.*
Object:	
Sudden Object:	In general, the sudden object should elicit a startle response. The object must appear suddenly and with sufficient intensity to induce a startle response. It may produce noise, but this is not required. The object should be neutral and not overly attractive to the animals. The size and colour of the selected object(s) must ensure clear visibility to both the animals and the cameras within the enclosure. A sufficient number of objects must be provided to ensure that all animals within the enclosure are expose to the sudden appearance of the object simultaneously. The object must be heavy enough to quickly reach the desired location within the enclosure, ensuring a sudden appearance. However, it must not be so heavy that it poses a risk of injury if it falls on an animal's head or body.

Requirements

✓ **Personnel (n = 3-6):**

Personnel	Number	Role
Testing assistant(s)	1-2	Responsible for the sudden object placement and wait times

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Camera manager(s)	1-2	Responsible of managing the camera operation (i.e., installing the cameras, validating the views and starting/stopping the recording)
Trained handler(s)	1-2	Responsible for handling of animals to and from the open field arena and for handling animals during entry into the arena

✓ **Materials Needed:**

- Personal protective equipment (PPE)
- Camera(s)
- Power source for camera(s) (i.e., extension cords, power bank with AC outlet)
- Object(s)
- Stopwatch
- Whiteboard
- Dry erased marker

General information

- **Location:** The familiar enclosure must meet criteria that ensure both animal safety and appropriate test conditions. It must be familiar to the animals (i.e., a pen or enclosure in which they are routinely housed, either temporarily or for prolonged periods). The enclosure must be enclosed by gates with an open surface area, and the floor must be non-slippery (e.g., covered with rubber mats or bedding material) to ensure secure footing and reduce the risk of injury. It must also be large enough to allow the free movement of all animals being tested.

- **Lighting and noise conditions:** The test environment must be sufficiently illuminated to ensure a clear video recording of the animal. To perform a reliable and reproducible test all potential distractions must be avoided, as such the presence of non-essential personnel and any unnecessary noise during testing is strictly prohibited.

- **Camera positioning:** Camera should be positioned to perfectly cover the full surface of the enclosure. Overhead and side views are suggested which helps to capture all angles of the animals.

- **Animal preparation**: Individuals within each group should be familiarized with one another prior to testing. In addition, a reliable identification method should be implemented to ensure that individual cows can be accurately identified from video recordings.

- **Test order preparation**: Animals housed in neighbouring pens should not be tested consecutively in order to preserve the novelty of the test. Animals housed in neighbouring pens may be tested simultaneously, provided they cannot interfere with or observe one another. Alternatively, they may be tested at sufficiently separated times, with an adequate interval between tests to minimize information transfer between animals. In the latter case, a predetermined and standardized testing order should be established and consistently followed across all testing sessions.

Procedure

1. A camera manager set up cameras to capture selected test pen, trained handlers ensure that the animals are confined within the designated testing area while a testing assistant starts a 30-minute acclimation period to allow the animals to settle and get familiar with the cameras before the test begins.

2. Each testing assistant picks up a sudden object and positions themselves in front of the pen, ensuring that the object remains out of the animals' sight until the start of the test.

3. Following the 30 minutes acclimation period, a camera manager initiates recording for all cameras and verifies that the field of view for each camera is correctly aligned to ensure full coverage of the test area surface.

4. A camera manager writes on the whiteboard the information that will allow to identify the video (animal ID/marking, test being conducted, date and time) and displays it in all camera views. While doing so, the camera manager verifies that the animals are standing, calm and have enough space to move freely; if any animals are asleep and/or lying, they should be gently encouraged to stand up. Animals are required to be standing for the test to proceed.

5. Once the camera manager and trained handler have exited the testing enclosure, a camera manager initiates a 2-minute waiting period to allow the animals to resume normal behaviour following human activity within the enclosure.

6. After the 2-minute wait period, a camera manager starts the test with a 1.5-minute period to record normal behaviours before the sudden event.

    - **If, any animals in the group lie down during this time,** trained handlers must enter the enclosure again and encourage them to stand. Once trained handlers are out of the enclosure, both the 2-minute and 1.5-minute wait times are restarted.

7. After the full 1.5-minute have elapsed, without any verbal clues or abrupt movements, testing assistants toss the objects at the same time aiming for the central area of the enclosure. When two objects are presented, they should be aimed at two separate locations within the central area, leaving sufficient space between the objects and from the enclosure walls to avoid spatial constraints or overlap between their respective areas of influence.

8. Once the object(s) have dropped suddenly in the enclosure, a camera manager starts a 1-minute timer.

9. After the 1-minute test period has elapsed, the test is concluded. The camera managers stop all recordings. Testing assistant(s) then retrieve the sudden object(s) from the enclosure.

10. Repeat this procedure until you have tested all selected groups.

Safety Note

All personnel should stand with proper distance from test animal to stay safe and not distract them. All personnel should remain calm and avoid sudden movements to prevent startling the animals.

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Microsoft 365 Copilot, an AI-based writing and editing tool was used to support language refinement, structural clarity, and consistency of technical descriptions.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

WELL-E Authorship Governance policy, adapted from the Contributor Roles Taxonomy (CRediT) proposed by Brand et al. (2015).

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* WELL-E authors and co-authors, who take accountability for the manuscript, must contribute to each stage of the paper. These stages are conceptualization/design, data analysis, and writing & editing.