Standard Operating Procedure

Sudden Object Test (SUD) - Open field – Group

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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 an open field arena, using one object presentation methods on a two or more animals simultaneously.*

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Terminology

Object presentation methods:	
Pulley system:	A system in which a rope passes through a ceiling hook positioned such that, when the object is on the ground, it allows for 360° exploration by the animals within the open field arena. The rope extends to an area behind the arena walls (Figure 1), ensuring that the object handler remains out of the animals' sight. The object may be pulled up or dropped.
Object placement:	System in which a remote-controlled object is positioned within the open field arena prior to the start of the test (Figure 2). The object must be positioned such that it does not physically or behaviorally impede the animals' entry into the arena (e.g., by inducing fear or reluctance), while still allowing for 360° exploration.

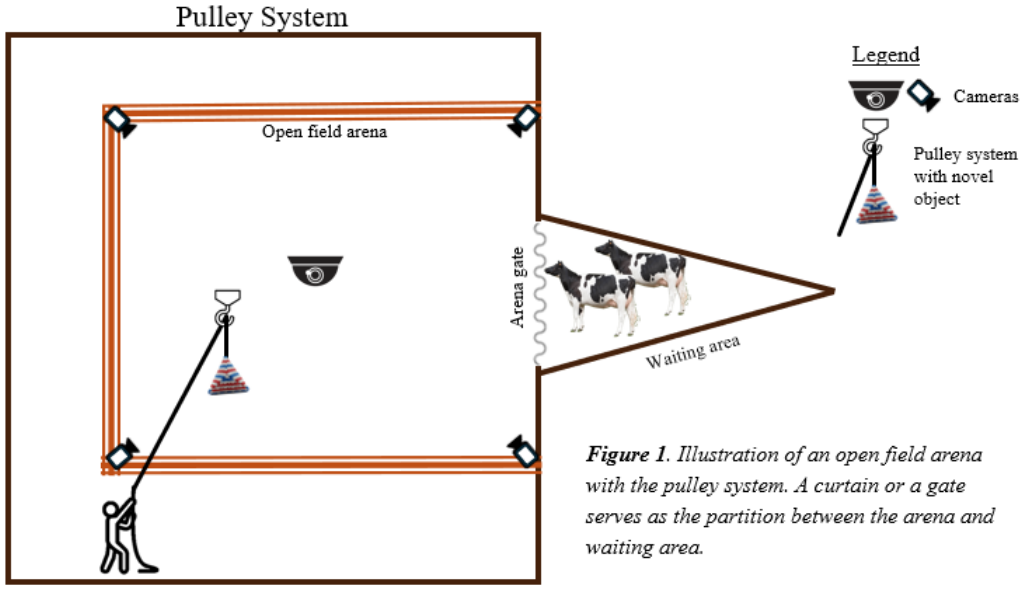


***Figure 1**. Illustration of an open field arena with the pulley system. A curtain or a gate serves as the partition between the arena and waiting area.*

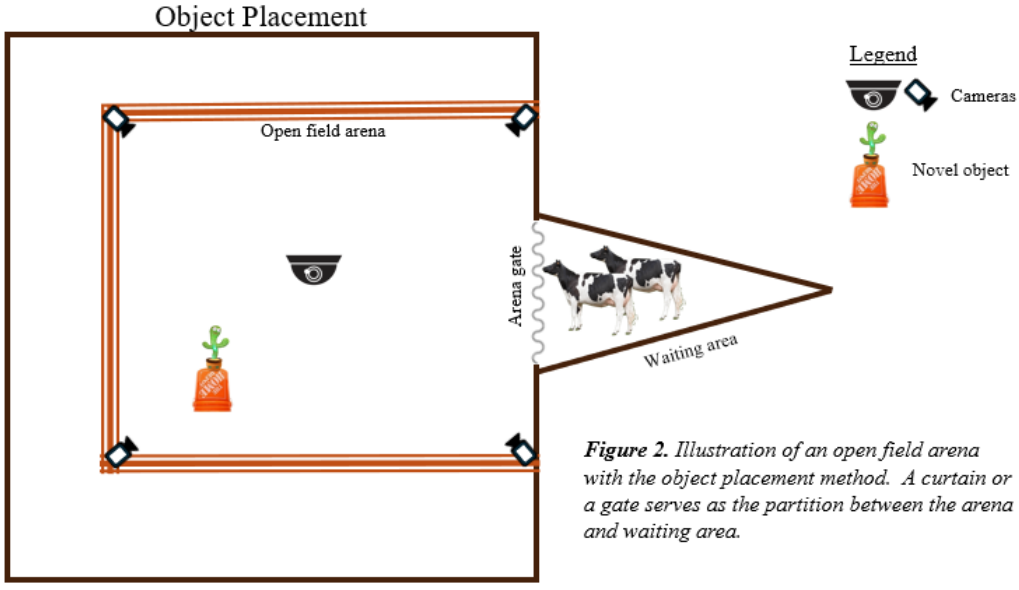


***Figure 2.** Illustration of an open field arena with the object placement method. A curtain or a gate serves as the partition between the arena and waiting area.*

Object:	
Sudden Object:	In general, the sudden object should elicit a startle response. The size and colour of the selected object(s) should ensure clear visibility to both the animals and the video cameras within the open field arena. Depending on the method used for object presentation, the following criteria apply: <u>Pulley system</u>: • *Object dropped down*: The object must be of sufficient weight to descend quickly, ensuring a sudden appearance. However, it must not be heavy enough to pose a risk of injury if it were to fall on the animals' head or body. • *Object pulled up*: The object should be familiar to the animals (i.e., present during arena familiarization). It should be neutral and not overly attractive, so that the animal is not motivated to interact with it prior to the test. It should be noted that when the object is raised, an approach-avoidance response cannot be reliably assessed. <u>Object placement:</u> As the object is already present in the arena, it should be familiar to the animals (i.e., present during arena familiarization). Upon command, it must move suddenly and with sufficient intensity to induce a startle response. The object may produce noise, but this is not required. It should be neutral and not overly attractive, so that the animals are not motivated to interact with it prior to the test.

Requirements

✓ **Personnel (n = 3-6):**

Personnel	Number	Role
Testing assistant(s)	1-2	Responsible for the sudden object placement and wait times
Camera manager(s)	1-2	Responsible of managing the camera operation (i.e., installing the cameras, validating the views and starting/stopping the recording) and wait times
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 open field arena must be designed to ensure both animal safety and appropriate test conditions. It must be enclosed by walls of sufficient height to prevent the animal from seeing over them or escaping. The walls must be opaque and may include a coloured pattern to provide visual contrast. The floor must be non-slippery (e.g., covered with rubber mats) to ensure secure footing and reduce the risk of injury. The arena should be located away from areas housing other animals to minimize external disturbances and distractions.

- **Group testing:** The size of the open field should be adapted to the number and size of the animals tested simultaneously. The arena must provide sufficient space for all individuals to move freely and express the full range of behaviours of interest, including locomotion, exploration, avoidance, and social interactions, without excessive spatial constraint or forced proximity. As a general principle, the available floor area should increase with group size and be sufficient to allow individuals to move independently while maintaining the opportunity for normal social interactions. For studies involving two or three animals, the arena should, at a minimum, allow all individuals to move simultaneously without physical interference and provide sufficient space for each individual to temporarily distance itself from the others. The adequacy of the arena should be assessed during pilot testing by verifying that animals are not persistently constrained by the walls or by the presence of other animals and that the behaviours of interest can be expressed without apparent restriction.

- **Lighting and noise conditions:** The test environment must be sufficiently and uniformly 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 are strictly prohibited.

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

- **Animal preparation**: Familiarization should encompass both the testing environment and the experimental procedures. To avoid confounding effects associated with novelty, animals must

be familiarized not only with the open-field arena and the individuals within each testing group, but also with the procedures involved in transportation to and placement within the arena (e.g., transport route, handling procedures, and any associated manipulations). The procedures used to move animals to the testing area should be carefully designed to minimize stress for both the animal and the handler and to ensure that exposure to potentially stressful factors is as consistent as possible across animals and testing sessions. Familiarization with the testing environment should include exposure to all components of the test. For object-placement tests, this includes exposure to both the arena and the object. When a pulley system and pull-up procedure are used, animals should also be familiarized with these elements. In addition, a reliable individual identification method must be implemented to ensure that animals can be accurately identified from video recordings during subsequent behavioural analyses.

- **Test order preparation**: If the test is to be repeated to improve efficiency and maintain a consistent schedule across tests, a predetermined random testing order should be established. However, if the test is conducted only once, establishing such an order may not be necessary.

Procedure

1. The testing assistant sets up the object according to the selected presentation method (i.e., pulley system or object placement, as defined in the terminology section). Only one method must be used for the test and any subsequent repetitions.

    - **If using the pulley system method:**

        - **Object pulled up:** a testing assistant attaches the object to the rope. The object must rest on the ground, and the rope should be retracted until all slack is eliminated, ensuring the rope is fully extended and ready for use. It should also be placed on the floor in the arena leaving space for 360º exploration.

        - **Object dropped down:** a testing assistant attaches the novel object to the rope and retracts the rope until the object is hanging just below the ceiling, out of view of the animal that will be tested. The object must be positioned such that, when lowered to the ground, it allows for 360° exploration within the arena.

    - **If using the object placement method:** a testing assistant places the object on the floor in the arena, ensuring there is sufficient space for 360° exploration and that the object does not obstruct the animals' entry into the arena.

2. 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 arena surface.

3. A camera manager writes on the whiteboard the information that will allow to identify the video (animals' ID/marking, test being conducted, date and time) and displays it in all camera views.

4. Trained handlers calmly walk the animals into the waiting area of the arena (Figure 1, 2). and then a testing assistant starts a 30-second wait period to allow the animals to settle.

5. After the 30-second wait period, a testing assistant opens the arena gate to allow the animals to enter the arena voluntarily. If the animals do not enter after 30 seconds, they can be encouraged to do so by a trained handler following the steps below. If the step is unsuccessful after 30 seconds, the handler moves onto the next step:

   Step 1: Use vocalization (i.e., talking to the animal with regular voice volume) and hand gestures (i.e., soft hand clapping).
   Step 2: Use gentle tactile stimulation (i.e., touching the animal on the rump).
   Step 3: Select one animal from the group and physically prompt the animal to move into the arena (i.e., putting hands on the animal's rump and using consistent forward motion to encourage the animal to move forward into the arena).
   Step 4: If the selected the animal is still unwilling to enter the arena, repeated step 3 on another animal in the group. If none of the animals are willing to enter the arena the group is walked back to its home environment and a second attempt is made later.

6. Once the animals are in the arena, one testing assistant closes the arena gate while another gets ready to manage the object, and the camera manager times a 20-second settling period. The camera manager monitors the live recording, signals the testing assistant when one of the animals is looking at or towards the object, and then records the time at which the signal is given.

7. When the testing assistant receives the signal from the camera manager,

   - **If using the pulley system method:** the testing assistant quickly pulls on the rope to bring the object near the ceiling or releases the rope to allow the object to fall to the ground

   - **If using object placement method:** the testing assistant activates the remote-controlled object via the remote for a duration of 3 seconds.

8. Once the object has suddenly moved, a camera manager starts a 1-minute test period.

9. After the 1-minute timer has elapsed, the test is concluded, a camera manager stops the recording, trained handlers walk the animals back to their home environment, and a testing assistant returns the object to its original position.

10. Before testing another group of animals, the arena must be cleaned, urine and/or feces must be removed when applicable, and the floor must be dried.

11. Repeat the procedure for each group.

Safety Note

All personnel should maintain proper distance from test animals to stay safe and not distract them. All personnel should remain calm and avoid sudden movements to prevent startling the animals. Surface of the arena should be safe (e.g., non-slippery, clean, even) and the gates be sturdy enough to withstand unpredictable behaviour of the animals.

AI ASSISTANCE DISCLOSURE

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.