Standard Operating Procedure

Sudden Object Test (SUD) – Tie Stall

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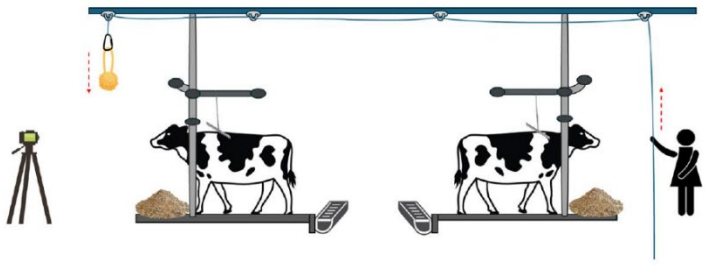
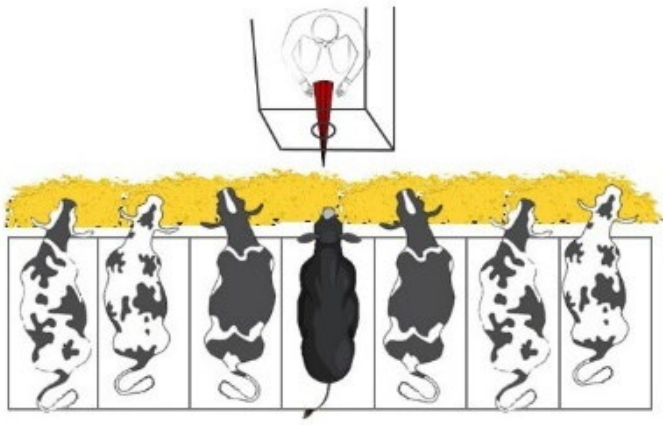
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Description/objective

The Sudden Object Test evaluates animals' behavioural reactions to sudden and unexpected stimulus presented within their environments. It is designed to assess startle responses, approach–avoidance tendencies, and recovery following the sudden stimuli. This test is adaptable to various environments and all age classes. The test is designed for both individual and group testing. *The test procedure described in this SOP is performed in a tie-stall environment, using either one of two object presentation methods on either a single animal or multiple animals simultaneously.*

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Terminology

Object presentation methods:	
Pulley system:	System in which, for each animal, the object is tied to a rope that passes through ceiling hooks from the feeding area of the test animal to the feeding area of the animal in the row behind (Figure 1). As such, the object handler remains out of sight from the test animal. The pulley system must be installed at least a day before the test day. This system can be installed for as many animals as needed. Therefore, multiple animals can be tested simultaneously.  **Figure 1.** An illustration of the stalls from a side view featuring the rope and pulley system. This method allows one or more objects to be lowered simultaneously, facilitating tests on multiple animals within a single blocked test period.
Object placement:	System in which the object and the testing assistant are hidden behind a shield in front of the animal before the test begins. The object suddenly appears in front of the animal from a whole in the shield (Figure 2).  **Figure 2.** In this system the testing assistant suddenly opens an umbrella through the whole in the shield.
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 animal's head or body. • *Object pulled up*: The object should be familiar to the animal (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 in the animal's view it should be familiar to the animal (i.e., present during familiarization period). 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 animal is not motivated to interact with it prior to the test.

Requirements

✓ **Personnel (n=2-4):**

Personnel	Number	Role
Testing assistant(s)	1-2	Responsible for the sudden object introduction and handling 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

✓ **Materials Needed:**

- Personal Protective Equipment (PPE)
- Camera(s)
- Power source for camera(s) (i.e., extension cords, power bank with AC outlet)
- Sudden Object(s) with or without shield(s)
- Stopwatch

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- Whiteboard
- Dry erased marker

General information

- **Location:** Tie-stall environment with a minimum distance of 200 cm between the manger wall and the wall in front of the stall.

- **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 are strictly prohibited.

- **Camera positioning:** Camera should be positioned to cover where the object will appear and the animals' head and body.

- **Animal preparation**: To avoid confounding effects, animals need to be familiar with equipment not usually in their environment (i.e., cameras, extension cords, etc.). Test animal(s) and their neighbours must be standing for this test to reduce the risk of injury, and they must be standing for a minimum of 1 minute before the test to ensure they are calm, balanced and have completed any elimination behaviours (e.g., defecation and/or urination).

- **Test order preparation**: Whether testing an individual animal or concurrent animals, do not immediately proceed to test any animals that were adjacent to or facing the subjects during their test. When testing concurrent animals, animals selected must not be immediate neighbours; animals should be separated by at least two animals. These neighboring animals must be given at least 30 minutes of recovery time to allow them to return to a neutral state after possible agitation. To maximize efficiency, a predetermined testing order should be established that skips these immediate neighbours. Testing should be conducted during a neutral period and not immediately after feeding or other activities that may influence animal behaviour.

Procedure

1. The camera managers set up cameras to capture selected test animals at least 30 minutes before the test to allow the animals to familiarize with the new equipment and verify that the field of view for each camera is correctly aligned to ensure full visibility of the selected animal' head and body, and the location where the object will land.

2. The testing assistants set up object(s) according to the chosen object presentation methods (i.e., pulley system or object placement; defined in the terminology section).

- **If using the pulley system method:** testing assistants attach the sudden object(s) to the rope at least 30 minutes before testing begins. The object(s) should be hanging above the stall, out of view of animal(s) being tested.

- **If using the object placement method:** a testing assistant installs the shield in front of the test animal(s) at least 30 minutes before the test begins and places the sudden object(s) through a hole in the shield.

3. Close to the start of the test, testing assistants ensure that the focal animal(s) is/are standing.

4. Close to the start of the test, a camera manager writes on the whiteboard the information that will allow to identify in the video the animal(s) being tested, the test being conducted, the date and the time. If testing multiple animals simultaneously, this step must be repeated for all animals.

5. When the familiarization period has elapse, a camera manager starts the recording for all cameras and displays the whiteboard in the animal's respective camera views. Once all cameras are set to record and whiteboard information displayed, the camera managers retreat from the testing area and signal a testing assistant to begin a 2-minute timer before the test begins.

6. During these 2 minutes, testing assistants prepare for lowering the object(s) with the pulley system or position itself behind the shield with the object picking out of the hole ready for sudden movement.

- **If focal or neighboring animals lie down during the 2-minute timer**, test assistants must gently encourage the animal to stand again and then restart a 2-minute timer once the animal(s) is/are standing.

7. After the 2-minute wait period, a testing assistant starts the test with a 1.5-minute period to record normal behaviours before the sudden event.

- **If, focal or neighboring animals lie down during this time,** testing assistants must again encourage them to stand. Once test assistants are out of camera view or back behind the shield, both the 2-minute and 1.5-minute wait times are restarted.

NOTE: If an animal lies down at any point before step 8, steps 6 and 7 need to be repeated for each animal that lies down.

8. After the full 1.5-minute have elapsed,

- **If using the pulley system method:** testing assistant(s) wait on the signal from the camera manager to let go of the rope(s) so that the object quickly drops in front of the animal. The animal must be looking in the direction of the object.

- **If using the object placement method:** testing assistant(s) look at the animal through the hole in the shield to ensure the test animal is looking at the object before suddenly moving it (in this example, opening of the umbrella). Confirm the animal is facing forward before the sudden movement—if she is grooming or has her head turned backward, wait until she faces forward before proceeding.

9. Once the object(s) have dropped or moved suddenly, a camera manager starts a 1-minute timer.

10. After the 1-minute test period has elapsed, the test is concluded. The camera managers stop all recordings. Testing assistant(s) then remove the sudden object(s) and the shield(s) if using the object placement method or detach the object(s) from the rope and retract the rope until the hook is positioned near the ceiling—out of the animal's reach and rope of sight.

11. Repeat this procedure until you have tested all selected subjects.

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. Gutter covers can be used as an additional safety measure to prevent injury from animals that may step back into the gutter when reacting to the object.

AI ASSISTANCE DISCLOSURE

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

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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.