



Standard Operating Procedure **Novel Object Test (NOT) – Tie Stall**

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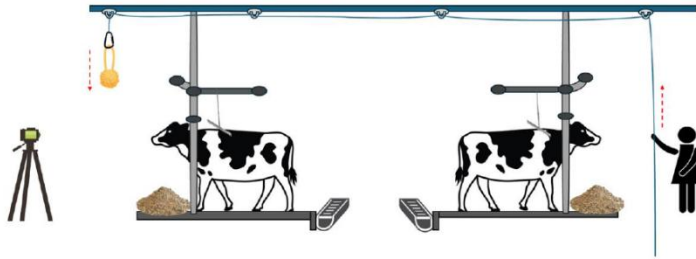
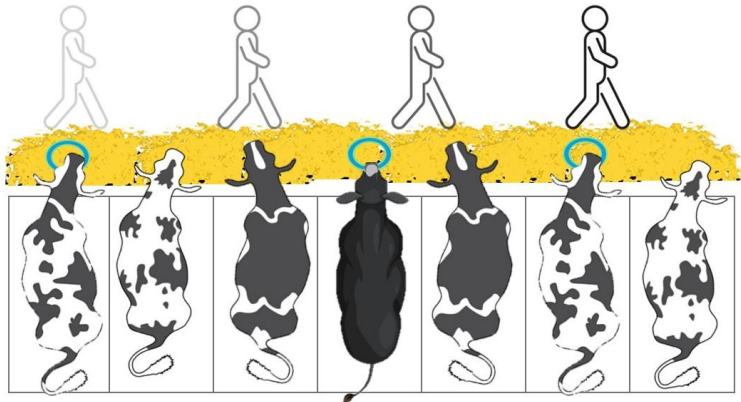
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Description/objective

The Novel Object Test measures animals' behavioural reactions to an unfamiliar object. It is designed to assess curiosity, exploration, and fear-related responses toward novel stimuli. This test can be performed in different environments and is designed to test a single animal or multiple animals in one testing session. It is adaptable to all age classes. *The test procedure described in this SOP is performed in a tie-stall environment, using either one of two different object presentation methods on either a single animal or multiple animals simultaneously.*

Terminology

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Object presentation methods:	
Pulley system:	System in which, for each animal, the novel 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 at the same time.  Figure 1. An illustration of the stalls from a side view featuring the rope and pulley system. This method enabled each testing assistant to simultaneously lower one or more objects, facilitating tests on multiple animals within a single blocked test period.
Object placement:	System in which the object is slowly dropped in the feed in front of the animal (Figure 2). Multiple animals can be tested at the same time.  Figure 2. The testing assistant walks with steady pace and places the novel object(s) in front of the animal(s)
Object:	
Novel Object:	In general, the novel object must be unfamiliar to the animal. If the test is repeated, the same object must not be reused to keep the novelty. However, all objects should be comparable in size, weight, and texture, and should offer similar opportunities for manipulation, even if they differ in shape or colour.

	Note that for the <u>pulley System</u> , the object must be of sufficient weight to descend slowly, ensuring a gradual appearance. At the same time, it must not be so heavy as to pose a risk of injury if it were to fall on the animal.
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Requirements

✓ Personnel (n = 2-4):

Personnel	Number	Role
Testing assistant(s)	1-2	Responsible for the novel objects and wait times. The number of testing assistants required will vary according to the number of animals tested simultaneously.
Camera manager(s)	1-2	Responsible of managing the camera operation (i.e., installing the cameras, validating the views and starting/stopping the recording)

✓ Materials Needed:

- Personal Protective Equipment (PPE)
- Camera(s)
- Power source for camera(s) (i.e., extension cords, power bank with AC outlet)
- Novel Object(s)
- Stopwatch
- 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 perfectly cover the object landing and the animals' head and body.

- **Animal preparation:** To avoid confounding effects, animals should be familiarized with any equipment that is not normally present in their environment (e.g., cameras, extension cords), except for the novel object being tested. When the pulley system is used for multiple animals simultaneously, it is recommended that it be installed at least one day before testing, as the installation process will disturb the animals' environment
- **Test order preparation:** When testing animals individually, a barrier must be installed at least 1 day before testing between adjacent animals to prevent neighboring animals from accessing the novel object. Animals adjacent to or directly facing the tested subject(s), whether during individual or group testing (e.g., half-row or full-row assessments), must not be tested immediately afterward. A minimum 30-minute recovery period must be provided to allow these animals to return to a neutral state and to preserve the novelty of the test stimulus. To improve testing efficiency, a predetermined testing order should be used to avoid sampling these neighboring animals until the recovery period has elapsed. 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's 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 assistant(s) attach the novel 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 gathers the number of objects required for the test and holds them out of view of animal(s) being tested.
3. About 2 minutes before the start of the test, testing assistants ensure that the focal animal(s) are not asleep, otherwise gently wake them up. The animal(s) does/do not need to be standing for the test.
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 and object set up (in the case of using the pulley system method) period has elapsed, a camera manager starts the recording for all cameras and displays the whiteboard in the animal's respective camera views. Once all cameras are recording and the whiteboard information is 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 assistant(s) prepare for lowering the object(s) with the pulley system or position itself for object(s) distribution.
 - **If during the first minute**, a test animal falls asleep (e.g., folds her head back) or appears to be asleep, one testing assistant should approach and gently wake the animal. The 2 minutes timer does not need to be restarted.
 - **If an animal attempts to sleep within the last 30 seconds of the 2 minutes**, one testing assistant should approach and wake the animal. After the testing assistant has left the camera view/testing area, an additional 30 seconds is added to the timer (i.e., in this case objects are distributed after 2 minutes 30 seconds instead of 2 minutes).
7. When the 2 minutes (or 2 minutes and 30 seconds) have elapse,
 - **If using the pulley system method:** testing assistant(s) gradually lower the novel object(s) in front of each animal at a consistent pace, ensuring that all objects land in front of the animals simultaneously, while also making sure that the objects remain as much as possible in the middle of the stall. All objects should be on the ground within 20 seconds.
 - **If using the object placement method:** The testing assistant passes the focal animal(s) at a steady pace (~5 seconds/animal), arching down to slowly release the object into the feed approximately 5 cm from the manger curb (or as close as safely possible) without breaking stride.
8. When all objects have landed on the ground or been distributed to the last animal in the sequence, the testing assistants retreat out of the testing area to minimize potential distraction for the animals, and one testing assistant starts a 5-minute timer. **Note:** In the object placement method, animals enter the test at different times because objects are distributed sequentially. An individual animal's test begins when the testing assistant has distributed the object to the second subsequent animal in the sequence. Therefore, the first animals to receive objects may have started the test before the last animals receive their



objects. The observation period remains 5 minutes for every animal. The start time of the test should be recorded for each animal.

9. After the 5-minute test period has elapsed, the test is concluded and the camera managers stop the camera recording and testing assistants retrieve the novel objects.
10. Repeat this procedure until you have tested all selected subjects. Before testing another animal, the objects must be cleaned.

Safety Note

All personnel should stand with proper distance from test animal(s) 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 back into the gutter when reacting to the object.

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.