Standard Operating Procedure

Open Field Test - Group

Tania Wolfe[1,2], Marjorie Cellier[1,2], Célia Julliot[1,2], Martha Krawiec[1,2], Nadège Aigueperse[2,3], Abdoulaye Baniré Diallo[2,4], Elsa Vasseur[1,2*]

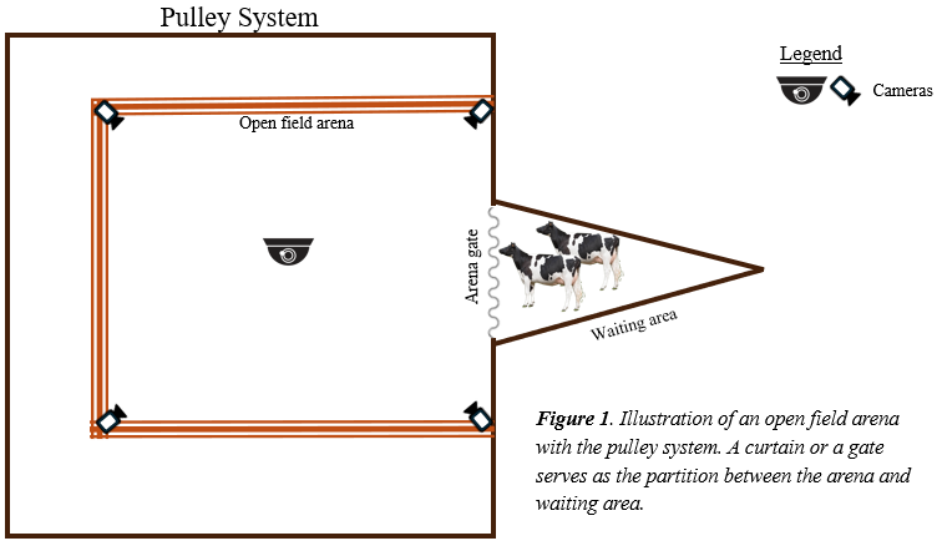
[1] Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, Quebec H9X 3V9, Canada
[2] Research and Innovation Chair in Animal Welfare and Artificial Intelligence (WELL-E), McGill University, Sainte-Anne-de-Bellevue, Quebec H9X 3V9, Canada
[3] INRAE, VetAgro Sup, UMR Herbivores, F-63122 Saint Genes-Champanelle, France
[4] Département d'informatique, UQAM, Montreal, Quebec, H2L 2C4, Canada
*Corresponding author: elsa.vasseur@mcgill.ca

Description/objective

The Open Field Test (OFT) evaluates animals' behavioral responses when placed in an unfamiliar environment. The test measures individual differences in exploratory behavior, activity levels, and responses to social contagion and novelty. This test can be performed in different environments and can be 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 an open field arena on two or more animals.*

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Terminology

Open-field arena	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; these walls must be opaque and may include a colored 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 other animals' housing areas to minimize external disturbances and distractions.  ***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.*

Requirements

✓ **Personnel (n = 4–5):**

Personnel	Number	Role
Testing assistant(s)	1-2	Responsible for time management of the test, open and close the gate and record animals' vocalization during the test.
Camera manager(s)	1	Responsible of managing the camera operation (i.e., installing the cameras, validating the views and starting/stopping the recording).
Trained handler	2	Responsible for handling animals during transfer to and from the open field arena and for placing animals into the arena at the start of testing. Assist with guiding animals into the arena when necessary.

✓ **Materials Needed:**

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

General information

- **Location:** Open field arena.

- **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 themselves 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 animals. 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:** Whenever possible, animals should be habituated to routine handling and to the transport route prior to testing, in order to minimize stress and variability associated with handling and transport. However, animals must not be exposed to the new environment prior to testing so that the novelty of the test environment is preserved.

- **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.

NOTE: For repeated testing, a different arena should be used whenever possible. If this is not feasible, the arena environment should be modified (e.g., with coloured tape, cardboard shapes, or other visual alterations on the walls) to maintain novelty.

Procedure

1. 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.

2. 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.

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

4. After 30 seconds has passed, testing assistant(s) open the gate to allow the animals to enter the arena.

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, it 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 1: Use vocalization (i.e., talking to the animals with regular voice volume) and hand gesture (i.e., soft hand clapping).
   Step 2: Use gentle tactile stimulation (i.e., touching the animals on the rump).
   Step 3: Select one animal from the group and physically prompt the animal to move into the arena (i.e., putting your 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 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 in the arena, a testing assistant closes the gate while another starts a 3-minute timer for the test.

7. After the 3-minute test period has elapsed, the test is concluded, a camera manager stops the recording, and trained handlers walk the animals back to its home environment.

8. Before testing another group, the arena must be cleaned, urine and/or feces must be removed when applicable, and the floor must be dried, and the arena gate must be closed.

9. 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 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).

Project Stage	Term	Individual
All Stages (or partial, dependent)	Supervision	Elsa Vasseur Nadège Aigueperse Marjorie Cellier Tania Wolfe
Conceptualization	Conceptualization - Research Area and Project Planning	Elsa Vasseur Abdoulaye B. Diallo Marjorie Cellier Nadège Aigueperse
Conceptualization	Conceptualization and Implementation	Nadège Aigueperse Marjorie Cellier Tania Wolfe Célia Julliot Martha Krawiec
Conceptualization	Methodology	Nadège Aigueperse Marjorie Cellier Tania Wolfe Célia Julliot Martha Krawiec
Conceptualization	Funding Acquisition	Elsa Vasseur Abdoulaye B. Diallo
Analysis	Software	N/A
Analysis	Validation	Nadège Aigueperse Marjorie Cellier Tania Wolfe Célia Julliot Martha Krawiec
Analysis	Formal Analysis	N/A
Writing	Writing -original draft	Shabnaz Mokhtarnazif Tania Wolfe

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Writing	Writing -Review and Editing	Elsa Vasseur Abdoulaye B. Diallo Marjorie Cellier Nadège Aigueperse Shabnaz Mokhtarnazif Tania Wolfe Célia Julliot Martha Krawiec
Writing	Visualization	N/A
Services	Resources	Tania Wolfe
Services	Project Administration	Nadège Aigueperse Marjorie Cellier Célia Julliot Martha Krawiec Tania Wolfe
Services/Training	Investigation	Marjorie Cellier Tania Wolfe Célia Julliot Martha Krawiec Georgiana Amarioarei Shabnaz Mokhtarnazif
Services/Training	Data Curation	N/A

* 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.