Example Calibration Procedure for WMicrotracker According to ISO 17025

Device:

WMicrotracker ONE

Revision Number: Rev 1.0 2024

Device Identification Number: SN#####

Device Description:

The WMicrotracker is a device that utilizes an infrared microbeam system to detect light refraction through the bodies of small animals, in order to record the locomotor activity of worms in real-time in liquid cultures. The equipment features a set of independent infrared detectors that allow simultaneous reading of 384 channels, and plate adapters that enable its use with 6, 24, 96, and 384-well culture plates. The equipment has an algorithm for adjusting electronic signals that self-calibrates internal parameters (LED power and phototransistor gain) according to the degree of optical absorbance of the culture medium each time a new experiment is conducted.

Recommended Testing Sheet:

The following calibration actions are recommended:

Type of Calibration	Recommended Frequency	Estimated Duration
Periodic Internal Check: Measurement Blank Verification	Every day, before starting the assay routine.	5 minutes
Periodic Internal Check: General Electrical Testing of the Device (TEST Device) and Proper Alignment of Microbeams	Every day, before starting the assay routine.	5 minutes
Internal Control for All Assays: Inclusion of Blank, Control, and 2 Standard Drugs.	Internal controls to include in all assays.	N/S
Calibration: Quarterly Check of Homogeneity and Sensitivity.	Quarterly or semi-annually, depending on usage.	1 hour + preparation of previous biological material.
Preventive Maintenance: Preventive Adjustment and Cleaning.	Quarterly or semi-annually, depending on usage.	15 minutes

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<u>Periodic Internal Check:</u> Measurement Blank Verification

Objective:

To verify that there are no exogenous sources or internal defects generating noise in the measurement.

Estimated Test Duration:

5 minutes

Procedure:

It is recommended to perform the electrical testing verification periodically, preferably on the day before starting the assay routine. For this measurement, the procedure detailed in Annex I should be followed. In summary, this assay is conducted using a 96-well plate (format according to the laboratory-defined standard) containing liquid medium, without animals. The plate is measured for 5 minutes.

Acceptance Criteria:

It is verified that the detected activity is 0 (maximum accepted threshold = 1) in all measured wells.

Contingency Plan for Failure:

In case of assay failure, verify possible sources of noise according to the details outlined in Annex I.

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<u>Periodic Internal Check:</u> General Electrical Testing of the Device (TEST Device) and Proper Alignment of Microbeams

Objective:

To verify that the detectors are functioning correctly electrically, and that the microbeam template is properly aligned with respect to the tray and adapter.

Estimated Test Duration:

5 minutes

Procedure:

It is recommended to perform the electrical testing verification periodically, preferably on the day before starting the assay routine. For this measurement, the procedure detailed in Annex II should be followed. In summary, this assay is conducted using the 96-well adapter (according to the U or Flat bottom format set up in the user laboratory), with the microperforated alignment guide plate. The 96-well plate is not placed. The "TEST Device" assay algorithm will perform a sequential testing protocol of all the WMicrotracker beams, in row and column patterns, emitting a PASSED or NOT PASSED report at the end.

Acceptance Criteria:

Test Report = PASSED

Contingency Plan for Failure:

In case of assay failure, repeat the assay to confirm the failure, and perform preventive maintenance.

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<u>Internal Control for All Assays:</u> Inclusion of Blank, Control, and 2 Standard Drugs

Objective:

To include an internal control in the assays that verifies the expected consistency of the data obtained with the WMicrotracker, and also serves as a biological control.

Procedure:

Included in the plate setup are: 2 wells containing only buffer, 2 wells containing untreated worms, 2 wells containing drug 1 ##uM, and 2 wells containing drug 2 ##uM. A guide to recommended drugs for assays of *Haemonchus contortus* can be found in Annex III.

Acceptance Criteria:

The control activity must be within the accepted range for a normal assay (predefined by the assay laboratory under its standard breeding, cultivation, and temperature conditions). Drug 1 and 2 should show a decrease within the expected range for the tested organism.

Contingency Plan for Failure:

The experiment is considered invalid and must be repeated. The condition of the biological material, environmental conditions, and preparation of drugs and solutions must be checked.

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Calibration: Quarterly Check of Homogeneity and Sensitivity

Objective:

To perform a check of the homogeneity of all equipment sensors and to verify detection sensitivity using a microorganism curve.

Procedure:

Calibration of the WMicrotracker is performed quarterly according to the laboratory's internal calibration procedure described in Annex IV. For this assay, a standard solution of nematodes is used (to be defined in the assay laboratory, whether C.elegans or haemonchus spp, and at what stage) to calibrate the accuracy and sensitivity of the infrared microbeam detection system. Additionally, the proper functioning of the data analysis software is verified.

Acceptance Criteria:

Measurement deviation +- 25% (see annex for explanation of calculation). Linear detection range between 10 and 50 worms/well. Absolute number of activity counts within the expected range.

Contingency Plan for Failure:

Verify the condition of the nematode plate under the microscope to qualitatively assess vitality and pipetting. Repeat the experiment with another batch of worms to verify the failure. If the deviation is between 25% and 45%, generate the calibration pattern file and recalculate. If it is higher, contact the manufacturer.

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<u>Preventive Maintenance:</u> Preventive Adjustment and Cleaning

Objective:

To clean the optical system and verify the adjustments of moving and fixing mechanical parts.

Procedure:

The preventive maintenance of the WMicrotracker is performed quarterly according to the laboratory's internal maintenance procedure described in Annex V. During preventive maintenance, the optical system is cleaned using aspiration, and the correct closure of the tray, the plugs, and the correct leveling of the equipment on the workbench are verified. After preventive maintenance, it is recommended to perform testing for:

- Measurement Blank Verification
- General Electrical Testing of the Device (TEST Device) and Proper Alignment of Microbeams
- Homogeneity and Sensitivity

Acceptance Criteria:

Checklist of preventive maintenance and OK of subsequent tests.

Contingency Plan for Failure:

Repeat the cleaning procedure. Contact the vendor's technical support for assistance in advanced mechanical adjustment.

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Maintenance and Calibration Record:

All maintenance and calibration activities are recorded in a specific maintenance and calibration log form for each equipment. The records should include the date of the activity, responsible personnel, the procedure used, and the results of the activity. These records are kept in a maintenance and calibration log file and should be available for review by internal and external audit personnel.

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ANNEX I: Measurement Blank Verification

To be performed before starting the assay routine of the day.

Procedure:

1. Use a 96-well plate (bottom format according to the laboratory-defined standard).
2. Pipette 100 µl of liquid medium (water, buffer), without animals.
3. Acquire the plate for 5 minutes in Mode 1_Threshold Avrg (Default.Agar&Liquid) - Threshold 1.8.
4. Generate the data report.

Acceptance Criteria:

Verify that the detected activity is 0 (maximum accepted threshold = 1) in all measured wells.

Contingency Plan for Failure:

In case of assay failure, verify possible sources of noise:

- Vibrations on the bench where the equipment is located.
- Exposure of the equipment to light rays.
- Defective power supply.

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ANNEX II: General Electrical Testing of the Device (TEST Device) and Proper Alignment of Microbeams

To perform the general electrical testing of the device, follow these steps:

1. Place the corresponding adapter and template on the equipment (w96F or w96U), without a plate.
2. Go to File/Test Device to initiate the examination.
3. Once the testing is completed, a report with the obtained results will be automatically generated.

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ANNEX III. Internal Control for All Assays: Inclusion of Blank, Control, and 2 Standard Drugs.

For each plate to be analyzed, include the following controls in duplicate:

- **Blank:** Medium only without nematodes.
- **Negative Control:** Nematodes in the medium used + x% DMSO (final concentration in drug-containing wells).
- **Positive Control:** Add at least two drugs known to have an effect on nematode activity. It is important to ensure that the selected drugs are appropriate for the type of nematode being evaluated and used at the correct concentrations.

Example of drugs that can be used as positive control in experiments with Haemonchus contortus: (Final concentration of drugs in the well 20 µM.)

- Monepantel (Zolvix; Elanco, Greenfield, IN, USA).
- Moxidectin (Cydectin; Virbac, Carros, France)

The procedure according to Taki et al: Pharmaceuticals 2021, 14(7), 616; https://doi.org/10.3390/ph14070616 recommends Monepantel 20uM to obtain a positive control with 50% activity, and Moxidectin 20uM to obtain a positive control with 20% activity.

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ANNEX IV: Calibration: Quarterly or Semi-Annual Check of Homogeneity and Sensitivity

Activity Detection Calibration:

- *For these tests, a standard solution of nematodes will be used (to be defined in the assay laboratory, whether C.elegans or Haemonchus spp, and at what stage).*
- *These protocols should be carried out in an air-conditioned room.*
- *All buffers should be at room temperature.*

Protocols using N2 Young Adult Worms in the tests.

A) HOMOGENEITY RECORD OF WELLS/CHANNELS_w96F:

Materials Needed:

- *2 NGM plates (10 cm) with synchronized adult worms.*
- *15 mL tube.*
- *96-well flat-bottom microplate.*
- *WMicrotracker.*
- *p20 / p1000 pipette.*
- *Multichannel pipette.*
- *Multichannel pipette reservoir.*
- *Tips.*

Protocol:

1. *Collect young adult worms from 2 plates using M9 buffer and transfer them to a sterile 15 mL tube.*
2. *Let the worms settle for approximately 3 minutes (adult worms will fall by gravity to the bottom of the tube).*
3. *Discard the supernatant using a P1000 pipette, being careful not to disturb the pellet.*
4. *Perform a wash with 5 mL of M9 buffer. Shake or briefly invert the tube.*
5. *Repeat the decantation step. Discard the supernatant and add 3 mL of M9 buffer.*

6. *Homogenize the suspension by shaking the tube by hand 3 times. Count the number of worms in 10 µl of solution. Repeat three times and calculate the average number of worms/10 µl.*
7. *Prepare a suspension to obtain [5 worms/10 µl]. Adjust the volume in M9 buffer supplemented with 1% OP50.*
8. *Transfer the worm suspension to the multichannel pipette reservoir.*
9. *Transfer 90 µl of worm solution to the 96-well microplate with a multichannel pipette, considering the pipetting recommendations (see appendix).*
10. *Before reading the plate, gently homogenize the worm population by shaking by hand (rotating the microplate on the bench) for 5 seconds.*
11. *Record the activity of the worms using WMicrotracker for 10 minutes.*
12. *Repeat the gentle plate homogenization by hand and then, rotate the plate so that well A1 is now on the lower right side of the equipment (inverted plate) and acquire the locomotor activity of the worms for another 10 minutes.*

Analysis:

13. *Generate the report for both acquisitions.*
14. *Calculate the average activity obtained for each well and channel in both acquisitions (right-side up and inverted plate).*

Acceptance Criteria:

- *Wells: The deviation between the maximum and minimum activity of the wells must be less than 25% of the average activity of the wells.*
- *Channels: The deviation between the maximum and minimum activity of the channels must be less than 35% of the average activity of the channels.*

Suggestions to Consider for Nematode Solution Pipetting:

- *Add an excess volume of 25% in the pipette reservoir.*
- *In the first pipetting, wash the pipette tip by pipetting the content up and down in the C. elegans culture at least four times.*
- *Agitate the pipette reservoir by gently rotating it before and during pipetting, using your free hand.*
- *Maintain the pipetting level a few millimeters from the bottom of the reservoir each time.*

- *Visually check for bubbles or empty tips present each time.*

B) WORM CURVE:

Materials Needed:

- *1 NGM plate (10 cm) with synchronized adult worms.*
- *15 mL tube.*
- *96-well flat-bottom microplate.*
- *WMicrotracker.*
- *p20/ p200 /p1000 pipette.*
- *Tips.*
- *Eppendorf.*
- *M9 Buffer + 0.05% BSA.*

Protocol:

1. *Collect young adult worms from the plate using M9 buffer and transfer them to a sterile 15 mL tube.*
2. *Let the worms settle for approximately 3 minutes (adult worms will fall by gravity to the bottom of the tube).*
3. *Discard the supernatant using a P1000 pipette, being careful not to disturb the pellet.*
4. *Perform a wash with 3 ml of M9 buffer. Shake or briefly invert the tube.*
5. *Repeat the decantation step. Discard the supernatant and add 2 ml of M9 buffer.*
6. *Homogenize the suspension by shaking the tube by hand 3 times. Count the number of worms in 10 µl of solution. Repeat three times and calculate the average number of worms/10 µl.*
7. *Prepare a suspension to obtain [10 worms/10 µl]. Adjust the volume in M9 buffer supplemented with 0.05% BSA.*
8. *Perform dilutions of the worm solution as indicated in the scheme:*

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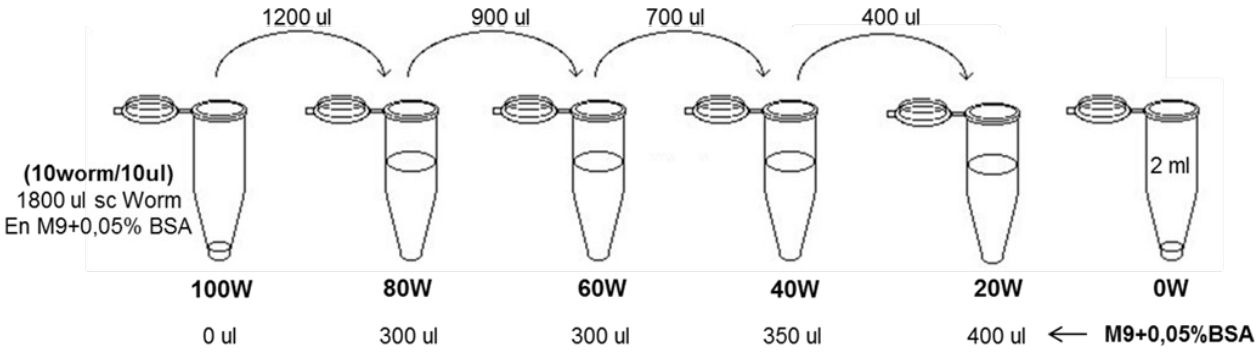

9. *Transfer 100 µl from each tube containing a different number of worms to a 96-well flat-bottom plate, as indicated in the scheme (in quadruplicate for each condition).*

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	0W	20W	40W	60W	80W	100W						
F												
G												
H												

10. *Record for 20 minutes using Mode 1_ Threshold Avrg (Default.Agar&Liquid) - Threshold 1.8*

Analysis:

11. *Generate the activity report.*
12. *Count the actual number of worms in each well and calculate the average number of worms per condition.*
13. *Plot worm activity versus the actual average number of worms per well on a scatter plot.*
14. *Evaluate the fit to a line of activity between 20 and 80 worms.*

Acceptance Criteria:

- *The fit of the line to the points should have an R2 greater than 0.9.*

Internal Temperature Probe Calibration

Materials Needed:

- *Calibrated reference thermometer.*

Procedure:

1. *Place the thermometer inside the equipment.*
2. *After 10 minutes, perform an acquisition on the equipment.*
3. *Record the temperature indicated by the software and the temperature of the thermometer.*

Acceptance Criteria:

The temperature difference between the temperature indicated by the device's temperature probe and the reference thermometer should not exceed 1°C.

Contingency Plan:

Recalibrate the temperature probe:

1. *Go to File/Advanced/Factory Settings.*
2. *Enter the temperature indicated by the reference thermometer in the Calibration T* box.*
3. *Click on SET.*

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<u>ANNEX V: Preventive Maintenance: Preventive Adjustment and Cleaning</u>

1. *Cleaning of the Optical System*

Materials Needed:

- *Mini vacuum cleaner Brigii Y120 Pro, rechargeable USB vacuum cleaner and pump.*
- *Adapter with 6mm diameter suction tube.*

Procedure:

Using a mini vacuum cleaner like Bridgii and the adapter tube, proceed to go over the microperforated surface, vacuuming any solid particles that may have accumulated on it.

2. *Verification of Proper Mechanical Door Closure*

Materials Needed:

- *Microperforated template aligner (for 96Flat or U-bottom well according to the assay).*

Procedure:

Verify that the door adjustment is adequate by aligning the microperforated template with the microbeams, using the grid generated by the provided proprietary software. If it is detected that the tray does not slide smoothly, lubricate the tray guide with Vaseline grease (similar to Aceitex Super Grease Vaseline).

Verification of the Condition of Casters and Equipment Level

Materials Needed:

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Bubble or air level.

Procedure:

1. *Verify that all 4 lower casters are in good condition.*
2. *Check the horizontality of the smooth plane where the equipment's casters rest using a bubble or air level.*
3. *Repeat the operation on the upper casing of the equipment.*
4. *Both should be correct.*

After preventive maintenance, functionality tests are performed according to the recommended periodic checks.

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