
	<p style="text-align: center;"><i>Standard Operating Procedure</i></p> 	Effective Date: 20 June 2013	Version: 002
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Standard Operation Procedure – Osmolality

1. Scope and Application

The Osmomat 3000 device is a non-invasive in-vitro (in an artificial environment rather than inside a living organism, e.g. in a test tube) diagnostic product used to determine the osmolality of aqueous solutions.

- Only use the device to measure aqueous solutions
- Never measure organic, saturated, or highly viscous solutions
- Only use accessories and consumable supplied by Gonotec for measurements.

2. Summary

Osmolality

- The device measures the total osmolality of any aqueous solution. The total osmolality indicates the concentration of all osmotically active substances (such as salts, dextrose, proteins) per kilogram of water.

The osmolality is specified in mOsmol/kg.

- The device determines the total osmolality of the sample solution based on the freezing point depression.
- Measure and compare the freezing point of distilled water and an aqueous solution are measured and compared. The osmolality of any solution is determined using a linear function by 2 or 3 point calibration.

Freezing point depression

- The freezing point of a solution is depressed by adding soluble or mixable substances.
- The freezing points of pure water and a solution are measured and compared. Whereas water has a freezing point of 0 °C, a solution with saline concentration of 1osmol/kg has a freezing point of -1.858°C. That means that one mol of a given non-dissociated substance (6.023×10^{23} parts diluted in one kilogram of water) lowers the freezing point of a solution by 1.858 °C.
- The following definitions are used in calculating osmolality:

$C_{\text{osm}} = \Delta T / K$	C_{osm} =osmolality[osmol/kg] T =freezing point depression[°C] K =1.858 °C kg/osmol freezing point constant
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3. Apparatus and Glassware

- Gonotec Osmomat 3000
- Touchscreen
- Upper cooling system(behind movable elevator cover)
- Thermistor probe with measuring vessel
- Lower cooling system
- Elevator
- Printer



- 50 ml glass beakers
- Testing vials

4. Interferences

- Never leave bottles of Standard solution uncapped, as prolonged exposure to the atmosphere will affect the solution's concentration.

5. Collection, Preservation and Storage

- Collect faecal samples in 1L plastic buckets.
- Preferably, analyse samples immediately after sampling.
- Store samples at 4 °C or freeze dry samples.
- Preserve wastewater samples by acidifying with concentrated sulphuric acid to pH 2 and faecal samples by freeze drying or freezing.
- Reclose the reagent bottles immediately after use.

6. Safety Precautions

- Handle concentrated acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed. After the analysis, clean bottles and beakers with clear water keep it for drying.
- Dispose the used gloves after completion of analysis.
- Clean the hands using antiseptic soap.
- Disinfect hands after washing with soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

7. Sample Preparation – Faecal Sludge

1. Weigh out 2.0000g of well-mixed faecal sludge sample.
2. Blend the weighed sample with 500ml of distilled water in a 1L blender for 30 seconds on the highest speed.
3. Add 250ml distilled water and blend on highest speed until the sample is homogenised (this could range from 30 to 60 seconds).
4. Transfer the blended mixture into a 1L volumetric flask.
5. Add 200ml of blender washings into the flask and top up to 1L with distilled water.
6. Transfer the 1L solution to a plastic bottle and store at 4 °C.

8. Reagents

- Soft paper towel
- Micropipette(50uL)
- Gonotec measuring vessel
- Filtered/diluted sample.

9. Calibration

The device is calibrated using a 3 point calibration method (using distilled water and 2 calibration standards).

- Clean thermistor probe using a soft wet the dry paper towel.
- Follow the instructions on the touch screen.
- Pipette 50ul distilled water into a cleaned measuring vessel.
- Click the measuring vessel on the thermistor probe with cove facing front.
- Slide elevator down with both hands. Zero point calibration starts automatically. Pat attention to the display on the screen.
- Move elevator up with both hands once the first measurement is complete.
- Slide the elevator up with both hands.
- Remove measuring vessel from thermistor probe.

- Dispose the measuring vessel and sample into a waste beaker.
- Clean the thermistor probe with a wet then dry soft paper towel. Failure to clean the thermistor probe immediately after measurement could result in carryover and incorrect measurement results.
- Following successful zero point measurement,press"1.Standard to start calibration using the first calibration standard.
- Continue the calibration with Standard 2 then start the sample measurements.

10. Procedure

1. **Press 'measure'** the measurement menu opens.
2. Pipette a sample volume of 50ul into an unused and clean measurement vessel (do not reuse vessels).
3. The sample must be pipetted without any air bubbles.
4. Click the measuring vessel on the thermistor probe with the cover facing front.
5. Press 'single sample' and enter the sample ID using the virtual key.
6. Slide the elevator down using both hands.
7. The measurement result then displays on the touchscreen.
8. Slide the elevator up with both hands.
9. Remove measuring vessel from thermistor probe.
10. Dispose the measuring vessel and sample into a waste beaker.
11. Clean the thermistor probe with a wet then dry soft paper towel. Failure to clean the thermistor probe immediately after measurement could result in carryover and incorrect measurement results.

Shut down

1. **Power down the device using the on/off switch on the rear side.**
2. **Click a measuring vessel on to the thermistor probe.**
3. **Wipe device using a moistened paper towel and cover with protective sleeve.**

11. Waste Disposal

- Flush down the sluice with excess water.

12. Data Quality

13. References

<http://www.gonotec.com/products/osmomat-3000>

APPROVAL OF STANDARD OPERATING PROCEDURE

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