pH Validation Dish
User manual

Table of contents

1 Introduction

This user manual describes the usage of the pH Validation Dish.

1.1 Intended use

The pH Validation Dish is used to equilibrate medium samples inside incubators before performing pH measurements.

1.2 Description of product

The silicone lid of the pH Validation Dish is attached to a non-sterile dish. The dish carries a MEA-tested label, which designates the dish as a “pH Validation Dish”. The silicone lid is designed to allow pH equilibration of a bicarbonate buffer system in a carbon dioxide incubator in less than eight hours. The lid limits evaporation (< 5% in 24 hours), even when the dish is incubated in a dry incubator, and it forms a watertight seal to prevent leakage during handling. The pH Validation Dish is non-embryotoxic.

1.3 Composition

The flexible lid is composed of silicone which complies with FDA 21 CFR 177.2600. The dish is made of an optical grade polystyrene, which complies with FDA 21 CFR 177.1640. The entire pH Validation Dish product comprised of the lid, dish and attached label has been tested for embryo toxicity using a 1-cell Mouse Embryo Assay. The result of the test was at least 80% expanded blastocysts after 96 hrs in an enclosed environment. (see also Certificate of Analysis)

2 Important restrictions and warnings

The following restrictions and warnings shall ensure the correct use of the pH Validation Dish by qualified clinic personnel.

All users must therefore agree to read and understand this user manual, observe the restrictions regarding use and read the following warnings.

WARNING
• Do NOT use the pH validation dish for incubating embryos. The dish is non-sterile and no images are acquired from it if used in a time-lapse incubator.

WARNING
• The pH value must be measured directly in the medium sample immediately after equilibration. Transferring the medium to another container will cause degassing and increase the pH value. • The pH Validation Dish must be turned briefly upside down immediately after removing it from the incubator to allow condensation droplets to re-equilibrate with the remaining media. After this procedure the pH measurements will be correct and match the pH value in culture dishes. • Measurements made without first turning the dish upside down will NOT represent the pH in oil covered culture dishes in the same incubator.

WARNING
• When removing a medium sample for pH measurement, carefully minimise exposure to ambient air and compensate for any degassing that might have occurred. It is recommended to always leave some medium in the dish to avoid the formation of bubbles during pipetting. • The lid must be affixed correctly during equilibration and subsequent handling. If leakage is observed then discard the pH validation dish and all related measurements.

WARNING
• The pH Validation Dish is intended for single use only and may NOT be re-used. Any attempt by the user at cleaning the dish may result in contamination with microorganisms or other risks of device failure such as leakage of medium or excessive evaporation if the sealing is compromised.

WARNING
• To avoid contamination with microorganisms, always place the pH Validation Dish in a sterile laminar flow hood while handling it.

3 Preparing the pH Validation Dish

Follow the below instructions to prepare a pH validation dish:

1. Remove the pH Validation Dish from the pouch.
2. Remove the silicone lid.

3. Fill the dish with 3.5 mL of culture medium.
4. Place the silicone lid on the dish and press the lid firmly into place.
5. Verify that the lid is closed correctly by pressing firmly down along the edge and ensure a smooth planar surface.
6. Insert the pH validation dish in an incubator and leave it to equilibrate for at least 8 hours and no more than 24 hours.

4 Performing pH measurements

When the equilibration is completed, the pH value should be measured directly in the medium sample contained within the pH Validation Dish.

General pH measurement procedure:

1. Remove the pH validation dish from the incubator.
2. Ensure that the lid is still correctly affixed by pressing firmly along the edges of the lid.
3. Immediately turn the pH validation dish briefly upside down.
4. Keep the lid on until the pH measurement is ready to be performed.
5. Remove the silicone lid and immediately measure the pH value directly in the pH validation dish. Insert a pH sensor into the medium or withdraw a sample for analysis without exposing it to degassing. Do not remove the lid until right before starting the measurement.

It is important to turn the dish briefly upside down as this will allow condensation droplets to re-equilibrate with remaining media for correct pH measurement.

It is important to measure the pH value as quickly as possible after removing the pH validation dish from the incubator (i.e. within less than 5 minutes). This time limit must be observed as carbon dioxide is lost to the atmosphere and the bicarbonate buffer system adjusts accordingly. The resulting pH increase begins immediately after removing the dish from the incubator and is greatly accelerated when the silicone lid is removed.

When turning the pH validation dish upside down it is important to watch for any signs of leakage. If leakage is observed then discard the pH validation dish and clean up any spill immediately to avoid any microbial growth. All measurements made in a leaking pH validation dish must likewise be discarded and the measurement repeated.

4.1 Measuring pH directly in medium sample

The pH value can be measured directly in the equilibrated medium sample by using a suitable pH electrode, provided that the sensor can be fully submerged in the medium sample.

It is important that both the sensing surface (usually pH glass) and the reference junction (usually a ceramic junction) are both fully submerged in the medium. The maximum immersion depth is 5-7 mm, depending on where the pH sensor is submerged in the pH Validation Dish.

Standard pH electrodes often require a minimal submersion depth of 15 mm or more. These can thus not be used. Instead, pH sensors that allow measurement in smaller volumes must be used. These sensors are often called mini-sensors or micro-sensors and are available from various vendors.

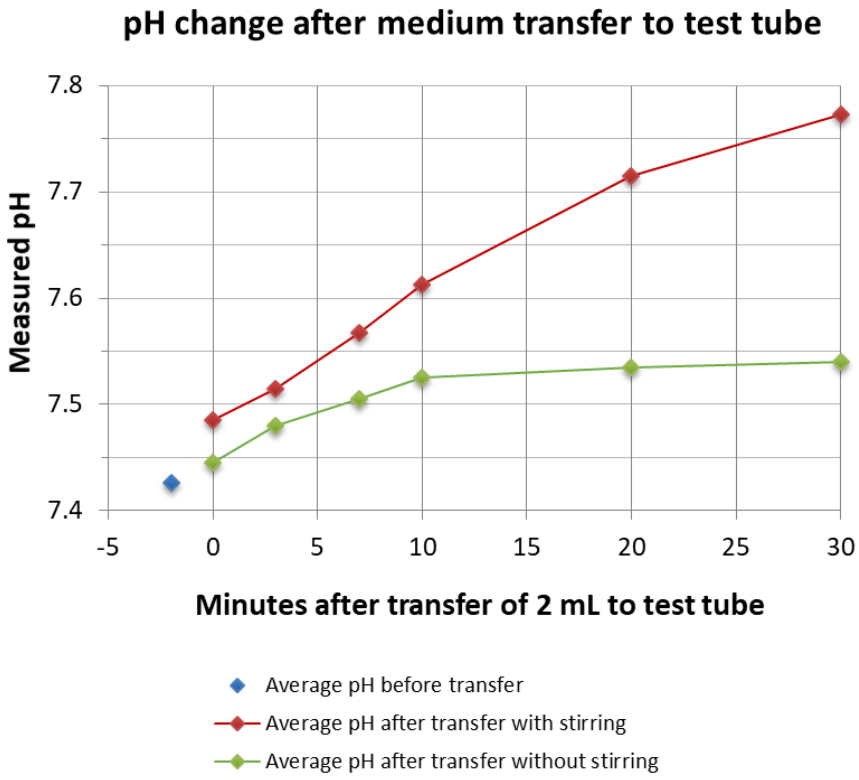
4.2 Withdrawing a sample for analysis

When removing a sample for external analysis by a blood analyser (e.g. the iSTAT handheld blood analyser by Abbott or a similar device), it is important to minimise degassing and resulting pH changes. This can be accomplished by using a glass or stainless steel syringe (e.g. a Hamilton syringe, Sigma Aldrich) or a glass Pasteur pipette. Alternatively, polymer pipette tips may be used (e.g. Eppendorf pipettes). However, potential degassing of CO_2 into the polymer pipette tip should be considered, and it is thus important to work quickly and efficiently. The sample should be contained at all times and analysed as quickly as possible.

It is important to avoid bubble formation and reduce degassing during handling by removing the sample in a single step and leaving most of the medium in the dish. It is recommended that the withdrawn sample is less than 0.5 mL. However, up to 2.5 mL may safely be removed from the pH Validation Dish, if necessary.

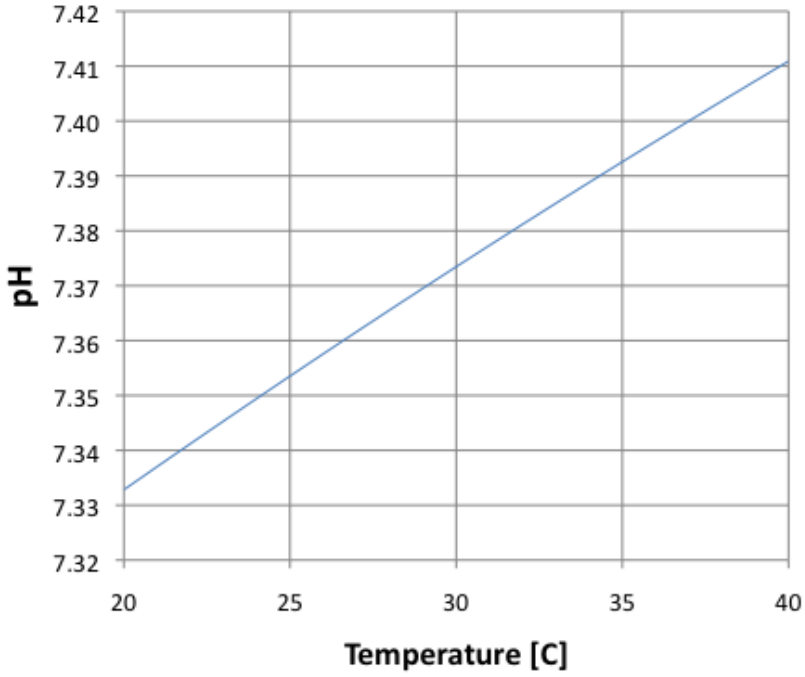
4.3 pH change after transferring medium to another container

We do NOT recommend to transfer the medium to another container such as a test tube for subsequent pH measurement. If the medium is transferred, significant changes in the pH value are likely to occur due to degassing. During the manufacturer's test, even a careful transfer procedure with attempts to minimise exposure and avoid bubble formation gave rise to a pH increase of 0.04 to 0.08, followed by a gradual pH increase by 0.1 per 10 minutes when the medium remained in the test tube.



4.4 Compensating for temperature changes

The equilibrium constants of the bicarbonate system are temperature dependent. Removing a closed container with medium from the incubator and reducing the temperature of the container will reduce the internal pH value (whereas any degassing of CO_2 in an open container will increase the pH value). The figure below shows the moderate effect of reducing the medium temperature to room temperature while maintaining 5% CO_2. Cooling the medium from 37°C to room temperature will decrease the pH value by ≈ 0.05. It is important to compensate for this to determine the actual pH value to which an embryo is exposed during an incubation process at 37°C.



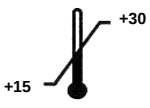
Make sure to compensate for any change in the pH value if the medium cools down from 37°C to room temperature during the measurement process.

Most blood gas analysers will automatically provide the equivalent pH value at 37°C and thus compensate for any cooling which may have occurred during handling and measurement.

Consult the manual for your pH measurement instrument to determine whether your instrument performs this type of correction.

5 Symbols and labels

5.1 Product information labels

Label	Description	Note
	Vitrolife catalogue number. The number 16452 specifies that this is the pH Validation Dish	-
	The product is not sterile	-
	Manufacturer name and logo: Vitrolife A/S Jens Juuls Vej 16, 8260 Viby J Denmark +45 7221 7900	-
	Use by date in the format YYYY (Year)-MM (Month)-DD (Day)	-
	Do not reuse	-
 E1-P8-YYMM-Batch	Batch code for the individual production lot. E1: pH Validation Dish P8: Polystyrene polymer YYMM: Production year and month Batch: Batch number in the current month	-
	Consult instructions for use	-
	Store at room temperature	

6 Contact information

Urgently need help? Call our service hotline for support:

+45 7023 0500

(available 24 hours a day, 7 days a week)

E-mail support: support.embryoscope@vitrolife.com

(response within two working days)

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