Public Health England

Protecting and improving the nation's health

Determination of pH in food and water samples

National Infection Service Food Water and Environmental Microbiology Standard Method

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Status of National Infection Service Food, Water and Environmental Microbiology Methods

These methods are well referenced and represent a good minimum standard for food, water and environmental microbiology. However, in using Standard Methods, laboratories should take account of local requirements and it may be necessary to undertake additional investigations.

The performance of a standard method depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Citation for this document:

Public Health England (2017). Determination of pH in Food and Water Samples. National Infection Service. Food, Water & Environmental Microbiology Standard Method FNES63 [P2]; Version 2.

Amendment history

Controlled document reference	**FNES63 [P2]**
Controlled document title	**Determination of pH in Food and Water Samples**

The amendments that have occurred since the previous version of this document are shown below. On issue of revised or new documents each controlled document should be updated by the copyholder in the laboratory.

Page	Section(s) involved	Amendment
All	All	Microbiology Services changed to National Infection Service throughout
6	Background	Reference annotation updated
8	5.0 Reagents	Abbreviation expanded
9	6.0 Calibration	Reference to section 4.1 changed to 6.2
10	6.3 Daily check	Clarification of use of calibration buffers for the daily check
12	8.3 Measuring pH	Point 4 expanded to recommend that flat end of probe is in full contact with the food, 30 second timeframe removed
13	9.0 Quality control	Reference to weekly and daily checks added.
15	References	Updated (CR11209)
16	Appendix 1	Table 2 title amended to reflect that the pH range are optimal for these bacteria

Introduction

Scope

This document describes the determination of pH value of food and water samples. The method is based on that described in Practical Food Microbiology[2], It is applicable to the measurement of pH in all food types.

Background

Monitoring the pH of foods during production may be a vital step in producing a safe, high quality food. Proper pH range is often essential in many of the physical and chemical reactions that take place during food processing. For example, pH control is required to ensure proper gel formation in jelly making. The correct pH is also needed to achieve successful fermentations in the production of many cheeses, pickles and other foods. The pH of foods may vary during their shelf life, as a result of changes taking place in the food, eg mould growth. Such a change in pH, ie a drop in pH, is indeed one of the preservation factors in some fermented sausages and mould ripened cheeses. (Appendix 1)[1].

It may be necessary to determine the pH of a food sample before microbiological examination as this can influence the range of examinations applied and organisms sought. In general, in foods with a pH below 4.5, pathogens would not be expected to survive; the organisms present would be limited to yeasts, moulds and a few acid tolerant bacteria (see Appendix 1).

The pH of water from swimming pools and other chlorinated or brominated water sources may have a significant impact on water quality and efficacy of the disinfectant. As such, determining the pH of water from these sources can add value to the results obtained.

1. Principle

A pH electrode is used to measure the pH either within a liquid or food homogenate, or at the surface of a solid food sample.

2. Definitions

pH

A measure of the concentration of hydrogen ions (ie acidity) in a solution.

pH value

A measure of the pH of a solution expressed as a number on a scale from 0 to 14.

3. Safety considerations

3.1 General safety considerations

Normal microbiology laboratory precautions apply[3].

All laboratory activities associated with this SOP must be risk assessed to identify hazards[4-5]. Appropriate controls must be in place to reduce the risk to staff or other groups. Staff must be trained to perform the activities described and must be provided with any personal protective equipment (PPE) specified in this method. Review of this method must also include a review of the associated risk assessment to ensure that controls are still appropriate and effective. Risk assessments are site specific and are managed within safety organiser.

Information note: throughout this method hazards are identified using red text. Where a means of controlling a hazard has been identified this is shown in green text.

3.2 Specific safety considerations

Food products must be handled with appropriate care, depending on their inherent risks. For example, sub-sampling of certain hard or tough food and feed products (eg dried meat) and the opening of containers, such as tins, may require the use of sharp

utensils. When using these utensils take special care to avoid injury, wear protective gloves, and use can-openers if available. The electrodes are made of glass and as such may be vulnerable to breakage. The pH buffers in use are alkaline and acidic and gloves must be worn when handling these substances.

3.3 Laboratory containment

All activities associated with this method can be performed in a Containment Level 2 Laboratory.

4. Equipment

Normal laboratory equipment require and in addition:

- Hanna pH meter (model various)
- pH electrode (general purpose)
- pH electrode (surface probe)
- Thermometer (digital or alcohol)

5. Reagents

Equivalent commercial dehydrated media may be used; follow the manufacturer's instructions.

pH Standard Buffers: Certified Traceable to National Institute of Standards and Technology (NIST) 4.0 +/- 0.01 @ 20°C
7.0 +/- 0.01 @ 20°C
10.0 +/- 0.01 @ 20°C

For use, transfer 15 ml to a sterile plastic universal, re-cap tightly.

In use solution should not be used for more than 2 weeks. Write the date of expiry on the universal.

Information note: unused pH solution must never be transferred back into the stock bottle.

- phosphate buffered solution (PBS)
- distilled/deionised Water (DW)
- storage solution
- filling solution

- general cleaning solution
- protein cleaning solution

6. Calibration

6.1 Calibration frequency

The instruments should be calibrated in the following circumstances:

- when the instrument is new
- whenever the pH electrode is replaced
- at least once per week or if the daily check is out of range (see section 6.2)
- after testing aggressive chemicals
- after the cleaning procedure
- after changing the standard buffers

Regular calibration will ensure greater accuracy of the results.

A 2-point calibration is recommended. However, a 3 and 1-point calibration can also be used. Refer to the instruction manual of the pH meter for 3 and one-point calibration.

The 2-point calibration is described below. It is recommended that pH 7 is chosen as the first point. Consider the likely pH of the sample when choosing the second point, pH 4 (acidic) or pH 10 (alkaline).

6.2 Calibration procedure

The calibration procedure will vary depending on the equipment model and regard must be given to the manufacturer's instructions for use.

1) Place the pH 7 and pH 4 or pH 10 reference buffers in a plastic universal
2) Rinse a digital or alcohol thermometer with a stream of deionised water and use a tissue to remove excess water.
3) Immerse the thermometer in the pH 7 buffer, stir gently and wait 1 or 2 minutes for thermal equilibrium. Read the temperature and remove.
4) Adjust the temperature on the secondary LCD of the pH meter with the arrow keys "▲ºC" or "▼ºC" to the temperature displayed on the thermometer.
5) Rinse the pH electrode with a stream of deionised water and use a tissue to remove excess water.

6) Immerse the electrode into the pH 7 buffer, stir gently and wait 1 or 2 minutes for thermal equilibrium.
7) Press CAL. The “CAL” and ”BUF1” indicators and the most common “7.01” buffer will be displayed on the secondary LCD.
8) If necessary, press “▲ºC” or “▼ºC” to select a different buffer value.
9) The “NOT READY” indicator will blink on the LCD until the reading has stabilised.
10) When the reading is stable and close to the selected buffer, “READY” and “CFM” will blink.
11) Press CFM to confirm calibration.
12) The meter stores the reading and the calibrated value is then displayed on the primary LCD and the secondary LCD will display the second expected buffer value.
13) Rinse the pH electrode with a stream of deionised water and use a tissue to remove excess water.
14) Immerse the electrode into the 2nd buffer (use pH 4 if measuring in the acid range or pH 10 if measuring in the alkali range) and stir gently.
15) If necessary, press “▲ºC” or “▼ºC” to select a different buffer value.
16) The “NOT READY” (pH211) or “□” (HI2122) indicators will blink on the LCD until the reading has stabilised.
17) When the reading is stable and close to the selected buffer, “READY” and “CFM” will blink.
18) Press CFM to confirm calibration.
17) The meter stores the reading and returns to normal operational mode, completing the calibration.

Calibration of the pH electrode should be documented in the applicable pH meter record.

Information note: if the value measured by the meter is not close to the selected buffer, “WRONG BUF” will blink on the display. In this case check if the correct buffer is being used, change the buffer or refresh the electrode by following the maintenance procedure. If the problem persists inform a senior member of staff.

6.3 Daily check

A 2-point check is recommended using the reference buffers described in section 6.2. However, a 3 and 1-point check can also be used for some probes. Immerse the probe in each buffer in turn and check that the reading is within the acceptable range and tolerance for each buffer.

7. Maintenance

7.1 Electrode storage

To minimize clogging and assure a quick response time, the glass bulb and the junction should be kept moist.

When not in use, store with the protective cap in place containing a few drops of storage solution or, in its absence store in a plastic universal filled with storage solution. If none of the above is available, tap water may also be used for a short period (couple of days). Store in a stable position, protected from being damaged.

Information note: never store the electrode in distilled or deionized water and never allow it to dry out.

7.2 Electrode cleaning

Slow response and non-reproducible measurements are signs that the electrode has become coated or clogged.

Soak the electrode in general cleaning solution for approximately 30 minutes. Rinse the electrode with distilled water and soak the electrode in storage solution for at least 1 hour before taking measurements.

Consider an additional soak in protein cleaning solution for 15 minutes after the general cleaning solution to remove visible films, dirt or deposits.

7.3 General maintenance

The electrode and the cables must be inspected each time before use.

The cable used for connection to the instrument must be intact. There must be no points of broken insulation on the cable or cracks on the electrode stem or bulb. Connectors must be clean and dry.

If any scratches or cracks are present the electrode must be replaced. If necessary, the meter will be placed out of use and a replacement electrode ordered.

For refillable electrodes refill the reference chamber with fresh electrolyte. Allow the electrode to stand upright for 1 hour. Ensure the electrode is immersed in storage solution.

7.4 Troubleshooting

If a problem is identified refer to the troubleshooting guide provided in the manufacturer's instructions.

8. Determining the pH of food

8.1 Sample preparation liquid foods

Using aseptic technique remove a portion of the sample to a sterile honey jar/universal, avoiding contamination of the bulk of the food.

8.2 Sample preparation solid foods

8.2.1 Surface probe

No sample preparation required. Select an area of food that is regular. If the surface of the food is irregular consider cutting it to expose a flat surface

8.2.2 Liquid probe

Using the method described in FNES26 (F2)[6], a 1 in 10 homogenate of the food is prepared, using sterile distilled water in place of buffered peptone water. A 10g sample of the food should be sufficient. Ensure that a representative portion of the food is taken. If the food is a composite, or if its properties are likely to be different at different locations in the sample (eg surface and core), it may be necessary to take separate portions of each area or component.

Allow the particulate matter to settle in the homogenate before decanting the fluid into a plastic universal for testing. Label the universal with the unique laboratory number, date and time.

If the pH is not measured immediately, store the sample in a refrigerator and test within 24 hours of homogenisation.

8.3 Measuring pH

Make sure the appropriate standard buffers have been tested and the instrument(s) has been calibrated before performing pH measurements.

1. Press the ON/OFF key
2. Remove the probe from the storage solution and rinse the electrode thoroughly with deionised water to remove salt deposits.
3. Blot dry with clean tissue.
4. Place the probe into the food sample or apply to the solid surface of the food ensuring full contact with the flat probe end.

Wait for the reading to settle before recording the result.

5. The machine automatically displays the result.
6. Rinse the probe with deionised water before continuing with the next sample.

The pH electrode should be returned to the storage solution after use and the pH meter should be switched off at the end of each day.

9. Quality control

Daily and weekly calibrations must be performed. It is recommended that all laboratories carrying out this procedure participate in an external proficiency scheme.

10. Calculation of results

Results are transferred to the StarLims system as described in Method FNES6 (Q12)[7] Sample processing and result entry in StarLims. The result is recorded directly on the system using single result entry. No calculations are required.

11. Reporting of results

Where indicated and if the test has been requested by the customer the result is reported using the StarLims system as described in method FNES17 (Q13)[8] Technical Validation and release of result in StarLims. The test report specifies the method used, all details necessary for complete identification of the sample and details of any incidents that may have influenced the result.

12. Acknowledgements and contacts

This Standard Method has been developed, reviewed and revised by National Infection Service, Food, Water and Environmental Microbiology Methods Working Group.

The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

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Appendix 1: pH values and foods

Table 1. pH range of foods

pH Range	Food	pH
Low Acid (pH 7.0 -5.5)	Milk	6.3-6.5
	Cheddar cheese	5.9
	Roquefort cheese	5.5-5.9
	Bacon	5.6-6.6
	Red meat	5.4-6.2
	Ham	5.9-6.1
	Canned vegetables	5.4-6.4
	Poultry	5.6-6.4
	Fish	6.6-6.8
	Crustaceans	6.8-7.0
	Butter	6.1-6.4
	Potatoes	5.6-6.2
	Rice	6.0-6.7
	Bread	5.3-5.8
Medium Acid (pH 5.5-4.5)	Fermented vegetables	3.9-5.1
	Cottage cheese	4.5
	Bananas	4.5-5.2
	Green beans	4.5-5.5
Acid (pH 4.5-3.7)	Mayonnaise	3.0-4.1
	Tomatoes	4.0
High Acid (pH <3.7)	Canned pickles & fruit juices	3.5-3.9
	Sauerkraut	3.1-3.3
	Citrus fruits	3.0-3.5
	Apples	2.0-3.3

Table 2. pH Limits for Optimal Bacterial Growth

Organisms	Optimum pH
Campylobacter jejuni	6.5-7.5
Vibrio cholerae	7.6
Vibrio parahaemolyticus	7.8-8.6
Staphylococcus aureus for growth (for enterotoxin production)	6-7 (7-8)
Clostridium perfringens	7.2
Escherichia coli	6-7
Bacillus cereus	6-7
Salmonella spp.	7-7.5
Listeria monocytogenes	7.0
Aspergillus flavus for growth	5-8

Appendix 2: Flowchart showing the process to determine pH in food and water