

NATIONAL STANDARD METHOD

DETERMINATION OF PEROXIDASE ACTIVITY (STORCH TEST) IN MILK

D 6

Issued by Standards Unit, Evaluations and Standards Laboratory
Specialist and Reference Microbiology Division

DETERMINATION OF PEROXIDASE ACTIVITY (STORCH TEST) IN MILK

Issue no: 2.4 Issue date: 03.05.05 Issued by Standards Unit, Evaluations and Standards Laboratory on behalf of the Group F,
W & E Co-ordinators Forum and the Environmental Surveillance Unit, CDSC. Page 1 of 8

Reference no: D 6i2.4

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AMENDMENT PROCEDURE

Controlled document reference	D 6
Controlled document title	Standard Operating Procedure for Determination of Peroxidase Activity (Storch Test) in Milk

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
5/ 03.05.05	2.3	2.4	1	Front page	Redesigned
			2	Status of document	Reworded
			4	Amendment page	Redesigned

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STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF PEROXIDASE ACTIVITY (STORCH TEST) IN MILK

INTRODUCTION

Scope

The procedure describes the determination of the presence or absence of the enzyme peroxidase in milk as a control of pasteurisation.

Background

The reference procedure specified in the Dairy Products (Hygiene) Regulations 1995¹ for determination of peroxidase activity is described in 91/180/EEC². The regulations require a positive reaction in the peroxidase test for pasteurised milk samples. The peroxidase enzyme present in raw milk is inactivated at 75 - 80°C. If the milk has been overheated (>75°C) during pasteurisation, inactivation of the enzyme will occur and give a negative peroxidase test.

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1.0 PRINCIPLE

The peroxidase enzyme decomposes hydrogen peroxide. The atomic oxygen liberated oxidises the colourless 1,4-phenylenediamine into the purple indophenol. The colour intensity is proportional to the enzyme's concentration.

2.0 DEFINITIONS

Peroxidase-positive reaction

If the milk is properly pasteurised a blue colour occurs within 30 seconds after mixing.

Peroxidase-negative reaction

No colour occurs within 30 seconds after mixing.

3.0 SAFETY PRECAUTIONS

1,4-phenylenediamine is toxic through skin absorption and a potential carcinogen. Hydrogen peroxide 30% and concentrated sulphuric acid are corrosive and extremely irritating to the respiratory system. Appropriate protective clothing should be worn and a fume cupboard used when handling these products.

4.0 REAGENTS

1,4-phenylenediamine solution

Dissolve 2.0 g of 1,4-phenylenediamine (C₆H₈N₂) in warm (50°C) water then make up to a volume of 100 ml. Keep the solution in a closed dark brown bottle and store in a cool, dark place. After preparation, the solution forms a sediment in 1-2 days; discard when this occurs.

Note: the solution can be aliquoted after preparation and kept frozen for up to 6 months.

Hydrogen peroxide solution

Dilute 9 ml of hydrogen peroxide 30% (100 volumes) in water and make up to 100 ml. Add 1.0 ml concentrated sulphuric acid to stabilise the solution. If kept in a closed bottle in a cool, dark place away from contact with organic compounds, the solution will remain stable for one month.

5.0 METHOD

Introduce 5 ml of the milk sample into a clean test tube with suitable closure. Add 5 ml of 1,4-phenylenediamine solution. Add 2 drops of hydrogen peroxide solution. Mix well, then examine for colour production within 30 seconds. Note: The blue reaction may occur before addition of hydrogen peroxide. If the colour production occurs more than 30 seconds after the addition of the reagents, the reaction is unspecific.

6.0 REPORTING OF RESULTS

If the colour production occurs within 30 seconds, report:

Peroxidase Test - Positive

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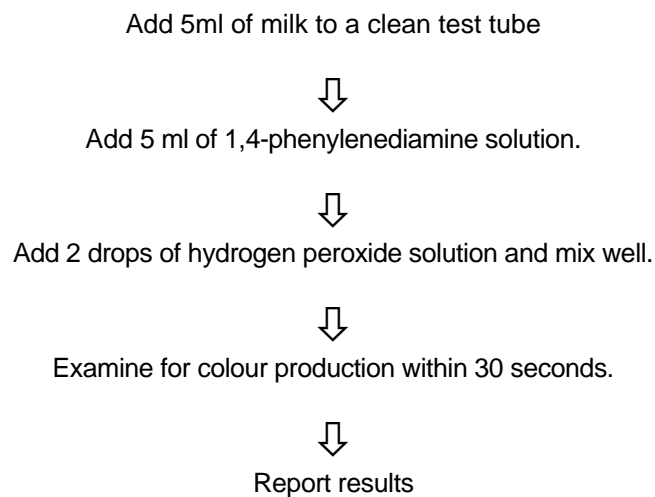
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If there is no colour production, report:

Peroxidase Test - Negative

Appendix: Flowchart showing the process for the determination of peroxidase activity (Storch test) in milk



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REFERENCES

- ¹ England and Wales. The Dairy Products (Hygiene) Regulations 1995. Statutory Instrument No 1086. London: HMSO, 1995
- ² Anon. 1991. Commission decision laying down certain methods of analysis and testing of raw milk and heat-treated milk (91/180/EEC). Official Journal of the European Communities L 93, 13.4.1991, 148

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