HOLOGIC®

AccuProbe®

LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST

(bioMérieux ref. 39500 / Hologic Cat. No. 102920)

INTENDED USE

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST is a rapid DNA probe test, which utilizes the technique of nucleic acid hybridization for the identification of *Listeria monocytogenes* isolated from culture.

A detection method is also proposed which has been certified by AFNOR Certification under the reference N° BIO 12/4-02/95 (refer to section on MICROBIOLOGICAL CONTROL paragraph C).

SUMMARY AND EXPLANATION OF THE TEST

Listeria monocytogenes is primarily a soil-borne micro-organism dispersed throughout the environment. It has been found in such sources as water, agricultural products, and in animals. Recognized as a human pathogen for more than 50 years, *Listeria monocytogenes* has been identified as the etiologic agent of listeriosis, causing meningitis, encephalitis, septicemia, endocarditis, abortion, abscesses and local purulent lesions in humans (2,4). Several major outbreaks of listeriosis within the last decade have been connected to consumption of contaminated food. Pregnant women, neonates, immuno-compromized patients and the elderly are at greatest risk of acquiring listeriosis (5).

Current identification methods rely on traditional physiological and biochemical methods. These include Gram stain morphology, catalase, motility, beta hemolysis on blood agar and sugar fermentation (8).

The ACCUPROBE LISTERIA MONOCYTOGENES TEST is a rapid and objective method for the detection of *Listeria monocytogenes*, which detects specific ribosomal RNA sequences within 35 minutes of sample preparation.

PRINCIPLE

Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes (6). The AccuProbe method uses a single-stranded DNA probe with a chemiluminescent label that is complementary to the ribosomal RNA of the target organism. After the ribosomal RNA is released from the target organism, the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The Selection Reagent allows for the differentiation of non-hybridized and hybridized probes.The light signal emitted by the DNA:RNA hybrids is measured by a Hologic luminometer. A positive result is a luminometer reading equal to or greater than the cut-off. A value below this cut-off is the negative result.

REAGENTS

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Reagents used for the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE LISTERIA MONOCYTOGENES KIT
(bioMérieux ref. 39500 / Hologic Cat. No. 102920)

Probe Reagent (P) (4 x 5 tubes)
Listeria monocytogenes.

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT
(bioMérieux ref. 39305 / Hologic Cat. No. 102800)

Reagent 1 (Lysis Reagent) (1) 1 x 10 mL
Buffered solution containing 0.04% sodium azide.

Reagent 2 (Hybridization Buffer) (2) 1 x 10 mL
Buffered solution.

Reagent 3 (Selection Reagent) (3) 1 x 60 mL
Buffered solution.

HOLOGIC DETECTION REAGENT KIT
(bioMérieux ref. 39300 / Hologic Cat. No. 201791)

Detection Reagent I (RI) 1 x 240 mL
0.1% hydrogen peroxide in 0.001 N nitric acid.

Detection Reagent II (RII) 1 x 240 mL
1 N sodium hydroxide.

WARNINGS AND PRECAUTIONS

A. For in vitro diagnostic use or industrial microbiology use only.
B. Observe usual safety precautions when performing this assay (3).
C. Use only for the identification of *Listeria monocytogenes* isolated from culture.
D. Use only supplied or specified disposable laboratory ware.
E. Some reagents used in this test contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. Upon disposal of these reagents, always dilute the material with a large volume of water to prevent azide buildup in the plumbing.
F. Avoid contact of Detection Reagents I and II with skin, eyes and mucous membranes. Wash with water if these reagents come in to contact with skin. If spills of these reagents occur, dilute with water before wiping dry.
G. To ensure optimal performance we recommend that prior to testing you inspect the tubes for dislodged material. If dislodged material is present, tap tube on the counter top in order to settle contents to the bottom of the tube.
H. Comply with Good Laboratory Practice (e.g. standard ISO 7218) (12).

STORAGE

Probe Reagent tubes must be stored in their foil pouches at 2° to 8°C. They are stable in the unopened pouches until the expiration date indicated. Once opened, the pouch should be resealed and the tubes should be used within two months and prior to the expiration date.

Other reagents used in the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST may be stored between 2° and 25°C and are stable until the expiration date indicated.

DO NOT FREEZE THE REAGENTS

SAMPLE PREPARATION

The ACCUPROBE LISTERIA MONOCYTOGENES TEST is designed to determine the identity of *Listeria monocytogenes* isolated from culture. The detection of *L. monocytogenes* in food products is performed after a pre-enrichment step.

CLINICAL IN VITRO DIAGNOSTIC USE

A. **Solid Media Method**. Growth isolated from appropriate solid media, such as 5% sheep blood, brain heart infusion, or chocolate agar may be tested. In addition, growth from selective media such as McBride Agar and LPM Agar (Remel) is also acceptable. Samples may be tested as soon as growth is visible but should be less than 72 hours old.
    1. Growth can be removed with a 1 µL disposable plastic loop, a wire loop, a disposable plastic needle or an applicator stick. Swabs should not be used due to the small volume of liquid in which the cells are subsequently resuspended.
    2. If a single colony is to be tested, it should be at least 1 mm in diameter. A 1 µL loopful of cells or several (3 or 4) smaller colonies can be tested.
    3. Avoid taking any of the solid media with the cells.
    4. The operator may elect to inoculate another culture plate at this time to confirm the purity of the isolate.

B. **Broth Culture Method**. Growth in broths, such as trypticase soy, brain heart infusion, or Listeria Enrichment Broth with turbidity equivalent to or greater than a McFarland I Nephelometer standard may be tested with the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST. Pipette a 50 µL sample from the well-mixed broth suspension into the probe reagent tubes as described below.

MICROBIOLOGICAL CONTROL

The procedure given in paragraph C of this section is certified by AFNOR Certification.

A. **Solid media method for Identification or detection**. Growth isolated from appropriate solid media (5% sheep blood, PALCAM, Oxford, McBride agars) may be tested. Samples may be tested as soon as colonies are visible, but should be less than 72 hours old.
    1. Growth can be removed using a 1 µL disposable plastic loop, a wire loop, a Pasteur pipette sealed or a disposable plastic needle. Swabs should not be used due to the small volume of liquid in which the cells are subsequently resuspended.
    2. It is possible to test either several small colonies (3 or 4), or a single colony of at least 1 mm in diameter (1 colony contains 10^{11} - 10^{12} bacteria), or to remove growth at the site of inoculation (1 µL loop).
    3. Avoid taking any of the solid media with the cells.
    4. Depending on the growth, collect either all the colonies present, or those in the inoculation zone.
    5. The operator may elect to inoculate another culture plate at this time to confirm the purity of the isolate.

B. **Broth culture method**. The test can be performed directly on an appropriate culture broth. According to studies performed on a selection of media (1, 7), only certain broths can be used

directly. Non-selective broths such as Brain Heart Infusion, Trypticase Soy, Listeria Enrichment Broth, Todd-Hewitt Broth, give good results, whereas certain more selective broths (L. PALCAMY, UVM, Fraser) reduce test sensitivity, leading to false negative results. Incubation should be extended for 48 hours at 30°C. **The use of Fraser, UVM, or L. PALCAMY broths requires subculturing on a solid medium**.

Pipette 50 µL of well-mixed culture broth into the Probe Reagent tube, as described in paragraph C of the TEST PROCEDURE section.

C. **PROCEDURE CERTIFIED BY AFNOR CERTIFICATION N° BIO 12/4-02/95 FOR ALL HUMAN FOOD PRODUCTS AND PRODUCTION ENVIRONMENTAL SAMPLES**.

D0 1. Mix X g of sample in 9X mL of Half Fraser Broth then incubate for 18 to 24 hours at 30° ± 1°C.
NB: within the framework of the NF VALIDATION mark, no test samples over 25g were tested.

D0 + 24h 2. After incubation of the enrichment broth, subculture to selective agar (Oxford agar for dairy products or PALCAM agar for all human food products and production environmental samples). Apply sample in wide streaks using a swab. Incubate for 18 to 24 hours at 37° ± 1°C (selective plate N°1).

3. Incubation of the Half Fraser Broth is extended for 18 to 24 hours at 30° ± 1°C.

D0 + 48h 4. After 18-24 hours incubation at 37°C ± 1°C of the selective plate N° 1, the detection of *Listeria monocytogenes* is performed on typical colonies from the selective agar, following the Solid Media method described in Paragraph A of this section and according to procedures identified in the TEST PROCEDURE section. In the absence of typical colonies on the plate or in the case of a negative AccuProbe result:

- continue incubation of the selective plate N° 1 for a further 18-24 hours at 37° ± 1°C
- inoculate a new selective agar plate (plate N° 2) using Half Fraser Broth incubated for 48 hours. Incubate the agar for 24 hours at 37° ± 1°C.

D0 + 72h 5. After 48 hours incubation of selective plate N° 1 or 24 hours incubation of selective plate N° 2, proceed with the detection of *L. monocytogenes* according to the protocol described in the section Microbiological Control, paragraph A. In the absence of typical colonies but in the presence of atypical colonies, perform the test by sampling from the point of inoculation.

Confirmation of positive results: According to the certified NF VALIDATION protocol, all positive results obtained by the AccuProbe method must be confirmed. Confirmation should be performed using one of the following three options :

- According to classical reference methods described by the CEN, ISO or AFNOR which includes a purification step on a nutritive agar.
- Using a chromogenic medium such as Listeria according to Ottaviani and Agosti described in the standard ISO 11290-1 or using a chromogenic medium which is included in an AFNOR validated method. Using a 1 µl loop, sample from the point of inoculation of the selective plate which gave the positive AccuProbe result and proceed with an isolation on the chromogenic medium. Incubate the plate at the temperature and time indicated by the manufacturer. The presence of isolated characteristic colonies confirms the AccuProbe result. The following chromogenic media have been tested during AFNOR validations performed in 2003 and 2007 : *Compass L. mono Agar (2003), Chromagar Listeria (2003), ALOA (2003), Rapid' L. Mono (2003 and 2007) and*

OAA (2007).

- Use of any other method certified by AFNOR Certification, which is based on a different principle than the method AccuProbe. The complete protocol described for the validated method must be used.

In case of discrepant results: positive by AccuProbe, unconfirmed by classical tests described in the standard CEN or ISO methods or after isolation on chromogenic media or by comparison with another method certified by AFNOR Certification, the laboratory is responsible for demonstrating the validity of the result reported.

In the case of chromogenic media being used, we recommend continuing the incubation for a further 24 hours or inoculating a different chromogenic medium from the positive selective plate (Palcam or Oxford).

MATERIALS PROVIDED

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST **(bioMérieux ref. 39500 / Hologic Cat. No. 102920)**

	20 Tests
Probe Reagent (P)	4 x 5 tubes

MATERIALS REQUIRED BUT NOT PROVIDED

1 µL plastic sterile inoculating loops, wire loops, Pasteur pipettes sealed or plastic needles for collecting colonies
Control culture strains
Incubator or water bath (37° ± 1°C)
Water bath or heating block* (60° ± 1°C)
Micropipettes (50 µL, 300 µL)
Re-pipettors (50 µL, 300 µL)
Vortex mixer

*Heating blocks in the dry heat bath should have wells that are correctly sized for 12 x 75 mm tubes. The use of Hologic dry heat bath is recommended.

AVAILABLE FROM YOUR HOLOGIC DISTRIBUTOR:

Hologic Leader 50i Luminometer
(bioMérieux ref. 39400 / Hologic Cat. No. 103100i)

Hologic Heating Block (60° ± 1°C)
(bioMérieux ref. 39406)

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT
(bioMérieux ref. 39305 / Hologic Cat.No. 102800)

HOLOGIC DETECTION REAGENT KIT
(bioMérieux ref. 39300 / Hologic Cat. No. 201791)

TEST PROCEDURE

A. EQUIPMENT PREPARATION

1. Adjust the incubator or water bath to 37° ± 1°C.
2. Adjust the water bath or heating block to 60° ± 1°C.
3. Prepare the Hologic luminometer for operation. Make sure there is sufficient volume of

Detection Reagents I and II to complete the tests.

B. CONTROLS

Positive and negative control strains should be tested routinely in each laboratory, according to local regulations. A culture of *L. monocytogenes* (ATCC 35152) may be used as the positive control while a culture of *L. grayi* (ATCC 19120) may be used as the negative control.

C. PREPARATION OF SAMPLES FOR HYBRIDIZATION

1. Open the foil pouch by cutting evenly across the top of the pouch. Remove enough Probe Reagent tubes to test the culture isolates and/or controls. Reseal the pouch by folding the opened edge over several times and securing with adhesive tape or a clip. **Leave the desiccant in the pouch**.
2. Label a sufficient number of Probe Reagent tubes to test the culture isolates and/or controls. Remove and retain the caps.
3. Pipette 50 µL of Reagent 1 (Lysis Reagent) into all Probe Reagent tubes. **If broth cultures are to be tested, do not add Reagent 1 to the Probe Reagent tubes**.
4. Transfer growth from the solid medium or 50 µL of a well-mixed broth culture into the labeled Probe Reagent tubes, as described in the SAMPLE PREPARATION section. If testing growth from solid media, twirl the loop or needle in Reagent 1 (Lysis Reagent) and mix thoroughly.
5. Recap the Probe Reagent tubes and incubate at 37° ± 1°C for 5 minutes in a water bath or 10 minutes in an incubator.

D. HYBRIDIZATION

1. Remove the Probe Reagent tubes from the water bath or incubator. Remove and retain the caps. Pipette 50 µL of Reagent 2 (Hybridization Buffer) into all Probe Reagent tubes.
2. Recap the Probe Reagent tubes and incubate for 15 minutes at 60° ± 1°C in a water bath or heating block.

E. SELECTION OF HYBRIDS

1. Remove the Probe Reagent tubes from the water bath or heating block. Remove and retain the caps. Pipette 300 µL of Reagent 3 (Selection Reagent) into each tube. Recap the tubes and vortex them to mix completely.
2. Incubate the Probe Reagent tubes for at least 5 minutes at 60° ± 1°C in a water bath or heating block.
3. Remove the Probe Reagent tubes from the water bath or heating block and leave them at room temperature for at least 5 minutes. Remove and discard the caps. **Read the results in the luminometer within 1 hour after removing from the water bath or heating block**.

F. DETECTION

1. Select the appropriate protocol on the luminometer, in compliance with the recommendations in the package insert of the device.
2. Using a damp tissue or paper towel, wipe each tube to ensure that no residue is present on the outside of the tube. Insert the tube into the luminometer and follow the instructions.
3. When the analysis is completed, remove the tube(s) from the luminometer.

PROCEDURAL NOTES

A. REAGENTS: Reagent 2 (Hybridization Buffer) may precipitate. Warming and mixing the solution at 35° - 60°C will dissolve the precipitate.

B. TEMPERATURE: Hybridization and Selection reactions are temperature-dependent. Therefore, it is

imperative that the incubator, water bath or heating block is maintained within the specified temperature range.

C. TIME:

1. The Hybridization Reaction should be started within 1 hour of adding the cells and Reagent 1 to the Probe Reagent tubes.
2. The Hybridization and Selection reactions are time-dependent. Hybridize for at least 15 minutes but no more than 20 minutes. Incubate the Probe Reagent tubes during the Selection Step for at least 5 minutes, but no more than 6 minutes.

D. WATER BATH: The level of water in the water bath should be maintained to ensure that the entire liquid reaction volume in the Probe Reagent tubes is submerged.

E. VORTEXING: It is critical to obtain a homogeneous mixture during the Selection Step, specifically after the addition of Reagent 3.

F. TROUBLE-SHOOTING

1. High negative control values (*L. grayi* ATCC 19120) greater than 20,000 RLU (Relative Light Units) in the Leader luminometer or 600 PLU (Photometric Light Units) in the AccuLDR (formerly PAL) luminometer can be caused by insufficient mixing after adding Reagent 3 (Selection Reagent), or by testing mixed cultures. Since mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.
2. Low positive control values (*L. monocytogenes* ATCC 35152) less than 50,000 RLU in the Leader luminometer or 1,500 PLU in the AccuLDR luminometer can be caused by insufficient cell numbers or by testing mixed or aged cultures. Since mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST are based on the following cut-off values. Samples producing signals greater than or equal to these cut-off values are considered positive. Signals less than these cut-off values are considered negative. Results in the equivocal range should be retested. If the second test still gives an equivocal result, the strain should be subcultured to check its purity.

	AccuLDR (formerly PAL)	**Leader**
Cut-off value	1,500 PLU	50,000 RLU
Equivocal range	1,200-1,499 PLU	40,000-49,999 RLU

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative controls (e.g. *L. grayi*, ATCC 19120) and positive controls (e.g. *L. monocytogenes*, ATCC 35152) should satisfy the following values:

	AccuLDR (formerly PAL)	**Leader**
Negative control	< 600 PLU	< 20,000 RLU
Positive control	> 1,500 PLU	> 50,000 RLU

If the controls are out-of-range, test results must not be taken into consideration.

LIMITATIONS

This method has been tested using fresh growth from solid media and from broth cultures listed in the SAMPLE PREPARATION section. The efficacy of this test has not been demonstrated on direct clinical specimen (as cerebrospinal fluid or blood samples).

Results from the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

EXPECTED VALUES

The ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST was compared to standard culture biochemical identification methods, using 175 isolates of *L. monocytogenes* species and 102 additional isolates representing 21 genera at 2 clinical sites. A second evaluation was undertaken with 296 strains isolated from suspected contaminated food materials. Isolates were categorized as either positive (> 50,000 RLU), or negative (< 40,000 RLU). The range of observations for negative cultures was 520 to 27,990 RLU and 77,088 to 1,283,789 RLU for positive cultures. A comparison of these results to standard culture identification methods is shown below:

ACCUPROBE / CULTURE IDENTIFICATION

AccuProbe / Culture	Pos / Pos	Pos / Neg	Neg / Pos	Neg / Neg	Sensitivity / Specificity	Percent Agreement
Site 1	175	0	0	102	100% / 100%	100%
Site 2	81	1	0	110	100% / 99.5%	99.7%
Total	256	1	0	212	100% / 99.7%	99.8%

One AccuProbe positive, culture negative isolate from Site 2 was retested with the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST and gave a negative result.

PERFORMANCE

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST was calculated by assaying three concentrations of ribosomal RNA isolated from *L. monocytogenes* using 10 replicates in a single assay.

Sample	A	B	C
Number of replicates	10	10	10
Mean Response (RLU)	132,370	72,720	41,074
Standard Deviation	9,981	3,126	3,837
Coefficient of variation	7.5%	4.3%	9.3%

B. BETWEEN-RUN PRECISION

The between-run precision was calculated by assaying the same three concentrations of *L. monocytogenes* ribosomal RNA using single determinations in 12 consecutive runs.

Sample	A	B	C
Number of replicates	12	12	12
Mean Response (RLU)	146,469	77,240	41,074
Standard Deviation	19,793	8,634	4,343
Coefficient of variation	13.5%	11.2%	10.8%

C. SPECIFICITY

A total of 97 ATCC strains were evaluated using the ACCUPROBE LISTERIA MONOCYTOGENES CULTURE IDENTIFICATION TEST. These isolates represented a total of 87 species from 53 genera. 15 isolates from 7 species of Listeria were tested (*L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. murrayi*, *L. seeligeri*, *L. welshimeri*). Only the isolates of *L. monocytogenes* produced positive results.

D. RECOVERY

Five serial dilutions of *L. monocytogenes* (ranging from 0 to 30 million cells per test) were assayed in the presence of 30 million cells of the following selected non-target species: *L. grayi, L. ivanovii, Erysipelothrix rhusiopathiae, Brochothryx thermophacta*. No interference or cross-reactions were observed.

E. SPECIFIC PERFORMANCE CHARACTERISTICS

Within the framework of the NF VALIDATION mark, the following results were obtained during the preliminary study:

- **Inclusivity/exclusivity:** all 50 strains of *Listeria monocytogenes* tested were detected. The study of 18 strains of Listeria (non *monocytogenes*) and 12 strains not belonging to the genus Listeria did not reveal any cross reactivity.
- **Relative detection level:** the AccuProbe method and the ISO 11290-1 reference method have the same 50% detection limit: between 0.3 and 5.2 CFU/25g.
- **Comparative study:** 346 samples were tested simultaneously using the AccuProbe method (Palcam agar) and the EN ISO 11290-1 method. The following results were obtained:

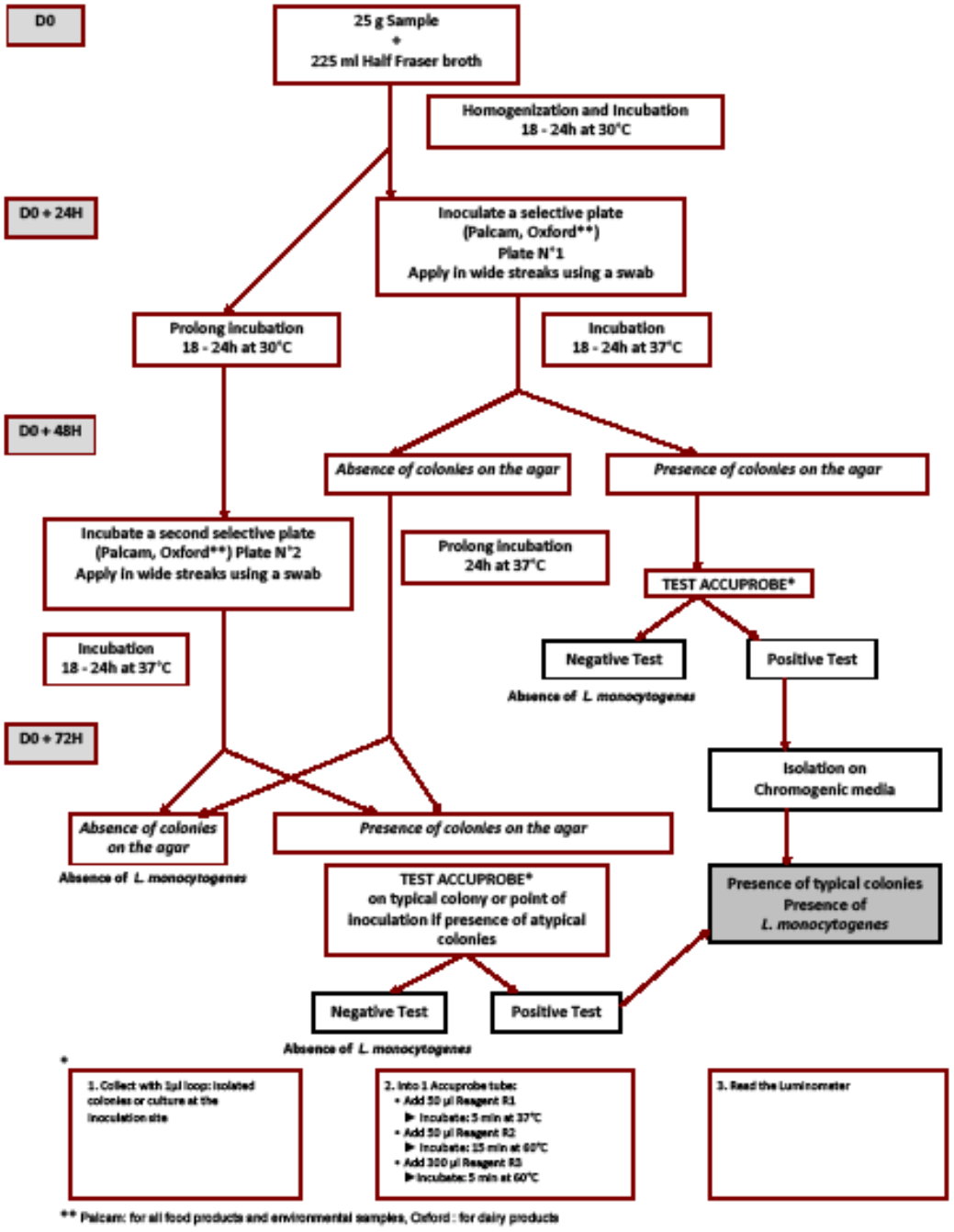
  -False negative results with AccuProbe method: 3
  -Additional positive results with AccuProbe method: 8
  -Concordant results: 335

The AccuProbe Listeria monocytogenes method has been certified by AFNOR Certification as an alternative method for the analysis of human food products and production environmental samples. This approval was obtained by comparison with the reference method described in the standard EN ISO 11290-1A1 and according to the protocol described in the standard EN ISO 16140.
The BIO 12/4-02/95 certification can be obtained from our Technical Assistance Department or from AFNOR Certification. The date of end of validity for the NF VALIDATION is indicated on the certificate.

BIO 12/4 - 02/95
METHODES ALTERNATIVES D'ANALYSE POUR L'AGROALIMENTAIRE
Certifié par AFAQ AFNOR Certification
www.afnor.org

APPENDIX
PROCEDURE FOR THE
« RAPID METHOD FOR THE DETECTION OF LISTERIA MONOCYTOGENES »
AFNOR* Certification N° BIO 12/4 - 02/95

*AFNOR: French Standardization Association



** Palcam: for all food products and environmental samples, Oxford : for dairy products

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12. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations - ISO 7218.

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