G.B. English

Product Number:
9871

NEOGEN®
ANSR®

for Listeria

BY AFNOR CERTIFICATION
NF VALIDATION
EN ISO 16140

NEO 35/03-01/16
Alternative Analytical
Methods For Agribusiness
nf-validation.afnor.org/en

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 101202

In an AOAC Research Institute Performance Tested Method℠ study, NEOGEN's ANSR *Listeria* assay was found to be an effective procedure for detection of *Listeria* species.

ANSR® for *Listeria*

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Intended Use

The ANSR® method for *Listeria* provides rapid and accurate detection of *Listeria* spp. in a wide variety of foods and environmental samples. All *Listeria* serovars can be detected with the ANSR method.

NEOGEN®'s ANSR for *Listeria* assay has been certified by NF VALIDATION as an alternative to the reference standard ISO 11290-1, according to the ISO 16140-2, for the detection of *Listeria* spp. in all food products for human consumption and in environmental samples. For more information about the end of the validity of the NF VALIDATION certification, please refer to the certificate NEO 35/03–01/16 available on the website: nf-validation.afnor.org/en or on request from NEOGEN.

Assay Principles

ANSR for *Listeria* is an isothermal, amplified nucleic acid assay. The ANSR for *Listeria* method is based on nicking enzyme amplification reaction (NEAR) technology preceded by the reverse transcription of 23s ribosomal RNA. Target complementary DNA is amplified through a mechanism of polymerization from the ends of nicks created in double-stranded DNA by the action of a specific endonuclease. Amplified target sequences are detected in real time using fluorescent molecular beacon probes.

A two-stage lysis reaction is performed, first at 37 ± 2°C for 10 minutes, then at 80 ± 2°C for 20 minutes. Next, a portion of the lysed sample is transferred to a strip tube containing lyophilized ANSR reagents. The tubes are sealed and incubated at 56 ± 1°C on the ANSR reader. Results are generated by the reader and displayed in the ANSR software within 18 minutes. Positive results may be confirmed from the enrichment cultures following standard procedures. Each tube of ANSR reagents contains an internal positive control, ensuring that the reagents are functioning properly.

Intended User

The ANSR for *Listeria* test is designed for use by personnel with appropriate training. Training in the use of the ANSR test system is available through NEOGEN.

Materials Provided

1. 12 strips of 8 cluster tubes, 1.2 mL
2. 12 strips of 8 reaction tubes, 200 µL, containing lyophilized ANSR for *Listeria* reagents in 2 sealed foil pouches with desiccant pack
3. 12 strips of 8 permanent caps for reaction tubes
4. 1 bottle lysis reagent suspension buffer, 60 mL
5. 3 vials containing lyophilized lysis reagents
6. 1 kit insert

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Equipment Required

1. ANSR reader (NEOGEN item 9828)
2. Computer and software for connection to ANSR reader (NEOGEN item 9832)
3. 2 heater blocks with lysis block inserts for 1.2 mL cluster tubes, 80 ± 2°C and37 ± 2°C (NEOGEN item 9836 or equivalent)
4. Pipettor, 20–200 µL (NEOGEN item 9276 or equivalent)
5. Pipettor, 100–1000 µL (NEOGEN item 9463 or equivalent)
6. Pipette tip rack, 100–1000 µL, sterile, 96 tips (NEOGEN item 9487 or equivalent)
7. Pipettor, 10–100 µL, 8-channel (NEOGEN item 9388 or equivalent)
8. Pipette tips, 100 µL, sterile, filtered, 96 tips (NEOGEN item 9389 or equivalent)
9. Stomacher or equivalent (optional)
10. Vortex, adjustable speed (NEOGEN item 9494 or equivalent)
11. 3 thermometers, traceable (NEOGEN item 9518 or equivalent)
12. Timer, 3-channel (NEOGEN item 9426 or equivalent)
13. Optional-for-use heater block with 0.2 mL reaction tube aluminum block insert, 56 ± 1°C (NEOGEN 9836 or equivalent)
14. Webcam (NEOGEN item WEBCAM)
15. ANSR Ethernet cable (NEOGEN item 9835 or equivalent)
16. 10 mL pipette pump (NEOGEN item 9277 or equivalent)
17. Pipettes, sterile serological (NEOGEN item 8686 or equivalent)
18. 40-slot, 20 mm test tube rack, autoclavable (NEOGEN item 9553 or equivalent)

Other Materials Required

1. Stomacher-type bags for sample enrichment. Filtered bags are recommended. (NEOGEN item 6827 or equivalent)
2. Graduated cylinder, 250 mL (NEOGEN item 9368 or equivalent)
3. 1 L purified water for preparation of LESS Plus enrichment medium
4. 20 L RO/deionized water for preparation of the µPREP® LESS Plus enrichment medium
5. µPREP Filter Unit for preparation of the µPREP LESS Plus enrichment medium (Lab M item MPA001)
6. Microbiological swabs in 1 mL Letheen broth (NEOGEN item 6651 or equivalent)
7. 1.5 mL microcentrifuge tubes (NEOGEN item 9372A or equivalent)

Precautions

1. For environmental testing in an industrial food manufacturing/preparation, or labeling enforcement context only.
2. Do not use any part of the test beyond the expiry date.
3. Do not open the foil pouch until just before use.
4. Ensure the foil bag is tightly sealed after removal of a device.
5. Always store the kit between 2–8°C (35–46°F). Avoid freezing.
6. Bring kit to room temperature 18–30°C (64–86°F) prior to use.

Media Enrichment Broth Required

1. LESS Plus medium, 500 g bottle (NEOGEN item NCM0202)
2. LESS Plus medium, 225 mL x 6 bottles (on request)
3. LESS Plus medium, 3 L x 3 bags (NEOGEN item NCM3400)
4. µPREP LESS Plus medium, 20 L x 5 bags (Lab M item NCM3206)

Storage

Store ANSR reagents at 2–8°C. After removing reaction tubes from the foil pouch, promptly reseal the pouch. Leave the desiccant pack in the pouch at all times.

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Precautions

1. Use good microbiology laboratory practices, such as ISO 7218.
2. Dispose of used pipette tips in a covered container containing a fresh solution of 10% bleach. The 10% bleach solution should be made fresh each day. Undiluted stock solutions of bleach should be used within 30 days after opening.
3. Discard bleach solution and tips as regular waste at the end of each day.
4. *Listeria monocytogenes* is a known hazard to pregnant women and immunocompromised individuals. Consult with your facility safety director for specific instructions.
5. Do not use reagents beyond the expiration date.
6. Use of enrichment media and incubation times or temperatures other than those specified may lead to erroneous results.
7. Remove reaction tubes from the foil pouch just before use and keep covered until heating process begins. Reseal the pouch containing the remaining reaction tubes to avoid prolonged exposure to light. More than 15 minutes of total exposure time may lead to erroneous results.
8. **Caution**: Do not, under any circumstance, remove caps from reaction tubes after the assay has been started. This is essential in order to prevent accidental contamination of the environment with amplification products.
9. Exercise care in all pipetting steps to avoid cross-contamination of samples.
10. Complete all assay steps in sequence, avoiding delays between steps.
11. Tap reaction tubes on bench top to make sure lyophilized reagents are at the bottom of the tube prior to adding lysed sample.
12. The laboratory equipment (pipettes, tubes, etc.) must not circulate from one work station to another.
13. Use powder-free gloves. Change gloves often, especially if you suspect they are contaminated.
14. Clean work spaces periodically with at least 10% bleach and another decontaminating agent.
15. It is strongly advised to work under a hood or a PCR workstation during lyses and amplification steps.

Preparation of enrichment broth (LESS Plus medium)

1. Dissolve 44 g of the medium in 1 L of purified water. Heat with frequent agitation to completely dissolve the medium, if necessary.
2. Autoclave at 110°C for 15 minutes.

Preparation of enrichment broth (µPREP LESS Plus medium)

1. Reconstitute 20 L bag of µPREP LESS Plus medium with 20 L of RO/deionized water via µPREP Filter Unit. Do not autoclave.
   Note: See Lab M MPB005 and MPA001 instructions.

Sample Preparation

1. Weigh x g sample in stomacher-type bag.
2. Dilute 1:10, x g or x mL of sample in 9 x x mL of LESS Plus medium (e.g. dilute 25 g or 25 mL of sample in 225 mL of LESS Plus medium).
   Note: For swab testing, the volume of medium should cover the swab sample.
   Note: In the context of NF VALIDATION mark, no samples of over 25 g were tested.
   Note: If needed, prepare samples according to standards for the product concerned (ISO 6887 series)
3. Homogenize (stomacher, etc.)

Sample Enrichment

1. Incubate the enrichment broth and samples at 30 ± 1°C for 25 ± 3 hours.
2. To test single samples, follow assay procedures.
3. To test pooled samples, a 1 mL aliquot of enriched LESS plus medium is combined with up to 9 additional 1 mL aliquots of enriched sample of the same or different matrix types, for a maximum pooled sample volume of 10 mL. Mix using a vortex then follow assay procedure.
   Note: Samples can be maintained at room temperature for two hours when carrying out the analysis.
   Note: It is possible to store the enriched LESS Plus medium between +2°C and +8°C for 72 hours maximum, following the last incubation at 30°C.

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Note: For specific matrices, contact NEOGEN support (out of the scope of NF VALIDATION). E.g. for morges testing, it is recommended to incubate the culture at 30 ± 1°C for 18–24 hours, pipette 1 mL of the enriched LESS Plus medium and dilute in 9 mL of LESS Plus medium supplemented with 100 µL of Amphotericin B (final concentration of 2.5 µg/mL) and 100 µL of Polymyxin-B (final concentration of 64000 IU/L). Incubate 6 ± 1 hours, then follow assay procedure.
Note: Do not shake the suspension before collecting the sample and avoid collecting large fragments of food debris. For food samples with a fatty surface layer, collect the sample from just below this layer.

LYSIS reagent SOLUTION PREPARATION

1. Reconstitute 1 vial of lyophilized lysis reagents with 18 mL of lysis reagent suspension buffer by adding the buffer to the reagent vial. Swirl gently to mix.
   Note: 1 vial of lysis reagents is enough for approximately 32 samples. Prepared lysis reagent solution can be stored at 2–8°C for up to 30 days.

ANSR Test Procedure

Prior to starting the assay:

1. Preheat one lysis heater block to 80 ± 2°C. Preheat the second lysis heater block to 37 ± 2°C. If using the optional single heater, preheat to 56 ± 1°C. Use the thermometer for the temperature reading.
2. Remove the foil pouch containing the reaction tubes from the refrigerator and allow them to warm at room temperature for 15 minutes. To avoid excess light exposure, leave reaction tubes in foil pouch until they are needed.
   Note: Keep the lysis reagent solution in the refrigerator until ready to use.
3. Connect the ANSR reader to the computer via USB or Ethernet and turn the computer on.
4. Turn on the ANSR reader. The reader will preheat to 56 ± 1°C.
5. Start the ANSR software and click the connect button. Input sample IDs, lot number, and user information.
   Note: For instructions on using the reader and software, see the user guide that came with the ANSR reader.
6. ANSR software versions up to 1.8.3 were in the context of NF VALIDATION. Please check with your technical representative for the latest version.

Assay Procedure

1. Add 50 µL enrichment culture (or pooled sample) to a 1.2 mL cluster tube(s) using a micro-pipette with 100 µL filtered tips. Ensure that only the tips are in contact with the broth or the stomacher bag and use a new pipette tip for each sample.
   Note: To reduce the risk of cross contamination, it is possible to pipette 1 mL from the enrichment bag into a tube using a regular pipette. Then using a micro-pipette remove 50 µL from the tube and carry out the assay.
   Note: Cluster tubes may be pulled apart to provide the number of tubes needed.
2. Add 450 µL lysis reagent solution to each cluster tube(s) containing culture. Be sure to switch pipette tips between samples.
   Note: Return the lysis reagent solution to the refrigerator after use (within 1 hour).
3. Place the cluster tube(s) into the 37 ± 2°C heater block and incubate for 10 minutes.
4. Immediately transfer the cluster tube(s) to the 80 ± 2°C heater block and incubate for 20 minutes.
   Note: The 80 ± 2°C incubation time may be extended to a total of 60 minutes for the purpose of managing staggered assay start times.
5. For 3 to 5 minutes before the end of the lysis step, preheat the ANSR reagents to 56 ± 1°C by placing the reaction tubes in the ANSR reader. Optional: A separate heat block can be used. It should be heated to 56 ± 1°C.
   Note: The strip of reaction tubes may be cut to provide the number of tubes needed. Keep all unused tubes in the sealed foil pack. Ensure the pellet in the reaction tube(s) is at the bottom by tapping the tubes gently on the bench top.
6. After the completion of the 20–60 minute lysis incubation, remove and discard the caps from the reaction tube(s) in the ANSR reader.
   Important: Proceed with steps 7–9 without delay. The transfer of the sample from the lysis tubes at 80°C to the reaction tubes should be completed within 1 minute.
7. Using an 8-channel pipette and 100 µL filtered tips, carefully transfer 50 µL from the top third of the lysed sample(s) in the cluster tube(s) to the reaction tube(s). Debris may accumulate at the bottom of the lysis tube(s) that will interfere with assay performance. Avoid transfer of debris by aspirating from the top third of the lysis tube(s). Do not prime the pipette tips and do not mix before aspirating. Place the provided permanent cap(s) on the reaction tube(s).

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Note: Lysed sample may be transferred from the same cluster tube a maximum of 3 times. CAUTION: Ensure that the pellet is not touched with the pipette when transferring the lysed sample as this can lead to erroneous results.

8. Remove the strip(s) of tubes from the reader (or 56 ± 1°C heat block if one was used) and vortex briefly (about 2 seconds), then place back into the reader without delay. Close the reader's lid.
   Note: The reader will not provide accurate results if the lid is open. Keep the lid closed at all times while the assay is running. Contamination may occur if the permanent caps are not placed on the reaction tubes and/or if the permanent caps are removed.
9. Click start in the ANSR software to begin the 18 minute assay.
10. Results will be displayed as positive, negative, or invalid by the software at the end of the assay. If the result is invalid, the test must be repeated from the lysed sample held at 80°C or if necessary after a 1:10 dilution of the lysate in lysis buffer prewarmed to 80°C or after a 1:10 dilution of the pre-enriched LESS Plus medium.

Interpretation of Results

Each tube of ANSR reagents contains an internal positive control. A positive control curve will develop in the case of a valid assay. In the case of an invalid result, the positive control curve should be examined and the assay repeated. The sample matrix may be tested for inhibitory effects, please see page 5, point 10 for details. The ANSR software will indicate the test results as positive or negative for the presence of *Listeria* spp. in the enriched sample. In addition, the real-time fluorescence curve generated from the assay can be viewed. If a positive result is detected from a pooled sample it is recommended to individually test each original sample, starting from the single enriched bags.

Confirmation

In the context of the NF VALIDATION certified method, all positive ANSR results need to be confirmed in one of two ways:

1. Using standard tests described in the standardized CEN or ISO methods (including the purification step). For the confirmation test, it is necessary to start from the LESS Plus enrichment medium after the full 25 ± 3 hours enrichment at 30°C.
2. Subculturing 0.1 mL from the LESS Plus enrichment medium onto a selective agar plate (Palcam (for example NEOGEN item NCM0111) or Agar *Listeria* according Ottaviani and Agosti (for example *Listeria* Chromogenic Agar NEOGEN item NCM1004). Incubate the plate following the kit instructions. The presence of typical colonies of *Listeria* spp. is sufficient to confirm the presence of *Listeria*.
   Note: In the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

Listeria Right Now™ Protocol (Excluded from NF VALIDATION SCOPE)

Listeria Right Now™ is an ANSR *Listeria* protocol for the detection of *Listeria* spp. from environmental surfaces without enrichment. The entire collected contents of the swab are subjected to sample processing. After expression of the swab in the lysis buffer, a two-stage lysis reaction is performed, first at 37°C for 10 minutes, then at 80°C for 20 minutes. Next, a portion of the lysed sample is transferred to a strip tube containing lyophilized ANSR reagents. The tubes are sealed and incubated at 56°C on the ANSR reader. Results are generated by the reader and displayed in the ANSR software within 18 minutes. Each tube of ANSR reagents contains an internal positive control, ensuring that the reagents are functioning properly.

Procedure

1. Collect environmental sample using the recommended microbiological swabs that are pre-moistened with Letheen broth.
2. Sample each surface by swabbing horizontally, vertically, and diagonally enough times in each direction to provide full coverage of the test area (up to an area of 10 cm x 10 cm).
3. Remove the remaining Letheen broth from the sample collection tubes.
4. After swabbing, place the swab back into the tube without Letheen broth.
5. Keep the swab sample collection tubes at 4 ± 2°C.
6. The sample should be tested the same day after collection. Testing should not be carried out if the time after collection exceeds 8 hours.
7. Remove swab sample from the tube and place into the microcentrifuge tube containing 1 mL of Lysis buffer.
8. Swirl swab and mix gently with up-and-down movements in the lysis reagent solution approximately 20 times, then press the swab tip against the inside of the tube to remove excess liquid. Remove swab and discard.
9. Close cap of the microcentrifuge tube, vortex to mix, and proceed with the ANSR assay procedure.

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Disposal

Enrichment cultures and used lysis tubes should be disposed of as biohazard waste. The preferred method of treatment for biohazard waste is autoclaving. Items that cannot be autoclaved may be disinfected with bleach solution. Consult with the safety advisor for your facility for detailed instructions.

Do not remove the permanent caps, for ANY reason, from the ANSR reaction tubes once the assay has started, even when disposing of them. Reaction tubes can be disposed of as non-biohazardous waste. It is recommended that they be placed in sealable plastic bags and immediately disposed of to protect against accidental opening.

Customer Service

NEOGEN Customer and Technical Services can be contacted through NEOGEN.com and product training is available by request.

SDS Information Available

Safety data sheets are available for all test kits at NEOGEN.com or by calling 800.234.5333 or 517.372.9200.

Terms and Conditions

NEOGEN's full terms and conditions are available online.

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