PRINCIPLE OF THE METHOD
See the BAX® System User Guide for an overview of how the BAX® System method uses automated, real-time Polymerase Chain Reaction (PCR) technology.

MATERIALS
BAX® System Real-Time PCR Assays – STEC suite
STEC Screening assay for Oxy and eae (Part kit KIT2021 [D1464964]); STEC Panel 1 assay for E. coli O26, O111, O121 (Part Kit KIT2008 [D14642970]); or STEC Panel 2 assay for E. coli O45, O103, O145 (Part Kit KIT2009 [D14642967]).

BAX® System start-up package (equipment and supplies for up to 152 tests):
 • BAX® System Q7ycler/detector and computer workstation
 • Heating blocks with inserts capable of maintaining 37.5°C and instruments
 • Cooling blocks with inserts
 • PCR tube holder
 • Capping/coping tools
 • Adjustable mechanical pipettes (5.5-50 µL)
 • Repeating pipette
 • Multi-channel pipette (8 channels – 5-50 µL)
 • Pipette tip box with caps and racks
 • Pipette tips with barriers
 • Powder-free nitrile gloves

Other-quarterly kits:
 • The Automated Thermal Block (Catalog No. MOCV223 [D1414522]) may be used in place of heating and cooling blocks.
 • Stomacher with bags
 • Incubator capable of maintaining directed enrichment temperatures between 39-42°C.

Note: AFNOR Certification standards require an incubator capable of maintaining ±1°C.

ENRICHMENT PROTOCOL
1. Prepare Enrichment Broth
Prepare enrichment broth according to the manufacturer’s instructions. See the BAX® System User Guide for common enrichment media recipes.

2. Collect and Enrich Samples
Method Approved by AFNOR
 • Raw ground beef (375 g): Homogenize sample with 1.5 L prewarmed (46°C) glucose-containing TSB with 2 mg/L novobiocin. Incubate at 41°C for 12-24 hours.
 • Raw ground beef with soy (375 g): Homogenize sample with 1.5 L prewarmed (46°C) glucose-containing TSB with 10 µL casamino acids and 8 mg/L novobiocin. Incubate at 41°C for 12-24 hours.
 • Raw beef trim (325 g): Gently massaged sample with 1.5 L prewarmed (37°C) mTSB media. Incubate at 41°C for 12-24 hours.
 • Raw beef meats (25 g): Homogenize sample with 1.5 L prewarmed (46°C) glucose-containing TSB. Incubate at 41°C for 12-24 hours.
 • Raw ground beef (325 g): Homogenize sample with 1.5 L prewarmed (46°C) glucose-containing TSB. Incubate at 41°C for 12-24 hours.
 • Raw ground beef (25 g): BW Media - Homogenize sample with 225 ml prewarmed (37°C) BW media. Incubate at 37°C for 10-24 hours.
 • Raw meat (25 g): BW Media - Homogenize sample with 25 ml prewarmed (37°C) mTBS with casamino acids. Incubate at 42°C for 10-24 hours.
 • Flour (25 g): Homogenize sample with 225 ml prewarmed (42°C) mTBS with 8 mg/L novobiocin. Incubate at 42°C for 24 hours.

Note: Incubation temperature must be maintained between 39 °C and 42 °C for this assay.

Method Approved by AFNOR
Test portions weighing more than 25 g have not been tested in the current enrichment isolation method. For preparation of initial suspensions, follow instructions of EN ISO 6887 standard.
 • Raw beef meats (25 g): BW Media - Homogenize sample with 225 ml prewarmed (41.5°C) BW media. Incubate at 41.5°C for 10-24 hours.
 • Raw meat (25 g): BW Media - Homogenize sample with 25 ml prewarmed (41.5°C) mTBS with casamino acids. Incubate at 42°C for 10-24 hours.
 • Raw daily products (25 g): Double strength (DS) BW Media - Homogenize sample with 225 ml prewarmed (41.5°C) DS BW media. Incubate at 41.5°C for 24-28 hours.

TEST PROTOCOL – ALL ASSAYS
3. Prepare Equipment
3.1 Turn on the heating blocks to 37°C and 95°C.
3.2 Make sure cooling blocks are chilled to 2-8°C.
3.3 If using the Automated Thermal Block, follow the instructions in the Thermal Block User Guide for running the Gram Negative program.
3.4 Power on the Q7 instrument and launch the BAX® System application.

4. Calibrate a rack file (See User guide for details) IMPORTANT NOTE FOR STEC SCREENING WITH “STX ONLY”:
An alternative targeted drop-down option is available for running the “stx only” program. See the BAX® System User Guide for details.

5. Hydrate PCR Tablets
5.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.
5.2 Place a PCR tube rack on a chilled (2-8°C) PCR cooling block.
5.3 Arrange strips of PCR tubes according to your rack file.
5.4 Remove the caps from the first strip of PCR tubes with the decapping tool.
5.5 Transfer 30 µL lysate (from step 4.8) into PCR tubes, then seal with flat optical caps.
5.6 Repeat with remaining strips of PCR until all PCR tablets have been hydrated.

Note: PCR tablets must be hydrated and re-sealed immediately after removing the caps from the PCR tubes.

6. Amplify and Detect
6.1 At the “Ready for Rack Load” prompt, click the NEXT button and open the instrument drawer.
6.2 Place the PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.
6.3 Close the drawer and click the NEXT button to begin automated processing.

Note: If desired, remaining lysate can be sealed and stored at 4°C for additional processing with the BAX® System STEC suite and/or real-time E. coli O157:H7 assay.

7. Review Quals
Quals results are displayed as a grid of color-coded icons in the top half of the screen.
CONFIRMATION
Method Approved by AOAC
The BAX® System results should be confirmed from the reference culture method appropriate for the sample type, such as:
- U.S. FDA Bacteriological Analytic Manual (BAM)
- USDA FSIS Microbiology Laboratory Guidebook (MLG)
- Health Canada Compendium of Analytical Methods
- International Organization for Standardization (ISO)

TECHNICAL ASSISTANCE
For questions or comments, please contact your local distributor. You can also contact Hygiena at 1-800-656-5300 outside the U.S., or email diagnostics.support@hygiena.com.

LIMITATION OF WARRANTY AND LIABILITY
NOTICE: READ THIS LIMITATION OF LIABILITY AND WARRANTY BEFORE USING THE BAX® System. The purchase of the BAX® System, or any component of the BAX® System, by user, assumes the risk of all results obtained from the use of the BAX® System. In particular, Hygiena disclaims any and all liabilities, including, without limitation, any warranty, express or implied, including, without limitation, any warranty of MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR ANY OTHER WARRANTY, WHETHER OR NOT BASED UPON THE NEGLIGENCE, STRICT LIABILITY OR BREACH OF WARRANTY, TORT, CONTRACT, STRICT LIABILITY, NEGLIGENCE OR OTHERWISE SHALL BE LIMITED TO THE FOLLOWING: (a) Should Equipment fail to conform with the Paragraph 2 warranty, Hygiena shall, at its option, repair or replace the non-conforming Equipment with new or refurbished (repaired or restored) equipment that is functionally equivalent Equipment or refund the purchase price. (b) Should BAX® Software fail to conform to the Paragraph 3 warranty, Hygiena will replace it free of charge. (c) In all other cases, claims for damages, and/or if they are foreseeable.

Screening Assay Results (stx and eae)
Positive result – Indicates that both stx and eae are present in that sample. The amplification plot shows a rise in the stx (blue) and eae (green) targets.

Negative result – Indicates that the combination of stx and eae is not present in that sample. If only one of the stx or eae targets is present, the sample is considered negative.

Screening Assay Results (“STX ONLY”)
Positive result – Indicates that stx is present in that sample. The amplification plot shows a rise in the stx (blue) target. The eae target is ignored.

Negative result – Indicates that stx is not present in that sample. The eae target is ignored.

*Using the Test Protocol above, stored lysates of positive Screening samples will be run with the Panel 1 and Panel 2 assays to identify specific “Big 6” serogroups, if present.

Panel 1 Assay Results (E. coli O26, O111, O121)
Positive result – Indicates that one or more of the Panel 1 targets are present in the sample:
- E. coli O26 - the amplification plot shows a rise in the O26 (gold) target
- E. coli O111 - the amplification plot shows a rise in the O111 (grey) target
- E. coli O121 - the amplification plot shows a rise in the O121 (purple) target

Negative result – Indicates that none of the Panel 1 targets are present in that sample.

Panel 2 Assay Results (E. coli O103, O145)
Positive result – Indicates that one or more of the Panel 2 targets are present in that sample:
- E. coli O103 - the amplification plot shows a rise in the O103 (brown) target
- E. coli O145 - the amplification plot shows a rise in the O145 (turquoise) target

Negative result – Indicates that none of the Panel 2 targets are present in that sample.

The BAX® System Real-Time PCR Assays for STEC (Screening, Panel 1 and Panel 2) have been evaluated according to the AOAC Research Council, Methods Performance Tested Method® 99-10301. The performance of these assays was reviewed by AOAC-R and was found to perform to the manufacturer’s specifications. Validation studies conducted according to EN ISO 16140-2 standard have been completed. Sensitivity and specificity equal to or better than the reference culture based methods.

The USDA Food Safety and Inspection Service (USDA-FSIS) has adopted the BAX® System STEC suite for monitoring meat products and carcass surfaces. See FSIS Microbiology Laboratory Guidebook (MLG) Method 58.54 for details and protocols. Please note that the enrichment and sample preparation protocols in the MLG may differ from those in the BAX® System documentation.

The BAX® System Real-Time PCR Assay for STEC, Screening (Panel 1 and Panel 2) has been evaluated according to NF VALIDATION rules. Validation studies conducted according to EN ISO 16140-2 standard found this test to be fit for purpose. To satisfy the NF VALIDATION rules for raw beef meats, raw dairy products and vegetables.

The version of software used during the NF Validation study is indicated in the NF VALIDATION certificate of the alternative method. For more information, including validity dates, please refer to certificate QUA 1811-12/20 available at http://nf-validation.afnor.org.

DISPOSAL
Decontaminate materials and dispose of biohazardous waste per your site practices and as required by federal, state and local regulations.

VALIDATION
The BAX® System Real-Time PCR Assays for STEC (Screening, Panel 1 and Panel 2) have been evaluated according to AOAC Research Council, Methods Performance Tested Method® 99-10301. The performance of these assays was reviewed by AOAC-R and was found to perform to the manufacturer’s specifications. Validation studies conducted according to EN ISO 16140-2 standard have been completed. Sensitivity and specificity equal to or better than the reference culture based methods.

The BAX® System Real-Time PCR Assay for STEC, Screening (Panel 1 and Panel 2) has been evaluated according to NF VALIDATION rules. Validation studies conducted according to EN ISO 16140-2 standard found this test to be fit for purpose. To satisfy the NF VALIDATION rules for raw beef meats, raw dairy products and vegetables.