Hygiena™
Lateral Flow System

E. coli O157 Test Kit
Part ASY2030

Contents

E. coli O157 test strips – 50

Package insert

Intended Use

Hygiena™ Lateral Flow System *E. coli* O157 tests have been designed to detect *Escherichia coli* O157 (including H7) in raw ground beef and raw boneless beef trim. The test kit permits presumptive detection and identification of the target pathogen when present at levels of one *E. coli* O157 organism per 25 grams of sample (0.04 cells/g). The test provides a simplified process with results in as few as 8 hours (for 25-g samples) or 10-15 hours (for 375-g samples). *E. coli* O157 test strips are designed to be used by trained technicians who follow good microbiology laboratory practices. Although the test strips are easy to use, the protocol involves the use of potentially hazardous microorganisms so appropriate safety practices must be observed.

Field of use: Data obtained from the Hygiena™ Lateral Flow System should not be used for human diagnostic or human treatment purposes. This product is not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. The Hygiena™ Lateral Flow System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Not approved for clinical diagnosis. Use only for research and development, including quality assurance and quality control testing, under supervision of technically qualified persons. Read the Limitation of Warranty and Liability before using product.

Principle of the Method

This immunoassay test uses a double antibody sandwich format. An antibody that is specific to *E. coli* O157 is sprayed and immobilized in a test line on the surface of a membrane. A second antibody reagent, also recognizing *E. coli* O157 and labeled with colloidal gold, is contained within a reagent pad upstream from the test line on the membrane.

As liquid sample moves by capillary action through the reagent pad, the antibody-gold reagent specifically binds to *E. coli* O157 and moves with the sample into the test membrane. Moving up the test membrane, the sample passes through the test line, where the immobilized *E. coli* O157 antibody captures the *E. coli* O157-antibody-gold complex, forming an antibody-*E. coli* O157-antibody sandwich. The test line then develops a red color. In the absence of *E. coli* O157, no antibody-*E. coli* O157-antibody sandwich is formed, and the test line does not turn red.

Reagents immobilized at the control line capture excess gold reagent passing through the test line. This causes the control line to develop a red color, which indicates that the test flowed correctly on the strip.

Therefore, a single (control) line on the membrane indicates a negative sample, and two (sample and control) lines indicate a positive sample.

Storage and Shelf Life

Store the Hygiena™ Lateral Flow System *E. coli* O157 test strips at room temperature (15-30°C) in the supplied canister, which contains a desiccant liner. Storage conditions higher than room temperature may adversely affect performance of the test strip.

After opening the canister, take care to re-seal the lid so that the strips are protected from moisture. A humidity indicator included in each canister displays blue dots, which turn pink if the strips are exposed to moisture. Do not use strips if any of the dots on the humidity indicator have turned pink.

Do not use test strips after the expiration date on the canister label.

Required Materials

Hygiena™ Lateral Flow System *E. coli* O157 Test Kit (ASY2030 – 50 test strips per kit)

Hygiena™ Lateral Flow System *E. coli* O157 Media (MED2007 - 500 g) – for LFS Media protocol

Modified Trypticase Soy Broth (Oxoid CM0989B or equivalent) with novobiocin plus casamino acids – for Standard Media protocol

Balance - 25 to 1000 grams with 0.2 g sensitivity

Stomacher with filtered bags (Seward Stomacher® 400 Circulator or equivalent)

Incubator capable of maintaining 42±1°C

Plastic test tubes (12 x 75 mm) with rack

Pipettes (400 µL) for transfers

Enrichment Protocol – Hygiena™ LFS Media

1. Prepare enrichment broth

1.1 Add 25.2±0.2 grams of Hygiena™ Lateral Flow System *E. coli* O157 Media to 1 liter deionized water. Shake vigorously until the media is dissolved.

1.2 Autoclave media at 121°C for 15 minutes. Alternatively, media may be filter sterilized (pore size of 0.2µm).

1.3 Prepared media may be stored for up to 2 weeks at 2-8˚C. For best results, use the media as soon as it is prepared.

Note: Enrichment broth may also be prepared by adding 25.2±0.2 grams Hygiena™ Lateral Flow System E. coli O157 Media to 1 liter pre-warmed (42°C) sterile, deionized water without autoclaving. If prepared with sterile water, media must be used within 3 hours or preparation.

2. Collect and enrich samples – 25 g

As part of good laboratory practice, we recommend that you run a positive and negative control along with your samples.

2.1 Place 25 g ground beef or beef trim in a sterile stomacher bag.

2.2 Add 225 mL pre-warmed (42°C) prepared enrichment broth to the bag, and stomach for 45 seconds.

2.3 Close the bag loosely and incubate 8-18 hours at 42°C with or without agitation.

Note: Sample bags should be closed loosely to allow air exchange during sample enrichment and optimize pathogen growth and antigenic expression.

2.4 After incubation, remove the bag from the incubator and use a gentle swirling motion to mix the contents.

2. Collect and enrich samples – 375 g

As part of good laboratory practice, we recommend that you run a positive and negative control along with your samples.

2.1 Place 375 g sample in a sterile stomacher bag.

2.2 Add pre-warmed (42°C) prepared enrichment broth to the bag, and stomach for 45 seconds.

- For ground beef, use 3.375 L enrichment broth.
- For beef trim, use 1.5 L enrichment broth.

2.3 Close the bag loosely and incubate at 42°C with or without agitation.

- For ground beef, incubate samples for 12-18 hours.
- For beef trim, incubate samples for 10-18 hours.

Note: Sample bags should be closed loosely to allow air exchange during sample enrichment and optimize pathogen growth and antigenic expression.

2.4 After incubation, remove the bag from the incubator and use a gentle swirling motion to mix the contents.

Enrichment Protocol – Standard Media

1. Prepare enrichment broth

1.1 Add 33 grams of mTSB and 10 grams of casaminoacids (casein acid hydrolysate) to 1 liter distilled water. Shake vigorously until the media is dissolved.

1.2 Autoclave media at 121°C for 20 minutes.

1.3 Cool media to approximately 50°C, then add 20 mg novobiocin to the media.

2. Collect and enrich samples – 375 g

As part of good laboratory practice, we recommend that you run a positive and negative control along with your samples.

2.1 Place 375 g ground beef or beef trim in a sterile stomacher bag.

2.2 Add 3.375 L pre-warmed (42°C) prepared mTSB to the bag, and stomach for 45 seconds.

2.3 Close the bag loosely and incubate 15-22 hours at 42°C with or without agitation.

Note: Sample bags should be closed loosely to allow air exchange during sample enrichment and optimize pathogen growth and antigenic expression.

2.4 After incubation, remove the bag from the incubator and use a gentle swirling motion to mix the contents.

Heat-Kill Step – optional for all samples

The Hygiena™ Lateral Flow System *E. coli* O157 test has been developed to detect both live and heat-killed organisms. To detect heat-killed organisms after incubation, use a sterile pipette to transfer 5 mL enriched sample to a glass tube. Heat in boiling water for 10 minutes, then allow tube to cool to room temperature before proceeding with the test protocol.

Test Protocol – all samples

3. Test samples

3.1 Arrange and mark tubes for each sample in a rack.

3.2 Transfer 1 mL enriched sample to a tube.

3.3 Remove the required number of test strips from the canister. Do not remove the label on the strip.

3.4 Insert the strip with **arrows facing downwards** into the tube.

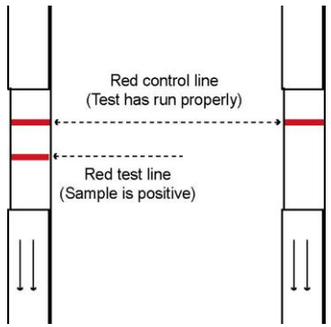
3.5 Allow the strip to develop for **10 minutes**, then check the results as follows:

a. At least one line, the control line, should always develop. A red line in this position indicates that the strip is functioning properly.

b. If at 10 minutes the test strip shows only a clearly visible control line, then the sample is negative for *E. coli* O157.

c. A red line appearing below the control line is the test line and indicates a positive result. If the test strip displays two (2) red lines, the test is complete and the sample is positive for *E. coli* O157

d. If no control line develops within 10 minutes, the test is invalid and needs to be repeated.



Note: Test strip results should always be interpreted after 10 minutes. Test strips interpreted after 20 minutes are invalid.

4. Confirm presumptive positive results

Use enriched samples to confirm presumptive positives according to USDA-FSIS Microbiology Laboratory Guidebook (MLG) or FDA Bacteriological Analytical Manual (BAM) methods for the detection of *E. coli* O157.

Disposal

Decontaminate used test strips, pipettes and enrichments by autoclaving or according to your site practices. Ensure all biohazardous waste is disposed of according to local, regional and national regulations.

Precautions

E. coli O157 has a very low infective dose (approximately 50 cells). Extreme care should be used in handling samples, enriched media and used test strips.

Validation

Samples of this test kit model were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specifications as stated in the test kit's descriptive insert. The producer certifies this kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in the Performance Tested Certificate number 010601.

Note: Although this test is capable of detecting target pathogen present in enrichment media at the detection level sensitivity of the test strip, the successful detection of the target pathogen in a specific food matrix is dependent upon the ability of the target pathogen to adequately reach the test's detection level in the enrichment media. This ability may be influenced by a variety of factors, including but not limited to competitive flora, sample matrix, sample size and condition of the target pathogen.

Technical Assistance

For questions or comments, please contact your local distributor. You can also call 302-695-5300 in the U.S. or email diagnostics.support@hygiena.com.

Limitation of Warranty and Liability

NOTICE: READ THIS LIMITATION OF WARRANTY AND LIABILITY BEFORE USING THE HYGIENA™ LATERAL FLOW SYSTEM. If the terms are not acceptable, notify Hygiena immediately and arrangements will be made for return of the unused test strips and/or media to Hygiena and for the refund of the purchase price, less shipping costs. USE OF HYGIENA™ LATERAL FLOW SYSTEM TEST STRIPS AND/OR MEDIA CONSTITUTES AN ACCEPTANCE OF ALL TERMS AND/OR CONDITIONS OF THIS LIMITATION OF WARRANTY AND/OR LIABILITY. Any additional or different terms in User's purchase form(s) are material alterations and hereby rejected.

Hygiena warrants that the Hygiena™ Lateral Flow System test strips and/or media will be free of defects in materials and workmanship when used in accordance with the applicable instructions to the expiration date marked on the product label. Application protocols published by Hygiena are intended as guidelines; each User is expected to validate the applicability of each protocol to their individual applications.

HYGIENA MAKES NO OTHER WARRANTY, EITHER EXPRESSED OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING.

The sole obligation of Hygiena with respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the Product(s) that proves defective in materials or workmanship within the warranty period, provided the User notifies Hygiena promptly of any such defect.

The accuracy of the Hygiena™ Lateral Flow System can be affected by factors over which Hygiena has no control, including, without limitation, the use of the test strips and/or media in a manner that is contrary to the conditions of use, the procedures or the instructions specified by Hygiena. Because of the large number of factors over which Hygiena has no control, Hygiena makes no promise or guarantee of the accuracy of results obtained from the use of the Hygiena™ Lateral Flow System. In particular, Hygiena disclaims any warranty or liability and assumes no responsibility whatever for the failure of the Hygiena™ Lateral Flow System due, in whole or in part, to User's failure to (a) properly maintain equipment, (b) maintain specified operating or storage conditions, (c) follow the specified instructions, or (d) use the proper microbiological techniques consistent with the standard of care accepted in the industry for the proper collection, storage, handling and preparation of the sample.

Externally caused failures, such as improper sample preparation, improper storage or loading of reagents, electrical outages, or out-of-specification environmental conditions are not covered under this warranty. Circumstances beyond the reasonable control of Hygiena, including fire, explosions, accidents, flood, labor trouble or shortage, war, act of or authorized by any government, inability to obtain suitable material, equipment, fuel, power or transportation, or acts of God are not covered under this warranty.

The Hygiena™ Lateral Flow System is designed to test only for the presence of the target organisms specified in the particular assay. Hygiena™ Lateral Flow System has been tested against many, but not all, strains of the target within the sample types specified in the package insert. Hygiena, therefore, cannot and does not make any representation or warranty that the Hygiena™ Lateral Flow System is capable of detecting every bacterium in the target genus, serotype, or species in any sample source. Accordingly, the Hygiena™ Lateral Flow System should not be used as the sole test for the release of User's products, nor should it be used as the sole basis for determining the safety of User's products.

USER ASSUMES ALL RISKS IN USING THE HYGIENA™ LATERAL FLOW SYSTEM. HYGIENA OR ITS AFFILIATES, SUPPLIERS, DISTRIBUTORS, ITS LICENSORS OR REPRESENTATIVES SHALL HAVE NO LIABILITY TO BUYER OR TO ANY OTHER PERSON OR ENTITY FOR ANY INDIRECT, INCIDENTAL, SPECIAL, OR CONSEQUENTIAL DAMAGES WHATSOEVER, INCLUDING, BUT NOT LIMITED TO, LOSS OF REVENUE OR PROFIT, LOST OR DAMAGED DATA OR OTHER COMMERCIAL OR ECONOMIC LOSS EVEN IF CAUSED BY THE NEGLIGENCE OF HYGIENA OR ITS AFFILIATES, SUPPLIERS, DISTRIBUTORS, ITS LICENSORS OR REPRESENTATIVES AND/OR IF HYGIENA HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND/OR IF THEY ARE FORESEEABLE.

Not an insurer: By selling Hygiena™ Lateral Flow System products or services, Hygiena or its representatives do not become an insurer of User's business. Hygiena or its representatives do not and cannot know all of the potential consequences to User's business of a failure of the Hygiena™ Lateral Flow System to perform as expected. That is why Hygiena does not agree to be responsible for User's incidental or consequential business losses in the event the Hygiena™ Lateral Flow System fails to perform as expected. For the same reason, Hygiena has limited its liability to the cost of the product. For additional protection against the risk of loss, User should consult an insurance broker and buy insurance suitable to the risks of its particular business.