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ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
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Solus *E. coli* O157 ELISA

ELISA Based Test System For the Detection of *E. coli* O157 in Foods and Environmental Samples

Product Number: EC-048S
This method is certified by AFNOR Certification for the detection of *E. coli* O157, including H7, in raw beef meat products (seasoned or not) raw milk and dairy products, vegetables and environmental samples (validation ref. no. SOL 37/03-10/15 valid until 14/10/2019).

1. INTRODUCTION

The rapid detection of pathogens in foods is essential to ensure the safety of consumers. *E. coli* O157 detection in samples is inherently difficult because often there is a small number of target bacteria found amongst a large number of very similar competing flora. Traditional methods for the detection of food borne bacteria use time consuming methods of growth in culture media followed by isolation, biochemical identification and serology. Consequently, results are obtained within 3 to 5 days.

The Solus *E. coli* O157 kit provides a negative or a presumptive positive result in 18 to 22 hours (including two hours for the assay), after a single enrichment step.

The ELISA part can be performed in less than two hours; therefore the presumptive results may be obtained as early as 18 hours.

2. INTENDED USE

The test method is easy to perform, however it requires laboratory facilities plus qualified and trained personnel. Basic training is recommended to first time users and is given by Solus Scientific Solutions Ltd.
The detection limit of the kit is in the range of 10^4 – 10^5 cells per ml in the enrichment broth.
Using the method includes compliance with Good Laboratory Practices (refer to EN ISO 7218).

3. REAGENTS PROVIDED

Kit components are supplied stabilised and ready to use at working concentration. Only the Washing Buffer has to be diluted 25 times in deionised water.
Each kit contains sufficient material for 1 x 93 determinations, plus controls.
The kit expiry date is displayed on each product label.

- 1 x 96 well microplate (in breakable strip format). Wells are coated with antibodies against *E. coli* O157.
- Negative Control (Green label). 3 ml in working dilution. Contains diluent with preservative.
- Positive Control (Red label). 3 ml in working dilution. Contains heat-killed *E. coli* O157 in diluent with preservative.
- Conjugate (Orange label). 11 ml in working dilution. Contains horseradish peroxidase-antibody conjugate in diluent with stabilisers.
- Substrate. (Blue label). 11 ml in working dilution. Contains 3,3',5,5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers. Solution should be clear or slightly faint blue solution.
- Stop Solution. (Yellow label). 11 ml in

working dilution. Contains 10% sulphuric acid.
- Washing Buffer Concentrate (25x). 6 x 10ml. Contains 0.25MTris-HCl/3.5M NaCl/ 1.25% Tween 20 and preservative, pH 8.0.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Refrigerator at 2-8°C.
- Deionised water
- Modified Tryptone Soya Broth + novobiocin (20mg/L)
- Measuring cylinder for 250 ml or 1L
- Sterile 10 ml test tubes suitable for selective enrichment culture
- Stomacher (or similar apparatus) and bags
- 3ml transfer pipettes (sterile)
- Test Tube for sample boiling (e.g. 5ml polypropylene rimless test tubes 13x75mm
- Vortex mixer
- Timer
- Incubator at 37±1°C
- Incubator at 41.5±1°C
- Water bath (capable of heating to 85-100°C)
- Pipettes and tips (1ml; 0.1ml)
- Microplate Washer
- Microplate Reader with 450 nm filter
- Autoclave for decontamination of samples

5. REAGENT PREPARATION

5.1. Wash Buffer:
Add the contents of a Wash buffer bottle (10ml) to 240ml of deionised water to prepare the wash buffer at assay concentration. Dispense and label as appropriate. The prepared solution can be stored at 2-8°C for a maximum of 4 weeks.
5.2. Culture Broth (growth medium):
Prepare the modified Tryptone Soya Broth following manufacturer's instructions. Allow to cool to room temperature before adding the novobiocin supplement at 20mg/L (mTSBn).

6. SAMPLE PREPARATION AND ENRICHMENT- standard method

25g of the sample to be tested is homogenised, if necessary by stomacher, and incubate for 16-20 hours in 225ml Modified Tryptone Soya Broth + novobiocin (20mg/L) at 41.5±1°C.
In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.
When the incubation period in mTSBn is completed transfer 1ml aliquot (free of sample debris) to a glass or polypropylene test tube.

The unboiled mTSBn samples should be kept for verification until ELISA results are obtained. If this is not possible keep the broths for 48 hours at 2-8°C prior to the ELISA test.

Heat the aliquot to 85-100°C for 15-20 minutes in the test tube. After heating, allow the sample to cool to room temperature. This may be accelerated by placing the test tubes in cold tap water for 5 minutes.

7. ELISA ASSAY PROCEDURE

7.1. Take the test kit from storage at 2-8°C one hour before use to allow the components to reach room temperature. Determine the number of wells required for the test. Take the required number of strips from the pouch and fit them to the frame provided. Unused strips should be returned to the pouch and stored at 2-8°C.
7.2. Prepare Wash Buffer as detailed in section 5.1.
7.3. Leave the first well in the strip empty to serve as a 'blank' for measuring the absorbance of the substrate.
7.4. Pipette 0.1ml of Negative Control (Green label) into the second well.
7.5. Pipette 0.1ml of Positive Control (Red label) into the third well.
7.6. Pipette 0.1ml of each boiled/ cooled sample separately into consecutive wells in the strip. If there are wells left over at the end of a test strip the Positive or Negative Controls may be repeated.
7.7. Incubate the plate (containing the strips) at 37±1°C for 30 - 35 min.
7.8. After incubation, aspirate the contents of the wells, removing as much of the liquid as possible. Wash the wells 5 times with wash

buffer ensuring complete filling and emptying of the wells through each wash cycle. The washing technique is critical to assay performance, hence it is recommended to use a microplate washer instrument.

If a manual washing technique is used we favour the use of a 'squeezy' wash bottle to fill the wells or alternatively a multichannel pipette can be used, then tap out the wells onto clean absorbent towel to remove excess wash buffer between all the washes.

7.9. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.
7.10. Incubate the plate at 37±1°C for 30 mins. (5 mins. extra incubation time is tolerated).
7.11. Repeat the wash cycles as detailed in section 7.8
7.12. Pipette 0.1ml of TMB Substrate (Blue label) into all wells, including the 'blank' well. 7.13. Incubate the plate at room temperature (18 - 30°C) for 10 mins. in the dark. Start stopwatch at well A1.
7.14. After incubation stop the reaction by adding 0.1ml of Stop Solution (Yellow label) to all wells including the 'blank' well. The stop solution will cause the blue colour in wells to change to yellow.
7.15. The optical densities need to be read within 10 minutes in a plate reader using a 450nm filter. Inspect the wells before reading for air bubbles and if present burst with a needle. The reader should be zeroed against the 'blank' well before the other wells are read. Do not use reference filter.

The use of Automatic ELISA equipment is preferred and should be set up and validated to this protocol.

8. INTERPRETATION OF RESULTS

Interpretation: Results are expressed as optical density (OD_{450}) measurements using a Microplate Reader.

Assay acceptance criteria:
Negative Control $OD_{450} < 0.100$
Positive Control $OD_{450} > 0.500$
Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be performed again. Typical values for the negative control are 0.001- 0.050 and for the positive control 0.900-1.700. The value of the blanking well (usually A1) should always be subtracted.

Samples with OD_{450} readings of less than 0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding non-boiled aliquot of mTSBn broth may be discarded following local regulations/guidelines.

Sample wells with $OD_{450} > 0.200$ are considered presumptive positive for *E. coli* O157. Presumptive positive results must be verified using a recognised culture method.

9. CONFIRMATION OF POSITIVE RESULTS FROM *E. coli* O157 ELISA

In the context of NF VALIDATION, all samples identified as positive by the alternative method must be confirmed by one of the following tests:
- Using the conventional tests described in the standardised methods by CEN or ISO from colonies. The confirmation step must start from the (non-heated) mTSB+n broth stored at 41.5°C or 2-8°C. (See ISO 16654:2001 Horizontal method for the detection of Escherichia coli O157).

- Streaking mTSB+n (10µL) onto CT-SMAC and a chromogenic agar plate (such as CHROMagar O157), and incubate the plates at 37°C±1° for 18-24 hours. Perform a serological identification of characteristic colonies, with or without a purification step, using an O157 latex test (Microgen E. coli O157 M44) and after a purification step using H7 latex test (Wellcolex E. coli O157:H7 R30959601).
-In the event of discordant results (presumptive positive ELISA results and a

negative culture result) an IMS step must be used as a confirmation step by taking 1 ml of mTSB+n (non-heated) and follow the method as described in ISO 16654:2001 prior to streaking the magnetic particles onto both agar plates. This method is more sensitive than direct plating and can help to confirm samples that contain lower levels of target organism.

In the event of discordant results (presumptive positive ELISA result, non-confirmed by one of the means described above and in particular for the latex tests) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

10. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. DO NOT FREEZE.
The kit expiry date is displayed on the kit box plus all of the kit components within the box.
Any unused diluted wash buffer can be stored at 2-8°C for up to 4 weeks.
Any unused microplate strips should be returned to the foil pouch with the desiccant sachet and the seal closed completely. They can be stored at 2-8°C for up to 4 weeks.

11. SAFETY

The Stop Solution contains sulphuric acid which is corrosive. Wash immediately with large quantities of water if the solution comes into contact with skin or mucous membranes.
While the procedures detailed are simple and easy to perform, they require laboratory facilities with qualified and trained personnel for the handling of potentially pathogenic organisms. Training is recommended to first time users and is provided by Solus Scientific Solutions Ltd. As a guide, the following precautions should be taken as a minimum:

- Protective clothing should be worn including safety glasses, mask and gloves where appropriate.
- Do not pipette by mouth.
- Avoid contact with the skin.
- Do not eat, drink or apply cosmetics in the laboratory.

12. PRECAUTIONS

- Reagents are provided at fixed working concentration. Optimum sensitivity and specificity will be reduced if reagents are modified or not stored under the recommended conditions.
- Do not mix different lots of reagents.
- Avoid microbial contamination of opened reagent bottles.
- Ensure that no cross contamination occurs between wells.
- It is essential for proper performance of the test that the enzyme-conjugated antibody is not allowed to contaminate other reagents and equipment.
- Ensure that kit components are not exposed to temperatures greater than 40°C.
- It is not recommended to use solutions containing sodium azide for cleaning of equipment especially washing devices (the peroxidase enzyme used in the kit is inactivated by sodium azide).
- Do not use for diagnostic purposes of medical specimens.

13. MSDS INFORMATION

Material safety data sheets (MSDS) are available for this test on request.

14. WARRANTY

Accurate results depend on the proper use of the kit by following the instructions for use carefully. If the kit fails to perform according to specification please contact:

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