CompactDry™ EC

Simple and Easy Dry Medium for Coliform and *Escherichia coli*

Background

It is important to detect and measure *Escherichia coli and* coliforms in foodstuffs and the food production environment to monitor the degree of cleanliness and sanitary safety and limit the possibility of food poisoning, especially as *E.coli* is an indicator of faecal contamination. Detecting and measuring *E.coli* and coliforms is required in food industry. The standard pour plate method is widely used to determine microbial count. However, the pour plate method is time consuming and labour intensive. To save operator time in performing microbial count without difficulty, Shimadzu Diagnostics Corporation developed CompactDry™ plates based on a new technology applicable to the food industry. CompactDry™ only requires a simple step to add a drop of sample onto the surface of a pre-prepared dehydrated media plate.

Features and Benefits

1. A compact media plate: Only requires a small space for storage and incubation.
2. Ready to use: No need to prepare culture medium, therefore reducing both waste and equipment required. It is also well adapted for emergency and field testing.
3. The sample diffuses passively and evenly across the dehydrated media sheet, rehydrating the dry medium into a gel within seconds.
4. 24-month shelf life at room temperature: Easy to store.
5. Clear-cut colour development: *E. coli* colonies are blue/blue purple in colour and other coliforms form red/pink colonies. Easy to interpret. Isolated colonies can be subculture individually.
6. Good correlation with the pour plate method: Maintains data comparability.

Intended Use

This product is intended for the enumeration of coliforms and *Escherichia coli* in food and related samples.

Certification by AOAC

CompactDry™ EC has been compared to AOAC *OfficiaI Method℠* **966.24** and certified by the AOAC Research Institute *Performance Tested Methods℠* Program (Certificate No. 110402) for enumeration of coliforms in raw meat (raw ground beef, raw ground pork, raw pork, raw lamb, and raw veal). A matrix extension comparing the CompactDry™ EC to ISO 4832:2006 and ISO 16649-2:2001 for cooked chicken, fresh pre-washed bagged shredded iceberg lettuce, frozen cod filets, instant non-fat dry milk, and pasteurized 2% milk was approved in 2015.
Incubate at 35 ± 1°C for raw meat or 37 ± 1 °C for all other matrices during 24 ± 2 h.
Follow guidelines provided in the Interpretation section of this document.

Certification NF VALIDATION by AFNOR Certification

The CompactDry™ EC methods has been certified "NF VALIDATION" as alternative analysis methods for the enumeration of coliforms (N°SDC 42/01-12/24) and *Escherichia coli* (N°SDC 42/02-12/24) in all human food products (by performing validation assays in a broad range of foods) and industrial production environmental samples.
This validation has been obtained according to EN ISO 16140-2: 2016 protocol and by comparison with both reference methods:

- NF ISO 4832:2006 for **coliforms;** two incubations temperatures are proposed (30°C and 37°C with a note on dairy), and this validation has been performed at 37°C only.
- NF ISO 16649-2:2001 for ***Escherichia coli.***

For more information about the end of validity of the "NF VALIDATION" certification, please refer to the certificates N°SDC 42/01-12/24 and N°SDC 42/02-12/24 available on the website http://nf-validation.afnor.org/en and/or on request asking Shimadzu Diagnostics Corporation.
Follow guidelines provided in the Interpretation section of this document.

Certification by MicroVal/NordVal

CompactDry™ EC has been validated (MicroVal 2008LR04/05) in accordance with ISO 16140-2: and is at least equivalent to the reference method ISO 16649-2:2001 for the enumeration of *E.coli* and ISO 4832:2006 for the enumeration of coliforms.
Scope of validation: Broad range of foods, environmental samples, pet food and animal feed. Validation has been performed on a broad range of foods (dairy products, fish products, fresh and processed produce, meat and meat products, poultry and poultry products), pet food and environmental samples. The validation report is available on the MicroVal website: www.microval.org

Test Kit Components

1. CompactDry™ EC Plates

Additional Reagents and Supplies Required, Not Provided

1. Butterfield's phosphate-buffered diluent (BPBD) – Prepare according to AOAC **966.24**
2. Maximum Recovery Diluent (MRD) – Prepare according to ISO 4832:2006 (AOAC)
3. Buffered Peptone Water (BPW) – Prepare according to ISO 6887 (NF VALIDATION)
4. Filtered Stomacher bags

Apparatus

1. Blender or Stomacher™ or equivalent for homogenizing sample
2. Sterile Pipette – 1 mL
3. Incubator

Operating Procedure

Sample preparation

For preparations of initial suspensions, follow instructions of EN ISO 6887 standards or ISO 18593 for environmental samples.

1. Sample preparation: Use appropriate sterile diluents: For further information, see section "**Specific Instructions for Validated Methods**"
2. Blend or homogenize sample (see section "**Specific Instructions for Validated Methods**")
3. If sample has a low or high pH, adjust the pH to 6.8 – 7.2 for an optimal growth and recovery of microorganisms

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General Instructions for CompactDry™ EC

Also refer to specific instructions for AOAC, NF validation, MicroVal and NordVal certifications

1. Open the aluminium pouch and take out a set of 4 plates and detach the number of plates required.
2. A set of four connected plates can be used for serial dilution of the same sample.
3. Enumeration range of CompactDry™ EC is 1–250 CFU/plate. Sample should be diluted in buffer to obtain a concentration level of less than 250 CFU/plate.
4. Remove cap from plate, pipette 1 mL of sample (diluted if necessary) in the middle of the dry sheet and replace the lid. The sample will diffuse passively and evenly across the dehydrated media sheet, rehydrating the dry medium into a gel within seconds.
5. Write the appropriate sample information in the label section.
6. Turn the plate over (lid down) and incubate for the appropriate time and temperature. (See specific instructions for validated methods**).** CompactDry™ plates are stackable up to 6 plates for incubation (NF validation).
7. Following incubation, count the number of coloured microbial colonies from the back of the plate. White paper placed under the plate can make colony counting easier.

Interpretation

1. CompactDry™ EC contains two chromogenic enzyme substrates, Magenta-gal and X-gluc. *E.coli* forms blue/blue purple colonies and coliforms (other than *E.coli)* form red/pink colonies. The total coliform count is the combined total of blue/blue purple colonies and red/pink colonies.
2. Refer to EN ISO 7218 standard for calculation and recording of results.
3. *E. coli* 0157 do not produce beta-glucuronidase and cannot be detected as *E. coli* on CompactDry™ EC. *E.coli* 0157 form red/pink coloured colonies on CompactDry™ EC.
4. During the inclusivity study (NF VALIDATION):
   - 2 strains, *Escherichia vulneris* and *Buttiauxella agrestis,* gave typical blue colonies using Compact Dry™ EC while no colony was present on TBX plates. The identification of these two strains has been confirmed.
   - 3 coliform strains (*Citrobacter braakii, Kluyvera intermedia* Adria 860*, Kluyvera intermedia* Ad3003), gave lower enumeration on Compact Dry™ EC than on VRBL.

Specific Instructions for Validated Methods

AOAC® Official Methods℠ 966.24 (Certificate No 110402)

Scope of validation: Broad range of food products
Sample preparation: Prepare appropriate diluent: Butterfield's phosphate-buffered diluent (BPBD) for raw meat products or MRD for other claim matrices. Autoclave to sterilise.
Viable count in solid foodstuffs:

- For raw meat, weigh 50g of sample and add 450 mL BPBD to the sample. Homogenize by blender for 2 min ± 15s.
- For cooked chicken, fresh lettuce or frozen fish; weigh 10g of sample and add 90 mL MRD to the sample. Homogenize by stomacher for 1 min ± 15 s.
- For milk powder, weigh 10g of sample and add 90 mL MRD pre-warmed to 45 ± 1°C. Slowly swirl and shake until sample is in suspension.
- Viable count in liquid foodstuff and pasteurised milk: either use undiluted or if bacterial count is potentially > 250 CFU/plate, dilute 1 mL in 9 mL MRD and vortex to mix.

Incubate CompactDry™ EC plates at: Incubate at 35 ± 1°C for raw meat or 37 ± 1 °C for all other matrices during 24 ± 2 h.
Follow guidelines provided in **Interpretation** section of this document.

NF Validation by AFNOR Certification

NF Validation certified methods for coliforms (SDC 42/01-12/24) and *Escherichia coli* (N°SDC 42/02-12/24) have been validated in compliance with ISO 4832:2006 and ISO 16649-2:2001 respectively.
Sample Preparation: Buffered Peptone Water (BPW) is prepared (according to ISO 6887 - Part 1 to 5) and used to dilute all liquid and solid food matrices: weigh 10g of sample and add 90mL of BPW and homogenize by blender.
Environmental sample: It is recommended to use CompactDry Swab BPW (450004-BPW-0500) available as an optional kit.
Incubation: 37 ± 1°C for 24 h ± 2 h.
Interpretation: Calculate the number of microorganisms present in the test sample according to ISO 7218 for one CompactDry™ EC plate per dilution. Estimates are outside of the scope of the NF Validation certification

MicroVal (2008LR04/05) Certifications: Compliant with the ISO 16140-2:2016 in comparison with the reference method 4832:2006
Scope of validation: Broad range of foods (five categories), pet food and environmental samples.

Sample Preparation: Samples were diluted in an appropriate diluent according to ISO 6887 and homogenised in a stomacher
Incubation: 37 ± 1°C for 24 ± 2h.
Interpretation: Following incubation, red and otherwise coloured colonies were counted, and the CFU/g was calculated for each sample, according to ISO 7218.

Precaution for use

1. Comply with Good Laboratory Practices (refer to EN ISO 7218 standard).
2. Do not use CompactDry™ EC for human or animal diagnostics.
3. To avoid microbial contamination, do not touch the surface of the medium sheet before or after inoculation.
4. During incubation, keep lid tightly closed to avoid dehydration.
5. Use of filtered stomacher bags is recommended to eliminate risks of carryover of foodstuffs onto the surface of the medium.
6. If more than 10^4 CFU/mL are inoculated onto a plate, no distinguishable coloured colonies will form and the entire plate will become homogeneously coloured.
7. If the nature of the sample could affect the reaction with the chromogenic medium, it may require dilution, pH adjustment or other. This may also include high viscosity samples, coloured sample, and sample which could react with the redox indicator due to high or low pH.

Warning and precautions for use

1. General precautions

1. Read and follow warnings and precautions for use described in the package insert and/or label.
2. Do not use the product after its expiration date. Quality of the product is not guaranteed after shelf-life date is expired.
3. Do not use CompactDry™ EC if the plate is contaminated, it has become artificially discoloured or has a damaged container.
4. Use plates as soon as possible after opening. Return any unused plates to the aluminium pouch, seal with tape to avoid light and moisture and store at RT.
5. CompactDry™ EC is sensitive to light, which will affect the colour development of colonies.
6. Replace cap tightly after inoculation to avoid dehydration of gelled medium.

2. Safety Precautions

1. If medium or reagent comes into contact with eyes or mouth, immediately wash with water and consult a physician.
2. Manipulation of microorganisms involves a risk of laboratory acquired infection. Manipulation should be carried out under the supervision of trained laboratory personnel with biohazard protection measures.
3. Treat any laboratory equipment, or medium that comes into contact with the sample, as infectious and sterilize appropriately.

3. Precautions for waste disposal

Sterilize any medium, reagent or materials by autoclaving after use, and then dispose as industrial waste according to local laws and regulations for disposal of such material.

4. User Responsibility

1. It is the user's responsibility in selecting any test method to evaluate a sufficient number of samples with particular foods and microbial challenges to satisfy the user that the chosen test method meets the user's criteria.
2. It is the user's responsibility to determine that any test methods and results meet its customers' or suppliers' requirements. The user must train its personnel in proper testing techniques.
3. It is the user's responsibility to validate the performance of this method for use with any non-certified matrix.

5. Limitation of Warranties

CompactDry™ plates are manufactured at an ISO 9001:2015 facility.
If any CompactDry™ plate is proven to be defective by fault of the manufacturer, or its authorized distributors, they may replace or, at their discretion, refund the purchase price of any plate.

Storage and Shelf life

Storage: Room temperature (1 – 30 °C).
Shelf life: Twenty-four (24) months after manufacturing.
Expiration date is printed on outer box label and aluminium pouch label.

Package

CompactDry™ EC 100 plates Code HS8781

References

1. ISO 4832:2006 - Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique
2. ISO 16649-2:2001 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide
3. ISO 18593:2018 Microbiology of the food chain — Horizontal methods for surface sampling
4. ISO 16140-2:2016. Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
5. ISO 6887 (Part 1 to 5). Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination
6. ISO 7218. Microbiology of the food chain - General requirements and guidance for microbiological examinations.

Tracking version

Date	Action	Name
July 2023	Issue	SDE-IFU-EC-2023-V01
February 2024	Revision	SDE-IFU-EC-2024-V02
October 2024	Revision	SDE-IFU-EC-2024-V03
November 2024	Revision	SDE-IFU-EC-2024-V04
December 2024	Revision	SDE-IFU-EC-2024-V05

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BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

SDC 42/01-12/24
SDC 42/02-12/24

ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

http://nf-validation.afnor.org/en

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