菌数測定用簡易培地 大腸菌・大腸菌群数測定用

* JUNGHHST" EC

*----- 開発の経緯および特徴 -----

食品の安全性を確保する上で、食品や環境中の微生物数を測定することは極めて 重要です。従来より行われている汚染指標菌の混釈培養検査は培地の準備や試料の 混釈操作に多くの労力と経験を必要としていました。

コンパクトドライ[™] E C は、このような負担を軽減し、誰でも、どこでも、簡便に 混釈培養が行えるよう開発した培地で、試料液を加えるだけの操作で菌数検査が実 施できます

「特 徴]

- 1) コンパクトなサイズなので場所をとりません。
- 2) 培地調製の必要がありません。
- 3)接種した試料は自然に均一に拡散します。
- 4) 室温で保存可能です。
- 5) コロニーの発色が明りょうで、釣菌も容易にできます。
- 6) 従来の混釈培養法のデータと整合性がとれます。

——操作法——

[試料の調製、接種方法]

1. 固形食品材料の菌数測定

材料に緩衝液を添加し、ストマッカーで均質化します。試料液 1 mL (必要に応じて希釈する)を取り、本品に接種します。

2. 水や液状食品の菌数測定

試料液1mL(必要に応じて希釈する)をそのまま本品に接種します。

**/*3. ふき取り材料の菌数測定

綿棒などで食品や環境材料をふき取ったふき取り液 $1\,\mathrm{mL}$ (必要に応じて希釈する)を本品に接種します。

簡易ふき取りキット (BPW) Ⅱ ガンマ線滅菌 (コード06544) 又は簡易ふき取りキット (PBS) CC Ⅱ ガンマ線滅菌 (コード06545) を使用すると便利です。

[使用法]

1)アルミ袋を開封し、4連のプレートを取り出します。

- 2)検査に必要な枚数のプレートを折り曲げて切り離します。段階希釈した試料液を接種するときは切り離さずそのまま使用すると便利です。
- 3) プレートのフタを開け、シートの中央部に試料液 $1\,\mathrm{mL}$ を接種します。試料液はシート全体(培地面積は $20\,\mathrm{cm}$)に均一に広がりゲル化します。
- 4) フタをした後、倒置しフラン器に入れて、35±1℃で24±2時間培養します。
- 5)発色したコロニーを裏面からカウントします。下に白い紙などを置いてカウントするとコロニーが見やすくなります。

----- 操作上の留意事項 -----

- 1) 試料の接種に際しては、落下菌による汚染や培地面に手指が触れるなどの汚染に注意してください。
- 2)培養中の乾燥を防ぐため、フタはしっかりと閉めてください。
- 3)食材片の持ち込みによる影響を防ぐため、なるべくフィルター付きストマッカー袋を使用してください。
- 4) 試料は1プレートあたり300 cfu以下になるように緩衝液などで希釈してから接種します。滅菌希釈液Ⅱ(コード01553)を使用すると便利です。
- 5) 1 プレートあたり10⁴ cfu以上の菌が接種されるとコロニーが形成されないため 発色コロニーが現れず、シート全体が着色したようになります。
- 6) 食品自体が培地の反応に影響を与えるものは、緩衝液などで希釈する等、その 原因を取り除いてから接種してください。
- 例:粘度の高いもの、濃く着色したもの、発色基質と反応するもの、pHが極端 に高いかまたは低いもの。

—— 判 定 法——

[判定法]

MAGENTA-GALおよびX-GLUの2種類の発色酵素基質が培地中に含まれており、 大腸菌は青~青紫色、大腸菌群はピンク~赤紫色に発色します。大腸菌群数として は両発色コロニー数の合計数で算出します。

「判定上の注意事項]

- 1)シート状培地の面積は20 cmです。また、プレート底面には計測に便利な格子 (1 cm×1 cm) を薄くつけてあります。菌数が多い場合は、代表的な格子内のコロニー数を算出して、その値に20を掛けて菌数を算出します。
- 2)大腸菌O157はβ-グルクロニダーゼを産生しないため、大腸菌としての検出はできず、ピンク~赤紫色コロニーを形成します。

─── 使用上または取扱い上の注意事項 ───

1. 一般的な注意事項

- 1)この添付文書をよく読み、記載された操作法、注意に従って使用してください。 2)使用期限を過ぎた製品は品質を保証できないので使用しないでください。
- 3) 使用前に容器の破損、異物混入、変色、吸湿等の異常が認められた培地は使用しないでください。
- 4) 残ったプレートは、アルミ袋に入れ、テープ止めをして防湿および遮光保存し、 早めに使用してください。

2. 危険防止上の注意事項

- 1) 試薬等が目や口に入った場合には、水で十分に洗い流し、医師に相談し、指示を受けてください。
- 2) 微生物の取り扱いは常に感染の危険があるので、取り扱いにあたっては熟練した人の指導のもとに、バイオハザード対策を実施したうえで使用してください。 3) 検体に接触した器材、培地等は感染の危険があるものとして取り扱いください。

3. 廃棄上の注意事項

使用済みの培地は高圧蒸気滅菌または十分に煮沸するなど殺菌処理をしたのちに 廃棄してください。

----- 貯法・使用期限 -----

[貯 法]

室温(1~30℃)に保存してください。

[使用期限]

製造後24カ月間。

外箱のラベルおよびプレートのアルミ袋に使用期限を表示してあります。

*── 包装単位 ──

*----- 問い合わせ先 -----

〒110-0005 東京都台東区上野 3-24-6

島津ダイアグノスティクス株式会社 カスタマーサポート担当

電話: 03(5846)5707

*製造販売元

島津ダイアグノスティクス株式会社

東京都台東区上野 3 - 24 - 6 〒110-0005 TEL 03(5846)5611(代)

**CompactDryTM EC

Simple and Easy Dry Medium for Coliform and Escherichia coli

* *Background

It is important to detect and measure coliforms and *Escherichia coli* in foodstuffs and the food environment to monitor the degree of exposure and limit the possibility of food poisoning, especially as *E.coli* is an indicator of fecal contamination. Measuring *E.coli* and coliforms simultaneously is needed in food market

The pour plate method has been widely used to determine microbial counts, however, it is time consuming and complicated, requiring operations such as preparation of hot agar maintained at $45 - 50^{\circ}$ C, and uniform mixing and dilution. To save operator time and allow a trained laboratory scientist to perform the microbial count test without difficulty, the CompactDryTM was developed based on new concept and technology applicable to the food industry. CompactDryTM requires a simple and easy manipulation to add a drop of specimen on the device.

Features and Benefits

- 1) Small and compact plate: Need only small physical spaces for storing, testing and incubating.
- 2) Ready to use and portable plate: No need to prepare medium, which eliminates the waste of medium as well as the apparatus to prepare the medium. Good for an emergency and field testing.
- 3) Sample diffuses automatically and evenly into the plate: No need for mixing and diluting after sampling.
- *4) Dried plate with 24 month shelf life at room temperature: Easy to store. Once a liquid sample is added, the dry coated medium transforms into a gel and the plate is ready to incubate.
- 5) *E. coli* colonies are blue/blue purple in color and other coliforms form red/pink colonies. Isolated colonies can be subcultured individually to other media.
- 6) Good correlation with pour plate method: Maintain the continuity of data accumulated.

Certification by AOAC

The CompactDry™ EC has been compared to AOAC *Official 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.

* * Test Kit Components

1) CompactDry™ EC Plates

Additional Reagents and Supplies Required, Not Provided

- Butterfield's phosphate-buffered diluent (BPBD) Prepare according to AOAC 966.24
- 2) Maximum recovery diluent (MRD) Prepare according to ISO 4832:2006
- 3) Filtered Stomacher bags

Apparatus

- 1) Blender or StomacherTM or equivalent for homogenizing sample
- 2) Pipets 1 mL
- 3) Incubator $-35 \pm 1^{\circ}$ C (raw meat products) or $37 \pm 1^{\circ}$ C (all other matrices)

Operating Procedure

Preparation of specimen

- 1) Prepare appropriate diluent: Butterfield's buffered phosphate diluent (BPBD) for raw meat products or Maximum Recovery Diluent (MRD) for other claimed matrices. Autoclave for sterilization.
- 2) 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 \pm 15 s. For cooked chicken, fresh lettuce, or frozen fish, weigh 10 g of sample and add 90 mL MRD. Homogenize by Stomacher for 1 min \pm 10s. For milk powder, weigh 10 g of sample and add to 90 mL MRD pre-warmed to $45\pm1^{\circ}$ C. Slowly swirl and shake until sample is dissolved.

3) Viable count in liquid foodstuffs

For pasteurized milk, use without dilution, dilute 1 mL in 9 mL MRD., or dilute further if viable count is >250 CFU/plate. Vortex to mix.

**4) Viable count in swab test sample (not included in AOAC PTM certification)
Use wiping solution (without dilution or diluted if necessary in MRD)
obtained from the cotton swab. It is recommended to use Swab Test ST25PBS (Code 06698) available as an optional kit.

Shelf li

**Package
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Direction for CompactDry™ EC

- 1) Open aluminum bag and remove a set of 4 plates.
- 2) Detach necessary number of plate(s) from a set of four by bending up and down while pressing the lid. Use a connected set of four plates when serial dilution measuring is intended.
- 3) Remove the cap ofrom the plate, pipette 1 mL of sample (to be diluted further if necessary) in the middle of the dry sheet, and replace cap. Specimen diffuses automatically and evenly over the entire sheet (total medium of 20 cm²) to transform it into gel within seconds.
- 4) Write the appropriate information on the memorandum section. Invert the capped plate and place in incubator at 35 \pm 1 °C for raw meat or 37 \pm 1 °C for all other matrices. Incubate 24 \pm 2 h.
- 5) From the backside of the plate, count the number of colored colonies in the medium. White paper placed under the plate can make colony counting easier. For large numbers of colonies, use the grids carved on the backside consisting of 1 cm x 1 cm, or 0.5 cm x 0.5 cm, at the four corners.
- 6) The enumeration range of the CompactDry™ EC is 1-250 CFU/plate. Specimen should be diluted in the appropriate diluent to obtain a concentration level in the countable range.

Precaution for use

- 1) Do not use CompactDry™ EC for human and animal diagnosis.
- 2) To avoid microbial contamination, do not touch the surface of the dry sheet medium during inoculation.
- 3) During incubation, keep cap tight to avoid any possible dehydration.
- 4) Use of filtered stomacher bags is recommended to eliminate risk of carry over of tiny pieces of foodstuffs onto the surface of the medium.
 5) If more than 10⁴ CFU/mL were inoculated onto a plate, no distinguishable
- 5) If more than 10⁴ CFU/mL were inoculated onto a plate, no distinguish colored colonies will form and the entire plate will become colored.
- **6) If the nature of sample affects the reaction of the medium, inoculate the sample only after the factor has been eliminated by means such as dilution, pH adjustment or other. This may include samples with high viscosity, that are colored, that react with the chromogenic enzyme substrate, or that have too high or too low pH.

Interpretation

- 1) The medium 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) *E. coli* O157 do not produce beta-glucuronidase and, thus, cannot be detected as *E. coli* on CompactDry™ EC. *E. coli* O157 form red/pink colored colonies on CompactDry™ EC.
- 3) The full plate size is 20 cm². The backside contains carved grids of 1 cm x 1 cm and 0.5 cm x 0.5 cm to make colony counting easier. If large numbers of colonies are present on the medium, the total viable count can be obtained by averaging the number of colonies per large grid (1 cm x 1 cm), counted from several grids, and multiplying by 20. Alternatively, the total viable count can be obtain by averaging the number of colonies per small grid (0.5 cm x 0.5 cm), counted from several grids, and multiplying by 80.

Warning and Direction for Use

1. General precautions

- 1) Read and follow precisely the warnings and directions 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 its shelf life.3) Do not use product that contains any foreign materials, is discolored or
- dehydrated, or has a damaged container.
 4) Use plates as soon as possible after opening. Any unused plates should be
- returned to the aluminum bag and sealed with tape to avoid light and moisture.

 5) Cap tightly after inoculation to avoid dehydration of gelled medium.

2. Safety Precautions

- 1) Wash immediately with water if medium or reagent comes into contact with eyes or mouth. Consult a physician.
- 2) Manipulations with microorganisms involve certain risks of laboratory-acquired infections. Practice manipulations under the supervision of trained laboratory personnel with biohazard protection measures.
- 3) Treat laboratory equipment or medium that comes into contact with the specimen as infectious.

3. Precautions for waste disposal

Sterilize any medium, reagent and materials by autoclaving or boiling after use, and then dispose as industrial waste according to local laws and regulations.

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
- 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

CompactDryTM plates are manufactured at an ISO 9001:2015 facility.

If any CompactDry™ plate is proven to defective by fault of the manufacturer or its authorized distributors, they may replace or, at their discretion, refund the purchase price of any plate. These are the exclusive remedies.

Storage: Keep at room temperature (1-30°C) Shelf life: Twenty-four (24) months after manufacturing

* Storage and Shelf life

Shelf life is printed on both label of outer box and aluminum bag.

* * Package CompactDry™ EC 40 plates ·······

**Further information

Customer Support Section Shimadzu Diagnostics Corporation

3-24-6, Ueno, Taito-ku, Tokyo 110-0005 JAPAN Tel: +81-3-5846-5707 contact@sdc.shimadzu.co.jp

** Manufactured by

Shimadzu Diagnostics Corporation

3-24-6, Ueno, Taito-ku, Tokyo 110-0005, Japan

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