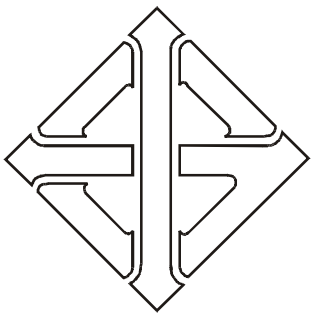

# มาตรฐานผลิตภัณฑ์อุตสาหกรรม

THAI INDUSTRIAL STANDARD

**มอก. 2240 เล่ม 2 – 2548**

**ISO/TS 11133 – 2 : 2003**

# จุลชีววิทยาของอาหารและอาหารสัตว์ – ข้อแนะนำในการเตรียมและผลิตอาหารเลี้ยงเชื้อ

## เล่ม 2 ข้อแนะนำในทางปฏิบัติสำหรับการทดสอบประสิทธิภาพอาหารเลี้ยงเชื้อ

MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS – GUIDELINES ON PREPARATION AND PRODUCTION OF CULTURE MEDIA

PART 2 PRACTICAL GUIDELINES ON PERFORMANCE TESTING OF CULTURE MEDIA

**สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม**

**กระทรวงอุตสาหกรรม**

ICS 07.100.30

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# มาตรฐานผลิตภัณฑ์อุตสาหกรรม

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**มอก. 2240 เล่ม 2 – 2548**

**สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม**
**กระทรวงอุตสาหกรรม ถนนพระรามที่ 6 กรุงเทพฯ 10400**
**โทรศัพท์ 0 2202 3300**

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**วันที่ 9 กุมภาพันธ์ พุทธศักราช 2549**

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| นายณฤทธิ์ ฤกษ์ม่วง | สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม |

การทดสอบทางจุลชีววิทยาที่เชื่อถือได้มีความจำเป็นต้องใช้อาหารเลี้ยงเชื้อที่ผ่านการตรวจสอบคุณภาพ การกำหนดเกณฑ์การยอมรับขั้นต่ำสำหรับอาหารเลี้ยงเชื้อทุกชนิดที่ระบุไว้ในวิธีมาตรฐานจึงเป็นสิ่งสำคัญเพื่อให้มั่นใจว่าอาหารเลี้ยงเชื้อนั้นเชื่อถือได้ ข้อแนะนำในทางปฏิบัติสำหรับการทดสอบประสิทธิภาพอาหารเลี้ยงเชื้อฉบับนี้ใช้สำหรับ

ก) อาหารเลี้ยงเชื้อพร้อมใช้หรืออาหารเลี้ยงเชื้อชนิดแห้งที่ผลิตขึ้นเพื่อการค้า

ข) อาหารเลี้ยงเชื้อที่เตรียมขึ้นเองจากส่วนประกอบเฉพาะโดยห้องปฏิบัติการ

การจัดทำเกณฑ์ขั้นต่ำในการทดสอบประสิทธิภาพเมื่อเป็นที่ยอมรับอย่างกว้างขวาง เป็นผลให้อาหารเลี้ยงเชื้อที่ผลิตในเชิงพาณิชย์มีคุณภาพสม่ำเสมอและห้องปฏิบัติการลดจำนวนการตรวจสอบคุณภาพอาหารเลี้ยงเชื้อลงได้

นอกจากนี้เกณฑ์การยอมรับขั้นต่ำที่ทดสอบโดยวิธีที่ระบุในมาตรฐานฉบับนี้ ห้องปฏิบัติการทางจุลชีววิทยาสามารถนำไปใช้ทดสอบคุณสมบัติอาหารเลี้ยงเชื้อด้านการผลิต การคัดเลือก และ/หรือ การเลือกใช้

การทดสอบคุณภาพอาหารเลี้ยงเชื้อในมาตรฐานนี้มีความสำคัญเป็นลำดับแรกสำหรับการทดสอบทางจุลชีววิทยาของอาหารและอาหารสัตว์

คณะกรรมการวิชาการคณะที่ 957 มาตรฐานอาหารและผลิตภัณฑ์เกษตรด้านจุลชีววิทยา รับผิดชอบงานของคณะอนุกรรมการคณะที่ 9 ภายใต้กรรมการวิชาการคณะที่ 34 ขององค์การระหว่างประเทศว่าด้วยการมาตรฐานเป็นผู้จัดทำมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้จัดทำขึ้นเพื่อให้ทันกับความต้องการของผู้ใช้ และจักได้แปลเป็นภาษาไทยในโอกาสอันสมควร หากมีข้อสงสัยโปรดติดต่อสอบถามที่สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

คณะกรรมการมาตรฐานผลิตภัณฑ์อุตสาหกรรมได้พิจารณามาตรฐานนี้แล้ว เห็นสมควรเสนอรัฐมนตรีประกาศตามมาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ. 2511

**ประกาศกระทรวงอุตสาหกรรม**

**ฉบับที่ 3434 ( พ.ศ. 2548 )**

ออกตามความในพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม

พ.ศ. 2511

เรื่อง กำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม

จุลชีววิทยาของอาหารและอาหารสัตว์–ข้อแนะนำในการเตรียมและผลิตอาหารเลี้ยงเชื้อ

เล่ม 2 ข้อแนะนำในทางปฏิบัติสำหรับการทดสอบประสิทธิภาพอาหารเลี้ยงเชื้อ

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อาศัยอำนาจตามความในมาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ. 2511 รัฐมนตรีว่าการกระทรวงอุตสาหกรรมออกประกาศกำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม จุลชีววิทยาของอาหารและอาหารสัตว์–ข้อแนะนำในการเตรียมและผลิตอาหารเลี้ยงเชื้อ เล่ม 2 ข้อแนะนำในทางปฏิบัติสำหรับการทดสอบประสิทธิภาพอาหารเลี้ยงเชื้อ มาตรฐานเลขที่ มอก. 2240 เล่ม 2–2548 ไว้ ดังมีรายการละเอียดต่อท้ายประกาศนี้

ประกาศ ณ วันที่ 31 ตุลาคม พ.ศ. 2548

**นายสุริยะ จึงรุ่งเรืองกิจ**

รัฐมนตรีว่าการกระทรวงอุตสาหกรรม

# มาตรฐานผลิตภัณฑ์อุตสาหกรรม
# จุลชีววิทยาของอาหารและอาหารสัตว์
# ข้อแนะนำในการเตรียมอาหารเลี้ยงเชื้อ
## เล่ม 2 ข้อแนะนำในทางปฏิบัติสำหรับการทดสอบประสิทธิภาพอาหารเลี้ยงเชื้อ

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดขึ้นโดยรับมาตรฐาน ISO/TS 11133-2:2003 Microbiology of food and animal feeding stuffs- Guidelines on preparation and production of culture media-Part 2: Practical guidelines on performance testing of culture media มาใช้โดยวิธีพิมพ์ซ้ำในระดับเหมือนกันทุกประการ

คุณลักษณะทางเทคนิคนี้กำหนดเกณฑ์และวิธีสำหรับการทดสอบประสิทธิภาพของอาหารเลี้ยงเชื้อ ซึ่งสามารถใช้สำหรับ

- หน่วยงานที่ผลิต และ/หรือ จัดจำหน่าย อาหารเลี้ยงเชื้อพร้อมใช้ หรือกึ่งสำเร็จรูป หรืออาหารเลี้ยงเชื้อชนิดแห้งในห้องปฏิบัติการทางจุลชีววิทยา
- หน่วยงานที่ไม่ได้ดำเนินการเชิงพาณิชย์ซึ่งเตรียมอาหารเลี้ยงเชื้อให้หน่วยงานอื่น
- ห้องปฏิบัติการทางจุลชีววิทยาที่เตรียมอาหารเลี้ยงเชื้อเพื่อใช้เองและทำการทดสอบประสิทธิภาพของอาหารเลี้ยงเชื้อ

ข้อกำหนดหลักในมาตรฐานฉบับนี้ประกอบด้วยบทนิยามที่มีรายละเอียดเป็นไปตาม มอก. 2240-2548 หลักเกณฑ์การควบคุมคุณภาพที่ทำเป็นประจำซึ่งประกอบด้วย เกณฑ์คุณภาพทั่วไป เกณฑ์คุณภาพด้านจุลชีววิทยา การทดสอบประสิทธิภาพและการแปลผลข้อมูล วิธีที่ใช้สำหรับการทดสอบคุณภาพอาหารเลี้ยงเชื้อ และการจัดทำเอกสารผลการทดสอบ

# 1 Scope

This Technical Specification sets criteria and methods for the performance testing of culture media. This Technical Specification applies to:

— commercial bodies producing and/or distributing ready-to-use or semi-finished reconstituted or dehydrated media to microbiological laboratories;

— non-commercial bodies supplying media to third parties;

— microbiological laboratories preparing culture media for their own use and evaluating the performance of these media.

# 2 Normative references

This Technical Specification incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this Technical Specification only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

ENV ISO 11133-1:2000, *Microbiology of food and animal feeding stuffs —Guidelines on preparation and production of culture media —Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory (ISO/TR 11133-1:2000).*

# 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ENV ISO 11133-1:2000 apply.

# 4 Criteria for routine quality control

## 4.1 General quality criteria

### 4.1.1 Quality of culture media

The quality of culture media depends on the quality of the basic ingredients, correct formulation, quality of preparation procedures, elimination of contaminant microbial agents and appropriate packaging and storage conditions (see ENV ISO 11133-1).

The manufacturer or producer in the laboratory shall comply with the physico-chemical characteristics of the culture media as specified in the corresponding standard. Furthermore, quality assessment shall ensure that the culture medium conforms to stated recommendations, including*:*

— distributed quantity and/or thickness;

— appearance, colour and homogeneity;

— gel consistency;

— moisture content;

— pH value;

— buffering capacity;

— microbial contamination.

The individual components and any nutritive or selective supplements shall also undergo suitable quality assessment procedures.

#### 4.1.2 Quality of basic media components

Culture media described in the International Standards were judged satisfactory; however, due to the variability of their quality, it may be acceptable for media manufacturers to modify the concentration of some basic biological ingredients, as listed below:

— peptones and meat or yeast extracts variable in their nutritive properties;

— agar variable in its gelling properties;

— buffering substances;

— bile salts, bile extract and desoxycholate, antibacterial dyes, depending on their selective properties;

— antibiotics depending on their activity.

### 4.2 Microbiological quality criteria

#### 4.2.1 General

The microbiological performance tests shall be carried out on a sample which is representative of a batch of end product.

#### 4.2.2 Microbial contamination

An appropriate quantity, depending on the size of the batch of culture medium, shall be tested for microbial contamination by incubation under appropriate conditions. Target limits for the percentage of contaminated plates or containers of liquid medium should be established for each medium or specified by the manufacturer. Manufacturers should draw up specifications based on media components, processing limits and type of packaging.

NOTE 1 The samples to be tested should be at least 1 plate or tube or 1 % of plates or tubes from the beginning and 1 plate or tube or 1 % of plates or tubes from the end of a pouring or dispensing process. The plates or tubes should be incubated for at least 18 h at 37ºC or under the incubation conditions which are used routinely for this medium according to the specific standard.

NOTE 2 For statistical sampling plans refer to the ISO 2859-1:1999.

#### 4.2.3 Growth

##### 4.2.3.1 General

To evaluate each batch of complete culture medium, nutrient components or supplements, growth shall be appropriately assessed by either:

a) quantitative; or

b) semi-quantitative; or

c) qualitative methods.

Quantitative, semi-quantitative or qualitative evaluations shall be performed by the methods described in this Technical Specification or by another generally accepted technique. For interpretation of the results of testing, it is necessary to compare the amount of growth on the test medium with that on a reference medium. The use of a specific reference medium is therefore mandatory for quantitative methods (see the specific standard or Annex B)

For semi-quantitative or qualitative methods, the use of a specific reference medium (see corresponding specific standard or Annex B) or a culture medium giving a "positive" reaction helps to interpret results. The reference medium must be of known good quality chosen from a recently released batch, or, if comparing long term stability, a recently released batch, a batch from another supplier, or a ready-to-use medium, etc.

In addition, growth of the target strains shall be typical in appearance, size and morphology of the colonies and growth of the non-target strains shall be partly or completely inhibited.

#### 4.2.3.2 Productivity

Solid, semi-solid or liquid culture media shall be inoculated with an appropriate inoculum (5.2.1.1) of the working culture of each of the defined test microorganisms using an appropriate device.

Productivity shall reach a defined minimum limit (see corresponding specific standard or Annex B).

For quantitative methods the Productivity Ratio $P_R$ (1) is determined as follows:

$$P_R = \frac{N_S}{N_O} \quad (1)$$

where

$N_s$ is the total colony count obtained on the culture medium under test (obtained from one or more plates);

$N_o$ is the total colony count obtained on the defined reference culture medium obtained from one or more plates, and shall be ≥ 100 cfu.

NOTE The Productivity Ratio of a non selective medium is at least 0,7 for microorganisms that can grow easily on that medium. The $P_R$ of the target microorganisms on a selective medium should be at least 0,1. These values are generally achievable, however less rigorous criteria can be accepted for certain combinations of media and test microorganisms (see corresponding specific standard or Annex B)

For semi-quantitative methods, the scores of consecutive sectors of a plate inoculated by the ecometric technique are summed to obtain the growth index $G_I$, which varies according to the culture medium. It is therefore important to compare them with previous indices and/or $G_I$ of a reference medium and to ensure that variations are not excessive. The expected range of variations for each culture medium can also be established once sufficient experience of the method has been gained.

Qualitative evaluations shall be carried out visually by allocating growth scores.

#### 4.2.3.3 Selectivity

To assess selectivity quantitatively, selective culture media and a reference medium are inoculated with an appropriate inoculum (5.2.1.2.) of the defined test microorganism using an appropriate device. Selectivity has to reach defined values (see corresponding specific standard or Annex B).

The Selectivity Factor $S_F$ (2), is calculated as follows:

$$S_F = D_O - D_S \quad (2)$$

where

$D_O$ is the highest dilution showing growth of at least 10 colonies on the reference medium;

$D_S$ is the highest dilution showing comparable growth on the test medium.

$S_F$, $D_O$ and $D_S$ are expressed in $\log_{10}$ units.

NOTE The $S_F$ of non-target microorganisms on a selective medium should be at least 2. This value is generally achievable. However, less rigorous criteria can be accepted for certain combinations of media and test microorganisms (see corresponding specific standard or Annex B).

For semi-quantitative and qualitative methods the growth of the non-target strain(s) shall be inhibited partly or completely.

#### 4.2.4 Biochemical and physiological characteristics (selectivity and specificity)

The colony morphology and the diagnostic features together with the degree of selectivity should be established in order to obtain a complete picture of the performance of a medium.

The essential characteristics of specificity shall be defined and achieved. For differential media the quality of biochemical / physiological characteristics of the target microorganism(s) and the degree of inhibition of non-target microorganisms should be determined with an appropriate set of test strains.

#### 4.2.5 Antimicrobial testing characteristics

The antimicrobial action of antibiotics depends upon their agar diffusion characteristics and any antagonistic effects from the components present. Media for testing the presence or absence of antimicrobial substances in food samples should conform to reference methods.

### 4.3 Performance evaluation and interpretation of results

A batch of culture medium performs satisfactorily if all the test microorganisms used perform according to the given specifications. It shall be accepted if both general and microbiological quality criteria are met.

## 5 Methods for use in performance testing of culture media

### 5.1 General

Examples of quantitative, semi-quantitative and qualitative testing methods for solid culture media and liquid media are described. In most cases in the user's laboratory semi-quantitative and qualitative techniques will meet the performance testing requirements of a batch of culture medium.

For special cases, e.g. evaluation of a new medium or a new manufacturer, etc., quantitative testing methods shall be performed by the user's laboratory.

Familiarity with general microbiological techniques is assumed and therefore the methods are not given in exhaustive detail.

Suitable test microorganisms are listed in Annex B (see also ENV ISO 11133-1).

NOTE It is the intention in the future, that new and revised individual standards for detection or enumeration of specific microorganisms or groups of microorganisms will describe the relevant test microorganisms to be used, together with the acceptance criteria for each culture medium in the standard.

In liquid media the interactions leading to the successful growth of microorganisms are more complex, hence defining performance testing methods is less straightforward than for solid media.

For the successful isolation of targeted microorganisms in a multistage method, for example detection of *Salmonella,* several complex interactions take place at each growth stage. Here a control test using appropriate samples, culture and reference materials should be set up, so that the productivity or the selectivity, respectively, of the whole method is demonstrated. This is in addition to demonstrating that each component medium is fit for purpose.

### 5.2 Test microorganisms

The appropriate reference strains of target (productivity) and non-target (selectivity) microorganisms for each culture medium are given in Annex B. The test microorganisms should meet the requirements given in 5.2.2 of ENV ISO 11133-1:2000, e.g. robust, weakly growing, biochemically unreactive or injured strains, as appropriate.

Guidance on the preservation and maintenance of reference strains is given in Annex B of ENV ISO 11133-1.

#### 5.2.1 Preparation of the working culture

Working cultures shall be prepared as a pure stationary phase culture in a non-selective broth from the reference stock culture.

Different techniques may be used, but shall guarantee the purity of the inoculum, as well as its standardisation which allows it to be used at a later stage.

NOTE Frozen inocula may be used if it can be shown that the microorganism can survive for the chosen period.

##### 5.2.1.1 Working culture for productivity testing

For quantitative tests of plate media for wanted microorganisms, an inoculum level of approximately $10^2$ cfu is used.

For semi-quantitative or qualitative tests of plate media, an inoculum level $10^3$ -$10^4$ cfu is need.

For productivity tests of liquid media, an inoculum level 10-100 cfu is used.

##### 5.2.1.2 Working culture for selectivity testing

For selectivity testing of culture media, a suspension of the non-target microorganism containing $10^4$ cfu to $10^6$ cfu is inoculated onto the plate or into the tube of medium.

##### 5.2.1.3 Incubation conditions

Incubate the inoculated culture media in accordance with the conditions described in the corresponding standard and given in the appropriate tables in Annex B.

### 5.3 Methods for solid culture media

#### 5.3.1 Quantitative plating method

##### 5.3.1.1 General

This is a general method suitable for most solid culture media. It may not be suitable for testing some types of moulds.

##### 5.3.1.2 Procedure

— Use working cultures as described in 5.2.1.

— Select an appropriate number of plates representative of each batch to be tested and ensure the surface of each plate is adequately dried. Plates of the reference medium should be similarly prepared (see 4.4.4 of ENV ISO 11133-1:2000).

— Spread onto the surface of the test and reference plates an inoculum of the diluted working culture to give counts that fall within the recommended limits given in 5.2.1.

NOTE 1 The modified Miles-Misra surface drop method and other dropping systems or a spiral plater may also be used.

NOTE 2 The pour plate method may also be used for culture media normally used for enumeration in this way.

— Incubate plates under appropriate conditions as defined in the individual standards.

— Count the colonies present on each plate or from each drop as appropriate. Assess the size and appearance of the colonies.

#### 5.3.1.3 Calculation

Based on the volume spread on the plates and the dilution factor, the mean count on the medium can be calculated. In the case of dropping methods the number of drops and their volume must be considered.

#### 5.3.1.4 Interpretation of results

To interpret the results, the Productivity Ratio $P_R$ (4.2.3.2), and where appropriate the Selectivity Factor $S_F$ (4.2.3.3), should be calculated.

### 5.3.2 Semi-quantitative streaking method based on ecometry

#### 5.3.2.1 General

The streaking method is suitable for performance testing of solid and liquid culture media but the method is only semi-quantitative. Growth indices are therefore only indicative and it can only be regarded as a supplementary test for solid culture media.

When using this method the culture media tested should be dried to the same degree and the whole procedure shall be standardized so that results of different batches can be compared.

#### 5.3.2.2 Procedure

— Agar plates are prepared in the usual manner with about 15 ml of agar. Media normally used for the pour plate technique, for example Plate Count Agar (PCA), may also be tested by surface plating on solidified media.

— Use working cultures as described in 5.2.1.

— The plates are streaked as shown in Figure 1 using a 1 µl loop. Four parallel lines are drawn with the loop at approximately 0,5 cm intervals over sector A. Streaking is repeated for sectors B and C and terminated in sector D with a single line. A template can be used beneath the plate to facilitate accurate streaking.

— The incubation times and temperatures stated in the standard methods are used.

NOTE Only the loop, not the wire, should be dipped in the culture. The loop should be completely filled with the culture. Excess liquid should be removed by pressing the wider part of the loop three times against the edge of the container. When streaking plates the angle between the loop and agar surface should be 20° to 30°. The pressure of the loop on the agar surface and the rapidity of streaking must be consistent throughout. Dipping the loop in the culture whilst foam and/or bubbles are on the surface of the broth should be avoided.

Normally the same loop is used for streaking all sectors from A to D without flaming the loop between streaks. In some cases where a lower growth index $G_I$ is expected to demonstrate distinct differences, changing or sterilising the loop between streaking sectors A and B may be appropriate.

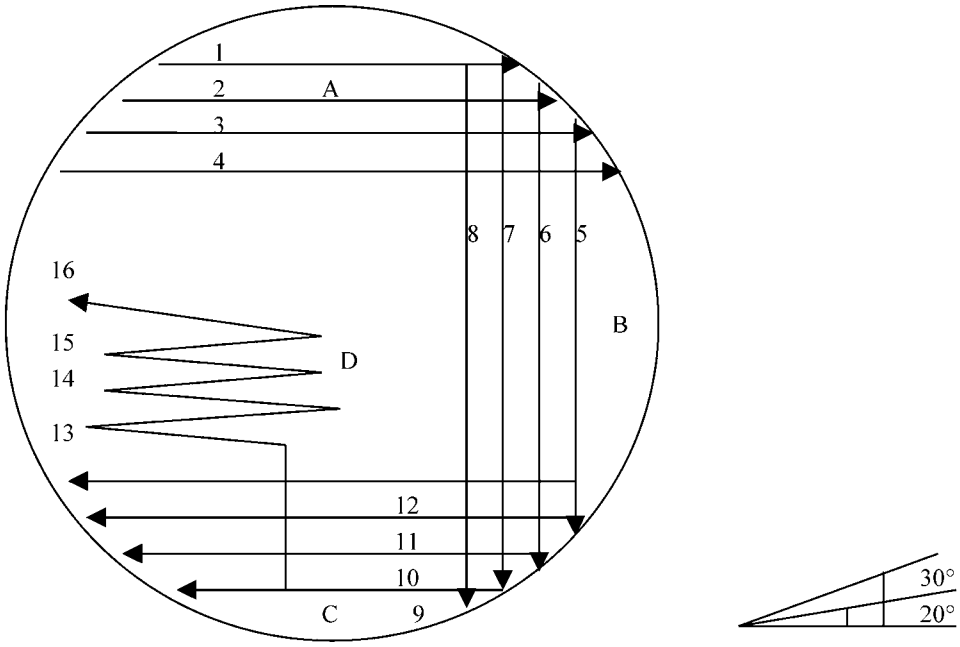


**Figure 1 — Pattern of inoculation by modified streaking method and angle of loop**

#### 5.3.2.3 Calculation

After incubation, the appearance, colony size and intensity of growth are assessed and the growth index $G_I$ calculated. Each streaking line showing growth is scored with 1. The maximum score per plate is 16. The streak is scored as 0,5 if growth only occurs along half of its length. A streak without growth or with scanty growth (less than half the length), is scored as 0. The scores are summed to obtain the $G_I$. For example, if growth was obtained in sectors A and B and in half of sector C the $G_I$ would be 10.

#### 5.3.2.4 Interpretation of results

The growth index $G_I$ given by a target strain should be at least 6 in order to conclude that the medium is acceptable. In the case of non selective media the $G_I$ is normally higher.

In addition, growth of the target strain shall be typical, and growth of non-target strains shall be partly or completely inhibited.

### 5.3.3 Qualitative streaking method

#### 5.3.3.1 General

The method is suitable for supplementary performance testing of solid culture media.

The method is only qualitative and scores are therefore only indicative.

#### 5.3.3.2 Procedure

— Agar plates are prepared in the usual manner with about 15 ml of agar. Media normally used for the pour plate technique, for example Plate Count Agar (PCA), may also be tested by surface plating on solidified media.

— Use working cultures as described in 5.2.1.

— The test microorganisms are streaked in parallel straight lines with a 1 µl loop on the surface of the test medium. Several test microorganisms can be streaked on the same plate without crossing.

NOTE Other standardized streaking techniques can be used.

— The incubation times and temperatures stated in the standard methods are used.

#### 5.3.3.3 Interpretation of results

The growth on the plates after incubation is assessed as:

— 0 corresponds to zero growth,

— 1 corresponds to weak growth, and

— 2 corresponds to good growth.

Target microorganisms shall score 2 and have typical appearance, size and colony morphology. The growth of non-target microorganisms shall be partly or completely inhibited (0 or 1).

### 5.4 Methods for liquid culture media

#### 5.4.1 General

To determine the productivity of a liquid medium an appropriate inoculum shall be used. The quantitative, semi-quantitative and qualitative methods described below assess productivity and selectivity. The proposed methods record the quantity of growth after appropriate incubation by plating or streaking from the liquid media onto agar media and enumerating colony forming units (cfu) or calculating scores from the liquid medium. For qualitative methods in liquid media the characteristic reactions are assessed visually.

#### 5.4.2 Quantitative dilution method for target and non-target microorganisms

The method is also appropriate for evaluation of new culture media or diluents.

#### 5.4.2.1 Procedure

— Select an appropriate number of tubes or 10 ml portions of each batch of liquid medium to be tested.

— Inoculation of target microorganisms: Inoculate test broth and reference broth for each test organism with a small number (e.g. 10 cfu to 100 cfu into each tube; for preparation of the inoculum see 5.2.1.) and mix.

— Inoculation of non-target microorganisms: Inoculate test broth and reference broth for each test organism with a higher number (>1000 cfu into each tube; for preparation of the inoculum see 5.2.1.) and mix.

— Inoculation of target and non-target microorganisms as a mixed culture: For testing mixed cultures in selective media inoculate test broth and reference broth with a small number of target microorganisms (e.g. 10 cfu to 100 cfu for every tube; for preparation of the inoculum see 5.2.1.) and in the same tube with a higher number of non-target microorganisms (> 1000 cfu into each tube; for preparation of the inoculum see 5.2.1.) and mix.

— Inoculation of target and non-target microorganisms in diluents and transport media: Inoculate diluents with test microorganisms (e.g. 100 cfu to 1000 cfu into each tube; for preparation of the inoculum see 5.2.1.) and mix.

— The incubation times and temperatures stated in the standard methods are used.

Diluents should be incubated for 45 min at room temperature and then plated out. Transport media should be incubated at an appropriate temperature and time according to normal usage and then plated out.

— Remove an aliquot volume or if necessary a dilution from each broth after the incubation step and spread to a non-inhibitory agar plate as described in 5.3.1.

NOTE 1 The modified Miles-Misra surface drop method, other dropping systems or a spiral plater can be used to give countable colonies on the plates.

NOTE 2 To test mixed cultures, spreading should be done when possible on non-selective agar plates which allow differentiation of the microorganisms in the mixed culture (e.g. Plate Count Agar with MUG for counting *Escherichia coli* and *Salmonella* spp.). When it is not possible to distinguish mixed cultures on non-selective agar, selective agar media should be used providing that their performance has been previously tested.

#### 5.4.2.2 Reading, calculation and interpretation of results

After incubation colonies of target and non-target microorganisms are counted, in the case of mixed cultures distinguishing the different types. Calculation and interpretation shall be done with respect to the aim of examination:

a) comparative interpretation between reference broth and test broth using $P_R$ and $S_F$ figures as described in 4.2.3.2 and 4.2.3.3:

   — for target microorganisms $P_R$ shall not be < 0,1 (the difference in growth does not exceed one order of magnitude);

   — for non-target microorganisms $S_F$ shall reach at least 2;

   — in mixed cultures the growth of target microorganisms shall not be inhibited by the non-target microorganisms, i.e. the target microorganisms shall always be the dominant population;

b) for other cases achieving fixed minimum counts for target microorganisms and maximum counts for non-target microorganisms is more appropriate:

   — target microorganisms shall reach $10^6$ cfu/ml to $10^8$ cfu/ml;

   — non-target microorganisms shall not exceed $10^4$ cfu/ml in selective broth.

c) for diluents and transport media neither reduced nor higher numbers of target and/or non-target organism are required. The number of microorganisms after incubation in these media shall be within ± 50% of the initial count.

NOTE The quality of a liquid medium with respect to optimal growth properties is indicated most appropriately in the early growth phase. Looking at the length of the log phase and growth in the early log phase gives the most sensitive information on productivity and selectivity of target and non-target microorganisms respectively in the test and reference broths. Therefore if only minor differences in the media quality are being sought, streaking from the liquid media onto the plates should be done after a shorter incubation period of e.g. 6 h or 12 h.

### 5.4.3 Semi-quantitative single tube method for target, non-target and mixed microorganisms

#### 5.4.3.1 Procedure

— Select an appropriate number of tubes or 10 ml portions of each batch to be tested (see 4.2.2).

— Inoculation of target and non-target organisms as a mixed culture: Inoculate 1 tube of test broth with about 10 cfu to 100 cfu of target microorganism and in the same tube inoculate with a higher number of non-target microorganisms (> 1000 cfu for every tube) and mix.

— Inoculation of non-target microorganisms: Inoculate one tube of test broth per microorganism with a higher number (> 1000 cfu) and mix.

— The incubation times and temperatures stated in the standard methods are used.

— Remove 10 µl from the mixed culture and streak on a plate of the specific selective medium for the target microorganism.

— Remove one loop (10 µl) from the culture of non-target microorganism and streak on a plate of a non-selective medium (e.g. TSA).

— Incubate both plates under appropriate conditions for a suitable time, as indicated in individual standards.

#### 5.4.3.2 Calculation and interpretation of results

Productivity of the liquid test broth is satisfactory if at least 10 colonies of the target microorganism have grown on the selective agar plate.

Selectivity of the liquid test broth is satisfactory if no growth (or less than 10 cfu) of non-target microorganisms occurs on the non-selective agar plate.

### 5.4.4 Qualitative single tube method

#### 5.4.4.1 General

The method is suitable for performance testing of liquid culture media. The method is only qualitative and scores are therefore only indicative. Turbid media, e.g. tetrathionate broth, cannot be tested by this method.

#### 5.4.4.2 Procedure

— for performance testing of liquid culture media the working cultures are directly inoculated into the medium being tested using a 1 µl loop;

— the incubation times and temperatures stated in the individual standard methods are used.

#### 5.4.4.3 Interpretation of results

Qualitative evaluation shall be carried out visually by allocating growth scores, e.g. from 0 to 2.

For tubes and bottles

— 0 corresponds to zero turbidity;

— 1 corresponds to very light turbidity;

— 2 corresponds to good turbidity.

The score of a target microorganism shall be 2.

NOTE 1 Sometimes the growth of microorganisms can only be observed as a cell aggregation/ deposit at the base of the tube or bottle. In this case careful shaking can improve assessment and interpretation.

NOTE 2 Other characteristics such as gas formation, colour change, etc. can also be assessed by this method.

## 6 Documentation of test results

### 6.1 Information provided by the manufacturer

The manufacturer or supplier of the culture media shall provide, on request, the specific microbiological growth characteristics and general information relating to the specific batch of culture medium, see 4.1.1 of ENV ISO 11133-1:2000.

### 6.2 Traceability

All the data from routine performance testing should be documented in an appropriate way and kept for a sufficient period of time according to the quality system in use. The use of control sheets for documenting and evaluating the results of the tests is recommended (see Annex A).

# Annex A
(informative)

## Example of card for recording test results of culture media prepared by the user laboratory

**Table A.1 — Example of a card**

| | | | | |
|---|---|---|---|---|
| Control card for internal quality testing of culture media | | | | |
| culture medium: | | volume prepared: | pouring date: | internal batch number: |
| dehydrated medium (& code): | supplier: | batch | amount: | date/signature: |
| Supplement: | supplier: | batch | amount: | date/signature: |
| Process details: | | | | |
| Physical quality control | | | | |
| expected pH-value: | measured pH: | quality confirmed: yes □ no □ | defects: | date/signature: |
| expected quantity filed and/or layer thickness: | observed: | quality confirmed: yes □ no □ | defects: | date/signature: |
| expected colour: | observed: | quality confirmed: yes □ no □ | defects: | date/signature: |
| expected clarity/presence of optical artefacts: | observed: | quality confirmed: yes □ no □ | defects: | date/signature: |
| expected gel stability / consistency / moisture: | observed: | quality confirmed: yes □ no □ | defects: | date/signature: |
| Microbial contamination | | | | |
| No. of tested plates or tubes: Incubation: | result: | quality confirmed: yes □ no □ | No. of contaminated plates or tubes: | date/signature: |
| Microbiological growth —Productivity | | Method of control: Quantitative □ | | Qualitative □ |
| strains: incubation: reference medium: | criteria: | result: | quality confirmed: yes □ no □ | date/signature: |
| Microbiological growth —Selectivity | | Method of control: Quantitative □ | | Qualitative □ |
| strains: incubation: reference medium: | criteria: | result: | quality confirmed: yes □ no □ | date/signature: |
| Microbiological growth —Specificity | | Method of control: Quantitative □ | | Qualitative □ |
| strains: incubation: reference medium: | criteria: | result: | quality confirmed: yes □ no □ | date/signature: |
| Release of the batch | | | | |
| Details of storage | | release of the batch yes □ no □ | | date/signature: |

# Annex B
(normative)

## Recommended test microorganisms for commonly used culture media (giving information on the culture medium, culture conditions, test microorganisms, culture collection number of test organisms and the expected reactions)

Tables B.1 to B.6 have been established taking into account the control strains used in the European Pharmacopoeia and the recommendations from the Pharmacopoeia on food microbiology for culture media (Working Party of ICFMH). These criteria will be included in specific standards when prepared or revised in the future (new standard or revision). A validated batch of media is a batch of media which has shown satisfactory performance. The use of the same strains from other reference collections is permitted (e.g. NCTC, CIP…). All cited media are described within EN and ISO standards.

**Table B.1 — Selective media for enumeration of microorganisms**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| Baird - Parker | S[a] | Coagulase positive Staphylo-cocci | EN ISO 6888-1 | Productivity | 24-48 h/ 37°C | S. aureus ATCC 6538<br>S. aureus ATCC 25923[b] | TSA | Quantitative | PR ≥ 0,5 | Black / grey colonies with clear halo (Egg yolk clearing reaction) |
| | | | | Selectivity | 48 h/37°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total inhibition | - |
| | | | | Specificity | 24-48 h/ 37°C | S. epidermidis ATCC 12228[b] | - | Qualitative | - | Black / grey colonies without egg yolk clearing reaction |
| RPFA | S | Coagulase positive Staphylo-cocci | EN ISO 6888-2 | Productivity | 24-48 h/ 37°C | S. aureus ATCC 6538 or 6538 P<br>S. aureus ATCC 25923[b] | TSA | Quantitative | PR ≥ 0,5 | Black / grey colonies with opacity halo |
| | | | | Selectivity | 48 h/37°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total inhibition | - |
| | | | | Specificity | 24-48 h/ 37°C | S. epidermidis ATCC 12228[b] | - | Qualitative | - | Black / grey colonies without opacity halo |
| Chloram-phenicol or OGA (OGY) | S | Yeasts / Moulds | ISO 7954 | Productivity | 3-5 days / 25°C | C. albicans ATCC 10231<br>A. niger ATCC 16404[b]<br>P. cyclopium ATCC 16025<br>S. cerevisiae ATCC 9763[b] | Media batch SDA (Sabouraud Dextrose Agar) or OGA or chloramphenicol agar | Quantitative | PR ≥ 0,5 | Characteristic colonies according to each species |
| | | | | Selectivity | 3-5 days / 25°C | E. coli ATCC 25922 or 8739[b]<br>B. subtilis ATCC 6633 | - | Qualitative | Total inhibition | - |

**Table B.1 (continued)**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| MRS | S | Lactic acid bacteria | ISO 15214 | Productivity | 72 h / 30°C | L. sake ATCC 15521[b]<br>Ped. damnosus ATCC 29358<br>Lc. lactis ATCC 19435[b] | Media batch MRS already validated | Quantitative | PR ≥ 0,5 | Characteristic colonies according to each species |
| | | | | Selectivity | 72 h / 30°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total inhibition | - |
| | | | | | | B. cereus ATCC 11778 | | | | |
| MYP | S | Bacillus cereus | EN ISO 7932 | Productivity | 24-48h / 30°C | B. cereus ATCC 11778[b] | TSA | Quantitative | PR ≥ 0,7 | Pink colonies with precipitation halo |
| | | | | Selectivity | 48h / 37°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total inhibition | - |
| | | | | Specificity | 48h / 37°C | B. subtilis ATCC 6633[b] | | | - | Yellow colonies without precipitation halo |
| Oxford | S | Listeria mono-cytogenes | EN ISO 11290 | Productivity | 48h / 37°C | L. mono 1/2a ATCC 19111 | TSA | Quantitative | PR ≥ 0,5 | Grey to black colonies with black halo |
| | | | | | | L. mono 4b ATCC 13932[b] | | | | |
| | | | | Selectivity | 48h / 37°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total inhibition | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | | |
| | | | | | | C. albicans ATCC 10231 | | | | |
| PALCAM | S | Listeria mono-cytogenes | EN ISO 11290 | Productivity | 48h / 37°C | L. mono 1/2a ATCC 19111 | TSA | Quantitative | PR ≥ 0,5 | Grey-green to black colonies with black halo |
| | | | | | | L. mono 4b ATCC 13932[b] | | | | |
| | | | | Selectivity | 72h / 30°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total inhibition | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | | |
| | | | | | | | | | | |
| TS(C) | S | Clostridium perfringens | EN ISO 7937 | Productivity | 20h / 37°C anaerobic atm. | Cl. perfringens ATCC 13124 | Media batch TS(C) already validated | Quantitative | PR ≥ 0,7 | Black colonies |
| | | | | | | Cl.perfringens ATCC 12916 | | | | |
| | | | | Selectivity TSC | 20h / 37°C anaerobic atmo. | E. coli ATCC 25922 or 8739 | - | Qualitative | Total inhibition | - |
| | | | | Specificity TS | | | - | Qualitative | - | White colonies |
| VRBG | S | Enterobac-teriaceae | ISO 7402 ISO 8523 | Productivity | 24h / 37°C | E. coli ATCC 25922 or 8739[b] | TSA | Quantitative | PR ≥ 0,5 | Pink to red colonies with or without precipitation halo |
| | | | | | | S. typhimurium ATCC 14028 | | | | |
| | | | | Selectivity | 24h / 37°C | E. faecalis ATCC 29212 or 19433[b] | - | Qualitative | Total inhibition | - |
| VRBL | S | Coliforms | ISO 4832 | Productivity | 24h / 30°C | E. coli ATCC 25922 or 8739[b] | TSA | Quantitative | PR ≥ 0,5 | Purplish colonies with or without precipitation halo |

**Table B.1 (continued)**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Selectivity | 24h / 30°C | E. faecalis ATCC 29212 or 19433[b] | - | Qualitative | Total inhibition | - |
| | | | | Specificity | 24h / 30°C | Ps. aeruginosa ATCC 27853 | - | Qualitative | - | Colourless to beige colonies |
| CT-SMAC | S | Escherichia coli O157 | ISO 16654 | Productivity | 24h / 37°C | E.coli O 157:H7 ATCC 43894 or 43895[b] (non-toxigenic) | TSA | Quantitative | PR ≥ 0,5 | Transparent colonies with a pale yellowish-brown appearance and a diameter of approx.1mm |
| | | | | Selectivity | 24h / 37°C | S. aureus ATCC 6538 or 25923[b] | - | Qualitative | Total inhibition | - |
| | | | | Specificity | 24h / 37°C | E.coli ATCC 11775 or 25922[b] | - | Qualitative | - | Pink colonies |
| BGBLB | L[c] | Coliforms | ISO 4831 | Productivity | 24-48h / 30°C | E. coli ATCC 25922 or 8739[b] C. freundii ATCC 43864 | - | Semi-quantitative | Turbidity 2 + Gas in 1/3 of Durham tube | Gas production and turbidity |
| | | | | Selectivity | 24-48h / 30°C | E. faecalis ATCC 29212 or 19433[b] | - | Qualitative | No growth | - |
| LST | L | Coliforms | ISO 4831 | Productivity | 24-48h / 30°C | E. coli ATCC 25922 or 8739[b] C. freundii ATCC 43864 | - | Semi-quantitative | Turbidity 2 + Gas in 1/3 of Durham tube | Gas production and turbidity |
| | | | | Selectivity | | E. faecalis ATCC 29212 or 19433[b] | - | Qualitative | No growth | - |
| EC | L | Escherichia coli | ISO 7251 | Productivity | 24-48h / 44°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Turbidity 2 + Gas in 1/3 of Durham tube | Gas production and turbidity |
| | | | | Selectivity | 24-48h / 44°C | Ps. aeruginosa ATCC 27853[b] | - | Qualitative | No growth | - |

a S = solid medium

b Strains to be used by the user laboratory (minimum)

c L = liquid medium

Note: For solid culture media, it is also possible to use a semi-quantitative plating method

**Table B.2 — Non selective media for enumeration of microorganisms**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| PCA | S[a] | Total flora | ISO 4833 | Productivity | 72h / 30°C | E. coli ATCC 25922 or 8739[b] | TSA | Quantitative | PR ≥ 0,7 | |
| | | | | | | S. aureus ATCC 6538 or 6538P | | | | |
| | | | | | | B. subtilis ATCC 6633[b] | | | | |

[a] S = solid medium
[b] Strains to be used by the user laboratory (minimum)

**Table B.3 — Selective enrichment media**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions of target microorganism |
|---|---|---|---|---|---|---|---|---|---|---|
| EE | L[a] | Enterobac-teriaceae | ISO 7402 ISO 8523 | Productivity | 24h / 37°C | E. coli ATCC 25922 or 8739[b] or S. typhimurium ATCC14028 | - | Semi-quantitative | >10 col. on VRBG | Pink to red colonies with or without precipitation halo |
| | | | | Selectivity | 24h / 37°C | + E. faecalis ATCC 29212 or 19433[b] | | Semi-quantitative | Total inhibition | |
| Half-Fraser | L | Listeria mono-cytogenes | EN ISO 11290-1 | Productivity | 24h / 30°C | L. mono 1/2a ATCC 19111 | - | Semi-quantitative | >10 col. on Oxford or PALCAM | Grey to black colonies with black halo |
| | | | | | | or L. mono 4b ATCC 13932[b] | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + E. faecalis ATCC 29212 or 19433[b] | | | | |
| | | | | Selectivity | 24h / 30°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | <100 colonies on TSA | |
| Fraser | L | Listeria mono-cytogenes | EN ISO 11290-1 | Productivity | 48h / 37°C | L. mono 1/2a ATCC 19111 | - | Semi-quantitative | >10 col. on Oxford or PALCAM | Grey to black colonies with black halo |
| | | | | | | or L. mono 4b ATCC 13932[b] | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |

**Table B.3 (continued)**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions of target microorganism |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | + E. faecalis ATCC 29212 or 19433[b] | | | | |
| | | | | Selectivity | 24-48h / 37°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | <100 colonies on TSA | |
| ITC | L | Yersinia enterocolitica | ISO 10273 | Productivity | 48h / 25°C | Y. enterocolitica ATCC 23715 or 9610[b] | - | Semi-quantitative | >10 col. on CIN or SSDC | Characteristic colonies according to each medium (see standard) |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + Ps. aeruginosa ATCC 27853[b] | | | | |
| | | | | Selectivity | 48h / 25°C | Ps. aeruginosa ATCC 27853[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | P. mirabilis ATCC 29906 | | | | |
| Park & Sanders | L | Campylobacter | ISO 10272 | Productivity | See standard | C. coli ATCC 43478* | - | Semi-quantitative | >10 col. on Karmali medium or other medium of choice | Characteristic colonies according to each medium (see standard) |
| | | | | | | or C. jejuni ATCC 33291 or 29428* | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + P. mirabilis ATCC 29906[b] | | | | |
| | | | | Selectivity | See standard | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | P. mirabilis ATCC 29906 | | | | |
| Preston | L | Campylobacter | ISO 10272 | Productivity | 18h/42°C | C. coli ATCC 43478[b] | - | Semi-quantitative | >10 col. on Karmali medium or other medium of choice | Characteristic colonies according to each medium (see standard) |
| | | | | | | or C. jejuni ATCC 33291 or 29428[b] | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + P. mirabilis ATCC 29906[b] | | | | |
| | | | | Selectivity | 18h/42°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | P. mirabilis ATCC 29906 | | | | |

**Table B.3 (continued)**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions of target microorganism |
|---|---|---|---|---|---|---|---|---|---|---|
| PSB | L | Yersinia enterocolitica | ISO 10273 | Productivity | 3-5 days / 25°C | Y. enterocolitica ATCC 23715 or 9610[b] | - | Semi-quantitative | >10 col. on CIN or SSDC | Characteristic colonies according to each medium (see standard) |
| | | | | | | | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + Ps. aeruginosa ATCC 27853[b] | | | | |
| | | | | Selectivity | 3-5 days / 25°C | Ps. aeruginosa ATCC 27853[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | P. mirabilis ATCC 29906 | | | | |
| MKTTn | L | Salmonella | ISO 6579 | Productivity | 24h / 37°C | S. typhimurium ATCC14028[b] | - | Semi-quantitative | >10 col. on XLD or other medium of choice | Characteristic colonies according to each medium (see standard) |
| | | | | | | or S. enteritidis ATCC 13076[b] | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + Ps. aeruginosa ATCC 27853[b] | | | | |
| | | | | Selectivity | 24h / 37°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | < 10 colonies on TSA | |
| Rappa-port Vassilia-dis | L | Salmonella | EN 12824 | Productivity | 24h / 41,5°C | S. typhimurium ATCC14028[b] | - | Semi-quantitative | >10 col. on BGA or other medium of choice | Characteristic colonies according to each medium (see standard) |
| | | | | | | or S. enteritidis ATCC 13076[b] | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739 | | | | |
| | | | | | | + Ps. aeruginosa ATCC 27853 | | | | |
| | | | | Selectivity | 24h / 41,5°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | < 10 colonies on TSA | |
| RVS | L | Salmonella | ISO 6579 | Productivity | 24h / 41,5°C | S. typhimurium ATCC14028 | - | Semi-quantitative | >10 col. on XLD or other medium of choice | Characteristic colonies according to each medium (see standard) |
| | | | | | | or S. enteritidis ATCC 13076[b] | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + Ps. aeruginosa ATCC 27853* | | | | |

**Table B.3 (continued)**

| Media | Type | Micro-organisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions of target microorganism |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Selectivity | 24h / 41,5°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | Total inhibition on TSA | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | < 10 colonies on TSA | |
| Selenite-cystine | L | Salmonella | EN 12824 | Productivity | 24h / 37°C | S. typhimurium ATCC14028[b] | - | Semi-quantitative | >10 col. On BGA or other medium of choice | Characteristic colonies according to each medium (see standard) |
| | | | | | | or S. enteritidis ATCC 13076[b] | | | | |
| | | | | | | + E. coli ATCC 25922 or 8739[b] | | | | |
| | | | | | | + Ps. aeruginosa ATCC 27853[b] | | | | |
| | | | | Selectivity | 24h / 37°C | E. coli ATCC 25922 or 8739[b] | - | Semi-quantitative | <100 colonies on TSA | - |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | | |

[a] L = liquid medium

[b] Strains to be used by the user laboratory (minimum)

**Table B.4 — Non selective enrichment media**

| Media | Type | Microorganisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| BHI | L[a] | Staphylococcus | ISO 6888 | Productivity | 24h / 37°C | S. aureus ATCC 25923[b] | | Qualitative | Turbidity 1 to 2 | - |
| Brucella | L | Campylobacter | ISO 10272 | Productivity | 2-5 days / 25°C | C. coli ATCC 43478 | - | Qualitative | Turbidity 1 to 2 | - |
| | | | | | | C. jejuni ATCC 33291 or 29428[b] | | | | |
| Peptone-salt | L | Dilution liquids | ISO 6887 | Diluent | 45 min / 20-25°C | E. coli ATCC 25922 or 8739[b] | TSA | Quantitative | +/- 50% col./T0 (+/- 50% of original count) | - |
| | | | | | | S. aureus ATCC 25923 | | | | |
| Thiogly-collate | L | Clostridium perfringens | ISO 7937 | Productivity | 24h / 37°C | Cl. perfringens ATCC 13124[b] | - | Qualitative | Turbidity 1 to 2 | - |
| | | | | | | | | | | |
| TSYEB | L | Listeria monocytogenes | ISO 11290 | Productivity | 24h / 25°C | L. mono 1/2a ATCC 19111 | - | Qualitative | Turbidity 1 to 2 | - |
| | | | | | | L. mono 4b ATCC 13932[b] | | | | |

[a] L = liquid medium
[b] Strains to be used by the user laboratory (minimum)

**Table B.5 — Selective isolation media**

| Media | Type | Microorganisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| Modified Butzler | S[a] | Campylobacter | ISO 10272 | Productivity | 24-72h / 42°C | C. coli ATCC 43478 | - | Qualitative | Good growth (2) | Characteristic colonies according to each medium (see standard) |
| CCDA | | | | | | | | | | |
| Karmali | | | | | | C. jejuni ATCC 33291 or 29428[b] | | | | |
| Preston | | | | | | | | | | |

**Table B.5 (continued)**

| Media | Type | Microorganisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| Skirrow | | | | Selectivity | 24-72h / 42°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total or partial inhibition (0 - 1) | No characteristic colonies |
| | | | | | | S. aureus ATCC 25923 | | | Total inhibition (0) | - |
| CIN | S | Yersinia enterocolitica | ISO 10273 | Productivity | 24h / 30°C | Y. enterocolitica ATCC 23715 or 9610[b] | - | Qualitative | Good growth (2) | Characteristic colonies according to each medium (see standard) |
| SSDC | | | | | | | | | | |
| | | | | Selectivity | 24h / 30°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total or partial inhibition (0 —1) | No characteristic colonies |
| | | | | | | S. aureus ATCC 25923 | | | Total inhibition (0) | - |
| brilliant green agar (BGA) | S | Salmonella | EN 12824 / ISO 6579 | Productivity | 24-48h / 37°C | S. typhimurium ATCC14028[b] | - | Qualitative | Good growth (2) | Characteristic colonies according to each medium (see standard) |
| | | | | | | S. enteritidis ATCC 13076 | | | | |
| XLD | | | | Selectivity | 24h-48h / 37°C | E. coli ATCC 25922 or 8739[b] | - | Qualitative | Total inhibition or low growth (0 - 1) | No characteristic colonies |
| | | | | | | E. faecalis ATCC 29212 or 19433 | | | Total inhibition (0) | - |

[a] S = solid medium

[b] Strains to be used by the user laboratory (minimum)

**Table B.6 — Non selective isolation media**

| Media | Type | Microorganisms | Standard | Function | Incubation | Control strains | Reference media | Method of control | Criteria | Characteristic reactions |
|---|---|---|---|---|---|---|---|---|---|---|
| Nutrient agar | S[a] | Enterobacteria-ceae | ISO 7402 ISO 8523 | Productivity | 24h / 37°C | E. coli ATCC 25922 or 8739[c] | - | Qualitative | Good growth (2) | - |
| | | Salmonella | EN 12824 ISO 6579 | | 24h / 37°C | S. typhimurium ATCC14028[c] | | | | |
| | | Yersinia enterocolitica | ISO 10273 | | 24h / 30°C | Y. enterocolitica ATCC 23715 or 9610[c] | | | | |
| TSYEA agar | S | Listeria monocytogenes | EN ISO 11290 | Productivity | 24h / 37°C | L. mono 1/2a ATCC 1911 or L. mono 4b ATCC 13932[b] | - | Qualitative | Good growth (2) | - |

[a] S = solid medium

[b] Strains to be used by the user laboratory (minimum)

[c] Strains free of choice according to the method used

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