TECHNICAL SPECIFICATION

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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

Microbiologie des aliments — Guide pour la préparation et la production des milieux de culture —

Partie 1: Guide général pour l'assurance de la qualité pour la préparation des milieux de culture en laboratoire

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed every three years with a view to deciding whether it can be transformed into an International Standard.

Attention is drawn to the possibility that some of the elements of this Technical Specification may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 11133-1 was prepared by the European Committee for Standardization (CEN) in collaboration with ISO Technical Committee TC 34, *Agricultural food products*, Subcommittee SC 9, *Microbiology*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Throughout the text of this part of ISO/TS 11133, read "...this European Prestandard..." to mean "...this Technical Specification...".

ISO/TS 11133 consists of the following parts, under the general title *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*

— *Part 2: Practical implementation of the general guidelines on quality assurance of culture media in the laboratory*

— *Part 3: Performance testing*

Annexes A, B and C of this part of ISO/TS 11133 are for information only.

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Foreword

The text of ENV ISO 11133-1:2000 has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Agricultural food products".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2000, and conflicting national standards shall be withdrawn at the latest by December 2000.

This draft European standard “Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media” consists of two parts :

- *Part 1 : General guidelines on quality assurance for the preparation of culture media in the laboratory*
- *Part 2 : Practical guidelines on performance testing of culture media in the laboratory*

Annexes designated as “informative” are given for information only. In this standard Annexes A, B and C are informative.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

In the microbiology laboratory many tests and procedures depend upon culture media being consistent and providing reproducible results. Culture media are used in all traditional cultural techniques and also for many alternative techniques. Many formulae of dehydrated culture media are commercially available and many more, designed for specific growth purposes, are described in the literature. Additionally, in laboratories carrying out the microbiological examination of food, the main objectives are to maintain, resuscitate, grow, detect and / or enumerate a wide variety of microorganisms. The requirements for media are specific to both the sample and the organisms to be detected. Culture media meeting established or minimal performance criteria are therefore a pre-requisite for any reliable microbiological work. Sufficient testing should be carried out to demonstrate i) the acceptability of each batch of medium ii) that the medium is ‘fit for purpose’ and iii) that the medium can produce consistent results.

These three criteria are an essential part of internal quality control procedures and, with appropriate documentation, will permit effective monitoring of culture media and contribute to the production of both accurate and precise data.

1 Scope

This European prestandard provides the general terminology related to quality assurance of the preparation of culture media and specifies the **minimum** requirements to be used for the microbiological analysis of products intended for human consumption or animal feeding.

These requirements are applicable to three categories of culture media used in laboratories that prepare and/or use culture media for performing microbiological analyses :

- commercially manufactured ready-to-use media ;
- media prepared from commercially available dehydrated formulations (either complete e.g. plate count agar or basal media to which supplements are added e.g. Baird-Parker agar) ;
- media prepared from its individual components.

2 Normative references

This European Prestandard 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 European Prestandard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN 1659:1996, *In vitro diagnostic systems – Culture media for microbiology – Terms and definitions.*

EN 12322:1999, *In vitro diagnostic medical devices – Culture media for microbiology – Performance criteria for culture media.*

ISO 8402:1994, *Quality management and quality assurance – Vocabulary.*[1)]

3 Terminology

3.1 General

This clause gives the general definitions related to quality assurance and provides different types of terminology related to culture media and to control cultures. Standards cited between brackets indicate that the text given is identical to that cited.

3.2 Terminology of quality assurance

3.2.1
quality assurance
all the planned and systematic activities implemented within the quality system and demonstrated as needed, to provide adequate confidence that an entity will fulfil the requirements for quality

[ISO 8402]

1) This is under revision and will be combined with ISO 9000-1:1994 to become ISO 9000:2000, *Quality management systems — Fundamentals and vocabulary*.

3.2.2
quality control
operational techniques and activities that are used to fulfil the requirements for quality

[ISO 8402]

3.2.3
internal quality control
a continuous control programme of the laboratory's work prepared by or for them, and based on control analysis together with follow-up and, if necessary, corrective actions

3.2.4
batch of culture media ; lot of culture media
fully traceable unit of a medium referring to a defined amount of bulk, semi-finished product or end product, which is consistent in type and quality and which has passed the requirements of production (in-process control) and quality assurance testing, and which has been produced within one defined production period, having been assigned the same lot number

[EN 12322]

3.2.5
performance of culture media
the response of a culture medium to challenge by test organisms under defined conditions

3.3 Terminology of culture media

3.3.1
culture medium
formulation of substances, in liquid, semi-solid or in solid form, which contain natural and/or synthetic constituents intended to support the multiplication, or to preserve the viability, of microorganisms

NOTE When used in connection with compound words, this term is often shortened into "medium" (e.g. enrichment medium).

[EN 1659]

3.3.1 Culture media classified by composition

3.3.2.1
chemically defined culture medium
culture medium consisting only of chemically defined constituents (i.e. of known molecular structure and degree of purity)

[EN 1659]

3.3.2.2
chemically incomplete culture medium
culture medium consisting entirely or partly of natural materials, processed or otherwise, the chemical composition of which is not completely defined

NOTE For the various chemically undefined components used in culture media, ISO/TC 34/SC 9 has specified harmonised designations - see Annex A.

3.3.2 Culture media classified by consistency

3.3.3.1
liquid culture medium
culture medium consisting of an aqueous solution of one or more constituents (e.g. peptone water, nutrient broth)

NOTE 1 In some cases, solid particles are added to the liquid culture medium.

NOTE 2 Liquid media in tubes, flasks or bottles are commonly called "broth".

[EN 1659]

3.3.3.2
solid culture medium and semi-solid culture medium
liquid culture medium containing solidifying materials (e.g. agar-agar, gelatine, etc.,) in different concentrations

NOTE 1 Due to the world-wide use of culture media solidified with agar-agar, the shortened term "agar" is often used synonymously for solid culture media and therefore in connection with nouns, e.g. "Plate count agar".

NOTE 2 Solid culture media poured into Petri dishes are commonly called "plates". Solid culture media poured into tubes that are kept in slanted positions while the media are solidifying are often called "slants".

[EN 1659]

3.3.3 Culture media classified by intent of use

3.3.4.1
transport medium
culture medium designed to preserve and maintain the viability of microorganisms for the time period between sample collection and laboratory processing of the sample

NOTE Transport media usually contain substances that do not permit multiplication of microorganisms but ensure their preservation (e.g. Stuart's or Amies' Transport medium).

[EN 1659]

3.3.4.2
preservation medium
culture medium designed to preserve and maintain the viability of microorganisms over an extended period, to protect them against the adverse influences which may occur during long-term storage and to allow recovery after this period (e.g. Dorset egg medium)

[EN 1659]

3.3.4.3
resuscitation medium
culture medium enabling stressed and damaged microorganisms to repair and recover their capacity for normal growth without necessarily promoting their multiplication

[EN 1659]

3.3.4.4
enrichment medium
predominantly liquid culture medium which, due to its composition, provides particularly favourable conditions for multiplication of microorganisms

[EN 1659]

3.3.4.4.1
selective enrichment medium
enrichment medium which supports the multiplication of specific microorganisms whilst partially or totally inhibiting the growth of other microorganisms (e.g. Rappaport-Vassiliadis medium)

3.3.4.4.2
non-selective enrichment medium
enrichment medium which supports the growth of most microorganisms (e.g. nutrient broth)

3.3.4.5
isolation medium
solid or semi-solid culture medium which supports the growth of microorganisms

3.3.4.5.1
selective isolation medium
isolation medium which supports growth of specific microorganisms, while inhibiting other microorganisms (e.g. PALCAM agar, MacConkey agar)

[EN 1659]

3.3.4.5.2
non-selective isolation medium
isolation medium which is not devised to selectively inhibit microorganisms (e.g. nutrient agar)

[EN 1659]

3.3.4.6
differential medium
culture medium which permits the testing of one or more physiological/biochemical characteristics of the microorganisms for their identification (e.g. Urea medium, Kligler agar)

NOTE Differential media which can be used as isolation media are referred to as isolation/differential media (e.g. xylose lysine desoxycholate (XLD) agar).

[EN 1659]

3.3.4.7
identification medium
culture medium designed to produce a specific identification reaction which does not require any further confirmatory test

NOTE Identification media which can be used as isolation media are referred to as isolation/identification media.

[EN 1659]

3.3.4.8
media having multiple uses
certain culture media may be assigned to several categories, e.g. Blood agar is a resuscitation medium according to 3.3.4.3, an isolation medium according to 3.3.4.5 and a differential medium according to 3.3.4.6 used for detection of haemolysis

3.3.4 Culture media classified according to preparation method

3.3.5.1
ready-to-use medium
culture medium which is supplied in containers in ready-to-use form (e.g. Petri dishes or tubes or other containers)

3.3.5.2
culture medium prepared from commercially dehydrated formulations
culture medium in dry form which is not ready for immediate use (e.g. powders, granules, lyophilised products). Rehydration will make one of two kinds of medium

- a complete ready-to-use medium ;

- an incomplete medium to which labile components are added at the time of use.

3.3.5.3 Culture medium prepared from individual components **in the laboratory**

3.4 Terminology for test organisms

3.4.1 General

These are microorganisms generally used for quality control and performance testing of culture media. They are defined according to their source as follows.

3.4.2
reference strain
microorganism defined to at least the genus and species level, catalogued and described according to its characteristics and preferably stating its origin

[EN 12322]

3.4.3
reference stocks
a set of separate identical cultures obtained in the laboratory by a single sub-culture from the reference strain either in the laboratory or from a supplier

[EN 12322]

3.4.4
working culture
a primary sub-culture from a reference stock (3.4.3)

4 Practices for quality control of culture media

4.1 Documentation

4.1.1 Documentation required from manufacturer

The following details should be available from the manufacturer:

- name of the medium, individual components and any supplements, and their product codes ;

- batch code ;

- pH value of the medium before use ;
- storage information and expiry date ;
- any performance evaluation and test organism used ;
- technical data sheet ;
- quality-control certificate ;
- safety and/or hazard data where needed.

4.1.2 Check list by the laboratory

Laboratory checks upon receipt of the medium:

- name of medium and batch code ;
- date of receipt ;
- expiry date ;
- condition and integrity of packaging ;

4.2 Storage

4.2.1 General

In all cases follow the manufacturer's instructions where available regarding storage conditions, expiry date and use.

4.2.2 Quality management and control for dehydrated media and supplements

Media are usually purchased from commercial manufacturers. They are delivered in dehydrated powdered or granulated form in sealed containers and supplements of different selective or diagnostic substances are supplied in either the lyophilised or liquid state. However, purchases should be planned to encourage a regular turnover of stock (i.e. first in-first out). To maintain an effective inventory further checks should include :

- re-checking of the seal ;
- date of first opening ;
- visual assessment of contents of opened containers.

Especially after opening a new container, the quality of the medium may depend on the storage environment. Loss of quality of dehydrated media is shown by change in flow characteristics of the powder, homogeneity, caking, colour changes etc.. Any dehydrated medium that has absorbed moisture or shows obvious changes in physical appearance should be discarded.

4.2.3 Commercially supplied ready-to-use media

Follow the manufacturer's instructions regarding storage conditions, expiry date and use.

4.2.4 Media prepared from commercially available dehydrated formulations and basic individual components

The shelf-life of these types of media varies. It is therefore difficult to state general time limits for storage of prepared media. Specific International or National Standards may stipulate specific conditions and shelf life.

Sterilised culture media dispensed in plates, tubes or bottles and reagents which are not used immediately shall be protected against light and desiccation.

Unless a validated expiry date has been established or is specified in the International Standard in question, sterile partially complete media, i.e. media to which final components are added immediately before use, shall be kept in a refrigerator for not more than 3 months or at room temperature for not more than 1 month under conditions which prevent their composition from being modified. However, it is recommended that media to which labile selective supplements have been added should be used on the day of preparation. Solid media containing chemically reactive and / or labile substances should not be stored in bulk for remelting.

Observe any colour change, sign of evaporation/dehydration or microbial growth. Batches of media showing such changes should not be used.

Prior to use or before further heating, it is recommended that the culture media be equilibrated to ambient temperature.

4.3 Laboratory preparation of media

4.3.1 General

The accurate preparation of culture media is one of the fundamental steps in microbiological examination and it shall be given special care.

Respect good laboratory practice and the manufacturer's instructions regarding the handling of dehydrated media and other components, particularly those containing hazardous materials i.e. bile salts or other selective agents.

Where media are prepared from dehydrated commercial formulations follow the manufacturer's instructions precisely. Document all relevant data, i.e. weights/volume, pH, date of preparation, sterilisation conditions, operator.

For media prepared from individual components, follow the recipe precisely and record all details as in (4.1.2) and, in addition, the full identity (i.e. code and batch number) of all the components used.

4.3.2 Water

The water used shall be distilled water or water of equivalent quality i.e. free from substances likely to inhibit or influence the growth of microorganisms under the test conditions. If the distilled water is prepared from chlorinated water, neutralise the chlorine prior to distillation.

The distilled water shall be stored in containers manufactured preferably from inert materials (e.g. neutral glass, polyethylene etc.) which shall be shown to be free of any inhibitory substances prior to their initial use.

NOTE In some cases, it may be necessary to use freshly prepared water, free of dissolved carbon dioxide.

In order to be considered as being of good quality, the distilled water shall have a resistivity of at least 300 000Ωcm.

WARNING Water processed through an ion exchanger (de-ionised), may have a high microorganism content ; it is therefore advisable not to use such water without verifying that the microorganism content of the water is low. Consult the manufacturer for the best way to minimise microbial contamination. Heavily

contaminated deionised water that has been filter sterilised may still contain substances inhibitory to the growth of some microorganisms.

4.3.3 Weighing and rehydration

Carefully weigh the appropriate amount of dehydrated medium (taking care not to inhale powder, especially with media containing toxic substances) and progressively add the required amount of water avoiding clumping.

4.3.4 Dissolution and dispersion

Dehydrated media needs rapid dispersion by instant and repeated stirring followed by heating, if necessary, to dissolve. Media containing agar should be allowed to soak for several minutes prior to heating with mixing to dissolve. For media prepared from individual components each component should be added separately and allowed to dissolve before finally making up to volume.

4.3.5 Measurement and adjustment of pH

Measure the pH using a pH meter and adjust if necessary i.e. for media prepared from individual components in the laboratory so that after sterilising and cooling to 25 °C the medium is at the required pH ± 0,2 pH units, unless otherwise stated. The adjustment is normally carried out using a solution of approximately 40g/l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) hydrochloric acid (HCl).

NOTE Commercially manufactured media may show significant changes in pH before and after autoclaving. However, provided good quality distilled or de-ionised water is used, pH adjustment prior to autoclaving should not be necessary.

4.3.6 Dispensing

Dispense the medium into appropriate containers having a volume 1,2 to 3 times that of the medium.

4.3.7 Sterilisation

4.3.7.1 General

The sterilisation of culture media and of reagents may be carried out by using sterilisation by moist heat (4.3.7.2) or sterilisation by filtration (4.3.7.3).

Certain media do not need sterilisation by autoclaving but can be used following boiling. For example, media for *Enterobacteriaceae* containing brilliant green are particularly sensitive to heat and light and should be rapidly cooled after boiling and protected from strong light. Also some reagents can be used without sterilisation (refer to appropriate International Standard or manufacturer's instructions).

4.3.7.2 Sterilisation by moist heat

Sterilisation by moist heat is performed in an autoclave or media preparator. Generally the autoclaving operation takes 15 min at 121 °C. For volumes greater than 1 000 ml, adapt the sterilisation cycle as necessary. In all cases follow the instructions given in the International Standard or the manufacturer's instructions. The performance of the autoclave should be monitored by temperature profiling using thermocouples and test strips under typical load conditions to ensure the desired temperatures can be reached.

NOTE Overheating can occur when large volumes of media (> 1 000 ml) are processed in an autoclave.

Control of the efficacy of sterilisation is essential.

After heating it is essential that media be cooled in a manner to prevent boiling over. This is particularly important for sensitive media e.g. *Enterobacteriaceae* and media in large volumes.

4.3.7.3 Sterilisation by filtration

Sterilisation by filtration can be performed under vacuum or pressurised conditions. Use membranes and filter elements with a pore diameter of 0,22 μm. They shall have been sterilised in the autoclave. Refer to the manufacturer's instructions regarding the use of filter elements or membranes that have been purchased in a sterile condition. Sterilise the different parts of the filtration apparatus, assembled or not, in the autoclave for 15 min at 121 °C. If necessary, aseptic assembly can be performed in a laminar flow cabinet after autoclaving.

NOTE Some filter membranes may retain proteins (such as antibiotics). In order to obtain the correct concentration the user should pre-wet the filter.

4.3.7.4 Monitoring

After autoclaving, boiling or filtration, all media should be monitored, in particular with respect to pH, colour, sterility and consistency.

4.3.8 Preparation of supplements

Manufactured supplements containing toxic agents, particularly antibiotics, must be handled with care avoiding dispersion of powder which may give rise to allergic or other reactions in laboratory personnel. Take appropriate precautions and follow the manufacturer's instruction when making solutions. Do not use beyond their stated shelf-life which, for antibiotic working solutions, is generally the same day. Under certain circumstances, antibiotic solutions may be stored frozen in suitable aliquots but should not be re-frozen after thawing. The potential loss of activity due to freezing should be discussed with the manufacturer or tested by the user.

4.4 Preparation for use

4.4.1 Melting of agar culture media

Melt a culture medium by placing it in a boiling water bath or by any other process which gives identical results (e.g. a steam flow-through autoclave). Media that have previously been autoclaved should be reheated for a minimum time to maintain media quality. Avoid over-heating and remove when it has melted. Cool the molten medium to 47 °C ± 2 °C in a thermostatically controlled water bath until such time as it is to be used. The time needed to reach 47 °C depends on the type of medium, the volume and the number of units in the water bath. Molten medium should be used as soon as possible but it is recommended that it should not be retained for more than 4 h.

4.4.2 De-aeration of culture media

If necessary, just prior to use, heat the culture medium in boiling water or under a flow of steam for 15 min, with lids or caps loose ; after heating, tighten the caps and cool down rapidly to the operating temperature.

4.4.3 Addition of supplements

Heat-labile supplements should be added to the medium after it has been cooled to 47°C ± 2°C. Allow the sterile supplement to come to room temperature before adding it to the agar medium. Cold liquids may cause agar to gel or form transparent flakes. Mix all supplements into the medium gently and thoroughly, then distribute into the final containers as quickly as possible.

4.4.4 Preparation and storage of media in Petri dishes

Pour the molten agar culture medium into Petri dishes so as to obtain a thickness of at least 2 mm (e.g. for 90 mm diameter dishes, 15 ml of agar are normally required). Allow the agar to cool and solidify by placing the Petri dishes with lids in place on a cool, horizontal surface.

NOTE During incubation, a loss of moisture of the agar media will occur. A loss of more than 15 % of the water content can adversely affect the growth of microorganisms in some circumstances. Factors influencing water loss are medium composition, amount of medium in the plates, the type of incubator i.e. fan-assisted or otherwise, humidity of the atmosphere in the incubator, the position and number of the plates in the incubator and the incubation temperature.

Use the solidified medium immediately or store under conditions which prevent its composition from being modified, i.e. in the dark and/or in the refrigerator at 4°C to 12°C in sealed bags for a maximum period of one week or as directed by the manufacturer or specific standard. Label the dishes on the base with date of preparation and/or expiry date and identity. Alternative coding systems meeting these requirements may be used.

The shelf-life of poured plates will increase if they are stored in sealed plastic bags. In order to avoid the occurrence of condensate, the plates must be cool before being placed into bags. Do not dry the surface of agar plates prior to chill storage.

In general, for the surface inoculation of a solid culture medium, dry the dishes, preferably with the lids removed and with the agar surface facing downwards, in an oven set at a temperature between 25 °C and 50 °C or in a laminar-flow cabinet, until the droplets have disappeared from the surface of the medium. Do not over-dry them. Commercially prepared ready-to-use agar plates should be stored and used according to the manufacturer's instructions.

4.4.5 Incubation

Do not stack dishes in piles more than 6 high and leave space for air circulation to allow medium to equilibrate to incubation temperature as rapidly as possible. For liquid media, time to reach incubation temperature is dependant upon a number of factors e.g. volume, loading, container, incubator type. In the case of anaerobic jars it may be necessary to stack plates in excess of 6 high.

4.5 Disposal of media

Both contaminated and unused media must be disposed of in a manner that is safe and meets any local or national Regulations.

5 Quality control of finished product

5.1 Physical quality control

Laboratory testing should include as a minimum :

- pH-value measured at between 20 °C and 25 °C ;

and by observation :

- quantity filled and/or layer thickness ;
- colour ;
- clarity/presence of optical artefacts ;
- gel stability / consistency / moistness.

5.2 Microbiological quality control

5.2.1 Contamination

An appropriate amount of each batch should tested for contamination.

5.2.2 Test organisms

A set of test organisms should only contain microorganisms with stable characteristics representative of their species and that have been shown to be reliable for the demonstration of optimal performance of a particular laboratory prepared medium. The test organisms should primarily comprise strains that are widely available in reference culture collections, but well-characterised strains isolated by the laboratory may also be included. The relevant cultural characteristics of the stock culture should be examined and recorded by the laboratory and the strain renewed should atypical characteristics occur. It is preferable to use strains that have originated from foods although not all culture collections provide such data on their origin.

The test organisms for each medium may include :

- robust positive strains with typical characteristics ;
- weakly growing positive strains (i.e. of a more sensitive nature) ;
- biochemically un-reactive strains e.g. those showing different fermentation or fluorescence reactions ;
- completely inhibited strains.

NOTE The International Committee for Food Microbiology and Hygiene's Working Party on Culture Media (WPCM) have prescribed a validated collection of test strains for media evaluation [1].

5.2.3 Ready-to-use media and reagents

Manufacturers of commercially available ready-to-use media especially if approved to ISO 9001 [2] or ISO 9002 [3] standards, will have a quality programme in place and may issue a quality certificate with the media they supply. Under those conditions the user may not need to carry out extensive testing on such media but should ensure that storage conditions are maintained.

5.2.4 Media prepared from commercially available dehydrated formulations

For the purposes of this part of ENV ISO/TR 11133-1, qualitative tests for each batch of prepared medium are the minimum requirements but, where quantitative examinations on samples are to be carried out, quantitative tests on each batch will give greater assurance of media quality. For those media which contain no indicators or selective agents the use of a single positive test strain is appropriate. For those media which do contain indicators or selective agents, strains which demonstrate the function of the indicator(s) and selectivity must be utilised. For complex media, i.e. with added supplements, each batch should be verified with strains with characteristics listed in 5.2.2. In the case of ready-to-use media to which laboratory-prepared supplements have been added the same applies.

5.2.5 Media prepared from basic individual components.

It is recommended that in addition to the qualitative tests described in 5.2.4 that some quantitative testing is carried out using techniques such as the modified Miles & Misra technique [1] or spiral plating in order to monitor trends in quality of basic materials, productivity of the medium and in-house laboratory production protocols.

NOTE These are minimal guidelines, in practice, foods may contain stressed microorganisms. The suitability of the medium with respect to the recovery of stressed cells should be taken into account. For information on preservation, maintenance techniques and reference culture collections see Annex B.

Annex A
(informative)
Designation of the components of the culture media in standards on microbiological analysis of food and animal feeding stuffs products

A.1 General

In order to harmonise the description of the various components in the composition of culture media in microbiological standard methods, ISO/TC 34/SC 9 “Food and agricultural products – Microbiology” decided the designations for the categories of components designated in A.2 to A.5.

A.2 Peptones

- enzymatic digest of casein[2)];
- enzymatic digest of soybean meal ;
- enzymatic digest of animal tissues[3)];
- enzymatic digest of heart ;
- enzymatic digest of gelatin ;
- enzymatic digest of animal and plant tissue.

A.3 Extracts

- meat extract ;
- brain-heart extract ;
- yeast extract ;
- ox bile for bacteriology ;
- bile salts ;
- bile salts No.3.

A.4 Agar

- bacteriological agar ;

A.5 Other

- egg yolk emulsion ;

2) This includes peptic digest of casein, tryptic digest of casein and tryptone.

3) This includes meat peptone, peptic digest of meat, pancreatic digest of meat.

- skim milk powder ;
- acid hydrolysate of casein.