INSTRUCTIONS FOR USE

DEOXYRIBONUCLEASE TEST MEDIUM

Dehydrated culture medium

DNAse Test Agar - from left:
S.aureus DNAse+, *K.pneumoniae* DNAse-

1 - INTENDED USE

In vitro diagnostic. For the differentiation of microorganisms by the ability to produce deoxyribonuclease enzyme.

2 - COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	20 g
Deoxyribonucleic acid	2 g
Sodium chloride	5 g
Agar	15 g

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Deoxyribonuclease Test Medium, prepared according to the formula of Jeffries, Holtman and Guse,[1] is a differential medium to test the ability of an organism to produce deoxyribonuclease (DNase) enzyme. The DNase test should be used in conjunction with other tests for the identification of *S.aureus* (+)[2] and is an aid in the differentiation and identification of non-pigmented *Serratia* strains (+) from *Klebsiella-Enterobacter* (-).[3]

Cunningham, Catlin and Garilhe[4] in 1956 demonstrated that coagulase positive, mannitol fermenters and chromogenic *S.aureus* strains, produce a calcium-dependent, thermostable, deoxyribonuclease capable of hydrolyzing the 5'-phosphodiester bonds of DNA. Weckman and Caltin[5] in a study with 87 staphylococcal strains of clinical origin showed that the deoxyribonuclease activity is well correlated with the production of coagulase and the coagulase test can be accompanied by the DNase test for the detection of pathogenic staphylococci.

Tryptose provides carbon and nitrogen for growth; sodium chloride maintains the osmotic balance; deoxyribonucleic acid enables the detection of deoxyribonuclease that depolymerizes DNA.

The depolymerization of the DNA may be detected by adding a hydrochloric acid solution to the plates and observing clear zones around the colonies. In the absence of DNase activity, the reagent reacts with the polymerized DNA, resulting in the formation of a cloudy precipitate.

The medium can be supplemented with mannitol, a pH indicator and dyes to obtain additional differential information or to avoid the use of hydrochloric acid to detect the DNase.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	whitish, opalescent
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Deoxyribonuclease Test Medium	Dehydrated culture medium	4013682	500 g (11,9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave and water-bath, sterile loops, incubator and laboratory equipment as required, ancillary culture media and reagents for the complete identification of the culture; Hydrochloric acid (HCl) 1 N solution.

8 - SPECIMENS

Deoxyribonuclease Test Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

9 - TEST PROCEDURE

Use a heavy inoculum and draw a line 3-4cm long from the rim to the centre of the DNase Test Agar plate.
Incubate aerobically at 35-37°C for 18-24 hours.

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10 - READING AND INTERPRETATION

After incubation flood the plate to a depth of a few millimetres of 1N HCl and leave the plate to stand for a few minutes to allow the reagent to absorb into the medium. Decant excess hydrochloric acid and then examine against a dark background. Polymerized DNA is precipitated and produces a white cloudy area in the agar because of the reaction of HCl with DNA.
Positive result: colonies or growth are surrounded by clear zones.
Negative result: no zone of clearing or cloudy precipitate around colony/growth and throughout DNase test agar plate.

11- DISCRETIONARY ADDITIVES

Deoxyribonuclease Test Medium may be supplemented with several compounds for obtaining additional differential information or to avoid the use of hydrochloric acid for detecting the production of DNase. The modified medium as described below can be incubated as previously described.
1. DNase Test Agar W/ mannitol (Coobe[6]): before sterilization, add 10 g/L of mannitol and 0.025 g/L of phenol red or bromothymol blue to the base medium; the modified medium allows the detection of mannitol fermentation (yellow colonies with yellow halo).
2. DNase Test Agar W/toluidine blue (Schreir[7]): add to the base medium, before sterilization, blue toluidine 0.1 g/L; the modified medium is blue in colour because of the formation of complexes with polymerized DNA and allows the detection of the production of DNase without the addition of HCl (DNase +: pink zone around the growth, DNase -: the medium remains blue). The medium with toluidine is inhibitory for Gram positive bacteria therefore it is suitable for the detection of the DNase of *Enterobacteriaceae* only.
3. DNase Test Agar W/methyl green (Smith[8]): add to the base medium, after sterilization at 47-50°C, 10 mL/L of a 0.5% solution of methyl green in water, repeatedly extracted with chloroform; the modified medium is green in colour because of the formation of complexes with polymerized DNA and allows the detection of the production of DNase without the addition of hydrochloric acid (DNase +: colourless zone around the growth, DNase -: the medium remains green).

12 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory.. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.aureus ATCC 25923	35-37°C / 18-24H / A	clear zones around the colonies (DNase +)
K.pneumoniae ATCC 27736	35-37°C / 18-24H / A	no zone of clearing (DNase -)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

13 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Deoxyribonuclease Test Medium is tested for performances characteristics comparing the results with a previously approved Reference Batch.
Pure colonies cultivated on Tryptic Soy Agar of 3 DNase positive strains (*S.aureus* ATCC 25923, *S.marcescens* ATCC 8100, *B.cereus* ATCC 11778) and 2 DNase negative strains (*K.pneumoniae* ATCC 27736, *S.epidermidis* ATCC 12228) are inoculated by drawing a line on the agar surface. After incubation at 35-37°C for 18-24 hours aerobically, 1N HCl reagent is added to each plate and the clear zones around the growth is observed. All strains show a reactivity according to the specifications for both batches tested.

14 - LIMITATIONS OF THE METHOD

- The detection of DNase enzyme is not a sufficient to speciate an organism; additional biochemical and serological tests must be performed.
- Other microorganisms such as *B.bronchispetica*, *P.vulgaris, P.mirabilis, C.diphteriae, Clostridium septicum, E.coli, P.aeruginosa, A.hydrophila, Vibrio, Bacillus, Streptococcus*, are either positive or variable for DNase production. [3]
- The addition of 1N HCl to the plate inactivates microbial growth and the plates cannot be further incubated or used for other diagnostic tests.
- Both *S.aureus* and *S.epidermidis* produce an extracellular DNase, however the quantity produced by *S.aureus* is much higher.[3]
- Some MRSA strains do not give positive DNase test results and some strains of coagulase-negative staphylococci such as *Staphylococcus capitis* may give weak reactions. [9]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

15 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- Apply Good Manufacturing Practice in the production process of prepared media.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.

- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

16 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (plates/tubes/bottles) and the storage method (temperature and packaging).

17 - REFERENCES

1. Jeffries CD, Holtman DF, Guse DG. Rapid method for determining the activity of microorganisms on nucleic acids. J Bacteriol 1957; 73: 590.
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3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Cunningham L, Catlin BW, de Garilhe MP. A deoxyribonuclease Micrococcus pyogenes. J Am Chem Soc,1956; 78:4642-4645.
5. Weckman BG, Catlin BW Deoxyribonuclease activity of micrococci from clinical sources. J. Bacteriol 1957; 73:747-753.
6. Coobe ER. The rapid recognition of Staphylococcus aureus: deoxyribonuclease and coagulase test in correlation with sensitivities and other properties. Ulster Med J 1968; 37:146-149.
7. Schreir JB. Modification of deoxyribonuclease test medium for rapid identification of Serratia marcescens. Amer J Clin Path 1969;51:711-716.
8. Smith PB, Hancock GA, Rhoden DL. Improved medium for detecting deoxyribonuclease-producing bacteria. *Appl Microbiol* 1969; 18: 991-994.
9. Deoxyribonuclease (DNase) Test: Principle, Procedure and results - Learn Microbiology Online.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/06
Revision 2	Update of “precautions and warnings” and “storage conditions and shelf life”	2022/03
Revision 3	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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