Technical Data Sheet

Legionella-GVPC selective Agar

Ordering number: 1.46710.0020 / 1.46710.0100

Legionella-GVPC selective Agar in 90 mm settle plates is designed for the isolation of Legionella from water samples particularly from hot water treatment plants, drinking water, natural water, as well as from clinical material such as sputum, tracheobronchial secretions, pleural effusion, exudates and other.

Ten settle plates each with a diameter of 90 mm are single-bagged in transparent, hydrogen peroxide impermeable sleeves (non-irradiated). The sleeves consist of polypropylene with a barrier of PE-EVOH-PE.

The composition of Legionella-GVPC selective Agar complies with ISO 11731.

Mode of Action

In Legionella-GVPC selective Agar the high amount of glycine and polymyxin B act against gram-negative, vancomycin against gram-positive bacteria of the accompanying bacterial flora. Cycloheximide acts fungicidal against both yeasts and molds. Thus, the selectivity of the medium is increased. Legionella-GVPC selective Agar is currently considered the best selective medium for the isolation of *Legionella pneumophila* from environmental samples.

Legionella have special demands on the culture conditions and require special growth factors. They are unable to grow on the usual routine agar, neither on blood agar.

The activated carbon in GVPC agar has an antitoxic effect due to the adsorption of fatty acids, but especially due to the breakdown of oxygen radicals and H_2O_2. Yeast extract provides the necessary proteins and nutrients. L-cysteine is an essential growth factor for all Legionella. The soluble iron (III) pyrophosphate [$Fe_4(P_2O_7)_3$] is used as a special iron source, whose quality significantly affects the growth of the Legionella. α-ketoglutarate stimulates the growth of legionella both as a carbon and nitrogen source (Krebs Cycle) as well as through mediation of the iron transport. Legionella grow optimally in a narrow pH range from 6.85 to 6.95. By addition of ACES buffer (N-2-acetamido-2-aminoethane sulfonic acid / KOH) this pH range is guaranteed.

Typical Composition

Activated Charcoal	2 g/l
Yeast Extract	10 g/l
ACES Buffer	10 g/l
$Fe_4(P_2O_7)_3$	250 mg/l
L-Cysteine	400 mg/l
Glycine	3 g/l
A-Ketoglutarate	1 g/l
Cycloheximide	80 mg/l
Vancomycin	1 mg/l
Polymyxin B	80,000 IU
Agar	17 g/l

The appearance of the medium is black and nearly non-transparent. The pH value is in the range of 6.6-7.0. The medium can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

Each plate is provided with a label including a data matrix code for paperless plate identification. The code consists of a two-dimensional 20-digit serial number, which harbors the following information:

digits 1-3: here code 226 (corresponds to article 146710); digits 4-9: lot number; digits 10-14: batch specific individual number; digits 15-20: expiration date (YY/MM/DD).

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid false positive results.

Legionella grow best at 36 ± 1 ° C in an atmosphere of 80-90 % humidity. They are aerobic and do not require an increased CO_2 content of the air. Incubation is carried out for at least 7 (sometimes 14) days, rapidly growing Legionella can be detected after just 1 to 2 days.

Note: NaCl solutions damage legionella. Keep Legionella culture media in the dark!

Temporary acidification of the sample to pH 2.2 or heating to 56 °C can significantly reduce any accompanying flora without influencing the growth of Legionella. The following procedures are recommended for clinical and environmental samples:

Heat treatment

Incubate specimen for 30 minutes in a water bath at 50 °C, then streak on plate.

Acid treatment

10 ml of specimen are centrifuged for 20 min at 2500-3000 r/min and the supernatant is drained off up to approximately 1 ml. Resuspend the pellet and incubate with 9 ml KCl/HCl buffer pH 2.2* for 5 min.

* KCl/HCl buffer: 3.9 ml of 0.2 M HCl
25.0 ml of 0.2 M KCl
pH 2.2 (adjusted with 1 M KOH)

The pre-treated samples and the untreated starting sample are plated on GVPC selective agar and BCYEα-agar. For samples from the respiratory tract a 1:10 dilution in Tryptic Soy Broth (article number 146432) is often advantageous before plating.

The small Legionella colonies can be viewed with a magnifying glass or a microscope after 1 to 2 days of incubation, some of them are already visible to the naked eye. The colonies show a speckled opalescent as frosted glass and are viscous which complicates the inoculation. The colonies often show a pink to blue-green iridescence during the early growth period that is lost by prolonged incubation. Legionella grow round, with a hem, convex, 1 to 4 mm in diameter and appear gray-white and a little opaque after prolonged incubation. Many *Legionella spp.* produce a brown melanin-like pigment. Under long-wave UV light (at 366 nm) several *Legionella spp.* fluoresce (autofluorescence) and glow blue and white*. L. pneumophila* exhibits no autofluorescence (see table).

In the case of suspected Legionella infection, the clinical samples, as well as water samples, should be checked with a direct Legionella immunofluorescence test (DFA (direct fluorescent antibody) labeled with FITC-monoclonal antibodies) after concentration by filtration or centrifugation. The grown colonies show in the Gram staining extreme polymorphisms with short rod-shaped or long filaments. They are gram-negative and show a weak positive catalase reaction. Not at all *Legionella spp.* show a positive oxidase reaction (see table).

A safe method is using the DFA in the grown colonies. For this purpose, test systems for *L. pneumophila* serogroup 1 and 2-14 as well as other *Legionella spp.* can be used. Additionally, latex agglutination test are available against this antigen combinations. The biochemical activity of Legionella is not sufficient for the usual identification reactions, such as for carbohydrate cleavage or urease reaction. For the elimination of any grown non-Legionella colonies a subculture on BCYEα- **and** blood agar, such as Columbia Blood Agar (article number 146559) has to be done. No growth on blood agar and growth on BCYEα-agar ensures the diagnosis of Legionella. The following table shows the most important reactions of Legionella species. 35 species are known so far.

Phenotypic properties of the moist common Legionella strains:

Strain	Number of serogroups	Isolated from		Oxidase	Hippurate-hydrolase
		Human	Environment		
L. pneumophila	14	+	+	V	+
L. bozemanii	2	+	+	V	-
L. gormanii	1	+	+	-	-
L. jordanis	1	+	+	+	-
L. longbeachae	2	+	-	+	-
L. micdadei	1	+	+	+	-

Strain	Gelatine-liquidation	Brown pigment	Autofluorescence	Motility
L. pneumophila	+	+	-	+
L. bozemanii	+	+	blue-white	+
L. gormanii	+	+	blue-white	+
L. jordanis	+	+	-	+
L. longbeachae	+	+	-	+
L. micdadei	-	-	-	+

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +4 °C to +12 °C.

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum	Incubation	Expected Results
Legionella pneumophila (serogroup 1)	33152	streak plate method	44-48 h at 35-37 °C	good growth; grey-whitish colonies
Legionella pneumophila (serogroup 5)	33216	streak plate method	44-48 h at 35-37 °C	good growth; grey-whitish colonies
Enterococcus faecalis	19433	streak plate method	44-48 h at 35-37 °C	growth inhibited
Candida glabrata	70614	streak plate method	44-48 h at 35-37 °C	growth inhibited
Escherichia coli	25922	streak plate method	44-48 h at 35-37 °C	growth moderately inhibited

Please refer to the actual batch related Certificate of Analysis.

Literature

Dennis, P.J., Bartlek, C.L.R. and Wright, A.E. (1984): Comparison of isolation methods for *Legionella sp.* In: Thornsbury, C. et al. (eds.) Legionella: Proceedings of the 2nd International DGHM (1985). Verfahrensrichtlinien für die Mikro-biologische Diagnostik. Kap. 1.3.

Ehret, W. (1992): Mikrobiologische Legionelladiagnostik. Immun. Infekt. **20**: 50-52.

ISO 11731 (1998): Water Quality – Detection and enumeration of Legionella.

Rodgers, F.G. (1998): Legionella. In: Collier, L., Balows, A., Süssman, M. (eds.): Topley & Wilson's. Microbiology and Microbial Infections. Vol. 2, Chapter 49, pp. 1147-1165. Arnold, London-Sydney-Auckland.

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Ordering Information

Product	Cat. No.	Pack size
Legionella-GVPC selective Agar	1.46710.0020	20 x 90 mm plates
Legionella-GVPC selective Agar	1.46710.0100	100 x 90 mm plates
ReadyPlate™ 55 GVPC Agar ISO 11731	1.46771.0020	20 x 55 mm plates
ReadyPlate™ 55 GVPC Agar ISO 11731	1.46771.0200	100 x 55 mm plates
ReadyPlate™ 55 Kit GVPC Agar ISO 11731	1.46773.0150	Kit (150 plates + 150 membrane filters)
Tryptic Soy Broth	1.46432.0100	100 x 9 ml tubes
Columbia Blood Agar	1.46559.0020	20 x 90 mm plates
Columbia Blood Agar	1.46559.0100	100 x 90 mm plates
EZ-Pak Filters MCE 0.45µm 47mm white gridded	EZHAWG474	4 x 150 pcs
S-Pak Filters MCE 0.45µm 47mm white gridded	HAWG047S6	4 x 150 pcs

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