

PP2267 CYE + GVPC AGAR

FORMULA*

* Formulation may be adjusted and/ or supplemented to meet performance criteria

Charcoal	2.0	gm per litre
Yeast Extract	10.0	
ACES Buffer	10.0	
Ferric Pyrophosphate	0.25	
L-Cysteine	0.40	
alpha-ketoglutarate	1.0	
Agar	13.0	
Glycine (ammonia free)	3.0	
Vancomycin	1.0	mg per litre
Polymyxin	80,000.0	IU
Cycloheximide	80.0	mg per litre
pH [^]	6.8 ± 0.2	

([^] at time of manufacture)

DESCRIPTION

CYE medium is based on the formulation of Edelstein. The sterile lyophilised supplement contains ACES buffer/potassium hydroxide, alpha-ketoglutarate, ferric pyrophosphate and L-cysteine HCl. When added to CYE agar base it stabilises the pH of the medium at 6.9 and provides essential growth factors (1). Inoculated plates are typically incubated at 35°C in a humidified atmosphere in ambient air. CO₂ levels higher than 5% may inhibit growth (2).

This medium was described in Australian and ISO Standards methods for the examination of waters (3, 4).

The antimicrobials glycine, vancomycin, and polymyxin will collectively inhibit most Gram-positive and Gram-negative bacterial growth. Cycloheximide will suppress the growth of fungi (1).

QUALITY CONTROL

Organisms:

<i>L. pneumophila</i>	WDCM 00107 (ATCC®33152™)
<i>L. longbeachae</i>	ATCC®33462™
<i>L. pneumophila</i>	ATCC®43111™
<i>P. aeruginosa</i>	WDCM 00024 (ATCC®10145™)
<i>E. coli</i>	WDCM 00013 (ATCC®25922™)
<i>E. faecalis</i>	WDCM 00087 (ATCC®29212™)

Microbial Load Evaluation:

Sample size and acceptance criteria determined in accordance with ASM Guidelines (5).

Incubated at 30°C for 3 days.

Inoculum:

As described in TFS MBD QSP1105, inoculate the specified test organism using 50-150cfu or working culture A (≥10⁴ cfu). Control plates are inoculated in parallel for the quantitative recovery calculations.

Incubation:

3-5 days / 35°C ± 2°C / aerobic.

Expected Results:

<i>L. pneumophila</i> WDCM 00107	≥50% recovery, grey/green colonies, sheen.
<i>L. longbeachae</i> ATCC®33462™	≥50% recovery, grey/green colonies.
<i>L. pneumophila</i> ATCC®43111™	≥50% recovery, grey/green colonies, sheen.
<i>P. aeruginosa</i> WDCM 00024	inhibited
<i>E. coli</i> WDCM 00013	inhibited
<i>E. faecalis</i> WDCM 00087	inhibited

Also Checked & Recorded

1. Batch number
2. Colour
3. Charcoal distribution
4. Gel strength
5. pH
6. Microbial Load
7. Correctly Labelled

STORAGE

A shelf life of 10 weeks from date of manufacture applies, when stored at 2°-8°C, unopened in its original packaging, and away from direct light.

REFERENCES

1. Bridson E. Y. 'The Oxoid Manual' 9th Edition 2006.
2. Edelstein P.H. *Legionella*. In: *Manual of Clinical Microbiology*, 10th edition. 2011. ASM Press, Washington DC.
3. AS/NZS 3896:2017. *Waters – Examination for Legionella spp. including Legionella pneumophila*. 2017. Standards Australia, Sydney.
4. ISO 11731: 2017. *Water quality – enumeration of Legionella*. 2017. International Standards Organisation, Geneva.
5. *Guidelines for Assuring Quality of Food & Water Microbiological Culture Media*. Current edition. Culture Media Special Interest Group, Australian Society for Microbiology, Inc.

WDCM is the World Data Centre for Microorganisms. See <http://refs.wdcm.org/home.htm> for more information.

ATCC is the American Type Culture Collection. Trade name and catalogue numbers are trademarks of the ATCC. www.atcc.org.

Inoculum levels as described in QSP1105 are in accordance with the ASM Guidelines and with AS5140/ ISO11133.

Expected results are in accordance with AS5140/ISO11133.

Shelflife determination is in alignment with the described parameters of AS5140/ ISO11133.

Dispose of all microbiological media –used or expired – in accordance with Australian Standard AS2243.3.