BD™ Yersinia Selective Agar (CIN Agar) • BD Aeromonas Yersinia Agar

INTENDED USE

BD Yersinia Selective Agar (=CIN Agar, Cefsulodin-Irgasan-Novobiocin Agar) is a selective differential medium for the isolation of *Yersinia enterocolitica*, and **BD Aeromonas Yersinia Agar** is a selective differential medium for the isolation of both *Yersinia enterocolitica* and *Aeromonas* spp. from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

BD Yersinia Selective Agar was first described by Schiemann as an alternative to MacConkey Agar and other commonly used media for isolation of *Yersinia enterocolitica*, a causative agent of gastroenteritis.[1] The medium has been found to be superior to MacConkey, SS, CAL or Y agars.[2] **BD Aeromonas Yersinia Agar** is a modification of **BD Yersinia Selective Agar** that, in addition to *Yersinia enterocolitica*, supports growth of *Aeromonas* species (which also may cause enteritis) since it has a reduced cefsulodin content.[3,4]

In both media, peptones provide nutrients. Fermentation of mannitol in the presence of neutral red results in a characteristic "bull's-eye" colony of *Y. enterocolitica*, colorless with red centers on both media. Selective inhibition of gram-negative and gram-positive organisms is obtained by means of crystal violet, sodium desoxycholate and the antimicrobial agents, cefsulodin, Irgasan® (Triclosan), and novobiocin. On **BD Aeromonas Yersinia Agar**, *Aeromonas* species produce pale colonies that have a rose to red center, similar to *Yersinia*. Both organisms may be differentiated from each other by means of the oxidase reaction (positive for *Aeromonas* only).[3-5]

BD Yersinia Selective Agar and **BD Aeromonas Yersinia Agar** may also be used for the isolation of *Yersinia* species other than *Y. enterocolitica*, e.g., for *Y. pseudotuberculosis*.[5]

REAGENTS

Formulas* Per Liter Purified Water

BD Yersinia Selective Agar

Pancreatic Digest of Gelatin	10.0 g	Magnesium Sulfate	0.001 g
Peptic Digest of Animal Tissue	5.0	Crystal Violet	0.001
Beef Extract	5.0	Neutral Red	0.03
Yeast Extract	2.0	Cefsulodin	0.015
Mannitol	20.0	Irgasan	0.004
Sodium Pyruvate	2.0	Novobiocin	0.0025
Sodium Chloride	1.0	Agar	12.0
Sodium Desoxycholate	0.5		

pH 7.4 +/- 0.2

Instead of 0.015 g of cefsulodin in **BD Yersinia Selective Agar**, **BD Aeromonas Yersinia Agar** contains only 0.004 g of cefsulodin per liter medium.

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates aerobically at 25 +/- 2° C or 35 +/- 2° C and read after 20 to 24 and 42 to 48 hours.

Strains	**BD Yersinia Selective Agar (CIN Agar)**	**BD Aeromonas Yersinia Agar**
Aeromonas hydrophila ATCC™ 7966	Inhibition partial to complete	Growth fair to good; colorless to pale rose colony with a rose to red center
Yersinia enterocolitica ATCC 9610	Growth good to excellent; pale rose colony with a dark red center ("bull's eye" colony)*	Growth good to excellent; pale rose colony with a dark red center ("bull's eye" colony)*
Escherichia coli ATCC 25922	Inhibition complete	Inhibition complete
Enterococcus faecalis ATCC 29212	Inhibition partial to complete	Inhibition partial to complete
Proteus mirabilis ATCC 14153	Inhibition partial to complete	Inhibition partial to complete
Pseudomonas aeruginosa ATCC 27853	Inhibition partial to complete	Inhibition partial to complete, fair growth acceptable
Staphylococcus aureus ATCC 25923	Inhibition partial to complete	Inhibition partial to complete
Uninoculated	Light pink, slightly opalescent	

* May be completely pink after 42 to 48 hours of incubation.

PROCEDURE

Materials Provided

BD Yersinia Selective Agar (CIN Agar) or **BD Aeromonas Yersinia Agar**, both provided in 90 mm **Stacker™** plates. Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

BD Yersinia Selective Agar is a differential selective medium for the isolation of *Yersinia enterocolitica*, and **BD Aeromonas Yersinia Agar** is a selective differential medium for the isolation of *Aeromonas* species and *Yersinia enterocolitica* from human stool specimens or rectal swabs (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Aeromonas and *Yersinia* species grow well between 25 and 37° C. The lower temperature is optimal for *Yersinia* and is recommended for primary isolation. Incubate plates at 25 to 32° C for 24 to 48 h.

A less selective medium, such as **BD MacConkey II Agar**, should also be inoculated with the specimen (and incubated at 35 +/- 2° C) for the detection of other pathogens involved in the infection.
A “cold enrichment” procedure may occasionally be necessary for the isolation of *Yersinia enterocolitica*, from stool specimens and other materials such as food: Inoculate about 1 gram of sample into 8 to 12 ml phosphate-buffered saline (pH 7.2) and hold at 4°C for up to 21 days.[5,6] Enrichment of *Aeromonas* can be performed in alcaline peptone water.[4,7] Periodically subculture from the respective enrichment onto plates of either medium described in this document, streaking for isolation. Incubate plates as stated above.

Results
Typical *Yersinia enterocolitica* colonies will have deep-red centers surrounded by a transparent, pale border giving the appearance of a "bull's-eye" on **BD Yersinia Selective Agar** and **BD Aeromonas Yersinia Agar** after 24 hours of incubation. After 42 to 48 hours of incubation, they are often completely pink. *Yersinia pseudotuberculosis* usually lacks the transparent zone around the colonies. *Aeromonas* produces paler colonies which also have a rose to red center and are oxidase positive. *Aeromonas* can be easily differentiated from *Yersinia* and other *Enterobacteriaceae* by a standard oxidase test (only *Aeromonas* spp. will give a positive result). Biochemical and serological confirmation is necessary for a complete identification of suspicious isolates.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
BD Yersinia Selective Agar (CIN) and **BD Aeromonas Yersinia Agar** are suitable for the isolation of *Yersinia enterocolitica* from human stool specimens or other materials.[1,2, 5-8] In addition, **BD Aeromonas Yersinia Agar** is used for the isolation of *Aeromonas*.[3-5, 7,8]
Although *Yersinia* can be recovered by direct plating, specimens with low viable counts may require cold enrichment (4°C) in phosphate-buffered saline.[5,7] However, cold enrichment may not be practical because of the long incubation time and because it selects for nonpathogenic strains of *Y. enterocolitica* and other *Yersinia* species.[5]
Enrichment of *Aeromonas* in alcaline peptone water may be helpful to isolate the organism form populations that shed low numbers of organisms, e.g., from carriers or convalescent-phase patients.[4,7]
On both media described here, also other *Yersinia* species, such as *Y. pseudotuberculosis, Y. frederiksenii,* and *Y. intermedia* will grow.[5] The pathogenic potential of the latter two species is controversal, but at this time, isolates should not be disregarded.[5]
The formulation of **BD Aeromonas Yersinia Agar** but not **BD Yersinia Selective Agar** is recommended for the selective isolation of *Yersinia pestis*.[5]
Enterobacteriaceae other than Yersinia may grow on these media, especially *Citrobacter* species. *Serratia* and *Citrobacter* cannot always be reliably differentiated from *Yersinia* by colony morphology alone. Therefore, biochemical and serological tests are necessary for confirmation and complete identification of the isolates.

Certain strains of *Aeromonas* may produce weak growth on **BD Aeromonas Yersinia Agar**. It is recommended to include additional isolation media for a recovery of the total population of these organisms.

REFERENCES

1. Schiemann, D.A. 1979. Synthesis of a selective agar medium for *Yersinia enterocolitica*. Can. J. Microbiol. 25:1298-1304.
2. Head, C.B., D.A. Whitty, and S. Ratnam. 1982. Comparative study of selective media for recovery of *Yersinia enterocolitica*. J. Clin. Microbiol. 16:615-621.
3. Altorfer, R., et al. 1985. Growth of *Aeromonas* spp. on cefsulodin-irgasan-novobiocin agar selective for *Yersinia enterocolitica*. J. Clin. Microbiol. 22: 478-480.
4. Abbott, S.L. 2003. *Aeromonas. In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

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6. Weissfeld, A.S. and A.C. Sonnenwirth. 1982. Rapid isolation of *Yersinia* spp. from feces. J. Clin. Microbiol. 15:508-510.
7. Kist, M., et al. 2000. Infektionen des Darmes. *In:* Mauch, H., Lüttiken, R., and S. Gatermann (eds.): MiQ - Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik, vol. 9. Urban & Fischer, Munich, Germany.
8. Downes, F.P., and K. Ito. 2001. Compendium of methods for the microbiological examination of foods. 4th edition. American Public Health Association (APHA). Washington, D.C. USA.

PACKAGING/AVAILABILITY

BD Yersinia Selective Agar (CIN)

Cat. No. 254056	Ready-to-use Plated Media, cpu 20
Cat. No. 254088	Ready-to-use Plated Media, cpu 120

BD Aeromonas Yersinia Agar

Cat. No. 254443	Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.

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