Differential Reinforced Clostridial Agar

Intended Use

Differential Reinforced Clostridial Agar is used for enumerating and cultivating sulfite-reducing clostridia.

Summary and Explanation

Differential Reinforced Clostridial Medium (broth) was developed by Gibbs and Freame in 1965.[1] The medium could be used to enumerate clostridia in foods using the Most Probable Number (MPN) method. Differential Reinforced Clostridial Agar (DRCA) is based on Differential Reinforced Clostridial Medium, but with the addition of agar.

The assay is performed using unheated and heat-shocked tubes of DRCA containing replicate dilutions of the test sample. Blackening of the medium is presumptive evidence for the presence of sulfite-reducing clostridia. In this method, heat-shocked tubes showing blackening are confirmatory for clostridia. Non-heat-shocked tubes showing blackening must be heat shocked to kill off vegetative cells and subcultured into DRCA to confirm the presence of sulfite-reducing clostridia.

Principles of the Procedure

Peptones, beef extract, yeast extract, starch and L-cysteine provide nutrients and co-factors required for good growth of clostridia. Dextrose is included in the medium as an energy source. Partial selectivity of the medium is achieved through the addition of sodium acetate. Agar is the solidifying agent. Anaerobiosis in the medium is detected by the redox indicator resazurin. The addition of ferric ammonium citrate to the medium is used to detect sulfite reduction. Blackening of the medium is due to the formation of iron sulfide.

User Quality Control

Identity Specifications

Difco™ Differential Reinforced Clostridial Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.25% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent while hot; upon cooling, solution becomes light red.
Prepared Appearance:	Light pink, clear to slightly opalescent without significant precipitate.
Reaction of 4.25% Solution at 25°C:	pH 7.1 ± 0.2

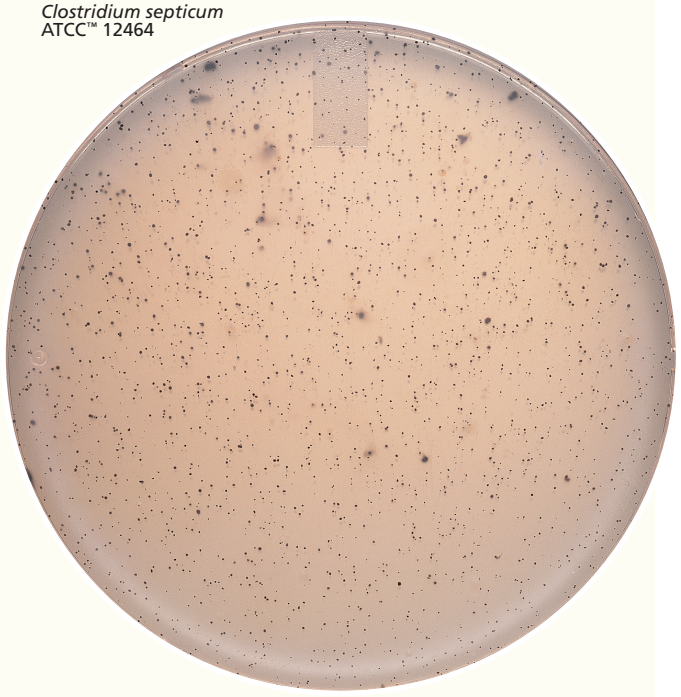
Cultural Response

Difco™ Differential Reinforced Clostridial Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C in an anaerobic atmosphere for 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	BLACK COLONIES
Clostridium bifermentans	638	10^2-10^3	Good	+
Clostridium perfringens	12924	10^2-10^3	Good	+
Clostridium septicum	12464	10^2-10^3	Good	+

Clostridium septicum
ATCC™ 12464



Formula

Difco™ Differential Reinforced Clostridial Agar

Approximate Formula* Per Liter		
Tryptone	5.0	g
Peptone	5.0	g
Beef Extract, Desiccated	8.0	g
Yeast Extract	1.0	g
L-Cysteine HCl	0.5	g
Starch	1.0	g
Dextrose	1.0	g
Sodium Acetate	5.0	g
Sodium Bisulfite	0.5	g
Ferric Ammonium Citrate	0.5	g
Resazurin	2.0	mg
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 42.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Prepare serial 10-fold dilutions of the sample in 1/4 strength Ringer's solution or 0.1% peptone water.
2. Depending on the amount of the initial sample, transfer 1 mL or 0.1 mL of the appropriate dilution, prepared in step 1, to the bottom of a molten (45-50°C) DRCA tube. Prepare a duplicate tube using the same procedure.
3. Tighten the caps on the tubes.
4. Heat one of the duplicate DRCA tubes prepared in step 2 to 80 ± 1°C for 10 minutes to kill vegetative cells.
5. Incubate both tubes, heat-shocked and non-heat-shocked, at 35 ± 1°C for 5 days; examine for sulfite reduction.

Non-heat-shocked cultures showing blackening must be heat shocked and subcultured to DRCA for confirmation.

Alternative Procedures

Inoculate samples onto the surface of agar plates using the streak plate or spread plate technique. Samples may be inoculated into DRCA using the pour plate technique. Medium in agar deeps may be inoculated using the stab technique. DRCA may be used to overlay the membrane filter in the membrane filter technique. Incubate plates and tubes at 35 ± 1°C for 24-48 hours under anaerobic conditions. Agar deeps may be incubated under aerobic conditions when following the Prickett tube method.[2]

Expected Results

The presence of clostridia is presumptively indicated by blackening in the medium. Heat-shocked tubes showing blackening should be considered confirmatory for the presence of sulfite-reducing clostridia.

References

1. Gibbs and Freame. 1965. J. Appl. Microbiol. *28*:95.
2. Miller, Gerrett and Prickett. 1939. Food Res. *4*:447.

Availability

Difco™ Differential Reinforced Clostridial Agar

Cat. No. 264120 Dehydrated – 500 g