RAPID FERMENTATION AGAR (RFA) w/ and w/o CARBOHYDRATES

INTENDED USE
Remel Rapid Fermentation Agar w/ and w/o Carbohydrates are semisolid media recommended for use in qualitative procedures for rapid detection of acid production by fastidious organisms.

SUMMARY AND EXPLANATION
In 1973, Kellogg and Turner developed a rapid carbohydrate fermentation procedure using a buffered salt solution with a pH indicator.[1] In 1974, Brown modified the previous procedure by decreasing the substrate volume in the fermentation tube and increasing the inoculum.[2] Rapid Fermentation Agar (RFA) is a modification of Cystine Tryptic Agar (CTA) originally described by Vera.[3]

PRINCIPLE
RFA medium contains cystine and casein peptone which supply essential nutrients for the growth of fastidious organisms. This medium produces rapid results in detecting acid production from various carbohydrates. When the high concentration of carbohydrate is exposed to a large number of organisms, a rapid reaction takes place which is indicated by an acid shift in the phenol red indicator. The indicator changes from an orange-red color to yellow, indicating a positive test. A negative carbohydrate test, indicated by a neutral orange color, results from the deamination of the peptone in the absence of utilizable carbohydrate.

REAGENTS (CLASSICAL FORMULAE)*

Casein Peptone	20.0	g	Sodium Sulfite	0.5	g
Sodium Chloride	5.0	g	Phenol Red	0.017	g
L-Cystine	0.5	g	Agar	3.5	g
			Demineralized Water	1000.0	ml

pH 7.4 ± 0.2 @ 25°C

The following carbohydrates are available per liter of medium:

Dextrose	20.0	g	Lactose	20.0	g
Fructose	20.0	g	Maltose	20.0	g
			Sucrose	20.0	g

*Adjusted as required to meet performance standards.

PROCEDURE
1. The performance of this medium is dependent on a properly prepared inoculum. Use a pure culture of the test isolate grown on nonselective medium to inoculate RFA tubes. Inoculate a tube of RFA Base Control for each test isolate and incubate in parallel with the selected RFA w/ Carbohydrate tubes. The control tube serves to indicate a system failure, such as carryover of carbohydrate from a primary isolation medium.
2. The inoculum should be well-mixed in the medium at the bottom of the tube. The amount of inoculum and the dispersion of the organisms in the medium will determine the speed of the reaction. A large inoculum must be used to achieve a rapid reaction.
3. Incubate RFA media aerobically at 33-37°C in an incubator or water bath for 2-4 hours with tightened caps. Continue incubation of nonreactive tests for up to 24 hours.
4. Observe for a yellow color development indicating acid production.

INTERPRETATION OF THE TEST
Positive Test - Yellow color development (acid)
Negative Test - Red (alkaline) to orange (neutral) color development

QUALITY CONTROL
All lot numbers of Rapid Fermentation Agar w/ and w/o Carbohydrates have been tested for performance and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. Control organisms should be selected that demonstrate a positive and negative reaction for each carbohydrate tested. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS
1. Incubation in a 33-37°C water bath, rather than in an incubator, has been shown to accelerate the rate of reaction.[4]
2. All isolates of *Neisseria gonorrhoeae* from medical legal cases should be confirmed with at least two methodologies.[4]

BIBLIOGRAPHY

1. Kellogg, D.S., Jr. and E.M. Turner. 1973. Appl. Microbiol. 25:550-552.
2. Brown, W.J. 1974. Appl. Microbiol. 27:1027-1030.
3. Vera, J. 1948. J. Bacteriol. 55:351.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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remel
12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128