MALONATE BROTH
(Ewing Modification)

INTENDED USE
Remel Malonate Broth (Ewing Modification) is a liquid medium recommended for use in qualitative procedures to differentiate members of the *Enterobacteriaceae* based on the utilization of sodium malonate.

SUMMARY AND EXPLANATION
In 1933, Leifson developed a liquid medium for differentiating *Escherichia coli* from *Enterobacter* spp.[1] This medium contained ammonium sulfate as the sole source of nitrogen, malonate as the sole source of carbon, and brom thymol blue as the pH indicator. *Enterobacter* grew and changed the indicator from green to blue (alkalinization). *E.coli* grew poorly and did not change the indicator. Ewing et al. modified Leifson's formulation by adding a trace amount of dextrose and yeast extract to stimulate growth of certain organisms that otherwise failed to grow, and permit observation of their reaction on malonate.[2,3] Malonate Broth is recommended for differentiation of certain subgroups of *Salmonella* from other *Enterobacteriaceae.*[4]

PRINCIPLE
An organism that utilizes sodium malonate as a carbon source and ammonium sulfate as a nitrogen source produces an alkaline reaction. This changes the brom thymol blue from green to light blue to Prussian blue. Some organisms which cannot utilize malonate will ferment dextrose, resulting in an acid reaction causing the pH indicator to change from green to yellow.

REAGENTS (CLASSICAL FORMULA)*

Sodium Malonate	3.0	g	Dipotassium Phosphate	0.6	g
Ammonium Sulfate	2.0	g	Monopotassium Phosphate	0.4	g
Sodium Chloride	2.0	g	Dextrose	0.25	g
Yeast Extract	1.0	g	Brom Thymol Blue	25.0	mg
			Demineralized Water	1000.0	ml

pH 6.7 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. The performance of this medium is dependent on a properly prepared inoculum. Inoculate Malonate Broth with growth from a pure, 18-24 hour culture. Use a light inoculum.
2. Incubate Malonate Broth aerobically with cap loosened at 33-37°C for up to 48 hours.
3. Observe tube at 24 and 48 hours for alkalinization.

INTERPRETATION OF THE TEST
Positive Test - Blue color development due to utilization of malonate

Negative Test - Medium remains green or yellow color develops due to the fermentation of dextrose

QUALITY CONTROL
All lot numbers of Malonate Broth (Ewing Modification) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Klebsiella pneumoniae ATCC® 27736	Aerobic, 48 h @ 33-37°C	Positive
Serratia marcescens ATCC® 8100	Aerobic, 48 h @ 33-37°C	Negative

LIMITATIONS
1. Some malonate-positive organisms produce only slight alkalinity. Compare the results of the test isolate tube with an uninoculated tube of Malonate Broth to verify weak-positive results. Any trace of blue color denotes a positive test at the end of 48 hours incubation. Do not discard test until the tubes have incubated 48 hours.[3,4]
2. Some malonate-negative strains produce a yellow color due to the fermentation of dextrose.[3]

BIBLIOGRAPHY

1. Leifson, E. 1933. J. Bacteriol. 26:329-330.
2. Ewing, W.H., B.R. Davis, and R.W. Reavis. 1957. Public Health Lab. 15:153-167.
3. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
4. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd 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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