

HERROLD'S MEDIUM w/ and w/o ADDITIVES

INTENDED USE

Remel Herrold's Medium w/ and w/o Additives is a solid medium recommended for use in qualitative procedures for selective isolation and differentiation of *Mycobacterium paratuberculosis*.

SUMMARY AND EXPLANATION

Johne's disease is a chronic granulomatous intestinal disease of ruminant animals caused by *M. paratuberculosis*. In 1895 Johne and Frothingham identified acid-fast bacilli in the intestinal tissues of cattle diagnosed with Johne's disease.¹ Twort successfully cultivated *M. paratuberculosis* in 1912, when he discovered *M. paratuberculosis* requires media supplemented with mycobactin for growth.² Mycobactins are endogenous siderophores that are produced by most mycobacteria other than *M. paratuberculosis*, allowing them to grow on Herrold's medium without mycobactin. *M. paratuberculosis*, however, is unable to produce siderophores and will not grow on media lacking mycobactins.³ Herrold's Medium with mycobactin J was found to produce colonies of *M. paratuberculosis* that appeared earlier and were more numerous than on media supplemented with other mycobactins.⁴ Diagnosis of Johne's disease is confirmed by the isolation of *M. paratuberculosis* from the feces of the affected animal. Because *M. paratuberculosis* is vastly outnumbered by other bacteria in fecal and intestinal tissue specimens, decontamination of specimens is recommended before inoculation of growth media.⁵

PRINCIPLE

Casein peptone supplies nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Beef extract supplies vitamins, carbohydrates, and nitrogenous compounds. The glycerol and egg mixture provide fatty acids and protein required for the metabolism of mycobacteria. Malachite green is a selective agent, which inhibits microorganisms other than mycobacteria. Amphotericin B inhibits fungal contaminants. Sodium pyruvate is a source of energy for bacterial metabolism and enhances the recovery of *M. paratuberculosis*. Mycobactin J is required for the growth of *M. paratuberculosis*.

REAGENTS (CLASSICAL FORMULA)*

Herrold's Medium:

Casein Peptone	9.0 g	Amphotericin B	50.0 mg
Sodium Chloride.....	4.5 g	Egg Yolks	100.0 ml
Beef Extract.....	2.7 g	Glycerol	27.0 ml
Malachite Green.....	0.1 g	Agar	15.3 g
		Deminerlized Water	1000.0 ml

pH 7.4 ± 0.2 @ 25°C

The following combinations of optional ingredients are available per liter of medium:

Herrold's Medium w/ Mycobactin J:

Mycobactin J

.....	2.0 mg
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Herrold's Medium w/ Mycobactin J and Pyruvate:

Mycobactin J.....	2.0 mg
Sodium Pyruvate	4.1 g

Herrold's Medium w/ Pyruvate:

Sodium Pyruvate.....	4.1 g
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*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is For Laboratory Use only. It is not intended for use in the diagnosis of human disease or other conditions.

PROCEDURE

1. Process the specimen/sample as soon as possible after it is received in the laboratory.
2. Consult appropriate references for recommended procedures for sample preparation and decontamination.^{4,7}
3. Incubate tubes in a slanted position at 33-37°C, tightening caps after the surface of the medium is dry.
4. Examine tubes weekly for growth for up to 16 weeks.

QUALITY CONTROL

Each lot number of Herrold's Medium w/ and w/o Additives has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers 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, sample results should not be reported.

CONTROL

Herrold's Medium:

<i>Mycobacterium paratuberculosis</i> ATCC® 19698	Aerobic, up to 16 weeks, 33-37°C	No growth
<i>Candida albicans</i> ATCC® 10231	Aerobic, up to 16 weeks, 33-37°C	No growth

INCUBATION

RESULTS

Herrold's Medium w/ Mycobactin J:

<i>Mycobacterium paratuberculosis</i> ATCC® 19698	Aerobic, up to 16 weeks, 33-37°C	Growth
<i>Candida albicans</i> ATCC® 10231	Aerobic, up to 16 weeks, 33-37°C	No growth

Herrold's Medium w/ Pyruvate:

<i>Mycobacterium paratuberculosis</i> ATCC® 19698	Aerobic, up to 16 weeks, 33-37°C	No growth
<i>Candida albicans</i> ATCC® 10231	Aerobic, up to 16 weeks, 33-37°C	No growth

Herrold's Medium w/ Mycobactin J and Pyruvate:

<i>Mycobacterium paratuberculosis</i> ATCC® 19698	Aerobic, up to 16 weeks, 33-37°C	Growth
<i>Candida albicans</i> ATCC® 10231	Aerobic, up to 16 weeks, 33-37°C	No growth

LIMITATIONS

1. Strains of *M. paratuberculosis* frequently lose mycobactin dependence upon subculture and no longer require complex media for growth.³

BIBLIOGRAPHY

1. Collins, M. and E. Manning. 2001. Johne's Information Center. Retrieved, January 12, 2005 from <http://www.johnes.org/index.shtml>.
2. Cocito, C., P. Gilot, M. Coene, M. de Kesel, P. Pascale, and P. Vannuffel. 1994. Clin. Microbiol. Rev. 7:328-345.
3. Merkal, R.S. and B.J. Curran. 1974. Appl. Microbiol. 28:276-279.
4. Whipple, D.L., D.R. Callihan, and J.L. Jarnagin. 1991. J. Vet. Diagn. Invest. 3:368-373.
5. World Organization for Animal Health. 2004. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Retrieved, January 12, 2005 from http://www.oie.int/eng/normes/mmanual/A_00045.htm.
6. Stabel, J.R. 1997. J. Vet. Diagn. Invest. 9:375-380.
7. Carter, G.R. and J.R. Cole, Jr. 1990. Diagnostic Procedures in Veterinary Bacteriology and Mycology. Academic Press, Inc. San Diego, CA.

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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