

Table 1. Metabolites identified in *M. gallisepticum* and *M. bovis* and mycoplasma broth (MB) medium analyses.

| Compound name | Compound ID | Detection platform | | MB Medium |
|----------------|-------------|-------------------------|-----------------|-----------|
| | | <i>M. gallisepticum</i> | <i>M. bovis</i> | |
| AMP | C00020 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | |
| ADP | C00008 | LC/MS* | LC/MS* | |
| ATP | C00002 | LC/MS* | LC/MS* | |
| UMP | C00105 | GC/MS+LC/MS* | LC/MS* | |
| UDP | C00015 | LC/MS | LC/MS | |
| UTP | C00075 | LC/MS | ND | |
| Xanthine | C00385 | LC/MS* | ND | GC/MS |
| Xanthosine | C01762 | GC/MS+LC/MS | GC/MS | |
| Thymine | C00178 | LC/MS | LC/MS | |
| dAMP | C00360 | LC/MS | LC/MS | |
| dCMP | C00239 | LC/MS* | LC/MS* | |
| Adenine | C00147 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | |
| Deoxyadenosine | C00559 | ND | LC/MS | |
| Deoxyguanosine | C00330 | ND | LC/MS | |

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|---------------|--------|--------------|--------------|-------|
| Deoxyuridine | C00526 | ND | LC/MS | |
| dTDP | C00363 | ND | LC/MS | |
| dTMP | C00364 | ND | LC/MS | |
| dUMP | C00365 | ND | LC/MS* | |
| GDP | C00035 | ND | LC/MS | |
| Guanine | C00242 | ND | LC/MS | |
| Orotidine-5P | C01103 | ND | LC/MS | |
| Thymidine | C00214 | ND | LC/MS | |
| Uridine | C00299 | ND | LC/MS* | |
| GMP | C00144 | LC/MS* | LC/MS* | |
| CMP | C00055 | LC/MS* | GC/MS+LC/MS* | |
| Guanosine | C00387 | GC/MS+LC/MS* | GC/MS+LC/MS* | GC/MS |
| Uric acid | C00366 | GC/MS+LC/MS* | GC/MS | |
| Adenosine | C00212 | GC/MS | GC/MS | |
| CTP | C00063 | LC/MS* | LC/MS* | |
| Deoxycytidine | C00881 | LC/MS* | LC/MS* | |
| Uracil | C00106 | LC/MS* | GC/MS+LC/MS* | |

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|------------------|----------------|--------------|---------------|--------|
| Cytidine | C00475 | LC/MS* | LC/MS* | |
| Pseudouridine | C02067 | GC/MS | GC/MS | |
| Inosine | C00294 | ND | ND | GC/MS |
| IDP | C00104 | LC/MS* | LC/MS* | |
| UDP-D-Galactose | C00052 | ND | LC/MS | |
| UDP-D-Glucose | C00029 | ND | LC/MS* | |
| D-Ribofuranose | C16639 (beta) | ND | GC/MS | |
| Sucrose | C00089 | GC/MS* | GC/MS* | GC/MS* |
| D-Glucose | C00267/C00221/ | GC/MS* | GC/MS* | GC/MS* |
| | C00031 | | | |
| Mannitol | C00392 | GC/MS+LC/MS* | GC/MS+LC/MS* | GC/MS* |
| Cellobiose | C00185 | LC/MS* | LC/MS* | |
| D-Erythrose | C01796 | LC/MS | LC/MS | |
| D-Mannose/Talose | C00159/C06467 | GC/MS* | GC/MS* | GC/MS* |
| D-Fructose | C00095 | GC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Arabinofuranose | C06115 (L) | GC/MS | GC/MS | GC/MS |
| D-Trehalose | C01083 | GC/MS* | GC/MS* | GC/MS* |

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|----------------------------------|---------------|---------------|---------------|--------|
| Raffinose | C00492 | | | GC/MS* |
| Glycerol | C00116 | GC/MS | GC/MS | GC/MS* |
| Myo-inositol | C00137 | GC/MS*+LC/MS | GC/MS*+LC/MS | GC/MS* |
| Allo-inositol | | GC/MS | GC/MS | GC/MS |
| D-Glucose-6P | C00092 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | |
| Sedoheptulose-7P | C05382 | GC/MS | GC/MS | |
| D-Glucopyranose-P | | GC/MS | GC/MS | |
| D-Sorbitol-6P | C01096 | GC/MS | GC/MS | |
| D-Mannopyranose-6P | C00275 | GC/MS | GC/MS | |
| D-Galactonofuranose-6P | | GC/MS | GC/MS | |
| sn-Glycerol-3P | C00093 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| D-Sedoheptulose-1,7P | C00447 | LC/MS | LC/MS | |
| 3-Phospho-D-glycerate | C00197 | GC/MS*+LC/MS | GC/MS* | |
| D-Ribose-5P | C00117 | LC/MS* | LC/MS* | |
| Dihydroxyacetonephosphate (DHAP) | C00111 | LC/MS | LC/MS | |
| DL-Glyceraldehyde-3P | C00118/C00661 | LC/MS* | LC/MS* | |
| Octoluse Bisphosphate | | LC/MS | LC/MS | |

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|---------------------------|----------------|---------------|---------------|--------|
| Fructose 1/6-bisphosphate | C00354 | LC/MS* | LC/MS* | |
| Fructose-6P | C00085 | GC/MS* | GC/MS* | |
| 2-Deoxy-D-ribose-5P | C00673 | LC/MS* | LC/MS* | |
| Ribulose-5P | C00199/C01101 | GC/MS* | GC/MS* | |
| Glycerate | C00258 | GC/MS*+LC/MS | GC/MS* | GC/MS* |
| Glyoxylate | C00048 | LC/MS | ND | |
| Phosphoenolpyruvate | C00074 | LC/MS* | LC/MS* | |
| (R)-Lactate | C00256 | GC/MS*+LC/MS | GC/MS*+LC/MS | GC/MS* |
| Pyruvate | C00022 | LC/MS | ND | |
| Citraconic acid | C02226 | LC/MS | LC/MS | |
| 3-Methylphenylacetic acid | | LC/MS | LC/MS | |
| 2-Dehydro-D-gluconate | C06473 | LC/MS* | LC/MS* | |
| Fumarate | C00122 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Malate | C00711/C00149/ | GC/MS+LC/MS* | GC/MS* | |
| | C00497 | | | |
| Isocitrate | C00311 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | |
| Citrate | C00158 | LC/MS* | LC/MS* | GC/MS* |

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|--------------------------|---------------|---------------|---------------|--------|
| Succinate | C00042 | ND | GC/MS* | |
| Acetic acid | C00033 | GC/MS* | GC/MS* | |
| D-Galacturonate | C00333 | LC/MS* | LC/MS* | |
| Gluconic acid | C00257 | GC/MS | | GC/MS |
| Glucuronic acid | C00191 | GC/MS | GC/MS | |
| D-Galactono-1, 4-lactone | C03383 | LC/MS* | LC/MS* | |
| Urea | C00086 | ND | ND | GC/MS* |
| Ornithine | C00077 | GC/MS*+LC/MS | GC/MS* | GC/MS* |
| Lysine | C00047 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Arginine | C00062 | LC/MS* | LC/MS* | |
| Leucine | C00123 | LC/MS* | LC/MS* | |
| L-Tryptophan | C00078 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| L-Valine | C00183 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Aspartic acid | C00049 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Phenylalanine | C00079 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Histidine | C00135 | GC/MS*+LC/MS* | LC/MS* | GC/MS* |
| Pyroglutamic acid | C01879/C02237 | LC/MS* | GC/MS+LC/MS* | GC/MS |

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|--------------------|--------|---------------|---------------|--------|
| Serine | C00065 | GC/MS*+LC/MS | GC/MS*+LC/MS | GC/MS* |
| L-Proline | C00148 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Glycine | C00037 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| L-Alanine | C00041 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Glutathione | C00051 | LC/MS* | LC/MS* | |
| Isoleucine | C00407 | GC/MS*+LC/MS* | GC/MS* | GC/MS* |
| Asparagine | C00152 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Citrulline | C00327 | GC/MS+LC/MS* | GC/MS+LC/MS* | |
| O-phospho-serine | C01005 | GC/MS+LC/MS* | GC/MS+LC/MS* | |
| L-Threonine | C00188 | GC/MS* | GC/MS* | GC/MS* |
| Homoserine | C00263 | GC/MS* | ND | |
| Methionine | C00073 | GC/MS* | GC/MS* | GC/MS* |
| L-Glutamic acid | C00025 | GC/MS+LC/MS* | GC/MS+LC/MS* | GC/MS* |
| Tyrosine | C00082 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | GC/MS* |
| Taurine | C00245 | GC/MS | ND | |
| D-Glucosamine-1P | C06156 | LC/MS* | ND | |
| 2-Keto-isovalerate | C00141 | LC/MS | LC/MS | |

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|-----------------------------|--------|-------------|--------|-------|
| N-Acetyl-glucosamine-1, 6-P | C04461 | LC/MS | LC/MS | |
| D-Glucosamine | C00329 | LC/MS* | LC/MS* | |
| NADH | C00004 | LC/MS* | LC/MS* | |
| NAD ⁺ | C00003 | LC/MS | LC/MS | |
| FAD | C00016 | LC/MS | LC/MS | |
| NADP ⁺ | C00006 | LC/MS | ND | |
| Nicotinamide | C00153 | GC/MS+LC/MS | GC/MS | |
| CoA | C00010 | LC/MS | LC/MS | |
| Lipoate | C00725 | LC/MS | ND | |
| Dephospho-CoA | C00882 | ND | LC/MS | |
| Acetylphosphate | C00227 | LC/MS | ND | |
| Cholesteryl sulfate | C18043 | LC/MS* | LC/MS* | |
| 4-Pyridoxic acid | C00847 | LC/MS* | LC/MS* | |
| Ascorbic acid | C00072 | ND | ND | GC/MS |
| Betaine | C00719 | LC/MS* | LC/MS* | |
| Biotin | C00120 | LC/MS* | LC/MS* | |
| Phosphoric acid | C00009 | GC/MS | GC/MS | GC/MS |

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|---------------------------|--------|---------------|---------------|--------|
| Pyrophosphate | C00013 | ND | GC/MS | |
| Myristic acid | C06424 | GC/MS*+LC/MS* | GC/MS*+LC/MS* | |
| Palmitic acid | C00249 | GC/MS* | GC/MS* | GC/MS* |
| Stearic acid | C01530 | GC/MS* | GC/MS* | GC/MS* |
| Glycerophosphorylglycerol | | GC/MS | GC/MS | |
| 2-Oxobutanoate | C00109 | LC/MS* | LC/MS* | |

Asterisks (*) indicate the metabolites were identified using targeted analysis with authenticated standards on LC/MS and metabolites identified with in house library on GC/MS. The level of identification for each compound, according to the standards proposed by Sumner et al 2007, is listed in Tables S5 and S6. For GC/MS, the fragmented ion and retention time is provided (Table S5), and for LC/MS the retention time, mass accuracy (m/z), obtained mass and ppm errors are provided (Table S6). For metabolites not marked with an asterisk, the level of identification is level 2.