Title: Isolated Methylmalonic Acidemia *GeneReview* – Biochemical Testing
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Biochemical Testing

The following testing methods can both establish the diagnosis and establish the specific enzyme subtype.

Cellular biochemical testing including ^{14}C-propionate incorporation studies followed in some cases by complementation analysis can be used to establish the diagnosis if no pathogenic variants are identified; however, these tests require a skin biopsy to construct a primary fibroblast cell line and none are widely available.

- **^{14}C propionate incorporation assay.** The in vitro cellular assay of propionate conversion indirectly measures the activity of the enzyme methylmalonyl-CoA mutase by assessing the incorporation of the ^{14}C radiolabel in the precursor, propionate, into macromolecules.

  The following methylmalonyl-CoA mutase deficiency enzymatic subtypes are recognized:

  - *mut^0* enzymatic subtype, in which enzymatic activity is non-detectable
  - *mut$^-$* enzymatic subtype, in which residual enzymatic activity is present

    Note: In addition, while an in vitro assay can provide some insight into responsiveness to exogenous administration of cobalamin, it is not always predictive of the in vivo response.
- **Complementation analysis.** This in vitro assay assigns a genetic group or class to the enzymatic block (i.e., *mut^0*/*mut$^-$*, *cblA*, *cblB*, *cblD*-MMA) using heterokaryon rescue or enzymatic cross-correction [Gravel et al 1975]. Assignment of the enzymatic block to a particular complementation group is especially important if the abnormality is not in *MUT*, the gene encoding L-methylmalonyl-CoA mutase. For more details about complementation analysis, see **Complementation Analysis**.
- **Cobalamin distribution.** This in vitro assay uses radioactive CN-[^{57}Co] cobalamin to assess uptake, intracellular amounts, and relative proportions of CN-Cbl, OH-Cbl, adenosyl-Cbl (AdoCbl), and methyl-Cbl (MeCbl) by HPLC [Fowler & Jacobs 1998].

Complementation Analysis

Note: The following information is provided by the authors listed above and has not been reviewed by *GeneReviews* staff.

The cell line from the affected individual is mixed with a panel of established cell lines of known status (e.g., *mut^0*, *cblA*) in the presence of polyethylene glycol (PEG). PEG causes the cells to fuse, allowing cross-correction to occur in vitro. The ^{14}C tracer assay described above is then repeated after fusion. A tester:cell line fusion that fails to rescue ^{14}C incorporation assigns a complementation group to the patient cell line. For example,

if a cell line from a patient with isolated methylmalonic acidemia has defective ^{14}C-propionate incorporation that is restored after fusion with *cblA- cblB-, cblH-,* and *cblD*-derived lines but not a mut class cell line, the defect can be assigned to the methylmalonyl-CoA mutase locus.

Note: (1) Interallelic interactions need to be considered in the assignment of complementation status. Because most cell lines harbor unique combinations of mutations, as many as four distinct mutated alleles can exist in the heterokaryon. If one mutated allele can partially rescue another, ^{14}C propionate flux can be restored in the heterokaryon and the patient line appears to complement the mutant line, even though the mutations reside at the same locus. In practice, a panel of different tester cell lines helps detect a false positive complementation result, which appears as a correction of some lines but not others of the same class. This phenomenon has been studied at the methylmalonyl-CoA mutase locus and may occur in other groups as well [Raff et al 1991]. The exact basis of this phenomenon is unknown. (2) Although leukocytes and EBV-transformed cell lines can be used for propionate fixation assays, fibroblasts are preferred because they are required for somatic cell complementation studies.

References

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