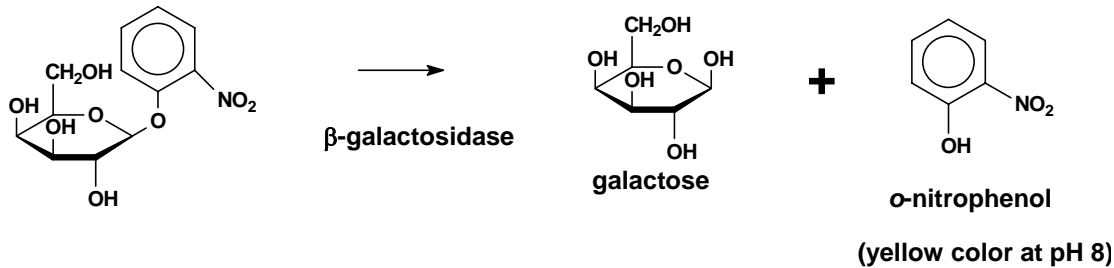
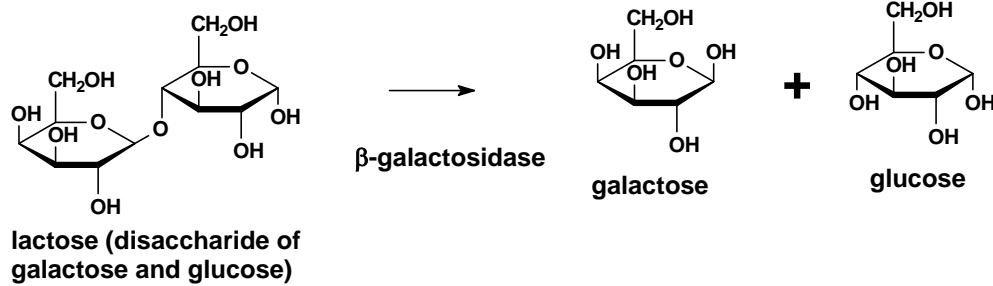


Biochemical Tests incorporated in the ColiLert Assay: ONPG and MUG

ONPG

β -galactosidase is an enzyme found in most coliform bacteria. It catalyzes the hydrolysis of the disaccharide lactose into galactose and glucose. The presence of this enzyme can be easily detected with the water-soluble substrate analog ONPG (see below). The formation of yellow color following incubation in the presence of the ONPG substrate is indicative of β -galactosidase activity and presumptive for coliform bacteria.

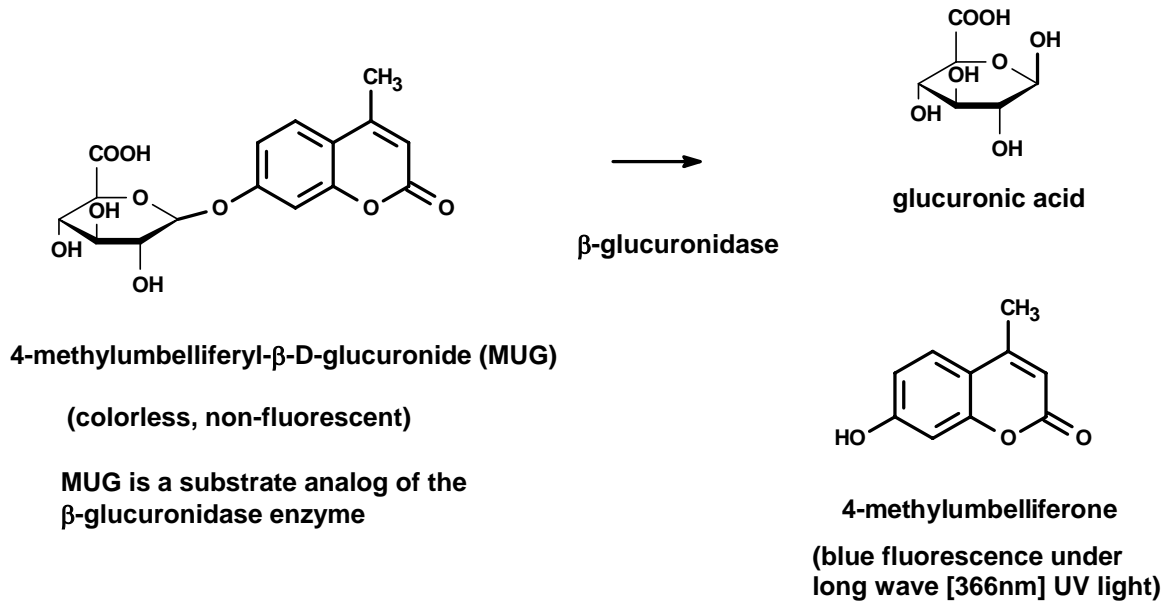


o-nitrophenyl- β -D-galactopyranoside
(ONPG, a colorless compound)

ONPG is a substrate analog of the enzyme β -galactosidase

MUG

β -glucuronidase is an enzyme found in most *Escherichia coli* strains (except for *E. coli* O157:H7), but is absent in most other enteric bacteria, including coliforms. The presence of this enzyme can be easily detected with the water-soluble substrate analog MUG (see below). The formation of a fluorescent compound (4-methylumbelliferone) following incubation in the presence of the MUG substrate is indicative of β -glucuronidase activity and presumptive for *Escherichia coli*. The blue fluorescence emitted by 4-methylumbelliferone can be detected by excitation with long-wave (366 nm) UV light. Do not look directly at the UV light source, only at the culture container being separated.



The ColiLert test combines both the ONPG test and the MUG test and is used to determine the presence or absence of coliforms (yellow color formation only) or the presence or absence of *Escherichia coli* (both yellow color formation and blue fluorescence). By itself the ColiLert test cannot determine numbers of coliforms or *Escherichia coli*, only their presence or absence.

However, with modification the test can be used to estimate the number of coliforms or *Escherichia coli* by using a “most-probable number” or MPN approach. Prior to incubation, if water sample containing the ColiLert growth medium and ONPG/MUG substrates is divided uniformly between a number of separate tubes or wells the MPN approach can be used. For this class we will take the water sample containing the ColiLert medium and reagents and distribute the suspension into multiple wells of a “QuantiTray” device. The separate wells will be individually sealed in a heat sealing device. The tray will then be incubated and the individual wells examined for yellow color or blue fluorescence. Some wells may be negative, others positive. Using the number of positive wells and a table that was developed using the appropriate probability and distribution equations, the “most-probable number” of coliforms or *Escherichia coli* cells that would give the results obtained can be estimated.