## Coliscan® Easygel® Colony Color Guide



## Specific colonies are numbered in picture above and their interpretation is described below. Refer to color photo for actual color reference.

The left half of the photo (#1-4) consists of colonies of *E. coli* (#1A, 1B) and *Enterobacter aerogenes* (#2, 3, 4) growing in/on Coliscan® Easygel® medium. The right half of the photo (#5, 6, 7) represents the appearance of organisms other than *E. coli* or coliforms.

- 1A- Two E. coli CFUs showing purple color with obvious pink diffused halos. Fecal coliform. (Glucuronidase +, Galactosidase +)
- 1B- E. coli CFUs blue/purple color with minimal pink halos. Fecal coliform. (Glucuronidase +, Galactosidase +)
- 2 Enterobacter aerogenes CFUs as dark, solid pink color. Non-fecal coliform. (Contain no blue/purple) (Glucuronidase -, Galactosidase +)
- 3 Enterobacter aerogenes CFUs as light pink color. Non-fecal coliform (Glucuronidase -, Galactosidase +)
- 4 Enterobacter aerogenes CFUs spread on surface of medium.(2 original colonies) Non-fecal coliform. (Glucuronidase -, Galactosidase +)
- 5 Teal green CFU growing on surface of medium. (Glucuronidase +) This colony type should not be counted as E. coli or coliform.\*
- 6 Teal green CFU growing in the medium. (Glucuronidase +) This colony type should not be counted as E. coli or coliform.\*
- 7 Colorless CFU (indicates no Glucuronidase or Galactosidase activity). This colony type should not be counted as E. coli or coliform.\*

\*These teal or colorless types of colonies may be significant other types of bacteria (such as *Salmonella spp.* or *Shigella spp.*) or even rarely found atypical *E. coli* or coliforms, but should never be counted as *E. coli* or coliform without further biochemical testing.

## Background information on Coliscan® Easygel®

Coliform bacteria are members of the family Enterobacteriaceae and are defined as gram negative, non-spore-forming rods which ferment the sugar lactose with the evolution of gas and acids. Many coliforms are normally found in soil and water and do not necessarily indicate the presence of fecal contamination, but *Escherichia coli* (*E. coli*) is a primary bacterium in the human and animal intestinal tract and its presence in food or water indicates fecal contamination. Therefore, *E. coli* is the coliform that is used as an indicator for fecal contamination. Other coliform genera include *Citrobacter*, *Enterobacter* and *Klebsiella*. The USEPA acknowledges that *E. coli* is the best indicator of health risk in fresh water and is currently recommending testing for *E. coli* instead of fecal coliforms. The term "fecal coliform" indicates coliforms which will grow at a temperature of 44.5° C. This is not an accurate designation as there are coliforms of non-fecal origin that will grow at 44.5° C.

Traditional tests for coliforms and *E. coli* or fecal coliforms require the inoculation of media containing lactose, incubation under carefully controlled temperatures, and examination for the presence of gas from lactose fermentation. Additional special media must then be inoculated and incubated at elevated, carefully controlled temperatures to confirm the presence of *E. coli* or fecal coliforms. All these require extra equipment and careful regulation of time and temperature. This approach is not only expensive and time consuming, but can be less than precise in indicating the numbers of specific organisms present.

As a result of the difficulties and lack of precision inherent in the older technology, new approaches have been developed and are being used very successfully. One of the best approaches is based on the fact that in order for coliforms to ferment lactose, they must produce certain enzymes which can be identified and used to verify the presence of the coliforms. General coliforms produce the enzyme galactosidase in lactose fermentation and *E. coli* produces the enzyme glucuronidase in addition to galactosidase.

Coliscan takes advantage of these facts to give you a simple, accurate and quantitative way to identify and differentiate coliforms and E. coli (true fecal coliform) from other bacteria in water or other types of samples. This patented method incorporates two special chromogenic substrates which are acted upon by the presence of the enzymes galactosidase and glucuronidase to produce pigments of contrasting colors. All that is needed to identify the presence and numbers of coliforms and E. coli is to add a test sample to the medium, pour it into a petri dish and incubate it at room temperature or at a higher controlled temperature (35° C is suggested). General coliforms will produce the enzyme galactosidase and the colonies that grow in the medium will be a pink color. E. coli will produce both galactosidase and glucuronidase and will therefore grow as dark blue to purple colonies in the medium. It is simple to count the blue/purple colonies (E. coli) which indicate the number of E. coli per sample. The pink colonies indicate the number of general coliforms per sample. The combined general coliform and E. coli number equals the total coliform number. Any non-colored colonies which grow in the medium are not coliforms, but may be members of the family Enterobacteriaceae. Since the Coliscan contains inhibitors, most other bacterial types will not grow. It is best for the Coliscan to be incubated at a temperature higher than room temperature so that the organisms will grow faster. The suggested temperature range is between 30-37° C (85-99° F). The coliform/E. coli organisms will grow faster at this temperature range than at room temperature, so that results can be counted at 24-48 hours incubation time instead of about 24 hours later if incubated at room temperature, 22-27° C (72-80° F). Micrology Laboratories can provide information on home made or inexpensive commercial incubators.

The beauty of the Coliscan method is that it uses proven and accepted technology to allow anyone to do effective coliform/E. *coli* testing. For water testing, you can add up to a 5 mL sample of water to the bottle of medium that makes one petri plate. This will detect as small a number of coliforms or *E. coli* as one living bacterium in five milliliters of water. The method is also easily adapted for large samples with membrane filter use. Beware of copycat methods by other manufacturers who claim similar red and blue colors for coliforms and fecal coliforms, but whose results are unreliable due to inferior technology. They cannot legally copy the patented Coliscan technology.

Coliscan has a shelf life of 6 months and should be kept frozen until used. You may refrigerate for up to 2 weeks, but freezing is best in order to maintain color intensity throughout the 6 month period.

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