

Expected reactions of various microorganisms with m-ColiBlue24®

Total Coliforms will produce a **red colony**. Specifically Enterobacter, Klebsiella and Citrobacter species are expected to produce a red colony. Not every strain of the above species has been tested. The following strains have been tested and may be recommended for use in quality control testing.

Enterobacter species -	<i>E. cloacae</i> ATCC #13047 <i>E. cloacae</i> ATCC #23355 <i>E. aerogenes</i> ATCC #13048
Klebsiella species -	<i>K. pneumoniae</i> ATCC #13883
Citrobacter species -	<i>C. freundii</i> ATCC #8090

Escherichia coli will produce a **blue colony**. Not every strain of *E. coli* has been tested; however, the following strains are known to give a positive reaction (blue colony).

E. coli ATCC #25922
E. coli ATCC #11775

E. coli O157:H7 is beta-glucuronidase negative and will not produce a blue colony, but will grow as a red colony.

Known **Negative reaction**/No growth after 24-25 hours:

Pseudomonas aeruginosa ATCC #27853
Proteus vulgaris ATCC #13315
Aeromonas hydrophila ATCC #7965
Aeromonas hydrophila ATCC #35654
Aeromonas hydrophila ATCC #49140

Some strains of the following microorganisms are known to produce a **false-positive** total coliform reaction (i.e. a red colony, but not a true total coliform):

<i>Serratia</i> species	<i>Yersinia enterocolitica</i>
<i>Hafnia alvei</i>	<i>Leclercia adecarboxylata</i>
<i>Vibrio fluvialis</i>	<i>Ewingella americana</i>
<i>Aeromonas</i> species	<i>Staphylococcus</i> species
<i>Proteus vulgaris</i> ATCC #6380	<i>Proteus mirabilis</i> ATCC #25933
<i>Providencia stuartii</i>	
<i>Pseudomonas aeruginosa</i> ATCC #9027 - variable reaction may be positive when incubated longer than 25 hours.	

PLEASE NOTE: Many of the bacteria listed above which cause false-positive reactions share key physiological similarities with the 4 traditional coliform* genera (*Escherichia*, *Enterobacter*, *Klebsiella*, and *Citrobacter*). In addition, m-ColiBlue24® was developed to provide optimal growth conditions for all of the strains of coliform bacteria, especially those stressed due to treatment methods for drinking

water. As a result, it is difficult to insure maximum recovery of these 4 target groups without allowing the growth of **some nontarget** microorganisms. The interference caused by these bacteria can be corrected by use of the standard cytochrome oxidase test. Total coliforms, including *E. coli*, are oxidase negative while some interfering bacteria are oxidase positive. The oxidase test is described in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, Section 9225 (p. 9-70). Additionally, two methods of oxidase confirmation are described in the Hach Analytical Procedure. The presence of total coliforms can also be confirmed using the ONPG test. Total coliforms will cleave ONPG producing a positive result, or yellow color. The ONPG test is described in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, Section 9020B (p. 9-10).

Results from the Specificity Study, conducted as a part of the USEPA approval process, show that a false-positive reaction is rare (only 2.5%) with *E. coli* (blue colonies), because the formation of blue colonies results from a very specific enzymatic reaction. There are some strains of bacteria, other than *E. coli*, that produce beta-glucuronidase, but these strains are not typically found in water.

Method Performance Characteristics (taken from the studies conducted for USEPA approval)

For <i>E. coli</i>	false positive error	2.5%
	false negative error	0.0%

Agreement between m-ColiBlue24® and reference methods was 98.8%.

For total coliform	false positive error	26.8%
	false negative error	1.6%

Agreement between m-ColiBlue24® and reference method m-Endo, was 86.2%.

m-Endo method has a false positive error of 29.6% and a false negative error of 3.4%.

*The definition of what constitutes a true coliform varies depending on regulatory agency. Certain agencies list true coliforms as Escherichia, Enterobacter, Klebsiella, Citrobacter, Serratia and Proteus. Some European water companies indicate true coliforms are Escherichia, Enterobacter, Klebsiella, Citrobacter, Serratia, Hafnia and Yersinia. Please contact your local regulatory agency for a their description of a true coliform.

m-ColiBlue24 is a registered trademark and patented product of Hach Company, US Patents 5,650,290 and 5,849,515