

KONFIRM WATER TEST

KS1000 - for 100 ml samples, 20 tests

PRINCIPLE / DISCUSSION:

KONFIRM is an MMO-MUG test to detect and confirm the presence of total coliforms or *E. coli* in drinking water samples. Konfirm will detect the occasional MUG negative *E. coli* not detected in other systems of this type. The active ingredients in Konfirm are incorporated into a tablet containing growth factors, indicators, and inert tableting ingredients, producing easily read reactions which will consistently indicate and confirm the presence of fecal contamination by simple, easy to read reactions. Non-coliform bacteria are inhibited and do not interfere with the test during the specified incubation times.

O-nitrophenyl -β-D-galactopyranoside (ONPG) and 4-Methylumbelliferyl -β-D-glucuronide (MUG) are enzyme substrates bound to specific indicators. When the enzyme galactosidase acts on colorless ONPG, it separates the substrate leaving the pure O-nitrophenyl, which is yellow.

The enzyme glucuronidase separates the MUG compound into its components, releasing 4 methylumbelliferone which is fluorescent in its uncombined state.

Tryptophane is an amino acid which is degraded to indole by some coliforms. The presence of indole is demonstrated by the addition of the developer provided with the kit. This important test is unique to KONFIRM and not offered by other systems of this type. (see INTERPRETATION)

MATERIAL SAFETY DATA:

The active ingredients of the tablets are Amphotericin B, 4-Methylumbelliferyl-β-D-glucuronide, O-nitrophenyl-β-D-galactopyranoside and Tryptophane. Though not known to be dangerous, the chemical properties of all of these have not been thoroughly investigated. Irritation may occur with prolonged contact. In case of accidental ingestion, wash out mouth with water provided person is conscious. Provide oxygen if breathing is impaired. Call a physician

The indole developer contains p-amino-dimethyl-cinnamaldehyde in hydrochloric acid. The developer is poisonous, mildly corrosive and stains fabric and hands. Handle with care. Consult a poison control center in case of ingestion.

MATERIALS REQUIRED:

Each kit contains the specified number of tablets and 1 bottle of developer (PEP reagent). In addition to the tablets provided you will need the following:

Incubator - 35C +/- 0.5

Long wavelength ultraviolet lamp, KS1699 or equal

Non-fluorescent container

Swabs (OPTIONAL)

Forceps

SAMPLE COLLECTION:

Water samples should be collected aseptically according to Standard Methods for the Examination of Water and Wastewater, 17th Edition. Sodium thiosulfate is not necessary but will not interfere with the test if present.

GENERAL INSTRUCTIONS:

Konfirm is a primary drinking water test and should not be used with any type of enhancement. Any method which concentrates the bacteria or encourages the growth of heterotrophs will invalidate the test and encourage false positive reactions.

Konfirm tablets and inoculated tests should be protected from prolonged exposure to direct sunlight.

Always use good laboratory technique when using Konfirm, as with any test for bacteria.

QUALITY CONTROL PROCEDURE:

Each lot of Konfirm should be tested with strains of known reactivity. We recommend:

Escherichia coli - ATCC 25922

Klebsiella pneumoniae - ATCC 33495

Pseudomonas aeruginosa - ATCC 27853

Label a sterile, non-fluorescent container for each of the three organisms. Follow the setup instructions using sterile distilled water. Inoculate the water by lightly touching a colony of pure culture with a sterile loop and mixing into the water.

Alternatively, you may make serial dilutions from a pure broth culture of the quality control organisms to obtain a predictable inoculum of 1-5 colony forming units per 100 ml. or water.

The expected results are:

E. coli: yellow color, blue fluorescence, blue/green indole test

Klebsiella pneumoniae: yellow color, no fluorescence, yellow indole test

Pseudomonas aeruginosa: no color, weak or no fluorescence, yellow indole test

SETUP PROCEDURE:

1) Place one tablet into a sterile, non-fluorescent container with the appropriate amount of water. For KS1000 and KS10008, use 100 +/- 10 ml. For KS1005, use 50 +/- 5 ml.

2) Cap the container and shake briefly to activate the tablet. Small bubbles should appear on the surface of the tablet as reaction begins. Rarely, the manufacturing process produces a tablet that is harder than normal. If this should occur see "Interpretation" for reading results. If desired, the tablet

may be crushed with the forceps when adding to the water. The tablets normally do not dissolve completely; a residue in the bottom is normal and does not affect the test. NOTE: Development of any shade of yellow or brown as the tablet dissolves is an indication of high concentrations of chlorine in the sample, and negates the test. If the water source is one that already shows a yellow or brown color, look for a deepening of the color as the tablet dissolves. (See notes under USE OF COMPARATOR)

3) Incubate for 24 hours at 35°C. If no incubator is available, let test stand 72 hours at room temperature between 60 and 75°F or for 48 hours at temperature between 75 and 90°F. Tests at temperature over 90°F will finish in the normal time frame. For optimal inhibition of organisms other than *E. coli*, higher temperatures (44°C) may be used.

USE OF COMPARATOR:

The comparators are located on a card in the tablet container. It shows the approximate color of the positive yellow ONPG, blue/green indole, and blue fluorescence. To observe the desired color of a positive ONPG test, hold the YELLOW dot behind the bottle of water and observe through the bottle both above and below the water surface. Water that is yellow or brown at onset should develop to this compared color or darker. To observe fluorescence, hold the Wood's lamp over the WHITE dot. A positive MUG will be only slightly duller than this color. For the true color of the fluorescence hold the Wood's lamp in front of the bottle and observe the dot through the bottle, above the water. A positive indole will progress to the color of the GREEN dot in 1-5 minutes.

AFTER 24 HOURS INTERPRET RESULTS:

1) At 24 hours, observe for bright yellow color. Colorless substrate with a dissolved tablet or colorless around the undissolved tablet is negative. **Any level of yellow color development is considered a positive test for total coliforms.** If the test is cream colored and the tablet shows no sign of disintegration after the 24 hours observe for a yellow color around the tablet itself as a positive ONPG. In this event, shake the bottle slightly then reincubate for 4 to 6 hours to intensify the color. No change after the additional incubation is negative.

2) If the ONPG is positive, test for fluorescence by holding it 2-4 inches from a U.V. lamp in a darkened room. Observe for a bright blue fluorescence, especially on the surface of the water. (See USE OF COMPARATOR). **Blue fluorescence along with yellow ONPG is presumptive for *E. coli*.**

3) Optional-If the ONPG is positive, do the indole test. Dip a sterile swab into the test and drop 2-3 drops of developer onto the tip of the swab. An alternate method is to shake the bottle, remove the cap and add the reagent to the liquid that clings to the inside of the lid. Development of a green to blue color is positive. A positive indole along with a positive ONPG and MUG is **confirmatory for *E. coli*; only *E. coli* is positive for all three substrates.**

A positive ONPG, negative MUG, and positive indole has still has a high probability of being *E. coli* whereas a negative indole is **unlikely** to be an *E. coli*. NOTE: All tests are usually complete when a positive ONPG is seen, however the indole test takes the longest to develop and may not be complete before the full incubation time. If reading before the full incubation time, reincubate negative indole tests.

STORAGE AND SHELF LIFE:

Store tablets with the desiccant at room temperature up to 76°F. Store away from light or heat sources. Konfirm is stable under these conditions up to the date provided on the label. The indole developer should be kept refrigerated when not in use.

DISPOSAL

Incubated Konfirm should be always be considered as biohazardous even if negative for coliforms. All used test materials should be decontaminated before disposal. Dispose of all materials used in the test according to EPA standards for biohazard waste. Call us for information on our recycling program for packaging materials.

TECHNICAL ASSISTANCE

If you have any questions, please call us at 1-800-THE-1-KEY (843-1539).

REFERENCES:

1) Manual of Clinical Microbiology, Fifth Edition, Chapter 36, "Enterobacteriaceae".

2) Bailey and Scott's Diagnostic Microbiology, Seventh Edition, Chapter 27 "Enterobacteriaceae".

3) Journal of Clinical Microbiology, June 1992, p.1402-1406, "Fluorogenic Substrates for Differentiation of Gram-Negative Non fermentative and Oxidase-Positive Fermentative Bacteria", Peter Kampfer, Isolde Kulies, and Wolfgang Dott.

4) Journal of Clinical Microbiology, Sept., 1986, p.368-371, "Comparison of β-Glucuronidase-Based Substrate Systems for Identification of *Escherichia coli*.", Stephen C. Edberg, and Christine M. Kontnick.

5) Standard Methods for the Examination of Water and Wastewater 17th Edition, APHA . AWWA . WPCF . Denver, CO.



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