



Aquagenx® CBT H₂S (Compartment Bag Test) Most Probable Number (MPN) Kit Instructions for Use: Drinking Water

Overview

The Aquagenx® CBT H₂S MPN Kit detects and quantifies the Most Probable Number (MPN) of hydrogen sulfide (H₂S) producing bacteria in a 100 mL sample. When the H₂S powder growth medium metabolizes H₂S-producing bacteria, the color of the water turns black, indicating the presence of H₂S-producing bacteria. Most Probable Number (MPN) test results are obtained by easy color match using the Aquagenx® color-coded MPN Table. H₂S-producing bacteria has been evaluated repeatedly as a fecal indicator bacterium for water quality testing. Many studies show H₂S provides both qualitative (presence or absence) and quantitative data (relative concentrations) that are comparable to and sometimes higher than those of the usual fecal indicator bacteria such as *E. coli* and thermotolerant (fecal) coliforms.






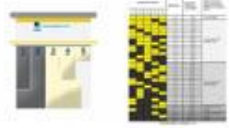

Shelf Life

Shelf life of H₂S growth medium is 2.5 – 2.9 years after date of manufacture.

Storage

Cold chain for H₂S growth medium not required. Recommended storage at 10°-25° Celsius. Protect growth medium from moisture, heat and bright light.

Summary of Test Procedures for CBT H₂S MPN Kit

<p>Collect 100 mL sample</p> 	<p>Add powder growth medium</p> 	<p>Pour sample into compartment bag</p> 	<p>Roll down Whirl-Pak seal and attach plastic clip</p> 
<p>Incubate 24-48 hours</p> 	<p>Score MPN test results</p> 	<p>Decontaminate sample</p> 	

How to Interpret Color-Change Test Results

Color of compartment in Compartment Bag	Yellow/Golden Brown in ambient light	Black/Black Gray in ambient light
H ₂ S-producing bacteria	Negative	Positive

Black (positive) presence of H₂S can look fuzzy. Dirt particles and debris in sample will have sharper edges and look like specs.

If only dark particles or debris are found, the compartment is negative for H₂S.

Basis of Aquagenx® CBT Most Probable Number (MPN) Table

The Aquagenx® CBT MPN Table (page four) is based on the World Health Organization “Guidelines for Drinking Water Quality,” 4th Edition. MPN of H₂S bacteria per 100 mL is estimated from the combination of positive (black color) and negative (no black color) compartments in the Aquagenx® Compartment Bag.

Procedural Notes

1. Prepare work area

- Sanitize work area with disinfectant cleaning solution, paper towels or wipes

2. Collect 100 mL water sample with Whirl-Pak™ Thio-Bag™

- White tablet in Whirl-Pak™ Thio-Bag™ is sodium thiosulfate, which neutralizes residual chlorine in sample. Do not remove.
- Wearing disposable, thin plastic gloves is recommended. If you don't have gloves, avoid touching inside of Thio-Bag with bare hands.
- Limit the collection of debris/particles in Thio-Bag as water sample is collected to avoid confusing debris/particles with potential positive black/gray results for H₂S.
- Fill Thio-Bag to 100 mL fill mark. Record sample details.

3. Add H₂S growth medium to sample in Whirl-Pak™ Thio-Bag™

- Open growth medium powder pillow with scissors and pour powder growth medium into Thio-Bag.
- Do not touch growth medium with bare fingers or hands.
- Close Thio-Bag with Whirl-Pak seal.
- Dissolve medium in sample. Powder medium is dissolved when you cannot see any white powder in sample. Sample liquid may turn pale yellow color.

4. Pour sample with dissolved medium from Thio-Bag into Aquagenx® Compartment Bag

- Label bag or attach barcode asset tag to compartment bag.
- Tear off perforated seam at top of bag.
- Rub top and sides of bag together in each compartment to open so water easily runs into compartments.
- Use white tabs at top of bag to pull compartment bag open.
- Slowly pour sample into bag while gently tilting and squeezing bag to distribute sample amongst five compartments.
- Fill evenly to the top of the fill line.

5. Seal compartment bag shut

- Roll down Whirl-Pak seal at top of compartment bag and fasten shut.
- Attach plastic seal clip across compartment bag to prevent water from leaking out of compartments. Place U-shape part of clip across width of compartment bag along the fill line but *below the compartment openings*. Place rod-shaped part of the clip on the opposite side of compartment bag and snap into U-shape to lock in place.

6. Incubation Period and Temperatures

- During the incubation period, CBTs can develop an odor. To control odor, place CBTs in another sealed plastic bag or container during the incubation period.
- Ambient temperature incubation works at 25°- 37°C.
- The CBT works at variable temperatures. Constant temperature control in an incubator is not required but is recommended in cooler temperatures if available.

Recommended Incubation Periods at Ambient Temperature Conditions:

35-37°C: Incubate 20-24 hours. If no color change, incubate another 24 hours to confirm negative result.

31-34°C: Incubate 24-30 hours. If no color change, incubate another 24 hours to confirm negative result.

25-30°C: Incubate 40-48 hours. If no color change, incubate another 24 hours to confirm negative result.

Below 25°C: Incubate in a portable incubator 35-37°C for 24 hours, or put in or near another heat source for up to 48 hours depending on the temperature

7. Score and record MPN test results

- Align compartments in correct sequence to Aquagenx® MPN Table on page four, hold bag up to read results
- Yellow/golden brown indicates negative (absence) compartment for H₂S
- Black/gray indicates positive (presence) compartment for H₂S
- Black (positive) presence of H₂S can look fuzzy in compartments. Dirt particles and debris in sample will have sharper edges and look like specs. If only dark particles or debris are found in a compartment, the compartment is negative for H₂S.
- Match color sequence of five compartments to one of 32 rows in Aquagenx® MPN Table to obtain MPN test results for H₂S-producing bacteria.
- Record test results.

8. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) or sufficient chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to Thio-Bag to provide about 200 milligrams of free chlorine.
- After 30 minutes, pour contents into a sink, toilet or hole in ground and safely dispose the empty compartment bag.

Aquagenx™ CBT Most Probable Number (MPN) Table

Align your compartment bag so compartment #1 is on the left and compartment #5 is on the right. Match the color sequence of your five compartments to one of these 32 rows. Additional scoring instructions are found below the MPN Table.



Compartment Number					MPN/100mL	Upper 95% Confidence Level/100mL	WHO Health Risk Category Based on MPN and Confidence Level
1	2	3	4	5			
10mL	30mL	56mL	3mL	1mL			
Yellow	Yellow	Yellow	Yellow	Yellow	0.0	2.87	Low Risk/Safe
Yellow	Yellow	Yellow	Black	Yellow	1.0	5.14	Intermediate Risk/ Probably Safe
Yellow	Yellow	Yellow	Black	Black	1.0	4.74	
Yellow	Yellow	Yellow	Black	Black	1.1	5.16	
Yellow	Black	Yellow	Yellow	Yellow	1.2	5.64	
Yellow	Yellow	Black	Yellow	Yellow	1.5	7.81	
Yellow	Yellow	Yellow	Black	Black	2.0	6.32	
Yellow	Yellow	Yellow	Black	Yellow	2.1	6.85	
Yellow	Yellow	Yellow	Black	Black	2.1	6.64	
Yellow	Black	Yellow	Black	Yellow	2.4	7.81	
Yellow	Black	Yellow	Black	Black	2.4	8.12	
Yellow	Black	Yellow	Black	Black	2.6	8.51	
Yellow	Black	Yellow	Black	Black	3.2	8.38	
Yellow	Black	Yellow	Black	Black	3.7	9.70	
Yellow	Black	Black	Yellow	Yellow	3.1	11.36	Intermediate Risk/ Possibly Safe
Yellow	Black	Black	Yellow	Black	3.2	11.82	
Yellow	Black	Black	Yellow	Black	3.4	12.53	
Yellow	Black	Black	Yellow	Black	3.9	10.43	
Yellow	Black	Black	Yellow	Black	4.0	10.94	
Yellow	Black	Black	Black	Yellow	4.7	22.75	
Yellow	Black	Black	Black	Black	5.2	14.73	
Yellow	Black	Black	Black	Black	5.4	12.93	
Yellow	Black	Black	Black	Black	5.6	17.14	
Yellow	Black	Black	Black	Black	5.8	16.87	
Yellow	Black	Black	Black	Black	8.4	21.19	
Yellow	Black	Black	Black	Black	9.1	37.04	
Yellow	Black	Black	Black	Black	9.6	37.68	
Yellow	Black	Black	Black	Black	13.6	83.06	High Risk/Possibly Unsafe
Yellow	Black	Black	Black	Black	17.1	56.35	High Risk/Probably Unsafe
Yellow	Black	Black	Black	Black	32.6	145.55	High Risk/Probably Unsafe
Yellow	Black	Black	Black	Black	48.3	351.91	High Risk/Probably Unsafe
Yellow	Black	Black	Black	Black	>100	9435.10	Unsafe

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Black (positive) presence of H2S can look fuzzy. Dirt particles and debris in sample will have sharper edges and look like specs.

If only dark particles or debris are found, the compartment is negative for H2S.