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# Comparative Evaluation Guide

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Kool Kount Assayer  
Comparative Evaluation Guide

## Laboratory procedures



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## Comparative Evaluation Guide

### Kool Kount Assayers

#### Technical Information

##### KOOL KOUNT Assayer (KKA)

KKA uses the biological reduction of 2,3,5 triphenyl tetrazolium chloride (TTC) by actively metabolizing microorganisms as its method. TTC is a redox indicator. The indicator TTC is typically used as a color indicator with TSB media, standard heterotrophic agar plates and dip slides. TTC is commonly referenced in various general laboratory procedures for total viable microbe measurements. In KKA, the rate of this indicator's reduction by microbes over time is used to measure microbial concentration. In 1996, this method was granted a patent, 5550032. This technique was submitted for ASTM method approval in 1995 after completing the ASTM required round robin evaluations for field test methods. In January 1998, ASTM subcommittee D19.24 approved the method

##### Intended Use

KKA is used for the quick field enumeration of a wide variety of microorganisms. The media used in the KKA conforms to formula specified in the US Pharmacopoeia XXIII (USP) and Code of Federal Regulations (21 CFR) for sterility testing of pharmaceutical products, biologics and devices.

The media in the KKA (Tryptic Soy Broth) is commonly referred to as Soybean-Casein Digest Medium, USP, and Fluid Soybean-Casein Digest Medium; it is commonly abbreviated as TSB.

##### Summary and Explanation

The KKA contains a general-purpose medium used for isolating fastidious and non-fastidious microorganisms. The media contained in the KKA was originally developed for use in determining the effectiveness of sulfonamides against pneumococci and other organisms. The medium contained in the KKA is often used to support growth of non-typical isolates such as *Brucella*, *Clostridia* and non-sporulating anaerobes grow luxuriantly in this broth when incubated under anaerobic conditions.

The USDA Animal and Plant Health Inspection Service chose the media used in the KKA for detecting viable bacteria in live vaccines. The KKA medium is also used in the coliphage detection procedure, a proposed methodology in "*Standard Methods for the Examination of Water and Wastewater*." TSB is recommended for testing bacterial contaminants in cosmetics and complies with established standards in the food industry. The National Committee for Clinical Laboratory Standards (NCCLS) recommends the media in the KKA for inoculum preparation when performing the disk diffusion sensitivity test, also known as the Kirby-Bauer method. The

rich nutritional base of the media contained in the KKA is often modified to provide varying growth environments. The medium is used as an enrichment broth in clinical applications and is an excellent blood culture medium when supplemented with SPS and CO<sub>2</sub>.

## **Principle**

The medium in the KKA contains casein and soy peptones that provide amino acids and other nitrogenous substances making it a nutritious medium for a variety of microorganisms. Dextrose is the energy source, and sodium chloride maintains the osmotic equilibrium. Dipotassium phosphate is added as a buffer to maintain pH.

## **Understanding The Test**

It is important to understand that KOOL KOUNT Assayer (KKA) is a very competent and flexible microbial measurement tool. Occasionally, first time users of the product do not understand the results of KKA when comparing the results previously obtained from often less reliable microbial testers such as agar paddle testers.

Unlike most microbial testers in its product class, KKA can perform both presence (total) and activity (untreated) measurements. KKA's normal mode, activity measurement, may or may not agree with presence measurements typical of agar pads, paddles and plates testers.

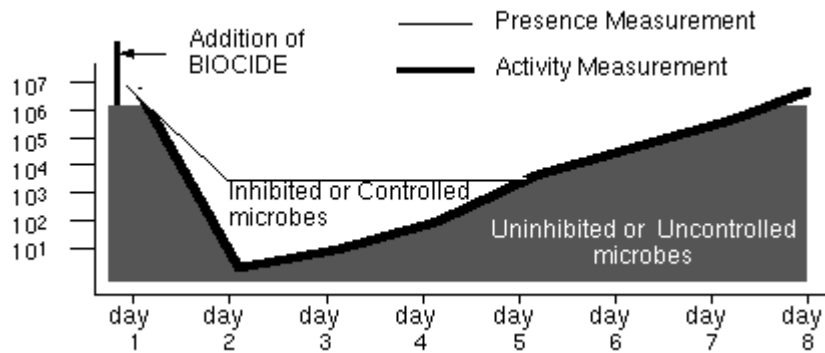
For these reasons, valid scientific cause exists for different readings when comparing KKA results to results from previously used microbial testers. Understand these differences and you can use the additional knowledge given by KKA to gain a competitive edge with your biocide treatment program.

**Presence Measurement** - The presence measurement is the total number of microbes present at the time of testing. The total presence concentration includes both viable, replicating microbes (active) and near death or incapacitated microbes (inactive).

**Activity Measurement** - The activity measurement is the number of microbes that are viable and replicating. This measurement is the count of microbes unaffected by the biocide. As the concentration of uninhibited microbes, the "active" microbe count is a good basis for determining when new biocide additions should be made. For example (see graph below), if the presence count by agar paddle is 10<sup>5</sup> and the activity count by KKA is 10<sup>2</sup>, the microbial counts are not in conflict but accurate. The cooling tower is in control. The difference between the presence and activity measurements in this case represents the count of microbes that have been inhibited by the biocide being used at that biocide's concentration level. Further additions of the same biocide at these low activity levels that do not decrease the activity count could increase the risk of bio-filming as the active or biocide tolerant microbes are apt to be stimulated to increase levels of polysaccharide production.

**Testing for Presence** - Neutralize the biocide in your water sample. Allow the sample to sit for 15 minutes and then use KOOL KOUNT Assayer as normal. By neutralizing the biocide, dying and incapacitated microbes will recover much like in a plate or paddle test and the KOOL KOUNT Assayer will detect them.

**Testing for Activity** - DO NOT neutralize the water sample. Simply use KOOL KOUNT Assayer as normal. This reading will not necessarily give the same result of the traditional paddle tester but it will give the count of microbes that are not being affected by the biocide treatment program.



• Figure 1 Presence / Activity

### **KOOL KOUNT Assayer**



# Biocide Neutralization and Sample Buffering

## Comparative Evaluation Guide

### Laboratory Evaluation

#### Neutralization of Chemical Biocides and Disinfectants

Complete neutralization of biocides or disinfectants in cooling water samples is important when using the KKA because biocide or disinfectant carryover can cause false no-growth or low-test results when compared to laboratory procedures using serial dilutions. The Kool Kount Assayer (P) incorporates D/E Neutralizing media to effectively neutralize the inhibitory effects of biocides or disinfectant carryover, allowing differentiation between bacteriostasis and the true bactericidal action of disinfectant chemicals. This is a critical characteristic to consider when evaluating biocide efficacy. The Kool Kount Assayer (P) is recommended for use when evaluating environmental and industrial water samples containing biocides or disinfectants.

#### **Kool Kount Assayer (P)**

Besides containing the basic Kool Kount Assayer media, the Kool Kount Assayer (P) contains a number of chemicals capable of neutralizing industrial biocides and disinfectants. The lecithin in the media neutralizes quaternary ammonium compounds. Polysorbate 80 in the medium neutralizes phenolics. Thioglycollate contained in the Kool Kount Assayer (P) neutralizes mercurials, and sodium thiosulfate contained in the media neutralizes chlorine. The sodium bisulfate in the medium neutralized out isothiazolones and glutaraldehyde.

The Kool Kount Assayer (P) is suited for environmental sampling, where neutralization of the chemical is important to determine bacterial activity. A bacteriostatic sample may contain bacteria held in bacteriostasis but which may still be viable and cause problems to the cooling system when biocide levels drop due to system bleed and makeup. The Kool Kount Assayer (P) can effectively recover these microorganisms increasing the overall recovery of bacteria in treated waters.

#### **IME Buffer Powder for the Kool Kount Assayer and Kool Kount Assayer (P)**

IME Buffer Powder is used for the pre-enrichment of damaged microorganisms. IME Buffer Powder helps to repair microorganisms contained in the environmental sample damaged by chemical disinfectants and biocides, increasing the recovery rate when compared to standard field tests.

Biocide treatment, high osmotic pressure and pH changes can cause sublethal injury to microorganisms contained in environmental samples. Preenrichment in a nonselective medium allows repair of cell damage and facilitates the recover of environmental microorganisms. When added to an environmental sample prior to testing, the IME Buffer Powder maintains a high pH over the preenrichment period and results in the repair of injured cells.

IME Buffer powder contains peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride in the powder maintains the osmotic balance. Phosphates buffer the medium.

**Precautions:**

1. **KEEP AWAY FROM CHILDREN**
2. **MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US)**  
Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.
3. **FIRST AID:** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

**Storage**

Store below 30°C. The medium is very hygroscopic. Keep tightly closed.

**Procedure**

**Materials Provided**

- IME Buffer Powder

**Materials required but not provided**

- Kool Kount Assayer or Kool Kount Assayer (P)
- Snapping Cup
- Laboratory Balance
- Incubator

**Test procedure**

1. Dissolve the medium in 50ml of sample water – 1gram
2. Incubate for 15-30min. at 30°C
3. Test sample with Kool Kount Assayer or Kool Kount Assayer (P)

## **Comparative Evaluation Guide**

### Laboratory Evaluation

#### **The Kool Kount Assayer Compared to Plate Counts and DipSlides**

It is important to remember during any evaluation of microbiology media/testing procedure, that different media and incubation temperatures can have dramatic effects on recovery. The Kool Kount Assayer and Kool Kount Assayer (P) contain TSB media, ideally, any comparative media should be of similar makeup. Tryptic Soy Agar is recommended when performing laboratory evaluations where pour plate or spread plate techniques are used. When membrane filtration techniques are used, it is recommended that m-TSB media is used.

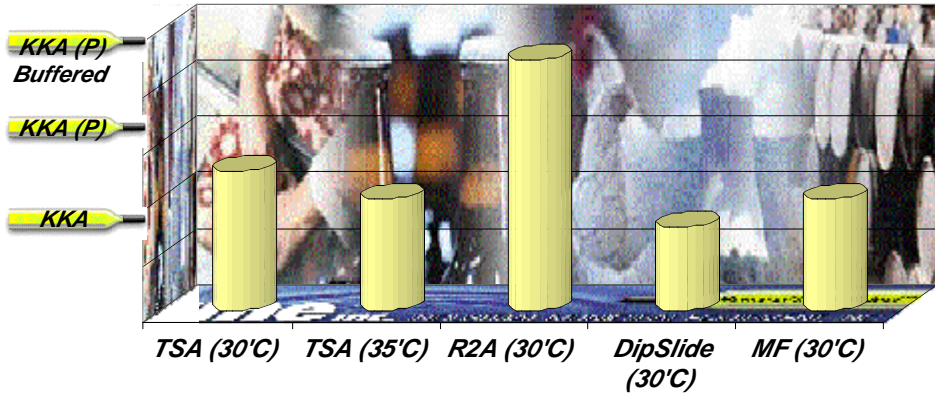
Incubation temperatures will also have a major impact in the comparative analysis. When used in conjunction with the AutoAnalyzer 2000 or AutoAnalyzer 2002-M the Kool Kount Assayer is incubated at 35°C, 5°C higher than standard environmental culture incubation. This is done in an attempt to maximize the bacterial growth rate to allow for the highest recovery in the shortest amount of time. Significant differences could be observed if incubation temperatures used in laboratory methods do not match.

Biocide neutralization and sample buffering are also an important consideration when performing a comparative analysis. Because the Kool Kount Assayer uses system water to re-hydrate the TSB media in the ampoule, samples containing disinfectant or biocides will produce inhibitory effects causing low or no-growth results.

Standard laboratory procedures such as plate counts and membrane filtration overcome the inhibitory effects of disinfectant carryover by sample dilution. Sample dilution will effectively reduce standard cooling system biocide levels to nominal levels, negating the need for neutralization. An example would be a sample of cooling water containing 100ppm of isothiazolone 1.5%, a common chemical encountered in cooling systems. This theoretical sample would contain 1.5ppm of the active ingredient, when diluted to a standard 1:100 dilution, the sample will only contain 0.015ppm of the active ingredient, not enough to cause any inhibition to the bacteria contained in the sample. Conversely, because the Kool Kount Assayer uses 7 milliliters of system water to re-hydrate the TSB medium, lack of neutralization would cause the test to be run in the presence of 100ppm of isothiazolone; this amount of biocide in the bacterial media could cause lower numbers or no-growth, when compared to dilute samples.

When a comparative analysis is performed to determine the usefulness of a Kool Kount Assayer using samples from cooling systems (or other uncontrolled sample sources) it is imperative that the Kool Kount Assayer (P) be used, preferably in combination with buffering. Laboratory studies have shown

that the Kool Kount Assayer (P) in combination with sample buffering will, on average, increase the recovery rate of bacteria in the sample to that higher than standard plate count procedures using TSA media incubated at 30°C for 72 hours. These laboratory studies have shown recovery rates (using the Kool Kount Assayer (P) in combination with IME Buffer Powder on field samples containing biocides) were, on average, comparable to counts obtained by using R2A agar incubated at 30°C (+/- 2°C) for 7 days (see figure 3.1).



• Figure 2 Comparison of KKA recovery

## DipSlides

DipSlides are hygiene contact slides used for assessing the microbiological contamination of surfaces or fluids. These field tests were originally designed to monitor the microbial flora of surfaces, walls, ceilings, equipment and sterile liquids as part of an action plan for achieving good manufacturing practices in factories handling foods, cosmetics or pharmaceuticals (Restaino, L, 1994; Scott, E., S. F. Bloomfield, and C. G. Barlow, 1984; Thomas, M. E. M., E. Piper, and I. M. Mauer, 1972). Because of their ease of use water treatment companies have incorporated them into their field-testing protocol to test for total aerobic bacteria.

DipSlides are supplied with a comparison chart that allows the individual conducting the test to visually judge the approximate bacterial level in the sample being evaluated. Because of the relatively high level of bacteria contained in cooling samples when compared to samples originally designed to be tested by DipSlides (i.e. urine, milk, sterile liquids), bacterial counts are rarely accurate when compared to standard methods designed to examine water and waste waters containing bacteria levels in excess of 10,000 CFU/ml.

The Kool Kount Assayer was designed and calibrated for testing industrial cooling waters containing high bacterial levels, as such the Kool Kount Assayer will routinely give higher, more reproducible results when compared to DipSlides. The Kool Kount Assayer test method was submitted for ASTM approval in 1995 after completing the ASTM required round robin evaluations for field test methods. In January 1998, ASTM subcommittee D19.24 approved the method.

## **R2A Agar**

R2A Agar is used for enumerating heterotrophic organisms in treated water. R2A Agar is a low nutrient medium used in combination with a lower incubation temperature and longer incubation times to stimulate the growth of stressed and chlorine-tolerant bacteria. Nutritionally rich media, such as Tryptone Glucose Yeast Extract Agar (TGEA) or Plate Count Agar (PCA) support the growth of fast-growing bacteria, but may suppress slow growing or stressed bacteria found in treated water. When compared to TGEA and PCA, R2A Agar has been reported to improve the recovery of stressed and chlorine-tolerant bacteria. R2A is recommended in *Standard Methods for the Examination of Water and Waste Water* for pour plate, spread plate and membrane filter methods for heterotrophic plate counts.

When comparing the Kool Kount Assayer to R2A media it is important to use the Kool Kount Assayer (P) preferably in combination with the IME Buffer Powder. When field samples are used for evaluation, the combination of these two products have shown similar recovery rates in 3<sup>rd</sup> party laboratory evaluations.

## **Summary**

As with any rapid field test certain allowances must be made. Rapid field tests are a tool to be used in an overall treatment and service package offered to your customers. Field Tests are not a substitute for more sophisticated laboratory methods performed by trained microbiologists and technicians in a laboratory environment. Whenever simple, rapid field tests are required, some amount of accuracy will inevitably be sacrificed. The Kool Kount family of biological testers are the only rapid total aerobic bacterial count field test with ASTM approval. The Kool Kount Assayers have been designed to minimize inaccuracies and provide high quality results in less time than traditional field microbiology test methods allowing you to give quality information to your customers faster.

Still, the Kool Kount biological tests are a tool that if used improperly will provide less than accurate results. When using the Kool Kount biological testers in the field or evaluating the Kool Kount biological testers in the laboratory environment, care must be taken to select the proper tester for the sample being evaluated. When properly selected and used, the Kool Kount biological testers deliver consistently higher quality results in less time and with a greater ease of use than traditional microbial field tests.

If you have any questions during your evaluation period, please contact a member of the IME technical staff and they will be happy to assist you in any way.

## Comparative Evaluation Guide

### Laboratory Evaluation

#### Laboratory Test Procedures

##### Introduction

Comparative evaluations of microbiology growth media and test procedures should be performed under a controlled environment. Test samples should be made with known densities of bacteria using McFarland Barium Standards and calibrated with a turbidimeter.

##### McFarland Barium Standards

McFarland Barium Standards are tubes of barium sulfate suspensions prepared as described by McFarland for use as standards in adjusting densities of bacterial suspensions and other turbid suspensions.

**Principle:** The density of bacterial and other turbid suspensions are determined by direct visual comparison of suspensions with the McFarland barium sulfate standards. In order to obtain reliable results the diameter of the tubes containing the test suspension must be of comparable size to the tubes used for the McFarland standard. It is also essential that the standards be thoroughly shaken to insure complete suspension of the barium sulfate each time they are used.

Visual comparison of turbidity is easily and accurately made by viewing the test suspensions and standard against a white background with a black line running horizontally at midpoint (see photo).

McFarland Turbidity Standard

Reference in:  
Gradwohl's Clinical  
Laboratory Methods  
and Diagnosis. In A.  
C. Sonnenwirth and  
L. Jarett (ed.). C. V.  
Mosby Company,  
1980 p. 1363



**Preparation:** In order to estimate the numbers of bacteria in suspensions from cultures, the density of the experimental suspension (absorbance or transmission) is compared to that of the set of reference tubes in the series. The reference tubes are prepared by adding the following amounts of stock solutions of 1% aqueous barium chloride and 1% aqueous sulfuric acid to tubes of a size appropriate for the test. Different proportions of barium chloride and sulfuric acid are used to prepare a range of barium sulfate concentrations for the McFarland turbidity standards.

The amounts of each stock and the corresponding densities of bacteria are given in the following table:

Parts of BaCl <sub>2</sub> (1%) in 1 ml	Parts of H <sub>2</sub> SO <sub>4</sub> (1%) in 1 ml	Corresponding number of bacteria (million/ml)
0.1	9.9	300
0.2	9.8	600
0.3	9.7	900
0.4	9.6	1200
0.5	9.5	1500
0.6	9.4	1800
0.7	9.3	2100
0.8	9.2	2400
0.9	9.1	2700
1.0	9.0	3000

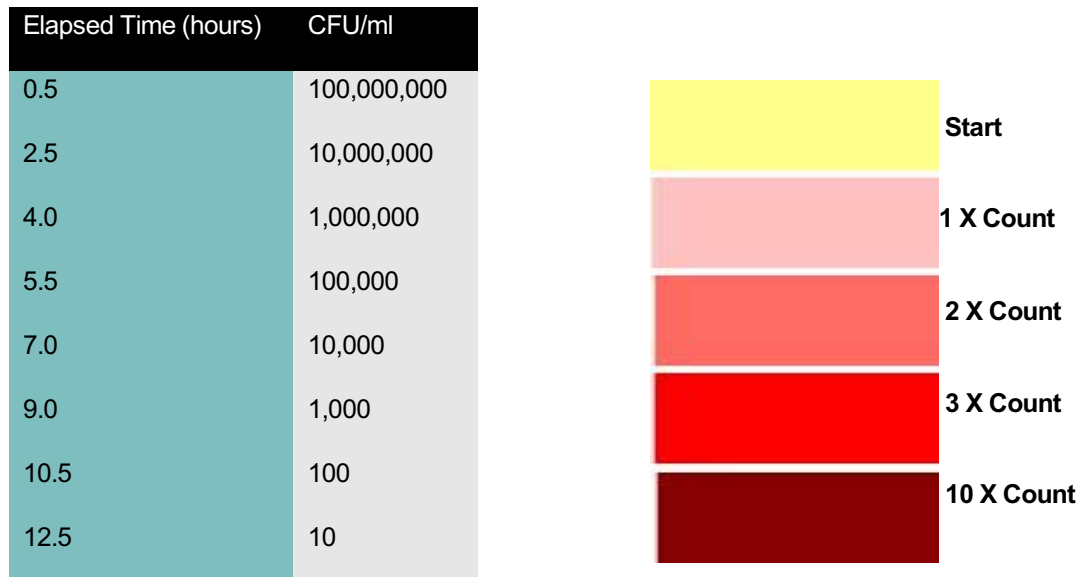
• Figure 3 McFarland Standards

By using McFarland standards, recovery rates of both plate count procedures and the Kool Kount Assayer can be properly judged. When field samples with unknown amounts of bacteria are used to judge accuracy, too many variables exist to properly evaluate the comparison.

### Kool Kount Evaluation

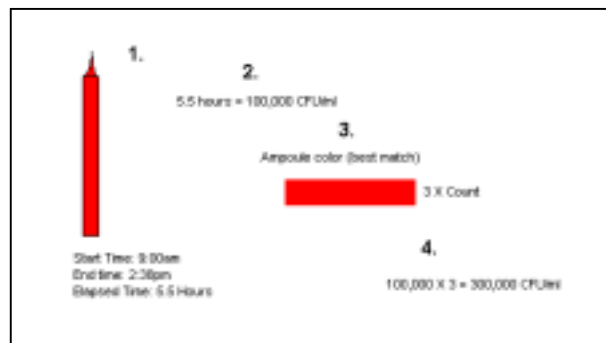
- **Control:** Prepare test sample by adding bacterial culture to sterile dilution water to obtain the desired McFarland density. Ideally, the sample and McFarland standard should be compared using a turbidimeter.
- Process a portion of the prepared and calibrated sample by approved laboratory methodology (i.e. pour plate, spread plate, membrane filtration) for the examination of water and wastewater, and incubate. If evaluation is being made with the AutoAnalyzer 2002M, incubate plates at 35°C.
- Process the remainder of sample using the Kool Kount Assayer.
- **Kool Kount Assayer:** Rinse the Snapping Cup three (3) times with sample.
- Fill Snapping Cup with sample to be tested.
- Let stand for two (2) minutes to eliminate entrained air.
- Fill the Kool Kount by snapping the ampoule with the point tip down under the water in the Snapping Cup.

- Gently mix the ampoule contents until the powder is completely dissolved.
- If using the AutoAnalyzer 2002M, place ampoule in analyzer and start test as outlined in operating instructions supplied with the unit.
- If using the visual method, record the start time and sample ID.
- Incubate the ampoule at the same temperature as the control test, preferably 35°C. Lower incubation temperatures will increase reaction time and may affect accuracy.
- Examine the ampoule periodically for a color change (refer to color chart supplied with the Kool Kount Assayer).
- When the color change is observed (light pink), record the elapsed time and follow the CFU/ml calculation chart.



• Figure 4 Kool Kount Assayer Calculation Chart

To obtain CFU/ml, determine elapsed time and obtain count. Compare ampoule color to the chart and multiply count by the factor indicated by color chart.



## Evaluation of Field Samples

As mentioned previously, it is never a good laboratory practice to conduct an **initial comparative evaluation** of microbiology media with uncontrolled field samples. This is certainly the case when testing industrial waters containing biocides or disinfectants. Industrial waters such as cooling waters being treated with chemical biocides are subject to bleed, makeup and periodic dosages of non-oxidizing biocides. Depending when the sample was taken, size of the system, holding time index and time of last biocide dosage, unknown amounts of inhibitory chemicals will be present in the sample. Also, depending on the treatment used and its performance in the system, bacterial levels will vary greatly.

Without knowing the amount of bacteria contained in the sample before testing it is hard to judge the accuracy of the control test, versus the test being evaluated. If the test being evaluated reports higher numbers than the control test, the evaluator must determine if the control test inhibits bacterial growth, if the evaluated test has higher recover due to superior performance or lack of accuracy. If the control test recovers more bacteria than the evaluated test is the discrepancy due to poor or improper media in the evaluated test, disinfectant carryover, and poor test procedures, ECT? Without controlling the test sample, proper evaluations cannot be made.

If field samples are to be used in the evaluation process as either the sole test sample or in conjunction with control samples, it is important to pick the proper Kool Kount Assayer. If the field sample contains a chemical biocide the Kool Kount (P) will produce better results due to its ability to neutralize chemical biocides. **USE OF THE KOOL KOUNT ASSAYER WITHOUT THE D/E MATERIAL WILL GIVE LOWER RESULTS THAN THE CONTROL IF CHEMICAL BIOCIDES ARE PRESENT IN THE SAMPLE.**

Field samples also contain injured or damaged microorganisms, the use of the IME Buffer Powder is highly recommended when testing field samples with chemical biocides present. The use of the buffer powder will increase the recovery amount by allowing the damaged microorganisms to repair themselves.

Once the proper Kool Kount Assayer is chosen, process the sample as described previously.

## Comparative Evaluation Guide

### Products and Application Guide

#### Application Guide (Cooling Systems)

Products	Cooling Towers	Makeup Water	Presence Tests	Activity Test	Closed Loop Systems	AirWashers
Kool Kount	✓	✓	✓	✓	✓	✓
Kool Kount (P)	✓		✓		✓	✓
Kool Kount (SRB)	✓		✓		✓	✓
IME Buffer Powder	✓	✓	✓		✓	✓

• Figure 5 Application Guide (Cooling Systems)

## Comparative Evaluation Guide

### Cooling Systems

#### Incubation Temperatures and Media Selection Guide

Products	TSA @ 35°C Field Sample	TSA @ 35°C Laboratory Control	TSA @ 30°C Field Sample	TSA @ 30°C Laboratory Control	M-TSB @ 35°C Laboratory Control	M-TSB @ 30°C Field Sample	R2A @ 30°C Field Sample	R2A @ 30°C Laboratory Control	DipSlide @ 30°C Field Sample	DipSlide @ 30°C Laboratory Control
Kool Kount		✓		✓	✓					✓
Kool Kount (P)	✓		✓			✓	✓	✓	✓	
IME Buffer Powder	✓		✓			✓	✓	✓	✓	

• Figure 6 Incubation Temperatures and Media Selection Guide

## Comparative Evaluation Guide

Kool Kount Assayer instructions

### Visual 3 Step Guide to Using The Kool Kount Assayer

## It's As Easy As 1,2,3!

### 1. Snap

hermetically sealed ampoule automatically fills with sample water.

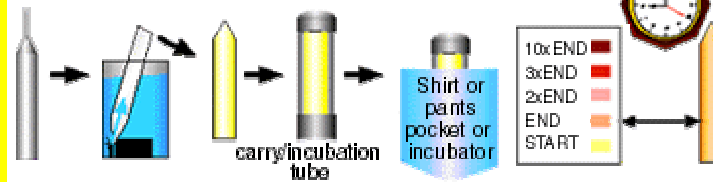
### 2. Incubate

Place ampoule in safety incubation tube in your pocket.

### 3. Check & Read

Record elapsed time to color change for concentration.

U.S. Patent #:  
5159799 & 5550032



### OR...

Do it automatically with the [Incubator/Analyzer 2002](#)

- Fully Automatic Incubation
- Automatic End-Point Detection
- Automatic Data Retention
- Digital Data Display
- Runs 2 to 4 tests Simultaneously
- Capable of Field (DC) & Lab (AC)

• Figure 7 Visual 3 Step Guide to Using the Kool Kount Assayer

## Comparative Evaluation Guide

### Spectrometers

#### Spectrophotometer Procedure

**Step 1** Follow provided directions for sampling and cracking the ampoule. Be sure to shake ampoule well to get all components of test into solution.

**Step 2** Remove contents (without rod) into a test tube. Contents can be removed aseptically from opening in top with a needle and syringe. Or the outside of the ampoule can be cleaned with alcohol and then the top cracked open with a sterilized tool.

**Step 3** Make a small ink mark at the top of the test tube.

**Step 4** Set spectrophotometer to 465nm.

**Step 5** Insert test tube into spectrophotometer with ink mark toward front and take a "time zero" reading of % Transmittance. Record this result along with the time.

**Step 6** Incubate at 35-37C.

**Step 7** Take a spectrophotometer reading every half hour making sure ink mark is toward the front. Record results. When there is a 10% reduction in %T the test is positive. (I.e.-75%T and 5 hours 65%T)

**Step 8** Add 1/2 hour to the time in Step 7 and use visual results timetable to determine cfu/ml in the sample.

**\*\*NOTE\*\***

This method works for counts of 10 to 1,000,000 cfu/ml. For counts higher than 1,000,000 cfu/ml it is best to use the visual test procedure or perform serial dilutions of the sample. Serial dilutions lower the bacterial count in the sample tested and enable you to use the spectrophotometer procedure. To obtain a final result simply multiply by the dilution factor. (Example- a 1/100 dilution yields a 10% reduction in %Transmittance after 5 hours; using the visual results timetable for 5.5 hours the count in sample tested is 100,000 cfu/ml; multiply this result by 100 to get a count of 10,000,000 cfu/ml in the actual sample)  
\*\*Please note that floating particulates in test sample can interfere with spectrophotometer readings. The sample can be filtered before beginning the test to remove particulates. Only filter sample if the floating matter is not necessary for your specific test. (I.e.-containing a biologic you are testing for) If the sample cannot be filtered please use the visual method.

## Comparative Evaluation Guide

### Deposit Samples

#### Activated Sludge

1. Obtain a representative sample of wastewater from an aerated basin or tank (mixed-liquor suspended solids, MLSS).
2. Dilute sample 1:10, 1:100 or greater with sterile, non-chlorinated water to reduce color interference from solids as well as possible chemical interferences\*. Use standard dilution bottles (100 ml capacity or greater) or other sterile containers with lids or caps. Dilution is also necessary to count viable active organisms in samples exceeding counts of  $10^8$  colony-forming units (CFU's) per milliliter (ml).
3. Mix diluted sample gently in the appropriate container for 20 to 30 seconds to evenly disperse floc particles.
4. Rinse snapping cup three times with sample or sterile water. Fill Kool Kount Assayer snapping cup with prepared sample and snap vial. Allow vial to fill completely before removing.
5. Incubate in a 35°C/95°F environment. This may be done in a shirt breast pocket or incubator. A special Carry/Incubation Tube (**Catalog #IM93357BK**) is also available to allow safe, convenient incubation.
6. To stop a test, simply refrigerate or put sample in an ice chest. To restart, simply incubate and subtract 0.5 hour from the total cumulative time to obtain final results. Read chart results times dilution factor for final results.

#### Facultative Wastewater Lagoons, Ponds, Etc.

1. Obtain a sample from the system to be tested. If sample is clear and is suspected to contain  $10^8$  CFU/ml or less, simply fill snapping cup and snap the Kool Kount Assayer ampoule until completely filled. If sample is suspected to contain higher counts, or contains chemical interferences or significant color, simply dilute per instructions in Steps 2 and 3 above. Control ampoules are also available for slight color adjustments.
2. Proceed to Step 4 above and complete final steps.

**\*Chemical interferences may be due to a strong presence of reducing compounds and high levels of ammonia.**



## Comparative Evaluation Guide

### Products and Application Guide

#### Water/Wastewater Testing – Laboratory Media Selection Guide

<i>Products</i>	<i>Standard plate Count</i>	<i>Total Coliforms</i>	<i>Fecal Coliforms / E. coli</i>	<i>Fecal Streptococcus</i>	<i>Salmonella</i>	<i>Stressed Organisms</i>	<i>Staphylococcus</i>
A-1 medium/A-Broth			✓				
Azide Dextrose Broth				✓			
BAGG Broth			✓				
Bile Esculin Agar				✓			
Bile Esculin Azide Agar				✓			
Bismuth Sulfite Agar					✓		
Brilliant Green Agar					✓		
Brilliant Green Agar Modified					✓		
Brilliant Green Bile Agar 2%		✓					
M Brilliant Green Broth					✓		

Products	Standard plate Count	Total Coliforms	Fecal Coliforms / E. coli	Fecal Streptococcus	Salmonella	Stressed Organisms	Staphylococcus
Desoxycholate Lactose Agar		✓					
m E Agar/M-E Agar Base				✓			
EC Medium/EC Broth			✓				
EC medium with MUG			✓				
m Endo Agar LES/ m Endo Broth		✓					
m Enterococcus Agar				✓			
Esculin Iron Agar				✓			
EVA Broth/Ethyl Azide Agar				✓			
m FC Agar			✓				
m FC Basal Medium			✓				
m FC Broth Base			✓				
m HPC Agar	✓						
Lactose Peptone Broth		✓					
Laural Tryptose Broth		✓					
Laural Tryptose Broth with MUG		✓					
Muller Kauffmann Tetrathionate Broth Base					✓		
Nutrient Agar W/ MUG			✓				
Plate Count Agar	✓						
Presence-Absence Broth		✓					
R2A Agar						✓	

## Appendix

### ***Comparing the Kool Kount Assayer to Dip Slides in a non-laboratory environment.***

Although it is never an ideal environment to do a comparative analysis, the Kool Kount Assayer can be evaluated in the field. This type of evaluation should be conducted with at least one known sample such as tap water as a control. The tap water sample is used to verify both tests in a known low-level contamination environment.

Results from the Dip Slide are obtained by comparing the test paddle to a chart that depicts similar paddles with contamination of varying degree. Rarely do these pictures match the scale of the test being examined and the final judgment is left to the examiner. In Tests conducted with over 30 individuals familiar with Dip Slide use on three separate occasions by an outside laboratory have shown that when a group of people individually examine the same Dip Slide, a different result is obtained from person to person more than 80 % of the time. Results obtained from the Kool Kount Assayer obtained in one of three ways that minimize this error.

1. **Timed method** where the examiner first determines the level of acceptable contamination, example 10,000 CFU/ml as specified in cooling systems by CTI. In this example the test would be run for 7 hours and the result would be recorded as <10,000 CFU/ml for a negative result or  $\geq 10,000$  CFU/ml for a positive result. This type of test has the advantage of being able to be conducted and recorded during 1 8-hour shift. Corrective action can be taken the same day as apposed to 48 hours later when compared to Dip Slides. When one considers the doubling time of some bacteria (15 min.) this is a distinct over other field tests when trying to control problem systems. When comparing this method to the to Dip Slides, record the Kool Kount Result and compare it to the Dip Slide result obtained 2 days later.
2. **Comparative method** is performed by checking the Kool Kount Assayer every 1 to 2 hours over an 18-hour period of time. When the end point is reached the time is checked with the supplied chart to determine the CFU/ml. When comparing this method to the to Dip Slides, record the Kool Kount Result and compare it to the Dip Slide result obtained 1.5 days later.
3. **Automated method** is performed by placing the Kool Kount in the AutoAnalyzer 2002M (sold separately) and allowing the analyzer to record the results. This method has the following advantages:
  - a. Automated, removing operator error from the test reading portion of the test.
  - b. More accurate results than visual method because the end point is judged as a loss of transmittance by a spectrometer rather than a color change by the operators eye.
  - c. Tests will be recorded in the absence of an operator. Rarely will an operator take a test home if the time to end point exceeds 8 hours. If the change happens shortly after leaving the operator will not know the proper result. If the analyzer is used the result will be stored so that it can be recorded the next morning.

In the first step of the field evaluation you will need to obtain a sample of clean tap water and decide which method of examining the Kool Kount Assayer best fits your needs. Most tap water will contain between 0-10 CFU/ml of bacteria, and in some cases 100 CFU/ml. The CFU/ml of municipal tap water will rarely exceed 1000 CFU/ml. First, test the tap water sample with both the Kool Kount Assayer and the Dip Slide and record the results. Normal results obtained from the Kool Kount will be 0-10 CFU/ml while the Dip Slide should show a negative result. This is because the Kool Kount Assayer is more accurate at low-level contamination and the Dip Slide is a semi-quantitative tester designed to record results obtained from liquid samples as:

- <1,000 CFU/ml
- 1,000 – 1,000,000 CFU/ml
- >1,000,000 CFU/ml

The ability of the Dip Slide to record low level contamination is extremely limited. This first test should give the examiner a “benchmark” for further tests.

Next, examine environmental sources comparing the results obtained from the Kool Kount Assayer to the ones obtained from the Dip Slide. Typically, the results should be similar with the Kool Kount Assayer giving results that are generally slightly higher. This is due to the following factors:

- **Shelf life** of product. Kool Kount Assayers have a much longer shelf life and do not degrade as rapidly as Dip Slides. Media deterioration can adversely affect the performance of the Dip Slide, reducing the number of bacteria capable of growing on the media.
- **Refrigeration**. The Kool Kount Assayer does not need refrigeration, the Dip Slide does. Typically, Dip Slide users carry the product in the trunk of their cars where the test is exposed to a wide range of temperature conditions. These conditions almost never match the required refrigeration requirements and reduce the shelf life of the product to <30 days and cause dehydration of the media. When a test is conducted with a Dip Slide that is dehydrated the media on the paddle tries to “recover” some of its lost water by absorbing some of the sample water. If the water contains any biocide, this inhibitory chemical will also be absorbed. If the Dip Slide is extremely dehydrated the paddle will not be able to absorb enough water to fully rehydrate itself and the media will not contain enough water to support bacterial growth. This can be checked by inspecting the unopened Dip Slide for moisture buildup at the bottom of the vile. This excess moisture is a result of water evaporating from the media and condensing on the vile. The amount of moisture contained in the vile is directly proportional to the amount of sample water the media will try to recover during the sampling portion of the test. If there is 1ml of evaporated water in the vile, the media will try to recover that 1ml. This could cause false high numbers as contaminated water is absorbed into the media or false low numbers as any pesticide or biocide contained in the water is absorbed into the media.

Evaluation of the Kool Kount can be done under uncontrolled environments such as the field, but do require a careful consideration of the sample source, condition of the Dip Slides used in the evaluation and finally the reading of the result. Not considering any one of these points could cause either test to out or under perform the other leading to an unfair evaluation. When evaluating any test, the key points to consider are:

1. **Objectivity**, do not try to enter into the evaluation assuming one test will fail or out perform the other. Let the results speak for themselves.
2. **Control** as many of the factors of the test as possible.
3. **Condition of the equipment.** Do not use out of date Dip Slides or Dip Slides with noticeable dehydration. Although this would favor the Kool Kount Assayer, it is not a fair evaluation. Pick a box of Dip Slides that have been refrigerated and have plenty of shelf life remaining.

We feel confident that when you try the Kool Kount Assayer you will find it is a cost effective, convenient and accurate field test. If you have any questions during your evaluation please do not hesitate to contact a member of the IME technical staff.