Bacteria Die-off Study

Houston-Galveston Area Council

Prepared in cooperation with the
City of Houston, Department of Health & Human Services
and the Texas Commission on Environmental Quality,
Clean Rivers Program

August 31, 2005

Executive Summary

Historically, it has been thought that *Escherichia coli* (*E. coli)* does not proliferate or replicate outside the body of warm-blooded animals and that concentrations in surface water decrease significantly 24-48 hours after being introduced into surface waters. But in nutrient rich water, such as in Houston area streams, it is suspected that the bacteria level is not only sustained for an extended period of time, but may even increase to a certain extent. The assumption that bacterial concentrations will follow the natural growth/die off cycle after storm events or following sewage influxes may also be incorrect thus leading to poor decisions regarding the reopening of swimming areas and other regulatory decisions. This study will help managers determine how the bacteria levels in the Houston area surface waters actually react.

Samples were collected from two sites on Buffalo Bayou over several months to compare how the bacteria concentrations changed under different lab scenarios and over time. One site had historically high bacteria numbers while the other had historically low concentrations with occasional high values. Five different scenarios were set up in the lab to closely mimic natural environmental conditions. A sampling event required collecting bayou water and testing samples for 4 consecutive days. A second part of the project looked at how bacteria concentrations changed at the two locations over time. Following a rainfall event, samples were collected in the field on 4 consecutive days for independent tests.

Results clearly indicated a relationship between temperature and longevity of bacteria. Samples kept at temperatures between 2 and 4°C had greatly enhanced survival rates of bacteria. Even though some die-off was seen, it was generally after leaving the sample in the refrigerator for several days. These results challenge the need to limit bacteria holding times to eight hours maximum.

Once bacteria are introduced to a warmer environment, they will die-off in a matter of days which contradicts an assumption that they remain viable for a long period of time or even proliferate. There was no relationship between bacteria concentration, die-off rate or nutrients. However, the rate of stirring or agitation did seem to be a factor in bacteria die-off. In the lab scenarios where the water was allowed to settle, the die-off rate was quicker then when agitated, even if the solids were re-suspended immediately before the sample was collected for testing. There did appear to be a general relationship between total suspended solids and bacteria. In most cases, as the solids decreased, the bacteria concentrations decreased as well.

Acknowledgments

This report summarizes the joint efforts of the TCEQ, the Houston-Galveston Area Council, the City of Houston, Department of Health & Human Services field office and North Laboratory, and the technical contributions from PBS&J consulting firm.

The following individuals are recognized for their ongoing contribution to the development and implementation of this project:

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Charles Roosevelt, Field Supervisor, Department of Health and Human Services
Paul Jensen, Vice President, PBS&J

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1.0 Introduction

Contact recreation is a "use" criteria assigned to all water bodies in the state of Texas except where water bodies have industrial uses associated with them – such as the Houston ship channel or have been designated as wildlife refuges. Support of the contact recreation use is based on a set of at least 10 bacteria samples. For routine monitoring bacteria data, 126 colonies /100 ml is the long-term geometric average standard for *Escherichia coli* (*E. coli)* and 394 colonies/100 ml is the criterion applied to individual samples. The contact recreation use is not supported if the geometric average of the samples collected exceeds the mean criterion or if the criteria for individual samples are exceeded more than 25 percent of the time. Throughout the Houston-Galveston region, 42% of the stream segments are on the State of Texas 303(d) – *List of Impaired Waters* due to bacterial contamination levels exceeding the water quality standard for "contact recreation" designated waters. Subsequently, the Texas Commission on Environmental Quality (TCEQ) has initiated a Total Maximum Daily Load (TMDL) study to address the causes of the bacterial contamination in Buffalo and White Oak Bayous.

Historically, it has been thought that *E. coli* does not proliferate or replicate outside the body of warm-blooded animals and that concentrations in surface water decrease significantly, possibly by 1 log, 24-48 hours after being introduced into surface waters. The stationary phase of the growth cycle usually leads quickly to the phase of decline or death because of the depletion of essential nutrients. But in nutrient rich water, such as in Houston area streams, it is suspected that the bacteria level is not only sustained for an extended period of time, but may even increase to a certain extent. The assumption that bacterial concentrations will follow the natural growth/die off cycle after storm events or following sewage influxes may also be incorrect thus leading to poor decisions regarding the reopening of swimming areas and other TMDL influenced decisions. This study will help managers determine how the bacteria levels in the Houston area surface waters actually react. This issue was the subject of a TMDL study conducted in 2001 by PBS&J, however it was restricted to having very limited mixing conditions and the results were inconclusive.

During the summer of 2001, the Bacteria TMDL chamber studies demonstrated initial *E. coli* concentrations of bayou water at about 10,000 MPN/100 ml. After a two-day period, the concentrations of all samples without mixing had dropped to about 10 MPN/100 ml. These results were well below the ambient levels seen in the bayous. The die-off co-efficient obtained from these data is about 2/day. A similar rate was used in the TMDL modeling; however, this may not be a representative rate if the mixing that is a part of the bayou's natural flow is accurately included.

Another variable to be considered in this study was the effect of storage at 4ºC over time. The "holding time until analysis" is an issue due to the inability of sampling staff to get samples delivered to the lab and analyzed within the 6 + 2 hours holding time. Some researchers have suggested 6 hours is too restrictive and bacterial concentrations remain constant for longer periods of time when kept at or below 4ºC. However, in the summer of 2001 bacteria chamber studies, the West District wastewater treatment plant (WWTP) disinfected effluent had "no detection of indicator bacteria" results initially. Un-refrigerated samples measured the next day had a concentration of over 100 MPN/100 ml indicating an increase over time.

The purpose of this study is to provide improved data on the die-off rate of indicator bacteria in Houston bayous. In the laboratory, different temperature and sample mixing conditions were created to better simulate various mixing conditions found in the natural environment.

There are basically two parts to this project. Part 1 was conducted in the laboratory and looked at bacteria die-off rates from two different sampling sites on Houston's Buffalo Bayou. Variables included the differences between sample mixing, no sample mixing, and the effect of two different speeds of continuous mixing. Another regime included in Part 1 was to help determine if bacteria concentrations change over time while being kept in the refrigerator. Containers of bayou water were kept in a refrigerator between 1ºC & 4ºC while other containers of water were held at room temperature and kept within a darkened fume hood. The front sash of the vent hood was covered to block majority of the ambient light except during sample processing.

The last variable explored in Part 1 had to do with the possible correlation between bacteria die-off rates and nutrient concentrations. The basic process employed in the study was to isolate a sample of water from two different sites and to track the concentration of bacteria in the isolated samples over a 4-day time period over different seasons. Nutrient data was compared to the seasonal die-off rate of bacteria to see if there was any relationship. The first site was chosen because that location had historically high bacterial results. The second site was located where bacterial results had been historically low but a few high numbers had been occasionally measured over the years.

Part 2 of the bacteria die-off study was designed to look at the bacteria response in the natural environment after a typical rainfall event that would cause an influx of bacteria into the water body via run-off. This portion of the study looked at how bacteria numbers changed at a given site over time following rainfall and whether that information correlates with nutrient levels in the water body. Rainfall preceding the sampling event had to register a peak of approximately1,000 cfs (cubic feet per second) or greater on the USGS stream flow gauge located at the Piney Point Road bridge over Buffalo Bayou. Full instructions were written up and included in the Appendix A of the Quality Assurance Project Plan (QAPP). Investigators acknowledged the information gathered would not look at the same water as it flows down the bayou but rather, the water quality at a physical location over time.

2.0 Method

Water samples were collected from two sampling locations. The first site was Buffalo Bayou at Piney Point Road, 4.3 miles west of Loop 610 West (site #11358), where bacterial results have been historically low but a few high numbers have been measured over the years. The second site was Buffalo Bayou at Voss Road (site #11356) where bacterial results have been historically high. Both sites are associated with a United States Geological Survey (USGS) gage station so flow data can be captured to assist with data interpretation (See Figure B1 in the QAPP in Appendix A). Rainfall data was gathered by the City of Houston, Department of Health & Human Services, using the Harris County Office of Emergency Management. Dry weather sampling was preceded by at least 7 days of no precipitation and when no discharge was occurring from Barker-Cypress reservoir.

Approximately 10 liters of water was collected from the two study sites on Monday of each test week. At the lab, bacteria analyses were performed and aliquots of sample water for testing conventional parameters were separated for analysis. Extra water was split into the five equal portions and used to set up the different testing scenarios in large flasks. Over the next four consecutive days, water samples were collected from each flask and analyzed for *E. coli* bacteria. On each day of testing, 100 ml was withdrawn from each container of water and two dilutions analyzed – 1:10 and 1:100 – using IDEXX colilert 18.

Part 1 required setting up five (5) different conditions in the lab and holding the sample water throughout the test week. The five holding conditions were as follows:

1. Approximately 2 Liters of water was placed in a sealed container (flask) in the refrigerator and stored at or below 4°C. Before a sample was collected for bacterial testing, the container was shaken to resuspend all particles that had settled out over time. The required 100 ml of water was pipetted from the container and analyzed. Afterwards, the container was resealed and returned to the refrigerator until the next day when testing was repeated. This test not only served as the control, but would address whether bacterial concentration increased or decreased in samples held at $\leq$4°C for longer than the prescribed holding time.

2. Approximately 2 Liters of water was placed in a darkened fume hood at room temperature and allowed to settle in a large flask. The container was covered but it was not sealed tight. Prior to each bacteria test, the container was sealed and shaken according to *Standard Methods*. Then, the required 100 ml of sample was pipetted from the holding container and analyzed. This test was expected to address bacteria die-off in an environment that began to replicate the field environment by occluding the light and more closely replicating warmer summer months.

3. Approximately 2 Liters of water was placed in a darkened fume hood at room temperature and allowed to settle in a large flask. The container was covered but it was not sealed tight. Each time 100 ml was removed for a bacteria test, the required aliquot of water was pipetted from the top 1-inch of the container. **NO** shaking or stirring of the flask occurred before the samples were removed. This test was used to track growth or die-off of *E. coli* concentrations on a daily basis in a laboratory controlled environment and also served as a control for a no flow condition.

4. Approximately 2 Liters of water was placed in a darkened fume hood at room temperature and was slowly stirred. The agitation was sufficient enough to keep all particles suspended or moving around the flask from top to bottom. The container had a cover but it was not sealed tight. Each time a bacteria test was analyzed, a 100 ml sample of water was pipetted from the container. The objective of this portion of the study was to track *E. coli* concentrations daily in an environment that more closely mimics a low flowing, light occluded stream situation. The conclusions from this test regime would aid in model development for the bacteria TMDL.

5. Approximately 2 Liters of water was placed in a darkened fume hood at room temperature and stirred at a high rate of speed. The sample was vigorously agitated so all particles were kept suspended and the water exhibited a well-defined vortex. The flask had a cover but it was not sealed tight. Each time a bacteria test had to be analyzed, a 100 ml sample of water was pipetted from the container. The objective of this portion of the study was to track *E. coli* concentrations daily in an environment that more closely mimic a high flowing, light occluded stream situation. The conclusions from this test regime should also aid in model development for the bacteria TMDL.

Part 2 of the bacteria die-off study involved collecting samples during or shortly after a single day storm event that "peaked" the stream flow gauge at 1,000 cfs or greater within the 24 hours prior to sampling. Rain events had to have been preceded by several days with no precipitation and occurred either late Sunday or early Monday to initiate additional sampling. The quantity of water collected was sufficient to analyze all regularly tested conventional parameters including nutrients and bacteria on the first day, plus enough water to set up the five different scenarios for bacteria testing each day for the rest of the week. Additionally, bacteria samples, TSS samples and field parameters were collected from both sites over the next three (3) consecutive days following the rainfall event. These tests were used to determine how bacteria concentrations changed at each sampling location following a rain event and to determine if there was a correlation to total suspended solids. A complete Quality Assurance Project Plan (QAPP) was written and approved for this project in late June 2004. See Appendix A for a copy of the approved QAPP.

The project goal was to complete a total of six sampling events – 4 dry and 2 wet. To complete Part 1, at least one sampling event needed to occur during the index period (March 15 thru October 15) and, if possible, at least one sampling event should occur during the critical period (July 1 through September 30). One event needed to be collected during the winter months (December thru February) to provide information on possible seasonal variations. Remaining samples could be scheduled as time and/or rainfall allowed. The goal for Part 2 was to collect storm events during the 11 months of the project's sampling period. If only one rainfall event was sampled, then that event should be collected during the index period. If no storm event occurred as the protocols required, all six events could be dry weather, low flow conditions.

3.0 Results

Of the desired six sampling events only five were completed – four dry weather events and one wet weather event. Two dry weather, low flow events were completed during the index period and one event was completed during the winter. No sampling events were completed during the critical period as originally hoped. One rainfall event was conducted in the index period – May 9, 2005. Appendix B contains two tables of all data results associated with the first day of each sampling event. As previously stated, the protocol for Part 1 required samples to be collected on Monday of each test week and analyzed for bacteria and conventional chemical parameters. Then, five different holding scenarios were set up for additional bacteriological testing throughout the week. Figures 1 – 10 illustrate the bacteria die-off rates for each scenario tested. Tables containing each week's bacteriological test results under the different holding conditions

are presented in Appendix C. Results from Piney Point Rd are separated from Voss Rd since the sites are not related other than both being located on Buffalo Bayou. Notice the scales on the graphs are different. Different scales were used to show the trend of the bacteria concentrations as opposed to comparing the bacteria results between the two locations. The sites were chosen based on a review of data to find one site with high historical bacteria and one with low bacteria levels.

The nutrient data found in Appendix B tables B-1 and B-2 show that ammonia levels remained fairly constant between all sampling events regardless of flow conditions. At these two locations, there appeared to be no difference between wastewater treatment plant dominated low flow, dry weather conditions and wet weather, high flow conditions. However, nitrate, ortho and total phosphorus was fairly constant except for the rain event which occurred on May 9, 2005. Nitrate levels dropped to < 1 mg/l after the rain event after fluctuating between 4.5 and 8.0 mg/l during the dry weather, low flow periods. Likewise, after the rainfall event total and ortho phosphate concentrations generally dropped to less than half of the levels previously found during dry weather conditions.

Flask 1 was chosen as the control since it included refrigeration of the samples during each sampling/testing event. Results from this scenario show that once samples are preserved in ice or kept in refrigerator at <4°C, the bacteria are preserved and die-off rates are very slow. With few exceptions, many die-off rates were minimal and some late week results showed the concentrations actually increased slightly. Graphically, some of the results from flask 1 seem to be all over the charts. Flask 2 on the other hand, generally exhibited an expected die-off trend. The differences between flasks 1 and 2 were the temperature in which the flasks were held over time. The cold environment preserved the bacteria while warm temperatures did not enhance the longevity of the bacteria at all. Rather, the majority of the results in flask 2 showed decreases between the first and final analysis even after re-suspension.

Flask 3 results indicate that die-off rates are enhanced by warm temperatures and settling. This scenario did not include shaking or stirring before testing so any bacteria present in the water appeared to “fall out” or die-off.

Flasks 4 and 5 were the closest in design to the natural bayou environment. Flask 4 was slowly stirred and mimicked the sluggish movement of the Houston bayous in the warm, dry months. In contract, flask 5 was chosen to imitate the turbulent waters found in a high flow situation. In each of the graphs of Figures 1 thru 10, the line representing the sluggish movement (flask 4) always had lower results than the vigorously stirred flask 5. There was a consistent die-off but bacteria appeared to live longer in the highly mixed scenario than in the slowly moving container.

Figure 1. Bacteria Die-off results for Piney Point Rd sample held under five different lab scenarios beginning October 4, 2004.

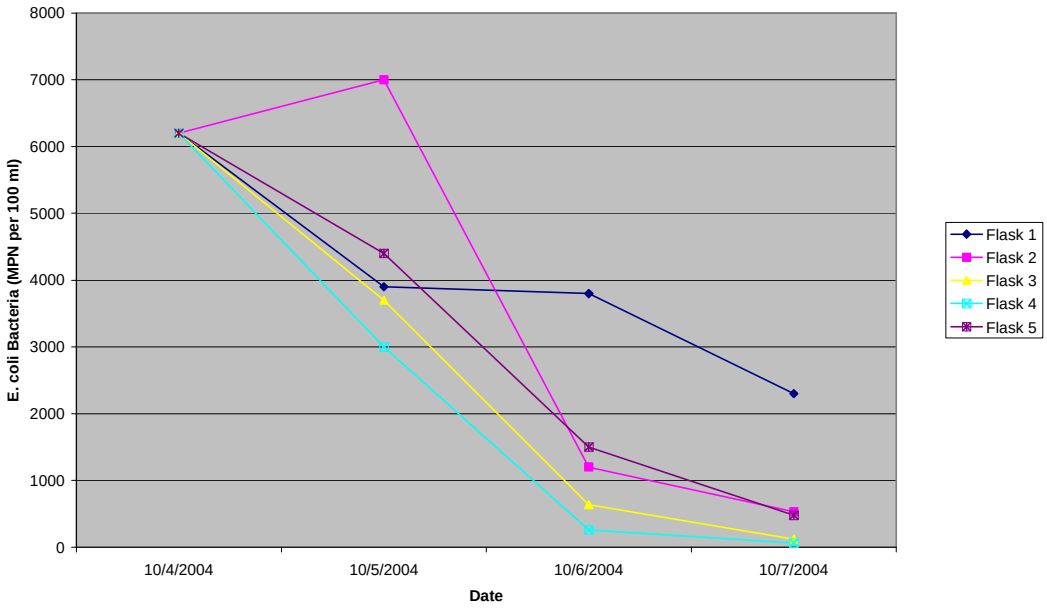
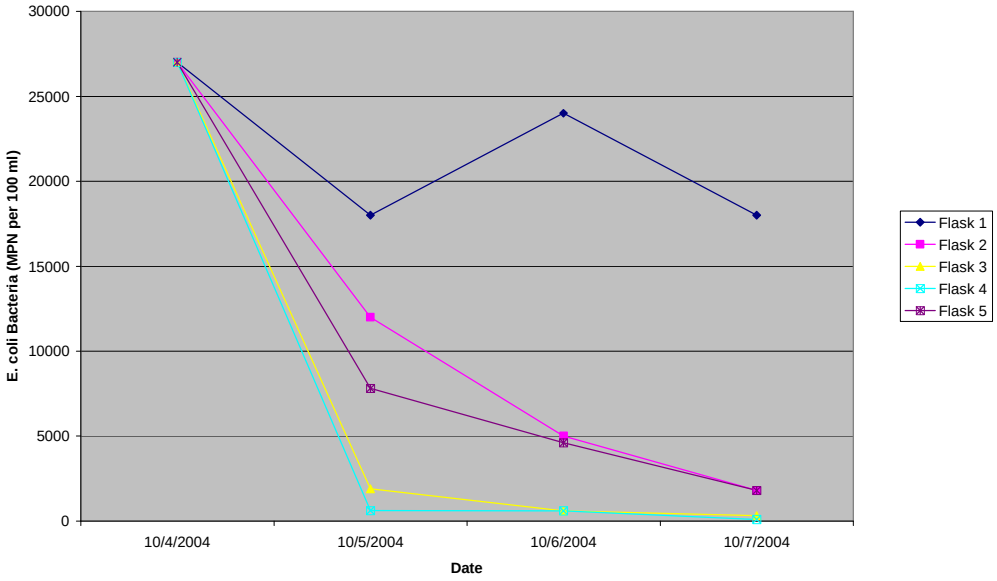


Figure 2. Bacteria Die-off results for Voss Rd sample held under five different lab scenarios beginning October 4, 2004.



Flask 1 - Sealed container, refrigerated, settled, reshaken for pipetting & testing

Flask 2 - Darkened fume hood @ room temperature, settled, reshaken for pipetting & testing

Flask 3 - Darkened fume hood @ room temperature, settled, NOT shaken or stirred before pipetting & testing

Flask 4 - Darkened fume hood @ room temperature, slowly stirred continuously, pipetted & tested

Flask 5 - Darkened fume hood @ room temperature, vigorously agitated/stirred continuously, pipetted & tested

Figure 3. Bacteria Die-off results for Piney Point Rd sample held under five different lab scenarios beginning January 10, 2005.

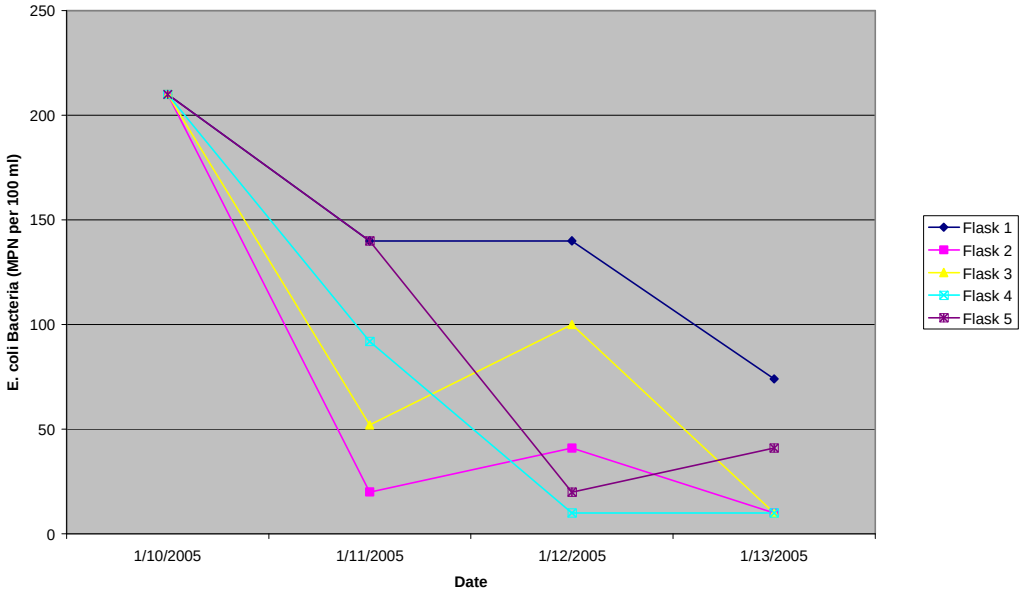
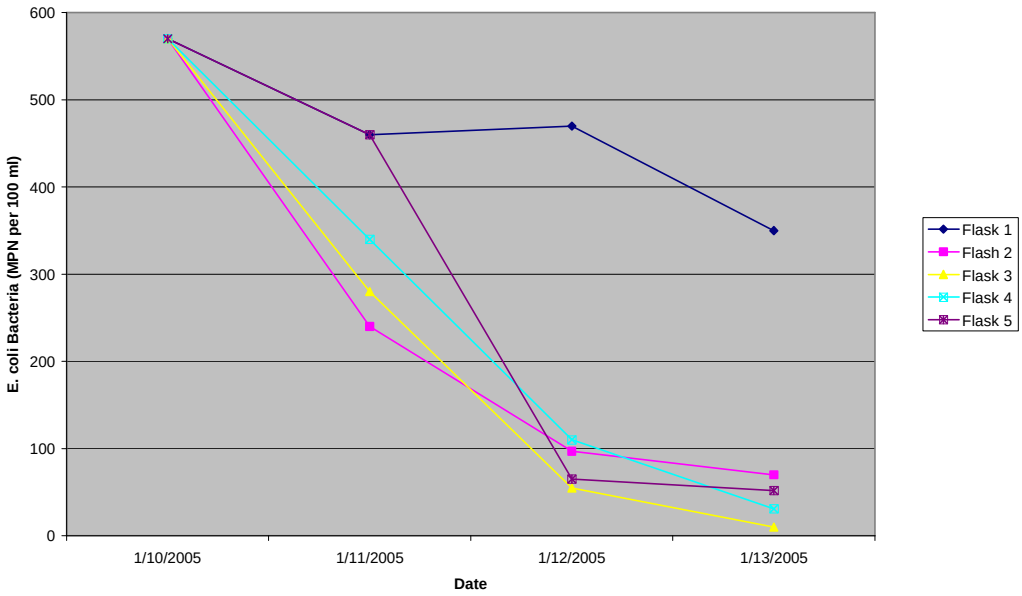


Figure 4. Bacteria Die-off results for Voss Rd sample held under five different lab scenarios beginning January 10, 2005.



Flask 1 - Sealed container, refrigerated, settled, reshaken for pipetting & testing

Flask 2 - Darkened fume hood @ room temperature, settled, reshaken for pipetting & testing

Flask 3 - Darkened fume hood @ room temperature, settled, NOT shaken or stirred before pipetting & testing

Flask 4 - Darkened fume hood @ room temperature, slowly stirred continuously, pipetted & tested

Flask 5 - Darkened fume hood @ room temperature, vigorously agitated/stirred continuously, pipetted & tested

Figure 5. Bacteria Die-off resutls for Piney Point Rd sample held under five different lab scenarios beginning March 14, 2005.

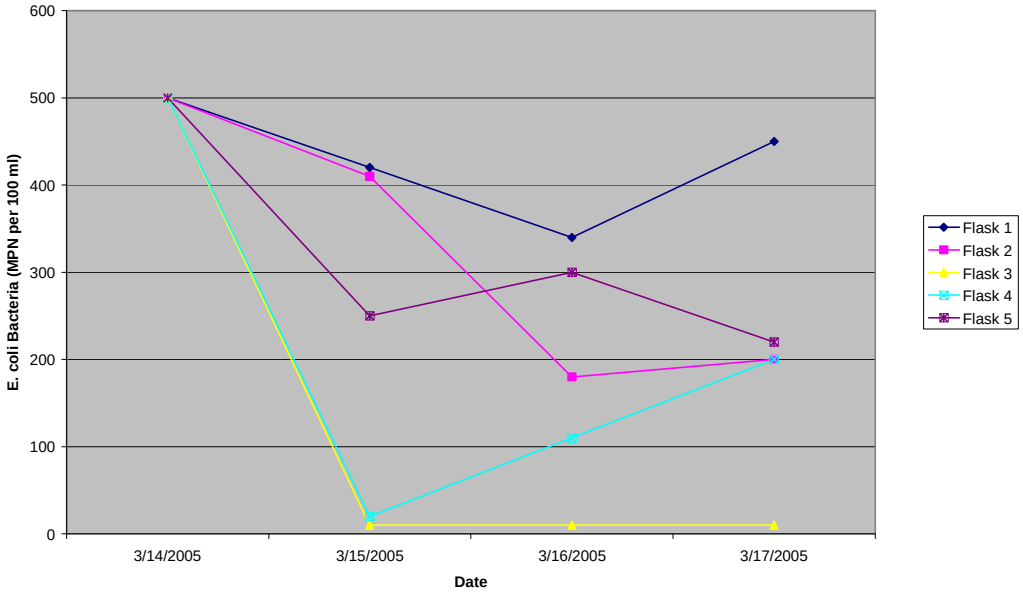
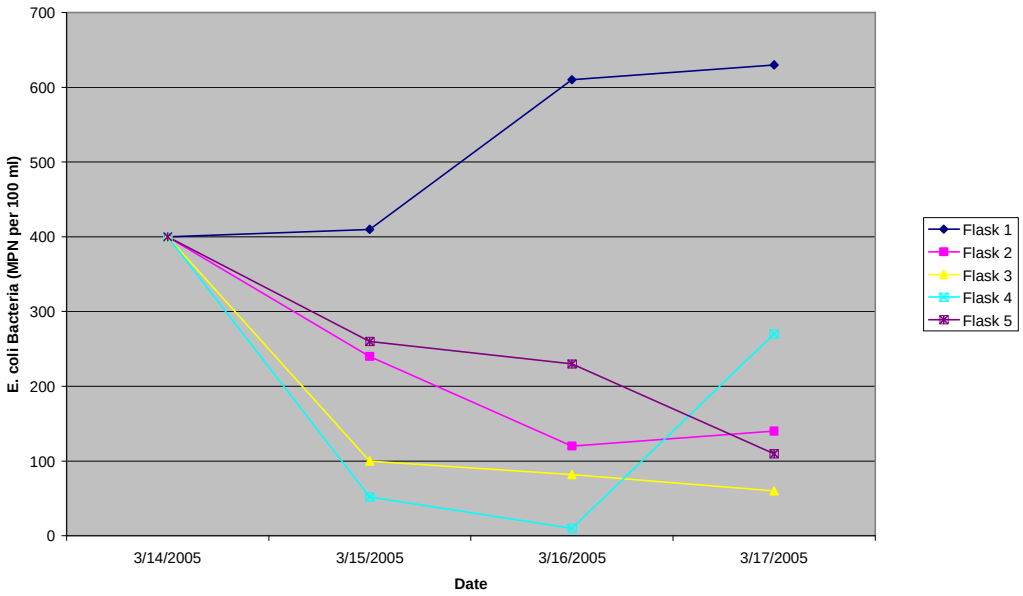


Figure 6. Bacteria Die-off results for Voss Rd sample held under five different lab scenarios beginning March 14, 2005.



Flask 1 - Sealed container, refrigerated, settled, reshaken for pipetting & testing

Flask 2 - Darkened fume hood @ room temperature, settled, reshaken for pipetting & testing

Flask 3 - Darkened fume hood @ room temperature, settled, NOT shaken or stirred before pipetting & testing

Flask 4 - Darkened fume hood @ room temperature, slowly stirred continuously, pipetted & tested

Flask 5 - Darkened fume hood @ room temperature, vigorously agitated/stirred continuously, pipetted & tested

Figure 7. Bacteria Die-off results for Piney Point Rd sample held under five different lab scenarios beginning April 18, 2005.

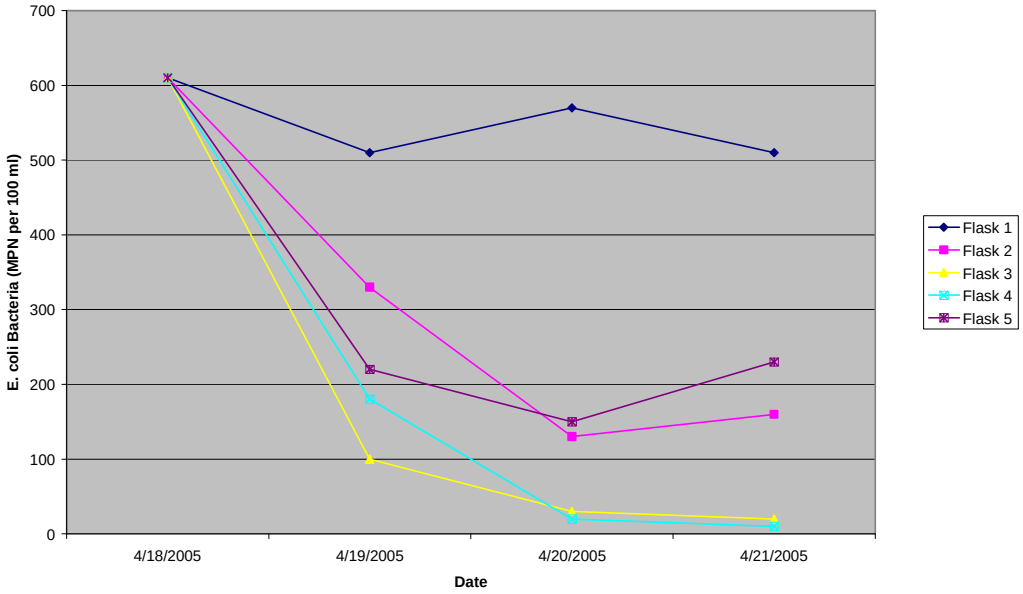
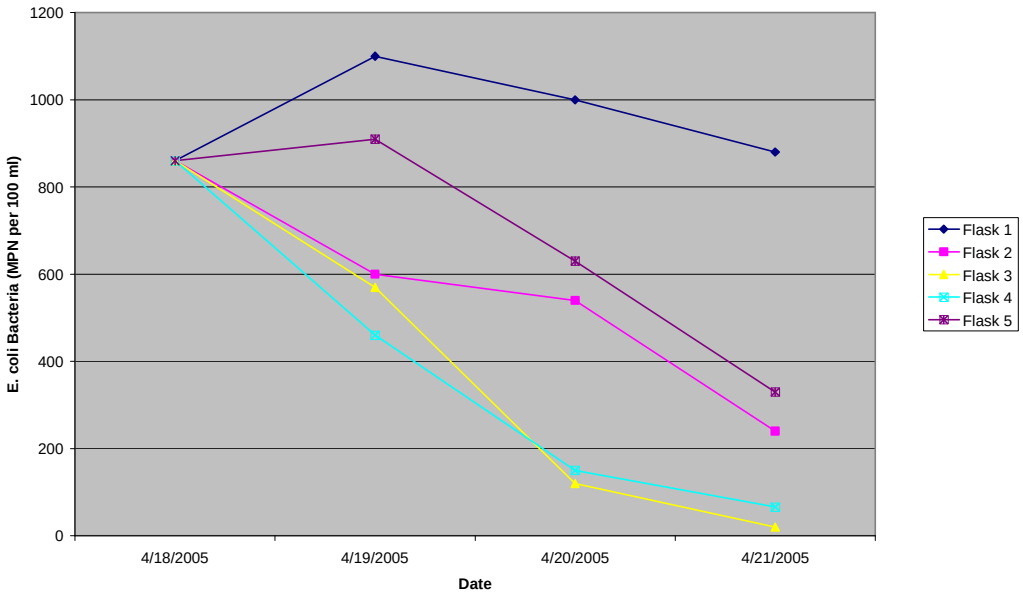


Figure 8. Bacteria Die-off results for Voss Rd sample held under five different lab scenarios beginning April 18, 2005.



Flask 1 - Sealed container, refrigerated, settled, reshaken for pipetting & testing

Flask 2 - Darkened fume hood @ room temperature, settled, reshaken for pipetting & testing

Flask 3 - Darkened fume hood @ room temperature, settled, NOT shaken or stirred before pipetting & testing

Flask 4 - Darkened fume hood @ room temperature, slowly stirred continuously, pipetted & tested

Flask 5 - Darkened fume hood @ room temperature, vigorously agitated/stirred continuously, pipetted & tested

Figure 9. Bacteria Die-off results for Piney Point Rd sample held under five different lab scenarios beginning May 9, 2005.

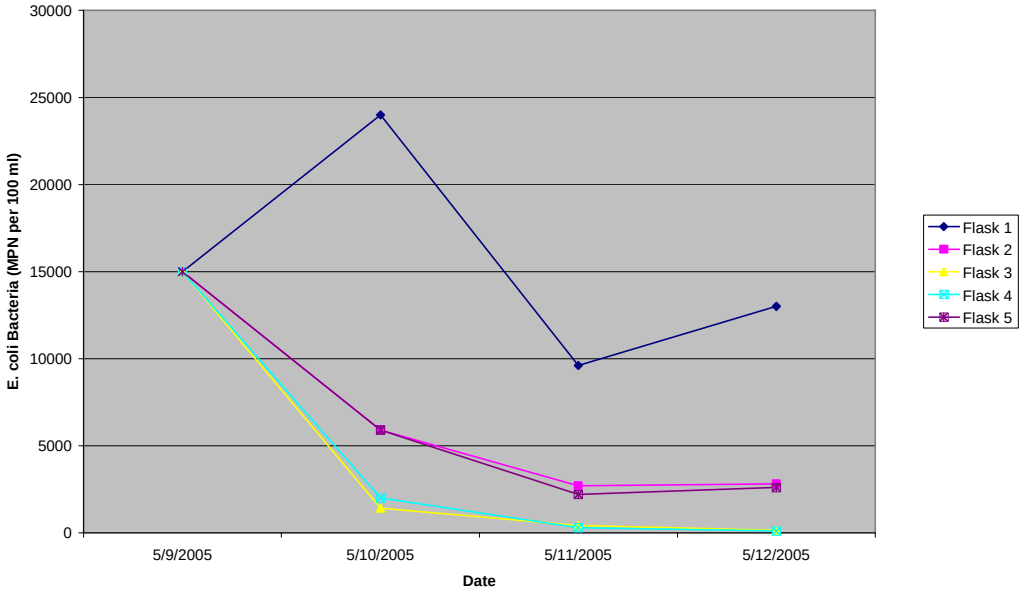
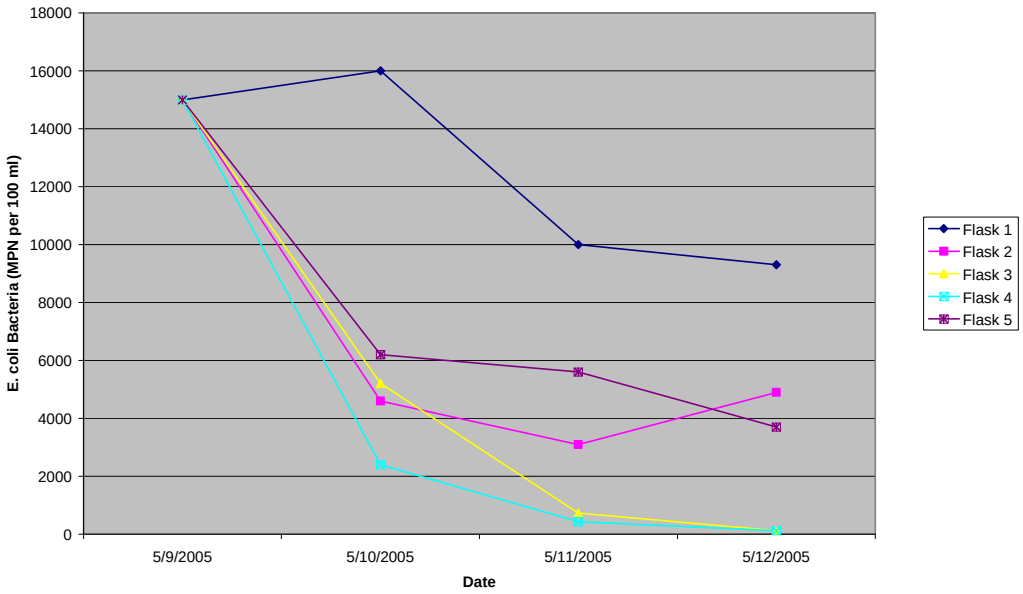


Figure 10. Bacteria Die-off results for Voss Rd sample held under five different lab scenarios beginning May 9, 2005.



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Flask 3 - Darkened fume hood @ room temperature, settled, NOT shaken or stirred before pipetting & testing

Flask 4 - Darkened fume hood @ room temperature, slowly stirred continuously, pipetted & tested

Flask 5 - Darkened fume hood @ room temperature, vigorously agitated/stirred continuously, pipetted & tested

Part 2 of the project looked at how bacteria levels changed over time at the two locations following a significant rainfall event less than 24 hours before samples were collected. The results for this one rainfall event are presented in Figure 11 with all associated data found in Table 1. As expected, the bacteria results were extremely high after the initial rainfall and decreased over time and there was a significant drop in bacteria concentration between day 2 and day 3. However, bacteria results still exceeded the single grab limit of 394 per 100 ml for *E. coli* even on the fourth day. Table 1 also shows how Total suspended solid results diminished over time at both the Piney Point Road and Voss Road locations following the rainfall event.

Figure 11. Bacteria results over time for Buffalo Bayou at two locations following a significant rainfall event.

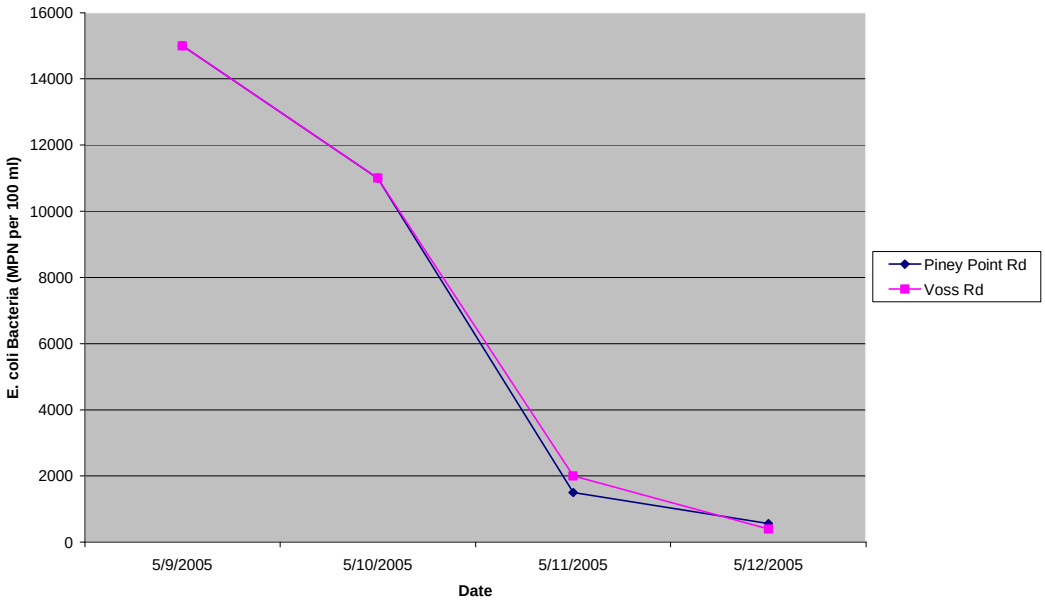

Table 1. Bacteria levels in Buffalo Bayou at two locations after a significant rainfall event.

Date sampled	Piney Point		Voss Road	
	E. coli per 100 ml	TSS mg/L	*E. coli* per 100 ml	TSS mg/L
5/9/2005	15000	458	15000	252
5/10/2005	11000	167	11000	192
5/11/2005	1500	69	2000	111
5/12/2005	560	47	400	60

4.0 Discussion/Conclusion

The data from this study provides several useful pieces of information. First, temperature between 2 and 4°C appears to greatly enhance the survival rate of bacteria. Even though some die-off was seen, it generally was between the first and second day after leaving the sample in the refrigerator. These results challenge the need for delivering bacteria samples to the lab within 6 hours and having all samples processed within another 2 hours for a maximum of 8 hours holding time. If the samples are properly iced and stored at <4°C, it looks like samples could easily be turned in or analyzed the day after they were collected and still have analysis produce viable data results. Removing or lessening the current time restrictions for delivering bacteria samples to the lab would greatly enhance scheduling flexibility and increase sample collection activities. Ultimately, more bacteria samples or more samples in general could be collected in a day if properly iced and adequately chilled until analysis occurred. This subject requires further investigation due to the small number of samples included in this study.

Comparing flask testing scenarios 1 and 2, it can be concluded that once bacteria are introduced to warmer temperatures in the environment, they will die-off in a matter of days. This was demonstrated by the identical tests in flasks 1 and 2, except for a temperature variation. Refrigerated flask 1 sustained higher bacteria concentrations for the length of the study week, while die-off began after the first day in the room temperature sample flasks. It would have been very interesting to learn how many days it would have taken for the bacteria levels to diminish to near or less than the single-grab standard of 394 MPN per 100 ml in the refrigerated samples.

Flask 3 had no stirring or shaking of the samples after sample water was placed in the containers. This scenario supports the assumption that bacteria will "fall out" or die-off over time if allowed to settle out of the water column. This scenario, along with flask 2, suggests that as long as the solids/sediment are not re-suspended, the die-off rate will be constant and fairly quick. Results for flask 3 seemed to drop dramatically between the second and third day in majority of the sampling events. This conclusion supports routing storm water flows through detention ponds for "settling" or "cleaning" before discharging to area waterways.

By comparing flask 4 and 5, one learns the bacteria have a definite die-off rate but the bacteria remains viable for a longer period of time in the vigorously agitated samples. Here again, the sluggish or slower moving waters of the bayou allow for bacteria and/or sediment to settle out.

In Part 2 of this study, results showed a general relationship between bacteria and total suspended solids (TSS). The relationship is not 1:1, nor is it a firm correlation. Rather, it can be used as a rule of thumb. In the samples collected after the rainfall event, the bacteria concentrations decreased over time as did the TSS results. However, as seen in the tables for both the rain event and the dry weather, low sampling events, the TSS data is not directly relatable. There was a particularly unusual exception for both sites sampled on October 4, 2004. While the bacteria numbers were very high, especially for Voss Rd, the TSS values were insignificant.

There was no relationship found between bacteria and any of the nutrients. These data seem to refute the idea that the nutrient rich waters of the Houston area might enhance the survival of

bacteria in the environment. Rather, the nutrient concentrations seemed to have no affect on the longevity of bacteria at all. Additionally, chloride results confirmed these sites were indeed fresh water sites with fluoride and sulfate results being “normal.”

In regard to seasonal variation, the results are inconclusive. Lowest overall bacteria concentrations were measured during January and March sampling events. However, in this area winter months are generally the wettest months and one would expect higher bacteria levels due to nonpoint source pollution runoff. In this study, all “dry weather, low flow conditions” were preceded by 7 days of no precipitation so the “playing field” appears to have been fairly equal between all sampling events. More data would be needed before speculating on this subject.

Appendix A

Approved
Quality Assurance Project Plan

Appendix J to the Houston-Galveston Area Council Clean Rivers Program FY 2004/2005 QAPP

Bacteria Die-off Study

Prepared by the Houston-Galveston Area Council

In Cooperation with the Texas Commission on Environmental Quality (TCEQ)

Effective Period
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S-A1 APPROVAL PAGE

The following signatures are required for this special study:

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Jean Wright Date
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Laurie Curra Date
TCEQ CRP Project Manager

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Sharon Coleman Date
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Laurie Curra Date
TCEQ CRP Project Quality Assurance Specialist

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Milburn “Earl” Breazeale Date
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Kim Phillips Date
City of Houston, North Laboratory Microbiologist

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Paul Jensen Date
PBS&J Project Advisor

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LIST OF ACRONYMS

As described in Section A2 of the H-GAC 2004/2005 Basin-wide QAPP

SS-A3 DISTRIBUTION LIST As described in Section A3 of the H-GAC 2004/2005 Basin-wide QAPP as follows (in *italics*):

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Austin, Texas 78711-3087

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The Houston-Galveston Area Council will provide copies of this project plan and any amendments or revisions of this plan to each person on this list and to each sub-tier project participant, e.g., subcontractors, other units of government, laboratories. The Houston-Galveston Area Council will document distribution of the plan and any amendments and appendices, maintain this documentation as part of the project's quality assurance records, and will be available for review.

SS-A4 PROJECT/TASK ORGANIZATION

Todd Running, H-GAC Project Manager

Role and responsibility as described for the H-GAC Project Manager in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP

Jean Wright, H-GAC Quality Assurance Officer

Role and responsibility as described for the H-GAC QAO in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP

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Role and responsibility as described for the H-GAC Data Manager in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP

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Role and responsibility as described for the TCEQ Project Manager in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP.

Sharon Coleman, Acting TCEQ Lead QA Specialist

Role and responsibility as described for the Lead QA Specialist in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP.

Laurie Curra, TCEQ Project QA Specialist

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Eric Reese, CRP Data Manager

Role and responsibility as described for the TCEQ Project Data Manager in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP.

Milburn "Earl" Breazeale, City of Houston CRP Project Manager

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Emina Marjanovich, City of Houston Technical Supervisor, Braeswood Lab

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John Hawley, Braeswood Laboratory QAO

Role and responsibility as described for the City of Houston CRP Braeswood Lab QAO in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP.

Larry Bagwill, City of Houston Technical Supervisor

Role and responsibility as described for the City of Houston, North Lab Technical Supervisor in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP.

Cindy Boule', North Laboratory QAO

Role and responsibility as described for the City of Houston, North Lab QAO in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP.

Kim Phillips, City of Houston Microbiologist

Performs all microbiological analyses, computes, quality-assures and reports data to the City of Houston Technical Supervisor. As the City of Houston Health and Human Services Laboratory Microbiologist, also serves in an advisory capacity for this project.

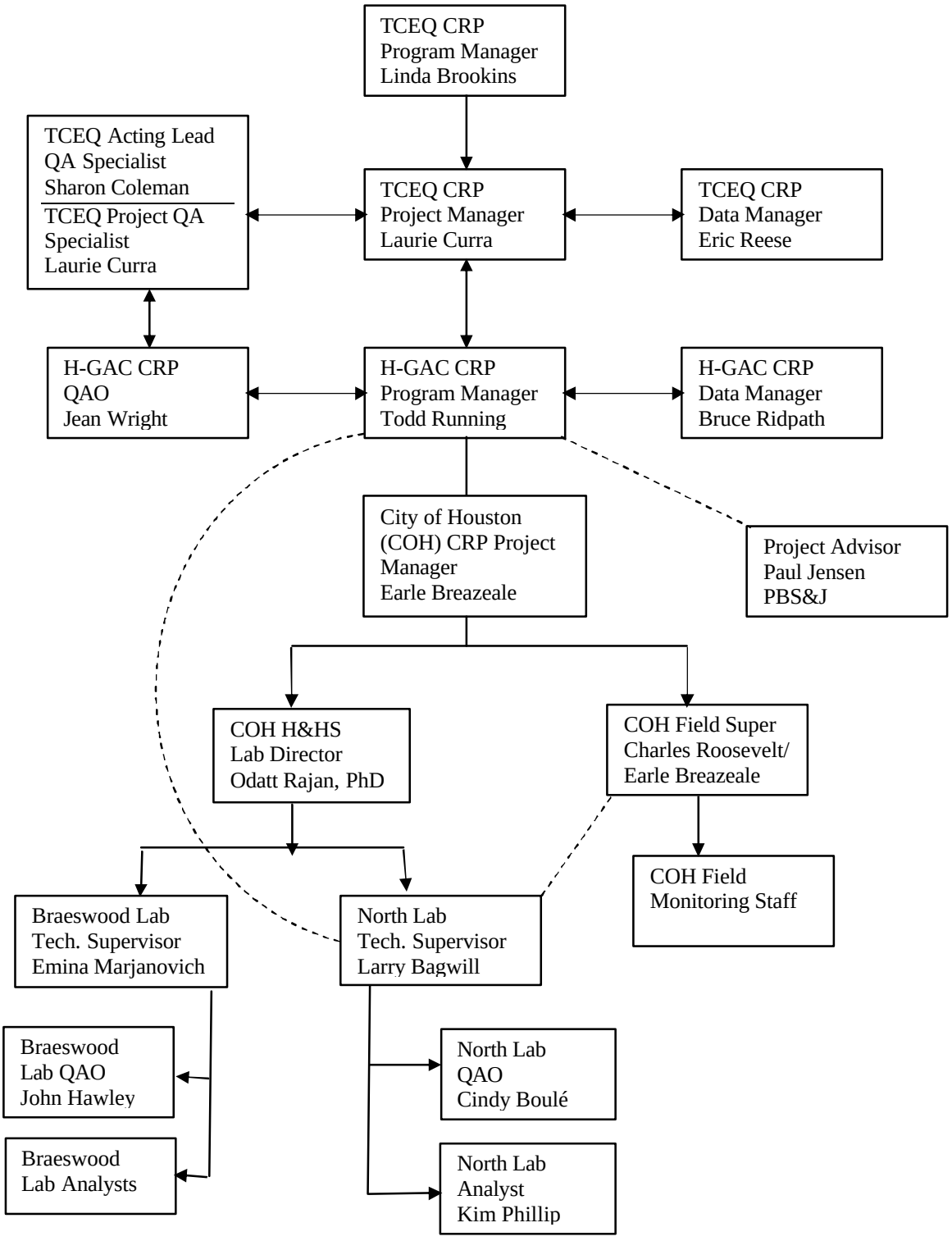
Charles Roosevelt, CRP QAO, CRP Data Manager, and Field Supervisor for Ambient Waters

Role and responsibility as described for the City of Houston CRP QAO, CRP Data Manager, and Field Supervisor for Ambient Waters in Section A4 of the H-GAC 2004/2005 Basin-wide QAPP

Paul Jensen, PBS&J Project Advisor

An engineering consultant for the City of Houston Public Works & Engineering Department working primarily with water and wastewater. He is one of the principals on the Bayou Bacteria TMDL that is being conducted by TCEQ on the Buffalo Bayou and White Oak Bayou watersheds in Houston, Texas, and serves in an advisory capacity for this project.

Organization Chart



Dashed lines signify direct lines of communication.

SS-A5 PROBLEM DEFINITION

Historically, it has been thought that *E. coli* does not proliferate (replicate) outside the body of warm-blooded animals and that concentrations in surface water decrease significantly (possibly by 1 log) 24-48 hours after being introduced into surface waters. The stationary phase of the growth cycle usually leads quickly to the phase of decline or death because of the depletion of essential nutrients. But in nutrient rich water, such as in Houston area bayous, it is suspected that the bacteria level is not only sustained for an extended period of time, but may even increase to a certain extent. The assumption that bacterial concentrations recorded after storm events or sewage influxes will follow the natural growth/die off cycle may be incorrect thus leading to poor decisions regarding the reopening of swimming areas and other TMDL influenced decisions. This study will help determine how the bacteria levels in the Houston area surface waters actually react. This issue was the subject of a TMDL study conducted in 2001 by PBS&J, however it was restricted to having very limited mixing conditions. The purpose of this study is to provide improved data on the die-off rate of indicator bacteria in Houston bayous.

Part 1 of this study will be conducted in the laboratory and will look at bacteria die-off rates from two different sampling sites on Houston's Buffalo Bayou. Variables to be explored in this part of the study includes the differences between sample mixing, no sample mixing, and the effect of two different speeds of continuous mixing. During the summer of 2001, the Bacteria TMDL chamber studies demonstrated initial *E. coli* concentrations of bayou water at about 10,000 MPN/100 mL. After a two-day period, the concentrations of all samples without mixing had dropped to about 10 MPN/100 mL. These results were well below the ambient levels seen in the bayous. The die-off co-efficient obtained from these data is about 2/day. A similar rate was used in the TMDL modeling; however, this may not be a representative rate if the mixing that is a part of the bayou's natural flow is accurately included. The purpose of these studies is to check die-off rates under mixing conditions that more nearly simulate what exists in the bayous.

Another variable to be considered in **Part 1** is the effect of storage at 4ºC over time. The "holding time until analysis" is an issue due to the inability of sampling staff to get samples delivered to the lab and anlyzed within the 6 + 2 hours holding time. Some researchers have suggested 6 hours is too restrictive and bacterial concentrations remain constant for longer periods of time when kept at or below 4ºC. However, in the summer of 2001 bacteria chamber studies, the West District wastewater treatment plant (WWTP) disinfected effluent had "no detection of indicator bacteria" results initially. Unrefrigerated samples measured the next day had a concentration of over 100 MPN/100 mL indicating an increase over time. One of the analysis regimes included in **Part 1** will help to determine if bacteria concentrations change over time while being kept in the refrigerator. Containers of bayou water will be kept in a refrigerator between 1 & 4ºC while other containers of water are held at room temperature and kept within a fume hood. The front sash of the vent hood will be covered by brown paper or foil to block majority of the ambient light except during sample processing.

The other variable to be explored in **Part 1** has to do with the possible correlation of die-off rates with nutrient concentrations. The basic process employed in the study is to isolate a sample of water from two different sites and to track the concentration of bacteria in the isolated samples over a 4-day time period over different seasons. Nutrient data will be compared to the seasonal die-off rate of bacteria to see if there is any relationship. The first site will be a location where bacterial results have been historically high. The second site will be located where bacterial results have been historically low but a few high numbers have been occasionally measured over the years.

Part 2 of the bacteria die-off study is designed to look at the bacteria response in the natural environment after a typical rainfall event that would cause an influx of bacteria into the water body via run-off. Assumptions that bacterial concentrations recorded after storm events or sewage influxes will follow the natural growth/die-off cycle may be incorrect, thus leading to poor decisions regarding the reopening of swimming areas and other TMDL influenced decisions. It is suspected that in Houston area bayous, the bacteria levels are not only sustained for an extended periods of time, but may even increase to a certain extent. This portion of the study will look at how bacteria numbers change at a given site over time following rainfall and whether that information correlates with nutrient levels in the waterbody. The rainfall event must register a peak of aproximately1,000 cfs or greater on the USGS stream flow gauge located at the Piney Point Road bridge over Buffalo Bayou. Full instructions have been written up and included in Appendix A of this document. The information gathered will not look at the same water as it flows down the bayou but rather, the water quality at a physical location over time.

SS-A6 PROJECT/TASK DESCRIPTION

In **Part 1** of the study, water samples will be collected from two sampling locations. The first will be a location where bacterial results have been historically high. The second site will be located where bacterial results have been historically low but a few high numbers have still been measured over the years. Site #11356 – Buffalo Bayou at Voss Road – is currently on the Coordinated Monitoring Schedule (CMS) for FY2004 and is the site where bacterial results have been high historically (See Figure SS-B1). Site #11358 – Buffalo Bayou at Piney Point Road, 4.3 miles west of Loop 610 West in Houston – is not on the current CMS but has been sampled by the City of Houston in the past and is the site where bacterial results have been historically low but a few high numbers have been recorded over the years. Both sites are associated with a United States Geological Survey (USGS) gage station so flow data can be captured to assist with data interpretation. Plus, rainfall data will be gathered by H-GAC from the Harris County Flood Control Division, the Harris County Office of Emergency Management, all wastewater treatment plants within close proximity of the sites and/or any other reliable sources identified before the final report is written.

Water samples from the two study sites will be collected on Monday each time. The quantity of water collected will be sufficient to analyze all conventional parameters including bacteria and nutrient parameters on the first day plus ample water to run five (5) bacteria tests over the next three consecutive days. On each day of bacteria testing, 100 mL will be withdrawn from each container of water and two dilutions analyzed – 1:10 and 1:100.

The basic project for **Part 1** will require setting up five (5) different conditions in the lab to hold the sample water. The five holding conditions are as follows:

1. Approximately 2 Liters of water will be placed in a sealed container in the refrigerator and stored at or below 4°C. Before a bacteria test is analyzed, the container will be shaken to resuspend all particles that have settled out over time. The required 100 mL of water will be pipetted from the container and analyzed. Afterwards, the container will be resealed and returned to the refrigerator until the next day's testing. This test will not only serve as the control, but will address whether bacterial concentration increase or decrease in samples held at $\leq$4°C for longer than the prescribed holding time.

2. Approximately 2 Liters of water will be placed in a darkened fume hood at room temperature and be allowed to settle in its container. The container will have a cover but it does not have to be sealed tight. Prior to each bacteria test, the container will be sealed and shaken according to *Standard Methods*. Then the required 100 mL of sample will be pipetted from the holding container and analyzed. This test will address bacteria die-off in an environment that begins to replicate the field environment by occluding the light and more closely replicating warmer summer months.

3. Approximately 2 Liters of water will be placed in a darkened fume hood at room temperature and be allowed to settle in its container. The container will have a cover but it does not have to be sealed tight. Each time 100 mL is removed for a bacteria test, the required aliquot of water will be pipetted from the top 1-inch of the container. **NO** shaking or stirring of the holding container will occur before the samples are removed. This test will be used to track growth or die-off of *E. coli* concentrations on a daily basis in a laboratory controlled environment and will also serve as a control for a no flow condition.

4. Approximately 2 Liters of water will be placed in a darkened fume hood at room temperature and will be slowly stirred. The agitation will be sufficient enough to keep all particles suspended or moving around the container from top to bottom. The container will have a cover but it does not have to be sealed tight. Each time a bacteria test is analyzed, a 100 mL sample of water will be pipetted from the container. The objective of this portion of the study will be to track *E. coli* concentrations daily in an environment that more closely mimics a low flowing, light occluded stream situation. The conclusions from this test regime will aid in model development for the bacteria TMDL.

5. Approximately 2 Liters of water will be placed in a darkened fume hood at room temperature and will be stirred at a high rate of speed. The sample will be vigorously agitated so all particles are kept suspended and the water exhibits a well-defined vortex. The container will have a cover but it does not have to be sealed tight. Each time a bacteria test is analyzed, a 100 mL sample of water will be pipetted from the container. The objective of this portion of the study will be to track *E. coli* concentrations daily in an environment that more closely mimic a high flowing, light occluded stream situation. The conclusions from this test regime will aid in model development for the bacteria TMDL.

Part 2 of the bacteria die-off study will involve collecting samples during or shortly after a single day storm event that "peaks" the stream flow gauge at 1,000 cfs or greater within the 24 hours prior to sampling. These rain events must occur either late Sunday or on Monday to initiate additional sampling. The quantity of water will be sufficient to analyze all regularly tested conventional parameters including nutrients and bacteria on the first day and enough for bacteria testing the rest of the week. Plus, additional bacteria samples **only** and field parameters will be collected from both sites over the next three (3) consecutive days. These tests will determine how bacteria concentrations changed at each sampling location following a rain event. See Appendix A for a detailed description of the sampling guidelines.

The project goal is to complete a total of six sampling events with all sampling and analysis completed and **results submitted to H-GAC by March 31, 2005**. For **Part 1**, at least one sampling event should occur during the index period (March 15 thru June 30 or October 1 thru 15), at least one sampling event should occur during the critical period (July 1 through September 30), and one should be collected during the winter months (December thru February) to provide information on possible seasonal variations. The

remaining samples can be scheduled as time and rainfall allows. The goal for **Part 2** is to collect a minimum of 1 storm event during the 11 months of the project's sampling period. If only one rainfall event is sampled, the event should be collected during the index period. No more than two (2) storm events will be accepted. If no storm event can be sampled, all six events will be dry weather, low flow conditions.

H-GAC will be responsible for writing the final report which is due on or before August 31, 2005. A draft final report is due July 15, 2005.

SS-A7 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

The measurement performance specifications to support the project objectives are specified in Table SS-A7.1.

Table SS-A7.1 - Measurement Performance Specifications

PARAMETER	UNITS	MATRIX	METHOD	STORET	AWRL	Lab Reporting Limit (RL)	RECOVERY AT RLs	PRECISION (RPD of LCS/LCS dup)	BIAS (%Rec. of LCS)	Lab
Field Parameters measured by City of Houston, Health & Human Services										
Conductivity	µS/cm	water	EPA 120.1 and TCEQ SOP	00094	NA*	NA	NA	NA	NA	Field
Days since last significant rainfall	Days	NA	TCEQ SOP	72053	NA*	NA	NA	NA	NA	Field
DO	mg/L	water	EPA 360.1 and TCEQ SOP	00300	NA*	NA	NA	NA	NA	Field
Flow measurement method	1-gage 2-electric 3-mechanical 4-weir/flume 5-doppler	water	TCEQ SOP	89835	NA*	NA	NA	NA	NA	Field
Flow, Instantaneous	cfs	water	TCEQ SOP	00061	NA*	NA	NA	NA	NA	Field
Flow severity (if no flow measured)	1-no flow, 2-low, 3-normal, 4-flood, 5-high, 6-dry	water	TCEQ SOP	01351	NA*	NA	NA	NA	NA	Field
pH	pH/ units	water	EPA 150.1 and TCEQ SOP	00400	NA*	NA	NA	NA	NA	Field
Present Weather	1-clear 2-partly cloudy 3-cloudy 4-rain	NA	NA	89966	NA	NA	NA	NA	NA	Field
Secchi Depth	meters	water	TCEQ SOP	00078	NA*	NA	NA	NA	NA	Field
Temperature	º C	water	EPA 170.1 and TCEQ SOP	00010	NA*	NA	NA	NA	NA	Field
Turbidity, Observed (if not lab tested)	1-low 2-medium 3-high	water	TCEQ	88842	NA*	NA	NA	NA	NA	Field
Water Clarity (if no secchi)	1-excellent 2-good 3-fair 4-poor	water	TCEQ	20424	NA*	NA	NA	NA	NA	Field

PARAMETER	UNITS	MATRIX	METHOD	STORET	AWRL	Lab Reporting Limit (RL)	RECOVERY AT RLs	PRECISION (RPD of LCS/LCS dup)	BIAS (%Rec. of LCS)	Lab
Field Parameters measured by City of Houston, Health & Human Services										
Water Color	1-brownish 2-reddish 3-greenish 4-blackish 5-clear 6-other	water	TCEQ	89969	NA*	NA	NA	NA	NA	Field
Water Odor	1-sewage 2-chemical 3-rotten egg 4-musky 5-fishy 6-none 7-other	water	TCEQ	89971	NA*	NA	NA	NA	NA	Field
Wind Intensity	1-calm 2-slight 3-moderate 4-strong	NA	NA	89965	NA	NA	NA	NA	NA	Field

PARAMETER	UNITS	MATRIX	METHOD	STORET	AWRL	Lab Reporting Limit (RL)	RECOVERY AT RLs	PRECISION (RPD of LCS/LCS dups)	BIAS %Rec. of LCS	Lab
Conventional and Bacteriological Parameters Collected by City of Houston, Health & Human Services										
Ammonia-N	mg/L	Water	EPA 350.1	00610	.02	.03	75-125	20	80-120	HH North
C-BOD5	mg/L	Water	SM 5210B	00310	2	4	NA	20	NA	HH North
Chloride	mg/L	water	EPA 300.0	00940	10	5	75-125	20	80-120	HH North
E. coli, IDEXX Colilert	MPN/100 mL	water	SM 9223-B	31699	1	1	NA	.5**	NA	HH North
Fluoride, total	mg/L	water	EPA 300.0	00951	.5	.2	75-125	20	80-120	HH North
Nitrate-N, total	mg/L	water	EPA 300.0	00620	.02	.2	75-125	20	80-120	HH North
Phosphorus, total	mg/L	water	EPA 365.3	00665	.06	.06	75-125	20	80-120	Braeswood Lab
Ortho phosphate Phosphorus (field filtered)	mg/L	water	EPA 365.1	00671	.04	.04	75-125	20	80-120	HH-North
Sulfate	mg/L	water	EPA 300.0	00945	10	5	75-125	20	80-120	HH North
TDS, dried at 180 degrees C	mg/L	water	EPA 160.1	70300	10	10	NA	20	NA	HH North
TSS	mg/L	water	EPA 160.2	00530	4	4	NA	20	NA	HH North

* Reporting to be consistent with SWQM guidance and based on measurement capability.

** Based on a range statistic as described in Standard Methods, 20th Edition, Section 9020-B, Quality Assurance/Quality Control – Intra-laboratory Quality Control Guidelines. This criterion applies to bacteriological duplicates with concentrations >10 MPN/100mL or 10 colonies/100mL.

References for Table A7.1:

United States Environmental Protection Agency (USEPA) Methods for Chemical Analysis of Water and Wastes, Manual #EPA-600/4-79-020
American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF), Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998
TCEQ SOP – "Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue."
American Society for Testing and Materials (ASTM) Annual Book of Standards, Vol. 11.02

Ambient Water Reporting Limits (AWRLs) As described in Section A7 of the H-GAC 2004/2005 Basin-wide QAPP as follows:

*"The AWRL establishes the reporting specification at **or below** which data for a parameter must be reported to be compared with freshwater screening criteria. The AWRLs specified in Table A7.1 are the program-defined reporting specifications for each analyte and yield data acceptable for routine water quality monitoring. The reporting limit is the lowest concentration at which the laboratory will report quantitative data within a specified recovery range. The laboratory will meet two requirements in order to report meaningful results to the Clean Rivers Program:*

- *The laboratory's reporting limit for each analyte will be at **or below** the AWRL.*
- *The laboratory will demonstrate and document on an ongoing basis the laboratory's ability to quantitate at its reporting limits."*

Precision As described in Section A7 of the H-GAC 2004/2005 Basin-wide QAPP as follows:

"Precision is a statistical measure of the variability of a measurement when a collection or an analysis is repeated and includes components of random error. It is strictly defined as the degree of mutual agreement among independent measurements as the result of repeated application of the same process under similar conditions.

Field splits are used to assess the variability of sample handling, preservation, and storage, as well as the analytical process, and are prepared by splitting samples in the field. Control limits for field splits are defined in Section B5

Laboratory precision is assessed by comparing replicate analyses of laboratory control standards or sample/duplicate pairs in the case of bacterial analysis. Precision results are plotted on quality control charts which are based on historical data and used during evaluation of analytical performance. Program-defined measurement performance specifications for laboratory control standard/laboratory control standard duplicate pairs are defined in Table A7.1."

Bias As described in Section A7 of the H-GAC 2004/2005 Basin-wide QAPP as follows:

"Bias is a statistical measurement of correctness and includes multiple components of systematic error. A measurement is considered unbiased when the value reported does not differ from the true value. Bias is verified through the analysis of laboratory control standards prepared with certified reference materials and by calculating percent recovery. Results are plotted on quality control charts, which are calculated based on historical data and used during evaluation of analytical performance. Program-defined measurement performance specifications for laboratory control standards are specified in Table A7.1."

Representativeness (This section is different than the Regional QAPP.)

In Part 1, the analysis regime is designed to mimic or represent the instream condition where bacterial die-off, replication, and/or re-growth may occur. The sampling of pertinent media according to TCEQ SOPs and use of only approved analytical methods will assure the measurement data represents the conditions at the site. Temporal representation will be addressed by duplicating the analyses throughout the year. In Part 2, the analysis regime will be conducted so that the bacterial concentrations will be representative of conditions at each site immediately following a rain event with run-off. Part 2 of this study will not be

conducted if a rainfall event does not occur as described within the study period. Both Part 1 and Part 2 sample regimes will involve **at least** one sampling event during the index period (March 15 through October 15). Then Part 1 will have at least one sampling event during the critical period (July 1 through September 30), and one event during the winter months to provide information on possible seasonal variations. The goal for meeting total representation of the water body will be tempered by the potential funding for complete representativeness.

Comparability As described in Section A7 of the H-GAC 2004/2005 basin-wide QAPP as follows:

Confidence in the comparability of the data sets for this project and for water quality assessments is based on the commitment of project staff to use only approved sampling and analysis methods and QA/QC protocols in accordance with quality system requirements and as described in this QAPP and in TCEQ SOPs. Comparability is also guaranteed by reporting data in standard units, by using accepted rules for rounding figures, and by reporting data in a standard format as specified in Section B10.

Completeness (This section is different than the Regional QAPP.)

The completeness of the data is basically a relationship of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data should be available. However, the possibility of unavailable data due to unfavorable weather conditions, accidents, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project(s) that 80% data completion is achieved. This percentage is based upon missing the rainfall event but having 100% delivery of the non-rain sampling events.

SS-A8 SPECIAL TRAINING NEEDS/CERTIFICATION (This section is different than the Regional QAPP.)

No special training is required for this project. However, the City of Houston will submit a letter as required by the Basin-wide QAPP before sampling commences confirming that all employees collecting samples for this project have been properly trained according to the requirements of the *SWQM Procedures Manual* and this QAPP.

SS-A9 DOCUMENTS AND RECORDS

As described in Section A9 of the H-GAC 2004/2005 Basin-wide QAPP for the City of Houston Health and Human Services Laboratory.

SS-B1 SAMPLING PROCESS DESIGN

The data collection design is summarized in Table SS-B1 (Sampling Sites and Monitoring Frequency). A monitoring schedule planner is presented in Appendix A of this document along with instructions for finding information about "Stream Flow" for determining a significant rainfall event. Figure SS-B1 (Sample Site Map) follows the monitoring schedule planner.

Table SS B1 Monitoring site description and maximum sampling frequency for the Bacteria Die-off study.

Region	Station ID	Station Description	Start Date	End Date	SC1/SC2	Program Code	Field	Conv.	Bacteria	Parameters
12	11356	Buffalo Bayou at Voss Road	7/1/2004	8/31/2005	HG/HH	RT	5	5	5	Conductivity, Days since last significant rainfall, DO,Flow, Flow severity,pH, present weather, secchi depth, temperature, water color, water odor, wind intensity, ammonia, C-BOD5, Chloride, *E.coli*, flouride, nitrate-N, total phosphorus, ortho phosphate (field filtered), sulfate, TDS, TSS, TOC, Turbidity
12	11356	Buffalo Bayou at Voss Road	7/1/2004	8/31/2005	HG/HH	SS	1	1	1	Conductivity, Days since last significant rainfall, DO,Flow, Flow severity,pH, present weather, secchi depth, temperature, water color, water odor, wind intensity, ammonia, C-BOD5, Chloride, *E.coli*, flouride, nitrate-N, total phosphorus, ortho phosphate (field filtered), sulfate, TDS, TSS, TOC, Turbidity
12	11356	Buffalo Bayou at Voss Road	7/1/2004	8/31/2005	HG/HH	SS	3	3	3	Conductivity, Days since last significant rainfall, DO,Flow, Flow severity,pH, present weather, secchi depth, temperature, water color, water odor, wind intensity, *E.coli*,
12	11358	Buffalo Bayou at Piney Point Rd 4.3 mi west Loop 610 in west Houston	7/1/2004	8/31/2005	HG/HH	RT	5	5	5	Conductivity, Days since last significant rainfall, DO,Flow, Flow severity,pH, present weather, secchi depth, temperature, water color, water odor, wind intensity, ammonia, C-BOD5, Chloride, *E.coli*, flouride, nitrate-N, total phosphorus, ortho phosphate (field filtered), sulfate, TDS, TSS, TOC, Turbidity
12	11358	Buffalo Bayou at Piney Point Rd 4.3 mi west Loop 610 in west Houston	7/1/2004	8/31/2005	HG/HH	SS	1	1	1	Conductivity, Days since last significant rainfall, DO,Flow, Flow severity,pH, present weather, secchi depth, temperature, water color, water odor, wind intensity, ammonia, C-BOD5, Chloride, *E.coli*, flouride, nitrate-N, total phosphorus, ortho phosphate (field filtered), sulfate, TDS, TSS, TOC, Turbidity
12	11358	Buffalo Bayou at Piney Point Rd 4.3 mi west Loop 610 in west Houston	7/1/2004	8/31/2005	HG/HH	SS	3	3	3	Conductivity, Days since last significant rainfall, DO,Flow, Flow severity,pH, present weather, secchi depth, temperature, water color, water odor, wind intensity, *E.coli*,


Segment 1014: Buffalo Bayou in West Houston
11356: Buffalo Bayou at Voss
11356
11191
Piney Point Drive
Memorial Drive
Voss
Briar Forest Drive
11357
Buffalo
Bayou
15846
11358
11358: Buffalo Bayou at Piney Point
10584-001 (Memorial Villages Mun. WWTP)
0
1,000
2,000
Feet
NORTH
Houston-Galveston Area Council 2004 www.h-gac.com/crp

Sample Design Rationale and Site Selection Criteria

The sample design rationale is based on the need to determine whether *E. coli* bacteria proliferate (replicate) in the warm, nutrient rich waters found around the Houston area. This two (2) part project will involve collecting water samples from two representative locations only. Historical data indicates bacteria numbers are frequently very high at the first site and frequently low or within acceptable ranges at the second site. Water samples will be collected for analysis during the index period, the critical period, and the winter season to look for seasonal variations. Five (5) different regimes will be created in the laboratory to hold samples for retesting on three (3) consecutive days. The various regimes will provide controls as well as better simulate mixing conditions within the stream. Subsequent testing will provide the data necessary to determine whether bacteria survive and proliferate in waters of the region. The overall goal for Part 1 is to obtain results from six sampling events. Results of the five regimes will be compared against each other and between the events to look survival. At least one of the six events should follow a rain event with accompanying run-off to complete Part 2.

Part 2 requires bacteria testing on the four (4) consecutive days following a rain event. A full suite of parameters will be collected on the Monday. Then, field parameters and bacteria samples only will be collected and analyzed on Tuesday, Wednesday, and Thursday of the same week. The rationale of this effort is to look at bacteria results at one location over time. Appendix A of this document includes a schedule planner and instructions for retrieving "Stream Flow" information to determine whether a rainfall event is significant.

In addition to the historical data available for the selected sites, both locations have USGS flow gages associated with them. Flow data will be used to interpret the results. Both parts of the study will provide valuable information for use in determining the die-off rate of indicator bacteria in the Houston area.

SS-B2 SAMPLING METHODS

Field Sampling Procedures.

As described in Section B2 of the basin-wide QAPP, all will be collected as required in the current SWQM Procedures, Volume 1 except for collecting and transporting the water sample used for the bacteria testing. There will be 10 L of water collected to be divided between the various bacteria holding scenerios.

Sample volume, container types, minimum sample volume, preservation requirements, and holding time requirements.

Table SS-B2 describes the sample containers, preservation methods, storage and handling requirements to complete this special study. The information presented is the same as found in Section B2 of the Basin-wide QAPP **except** for the volume of water initially collected for the bacteria analysis and subsequent testing. As explained in the project/task description, on the first day a large volume of water will be collected for the bacteria tests. Specifically, several buckets of bayou water will be co-mingled in a 10-liter, autoclaveable carboy with spigot and transported on ice in a cooler to the lab. Then, after water is removed for the first day's tests, the remainder of the water will be divided and held under the five (5) different regimes over the next four (4) days. The bacteria test will be repeated each of those days by withdrawing 100 mL from each holding container to analyze as a normal water sample.

Table SS-B2. Sample Storage, Preservation, and Handling Requirements

Parameter	Matrix	Container	Preservation	Sample Volume	Holding Time
Ammonia-N	water	Plastic	Cool to 4°C H_2S0_4 to pH <2	100 mL*	28 days
C-BOD5	water	Plastic	Cool to 4°C	1000 mL	48 hours
Chloride	water	Plastic	Cool to 4°C	100 mL	28 days
E. coli	water	Sterile Plastic	Cool to 4°C	10 Liters**	Variable***
*E. coli*****	water	Sterile Plastic	Some held at room temperature	10 Liters**	Variable***
Fluoride, total	water	Plastic	Cool to 4°C	100 mL	28 days
Nitrate – N, total	water	Plastic	Cool to 4°C H_2S0_4 to pH <2	100 mL*	48 hours
Ortho phosphate Phosphorus	water	Plastic	Cool to 4°C	100 mL	48 hours
Phosphorus, total	water	Plastic	Cool to 4°C H_2S0_4 to pH <2	100 mL	28 days
Sulfate	water	Plastic	Cool to 4°C	100 mL	28 days
TDS	water	Plastic	Cool to 4°C	100 mL	7 days
TSS	water	Plastic	Cool to 4°C	100 mL	7 days

*The required amount of liquid is removed from a large container of water (~1000mL) intended for several different parameters.
**10-Liter, autoclaveable carboy with spigot.
*** Each 10 Liters of water will be subdivided into 2 L beakers to set up the 5 different "holding" scenerios described in the SS-A6 Project/ Task Description.
****This *E.coli* entry corresponds to the samples held over for testing after the initial collection and analysis. These results will not be sent to TCEQ for inclusion in the TRACS database.

Sample Containers

With the exception of the container used to collect the bacteria test, it is as described in Section B2 of the basin-wide QAPP. An extra large volume of water (10 Liters) will be collected in a 10 L, autoclaveable carboy with spigot to have enough water for the additional testing later in the week.

Processes to Prevent Contamination

As described in Section B2 of the basin-wide QAPP.

Documentation of Field Sampling

Forms used to document field activities will remain the same as described in Section B2 of the basin-wide QAPP. Sections of the form that are not used or completed on any particular day will be closed out with single lines, initials, and the date of closeout.

Recording Data

As described in Section B2 of the basin-wide QAPP.

Deficiencies, Non-conformances and Corrective Action Related to Sampling Requirements

As described in Section B2 of the basin-wide QAPP.

SS-B3 SAMPLING HANDLING AND CUSTODY

Chain-of-Custody

As described in Section B3 of the basin-wide QAPP.

Sample Labeling

As described in Section B3 of the basin-wide QAPP.

Sample Handling

Sampling handling will be performed as described in Section B3 of the basin-wide QAPP except for the bacteria analyses performed on Tuesday thru Thursday. As part of the study, the water being tested between Tuesday and Thursday will be collected on Monday, divided into 5 different holding methods in the lab and tested on four subsequent days of the week. One hundred milliliters of sample will be pipetted from each of the five different containers of water and analyzed for *E. coli* bacteria. The holding regimes details are described in the project/task description (SS-A6) beginning on page 9.

As indicated in table SS-A7.1, total phosphorus samples are analyzed at the Braeswood lab. The sample containers are delivered in the following manner: a cubi-tainer is filled and preserved in the field by the sampler, then the container is transported in ice and delivered to the north lab where it is logged into the laboratory. Next, several aliquots of samples are removed from the cubi-tainer to test for other parameters listed in table SS-B2, afterwards the semi-filled container is refrigerated. Once per week, all total phosphorus samples (partially filled cubi-tainers) are transported to the Braeswood lab – preserved samples have a 28 days holding time. Samples are transported in a cooler with ice and released into the custody of the Braeswood lab.

Deficiencies, Non-conformances and Corrective Action Related to Chain-of-Custody

As described in Section B3 of the basin-wide QAPP.

SS-B4 ANALYTICAL METHODS

The analytical methods, associated matrices, and performing laboratories are listed in Table SS-2 of Section SS-A7. The authority for analysis methodologies under the Clean Rivers Program is derived from the TSWQS (§§307.1 - 307.10) in that data generally are generated for comparison to those standards and/or criteria. The Standards state, "Procedures for laboratory analysis will be in accordance with the most recently published edition of *Standard Methods for the Examination of Water and Wastewater*, the latest version of the *TCEQ Surface Water Quality Monitoring Procedures Manual*, 40 CFR 136, or other reliable procedures acceptable to the executive director." Copies of laboratory SOPs are retained by H-GAC and are available for review by the TCEQ. Laboratory SOPs are consistent with EPA requirements as specified in the method.

Standards Traceability

As described in Section B4 of the basin-wide QAPP.

Analytical Method Modification

Not applicable to this QAPP.

Deficiencies, Nonconformances and Corrective Action Related to Analytical Methods

As described in Section B4 of the basin-wide QAPP.

SS-B5 QUALITY CONTROL

Sampling Quality Control Requirements and Acceptability

As described in Section B5 of the basin-wide QAPP.

Laboratory Measurement Quality Control Requirements and Acceptability Criteria

As described in Section B5 of the basin-wide QAPP revision 2.

Failures in Field and Laboratory Quality Control and Corrective Action

As described in Section B5 of the basin-wide QAPP revision 2.

SS-B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

As described in Section B6 of the basin-wide QAPP.

SS-B7 INSTRUMENT CALIBRATION AND FREQUENCY

As described in Section B7 of the basin-wide QAPP.revision 2.

SS-B8 INSPECTION/ACCEPTANCE REQUIREMENT FOR SUPPLIES AND CONSUMABLES

As described in Section B8 of the basin-wide QAPP.

SS-B9 NON_DIRECT MEASUREMENTS

As described in Section B9 of the basin-wide QAPP flow data that will come from USGS maintained gauging station located on Buffalo Bayou at Piney Point. As described in Section SS-A6 Project/Task Description, rainfall data will be gathered by H-GAC from either the Harris County Flood Control

Division, the Harris County Office of Emergency Management, a wastewater treatment plants within close proximity of the sites and/or any other reliable sources identified before the final report is written. Rainfall data will be used in conjunction with flow data to determine when the rainfall sampling is appropriate.

SS-B10 DATA MANAGEMENT

The data collected on the first day of each study "week" is similar to ambient data routinely collected and will be submitted as described in Section B10 of the basin-wide QAPP. A copy of the first day's data as well as all other data generated during each study "week" will be managed and submitted separately by the North Laboratory Supervisor. Data will be submitted in both hard copy and electronic format for H-GAC within 30 days of each sampling event. H-GAC is responsible for summarizing and writing the reports. Electronic data with a program code of "RT" will be submitted to the TCEQ along regularly submitted data.

SS-C1 ASSESSMENTS AND RESPONSE ACTIONS

As described in Section C1 of the basin-wide QAPP.

Corrective Action

As described in Section C1 of the basin-wide QAPP.

SS-C2 REPORTS TO MANAGEMENT

Reports to Planning Agency Project Management

As described in Section C2 of the basin-wide QAPP.

Reports by TCEQ Project Management

Quarterly reports will be submitted as described in Section C2 of the basin-wide QAPP. The draft final report will be submitted to TCEQ by June 15, 2005, with the final report being submitted on or before August 31, 2005.

SS-D1 DATA REVIEW, VERIFICATION, AND VALIDATION

Only ambient data submitted to TCEQ for inclusion in TRACs will be reviewed, verified, and validated as described in Section D1 of the basin-wide QAPP. All other data will be reviewed, verified, and validated for accuracy but not sent to TCEQ for inclusion in TRACs because it will not be considered "ambient" data. This includes the Monday data where sample collection targets a storm event. Rather, this data will be looked at to determine whether there is confirmation of the hypothesis that *E.coli* bacteria can survive and possibly proliferate outside of the human body in the warm, nutrient rich waters of the Houston area.

SS-D2 VERIFICATION AND VALIDATION METHODS

As described in Section D2 of the basin-wide QAPP.

SS-D3 RECONCILIATION WITH USER REQUIREMENTS

The data collected on the first day of each "study week" will be analyzed and reconciled as described by Section D3 of the basin-wide QAPP. This is justified because the data is ambient water quality data collected and analyzed according to approved Standard Methods, TCEQ SWQM procedures, and described in the basin-wide QAPP. Data results from bacteria tests performed on subsequent days or associated with stormwater events will not be reconciled for any uses described in the basin-wide QAPP. Nor will the data be analyzed statistically. Rather, bacteria die-off rates will be evaluated against themselves and between holding regimes only. This special study is a research project comparing the die-off rate produced by different holding regimes. Results will be used to verify a die-off rate value used in future bacteria modeling. The primary goal of this study is to answer the vital question regarding how and if *E. coli* bacteria survive and reproduce in the warm, nutrient rich waters found in Houston area bayous. H-GAC expects to see differences between holding regimes and replication of results between subsequent sampling events.

Appendix A

Guidelines for collecting samples for Bacteria Die-Off Study

- All "events" will be collected on a Monday. Extra bacteria samples will be collected on Tuesday, Wednesday, &Thursday following a rainfall event only.
- One (1) event during the Index Period from March 15 thru October 15
- One (1) event during the Critical Period from July 1 thru September 30
- One (1) event during the Winter season: December thru February
- One (1) event during or after a rainfall event followed by 3 days of bacteria testing (+field parameters) – any month
- One (1) free choice – any month
- A second rainfall event w/ extra bacteria testing OR free choice – any month

Table to help plan sampling events.

	May 2004	June	July	August	September	October	November	December	January 2005	February	March*
Index Period						thru 15th					
Critical Period			Beginning the 1st		thru 30th						
Winter Season											
Free Choice											
Rain Event #1											
Rain Event #2 Or Free Choice											

A "rainfall event" will be determined by looking at the USGS Instantaneous StreamFlow web site at www.usgs.gov (See attached instructions) **AND** using the following rough guidelines:

1. The flow at Buffalo Bayou & Piney Pt. peaked at about 1,000 cfs or greater in the 24 hours before the Monday samples are collected,
2. The water on Monday had the brown color characteristic of fresh runoff,
3. The flow was not constant at about 2,000 cfs suggesting that reservoir releases are controlling or contributing to the flow, and
4. There are no major rains forecasted for the next few days.

* Try NOT to sample in this month if possible because all data is due to H-GAC by March 31, 2005

Looking up Instantaneous Flow
for Buffalo Bayou at Piney Pt, Houston, TX

Type: www.usgs.gov enter

Choose: **Our Science** choose: **Water**

In box titled: "NWISWeb Water Data" OR "Water Data – NWISWeb"
Choose: **Real-time** double click

In upper right area of screen "Data Category" = Real-time and "Geographical Area" = Texas go

Double Click on: **Statewide Streamflow Table**

Scroll down under the heading: **San Jacinto River Basin** to **Site 08073700** --double click on site number

A graph will appear displaying the most recent seven (7) days of discharge data.

In "Days" category type in 31 enter

Review 31 day graph to look for peak discharge of **approximately** 1000cfs or greater in the last twenty-four (24) hours – give or take. If the peak follows several days of dry weather, then plan to sample bacteria again on Tuesday, Wednesday, and Thursday of the same week.

Note: If the graph has the acceptable peak and preceding dry weather AND bacteria samples were collected on the following days, then call Jean Wright at 713-499-6660 or Todd Running at 713-993-4549 to notify H-GAC that extra bacteria samples were collected that week. H-GAC will print out a graph for use in the final report. Thank you for the help

Appendix B

Water Quality Data for Piney Point and Voss Roads Sample Sites

Table B-1. Water Quality Sample Results for Buffalo Bayou at Piney Point Rd, Houston, Texas.

Sample Date	E coli - MPN	pH	DO	Temp °C	Conductivity	TSS	TDS	CBOD5	N-NH3	N-NO3	T Phos	P-Ortho	Cl^-	Fl^-	SO4
10/4/2004	6,200		6.15	26.83	722	29	474	< 4	0.18	7.36	1.57	1.5	94	0.38	31
1/10/2005	210	7.62	8.41	18.32	823	13	473	no data	0.17	8.08	1.52	1.42	104	0.38	38
3/14/2005	500	7.56	6.94	19.31	487	55	401	< 4	0.11	4.03	0.86	0.585	52	0.28	20
4/18/2005	610	7.61	6.94	21.21	790	34	496	< 4	0.22	6.66	1.46	1.23	116	0.4	34
5/9/2005	15,000	8.22	7.14	20.06	195	458	162	< 4	0.23	0.785	0.46	0.21	21	0.24	12

Table B-2. Water Quality Sample Results for Buffalo Bayou at Voss Rd, Houston, Texas.

Sample Date	E coli - MPN	pH	DO	Temp °C	Conductivity	TSS	TDS	CBOD5	N-NH3	N-NO3	T Phos	P-Ortho	Cl^-	Fl^-	SO4
10/4/2004	27,000		4.94	26.49	514	41	334	< 4	0.08	4.5	0.96	0.89	60	0.33	25
1/10/2005	570	7.64	8.77	17.56	824	10	460	no data	0.07	7.23	1.45	1.27	105	0.38	38
3/14/2005	400	7.58	6.91	18.99	490	55	404	< 4	0.11	4.09	0.8	0.52	52	0.28	20
4/18/2005	860	7.65	6.64	21.16	791	56	464	< 4	0.08	5.92	1.37	1.08	118	0.4	32
5/9/2005	15,000	8.12	6.93	19.24	203	252	163	< 4	0.22	0.78	0.42	0.19	22	<0.2	10

Appendix C

Bacteria Die-off Data for two locations on Buffalo Bayou

Table C-1. Bacteria Die-off results for samples collected from Buffalo Bayou at Piney Point Rd. and held in laboratory under five different conditions to simulate environmental conditions.

Date	Description	Initial Results	Flask 1	Flask 2	Flask 3	Flask 4	Flask 5
October 4, 2004	Initial Sample	6200	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	3900	7000	3700	3000	4400
	Day 3	N/A	3800	1200	640	260	1500
	Day 4	N/A	2300	530	120	63	480
January 10, 2005	Initial Sample	210	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	140	20	52	92	140
	Day 3	N/A	140	41	100	10	20
	Day 4	N/A	74	<10	<10	<10	41
March 14, 2005	Initial Sample	500	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	420	410	10	20	250
	Day 3	N/A	340	180	10	110	300
	Day 4	N/A	450	200	<10	200	220
April 18, 2005	Initial Sample	610	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	510	330	100	180	220
	Day 3	N/A	570	130	30	20	150
	Day 4	N/A	510	160	20	10	230
May 9, 2005	Initial Sample	15000	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	24000	5900	1400	2000	5900
	Day 3	N/A	9600	2700	410	280	2200
	Day 4	N/A	13000	2800	140	98	2600

Flask 1 - Sealed container, refrigerated, settled, reshaken for pipetting & testing

Flask 2 - Darkened fume hood @ room temperature, settled, reshaken for pipetting & testing

Flask 3 - Darkened fume hood @ room temperature, settled, NOT shaken or stirred before pipetting & testing

Flask 4 - Darkened fume hood @ room temperature, slowly stirred continuously, pipetted & tested

Flask 5 - Darkened fume hood @ room temperature, vigorously agitated/stirred continuously, pipetted & tested

Table C-2. Bacteria Die-off results for samples collected from Buffalo Bayou at Voss Rd. and held in laboratory under five different conditions to simulate environmental conditions.

Date	Description	Initial results	Flask 1	Flask 2	Flask 3	Flask 4	Flask 5
October 4, 2004	Initial Sample	27,000	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	18,000	12,000	1,900	610	7,800
	Day 3	N/A	24,000	5,000	590	600	4,600
	Day 4	N/A	18,000	1,800	320	97	1,800
January 10, 2005	Initial Sample	570	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	460	240	280	340	460
	Day 3	N/A	470	97	55	110	65
	Day 4	N/A	350	70	10	31	52
March 14, 2005	Initial Sample	400	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	410	240	100	52	260
	Day 3	N/A	610	120	82	10	230
	Day 4	N/A	630	140	60	270	110
April 18, 2005	Initial Sample	860	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	1100	600	570	460	910
	Day 3	N/A	1000	540	120	150	630
	Day 4	N/A	880	240	20	66	330
May 9, 2005	Initial Sample	15,000	N/A	N/A	N/A	N/A	N/A
	Day 2	N/A	16,000	4,600	5,200	2,400	6,200
	Day 3	N/A	10,000	3,100	730	440	5,600
	Day 4	N/A	9,300	4,900	120	120	3,700

Flask 1 - Sealed container, refrigerated, settled, reshaken for pipetting & testing

Flask 2 - Darkened fume hood @ room temperature, settled, reshaken for pipetting & testing

Flask 3 - Darkened fume hood @ room temperature, settled, NOT shaken or stirred before pipetting & testing

Flask 4 - Darkened fume hood @ room temperature, slowly stirred continuously, pipetted & tested

Flask 5 - Darkened fume hood @ room temperature, vigorously agitated/stirred continuously, pipetted & tested