3. Water Sampling and Laboratory Assessment

As an element of this assessment, the sampling and analytical protocol typical of that on January 26, 2015 was reviewed in detail. City sample locations and sample collection procedures were evaluated by a certified auditor on February 11, 2015 and by AECOM (unannounced) on February 25, 2015. Sample locations were generally clean. General findings during these reviews are provided below.

The bacterial sample reception and processing procedures for the analysis of EC and TC by QuantiTray (IDEXX) and HPC by APHA 9215B were also evaluated by a certified auditor on February 17, 2015 and by AECOM (unannounced) on February 25, 2015. Reports of the analysis performed by the contract laboratory are provided in **Appendix B**. Findings during these reviews are provided below.

3.1 Sampling Protocol Review

On February 25, 2015, AECOM met with a sample collector at sample location NE-07 at approximately 10:00 am. This was an unannounced review, and the sample collector was met at the 4th of 14 sample stops. The AECOM rode with the sample collector to the end of the route, and followed the samples into the laboratory for analysis.

The sample protocol followed was consistent, but there were several areas of improvement that were noted during the review:

-
- Many sample taps had aerators which could not be removed by the sample collector. This included “tamper proof” aerators which required a special tool for removal (which the sample collector did not have). Microbiological sampling from taps with aerators attached is not recommended.
- Inability to remove aerators affects the amount of water flowing from the faucet, and thus extends flushing time required to clear the service line back to the main. Temperature stability was used as an indication when the sample line had been adequately flushed. The City should consider a volumetric requirement for flushing specific to each sample location, with sample collectors checking flowrates at maximum flush volume to assure the flush time and volume is adequate to clear the sample line.

A detailed list of recommendations has been provided to the City for consideration, and is provided in **Appendix B**.

3.2 Laboratory Protocol Review

AECOM accompanied the sample collector to the contract laboratory. Samples were received at a common reception desk for all samples arriving at the laboratory. Samples from City were labeled, arranged by sample ID number, analyzed for HPC, prepared for analysis by Colilert QuantiTray technique for TC and EC, placed into QuantiTrays, and incubated. After the samples were processed for HPC, the labels were stamped noting HPCs had been completed, and the samples were transferred to the Colilert preparation bench. At this point, any excess water above the 100 mL mark on the sample bottle was decanted using a vacuum tube. Samples were then transferred to the bench where the Colilert media was added, and the QuantiTray was sealed. Sealed QuantiTrays were placed in an incubator.

The overall technique of the four lab personnel involved in the analytical process was methodical and clean. Areas for improvement were observed and are noted below:

- The order of analysis was the order provided by the City on the chain of custody sheet. The samples should be ordered from “cleanest” to “least clean”, based on the source of the sample. Under this protocol, raw water samples and construction-related samples would be numbered to be at the end of the analytical protocol, reducing the possibility that the cleaner samples are contaminated by less clean samples.
- The City’s drinking water samples may be analyzed in a batch with samples from other sources. The City should request that their samples be run as a separate batch, isolated from any other samples of unknown source.
- The vacuum decantation step used to adjust the volume of sample in the sample bottle after HPC analysis and before TC/EC analysis should not be used on the City’s samples. This technique as observed increases the risk of contamination between the HPC step and the TC/EC step in the analytical process. Samples that are de-chlorinated (as are all DS samples) are particularly susceptible to contamination during sample handling. The City and lab should consider reviewing this process step to minimize the risk of sample contamination.
- The chain of custody sheet (or similar formal documentation) should be initialed by the analyst at the end of each analytical step in the process to allow retrospective analysis of results.

A detailed list of recommendations regarding the analysis of the City samples has been provided to the City and the lab, and is provided in **Appendix B**.

3.3 External Reports

In addition to this report, independent reports related to the January 26, 2015 event were provided by:

- Dr. Jared **Bullard** (Associate Medical Director, Cadham Provincial Laboratory (CPL)) regarding genotype analysis of the 5 positive EC samples from the January 26, 2015 event analyzed at CPL on February 5, 2015.
- W. **Lipinsky**, (Assessor, Independent Consultant) regarding a February 11, 2015 assessment of sampling procedures and conditions at the 6 positive sites from January 26, 2015 event.
- M. H. **Brodsky** (Assessor, Brodsky Consultants) regarding laboratory procedural protocols used on the microbial samples collected on January 26, 2015 and analyzed by the ALS laboratory, and a subsequent report on AECOMs protocol review.
- Lisa **Richards**, MD MSc FRCPC (Medical Officer of Health, Winnipeg Regional Health Authority), March 13, 2015, regarding public health disease records and observations following the 3 EC positive sample events from January 1, 2013 through January 26, 2015.

Lipinski and Brodsky were retained by the City to conduct analyses independent of AECOM. Each of these reports is provided in **Appendix C**, and are summarized below.

Bullard’s report provides results of genotype differentiation from 4 of the 5 EC positive samples from January 26, 2015. A total of 7 EC isolates were analyzed for genotypes: 4 from sample location NE-07 (9 total QuantiTray cells positive for EC), and one each from the remaining 3 sample locations. In addition, the ALS laboratory EC positive control was analyzed, as were triplicates of the CPL method control, for a total of 11 traces on the analytical run. Results indicated each of the 7 isolates analyzed from the City

samples was genotypically different, and none of these matched the genotypic pattern of the ALS control. Bullard concluded that:

> *"…the isolation of 7 different E.coli strains is inconsistent with a common source lab error or systematic lab contamination. The combination of one collector, plus one side of the city, plus no apparent contamination in any other trays that day, in addition to multiple genotypes of E.coli (likely representing multiple sources of contamination rather than a single one) suggests, in a balance of probabilities, a collection/specimen handing (pre-analytic) issue rather than an analytic or post-analytic issue."*

In Bullard's conclusion, the term "pre-analytic" refers to any activity prior to when the sample (with Colilert reagent added) is sealed in the QuantiTray. Thus, his statement above implies that the source of the TC/EC detected in the QuantiTray likely originated prior to the sample being sealed in the QuantiTray, which includes the DS, the sampling process, and the laboratory process up to the point of the sample being sealed in the QuantiTray.

Lipinsky's report was requested by the City to provide opportunities for improvement in microbial sampling techniques. He identified 12 action items and 8 recommendations regarding the sampling procedure. The action items refer to documentation, handling and storage of sample containers, clean sampling procedures, and a review process for procedural changes in sampling. The action items indicated opportunities for improvement.

Lipinski held interviews with sampling staff and supervisory staff, including an interview with the technician who sampled all of the positive samples. He noted that the technician is a qualified and trained drinking water sample collector and is aware of the procedures and importance of water testing. The technician was unable to identify anything that could have resulted in potential contamination on January 26, 2015 and indicated that it was another routine sampling day.

He concluded that:

> *"Based on what was observed during this assessment, there is a low probability that positive results can occur as a result of contamination at the time of sampling. This is supported by test results from the 2014 sampling season where there was only one positive E.coli sample. There are a number of potential sources for positive coliform results in drinking water other the sampling phase. To ensure that the probability of contamination during sampling is eliminated, a number of continual improvement items have been recommended".*

Lipinsky's list of action items and recommendations were consistent with recommendations by AECOM.

Brodsky's report indicated that the laboratory providing the analysis of samples on January 26/27, 2015 followed Standard Operating Procedures (SOP) and procedures appropriate for the analysis. He recommended two items that deal with additional documentation in the area of Control of Records that could be improved. He specifically focused on the HPC and IDEXX Colilert QuantiTray methodology, although he did not provide reference or details regarding the IDEXX QuantiTray method. Brodsky reviewed only the methods presented in SOPs. Methods not in the SOP were not reviewed, and he did not comment on the specific handling of samples for volume reduction prior to analysis for TC/EC. He concluded that:

> *"My observations of their analytical and QC records indicated that the laboratory followed sample handling, preparation and analytical protocols and procedures as per their Quality Management*

System. The evidence I examined indicated that the samples in question were not contaminated with coliforms and E. coli by cross contamination in the laboratory."

Brodsky did not provide detailed descriptions of sample handing during the HPC and TC/EC analyses. In a subsequent report, he later clarified that he did not observe the vacuum-siphon volume reduction step between the HPC and TC/EC steps observed by AECOM. Brodsky stated:

"The analysts who processed the samples indicated that they did not use the syphoning. Based on all this evidence I concluded that the syphoning technique was not used in the processing of the samples in question and therefore was a non-issue with respect to the possibility of cross contamination of those samples. In addition, if this was a high risk procedure, there would have been historical evidence of cross contamination in blank sample controls whenever this procedure was used. There was no such evidence. This conclusion was supported by QC data recently provided on the syphoning equipment and technique that showed that it does not contribute to microbial contamination of the samples."

Richard's report of March 13, 2015 comments on the lack of unusual disease activity during the period following the 3 EC positive sample events since January 26, 2013. In her report she states that:

"In the case of a major contamination of the City's water supply, a rapid and dramatic increase of case counts would be expected (e.g., hundreds of cases), and would likely be preceded by a similarly dramatic rise in emergency room visits for GI complaints. The number of reported cases of infections that could typically be caused by waterborne pathogens from January 1, 2013 to February 28, 2015 was reviewed. The observed reports of GI infections during this time period did not indicate that there was a GI outbreak related to any of the three events listed above."

3.4 City Response to Recommendations

Lipinski's audit of the City SOP for bacteriological sampling and monitoring program identified 12 action items and 8 items of recommendation for improvement. The City has reviewed all action items from the audit, generally categorized as management items (process review, quality assurance, and technical issues), and sampling methods items (missing process steps, errors in sampling, and suggestions for improvement).

The 4 management items will all be addressed over a prioritized 3 year period, noting that those items requiring significant resources and upgrades to the Analytical Serviced Branch will require more time to implement. Of the action items for the Sampling Methods, all of the 9 items have been reviewed and a procedure for implementing action items is in place. These changes will be included in the revised SOP for Bacteriological Monitoring and Sampling and related Supporting Work Instructions (SWI), which will be in effect no later than April 30, 2015.

Regarding recommendations for improvement from Lipinski's audit, the City has reviewed these recommendations categorized as management items (quality control (QC) and technical issues), and sampling methods items (documentation, additional process steps). Of the management issues, items related to additional studies and QC samples (field blanks, duplicates) have been addressed through validation studies and the QC program is reviewed annually (next review scheduled in October/November 2015). The handling of samples and sample procedures within the contract lab will be reviewed and modifications, if required, will be in place by June 30, 2015.

Other items for improvement are to be covered in review and update of sampling SOPs scheduled for completion in 2015. Two recommended items regarding sample locations in privately owned sample locations open to public access are beyond the City's ability to control (cleanliness of sample sink and removable aerators), and a modified version of this recommendation is being considered.

A detailed listing of the specific action and recommendation items and the City's response is provided in **Appendix B**.