Journal of Hydrology 534 (2016) 524–533

Contents lists available at ScienceDirect

Journal of Hydrology

journal homepage: www.elsevier.com/locate/jhydrol

Uncertainty in monitoring *E. coli* concentrations in streams and stormwater runoff

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ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form 4 November 2015
Accepted 19 January 2016
Available online 27 January 2016
This manuscript was handled by Laurent Charlet, Editor-in-Chief, with the assistance of Rizlan Bernier-Latmani, Associate Editor

Keywords:
E. coli
Uncertainty
TMDL
Error propagation
Monitoring

SUMMARY

Microbial contamination of surface waters, a substantial public health concern throughout the world, is typically identified by fecal indicator bacteria such as *Escherichia coli*. Thus, monitoring *E. coli* concentrations is critical to evaluate current conditions, determine restoration effectiveness, and inform model development and calibration. An often overlooked component of these monitoring and modeling activities is understanding the inherent random and systematic uncertainty present in measured data. In this research, a review and subsequent analysis was performed to identify, document, and analyze measurement uncertainty of *E. coli* data collected in stream flow and stormwater runoff as individual discrete samples or throughout a single runoff event. Data on the uncertainty contributed by sample collection, sample preservation/storage, and laboratory analysis in measured *E. coli* concentrations were compiled and analyzed, and differences in sampling method and data quality scenarios were compared. The analysis showed that: (1) manual integrated sampling produced the lowest random and systematic uncertainty in individual samples, but automated sampling typically produced the lowest uncertainty when sampling throughout runoff events; (2) sample collection procedures often contributed the highest amount of uncertainty, although laboratory analysis introduced substantial random uncertainty and preservation/storage introduced substantial systematic uncertainty under some scenarios; and (3) the uncertainty in measured *E. coli* concentrations was greater than that of sediment and nutrients, but the difference was not as great as may be assumed. This comprehensive analysis of uncertainty in *E. coli* concentrations measured in streamflow and runoff should provide valuable insight for designing *E. coli* monitoring projects, reducing uncertainty in quality assurance efforts, regulatory and policy decision making, and fate and transport modeling.

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1. Introduction

The presence of pathogens in surface waters is increasingly a concern in the United States and worldwide, with fecal indicator bacteria (FIB) typically being used to indicate the presence of fecal matter in surface waters and the associated risk of pathogen contamination. Case in point, more stream and river miles were impaired due to pathogens (as inferred by high FIB concentrations) than any other pollutant in the United States Environmental Protection Agency's (USEPA) national summary of data collected from states under sections 305(b) and 303(d) of the Clean Water Act (USEPA, 2014). Since 1995, this has led to more Total Maximum Daily Loads (TMDLs) being developed in the United States for indicator bacteria than any other impairment (USEPA, 2014). Such pollution is not unique to the United States, with similar concerns being present from Australia's Yarra River (Daly et al., 2013) to the Seine River Estuary in France (Garcia-Armisen et al., 2005).

Modeling is a primary component of TMDL development, and similar watershed management plan development worldwide, with models being calibrated and validated using field-collected flow and water quality data. The output from these efforts is used for determining source load allocations (i.e., allowable pollutant

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http://dx.doi.org/10.1016/j.jhydrol.2016.01.040
0022-1694/Published by Elsevier B.V.

loads exported to the impacted surface water by various sources in the watershed). There are inherent errors associated with field monitoring, and TMDLs are required to include some margin of safety in these source load allocations due to the uncertainty present in these data (40 CFR 130.7). Further, optimal water quality monitoring can only be achieved if uncertainty in measurements and alternatives to reduce it are considered in sampling design and implementation (Beven, 2006; Harmel et al., 2006a, 2006b; Rode and Suhr, 2007). This is rarely the case with routine monitoring conducted by regulatory entities, despite the recognition of the importance of measurement uncertainty. In addition, little research has been performed to determine the uncertainty associated with monitoring FIB in streams and stormwater runoff. Due to this lack of information, relatively arbitrary margins of safety are currently employed to account for variability. Studies such as Hession et al. (1996) have indicated that uncertainty and risk analysis are a vital part of TMDL development. Thus, defining the uncertainty associated with FIB monitoring is a critical need that will improve the scientific basis of pathogen regulation, policy, modeling, and watershed plan development and implementation.

Fecal indicator bacteria are generally used instead of specific pathogens because of the large number of potential waterborne pathogens, substantial time required and expense of pathogen analyses, analytical expertise required to perform such analyses, difficulty determining which pathogens to target, and longer survivability of indicators (EPA, 2003). Various FIB, including fecal coliform, *Escherichia coli*, and enterococci, are utilized to assess compliance with water quality standards related to fecal contamination with the FIB of choice varying regionally and by water body type. In 1986, the USEPA published a report recommending *E. coli* or enterococci as a preferred FIB for fresh waters (USEPA, 1986). Subsequently, *E. coli* has been more frequently utilized and researched in fresh waters and is the focal point of this study.

Previous efforts to elucidate the uncertainty associated with water quality sampling and analysis have focused on nutrients and sediment (Harmel et al., 2006b, 2009). Harmel et al. (2006b) compiled error sources associated with flow measurement, sample collection, sample preservation/storage, and laboratory analysis for total suspended solids and various nutrient species. The total error accompanying these elements was compiled using the root mean square error propagation methodology (Topping, 1972). Harmel et al. (2006b) estimated the uncertainty of storm concentrations to be ±15% for total suspended sediment, ±14% for NO_3-N, ±20% for PO_4-P, ±27% for total N, and ±29% for total P. Using a similar methodology, McCarthy et al. (2008) conducted the only known comprehensive uncertainty analysis of field-collected *E. coli* data. Their results showed an average uncertainty of ±33% and a range of ±15–67%. However, because uncertainty varies based on the method of data collection, storage, and analysis, further research is needed to understand the uncertainty of additional monitoring regimes not analyzed by McCarthy et al. (2008). The Harmel et al. (2006b) and McCarthy et al. (2008) studies noted that "random" effects or sources of uncertainty are typically bi-directional and appropriately represented by the normal distribution.

The objective of this study was to expand on previous urban stormwater work by McCarthy et al. (2008) by compiling a more comprehensive collection of uncertainty data related to *E. coli* concentrations measured in streamflow and runoff. Specifically, uncertainty contributed by sample collection, sample preservation and storage, and laboratory analysis in measured *E. coli* data were compiled and presented using the theoretical framework established by Harmel et al. (2006b) and McCarthy et al. (2008). Similarly, the differences in sampling method and sample type (individual discrete and runoff event) were compared.

Similar to Harmel et al. (2006b), the analysis applies principally to edge-of-field runoff (<50 ha) and streamflow in small watersheds (<10,000 ha). On larger streams and rivers with perennial flow, additional considerations such as diurnal fluctuations, groundwater contribution, freshwater and saltwater interaction, and point sources such as waste water treatment plant outfalls would need to be considered. Lastly, the terms "error" and uncertainty are used synonymously herein to represent random and systematic statistical variation. Human error and equipment malfunction are not considered.

2. Materials and methods

2.1. Compilation of uncertainty data

An exhaustive literature search was performed to collect and compile data pertaining to measurement uncertainty for determination of *E. coli* concentrations in runoff and streamflow from small watersheds (inclusion of sources of spatial and temporal variability that contribute to uncertainty in data sets from long-term and/or multi-location monitoring projects was outside the scope of the present analysis). Then uncertainty estimates were determined as described in Table 1. These data/results were used to populate Tables 2–4, which present uncertainty estimates for steps/procedures within the major procedural categories (i.e., sample collection, sample preservation/storage, laboratory analysis) established by Harmel et al. (2006b). The distributional parameters presented in Tables 2–4 (usually the average and standard deviation) were used in the subsequent estimation of uncertainty contributed by each of the procedural categories and in the overall measured *E. coli* concentrations.

Harmel et al. (2006b, 2009) assumed that measurement uncertainty in water quality data collection was random, bi-directional (equally likely to be positive or negative), and normally distributed. These assumptions are valid for sources of "random" uncertainty in the present analysis of the uncertainty associated with individual *E. coli* concentrations, whether individual discrete samples or throughout a single storm runoff event. *It is important to note that this assumption does not apply to populations or sets of E. coli data, which are often asymmetric.* In contrast to Harmel et al. (2006b), the present analysis also assessed several sources of "systematic" uncertainty that introduced directional bias and are not appropriately represented by the normal distribution. To accommodate both types of uncertainty, uncertainty sources were separated based on whether they introduce random or systematic uncertainty (Tables 2–4).

2.2. Estimation of uncertainty in each procedural category and in measured E. coli concentrations

With the uncertainty estimates for individual steps or procedural categories, the random uncertainty in each procedural category and in measured *E. coli* concentrations was estimated with the method of Topping (1972) adapted as shown in Eq. (4). These results represent the cumulative random uncertainty such that over-estimation and under-estimation are equally likely; therefore, the resulting uncertainty is presented as ±%.

$$\pm\%\ unc. = \frac{\Delta E.\ coli}{E.\ coli} = \sqrt{\left(\frac{\Delta x_1}{x_1}\right)^2 + \left(\frac{\Delta x_2}{x_2}\right)^2 + \left(\frac{\Delta x_3}{x_3}\right)^2 + \cdots + \left(\frac{\Delta x_n}{x_n}\right)^2} \quad (4)$$

Then, the influence of systematic uncertainty was included as the sum of uncertainty in individual steps or processes that contributed to over- or under-estimation. The systematic uncertainty thus shifted the random uncertainty by the appropriate direction to achieve an overall uncertainty estimate.

Table 1
Summary of methods used to determine uncertainty estimates from available literature and data sets.

Uncertainty estimation method	Comments	Equation
1. Used uncertainty estimate as directly reported	– Rarely were these estimates available	–
2. Used methods of Taylor and Kuyatt (1994) and McCarthy et al. (2008) (Eq. (1)) or Harmel and Smith (2007) to estimate uncertainty	– For random uncertainty – Used if necessary summary statistics (e.g., mean, standard deviation, number of samples collected) were reported	$\pm\%\ unc. = \frac{\Delta x_i}{x_i} \approx \frac{2u(x_i)}{x_i}$ (1)[a]
3.1. Used Eq. (1) to estimate uncertainty	– For random uncertainty – Used for raw data sets, after determination of mean and standard deviation	Eq. (1)
3.2. Used Eq. (2) to estimate uncertainty	– For systematic uncertainty – Used for paired values (a_i, b_i) with a_i assumed to be the "true" value	$\pm\%\ unc. = \frac{(a_i - b_i)}{a_i}$ (2)[b]
3.3. Used Eq. (3) to estimate uncertainty	– For random uncertainty – Used for paired values with no "true" value	$\pm\%\ unc. = \frac{\lvert a_i - b_i \rvert}{avg(a_i, b_i)}$ (3)
3.4. Used best professional judgment to assign an uncertainty estimate based on data for another constituent such as total suspended solids	Used when no data relevant to *E. coli* were available. – Used only as a contingency for knowledge gaps present for critical elements of *E. coli* monitoring; accounting for these uncertainty sources was necessary for a comprehensive uncertainty analysis	–

[a] Where x_i is the sample mean of a given data series, $\Delta x_i/x_i$ is the relative uncertainty of a quantity x_i, and $u(x_i)$ is the standard deviation of the mean.
[b] Where a_i and b_i are paired values.

For this analysis, three "data quality" scenarios were created (good, average, poor) using a method similar to that of Harmel et al. (2006b). To determine differences in the uncertainty for these scenarios, relevant uncertainty data for each data collection step/procedure were selected from Tables 2–4. For example in the good scenario, which represents concerted quality assurance/quality control (QA/QC) effort and good sampling conditions (e.g., well-mixed, small streams), the lowest uncertainty estimates were selected when multiple options were available. For the poor scenario, which represents reduced QA/QC emphasis and larger and/or less uniform stream cross-sections, uncertainty estimates appropriately representing that scenario were selected. When only a single uncertainty estimate was available (e.g., Position within cross section – Across transect in Table 2), best professional judgment was used to assign a reasonable estimate based on the published mean and standard deviation.

Then, the random and systematic uncertainty for each procedural category (sample collection, sample preservation/storage, laboratory analysis) and each sampling method (manual grab, manual integrated, automated) were analyzed for the three data quality scenarios (good, average, poor). Similarly, differences in uncertainty for individual samples and for samples collected throughout runoff events (whole event sampling) were compared for each procedural category and each sampling method.

3. Results and discussion

3.1. Compilation of E. coli uncertainty data

3.1.1. Sample collection

Samples are typically collected from surface waters with either manual or automated sampling techniques. For manual sampling, which is designed to sample at a discrete moment in time, uncertainty is introduced by the timing and location of sample collection. With manual grab sampling, a sample is taken at a single location in the stream, but with integrated manual techniques a sample is collected throughout the stream cross section. With automated sampling, samples are typically taken from a fixed point in the stream. In the only known direct comparison of *E. coli* sampling techniques, Galfi et al. (2014) found automated flow-weighted discrete samples had a mean *E. coli* concentration of 3120 CFU/100 ml, whereas corresponding grab samples taken at the same time had a mean of 3438 CFU/100 ml. Although the geometric means were not statistically different, typical results showed slightly lower concentrations for samples collected by the autosampler.

Several studies have evaluated the effect of sampling location within the cross-section on uncertainty of water quality constituents including nutrient and sediment concentrations (Martin et al., 1992; Ging, 1999; Harmel et al., 2010). Similarly, uncertainty estimates related to *E. coli* sample collection, time of day, and location were published by Traister and Ansifeld (2006), Whitman and Nevers (2004, 2008), and Ibekwe et al. (2011).

In terms of sampling location within the flow cross section, McCarthy et al. (2008) determined no statistically significant differences in *E. coli* concentrations from samples collected in the top and bottom of the water column (Fig. 1). Karthikeyan (unpublished data) conducted a similar analysis comparing stream *E. coli* samples collected from the top 1–2 cm to samples collected near the stream bottom. The resulting p value (0.058) indicated a likely significant effect on the mean, and the uncertainty or the difference between the two samples (Avg. = −509%) was much larger than that shown by McCarthy et al. (2008). Quilliam et al. (2011) sampled across a river transect to determine the most representative sampling location relative to public use areas in a UK river/estuary system. Three of four transects, with 3–5 samples each, exhibited significant differences in *E. coli* concentrations within the transect, and the uncertainty averaged ±62%. The flow conditions in the stream or pipe (laminar or turbulent flow) likely influence the amount of mixing, and thus the consistency of concentrations, therein. Thus, these effects likely vary based on location.

With automated sampling, uncertainty is also introduced by the location of the sampler intake (discussed previously), frequency of sampling (or sampling interval), and the minimum flow threshold (if storm sampling). Each of these sources of uncertainty are summarized in Harmel et al. (2006b) based on previous research such as Miller et al. (2000, 2007), Harmel et al. (2002), Harmel and King (2005), and King and Harmel (2003); however, none of these evaluated automated *E. coli* sample collection. Therefore, in the absence of comparable data for pathogens, we used values for nutrients and sediment as rough estimates for *E. coli*. McCarthy et al. (2008) followed a similar approach where continually measured turbidity data were used to estimate the error introduced into *E. coli* data by sampling interval; they found that sampling interval errors ranged between 7% and 9%. In contrast, the uncertainty introduced by the sampler set up, specifically the effect of residual fecal coliform in autosampler tubing, has been evaluated

Table 2
Uncertainty in *E. coli* sample collection.

Sample collection technique	Random uncertainty	Systematic uncertainty	Reference	Unc. Est. method
Location (immediately below bridge)				
Large population of nesting birds	–	Avg. = +1017%; Std. = 919%	Wolfe (unpublished)	3.2
Large population of nesting birds	–	Avg. = +1264%; Std. = 1286%	Pendergrass et al. (2015)	3.2
Small population of nesting birds	–	Avg. = +43%; Std. = 65%	Wolfe (unpublished)	3.2
Small population of nesting birds	–	Avg. = +139%; Std. = 202%	Pendergrass et al. (2015)	3.2
Small population of nesting birds	–	Avg. = +69%; Std. = 59%	Karthikeyan (unpublished)	3.2
Sample location and timing				
Sample timing				
Repeated sampling (1 min apart)	Avg. = ±23%; Std. = 16%	–	Pendergrass et al. (2015)	3.1
Position within cross section				
Surface sample vs. bottom	–	Avg. = −1%; Std. = 27%	McCarthy et al. (2008)	3.3
Surface sample vs. bottom	–	Avg. = −509%; Std. = 927%	Karthikeyan (unpublished)	3.2
Surface sample vs. cross-sectionally integrated sample (assume similar to TSS)	–	Median = −20%	Martin et al. (1992)	
Across transect	Avg. = ±62%; Std. = 30%	–	Quilliam et al. (2011)	3.1
Automatic sampling				
Sampling interval				
Assume similar to nutrients and sediment	±15%	–	King and Harmel (2003), Harmel and King (2005), Miller et al. (2000)	3.4
Assumed similar to turbidity measurements	Avg. = ±8.5% Std. = 12%	–	McCarthy et al. (2008)	
Minimum flow threshold				
Assume similar to nutrients and sediment	±10%	–	Harmel et al. (2002)	3.4
Intra-event residual in tubing (30 min between samples)				
Straight tubing	–	Avg. = +5.5%; Std. = 0.05%	Hathaway et al. (2014)	3.2
Tubing with loop	–	Avg. = +4.5%; Std. = 0.04%	Hathaway et al. (2014)	3.2
Sloped tubing	–	Avg. = +1.7%; Std. = 0.02%	Hathaway et al. (2014)	3.2
Tubing with dip	–	Avg. = +2.7%; Std. = 0.02%	Hathaway et al. (2014)	3.2
Inter-event residual in tubing (7 days between samples)				
Straight tubing	–	Avg. = +0.2%; Std. = 0.001%	Hathaway et al. (2014)	3.2
Tubing with loop, sloped tubing, tubing with dip	–	Avg. = +0.1%; Std. = 0.001%	Hathaway et al. (2014)	3.2

Table 3
Uncertainty in *E. coli* sample preservation/storage.

Preservation/storage technique	Random uncertainty	Systematic uncertainty	Reference	Unc. Est. method
In field conditions (up to 24 h)	Avg. = ±25%; Std. = 14%;	–	McCarthy et al. (2008)	2
In field conditions for 4 h (3 sites, 2 events with 5 reps each)	–	Avg. = +15%; Std. = 39%	McCarthy et al. (2008)	3.2
In field conditions for 8 h (3 sites, 2 events with 5 reps each)	–	Avg. = +12%; Std. = 54%	McCarthy et al. (2008)	3.2
In field conditions for 24 h (3 sites, 2 events with 5 reps each)	–	Avg. = −15%; Std. = 40%	McCarthy et al. (2008)	3.2
At 5 °C 4 h (1 site, 5 reps)	–	Avg. = +13%	McCarthy et al. (2008)	3.2
At 5 °C 8 h (1 site, 5 reps)	–	Avg. = +13%	McCarthy et al. (2008)	3.2
At 5 °C 24 h (1 site, 5 reps)	–	Avg. = −22%	McCarthy et al. (2008)	3.2
At 5 °C (2 h) vs. <1 h	–	Avg. = +15%; Std. = 52%	Karthikeyan (unpublished)	3.2
At 5 °C (3 h) vs. <1 h	–	Avg. = −15%; Std. = 14%	Karthikeyan (unpublished)	3.2
At 5 °C (6 h) vs. <1 h	–	Avg. = −3%; Std. = 27%	Karthikeyan (unpublished)	3.2
At 5 °C (12 h) vs. <1 h	–	Avg. = −17%; Std. = 10%	Karthikeyan (unpublished)	3.2
At 5 °C (24 h) vs. <1 h	–	Avg. = −5%; Std. = 16%	Karthikeyan (unpublished)	3.2
At 5 °C (48 h) vs. <1 h	–	Avg. = −8%; Std. = 18%	Karthikeyan (unpublished)	3.2
At 15 °C (6 h) vs. 5 °C	–	Avg. = +6%; Std. = 8%	Karthikeyan (unpublished)	3.2
At 25 °C (6 h) vs. 5 °C	–	Avg. = +8%; Std. = 18%	Karthikeyan (unpublished)	3.2
At 1–4 °C (24 h) vs. 8 h	–	Avg. = −4%; Std. = 5%	TCEQ (2008)	3.2
At 1–4 °C (30 h) vs. 8 h	–	Avg. = +1%; Std. = 5%	TCEQ (2008)	3.2
At 1–4 °C (48 h) vs. 8 h	–	Avg. = −2%; Std. = 6%	TCEQ (2008)	3.2
At < 10 °C (6 h) vs. 0 h	–	Avg. = +1%; Std. = 16%	USEPA (2006b)	3.2
At < 10 °C (24 h) vs. 0 h	–	Avg. = −20%; Std. = 42%	USEPA (2006b)	3.2

by Solo-Gabriele et al. (2000), Line et al. (2008), and Boyer and Kucznska (2003) to determine the potential for cross contamination of samples. Because of potential contamination, Hathaway et al. (2010) removed, washed, rinsed with deionized water, autoclaved, and reinstalled all sample tubing between storm events. This prompted additional study to quantify the contribution of residual *E. coli* concentrations in autosampler tubing (Hathaway et al., 2014). This study found little contamination in tubing after seven days of dry conditions between events (<+1%), but higher contamination within events with a 30 min sampling interval (+2–6%). Similarly, studies such as Galfi et al. (2014) show the potential for contamination when subsequent samples have highly variable concentrations of *E. coli*. Galfi et al. (2014) also showed the influence of sample tubing length on contamination, with longer

Table 4
Uncertainty in laboratory *E. coli* sample analysis.

Laboratory analysis methodology	Random uncertainty	Systematic uncertainty	Reference	Unc. Est. method
EPA 1103.1 (mTEC)				
PBS[a] low level spike (1620–4670 CFU/100 ml)	Avg. = ±31.3%; Std. = 15.7%	–	USEPA (2008)	2
PBS high level spike (1,620,000–4,670,000 CFU/100 ml)	Avg. = ±41.2%; Std. = 42.5%	–	USEPA (2008)	2
CSO[b] unspiked (6–430,000 CFU/100 ml)	Avg. = ±23.5%; Std. = 18.7%	–	USEPA (2008)	2
CSO spiked (51–8857 CFU/100 ml)	Avg. = ±29.4%; Std. = 11.0%	–	USEPA (2008)	2
EPA 1603 (modified mTEC)				
Multi-lab precision – disinfected wastewater spiked in lab	Avg. = ±9.2	–	USEPA (2006a)	1
Multi-lab precision – PBS spiked in lab	Avg. = ±6.9	–	USEPA (2006a)	1
PBS low level spike (1620–4670 CFU/100 ml)	Avg. = ±30.5%; Std. = 22.5%	–	USEPA (2008)	2
PBS high level spike (1,620,000–4,670,000 CFU/100 ml)	Avg. = ±30.3%; Std. = 37.4%	–	USEPA (2008)	2
CSO unspiked (5–680,000 CFU/100 ml)	Avg. = ±28.0%; Std. = 16.5%	–	USEPA (2008)	2
CSO spiked (35–8189 CFU/100 ml)	Avg. = ±33.0%; Std. = 27.5%	–	USEPA (2008)	2
Includes uncertainty contributed by field splitting	Avg. = ±21%; Std. = 22%	–	Gregory et al. (2012, 2013)	3.3
Test versus Bioball (24 CFU/100 ml)	Avg. = ±8%; Std. = 15%	–	Gregory et al. (2012, 2013)	3.3
Includes uncertainty contributed by lab splitting	Avg. = ±19%; Std. = 26%	–	Wagner (unpublished)	3.3
Comparative Study to Method 1604	Avg. = ±14%	–	Brenner et al. (1993)	2
EPA 1604 (MI Agar)				
Comparative Study to Method 1603	Avg. = ±10.5%	–	Brenner et al. (1993)	2
Single lab precision	Avg. = ±8.2%; Std. = 7.3%	–	USEPA (2002)	2
Multi-lab precision	Avg. = ±5.1%; Std. = 3.8%	–	USEPA (2002)	2
IDEXX Colilert				
Conducted by manufacturer	Avg. = ±27%	–	IDEXX (2004)	1
Colilert 24 (4 groups of 5 reps, 3 sites, twice per site)	Avg. = ±22%; Std. = 15%	–	McCarthy et al. (2008)	2
Colilert-18 or -24	Avg. = ±36%; Std. = 14%	–	TCEQ (2008)	2
Colilert-18 (conducted by method developer)	Avg. = ±9%	–	Noble et al. (2010)	2
Colilert-18 (conducted by independent laboratories)	Avg. = ±12%	–	Noble et al. (2010)	2
qPCR				
qPCR with bead beating (conducted by method developer)	Avg. = ±32%	–	Noble et al. (2010)	2
qPCR with bead beating (conducted by authors)	Avg. = ±12%	–	Noble et al. (2010)	2

[a] PBS – Phosphate Buffered Saline.
[b] CSO – Combined Sewer Overflow.

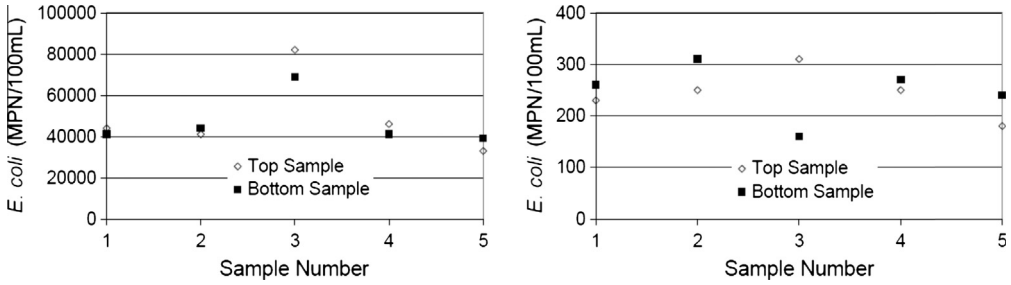

Fig. 1. *E. coli* concentrations taken simultaneously from the top and bottom of the water column (from McCarthy et al., 2008).

tubing showing higher levels of contamination. These studies confirm the importance of field QA/QC procedures when monitoring indicator bacteria such as ensuring positive drainage from the tubing between samples (i.e., ensure the tubing is sloped to allow complete draining between sample events).

Another potentially large source of sample collection uncertainty is sampling location relative to bridges with nesting birds. Sampling immediately below bridges with high bird populations can contribute >1000% systematic uncertainty according to Pendergrass et al. (2015) and Wolfe (unpublished); however, this uncertainty source was not included in the present analysis because it would not occur in most sampling locations.

3.1.2. Sample preservation/storage

The uncertainty contributed by sample preservation/storage typically receives considerable attention in quality assurance efforts to reduce uncertainty in water quality data whether sampling for nutrients (e.g., Kotlash and Chessman, 1998) or FIB (McCarthy et al., 2008; Hathaway et al., 2010). Sample preservation and storage protocols may be even more critical for microbial samples due to their transient nature and susceptibility to environmental conditions (Crane and Moore, 1986).

Typical preservation procedures involve placing the sample on ice after collection and transporting it to a refrigerator such that storage time between collection and analysis is minimized to the extent possible. For non-potable water compliance analyses, the standard storage time between collection and processing is $\leqslant$8 h with the sample held below 10 °C during this period (APHA, AWWA, and WEF, 1998). For non-compliance sampling, the standard hold time given by APHA, AWWA, and WEF (1998) is 24 h with the sample held below 10 °C during storage. However, utilizing hold times longer than 8 h for fecal indicator bacteria is supported by studies such as Pope et al. (2003) and Selvakumar and Borst (2006). Thus, numerous research studies have utilized 24 h as a hold time threshold (e.g., Solo-Gabriele et al., 2000; Characklis et al., 2005; Hathaway et al., 2010).

As logistical, economic, technical, and personnel barriers frequently prevent short hold times, studies have been performed to determine the changes in concentrations associated with various hold time durations and conditions under which samples are

held (Pope et al., 2003; Selvakumar and Borst, 2006). However, many of these studies do not calculate or provide sufficient data to determine the uncertainty related to sample preservation and storage (e.g., Canteras et al., 1995; Medema et al., 1997). Karthikeyan (unpublished data) showed that *E. coli* concentrations increased an average of 15% in the first 2 h following collection, but decreased from 3% to 17% for 3–48 h. Karthikeyan (unpublished data) also showed slightly higher average uncertainty when samples were held at 25 °C compared to 15 °C (analyzed after 6 h). A Texas Commission on Environmental Quality (TCEQ) study (2008) showed small decreases in *E. coli* concentrations after 24 h and 48 h relative to those after 8 h, but concentrations increased slightly after 30 h. In a comparative study of *E. coli* concentrations determined after hold times of 6 h and 24 h, changes after 6 h ranged from −21% to +30% (Avg. = 1%); however, decreases occurred in most of the samples by 24 h (Avg. = −20%, although one sample increased by 61%) (USEPA, 2006b).

McCarthy et al. (2008) built upon this literature by testing the uncertainty associated with samples stored outside in "environmental conditions" for up to 24 h. McCarthy et al. (2008) determined that the number of hours a sample is stored in the field was not statistically significant with an average random uncertainty of ±25%. However, when comparing holding times for samples stored in environmental conditions, McCarthy et al. (2008) reported initial increases in *E. coli* concentrations (4 and 8 h) but decreases after 24 h. Although preservation and storage does introduce systematic uncertainty in measured *E. coli* concentrations, the direction of that uncertainty is quite variable based on these studies.

3.1.3. Laboratory analysis

Similar to sample preservation/storage, laboratory analysis is a focal point in efforts to reduce measurement uncertainty in nutrient (Jarvie et al., 2002) and FIB analyses. *E. coli* can be analyzed by various methodologies, including both culture and genetic techniques. Membrane filtration techniques are common for *E. coli* analysis, utilizing such growth media as membrane thermotolerant *E. coli* (mTEC), modified mTEC, or MI agar. Also increasingly common is the usage of the Defined Substrate Technology® present in IDEXX's Colilert product due to its ease of use. Uncertainty in literature was found to vary based on three elements: analysis methodology, concentration of *E. coli* in the sample, and between-lab errors. Variability in analysis methodologies ranged from 5% to 41%, with the least variability noted for MI agar being processed by multiple laboratories (USEPA, 2002). The greatest uncertainty involved utilizing the mTEC method to measure high concentrations of *E. coli* added to Phosphate Buffered Saline (1,620,000–4,670,000 CFU/100 ml) (USEPA, 2008). IDEXX Colilert and the modified mTEC analyses showed similar uncertainty, varying from ±9% to 36% and ±8% to 33%, respectively (USEPA, 2006a; IDEXX, 2004; McCarthy et al., 2008). MI Agar showed the least average uncertainty (between ±5% and 11%), while mTEC was typically the most uncertain (±24% to 41%). Finally, qPCR uncertainty was similar to that of IDEXX Colilert and the modified mTEC method based on a limited number of data points (±12–32% average uncertainty). Data from USEPA (2008) for both the mTEC and modified mTEC methods also suggested that uncertainty is relatively consistent despite differences in *E. coli* concentration.

Genetic based methodologies (qPCR) were compared to membrane filtration for enumeration of *E. coli* by Noble et al. (2010). In this study, both the developer of the qPCR techniques and microbiologists from a municipal public health laboratory (City of Los Angeles) determined *E. coli* concentrations via both analysis methodologies. Uncertainty was not consistently different among the two methodologies, and the qPCR techniques did not show substantially different variability from the culture techniques.

Wagner (unpublished data) and Gregory et al. (2012, 2013) split samples to assess the uncertainty contributed by the EPA Method 1603 analytical procedure. The uncertainty in Wagner (unpublished data) samples averaged ±19%, and the uncertainty in Gregory et al. (2012, 2013) samples averaged ±21%. It should be kept in mind that these results are affected to some degree by the uncertainty in obtaining two identical samples in the physical process of field or lab splitting (subsampling). Thus, Gregory et al. (2012, 2013) used a calibrated BioBall® to produce a known *E. coli* concentration and thus directly evaluated the analytical uncertainty associated with EPA 1603. The resulting analytical uncertainty averaged ±8%. As shown in the discussion of analytical uncertainty and in Table 4, analysis of *E. coli* samples introduces random uncertainty (equally likely to be positive or negative).

3.1.4. Comparison of uncertainty contributed by each procedural category

As shown in Tables 4 and 5, sample collection contributed a majority of both random and systematic uncertainty in many of the data quality scenarios. The relatively large uncertainty in sample collection results mainly from vertical gradients and to some degree horizontal gradients in *E. coli* concentrations within stream cross-sections and to concentration differences over time (Table 2). It is also important to recognize that aqueous bacteria samples are heterogeneous suspensions. Unlike chemical constituents dissolved in solution, micro-organisms are not uniformly distributed within a sample. Instead they tend to clump forming aggregates with suspended clay particles and/or soluble and particulate organic matter (Lind and Davalos, 1990). In turbid waters, clay-organic matter aggregates are heavily colonized by bacteria whose numbers and biomass increase with turbidity (Lind et al., 1992). Suspended clay particles often carry the largest concentrations of pathogenic bacteria (Kunkel et al., 2013). Laboratory evidence suggests that bacteria are clustered to some degree, even within small volume samples (Gale, 1996). The heterogeneous nature of bacterial suspensions explains some of the extreme variation and uncertainty observed in *E. coli* sampling and analysis.

Preservation/storage also introduced notable systematic uncertainty in measured *E. coli* concentrations (Tables 5 and 6); however, the direction of the systematic shift varied (Table 3). Of the data reported in USEPA (2006b), TCEQ (2008), McCarthy et al. (2008), and Karthikeyan (unpublished), ~40% showed increases in *E. coli* concentrations post collection, and ~60% showed decreases. In spite of variability in the direction of systematic uncertainty, there seemed to be a shift in holding time influence that occurred in the 8–12 h time frame. For 2–12 h holding times, the average systematic uncertainty was +3%, and 66% of the *E. coli* concentrations increased during this time. In contrast, the average systematic uncertainty was −10% for 24–48 holding times, and 88% of the concentrations decreased. Similar results to these occurred when 2–8 h holding times were compared with 12–48 h.

The uncertainty in laboratory analysis was always random and ranged from ±8% for EPA 1604; to approximately ±20% for EPA 1603, IDEXX Colilert, and qPCR; and to ±31% for EPA 1103.1 (Table 4). As such, laboratory analysis typically contributed less random uncertainty than did sample collection (Tables 5 and 6). It is important to note that this study was not meant to compare the quality of laboratory *E. coli* analysis techniques. Thus, these results should not be used to justify the use of any method compared to another. The number of studies, samples utilized in each study, and conditions of each study performed to evaluate laboratory analysis methodologies varied, resulting in only preliminary estimates of their associated uncertainty for use herein.

It should also be kept in mind, that the uncertainty estimates presented in Tables 4 and 5 apply to general cases represented by good, average, and poor data quality scenarios. In the absence

Table 5
Random and systematic uncertainty in individual measured *E. coli* concentrations for various data quality scenarios and various sampling methods.

	Integrated			Near surface grab			Automated		
	Good (%)	Avg. (%)	Poor (%)	Good (%)	Avg. (%)	Poor (%)	Good (%)	Avg. (%)	Poor (%)
Random uncertainty[a]									
Sample collection									
Sampling interval/timing	±7	±23	±39	±7	±23	±39	±7	±23	±39
Horizontal gradient	±5	±10	±20	±32	±62	±92	±32	±62	±92
Min. flow threshold	±0	±0	±0	±0	±0	±0	±0	±0	±0
Subtotal for collection	±9	±25	±44	±33	±66	±100	±33	±66	±100
Laboratory analysis									
Analysis method	±8	±20	±31	±8	±20	±31	±8	±20	±31
Total random uncertainty	±12	±32	±54	±34	±69	±105	±34	±69	±105
Systematic uncertainty[b]									
Sample collection									
Vertical gradient	0	0	0	−1	−20	−254	+1	+20	+254
Within-event residual	0	0	0	0	0	0	+2	+3	+6
Between-event residual	0	0	0	0	0	0	0	0	0
Subtotal for collection	0	0	0	−1	−20	−254	+3	+23	+260
Preservation/storage									
Storage–time, temp	+3	−10	−10	+3	−10	−10	+3	−10	−10
Total systematic uncertainty	+3	−10	−10	+2	−30	−264	+6	+13	+250

[a] Random uncertainty was estimated with Eq. (4).
[b] Systematic uncertainty was estimated as the sum of individual sources of uncertainty.

Table 6
Random and systematic uncertainty in *E. coli* concentrations measured throughout runoff events (whole event sampling) for various data quality scenarios and various sampling methods.

	Integrated			Near surface grab			Automated		
	Good (%)	Avg. (%)	Poor (%)	Good (%)	Avg. (%)	Poor (%)	Good (%)	Avg. (%)	Poor (%)
Random uncertainty[a]									
Sample collection									
Sampling interval/timing	±55	±71	±71	±39	±55	±71	±7	±23	±39
Horizontal gradient	±5	±10	±20	±32	±62	±92	±32	±62	±92
Min. flow threshold	±0	±0	±0	±0	±0	±0	±5	±10	±15
Subtotal for collection	±55	±72	±74	±50	±83	±116	±33	±67	±101
Laboratory analysis									
Analysis method	±8	±20	±31	±8	±20	±31	±8	±20	±31
Total random uncertainty	±56	±74	±80	±51	±85	±120	±34	±70	±106
Systematic uncertainty[b]									
Sample collection									
Vertical gradient	0	0	0	−1	−20	−254	+1	+20	+254
Within-event residual	0	0	0	0	0	0	+2	+3	+6
Between-event residual	0	0	0	0	0	0	+0.1	+0.1	+0.2
Subtotal for collection	0	0	0	−1	−20	−254	+3	+23	+260
Preservation/storage									
Storage–time, temp	+3	−10	−10	+3	−10	−10	+3	−10	−20
Total systematic uncertainty	+3	−10	−10	+2	−30	−264	+6	+13	+240

[a] Random uncertainty was estimated with Eq. (4).
[b] Systematic uncertainty was estimated as the sum of individual sources of uncertainty.

of project-specific uncertainty data, these results can serve as reasonable estimates for *E. coli* concentrations measured in field runoff and streamflow from small watersheds; however, if available project-specific uncertainty estimates should be utilized to the extent possible.

3.2. Comparison of uncertainty associated with each sampling method

The random and systematic uncertainty contributed by each sampling method varied considerably. For integrated sampling, no systematic uncertainty is introduced by sample collection (Tables 5 and 6) because this method collects the sample throughout the stream cross-section and thus minimizes the influence of vertical and horizontal gradients in *E. coli* concentrations. Integrated sampling produces the best measurement of *E. coli* concentrations for individual samples (Fig. 2) contributing random uncertainty ranging from ±12% to ±54% for good to poor data quality scenarios (Table 5). This method does, however, introduce more random uncertainty for whole event sampling because the substantial time required for personnel to collect a single sample makes it difficult to collect frequent samples (Table 6). In other words, integrated sampling effectively captures cross-sectional concentration variability for individual samples but is not typically able to capture temporal variability, especially at multiple sampling sites.

For near surface grab sampling, which is commonly used to collect *E. coli* samples, both the random and systematic uncertainty

contributed by sample collection increases substantially for the average and poor scenarios (Tables 5 and 6, Fig. 2). This potential large increase in uncertainty results from the difficulty of capturing cross-sectional concentration gradients with a single grab sample. With this method it is also difficult for staff to be onsite to collect samples throughout runoff events such that temporal variability is sufficiently captured; therefore, the uncertainty is higher for event sampling than for individual measurements.

Automated sampling produces the lowest uncertainty when samples are collected throughout runoff events to determine the event mean (Table 6, Fig. 2), which is logical as automated samplers were developed for this purpose and thus best capture temporal variability. However, automated sampling, like grab sampling, is not able to capture the variability in *E. coli* concentrations in stream cross-sections (Quilliam et al., 2011), although modifications have been developed to reduce the influence of vertical stratification (e.g., Selbig et al., 2012). Whereas grab sampling underestimates sediment and *E. coli* concentrations because samples are typically collected near the surface, sample intakes of automated samplers are often mounted near the bottom of channels, thus overestimating sediment and *E. coli* concentrations. This difference can be clearly seen in Fig. 2 for the average and poor scenarios. The substantial increase in uncertainty contributed by sample collection in the average and poor scenarios supports previous recommendations of the importance of site selection when utilizing automated sampling (e.g., Harmel et al., 2006a).

3.3. Comparison of uncertainty in *E. coli* concentrations relative to other water quality constituents

Harmel et al. (2006b) published the following estimates of uncertainty, which apply to typical "average" conditions and sample collection throughout storm events with an autosampler: Q (±10%); total suspended sediment (±15%); NO_3-N, NH_4-N, and PO_4-P (±14–30%); total N and total P (±27–29%). Based on the present research, the random uncertainty associated with *E. coli* concentrations was greater than that of flow as well as sediment, and nutrient concentrations (Fig. 3). For *E. coli* concentrations, the uncertainty for the average scenario was ±70% and ranged from ±34% to ±106% for the good and poor scenarios. However, with careful attention to proper QA/QC and selection of good sampling sites (e.g., well-mixed, small streams), the ±33% average uncertainty of McCarthy et al. (2008) and the ±34% uncertainty of the

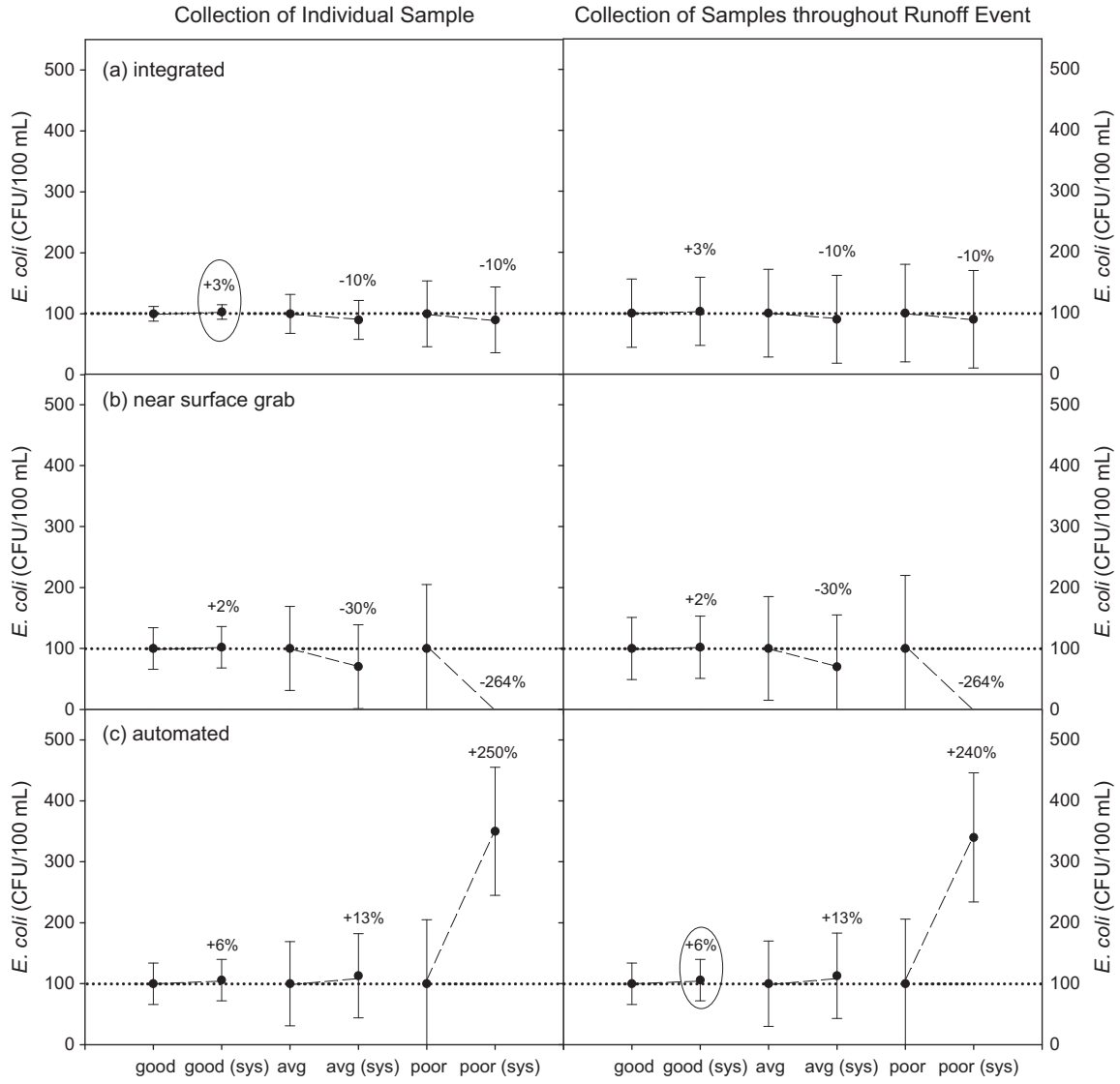

Fig. 2. Random and systematic uncertainty assuming a true *E. coli* concentration of 100 CFU/100 mL (errors bars represent random uncertainty, and the – or + value indicates the direction and magnitude of the shift due to systematic uncertainty) for: (a) integrated sampling, (b) near surface grab sampling, and (c) automated sampling.

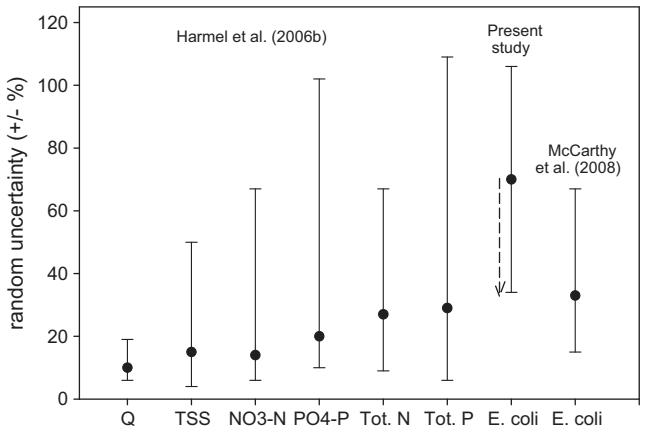

Fig. 3. Total random uncertainty ranges for flow and sediment, N, P, and *E. coli* concentrations (the whiskers represent the maximum and minimum for the typical data quality scenario and the black dot represents the average for the typical data quality scenario).

"good" data quality scenario in the present study are readily achievable (Fig. 3).

In the absence of project specific uncertainty estimates, the present results can be used to establish reasonable estimates for uncertainty introduced by sample collection, sample preservation/storage, laboratory analysis and for the resulting measured *E. coli* concentrations. However, the reader is encouraged to apply uncertainty estimates that correspond to specific data sets, if that information is available.

4. Conclusions

- The reduction of random and systematic uncertainty in *E. coli* concentrations measured with manual integrated sampling is an important result; however, it applies only to individual measurements such as commonly taken in periodic or routine sampling programs. In contrast, when *E. coli* data are collected throughout runoff events to determine the event mean, automated sampling typically produces the lowest uncertainty.
- The sample collection procedural category contributed the highest amount of uncertainty in all data quality scenarios due to the presence of vertical and horizontal gradients in *E. coli* concentrations. Various collection methodologies employ either fixed or distributed sampling locations and are thus biased depending on the strength and direction of these gradients.
- The total random uncertainty associated with *E. coli* concentrations for the average scenario was ±70% and ranged from ±34% to ±106% for the good and poor scenarios, which is larger than generally associated with flow and concentrations of sediment and nutrients. However, uncertainty can be readily reduced to ±33–34% with careful attention to QA/QC and selection of good sampling sites (e.g., well-mixed, small streams).

4.1. Gaps in scientific knowledgebase

Several issues related to *E. coli* sampling are not well understood and warrant further study to support improved data collection methodology; these include:

- Sampling immediately downstream of bridges with nesting birds. Additional study is needed to improve understanding of the magnitude, frequency of occurrence, and spatial differences and supplement information provided by Pendergrass et al. (2015), Karthikeyan (unpublished), and Wolfe (unpublished); however, the potential for significant increases in *E. coli* concentrations immediately downstream of bridges with nesting birds should be carefully weighed when determining sampling locations.
- Conditions under which *E. coli* concentrations increase or decrease in water samples after sample collection.
- Differences in the magnitude of within event cross-sectional variability and temporal variability.
- Influence of sampling interval, minimum flow threshold, and the use of refrigerated samplers.
- Vertical and horizontal gradients of *E. coli* concentrations in streams and the degree to which these gradients are affected by flow conditions (laminar vs. turbulent).

Acknowledgments

The continued support by the Texas State Soil and Water Conservation Board in related projects to better understand bacteria fate and transport and mitigate its negative impacts is appreciated.

USDA is an equal opportunity employer and provider.

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