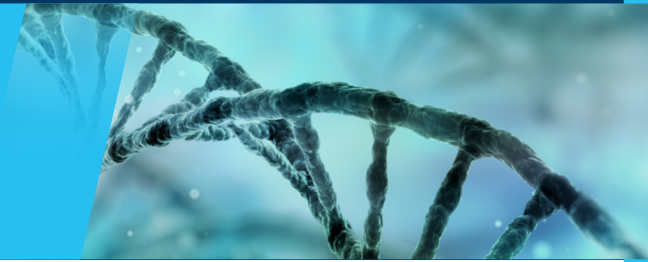


## IF SOMETHING SOUNDS TOO GOOD TO BE TRUE, IT PROBABLY IS...



When molecular biological tools (MBTs) like quantitative polymerase chain reaction (qPCR) are offered at a significantly lower price, you can be certain that corners are being cut to provide that discount.

### What corners are being cut?

- **Expertise of Laboratory Personnel: There is no substitute for experience.**

- At Microbial Insights, the staff scientists in our DNA Laboratory have performed DNA or RNA extractions on more than 20,000 true environmental samples (e.g., groundwater and soil from pollutant contaminated sites). Experience counts - DNA extraction from these types of environmental samples is much more challenging than the relatively “clean” samples for testing food, cooling water, or potable water wells.
- Many MBT start-ups and divisions at established laboratories have been launched over the years by self-described MBT experts that continue to jump from lab to lab yet have never actually performed any DNA based analyses. Laboratory managers and technicians with no real MBT experience attempting to duplicate methods from peer reviewed publications is simply not enough to ensure accurate and reliable results. These labs fail quickly but at the cost of the client’s samples.
- In many cases, discounted analysis is subcontracted to university laboratories and actually performed by students not dedicated professionals. The students have minimal experience in performing MBTs and may lack any experience with DNA extraction from real world samples and the QA/QC procedures that are so important with MBTs.
- Even professional technicians at established commercial laboratories attempting to launch a DNA division or add a qPCR assay to a lineup of traditional testing will likely lack the experience required to produce accurate MBT results.
- At Microbial Insights, our Laboratory Directors and Research Scientists are truly experts with extensive, hands-on experience in the design, validation, performance, and QA/QC parameters of the MBT analyses offered by their laboratories to ensure that we produce the most accurate and precise data day in and day out.

- **Quality Control and Quality Assurance (QA/QC): QA/QC parameters must be performed with every extraction set and qPCR plate to ensure the integrity of the sample results.**

- As discussed in *Questions to Ask the Laboratory*, extraction blanks and appropriate positive and negative controls must be performed to guard against and detect any amplification issues or cross-contamination. While critical for reliable results, performing blanks and running controls costs money and is often neglected by start-ups for the sake of cost and convenience.
- **Reagent Quality? Analysis of DNA which is present in all living material requires incredibly pure reagents that are expensive.**
  - Laboratories that offer MBTs at substantial discounts may be using inferior or even “homemade” reagents. Lower analysis prices are not the result of volume discounts on molecular biology grade reagents. Microbial Insights uses the highest quality reagents and since we process more samples per year for MBT analyses than any other laboratory we receive the largest volume discount on molecular biological grade reagents in the industry.

## Can You Trust Your qPCR Results?

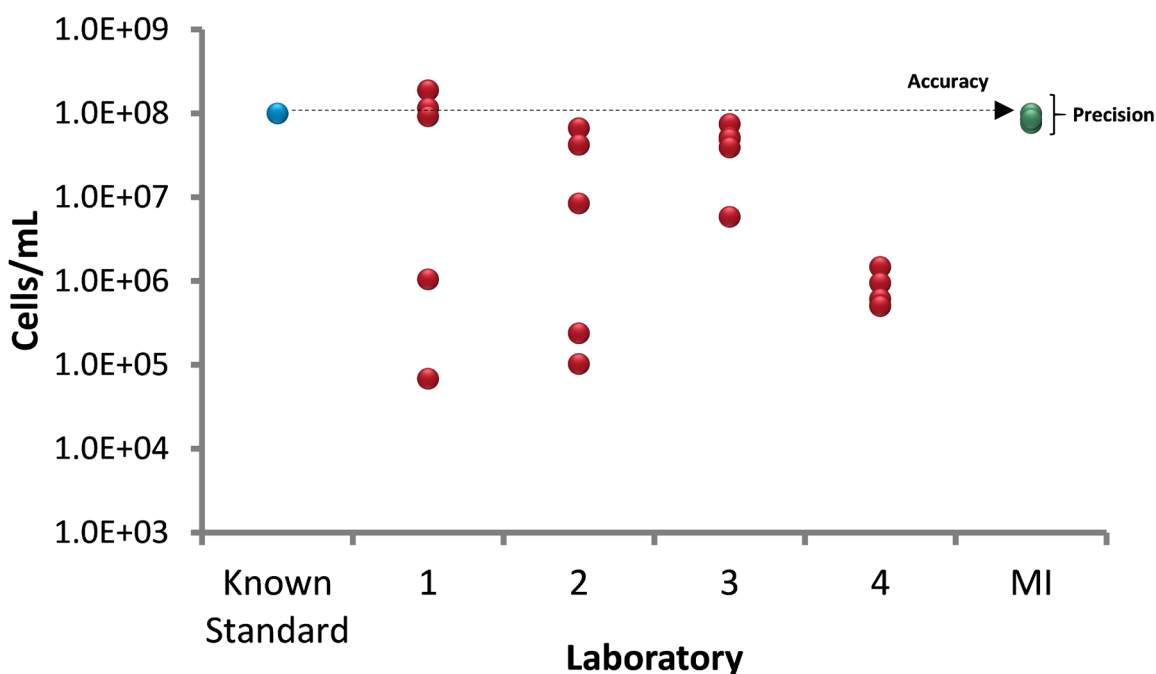
The answer may be that “You get what you pay for.”

### The *Dehalococcoides* Round Robin

As part of our commitment to data quality, Microbial Insights (MI) participated in a federally funded qPCR study where a standard of known *Dehalococcoides* (DHC) concentration was sent to academic laboratories, a national laboratory, a North American competitor, and MI for analysis. Each laboratory analyzed five true replicates of the known standard to test accuracy and precision. If a lab produces both accurate and precise data, their reported DHC concentrations would match the known standard (●) for all five samples despite five separate DNA extractions and qPCR runs.

As shown in the figure, qPCR results from other laboratories (●) were as many as three orders of magnitude lower than the known concentration of the standard (●) and reproducibility was often poor.

**The MI results (●) were not only extremely accurate but also very precise. Note that the five green data points from MI are so close that it is difficult to even tell that results for all five samples are plotted!**

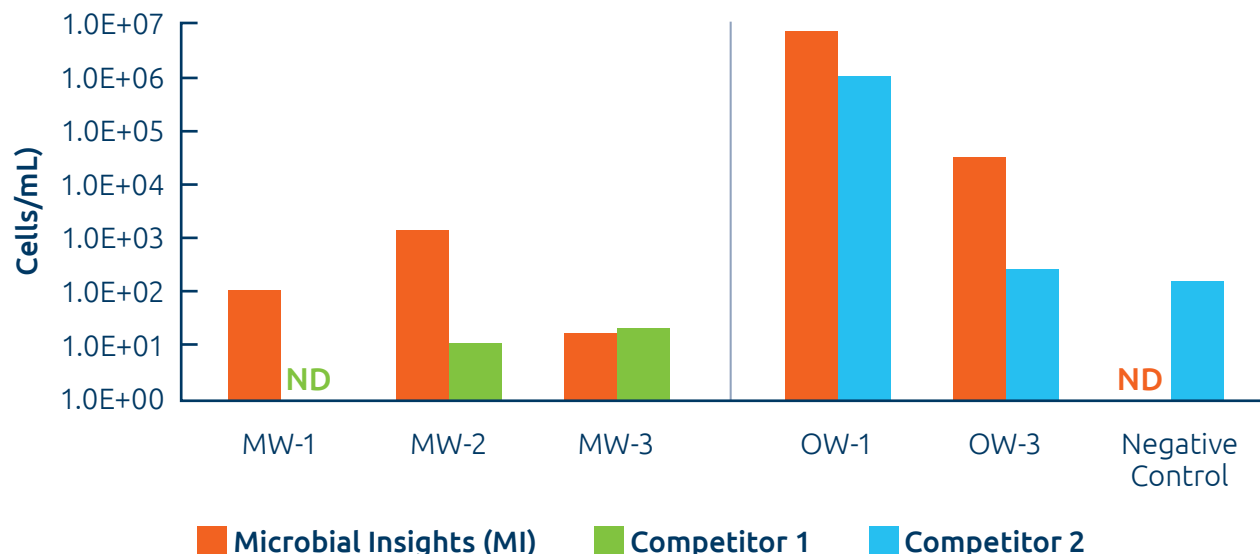


More specifically, all five of the MI results (●) were within 0.1 log of the known DHC concentration of the standard (●). Two of the five replicates matched the independently measured concentration of the standard exactly.

### Why You Can Trust Microbial Insights Results

The round robin confirms the fact that qPCR is not a trivial procedure. When even national laboratories couldn't match MI's accuracy, how would start-ups, discount, and any labs new to molecular biological tools fare in a round robin comparison? The accuracy of MI's data is due to the quality of our assays, continued investment in instrumentation, the experience and training of our staff scientists, and our rigorous QA/QC program. These QA/QC measures cost money and are the first corners cut by laboratories offering discounted pricing. At MI however, we will not compromise on data quality because we stake our reputation on our results every day.

At Microbial Insights, we are confident in the results that we report and encourage our clients to split field samples between different laboratories for comparisons. The graph below shows results of split samples analyzed by MI (■), a US competitor (■), and a European competitor (■).



### Competitor 1: Accurately quantified *Dehalococcoides* (DHC) in only 1/3<sup>rd</sup> of the samples and reported a false negative for MW-1.

- **MW-1:** Competitor failed to even detect DHC despite a concentration of  $10^2$  cells/mL. False negatives in the lab lead to unnecessary bioaugmentation in the field.
- **MW-2:** Competitor results were below the true DHC concentration by two orders of magnitude. Results that are consistently biased low can falsely reduce stakeholder confidence and prompt more aggressive or supplemental site activities such as additional electron donor injections.
- **MW-3:** Getting it right in **one out of three** samples is not acceptable.

### Competitor 2: Results are biased low and DHC detection in a negative control invalidates all their results.

- **OW-1:** Even in a sample with a high DHC concentration, the result from the competing laboratory was low by nearly an order of magnitude – a substantial difference.
- **OW-3:** Low by two orders of magnitude. The actual DHC concentration ( $10^4$  cells/mL) is the threshold concentration recommended for effective reductive dechlorination rates. In this case, the inaccurate results reported by Competitor 2 would almost certainly raise concerns about the effectiveness of the biostimulation potentially leading to unnecessary electron donor injections.
- **Negative Control:** Very troubling. Detection of DHC in the negative control is direct evidence of cross-contamination in the lab and **invalidates all of their results.**

### Accurate and Precise Data Leads to Good Decisions

Simply put, the inaccurate qPCR results reported by these competing laboratories would adversely impact site management decisions wasting time and money. Accurate qPCR data allows sound decisions and at Microbial Insights, we have proven the accuracy of our data during ISO accreditation, with round robins, with split samples, and with our QA/QC program for nearly 30 years.