

## *Standard Addition*

Standard Addition is a technique that helps qualify dubious test results. However, before we discuss it in detail, let us define some new terms and review the concepts of **STANDARD** and **BLANK**.

### New Terms

**Analyte** - The chemical you are testing for. For example, when testing for Ammonia, Ammonia is the “analyte”. When a doctor takes a blood sample for a cholesterol test, cholesterol is the “analyte”.

**Interferences** - Chemicals in the water that inadvertently interfere with our test.

**Method** - The chemicals, procedures, and Hach machine settings which define a particular test. For example, there are many methods for testing Ammonia; the one we use is the “Nessler Method”. Any given method is accurate over a finite range of analyte concentration, from zero to some maximum value called a “ceiling”.

**Reagent** - The Hach chemicals we add to the samples that make them change color. For example, when testing for Nitrate, the NitrVer5 powder pillow is the reagent.

**Standard Addition** - A test technique for investigating suspicious test results.

**Titration** - A method of determining the unknown concentration of an analyte in a solution, by adding to it a reagent of known concentration in carefully measured amounts until a reaction of known proportion is completed, as shown by a color change, and then calculating that unknown concentration. Titration provides the foundation for our test procedures.

### What is a “standard”?

In general, a standard is some official yardstick by which other things are measured. The dictionary definition is “something set up and established by authority as a rule for the measure of weight, etc.” For example, a scale company, by law, must use “standard” weights to calibrate its scales. These are expensive, extremely precise bronze weights that would be engraved with an official seal such as “1.00 U.S. pounds NIST-certified”.

We use similar standards in our water testing. The “standard solutions” in the large plastic bottles are guaranteed by Hach to contain exactly 1.00mg/L of an analyte (e.g. Ammonia).

What is a “blank”?

In contrast, a blank represents nothingness. Its dictionary definition is “devoid of content”; in our use, it means “devoid of analyte”. It is nothing but pure water and reagent.

Thus, the **BLANK** calibrates the Hach spectrophotometer by establishing its zero-point, and the **STANDARD** verifies that the system (machine, chemicals, and test procedures) are all working properly. The **BLANK** and method ceiling establish the left and right boundaries of the system - the **BLANK** is the minimum (0.0) and the method ceiling is the maximum. Ideally, all samples should fall between these values (i.e. 0...ceiling); the **STANDARD** always will. Mathematically, this is expressed as **BLANK** ≤ sample ≤ **method ceiling**. Method ceilings are explained in more detail in the following pages.

Example

In the following table, we compare the calibration of the Hach machine to that of a weighing scale, in a step-by-step manner. A scale was chosen for this example because it is analogous to the Hach tester.

Like the Hach tester, every scale (such as a bathroom, kitchen, or deli scale) has a zero point, a maximum point (“ceiling”), and a linear region in between; this is true for older mechanical scales as well as newer digital ones. Within that linear region, a scale will read the correct weight; outside that region, all bets are off. Ditto for the Hach.

In this example, imagine you are working the deli counter at Jewel. A customer brings a Tupperware container from home and asks you to fill it with six ounces of potato salad. Since the Tupperware itself weighs several ounces, you must think of some way to cancel out that weight, so that the customer will be charged only for the potato salad, not for the potato salad and Tupperware.

<b>Procedure for using Hach tester</b>	<b>Procedure for using a deli scale</b>
1. Place the <b>BLANK</b> in the tester and press ZERO button	1. Place the blank (empty) Tupperware container on the scale and press ZERO button
2. The tester should read “0.0”	2. The scale should read “0.0”
3. Place the <b>STANDARD</b> in the tester	3. The customer doesn’t trust your scale, so she goes to aisle five and returns with a standard one-pound box of C&H sugar, which she asks you to pour into the Tupperware container
4. The tester should read “1.0”	4. The scale should read “1.0”
5. The tester is now ready to test river samples	5. The scale is now ready to weigh potato salad

### End of Story?

No! The **BLANK** and **STANDARD** have only assured us that the system is working correctly at two points - but, they say nothing about its accuracy at other points. In other words, it is possible to prepare a perfect **BLANK** and a perfect **STANDARD**, and yet have erroneous sample data. It is for this reason that you must review your results to see if they look accurate after testing for each analyte.

One way to do so is to compare each result with that of other tests. For example, after testing for Nitrate, look at the result for WB2.0, and ask yourself these two questions:

1. Is this result much higher/lower than WB2.0 Nitrate in previous months? (If you have an Internet connection, it may be helpful after each test to look at the graphs on the RPG web site to see where your data falls (or, print them out ahead of time). Otherwise, page through the log books.)

2. Is this result much higher/lower than today's WB1.0 & WB3.0 Nitrates?

If the answer is Yes to either/both of these questions, then there are four possibilities why your test result is off. Note that the first two are legitimate causes for deviant data, while the last two are testing errors.

1. Normal Deviation. The amount of chemicals in the river will vary slightly from month-to-month and site-to-site. This is normal, and will vary from analyte-to-analyte.
2. Seasonal Variation. This primarily affects Chloride in the snowy months. For all other analytes, there may be gradual seasonal variation, but little month-to-month variation.
3. Operator Error. You made a mistake in the preparation of the sample. Double-check the procedure and redo it. Or, if the **STANDARD** was less than 0.90 or greater than 1.10, then redo the entire test, as there is something fundamentally wrong (an exception to this rule is Chloride).
4. Interferences. There may be other chemicals in the water which are interfering with your test, causing the region between 0.0 and the method ceiling to become nonlinear, and thus, erroneous. A Standard Addition test will reveal this.

To ensure the integrity of your test (and of the RPG River Monitoring Program as a whole), a bad test cannot simply be redone at a later date. Thus, it is your responsibility to decide, while testing, if your data excessively deviates from normal, and if it does, which of these four reasons the deviation can be attributed to. If you can rule out the first three, then you should consider testing for interferences by performing a Standard Addition test.

### Interferences and Standard Addition

Before we discuss how Standard Additions can uncover Interferences, we need to review reagents, the Hach machine, and how the two work together.

#### Reagents

A reagent is a chemical that reacts to the presence of another chemical. For our tests, Hach has chosen chemicals which produce a specific color in the presence of another chemical. Furthermore, not only is a specific color produced, but the *amount* of color produced is in direct proportion to the amount of analyte present. For example, if Sample X contains twice as much Phosphorus as Sample Y, then X should be twice as blue as Y. This is tricky business, and plus,

there are practical limits: if Sample Z contains 10,000 times as much Phosphorus as Sample Y, how can it be made 10,000 times bluer than Y?? It can't, and so, Hach establishes a method ceiling for every test method. The ceiling is a limit imposed by the method components (machine, reagent, and procedures), and is different for every test method. The ceiling specifies the maximum amount of analyte that the system can accurately measure. Beyond that, things go nonlinear and measurements are no longer accurate. The following table lists the ranges for our four test methods:

Analyte	Method Ceiling (mg/L)	Test Range (mg/L)
Phosphorus	2.5	0 ... 2.5
Nitrate	4.5	0 ... 4.5
Ammonia	2.5	0 ... 2.5
Chloride	20.0	0 ... 20.0

Thus, our reagents are not magic. They are simply chemicals which are known to change color very precisely and in proportion to the presence of some other chemical (the analyte). For example, Mercuric Thiocyanate reacts with Chloride to produce an orange color, while Nessler reagent reacts with Ammonia to produce a yellow color. Hach carefully chose these reagents and then designed the tester to work hand-in-hand with them.

#### Hach Spectrophotometer

Our Hach tester is a "spectrophotometer", a computer that measures the intensity of light in a given spectrum; that is, it measures the amount of color in a water sample. When you insert your sample bottle into the machine, a laser shines through the sample to a detector on the opposite side. The detector measures the amount of colored light that passed through the sample, and from that, the computer calculates the amount of analyte in your sample and displays it on the screen. For example, when you enter program #70 into the machine, you are telling it to test for Chloride, by measuring how orange your samples are. Actually, it does so using orange's complimentary color - blue, which is why you must tune it to "455" (the wavelength of blue = 455nm<sup>1</sup>). Likewise, when you enter program #380, you are telling it to test for Ammonia, by measuring the amount of yellow in your samples. Again, you tune the machine to "425", because violet is the complimentary color of yellow, and the wavelength of violet is 425nm.

#### Interferences

Hach guarantees several key items: it guarantees that a Standard Solution contains exactly 1mg/L of a given chemical, it guarantees that a reagent will produce the correct color in the presence of a given chemical, and it guarantees that the machine will work properly with those items. However, Hach *cannot* guarantee that a reagent will respond to one and only one chemical. There are millions of different chemicals in the world (several hundred of which may be in the river), and it is impossible to design a reagent that will react to only one of them. So, while Hach has carefully chosen reagents which are unlikely to be disturbed by chemicals commonly encountered in surface water testing, it will occasionally happen, and this leads to the problem of *interference*.

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<sup>1</sup>"nm" = nanometer, which is one-billionth of a meter (0.000000001 meters)

An interference is a chemical in the water which inadvertently reacts with a reagent. How it exactly reacts with the reagent is unknown to us (we don't really care, anyway); the important thing is that it will interfere in some way with the test by adding or subtracting color, and thus, it will ruin the results.

For example, we know that Mercuric Thiocyanate reacts with Chloride in a well-defined manner. What we *don't* know, however, is what *else* Mercuric Thiocyanate reacts with. For all we (or Hach) know, it might also react with zinc (or calcium or even something more complex like motor oil) and thus, if your river sample contains both zinc and Chloride, the sample might turn too orange, and the Hach meter will unwittingly display a false reading. Note that this will occur *regardless* of how well you prepared the **BLANK** and **STANDARD!**

That said, note that interferences are rare, and it would be even rarer for more than one interference to be in the river. Thus, if data from more than one test appears bad, then interferences are not to blame. For example, if your Phosphorous and Chloride results are questionable, then the problem is not with interferences.

Conversely, if the river contains an interference for one of the reagents, it will not likely interfere with the other reagents. For example, if it is found that the river contains a chemical that is interfering with the Nitrate test, Ammonia, Phosphorous, and Chloride tests will not be affected and their data will be fine.

Hach has published a list of chemicals that are known to interfere with its products. An abridged list is provided in the appendix at the end of this report.

So, how do you uncover the problem of a hidden interference? Through the use of a Standard Addition.

### Standard Addition

The technique of Standard Addition consists of adding a known amount of a Standard Solution to a river sample, and then testing it as usual.

If the result does not match that which you calculated, then there may be an interference in the river that is ruining your test. There is nothing you can do to correct this, so note it on the worksheet and logbook, and then contact the test coordinator.

On the other hand, a result that agrees with your calculation says nothing about the source of bad data; it simply rules out interferences. By process of elimination, though, it does suggest that the deviation might be due to natural variation or careless mistakes.

Therefore, the Standard Addition technique only indicates if an interference might be to blame for bad test data. (To a lesser extent, it can also help identify spoiled reagents, a faulty machine, or impure distilled water.) It cannot, however, determine if bad test data was caused by natural variation or careless mistakes.

Standard Addition works because the system is linear. Let's revisit the previous example to understand how this technique works, keeping in mind that it applies to the Hach tester as well.

### Example

As noted previously, you are working the deli counter at Jewel. Earlier, you sold six ounces of potato salad to a customer in a Tupperware container, and now she is back to complain that "it doesn't look like six ounces". Based on a similar portion of pasta salad you just sold, you agree that it doesn't look quite right.

Earlier, you had both witnessed that the scale properly compensated for the empty Tupperware (the "blank") with a "0.0" reading, and that it properly measured the one-pound box of sugar (the "standard") with a "1.0" reading. Now, the two of you agree that just because the scale works properly *at* two points does not guarantee that it works properly *between* the two points. How will you investigate this?

Using the technique of Standard Addition, you will investigate how the scale responds to the addition of a known weight. From its response, you will be able to determine if the scale is faulty.

At this suggestion, the customer runs back to aisle five, and returns with an identical Tupperware container and a two-ounce jar of Mrs. Dash. Repeating your previous procedure, you place the empty container on the scale, zero it, and add potato salad until it reads "6.0 oz". Next comes the crux of the experiment: you pour the two-ounce jar of Mrs. Dash on top of the six ounces of potato salad. If the scale reads "8.0 oz", then it is working perfectly! However, if it reads something goofy like "6.2 oz" or "9.0 oz", then you know that something is interfering with it!

Note that this trick will *always* work, regardless of the amount of potato salad, because the two-ounce jar of Mrs. Dash is a fixed, known quantity, and adding it to X ounces of potato salad will always make a scale read X+2 ounces. This is yet another advantage of a scale's linear nature.

### Dimensional Analysis

While the potato salad example illustrated the general concept of Standard Addition, its actual use in water testing is a bit more complicated.

Much of the difficulty in understanding Standard Additions lies in the extremely tiny quantities we are measuring. For example, an Ammonia test might yield a result of 0.5mg/L. In real terms, this equates to 0.00000103 pounds of Ammonia in a quart of river water. The amount of Ammonia in your little 25ml sample jar is even less - about 0.000000027 pounds (twenty-seven *billionths* of a pound). Such awkward quantities highlight the need for an appropriate set of dimensions.

Dimensions are units of measure. For example, the dimensions of a living room might be given as "15 x 20 feet", the dimension being feet. In our tests, the dimensions are milliliters (ml), milligrams (mg), liters (L), and together, mg/L (concentration). The prefix "milli" means one-thousandth (1/1000 or 0.001), not one-millionth. Thus, a milliliter (ml) is one-thousandths of a

liter and a milligram is one-thousandths of a gram (to get a notion of grams and milligrams, check the label on a bottle of aspirin or vitamins).

Dimensional Analysis, then, is the mathematics of dimensions; i.e. converting from one unit to another. This is a key component of the Standard Addition calculations. If you can get past that hurdle, you will be well on your way to understanding and using Standard Additions.

### Performing Standard Addition

Standard Addition consists of three distinct steps: calculate the theoretical concentration, measure the actual concentration, and then compare the two results.

#### 1. Calculate Theoretical Concentration of Spiked Sample

Using dimensional analysis, reduce concentration (mg/L) to weight (mg), to simplify the Standard Addition calculations (this is the least messy and most intuitive manner of understanding the math involved). Then, calculate the total weight of the analyte (i.e. river water + Standard Addition) in the small bottle. Finally, scale that value back up to mg/L. Note that the concentration value used here is from the bad data you are attempting to validate.

#### 2. Measure Actual Concentration of Spiked Sample

To a clean, dry bottle, add the normal amount of river water (note that this is a “fresh” sample from the mason jar). Then “spike” it with the proper amount of Standard Solution (taken from the large plastic Hach bottle, not from your little **STANDARD!**) Then, test it as usual - add the normal reagents, wait for the specified time, and place it in the Hach machine for measuring. This step is analogous to the Mrs. Dash example.

#### 3. Compare the Results from #1 and #2

If the measured result differs from that calculated by more than 10% or so, it indicates that your original data was corrupted by interferences.

For whatever reason, if you choose not to redo the test, then your original “bad” data is recorded on the worksheet and logbook. The Standard Addition data from #1 and #2 is for diagnostic purposes only - it is not to be recorded!!!

In summary, the technique of Standard Addition consists of three distinct steps:

1. do the math
2. spike a fresh river sample and then test it as usual
3. compare the results of #1 and #2, and then discard them

Thus, this technique does not attempt to deliver the correct test values; rather, it is simply a Yes/No indicator as to whether your original test was ruined because of interferences in the river water. For this reason, we “discard” the results.

The amounts of river water and Standard Solution must be precisely measured, and are given in the following table:

Analyte	Container	Add this much...			Multiply <sup>2</sup> Hach reading by
		River Water	Std Soln	Distilled H <sub>2</sub> O <sup>1</sup>	
Phosphorus	10ml glass bottle	2.0ml	3.0ml	5.0 ml	2
Nitrate	25ml plastic bottle	5.0ml	5.0ml	15.0ml	2.5
Ammonia	25ml plastic bottle	15.0ml	10.0ml	none	1
Chloride	25ml plastic bottle	1.0ml	5.0ml <sup>3</sup>	19.0ml	4.167

notes:

<sup>1</sup> no need to actually measure distilled H<sub>2</sub>O, just add river water & std soln and then fill to the line as usual

<sup>2</sup> scaling factor compensates for dilution:  $\frac{\text{river water} + \text{std soln} + \text{distilled water}}{\text{river water} + \text{std soln}}$

<sup>3</sup> this is from the diluted standard solution you mixed in the 500ml flask



### Standard Addition Examples

For this report, I collected a sample of river water from WB2.0 and then tested it in the normal manner. Then, I re-tested it using the Standard Addition technique. These tests were performed only for this report - there was no suspicion of interferences in the river water, and the test results will confirm that.

In the following pages, each analyte from that test will be presented, along with its calculations. By substituting your own test values in step 1A., you should be able to perform Standard Addition tests of your own.

### Sample Worksheet

As usual, I filled out a worksheet during my test. The pink rows represent my normal test results (i.e. the amount of analyte presumably in the river water), which, for the purposes of this example, we will assume are “bad” because they deviate from past WB2.0 results. The green rows represent my Standard Addition tests. Again, note that a Standard Addition test is merely an analytical procedure, not a measurement of river water. Thus, there is no use comparing the pink rows to the green rows; rather, the green rows will be compared to the theoretical calculations on the following pages to determine if interferences are to blame for my “bad” data.

Phosphorus - 10ml test - program 490			
Sample:	Amount of Sample:	Reading:	Result:
WB2.0	2.0 ml	1.41	x 5 = 7.05
WB2.0 - Spiked	2.0 ml + 3.0 ml	1.81	x 2 = 3.62
Nitrate MR - 25ml test - program 353			
Sample:	Amount of Sample:	Reading:	Result:
WB2.0	5.0 ml	1.8	x 5 = 9.0
WB2.0 - Spiked	5.0 ml + 5.0 ml	2.0	x 2.5 = 5.0
Ammonia - 25ml test - program 380			
Sample:	Amount of Sample:	Reading:	Result:
WB2.0	straight	0.31	0.31
WB2.0 - Spiked	15.0 ml + 10.0 ml	0.62	0.62
Chloride - 25ml test - program 70			
Sample:	Amount of Sample:	Reading:	Result:
WB2.0	1.0 ml	6.1	x 25 = 152.5
WB2.0 - Spiked	1.0 ml + 5.0 ml	7.9	x 4.167 = 32.92

## Standard Addition - Phosphorus

### 1. Calculate Theoretical Concentration of Spiked Sample

Given: From the table on page 8, we see that our 10ml glass bottle will contain 2.0ml of river water, 3.0ml of Standard Solution, and the remainder (5.0ml) is distilled H<sub>2</sub>O.

A. Calculate the actual amount of Phosphorus in the river sample that yielded your bad test data.

As an example, let's use the "bad" reading (pink row) from my worksheet:

<u>Sample</u>	<u>Amt of Sample</u>	<u>Reading</u>	<u>Result</u>
WB2.0	2.0ml	1.41 × 5	= 7.05mg/L

Express this concentration as mg/ml:  $\frac{7.05\text{mg}}{\text{L}} \equiv \frac{7.05\text{mg}}{1000\text{ml}} = \frac{0.00705\text{mg}}{\text{ml}}$

Calculate actual amount of Phosphorus:  $\frac{0.00705\text{mg}}{\text{ml}} \times 2\text{ml} = 0.0141\text{mg}$

B. Calculate the actual amount of Phosphorus in 3.0ml of Standard Solution. Hach guarantees its concentration is 1mg/L.

Express this concentration as mg/ml:  $\frac{1.0\text{mg}}{\text{L}} \equiv \frac{1.0\text{mg}}{1000\text{ml}} = \frac{0.001\text{mg}}{\text{ml}}$

Calculate actual amount of Phosphorus:  $\frac{0.001\text{mg}}{\text{ml}} \times 3\text{ml} = 0.003\text{mg}$

C. Add these two results to get total amount of Phosphorus in the glass bottle.

$$0.0141\text{mg} + .003\text{mg} = 0.0171\text{mg}$$

D. Convert this back to concentration (mg/L), noting that only 5ml of liquid contributes to the total in step C (the 5ml of distilled H<sub>2</sub>O contributes nothing).

$$\frac{0.0171\text{mg}}{5\text{ml} \times \frac{\text{L}}{1000\text{ml}}} = \frac{0.0171\text{mg}}{0.005\text{L}} = 3.42\text{mg/L}$$

Thus, the theoretical concentration of our spiked sample should be 3.42mg/L.

## Standard Addition - Phosphorus (cont'd)

### 2. Measure Actual Concentration of Spiked Sample

Note that, with the exception of step B, this procedure is exactly the same as a normal test.

Procedure:

- A. Add 2.0ml of river water to a clean, dry 10ml glass bottle. Note that this is a “fresh” sample from the large mason jar.
- B. Spike it! Add 3.0ml of Phosphate Standard Solution (from the large plastic 500ml Hach bottle). Use a new pipette for this.
- C. Fill to the 10ml line with distilled H<sub>2</sub>O.
- D. Add the PhosVer3 powder pillow, swirl, and wait two minutes.
- E. Place the sample bottle into the Hach tester and note the reading.

### 3. Compare the Results from #1 and #2

Do the results from step 1D. and 2E. match? If they differ by more than 10% or so, it suggests that your original data may have been corrupted by interferences.

In my own example, the Hach machine reported that my spiked sample was 1.81 (green row on worksheet); per the table on page 8, multiplying by two yields 3.62mg/L, which is close to the theoretical result in step 1D. Therefore, there were no Phosphorous interferences in the West Branch DuPage River, so something else is to blame for my bad data.

Finally, note that results you receive in steps 1D. and 2E. are for a spiked sample, not a real river sample. Therefore, regardless of what you concluded in step 3, the results do not reflect the amount of Phosphorus in the river, and thus, are not recorded in the log book.

## Standard Addition - Nitrate

### 1. Calculate Theoretical Concentration of Spiked Sample

Given: From the table on page 8, we see that our 25ml plastic bottle will contain 5.0ml of river water, 5.0ml of Standard Solution, and the remainder (15.0ml) is distilled H<sub>2</sub>O.

A. Calculate the actual amount of Nitrate in the river sample that yielded your bad test data.

As an example, let's use the "bad" reading (pink row) from my worksheet:

<u>Sample</u>	<u>Amt of Sample</u>	<u>Reading</u>	<u>Result</u>
WB2.0	5.0ml	1.8 × 5 =	9.0mg/L

Express this concentration as mg/ml:  $\frac{9.0mg}{L} \equiv \frac{9.0mg}{1000ml} = \frac{0.009mg}{ml}$

Calculate actual amount of Nitrate:  $\frac{0.009mg}{ml} \times 5ml = 0.045mg$

B. Calculate the actual amount of Nitrate in 5.0ml of Standard Solution. Hach guarantees its concentration is 1mg/L.

Express this concentration as mg/ml:  $\frac{1.0mg}{L} \equiv \frac{1.0mg}{1000ml} = \frac{0.001mg}{ml}$

Calculate actual amount of Nitrate:  $\frac{0.001mg}{ml} \times 5ml = 0.005mg$

C. Add these two results to get total amount of Nitrate in the plastic bottle.

$$0.045mg + 0.005mg = 0.05mg$$

D. Convert this back to concentration (mg/L), noting that only 10ml of liquid contributes to the total in step C (the 15ml of distilled H<sub>2</sub>O contributes nothing).

$$\frac{0.05mg}{10ml \times \frac{L}{1000ml}} = \frac{0.05mg}{0.010L} = 5.0mg/L$$

Thus, the theoretical concentration of our spiked sample should be 5.0mg/L.

## Standard Addition - Nitrate (cont'd)

### 2. Measure Actual Concentration of Spiked Sample

Note that, with the exception of step B, this procedure is exactly the same as a normal test.

Procedure:

- A. Add 5.0ml of river water to a clean, dry 25ml plastic bottle. Note that this is a “fresh” sample from the large mason jar.
- B. Spike it! Add 5.0ml of Nitrate Standard Solution (from the large plastic 500ml Hach bottle). Use a new pipette to do this.
- C. Fill to the 25ml line with distilled H<sub>2</sub>O.
- D. Add the NitraVer5 powder pillow, shake for one minute, and then wait five minutes.
- E. Place the sample bottle into the Hach tester and note the reading.

### 3. Compare the Results from #1 and #2

Do the results from step 1D. and 2E. match? If they differ by more than 10% or so, it suggests that your original data may have been corrupted by interferences.

In my own example, the Hach machine reported that my spiked sample was 2.0 (green row on worksheet); per the table on page 8, multiplying by 2.5 yields 5.0mg/L, which is identical to the theoretical result in step 1D. Therefore, there were no Nitrate interferences in the West Branch DuPage River, so something else is to blame for my bad data.

Finally, note that results you receive in steps 1D. and 2E. are for a spiked sample, not a real river sample. Therefore, regardless of what you concluded in step 3, the results do not reflect the amount of Nitrate in the river, and thus, are not recorded in the log book.

## Standard Addition - Ammonia

### 1. Calculate Theoretical Concentration of Spiked Sample

Given: From the table on page 8, we see that our 25ml plastic bottle will contain 15.0ml of river water, 10.0ml of Standard Solution, and no distilled H<sub>2</sub>O.

A. Calculate the actual amount of Ammonia in the river sample that yielded your bad test data.

As an example, let's use the "bad" reading (pink row) from my worksheet:

<u>Sample</u>	<u>Amt of Sample</u>	<u>Reading</u>	<u>Result</u>
WB2.0	25.0ml	0.31	0.31mg/L

Express this concentration as mg/ml:  $\frac{0.31mg}{L} \equiv \frac{0.31mg}{1000ml} = \frac{0.00031mg}{ml}$

Calculate actual amount of Ammonia:  $\frac{0.00031mg}{ml} \times 15ml = 0.00465mg$

B. Calculate the actual amount of Ammonia in 10.0ml of Standard Solution. Hach guarantees its concentration is 1mg/L.

Express this concentration as mg/ml:  $\frac{1.0mg}{L} \equiv \frac{1.0mg}{1000ml} = \frac{0.001mg}{ml}$

Calculate actual amount of Ammonia:  $\frac{0.001mg}{ml} \times 10ml = 0.010mg$

C. Add these two results to get total amount of Ammonia in the plastic bottle.

$$0.00465mg + .010mg = 0.01465mg$$

D. Convert this back to concentration (mg/L), noting that all 25ml of liquid contributes to the total in step C.

$$\frac{0.01465mg}{25ml \times \frac{L}{1000ml}} = \frac{0.01465mg}{0.025L} = 0.586mg/L$$

Thus, the theoretical concentration of our spiked sample should be 0.586mg/L.

## Standard Addition - Ammonia (cont'd)

### 2. Measure Actual Concentration of Spiked Sample

Note that, with the exception of step B, this procedure is exactly the same as a normal test.

Procedure:

- A. Add 15.0ml of river water to a clean, dry 25ml plastic bottle. Note that this is a “fresh” sample from the large mason jar.
- B. Spike it! Add 10.0ml of Nitrogen Ammonia Standard Solution (from the large plastic 500ml Hach bottle). Use a new pipette to do this.
- C. Add Mineral Stabilizer, etc. etc., and then wait one minute.
- D. Place the sample bottle into the Hach tester and note the reading.

### 3. Compare the Results from #1 and #2

Do the results from step 1D. and 2D. match? If they differ by more than 10% or so, it suggests that your original data may have been corrupted by interferences.

In my own example, the Hach machine reported that my spiked sample was 0.62mg/L (green row on worksheet), which is close to the theoretical result in step 1D. Therefore, there were no Ammonia interferences in the West Branch DuPage River, so something else is to blame for my bad data.

Finally, note that results you receive in steps 1D. and 2D. are for a spiked sample, not a real river sample. Therefore, regardless of what you concluded in step 3, the results do not reflect the amount of Ammonia in the river, and thus, are not recorded in the log book.

## Standard Addition - Chloride

### 1. Calculate Theoretical Concentration of Spiked Sample

Given: From the table on page 8, we see that our 25ml plastic bottle will contain 1.0ml of river water, 5.0ml of the diluted Standard Solution you mixed in the 500ml flask, and the remainder (19.0ml) is distilled H<sub>2</sub>O.

A. Calculate the actual amount of Chloride in the river sample that yielded your bad test data.

As an example, let's use the "bad" reading (pink row) from my worksheet:

<u>Sample</u>	<u>Amt of Sample</u>	<u>Reading</u>	<u>Result</u>
WB2.0	1.0ml	6.1 × 25	= 152.5mg/L

Express this concentration as mg/ml:  $\frac{152.5\text{mg}}{L} \equiv \frac{152.5\text{mg}}{1000\text{ml}} = \frac{0.1525\text{mg}}{\text{ml}}$

Calculate actual amount of Chloride:  $\frac{0.1525\text{mg}}{\text{ml}} \times 1\text{ml} = 0.1525\text{mg}$

B. Calculate the actual amount of Chloride in 5.0ml of the diluted Standard Solution, whose concentration is 10mg/L.

Express this concentration as mg/ml:  $\frac{10.0\text{mg}}{L} \equiv \frac{10.0\text{mg}}{1000\text{ml}} = \frac{0.01\text{mg}}{\text{ml}}$

Calculate actual amount of Chloride:  $\frac{0.01\text{mg}}{\text{ml}} \times 5\text{ml} = 0.05\text{mg}$

C. Add these two results to get total amount of Chloride in the plastic bottle.

$$0.1525\text{mg} + .05\text{mg} = 0.2025\text{mg}$$

D. Convert this back to concentration (mg/L), noting that only 6ml of liquid contributes to the total in step C (the 19ml of distilled H<sub>2</sub>O contributes nothing).

$$\frac{0.2025\text{mg}}{6\text{ml} \times \frac{L}{1000\text{ml}}} = \frac{0.2025\text{mg}}{0.006L} = 33.75\text{mg/L}$$

Thus, the theoretical concentration of our spiked sample should be 33.75mg/L.



## Standard Addition - Chloride (cont'd)

### 2. Measure Actual Concentration of Spiked Sample

Note that, with the exception of step B, this procedure is exactly the same as a normal test.

Procedure:

- A. Add 1.0ml of river water to a clean, dry 25ml plastic bottle. Note that this is a “fresh” sample from the large mason jar.
- B. Spike it! Add 5.0ml of the diluted Standard Solution from the 500ml flask.
- C. Fill to the 25ml line with distilled H<sub>2</sub>O.
- D. Add the Mercuric Thiocyanate, etc. and then wait two minutes.
- E. Place the sample bottle into the Hach tester and note the reading.

### 3. Compare the Results from #1 and #2

Do the results from step 1D. and 2E. match? If they differ by more than 10% or so, it suggests that your original data may have been corrupted by interferences.

In my own example, the Hach machine reported that my spiked sample was 7.9 (green row on worksheet); per the table on page 8, multiplying by 4.167 yields 32.92mg/L, which is close to the theoretical result in step 1D. Therefore, there were no Chloride interferences in the West Branch DuPage River, so something else is to blame for my bad data.

Finally, note that results you receive in steps 1D. and 2E. are for a spiked sample, not a real river sample. Therefore, regardless of what you concluded in step 3, the results do not reflect the amount of Chloride in the river, and thus, are not recorded in the log book.

## Appendix A - Known Interferences

The following table lists some of the chemicals which will interfere with our tests. For a complete list, refer to the Hach manual.

<b>Analyte</b>	<b>Interferences</b>
Phosphorus	Copper > 10mg/L Iron > 100mg/L Zinc > 80mg/L large amounts of turbidity extreme pH
Nitrate	nitrites ferric iron chloride > 100mg/L <sup>1</sup> extreme pH
Ammonia	iron sulfide chlorine alcohol
Chloride	extreme pH
<sup>1</sup> causes low readings	