Analysis of Free Chlorine in Poultry Chiller Water (Red Water) using DPD Free Chlorine Reagent

Guidance for improving performance of free chlorine measurements

Poultry processors are required to maintain a free chlorine level sufficient to control pathogenic bacteria found on bird carcasses. Bird carcasses are chilled at 32–34 °F in a continuous counter-flow chlorinated water system. The challenge to the processor is to maintain a free chlorine level of 0.5 - 5.0 mg/L free chlorine in a chiller water which has a huge and changing chlorine demand load due to fresh carcasses being constantly added. The chlorine demand comes from the blood, fat, and proteins originating from the carcasses. Chlorine is continuously fed into the chiller system to counterbalance this incoming organic load in order to maintain the free chlorine residual.

Theoretically, the chlorine demand must be totally met before a free chlorine residual can be obtained. This is classical breakpoint chlorination theory and is well documented in the literature. However, these high organic-loaded chiller waters are not truly through breakpoint and free chlorine, monochloramine, dichloramine and excess organic chloramines are all present at any one time in a sort of “dynamic equilibrium” that is constantly changing and non-uniform throughout the chiller water system. These systems are optimized at pH 5.5 – 6.5 where chlorine present as hypochlorous acid is most efficient at destroying the pathogenic bacteria. The current understanding is that the residual “free chlorine” residing in these complex chiller water matrices is effective in controlling bacteria even though other competing chlorine demand species are still present.

Background

The analysis of free chlorine in these chiller “red” waters is challenging and somewhat controversial as to whether the analysis is accurate. In order to attack this question there are multiple variables that need to be addressed and some assumptions that must be made.

First, the free chlorine concentration is variable throughout the system. The free chlorine concentration will be much higher at the point of chlorination than it will be at the opposite end of the chiller where fresh carcasses are being added. The chlorine concentration will vary at sampling sites between these two points. This needs to be taken into consideration when making chlorine additions or when comparing analysis results between different samples or sampling sites.

Second, the chiller waters are cold and the reaction rate of chlorine with the organic matter present is slow. The rate of reaction depends on the type and structure of the organic matter, temperature and chlorine concentration. It is important to recognize that the “dynamic equilibrium” existing in the sample may be disrupted or shifted when the sample is removed from the system. A sample removed will slowly warm with the residual free chlorine becoming more reactive. This leads to further reaction of the free chlorine with any existing chlorine demand and hence will lead to decreased free chlorine values if the free chlorine analysis is delayed. This becomes important when samples are transported to a lab or if samples are being analyzed on-line by a process analyzer. This can be illustrated by taking a sample and analyzing for free chlorine. Allow the remaining portion of sample to remain at room temperature for 15 – 30 minutes. Reanalyze the sample. The free chlorine values will usually be lower or entirely gone. This is often interpreted as the free chlorine test not giving reproducible results when, in fact, the free chlorine concentration in the sample has actually changed since the original analysis.

Third, DPD reagents for free chlorine react slowly with other inorganic and organic chlorine species present in the sample. The rate at which they react or “interfere” in the free chlorine determination depends on the form and concentration of these compounds present. The instructions for determining free chlorine using DPD Free Chlorine Reagent are written to read the sample as soon as possible after reagent addition and always to read the sample before 1 minute. Free chlorine reacts immediately with DPD Chlorine Reagent. The reaction time can be extended to one minute to allow any bubbles to dissipate or for particles present in the sample to settle before taking the reading. This effect of interfering compounds is illustrated when a sample is read at 1 minute after reagent addition and then reread again after 5 or 10 minutes. The free chlorine value will be higher after 5 or 10 minutes. This is often interpreted as “My free chlorine value keeps increasing!” This continual drifting higher of the free chlorine value should actually be attributed to interference from the other chlorinated compounds present in the sample and the value obtained at one minute or less is considered most accurate.

Fourth, the physical aspects of the sample such as temperature, background red color, particles and turbidity need to be addressed. The colorimeter or spectrophotometer must always be zeroed on the sample or the sample dilution being analyzed before addition of the DPD reagent. This will automatically subtract the background due to color and turbidity. One precaution should be noted. If bubbles or large particles are present in the sample, these should be allowed to dissipate or settle out before zeroing the instrument.

Fifth, sampling technique must be considered. Contamination from chlorine demand from previous samples will cause low results. Fats and other organics adhere to container and sample cell walls, reacting with any free chlorine present in the sample. Sampling containers should not be reused unless pretreated for chlorine demand. Glass containers are preferred, but often are not allowed in the processing area. Pre-treat the sample containers to remove any chlorine demand by soaking the container in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse each container or cell thoroughly with deionized or organic-free water before using. After testing is completed, rinse the sample cells and sampling containers with deionized water and fill with the weak bleach solution. This will insure that the cells and bottles are ready to use for the next testing period.

These strategies need to be incorporated into all testing protocols in order to develop confidence in the test results and to ensure that the results are useful in making process adjustments and controlling pathogenic bacteria levels.

The procedure below includes a Standard Additions step to help develop confidence in the free chlorine value determined on the chiller water sample. The theory behind the Standard Additions technique is that if a known amount of chlorine is added to a sample and is recovered by analysis, then the original value obtained is likely correct. If the Standard Addition is not recovered, then the original free chlorine value determined has to be assumed to be incorrect or needs to be further investigated.

Procedure: Measuring Free Chlorine in Chiller Water Samples (1:10 dilution method)

1. Collect sample in a clean chlorine demand free container. Rinse the container several times with the sample to be tested. Fill the container to overflowing to exclude any headspace and cap to eliminate exposure to air.
2. Place the container in an ice bath or chilled water bath to maintain sample temperature conditions similar to the chiller bath being tested.
3. Fill a clean instrument sample cell with 9.0 mL of *chilled deionized* or chlorine-demand free water. Use a TenSette Pipet or a serological pipet to measure the deionized water. The sample cell should be chlorine demand free and rinsed several times with the deionized water before filling.
4. Select the LR Chlorine procedure on the Hach instrument being used to measure the chlorine concentration.
5. Using a TenSette Pipet, add 1.0 mL of the chiller water sample to the deionized water in the sample cell. Swirl gently to mix.
6. Place the sample cell into the instrument and **ZERO** the instrument. The instrument display will read: **0.00** mg/L Cl_2. (Allow any bubbles to dissipate or large particles to settle before zeroing.) This step blanks out any background color or turbidity present in the sample.
7. Remove the sample cell and add one DPD Free Chlorine Powder Pillow to the sample. Swirl to dissolve the powder.
8. Place the sample cell back into the instrument and read the chlorine concentration from the display. (Allow any bubbles to dissipate or large particles to settle.) Read before one minute.
9. Multiple the displayed chlorine concentration by 10 (to adjust for the sample dilution) to obtain the free chlorine concentration present in the original sample. Record this value.
10. Note: To validate the free chlorine value obtained in Step 8, the following Standard Addition procedure is recommended for confirming test results.
11. Repeat Steps 1-6 above with a new sample.
12. Open a Chlorine Standard Solution Ampule and using a TenSette Pipet and a new pipet tip add 0.1 mL of chlorine standard to the sample. Swirl gently to mix.
13. Immediately add one DPD Free Chlorine Powder Pillow to the spiked sample. Swirl to mix.
14. Place the sample cell into the instrument and read the chlorine concentration. Record the value.
15. Calculate the concentration of chlorine added by the 0.1 mL standard addition.

$$\text{mg/L Chlorine} = \frac{0.1\text{ mL (vol. of standard added)} \times \text{Certificate value (mg/L } Cl_2)}{10.1\text{ mL (sample + standard)}}$$

16. The spiked sample result obtained (Step 14) should reflect the analyzed sample result (Step 8) plus the added calculated chlorine concentration (Step 15).
17. If the expected chlorine increase is recovered, then the original value calculated in Step 9 is believed to be correct and representative of the free chlorine concentration present at the sampling site in the chiller bath.

Example:

The value determined in Step 8 is 0.20 mg/L Cl_2.

The Chlorine Standard Solution Ampule has a certificate value of 60.6 mg/L Cl_2.

A 0.1 mL standard addition was added to 10 mL of the diluted chiller water sample.

$$\text{mg/L Chlorine} = \frac{0.1\text{ mL} \times 60.6\text{ mg/L } Cl_2}{10.1\text{ mL}} = 0.60\text{ mg/L } Cl_2 \text{ added}$$

The chlorine added by the 0.1 mL standard addition is 0.60 mg/L Cl_2.

Therefore the value obtained on the standard addition sample (Step 14) should be 0.20 + 0.60 = 0.80 mg/L Cl_2.

The value obtained in Step 14 was found to be actually 0.70 mg/L Cl_2. This would be considered an acceptable recovery.

This would support the calculated value in Step 9 of 0.20 x 10 = 2.0 mg/L in the original undiluted chiller water sample.

Problems: Low Recovery of Chlorine Standard Addition

When little or no recovery of the chlorine spike is obtained then the original value obtained on the sample in Step 8 is suspect and likely incorrect. Low values of 0.05 mg/L or less in Step 8 are subject to interference from background color, turbidity or breakthrough of interfering organic chloramines even though every attempt has been made to minimize these effects. It is best to confirm if these low values are correct.

Three troubleshooting procedures can be used to help resolve if free chlorine is present in the original sample.

- Add the 0.1 mL of the Chlorine Standard Solution Ampule to 10 mL of deionized water and analyze for free chlorine. Use the above equation to calculate the amount of chlorine added. Total recovery of the chlorine spike should be achieved which confirms that the instrument, reagents and technique used are all correct and working properly.
- Add 0.2 mL or 0.3 mL of the Chlorine Standard Ampule Solution to 10 mL of the diluted chiller water sample and analyze. Calculate the amount of chlorine added by using the equation and using the 0.2 mL or 0.3 mL factor where required. If little or no free chlorine is recovered in the samples with the additional standard added, then the original value is likely 0 and no free chlorine is present in the chiller water - sample.
- When extremely low values of less than 0.05 mg/L or high values of greater than 2.00 mg/L are obtained in Step 8, use a smaller dilution in Step 5 for low values or a larger dilution for high values. Apply the correct dilution factor used in Step 9.

Reagents:

DPD Free Chlorine Reagent, 100/pk	2105569
Chlorine Standard Solution Ampules 50-75 mg/L Cl_2 20/pk	1426820