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**Ag Health Labs now offering Starch Analysis!**

**April 2009**

After extensive research, multiple consultations, and several modifications to methods we are happy to report we have developed a starch method which is accurate, repeatable and very efficient. Starch analysis is a fairly fickle procedure which requires a great deal of precision and care.

While there are test kits available to simplify the procedure, these are not fool proof and are more costly than the method we are using. The method we are currently using is a Sodium Acetate / Glucose Oxidase Peroxidase Method developed largely by Mary Beth Hall, PhD, at USDA-ARS U.S. Dairy Forage Research Center Madison, Wisconsin. Dr. Hall has been one of, if not the leader in starch and sugar analysis in the US.

**NEW Suspect Myco Test!**

Ag Health Labs is now performing a Digitonin Sensitivity Test to differentiate:  
**Mycoplasma sp.**  
 Vs.  
**Acholeplasma sp.**

**NFTA Certified for 2009**

Ag Health Feed Lab certified once again by the National Forage Testing Association  
 Details on pg. 3

**Suspect Mycos?**

Many of you have already received results for Suspect Mycos on your Mycoplasma culture results. This is a new classification we began using at Ag Health to indicate we are performing a secondary test on a low level positive mycoplasma culture. The secondary test is a new assay, called a **Digitonin Sensitivity Test**, which allows us to differentiate between Mycoplasma organisms and Acholeplasma organisms.



**What is Acholeplasma?**

Acholeplasma is an organism from the family Mycoplasmataceae. This family also includes the genera Mycoplasma. Acholeplasmas are present in the environment and will grow on Mycoplasma agar if present in a contaminated milk sample. The most common isolate in milk is Acholeplasma laidlawii. A. laidlawii is not pathogenic to cattle and does not cause mastitis. These organisms are not contagious to other cows and should be regarded as a contaminant in the milk sample, and not a threat to the cow or her milk quality.

As contaminants, Acholeplasmas are almost always seen in low numbers (one or two plus) and are commonly found on screening samples from non-mastitic cows such as fresh cows. In the past Acholeplasmas could not be differentiated from Mycoplasmas and were  
 See **Digitonin** pg. 2

**Note regarding Pricing:**

Due to rising cost of materials required to perform water analysis we are announcing a price increase for **Colilert** water tests (Coliform presence/ absence) to \$21.00. This price change is effective as of 04/01/09.

We apologize for any inconvenience this may cause during turbulent economic times.

**Nitrate** analysis will remain at the current price of \$30.00.

**Need to get Samples to Ag Health?**

**Walco has agreed to courier samples from outside the Yakima Valley to Ag Health Labs. Talk to your Walco Rep or Ag Health for Details.**

**Contacts:**  
*Fred J. Muller, DVM*  
*Crystal Maiden, BS*

## New Digitonin Sensitivity Test!

therefore identified on cultures as Mycoplasma. This winter we learned from Dr. Allan Britten of a Digitonin Sensitivity Test which can be used to differentiate Acholeplasmas from Mycoplasmas. Over the past 3 months we have been using the Digitonin tests on suspect Myco cultures and validating the results with known samples and Myco typing results from UC Davis and Cornell University. At this point we are confident in the Digitonin test and while no test is 100 percent accurate we have found it very helpful in differentiating Mycoplasma sp. and Acholeplasma laidlawii.

### Why is the Digitonin Sensitivity Test important?

Many producers have had the experience in the past of receiving a + or ++ Mycoplasma culture on a beautiful fresh heifer with no signs of Mastitis. The thought of sending that animal to slaughter did not sit well with many of you, but we had no way to differentiate the positive result from any contaminant or false positive. Many low positive Myco shedders will develop into high shedding mastitis cows and risk contaminating many other cows. Some producers chose to re-test those low positive cows, some thing we discouraged because it's possible for truly positive Myco cows to test negative at times, only to begin shedding again when stressed or during her next calving event. Now we have a test to accurately identify false positive Mycos which are truly samples contaminated with Acholeplasma .

### The Digitonin Sensitivity Test.

### How does the Digitonin Sensitivity test work?

When a low positive Myco culture occurs on a screening sample, we will report the result as a suspect. We then transfer the colony to an enrichment broth for 2 days, followed by replating the broth on a full Myco agar plate with a digitonin disk present on it, culture the plate for 3 days and read the culture on day three. For most suspects it will take 5 days to receive a confirmatory result. On some samples we will incubate them an additional 3 days before reporting a result.

### What does the Digitonin Sensitivity Test cost?

While we have invested a lot of time and money into validating the Digitonin Sensitivity Test, we need to begin charging for the additional test on suspect Mycos. The Digitonin Sensitivity test requires additional medias as well as multiple steps by technicians so it's not cheap at \$12.50 per test. However, retaining a good producing cow that's truly negative for Mycoplasma is a savings of hundreds of dollars.

### How do I prevent Acholeplasma contamination?

We do not currently know what conditions favor growth of Acholeplasma species in the environment. While we may learn more in this area, it's difficult to spend money doing research on an organism which is not pathogenic to cows. *However, we believe the amount of Acholeplasma on the teat at sampling can be dramatically reduced by alcoholing teat ends prior to collecting milk samples.*

Many dairies have stopped alcoholing teats if they are not doing blood agar cultures on cows because the Myco media prevents growth of common organisms like E. coli, or Strep sp.. However, Acholeplasma can still grow as a contaminant from the teat surface. It's worth noting we see many suspect Mycos/Acholeplasmas in some herds and very few in others. I'm confident this is primarily related to sampling technique more than environmental differences.

**See Sample Collection  
Procedures Page 3**

### Other considerations on Suspect Mycos

You should consult your herd veterinarian or Dr. Fred Muller regarding how to handle suspect Mycos, Acholeplasma positives, or other related questions. Each producer may deal with suspects differently while waiting for confirmation. Note that Ag Health Labs will not run Digitonin Sensitivities on high shedding Myco positives or mastitis cows as these are almost always true Mycoplasmas and not contaminants. If you wish for us to run a Digitonin Sensitivity on a positive Myco result please contact us and we can perform the additional test at the additional cost, but we will suggest you discuss the decision with your veterinarian.

# Milk Sample Collection Technique

## Aseptic Milk Sample Collection

### REQUIRED SUPPLIES

- Sterile milk tubes
- Permanent marking pen
- Alcohol and gauze 4x4's
- Disposable latex or nitrile gloves



## Step One: Label milk tube

- Label tube with cow number first with dry hands
- Use a permanent marking pen
- Write collection date



## Step Two: Swab teat with alcohol soaked 4x4

- If composite sample swab far teats first, then near teats
- Concentrate on teat end



## Step Three: Discard 1-2 squirts of milk

- Clears teat canal of potential microbial contaminants
- Helps reduce false positive results
- Do not get milk on glove surface



## Step Four: Take milk sample

- If composite sample take equal amount from each quarter
- Sample near teats first, far teats last
- Do not fill tube more than 3/4 full



## Step Five: Refrigerate or Freeze samples immediately



## Ag Health Labs NFTA Certified for Forage Analysis for 2009!

National Forage Testing Association Certification Grades				
NFTA Sample ID	Dry Matter	Crude Protein	ADF	NDF
2008-AH-01	B	A	B	A
2008-AH-02	A	A	A	A
2008-AH-03	A	A	A	A
2008-GH-04	B	B	A	A
2008-AH-05	C	A	B	A
2008-CS-06	A	B	B	A