



UNITED STATES
SUGAR
CORPORATION

SOUTHERN
GARDENS
CITRUS

Southern Gardens Diagnostic Lab

Southern Gardens Diagnostic Lab

- Partnership between USSC/SG and IFAS
- USSC/SG providing facilities, equipment, oversight, and some labor
- IFAS providing a technician and some supplies
- FCPRAC also supplying some supplies and equipment
- No charge for testing
- Types of samples:
 - Nurseries
 - Groves
 - Research

Southern Gardens Diagnostic Lab

- **Will accept samples from anyone provided:**
- Legal disclaimer is signed by the grove/nursery owner (only need one on file)
- Samples arrive in good condition
- Sample Submission Form is completed

Sampling Protocol for Submission of Huanglongbing (syn= Greening or HLB) Samples to the Southern Gardens Diagnostic Laboratory



For more information contact:

Mike Irely
United States Sugar Corporation
Technical Operations
111 Ponce de Leon Ave.
Clewiston, FL 33440
863-902-2249
msirely@ussugar.com

Southern Gardens Diagnostic Lab

- Results will be sent to:
 - Grove/Nursery owner
 - Division of Plant Industry
 - Other agencies will have to access the data through DPI
- This is necessary to protect confidentiality of grove owners

Sampling Protocol for Submission of Huanglongbing (syn= Greening or HLB) Samples to the Southern Gardens Diagnostic Laboratory



For more information contact:

Mike Irely
United States Sugar Corporation
Technical Operations
111 Ponce de Leon Ave.
Clewiston, FL 33440
863-902-2249
msirey@ussugar.com

Legal Disclaimer

DISCLAIMER, INDEMNIFICATION AND HOLD HARMLESS AGREEMENT

Date: _____ ("Effective Date")

Company Name: _____ ("SUPPLIER")

SUPPLIER Mailing Address: _____

WHEREAS, SUPPLIER desires to have UNITED STATES SUGAR CORPORATION (U.S. SUGAR) conduct diagnostic assays ("PCR TESTING") for the presence of *Xanthomonas* sp., the causal agent of Huanglonging (citrus greening) in citrus samples supplied by the SUPPLIER.

WHEREAS, SUPPLIER is agreeing to assume all responsibility and liability for the PCR TESTING, and to indemnify and hold U.S. SUGAR and its subsidiaries, and its and their officers, directors, employees, agents, and property harmless from all such responsibility and liability in connection with and in exchange for the PCR TESTING pursuant to the provisions of this Agreement.

NOW THEREFORE, in consideration of the mutual covenants and promises contained herein, which the parties acknowledge to be good and valuable consideration for their obligations hereunder, the parties hereby agree as follows:

1. Definitions.

1.1. The term "Proceedings" shall include, without limitation, any threatened, pending, or completed claim, action, suit, or proceeding, whether of a civil, criminal, administrative, or investigative nature, in which U.S. SUGAR or its subsidiaries, or any party or other party, directly or indirectly in connection with any PCR TESTING performed by U.S. SUGAR or on behalf of SUPPLIER, the use by SUPPLIER of the information generated by PCR TESTING, or by reason of any action or inaction of SUPPLIER, its officers, directors, employees, agents, representatives, contractors, or sub-contractors, related in any manner whatsoever to the PCR TESTING.

1.2. The term "Expenses" shall include, without limitation: expenses of investigations, judicial or administrative proceedings or appeals; amount of judgments, fines or penalties; losses, damages; liabilities; amounts paid in settlement by or on behalf of U.S. SUGAR; attorney's fees and disbursements; and any expenses of establishing a right to indemnification under this Agreement.

Page 1 of 4

2. **General Disclaimer, No Representations or Warranties.** PCR TESTING IS A RESEARCH PROTOCOL THAT MAY PRODUCE INCONCLUSIVE RESULTS OR INFORMATION. SHOULD SUPPLIER RELY UPON THE INFORMATION GENERATED BY PCR TESTING, SUPPLIER ACKNOWLEDGES THAT SUPPLIER HAS ASSUMED ALL RISKS AND LIABILITY IN DOING SO. SUPPLIER ACKNOWLEDGES THAT U.S. SUGAR HAS NOT MADE ANY REPRESENTATIONS OR WARRANTIES CONCERNING THE PCR TESTING, THE INFORMATION GENERATED BY PCR TESTING, OR ANY COMPONENT OR CONSTITUTE THEREOF, INCLUDING, WITHOUT LIMITATION, THE COMPLIANCE OF THE PCR TESTING OR ANY PART THEREOF WITH ANY GOVERNMENTAL REQUIREMENTS OR SPECIFICATIONS. SUPPLIER DOES HEREBY DISCLAIM ANY AND ALL WARRANTIES THAT MAY BE DUE FROM U.S. SUGAR TO SUPPLIER. U.S. SUGAR SHALL NOT BE LIABLE TO SUPPLIER, OR ANY OTHER PERSON OR ENTITY, FOR ANY DAMAGES ARISING FROM, RELATED TO, OR CAUSED BY THE PCR TESTING AND/OR THE USE OF THE INFORMATION GENERATED BY PCR TESTING, INCLUDING BUT NOT LIMITED TO, ANY INCIDENTAL, SPECIAL, CONSEQUENTIAL, OR PUNITIVE DAMAGES. U.S. SUGAR MAKES NO WARRANTIES, EXPRESS OR IMPLIED, REGARDING THE VIABILITY OF ANY MATERIALS SUBMITTED OR FURNISHED TO U.S. SUGAR PURSUANT HERETO AND STORED AT U.S. SUGAR'S FACILITIES.

3. **Assumption of Liability and Indemnification.** SUPPLIER shall indemnify and hold U.S. SUGAR and its subsidiaries, and its and their officers, directors, employees, representatives, and property free and harmless from any and all Expenses arising out of, resulting from, in connection with, or related in any manner whatsoever to any Proceedings.

4. **Right of Indemnification.** Any indemnification hereunder shall be made no later than thirty (30) days after receipt by SUPPLIER of a written request from U.S. SUGAR.

5. **Enforcement.** The right to indemnification as provided for in this Agreement shall be enforceable by U.S. SUGAR in an action in any court of competent jurisdiction. In such an action, the burden of proving that indemnification is not required hereunder shall be on SUPPLIER. The termination of any Proceedings by judgment, order, or settlement, whether with or without court approval, shall not create a presumption that indemnification hereunder is not permitted by applicable law. The indemnification provided under this Agreement shall not be deemed exclusive of any other rights to which U.S. SUGAR may be entitled.

6. **Savings Clause.** If this Agreement or any portion hereof shall be invalidated on any ground by any court of competent jurisdiction, SUPPLIER shall nevertheless indemnify and hold U.S. SUGAR and its subsidiaries, and its and their officers, directors, employees, representatives, and property harmless as to Expenses with respect to any Proceedings to the full extent permitted by any applicable portion of this Agreement that shall not have been invalidated.

7. **No Advertising.** SUPPLIER agrees that the use of information generated by PCR TESTING is for SUPPLIER'S use only. SUPPLIER shall not identify or use the information generated by PCR TESTING or the name or logo of U.S. SUGAR or its subsidiaries, or its and their officers, directors, employees, or representatives in any advertisement, public announcement, marketing material, or other similar documentation.

Page 2 of 4

8. **Storage of Samples Submitted.** U.S. SUGAR shall not retain samples submitted for PCR TESTING longer than two (2) weeks after the completion of testing. Upon expiration of said storage period, all such samples shall be destroyed and U.S. SUGAR shall have no liability whatsoever for such destruction.

9. **Breach.** Breach of any terms contained herein or failure by SUPPLIER to perform any covenant, condition, or agreement contained in this Agreement shall be considered a default and shall entitle U.S. SUGAR to immediately pursue any remedy available hereunder or other remedy available at law or in equity; provided, however, that failure of U.S. SUGAR to enforce any default shall not be considered a waiver of the requirement, or the enforceability of this Agreement or any of its terms.

10. **Successors.** This Agreement shall be binding on and shall inure to the benefit of the heirs, executors, administrators, successors, and assigns of the parties hereto; provided, however, that SUPPLIER may not assign its rights and obligations hereunder without the prior written consent of U.S. SUGAR, which consent may be withheld in U.S. SUGAR'S sole discretion. Additionally, in the event of a merger, consolidation, or reorganization in which SUPPLIER is not the surviving entity, any sale of all or substantially all of the assets of SUPPLIER, or any liquidation of SUPPLIER, SUPPLIER shall have the obligations of SUPPLIER under this Agreement expressly assumed by the survivor, purchaser, or successor, as the case may be, or SUPPLIER shall otherwise adequately provide for the satisfaction of SUPPLIER'S obligations under this Agreement in a manner acceptable to U.S. SUGAR, in U.S. SUGAR'S sole discretion.

11. **Governing Law and Jurisdiction.** The terms and provisions of this Agreement shall be construed and enforced in accordance with the laws of the State of Florida. If a dispute shall arise between the parties hereto under this Agreement, the parties voluntarily submit to the jurisdiction of the state and federal courts in Florida.

12. **Entire Agreement.** This Agreement sets forth the entire understanding between the parties hereto regarding the subject matter contained herein.

13. **Notice.** All notices required under this Agreement shall be deemed duly given if in writing and delivered by courier or certified mail, return receipt requested as follows:

If to U.S. SUGAR: c/o Legal Affairs Department, United States Sugar Corporation
111 Ponce de Leon Avenue, Cleveland, Florida 33440

If to SUPPLIER: At the mailing address listed hereinabove.

Each party has a duty to report to the other parties any changes in their mailing address.

14. **Amendment.** No modification or amendment of this Agreement shall be valid unless in writing and signed by both parties.

Page 3 of 4

15. **Full Power and Authority.** The signatories to this Agreement represent and warrant, each to the other, that the signatory: (a) has read and understands this Agreement; (b) has full authority to bind the party to this Agreement represented by the signatory and that the party has full authority to enter into this Agreement and be bound by the terms and conditions of this Agreement; and (c) sets the signatory's hand hereunto with the intention of legally binding the party to this Agreement represented by the signatory.

16. **Survival.** The provisions in paragraphs 2, 3, 7, and 10 of this Agreement shall survive the expiration or termination of this Agreement.

17. **Counterparts.** This Agreement may be executed in any number of identical counterparts. If so executed, each of such counterparts is to be deemed an original for all purposes and all such counterparts shall, collectively, constitute one agreement, but, in making proof of this Agreement, it shall not be necessary to produce or account for more of such counterparts than are required to show that each party hereto executed at least one such counterpart. A facsimile or other reproduction of this Agreement may be executed by the parties, in counterparts or otherwise, and shall be considered valid, binding, and effective for all purposes.

UNITED STATES SUGAR CORPORATION

By: _____

Print Name: _____

Title: _____

SUPPLIER

By: _____

Print Name: _____

Title: _____

Page 4 of 4

- 4 pages of legal language to protect USSC/SG and the people doing the testing
- Unfortunately a necessity
- Only one needs to be on file for each owner

Sample Submission Form

- Needs to be complete
- If many samples from same area, suggest filling out basic info and making copies
- If many samples from same location, fill one out completely and rest can just have block, row, tree, section/township/range
- Goal is to collect enough info to go back to the specific tree if possible at a later date.

Huanglongbing (Greening) Sample Submission Form (▶ Required Information)

Submission Information

▶ Date Sample Collected: _____ ▶ Date Sent: _____
▶ Submitter name: _____ Affiliation: _____
▶ Address: _____
▶ City: _____ ▶ State: _____ ▶ Zipcode: _____
Email address: _____
Phone Number: _____ Fax Number: _____
▶ Results To Be Returned By: Mail Email Fax

Grove/Nursery/Sample Information

▶ Grove/Nursery Name: _____
▶ Address/Location/Map Section/Map Collected: _____

**Need STR and either GPS
or block, row and tree!**

**Please write
STR in correct
order!**

Time of Year

Sampling Protocol for Submission of Samples to the Southern Gardens Diagnostic Laboratory

Collection of Samples

Time of Year

1. If possible, samples should be collected from September through March although samples will be accepted throughout the year. *Samples collected during non-optimal times of the year may not give an accurate representation of the disease status of the trees sampled.*
2. Avoid collecting samples during leaf flushes (stop when feather flushes are approximately 2-3 in long, resume sampling when leaves from the most recent flush are fully expanded and beginning to harden off).

Asymptomatic Field Trees

What Tissue to Sample

Asymptomatic Field Trees (no symptoms at all on the trees):

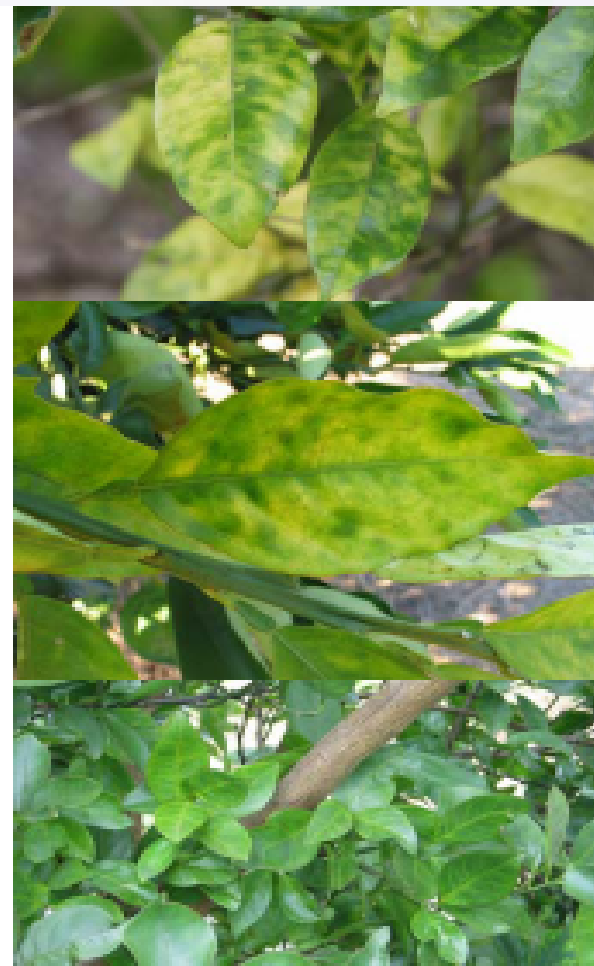
1. Samples should be collected from branches that are dark green and angular to slightly rounded in cross section. The bark should be green and not brown.
2. Leaves from the sampled branch/twig should be fully expanded and hardened.
3. Collect 6-8 leaves (including the leaf petiole) from around the tree.
4. Alternatively, cut 6-8 branches (at least 4 inches long) and trim them so that only the angular (to slightly rounded), dark green section of the branch/twig containing fully expanded hardened leaves is left. Leave the leaves on the twig.
5. Place the leaves/twigs into a sealable (e.g. zip lock) plastic bag and keep the sample cool and out of the sunlight.



Rounded Angular

Symptomatic Field Trees

3. If a variety of symptoms are present, the preferred samples (in order of preference) would be:
 - a. Branches with mottled leaves
 - b. Branches that contain shoots that are almost entirely yellow
 - c. Branches that have leaves with yellow veins
 - d. Branches with leaves that have either green islands on a yellow background or yellow islands on a green background
 - e. Branches with nutrient deficiencies that have a "rabbit ear" appearance (small, upright leaves)
 - f. Branches with leaves that show chlorosis and "vein corking"
 - g. Branches with zinc and/or iron deficiencies that are not related to blight or other known causes
4. Place the leaves/twigs into a sealable (e.g. ziplock) plastic bag and keep the sample cool and out of the sunlight.



Asymptomatic Budwood Trees

Asymptomatic Budwood Trees (scion trees, either planted in-ground or in large pots)

1. Samples should be collected from branches that are dark green and angular to slightly rounded in cross section. Essentially the samples should come from the same area of the tree where you would cut budwood that would be suitable for use in liners.
2. Leaves from the sampled location should be fully expanded and hardened.
3. Cut 4-6 branches and trim them so that only the angular (to slightly rounded), dark green section of the branch/twig is left (at least 4"). Leaves can be left on or removed.
4. Place the leaves/twigs into a sealable (e.g. ziplock) plastic bag and keep the sample cool and out of the sunlight.

Nursery Increase Blocks

Nursery Increase Blocks

1. Samples from nursery increase blocks should consist of fully expanded and hardened leaves.
2. Leaves should be collected from branches that are dark green and angular to slightly rounded in cross section. The samples should come from the same area of the tree where you would cut budwood that would be suitable for use in liners.
3. Approximately 10% of the plants should be sampled. Samples should be more or less evenly distributed across the entire increase block. If there are symptomatic plants, it is permissible to sample this area more intensively.
4. One leaf per plant should be collected and all leaves from each individual nursery increase block can be combined into one bag and submitted as a single sample.
5. Place the leaves into a sealable (e.g. zip lock) plastic bag and keep the sample cool and out of the sunlight.

Sample Handling and Shipping

Sample Handling and Shipping

1. Each sample should be in an individual sealable plastic bag (zip lock).
2. A completed sample submission form should be included in the bag (or stapled) with each sample
3. Samples should be double bagged.
 - a. When multiple samples are being submitted, multiple samples can be placed in one or more larger sealable bags, however each individual sample needs to be bagged individually and have its own individual completed sample submission form attached.
 - b. If a large number of samples are being submitted from a nursery (i.e. a nursery submission from an increase block) it is permissible to use one form to cover each bench/block.
4. Samples should be kept cool and out of direct sunlight until shipping (i.e. cooler with ice, cooler or box w/o ice kept in the shade, etc.).
5. If at all possible, the samples should be shipped or hand delivered the same day that they are collected. If same day shipment/delivery is not possible, samples should be shipped/delivered the following day provided the samples are kept cool (but not frozen).
6. **Samples should be shipped by overnight mail or courier and must not be shipped or held over the weekend.**
7. Sample shipment/delivery can and should be coordinated with Mike Irey (msirey@ussugar.com, 863-902-2249).
8. If samples are received in poor condition, the samples will not be processed and the submitter will be asked to re-submit the samples.

Sample Submission - Recap

- Select the most symptomatic tissue on the tree to submit
 - Most cases – leaves
 - Budwood trees - budsticks
- Break or cut the shoot off (5-10 leaves) and place in a ziplock bag. Then double bag (don't have to double bag each sample)
- Label the bag (block, row/tree or id number)
- Keep the sample cool (can store in refrigerator up 2 weeks)

Sample Submission - Recap

- Fill out the sample sheet (legible and complete)
- If submitting many samples per block (STR), don't need a form for each sample, just one for each block
- If sampling many blocks, keep all samples from each block together (each sample in an individual bag [properly labeled], all samples from one block then in one bag [i.e. double bagged], with 1 submission form for each block)
- Ship overnight (FedEx, UPS) or hand deliver
- Include disclaimer if first time submitting samples

Reporting of Results

Reporting of Results

1. Results will be sent directly to the grove owner/caretaker of the grove from which the samples were collected, even if samples were collected and submitted by a second party.
2. All test results will be made available to the Florida Department of Agriculture and Consumer Services-Division of Plant Industry, the Florida Department of Agriculture and Consumer Services-Bureau of Budwood Registration, and the United States Department of Agriculture.
3. Results will not be released to any other agencies or individuals.

Interpretation of Results

Interpretation of Results

1. Test results will be reported as falling in one of three categories:
 - a. HLB positive – test results indicate that *Candidatus Liberibacter* sp. was detected in the sample.
 - b. No HLB found – test results did not indicate that *Candidatus Liberibacter* sp. was present in the sample.
 - c. Inconclusive/retest- test results were inconclusive, therefore re-testing should be considered

Real-Time Polymerase Chain Reaction = RTPCR

- DNA based
- Not a trivial process
- Requires specialized equipment
 - Expensive
 - Not available in all labs
- Requires more lab experience than some of the more common tests
- Reagents are expensive
- Cost (depends on lab throughput) - \$5.74/sample not including capital costs, depreciation, or overhead costs

Process - Sample delivery and logging

- Sample delivery
 - In person
 - Overnight courier (prefer FedEx, then UPS)
- Try to have them arrive Tuesday through Friday, avoid weekends and holidays
- Bagged AND WITH PROPER PAPERWORK and disclaimer
 - Legible
 - Pencil or ball point pen (not roller ball)
 - Electronic

Huanglongbing (Greening) Sample Submission Form
(▶ Required Information)

Submission Information

▶ Date Sample Collected: 3/12/2008 ▶ Date Sent: 3/17/2008
▶ Submitter name: Holly Chamberlain Affiliation: AGRI-DEL/ INC
▶ Address: 1025 CR 17 N

▶ City: Lake Placid ▶ State: FL ▶ Zipcode: 33852
Email address: hlchamberlain@embarqmail.com
Phone Number: 863-990-7268 Fax Number: 863-465-7301
▶ Results To Be Returned By: Mail Email Fax

Grove/Nursery/Sample Information

▶ Grove/Nursery Name: Nursery wide/ Old Grove/ Small Groves
▶ Address/Location Where Specimen Was Collected: Kelly Rd & SR 27
▶ City: Lake Placid ▶ County: Highlands
▶ Block/Row/Tree Designation (must provide enough information to be able to locate the specific tree sampled): Rows: 2, 20
Latitude: _____ Longitude: _____
▶ Section/Township/Range: 17/38/30 Sample Id (local id): _____
▶ Host Plant Name/Variety: Valencia
Tree Age Tree size Symptomatic
Additional Comments: _____

Do not write in this area
Date Received: 3-18-08 Lab Id: 39544-39545

Process - Sample delivery and logging

PDM SCOUTING SERVICE

SMOAK GROVES
OLD GROVE AREA

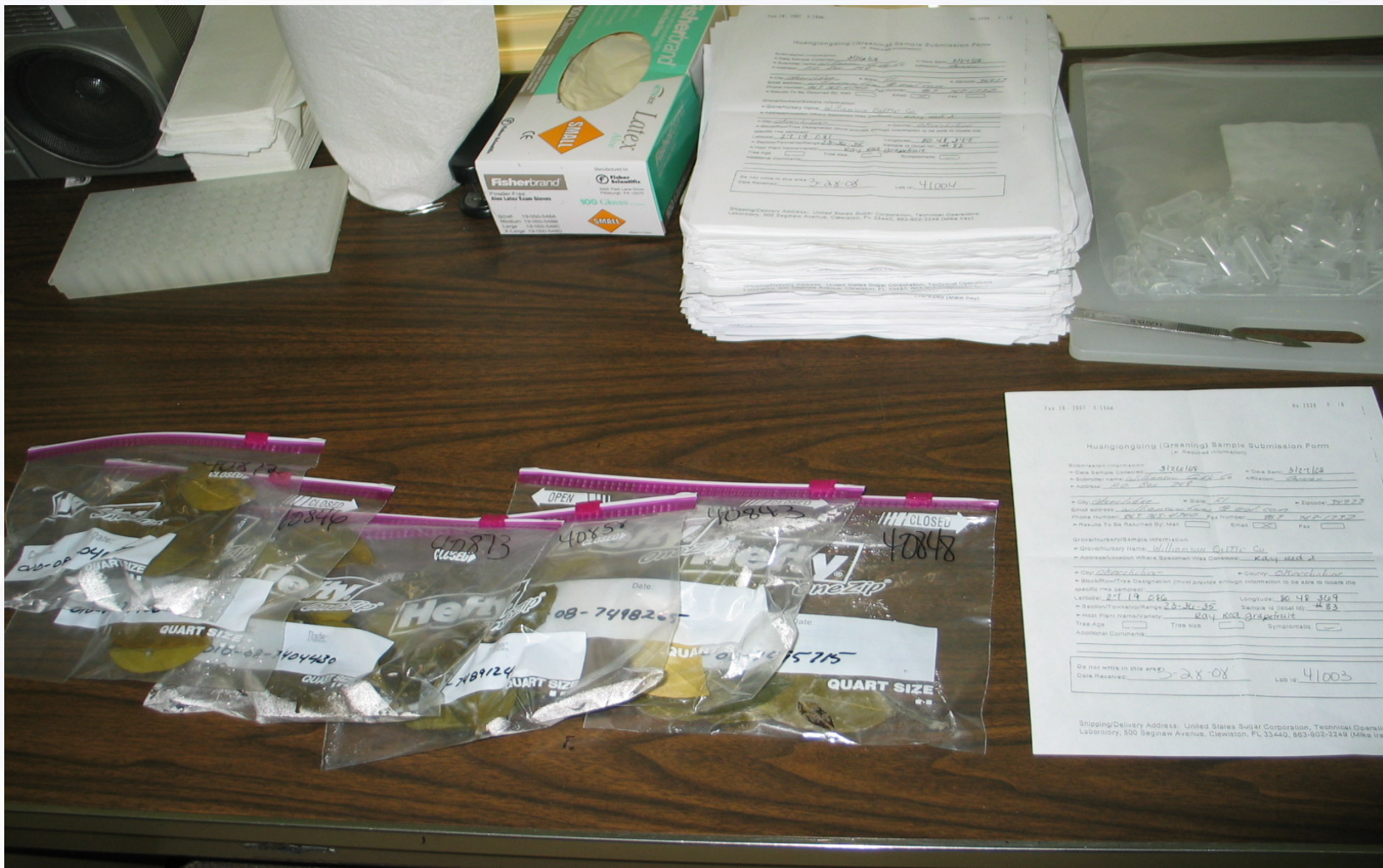
2008

Farm	Field	Block	Rating	ID	LAND ID	Inspector	Row#	Tree#
Smoak Groves	Old Grove (1)	Nursery Wide	Sampled	0312081201558	17-38-30	Norma	20	38
Smoak Groves	Old Grove (2)	Nursery Wide	Sampled	0312081201557	17-38-30	Elva	2	9

(1) 39544

(2) 39545

Process – The sample itself.....

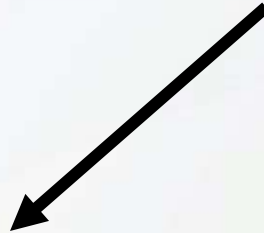


Sample processing

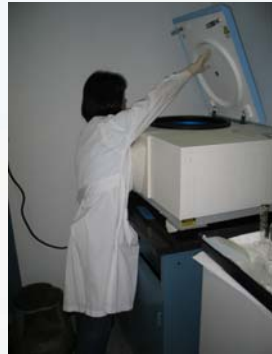


Frozen until processed
180 mg of tissue - petioles

Sample processing



DNA Extraction

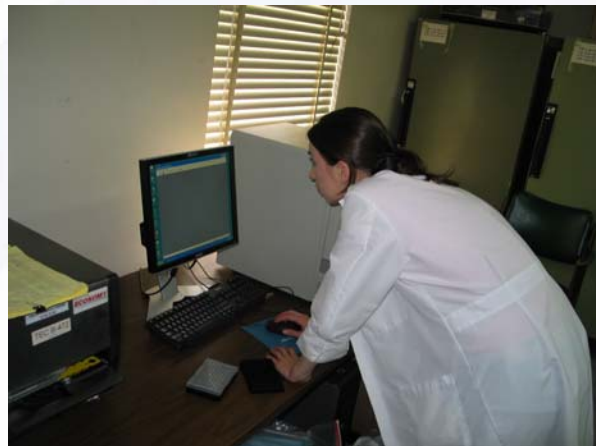


- Add detergent
- Incubate at 60 C
- Add potassium acetate
- Incubate at 4C
- Centrifuge
- Remove supernatant
- Add ice cold isopropanol to precipitate DNA
- Centrifuge
- Wash with 70% ethanol
- Dry DNA pellet
- Dissolve DNA in water

Completed Sample Prep



Real Time PCR

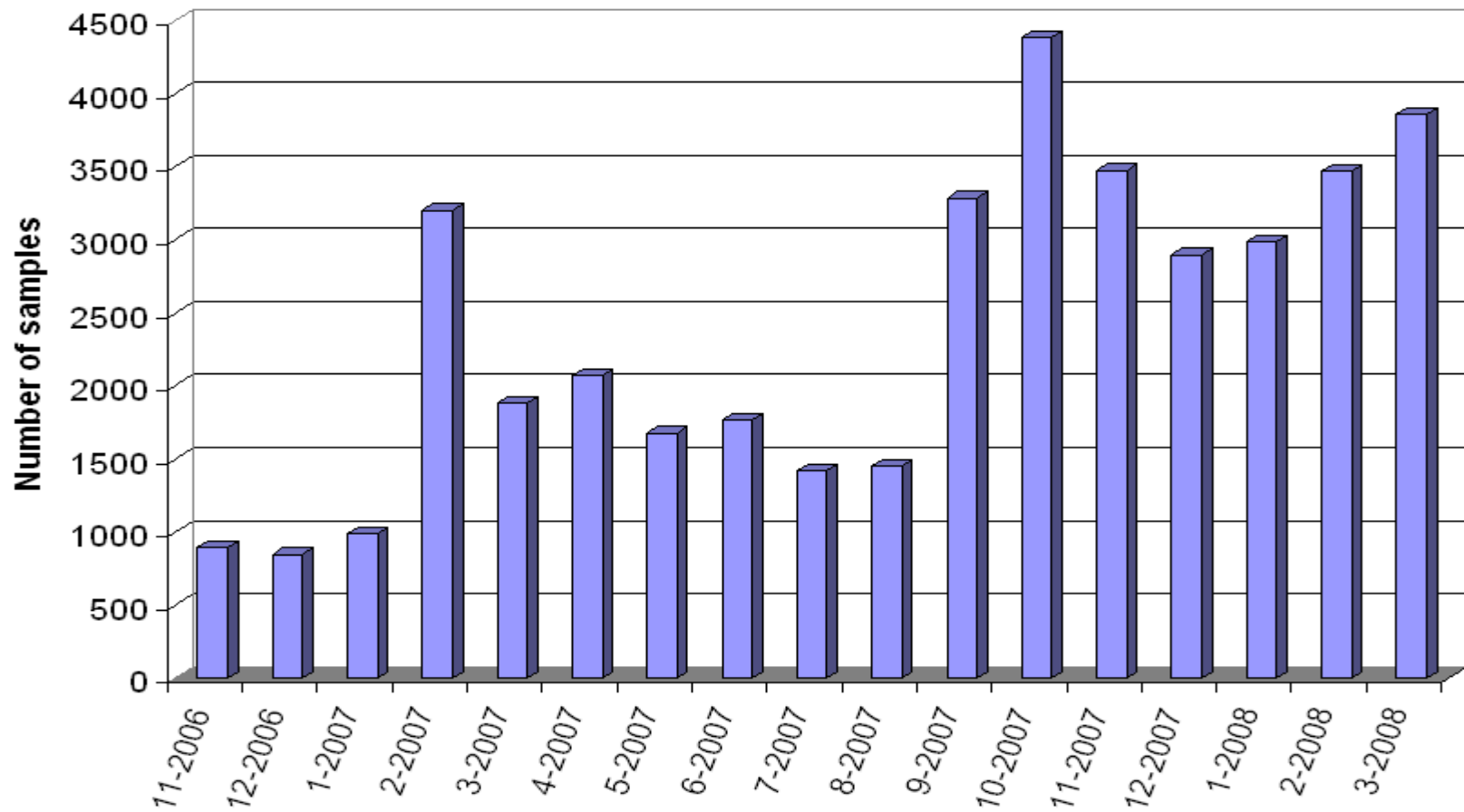


Can't test every tree in the grove
Please be reasonable with samples

Number of Samples Processed

- To date: 40,545 grower samples + ~15,000 internal samples

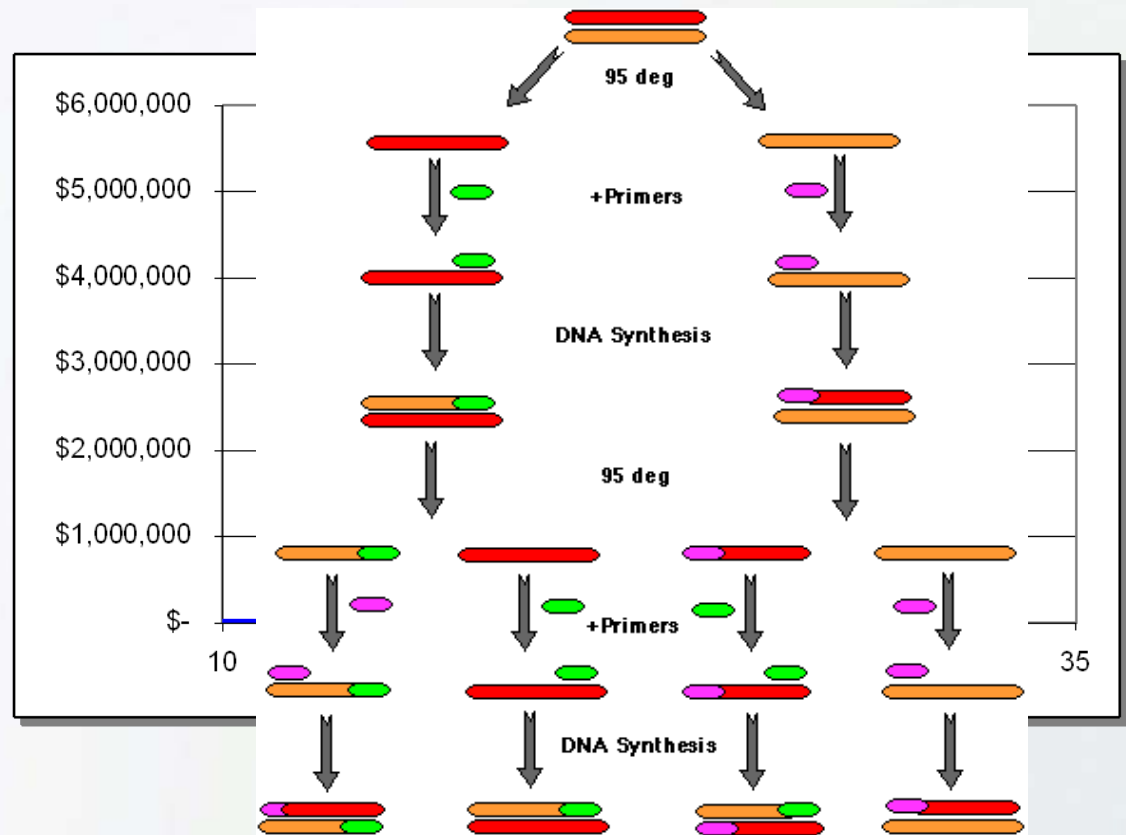
Number of Grower Samples Tested per Month



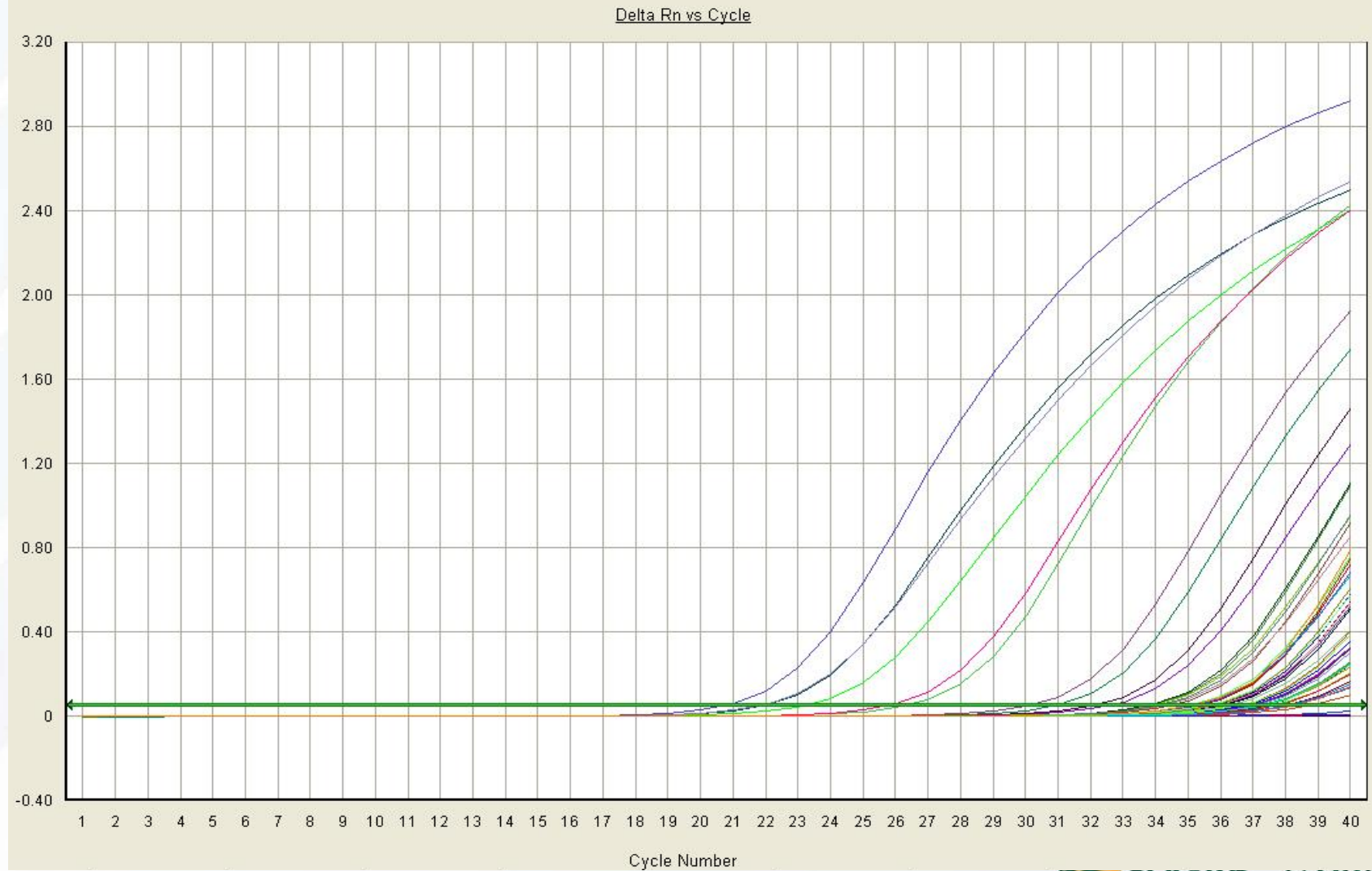
A Penny a Day and then Doubled....

Would you rather have a check for \$100K or \$0.01 to start and then doubled each day for 30 days?

Day	Amount
1	\$ 0.01
2	\$ 0.02
3	\$ 0.04
4	\$ 0.08
5	\$ 0.16
6	\$ 0.32
7	\$ 0.64
8	\$ 1.28
9	\$ 2.56
10	\$ 5.12
11	\$ 10.24
12	\$ 20.48
13	\$ 40.96
14	\$ 81.92
15	\$ 163.84
16	\$ 327.68
17	\$ 655.36
18	\$ 1,310.72
19	\$ 2,621.44
20	\$ 5,242.88
21	\$ 10,485.76
22	\$ 20,971.52
23	\$ 41,943.04
24	\$ 83,886.08
25	\$ 167,772.16
26	\$ 335,544.32
27	\$ 671,088.64
28	\$ 1,342,177.28
29	\$ 2,684,354.56
30	\$ 5,368,709.12



Real Time PCR



Lab Report

HLB Testing Report

Fax:
Email:

Assay Date Range:

4/11/2007 to 4/11/2007

USSC/Southern Gardens Diagnostic Lab

County

Diagnosis		HLB Positive							
Block	Row	Tree	CT	Local Id:	Lab Id	Collected	Received	Assayed	Sample submitted by:
	5	70	22.50	03-29-63	7741	3/27/2007	4/4/2007	4/11/2007	
	8	56	25.36	03-29-63	7746	3/27/2007	4/4/2007	4/11/2007	
	8	58	23.14	03-26-07	7745	3/27/2007	4/4/2007	4/11/2007	
	11	71	26.66	03-29-63	7742	3/27/2007	4/4/2007	4/11/2007	

Diagnosis		No HLB Found							
Block	Row	Tree	CT	Local Id:	Lab Id	Collected	Received	Assayed	Sample submitted by:
	1	82	33.83	03-09-29	7743	3/27/2007	4/4/2007	4/11/2007	
	12	74	39.87	03-29-24	7740	3/27/2007	4/4/2007	4/11/2007	

Diagnosis		Questionable							
Block	Row	Tree	CT	Local Id:	Lab Id	Collected	Received	Assayed	Sample submitted by:
	3	19	31.33	03-29-37	7744	3/27/2007	4/4/2007	4/11/2007	

Number Positive 4

Number Questionable 1

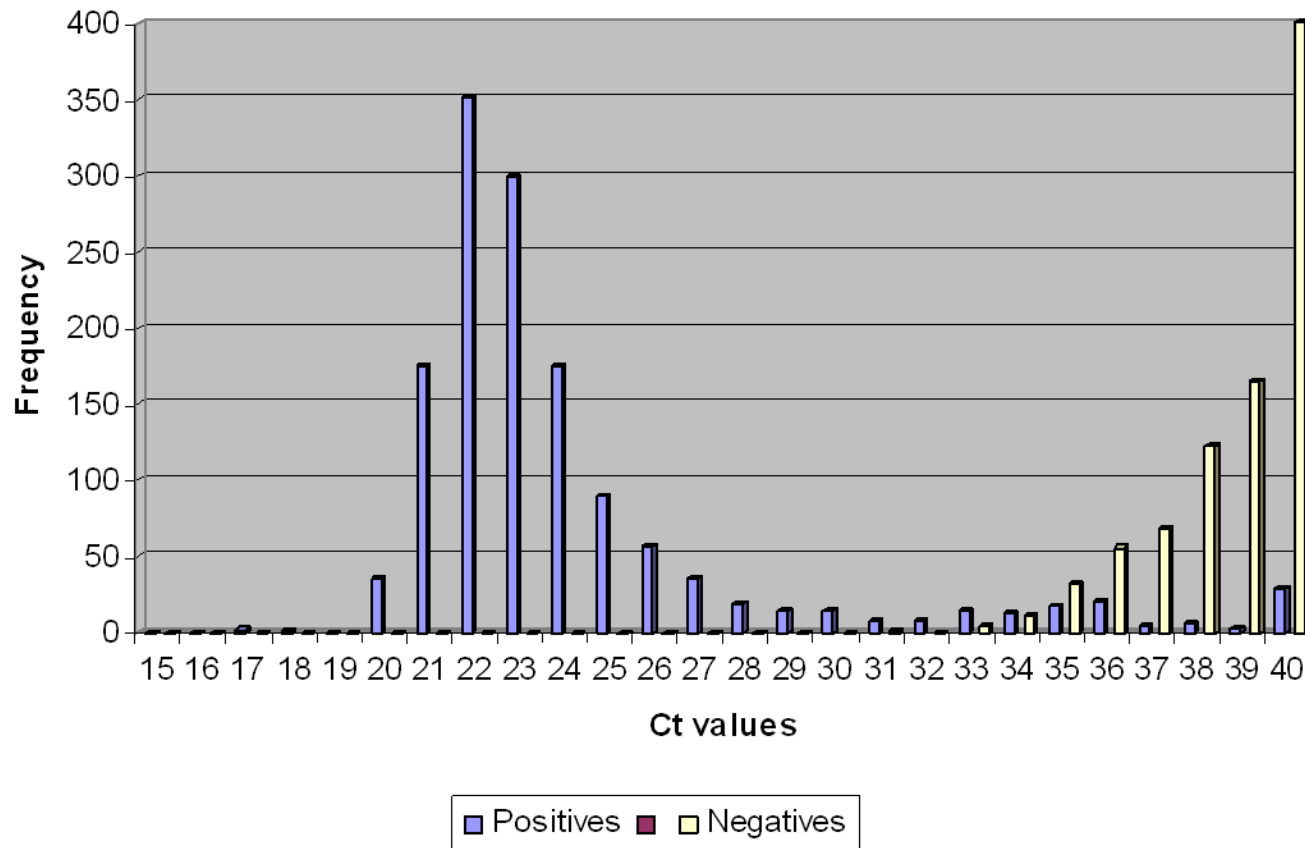
Total samples 7

Total Positive (+ and questionable) 71.4%

CT < 30 Positive
CT between 30-32 Questionable
CT > 32 No HLB Found

Threshold Determination

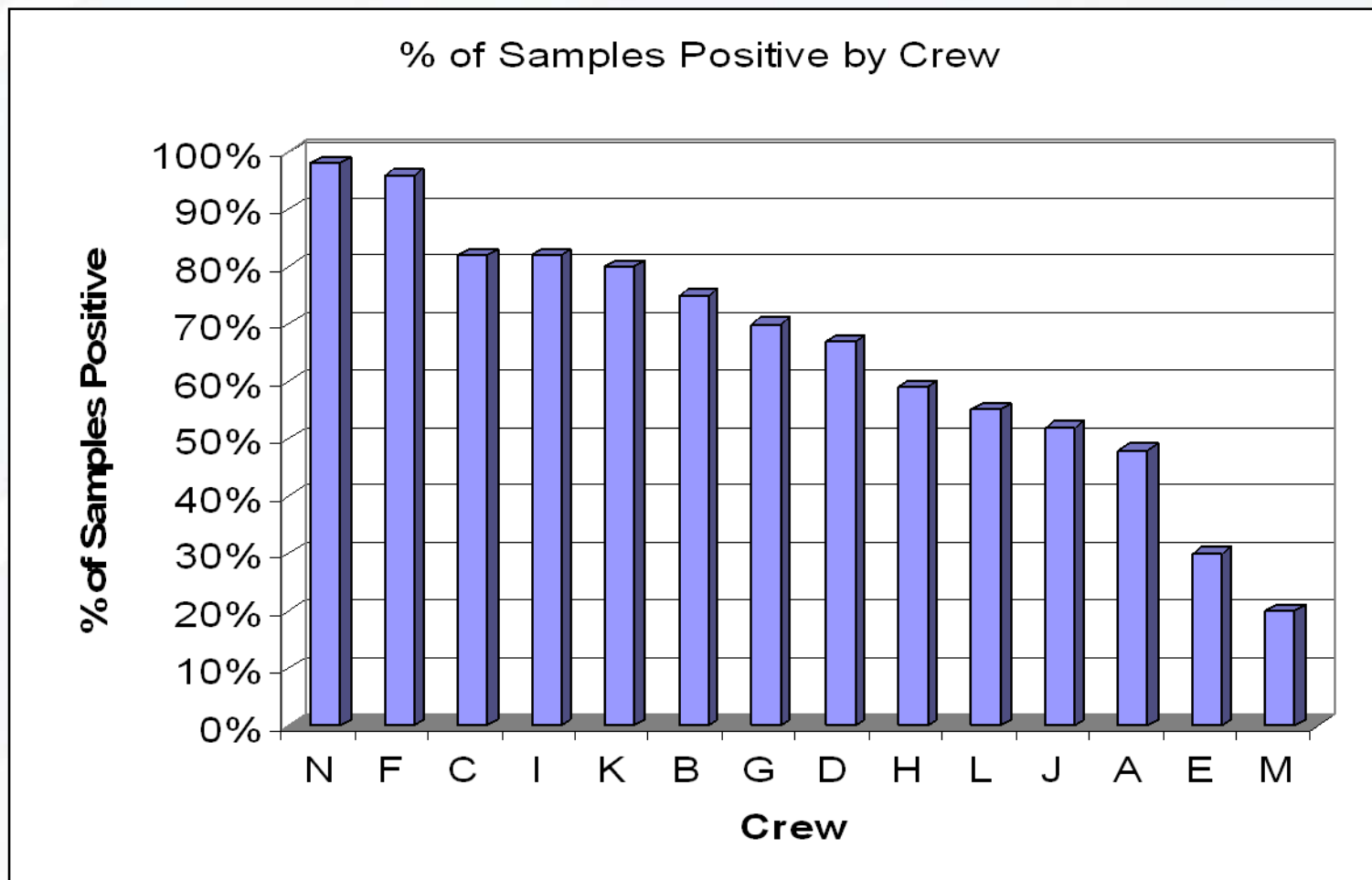
Distribution of CT values



Reasons to Send in Samples

- Determine if you have HLB – get on a control/management program
- Train your scouts
- Quality control of the scouting crews
- Research

Differences Between Crews and Areas



Questions

- Contact Information:

Mike Irely

United States Sugar Corporation

Technical Operations

111 Ponce de Leon Ave.

Clewiston, FL 33440

863-902-2249

863-233-1581 (cell)

msirey@ussugar.com

- New Shipping Address:

Technical Operations Laboratory

United States Sugar Corporation

1500 South W.C. Owen Ave

Clewiston, FL, 33440

<http://www.flcitrusmutual.com/files/712432d4-2860-44d1-a.pdf>

<http://www.hccga.com/posgreen/061221mikeirey.pdf>