

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



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## **Sampling Protocol for Submission of Huanglongbing (HLB) Samples to the Southern Gardens Diagnostic Laboratory**

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## Sample Documentation

1. **No sample will be accepted without a properly filled out sample submission form!**
2. **No samples will be accepted or processed without the submission of a properly executed Disclaimer and Hold Harmless Agreement.** Only one Disclaimer and Hold Harmless Agreement needs to be submitted for each organization/grove/nursery that is submitting samples (i.e. once the Disclaimer is on file, an unlimited number of samples can be submitted under the agreement for that organization). Either include the Disclaimer in with the first set of samples or send it to:  

**Mike Irey  
United States Sugar Corporation  
Technical Operations  
111 Ponce de Leon Ave.  
Clewiston, FL 33440  
863-902-2272 fax**
3. Enough information must be provided in the “address where specimen was collected” and the “block/nursery designation” portions of the form to enable a state inspector to locate the row and tree or nursery block that was tested should subsequent testing by official sources be required.

- 4. The Township, Range, and Section numbers MUST be included on the sample submission form.**
  
- 5. Although not a requirement, we would like to have sample locations documented by GPS coordinates.**

# 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).

### *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**

1. Samples should be collected from the symptomatic areas/branches of the trees.
2. Samples should consist of short sections (4-6 inches or greater) of symptomatic branches with the attached leaves. If fruit are present on the branches, the fruit can either be left on or they can be trimmed off. If the fruit

- are trimmed off, please leave the fruit stem on the sample (i.e. trim the fruit off as close to the button as possible leaving the stem on the branch).
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 (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**

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**

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](mailto: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.

### **Shipping/Delivery Address:**

United States Sugar Corporation  
Technical Operations  
Attention: Mike Irey  
111 Ponce de Leon Ave  
Clewiston, FL 33440

If using Fed Ex, samples can also be shipped to:

United States Sugar Corporation  
Technical Operations Laboratory  
500 Saginaw Avenue  
Clewiston, FL 33440

### **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**

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. HLB Questionable- test results were inconclusive, therefore re-testing should be considered
2. No testing procedure is completely accurate. Therefore if a sample is designated as “No HLB Found”, this does not mean that the tree/plant from which the sample was taken is disease-free. A “No HLB Found” designation



means only that no *Candidatus Liberibacter* was detected in the sample. This could be due to several reasons including but not limited to:

- a. No *Candidatus Liberibacter* was present.
- b. *Candidatus Liberibacter* was present but below the limit of detection.
- c. *Candidatus Liberibacter* was present but the sample was inadequate for testing (sample in poor condition, wrong type of tissue sampled, wrong time of year, etc.).
- d. The test failed.

## Summary of Samples by Tree Type

<b>Type of Tree</b>	<b>Desired Sample</b>
Field tree – Asymptomatic	Leaves or twigs randomly collected around tree
Field tree – Symptomatic	Leaves or twigs from affected area of tree
Budwood tree	Twigs randomly collected around the tree
Nursery increase blocks	Leaves randomly collected across block

# 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 Where Specimen Was Collected: \_\_\_\_\_

▶ City: \_\_\_\_\_ ▶ County: \_\_\_\_\_

▶ Block/Row/Tree Designation (*must provide enough information to be able to locate the specific tree sampled*): \_\_\_\_\_

Latitude: \_\_\_\_\_ Longitude: \_\_\_\_\_

▶ Section/Township/Range: \_\_\_\_\_ Sample Id (local id): \_\_\_\_\_

▶ Host Plant Name/Variety: \_\_\_\_\_

Tree Age  Tree size  Symptomatic

Additional Comments: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

***Do not write in this area***

Date Received: \_\_\_\_\_ Lab Id: \_\_\_\_\_

Shipping/Delivery Address: United States Sugar Corporation, Technical Operations  
111 Ponce de Leon Ave, Clewiston, FL 33440, 863-902-2249 (Mike Irey)

# DISCLAIMER, INDEMNIFICATION AND HOLD HARMLESS AGREEMENT

Date: \_\_\_\_\_ (“Effective Date”)

Company Name: \_\_\_\_\_ (“SUPPLIER”).

SUPPLIER Mailing Address: \_\_\_\_\_  
\_\_\_\_\_

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WHEREAS, SUPPLIER desires to have UNITED STATES SUGAR CORPORATION (U.S. SUGAR) conduct diagnostic assays (“PCR TESTING”) for the presence of *Liberibacter* sp., the causal agent of HuanglongBing (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 is or may have been involved as a party or otherwise, directly or indirectly in connection with any PCR TESTING performed by U.S. SUGAR for 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; attorneys’ fees and disbursements; and any expenses of establishing a right to indemnification under this Agreement.

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 CONSTITUENT 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 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.

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. Notices. 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, Clewiston, 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.

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: \_\_\_\_\_