Oct 8, 2013

Dear Participant,

The 2013 Proficiency Test (PT) panel for certification of diagnosticians/labs conducting molecular identification of the pathogen causing Huanglongbing (HLB), also known as Citrus Greening, in regulatory samples is being prepared. There are two differences to note concerning 2013 PT and testing: 1). The current Work Instruction for screening testing of HLB (WI-B-T-D-2) has been revised to combine *Candidatus* Liberibacter asiaticus and L. americanus tests into a single multiplex reaction. This WI can be accessed by registration to the CPHST-Beltsville Work Instruction document website (www.nahln.org). A separate WI for processing and testing of the PT panel samples will be provided to all participants during PT distribution. The associated WIs for DNA extraction of plant tissue samples for the PT and for USDA regulatory samples are currently unchanged. 2). HLB 2013 PT distribution will occur from mid-November 2013 through February, 2014.

The USDA HLB PT program began in 2007 in support of PPQ Citrus Health Response Program. Like the earlier years, the 2013 HLB PT panel consists of DNA samples requiring PCR analysis and lyophilized tissue samples requiring DNA extraction and PCR analysis. Participating laboratories should possess a valid USDA permit for interstate diagnostic samples to receive the PT panel. Information and applications for permits can be viewed at the following USDA-APHIS-PPQ website: http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml. When sent, HLB positive and healthy DNA controls for PCR will be provided for use with each panel.

To ensure the PT panels are of sufficient quality prior to shipment, the panel samples have been extensively tested according to USDA-approved diagnostic protocols. Individual, randomly selected aliquots of each DNA panel sample are tested periodically to ensure DNA stability. DNA is also extracted from randomly selected lyophilized tissue samples at regular time intervals and tested by PCR to ensure tissue stability and uniformity.

It is suggested that primers/probes/reagents (see tables below) are ordered in advance so that all required reagents are on hand before panel receipt. Please be aware that fluorescent probes can take up to14 days to receive. For those who have been previously HLB certified, it is strongly suggested that a new set of primers and probes is ordered, since degradation is one of the major reasons for failure or low efficiency of PCR. Purchase of a new DNeasy® Plant Mini Kit (Qiagen® cat. # 69104) is strongly recommended since using expired kit components contributes to low DNA extraction efficiencies, often resulting in out-of-range real-time PCR values and faint or missing conventional PCR product bands.

Please Note: PT Work Instructions will be provided with the panel. While these PT Work Instructions use the same probes/ primers and reagents as the Work Instructions for regulatory samples, the PT Work

Instructions include additional guidelines for handling and information specific to the processing of PT panel samples only. Likewise the PT work instructions should not be used for testing field samples.

In addition to the PT Work Instructions, results reporting forms will be provided electronically for PT results submission. Panel results submitted in an unacceptable format (other than the reporting forms provided) will be returned for reformatting and resubmission, thereby delaying the results evaluation. Upon receiving properly submitted results, panel results evaluation and prompt feedback will be provided by NPPLAP within one month.

Please, use the attached form to request a panel (s). Each individual participant should choose a preferred month (mid-November through February) to receive a panel(s), fill in the attached form and promptly return the completed form by e-mail to Vessela.A.Mavrodieva@aphis.usda.gov.

Diagnosticians or laboratories new to the PT program, should contact NPPLAP as soon as possible in order to complete the required documentation before receiving a 2013 PT panel.

Sincerely,

Patrick J. Shiel, Coordinator
National Plant Pathogen Laboratory Accreditation Program
USDA-APHIS-PPQ
Center for Plant Health Science and Technology (CPHST)
Phone #: 919-855-7416
Fax #: 919-855-7480

Vessela Mavrodieva, NPPLAP Proficiency Testing Manager
CPHST Beltsville Laboratory
USDA APHIS PPQ
Bldg. 580, BARC-East, Powder Mill Road
Beltsville, MD 20705
Phone #: 301-313-9208
Fax #: 301-313-9232

Primers and Probes used in ITS-based Real-Time PCR assay for HLB screening diagnostics:

Primer Name	Primer Mix Name	Primer Sequence 5'-3' (synthesized by Integrated DNA Technologies, Inc., Purification: Standard Desalting)	Target Gene	Specific to:
HLBas primer	**HLB**as primer mix	5'-TCG AGCGCGTATGCAATACG-3'	16S rRNA	HLB L. asiaticus (Las)
HLBam primer	**HLB**am primer mix	5'-GAGCGAGTACGCAAGTACTAG -3'	16S rRNA	HLB L. americanus (Lam)
HLBr[1] primer	**HLB**r primer	5'-GCGTTATCCCGTAGAAAAAGGTAG-3'	16S rRNA	HLB (all three species)
COXf primer	**COX**[2] primer Mix	5'-GTATGCCACGTCGCATTCCAGA-3'	COX	COX
COXr primer		5'-GCCAAAACTGCTAAGGGCATTC-3'	COX	COX

[1]The **HLBr** Primer is the same for all species PCR.
[2] COX refers to a cytochrome C oxidase gene in plants and is based on the COX gene from Citrus spp.
NOTE: The "r" in the primer name HLBr and COXr denotes the reverse primer; COXf denotes the forward primer.

Probe Name*	**Sequence 5'- 3'** (Synthesized by Integrated DNA Technologies, Inc., Probe Purification: HPLC)	**Target Gene**	**Specific to:**
HLBp probe	5'6-FAM/AGACGGGTGAGTAAC GCG/3'BHQ-1	16S rRNA	HLB-Las or HLB-Lam
COX-P	5'TET/ATC CAG ATG CTT ACG CTG G/3'BHQ-2	COX	COX

***Note: Probes take 7 to 14 business days to arrive, once ordered.**

Primers and Probes used in confirmatory diagnostics by Federal Labs will be announced later.

Reagents used in both the conventional PCR assays and Real-time PCR assays:

Platinum *Taq* DNA polymerase Note: Platinum *Taq* is supplied in a kit with 10X PCR Buffer and 50mM $MgCl_2$	10966-034, Invitrogen
dNTP MIX (10 mM solution, PCR reagent)	D-7295, Sigma

Contact Information:

If you have any questions concerning the NPPLAP or the PT in general, or to request extension of the PT results submission please contacts Dr. Patrick Shiel at (919) 855-7416 or Patrick.J.Shiel@aphis.usda.gov.

Questions concerning the PT panel distribution / receipt, or PT panel results formatting or submission (with the exception of extension requests) should be directed to Dr. Vessela Mavrodieva at (301)-313-9208 or Vessela.A.Mavrodieva@aphis.usda.gov.

National Plant Protection Laboratory Accreditation Program
2013 Huanglongbing (HLB) Proficiency Test Panel

Lab name: Full name
Fed-Ex address information, including phone number: SAMPLE FORM – PLEASE REPLY USING THE FORM ATTACHED TO THIS MAILING

Name of Diagnostician Requesting an HLB PT Panel	**Send PT Panel (Indicate Month)** **Nov, Dec, Jan, Feb)**	**Direct E-mail and Contact Information of Participating Diagnostician**
Ex. Chris R. Diagnostician	*Oct*	Ex. C.R.Diagnos1@partlab.com 202-555-1212

Please complete the form provided with this email and return to Vessela.A.Mavrodieva@aphis.usda.gov