December 17, 2012

Dear Participant,

The proficiency test (PT) panel for the 2013 *P. ramorum* National Plant Protection Laboratory Accreditation Program (NPPLAP) is expected to be ready for distribution from third week of January 2013 through April 2013.

The 2013 *P. ramorum* PT panel is a single format for all participants and contains two sample types: DNA samples requiring PCR analysis and lyophilized tissue samples requiring DNA extraction and PCR analysis. The lyophilized tissue samples are designed to measure proficiency for two critical stages in the *P. ramorum* diagnostic process: DNA extraction and PCR analysis. *P. ramorum* positive and healthy DNA controls for PCR will be provided with each panel. Because the tissue samples require USDA permits to receive out-of-state diagnostic samples, please make sure that these permits are up-to-date. Information and applications for permits can be viewed at the following USDAAPHIS-PPQ website: http://www.aphis.usda.gov/plant_health/permits/organism/index.shtml.

To ensure PT panels are of sufficient quality prior to shipment, samples are extensively tested using both real-time PCR and conventional PCR according to USDA-approved protocols. Individual, randomly selected aliquots of each DNA panel samples are tested periodically to ensure DNA stability. Lyophilized tissue integrity (stability and uniformity) is ensured by extraction of DNA from randomly selected lyophilized tissue samples at regular time intervals and tested by real-time and conventional PCR.

Information on Re-Accreditation by the NPPLAP

Periodic re-accreditation is a common feature in quality management systems, and re-accreditation audits are used for this purpose by the International Organization of Standardization (ISO), American Association of Veterinary Laboratory Accreditation (AAVLD) and the developing NPDN System for True, Accurate and Reliable Diagnostics (STAR-D).

Re-accreditation ensures the molecular diagnostic laboratory performing the regulatory testing is providing results that would be achievable and consistent with any other laboratory involved in the program. Significant changes in facilities and processes of the testing laboratory can affect regulatory results. Thus, re-accreditation provides reassurance to all stakeholders who rely on those results that the services the laboratory provides are contiguous and expertise in the laboratory is harmonized to achieve the highest level of quality.

Prior to applying for certification, it is important that your Laboratory Management ensures that accreditation up to date. If any of the following has occurred, your laboratory will need to be re-accredited:

- New instrumentation is being used to generate regulatory test results
- Inconsistent laboratory results cannot be resolved
- Process flow has changed significantly
    - The laboratory has moved to a different room or building
    - The laboratory has merged with another department
    - The laboratory has expanded to include additional workspace

- Management has changed
- A large amount of time has elapsed from the previous accreditation

Please review your original documentation associated with your NPPLAP Accreditation and contact Pat Shiel at Patrick.j.shiel@aphis.usda.gov if you have any questions concerning this initiative. The NPPLAP staff will review currently active NPPLAP labs to discern whether any of the above changes have occurred and if they may affect laboratory performance. A NPPLAP representative may contact you to schedule a visit for re-accreditation. The NPPLAP and its stakeholders values your professional knowledge and wishes to provide the highest level quality of results to all stakeholders affected, including PPQ, commercial growers and nurseries, and the public.

The above criteria for re-accreditation will also be taken under consideration when distributing proficiency tests to participants.

As with the original NPPLAP accreditation, the re-accreditation process includes submission of supporting documentation prior to a scheduled visit. This will minimize the amount of time needed during a visit by NPPLAP personnel to establish re-accreditation. The table below lists examples relative to the previous changes mentioned. These are intended as a guideline for the laboratory and are not inclusive for all lab situations.

Change	Needed Documentation
A) New instrumentation is used	Data* generated to show consistency in performance, and staff training history and background expertise
B) Inconsistent laboratory results	A summary of all attempts to reconcile the inconsistent results, with all relevant data* attached with enough detail to confirm the attempt was successful
C) Process flow has changed significantly	New schematics of the laboratory setup, detailing the location of instruments and process flow, and updated staff descriptions including training and background expertise
D) Management has changed	Updated staff descriptions and roles, including training, and all available supporting documentation which describes laboratory management (cleaning protocols, laboratory management quality policies, etc.)
E) Significant lapse in time from a prior accreditation	All documents required in C) and D).

*Data must be in .csv, .xls, or .xlsx format and contain enough detail to confirm the conclusion drawn by the laboratory.

Testing Requirements based on the Type of *P. ramorum* Certification Selection

Diagnostic laboratories may choose options from among three diagnostic protocols for processing regulatory samples. The table below describes the diagnostic protocol(s) to be used to analyze the PT2013 panel and therefore the equivalent Certification choice for 2013.

Certification Category	Selected Diagnostic Protocol	PT Option to request	PCR tests required to be performed	Inconclusive results follow-up	Comments
Screening	Conventional PCR only	**C**	Nested and Multiplex Conventional	As per work instruction	
Screening	Conventional and ITS Real-time PCR	**R**	Nested and Multiplex Conventional; ITS Real-time	As per work instruction	
Screening	Dual Real-time PCR only	**D**	ITS and Elicitin Real-time	As per work instructions for ITS and Elicitin PCR	**Conventional PCR is NO longer required to resolve inconclusive results; however, analysts must describe further diagnostic options required to resolve inconclusive results.**
Screening	Conventional PCR and Dual Real-time PCR	**B[1]**	Nested and Multiplex Conventional; ITS and Elicitin Real-time for all 12	As per work instructions	**Conventional and/or dual-real-time PCR certification**

Confirmatory	Dual Real-time PCR and Conventional PCR	**B-Conf.**[2]	Nested and Multiplex Convention al; ITS and Elicitin Real-time for all 12	As per work instructions	**All tests must be successfully completed for this certification.**

[1] Diagnosticians that choose option B in their panel request will be certified for conventional and/or dual real-time PCR based on PT performance for performing USDA regulatory diagnosis according to the Potentially Actionable Suspect Sample Policy for *P. ramorum*: http://www.aphis.usda.gov/plant_health/cphst/downloads/npplap/PASS%20policy_mar08.pdf

[2] The PPQ CPHST Beltsville federal laboratory must choose option B-Conf to maintain "Confirmatory" certification.

The option you select will determine the method(s) you intend to use and therefore the primers/probes that you will need. It is strongly suggested that you order a new fresh set of primers and probes, since degradation is one of the major reasons for failure or low efficiency of PCR. To schedule your panel receipt appropriately, please be aware that fluorescent probes can take a minimum of 14 days to receive. The DNA sequences of the primers and probes you will need to order to analyze the 2013 PT panel are listed in tables at the bottom of this letter. In addition, participating laboratories should not use DNA extraction kits that have expired since this may affect the quality of the DNA you extract and further the quality and outcome of your PCR analysis.

Change in the reporting time:
Results should be reported within three (3) weeks of receipt of the panel. Based on continued monitoring of the performance for the PT and data from real diagnostic samples turnover time, the following aspects regarding results reporting have been observed:

- DNA extraction of 8 samples with consequent analysis of all 12 samples (including DNA samples) plus controls using all listed diagnostic assays could be accomplished in 3-4 days based on current PT work instructions.
- Proficiency Test sample processing and results reporting should be conducted as closely as possible to regulatory sample submissions to any lab, i.e.: as if samples were customer submitted unknowns.
- Composite data on the PT panel performance, including response time, is used by U.S. emergency response agencies to evaluate laboratory preparedness.
- Panels are shipped on schedule at the Diagnosticians' request. Therefore Diagnosticians are expected to have equipment and reagents ready as well as a scheduled availability to begin working on panel samples as soon as they arrive.

Each individual participant should choose a preferred month to receive a panel(s) using the attached form and promptly return to the email address listed. We will make every effort to accommodate your request(s). If you or your lab diagnostician is new to the program, please contact the NPPLAP program to complete required documentation to participate in the PT panel for 2013.

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

Primers and Probes used in ITS-based real-time PCR assay for *P. ramorum* diagnostics:

Primer Name	**Primer Sequence** (synthesized by Integrated DNA Technologies, Inc., Purification - Standard Desalting)
Pram-114F	5' – TCA TGG CGA GCG CTT GA -3'
Pram-1527190R	5' – AGT ATA TTC AGT ATT TAG GAA TGG GTT TAA AAA GT – 3'
COX-F	5' – CGT CGC ATT CCA GAT TAT CCA – 3'
COX-RW	5' – CAA CTA CGG ATA TAT AAG RRC CRR AAC TG – 3'
Probe Name*	**Probe Sequence** (synthesized by Integrated DNA Technologies, Inc.)
	(Purification - Dual HPLC purification preferred if available)
Pram1527-134T	5' -/6-FAM/ TTC GGG TCT GAG CTA GTA G/BHQ-1/ - 3'
COX-P*	5'- /TexRd-X/or/VIC/ AGG GCA TTC CAT CCA GCG TAA GCA /BHQ-2/ - 3'

* Please, consider your real-time PCR instrument probe label requirements when ordering probes.

Primers and Probes used in Elicitin-based real-time PCR assay for *P. ramorum* diagnostics:

Primer Name	**Primer Sequence** (synthesized by Integrated DNA Technologies, Inc., Purification - Standard Desalting)
Prameli-102U-F	5' – TTC AAC CAG TGC GCG ACC – 3'
Prameli-259L-R	5' – GGC ACA GTC AGC TCG CAG TC – 3'
5.8S-F	5' – TGT CTA GGC TCG CAC ATC GA– 3'
5.8S-R	5' – GAT GAC TCA CTG AAT CCT GCA ATT – 3'
Probe Name*	**Probe Sequence** (synthesized by Integrated DNA Technologies, Inc.)
	(Purification - Dual HPLC purification preferred if available)
Elicitin probe Taq195eli-R	5' - /6-FAM/ CCG TGG ACG CGC ACA TGA GCG AGT AC /BHQ-1/ - 3'
5.8S internal control (**IC**) probe	5'- /TET/ ACG CTG CGA ACT GCG ATA CGT AAT GC /BHQ-1/ - 3'

* Please, consider your real-time PCR instrument probe label requirements when ordering probes

Primers used in Conventional PCR for *P. ramorum* diagnostics:

Nested PCR Primers:

Primer Name	**Primer Sequence** (synthesized by Integrated DNA Technologies, Inc., Purification - Standard Desalting)
Phyto1 primer	5'-CATGGCGAGCGCTTGA-3'
Phyto2 primer	5'-AAAGCCAAGCCCTGCAC-3'
Phyto3 primer	5'-GGTGGATGGGGACGTG-3'
Phyto4 primer	5'-GAAGCCGCCAACACAAG-3'

Multiplex PCR Primers:

Primer Name	**Primer Sequence** (synthesized by Integrated DNA Technologies, Inc., Purification - Standard Desalting)
Platf primer	5'-TTAGTTGGGGGCTTCTGTTC-3'
Platr primer	5'-AGCTGCCAACACAAATTTC-3'
NS1 primer	5'-GTAGTCATATGCTTGTCTC-3'
NS2 primer	5'-GGCTGCTGGCACCAGACTTGC-3'

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
D-(+) –Trehalose dehydrate	T9531-10G, Sigma

Contact Information:

If you have any questions concerning the provisional approval process in general or the proficiency-testing program such as PT panel submission extensions please contact 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 vessela.a.mavrodieva@aphis.usda.gov.