

Diagnostic Services and Account Set-up Procedures

About TriCal Diagnostics

We are a team of highly experienced diagnosticians with extensive knowledge of field plant pathology and nematology, microbiological and molecular identification techniques, research methodologies, soilborne pathogen and nematode detection methods, and production agriculture and horticulture. The first step in managing plant diseases is identifying the cause of such problems. Therefore, disease and nematode management relies on careful diagnosis. We offer disease and plant problem identification services to growers, field personnel, pest control advisors, general public, and others throughout California and the US. We value service to the customer, provide accurate and timely diagnoses, and offer experienced advice on disease management and IPM practices.

Director **Steven Koike** worked for 28 years as an extension plant pathologist with the University of California, conducting applied research, presenting plant pathology information via talks and publications, and operating a plant pathology diagnostic lab. He has published extensively and is the recipient of numerous honors, including the national Excellence in Extension Plant Pathology award from the American Phytopathological Society and the Outstanding Contribution to Agriculture Award from the California Association of Pest Control Advisors (CAPCA).

Manager Dr. **Hanane Stanghellini** received her Ph. D. in Plant Pathology and Soil Ecosystems from the University of Bonn, Germany. She has over 20 years of university research and private-sector experience in custom diagnosis of fungi and bacteria, microbial community structures, molecular analysis and functional genomics of nematodes, and disease resistance screening.

Molecular Specialist **Cayla Tsuchida** received her M. S. in Plant Pathology from the University of California, Davis where she investigated the variability of lettuce downy mildew in California and Arizona. She has 3 years of experience in diagnosing plant diseases and 5 years in conducting plant pathology research. Cayla has extensive experience in Next Generation and Sanger sequencing, gene editing, gene expression, and marker assisted selection for plant breeding. With Trical Diagnostics, Cayla focuses on diagnostics using molecular techniques. She will also apply similar methods for optimizing and developing DNA-based tests for diagnostic and research purposes.

Senior Researcher and Nematologist Dr. **Kristi Sanchez** received her Ph. D. in Plant Pathology, specializing in Nematology, from the University of California, Davis. She has over a decade of combined research and field experience while working at UC Davis, the agricultural industry, and the Plant Pest Diagnostics Center with the California Department of Food and Agriculture. Kristi's research background includes pest management of nematodes, morphological and molecular diagnostics of plant parasitic nematodes, and the development of experimental assays for nematode studies in the lab and field. With TriCal Diagnostics Kristi focuses on nematode assessments (sampling, detection, identification, quantification) and field research.

Lab Technician **Jennifer Guerrero** received her B. S. degree in Chemistry with a minor in Environmental Science from the University of California, Merced. While at UC Merced, she worked as a research assistant in a Soil Biogeochemistry Lab exploring how physical disturbances in the environment affect soil organic matter dynamics. She was a lab technician at an analytical lab for 5 years where she was responsible for sample preparation and analysis, data recording, and report writing. She is proficient with various analytical instruments and is experienced with developing SOPs and related documentation.

2023

Diagnostic Services: Plant Materials

• Custom diagnostics and problem-solving for plant samples:

 Plant samples are analyzed and tested using microbiological culturing methods, examination with microscopes, lab incubations, serology (ELISA and other tools), and other techniques. Pathogens and other crop issues (insect/mite, nutritional, genetic, abiotic) are identified. Implications of the problem and advice on management strategies are provided.

• Molecular diagnostics of plant samples:

- We use molecular methods including conventional PCR (polymerase chain reaction), qPCR (quantitative PCR), and RPA (recombinase polymerase amplification).
- o Examples of assays include:
 - RPA to identify *Verticillium dahliae*, *Macrophomina phaseolina* (strawberry strain), *Fusarium oxysporum* f. sp. *fragariae* (strawberry) and *Phytophthora*
 - RPA to identify Fusarium and Verticillium wilt pathogens of lettuce
 - qPCR screening of grape powdery mildew for QoI (Strobilurin) resistance
 - PCR race identification of *Fusarium oxysporum* f. sp. *apii* from celery
 - PCR race identification of Verticillium dahliae
 - qPCR to detect the tomato canker pathogen (*Clavibacter*)
 - qPCR/RT-PCR detection of viruses
 - Sequence-based identification of pathogens
- Molecular specialist Cayla Tsuchida (ctsuchida@trical.com) can talk with you about specialized assays and molecular-based research projects

Contact us for pricing and other information.

Diagnostic Services: Soil and Water

• Single soilborne strawberry pathogen qPCR assay:

• We use this rapid DNA-based method to detect single pathogens in soil: Verticillium dahliae or Macrophomina phaseolina (strawberry strain) or Fusarium oxysporum f. sp. fragariae (strawberry strain). Test results measure how much Verticillium or Macrophomina is present (quantitative) or will indicate if the strawberry Fusarium pathogen is present (yes/no: qualitative only).

• Comprehensive qPCR package for 3 major soilborne strawberry pathogens:

• We also offer discounted prices if using this qPCR method to detect all three pathogens: Verticillium dahliae, Macrophomina phaseolina (strawberry strain), and Fusarium oxysporum f. sp. fragariae (strawberry) in soil. Test results measure how much Verticillium and Macrophomina are present (quantitative) and will indicate if the strawberry Fusarium pathogen is present (yes/no: qualitative only).

• Soil plating for *Verticillium* and/or *Macrophomina*:

Agar plate test for culturable Verticillium and/or Macrophomina species. This test detects
various species and provides an overall count of viable Verticillium and or Macrophomina
propagules in soil. Results are available in approx. 5 to 6 weeks.

• Fusarium oxysporum f. sp. lactucae (lettuce):

o Agar plate test for the lettuce Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *lactucae*. Results are available in approx. 2 to 3 weeks.

• Phytophthora/Pythium test:

A baiting test is used to determine if *Phytophthora/Pythium* ("water molds") are in a soil sample. This assay indicates if the potential pathogen is present (yes/no: qualitative only). For species identification of *Phytophthora/Pythium*, molecular analysis is available for an additional fee.

• Irrigation water testing for pathogens and other microorganisms:

• Water can be tested for various soilborne pathogens and other microorganisms by using direct culture, filter, or bait methods.

Contact us for pricing and other information.

Diagnostic Services: Nematode Identification and Quantification

• Testing for nematodes in soil, roots, plants:

 Nematode extraction methods are used on soil, root, and other plant tissue samples when identification (to nematode genus) and enumeration of plant parasitic nematodes are needed. Methods include Baermann funnel, sugar flotation, and screening.

• Nematode species identification

O Species identification of plant parasitic nematodes can be determined using molecular (PCR) methods. Free-living nematodes, as indicators of soil health, can also be identified and quantified.

Contact Kristi Sanchez (ksanchez@trical.com) for pricing and other information.

Screening Plants for Pathogen Resistance

• Screening lettuce for resistance/susceptibility to *Bremia lactucae* (Bl:US races 7-9)

• We offer indexing and screening services for lettuce downy mildew disease.

Contact Hanane Stanghellini (hstanghellini@trical.com) for pricing and other information.

Special Projects and Research in Plant Pathology

TriCal Diagnostics is available to assist with specialized plant pathology projects:

Field problem solving investigations

Fungicides and biologicals efficacy testing (lab, greenhouse, field)

Production of pathogen inoculum for research

Collaborations on projects and grants

Expert witness information

Contact Steve Koike (skoike@trical.com) for pricing and other information.

Account Setup and Shipping Samples

Payment details / Setting up of accounts

An invoice will be generated when sample analyses are completed. Clients can set up accounts and will be billed monthly. To set up an account, fill out the two new account forms and send them to Hanane Stanghellini (hstanghellini@trical.com) or include them with your first submission of samples.

Documents available from our website (www.tricaldiagnostics.com)

- New Account application form
- New Account payment information form
- Guidelines for collecting and submitting samples: plants
- Guidelines for collecting and submitting samples: soil
- Guidelines for collecting and submitting samples: nematodes
- Lettuce downy mildew indexing and screening services
- Sample submission form
- CDFA permit for samples shipped to TriCal Diagnostics from within CA.
- Federal APHIS permits for samples shipped to TriCal Diagnostics from
 - o 1. Continental USA, or
 - o 2. International locations

Procedures for mailing/shipping samples to our Lab on Highway 25:

Collect samples according to the suggested guidelines.

Fill out and include the sample submission form.

Include a copy of the appropriate regulatory permit:

State CDFA permit (if sample is from within CA)

Federal APHIS permit 1 (if sample is from outside of CA but within continental USA)

For best results send the samples by overnight/next day delivery. If the sample is particularly perishable, include ice packets in the shipping container.

Do not ship samples on a Friday or weekend (delivery will be delayed until the following week).

Special instructions for international samples

Do not send samples until speaking with Steve, Hanane, or Lilly.

Samples originating from outside of the USA require special labeling (red & white APHIS labels).

Federal International APHIS permit 2 must also be included with the shipment.

See our instruction page for shipping international samples to TCD.

Mail or ship the sample (with submission form and appropriate permit) to:

TriCal Diagnostics Lab 8770 Highway 25 Hollister, California 95023

Note: US Post does not ship to this address; one must use FedEx, UPS, or other carrier.

Hand-delivered drop-off samples can be left at the following locations:

TriCal Diagnostics Lab

8770 Highway 25

Hollister, California 95023

For sample drop-off, open Monday through Friday, 8:00 a.m. through 4:00 p.m. Closed weekends and holidays.

Salinas drop-off location:

Grower-Shipper Association (refrigerator at back of mail/copy room)

512 Pajaro Street

Salinas, California 93902

For sample drop-off, open Monday through Friday, 8:00 a.m. through 4:00 p.m. Closed weekends and holidays.

Watsonville drop-off location:

Perry Laboratory (831-722-7606)

424 Airport Blvd

Watsonville, California 95076

For sample drop-off, open Monday through Friday, usually 9:00 a.m. through 5:00 p.m. Closed weekends and holidays. Call for specific hours of operation.

Contact Information

Steve Koike (408-612-6729) skoike@trical.com

Hanane Stanghellini [operations, accounts, billing, lettuce downy mildew] hstanghellini@trical.com

Cayla Tsuchida [molecular testing] ctsuchida@trical.com

Kristi Sanchez [nematology] ksanchez@trical.com

Lilly Cosio [administrative questions, sample shipping/delivery] lcosio@trical.com