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Guidelines for Selection of Tissues for Electron Microscopy Confirmation of *Candidatus*Liberibacter spp. in Huanglongbing-affected Citrus

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Polymerase chain reaction (PCR) with the pathogen-specific primers and electron microscopy are the two techniques of choice that have been used for detection and identification of Candidatus Liberibacter spp. in the Huanglongbing (HLB)-affected citrus. Due to the low population and uneven distribution of Liberibacter in the diseased citrus trees finding the bacteria with transmission electron microscopy has been a challenge. Work with samples from HLBaffected citrus during the past 5 years has resulted in certain guidelines for the selection of plant tissues that have led to success in visualization of the bacteria. With PCR-positive field citrus trees, the best source is phloem tissue from seed coats of developing seeds in young fruit supported by leaves with blotchy mottle symptoms. These half mature seeds have been shown to contain large numbers of intact bacteria. However, when working with young potted trees there are usually no fruit to sample. In these trees we had some success in finding bacteria in petioles or midveins of PCR-positive, young developing leaves (2/3 to fully expanded, but not hardened) sampled above leaves showing blotchy mottle symptoms. However, we have found larger numbers of bacteria in roots of the potted diseased trees. We have experienced the most success in sampling root tissue in areas where the phloem has just completed differentiation, in PCRpositive small pioneer and fibrous roots showing primary and secondary growth.