PEST SURVEY CARD

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Pest survey card on *Candidatus* Liberibacter solanacearum

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Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137), at the request of the European Commission. The purpose of the document is to assist the Member States to plan annual survey activities of quarantine organisms using a statistically sound and risk-based pest survey approach, in line with current international standards. The data requirements for such an activity include the pest distribution, its host range, its biology and risk factors as well as available detection and identification methods. This document is part of a toolkit that consists of pest-specific documents, such as the pest survey cards, and generic documents relevant for all pests to be surveyed, including the general survey guidelines and statistical software such as RiBESS+.

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Keywords: *Bactericera cockerelli*, *Candidatus* Liberibacter solanacearum, haplotype A, haplotype B, haplotype F, Lso, risk-based surveillance

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Introduction

The information presented in this pest survey summarised from the EPPO pest risk analysis on *Candidatus* Liberibacter solanacearum (EPPO, 2012), the FERA Rapid Pest Risk Analysis for the bacterium (FERA, online), the EPPO datasheets on *Candidatus* Liberibacter solanacearum and *Bactericera cockerelli* (EPPO, 2013a, b), the EPPO National Regulatory Control System for *Candidatus* Liberibacter solanacearum (EPPO, 2017), and other documents.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys of *Candidatus* Liberibacter solanacearum (hereinafter referred to as 'Lso') in EU Member States (EFSA, 2018). It is part of a toolkit that is being developed to assist Member States with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention guidelines for surveillance (FAO, 2016). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card on *Candidatus* Liberibacter solanacearum.[1]
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual available online[2]
   c. The statistical tools RiBESS+ and SAMPELATOR which are available online[3] with open access after registration.

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Candidatus* Liberibacter solanacearum

Class: Alphaproteobacteria **Order**: Rhizobiales **Family**: Phyllobacteriaceae **Genus**: *Liberibacter*

Synonym(s): *Liberibacter psyllaurous* Hansen, Trumble, Stouthamer & Paine

Common name of the pest: Zebra chip disease

Many different haplotypes of the bacterium have been described (haplotypes A, B C, D, E, F and U) (Table 1). This document provides an overview of all the haplotypes and known vectors of Lso identified so far.

However, in agreement with the European Commission, **the focus of this survey card is on the Lso haplotypes A, B, and F**. These are not present in the EU and are known to only infect solanaceous species.

Bactericera cockerelli (synonym *Paratrioza cockerelli* or *Trioza cockerelli*) is an insect (Hemiptera) commonly named as the potato psyllid or tomato psyllid that has been found to be associated with the bacterium *Candidatus* Liberibacter solanacearum (EPPO, 2013a,b). In particular, the insect has been identified as a vector of the three haplotypes of Lso under scrutiny (A, B and presumably F).

[1] The content of this EFSA Supporting Publication is reproduced as a live document available at: https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489 where it will be updated whenever relevant new information becomes available.
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://websso-efsa.openanalytics.eu/auth/realms/efsa/protocol/openid-connect/auth?response_type=code&client_id=shiny-efsa&redirect_uri=https%3A%2F%2Fshiny-efsa.openanalytics.eu%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce-237f192a72d5&login=true&scope=openid

1.2. EU pest regulatory status

Lso is not regulated under Council Directive 2000/29/EC[4].

Bactericera cockerelli, the vector of haplotypes A, B, and presumably F, is listed in Annex I A1 of Council Directive 2000/29/EC.

Since *Solanum tuberosum* is a major host of Lso A, B and F and of *B. cockerelli*, it is relevant to mention that the import of seed potatoes from third countries (other than Switzerland) is prohibited as laid down in Council Directive 2000/29/EC, Annex III Part A. Import of ware potatoes is also prohibited, except from a limited number of Mediterranean and European countries where Lso A, B and F are not present.

1.3. Pest distribution

1.3.1. Distribution of *Candidatus* Liberibacter solanacearum

Figure 1 shows the global distribution of all known haplotypes of Lso. Table 1 provides more detail on the reported distribution of the different haplotypes.

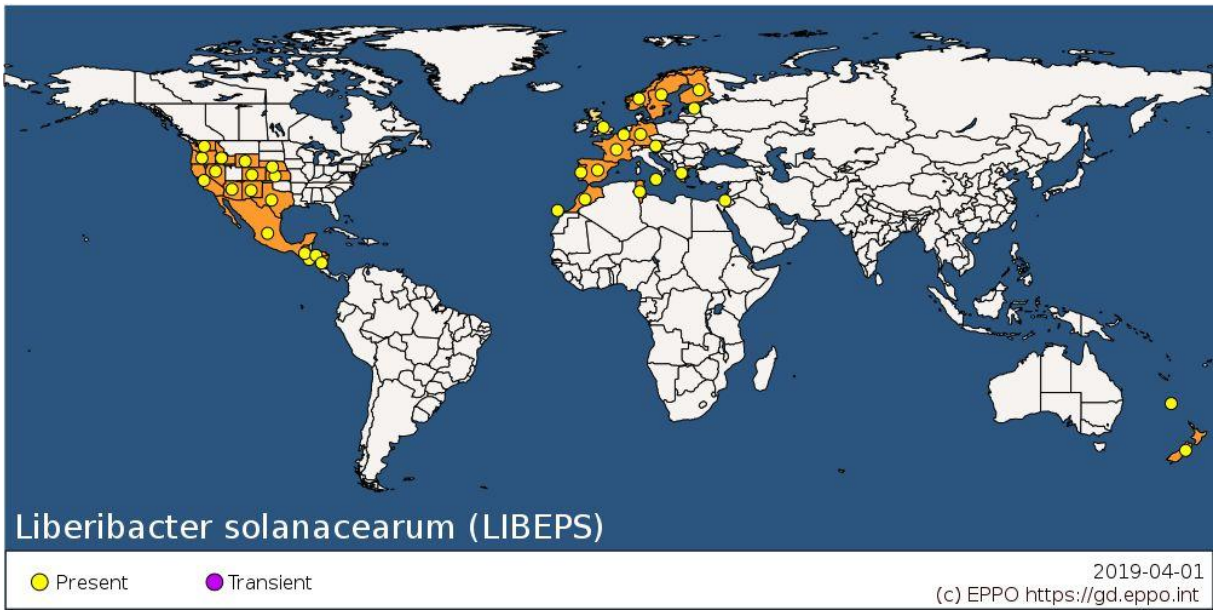

Figure 1: Global distribution of *Candidatus* Liberibacter solanacearum. This world distribution displays records for all known haplotypes without distinction of their different distribution (Source: EPPO global database, www.eppo.int)

[4] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1–112. Consolidated version of 01/04/2018

Lso haplotypes A, B and F

Lso haplotypes A, B and F are not present in the EU. Lso haplotypes A and B occur in Central and North America in solanaceous species (haplotype A occurs in El Salvador, Nicaragua, Honduras, Guatemala, western Mexico, western USA; haplotype B occurs in eastern Mexico up to Texas). Haplotype A is also found in New Zealand and Norfolk Island. In 2017, haplotype F was described in one potato tuber by Swisher Grimm and Garczynski (2019) in southern Oregon, USA (EPPO, 2017).

Lso haplotypes C, D and E

Lso haplotype C is associated with a disease in carrots in northern Europe (Austria, Estonia, Finland, Norway, Sweden, United Kingdom and Germany (EPPO, 2017; EPPO Global Database; Haapalainen et al., 2018a)). Lso haplotypes D and E are found in association with apiaceous species in several countries in southern Europe (Belgium, France, Greece, Italy, Portugal, Spain including the Canary Islands), in North Africa (Morocco and Tunisia) and in Israel (EPPO Global Database). These two haplotypes have only been reported on a few occasions or in a restricted distribution (Figure 1).

In a survey of Lso in historical seeds from collections of carrot and related Apiaceae species (seeds analysed dated from 1973 to 2006), the bacterium was detected in seeds originating from countries which had not already been reported as having it: Czechia (parsnip), Denmark (carrot, parsley), Egypt (carrot), Japan (carrot), Lebanon (wild carrot and *Daucus aureus*), the Netherlands (carrot, celery, celeriac, parsnip) and Syria (carrot). This information suggests that the distribution of Lso could be vaster than that already described (Monger and Jeffries, 2017).

Though haplotype C was found on asymptomatic potato tubers (Haapalainen et al., 2018a) and haplotype E on symptomatic ware potatoes in Spain (Palomo et al., 2014), transmission between different host plant families is limited since vectors do not feed on both Apiaceae and Solanaceae. The finding on potatoes is therefore considered to be a sporadic event (Palomo et al., 2014).

Lso haplotype U

In 2018, Haapalainen et al. described haplotype U in *Urtica dioica* and *Trioza urticae* in Finland (Haapalainen et al., 2018b).

1.3.2. Distribution of vectors of Lso

As shown in Table 1, *B. cockerelli* is the main psyllid vector of Lso in solanaceous species. *Trioza apicalis* is the main vector in carrots in the north of Europe whereas *B. trigonica* is the main psyllid vector in apiaceous species in the south of Europe and the Mediterranean Basin.

Vector of Lso haplotypes A, B and presumably F

Bactericera cockerelli, the psyllid vector in solanaceous species, is present in the countries where haplotypes A, B and F have been reported. It is absent from Europe (see Figure 2).

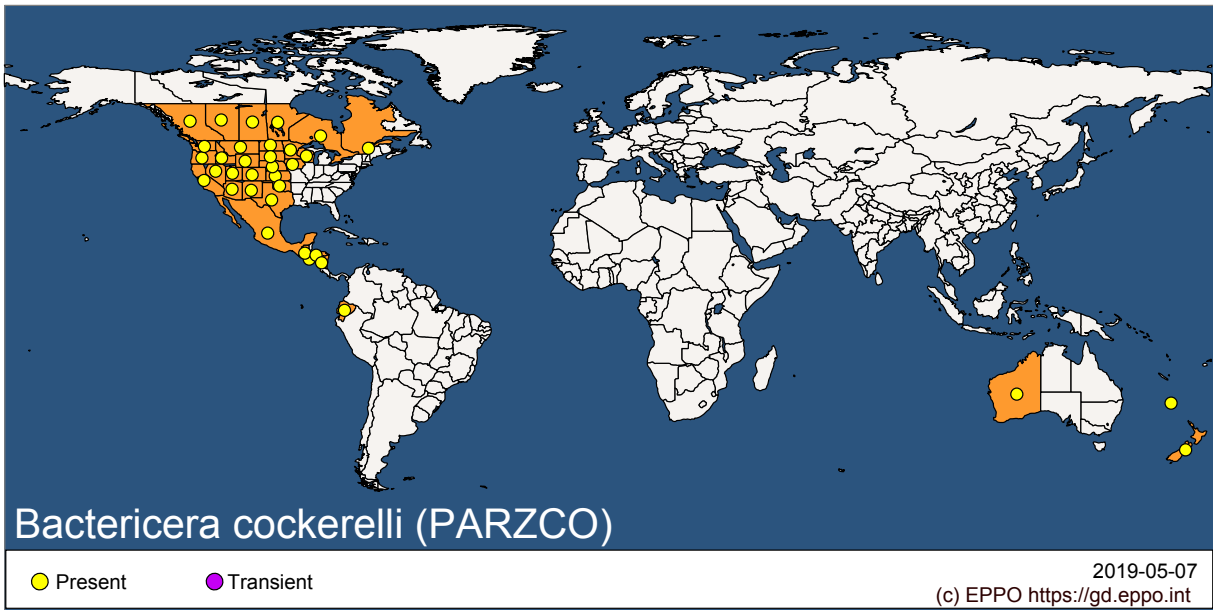

Figure 2: Global distribution of *Bactericera cockerelli.* (Source: EPPO global database, www.eppo.int)

Vectors of Lso haplotypes C, D, E and U

Trioza apicalis is the main psyllid vector in northern Europe in carrot fields. Carrot plants are a host for Lso haplotype C. This vector is present in Europe and favours spruce as an overwintering host (Kristoffersen and Anderbrant, 2007).

Bactericera trigonica is the main psyllid vector in southern Europe and the Mediterranean Basin in apiaceous species. Apiaceous species are host plants for Lso haplotypes D and E. The vector is also present in other Mediterranean countries outside the EU and in the Middle East.

Haplotype U has been found in *Trioza urticae* in Finland (Haapalainen et al., 2018b).

Bactericera nigricornis is present in Asia and western Europe, but its ability to transmit the bacterium has never been demonstrated. For *Bactericera tremblayi*, which is present in Greece, Iran, Italy, Serbia, Switzerland and Turkey (Ouvrard, 2019), it could be shown (using potato, carrot and leek as host plants) that it is not able to transmit Lso (Antolinez et al., 2017). Haapalainen et al. (2018b) found haplotype C in *Trioza anthrisci*, but state that, 'It seems likely that the CLso found in *T. anthrisci* and *A.* (*Anthriscus*) *sylvestris* do not currently form a major source of CLso infections in carrots, parsnips, or potatoes.' Therefore, this psyllid is not listed in Table 1.

1.4. Life cycle

Lso is transmitted by psyllid insect vectors to new host plants (FERA, online). In addition, it can be transmitted by propagative plant material and as shown in an experimental setup, it can also be transmitted by *Cuscuta campestris* (dodder) to *Catharanthus roseus* (periwinkle) and other herbaceous plants (Bertolini et al., 2015).

The psyllids transmit Lso to the host plants (Figure 3) and the transmission depends on their life cycle, as described in this section.

The psyllids' life cycle begins with the mating of two adults, followed by oviposition by the female on the host plants. After hatching, the psyllids have five nymphal stages. They feed on the host plant

foliage. After metamorphosis, the adults emerge. The new adults can then fly to new host plants or to overwintering hosts and begin a new life cycle. The length of the life cycle, the number of eggs, the flight distance, the number of generations per year and the overwintering hosts are dependent on the psyllid species and the environmental conditions (Haapalainen, 2014). *Bactericera cockerelli* usually has three to seven generations per year with one generation being completed in 3 – 5 weeks (EPPO, 2013b).

When a psyllid feeds on the phloem sap of an Lso-infected plant, the bacteria can be ingested by the psyllid. The bacterial cells must pass through the alimentary canal wall, move through the haemolymph, and finally reach the salivary glands. The bacteria can then be transmitted with salivary secretions into a new host plant during psyllid feeding. The latent period is the time between the pathogen acquisition and the potential for the psyllid to transmit it to a new plant (Haapalainen, 2014). The bacteria can also be transmitted to the offspring of an infected female psyllid by transovarial transmission (Hansen et al., 2008). When the concentration of the bacteria in the plant is low, plants may remain asymptomatic (EPPO, 2012). According to Rondon et al. (2017), trapping of psyllids should start at the beginning of the potato season. In a study by Klein and Rondon (2019), sampling was conducted for 10 – 12 weeks.

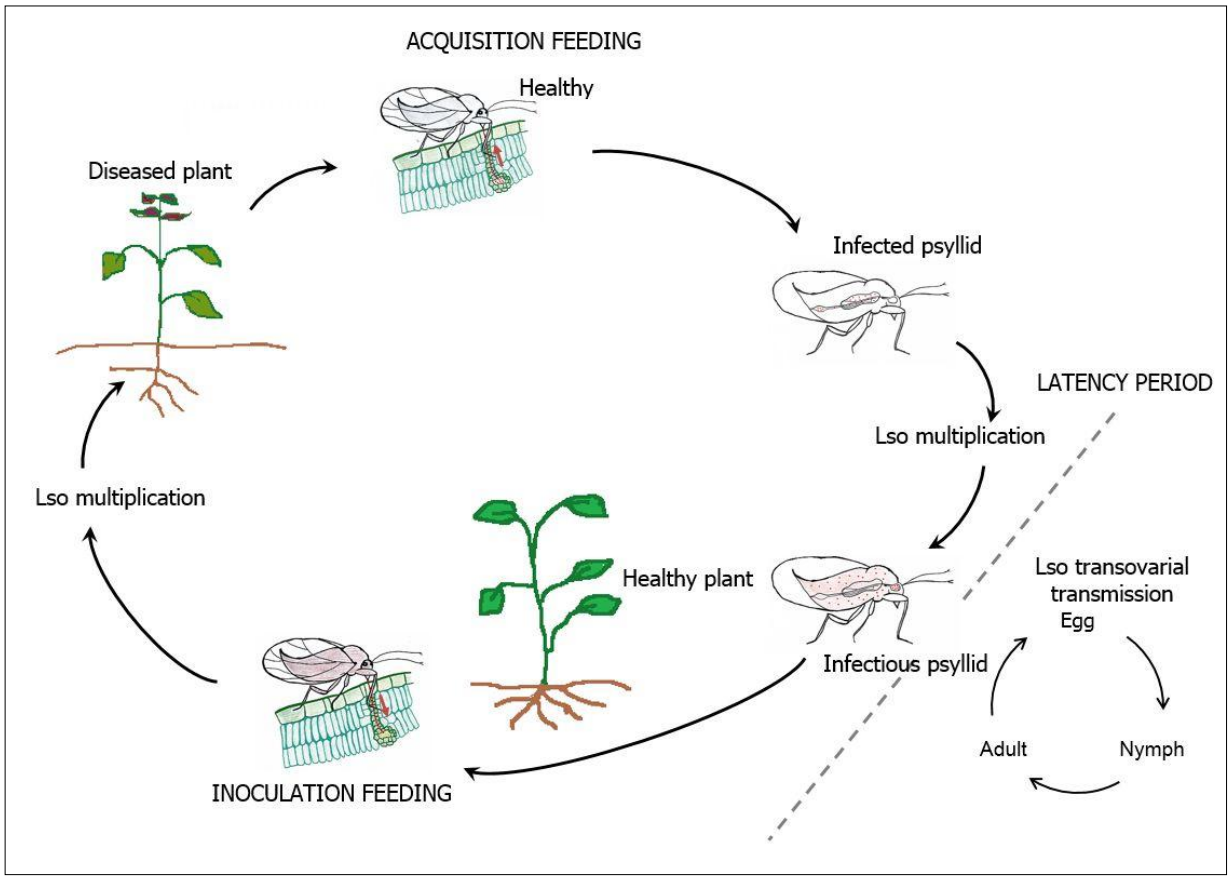

Figure 3: Disease cycle of *Candidatus* Liberibacter solanacearum and its psyllid vector

1.5. Host range and main hosts

The European and Mediterranean Plant Protection Organization (2017) provides some information on the different haplotypes of the bacterium and their host plants.

Lso haplotypes A and B, the so-called 'solanaceous haplotypes', can cause disease in solanaceous plants (e.g. *Solanum tuberosum* ('zebra chip disease'), *S. lycopersicum*, *Capsicum annuum*, *Nicotiana* spp.). In the Americas, it has been identified on various wild hosts such as yellow nightshade (*Solanum elaeagnifolium*), wolfberry (*Lycium barbarum*) or black nightshade (*S. ptychanthum*) (Wen et al. 2009). In 2017, haplotype F was described in one potato tuber in the USA (Swisher Grimm and Garczynski, 2019).

Lso haplotypes C, D and E have been found in several EU countries and in the Mediterranean Basin (for details see Section 1.3 'Pest distribution') in plants of the Apiaceae family. All three can be associated with carrots (*Daucus carota*), while Lso haplotypes D and E are also associated with other apiaceous species – celery (*Apium graveolens*), chervil (*Anthriscus cerefolium*), fennel (*Foeniculum vulgare*), parsley (*Petroselinum crispum*) and parsnip (*Pastinaca sativa*) (Alfaro-Fernández et al., 2017; Hajri et al., 2017). Lso haplotype C was detected in a few asymptomatic volunteer potatoes in Finland (Haapalainen et al., 2018a) and haplotype E in symptomatic potato tubers in Spain (Palomo et al., 2014; NPPO Spain, 2017).

Lso haplotype U has been reported in stinging nettle (*Urtica dioica*) (Haapalainen et al., 2018b).

A general overview of the different Lso haplotypes is given in Table 1.

Table 1: Overview of the different haplotypes of *Candidatus* Liberibacter solanacearum including their distribution, host plants, vectors, and distribution of vectors

Haplotype	Haplotype present in	Host plant	Vector	Vector present in	References (selection)
A	El Salvador, Nicaragua, Honduras, Guatemala, western Mexico, western USA, New Zealand, Norfolk Island	Solanaceous species	*Bactericera cockerelli*	Canada, USA, Guatemala, Honduras, El Salvador, Mexico, Nicaragua, Australia, New Zealand	Swisher Grimm and Garczynski, 2019; EPPO, 2017, EPPO Global Database
B	Eastern Mexico up to Texas				
C	Austria, Estonia, Finland, Norway, Sweden, United Kingdom and Germany	Carrots	*Trioza apicalis* (favours spruce as an overwintering host)	Mongolia, Austria, Czechia, Denmark, Finland, France, Germany, Italy, Latvia, Sweden, Poland, United Kingdom, Norway, Switzerland, Russia, Ukraine	EPPO Global Database, Haapalainen et al. 2018a
D	Belgium, France, Greece, Italy, Spain, Morocco, Tunisia, Israel	Apiaceous species	*Bactericera trigonica*	Algeria, Egypt, Morocco, Tunisia, Iran, Israel, Cyprus, Czechia, France, Greece, Hungary, Italy, Malta, Portugal, Spain, Serbia, Switzerland, Turkey	EPPO, 2017, EPPO Global Database
E	France, Italy, Portugal, Spain, Morocco, Tunisia				
F	Southern Oregon	Potato tubers	*Bactericera cockerelli* (assumed)	Canada, USA, Guatemala, Honduras, Mexico, Nicaragua Australia, New Zealand	Swisher Grimm and Garczynski, 2019
U	Finland	*Urtica dioica*	*Trioza urticae* (The ability to transmit the bacterium has not yet been demonstrated)	Afghanistan, Algeria, Asia-temperate, Austria, Belarus, Caucasus, China, Czechia, Denmark, Finland, France, Great Britain, Greece, Hungary, India, Iran, Ireland, Israel, Japan, Lebanon, Lithuania, Madeira, Mongolia, northern Africa, Russian Far East, Slovakia, Slovenia, Sweden, Tajikistan, Turkey, West Himalaya	Haapalainen et al., 2018b, Ouvrard, 2019

The host range of *B. cockerelli* is much wider than that for Lso. This psyllid is polyphagous and can be found on species in 20 plant families (in particular Solanaceae, Convolvulaceae and Lamiaceae) (EPPO, 2012) with a clear preference for tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), aubergine (*Solanum melongena*), and peppers (*Capsicum* spp.) (Biosecurity Australia, 2009; Yang and Liu, 2009). The insect vector feeds on the green parts of the plants, and could therefore be associated with the fruit of tomato, aubergine and pepper when imported from areas where the potato psyllid is present. Moreover, Lso haplotypes A, B and F are hosted by solanaceous species, in particular, potato tubers, and tomato and pepper plants.

In conclusion, when surveying for detection of Lso haplotypes A, B and F, surveillance should be carried out on solanaceous species, in particular, potato tubers, and tomato and pepper plants.

1.6. Environmental suitability

The bacterium is present across a wide geographical range, demonstrating that it can survive in different climates (see Figure 1). The limiting factor is therefore the presence of the vector. Focusing here on the haplotypes that are vectored by *B. cockerelli*, there may be a restriction to the milder climates that are needed for establishment of this psyllid (EPPO, 2012).

Taking into account the distribution of *B. cockerelli* in the Americas and in New Zealand, it is assumed that *B. cockerelli* would be able to become permanently established outdoors in the southern and central parts of the EU, as well as in northern areas that have mild winters, comparable to the climatic conditions in New Zealand. Establishment in the east of Poland and further north is unlikely, but transient populations could be possible (EPPO, 2012).

In addition, throughout the EU, glasshouse production sites where solanaceous plants are grown would provide good conditions for the establishment of *B. cockerelli.*

1.7. Spread capacity

Natural spread

Natural spread of Lso depends on the spread of its vectors. Rapid spread can be expected if a vector is present or introduced together with the bacterium. In New Zealand, Lso was found to spread over more than 1000 km within four years of the introduction of the vector, by both natural and human-assisted spread (Teulon et al., 2009). *Bactericera cockerelli* has been spread by wind over long distances during migrations in North America (Abdullah, 2008).

Human-assisted spread

With regard to potatoes, Pitman et al. (2011) found transmission of Lso by tubers. However, Munyaneza et al. (2011) concluded from their study that the risk of Lso transmission by tubers is weak. Few tubers transmit the bacterium and the potato plants issued from those tubers are weak and die rapidly (EPPO, 2013a). Therefore, the contribution of the potato tubers and potato production to the spread of Lso A, B and F might be limited.

The risk of Lso establishment from infected carrot seed is uncertain (FERA, online). Several experiments on seed transmission in carrot and other Apiaceae were conducted but the results of Bertolini et al. (2014) supporting seed transmission could not be confirmed in more recent experiments (e.g. Loiseau et al., 2017a,b; Oishi et al., 2017; Mawassi et al., 2018).

Lso could be spread by the movement and trade of infected plants for planting (especially with tomato plants and potato tubers), or by the grafting of young tomato or pepper plants (Crosslin et al., 2010; Crosslin and Munyaneza, 2009). New foci, especially for solanaceous species, however, will only become established if a vector on solanaceous crops is present in the new areas (EPPO, 2012).

Vectors could be spread by the trade of host plants for planting or by solanaceous fruits that still have green parts (e.g. vine tomatoes). Transport on items like clothes may rather lead to local spread. Adult psyllids can stay in an active state without feeding for only a short period of a few days. In New Zealand, spread was assumed through infested host material and goods (e.g. clothing) (Teulon et al., 2009; EPPO, 2012).

In conclusion, the movement of host plants for planting, including potato tubers and seedlings of tomato and pepper, is a pathway for introduction into the EU and a mechanism of spread within the EU of Lso A, B and F haplotypes. In addition, should infected *B. cockerelli* be introduced to the EU, the psyllid would spread the Lso A, B or (presumably) F by the movement of host plants on which the psyllid is present through trade or by dispersal assisted by wind.

1.8. Risk factor identification

The identification of the risk factors and their relative risk estimation is essential for performing a risk-based survey. It needs to be tailored to the situation in each Member State.

A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest in the area of interest. The risk factors that are relevant for surveillance are those that have more than one level of risk for the target population. The risk factors that will be considered for the surveys need to be characterised by their relative risk and the proportion of the overall plant population on which they apply. For the delimitation of the risk areas to be surveyed as a priority, it is necessary to first identify the risk activities that could contribute to the introduction or the spread of Lso. These activities should then be connected to specific locations, also called 'risk locations'. By considering the spread capacity of the pest and the availability of host plants around these locations, risk areas can be defined.

Imports of potato tubers are prohibited from countries where Lso A, B or F are present, as laid down in Annex III of Council Directive 2000/29/EC. Therefore, the import of potatoes is not regarded as a risk activity.

The import of plants for planting of tomato, pepper and aubergine from areas where the bacterium Lso A, B, F and/or their vector *B. cockerelli* occur can be considered to be a risk activity. The accidental introduction of specimens of the regulated psyllid *B. cockerelli* cannot be excluded, as the psyllids are tiny insects that could be overlooked. The nurseries, garden centres and other premises where such plants for planting of solanaceous species are traded, stored or further distributed can be defined as the risk locations. The nurseries themselves are already subject to the obligatory regular official examinations performed by the EU Member States (Council Directive 2000/29/EC Article 6 paragraph 5). This is particularly the case for the nurseries that import plant material of host plants. The risk areas could be defined by the solanaceous crops contiguous to these locations.

For *B. cockerelli*, EPPO (2017) also suggests that premises where host fruits from countries where the pest is known to occur are imported or packed are regarded as risk locations. In these cases, the risk areas could also be defined by the solanaceous crops contiguous to these locations.

For the estimation of the relative risks corresponding to the risk areas, the interception data of Lso A, B and F and their vector can be taken into account in terms of:

- Origins of interceptions
- Commodities intercepted
- Trade volumes of the commodity
- Destination of commodities intercepted.

In addition, the importance of the solanaceous crops grown in the risk areas should also be considered in terms of the concentration of farmers of such crops, the number of greenhouses or number of hectares.

2. Detection and identification

2.1. Visual examination

The detection of Lso should be carried out on symptomatic plants. It can also be carried out on asymptomatic plants (leaves and stems). Detection on asymptomatic potato tubers, however, will be less reliable and is not recommended, while tubers with recognisable zebra chip symptoms will result in reliable detection (FAO, 2017).

2.1.1. Symptoms

Symptoms caused by Lso

Figure 4 shows the symptoms of Lso on solanaceous species. The symptoms described below are cited from Munyaneza (2012), EPPO (2013a, citing others), Haapalainen (2014), Teresani et al. (2014) and FAO (2017).

On potato plants

Symptoms of Lso on potato plants are described as being similar to the phytoplasmas potato purple top (synomym: aster yellows) and psyllid yellows (EPPO, 2013b, see also below), making it difficult to distinguish the symptoms caused by Lso from those caused by the psyllid, including chlorosis, zigzag-shaped stems, axillary bud proliferation, swollen nodes, internodes shorter than usual, aerial tubers, vascular discoloration, leaf scorching, wilting of the plants.

On potato tubers

Underground symptoms manifest as smaller tubers, an increase in the number of tubers and short stolons. In addition, tubers tend to be deformed, have rough skin and loss of dormancy, resulting in premature germination. The germs are slender, hairy and very weak. These tubers are unusable for planting. A brown discoloration of the vascular ring is observed with necrotic flecking of the inner tissues and streaking of the medullary ray tissues – these symptoms are more marked on fried potatoes, which gave the name of 'zebra chip' to the disease in potato.

On solanaceous plants (above ground) in general, including pepper and tomato plants

In general, on Solanaceae, symptoms vary in severity and are influenced by the host cultivar, temperature, and growing conditions – they can be weak and are not very characteristic, and plants can also remain asymptomatic. Symptoms comprise stunting, uprightness of young leaves, chlorosis, purple leaves with basal cupping, upward rolling of leaves, rosetting, elongated nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit setting, and production of a high number of small, misshapen fruits of low quality.

Figure 4: Symptoms of Lso on solanaceous species: a) on a potato plant; b) on potato tubers (left hand side: raw, right hand side: fried); c) on a pepper plant; d) on a tobacco plant and e) on a tomato plant (Source: Joseph Munyaneza, USDA)

Symptoms caused by *B. cockerelli*

On the above-ground plant parts of potatoes and tomatoes, *B. cockerelli* may cause characteristic uprightness of new leaves, retardation of growth, chlorosis, purpling and basal cupping of new leaves, upward rolling of leaves, shorter and thicker terminal internodes causing rosetting, enlarged nodes, axillary branches or aerial potato tubers, no or numerous production of small, low quality fruits. Below ground, the psyllid may cause excessive numbers of very small misshapen potato tubers or chain tubers, and an early breaking of dormancy of tubers (List, 1939; Pletsch, 1947; Daniels, 1954; Wallis, 1955).

Symptoms on tomato are called psyllid yellows (EPPO 2013a), even if not caused by *B. cockerelli*, because the symptoms resemble those that are caused by a toxin when psyllid nymphal instars are feeding on the plants (EPPO, 2013b). They comprise spiky, chlorotic apical growth, mottling of leaves, curling of mid-veins, stunting of plants, and fruit deformation in some cultivars.

2.1.2. Morphological identification of the vector

Adults are about 2.5–2.75 mm long with two pairs of clear wings, of which the front wings are noticeably larger than the hind wings. Antennae are approximately the length of the thorax. At emergence, adults are pale green, and become dark green or brown within 2–3 days, and then grey or black. Head and thorax are white or yellow. Characteristically for *B. cockerelli*, the first abdominal segment has a broad, transverse white band and the last abdominal segment has an inverted V-shaped white mark (Pletsch, 1947; Wallis, 1955) (Figure 5).

From above, nymphs are elliptical and very flattened in profile, almost scale-like. They can be confused with the nymphs of whiteflies, but the psyllids move when disturbed. The five nymphal instars are morphologically very similar, but differ in size, ranging from 0.23 to 1.60 mm. First, the nymphs are orange, but when maturing they become yellowish-green and then green. The prominent compound eyes are reddish. Wing pads start to show with the third instar and become more pronounced with each further moult. Along the lateral margins of the body, there is a short border of wax filaments (EPPO, 2017 citing others) (Figures 5, 6 and 7).

Bactericera cockerelli (and other psyllid vectors of Lso) can be morphologically identified with the help of identification keys by Ossiannilsson (1992) and Carnegie et al. (2017). The latter includes illustrations and photos and is accessible online.

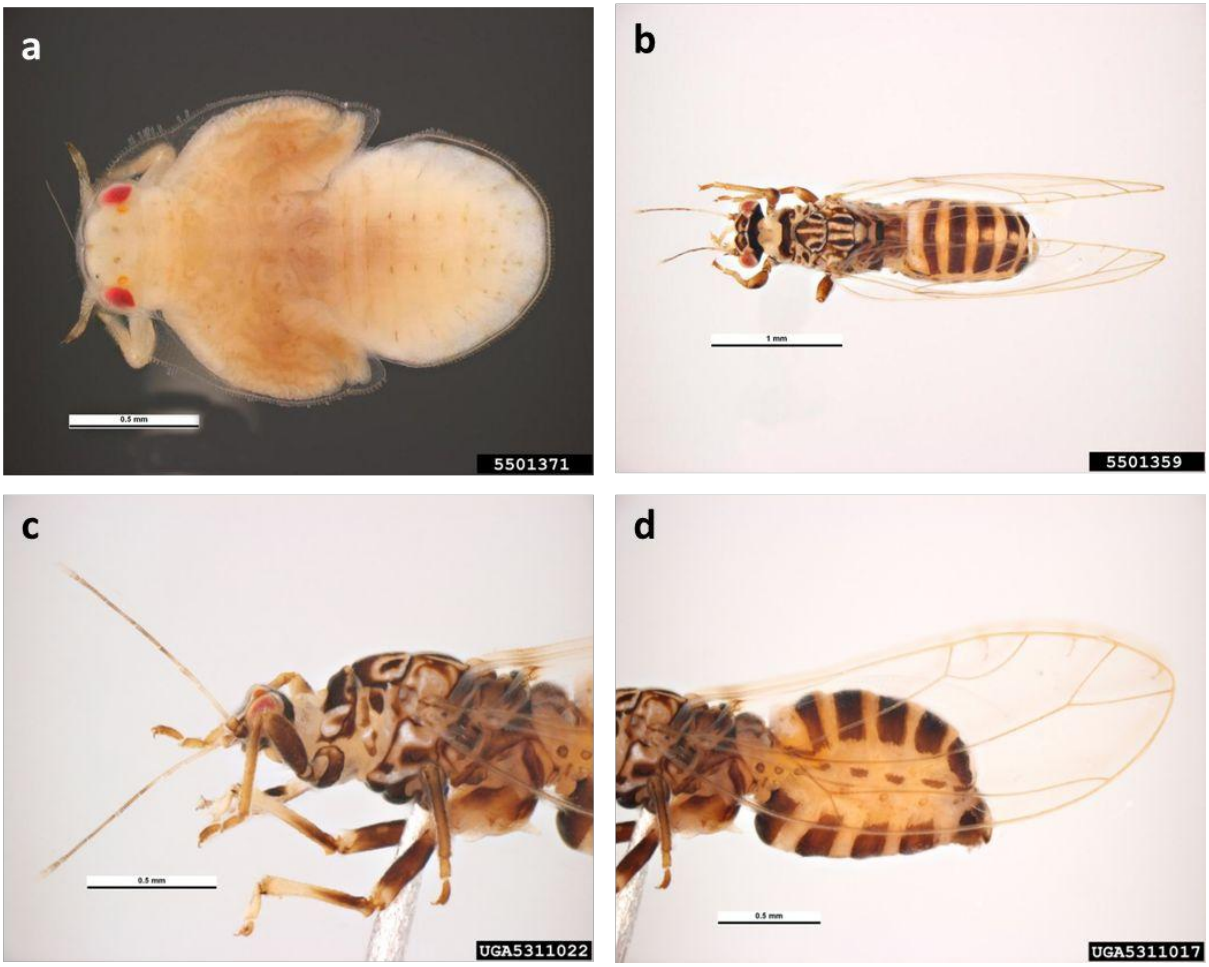

Figure 5: Potato/tomato psyllid (*Bactericera cockerelli*). a) dorsal view of nymph; b) dorsal view of adult; c) lateral view of adult and d) lateral view of adult (Images: Pest and Diseases Image Library, Bugwood.org)

Figure 6: Nymph of the potato psyllid (*Bactericera cockerelli*) on a potato (*Solanum tuberosum* L.) (Image: Eugene E. Nelson, Bugwood.org)

Figure 7: Dorsal view of nymph and teneral adult potato psyllid (*Bactericera cockerelli*) (Image: Whitney Cranshaw, Colorado State University, Bugwood.org)

2.2. Sampling

Plant material sampling

The distribution of Lso in plant parts may be heterogeneous depending on the plant species and consequently appropriate sampling is required to improve detection (FAO, 2017).

Three to five leaves and/or stems from symptomatic parts of the plant should be collected. Below-ground plant parts such as tubers, roots and stolons can also be used to detect Lso (but see comment under Section 2.1) (FAO, 2017).

Potato tubers showing obvious zebra chip symptoms should be tested individually. The tuber is cut and symptomatic tissue from the vascular area and the heel ends is sampled (FAO, 2017).

Before extraction, all plant material is subsampled so that the material used contains as much vascular tissue as possible (e.g. petioles, leaf midribs, cambium, and the heel end or vascular ring of potato tubers) (FAO, 2017).

Vector sampling

Early in the season, psyllid abundance is higher on field edges than in the interior of fields, but as the season progresses, they are more evenly distributed over the field (Rondon et al., 2017). For crops grown under protection, traps may also be located near potential points of pest entry (EPPO, 2017).

Collecting adult *B. cockerelli*

Adults of *B. cockerelli* can be hand-collected from symptomatic or asymptomatic plants. Egg and nymphal sampling requires visual examination of the foliage (EPPO, 2017).

Trapping adult *B. cockerelli*

The most effective way to sample adult *B. cockerelli* is by yellow sticky traps or yellow water traps. Lower set traps (i.e. just below the plant canopy) seem to give better results (EPPO, 2017). They need to be put higher up with the growing crop and should be replaced weekly. In addition, an inverted leaf blower or sticky cards can effectively monitor *B. cockerelli* but is more labour-intensive. Monitoring may also include non-host crops if volunteer potatoes are present (Klein and Rondon, 2019).

Hodge et al. (2019) confirmed the effectiveness of yellow sticky traps to also monitor *B. cockerelli* in tomatoes in glasshouses. They state that consistency in trapping could be improved by placing the traps at a constant height, illuminating them with ultraviolet light, and limiting the assessments to the centre of the traps.

See also Horton et al. (2019) for the development of a novel insect trap constructed with three-dimensional printing technology.

2.3. Laboratory testing and pest identification

2.3.1. Testing plant material

The FAO (2017) provides the diagnostic protocols suitable for all host plant species, and describes different molecular methods for Lso identification (PCRs). It is important to note that EPPO is currently preparing a detailed diagnostic protocol for this bacterium and its psyllid vectors.

Two real-time PCR tests are recommended for the detection of Lso in plant material or in vectors (Li et al., 2009 and Teresani et al., 2014).

The haplotype can be determined by amplifying and sequencing three genomic regions. The tests of Li et al. (2009) target the 16S rRNA gene region, Ravindran et al. (2011) target a region of the 16 –

23S rRNA intergenic spacer and Munyaneza et al. (2009) target a region of the rplL-rplJ gene region (50S rRNA). Amplicons of these three PCRs should be sequenced to determine the species and the haplotype of the bacterium in suspect samples. These PCRs can also be used as screening tests in symptomatic material. However, it should be noted that real-time PCR is recommended because of its better analytical sensitivity.

The minimum identification requirement for Lso is a positive result from one of the PCR tests. Confirmation is recommended for critical cases after Lso is detected by one rapid screening test. A second PCR should be performed. For conventional end-point PCR, the product should be sequenced. For the sequence to be considered as the same species as Lso, it should be ≥98% identical to the sequence from the reference isolate (GenBank accession number EU834130).

According to Levy et al. (2011), Lso may go undetected by molecular methods, if plants are tested within the first three weeks from infected vector feeding.

2.3.2. Testing vectors

Lso can be reliably detected in *B. cockerelli* by conventional and real-time PCRs recommended for plant material. Crosslin et al. (2011) recommend the use of bulks of 30 laboratory-reared adult psyllids for testing, while EPPO (2017) states that bulks should be limited to 10 psyllids if they are sampled from the field by either sticky traps or hand collection (EPPO, 2017).

3. Key elements for survey design

Based on the analyses of the information on the pest–host plant system, the different units that are needed to design the survey have to be defined and tailored to the situation of each Member State. The size of the defined target population and its structure in terms of the number of epidemiological units need to be known. When several pests have to be surveyed in the same crop, it is recommended that the same epidemiological and inspection units are used for each pest in order to optimise the survey programme as much as possible. This would optimise field inspections since they are organised per crop visit and not by pest. Table 2 shows an example of these definitions.

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for a survey for *Candidatus* Liberibacter solanacearum in solanaceous species

	Definition	**Unit**
Target population	Total number of potato lots, fields, hectares, or glasshouses in a Member State with solanaceous species	A potato lot or a field or a hectare or a glasshouse
Epidemiological units	Lots or hectares or glasshouses in a Member State with at least one host plant	A potato lot or a field or a hectare or a glasshouse
Inspection units (host)	Solanaceous plants, leaves of solanaceous plants	Number of plants or leaves
Inspection units (vector)	Individual plants used for hand collection or traps	Number of plants or traps

The general guidelines for risk-based statistically sound surveillance are presented in a separate document and describe the process of the survey design step by step and include:

1/ the choice of the type of survey to develop depending on the objectives of the survey

2/ a manual for guiding the user through the statistical tools for sample size calculations

3/ essential considerations when:

- choosing the sampling sites and taking samples
- collecting the data
- reporting the data and the survey results.

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Glossary

TERM	DEFINITION*
Buffer zone	An area surrounding or adjacent to an area officially delimited for phytosanitary purposes in order to minimise the probability of spread of the target pest into or out of the delimited area, and subject to phytosanitary or other control measures, if appropriate (ISPM 5: FAO, 2019).
Component (of a survey)	A component is a survey entity which can be distinguished based on its target population, the detection method (e.g. visual examination, laboratory testing, trapping) and the inspection unit (e.g. vectors, branches, twigs, leaves, fruits). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components.
Confidence	Sensitivity of the survey. Is a measure of reliability of the survey procedure (Montgomery and Runger, 2010).
Design prevalence	It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence. In 'freedom from pest' approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the 'design prevalence'. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence. (EFSA, 2018)
Detection survey	Survey conducted in an area to determine if pests are present (ISPM 5: FAO, 2019).
Delimiting survey	Survey conducted to establish the boundaries of an area considered to be infested by or free from a pest (ISPM 5: FAO, 2019).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016).
Epidemiological unit	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same epidemiology, should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a geographical area. They are the units of interest, on which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2019).
Inspection unit	The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place. (EFSA, 2018).
Inspector	Person authorized by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2019).
Method sensitivity	The conditional probability of testing positive given that the individual

	is diseased (Dohoo et al., 2010). The method diagnostic sensitivity (DSe) is the probability that a truly positive epidemiological unit will give a positive result and is related to the analytical sensitivity. It corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and confirmed as positive.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2019).
Pest freedom	An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM 5: FAO, 2019).
Population size	The estimation of the number of plants in the region to be surveyed (EFSA, 2018).
Potato lot	A potato crop identifiable by its homogeneity of composition (same cultivar), origin (same field), etc., or A number of potato tubers identifiable by their homogeneity of composition (same cultivar), origin (same field, same crop) and with traceability to the field in which they were produced.
Relative risk	The ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group (Dohoo et al., 2010).
Representative sample	A sample that describes very well the characteristics of the target population (Cameron et al., 2014).
RiBESS+	An online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of freedom from disease. Free access to the software with prior user registration is available at: https://shiny-efsa.openanalytics.eu/
Risk assessment	Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2019).
Risk factor	A factor that may be involved in causing the disease (Cameron et al., 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared to a baseline with a level of 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas where the highest probabilities exist to find the pest should the pest be present.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005).
Survey	An official procedure conducted over a defined period to determine the presence or absence of pests, or the boundaries or characteristics of a pest population, in an area, place of production or production site (ISPM 5: FAO, 2019).
Target population	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are:

	• Definition of the target population – the target population has to be clearly identified • Target population size and geographic boundary. (EFSA, 2018)
Test	Official examination of plants, plant products or other regulated articles, other than visual, to determine if pests are present, identify pests or determine compliance with specific phytosanitary requirements (ISPM 5: FAO, 2019).
Test specificity	The conditional probability of testing negative given that the individual does not have the disease of interest (Dohoo et al., 2010). The test diagnostic specificity (DSp) is the probability that a truly negative epidemiological unit will test negative and is related to the analytical specificity. In freedom from disease it is assumed to be 100%.
Visual examination	Examination using the unaided eye, lens, stereoscope or other optical microscope (ISPM 5: FAO, 2019).

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