Report Cover Page

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Title
Post-border surveillance techniques: review, synthesis and deployment - subproject 2e Proof-of-freedom toolbox
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Summary

A wide range of tools and methodologies that are available to prove the pest or disease status of a country or region was reviewed in ACERA 1004. The review highlighted the set of tools that could be used to achieve or maintain market access. Most were used for 'proof of freedom' claims and were classified as relating to either survey design or to scenario-tree modelling, however, not all of these have been developed into readily applicable tools that could be easily and accurately applied by biosecurity managers with limited prior knowledge of the methodology. In this document we have detailed three survey design tools that have been developed from accepted statistical formulae and are well accepted in animal-disease surveillance: *FreeCalc*; *EpiTools*; and *Survey Toolbox*. We recommend that EpiTools be chosen for undertaking survey design and analysis.

After additional investigation into scenario tree modeling (including Bayesian Belief Networks) and associated generic software, the authors deem that this method requires too much prior knowledge and is not sufficiently clear that biosecurity managers or scientific staff could be reasonably expected to use and master it quickly. It appears unlikely that a tool will ever be developed that can effectively replace the amount of training that would be required to master scenario-tree modeling. If scenario-tree modeling were required to make a proof-of-freedom claim, then it would be advisable to employ an expert who has had training in the use of this methodology, to undertake the analysis.

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1. Executive Summary

A range of tools and methodologies are available to provide evidence about the pest or disease status of plants and animals in order to maintain market access for agricultural products, and these were reviewed in Hester *et al.* (2010). In the context of market access, the set of readily applicable tools had mostly been used for 'proof of freedom' claims and were classified as relating to either survey design or to stochastic scenario-tree models. The aim of this project was to extend the review of proof-of-freedom tools discussed in Hester *et al.* (2010), to give specific details of how these tools could be applied to solve routine proof-of-freedom problems. The report is aimed at biosecurity managers and other scientific staff who do not possess expertise in statistics, risk analysis or Bayesian inference, but who are still regularly faced with having to design surveys and analyse survey results in order to secure or maintain access to export markets for their products, or to justify their own import restrictions.

On further investigation, the methodology and associated software relating to scenario-tree modeling (including Bayesian Belief Networks) was deemed to require too much prior knowledge to be something that a biosecurity manager could be reasonably expected to use and master quickly, and were not considered appropriate for this 'proof-of-freedom toolbox'.

This report reviews three survey-design tools, namely *FreeCalc*, *EpiTools* and *Survey Toolbox*, by providing practical information on the circumstances in which each tool could be applied, any advantages and disadvantages, information requirements of each, and examples showing how each tool may be used in the proof-of-freedom context. Key references to sources that explain the methodology and to applications of each tool are also supplied. Given the interrelated nature of these tools and changes to operating systems on which they operate, *EpiTools* is suggested as the preferred tool for developing plant- and animal-health survey-designs to solve proof-of-freedom problems.

2. Introduction

One of the key purposes of post-border surveillance is to secure or maintain market access for agricultural products by providing evidence about the pest and disease status of a country, or regions within a country. For those countries that are members of the World Trade Organization (WTO), participation in international trade in plant and animal products is governed by rules contained in the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and the Agreement on Technical Barriers to Trade (TBT Agreement, see WTO 1998 and WTO n.d. for more details on these agreements). Under this agreement, principles that can be used as a guide to market access negotiations and arrangements for animals and animal products are documented in *The Terrestrial Animal Health Code* (OIE 2010a) and the *Aquatic Animal Health Code* (OIE 2010b). The corresponding principles for trade in plants and plant products are contained in International Standards for Phytosanitary Measures (ISPMs) developed by the International Plant Protection Convention. The relevant guidelines are contained in IPSM 4, 6, 8 and 10 (FAO 1996, 1997, 1998b and 1999).

Proving pest or disease status may be necessary in the following circumstances:

- when a trading partner requires evidence that a jurisdiction, and thus its export products, are free of a particular pest or disease before it will approve the import of a commodity;
- when an area of low pest prevalence is proposed as a phytosanitary measure to enable trade to continue (plants only);
- when a jurisdiction needs to provide evidence that it is free of a quarantine pest before imposing its own restrictions on imports; and
- to prove eradication has occurred and to support a subsequent declaration of freedom.

When establishing and maintaining area freedom is the imperative, biosecurity managers need surveillance tools that allow them to use appropriate statistical practices to answer questions similar to the following:

- how should a survey be designed in order that enough host animals/plants/sites are checked to provide confidence that if the disease is present, it would be found with high probability?
- how can survey information be used to obtain a robust estimate of probability that the pest/disease isn't present?
- how can information from local landowners, private industry organizations, community groups and emergency 'hotlines' be used to support area freedom?

Numerous tools and methodologies available for demonstrating 'proof-of-freedom' were reviewed in ACERA 1004 (see Hester *et al.* 2010). The authors highlighted a set of readily applicable tools that could be used for post-border surveillance to achieve market access (see Table 1). The tools ranged from procedures and formulae to specialised software packages and web-based statistical calculators, and were classified as relating to either survey design or stochastic scenario-tree analysis. A scenario tree is a way of representing a hierarchy of information about a system. In analysing surveillance systems, scenario tree analysis has several functions, including visualisation and documentation of the logical and practical structure of the surveillance process; clarification and description of the steps involved in analysis of the surveillance system component; and definition of the interrelationships of factors affecting the probability of infection and the probability of detection (AusVet Animal Health Services n.d.b). Representing complex surveillance systems leads to the construction of large scenario trees and specialist computer software must be used. While the implementation of large trees can be represented in a simpler manner using matrix algebra or Bayesian belief networks (see Hood *et al.* 2009), we concluded that the time and resources required to understand the methodology and competently apply the associated software are too high for these tools to be classed as 'readily applicable tools'. If scenario-tree modeling were required to make a proof-of-freedom claim, then it would be advisable to employ an expert to undertake the analysis[1].

In addition, the tool cited in Hester *et al.* (2010) as being available and which was suitable for creating simple scenario trees (namely Ausvet's Freedom software), is no longer functional. Thus, we were not able to identify any suitable stochastic scenario-tree analysis tools, and the discussion of proof-of-freedom tools will be limited to those dealing with survey design.

The objective of this research is to explain these tools in more detail, providing practical advice on the appropriate use of each tool and, in effect, creating a 'proof-of-freedom toolbox' to assist biosecurity decision-making. The toolbox is particularly aimed at assisting decision makers in determining proof-of-freedom for invasive species.

In order to provide practical guidance with decision-making for proof-of-freedom claims, the following information will be provided for the tools outlined in Table 1:

- How to use each tool in the 'proof-of-freedom' context
- Circumstances in which each tool could be applied
- Information requirements
- Advantages and disadvantages of each approach

[1] For more information on application of the scenario-tree methodology to biosecurity issues, readers are referred to Martin et al. (2007a, b), Barrett et al. (2009), and Jarrad et al. (2010).

- Key references and previous applications

In addition, technical terms used in the discussion of each tool will be linked to their definition in a Glossary. Here we propose a structure for discussing the tools that can assist with proof-of-freedom claims.

Table 1. Surveillance methods and tools for proof-of-freedom (modified from Hester *et al*. 2010).

Method	Use of technique	Available tools	Documented Application	Reference
Survey design	Evidence of area freedom when sensitivity and specificity < 1, or sampling with replacement	FreeCalcV2 Epi Tools Suite Survey toolbox for livestock diseases	Area surveys of livestock animals	Cannon (2001); Cameron and Baldock (1998a); Cameron and Baldock (1998b); Cannon and Roe (1982)
	To provide evidence of freedom from a disease	Survey toolbox for aquatic animal diseases	Aquatic animal disease surveys (fish, crustaceans and molluscs)	Cameron (2002)
Stochastic scenario tree models	Evidence of area freedom	Scenario Trees to Quantify Confidence in Freedom from Disease (Freedom Tool)*	Classical swine fever, Denmark	Martin *et al.*.(2007a, b)
			Survey of an invertebrate, Barrow Island, WA	Barrett *et al.*(2009) Jarrad *et al.* (2010)
Bayesian belief networks	Evidence of area freedom in multiple component systems	Netica, GeNie, and others	FMD Danish CSF	Hood *et al.* (2009)

*The Freedom Tool is no longer functional and unlikely to become functional in the future.

3. Survey design tools

With this set of tools, making claims of area freedom is based around structured surveys that involve looking for pests and diseases that are not known to be present. The objective of surveillance is to provide evidence that a pest or disease was not detected using a survey that has a high degree of confidence of detecting the pest, if it were present, at or above an acceptably low prevalence, depending on the epidemiology of the specific pest or disease.

For some industries, regulations establish how disease freedom should be demonstrated and there may be little scope for deviation from these. When pest- or disease-specific guidelines for a structured population-based survey have not been given, appropriate statistical practices should be followed and documented. Many sampling techniques and formulas for sample size exist, and it is important to understand the appropriate circumstances for their use. This information can be found in standard sampling theory text books (e.g Cochran 1977, Krebs 1998 and Schreuder *et al.* 1993).

Hypothesis testing is an important part of survey-design and begins with some assertion about the value of a particular parameter, which, in the case of area freedom surveys, is usually the prevalence (proportion of the population infested at a particular point in time). Typically, the null hypothesis is that the pest or disease is present at a level equal to or greater than a specified (minimum) prevalence. The aim is to find evidence from the survey that rejects the null hypothesis in favour of the alternative hypothesis, that the disease is present at a level lower than the specified prevalence. There is, of course, the risk that an incorrect conclusion might be reached. The survey results might show no signs of a pest or disease in the population, when in fact the pest or disease is present (the null hypothesis is rejected when it should be accepted), this is known as a Type I error. Alternatively, analysis of the survey results might show the presence of a pest or disease when in fact this is not the case (null hypothesis is not rejected when it is false), known as a Type II error. Type II errors can only occur where test specificity is imperfect (less than 100%). In most proof of freedom situations the cost of a Type II error (usually in terms of lost trade) is so high that any positive results are followed up with additional testing and other investigation to ensure that the specificity of the system is effectively 100%, so that Type II error is 0. The value of a Type I error is usually set at 0.05 (there is a 5% chance of incorrectly assuming that the population is free of the pest or disease) and Type II errors (where they are permitted) are usually set at 0.05 or less.

Several online tools are now available to assist with survey design in the proof-of-freedom context, namely, FreeCalc, Epitools, *Survey toolbox for Livestock Diseases*, and *Survey Toolbox for Aquatic Animal Diseases* – and these tools are reviewed in this chapter. In

addition, thorough and practical accounts of the procedures involved in designing surveys and analyzing results are provided by McMaugh (2005) for plants, Cameron (1999) for diseases of livestock animals, and Cameron (2002) for diseases of aquatic animals. These resources are also discussed in this chapter.

3.1. FreeCalc

FreeCalc is an epidemiological probability calculator that was designed to assist with the planning and analysis of surveys to demonstrate freedom from disease in livestock (Ausvet n.d.a). FreeCalc version 2 was developed by Ausvet Animal Health Services and can be freely downloaded as shareware from http://www.ausvet.com.au/content.php?page=software#freecalc or from the Ausvet homepage http://www.ausvet.com.au/: click on the **Tools** menu item, then **Software** (Figure 1).

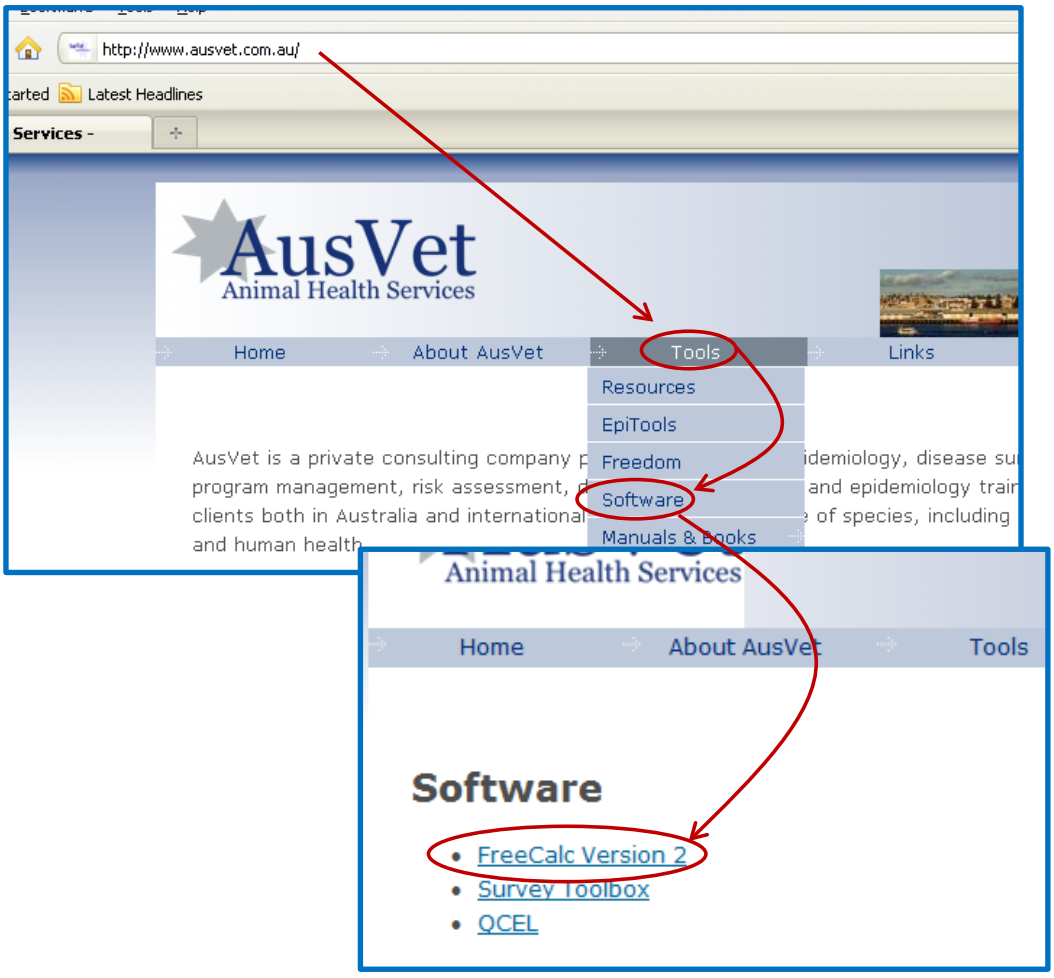


Figure 1. How to locate FreeCalc from the AusVet homepage.

Key references

Cameron, AR and Baldock, FC (1998) A new probability formula for surveys to substantiate freedom from disease. *Preventive Veterinary Medicine,* 34(1):1-17

Cameron, AR and Baldock, FC (1998) Two-stage sampling in surveys to substantiate freedom from disease. *Preventive Veterinary Medicine,* 34(1):19-30

Cameron (1999): *Survey Toolbox for Livestock Diseases - A practical manual and software package for active surveillance of livestock diseases in developing countries.* Australian Centre for International Agricultural Research, Canberra, Australia.

When to apply

The tool can be used to determine sample size for surveys and to analyse the results of surveys when sensitivity and specificity are known and imperfect (less than 100%) and when the population size is known or where it is possible to estimate all elements of the population.

Information requirements

To determine sample size the following information is required:

- Size of the population being sampled (e.g., the number of animals or number of host plants)
- Test sensitivity (%)
- Test specificity (%)
- Design prevalence
- Desired type I error
- Desired type II error

To analyse data in the proof-of freedom context the following information is required:

- Sample size used in the survey
- Test sensitivity and specificity (%)
- Population size (no. animals)
- Minimum expected (maximum acceptable) prevalence of disease, if present
- Required Type I error
- Required Type II error
- Number of positive test results detected

How does FreeCalc work?

Using an iterative procedure, FreeCalc calculates the number of animals (or host plants) that must be tested in order to provide evidence, at the specified level of confidence, that disease

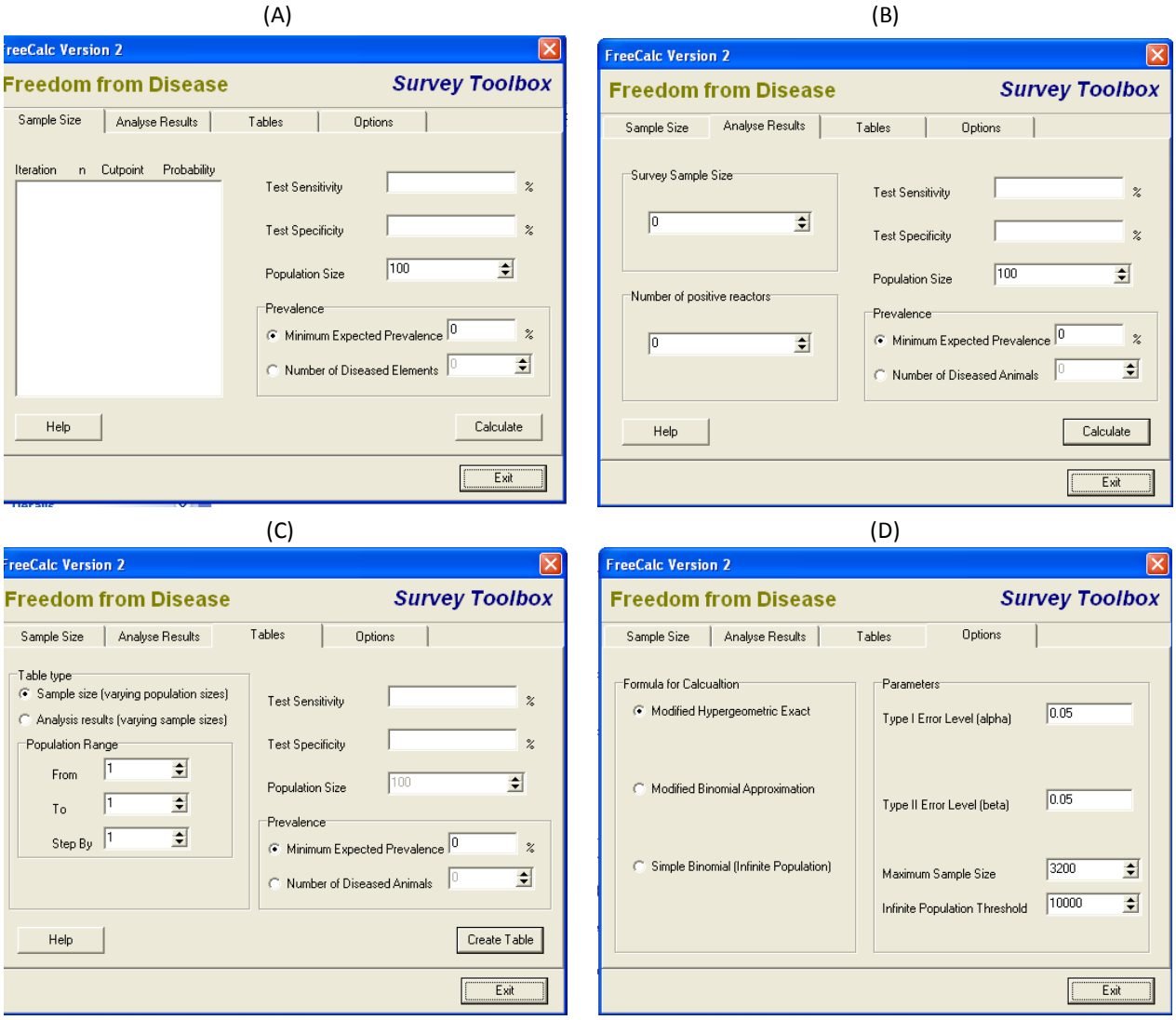


Figure 2. Screen views from FreeCalc: (A) Sample size; (B) Analyse Results; (C) Tables and (D) Options.

is not present. While in proof of freedom surveys it is usually assumed that the test specificity is 100%, FreeCalc does allow values of test specificity to be lower than 100%. Then, the maximum number of false-positive results that can be observed while still being able to conclude the population is free from disease (cutpoint number of positive test results) will be reported. Users will typically work from one of four screen-views (Figure 2).

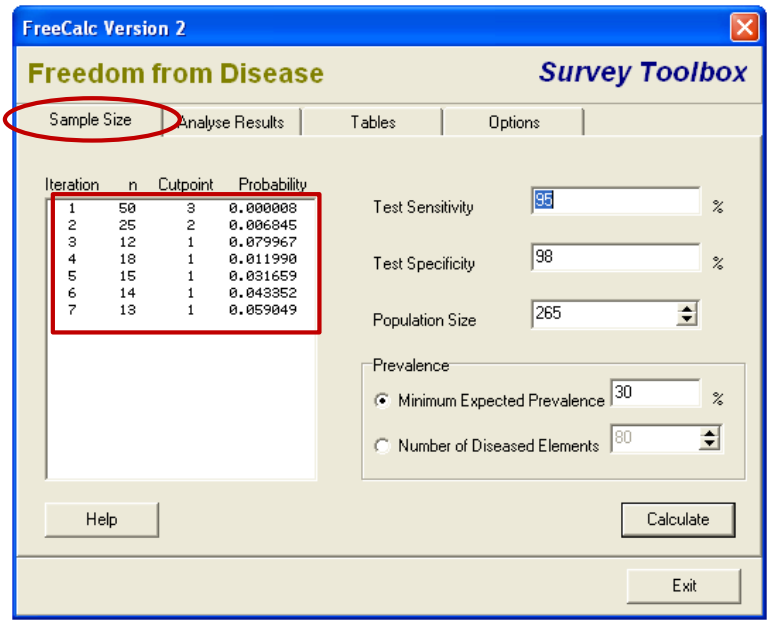
Example 1: determining sample size

Here we replicate the foot and mouth disease (FMD) example given in Cameron and Baldock (1998a) to show how the sample size for a survey to prove disease freedom is calculated using FreeCalc.

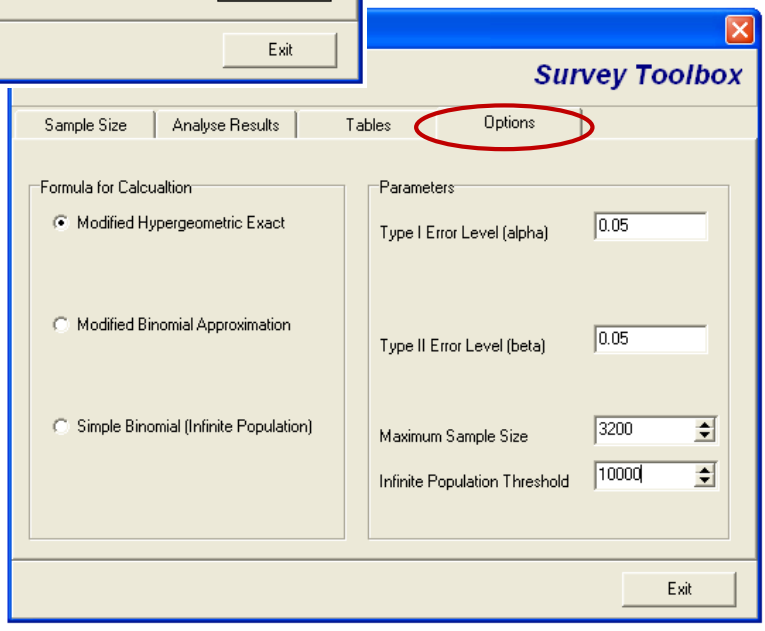
It is assumed that a FMD outbreak occurred in a previously FMD-free area but was subsequently eradicated. Surveys now need to be undertaken to demonstrate that the

disease is no longer present. In the case of this and similar surveys to prove freedom from a pest or disease, the null hypothesis is that FMD seroprevalence is equal to or greater than a specified (minimum) prevalence. The alternative hypothesis is that the seroprevalence is lower than the specified prevalence. For this example it is assumed that if FMD were circulating in the population it is most easily detected by serological testing and that if this were the case the expected seroprevalence would be equal to or greater than the design prevalence.

Test sensitivity and specificity are usually estimated from published literature or expert opinion. For this analysis they are assumed to be 95% and 98% respectively. If unsure of these values more conservative (lower) values can be used or a sensitivity analysis of values undertaken. If FMD has passed through the herd, the presence of antibodies to the disease would show in at least 30% of animals, so the minimum expected prevalence is 30%. The



(A)



(B)

unaffected herd being tested contains 265 animals. Information on test specificity, sensitivity, minimum expected prevalence and population size is put into the *Sample Size* tab within FreeCalc (Figure 3 A).

Additional information is required on the probability of falsely rejecting the null hypothesis and concluding that the population is not diseased when in fact it is (a type 1 error, α), and the probability of falsely accepting it, and concluding that the population is diseased when in fact it is not (type II error, β). Both error rates are specified as 0.05 and values are entered in the Options page within FreeCalc (Figure 3 B). The Modified Hypergeometric Exact Formula for Calculation should then be selected. The user should return to the *Sample Size* tab and press **Calculate**.

Results appear in a new screen (Figure 4) and on the left-hand side of the *Sample Size* screen (Figure 3A). For the given parameters, the results indicate that the sample size should be 14 animals, with a cutpoint number of positive tests equal to 1. The results can be interpreted as follows (from Cameron and Baldock 1998a):

i. If the herd is uninfected, the probability of observing one or less infected animals from the sample of 14 is equal to or just greater that 0.95 (there is less than a 5% chance of declaring the herd infected when it is not (a type II error); and

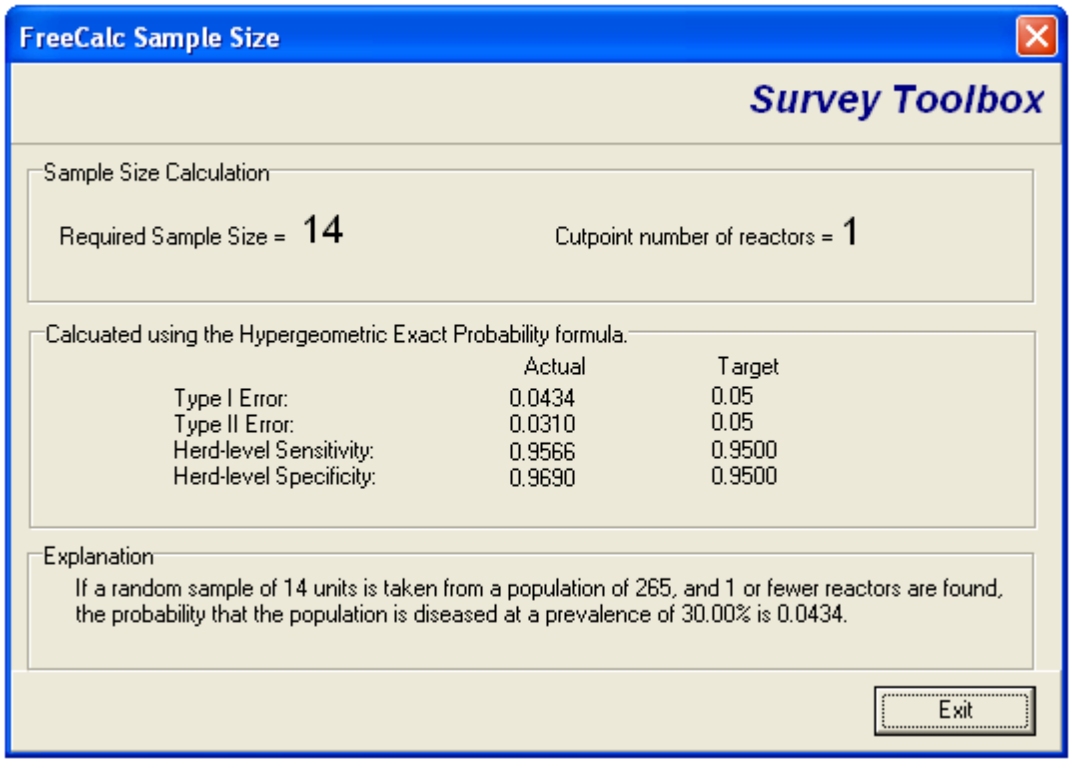


Figure 4 Results produced from FMD example taken from Cameron and Baldock (1998a)

ii. If the herd is infected with a prevalence of 30%, the probability of observing 1 or fewer reactors is equal to 0.0434, so confidence of detection is greater than 95% for a design prevalence of 30%.

Additional runs of FreeCalc with different values of sensitivity, specificity, minimum expected prevalence and population size are very easily done (press cross in top right hand corner of results page before each new run). The three main reasons for performing extra runs are to 1) undertake a sensitivity analysis, where you might vary specific inputs that you are uncertain about to see how much impact they have on the result; 2) to try and reduce sample size to meet a budget; and 3) because population sizes differ, such as when multiple or different herds/farms are being analysed. Examples of additional runs that could be undertaken in the current example are:

- if the diagnostic test for FMD were perfect (so both test sensitivity and specificity = 100%), the required sample size would drop to 9 animals;
- If the population were infinite (large, but exact size unknown), with the imperfect test (original values of specificity and sensitivity), then the sample size is again 14.
- Dropping the minimum expected prevalence to 10% (other parameters at their original values) yields a much larger sample size of 64 animals, with a cutpoint number of positive tests of 3.
- Using this lower prevalence with a perfect test drops the sample size to 28;
- Using this lower prevalence, the imperfect test and an infinite population yields a sample size of 67; and
- Using the lower prevalence, a perfect test and an infinite population requires a sample size of 29 animals.

Example 2: analysing data

Continuing with the FMD example given in Cameron and Baldock (ibid), assume that the minimum expected prevalence was assumed to be 10%, with an imperfect test and population size of 265. A sample of 64 animals was taken from the herd and two animals showed a positive reaction to the test. To understand what this means, FreeCalc's analysis module is used to interpret the results. The user works from the *Analyse Results* tab and enters the values for test sensitivity, test specificity, minimum expected prevalence and population size previously used, followed by the sample size (64) and the number of positive reactors (2) (Figure 5A). When the **Calculate** button is pressed, results appear in a new

screen (Figure 5B) and in the current example, they show that the probability of observing 2 or fewer positive tests under the null hypothesis (that the disease is present at a prevalence of 10%) is equal to 0.013. From this it can be concluded with a high level of confidence (1-0.013 = 0.987 or 98.7%) that the herd is not diseased, and that the two positive reactors were probably due to the imperfect specificity of the test (Cameron and Baldock 1998a). In regulatory or trade-related programs these positives would invariably be further investigated to determine their true status. However, if there is no specific requirement for such follow-up, it is not necessary to do so and the population can be concluded to be free of disease at the specified design prevalence and with appropriate level of confidence. A good example of this approach is provided by Dukpa et al. (2012), who demonstrated a high level of confidence that if FMD was present it was below the specified design prevalence, for a specific district of Bhutan without further follow-up of individual positive animals.

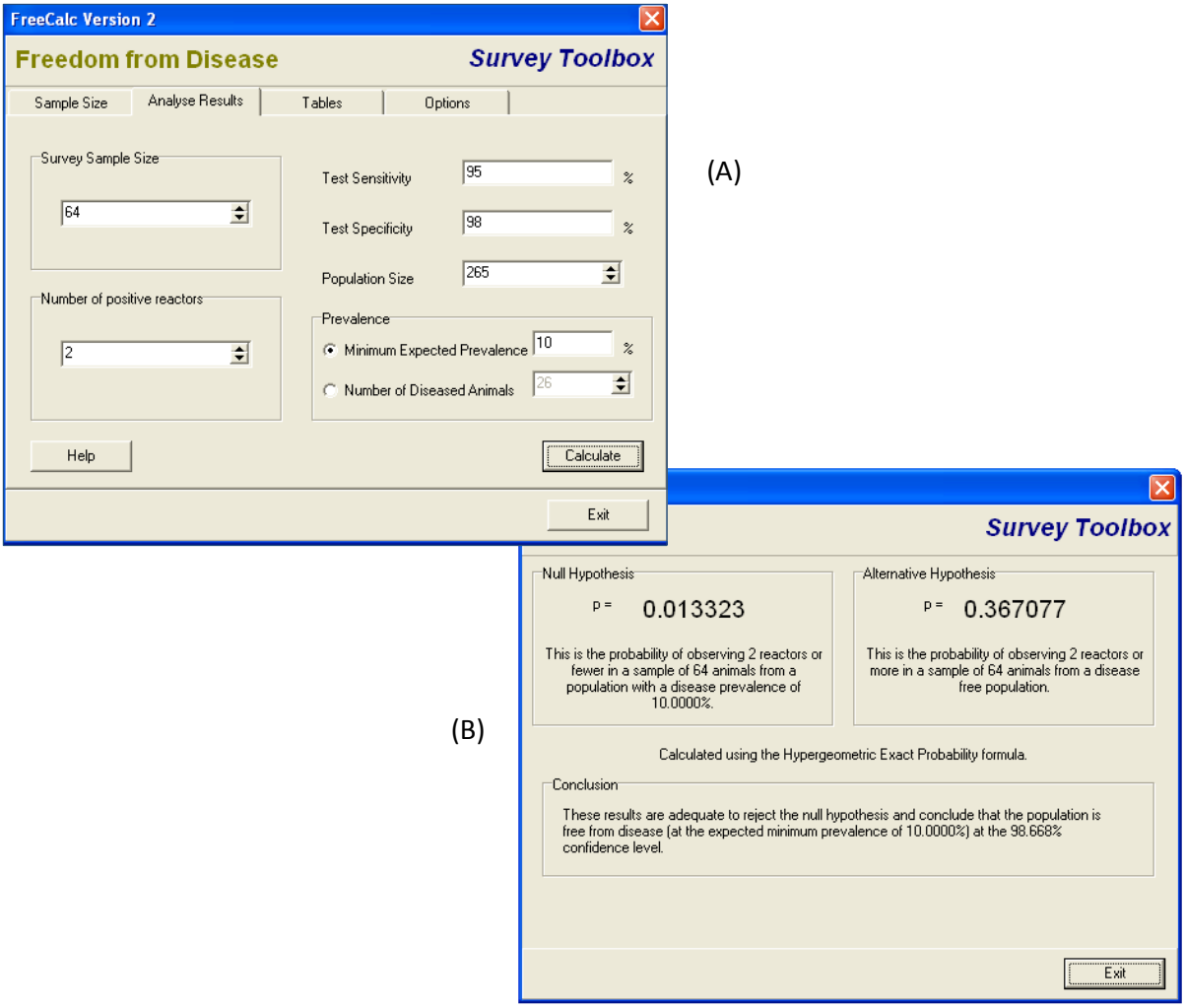


Figure 5 Use of the Analyse Results tab (A) to analyse results of FMD tests (B)

Advantages and disadvantages

FreeCalc is one of the few tools that can be used with imperfect test specificity, but it is rarely used in this way for regulatory or trade-related surveillance to the issue of having unresolved positives.

Previous applications of FreeCalc to proof-of-freedom claims

FreeCalc has been widely used in animal health, including in the following studies:

Kampen AH, Hopp P, Grøneng GM, Melkild I, Urdahl AM, Karlsson A and Tharaldsen J (2012) No indication of *Coxiella burnettii* infection in Norwegian farmed ruminants. *BMC Veterinary Research,* 8(1):59*.*

Makita K, Fèvre EM, Waiswa C, Eisler MC, Thrusfield M and Welburn SC (2011) Herd prevalence of bovine brucellosis and analysis of risk factors in cattle in urban and peri-urban areas of the Kampala economic zone, Uganda. *BMC Veterinary Research*, 7(1):60.

Reid, SA and Copeman, DB 2000 Surveys in Papua New Guinea to detect the presence of *Trypanosoma evansi* infection. *Australian Veterinary Journal*, 78(12): 843-845.

Rodríguez NF, Tejedor-Junco MT, González-Martín M, Santana del Pino A, Gutiérrez C (2012) Cross-sectional study on prevalence of *Trypanosoma evansi* infection in domestic ruminants in an endemic area of the Canary Islands (Spain). *Preventive Veterinary Medicine*, 105(1-2):144-8.

Ryan EG, Leonard N, O'Grady L, More SJ and Doherty ML (2012) Herd-level risk factors associated with *Leptospira* Hardjo seroprevalence in Beef/Suckler herds in the Republic of Ireland. *Irish Veterinary Journal*, 65(1):8

Dukpa K, Robertson ID and Ellis TM (2012) Serological and clinical surveillance studies to validate reported foot-and-mouth disease free status in Tsirang district of Bhutan. *Preventive Veterinary Medicine*, 104: 23-33.

Other information

The current version of the software is FreeCalc V2, and this is now more than 10 years old. The software is not being maintained or updated.

FreeCalc has been replicated in EpiTools (see 3.2) and it is most likely that further development will happen within that set of tools.

3.2. Epitools

Epitools is a set of web-based tools that were originally developed to recommend survey designs for use in estimating disease prevalence or demonstrating freedom from diseases in animal herds. Epitools is equally applicable in the plant-surveillance context – herds/farms map to backyards or orchards and animals map to trees. EpiTools was developed by Ausvet Animal Health Services. It is an open access tool, and can be freely accessed from

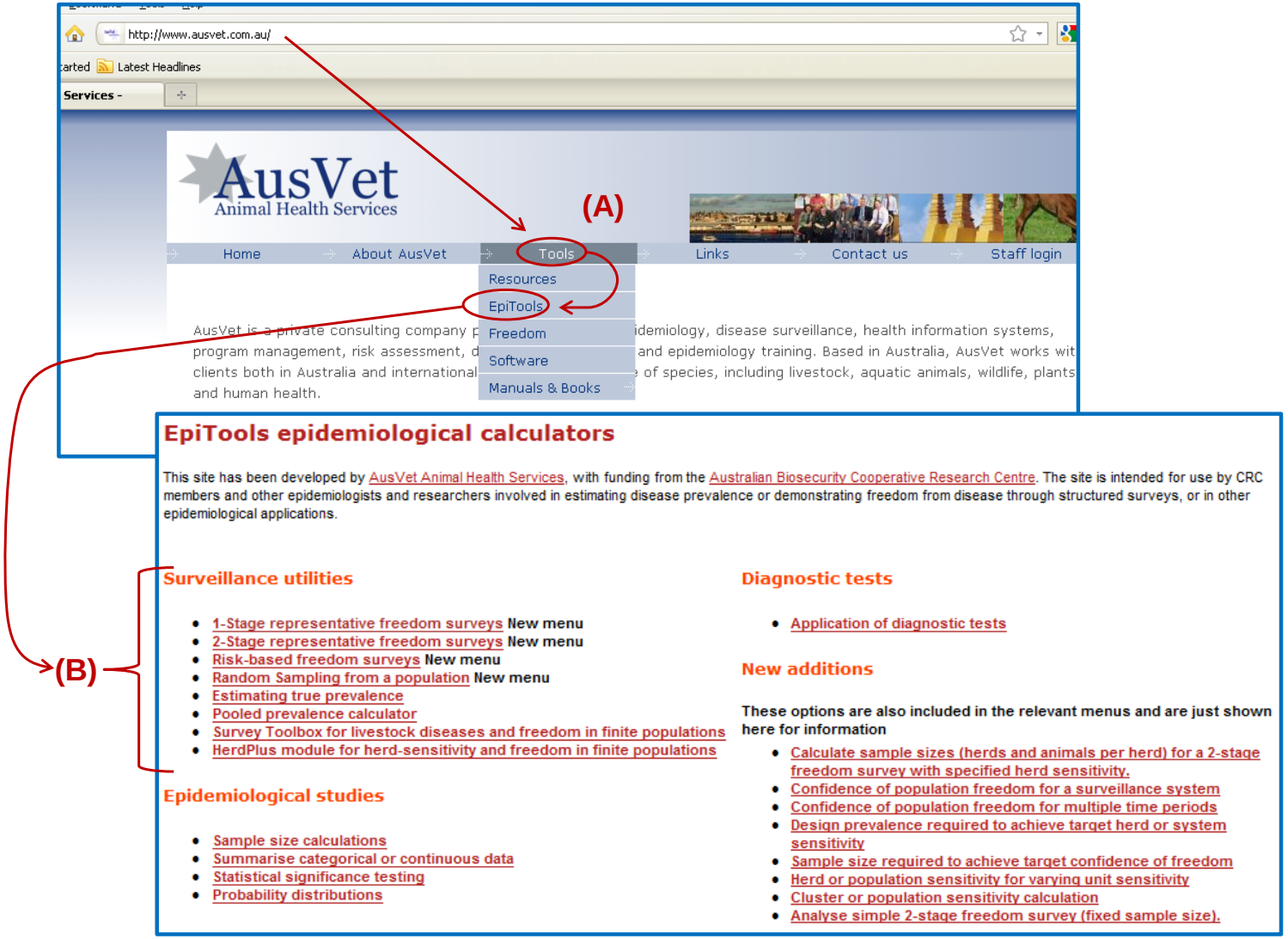


Figure 6. (A) shows the location of EpiTools on the AusVet home page (B) shows the front page of EpiTools

http://epitools.ausvet.com.au/content.php?page=home or from the Ausvet homepage http://www.ausvet.com.au/: click on the **Tools** menu item, then **EpiTools** (Figure 5A). Those tools relevant to the proof of freedom context are listed under **Surveillance utilities** (Figure 5B).

Key references

Sergeant ESG (2009) *Epitools epidemiological calculators*. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease. http://epitools.ausvet.com.au.Cameron AR and Baldock FC (1998) A new probability formula for surveys to substantiate freedom from disease. *Preventive Veterinary Medicine*, 34:1-17.

Cameron AR (1999) Survey Toolbox for Livestock Diseases - A practical manual and software package for active surveillance of livestock diseases in developing countries. Australian Centre for International Agricultural Research, Canberra, Australia.

Jordan D, McEwen SA (1998) Herd-level test performance based on uncertain estimates of individual test performance, individual true prevalence and herd true prevalence. *Preventive Veterinary Medicine*, 3:187-209.

MacDiarmid SC (1988) Future options for brucellosis surveillance in New Zealand beef herds *New Zealand Veterinary Journal* 36: 39-42.

Martin SW, Shoukri M and Thorburn MA (1992) Evaluating the health status of herds based on tests applied to individuals. *Preventive Veterinary Medicine*. 14:33-43.

The full reference list for all EpiTools functions can be found at http://epitools.ausvet.com.au/content.php?page=References]

When to apply

In the context of proof-of-freedom, EpiTools may be used to design survey strategies that can provide a level of confidence that a disease or pest is not present at a given confidence level above a specified, low, prevalence. The pest or disease may or may not be clustered (ie. in groups of plants or animals scattered across a landscape) and the population size may or may not be known (ie. we may be able to identify each animal in a herd but not each plant in an orchard).

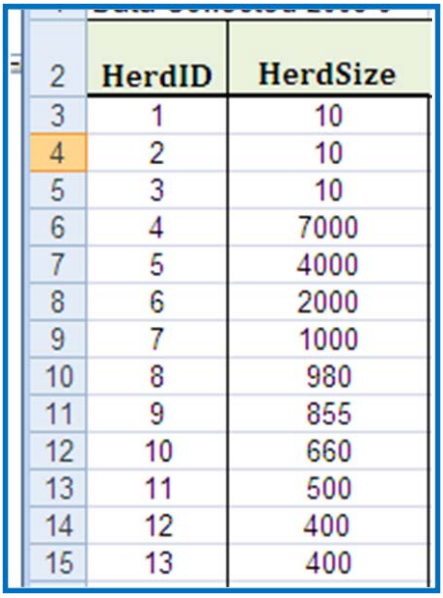
EpiTools may be used for risk-based surveying, where identifiable parts of the population are higher risk. Martin *et al.* (2007a and b) explain a methodology that is useful for undertaking risk-based surveying. This methodology is complex and beyond the scope of this document.

Table 2. Definitions and values of key concepts used by EpiTools in a plant-disease context

Parameter, animal-surveillance context (as in EpiTools)	Parameter, plant-surveillance context	Description in the plant-disease context	Symbol
Test sensitivity	Test sensitivity	The diagnostic sensitivity of a test. This is the probability that an individual diseased tree or plant will be correctly identified as positive by the test. Also called True Positive Rate (of a test). When calculating system sensitivity or number of orchards to sample for two-stage sampling, use location (a population of trees in a defined space) or orchard sensitivity (see below).	Se
Herd sensitivity	Location or orchard sensitivity	The probability that an infected orchard/location will give a positive result following a particular testing protocol, given that the disease is present in trees or plants at a prevalence equal to or greater than the design prevalence.	SeH
Herd-level design prevalence	Orchard-level design prevalence	The hypothetical minimum proportion of diseased orchards or properties that a survey is designed to detect (assuming each property is diseased at or above the tree-level design prevalence).	P*
Animal-level design prevalence	Tree-level design prevalence	The hypothetical minimum proportion of diseased trees in a population (either a specific location or property or a broader population of trees) that a survey is designed to detect.	P^*_T
System sensitivity	System sensitivity	The overall probability (level of confidence) of detecting disease if it is present in the population at the specified design prevalence(s). May be specified as a target to be achieved or calculated as the actual level achieved by the survey.	SSe
Population size	Population size		N

Information requirements

The information requirements vary depending on the problem. The definitions and values of key parameters used in EpiTools for both the animal-health and plant-health contexts are shown in Table 2. Data to be used with EpiTools should be placed in an Excel spreadsheet. Several of the tools explained below require the data to be organised so that a column containing a location identifier and a column containing the number of trees (or animals) at the location are placed next to each other (Figure 7). Additional columns of data can be included if desired (after the two columns of HerdID and HerdSize) and rows can be in any order. Detailed outputs will be in the same order as input and will also include any additional columns provided.



	HerdID	HerdSize
2		
3	1	10
4	2	10
5	3	10
6	4	7000
7	5	4000
8	6	2000
9	7	1000
10	8	980
11	9	855
12	10	660
13	11	500
14	12	400
15	13	400

Figure 7. An example of how data should be organised for use in some of the tools in EpiTools.

How does EpiTools work?

Hester *et al.* (2012) give detailed examples of key statistical functions provided in EpiTools for designing proof-of-freedom surveys for citrus canker through a series of five examples (Figure 8). Rather than repeat each example, we summarise only Examples 1 and 4 (method 2), and remove references to citrus canker.

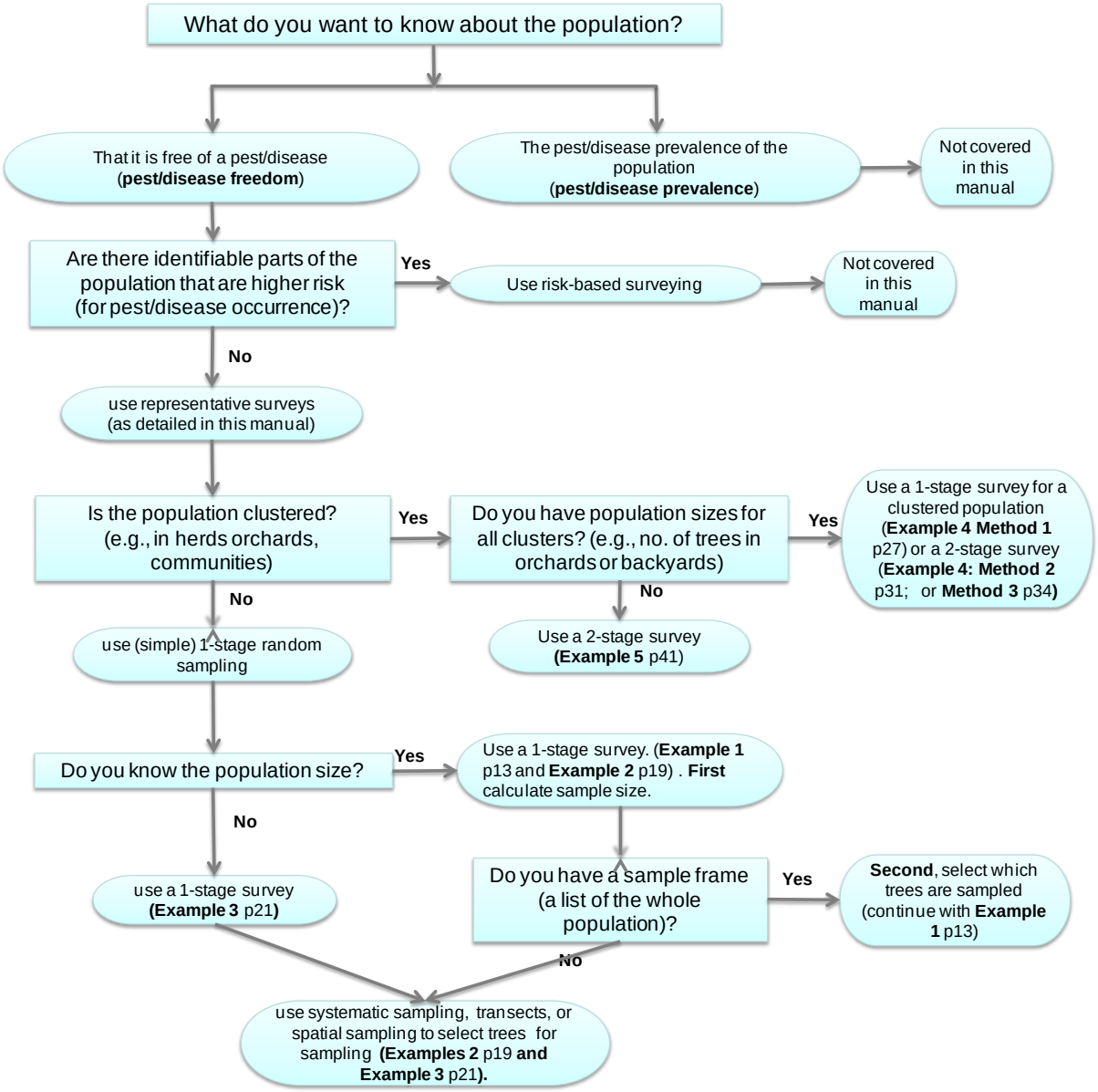


Figure 8. Event tree describing the various ways EpiTools may be used to construct proof-of-freedom surveys in the plant-health surveillance context. Taken from Hester *et al.* (2012), page numbers match up to that document.

Example 1: Determining sample size for a single population of hosts, known population size, no clustering of hosts, with a sampling frame

In this example we have a single orchard of 5,000 trees (*N*) that could contain a particular disease. We would like to know how many trees we should test (*n*) in order to be 95% confident of detecting the pathogen (*SeH*), given that our test sensitivity (*Se*) is 50% and our design prevalence (*P**) is 1%. Key information on the scenario is given in Table 3.

Table 3. Key information for Example 1

Parameter	Description	Value	Comment
N	Population size	5,000	
n	Sample size	?	
*P**	Design prevalence	0.01	This is equivalent to 50 infected trees in total
Se	Test sensitivity	0.5	
SeH	System sensitivity	0.95	This is how confident we want to be that if the pathogen was there at *P** we would find it

A one-stage survey is appropriate for the current scenario where the sampling frame exists (every member of the population is known) using the following steps:

1. Select **1-stage representative freedom surveys** from the EpiTools home page (Figure 9A);
2. Select **Sample size assuming perfect test specificity** (Figure 9B).
3. Insert values for **design prevalence** (0.01), **test sensitivity** (0.5), **desired herd sensitivity** (0.95) and **population size** (5000) into the appropriate input box (Figure 9C). Note that reducing the design prevalence to 0.001 or even 0.005 might be more realistic, but would result in much higher sample sizes.
4. Press **Submit**.

EpiTools calculates the required sample size (*n*) as 582 trees (Figure 9D). This can be interpreted as the minimum sample size that would enable us to be 95% confident of detecting a particular disease if it was present in 1% of trees in the population, and necessarily higher than 95% if the true prevalence is higher than 1%.

The next step is to actually select 582 trees from the orchard, as follows:

5. Select **Survey Toolbox for livestock diseases and freedom in finite populations** from the EpiTools home page (Figure 10A);

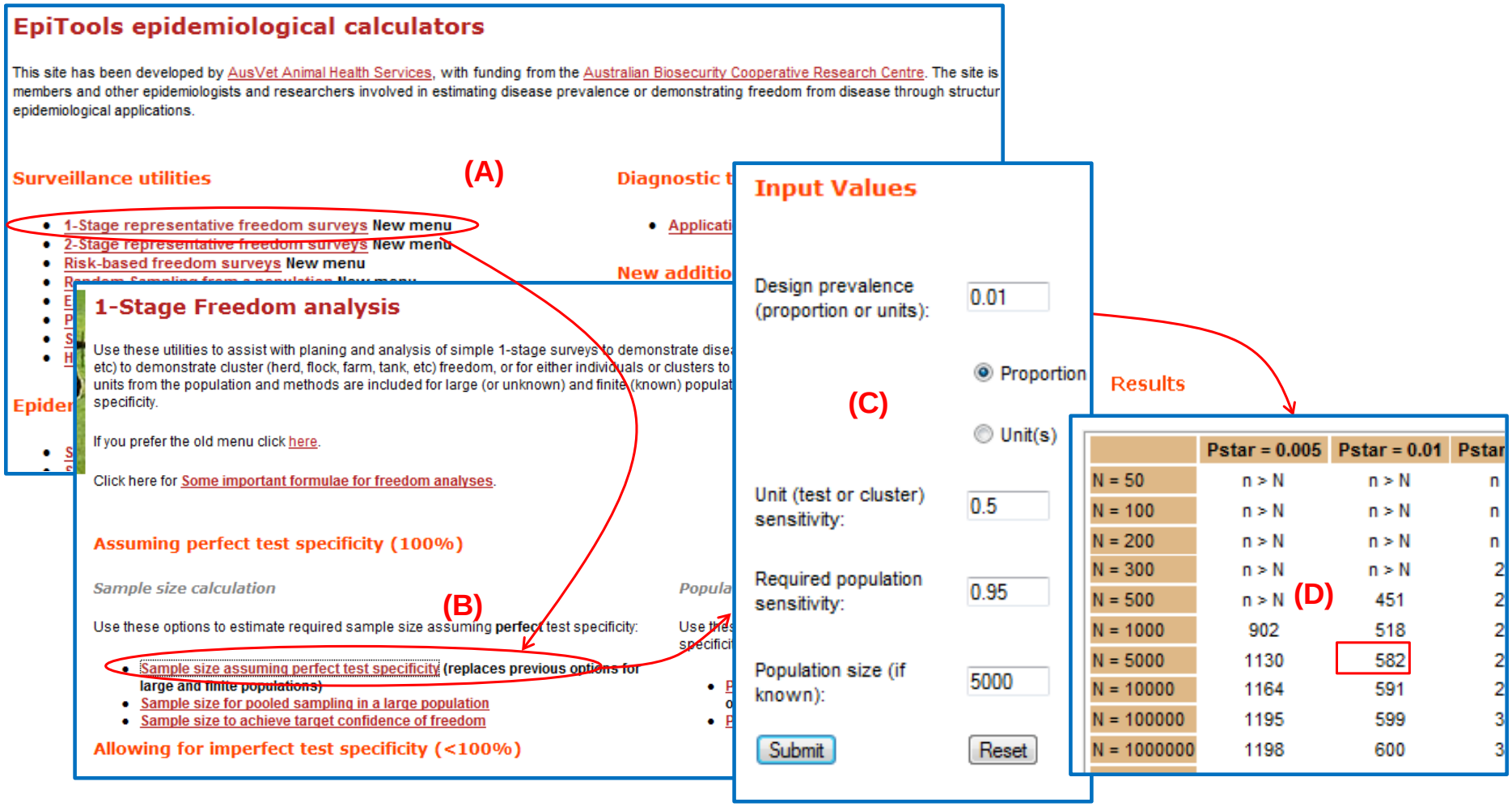


Figure 9. Screen grabs of the various steps involved in determining sample size for a single population of hosts with a known population size, no clustering of hosts, and with a sampling frame (Example 1 from Hester *et al.*, 2012)

6. Select **Generate a list of random numbers from a specified range of from a list** from the list of options that appears (Figure 10B);
7. Input the sample size determined earlier (**582**) (Figure 10C) and select **sampling without replacement**. Select **Specified range** for the random number source and then the minimum and maximum value for the desired range of random numbers in the boxes that should now appear (Figure 10D). In our example we want 582 numbers from our population of 5,000 trees, so the minimum is 1 and the maximum is 5,000.
8. Press **Submit.**

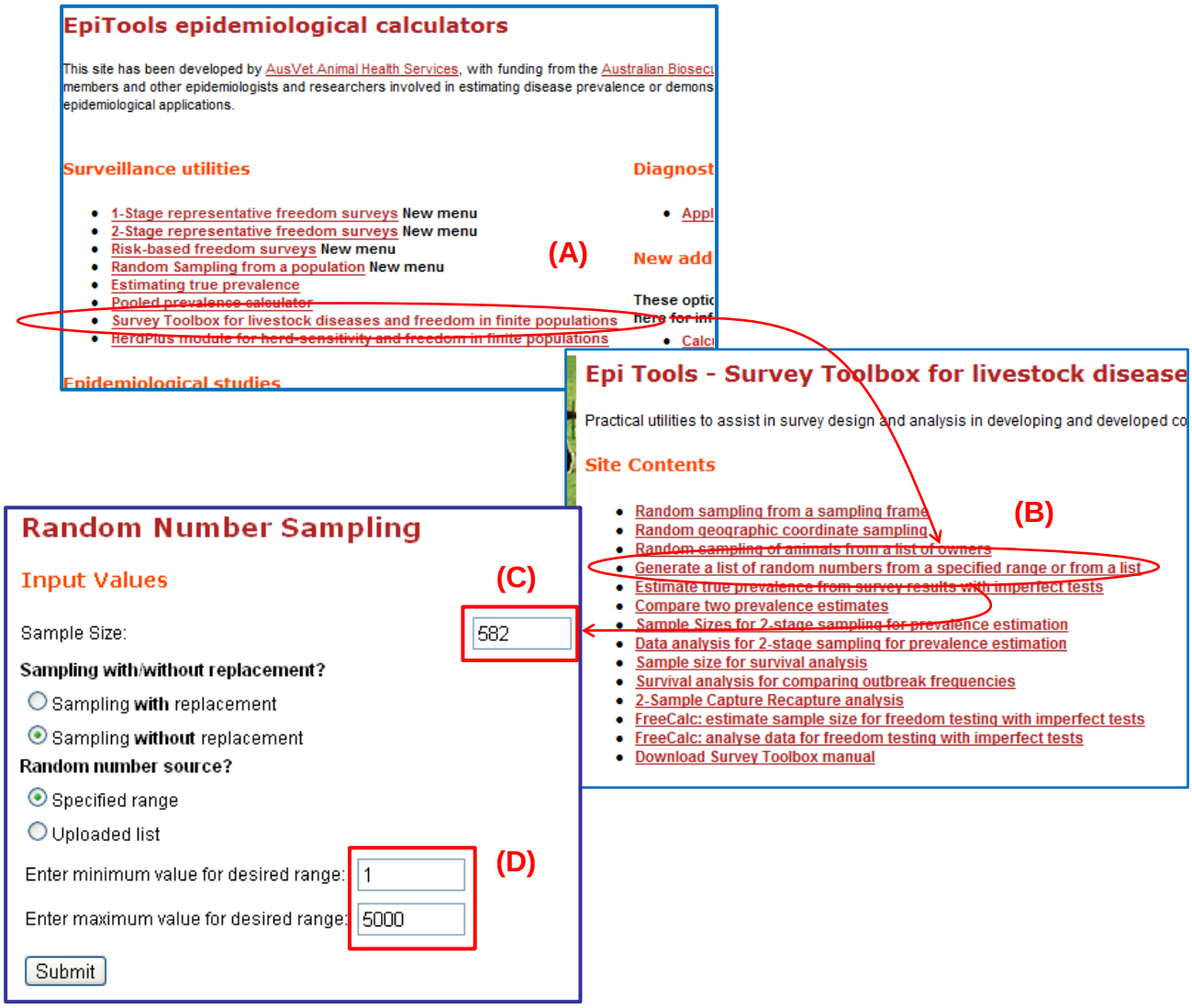


Figure 10. Screen view of the steps involved in determining which trees to sample when a sampling frame exists.

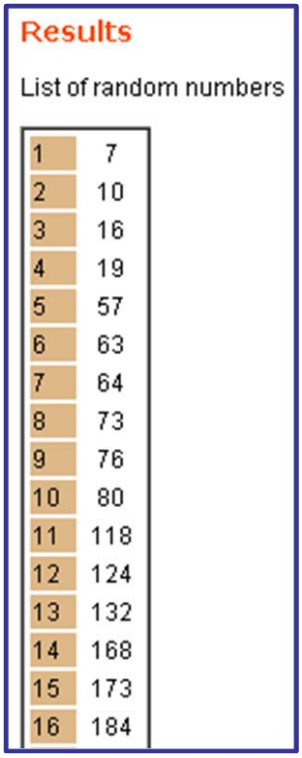


Figure 11 Partial view of the list of 582 random numbers generated at Step 8

The identification of which trees should be sampled appears as a list of 582 random numbers (Figure 11). The data can be transferred into an Excel spreadsheet by scrolling down to the bottom of the page and clicking on **Detailed Results.**

When the on-ground surveying actually takes place, the survey officer should choose a logical starting point and select the trees in the order listed in the results. If it happens that a tree is missing at the location, a nearby tree should be selected. Alternatively, the survey officer could **randomly** choose 582 trees at the location by some other sampling method, for example, systematic sampling.

Example 2: Multiple locations, clustered data, known cluster sizes

In this example we have 408 distinct locations containing host trees for a particular disease, ranging from several thousand trees in commercial orchards to small numbers of trees that have been planted in backyards. We will treat each location as an individual cluster and we also have information on the population size of each cluster. We would like to know the number of locations to sample and the number of trees that should be sampled at each selected location in order to be 95% confident at the orchard/location level (*SeH*) and 95% confident at the system-level (*SSe*) that the disease would be found at a design prevalence (*P**) of 1%. In this example our test sensitivity is the tree-level sensitivity value of 0.5. Key information about this scenario is given in Table 4.

A one-stage survey for a clustered population and a two-stage survey are both appropriate for this example, but below we explain the latter. A two-stage survey involves sampling at two levels: firstly a sample of properties is selected from a list (the sampling frame) of all properties that have susceptible species; and secondly a sample of trees is selected within each selected property.

Table 4. Key information for Example 2

Parameter	Description	Value	Comment
N	Population size	408	This is the number of clusters
n	Sample size	?	
P^*_T	Tree-level design prevalence	0.01	This is equivalent to 1 infected tree per 100 trees at infected locations
*P**	Orchard/location-level design prevalence	0.01	This is equivalent to 4 infected locations out of 408
Se	Test sensitivity	0.5	This is tree level sensitivity
SeH	Orchard/location sensitivity	0.5	This is how confident we want to be that if the pathogen was there at p* we would find it at each location
SSe	System sensitivity	0.95	This is how confident we want to be that if the pathogen was there at p* we would find it for the whole population

In this example, a target location/orchard-level sensitivity (confidence of detecting infection if present at a specific location at the design prevalence) is specified. The target orchard sensitivity is often, but not necessarily, set at 95% to provide a very high level of confidence of detection for individual locations sampled. This desired orchard sensitivity is then used to calculate sample sizes for both stages: number of locations to sample and number of trees to sample at each selected location. The following steps detail how to undertake a two-stage survey for a particular disease:

1. Select **2-Stage representative freedom surveys** from the list of options contained in the EpiTools home page (Figure 12A)
2. Select **Sample sizes for specified herd (cluster) sensitivity** from the list of options that subsequently appears (Figure 12B).
3. Design prevalence (the specified level of disease to be detected) must be specified at both animal (tree) and herd (location/orchard) levels. In this example the **animal-level design prevalence** (0.01) and **herd-level design prevalence** (0.01 - the proportion of infected locations that you wish to be able to detect), should be entered into the appropriate place within the input box that should now appear (Figure 12C). Both should be selected as **proportions**.
4. Next, enter the **test sensitivity** (50%), select a target value for **location/orchard sensitivity** (we use 50% but may be more or less), overall **system sensitivity** desired from the survey (usually 95%) (Figure 12C) and a value for the **number of herds in the population** (408 – the total number of locations).
5. Press **Submit**

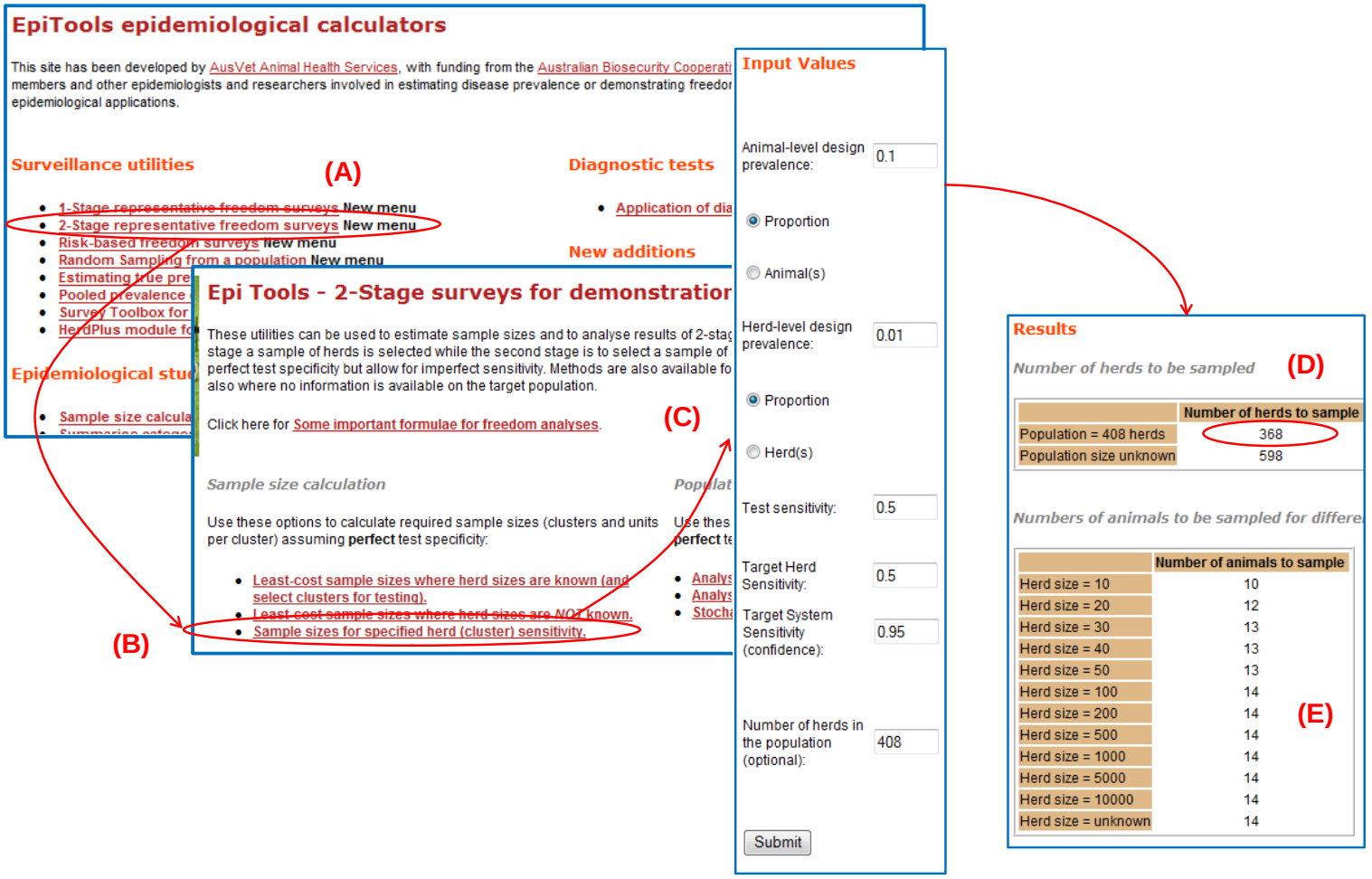


Figure 12. Screen views of the various steps involved in undertaking a 2-stage survey to determine sample size when there are multiple locations, clustered hosts and cluster sizes are known.

The required sample size for the specified level of confidence, design prevalence test sensitivities, and population size, is given as 368 (Figure 12 D). This is the number of locations to be surveyed.

To find which 368 locations (out of a total of 408) should be tested, either:

- Generate a list of 368 random numbers between 1 and 408 and select the corresponding locations from the list (as in Example 1), or
- Generate a random selection of locations from the sampling frame using EpiTools. Select **Survey Toolbox for livestock diseases and freedom in finite populations** from the EpiTools home page, then **Random sampling from a sampling frame**, enter the required **sample size** (368), select **simple random sampling**, **sampling without replacement**, ignore stratification and sub-grouping, paste the sampling frame data into the input box and click on **Submit**.

The next step is to actually select and sample the required number of trees at each location. The number of trees that should be sampled for a given orchard/location size were also given in the EpiTools results (Figure 12E) - for orchards that contain 10 trees each tree should be sampled, for orchards containing 20 trees, 12 of these should be sampled and so on. When not all trees at a location are to be sampled, those that are should be selected randomly. Instructions for doing this are found in Hester *et al*. (2012) Example 1 (known sampling frame) and Example 2 (unknown sampling frame).

Advantages and disadvantages

Open access, freely available

Previous applications of EpiTools proof-of-freedom claims

European Food Safety authority (2009) Porcine brucellosis (*Brucella suis*). Scientific opinion of the Panel on animal Health and Welfare. *The EFSA Juournal*,1144: 1-112.

Hester S, Sergeant E and Robinson A (2011) *EpiTools: Pest/Disease Freedom Application, Software Manual*, Australian Centre of Excellence for Risk Analysis Project 1004A.

Other information

Maintained, but on an *ad hoc* basis.

3.3. Survey Toolbox

This software has been developed to assist with survey design, implementation and analysis of diseases of livestock and aquatic animals in developing countries, although would be applicable to any part of the world. Very detailed and easy-to-understand manuals have been written for using the Survey Toolbox for surveys of livestock diseases (Cameron 1999) and aquatic animal diseases (Cameron 2002). The survey toolbox can be freely downloaded as shareware from http://www.ausvet.com.au/content.php?page=software#st or from the Ausvet homepage at http://www.ausvet.com.au/content.php?page=home (Figure 14). Once on the homepage, select **Tools**, click on **Software** (Figure 14A) and the **Survey Toolbox** menu option will appear (Figure 14B).

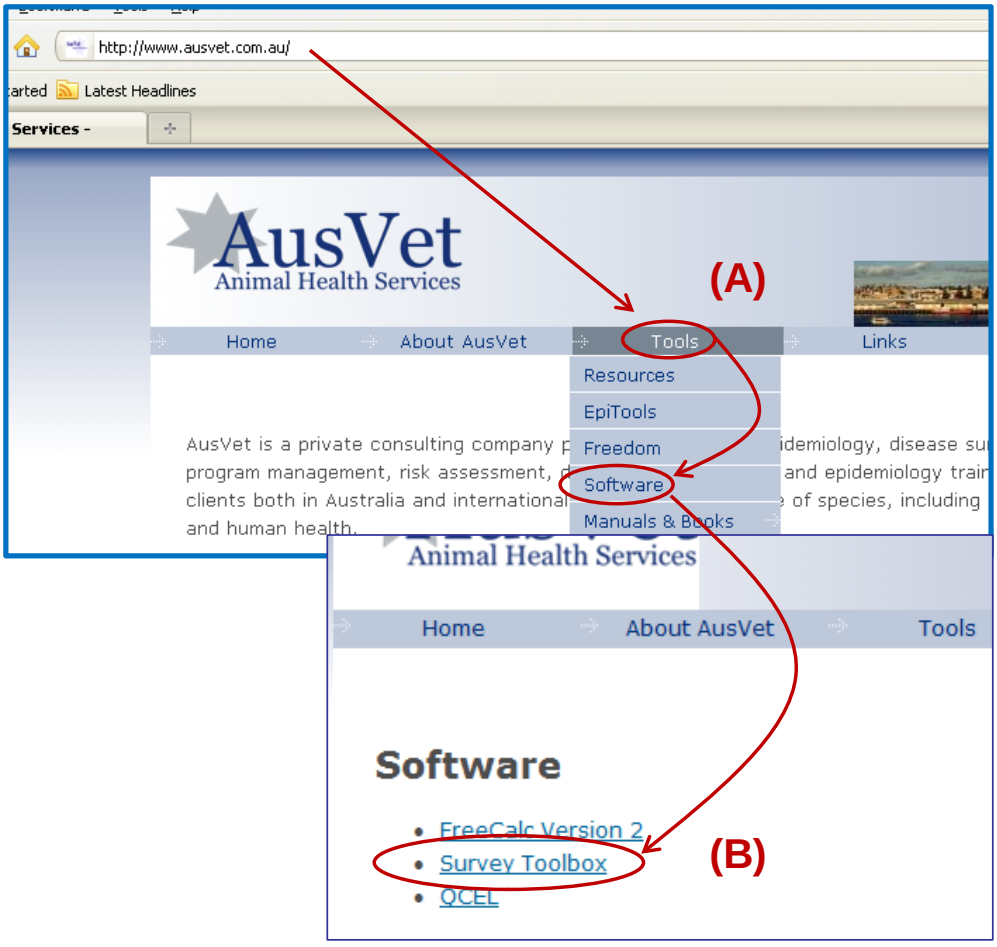


Figure 13. The Survey Toolbox is listed on the AusVet home page under Tools (A) then Software (B).

Key references

Cameron, A.R. (1999) *Survey Toolbox – A practical manual and software package for active surveillance of livestock diseases in developing countries*. ACIAR Monograph 54, 330 p:

This publication maybe downloaded from http://aciar.gov.au/publication/MN054. Chapter 9 of the publication discusses sample size calculations when demonstrating freedom from disease (Figure 14).

Cameron, A. (2002) *Survey Toolbox for Aquatic Animal Diseases. A Practical Manual and Software Package.* ACIAR Monograph No. 94, 375p.

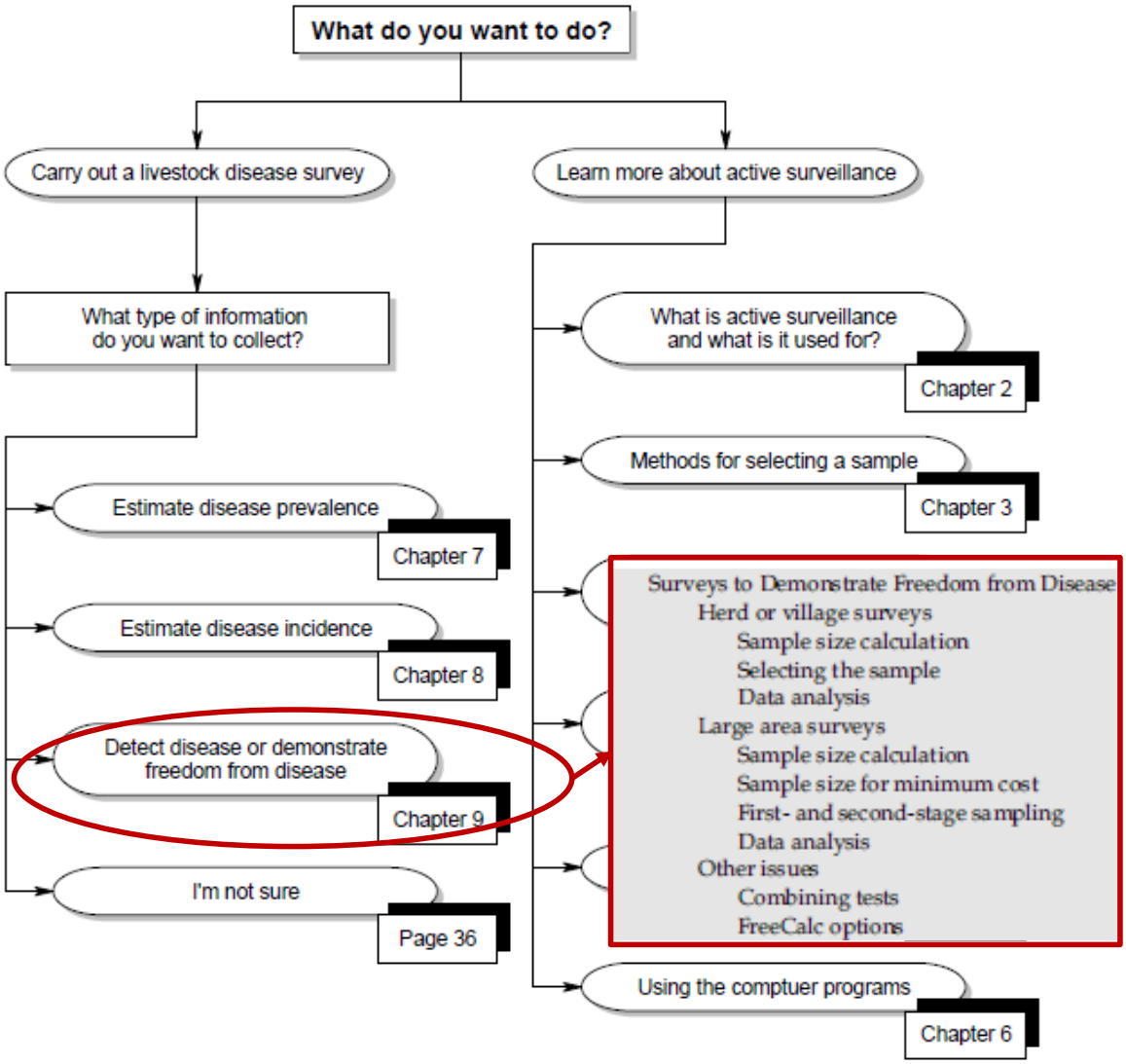


Figure 14. Details of Chapter 9 of Cameron (1999).

When to apply

The Survey Toolbox may be used when structured surveys are required to survey a relatively small single group (village, herd, shipment) or a large area (province, state, country) containing many animals, trees or plants.

Information requirements

To determine sample size the following information is required:

- Size of the population being sampled (e.g., the number of animals or number of host plants)
- Test sensitivity (%)
- Test specificity (%)
- Design prevalence (minimum expected prevalence, or maximum acceptable prevalence)
- Desired type I error
- Desired type II error

Key aspects of software for 'proof-of-freedom' claims

Calculations of sample size within the Survey Toolbox use FreeCalc (see Section 3.1). For surveys of a relatively small group of hosts one-stage sampling is explained in the manual, and for larger areas of hosts two-stage surveys are explained (Figure 15) (see Section 3.2 of this manual for an EpiTools example). Two-stage sampling is applicable in those situations where only a few locations will be affected by a pest or disease if it is present, but at those affected locations there is likely to be relatively high level of the pest or disease. The number of locations to be sampled is selected, followed by the number of units at each location.

If cost of sampling is an issue, then two-stage sampling can be used to find the minimum cost sample size. A variety of different sample size combinations at the location- and unit-level are found, each providing the same level of evidence for freedom from disease, but each having a different cost. The cost of sampling is based on the cost of testing locations and individual units at each location and how much testing is required at each level. The Survey toolbox explains how to find the minimum cost sample size using trial and error with FreeCalc.

Advantages and disadvantages

Easy to use, freely available

Other information

The current release of Survey Toolbox is Version 1.04. This is a minor upgrade to the previous version 1.03. Survey Toolbox does not run on operating systems that follow

Windows XP. Fortunately both FreeCalc and Survey Toolbox are now available through EpiTools where operating system is not an issue.

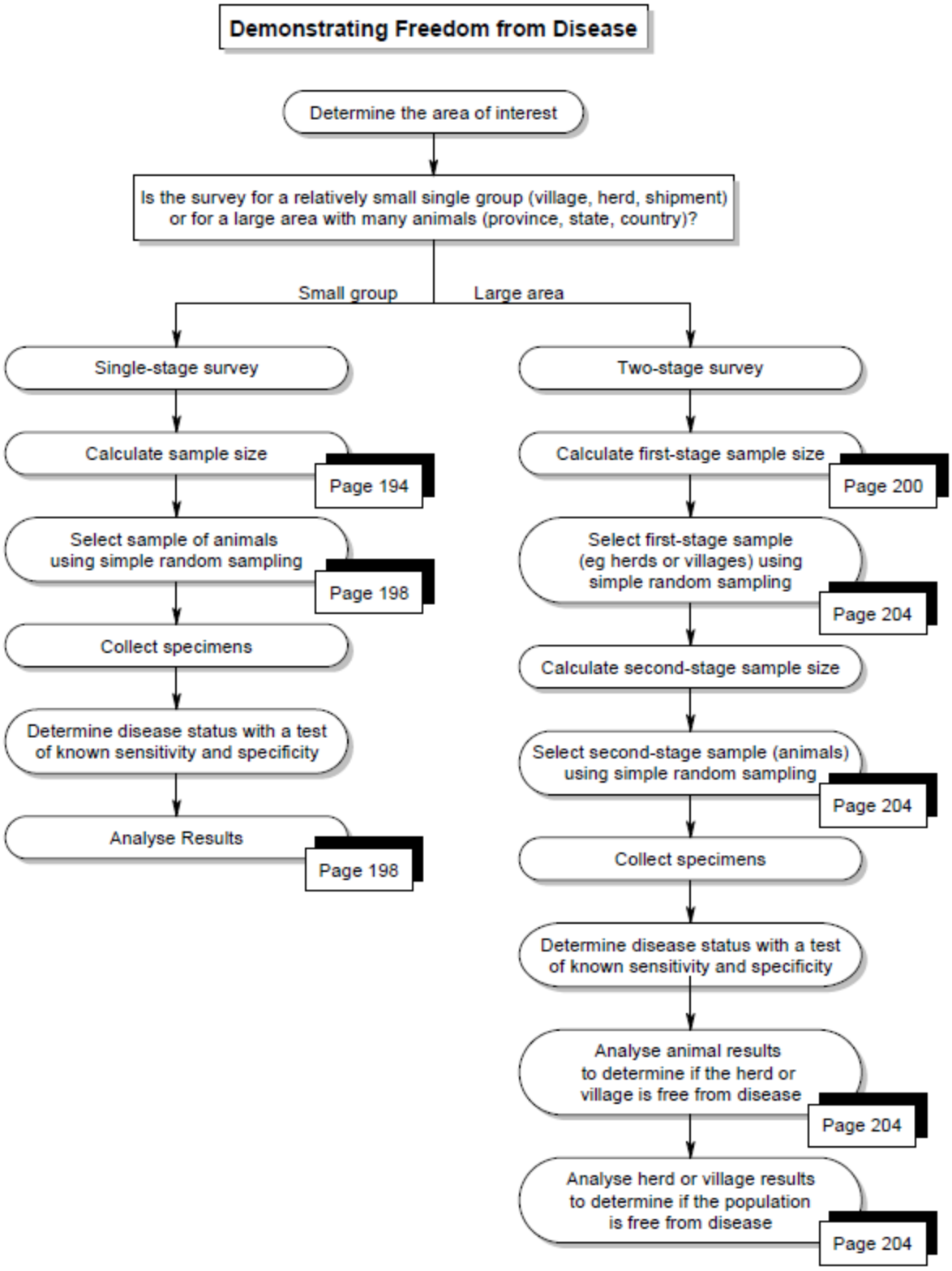


Figure 15. Chapter 9 of the Survey Toolbox (source: Cameron 1999)

4. Conclusion

While there is a range of methodologies available for demonstrating proof-of-freedom (see Hester *et al.* 2010) not all of these have been developed into readily applicable tools that could be easily and accurately applied by biosecurity managers with limited prior knowledge of the methodology. In this document we have detailed three survey design tools that have been developed from accepted statistical formulae and are well accepted in animal-disease surveillance: *FreeCalc*; *EpiTools*; and *Survey Toolbox.* Given that EpiTools contains FreeCalc and that Survey Toolbox does not operate on the most recent Windows operating system we recommend that EpiTools be chosen for undertaking survey design and analysis.

In this proof-of-freedom toolbox we had hoped to also detail tools associated with scenario tree modeling which have recently been used in the biosecurity context (e.g Martin *et al*. 2007a and b, Barrett *et al*. 2009). Use of this methodology allows information from multiple sources (landowners, private industry organizations, community groups and emergency 'hotlines') be used to support claims of area freedom. However, after additional investigation into scenario tree modeling (including Bayesian Belief Networks) and associated generic software, the authors deem that this method requires too much prior knowledge and is not sufficiently clear that biosecurity managers or scientific staff could be reasonably expected to use and master it quickly. It appears unlikely that a tool will ever be developed that can effectively replace the amount of training that would be required to master scenario-tree modeling. If scenario-tree modeling were required to make a proof-of-freedom claim, then it would be advisable to employ an expert who has had training in the use of this methodology, to undertake the analysis.

Risk-based surveillance, where survey efforts are focused on areas and populations where risk factors for a given pest or disease are highest, is an increasingly common approach to surveillance, where scenario trees are commonly used for analysis of the resulting data. Because stand-alone tools for analysis of risk-based data are still not well developed risk-based surveillance was also considered to be out of the scope of this report. This type of surveillance, whilst complicated, should also be brought to the attention of biosecurity managers, particularly given that resources for undertaking surveys are limited. In addition, as risk-based surveillance concepts are now reasonably well established, there are opportunities for developing appropriate tools and supporting documentation for this approach.

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6. Glossary

Area of low pest prevalence (ALPP): Establishing an ALPP is an option used to maintain or reduce a pest population below a specified level in an area, while at the same time facilitating exports or limiting pest impact in the area

Confidence of detection: The probability that disease would be detected if it was present in the population at a level equal to or exceeding the design prevalence.

Cutpoint number of positive test results: The specified number of positive test results above which a population will be considered to be infected.

Design (Target) Prevalence: the minimum pre-survey hypothetical level of disease or pest that a survey is designed to detect with a certain level of statistical confidence. It is the plant- or animal-level prevalence of a pest or disease to be used in calculating sample size. It is expected that the design prevalence (and actual prevalence) are low, depending on the epidemiology of the pest or disease, when claiming area freedom is the objective. A value for design prevalence will either be estimated (see McMaugh 2005 for further details) or a level chosen that is acceptable to all parties.

Estimated prevalence: This is the prevalence that was found as the result of the survey. Ideally the result is a good estimate of the actual prevalence, although this may not be the case if survey methods have poor accuracy or sensitivity.

Minimum expected prevalence: The lowest pest or disease prevalence that the survey can be expected to reliably identify. This level is chosen based on knowledge of the epidemiological behaviour of the disease

Phytosanitary measures: measures to protect plant health

Scenario-tree: A scenario tree is a way of representing a hierarchy of information about a system. It is a branching quantitative model and in biosecurity it is used for the analysis of surveillance systems components.

Sensitivity: the proportion of truly positive units (ie. diseased animals or plants) in a population that are correctly identified as positive (diseased) by the test.

Specificity: the proportion of truly negative units (ie. Non-diseased animals) in the population that are correctly identified as negative (non-diseased) by the test.

Surveillance: the collection, collation, analysis, interpretation and timely dissemination of information on the presence, distribution or prevalence of pests or diseases and the plants or animals that they affect.

Survey: An investigation, in which information is systematically collected, usually carried out on a sample of a defined group or area, within a defined time period

Type I error: the probability that the results of the survey will conclude that the population is not diseased when in fact it is. This is also known as the significance of the results, and is equal to 1 minus the level of confidence (Cameron 1999).

Type II error: the probability that the survey will conclude the population is diseased, when in fact it is not.