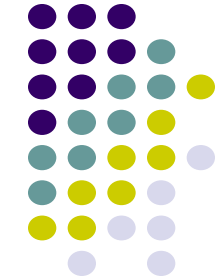
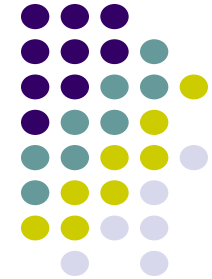
Assessing disease (pathogen) freedom



- Background and trade importance
- Freedom from disease
  - Single population (e.g. herd)
    - Example: Johne's disease
  - Country or region (multiple herds)
    - Examples: Bluetongue, PRRS, ND
- Software – Freecalc

OIE pathways for disease freedom



- Currently recognized pathways
  - Rinderpest
  - Foot-and-mouth disease (FMD)
  - Bovine spongiform encephalopathy (BSE)
  - Contagious bovine pleuropneumonia (CBPP)

- Emphasis in this presentation is on the underlying principles and concepts

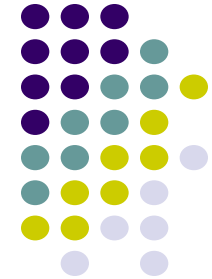
Definition

- “**Pathogen freedom”**
  - For non-OIE listed diseases, sometimes considered to be a prevalence < threshold value, sometimes termed the “design prevalence”, rather than zero prevalence i.e. disease is well controlled

    e.g. cattle disease in the EU – threshold is

    < 0.2% (2 in 1000) positive herds

Definition



- **“Pathogen freedom”**

  – strictly speaking means freedom from the pathogen in animals, the environment, potential wildlife reservoirs, vectors etc

  - relevant only at the time the evidence was collected

Demonstration of disease freedom

“Proof” theoretically requires:

1. Perfectly sensitive test
2. Testing of all animals in a population (e.g. herd, state, country, etc especially if diseases can exist at low prevalence)

“Absolute proof” is unrealistic; provision of **“relative proof” or “supportive evidence”** should be the goal

Relevance is time dependent

Bottom line……

- Be very clear about the meaning of “disease freedom” in the context of disease of interest
  - clinical disease or pathogen?
  - livestock or all possible sources (vectors, environment, wildlife reservoirs etc)?
  - does it mean “zero” or is a low prevalence acceptable?

Bottom line……

- Much easier to make definitive conclusions about disease freedom when
  - pathogen is highly contagious and prevalences always exceed say 30%

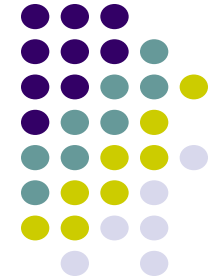
    Question: Is prev = 0% vs. ≥ 30%?
  - clinical signs occur in most infected animals and signs are not masked by vaccination
  - pathogen does not survive well outside of the host

Bottom line……

- Most difficult scenario
  - pathogen is not very contagious and prevalences can be low, say 0.1% to 10%

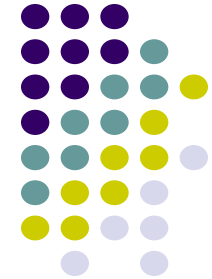
    Question: Is prev = 0% vs 0.1-10%?
  - clinical signs are rare
  - pathogen survives well outside of the host (e.g. environment and reservoir species)

Example: Single population



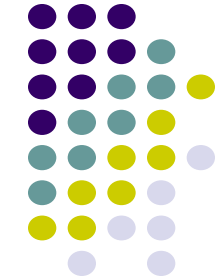
- What evidence would be necessary to demonstrate that a dairy herd of 100 cows was free of Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis)*?

Example: Single population



- **Considerations:**
  - Zero or low prevalence?
  - Sensitivity (and specificity) of tests to be used
  - Other issues
    - previous clinical and testing history
    - biosecurity; closed vs. open herd and testing of replacement females, if herd is open
    - Johne's status of neighboring herds
    - data for similar herds in same geographic area

Example: Single population



- Suppose that all 100 cows were tested and were ELISA negative (Biocor/Prionics test), what should you realistically conclude based on test results alone?

- Would your conclusion be the same if one cow was ELISA positive but fecal culture negative and the other 99 cows were ELISA negative?

Interpretation of test results

- **Approach 1:**

  Calculate apparent (sero)prevalence (AP)

  AP = 0 (upper 95% confidence limit ≈ 3/100 ≈ 0.03)

  (*The usual formula that we use for calculating a CI for a proportion base on the normal distribution does not work when 0 positives*)

  Interpretation: Herd has <3% seropositive cows with 95% confidence

  Problem: Really need true prevalence estimate because tests change over time or among laboratories

Interpretation of test results

- **Approach 2:**

  Calculate true prevalence and a 95% CI

  Need Se/Sp estimates for the ELISA

  Collins et al. (2005) showed that Se = 0.29 and Sp =0.997 for the Biocor ELISA

Single population

Approach 2:

True prev = (AP + Sp – 1)/ (Se + Sp -1)

= (0 + 0.997–1)/(0.29+0.997-1)

= - 0.003 / 0.287

= **- 0.01 (impossible)**

Interpretation: Herd has a negative prevalence

Problems:

Does not capture any uncertainty Se/Sp estimates

Does not really answer the key question

Is the prevalence = 0% given the data?

Qualitative solution……

Informally make a judgment based on:

1. Negative test results
2. Additional herd information
   - risk assessment data, biosecurity practices, previous test results
3. Data for similar herds – size and management in the state or local geographic area
4. Johne's status of neighboring herds, if available

One quantitative solution……

Use of the FreeCalc software

- incorporate test Se/Sp
- compares 2 hypotheses

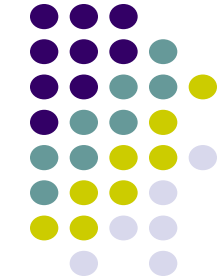
e.g. prevalence = 0% vs.

prevalence ≥ MDP

(minimum detectable prevalence)

FreeCalc2.exe

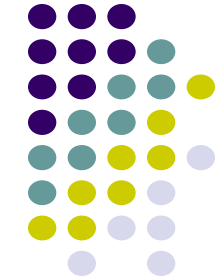
FreeCalc applications



- **Analysis of survey data** - what confidence do we have in the survey results given a finding of “x” reactors in a tested population of size “n” and certain test performance characteristics?

- **Survey planning** – find the appropriate sample size to designate “freedom” with high confidence

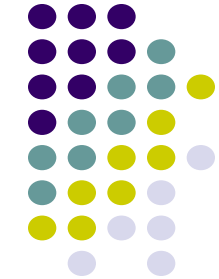
FreeCalc: analysis of survey data



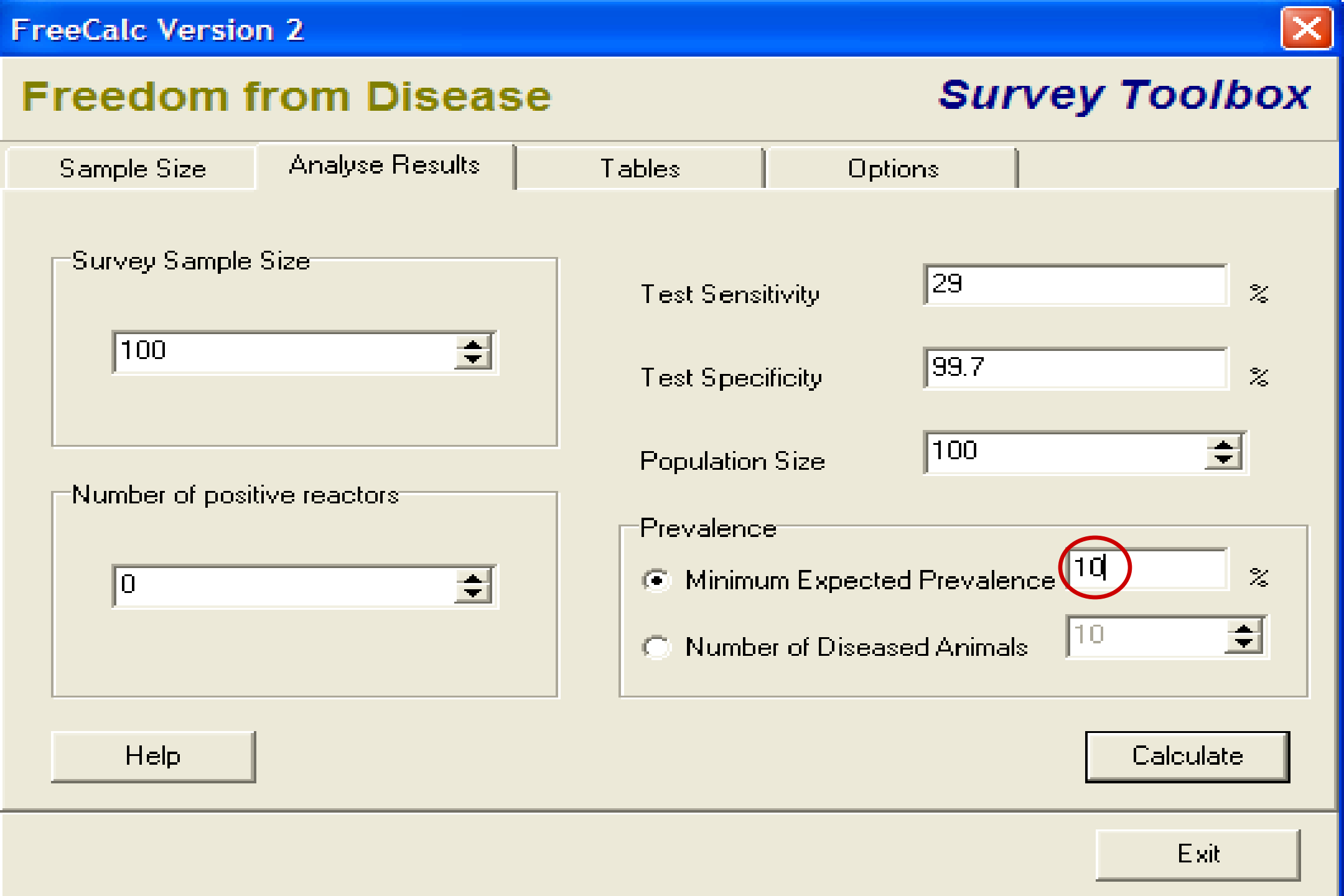
- **Null hypothesis** is that the herd is infected with the minimum detectable (“design”) prevalence
- **Alternative hypothesis** is the herd is not infected

- Opposite way to usual hypothesis testing format
- Justification is that we need to provide strong evidence against the null hypothesis (infection is present at a given at least the design prevalence)

FreeCalc: analysis of survey data



- Based on representative sample of animals in a herd to give high confidence of “freedom”
- Considerations
  - confidence level (1-α),
  - power (1-β),
  - test performance (sensitivity and specificity),
  - population size,
  - minimum detectable prevalence


FreeCalc Version 2
Freedom from Disease
Survey Toolbox
Sample Size
Analyse Results
Tables
Options
Survey Sample Size
100
Number of positive reactors
0
Test Sensitivity
29
%
Test Specificity
99.7
%
Population Size
100
Prevalence
Minimum Expected Prevalence
10
%
Number of Diseased Animals
10
Help
Calculate
Exit

FreeCalc Analysis

Survey Toolbox

Null Hypothesis

p = 0.024840

This is the probability of observing 0 reactors or fewer in a sample of 100 animals from a population with a disease prevalence of 10.0000%.

Alternative Hypothesis

p = 1.000000

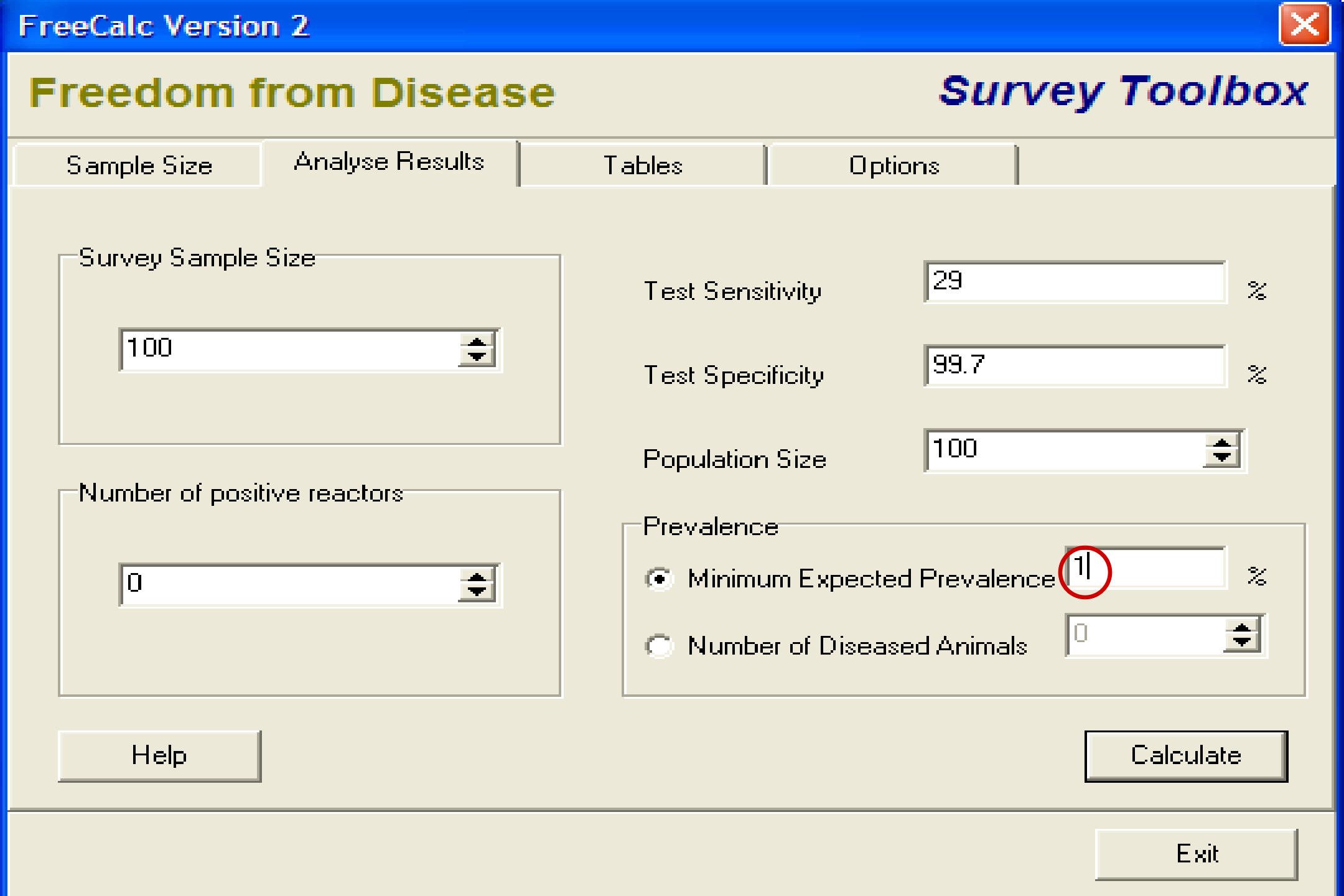
This is the probability of observing 0 reactors or more in a sample of 100 animals from a disease free population.

Calculated using the Hypergeometric Exact Probability formula.

Conclusion

These results are adequate to reject the null hypothesis and conclude that the population is free from disease (at the expected minimum prevalence of 10.0000%) at the 97.516% confidence level.

Exit


FreeCalc Version 2
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Survey Toolbox
Sample Size
Analyse Results
Tables
Options
Survey Sample Size
100
Number of positive reactors
0
Test Sensitivity
29
%
Test Specificity
99.7
%
Population Size
100
Prevalence
Minimum Expected Prevalence
1
%
Number of Diseased Animals
0
Help
Calculate
Exit

FreeCalc Analysis

Survey Toolbox

Null Hypothesis

p = 0.527326

This is the probability of observing 0 reactors or fewer in a sample of 100 animals from a population with a disease prevalence of 1.0000%.

Alternative Hypothesis

p = 1.000000

This is the probability of observing 0 reactors or more in a sample of 100 animals from a disease free population.

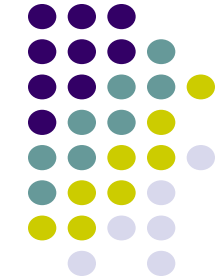
Calculated using the Hypergeometric Exact Probability formula.

Conclusion

These results are not adequate to reject the null hypothesis and conclude that the population is free from disease. The sample size was too small to distinguish a population with prevalence of 1.0000% from a disease free population.

Exit

FreeCalc



- Major limitations of analytic approach
  - Sensitivity and specificity considered known, fixed values
  - Does not consider probability of disease before the survey/testing
  - Other considerations not formally incorporated
- New software (Bayesfreecalc) resolves these issues but is more complicated to use
  - www.epi.ucdavis.edu/diagnostictests/software.htm

Multiple populations

- Major differences in approach when scale of freedom assessment is larger e.g. state or country
  - need to base evidence on sample of animals rather than a census (i.e. whole herd test)
  - possibility of geographic clustering must be considered
  - consideration of other issues e.g. trading patterns can be much more complex

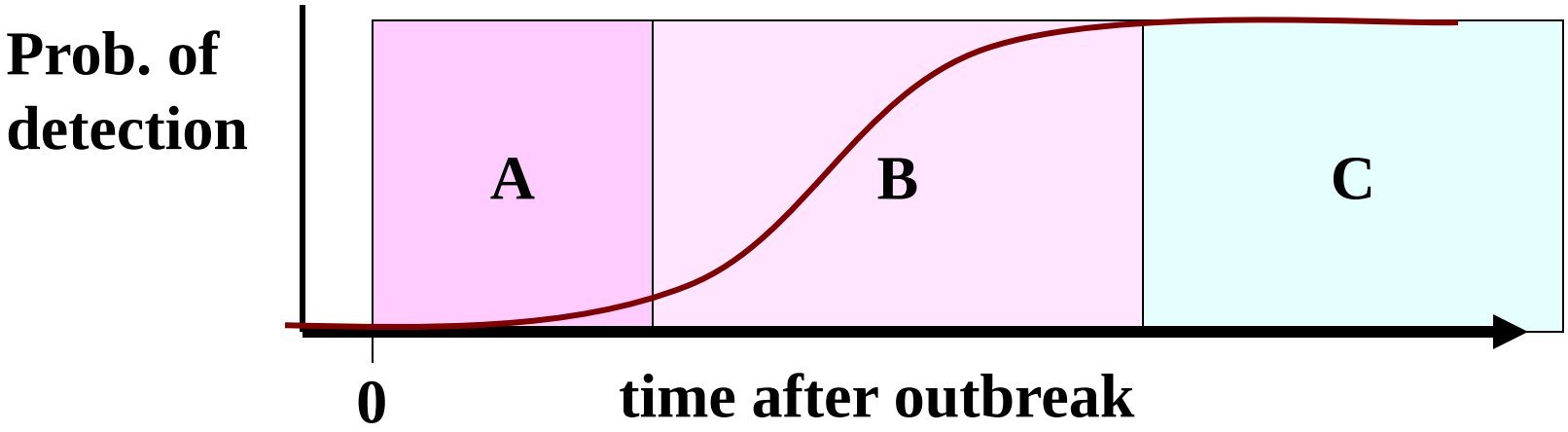
Multiple populations

Based on considerations such as:

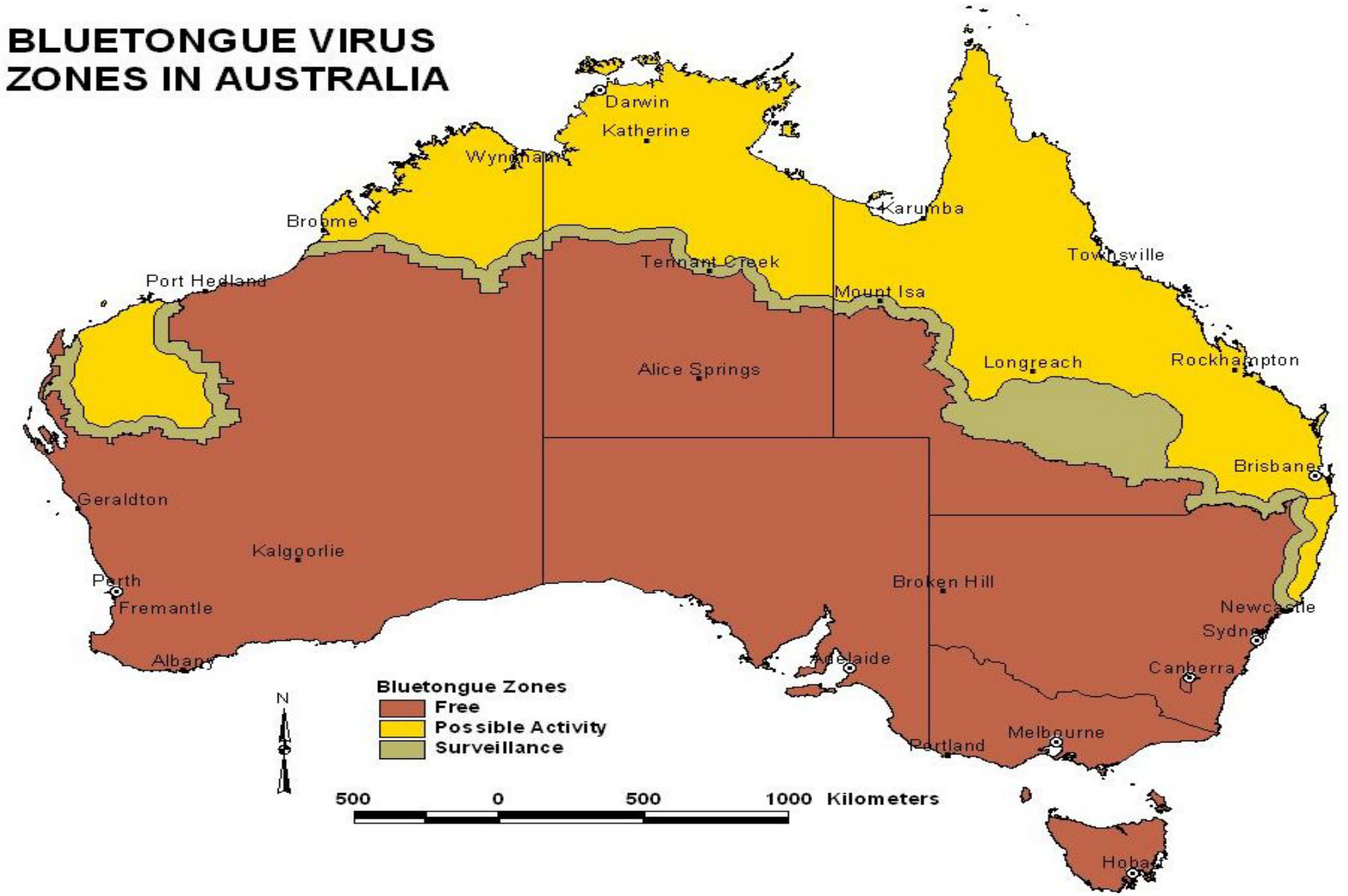
1. **Surveillance system** – neg. results
2. **Population survey** that yields neg. results
3. **Other factors**, including:
   - quality of veterinary services,
   - quality/reliability of surveillance system
   - diagnostic laboratory system
   - historic performance in detection of disease
   - usually, no more vaccine use
   - GIS/ mapping (vector-borne diseases)

Time aspect of surveillance systems

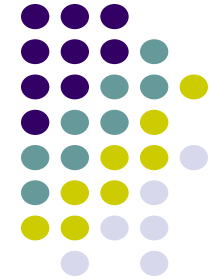
- Prospective view
  - 3 phases of probability of detection of an outbreak



  - A: outbreak not yet detectable ("latent period")
  - B: outbreak detectable ("apparent period")
  - C: outbreak most likely confirmed ("outdated period")

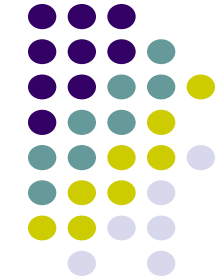

BLUETONGUE VIRUS
ZONES IN AUSTRALIA
Darwin
Katherine
Wyndham
Broome
Karumba
Townsville
Tennant Creek
Port Hedland
Mount Isa
Alice Springs
Longreach
Rockhampton
Brisbane
Geraldton
Kalgoorlie
Perth
Fremantle
Broken Hill
Albany
Adelaide
Canberra
Melbourne
Portland
Hobart
N
Bluetongue Zones
Free
Possible Activity
Surveillance
500 0 500 1000 Kilometers

Multiple populations



- **Evidence ("relative proof")** provided to trading partners who make judgements about trade in animals and animal products
  - no trade
  - trade with mitigations
  - unrestricted trade
- Usually evidence provided to trading partners formally incorporated into a **risk analysis**

Scenarios for disease freedom

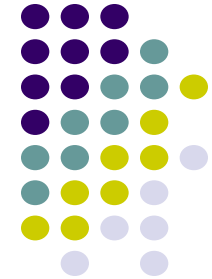


Country has historically been “free” of pathogen e.g. Australia and PRRS in pigs – geographical isolation, strict quarantine

Pathogen is being eradicated or has been eradicated

e.g. brucellosis in U.S; rinderpest in Africa

Both scenarios …..



- For many diseases, need **statistically valid population-based survey** with internationally-recognized test to increase confidence in the negative surveillance results
- Tests – usually serologic (Se and Sp < 1)
- Expect to find 0 reactors in the survey

Survey caveats……

- No survey of a sample of herds (animals) is able to “guarantee” that a population is free of a pathogen or agent

- Possible that a very small number of (or even a single) infected animal exists in a population and was not selected in a sample

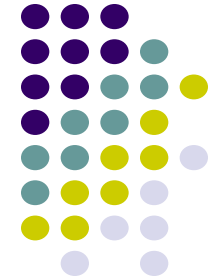
Survey result as a test

Survey Result	Infected	Not infected
+	**Power**	Type I error
-	Type II error	**Confidence**

Sensitivity of survey = power (usually >95%)

Specificity of survey = confidence (usually > 95%)

Interpreting a negative survey result



Pr (freedom | negative result) depends

- **Pr (freedom) before survey**
- **Sensitivity and specificity of survey**

Sero-prevalence surveys

PRRS July 1996 (Nicole Canon)

- 5 serum samples per pig herd at slaughter
- 108 herds
- **ELISA test** (IVI, Denac et al., 1997)

NCD 1997 (Daniela Gohm)

- 30 serum samples per hen flock at slaughter
- 260 herds
- **ELISA test** (SVANOVIR)

Survey outcomes

PRRS → No. test-positive sera & herds

NCD

↓

4 flocks classified as positive

Flocks	Test-positive sera
194	0
50	1
9	2
3	3
2	10
1	14
1	22
260	

DISEASE or FREEDOM FROM DISEASE ?

FreeCalc

- Analysis module for test results for PRRS data – 0/5 positive in all 108 herds

- Min exp. 60%; Se = 100%, Sp = 99%

FreeCalc Analysis

Survey Toolbox

Null Hypothesis

p = 0.009592

This is the probability of observing 0 reactors or fewer in a sample of 5 animals from a population with a disease prevalence of 60.0000%.

Alternative Hypothesis

p = 1.000000

This is the probability of observing 0 reactors or more in a sample of 5 animals from a disease free population.

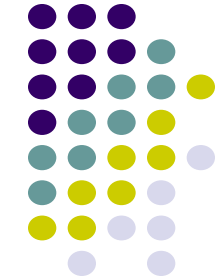
Calculated using the Hypergeometric Exact Probability formula.

Conclusion

These results are adequate to reject the null hypothesis and conclude that the population is free from disease (at the expected minimum prevalence of 60.0000%) at the 99.041% confidence level.

Exit

FreeCalc



- Analysis module for test results for ND data – example herd with 3 positive ex 30 tested

- Min exp. 10%; Se = 98%, Sp = 99%

FreeCalc Analysis

Survey Toolbox

Null Hypothesis

p = 0.597484

This is the probability of observing 3 reactors or fewer in a sample of 30 animals from a population with a disease prevalence of 10.0000%.

Alternative Hypothesis

p = 0.003318

This is the probability of observing 3 reactors or more in a sample of 30 animals from a disease free population.

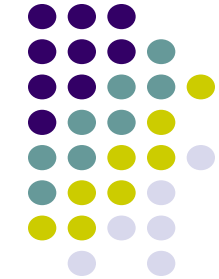
Calculated using the Hypergeometric Exact Probability formula.

Conclusion

These results are not adequate to conclude that the population is free from disease (at the expected minimum prevalence of 10.0000%). The confidence level is only 40.252%. We may conclude that the population is diseased at a confidence level of 99.668%.

Exit

FreeCalc: sample size module



- Example: PRRS survey planning
  - Minimum exp. prevalence = 60%
  - ELISA (Se = 0.98 - 1.0, Sp = 0.97 - 1)

FreeCalc Version 2

Freedom from Disease

Survey Toolbox

Sample Size | Analyse Results | Tables | Options

Iteration	n	Cutpoint	Probability
1	50	2	0.000000
2	25	1	0.000000
3	12	1	0.000267
4	6	1	0.038705
5	3	0	0.061820
6	4	0	0.024370

Test Sensitivity: 100 %

Test Specificity: 99 %

Population Size: 1000

Prevalence

- (•) Minimum Expected Prevalence: 60 %
- ( ) Number of Diseased Elements: 600

Help

Calculate

Exit

FreeCalc Sample Size

Survey Toolbox

Sample Size Calculation

Required Sample Size = **4**

Cutpoint number of reactors = **0**

Calcuated using the Hypergeometric Exact Probability formula.

	Actual	Target
Type I Error:	0.0244	0.05
Type II Error:	0.0394	0.05
Herd-level Sensitivity:	0.9756	0.9500
Herd-level Specificity:	0.9606	0.9500

Explanation

If a random sample of 4 units is taken from a population of 1000, and 0 or fewer reactors are found, the probability that the population is diseased at a prevalence of 60.00% is 0.0244.

Exit