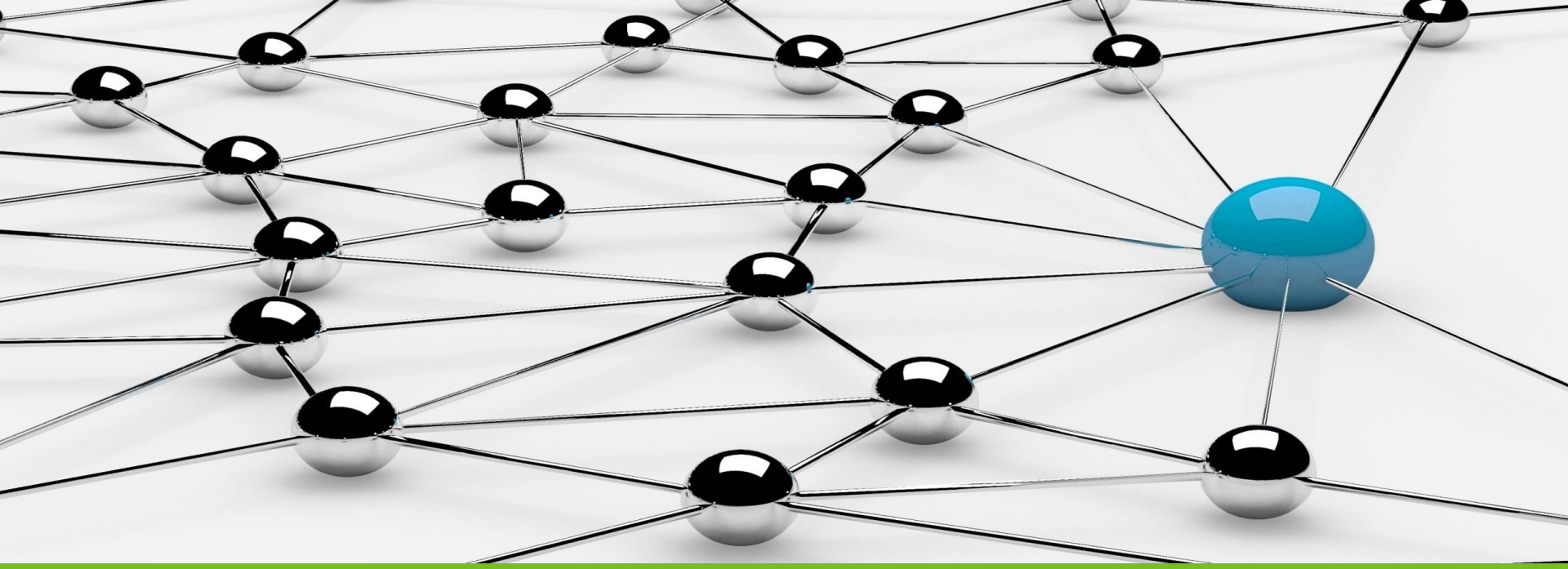


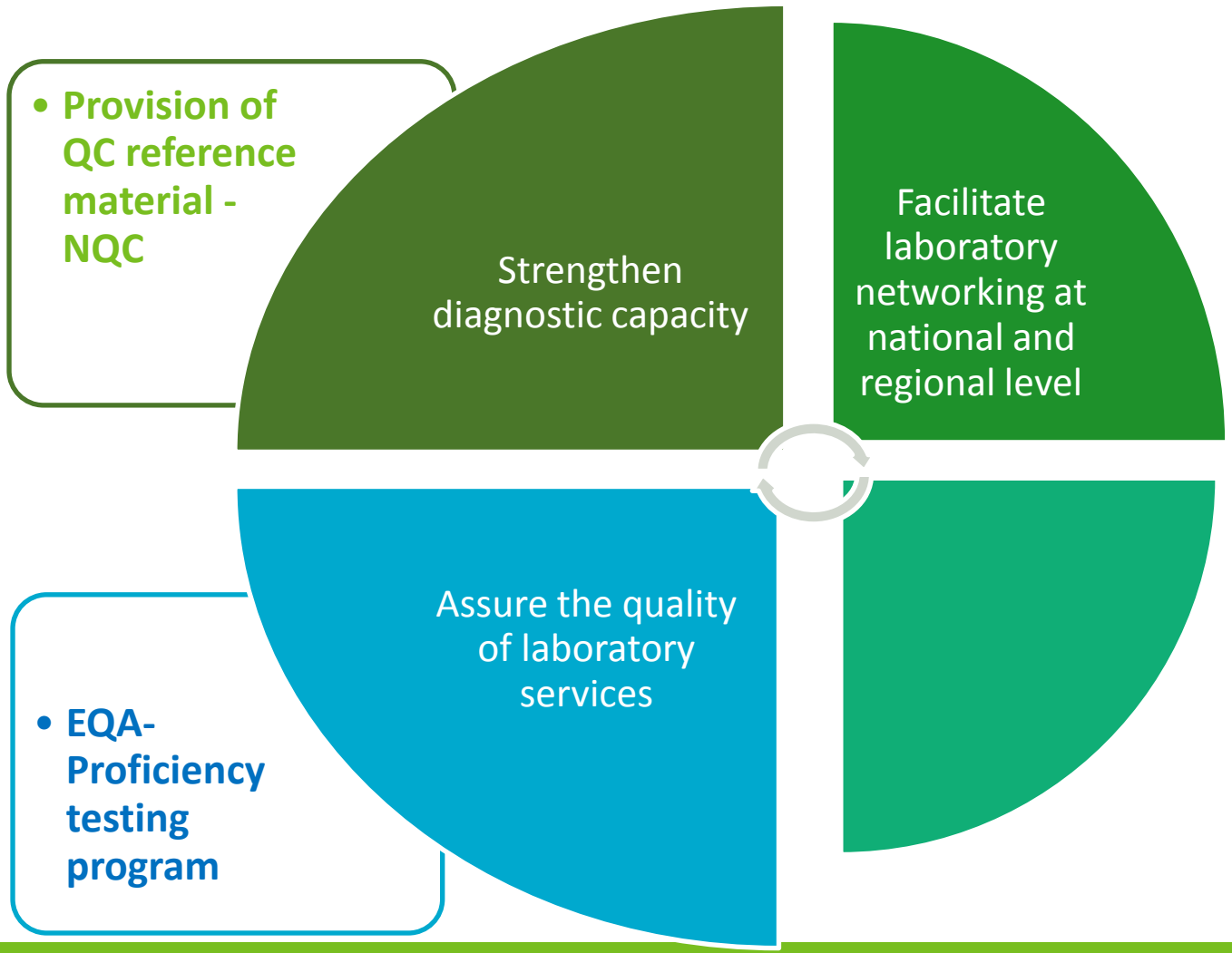
Proficiency Testing a network approach

Strengthening veterinary diagnostics

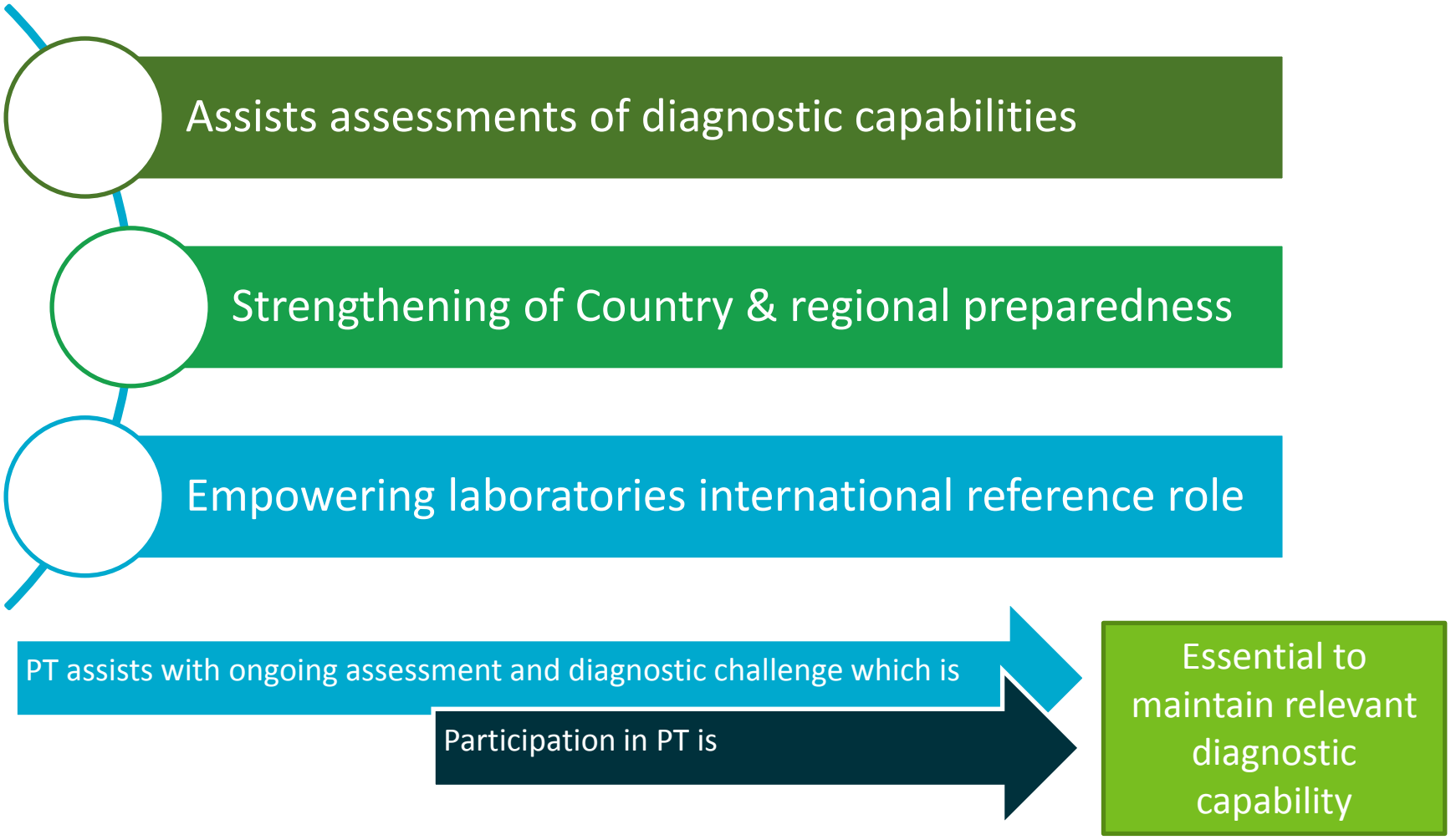
Gemma Carlile| PT Coordinator

17 November 2016

Where does PT fit into Veterinary Diagnostics?



Impacts of PT program across the network



AAHL Proficiency Testing (PT)

- Accredited to ISO17043 and incorporate ISO 13528
- Existing PT program services:
    - Laboratories for Emergency Animal Disease Diagnosis and Response - LEADDR
    - South east Asia – OIE/FAO sponsored project
- Provision of test panels and quality controls to laboratories, who report results and are critically assessed and compared.

ISO 13528:2015 - Statistical methods for use in proficiency testing by interlaboratory comparison

ISO/IEC 17043:2010 - General Requirements for Proficiency Testing

AAHL PT program and EQA has…

- Through LEADDR
  - Contributed to building laboratory network capacity and preparedness across Australia for EAD outbreaks.
- Internationally
  - Contributed to building laboratory capacity and strengthening veterinary diagnostic services across SEA for the detection of Highly Pathogenic Emerging Diseases (HPED) and Zoonotic Diseases.
- Supporting and promoting biosecurity through working partnerships
  - within the Australian laboratory network, and
  - throughout network laboratories in SEA.

Australian National Network Harmonization

- June 2010

Table 4 Comparison based on reported qualitative interpretation of Type A TaqMan results

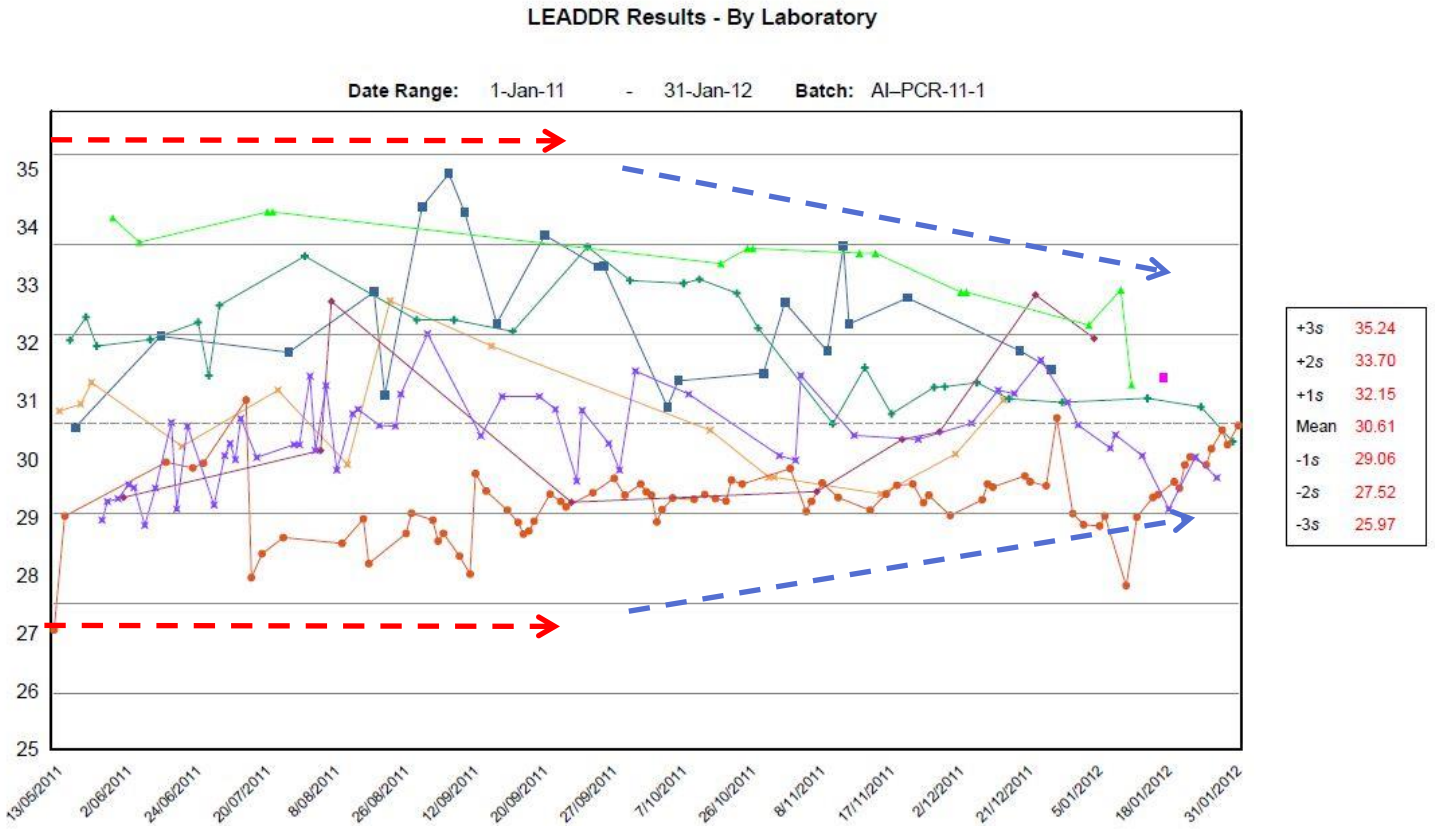
Sample	Identity								Agreement %
1	H1N1 (10^{-4})	Positive	Positive	Positive	Positive	Positive	Positive	Negative	86%
2	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	100%
3	H5N1 (10^{-2})	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
4	NDV (10^{-2})	Negative	Negative	Negative	Negative	Negative	Negative	Negative	100%
5	H3N8 (10^{-3})	Positive	Positive	Positive	Negative	Negative	Positive	Negative	57%
6	H5N1 (10^{-4})	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
7	H5N1 (10^{-7})	Positive	Positive	Positive	Negative	Negative	Positive	Negative	57%
8	H5N1 (10^{-4})	Positive	Positive	Positive	Positive	Positive	Positive	Negative	86%
9	H7N3 (10^{-3})	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
10	H5N1 (10^{-7})	Positive	Positive	Positive	Negative	Negative	Positive	Negative	57%

- Jan 2012

Table 3 Comparison based on reported qualitative interpretation of Type A TaqMan RT-PCR results.

Sample	Virus ID	Dilution									Agreement
1	H5N1 (Viet)	10-6	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
2	H3N8	10-3	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
3	H5N1 (Viet)	10-5	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
4	H5N1 (Wates)	10-3	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
5	None	-	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	100%
6	H7N3	10-4	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
7	H5N1 (Viet)	10-5	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
8	H5N1 (Viet)	10-3	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
9	H1N1	10-4	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
10	H5N1 (Viet)	10-6	Positive	Positive	Positive	Indeterminate	Positive	Positive	Positive	Positive	88%

SEA Network Harmonization



Molecular AIV PT – OFFLU Ref Labs

- **Type A Panel**
  - Sensitivity and Specificity
    - Differential & Negative samples to test assay specificity
    - Dilutions to assess assay sensitivity (use in surveillance)
  - Different H types to assess detection (H7 & H9, generic H-typing?)
  - Different N types to assess detection (N9 & N2, N6, N8, generic N-typing?)

- **H5 Panel**
  - Sensitivity and Specificity
  - Different H5 subtypes from various clades to assess detection (including H5Nx)

Objectives of a PCR PT for OFFLU Ref Labs

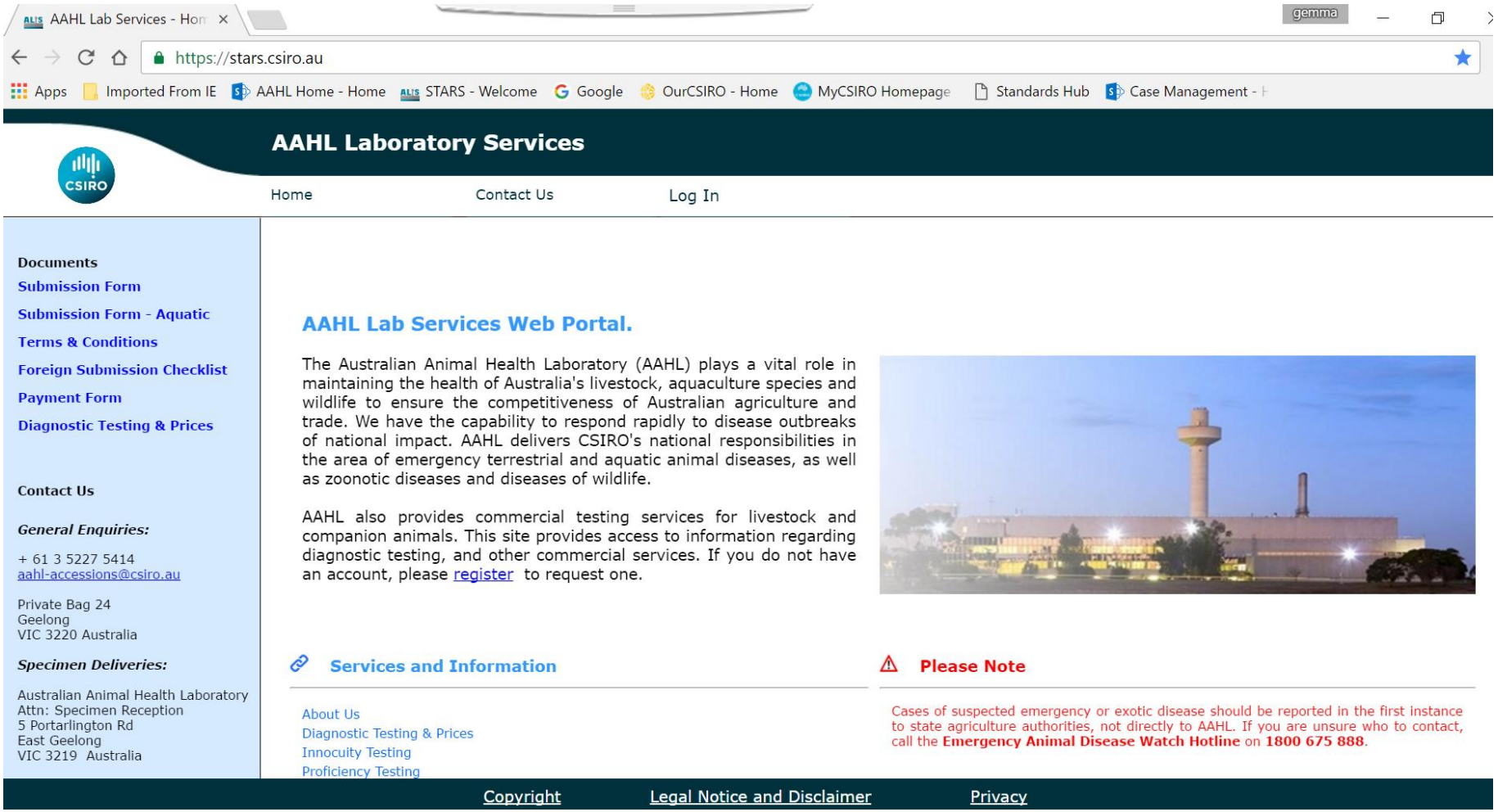
- Challenge National/Regional Systems across the OFFLU Ref Lab Network for the diagnosis of Emergency Animal Disease AIVs that may emerge from any geographical region.

- Expose Ref Labs to regionally specific HP (and LP) AIVs

  (eg. Eurasian/East Asian/Austra-Asian/American H9N2s)

- Leverage Ref Labs contributions to a network-supported (ie. OFFLU) national/regional surge capacity for AIV incursions or outbreaks.

  (eg. sharing of efficacious assays on OFFLU website for H7N9, H5Nx, etc)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Apps
Imported From IE
AAHL Home - Home
STARS - Welcome
Google
OurCSIRO - Home
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Case Management -
AAHL Laboratory Services
CSIRO
Home
Contact Us
Log In
Documents
Submission Form
Submission Form - Aquatic
Terms & Conditions
Foreign Submission Checklist
Payment Form
Diagnostic Testing & Prices
Contact Us
General Enquiries:
+ 61 3 5227 5414
aahl-accessions@csiro.au
Private Bag 24
Geelong
VIC 3220 Australia
Specimen Deliveries:
Australian Animal Health Laboratory
Attn: Specimen Reception
5 Portarlington Rd
East Geelong
VIC 3219 Australia
AAHL Lab Services Web Portal.
The Australian Animal Health Laboratory (AAHL) plays a vital role in maintaining the health of Australia's livestock, aquaculture species and wildlife to ensure the competitiveness of Australian agriculture and trade. We have the capability to respond rapidly to disease outbreaks of national impact. AAHL delivers CSIRO's national responsibilities in the area of emergency terrestrial and aquatic animal diseases, as well as zoonotic diseases and diseases of wildlife.
AAHL also provides commercial testing services for livestock and companion animals. This site provides access to information regarding diagnostic testing, and other commercial services. If you do not have an account, please register to request one.
Services and Information
About Us
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Innocuity Testing
Proficiency Testing
Please Note
Cases of suspected emergency or exotic disease should be reported in the first instance to state agriculture authorities, not directly to AAHL. If you are unsure who to contact, call the Emergency Animal Disease Watch Hotline on 1800 675 888.
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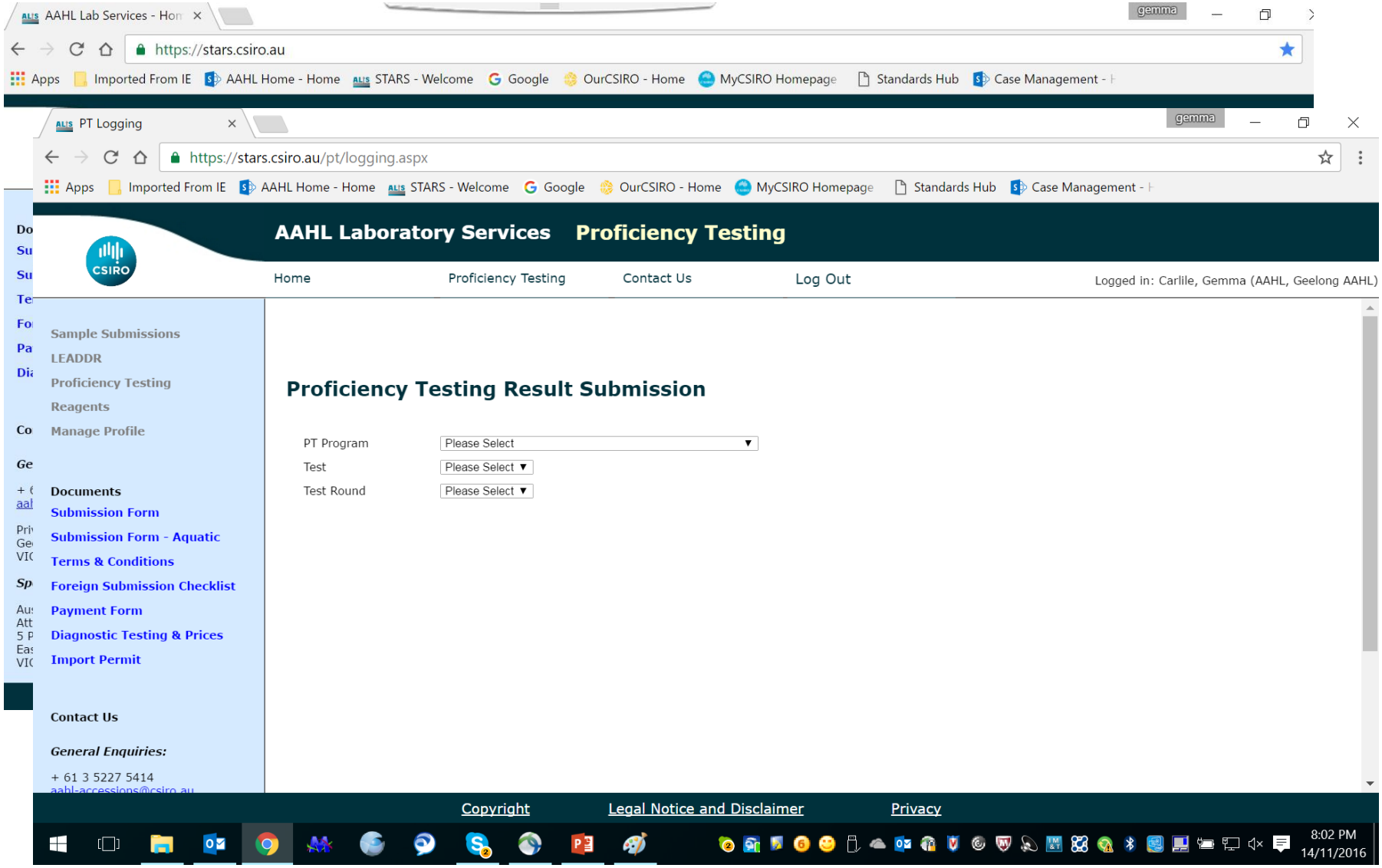
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Standards Hub
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PT Logging
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AAHL Laboratory Services
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Home
Proficiency Testing
Contact Us
Log Out
Logged in: Carlile, Gemma (AAHL, Geelong AAHL)
Sample Submissions
LEADDR
Proficiency Testing
Reagents
Manage Profile
Documents
Submission Form
Submission Form - Aquatic
Terms & Conditions
Foreign Submission Checklist
Payment Form
Diagnostic Testing & Prices
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Proficiency Testing Result Submission
PT Program
Please Select
Test
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8:02 PM
14/11/2016

WHEN and HOW?

One panel per year – 11 Laboratories

AAHL will facilitate the PT in-kind (but participants pay for shipment?)

First panel will be later this year (~Aug 2017)

Thank you

Dr Gemma Carlile
AAHL
Proficiency Testing Manager
t +61 2 9123 5607
e gemma.carlile@csiro.au
w https://www.csiro.au/en/Research/Facilities/AAHL

AAHL PT program under ISO 17043

PT Panel Composition

- Proficiency testing involves laboratories performing the same test on the same quality controlled samples and comparing results.

- Key requirement:
  - Samples are homogenous
  - Stable and
  - Suitable

- The number of samples chosen for inclusion in PT panel are dependent on;
  - Aim of PT scheme
  - Scope of PT
  - Analysis to be undertaken
  - Availability of samples
  - Test being performed

Considerations for test samples

- Test samples chosen for inclusion into PT panels are based on;
  - an ability to provide information about a laboratories capability

    - Repeatability – test-retest reliability (Intra-assay)
    - Reproducibility – provide consistent results (Inter-assay)
    - Sensitivity – analytical vs. diagnostic
    - Specificity – differentials

intra- and inter- assay variance are important indicators of assay performance and these measurements help to validate the performance of a method

All help to determine test reliability

Considerations for test samples

- Additional considerations for inclusion into PT panels is also based on;
  - the aims and objectives of the PT scheme
  - the participants competency
  - the test being assessed (new vs. established)
  - whether the test is harmonised or standardised

The composition of the PT panel is designed to yield the maximum amount of information to enable the analysis of results and assessment of test performance

Test Sample Preparation

- Test Sample preparation includes;
  - Acquisition and Collection
    - samples can be sourced externally and/or produced internally
    - where possible samples should be inactivated i.e. Non-infectious
  - Preparation and handling
    - samples should undergo pre-testing to assess eligibility and establish target test values – should match concentrations routinely encountered during diagnostic testing
    - prepared in bulk with minimum number requirements considered
    - aliquoted into distribution volumes and labelled

PRE-TESTING

Initial sample testing to identify appropriate dilutions to achieve target test value

Test Sample Assessment

- As PT involves a group of laboratories performing the same analyses on the same samples and comparing results, a key requirement is that the samples are ***homogenous*** and ***stable***.
- Achieved through;

Homogeneity testing

AND

Stability testing

Samples are sent for **homogeneity** testing (once of)

Samples are sent for **stability** testing(on-going)

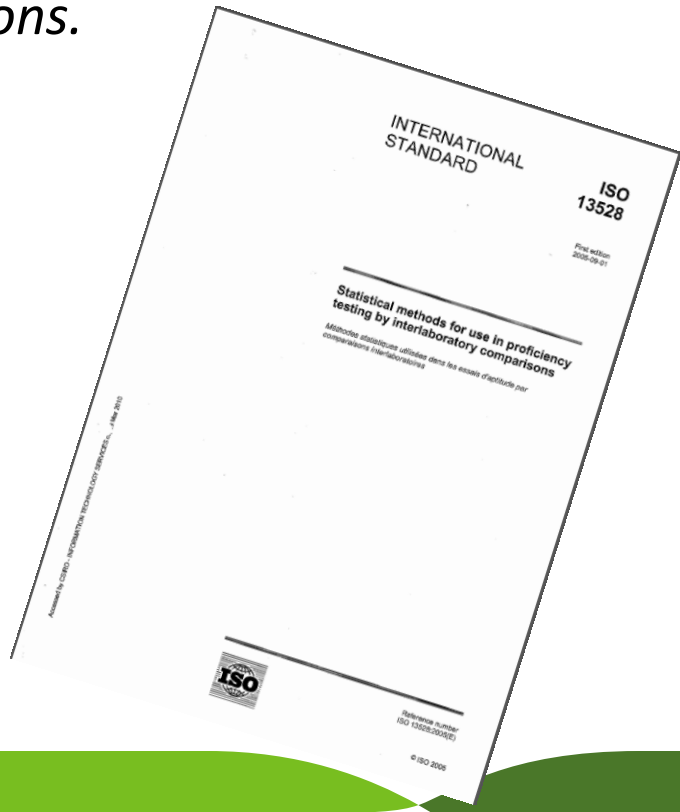
Homogeneity Testing

- Homogeneity testing of the test samples should occur as soon as possible after packaging in their final state.

- This is done according to ISO 13528 *Statistical methods for use in proficiency testing by interlaboratory comparisons.*

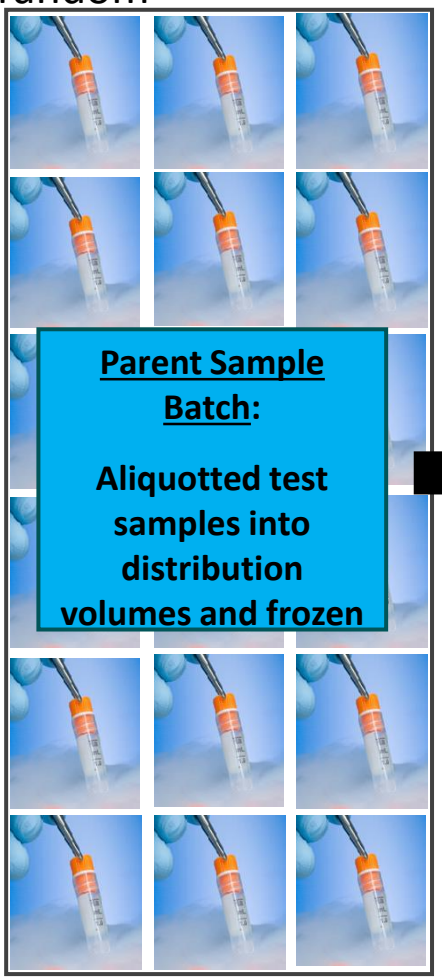
  - The procedures used for homogeneity testing must be documented.

  - The procedures used to establish homogeneity of the test samples must be demonstrated and documented before a test sample is approved for dispatch to participating laboratories.



Homogeneity Testing

A minimum of 10 of the test samples are chosen at random



Parent Sample Batch:

Aliquotted test samples into distribution volumes and frozen

- Random Sample Aliquot 1
- Random Sample Aliquot 2
- Random Sample Aliquot 3
- Random Sample Aliquot 4
- Random Sample Aliquot 5
- Random Sample Aliquot 6
- Random Sample Aliquot 7
- Random Sample Aliquot 8
- Random Sample Aliquot 9
- Random Sample Aliquot 10

- Data point 1A
- Data point 1B
- Data point 2A
- Data point 2B
- Data point 3A
- Data point 3B
- Data point 4A
- Data point 4B
- Data point 5A
- Data point 5B
- Data point 6A
- Data point 6B
- Data point 7A
- Data point 7B
- Data point 8A
- Data point 8B
- Data point 9A
- Data point 9B
- Data point 10A
- Data point 10B

Statistical Analysis to assess adequate homogeneity

Homogeneity Testing

- If this criteria is not met than;
  - The sample preparation is reviewed to see if improvements are possible

  Or

  - if the samples are the best available and meet the panel objectives then the data is reviewed and outliers removed as recommended by Fear and Thompson (2001).

Stability Testing

- To demonstrate that test samples will not significantly change
- And to distinguish between unexpected results and whether they are
  - due to participant variation OR
  - inherent instability of the test samples
- The stability of a sample batch is determined by the criteria set by the PT provider
- Prepared test samples need to be assessed for

1. ‘fit-for-purpose’ analysis needs to be undertaken to confirm that the sample type will perform satisfactorily for use
2. Ongoing establishment of test sample performance pre- and post- PT distribution

Pre- & Post-stability Testing

- Test samples are tested prior to distribution and after the deadline for submission of results from participants (as per ISO 13528).

- The time frame for pre-testing of test samples is set by the PT provider e.g.
  - 2 weeks before sending to laboratories
  - 1 week after result submission

- Post-stability testing is required at the completion of a PT round;
  - Demonstrates that the test sample has not significantly changed over the time course of the PT round

- The number of test samples tested for pre- and post-stability testing is **3** (ISO 13528).

Stability Testing

- The stability testing (and homogeneity testing) is reported in PT round reports to participating laboratories

- Instability of test samples can be controlled by;
  - limiting the time of testing for participating laboratories
  - giving specific directions on how samples should be stored
  - controlling how test samples are sent and the delivery time

Appendix III Homogeneity and stability

Each sample set was tested for homogeneity before shipping to participants. Homogeneity was calculated according to ISO 13528. Homogeneity was calculated for each of the 10 sets of samples and where between-sample standard deviation (s_S) for the standard deviation for proficiency assessment ($\hat{\sigma}$) of $s_S \leq 0{,}3\hat{\sigma}$ is considered to be acceptable. Only samples passing homogeneity are utilised for proficiency testing,

Stability is calculated by on the basis of the methods outlined in the AAHL QA manual 22.5. If a sample fails stability testing, the cause will be investigated and the sample will be further tested and monitored. Samples not meeting stability criteria may be considered for removal from the panel and subsequent analysis if the instability is considered by the PT coordinator to have adversely affected PT panel results. All samples were considered adequately stable for the purposes of this PT round.

PT Analysis

Considerations and analysis for PT Panel Results

What Statistical Analysis to choose?

- Statistical methods used to analyse proficiency testing results need to be tailored to match the design of each scheme.
- The statistical analysis needs to take into account:
  - whether consensus or reference values are used;
  - the test methods being used by participants and whether they are compatible and comparable;
  - the number of participating laboratories; and
  - the aim of the test round to assess sensitivity and repeatability

What to assess?

- There are 2 main sources of variability in the results for PT:
  - variation between laboratories and
  - variation within laboratory
- The aim during analysis is to evaluate and provide feedback on both of these types of variation.
- In order to do this participants must perform the same testing on the same test item.
- The program is designed so that pairs of related results are obtained – split sample pairs or uniform sample pairs

Related Sample Pairs

- Uniform sample pairs
  - identical blind duplicates (where the results are expected to be the same)

- Split sample pairs
  - slightly different blind duplicates (where the results should be slightly different e.g. dilution of the same sample isolate)

- The statistical analysis of paired samples is the same for both types of pairs (uniform or split), but the interpretation is slightly different.

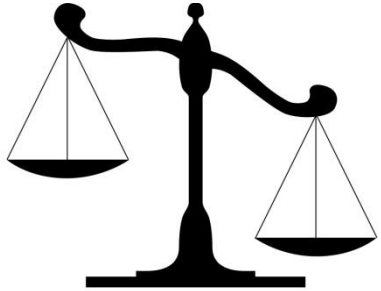
Data Preparation

- Prior to commencing the statistical analysis, the data should be checked to ensure:
  - that the data collected is accurate and appropriate for analysis
  - no gross errors and/or potential problems with the data.
- In some cases the data may need to be transformed e.g.
  - microbiological raw count data is transformed to log 10 results.
  - HI test analysis is usually carried out with the Log titre i.e. dilution 1/32 = 8

Statistical Analysis

- It is possible to use any statistical analysis as long as it is relevant to the test being assessed.
- Examples include:
  - % agreement between positive and negative results; consensus values
  - Summary statistics
  - Z-score analysis (robust)
  - Youden plots
  - Bar graphs

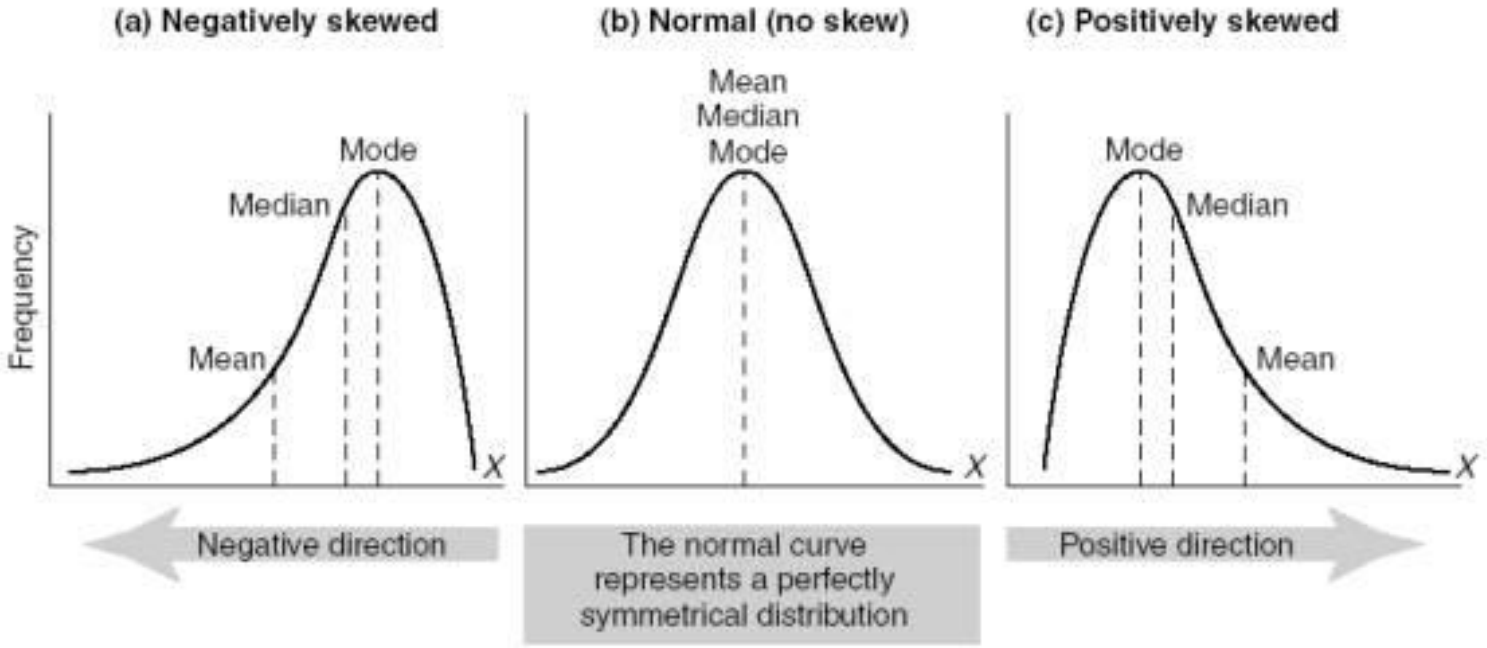
Consensus values



- Advantages;
  - Low cost – the assigned value does not require additional analytical work to assess measurement uncertainty
  - No one laboratory is accorded higher status
  - The calculation of consensus values is usually straightforward
- Disadvantages;
  - Consensus values are not independent of the participant results.
  - Does not account for results bias and therefore participants whose results are unbiased may unfairly receive extreme z-scores.

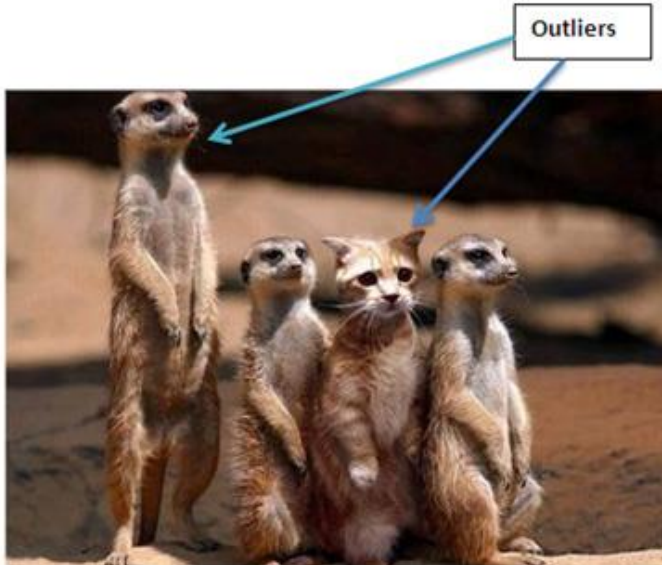
Robust statistics

- Robust statistics are statistics which are not highly affected or influenced by the presence of extreme results.
- Assumptions are made that the data are a sample from an essentially normal distribution contaminated with a small proportion of outliers.



Robust statistics

- Robustness is the “ability” of a statistical method to be unaffected by outliers e.g.
  - Median – a measure of the centre, minimises any effects due to extreme (very high or very low) results
  - Normalised Inter Quartile Range (IQR) – a measure of the spread
- They are similar to the mean and standard deviation, but these measurements are not robust



Important Considerations

- It is important to note that with any statistical application there are limitations that need to be considered when interpreting the summarised data.
  - If the data is skewed, biased or affected by methodology, the median result and the spread of results may not truly reflect an acceptable range of results.
  - If the number of participants are too few, the calculated acceptable spread of results may not accurately reflect a realistic spread of results in the field.
- It is important that scheme protocols are available so that participating laboratories can understand the statistics applied to each PT scheme.

Summary Statistics

- Once the data has been compiled, summary statistics are calculated to describe the data.

- Summary statistics include:
  - The Number of results (**N**)
  - Median
  - Normalised Inter Quartile Range (IQR_N) is a measure of the variability of the results.
  - Robust Coefficient of variation (CV) allows for the variability in different specimens/tests to be compared, expressed as a percentage
  - The minimum value
  - The maximum value
  - The Range is the difference between the minimum and maximum

Analysis of results is done so that it compares each individual result with the consensus of the entire group.

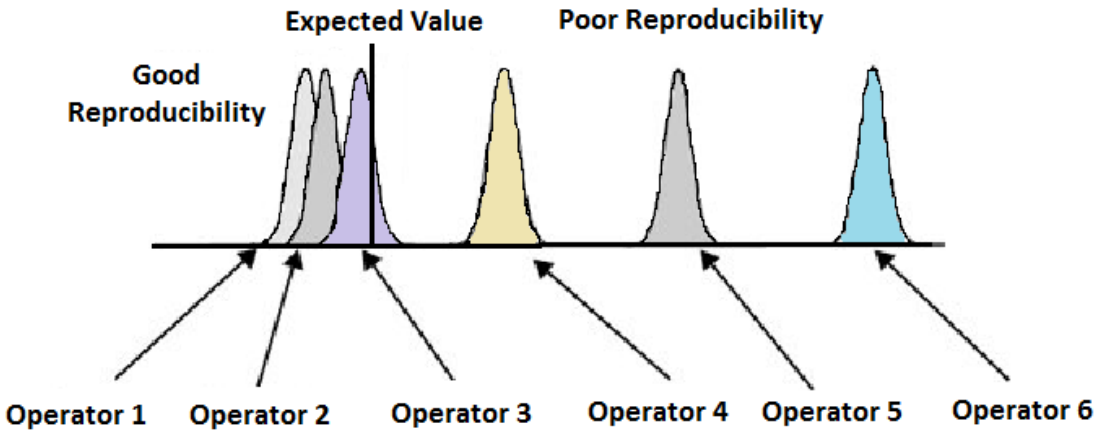
Robust Z-score

- Z-score - a normalised value which assigns a “score” to the result(s), relative to the other numbers in the group.
- **Robust Z-scores** are calculated by replacing the mean and standard deviation in the “classical” Z-score with the median and normalized IQR, respectively.
- Z-scores describe results with significant variations and also advise us on the type of variation.
- This is done by assessing both within-laboratory and between-laboratories variation for a pair of results.

Robust Z-score – between-laboratory

- *Between laboratory* Z-scores are based on the sum of the results and describe the variation between all laboratory results (**reproducibility**).

- A between laboratory outlier indicates results that demonstrate significant variation from the other laboratories.



Robust Z-score – within-laboratory

- *Within laboratory* variation is based on the difference between the sample pair and describes the variation within a laboratory (**repeatability**).
- A within-laboratory outlier indicates variation between the individual results submitted for the related sample pair by that laboratory and low precision
- Positive within laboratory Z-scores indicates the difference between the laboratory's sample pair is overestimated.
- Negative within laboratory Z-scores indicates the difference between the laboratory's sample pair is underestimated or has estimated the difference to be in the opposite direction to the median difference.

Robust Z-score

- ‘Scoring’ converts a participant’s raw result into a standard form that adds judgemental information about performance relative to the consensus result.
- Judgement is based on criteria – predefined **action limits.**
- The further from zero the Z-score is, the worse the result.

Z-score Action Limits

- A Z-score close to zero means that a result agrees well with the median consensus. No action is required.

  $|z| \leq 2$ satisfactory

- A Z-score greater than or equal to 2 but less than 3 identifies a result that is questionable and should be investigated.

  $|z| \geq 2$ but < 3 ($|z| \leq -2$ but > -3) questionable

- A Z-score greater than or equal to ±3 ie. $Z \geq 3$ or $Z \leq -3$, identifies a result which demonstrates significant variation from the other laboratory results (unacceptable result) and corrective action should be taken. These results are identified as outliers.

  $|z| \geq 3$ ($|z| \leq -3$) unsatisfactory

- Z-scores should: **initiate discussion** & **implement corrective actions**

Z-score Bar Charts

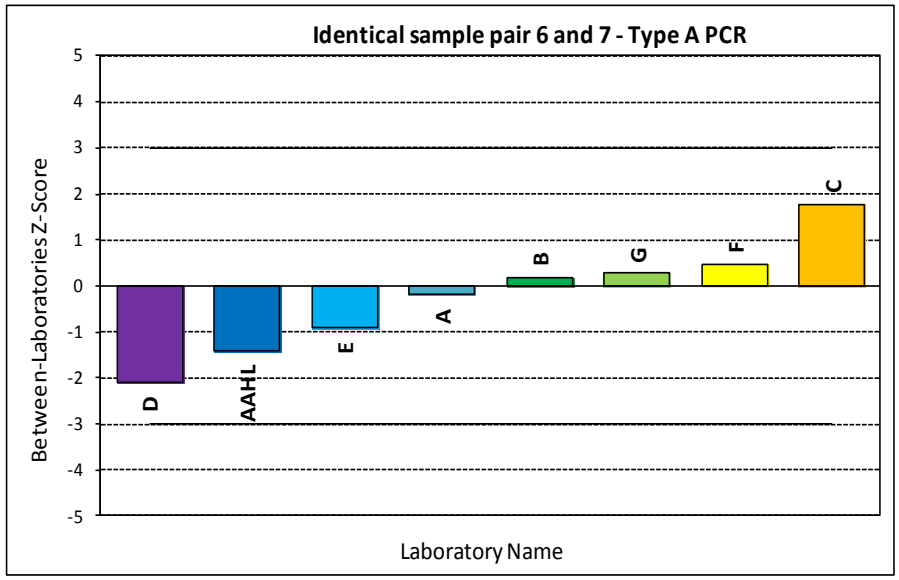
- The Z-score results are presented in summary tables and graphically in Z-score bar charts.

- Two bar charts are generated during analysis, a between-laboratory bar chart and a within-laboratory bar chart.

Table 5: PCR Assay – Within and between laboratory analyses for identical sample pair 3 and 7 using Influenza Type A TaqMan PCR.

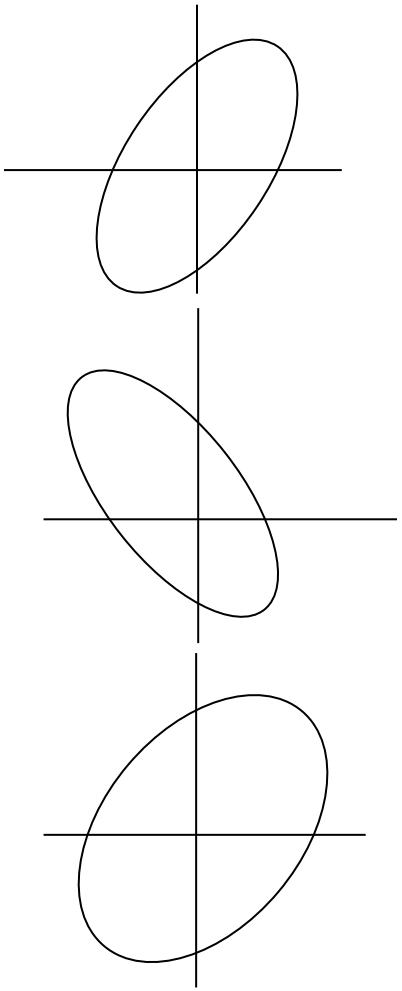
Laboratory name	Transformed Results		Between-Laboratory Z-Score	Within-Laboratory Z-Score
	Sample 3	Sample 7		
A	23.29	21.03	-0.06	1.05
B	20.61	20.91	-0.93	-0.29
C	23.30	19.25	-0.61	1.98
D	25.99	23.31	1.50	1.27
E	21.98	22.70	0.06	-0.51
F	24.28	24.18	1.24	-0.08
G	20.34	20.53	-1.13	-0.23
H	23.20	22.79	0.47	0.08

The between-laboratories and within-laboratory Z-scores are for the related pair. A Z-score between 0 and ±2 is "acceptable". A Z-score between ±2 and ±3 is "questionable" and **§** denotes an outlier, i.e. |z-score| ± > 3.



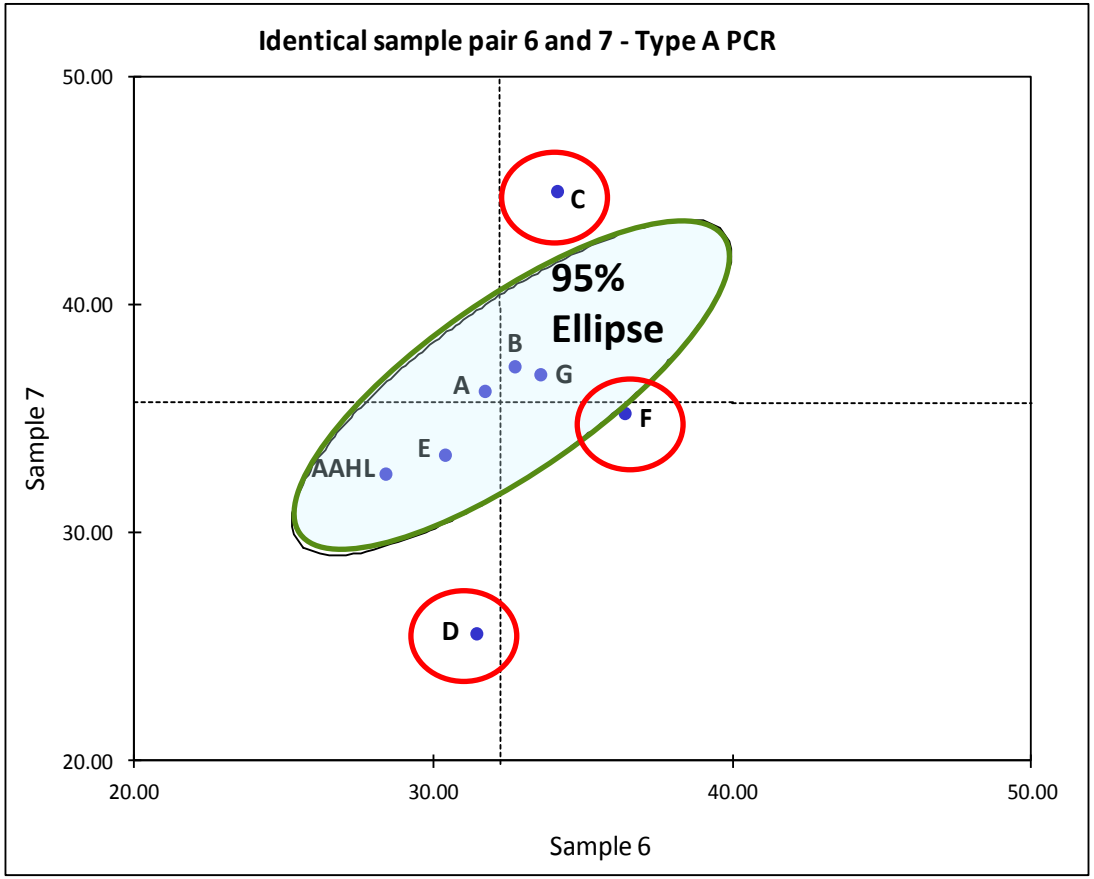
Youden Plot

- Youden plots can also be used to represent the data.
- The Youden plot is only relevant for related sample pairs i.e. split-level or uniform samples.
- Youden plots show the result of one sample as a function of the result of the other sample in a sample pair.
- The Youden plot gives an idea of the dominating sources of error in the results.
- An acceptable range of variation for the results from all laboratories is plotted in a 95% confidence ellipse



Youden Plot

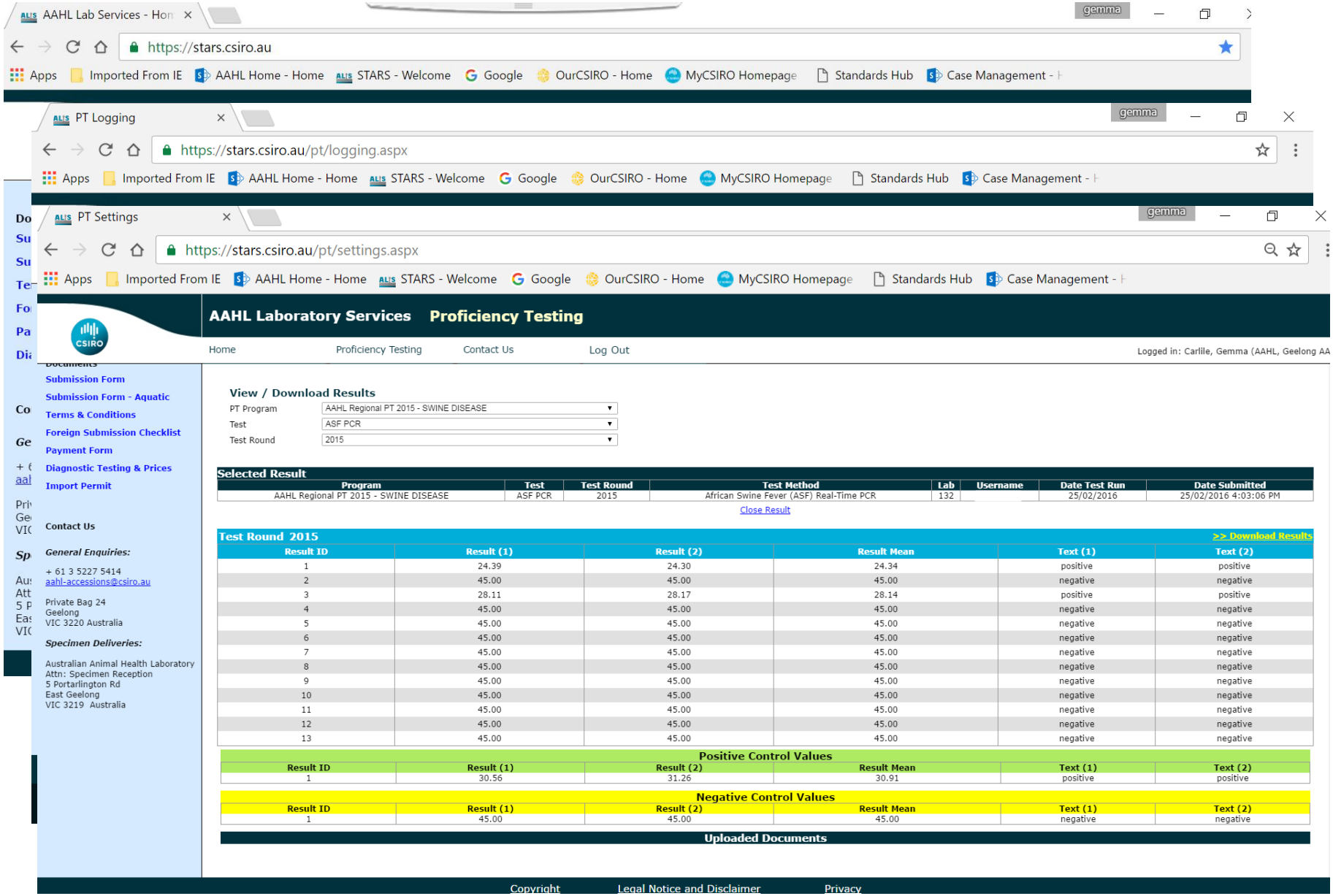
- 95% confidence ellipse with dashed lines indicating median values for each of the samples
- Laboratories with significant variation appear outside the ellipse



Assessment Key

The overall rating for each laboratory will reflect the most significant findings made, but less significant findings will also be noted.

Qualitative Assessment	Comment
Acceptable	Agreement with assigned results. No statistical differences were noted for the sample pair assessed. No specific follow-up is recommended.
Acceptable with observation	Qualitative results are acceptable. Minor statistical differences are noted but not considered significant. No specific follow-up is recommended.
Acceptable with condition	Qualitative results are acceptable. Statistical discrepancies are noted that warrant review.
Unacceptable	Results do not agree with assigned values. Review of procedures is highly recommended.
Observation	Observations noted about test results for additional assays performed beyond the scope of the PT panel.


AAHL Lab Services - Hon
https://stars.csiro.au
Apps Imported From IE AAHL Home - Home STARS - Welcome Google OurCSIRO - Home MyCSIRO Homepage Standards Hub Case Management -
gemma
PT Logging
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PT Settings
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AAHL Laboratory Services Proficiency Testing
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Logged in: Carlile, Gemma (AAHL, Geelong AA
Submission Form
Submission Form - Aquatic
Terms & Conditions
Foreign Submission Checklist
Payment Form
Diagnostic Testing & Prices
Import Permit
Contact Us
General Enquiries:
+ 61 3 5227 5414
aahl-accessions@csiro.au
Private Bag 24
Geelong
VIC 3220 Australia
Specimen Deliveries:
Australian Animal Health Laboratory
Attn: Specimen Reception
5 Portarlington Rd
East Geelong
VIC 3219 Australia
View / Download Results
PT Program AAHL Regional PT 2015 - SWINE DISEASE
Test ASF PCR
Test Round 2015
Selected Result
Program Test Test Round Test Method Lab Username Date Test Run Date Submitted
AAHL Regional PT 2015 - SWINE DISEASE ASF PCR 2015 African Swine Fever (ASF) Real-Time PCR 132 25/02/2016 25/02/2016 4:03:06 PM
Close Result
Test Round 2015
>> Download Results
Result ID Result (1) Result (2) Result Mean Text (1) Text (2)
1 24.39 24.30 24.34 positive positive
2 45.00 45.00 45.00 negative negative
3 28.11 28.17 28.14 positive positive
4 45.00 45.00 45.00 negative negative
5 45.00 45.00 45.00 negative negative
6 45.00 45.00 45.00 negative negative
7 45.00 45.00 45.00 negative negative
8 45.00 45.00 45.00 negative negative
9 45.00 45.00 45.00 negative negative
10 45.00 45.00 45.00 negative negative
11 45.00 45.00 45.00 negative negative
12 45.00 45.00 45.00 negative negative
13 45.00 45.00 45.00 negative negative
Positive Control Values
Result ID Result (1) Result (2) Result Mean Text (1) Text (2)
1 30.56 31.26 30.91 positive positive
Negative Control Values
Result ID Result (1) Result (2) Result Mean Text (1) Text (2)
1 45.00 45.00 45.00 negative negative
Uploaded Documents
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Laboratory Network Possibilities

- We can move from simply cooperation with trusted counterparts to sharing and relying on information for planning and making decisions
- Work-sharing with trusted counterparts (true leveraging of resources)
- Obtain better and more robust information to help make better decisions
- Strengthen areas in which we can better collaborate to leverage/create synergies with scientific and other resources
- Move from cooperation to confidence
- Achieve more rapid response

Resource Identification & Limitations

- Development of LEADDR and current activities are supported (absorbed) from existing funds across the network.
- Budget constraints - long term viability and efficiency requires identification of a funding source.
  - Equipment
  - Staff
  - Training
  - Facilities
  - Information management (STARS)
  - Planning and Management

EAD Preparedness: A Shared Responsibility

- All stakeholders have a shared responsibility in regards to preparedness for an EAD outbreak.

- The benefits of an appropriately funded, coordinated emergency response laboratory network in responding to an EAD emergency need serious consideration.

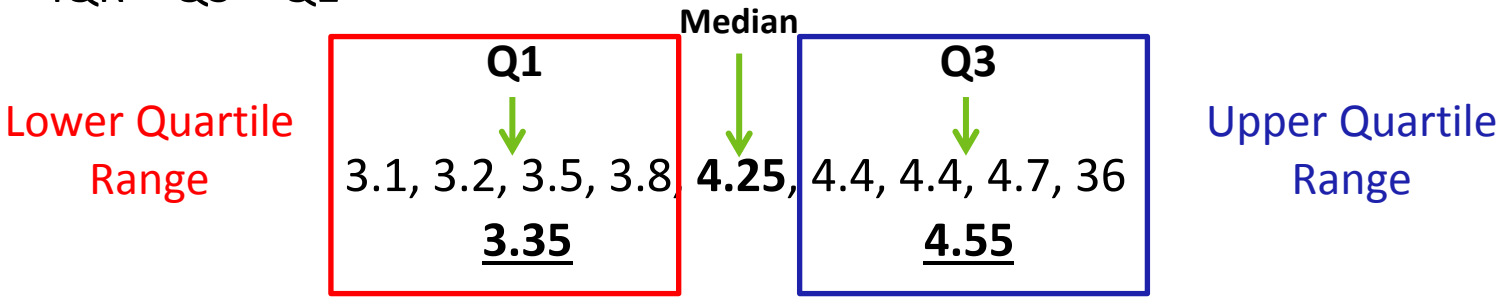
Summary Statistics

- Normalised Inter Quartile Range (IQR_N) is a measure of the variability of the results.

$$IQR_N = (IQR) \times 0.7413$$

- IQR = Q3 – Q1

IQR_N = 0.89



- **Q1**: The lower quartile is the value below which, as near as possible, a quarter of the results lie. The results corresponding to the first quartile (first 25% when ranked in order) i.e. Q1 = (N+1)/2

- **Q3**: The upper quartile is the value above which a quarter of the results lie. The results corresponding to the 3rd quartile (first 75% when ranked in order) i.e. Q3 = (N+1)/2

Summary Statistics

- Robust Coefficient of variation (CV) allows for the variability in different specimens/tests to be compared, expressed as a percentage

$$CV = 100 \times (IQR_N / Median)$$

- The greater the number, the greater the spread of results
- As a rule of thumb an example of a good spread of participant results in;
  - Real-time PCR proficiency testing is less than 5%
  - ELISA proficiency testing is less than 15%

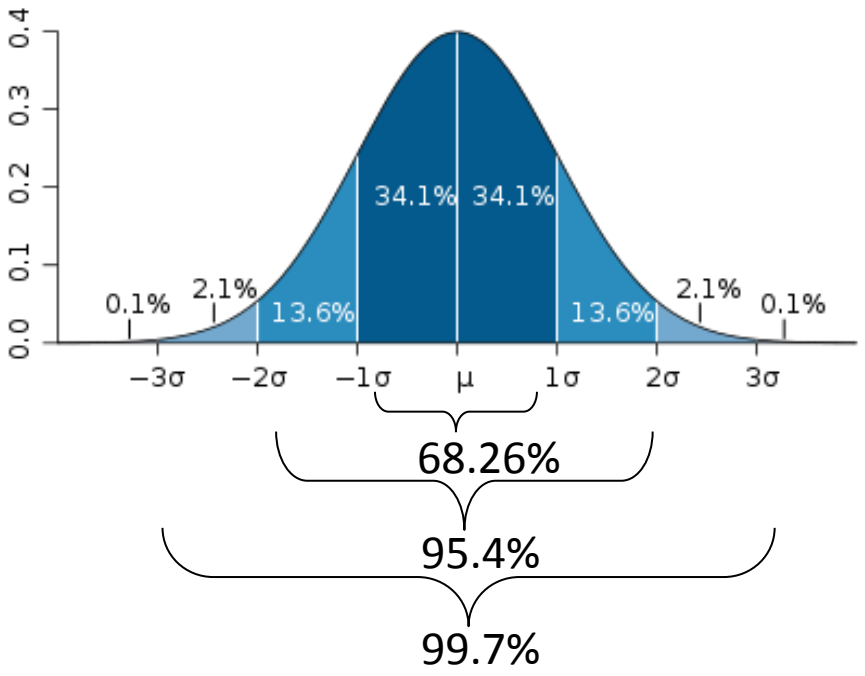
Summary Statistics

- The minimum is the lowest value X[1]
- The maximum is the highest value X[N]
- The Range is the difference between them (X[N] – X[1])

Analysis of results is done so that it compares each individual result with the consensus of the entire group.

Z-score

- z-scores are used to assess results from each individual laboratory
- z-scores are a normalized value that gives a score to each result relative to the other numbers in the group, e.g. median and normalized IQR
- a z-score value close to zero and less than 3 means that a result agrees well with those from other laboratories
- a z-score value Z < - 3 or Z > + 3 identifies an outlier with significant variation



Robust Z-score – between-laboratory

- The between laboratory and within laboratory robust Z-scores are based on the standardized sum and standardized difference of the pair of results, calculated by;

Between-laboratory Z-score (ZB)

$$ZB = \frac{S - median(S)}{IQR_N(S)}$$

S – Standardised sum

$$S = \frac{(A + B)}{\sqrt{2}}$$

A and B are the pair of results

Between laboratory comparison

- Between laboratory Z-scores are based on the sum of the results and describe the variation between all laboratory results (reproducibility). A between laboratory outlier indicates results that demonstrate significant variation from the other laboratories.
- Positive between laboratory Z-scores indicate results are above the median value. An outlier with a positive >3 Z-score indicates significant decreased sensitivity (ct values).
- Negative between laboratory Z-scores indicate results are lower than the median value. An outlier with a negative <-3 indicates significant increased sensitivity (ct values).

Robust Z-score – within-laboratory

Within-laboratory Z-score (ZW)

$$ZW = \frac{D - median(D)}{IQR_N(D)}$$

D – Standardised difference

$$D = \frac{(A - B)}{\sqrt{2}}$$

If the median (A) < the median (B);

$$D = \frac{(B - A)}{\sqrt{2}}$$