Proficiency Testing: a network approach

Strengthening veterinary diagnostics

AUSTRALIAN ANIMAL HEALTH LABORATORY (AAHL)
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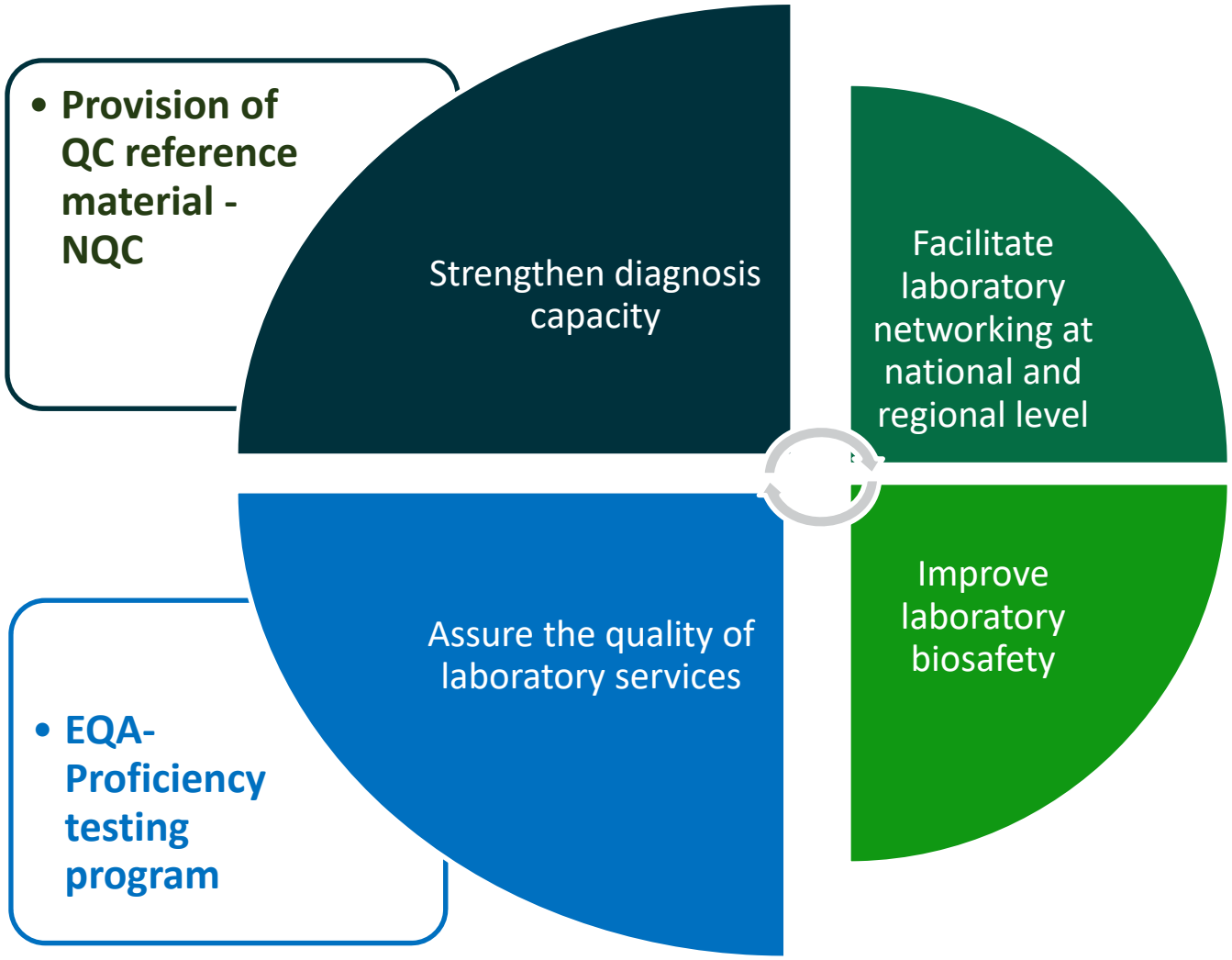
Where does PT fit into Veterinary Diagnostics?



Proficiency Testing (PT)

Important to participate in Proficiency Testing

International Standard ISO/IEC 17025 "General requirements for the competence of testing and calibration laboratories" Third Edition 2017-11

7.7.2 The laboratory shall monitor its performance by comparison with results of other laboratories, where available and appropriate. This monitoring shall be planned and reviewed and shall include, but not be limited to, either or both of the following:

a) participation in proficiency testing;

b) participation in interlaboratory comparisons other than proficiency testing

AAHL Proficiency Testing (PT)

- Accredited to ISO 17043 and incorporate ISO 13528
- AFDL program services:
    - Laboratories for Emergency Animal Disease Diagnosis and Response - LEADDR
    - South east Asia: Australian-government sponsored project
    - Chile: SERNAPESCA-sponsored PT Program
    - Australia: collaboration with ANQAP to provide PT
    - Evaluation panels for laboratories to implement capability
- Provision of test panels and quality controls to laboratories, who report results and are critically assessed and compared.

Impacts of PT program across the network

PT assists with ongoing training which is

Participation in PT is

Essential to maintain diagnostic services

Why a Networked Approach

- Networks offer a way to weave together and create capacities that get better leverage, knowledge, performance and results.
- Networks leverage and link expertise leading to efficiencies (everything doesn't have to be under one roof)
- Networks optimize use of assets (equipment, buildings etc.) that already exist in a system by connecting them to each other.
- Networks can build remarkable capacities because they mobilize diverse and flexible individuals and organizations.

Benefits of PT

Provides Confidence in Results;

- Test methods are being followed
  - detect any difficulties a laboratory may have with analyses
- Test results are accurate and precise
  - demonstrate repeatability and reproducibility
  - and test reliability
- Training is appropriate
  - identify training needs

- Systematic variations are identified
  - Provides information that can assist in future planning for equipment upgrades and staff training
- Consistency between other labs (harmonisation vs. standardisation)
- Credibility and compliance

PT can also provide laboratories with the necessary information to:

- Maintain and improve analytical quality
- Improve inter-laboratory agreement and raise standards
- Detect equipment faults, identify reagent problems and review staff training
- Initiate and evaluate corrective actions
- Compare performance to different analytical methods – assess harmonisation
- Ongoing monitoring of PT performance using an effective PT scheme will help to reduce laboratory errors, produce accurate test results and most importantly improve diagnostic testing

LEADDR – Current Diseases of Interest

- Avian Influenza - 2009
- Hendra Virus (HeV) - 2010
- Blue Tongue Virus (BTV) - 2011
- Newcastle Disease (NDV) - 2012
- Foot and Mouth Disease (FMD) – 2013

- White Spot Syndrome Virus (WSSV) - 2012
- Ostreid Herpes Virus (OsHV-1) - 2012
- Yellow Head virus (YHV) - 2012
- Megalocytivirus - 2012

- The provision of PT has resulted in **improved network harmonization of test methods and confidence in the network.**

A REGIONAL PROFICIENCY TESTING PROGRAM FOR AQUATIC ANIMAL DISEASE DIAGNOSTIC LABORATORIES IN ASIA-PACIFIC

	Disease pathogen	Label	OIE Listed
1	White spot syndrome virus (WSSV)	WSSV	Yes
2	Yellow head virus (YHV)	YHV	Yes
3	Taura syndrome virus (TSV)	TSV	Yes
4	Infectious myonecrosis virus (IMNV)	IMNV	Yes
5	Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	IHHNV	Yes
6	*Macrobrachium rosenbergii* nodavirus (*Mr*NV and XSV)	MrNV	Yes
7	Nervous necrosis viruses (NNV)	NNV	No
8	Koi herpesvirus (CyHV-3)	KHV	Yes
9	Megalocytiviruses (RSIV, ISKNV, GIV etc.)	RSIV	Yes
10	Spring viraemia of carp virus (SVCV)	SVCV	Yes

- 41 laboratories in 12 countries (not all laboratories tested for every pathogen)
- Four rounds of testing (program has been extended to 2022)

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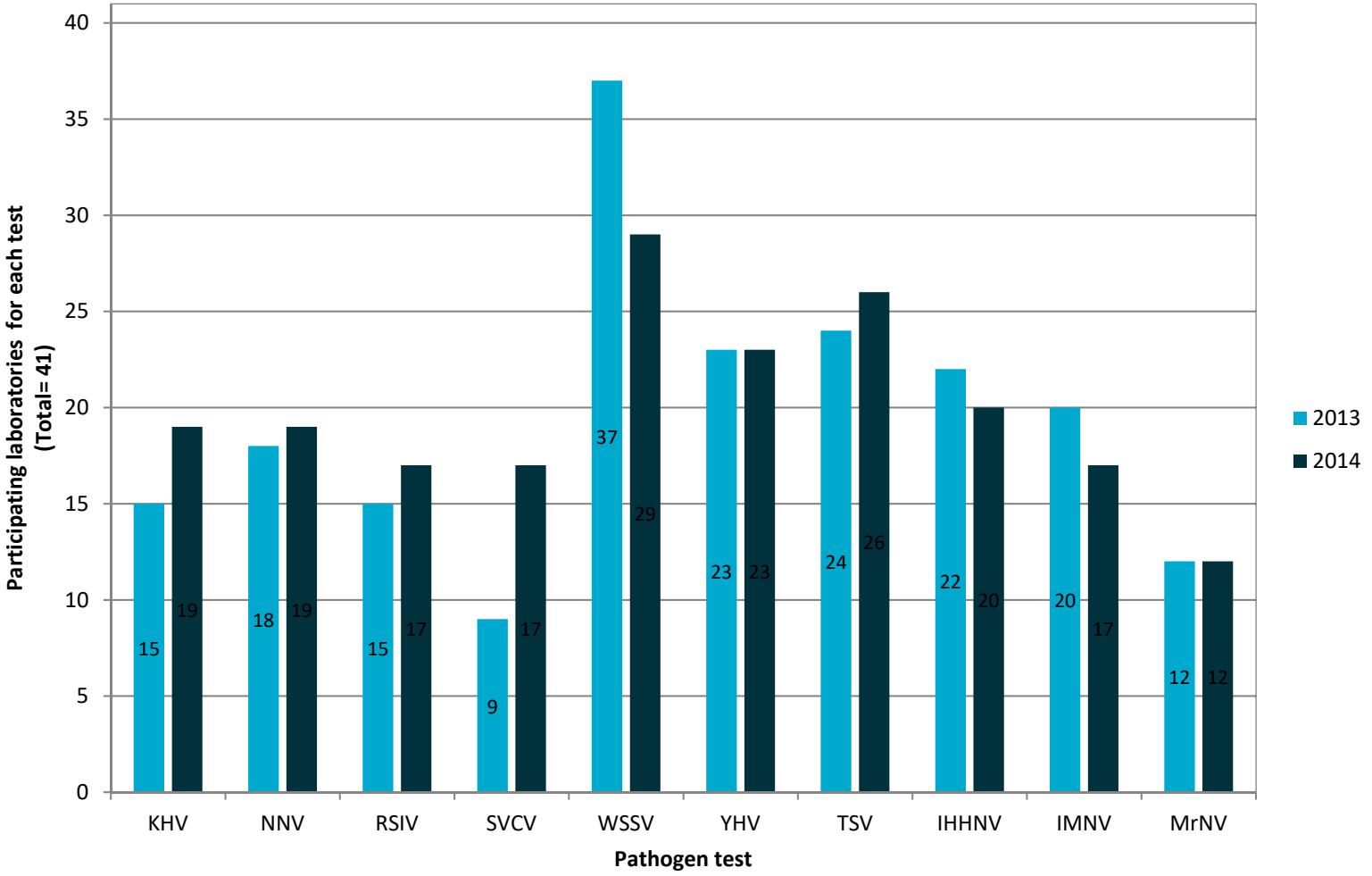

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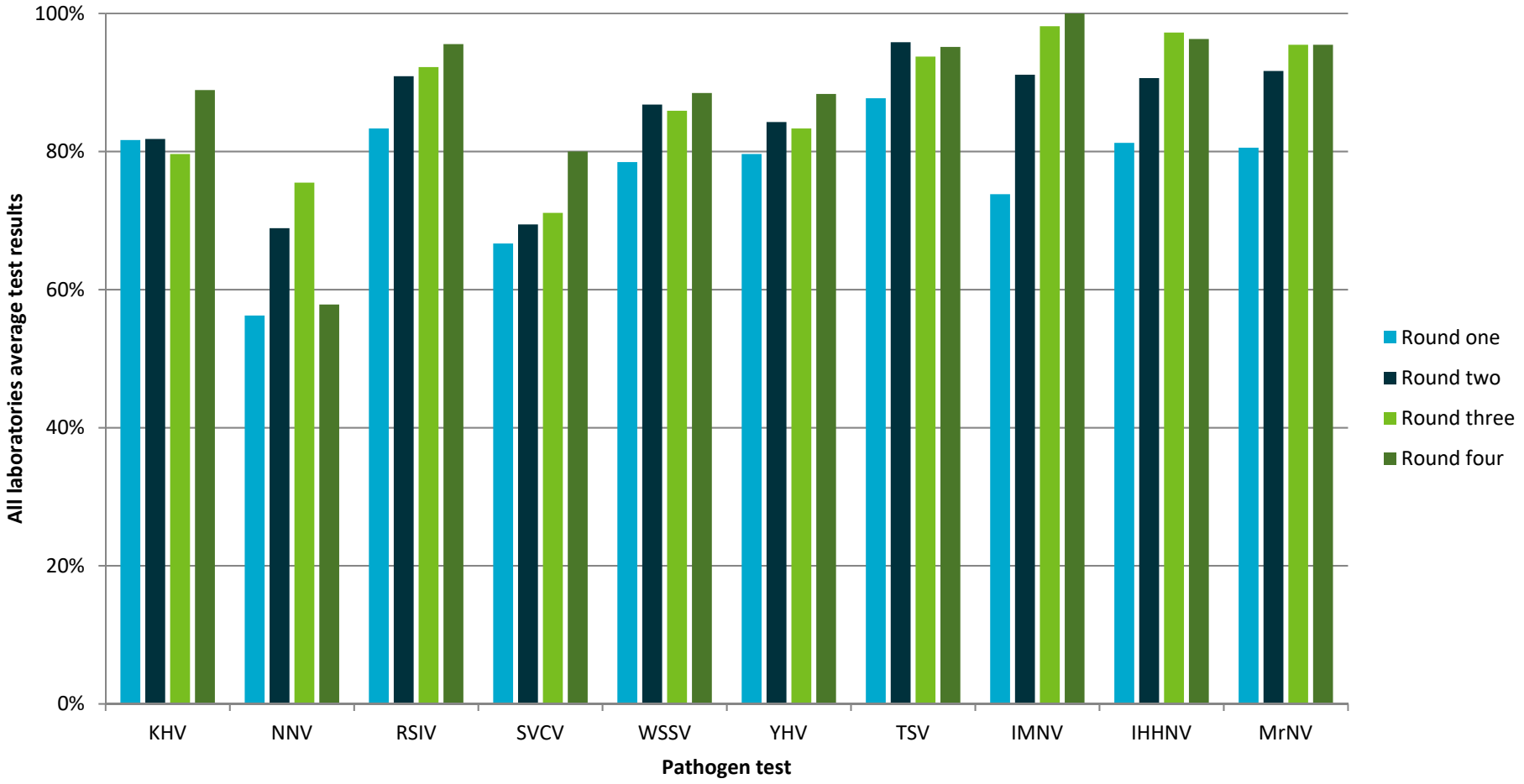


A REGIONAL PROFICIENCY TESTING PROGRAM FOR AQUATIC ANIMAL DISEASE DIAGNOSTIC LABORATORIES IN ASIA-PACIFIC

Table 2: Summary of Round 4 PCR results for KHV.

Laboratory No.	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Round 4 Results	R4 Score	R3 Score	R2 Score	R1 Score
Expected Interpretation	Moderate Pos	Moderate Pos	Neg	High Pos	Low Pos	Neg					
30	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	6	-	-
34	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	2	2	3
36	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	6	-	-
40	Neg	Neg	Neg	Pos	Neg	Neg	V	3	3	6	-
41	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	6	5	5
46	Pos	Pos	Neg	Pos	Neg	Neg	V	5	5	-	-
47	Pos	Neg	Neg	Pos	Neg	Neg	V	4	4	-	-
48	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	3	5	4
49	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	6	6	6
51	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	6	4	4
55	Pos	Neg	Pos	Pos	Pos	Neg	V	4	3	6	6
57	Pos (Ct 29.73)	Pos (Ct 32.76)	Neg (Ct >45)	Pos (Ct 22.50)	Pos (Ct 36.51)	Neg (Ct >45)	PASS	6	6	6	6
59	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	5	-	-
60	Pos	Pos	Neg	Pos	Neg	Pos	V	4	4	4	6
62	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	6	-	-
63	Pos	Pos	Pos	Pos	Pos	Neg	V	5	6	6	6
64	Pos	Pos	Neg	Pos	Pos	Neg	PASS	6	6	-	-
66	Pos	Pos	Pos	Pos	Pos	Neg	V	5	3	4	3
69	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	-	-	-	-	-
Expected Interpretation	Moderate Pos	Moderate Pos	Neg	High Pos	Low Pos	Neg					

Results were not received from laboratory 69.

PASS All results for test round correct
V Variation in expected result for at least one sample. Review and possible corrective action recommended.
NR No results submitted.

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
  - 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

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

    - Specificity – differentials

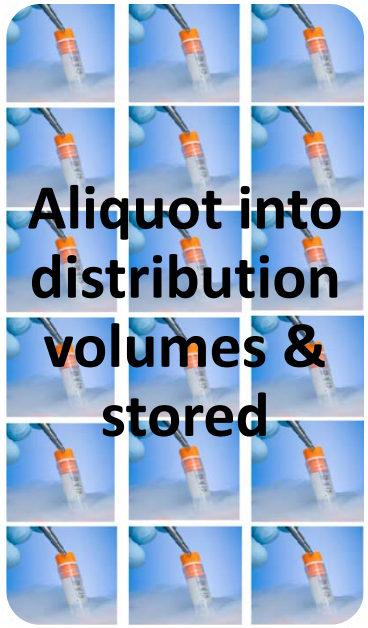
All help to determine test reliability

Test Sample Preparation

- Test Sample preparation includes;
  - Acquisition and Collection
    - samples can be sourced externally and/or produced internally
    - samples must inactivated (i.e. non-infectious)
  - Preparation and handling

PRE-TESTING

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



Test Sample Preparation

	Pathogen	Source Tissue	Material used for PT
1	WSSV	Ethanol-fixed prawns	Finely ground tissue homogenate (food processor → fine steel sieves) maintained in 70% ethanol. Sample provided in 70% ethanol, centrifuged and the pellet extracted and tested.
2	YHV	Ethanol-fixed prawns	
3	TSV	Ethanol-fixed prawns	
4	IMNV	Ethanol-fixed prawns	
5	IHHNV	Ethanol-fixed prawns	
6	MrNV	Ethanol-fixed prawns	
7	NNV	Cell culture supernatant	Fixation of the cell culture supernatant in 70% ethanol. Sample provided in 70% ethanol, centrifuged and the pellet extracted and tested.
8	KHV	Cell culture supernatant	
9	RSIV	Cell culture supernatant	
10	SVCV	Cell culture supernatant	

- Need to make sure the material is non-infectious (exotic pathogens)
  → heat degrades (destroy) target nucleic acid
  → need to comply with national and international regulations
  → changing to gamma-irradiation (best option)

Test Sample Preparation

A test panel of 5-10 samples is commonly chosen with the following in mind:

- A panel must contain a minimum of 5 samples
- One sample will produce an unequivocal strong positive (++) result
- One sample will produce an unequivocal weak positive (+) result
- One sample will produce an unequivocal negative (-) result
- One sample from a related agent (different serotype or genotype)
- The remaining test samples to be selected from any combination of the above categories with consideration given to the inclusion of pairs of related samples (**split** or **uniform**) to be included for statistical analysis.

- Use of paired samples gives an estimate of within panel variability

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)

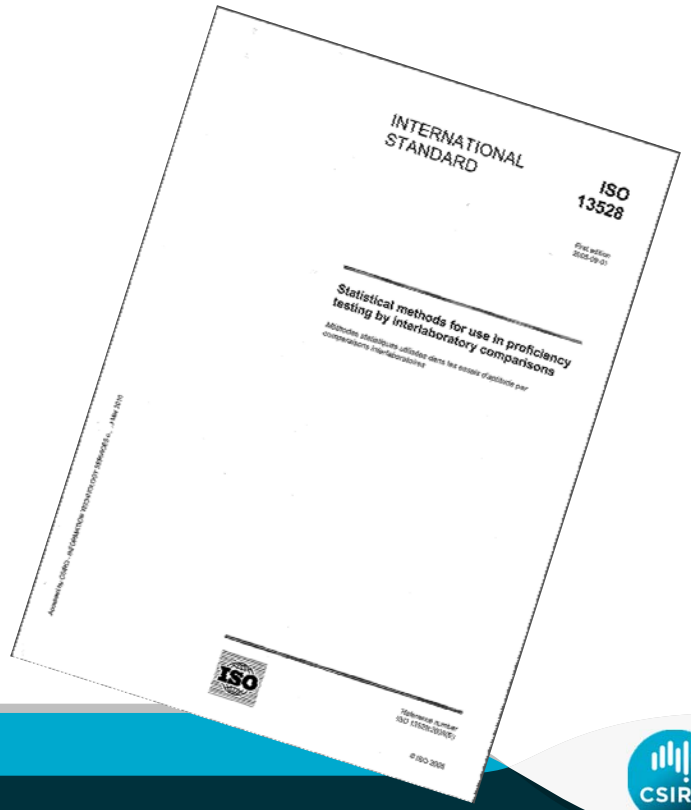
Homogeneity Testing

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

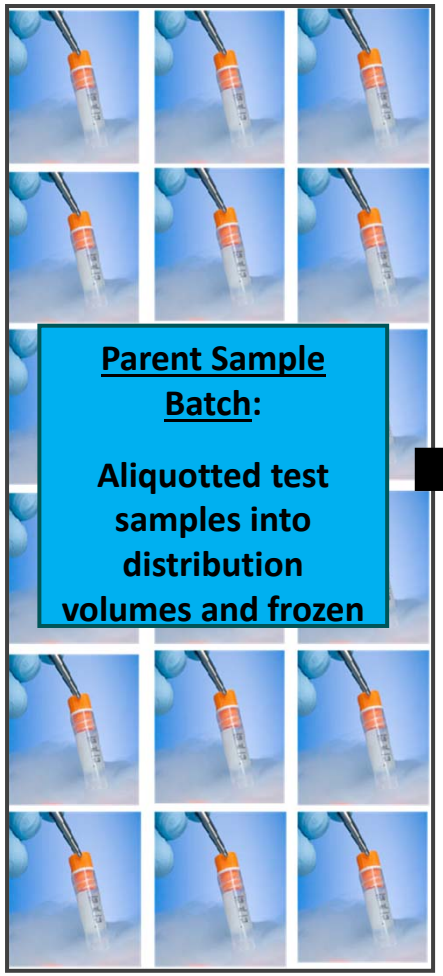
- This is done according to ISO 13528 *Statistical methods for use in proficiency testing by interlaboratory comparisons.*

  - 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



Parent Sample Batch:

Aliquotted test samples into distribution volumes and frozen

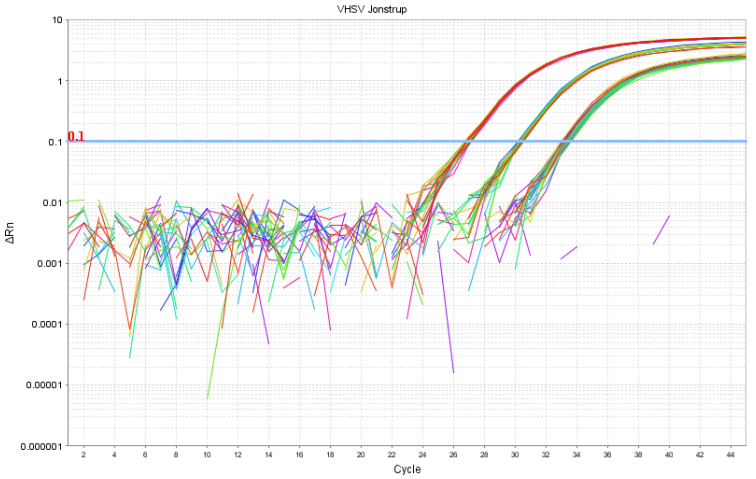
- Random Sample Aliquot 1
- Random Sample Aliquot 2
- Random Sample Aliquot 3
- Random Sample Aliquot 4
- Random Sample Aliquot 5
- Random Sample Aliquot 6
- Random Sample Aliquot 7
- Random Sample Aliquot 8
- Random Sample Aliquot 9
- Random Sample Aliquot 10

- 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

- Prior to distribution of the PT panel
- Calculated according to ISO 13528
- 10 samples chosen at random for each different positive sample
- Real-time PCR = CoV $\leq$ 5%



Homogeneity Testing

PATHOGEN	SAMPLE	CoV
IHNV	Strong	0.21
IHNV	Moderate	0.42
IHNV	Weak	0.00
VHSV	Strong	0.31
VHSV	Moderate	0.16
VHSV	Weak	0.35
KHV	Strong	0.65
KHV	Moderate	0.61
KHV	Weak	0.46
SVCV	Strong	0.42
SVCV	Moderate	0.00
SVCV	Weak	0.43
EHNV	Strong	0.30
EHNV	Moderate	0.23
EHNV	Weak	0.25
SAV	Strong	0.31
SAV	Moderate	0.27
SAV	Weak	0.00
OsHV-1	Strong	0.48
OsHV-1	Moderate	0.40
OsHV-1	Weak	0.26

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

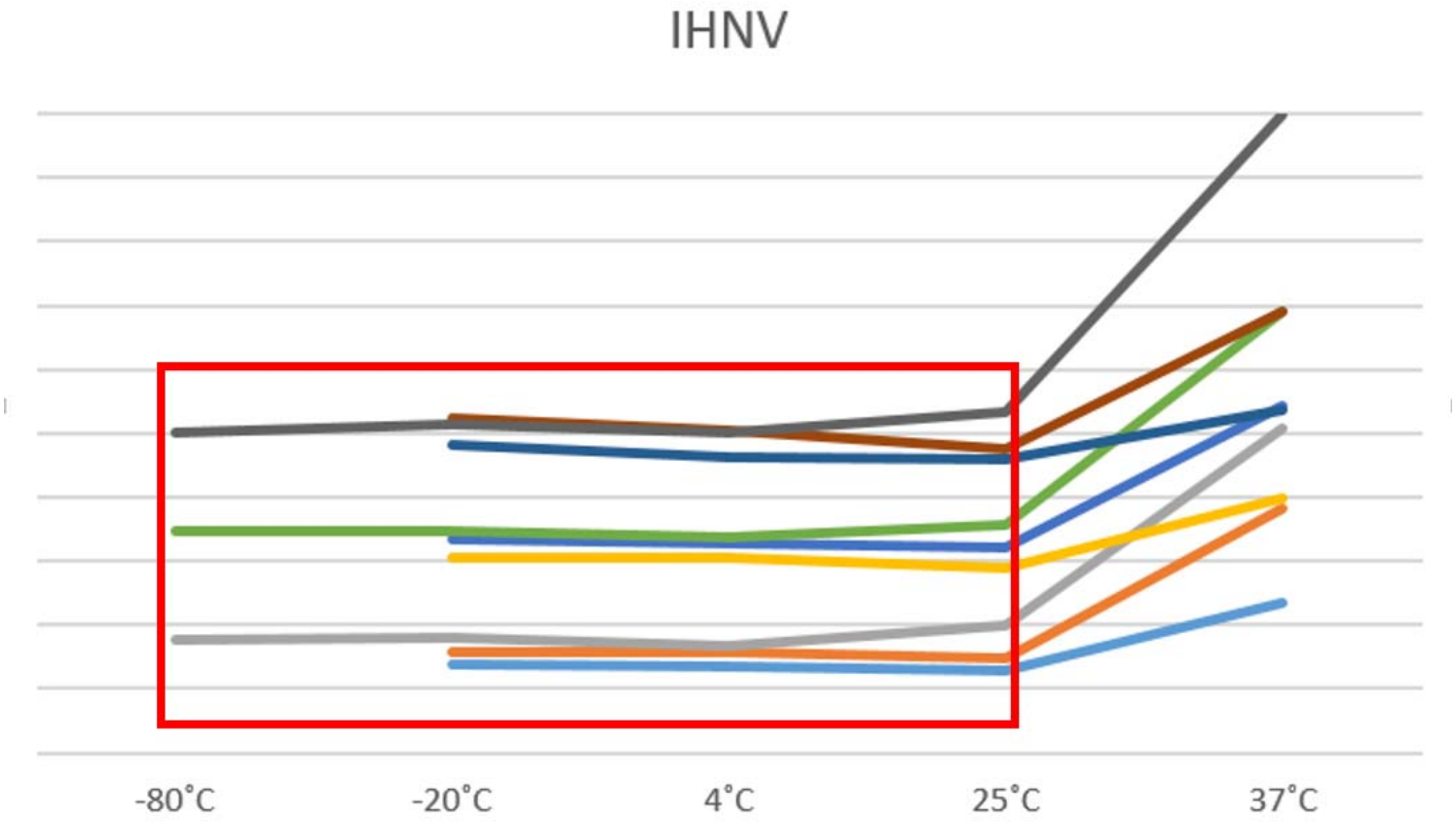
1. ‘fit-for-purpose’ analysis needs to be undertaken to confirm that the sample type will perform satisfactorily for use
2. Ongoing establishment of test sample performance pre- and post- PT distribution

Stability Testing

- Performed for all pathogens in PT1 and PT2
- At -20°C, 4°C, 25°C and 37°C
- Set time period

Stability Testing

- Results after 2, 5 and 10 weeks at each temperature



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

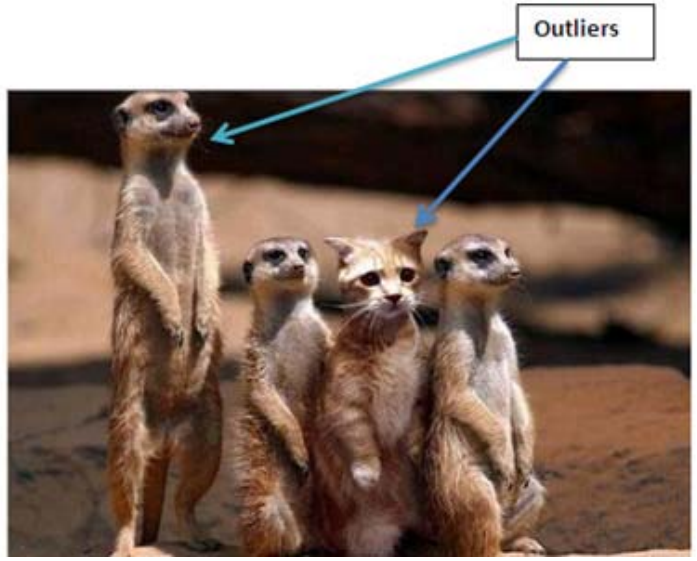
- Prior to commencing the statistical analysis, the data should be checked to ensure:
  - that the data collected is accurate and appropriate for analysis
  - no gross errors and/or potential problems with the data.
- In some cases the data may need to be transformed e.g.
  - microbiological raw count 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



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.

Pathogens for PT

- Round 1: VHSV and IHNV (SAV and PMCV under consideration)
- Round 2: KHV, SVCV and Megalocytivirus

- Participants?
- Requirements for positive control plasmids TBC

Thank you

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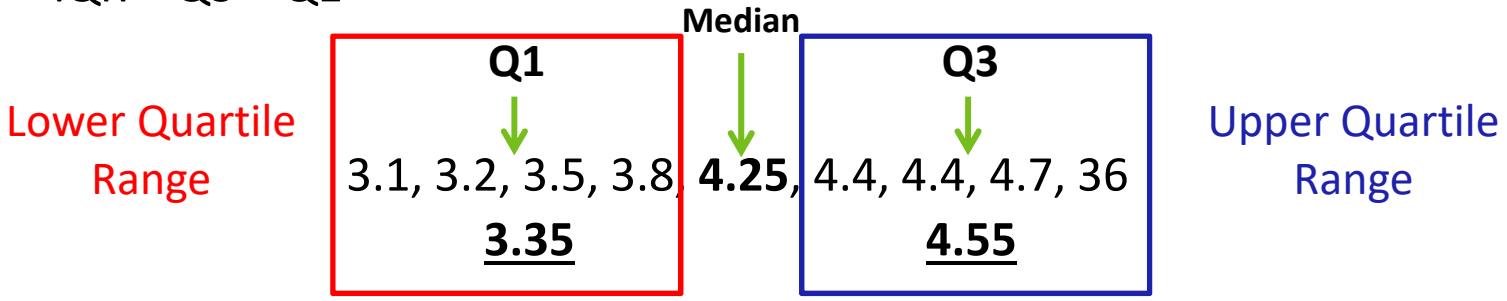
Summary Statistics

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

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

$IQR_N = 0.89$

- IQR = Q3 – Q1



- **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