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UNCERTAINTY AND VALIDITY OF METHODS USED IN EVALUATION OF LIVE POULTRY VACCINE IN CLEVB

By

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ABSTRACT

Measurement of validity and uncertainty for quality control results of four live poultry vaccines were studied for calculated the difference between uncertainty measurements for different routes of inoculation, accuracy, precision attributed to different factors; personal and inoculation site.A total of (80) samples were used in this study represent to four types of vaccines; three of them were egg adapted vaccine and last one was tissue culture (TC) adapted. Four analysts were involved to assess; the repeatability, reproducibility, bias, to determine uncertainty as well as estimate the stability and homogeneity of vaccine sample. The mean titer for (Infectious bursal disease vaccine (IBD), Fowl pox (FP), Avian Encephalomyelitis (AE) and tissue culture adapted Infectious bursal disease vaccine(IBDTC) were $10^{5.43}\pm^{0.403}$,$10^{3.58\ \pm0.256}$,$10^{3.44\pm0.326}$ and $10^{5.02\pm0.492}$ respectively. Homogeneity and stability results for all types of vaccines were accepted according to TS/ ISO22117.The reproducibility component of TC technique higher and the Bias was lower than all other egg inoculation routes.

CONCLUSION

The accuracy of TC technique is better than egg inoculation technique that reflected on the uncertainty. There is no significance difference between uncertainty measurements of 3 different routes of egg inoculation, despite significant difference in bias (accuracy) and reproducibility (precision) attributed to different factors; personal and inoculation site.

Key words:

Live poultry vaccines; uncertainty, bias, reproducibility.

INTRODUCTION

Third-party accreditation is a valuable tool to demonstrate a laboratory's competence to conduct testing. However, accreditation is only 1 part of establishing data credibility. A validated test method is the first component of a valid measurement system. Validation is defined as confirmation by examination and the provision of objective evidence that, the particular requirements for a specific intended use are fulfilled. The international and national standard ISO/IEC 17025 **(ISO/IEC (17025), 2005)** recognizes the importance of validated methods and requires that laboratory-developed methods or methods adopted by the laboratory be appropriate for the intended use. Validated methods are therefore required and their use agreed by the client (i.e.,end users of the test results such as veterinarians, animal health programs, and owners). ISO/IEC 17025 **(ISO/IEC (17025). 2005**) also requires that the introduction of methods developed by the laboratory for its own use be a planned activity conducted by qualified personnel with adequate resources. This article discusses considerations and recommendations for the conduct of veterinary diagnostic test method development, validation, evaluation, approval and transfer to the user laboratory in the ISO/IEC 17025 environment. These recommendations are based on those of nationally and internationally accepted standards and guidelines, as well as those of reputable and experienced technical bodies. They are also based on the author's experience in the evaluation of method development and transfer projects, Validation data, and the implementation of quality management systems in the area of method development. The performance of a test is defined by two independent measures: precision and accuracy. Precision refers to the closeness of agreement among repeated measurements of the same sample under prescribed conditions and accuracy to the closeness of agreement between the result of measurement and the value of the analyte measured **(ISO/IEC (17025 ,2005**).Every measurement made has an error associated with it and without a quantitative statement of the error it lacks worth. The parameter that quantifies the boundaries of the error of the measurement is called measurement uncertainty or (MU) **(NATA, 2009)**. The OIE quality standard, (2016) **(OIE, 2016)** defines measurement uncertainty as a parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the analyte measured. In the context of testing, MU provides quantitative estimates of the level of confidence that a laboratory has in the analytical precision of test results, and is therefore an essential component of a quality system for veterinary diagnostic

laboratories. MU can be regarded as a combined measure of precision and bias, where precision measures the ability to repeat the result each time. The same sample is tested and bias measures the ability to produce a 'true 'result. **(NPAAG, 2009)**. The Laboratory undertaking the uncertainty and validation study should have the qualified staff, skills and infrastructure to perform the method and should have a quality assurance system in place to provide adequate confidence in the results of the validation study.There are many possible sources in measurement of uncertainty and validation. These sources are not only measurement equipment and test methods but also by the person performing the test, data analysis, the environment and a host of other factors **(Guidance G104**, **2014).** Laboratories shall have and apply procedures for estimating uncertainty of measurement according to ISO/IEC 17025. Measurement of uncertainty is the parameter associated with the result of a measurement that characterized the dispersion of the values that could be attributed to the measured. Calculation of infected dose fifty is specific methods used in evaluation of live poultry vaccine. Measurement of uncertainty reflects to accuracy and perfect for the reliability of result to unable decision maker for accepts or refuse the goods. Quality assurance is important for verification of the accuracy and precision in the formation obtained from analysis ensuring that, the data obtained from analysis are suitable for use in decision making, ensuring the correctness of data and ensuring proper functioning to decrease maintaining equipment failure. The study aims to evaluate methods applied for quality control of live poultry vaccines by different technique (egg inoculation and tissue culture technique).

MATERIAL AND METHODS

Ethical approval:

Institutional Animal Ethics committee has accorded permission for conducting this trial.

*** Vaccine Samples:**

A total number of eighty samples representing four types of live attenuated poultry vaccines, 20 samples from different batches for each type were used. . Three types of vaccines represent egg adapted vaccine were infectious bursal disease vaccine, Avian Encephalomyelitis and Fowl pox and the fourth one was tissue culture adapted vaccine as infectious bursal disease.

*** Specific pathogen free -embryonated chicken egg (SPF-ECE):**

These eggs were obtained from specific pathogen free (SPF) egg production Farm, Koum Osheim, El-Fayoum, Egypt. Eggs were kept in a calibrated egg incubator at 37˚C with humidity 40-60%. The eggs were tested for contamination with any; IBD, ILT, Reo and Fowl

Pox antibodies, HA agent, *Mycoplasma Synovia, Mycoplasma gallisepticum*, *Salmonella* pullorum antibodies, leucosis virus, Chicken Anaemia Virus and reticuloendothelioses antibodies (REV) antigen. It was used in vaccine titration for egg adapted vaccines **(CFR USA, 2015**) and the embryo infected dose (EID_{50}) **(Reed and Muench, 1938)** had been calculated**.**

**** Tissue culture (TC) and media*:**

Primary chicken embryo fibroblast cell (CEF) was obtained and prepared according to **(Schat and Purchase, 1989)**, the method used for inoculation in the microtiter plates was done **(Villega, 1990)**. Infected dose ($TCID_{50}$) was calculated **(Reed and Muench, 1938)**.

***Reference material (strain control):**

The study used reference strains obtained from reference strain bank (CLEVB) with known titer (+ve&-ve) to ensure the reliability of result obtained from study **(ISO Guide 30, 1992 and Eurochem Guide, 1998).**

*** Quality assurance (ISO/IEC (17025) , 2005):**

Performance of equipment: (ISO/FDIS 7218, 2013).

All equipment used was kept clean and in good working condition before use. All equipment and monitoring devices used were calibrated to traceable national standards.

Equipment was monitored according to working conditions and the accuracy demanded for the results.

****Environmental monitoring:***

The temperature was checked periodically. The microbiological quality of air was checked before beginning of the study. The microbiological quality of surface was checked before analysis of the samples by using swab technique with acceptable limit <20 CFU/plate. The environmental control offers reasonable assurance that, the environment is not a source of contamination of sample under test **(ISO/FDIS 7218, 2013)**.

***Media and reagent performance (ISO/TS 11133-1, 2014):**

Media used in this study were tested before using to validate their efficacy. Media were prepared carefully and validated for performance including the measurement of productivity and selectivity to be sure that, the media were suitable for each purpose.

***Negative analytical controls:**

Control SPF egg to ensure freedom from certain avian viral and bacterial contamination or antibodies to be used in evaluation of avian vaccines was included. Media controlled by

sterility testing to ensure that analytical medium is not contaminated with the analyte and checked during preparation as schedule for quality control.

***Criteria of validation: (ISO/TS34SC9W03/16140, 2017):**

The measurement of uncertainty was calculated according to NORDES 537as follow:

•**Measurement of uncertainty and Statistical Methods (Dimech *et al.* ,2007 and ISO/TS 11133-2 ,2014):**

According to requirement of ***ISO/IEC (17025)*** the laboratory shall be select the test method.

•**Software Quality Control Chart (X chart) (Excelkontrol (on):**

The measurement of uncertainty depends on the following tools for start evaluation reproducibility, competent and Bias competent which is based on mean, standard deviation, true value, bias uncertainty, Ref value uncertainty, $(Bais)^2$, bias uncertainty, relative standard deviation (RSD) and RSD ***%.*** **(Excerkantral Version 2.1, 2008)**. Rules for out of control were checked **(ISO 8258, 2005 and Nordtest TR 569, 2006**).

•**Validated Software (Nordtest technical report 537) (Nordtest TR 537 , 2004):**

The MU is by using validated software from estimates of reproducibility and method Bias using control charts. Proficiency tests and Certified Reference Material (CRMs) **(Nordtest TR 537, 2004).** According to the following equation as follow:

1-Bias = (mean - true value) * 100 / true value.

2-RSD% = (STD / mean) * 100.

3-Recovery = (mean / true value) * 100.

4-Ref value uncertainty =100 * (error / 1.96 / true value).

5-Bias component= [sqrt {$(Bias)^2$+ (bias uncertainty) ^{2}+ (Ref value uncertainty) ^{2}}] ^{2}

6-Measurement Uncertainty =2*sqrt $(Sr)^2$ + (bias component) ^{2}

7-error= sqrt of [(uncertainty of micro pipette) ^{2} + (uncertainty of balance) ^{2}+ (uncertainty of reference strain) ^{2}).

Homogeneity of test material:

Five to ten randomly selected test materials were analyzed in duplicate for each analyte. The statistical tests initially check the data for any widely discrepant pairs using Cochran's test. If found such data are removed.Thereafter the remaining data are subject to analysis of variance (ANOVA) to estimate the sampling and analytical variances **(Fearn and Thompson, 2001**).

Stability of test material (ISO/IEC17043 (2010):

Sample to be used for study shall be stable, at least for the period from start date of examination to the end date of examination. The stability done according to ISO/TS22117 **(ISO 22117, 2010)** and results reported on (Table 5).

RESULTS

Table (1): Result of virus titer content for Egg adapted vaccine as Infectious Bursal disease (IBD), Fowl pox (FP) and Avian Encephalomyelitis (AE) and tissue culture adapted vaccine as infectious bursal disease (IBD T.C) for different batches.

Vaccine	Titer (log_{10} EID $_{50}$ / dose)			Titer ($log_{10}TCID_{50}$/dose)
batches	IBD	Fowl Pox	AE	IBDTC
1.	5.5	3.6	3.4	5
2.	5.3	3.6	3.4	5
3.	5.3	3.5	3.4	5.2
4.	5.5	3.6	3.4	5
5.	5.5	3.5	3.5	5
6.	5.3	3.6	3.5	5
7.	5.5	3.6	3.5	5
8.	5.5	3.6	3.5	4.8
9.	5.5	3.6	3.4	5
10.	5.3	3.6	3.4	5.2
11.	5.5	3.6	3.4	5
12.	5.5	3.6	3.4	5
13.	5.5	3.5	3.4	5
14.	5.3	3.6	3.4	5
15.	5.3	3.5	3.5	5
16.	5.5	3.6	3.5	4.8
17.	5.5	3.6	3.5	5
18.	5.3	3.6	3.5	5.2
19.	5.5	3.6	3.4	5
20.	5.5	3.6	3.4	5.2

NB: EID50: The Embryo Infective Dose, TCID50: Tissue culture Infective Dose (ID_{50}) estimated according to method (8). The virus titer of tested vaccines as shown in (Table 1) obtained from X. chart with confidence limit at 95% were used in statistical calculation to estimate of reproducibility % and bias %.

Table (2): Statistical Evaluation of results for egg adapted vaccines as Infectious Bursal disease (IBD), Fowl pox (FP) and Avian Encephalomyelitis (AE) and tissue culture adapted vaccine as infectious bursaldisease (IBDT.C) fordifferent batches.

Statistical parameters	Kind of Vaccine			
	IBD	Fowl Pox	AE	IBD TC
Mean	5.43	3.58	3.44	5.02
Standard deviation	0.098	0.04	0.05	0.11
Number	20	20	20	20
true value	5.43	3.57	3.46	4.98
Error	0.35	0.28	0.28	0.24
Bias	-1.63569E-14	0.280112045	-0.58	0.80
Bias uncertainty	0.40	0.26	0.33	0.49
Ref value uncertainty	3.29	4.00	4.13	2.46
$(Bias)^2$	2.67547E-28	0.08	0.33	0.65
$(bias\ uncertainty)^2$	0.16	0.07	0.11	0.24
$(Ref\ value\ uncertainty)^2$	10.82	16.0	17.05	6.05
Bias component	3.31	4.02	4.18	2.63
RSD	0.02	0.01	0.01	0.02
RSD%	1.80	1.15	1.46	2.20

Statistical evaluation for each parameter according to Validated Software (Nordtest technical report 537).

Table (3): Estimated of measurement uncertainty (MU) for different types of inoculation.

Type of vaccines	Reproducibility component	Bias component	Measurement Uncertainty =2sqrt (Sr)2 + (Bias component)2
IBD [via allantoic cavity]	**3.25**	**10.98**	**7.54%**
Fowl pox [on chorioallantoic membrane]	**1.31**	**16.16**	**8.36%**
AE [intra the yolk sac]	**2.13**	**17.49**	**8.86%**
IBD TC[primary chicken embryo fibroblast cell (CEF)]	**4.85**	**6.93**	**6.86%**

(Table 2,3) showed statistical calculation obtained from X chart to determine reproducibility and bias component which indicate that, the reproducibility component [precision] for vaccine IBD (TC) was 4.85% higher than other king of vaccines egg inoculation technique which were 3.25% ,1.31% and 2.13% for IBD, FOWL POX AND AE respectively. While bias component [Accuracy] for tissue culture technique was 6.93 % lower than egg inoculation technique for IBD, fowl pox and AE were 10.98 %, 16.16 % and 17.49% respectively.

The final measurement uncertainty for IBD (TC) was 6.86 % significant lower than other rout of egg inoculation as follow 7.54% for IBD, 8.36% for fowl pox and 8.86 % for AE.

Table (4): Homogeneity of test material of egg adapted vaccines as Infectious Bursal disease (IBD), Fowl pox (FP) and Avian Encephalomyelitis (AE) and tissue culture adapted vaccine as infectious bursal disease (IBD T.C) for different batches.

	IBD		Fowl Pox		AE		IBD TC	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	5.5	5.3	3.6	3.6	3.4	3.5	5	5
2	5.3	5.5	3.6	3.6	3.4	3.5	5	5
3	5.3	5.5	3.5	3.6	3.4	3.5	5.2	4.8
4	5.5	5.5	3.6	3.6	3.4	3.4	5	5
5	5.5	5.3	3.5	3.6	3.5	3.4	5	5.2
Mean	5.42		3.58		3.44		5.02	
No	10		10		10		10	
Standard deviation [σ_0]	0.10328		0.042164		0.05164		0.113529	
S am	0.016		0.002		0.004		0.02	
S am 2	-0.006		-0.00025		-0.0015		-0.008	
σ_{all}^2	0.00096		0.00016		0.00024		0.00116	
Critical	0.017965		0.002321		0.004491		0.022381	
S_{am}^2< Critical	Accept		Accept		Accept		Accept	

Homogeneity of different batch of each kind of vaccine was determined (23) and the results are recorded in (Table 4).

In (Table 4) we examine the power of the test by calculating the probability of rejecting the hypothesis of sufficient homogeneity when it is indeed true that $S_{am}^2 > \sigma all^2$. This probability depends, naturally, on the amount by which S_{am}^2 exceedsσall^2.If S_{am}^2<Critical indicated accept results.

Table (5): Stability of test material for different kinds of vaccine.

Accepted criteria	T1		
	up to 9.3	≤ 2	
❖Vaccine Infectious Bursal disease[IBD] egg adapted			
Titration of virus by using egg inoculation [via allantoic cavity]	**0.007**	**0.001**	**Accepted**
❖Fowl Pox [FP] Vaccine			
Titration of virus by using egg inoculation [on chorioallantoic membrane]	**0.001**	**0.0005**	**Accepted**
❖Avian Encephalomyelitis [AE] Vaccine			
Titration of virus by using egg inoculation [intra the yolk sac]	**0.001**	**0.0004**	**Accepted**
❖Tissue culture vaccine of Infectious Bursal disease [IBD TC]			
Titration of virus by using [primary chicken embryo fibroblast cell (CEF)]	**0.01**	**0.00**	**Accepted**

The stability of different batches of each kind of vaccinePerformed from start of examination until end of study were within accepted criteria of T1 up to 9.3 and T2 ≤ 2.

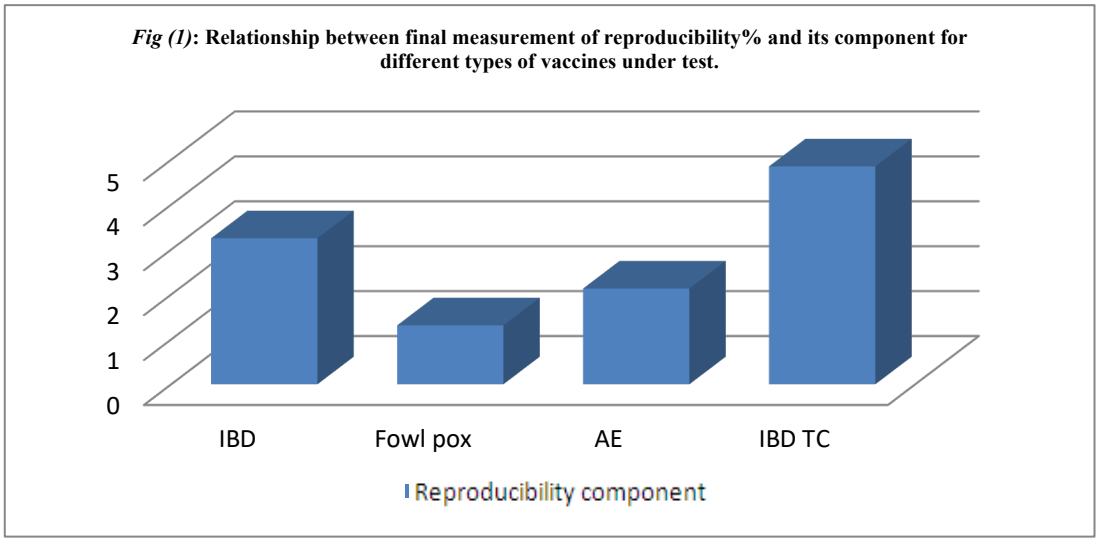


***Fig (1)*: Relationship between final measurement of reproducibility% and its component for different types of vaccines under test.**

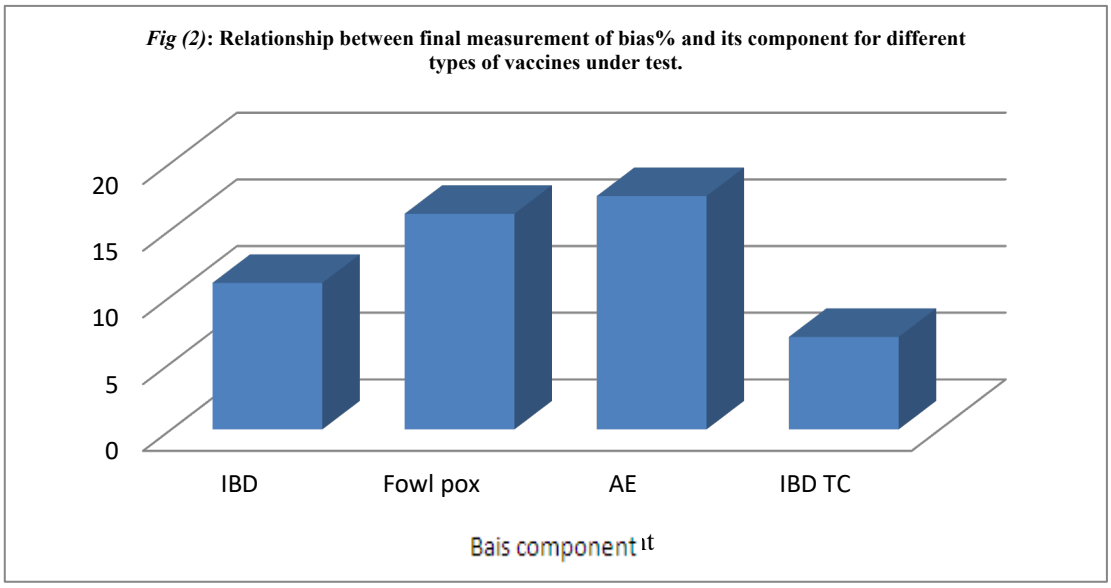


***Fig (2)*: Relationship between final measurement of bias% and its component for different types of vaccines under test.**

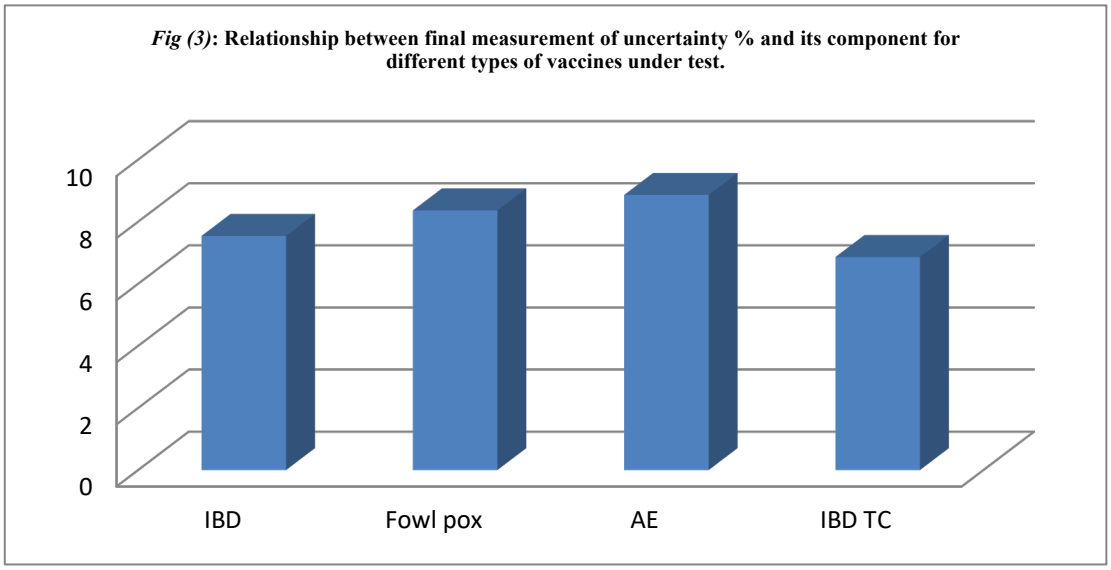


***Fig (3)*: Relationship between final measurement of uncertainty % and its component for different types of vaccines under test.**

The reproducibility component of TC technique was higher (4.85) than other different rout of the egg inoculation.

Bias % component elevated in (AE) than anther types of vaccines with different routs of inoculation.

MU for Fowl pox [on chorioallantoic membrane] and AE [intra the yolk sac] are equal and more than (IBD) either TC or egg types.

Fig (4): **Relationship between final measurement of reproducibility, bias and uncertainty% for egg adapted Infectious Bursal disease vaccine (IBD), under test.**

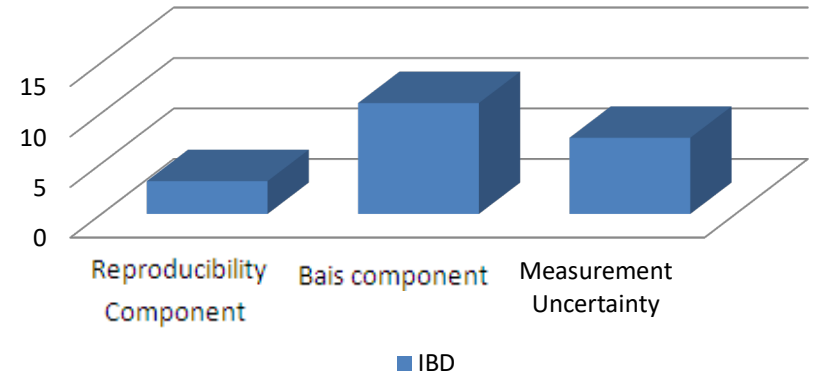


Fig (5): **Relationship between final measurement of reproducibility, bias and uncertainty % for egg adapted Fowl pox vaccine (FP), under test.**

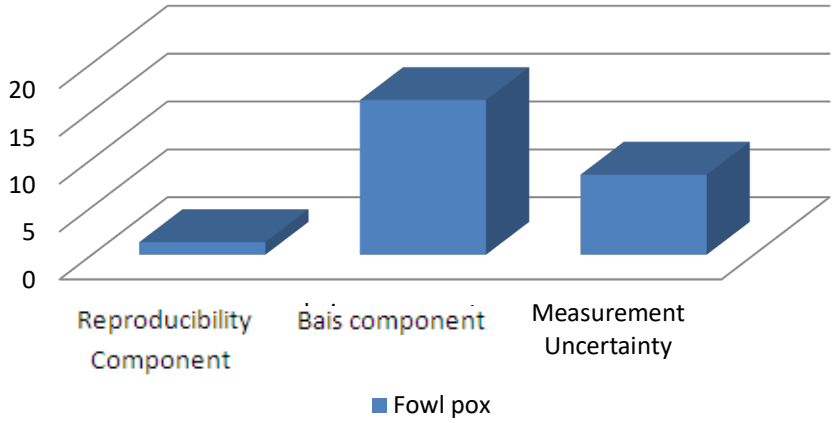


Fig (6): **Relationship between final measurement of reproducibility, bais and uncertainty% for Egg adapted Avian Encephalomyelitis vaccine(AE), under test.**

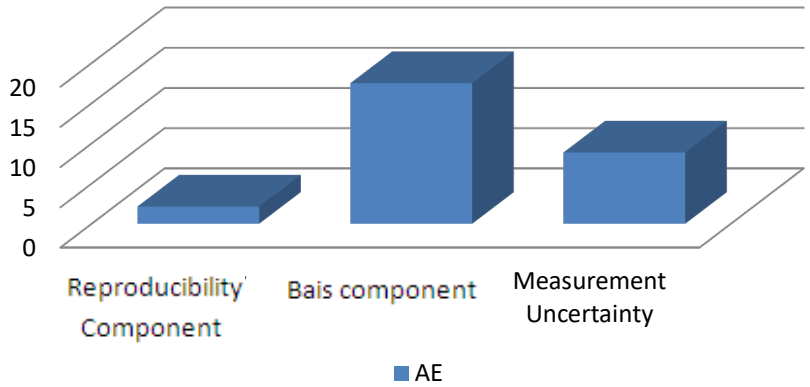
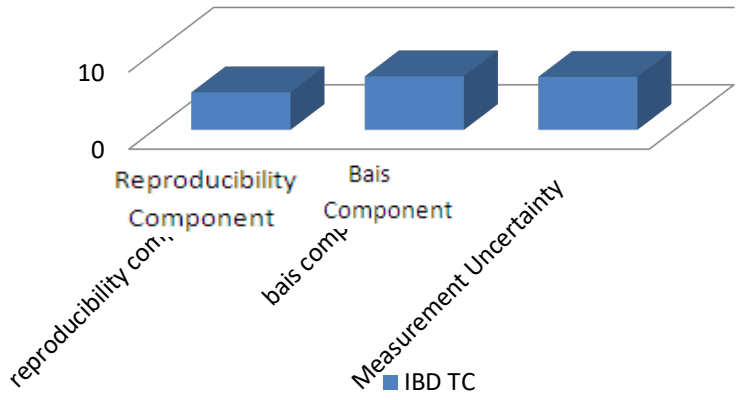


Fig (7): **Relationship between final measurement of reproducibility, bias and uncertainty% for Tissue culture adapted Infectious Bursal disease vaccine (IBD TC), undertest**



DISCUSSION

Measurement of uncertainty (MU) in veterinary diagnostic testing is process is not entirely reproducible; there is no exact value that can be associated with the measured analyte. So, the result is most accurately expressed as an estimate together with an associated level of imprecision. This imprecision is the MU. That is limited to the measurement process. It is not a question of whether the measurement is appropriate and fit for whatever use to which it may be applied. It is not an alternative to test validation, but is rightly considered a component of that process. Because there is MU associated with serological and other diagnostic measurements, the question is how to best estimate the MU. The framework against which MU must be applied is given by the standard against which the laboratory is accredited. The ISO/IEC 17025:2005 **(ISO/IEC (17025) , 2005)** standard, General requirements for the competence of testing and calibration laboratories specifies the standards to which laboratories must operate if they achieve accreditation according to requirement of ISO/IEC 17025:2005 (**ISO/IEC (17025) , 2005**) clause method validation [5,4,5], Estimation of uncertainty of measurement [5,4,6], and clause test report [5,10,3].

According to requirement of ISO/IEC (17025) the laboratory shall be select the test method. To ensure the following requirements:

-A review and setting of priorities by the laboratory or its organization.

-Accurate identification of the client's needs. ISO/ IEC 17025 states ''shall include a clear - specification of the client's requirements.'' Factors such as use, cost, desired performance

characteristics, and turnaround time should be included in discussions with the client. Of these, the proposed use of the method is probably the most important consideration.

-Establishment of criteria and implementation of procedures for submission of proposals for development, to ensure adequate information for decisions.

-Review of proposals by an informed, qualified group that represents the interests of the laboratory and possibly also of the client.

-Criteria and procedures for the selection of proposals for development.

There are two main approaches to estimate MU according to **(Nordtest TR 537, 2004).**

The 'components' or 'bottom-up' approach identifies all sources of uncertainty individually in a 'fish-bone' diagram. Chemical and physical testing laboratories tend to follow this approach because potential sources of uncertainty are usually readily identifiable, and their magnitudes can be estimated and combined. There are also published attempts to validate this approach in the medical testing field. For example, for serology, the uncertainties for time, temperature, volume, reading (OD), operator and reagent batch were identified to estimate the overall MU of the method **(Dimech *et al.*, 2007**). The advantage of this approach is that the major sources of uncertainty are clearly identified and weighted individually, which indicated that reagent batch-to-batch, lab-to-lab and operator variation contributed significantly to the total variation whereas reading, volume and temperature contributed to a lesser extent. The disadvantage is that it is a time-consuming process because it requires a complex statistical model and repeated measurements of each component.

The 'control sample' or 'top-down' approach is suitable for medical and veterinary diagnostic test methods because of the availability of quality control samples, which can be used to monitor whole-of-procedure-performance and directly estimate the combined MU of the test procedure. Upper and lower limits to approve or reject MU will depend on the purpose of the test. If the MU goal is not met it may be necessary to analyses the procedure to identify and modify uncertainty sources using the bottom-up approach. The advantage of this approach is the availability of repeatability data in diagnostic testing laboratories and simple calculations. The disadvantage is that, the result is a global MU for the entire procedure and it fails to differentiate between individual contributing components. Validated methods according to **(ISO/TS34SC9W03/16140, 2017**) provide information about precision for example repeatability; reproducibility; accuracy;analytical; diagnostic sensitivity and specificity within established limits. Therefore, MU is an important aspect of test validation but cannot replace

it. Currently, MU is used for test methods that produce quantitative results. This includes tests, where numeric results are calculated and then are expressed as a positive or negative result at a cut-off value. Suitable statistical measures to express MU are mean values plus/minus 2 standard deviations (95% C **ISO/IEC (17025), 2005**) relative standard deviation (rsd) or coefficient of variation (CV). MU is applied to the analytical procedure and not to pre- or post-analytical errors such as sample suitability, collection, transport and transcription or reporting errors. Also excluded are interfering biological factors of the animal such as sex, breed, and co-infection with other agents, age, body condition, pregnancy and immunity. Most of these components are included in the validation process. Our results obtained in (Tables 1, 2 and 3) indicated the mean titer for IBD, F Pox, AE and IBD (TC) were ($10^{5.43}\pm^{0.403}$,$10^{3.58\ \pm0.256}$,$10^{3.44\pm0.326}$ and $10^{5.02\pm0.492}$); respectively. These results agree with **(OIE ,2016)** who mentioned that the IBD vaccine to be satisfactory should be contained not less than $10^{3.5}$ EID_{50} /dose for egg type and $10^{3.5}$ $TCID_{50}$ /dose for TC type, while $10^{3.0}$ EID_{50} / dose for AE&FP. Homogeneity and stability results for all types of vaccines under test are accepted as obtained in (Tables 4,5). Our results agree with **(Fearn and Thompson, 2001), (ISO 22117, 2010*)* and (ISO 22117, 2010) 3, 24&25)** that mentioned that five to ten randomly selected test materials were analyzed in duplicate for each analyte. The statistical tests initially check the data for any widely discrepant pairs using Cochran's test. If found such data are removed. Thereafter the remaining data are subject to analysis of variance (ANOVA) to estimate the sampling and analytical variances. Samples to be used for proficiency testing shall be stable, at least for the period from preparation (by the provider) to the date of the study or the end of the time period allowed. The minimum period for stability testing should be the time between preparation of the materials and the specified date or time period of analysis. The results shown in Fig. (1,2 and 3) describe the relationship of reproducibility and Bias component among different analytical technique applied in this study. The first impression: the reproducibility component of TC technique was higher (4.85) while the Bias was lower (6.93) than other different rout of the egg inoculation. So the accuracy of test by TC was better than different routes of egg inoculation. The result shown in Fig. (4,5and6) describe that,the variance between bias and reproducibility components in different egg inoculations. The bias components for IBD, FP&AE were 10.98%.16.16 % and 17.49% respectively, while the reproducibility were 3.25%, 1.31% and 2.13% respectively. The results indicated that, the accuracy of egg inoculation which

represented by bias is low and precision is very high which reflected by reproducibility component due to precision of staff and tools applied in the performance of test. Quality oriented laboratories are always interested in monitoring the performance of their diagnostic tests for continual improvement. The use of internal quality controls over a range of expected results has become part of daily quality control and quality assurance operations of accredited facilities. Results provide relevant information about different aspects of repeatability, e.g. intra- and inter-assay variation1, intra- and inter operator variation, intra- and inter batch variation and inform about the level of robustness of a test procedure. The level of variation of a test result becomes increasingly important the closer the test values is to the cutoff value used to designate a test result as positive or negative. On the other hand, normally have little doubt about test results that are on the extreme ends of the measurement scale and if reference standards or calibrated controls against reference standards are used. Sub-Committee on Animal Health Laboratory Standards tends to call these results 'strong positive' or 'strong negative'. It is good laboratory practice to define a range of inconclusive, intermediate, suspicious, borderline, grey zone or equivocal test values falling between the positive and negative cutoffs**. (Reed and Muench, 1938)** Described an intermediate range and respective confidence intervals for test values for sero diagnostic tests falling around the cut-off. This range of values is considered as a borderline range for the clinical interpretation of test results. The proportion of the measurement range that gives unambiguous test results can be expressed using the intermediate range as the valid range proportion. In this study, the relevant information that MU provides for diagnostic test results is that it gives an estimate about the extension of this range of values around the cut-off. Once this range has been established, the diagnostician need to develop a test algorithm which describes how to follow-up samples which fall in the MU range. This can be a retest of the same sample or of a second sample and depends on the purpose of the test and its performance characteristics, in particular precision and accuracy1. Results from internal quality controls can be easily applied to estimate MU using a top-down approach with a minimum of additional testing and fulfill the requirements of ISO 17025.

CONCLUSION

So, the result also confirmed that, the accuracy of TC technique is better than egg inoculation technique which will reflect on the measurement of uncertainty.

There is no significance change of the final measurement uncertainty of different routes of

egg inoculation While there is variance between bias (accuracy) and reproducibility (precision) due to the equation of measurement of uncertainty depend all process performed in test accuracy and precession.

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