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Limit of detection of bacterial contaminant in living bacterial vaccine

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ABSTRACT

A total number of 30 vaccine vials including 3 batches of 3 different types of live bacterial poultry vaccines (Mycoplasma red vaccine, Mycoplasma yellow vaccine and non-colored Mycoplasma vaccine) were experimentally contaminated with 1,5,10 CFU of *Escherichia coli* reference strain (ATCC25992) /dose then subjected for sterility testing as referenced in world organization of animal health(OIE). The sterility is defined as the absence of viable microorganisms, includes viruses. It should be achieved by the use of aseptic techniques and validated sterilization methods, including heating, filtration, chemical treatments and irradiation that fit the intended purpose. Further dilution was carried out of vaccines showing negative results. Kappa index in different contamination levels in all tested vaccines after dilution was ranged between high Kappa index and almost total. In this study, it concluded that the applied testing method is appropriate for testing sterility of living bacterial poultry vaccines and it is fit to use.

1. INTRODUCTION

Bacterial infections of poultry are a worldwide important factor in terms of the economic losses and public health. The control of these diseases depends on high-cost sanitary measures and medical treatment. The protection effect of bacterial vaccines depends on the immune response of the host towards different antigenic components of the bacteria (Nagwa and Zeinab,2020).

The sterility is defined as the absence of viable microorganisms, includes viruses. It should be achieved by the use of aseptic techniques and validated sterilization methods, including heating, filtration, chemical treatments and irradiation that fit the intended purpose. Free contamination is defined as the absence of specified viable microorganisms. Adequate assurance of sterility and freedom from contaminating microorganisms can only be achieved by proper control of the primary materials used and their subsequent processing. Tests on intermediate products are necessary throughout the production process to check that this control has been achieved (OIE, 2019).

In the validation process was estimated by repeatability and reproducibility of the test method (Annex -1, 2017) aiming to secure that the test methods are good enough to qualify the tested vaccines. large economic values as well as considerable health, safety and environmental issues are involved, much more emphasis must be paid to the validation of the test methods. The frequency of the tested method should also be considered when determining the extent of validation. The total consequences of wrong results are of course larger for methods in extensive use than for test methods used occasionally (Excel kontrol, en 2008 and Excel control version 2.1 (2008).

This study aimed to assess the validity of the used method for testing the sterility of live bacterial poultry vaccines

2. MATERIAL AND METHODS

2.1. Samples

Thirty vaccine vials including 3 batches (3 vials of each batch) of live attenuated bacterial poultry vaccines including Mycoplasma (red colored) vaccine, Mycoplasma (yellow)vaccine and Mycoplasma (non-colored) vaccine were kindly obtained from bacterial sterility department, Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Cairo

2.2. Spike

Escherichia coli reference strain (ATCC25992) was kindly obtained from the Reference Strain Bank, Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) Abbassia, Cairo.

2.3. Preparation of the inoculum

Three beads of *E.coli* strains were enriched in 3 tubes of peptone water and incubated at 37°C overnight. These broth cultures were tenfold serially diluted to be used in spiking the tested vaccines at different 3 levels of contamination according to Reynolds (2005) as follow:

1st level reveals 1CFU/dose in vaccine vial.
2nd level reveals 5 CFU/ dose in vaccine vial.
3rd level reveals 10CFU/ dose in vaccine vial

2.4. Testing method

The used sterility testing method was carried out following up the directions of OIE (2019) where each vaccine vial was dissolved in 2 ml sterile distilled water then inoculated to thioglycolate broth produced by Sigma aldrich Company, fluid thioglycolate+0.5 beef extract produced by Sigma aldrich Company, Soyabean digest medium produced by Sigma aldrich Company in a ratio 1:15, followed by experimentally contamination with a level of bacterial

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concentration then incubated at 35°C and 25°C for 14 days and examined for turbidity and subculture at 3rd to 11th day by transferring 0.1 ml from turbid sample to : Brain heart infusion agar produced by Sigma Aldrich Company Tryptose Soya agar produced by Sigma Aldrich Company and Mycoplasma agar produced by Oxoid Company for typical growth or atypical growth for Mycoplasma

2.5. Media and reagent performance

According to ISO /11133-1 (2014), the used media in this study were tested before inoculation to validate their efficacy by measuring the productivity and selectivity.

2.5. Criteria of validation

The validation study depends on different parameters and tools to evaluate if the method is fit to use in check vaccine sterility or not. The parameters used were selectivity, trueness, sensitivity, specificity, concordance, repeatability and reproducibility according to the requirement of ISO 16140-6 (2019) tacking in consideration that:

Trueness = (a + d)/n *100

Sensitivity = a / (a + b) *100

Specificity = d/(c + d) *100

Concordance-Index Kappa=2(ad - bc) {(a +c) (c +d) + (a +b) (b +d)}. Where**:** a= Number of positive agreements. b= Number of false negatives. c= Number of false positives. d= Number of negative agreements. n= Total number of results.

Table 1 Concordance-Index kappa according to ISO/TC 34/SC 9/WG3 N027

Kappa value	Kappa index
>0.1	No
0.1-0.4	Low
>0.4-0.6	Distinct
>0.6-0.8	High
>0.8-1	Almost total

3. RESULTS

The contamination was able to be detected in all spiked batches of Mycoplasma (red colored) vaccine at all levels of contamination (level1 “1 CFU/dose”, level 2 “5 CFU/dose” and level 3 “10 CFU/dose”) (Table 2).

Table 2 Limits of Detection (LOD) for Mycoplasma (red colored) vaccine

Levels of contamination	Tested vaccines		
	Batch 1	Batch 2	Batch 3
Level 1 (1CFU/vaccine vial)	+ve	+ve	+ve
Level 2 (5CFU/vaccine vial)	+ve	+ve	+ve
Level 3 (10CFU/vaccine vial)	+ve	+ve	+ve

Table 3 showed that all spiked batches of Mycoplasma (yellow)vaccine showed the contamination could not be detected at 1 out of 3 batches of vaccine vials in level 1 (1CFU/dose) while such contamination was detected with level 2(5 CFU/dose) and level 3 (10 CFU/dose) in all tested batches.

Table 3 Limits of Detection (LOD) for Mycoplasma (yellow)vaccine

Levels of contamination	Tested vaccines		
	Batch 1	Batch 2	Batch 3
Level 1 (1CFU/vaccine vial)	+ve	+ve	-ve
Level 2 (5CFU/vaccine vial)	+ve	+ve	+ve
Level 3 (10CFU/vaccine vial)	+ve	+ve	+ve

Table 4 showed that in all spiked batches of Mycoplasma (non-colored) vaccine, the contamination could not be detected at 1 out of 3 batches of vaccine vials with level 1 (1 CFU/dose) and while it could be detected at level 2(5 CFU/dose) and level 3(10CFU/vaccine vial) in all batches.

Table 4 Limits of Detection (LOD) for Mycoplasma (non-colored) vaccine

Levels of contamination	Tested vaccines		
	Batch 1	Batch 2	Batch 3
Level 1 (1CFU/vaccine vial)	+ve	+ve	-ve
Level 2 (5CFU/vaccine vial)	+ve	+ve	+ve
Level 3 (10CFU/vaccine vial)	+ve	+ve	+ve

The obtained results revealed that the Mycoplasma (red colored) vaccine; the specificity, sensitivity, trueness and concordance where 100, 100, 100 and 1 consequently reflecting the validity of the used method in this vaccine type, while in colored and non-colored Mycoplasma vaccines at level 1 contamination the specificity; sensitivity, trueness and concordance were 100, 83.3, 50,88.9,66.7 and 0.88,0.57 consequently as shown in table (5). These results were considered of low validity for the used method in these types of vaccines.

Dilution of tested vaccine at level 1 contamination indicated that the Mycoplasma(red colured) vaccine the contamination could not be detected at second dilution (10ml) while could be detected in the other 2 dilutions, while in the Mycoplasma (yellow)vaccine and Mycoplasma (non-colored) vaccine the contamination was detected in second dilution (10 ml) as shown in Table (6).

The results of tested vaccines after dilution showed that in case of Mycoplasma (red colored) vaccine; the specificity, sensitivity, trueness and concordance were 100,100,100 and 1 consequently while in case of Mycoplasma (yellow)vaccine and Mycoplasma (non- colored) vaccine the specificity, sensitivity, trueness and concordance where 100,80.5,90.5 and 0.91consequently as tabulated in Table (7).

Table 5 Criteria of validation study for tested vaccines at level 1 of contamination (1 CFU/dose)

Tested vaccine	Criteria of validation				
	Specificity	Sensitivity	Trueness	Concordance Value	Kappa Index
Mycoplasma(red colured) vaccine	100	100	100	1	Almost total
Mycoplasma (yellow)vaccine	100	83.3	88.9	0.88	Almost total
Mycoplasma (non-colored) vaccine	100	50	66.7	0.57	Distinct

Table 6 Limits of Detection (LOD) for diluted tested vaccines

Tested vaccine	Detection of contamination with vaccine dilutions		
	5ml	10ml	20ml
Mycoplasma (red colored) vaccine	+ve	-ve	+ve
Mycoplasma (yellow) vaccine	+ve	+ve	+ve
Mycoplasma (non-colored) vaccine	-ve	+ve	+ve

Table 7 Criteria of validation study for all contamination levels in examined living bacterial vaccines after dilution

Tested vaccine	Criteria of validation				
	Specificity	Sensitivity	Trueness	Concordance value	Kappa Index
Mycoplasma (red colored) vaccine	100	100	100	1	Almost total
Mycoplasma (yellow)vaccine	100	100	100	1	Almost total
Mycoplasma (non- colored) vaccine	100	80.5	90.5	0.91	Almost total

4. DISUCSSION

It was stated that sterility testing is a process that confirm the presence or absence of extraneous viable contaminating microorganisms in biologics (Lee, 1990) who described a detailed sterility investigation that included the identification of the contaminant, reviews of documents, training records, gowning practices, environmental monitoring records, lab procedures and other critical controls as well as validity for any method used to detect bacterial contamination (OIE, 2019)

In this study, the obtained results revealed that the used method for detection of bacterial contamination of poultry bacterial vaccines; is fit to fulfill such purpose was in agreement with that obtained by Kamboh *et al.*, (2009) who found that lower bacterial contamination in poultry vaccines.

All the obtained result shown in table(1),(2),(3),(4),(5),(6) and (7) was agreed with the result obtained by Hanan M. Ibrahim, *et al.* (2019) who found there is a significant difference between direct inoculation of the vaccines and their dilutions. It could be concluded that the applied testing method is appropriate for testing sterility of living attenuated poultry viral vaccines but testing of some of such vaccines need further dilutions to obtain accurate results. All results through the present study indicate that the used method of bacterial contamination of bacterial vaccines; is fit to fulfill such purpose. As the result agreed with (CRF, 2015).

These findings secure that the used test method is good enough to qualify the tested vaccines (Excel kontrol, en 2008 and Excel kontrol version 2.1 (2008).

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