



Method validation for moisture content in combined live Newcastle Disease and Infectious Bronchitis vaccines by vacuum oven method

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BACKGROUND

The appropriate moisture content is critical for the viability of the vaccine, which affects to vaccine efficacy. The ASEAN standard for animal vaccines, the moisture content in freeze dried vaccines must not exceed 4%. Moisture content in freeze-dried vaccines is commonly determined using Karl Fischer titration and vacuum drying. Currently, the Veterinary Biologics Assay and research Center uses a vacuum oven method to determine moisture content, under conditions of $60 \pm 3^\circ\text{C}$, vacuum < 25 millibar, at least 3 hours drying time and room relative humidity (RH) $< 45\%$. Therefore, this study conducted a validation of the vacuum oven method for moisture content by reducing the drying time from 3 to 2 hours to time-saving and cost-effective method for determining the moisture content in freeze dried live vaccines.



METHOD

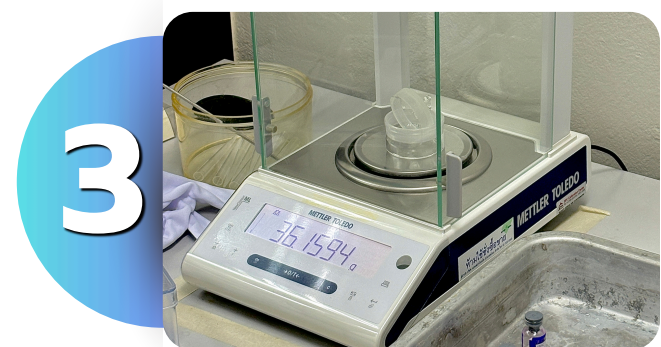
Ten batches of combined live Newcastle disease and Infectious Bronchitis vaccine were tested using vacuum oven at 60°C , 24 millibar, RH $< 45\%$ for duration time 2 and 3 hours. For precision, 100 μl of DW was dispensed in to weighed container and weighed. Samples were dried in vacuum oven at 60°C , 24 millibar, RH $< 45\%$ and reweighed. Weight difference before and after drying was used to calculate % moisture and the acceptance was determined using the F-test and t-test. Accuracy was tested by adding 100 μl of DW to moisture-free combined live Newcastle disease and infectious bronchitis vaccine and determine the acceptance of the method using % Recovery. Method validation was performed using ten batches of combined live Newcastle disease and Infectious Bronchitis vaccine, acceptance of the method variability was assessed using the F-test and t-test.



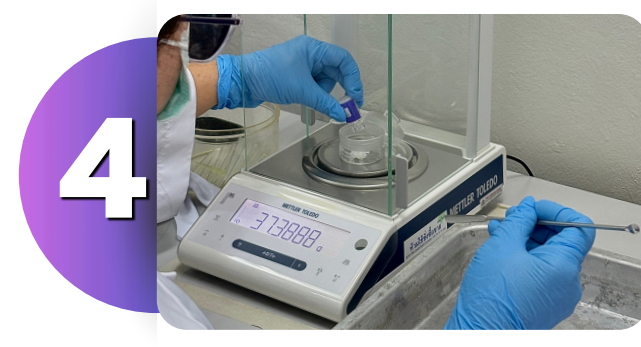
Samples



containers



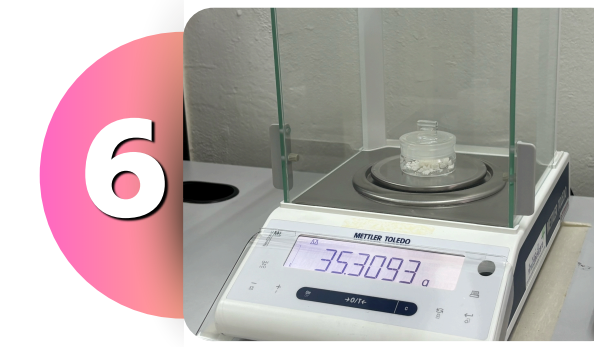
Weighing container (W_c)



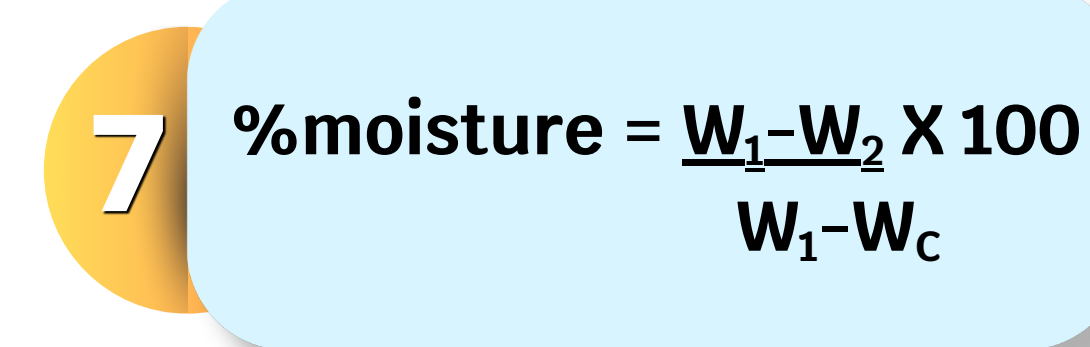
Weighing container + Sample (before) (W_1)



Incubate at 60°C , 24 millibar 2 and 3 hrs



Weighing container + Sample (after) (W_2)



$$\% \text{moisture} = \frac{W_1 - W_2}{W_1 - W_c} \times 100$$

calculate % moisture



RESULTS AND DISCUSSIONS

At 2 and 3 hours, the precision average moisture content was showed $100.04 \pm 1.05\%$ and $100.05 \pm 1.34\%$ with F_{cal} of 0.79 and $t_{\text{cal}} = -0.02$. Accuracy average moisture content was 101.23% and 101.39% and %R were 101.23 and 101.39. The method validation average moisture content was $3.09 \pm 0.68\%$ and $3.00 \pm 0.44\%$ with $F_{\text{cal}} = 1.54$ and $t_{\text{cal}} = 0.26$ ($n = 10$, $F_{\text{crit}} = 3.18$, $t_{\text{crit}} = 2.10$, $p = 0.05$). This results within the acceptable range according to AOAC (1993) and method met the validation of $F_{\text{cal}} < F_{\text{crit}}$ and $t_{\text{cal}} < t_{\text{crit}}$ at 95 % confidence level.

Result	Precision		Accuracy		Validation	
	2 hrs	3 hrs	2 hrs	3 hrs	2hrs	3 hrs
Mean	$100.04 \pm 1.05\%$	$100.05 \pm 1.34\%$	101.23%	101.39%	$3.09 \pm 0.68\%$	$3.00 \pm 0.44\%$
F_{cal}	0.79		-		1.54	
t_{cal}	-0.02		-		0.26	
%Recovery	-		101.23	101.39	-	

$n = 10$, $F_{\text{crit}} = 3.18$, $t_{\text{crit}} = 2.10$, $p = 0.05$, %Recovery to AOAC 97 - 103



CONCLUSION

Validation results of the moisture content in combined live Newcastle disease and Infectious Bronchitis vaccines by vacuum oven method at temperature of 60°C , vacuum 24 millibar and drying time of 2 hours can be used as a reliable method for determining moisture content in combined live Newcastle disease and infectious bronchitis vaccines.



REFERENCE

- ASEAN Secretariat. 1998. Appendix 4 General requirement for veterinary vaccine. In Manual of ASEAN standard for animal vaccine, Livestock publication series 2A, 2nd edition. Penehar Swadaya, Indonesia. pp. 105.
- AOAC International. 1993. AOAC Peer-verified Methods Program: Manual on Policies and Procedures. Association of Official Analytical Chemists, Gaithersburg, MD. pp. 35.
- The International Cooperation on Harmonization of Technical Requirements for the Registration of Veterinary Medicinal Products (VICH). 2003. Testing of Residual Moisture - Guidance for Industry (VICH GL 26). Canadian Food Inspection Agency (CFIA). (Online). Available: <https://inspection.canada.ca/en/animal-health/veterinary-biologics/guidelines-forms/vich/residual-moisture>.



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