

Stability of Inactivated Influenza Vaccine in Polypropylene Syringes under Various Storage Conditions

Influenza immunization is an effective strategy to reduce morbidity in health care providers and hospitalized patients.¹ When the vaccine is not provided in prefilled glass syringes, pharmacies prepare doses in polypropylene syringes, from multi-dose vials, to facilitate administration during vaccination campaigns. The *Canadian Immunization Guide* recommends delaying the process of loading syringes until it is time to vaccinate the patient, because of the lack of data about vaccine stability in syringes.² Data relating to room temperature storage are limited, and nonrefrigerated storage could result in reduced vaccine efficacy or adverse effects.^{2,3} However, refrigerators may not be consistently available during vaccination campaigns. Previous reports suggested that an influenza vaccine in its original prefilled glass syringe packaging can be stored for a period from 72 h to 14 days at room temperature without any effect on product quality.^{3,4} We sought to determine whether the influenza vaccine is stable in polypropylene syringes with refrigeration and at room temperature.

Vaccine stability testing includes determination of changes in vaccine structure, followed by immunologic assays to assess potency and biological activity.⁵ In the hemagglutination (HA) assay, the hemagglutinin protein protruding from the influenza vaccine envelope binds to red blood cells, causing them to agglutinate.⁶ This functional qualitative assay provides information about the physical stability of the vaccine.⁷ The current study used the HA assay to evaluate the stability of hemagglutinin, in

terms of binding to its receptor, after storage of vaccine in polypropylene syringes.

Samples of the inactivated split-virion, trivalent influenza vaccine for the 2016/2017 season in the northern hemisphere (GlaxoSmithKline Inc, Mississauga, Ontario; lot 22TC5, expiry May 2017) were loaded into polypropylene syringes (Becton, Dickinson and Company, Franklin Lakes, New Jersey) and subjected to various storage conditions (all with protection from light). A 50- μ L sample from each preparation was used to make serial 2-fold dilutions with phosphate-buffered saline in a 96-well round-bottom plate. Phosphate-buffered saline was used as the negative control, and freshly drawn-up vaccine was used as the positive control. Two drops of a 0.5% chicken red blood cell suspension were added to each well, and the plates were examined after 60 min at 4°C. A diffuse red layer at the bottom of the well was interpreted as indicating HA. In the absence of HA, the red blood cells settled as a “button”. Results were recorded by the study investigators, who were blinded to storage conditions. Results of HA activity are reported as geometric mean titres (GMTs), defined as the inverse of the highest dilution with complete HA. The GMT is a sensitive parameter used in immunohematological studies to detect differences in antibody effects.^{8,9} A GMT that is more than 2-fold lower than the positive control is interpreted as indicating a decrease in HA.⁷

Two groups of investigators completed the experiment (Table 1). As expected, heated samples did not display any HA. For samples stored at room temperature for 7 days, the HA GMT for group 2 suggests that titres dropped during the storage period, although a 4-fold decrease in HA titre was observed in only 1 of 6 samples overall. The GMTs after storage under other conditions (refrigerated storage for 72 h, followed by room temperature

Table 1. Hemagglutination Activity of Inactivated Influenza Vaccine Loaded in Polypropylene Syringes and Stored under Various Conditions, as Tested by 2 Investigator Groups

Storage Condition	Investigator Group 1		Investigator Group 2	
	No. of Samples	HA Titres (GMT and Range)	No. of Samples	HA Titres (GMT and Range)
Room temperature* for 7 days	3	5 161 (4 096–8 192)	3	813 (512–1 024)
Refrigerated† for 72 h, then room temperature* for 24 h	3	10 321 (8 192–16 384)	4	2 048 (2 048)
Refrigerated† for 72 h	3	5 161 (4 096–8 192)	4	1 722 (1 024–2 048)
Room temperature* for 30 h	3	5 161 (4 096–8 192)	3	1 024 (1 024)
Room temperature,* prepared morning of assay (positive control)	4	4 096 (2 048–8 192)	6	2 048 (2 048)
Heated‡ for 30 min	3	No HA	3	No HA

GMT = geometric mean titre, HA = hemagglutination.

*Room temperature: between 20°C and 25°C.

†Refrigeration: between 2°C and 8°C.

‡Heating: 80°C.

storage for 24 h; refrigerated storage for 72 h; and room temperature storage for 30 h) were comparable to the GMTs of samples prepared the morning of the experiment and held at room temperature.

The apparent decrease in HA titre after room temperature storage for 7 days may reflect recognized inter-rater variability in end-point detection of HA activity, or it may suggest that the structure of hemagglutinin is affected by extended exposure to room temperature. It would be of interest to test HA activity at intermediate times between 30 h and 7 days. However, storage in polypropylene syringes in select conditions (including refrigeration for 72 h and room temperature storage for 30 h) did not appear to reduce hemagglutinin activity, which indicates that storage in plastic did not lead to changes in protein structure. This finding suggests that potency would be retained, but quantitative serology assays, such as single radial immunodiffusion or viral neutralization assays, are required to confirm immunogenicity and clinical effect.⁵ Other limitations of our study include the use of a single brand of influenza vaccine from one season and a single brand of syringes. Considering the available evidence, storage of influenza vaccine samples in polypropylene syringes under refrigeration for 72 h and up to 30 h at room temperature maintains the ability of hemagglutinin to bind to its receptor, suggesting preservation of protein structure. These storage conditions could therefore facilitate vaccine preparation and administration during vaccination campaigns.

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