

Letter to the Editor

Ovalbumin content in 2009 to 2010 seasonal and H1N1 monovalent influenza vaccines*To the Editor:*

For the 2009 to 2010 influenza season, 34 manufacturers in 19 countries are manufacturing influenza vaccines. In the United States, there are 5 manufacturers producing 1 nasal and 5 injectable Food and Drug Administration (FDA)–approved seasonal influenza vaccines. These manufacturers are also producing 1 nasal and 4 injectable H1N1 (hemagglutinin 1, neuraminidase 1) monovalent vaccines. Because influenza vaccines are developed by inoculating embryonated chicken eggs with virus, the final product has always contained ovalbumin, which can pose a risk in children and adults with egg allergy. However, because manufacturers do not report final lot ovalbumin content, allergists are directed to start with skin prick and intradermal skin testing to the vaccine followed by either a 2-dose or a graded multidose vaccine administration protocol.¹ However, these recommendations may be too conservative, because all but 1 manufacturer for the 2009 to 2010 FDA-approved influenza vaccines state a maximum ovalbumin content of ≤ 1 μg per dose (≤ 2 $\mu\text{g}/\text{mL}$), and improvements such as ultracentrifugation have resulted in lower ovalbumin content in vaccines like FluMist (MedImmune LLC, Gaithersburg, Md).²

The goal of this study was to determine the ovalbumin content in available 2009 to 2010 influenza vaccines. Because manufacturers express the maximum ovalbumin content as micrograms per dose, and there are 3 possible doses (0.2 mL for the nasal vaccines or 0.25 mL or 0.50 mL for the injectable vaccines), we chose a single concentration description of micrograms per milliliter to compare better the ovalbumin content of the vaccines against the manufacturers' stated content. A commercial ovalbumin ELISA kit (Chicken Egg Ovalbumin; Alpha Diagnostic International, San Antonio, Tex) that has a sensitivity range of 0.5 to 4 ng/mL was used. The detailed methods are provided in this article's [Methods](#) section of the Online Repository at www.jacionline.org. These results are presented in [Tables I and II](#).

In our opinion, this information is clinically important for 3 reasons: (1) manufacturers do not report final lot ovalbumin content, (2) routine measurement of ovalbumin is labor-intensive and generally inaccessible by practicing allergists, and (3) knowing the ovalbumin content guides current vaccine administration protocols. For children and adults with egg allergy, 3 clinical courses are generally recommended: vaccine avoidance, vaccine skin testing with a 2-dose vaccine administration protocol, or vaccine skin testing with a multidose graded vaccine administration protocol.¹

The 2-dose protocol (10% of the dose is given initially, and if there is no reaction in 30 minutes, the remaining 90% of the dose is given, with an additional observation period afterward) is based on a single study demonstrating the safety of this approach with vaccines containing no more than 1.2 $\mu\text{g}/\text{mL}$ ovalbumin in 83 children and adults with egg allergy.³ The multistep (usually 4-6 injections) graded challenge for patients with positive vaccine skin test results has been successful, although it has not been compared directly with a 2-step or single-step challenge.⁴ A recent abstract described success using a multidose protocol in 58 patients with egg allergy with positive vaccine skin test results, with only minor reactions, although the specific ovalbumin content was not reported.⁵ Also, since 1997, a virosomal adjuvanted influenza vaccine, Inflenza V (Berna Biotech, Berna, Switzerland), that is approved in Europe contains less than 50 ng of ovalbumin per dose. A recent study demonstrated the safety of this vaccine, administered as a single dose, in 44 children with egg allergy.⁶

Our study found the actual ovalbumin content of the brands and lots of vaccines tested from manufacturers claiming a maximum ovalbumin content of 2 $\mu\text{g}/\text{mL}$ was actually much lower than this level, specifically 0.001 to 0.007 $\mu\text{g}/\text{mL}$ for the nasal vaccines and 0.018 to 0.41 $\mu\text{g}/\text{mL}$ for the injectable vaccines. The ovalbumin content in the vaccines from the manufacturer claiming a maximal ovalbumin content of 10 $\mu\text{g}/\text{mL}$ was also lower than the stated maximum (0.064-1.411 $\mu\text{g}/\text{mL}$) but higher than the vaccines from the other manufacturers. Some limitations to

TABLE I. Ovalbumin content in FDA-approved 2009 to 2010 seasonal influenza vaccines

Brand name	Manufacturer	Stated ovalbumin content	Lot no.	Actual ovalbumin content ($\mu\text{g}/\text{mL}$) [‡]
Alfuria	CSL Limited (Parkville, Victoria, Australia)	≤ 2 $\mu\text{g}/\text{mL}$ *	07049111A	0.021
			01749211A	0.018
			08049111A	0.020
Fluzone	Sanofi Pasteur (Swiftwater, Pa)	≤ 10 $\mu\text{g}/\text{mL}$ †	U3167FA	0.716
			U3214AA	1.083
			UT3172AA	1.238
			U3176AA	1.421
FluMist	MedImmune, LLC	≤ 1.2 $\mu\text{g}/\text{mL}$ †	500685P	0.007
			500693P	0.001
Fluarix	GlaxoSmithKline (Dresden, Germany)	≤ 2 $\mu\text{g}/\text{mL}$ *	NP	NP
Flulaval	ID Biomedical Corp of Quebec (Quebec City, QC, Canada)	≤ 2 $\mu\text{g}/\text{mL}$ *	NP	NP
Fluvirin	Novartis (Speke, Liverpool, UK)	≤ 2 $\mu\text{g}/\text{mL}$ *	NP	NP

NP, Not performed.

*Per manufacturer's package insert.

†Obtained by communication with manufacturer.

‡Ovalbumin measured by ELISA.

TABLE II. Ovalbumin content in FDA-approved 2009 to 2010 influenza A H1N1 monovalent vaccines

Brand name	Manufacturer	Stated ovalbumin content	Lot no.	Actual ovalbumin content (μg/mL) [‡]
H1N1 monovalent	Novartis	≤2 μg/mL*	100739 2P	0.041
H1N1 monovalent	Sanofi Pasteur	≤10 μg/mL†	UP010AA	0.064
H1N1 monovalent	MedImmune, LLC	≤1.2 μg/mL†	500778P	0.003
H1N1 monovalent	CSL Limited	≤2 μg/mL*	NP	NP
H1N1 monovalent	ID Biomedical Corp of Quebec	≤2 μg/mL*	NP	NP

NP, Not performed.

No specific brand names have been given by manufacturers for H1N1 monovalent vaccines approved for use in the United States.

*Per manufacturer's package insert.

†Obtained by communication with manufacturer.

‡Ovalbumin measured by ELISA.

our study include the fact that not all available influenza vaccines or their respective lots were analyzed. For the seasonal influenza vaccine alone, 248 lots from 6 FDA-approved seasonal influenza vaccines have been released. Although some experts are concerned about the possibility of lot-to-lot variability, ranging from 1 to 42 μg/mL,⁷ current manufacturing standards and our analysis suggest very little lot-to-lot variability.

The 2009 American Academy of Pediatrics Red Book recommends that anyone with systemic anaphylactic symptoms (generalized urticaria, hypotension, or manifestations of upper or lower airway obstruction) generally not receive the influenza vaccine, whereas the British Society of Allergy and Clinical Immunology recommends using only vaccines with a maximal egg content of 1.2 μg/mL in patients with egg allergy.^{8,9} The influenza vaccine administered to subjects with egg allergy in the 2-dose protocol described here had a maximum ovalbumin content of 1.2 μg/mL, and all subjects tolerated the entire dose. Thus, one should consider administering any vaccine containing less than this amount to subjects with egg allergy as a single dose, without previous vaccine skin testing.

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METHODS

A commercial ovalbumin ELISA kit (Chicken Egg Ovalbumin; Alpha Diagnostic International, San Antonio, Tex) that has a sensitivity range of 0.5 to 4 ng/mL was used. Antibodies used in this kit are monospecific for ovalbumin and have no cross-reactivity to other egg proteins. A total of 1 mL of each vaccine lot was obtained to perform testing in triplicate. A total of 200 μ L of a wash solution was added to each well of a 96-well plate that contained immobilized ovalbumin antibodies. After 10 minutes, the liquid was removed,

and 100 μ L of each vaccine was added to predetermined wells and incubated at room temperature for 1 hour. Wells were washed 4 times, and 100 μ L antiovalbumin enzyme conjugate was added with a 30-minute incubation period. After 5 additional wash steps, 100 μ L substrate was added with incubation for 15 minutes in the dark. Finally, a stop solution was added, and the OD at 450 nm was measured. A standardized curve (0.5-4 ng/mL; $r^2 = 0.97$) was included for each plate. Vaccine dilutions ranged from 1:10 to 1:1000 to determine ovalbumin content by using the standardized curve.