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Quantitation of Residual Host Protein in Chicken Embryo-Derived Vaccines by Radial Immunodiffusion

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Quantitation of soluble residual host protein in chicken embryo-derived vaccines was performed rapidly, economically, and accurately by radial immunodiffusion.

Feinberg (2) initially described a quantitative gel diffusion technique with antibody incorporated into the agar. This technique was further developed by Mancini et al. (4) and Fahey and McKelvey (1).

To avoid the problems of allergic sensitization against egg protein or of eliciting immediate or delayed hypersensitivity in a previously sensitized host, Reimer (5) suggested the use of radial immunodiffusion, with ovalbumin as the reference protein, to quantitate residual host protein in chicken embryo-derived vaccines.

A 10 mg/ml stock solution of ovalbumin (crystalline 3×, salt-free; Nutritional Biochemicals Corp., Cleveland, Ohio) was prepared as the standard reference antigen. To prepare antiserum against chicken embryo protein, the shells of twelve 10-day-old embryonated eggs were thoroughly washed in 70% ethanol. The embryos, including shells, were placed in a sterilized Waring Blendor and homogenized for 10 to 15 sec. Penicillin and streptomycin were added to a final concentration of 100 units/ml and 200 μg/ml, respectively. The egg material was then homogenized until the shell fragments were completely homogenized. The embryo homogenate was placed in 1.0-ml samples and stored at −20 C. A National Institutes of Health albino rabbit was injected subcutaneously with 1.0 ml and intraperitoneally with 2.0 ml of embryo homogenate twice a week for 4 weeks. Two weeks after the last inoculation the rabbit was exsanguinated by cardiac puncture and its serum was collected and stored at −20 C in 1.0-ml samples. By immunoelectrophoresis (3, 6), this antiserum showed antibody to seven distinct proteins of the embryo homogenate (diluted 1:10). When the embryo homogenate (diluted 1:120) and ovalbumin were reacted immunoelectrophoretically with the antiserum, only one precipitin arc was observed for each antigen. Both precipitin arcs appeared to be identical. This showed that the antigen having the highest concentration in the embryo homogenate was ovalbumin and ovalbumin could be used as the reference protein. When testing vaccines for the presence of contaminating chicken embryo

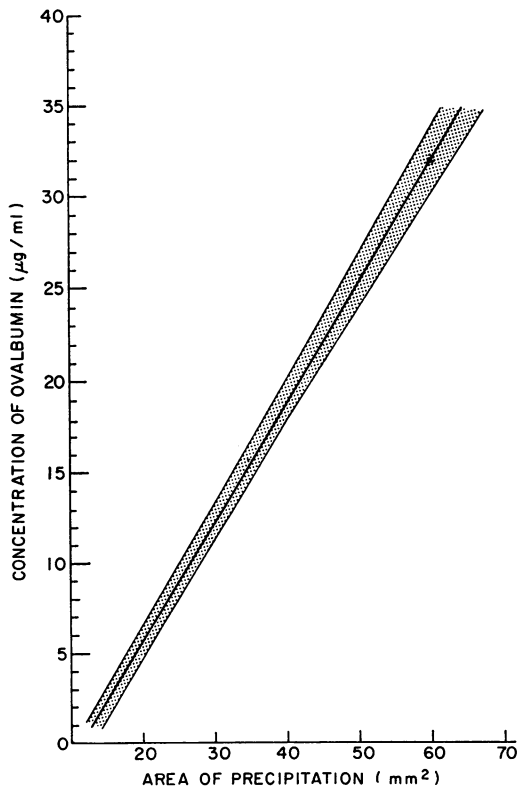

FIG. 1. *Calibration curve for the determination of residual chicken embryo protein in vaccines by radial immunodiffusion with confidence belts at the 95% level.*

protein by radial immunodiffusion, this antiserum will obviously react with a variety of soluble proteins. However, this test will not detect the presence of particulate or aggregated residual host protein when diffusion in gels is inhibited by particle size. We acknowledge that radial immunodiffusion might not identify the specific proteins contaminating a preparation. The use of an antiserum containing antibodies to many chicken embryo proteins would be more advantageous than an antiserum prepared against a single protein. A 1% agar-gel was dispensed into glass tubes in 9.0-ml amounts and allowed to cool to 50 C in a water bath. Undiluted rabbit antiserum (1.0 ml) heated to 50 C was added to the agar-gel to make a final concentration of 10%, mixed carefully with a warm pipette, and poured evenly on microscope slides (25 by 75 mm). After solidifying in a humid chamber, circular 3-mm wells were punched out in the gel. Using micropipettes, reference ovalbumin antigen and influenza vaccines were added up to the top of the well without allowing the sample to overflow onto the agar surface. Slides were incubated in a humid chamber for 28 hr, immediately immersed in distilled water in a petri dish, and photographed with a Polaroid MP-3 camera with a macrolens (220) attachment. Measurements of the size of the precipitate rings were made from the photographs. Since the form of the precipitates may occasionally depart from the ideal circular shape (4), two measurements of the diameter were taken at right angles to each other, these were added, and the sum was multiplied by a correction factor (to allow for the difference between the actual width of the slide and the width of the slide in the photograph) and divided by two. Reference antigen and influenza vaccines were also added to wells in agar which did not contain rabbit antiserum to test for nonspecific precipitin reactions.

Six replications each of the following concen-

TABLE 1. *Expected areas and upper and lower confidence (95%) limits*

Ovalbumin (μg/ml)	Lower limit	Calculated (expected) areas (mm²)	Upper limit
35	60.9	64.2	67.4
30	53.9	56.6	59.3
20	39.9	41.6	43.2
15	32.8	34.0	35.3
10	25.3	26.5	27.7
9	23.8	25.0	26.2
8	22.3	23.5	24.7
7	20.7	22.0	23.2
6	19.1	20.5	21.8
5	17.6	19.0	20.4
4	16.0	17.5	18.9
3	14.4	16.0	17.5
2	12.8	14.4	16.1
1	11.2	12.9	14.7

TABLE 2. *Quantitative radial immunodiffusion of chicken embryo-derived vaccines and embryo homogenates*

Vaccines or embryo homogenate	Vaccine type[a]	Dilution	Area of precipitation (mm²)	Ovalbumin (μg/ml)
Influenza 1967	S	Undiluted	22.6	7.4
Influenza 1968	S	Undiluted	22.6	7.4
Influenza 1969 no. 1	S	Undiluted	9.4	<1.0
Influenza 1969 no. 2	S/Z	Undiluted	12.8	1.0
Influenza 1969 no. 3	S/Z	Undiluted	12.8	1.0
Influenza 1969 no. 4[b]	S-$AlPO_4$	Undiluted	12.6	1.0
Influenza 1969 no. 5	S	Undiluted	14.9	2.4
Influenza 1969 no. 6[b]	S/HA-$AlPO_4$	Undiluted	8.4	<1.0
Influenza 1969 no. 7	S/HA	Undiluted	12.6	1.0
Measles		Undiluted	ND[c]	
Smallpox		Undiluted	50.3	25.8
Yellow fever		Undiluted	35.1	15.6
Embryo homogenate		1:40	33.6	14.6
Embryo homogenate		1:80	26.1	9.6
Embryo homogenate		1:160	16.8	3.5
Embryo homogenate		1:320	14.0	1.8
Embryo homogenate		1:640	11.6	1.0

[a] Vaccine manufacturing process: S = sharples; Z = zonal ultracentrifugation; HA = ether-extracted hemagglutinin; $AlPO_4$ = vaccine adsorbed on aluminum phosphate.

[b] Influenza vaccines (no. 4 and no. 5) and (no. 6 and no. 7) are identical pairs, except that one vaccine of each pair was adsorbed in $AlPO_4$. $AlPO_4$ vaccines were not treated to dissolve the $AlPO_4$, which probably inhibits protein diffusion in agar and results in lower protein estimates.

[c] Not detectable.

trations of ovalbumin (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, and 35 μg/ml) were tested by radial immunodiffusion. Regressions were computed by using the area measurement of response. It was found that the six replications could be fitted to a straight line separately. A Bartlett's test for homogeneity of the variances of the six replications was barely significant at the 5% level. A covariance of the six replications showed no significant difference in the slopes, nor the adjusted means, so the data of the six replications were pooled to form a single straight line. The pooled single line and its confidence belt (95%) are shown in Fig. 1. Table 1 shows the expected areas for each microgram per milliliter amount of ovalbumin along with the upper and lower confidence limits. Influenza virus vaccines from six manufacturers (1967, 1968, and 1969 formulations), measles virus vaccine, avianized smallpox virus vaccine, yellow fever virus vaccine, and dilutions of chicken embryo homogenate were analyzed for their concentration of egg protein (Table 2). All vaccines tested were undiluted.

Quantitative radial immunodiffusion is an economical, rapid, and accurate method for the detection of soluble residual host protein in chicken embryo-derived vaccines.

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