Influenza vaccines: the effect of vaccine dose on antibody response in primed populations during the ongoing interpandemic period. A review of the literature

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Health authorities tend to favour an increase of the antigen dose in inactivated influenza vaccines from ≤10 μg haemagglutinin (HA) per vaccine strain to 15 μg HA/strain. The increased dose is expected to yield a meaningful increase in the number of subjects to be protected after vaccination. To verify this expectation, we have reviewed 20 published reports (1978–1991) of serological studies in which anti-HA-IgG antibody after different doses was measured. In the review, stratification groups of previously primed subjects were formed and the antibody response was estimated for doses of 10 and 15 μg HA by linear k*2-χ^2 model. Despite a considerable heterogenicity of study populations, study designs, vaccine types and strains, and antibody assays, the results were consistent in revealing high protection rates (≥75%) for a 10 μg HA dose of influenza A vaccine components. For both response and protection rates, an increase of the antigenic load from 10 to 15 μg HA was not associated with a meaningful increase of seroresponse: in 38 out of 39 stratification groups, the increase of response and/or protection rate varied between −9% and +8%, with a median of 1.5%. These results do not justify the expectation that a vaccine dose of 15 μg HA per strain would be clinically superior to a dose of 10 μg HA. Only in a group of immune-compromised patients on chronic intermittent haemodialysis were results in favour of a higher dose found, which may justify further evaluation in this special population.

Keywords: Influenza virus; vaccine; dose; seroresponse

Infections of influenza A and B viruses cause significant mortality in the elderly and in subjects with chronic diseases and disabilities. Since their introduction in the 1940s, inactivated vaccines based on virus material grown in fertile hens' eggs have clearly been proven to be effective in decreasing mortality in these populations at risk whenever there is a sufficient match between the vaccine strain and the epidemic wild virus[1,2]. The World Health Organization maintains a world-wide surveillance on influenza epidemiology and annually reviews the strain composition of the vaccine[3]. Nowadays, highly purified vaccines without serious side-effects are available. Therefore, in many parts of the world, national and international health authorities recommend annual vaccine administration in subjects at risk for influenza-associated complications. Despite the availability of influenza vaccines for half a century, uncertainty has existed for a long time about the standard vaccine dose. In the course of harmonization, doses have been standardized internationally. In the United States of America, vaccines containing 7.5 μg HA of each of the components until 1980; since then, the standard dose has been doubled to 15 μg HA. In most European countries, 10 μg HA has been used until 1991. From 1992 onwards, European influenza vaccines will also contain 15 μg HA per strain, according to the new European 'Harmonization of Requirements For Influenza Vaccines'[4]. Australian influenza vaccines also currently contain 15 μg HA per strain.

Apparently, the preference of health authorities for increased vaccine dosages is based on the established graded positive relationship between the vaccine dose and the resulting IgG antibody response in previously primed subjects[5–7]. Because high serum titres of IgG antibody against viral HA protect against infection with a homologous virus[8–11], the serological response of this antibody upon immunization has become the most useful surrogate marker of vaccine efficicy (regardless of the fact that, to various degrees, additional immunological factors, such as local antibody and cellular immunity, contribute to protection).

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The arguments underlying the preference for the higher vaccine doses do not, however, address three important issues: first, the dose choice of pharmaceutical products should, in principle, be based on the concept of 'the lowest effective dose' and the benefit–risk ratio of the compound; second, vaccine production using fertilized hens' eggs is very laborious, involving a variety of logistical and environmental problems (risky dependency on a biological product, short time interval between the availability of annual vaccine strains and the beginning of the vaccination season); third, to counter the present, generally low, vaccination rates in high-risk patients, public campaigns are expected to increase the awareness of influenza and its prevention during the coming years. The expected increase in vaccine demand will put an enormous pressure on the current production capacity. The dependence on hens' eggs for vaccine production may then become a limiting factor.

In the light of these considerations, a standard dose of 15 μg HA or higher is justified only if an increase of vaccine dose from 10 to 15 μg HA has been shown, by scientific means, to be associated with a meaningful increase in the number of protected subjects. In this case, the three practical elements would be of lesser order.

From our own work[12–14], we doubted that an increase in vaccine dose from 10 to 15 μg HA would have an influence on the protection rate to such an extent that a decrease of available vaccine dosages is warranted. However, these vaccination trials may have suffered from a great variety of factors, such as prior experience of the study subjects with other influenza strains, state of health, or genetic conditions which may have superimposed a dose-effect in a single, limited study[15–17]. We therefore felt it necessary to review all available recent dose–response trials in the international scientific literature. We took into consideration the ongoing epidemiological situation where both influenza A (H3N2) and A (H1N1) subtypes have been circulating for many years (since 1968 and 1978, respectively) and vaccination is usually offered to subjects already primed for these subtypes.

MATERIALS AND METHODS

Source of literature and selection of papers

The databases Biosis Previes (Philadelphia, PA, USA), Medline (National Library of Medicine, Bethesda, ML, USA), and Embase (Excerpta Medica, Amsterdam, The Netherlands) were searched for various combinations of the keywords 'influenza', 'vaccine' (vaccination), 'immunisation' (immunization) and 'dose' (doses, dosing, dosage, dosages) in papers written in English. The search was undertaken in January 1992 and covered the period January 1978 through December 1991.

Serological studies using the single-radial immunodiffusion (SRD) method to determine the antigen dose were selected for this review. The year 1978 was chosen for the following reason: since 1967, the antigenic contents of vaccines had been estimated by comparison with an international influenza A standard preparation and expressed in chick red blood cell agglutination (CCA) units. This method was not always reliable as it tended to underestimate vaccine contents when whole-virus particles were aggregated. Moreover, with the development of split and subunit vaccines, this technique became impractical, because it produced extremely high values. Free haemagglutinin molecules can agglutinate erythrocytes more effectively than virus-bound haemagglutinin[18]. In 1978, the World Health Organization replaced the CCA method by the SRD test which expresses antigenic vaccine contents as μg HA[19]. Dose–response studies thus became more reliable[20].

Studies were selected in which at least two doses of an inactivated, aqueous influenza vaccine (whole-virus, WV; split, SPL; subunit, SU) without adjuvant were administered, in a randomized fashion, by the intramuscular or subcutaneous route. Any study design was accepted which included the sampling of two blood specimens (one before and a second after immunization), and the detection of IgG antibody against viral haemagglutinin by an appropriate assay. Tests measuring antibodies directed against other viral proteins (neuraminidase, M-protein) or measuring cellular immunity were excluded, in view of their unknown quantitative association with protection. Some studies include booster doses several weeks after the first immunization. In this review, data were selected from either the first or the second vaccination in view of the following: in subjects already primed for a given subtype, a booster vaccination will generally not increase antibody titres after the first vaccination with an influenza strain belonging to that same subtype. On the other hand, subjects not previously exposed to a given subtype (i.e. very young children, and defined age groups after first occurrence of pandemic strains), respond insufficiently to a first immunization and need a booster dose to reach an antibody level comparable to primed subjects. Obviously, in previously unprimed subjects, the first dose serves as primer which enables an adequate antibody production after booster dose[21–25]. As we were interested in dose–response statements in primed populations, we selected data on first vaccination in primed subjects, and on booster vaccination in previously unprimed subjects.

Measures of serological response, and statistical analysis

In the selected publications which used the haemagglutination inhibition (HI) test or the indirect immunofluorescence (IIF) test, seroresponse was expressed by using one or more of the following measures: (1) the pre- and postvaccination geometric mean titres (GMT) and/or the difference between the logarithms of post- and pre-GMT (mean fold increase, MFI), (2) the protection rate (PR), i.e. the proportion of subjects exceeding a given protection threshold after vaccination, (3) the response rate (RR), i.e. the proportion of subjects showing at least a fourfold titre increase after vaccination. The data were taken directly from the selected papers, recalculated from their original data if given, or derived from appropriate original tables or figures. For calculation of the protection rate, a titre threshold of 40[8,16,24] was used, if not mentioned otherwise by the authors.

One paper in which a single radial haemolysis test is described[25] presented pre- and postvaccination antibody concentrations as haemolysis areas (in mm^2), which were treated as GMT values in our analysis except for the logarithmation step. Post-GMT or MFI values could not be statistically analysed any further by us given the lack of original individual data. The significance level of differences between dose groups was taken from the original paper is given.

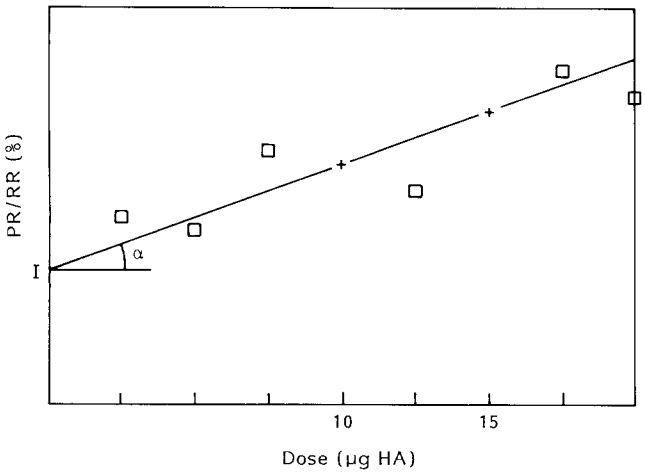

Figure 1 An example of the method used to estimate protection and response rates for doses of 10 and 15 μg HA from observed rates, per stratification group, by the linear k*2-χ^2 model. □, Observed values for doses other than 10 and 15 μg HA; α, I, straight line, calculated from observed values, with slope tan α and y-intercept I; +, estimated values for 10 and 15 μg HA

PR and RR values were subjected to probit analysis[26] (PROBIT in SAS 6.06) as it is the procedure often practised in literature. However, this method has preconditions which may not be met by all studies. In order to use a uniformly well-fitting method for all studies, we also applied the linear k*2-χ^2 model[27] which has fewer preconditions on the data than probit analysis. With either method, protection and response rates were estimated for the 10 and 15 μg HA doses, as exemplified in *Figure 1*.

The protection rate was supposed to be the most meaningful response measure. We assumed that an influenza vaccine should produce protection in at least 75% of primed but unprotected subjects, during the interpandemic period.

RESULTS

Papers reviewed (*Table 1*)

The literature search for the period 1978 through 1991 produced 180 English-language papers. From these, the titles of 84 papers revealed that they dealt with subjects other than trials with inactivated influenza vaccines in humans, for example trials with experimental live vaccines, or trials in animals. The remaining 96 papers were read for the presence of dose-comparison data in primed populations, which were found in 32 papers. Of these, 12 papers were not included: two[20,28] were, respectively, a summary and a part of a national trial which is described in three included papers[29–31]; one study (Ref. 32) described the same trial as Ref. 33 using a different serological technique; another[34] covers a selection of the study population which is described in the included paper[35]; one study[36] does not describe a previously planned trial with at-random allocation, but an accident during a mass vaccination campaign, and seven papers[37–43] used the obsolete CCA test to determine the vaccine dose.

Table 1 presents the first authors and the years of publication of the selected papers, as well as the years and places of performance. Eleven studies were conducted in Northern America, and nine in Europe. The first six trials were performed in 1978 using the A/USSR/77 (H1N1) virus. The virus had been detected in November 1977 in the Soviet Union and was antigenically closely related to influenza A-H1N1 strains which had circulated 30 years earlier[57,58].

Characteristics of study populations (*Table 1*)

Size and health state. The size of the study populations varied widely between 30[56] and 2062 subjects[54]. A total of 7328 subjects entered the 20 selected studies but, in many papers, not all these subjects could be included in this review for various reasons (for example, insufficient data, interference with natural influenza during vaccination campaign, or subgroups which were not offered different vaccine doses). The reasons for exclusion are described in detail in *Appendix 1*.

Four papers[12,25,33,48] included exclusively young adults. Children and adolescents (≤15 years of age) were included in four papers[31,35,46,54], and elderly (≥65 years of age) in nine papers[29,30,35,44,45,50–52,55]. Most papers dealt with 'apparently healthy' volunteers, typically pupils, university students and employees. None of these papers established the absence of illness in these subjects by clinical or laboratory measures. One study[29] included both healthy and chronically ill subjects, and five studies were conducted in chronically ill patients at risk for influenza complications, such as sufferers from cystic fybrosis[46], renal diseases demanding haemodialysis[55], and various chronic geriatric diseases[45,50,52]. Of these chronically ill populations, only patients on haemodialysis have been established as suffering from a compromised humoral immunity and to show an impaired seroresponse to vaccines[59–62].

Previous exposure to influenza. Of the 20 papers, eight[25,29,35,48,49,51,55,56] did not take into account the influence of homologous prevaccination antibody titres on the seroresponse. In the other studies, this issue was addressed, although in different ways. Two papers described a prescreening of subjects for low pre-existing antibody titres before intake[12,33]. Five papers retrospectively stratified for prevaccination antibody[30,44–47], two papers excluded all previously seropositive subjects[31,54], and two excluded retrospectively all subjects with high (protective) prevaccination titres[12,50]. Two studies[52,53] included prevaccination titres in a statistical model and adjusted for this factor by regression analysis or analysis of covariance.

Seven papers[12,29,30,45,47,51,52] gave information about the history of previous vaccination against influenza, but drew different consequences from that information; study designs varied considerably from exclusion of all previously vaccinated subjects[12] to inclusion of up to 74%[52] or 82%[29] of previously vaccinated subjects in the study samples.

All six studies performed in 1978 to test the new influenza A/USSR/77 (H1N1) strain addressed correctly the H1N1-priming period by stratifying for age. Of the 14 remaining, more recent studies, five[12,33,46,47,54] addressed priming periods by a restricted prospective age selection of the participants, or by stratification for age.

Characteristics of study designs (*Table 1, Appendix 1*)

Randomization. An essential requirement of a dose-comparative study to validate its statistical analysis

Table 1 Characteristics of the 20 publications selected for review

					Study populations						Study design					Serological measures				
Ref. no.	First author	Year of publication	Year and place of study		Initial size	Age range	Health state	Pre-vacc.	Vacc. hist.	Priming	Randomization	Blindness	Booster	Placebo	Serological test	Pre-GMT	Post-GMT	Protection rate threshold	Response rate	Statistics
35	Nicholson	1979	1978	GB	1335	12–85	Healthy	–	–	Yes	Yes	db	Yes	–	HI	Yes	Yes	Yes (40)	–	–
44	Lavergne	1980	1978	CAN	667	18–73	Healthy	Yes	–	Yes	Yes	db	–	–	HI	–	Yes	–	Yes	conv
45	Brandriss	1981	1978	USA	137	41–93	Chr.ill, n-inst.	Yes	Yes	Yes	Yes	db	Yes	Yes	HI	–	Yes	–	–	conv
29	Quinnan	1983	1978	USA	517	16–83	–	–	Yes	Yes	–	db	Yes	Yes	HI	Yes	Yes	Yes (40)	Yes	conv
30	Cate	1983	1978	USA	292	20–88	Healthy	Yes	Yes	Yes	–	–	Yes	Yes	HI	–	–	Yes (40)	–	conv
31	Wright	1983	1978	USA	1034	3–25	Healthy-chr.ill	Yes	–	Yes	No?	–	Yes	Yes	HI	Yes	Yes	Yes (40)	–	conv
46	Cross	1982	1980	USA	80	3–33	Chr.ill, inst.	Yes	–	Yes	Yes	db	Yes	–	HI	Yes	Yes	Yes (40)	Yes	conv
47	Moffat	1982	1980	GB	108	17–63	Healthy	Yes	Yes	Yes	Yes	db	–	–	HI	Yes	Yes	Yes (40)	Yes	conv
33	Goodeve	1983		GB	119	18–19	Healthy	Yes	–	Yes	Yes	db	–	Yes	HI	Yes	Yes	Yes (40)	Yes	–
48	Rudenko	1985		USSR	80	18–25	Healthy	–	–	–	Yes	–	Yes	Yes	HI	Yes	Yes	–	Yes	–
25	Jennings	1985	1983	GB	132	YA	Healthy	–	–	–	Yes	db	–	Yes	SRH	Yes[a]	Yes[a]	–	Yes[b]	conv
49	Clarke	1985		GB	100	18–61	Healthy	–	–	–	Yes	–	–	–	HI	Yes	Yes	Yes (40)	–	–
50	Arden	1986	1984	USA	50	58–99	Chr.ill, inst.	Yes	–	–	Yes	–	–	–	HI	Yes	Yes	Yes (40)	–	conv
51	Gross	1988	1984	USA	169	Elderly	Healthy	–	Yes	–	Yes	sb	–	–	HI	Yes	Yes	–	Yes	conv
12	Beyer	1986	1985	NL	94	20–35	Healthy	Yes	Yes	Yes	Yes	sb	–	–	HI	Yes	Yes	Yes (100)	Yes	conv
52	Peters	1988	1985	USA	131	70–96	Chr.ill, n-inst.	Yes	Yes	–	Yes	db	–	–	HI	Yes	Yes	Yes (32)	Yes	adv
53	Sullivan	1990	1985	USA	140	18–64	Healthy	Yes	–	–	Yes	db	–	–	HI	Yes	Yes	–	–	adv
54	Subbotina	1988		USSR	2062	9–22	Healthy	Yes	–	Yes	No?	–	–	–	HI	Yes	Yes	–	Yes	conv
55	Rautenberg	1989	1987	FRG	51	22–77	Haemodialysis	–	–	–	–	–	Yes	–	IIF	Yes	Yes	Yes (2555)	Yes	conv
56	Guarnaccia	1990		USA	30	20–50	Healthy	–	–	–	–	–	–	–	HI	Yes	Yes	–	–	adv

YA, young adults; chr.ill, chronically ill; inst., institutionalized; n-inst., non-institutionalized; Pre-vacc. prevaccination state (amount of homologous antibody before vaccination) addressed; Vacc.hist., history of previous vaccinations addressed; Priming, priming periods addressed; Randomization: no?, randomization doubtful (see *Appendix 1*); db, double-blind; sb, single-blind; HI, haemagglutination inhibition; SRH, single radial haemolysis; IIF, indirect immunofluorescence; conv, conventional; adv, advanced; not given or not addressed

[a]Haemolysis area in mm^2

[b]Based on subjects with an increase of haemolysis area >45%

is the random allocation, per stratification group, of the different doses to subjects. Fourteen papers addressed random allocation explicitly, but six other papers[29–31,54–56] did not report on this point. Considering the context and background of the institution, one may assume, however, that randomization had been performed, except possibly for two papers[31,54].

Blinding and placebo control. Double- or single-blinding and placebo control are essential when scoring for local and systemic adverse effects of the vaccines. When the objective endpoint is the induction of antibody, blinding is less critical. However, a placebo group is advisable when vaccine trials are done during a period when an outbreak of natural influenza may occur. Indeed, two studies[25,35] reported such an event. Seven studies[25,29–31,33,45,48] included a placebo.

Techniques and calculations (*Table 1*)

Serological tests. In all but two papers[25,55], the HI test was used to detect homologous antibodies against the viral vaccine components, performed in a microtitre fashion. This test has many variations (for instance, pretreatment of sera, incubation periods, type of erythrocytes, treatment of test antigen, recording of agglutination patterns). Moreover, the concentration of the test antigen (measured in haemagglutination units, HAU) affects the outcome of the test: a low concentration (3 HAU, Ref. 12) increases absolute titre values and may detect even small amounts of antibody with a higher chance of false-positive results (high sensitivity, low specificity); a high concentration (8 HAU, Refs 33, 35) results in a lower sensitivity and lower absolute titre values, but in better reproducibility. Most studies did not mention the amount of HAU used but referred to previous publications which were also consulted in this review. From this, it can be concluded that, in most cases, 4 HAU had been used in the HI tests.

Measures of serological response. *Table 1* presents also the measures of serological response which we could review. The pre- and postvaccination geometric mean titres (GMT), or measures derived from them, were the most common parameters to describe quantitatively the antibody response to the vaccine. Many papers also reported numbers, percentages or proportions of subjects under and beyond a threshold titre believed to correlate with protection. The threshold was a titre of 32–40 for those studies which used a test-antigen concentration of 4–8 HAU in the HI test, and higher (100 and 200, for influenza A and B, respectively) in the study which used 3 HAU[12]. The indirect immunofluorescence assay used in Ref. 55 had a threshold of 2555. Numbers, percentages or rates expressing 'response' (titre rise of at least fourfold) were also often given. The paper in which the single radial haemolysis test was used[25], also presented response rates (based on the number of subjects within an increase of more than 45% between pre- and postvaccination haemolysis areas). Seven papers presented pre- or postimmunization titres in cumulative tables for discrete titre intervals[31,33,35,44,49,51,52].

Statistical analysis. The final column of *Table 1* provides an indication of the statistical methods used to assess the significance of differences found between dose groups. No formal statistical analysis at all was applied in four studies[33,35,48,49]. In these studies, the absolute differences between groups were presented without addressing factors such as group size and probability calculations, i.e. statistical considerations which could affect the interpretation of the outcome. Other papers[12,25,29–31,44–47,50,54,55] used conventional statistical methods such as χ^2, Fisher's exact, t, or Wilcoxon-rank tests where thought appropriate. Three of the most recent studies[52,53,56] applied more advanced statistical procedures such as regression analysis, or analysis of variance. None of the published studies presented data indicating the amount of variation within the study groups for the observed values, such as ranges of observed titre values, or 95% confidence intervals with the reported dose differences in means and rates.

Stratification groups, doses and serological data (*Table 2*)

Many of the 20 papers included more than one vaccine type (WV and SU[35], WV and SPL[29–31,45]) or more than one influenza strain[12,25,29–31,44,45,47–49,51,53–56]. Subjects were stratified, by the authors, according to various criteria. For this review, the groups for dose-comparisons were either adopted as reported in the original papers, or restratified as described in detail in *Appendix 1*. Data on different vaccine types were pooled by us where they showed no significant difference in antibody titres, according to the authors. Data on different influenza strains, however, were never pooled.

Table 2 presents the results of this restratification procedure. Fifty stratification groups (SG) were derived from the 20 studies. SPL, WV and SU vaccines were used in 27, 22 and nine stratification groups, respectively (double scoring possible). Two papers[52,56] did not report the type of their vaccines. Many of the vaccine strains belonged to the influenza A-H1N1 subtype (20 SG) with the A/USSR/92/77 virus as the most frequent single strain (nine SG); the remaining strains consisted of influenza B (16 SG) and A-H3N2 (14 SG) viruses.

The sample size of the stratification groups varied widely (between 29 and 1137 subjects). In bi- and trivalent vaccine studies, the same subjects are scored two or three times, separately for each strain. The total number of subjects in *Table 2* (8921 subjects) is therefore considerably larger than the actual number of included subjects (7328 subjects). This assumes that after administration of bi- or trivalent vaccines, the antibody induction in any one subject is independent for each vaccine component. For further calculations, each stratification group is assumed to be a single, independent dose–response experiment.

The final column of *Table 2* shows the vaccine doses tested for each of the 50 stratification groups, varying between 1.5 and 94 μg HA. Some stratification groups contain only two doses, others up to six. A complete list of available serological data for each dose of each stratification group, which was used for the collection of raw data for this review, is given in *Appendix 2*.

Dose comparisons

***Dose comparisons of quantitative parameters* (Table 3).** It was not possible for us to apply statistical procedures on pre-GMT, post-GMT, or MFI values, because no reference was made to within-group variances, and the original raw data were not available.

Table 2 Characteristics of 50 stratification groups, derived from 20 studies

Ref.	Stratification group	Vaccine type	Strain	Size	Dosages (μg HA)
35	1	WV	A/USSR/92/77 (H1N1)	175	5, 9, 16, 32, 47, 94
	2	WV	A/USSR/92/77 (H1N1)	128	5, 9, 16, 32, 47, 94
	3	SU	A/USSR/92/77 (H1N1)	61	5, 18, 66
44	4	WV	A/Texas/1/77 (H3N2)	590	7.5, 15.5
	5	WV	A/USSR/92/77 (H1N1)	306	6, 10.5
	6	WV	B/Hong Kong/8/72	590	19.5, 33.5
45	7	WV, SPL	A/USSR/92/77 (H1N1)	137	7, 20
	8	WV, SPL	A/Texas/1/77 (H3N2)	137	7, 20
29	9	WV, SPL	A/USSR/92/77 (H1N1)	132	7, 20
	10	WV, SPL	A/USSR/92/77 (H1N1)	185	7, 20, 60
	11	WV, SPL	A/Texas/1/77 (H3N2)	260	7, 20
	12	WV, SPL	B/Hong Kong/8/72	260	7, 20
30	13	WV, SPL	A/USSR/92/77 (H1N1)	57	7, 20
	14	WV, SPL	A/Texas/1/77 (H3N2)	218	7, 20
	15	WV, SPL	B/Hong Kong/8/72	218	7, 20
31	16	WV, SPL	A/USSR/92/77 (H1N1)	355	2.3, 7, 20
	17	WV, SPL	A/Texas/1/77 (H3N2)	111	2.3, 7, 20
	18	WV, SPL	B/Hong Kong/8/72	229	2.3, 7, 20
46	19	SPL	B/Singapore/222/79	47	7, 60
47	20	WV	A/Brazil/11/78 (H1N1)	86	7, 10
	21	WV	B/Singapore/222/79	103	7, 15
33	22	SU	B/Hong Kong/?/73	96	5, 10, 20, 40
48	23	SU	A/Khabarovsk/74/77 (H1N1)	59	7.5, 15, 20
	24	SU	A/Texas/1/77 (H3N2)	59	7.5, 15, 20
25	25	SU	A/Bangkok/1/79 (H3N2)	95	6, 12, 24
	26	SU	A/Brazil/11/78 (H1N1)	95	6, 12, 24
49	27	SPL	A/Chile/1/83 (H1N1)	96	10, 15
	28	SPL	B/USSR/100/83	96	10, 15
50	29	SPL	B/USSR/100/83	50	15, 60
51	30	SPL	A/Philippines/2/82 (H3N2)	72	15, 30, 45
	31	SPL	A/Chile/1/83 (H1N1)	72	15, 30, 45
	32	SPL	B/USSR/100/83	72	15, 30, 45
	33	WV	A/Philippines/2/82 (H3N2)	75	15, 30, 45
	34	WV	A/Chile/1/83 (H1N1)	75	15, 30, 45
	35	WV	B/USSR/100/83	75	15, 30, 45
12	36	SPL	A/Philippines/2/82 (H3N2)	84	10, 15
	37	SPL	A/Chile/1/83 (H1N1)	76	10, 15
	38	SPL	B/USSR/100/83	82	10, 15
52	39	–	B/USSR/100/83	129	15, 60
53	40	SU	A/Philippines/2/82 (H3N2)	140	7.5, 15, 30
	41	SU	A/Chile/1/83 (H1N1)	140	7.5, 15, 30
	42	SU	B/USSR/100/83	140	7.5, 15, 30, 45
54	43	WV	A/Kiev/59/79 (H1N1)	1137	7, 14
	44	WV	A/Leningrad/385/80 (H3N2)	1137	7, 14
55	45	SPL	A/Singapore/6/86 (H1N1)	51	15, 30
	46	SPL	A/Leningrad/360/86 (H3N2)	51	15, 30
	47	SPL	B/Ann Arbor/1/86	51	10, 20
56	48	–	A/Leningrad/360/86 (H3N2)	29	1.5, 3, 15
	49	–	A/Taiwan/1/86 (H1N1)	29	1.5, 3, 15
	50	–	B/Ann Arbor/186	29	1.5, 3, 15

WV, whole-virus vaccine, SPL, split vaccine, SU, subunit vaccine

Thus, the papers were checked for statistical calculations computed by the authors themselves. *Table 3* presents those 39 stratification groups where the result of a statistical test on post-GMT or MFI had been reported by the authors. In column 'Result' of *Table 3*, '+' indicates that the authors found a statistically significant difference between doses ($\alpha < 0.05$), and '−' means its absence. This descriptive review procedure revealed 11 significant stratification groups out of 39 (28%). There was no clear association between the result of the significance tests and the size of the stratification groups or the subtype/type of the vaccine strain (not shown). Very small total dose ranges ($\leqslant 7$ μg HA) showed no significant dose differences (SG 5, 20, 36–38, 43, 44), but the other dose ranges did not correlate with the outcome; there were stratification groups with a large dose range which could not detect a significant dose–response relation (SG 10, 7–60 μg HA, SG 29 and SG 31, 15–60 μg HA).

***Dose comparisons of protection rates* (Table 4).** Protection rates as reported in, or derived from, the reviewed papers, were subjected to statistical analysis, i.e. a weighted linear model based on the k*2-χ^2 test, and probit analysis. *Table 4* presents, subdivided for influenza subtype/type and immune status, the results of these calculations for 26 stratification groups for which protection rates were available. For each stratification group with more than two doses, both methods were tested for model-fitting. For an acceptable fit, the test value should be greater than 0.10, which was not true for one stratification group (SG 1). Results from another five stratification groups (SG 20, 29, 39, 45, 46) should be interpreted cautiously since their doses do not include

Table 3 Significance of dose differences in post-geometric mean titres, or mean fold increase values, in studies that performed and reported statistical tests

Ref. no.	Stratification group	Size	Dose (μg HA)		Dose range (μg HA)	Dose number	Result[b]
			Minimum	Maximum			
44	4	590	7.5	15.5	8.0	2	–
44	5	306	6.0	10.5	4.5	2	–
44	6	590	19.5	33.5	14.0	2	+
45	7	137	7.0	20.0	13.0	2	+
45	8	137	7.0	20.0	13.0	2	+
29	9	132	7.0	20.0	13.0	2	–
29	10	185	7.0	60.0	53.0	3	–
29	11	260	7.0	20.0	13.0	2	–
29	12	260	7.0	20.0	13.0	2	–
31[a]	16	355	2.3	20.0	17.7	3	–
31[a]	17	111	2.3	20.0	17.7	3	–
31[a]	18	229	2.3	20.0	17.7	3	–
46	19	47	7.0	60.0	53.0	2	+
47	20	86	7.0	10.0	3.0	2	–
47	21	103	7.0	15.0	8.0	2	+
25	25	95	6.0	24.0	18.0	3	–
25	26	96	6.0	24.0	18.0	3	–
50	29	50	15.0	60.0	45.0	2	–
51	30	72	15.0	45.0	30.0	3	–
51	31	72	15.0	45.0	30.0	3	+
51	32	72	15.0	45.0	30.0	3	–
51	33	75	15.0	45.0	30.0	3	–
51	34	75	15.0	45.0	30.0	3	–
51	35	75	15.0	45.0	30.0	3	–
12	36	84	10.0	15.0	5.0	2	–
12	37	76	10.0	15.0	5.0	2	–
12	38	82	10.0	15.0	5.0	2	–
52	39	129	15.0	60.0	45.0	2	–
53	40	140	7.5	30.0	22.5	3	–
53	41	140	7.5	30.0	22.5	3	+
53	42	140	7.5	45.0	37.5	4	–
54[a]	43	1137	7.0	14.0	7.0	2	–
54[a]	44	1137	7.0	14.0	7.0	2	–
55	45	51	15.0	30.0	15.0	2	+
55	46	51	15.0	30.0	15.0	2	+
55	47	51	10.0	20.0	10.0	2	–
56	48	29	1.5	15.0	13.5	3	+
56	49	29	1.5	15.0	13.5	3	+
56	50	29	1.5	15.0	13.5	3	–

Note that, for some stratification groups, the sizes may differ in *Appendix 2* and in *Tables 3–5*, due to differences between inclusion criteria in the original papers and those by us (see *Appendix 1*)

[a] Possibly not randomized

[b] +/–, authors found/did not find a statistically significant difference between dose groups

10 and 15 μg HA. These doses had to be extrapolated with possibly doubtful validity.

Table 4 also presents intercept and slope of the linear model. Interestingly, six out of 26 stratification groups (23%) showed a negative slope, and one a slope equal to zero. In these studies, higher vaccine doses were associated with lower or equal protection rates. Of the remaining 19 positive slopes, only three were significantly different from zero, thus showing a significant dose–response relationship (SG 16, 19, 22).

Intercept and slope were used to estimate protection rates for 10 and 15 μg HA doses. Despite the considerable differences between studies and the significance levels of the model parameters, these results were very consistent for influenza A: all four stratification groups with A-H3N2 vaccine strains, and nine from ten stratification groups with A-H1N1 strain had protection rates greater than 75% at a dose of 10 μg HA. The median PR for all 14 influenza A strains was 81.5% with a range of 70–93%. For influenza B (9 SG), protection rates at 10 μg HA were more heterogeneous and generally lower than for the influenza A strains (range 51–97%, median 68%, six out of nine SG lower than 75%). This may reflect differences in laboratory techniques (some studies[12,50,52,53] used ether-treated test antigen in the HI test, others did not), or in the definition of a protection threshold.

The estimated PR values at 15 μg HA were not much different from those at 10 μg HA in non-immune-compromised populations. The difference between the protection rates varied between −3 and 6% in 22 out of 23 stratification groups with a median of 1%. The three stratification groups with a significantly positive slope did not differ from this pattern. Only SG 20, one of the stratification groups which should be interpreted cautiously, had a higher difference between the two doses (11%) but this may be meaningless, as this is the only stratification group with an extremely narrow dose range which makes extrapolation very unreliable (in the study, doses of 7 and 10 μg HA were compared). Moreover, the estimated (and real) PR value at 10 μg HA is already very high (91%), and the extrapolated PR value at 15 μg HA would exceed 100%. For the immune-compromised population represented in this review, patients on

Table 4 Estimation of protection rates for doses of 10 and 15 μg HA

Ref. no.	Stratification group	Subtype	Size	Doses			Linear $k*2$ χ^2 analysis						Probit analysis		
				No.	Min.	Max.	Model fit	Intercept	Slope	Dose (μg) 10	Dose (μg) 15	Difference	Model fit	Dose (μg) 10	Dose (μg) 15
Non-immune-compromised populations															
29	11	H3N2	185	2	8.8	22.5		0.769	0.0012	0.78	0.79	0.01		0.78	0.79
30	14	H3N2	141	2	7.0	20.0		0.814	−0.0066	0.75	0.71	−0.04		0.74	0.70
31[a]	17	H3N2	111	3	2.3	20.0	0.22	0.757	0.0008	0.76	0.77	0.01	0.21	0.77	0.76
12	36	H3N2	84	2	10.0	15.0		0.785	0.0126	0.91	0.97	0.06		0.91	0.97
35	1	H1N1	172	6	5.0	94.0	0.01	0.777	0.0032	0.81	0.83	0.02	0.04	0.80	0.85
35	2	H1N1	103	6	5.0	94.0	0.41	0.920	0.0000	0.92	0.92	0.00	0.53	0.91	0.92
35	3	H1N1	50	3	5.0	66.0	0.44	0.854	0.0024	0.88	0.89	0.01	0.59	0.89	0.93
29	9	H1N1	68	2	5.8	16.8		0.738	0.0052	0.79	0.82	0.03		0.80	0.82
29	10	H1N1	79	3	5.8	52.0	0.67	0.782	0.0024	0.81	0.82	0.01	0.44	0.81	0.83
30	13	H1N1	76	2	7.0	20.0		0.728	0.0096	0.82	0.87	0.05		0.85	0.89
31[a]	16	H1N1	355	3	2.3	20.0	0.50	0.607	0.0092[b]	0.70	0.75	0.05	0.14	0.72	0.76
47	20	H1N1	86	2	7.0	10.0		0.703	0.0210	0.91	1.02	0.11		0.91	0.96
49	27	H1N1	59	2	10.0	15.0		0.926	0.0004	0.93	0.93	0.00		0.93	0.93
12	37	H1N1	76	2	10.0	15.0		0.937	−0.0056	0.88	0.85	−0.03		0.88	0.85
29	12	B	169	2	7.8	24.0		0.594	0.0012	0.61	0.61	0.00		0.61	0.62
30	15	B	149	2	7.0	20.0		0.561	−0.0002	0.56	0.56	0.00		0.56	0.56
31[a]	18	B	229	3	2.3	20.0	0.18	0.575	−0.0002	0.57	0.57	0.00	0.19	0.57	0.57
46	19	B	36	2	7.0	60.0		0.774	0.0038[b]	0.81	0.83	0.02		0.94	0.99
33	22	B	80	4	5.0	40.0	0.54	0.572	0.0104[b]	0.68	0.73	0.05	0.70	0.72	0.81
49	28	B	69	2	10.0	15.0		1.020	−0.0052	0.97	0.94	−0.03		0.97	0.94
50	29	B	30	2	15.0	60.0		0.443	0.0062	0.51	0.54	0.03		0.44	0.54
12	38	B	82	2	10.0	15.0		0.850	0.0062	0.91	0.94	0.03		0.91	0.94
52	39	B	72	2	15.0	60.0		0.738	−0.0022	0.71	0.70	−0.01		0.73	0.70
Immune-compromised populations															
55	45	H1N1	51	2	15.0	30.0		0.229	0.0168	0.40	0.48	0.08		0.33	0.48
55	46	H3N2	51	2	15.0	30.0		0.015	0.0124	0.14	0.20	0.06		0.12	0.20
55	47	B	50	2	10.0	20.0		−0.058	0.0182	0.12	0.22	0.10		0.12	0.22

Note that, for some stratification groups, the sizes may differ in *Appendix 2* and in *Tables 3–5*, due to differences between inclusion criteria in the original papers and those by us (see *Appendix 1*)

[a]Possibly not randomized

[b]Slope significantly different from 0

haemodialysis[55], the pattern appeared different. The PR values at 10 μg HA were, for all three vaccine components, very low (12–40%), and showed an increase of 6–8% at a dose of 15 μg HA.

Results yielded by probit analysis were similar to those of the linear model (last two columns of *Table 4*). Only one stratification group (SG 19) showed a major discrepancy between both methods (PR at 10 μg HA: 81% by linear model, and 94% by probit), obviously related to the fact that, of all stratification groups with only two doses, SG 19 had the largest dose range (7–60 μg HA).

***Dose comparisons of response rates* (Table 5).** Thirty stratification groups could be analysed for response rates (RR) (*Table 5*). Both methods had insufficient model fitting in two cases (SG 25 and 33). In eight cases (SG 30, 33, 5, 20, 32, 35, 45, 46), the doses did not include 10 and 15 μg HA. The estimations for intercept, slope, and RR values at doses of 10 and 15 μg HA were very similar for both methods. With the linear model, nine stratification groups (30%) had a negative slope; of the remaining 21 positive slopes, four were significantly different from zero (SG 26, 6, 22, 47). Estimation of the RR at 10 μg HA, in non-immune-compromised populations, revealed a higher variability than for the protection rates, for all three virus subtypes/types (A-H3N2 34: 96%, median 85%; A-H1N1 39: 87%, median 76%; B 19: 98%, median 60%). Reasons for this variability may be related to the limitations of the response rate as a measure of seroresponse, and are addressed in the Discussion section of this review. Overall, the differences between RR values at doses of 10 and 15 μg HA varied between −9 and 8% in 26 out of 27 stratification groups, with a median of 1%. Again, SG 20 had a higher difference between the two doses (16%).

For patients on haemodialysis, the influenza B component (SG 47) showed the only huge increase, from an RR value of 20% at 10 μg HA to 41% at 15 μg HA.

DISCUSSION

Until recently, dose requirements for influenza vaccines in various countries were different from each other, which indicated a lack of consensus on the 'lowest effective dose', despite the great number of studies done to investigate this question. This may be related to the biological or experimental difficulties in establishing such a dose. The lowest effective dose may vary for different virus strains, different epidemiological situations, or different target groups, or may be dependent on the selected efficacy parameters to assess dose-effects. Dose-effects on the true efficacy parameters (influenza attack-rate, reduction in influenza-associated morbidity and mortality) can only be derived from field or challenge

Table 5 Estimation of response rates for doses of 10 and 15 μg HA

Ref. no.	Stratification group	Subtype	Size	Doses			Linear $k*2$ χ^2 analysis						Probit analysis		
				No.	Min.	Max.	Model fit	Intercept	Slope	Dose (μg) 10	Dose (μg) 15	Difference	fit	Dose (μg) 10	Dose (μg) 15
Non-immune-compromised populations															
44	4	H3N2	590	2	7.5	15.5		0.859	0.0018	0.88	0.89	0.01		0.88	0.89
29	11	H3N2	260	2	8.8	22.5		0.462	0.0070	0.53	0.57	0.04		0.54	0.58
48	24	H3N2	59	3	7.5	20.0	0.25	1.065	−0.0184	0.88	0.79	−0.09	0.18	0.91	0.79
25	25	H3N2	95	3	6.0	24.0	0.08	0.874	0.0030	0.90	0.92	0.02	0.06	0.91	0.92
51	30	H3N2	72	3	15.0	45.0	0.97	0.291	0.0046	0.34	0.36	0.02	0.92	0.31	0.36
51	33	H3N2	75	3	15.0	45.0	0.07	0.539	−0.0060	0.48	0.45	−0.03	0.04	0.49	0.44
12	36	H3N2	84	2	10.0	15.0		0.867	0.0088	0.96	1.00	0.04		0.96	1.00
53[a]	44	H3N2	956	2	7.0	14.0		0.864	−0.0038	0.82	0.81	−0.01		0.82	0.81
44	5	H1N1	306	2	6.0	10.5		0.749	−0.0048	0.70	0.68	−0.02		0.70	0.69
29	9	H1N1	89	2	5.8	16.8		0.779	−0.0012	0.77	0.76	−0.01		0.76	0.76
29	10	H1N1	185	3	5.8	52.0	0.30	0.424	0.0048	0.47	0.50	0.03	0.77	0.48	0.52
47	20	H1N1	86	2	7.0	10.0		0.554	0.0314	0.87	1.03	0.16		0.87	0.94
48	23	H1N1	59	3	7.5	20.0	0.94	0.926	−0.0160	0.77	0.69	−0.08	0.84	0.76	0.68
26	26	H1N1	90	3	6.0	24.0	0.85	0.628	0.0124[b]	0.75	0.81	0.06	0.38	0.79	0.85
51	31	H1N1	72	3	15.0	45.0	0.69	0.345	0.0048	0.39	0.42	0.03	0.80	0.35	0.41
51	34	H1N1	75	3	15.0	45.0	0.28	0.472	−0.0006	0.47	0.46	−0.01	0.28	0.45	0.45
12	37	H1N1	76	2	10.0	15.0		0.807	0.0050	0.86	0.88	0.02		0.86	0.88
54[a]	43	H1N1	993	2	7.0	14.0		0.770	0.0010	0.78	0.79	0.01		0.78	0.79
44	6	B	590	2	19.5	33.5		0.640	0.0052[b]	0.69	0.72	0.03		0.64	0.70
29	12	B	260	2	7.8	24.0		0.330	0.0056	0.39	0.41	0.02		0.39	0.42
46	19	B	47	2	7.0	60.0		0.659	0.0010	0.67	0.68	0.01		0.68	0.69
47	21	B	103	2	7.0	15.0		0.456	0.0148	0.60	0.68	0.08		0.62	0.68
33	22	B	96	4	5.0	40.0	0.29	0.532	0.0118[b]	0.65	0.71	0.06	0.87	0.71	0.81
51	32	B	72	3	15.0	45.0	0.98	0.172	0.0044	0.22	0.24	0.02	0.88	0.20	0.24
51	35	B	75	3	15.0	45.0	0.48	0.194	−0.0002	0.19	0.19	0.00	0.48	0.18	0.18
12	38	B	82	2	10.0	15.0		0.990	−0.0012	0.98	0.97	−0.01		0.98	0.97
52	39	B	129	2	15.0	60.0		0.498	0.0002	0.50	0.50	0.00		0.50	0.50
Immune-compromised populations															
55	45	H1N1	51	2	15.0	30.0		0.591	0.0060	0.65	0.68	0.03		0.62	0.68
55	46	H3N2	51	2	15.0	30.0		0.422	0.0038	0.46	0.48	0.02		0.45	0.48
55	47	B	51	2	10.0	20.0		−0.215	0.0416[b]	0.20	0.41	0.21		0.20	0.43

See footnote to *Table 4*

studies, the latter being feasible only in healthy adults for ethical reasons. Hence, for practical reasons, the choice for the recommended vaccine doses has mainly been based on serological studies where homologous antibody titres are used as surrogate markers of efficacy.

The 20 studies selected for this review covered doses from 1.5 to 94 μg HA. We found a fair amount of variation in study designs, study samples, priming and vaccination history of the vaccinees, group sizes, vaccine types, virus strains, laboratory techniques and statistical procedures for data analysis. We applied strict *post hoc* criteria to each study to form 50 stratification groups of subjects already primed for the vaccine strains, collected the data on seroresponse measures as given in the studies, and performed calculations to detect dose-effects within the stratification groups and to estimate the seroresponse at doses of 10 and 15 μg HA.

Quantitative seroresponse measures

There was a significant dose–response relationship based on post-GMT or mean fold increase (MFI) values in 11 out of 39 stratification groups. Of course, detection of such a relationship is dependent on group size and the range of doses included in the experiment, but even after exclusion of seven stratification groups with a very small dose range (≤7 μg HA), the number of significant results appears small (11/32, 34%), particularly since some stratification groups with a very large dose range are included. This finding could suggest that a real relationship between antibody development after vaccination and vaccine dose does not exist at all, or is, at least, 'shallow'[35]. However, 34% with a significant result is far more than one would expect by chance in the absence of a real dose–response relationship. More likely, in many studies other variables, insufficiently controlled, may have masked the dose-effect. The strongest determinant of postvaccination titre is the prevaccination (or baseline) titre[63,64] which was recognized and *addressed* in the majority of papers (*Table 1*) but actually *controlled* only in two[52,53] where it was treated as a confounding factor in a statistical model.

Response rate

Another frequently used seroresponse measure in the papers reviewed was the RR used in 30 stratification groups (60%). Since RR is a dichotomous derivate of the MFI, it is also affected by the prevaccination antibody titre. In our opinion, this measure is mathematically inappropriate (even after statistically correcting for prevaccination titre), has no clear immunological or clinical implication, and should be avoided[14]. Our theoretical considerations are confirmed by the large variability of the RR values when estimated for a dose of 10 μg HA (19–98%, *Table 5*).

Protection rate

Clinically, the most meaningful seroresponse measure is the protection rate (PR). It is not strongly dependent on prevaccination titre as its calculation is based on subjects unprotected prior to vaccination (i.e. subjects with negative or low baseline). Therefore, this measure is also useful in studies which are insufficiently controlled for prevaccination titre. Indeed, the PR values estimated for a dose of 10 μg HA were much more consistent than the RR values. Virtually all stratification groups with influenza A had PR values equal to or higher than 75%. The papers consistently show that a dose of 10 μg HA of influenza A vaccine induces an antibody titre beyond the protection level in most primed and non-immune-compromised subjects.

For influenza B, the variation in PR values was larger (51–97%), and fewer stratification groups exceeded a PR of 75% (3/9, 33%). It may be assumed that influenza B antigens are less immunogenic than influenza A. However, the observed lower PR values may be related to artefacts of the laboratory techniques, especially the haemagglutination inhibition test which tends to produce low titres with influenza B antigens[65]. Some authors ignored this problem, others addressed it by treating the antigen with ether to enhance its avidity, and again others by using a lower protection threshold than for influenza A antigens (for instance, 20 instead of 40). All this may have contributed to the higher variability of PR values for influenza B; the reason for some less favourable findings could therefore be of a technical nature.

Differences between PR and RR values for doses of 10 and 15 μg HA

Surprisingly, despite the limitations of the RR as a measure of seroresponse, and the PR in the case of influenza B, virtually no stratification groups for either RR or PR show a meaningful improvement when comparing their estimates for 10 and 15 μg HA. The stratification groups with a significantly positive slope show an increase of 2–6%, and the increases of other stratification groups oscillate around zero in a range from −9 to 8% (except SG 47). This is in good agreement with our own dose–response study in 544 young and elderly subjects, vaccinated with doses of 0, 10, 20 and 60 μg HA per strain[66]. When treating the data by the same method as used in this review to interpolate PR values for 10 and 15 μg, we found the differences for three vaccine components and two age groups to vary between 1 and 3% only.

These results suggest that, at a dose level of 10 μg HA, the (sigmoid) dose–response curve has already passed the zone of its largest increase. An additional increase in the vaccine dose from 10 to 15 μg HA virtually does not improve the seroresponse as measured by protection or response rates. The expectation expressed in the European 'Harmonization of Requirements For Influenza Vaccines'[4] that a vaccine dose of 15 μg HA per strain should be clinically superior to a dose of 10 μg HA per strain is not justified in vaccinees who are primed and have no manifest immunological disorders.

Immune-compromised subjects

One paper[55] dealt with a group of 51 patients on chronic intermittent haemodialysis with a manifest impairment of humoral immunity. There have been several unsuccessful attempts to overcome the low antibody production after influenza vaccination, for instance booster vaccinations some weeks later[67] or simultaneous administration of an immunomodulator[62]. The results reported in Ref. 55 suggest that these patients might have benefited from a higher amount of antigen (for example, the simultaneous injection of two standard doses), and should stimulate more trials in this and similar immune-compromised groups.

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APPENDIX 1

In the following comments on the 20 papers reviewed, numbers of pages, figures or tables mentioned refer to the original papers, unless italicized.

Nicholson *et al.* (Ref. 35)

The authors describe a complex vaccination trial in nine groups of volunteers with different age distributions and in different locations, using four different vaccine preparations containing the A/USSR/77 (H1N1) strain, two different modes of vaccine administration, and many dosages. After 1 month, the young age groups received a booster injection with the same vaccine and dosage as the first vaccination. Of the 1335 subjects entering the study, the authors themselves exclude one group of unknown size because of assumed occurrence of natural influenza in this group during the trial. Prevaccination HI antibody titres (as cumulative percentages) are presented for 980 subjects (Table 1). On page 130 the authors state that they determined the HI antibody responses to vaccination for 972 subjects, but Tables 2–4 present only 938 subjects. No comment is given on the missing subjects. From Tables 2–4, the distribution of subjects shown in *Table A1* can be derived.

Lavergne *et al.* (Ref. 44)

Immunizations were performed in 667 healthy volunteers with two doses of a trivalent WV vaccine containing A/Texas/1/77 (H3N2), A/USSR/92/77 (H1N1), and B/Hong Kong/2/73. Postvaccination sera of 77 subjects were not available. Serological data are presented for previously seronegative volunteers (Tables IVa–c) and all volunteers (Tables Va–c), stratified for three age groups (18–25, 26–50, 51–73 years of age). As the study population was regarded to be primed for influenza A (H3N2) and B, data of Tables Va and Vc were analysed in this review. For A/USSR/77, the data of age groups $\geqslant 26$ years of age (primed), but not <26 (unprimed, no booster vaccination performed) were used here. In Table Vc, the total postvaccination GMT for the lower dose group is not 73 but 75. As no serological data on prevaccination sera were presented, no PR values could be analysed.

Table A1

Age groups (years)	Mode of administration	WV	Aqueous CTAB SU	Aqueous Triton SU	Adsorbed Triton SU
12–25	Subcutaneous	175	72[a]	29[b]	126[c]
	Intradermal	44[d]	39[a]	–	–
⩾26	Subcutaneous	128	46[a]	61	94[c]
	Intradermal	48[d]	26[d]	50[d]	–
Totals		395	183	140	220

Reasons for exclusion from this review:
[a]The authors report that they had tested vaccine potency before delivering to and after returning from the study centres. The aqueous CTAB subunit vaccine lost all (low dosage) or much (high dosages) of its detectable HA contents, and data on antibody response were not reliable. For this review, those data are excluded
[b]The 29 young subjects receiving aqueous Triton SU vaccine are excluded because only one dosage (9 μg HA) was given in this group
[c]Only aqueous, but not adjuvant vaccine preparations (in this case $Al(OH)_3$-adsorbed), are accepted for this review. The remaining 364 subjects form three different stratification groups
[d]According to our selection criteria, only subjects with subcutaneous or intramuscular vaccine administration are included

Brandriss *et al.* (Ref. 45)

These authors undertook a vaccination trial in 137 chronically ill, but not institutionalized, elderly, with monovalent or trivalent WV or SPL vaccines, in two dosages, and with booster vaccinations 3 weeks later. The authors did not present tables or figures showing the serological response on different doses, but only stated: '. . . when the subjects were combined according to dose (7 μg or 20 μg) . . . the numbers were sufficient to suggest some significant differences in response. The postvaccination geometric mean titers to A/USSR/77 were higher after the 20 μg dose than after the 7 μg dose (1:144 versus 1:88.2, $p<0.01$) and were also higher to A/Texas/77 after 20 μg (1:87 versus 1:59.7, $p<0.05$). There were no significant differences between the 7-μg and the 20-μg preparations in the percentage of responders or the percentages of subjects with postvaccine HAI titers equal to or greater than 1:40.'

Quinnan *et al.* (Ref. 29)

A complex study is described which includes 517 subjects, with placebo (91 subjects) or monovalent and trivalent vaccine preparations from four manufacturers (WV and SPL), and booster injections for A/USSR/77 (H1N1) with the same vaccine preparation and dose as the first vaccination. Numbers given in the tables do not always correlate with numbers in the text. There were no titre rises in the placebo subjects, suggesting absence of natural influenza during the performance of the trial. Data for the different vaccine preparations were pooled by the authors. Instead of the numerical vaccine dosages (7, 20, 60 μg HA), the real mean dosages as given in Tables 1 and 2 are used in this review. Table 1 (A/USSR/77) includes two age groups (16–25 years of age, unprimed, $n=132$ subjects, and $\geqslant 26$ years of age, primed, $n=185$ subjects). Stratification has been done, by the authors, for previous vaccination with A/NJ/76 (Table 3), but the numbers cannot be correlated to those in Table 1. Therefore, this stratification is not included here. The authors found no major differences between subjects previously vaccinated and those not vaccinated

Table A2

Groups by authors (according to *Table 4*)				Stratification groups for review	
Strain	Age group	Vaccine type and dose	*n*	Group	Comments
A/USSR	Young adults	7 μg WV	17	13-1	A/USSR unprimed 7 μg ($n=41$)
A/USSR	Young adults	7 μg SPL	24		Pooled with 13-1
A/USSR	Young adults	20 μg WV	18	13-2	A/USSR unprimed 20 μg ($n=39$)
A/USSR	Young adults	20 μg SPL	21		Pooled with 13-2
A/USSR	Older adults		98		Excluded because authors pooled 20 and 60 μg HA doses
A/Tex	Young adults	7 μg WV	25	14-1	A/Tex 7 μg ($n=92$)
A/Tex	Young adults	7 μg SPL	28		Pooled with 14-1
A/Tex	Young adults	20 μg WV	23	14-2	A/Tex 20 μg ($n=126$)
A/Tex	Young adults	20 μg SPL	26		Pooled with 14-2
B/HK	Young adults	7 μg WV	25	15-1	B/HK 7 μg ($n=92$)
B/HK	Young adults	7 μg SPL	28		Pooled with 15-1
B/HK	Young adults	20 μg WV	23	15-2	B/HK 20 μg ($n=126$)
B/HK	Young adults	20 μg SPL	26		Pooled with 15-2
A/Tex	Older adults	7 μg WV	19		Pooled with 14-1
A/Tex	Older adults	7 μg SPL	20		Pooled with 14-1
A/Tex	Older adults	20 μg WV	40		Pooled with 14-2
A/Tex	Older adults	20 μg SPL	37		Pooled with 14-2
B/HK	Older adults	7 μg WV	19		Pooled with 15-1
B/HK	Older adults	7 μg SPL	20		Pooled with 15-1
B/HK	Older adults	20 μg WV	40		Pooled with 15-2
B/HK	Older adults	20 μg SPL	37		Pooled with 15-2

by A/NJ/76. For the unprimed age group, values after second vaccination are included. There are different total numbers of volunteers after the first and second vaccinations, but the percentages of protected subjects and the GMT values before vaccination are not given for the booster groups. For calculations, the respective values were adapted from the groups after first vaccination. Table 2 (A/Texas/77 (H3N2) and B/Hong Kong/72) presents the cumulated data of all ages, for 260 subjects.

Cate *et al.* (Ref. 30)

A study design was used similar to that described in Ref. 29, with monovalent and trivalent vaccines from four manufacturers (WV and SPL), two (7, 20 μg HA) or three doses (7, 20, 60 μg HA), and booster injections for A/USSR/77 (H1N1) after 1 month, in two groups of volunteers (age range 20–33 years of age, $n=154$, and 45–88 years of age, $n=138$). On page 738, the age range of the first group is wrongly given as 20–23. No information is given about the serology of the placebo groups. Unfortunately, data for 20 and 60 μg HA in the older adult age group vaccinated with A/USSR/77 (H1N1), were pooled by the authors as they found 'no major differences' between those dosages. These stratification groups had to be excluded as we were especially interested in those differences. Table 3 presents, according to priming periods, the immune response to A/USSR/77 (H1N1) only for 20–25-year-old and 55–88-year-old subjects. Table 4 gives the protection and response rates, but not the pre- and post-GMT-values, for all young and older subjects for all three antigens. This latter table was used for our review. Response rates, however, could not be used, as they were based on an unknown number of subjects with a prevaccination titre $\leqslant 20$. In total, 80 subjects could be included for the A/USSR/77 strain, and 218 subjects for A/Texas/77 and B/Hong Kong/72, respectively. We pooled the data for WV and SPL preparations. For A/Texas and B/Hong Kong, data for both age groups were pooled.

Table A2 shows the comparison groups presented by the authors, and the pooling which has been made for this review.

Wright *et al.* (Ref. 31)

The authors use a study design similar to that in Ref. 29 with monovalent and trivalent SV and SPL vaccine preparations from four manufacturers, three dosages (2.3, 7, 20 μg HA), and booster injections for A/USSR/77 (H1N1). There were 358 children and 676 young adults (healthy and chronically ill, total 1034, of whom 235 received placebo). No information is given about random allocation of vaccine doses to subjects. Whether allocation was really done is doubtful, at least for the lowest dose, as the authors stated: 'It may also reflect the fact that the lowest vaccine dose (pediatric), which contained ± 2.3 μg of antigen, was selectively given to younger children, . . .'. Table 6 presents the antibody response to A/USSR/77 (H1N1) of initially seronegative subjects. For our review, data of different age classes (3–6, 7–12 and 13–25 years of age) and vaccine preparations were pooled to form a group of unprimed subjects. Of these, 355 received a booster dose; those data were included here. Table 7 presents the antibody responses to A/Texas/77 and B/Hong Kong/72 of initially seronegative subjects for the age classes 3–6, 7–12, and 13–25 years. The first age class was supposed to be unprimed, and its data were not included, as booster vaccination had not been done for those vaccine strains. The other two age classes, although seronegative prior to vaccination, were supposed to be primed, and their data were pooled and included here.

Gross *et al.* (Ref. 46)

This study was conducted in patients with cystic fibrosis (children, young adults), in part from St.

Vincent's Hospital, as was the case in Ref. 28. It can be assumed that some in this study group also participated in that former study, but information about previous vaccination is not given. Two doses (7, 60 μg HA) of B/Singapore/79 are compared, given in a trivalent vaccine containing also 7 μg HA/Bangkok/1/79 (H3N2) and 7 μg A/Brazil/78 (H1N1). Dose groups were subdivided into subjects with negative and positive prevaccination titres for B/Singapore/79. The authors assume that previously seronegative subjects were unprimed for influenza B, which has been confirmed by a neutralization test. After 1 month most subjects in both dosage groups received a trivalent booster vaccine containing 7 μg HA of each strain. As this latter booster vaccination did not contain different dosages, only the data of the first vaccination in primed subjects were included in this review.

Moffat *et al.* (Ref. 47)

A trivalent WV vaccine was used to immunize 108 adult volunteers of whom 105 completed the study. The vaccine contained either 7 μg HA of each strain (A/Texas/1/77 (H3N2), A/Brazil/11/78 (H1N1), B/Singapore/222/79), or 7 μg HA A/Texas/1/77 (H3N2), 10 μg HA A/Brazil/11/78 (H1N1) and 15 μg HA B/Singapore/222/79. Thus, dose comparisons could be made for A/Brazil (7 versus 10 μg HA) and B/Singapore (7 versus 15 μg HA). Table 2 addresses the different H1N1-priming state for subjects born before and after 1956, for A/Brazil; no significant difference was found. Tables 1 and 3 present data on A/Brazil and A/Singapore, respectively, stratified for all participants, and for those with prevaccination titres <40; the latter stratification was chosen here. For the influenza B strain, the authors did not choose a protection threshold.

Goodeve *et al.* (Ref. 33)

After determining the serostatus of 119 healthy students, Goodeve and colleagues tested four dosages of a B/Hongkong/73 SU (CTAB) vaccine and placebo. No response occurred in the placebo group ($n=23$), thus no natural influenza was present After 4 weeks, volunteers were challenged with attenuated live virus. Tables 2 and 3 give data on the seroresponse to vaccination. In our review, the protection rates were calculated from Table 2 (subjects with titres >40, i.e. $\geqslant 60$, were supposed to be protected), and GMT values from Table 3.

Rudenko *et al.* (Ref. 48)

Eighty healthy volunteers were vaccinated with a trivalent SU vaccine containing A/Texas/1/77 (H3N2), A/Khabarovsk/74/77 (H1N1) and B/Hong Kong/8/73, on three dose levels and placebo-controlled, and with booster vaccination 1 month later. Serological data are given in Tables 1 and 2. The authors used the term 'seroconversion' rate without definition; we supposed it to be the response rate. We did not include data on influenza B, as titrations were done with a strain different from the vaccine strain (B/Leningrad/369/75). The vaccine series '01 i.m.' and '07 s.c.' were pooled by us, as they both contained 15 μg HA of each strain.

Jennings *et al.* (Ref. 25)

The authors evaluated three doses of a trivalent SU vaccine containing A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Lyon/1847/79, in young adults in a placebo-controlled study. The number of the volunteers entering the study is not given in the text of the paper; Table 1 reports on 132 subjects, Table 2 on 127 subjects including those receiving placebo. During the trial, there were natural influenza B infections among the participants (five out of 32 subjects receiving placebo); the data on the influenza B vaccine component were, therefore, not included here. Pre- and postvaccination antibody concentrations, presented as mean areas of haemolysis (in mm^2) were adapted from Table 2. In the text, the differences in postvaccination mean areas between the doses were described as not significant for the H3N2 vaccine strain. About the H1N1 vaccine strain, the text said: 'High postimmunization mean SRH antibody levels were also observed against A/Brazil, irrespectively of vaccine dosage administered'. This was interpreted by us as absence of significant differences between dosages for that strain as well. RR values were adapted from Table 1. Protection rates could not be calculated as the number of subjects protected before vaccination was not given.

Clarke *et al.* (Ref. 49)

Using the vaccine composition recommended for the season 1984/1985, two dosages of trivalent SPL vaccine were administered: A/Philippines/82 (H3N2) 10 versus 10, A/Chile/83 (H1N1) 10 versus 15, B/USSR/83 10 versus 15 μg HA. Thus, since for A/Philippines/82 (H3N2) no dose–response data are available, this strain has not been included in our review. Seroconversion is defined dependent on serostatus (seronegative subjects reaching a titre >40 after vaccination, seropositive subjects showing a fourfold increase), thus a combination of response and protection rate (Table 1). Those data are not included in our review. Protection rates and GMT values are derived from Table 3.

Arden *et al.* (Ref. 50)

Elderly, chronically ill subjects were vaccinated with trivalent SPL vaccine (15 μg HA each of A/Philippines/82 (H3N2), A/Chile/83 (H1N1) and B/USSR/83), and with either placebo or 45 μg HA B/USSR/83, thus a comparison between 15 and 60 μg HA B/USSR/83 was made. GMT values are described in the text. The authors tested the difference between the MFI values for the two dose groups and found $p=0.956$ by Wilcoxon's rank sum test. This was regarded as not significant by the authors, and in this review. Table 1 presents the protection rates for subjects unprotected before vaccination (<40). The authors used a *one*-tailed Fisher's exact test and regarded the difference between the protection rates as significant ($p<0.05$). In a *two*-tailed fashion this test would give $p=0.0816$, thus a non-significant difference for $\alpha=0.05$.

Gross *et al.* (Ref. 51)

A comparison is made between three dosages of two trivalent vaccine preparations (split, WV), thus 18 groups, in healthy, ambulatory elderly (health state well defined, mean ages given as 71–74, no age range reported). Of the subjects, 56–76% had been vaccinated

previously. Correction for prevaccination state is not discussed. The total number of volunteers is given as 148 in the text, but as 147 in Table 1. The numbers for split/Chile groups and WV/Chile groups are obviously confused in Table 1. The protection rates cannot be calculated because prevaccination data are not given. The authors report a significant difference in post-vaccination GMT between groups split/Chile/15 μg and split/Chile/45 μg, but when calculating MFI values, this difference is smaller because the prevaccination GMT values differ in these groups. Whether actual MFI values differ significantly cannot be decided here. The 15 μg HA dose groups are compared with a group of children with cystic fibrosis and young adults ($n=21$); these data are not included here.

Beyer *et al.* (Ref. 12)

Data are presented on healthy young students vaccinated with trivalent SPL vaccine, either 10 or 15 μg HA of each strain. The volumes of both doses differed per syringe (0.50 versus 0.75 ml), thus it can be assumed that the administration of vaccines was single-blinded but not double-blinded, as is stated in the text. Subjects were pretested for low prevaccination antibody titres. For calculations, subjects with high (protective) pre-vaccination antibody titres (influenza A >100, influenza B, ether-treated >200) were excluded. Absolute pre- and post-GMT values were recalculated from logarithm values. Table 4 also presents heterologous response to four other strains, and, as parameters, MFI and protection rates of responders only. These data were not included in this review.

Peters *et al.* (Ref. 52)

The authors describe a trial in elderly, some chronically ill, non-institutionalized subjects receiving trivalent vaccine (no information about vaccine type) each containing 15 μg HA of A/Philippines/82 (H3N2) and A/Chile/83 (H1N1), and either 15 μg B/USSR/83 (group I) or 60 μg B/USSR/83 (group III) into the right upper arm and, into the left upper arm, either placebo (group I and III) or 45 μg HA B/USSR/83 (group II). Numbers of subjects for pre- and first postvaccination sera: Group I, 42, group II, 44, group III, $45-2=43$. The data of groups II and III, receiving a total amount of 60 μg HA, have been pooled here. A second postvaccination sample (after 5 months) was drawn, but data are not presented here. Also, data about heterologous response were not included (they showed a difference between doses). The authors analysed the data by a regression model including age (not dependent), prevaccination state (dependent), and previous vaccinations (dependent).

Sullivan *et al.* (Ref. 53)

Healthy university personnel and students received a trivalent Triton-split vaccine containing A/Philippines/82 (H3N2), A/Chile/83 (H1N1) and B/USSR/83 with 7.5, 7.5, 7.5, or 15, 15, 15 or 30, 30, 30, or 15, 15, 45 μg HA. The authors combine the data of A/Philippines/82 (H3N2) and A/Chile/83 (H1N1) of the second and the fourth group. The authors looked at the data on response and protection rates ($\geqslant 32$), but, unfortunately, did not present these data as they did not find significant differences between dose groups for any strain.

Subbotina *et al.* (Ref. 54)

A mass vaccination trial is described in 2062 children and young adults (9–22 years of age) with a bivalent WV vaccine containing A/Kiev/59/79 (H1N1) and A/Leningrad/385/80 (H3N2). The vaccine doses were reported as 6–8 and 12–16 μg HA; we used 7 and 14 μg HA, respectively, for calculations. Whether a random allocation had been applied could be doubted considering the following statement: 'Vaccinations were conducted step by step starting with the oldest group to the youngest, first with a 6–8 μg HA dosage and then with the 12–16 μg HA dose'. Table 2 presents serological data for different age groups (pooled by us). The term 'seroconversion' is used but not defined by the authors; we supposed it to be an at least fourfold titre increase in subjects seronegative before vaccination.

Rautenberg *et al.* (Ref. 55)

Fifty-one patients on chronic intermittent haemodialysis were immunized with either a conventional dose (plus booster dose 5 weeks later), or a double dose of a trivalent SPL vaccine containing A/Singapore/6/86 (H1N1), A/Leningrad/360/86 (H3N2) and B/Ann Arbor/1/86. They used an indirect immunofluorescent test for IgA and IgG antibodies. Data were presented in Figures 1 and 2, and read, by us, for weeks 0 and 5 after (first) vaccination although sometimes peaks were reached at week 6. The presentation of the GMT values (as negative potency to the base 2) was changed by us (as reciprocal).

Guarnaccia *et al* (Ref. 56)

This study evaluated the practice of clinicians who diluted vaccine when immunizing subjects with allergy to egg proteins. In 29 healthy subjects (intake: 30), commercially available trivalent vaccine of unknown type was tested as original volume (0.5 ml), and as 1:5 and 1:10 dilutions. No information about contents in μg HA was given. Assuming 15 μg HA dose for the undiluted vaccine, as used in the United States at the time of performance of this study, the dosage groups would be 1.5, 3 and 15 μg HA. Little information on study design and no data about response or protection rates are provided. Pre- and 28-day-post-GMT-values are derived from Figures 1–3 according to $GMT=\exp(L*0.07+1.6)$, where L is the length of bar in mm. According to ANOVA done by authors, post-GMT of high and lower dosages differ significantly for the influenza A strains, but not for influenza B. However, pre- and post-vaccination titres *together* formed the dependent variable of the analysis, while the dosage was an independent variable. Whether the differences between the *MFI* values for influenza A strains were also significant cannot be judged here.

APPENDIX 2

Serological data from 20 dose–response studies

Ref.	Stratification group	Dose (μg HA)	N1	Pre GMT	Post GMT	MFI	Pre prot	Post prot	PR	N2	N Resp	RR
35	1	5.0	21	5	58	1.06	0	14	0.67	–	–	–
35	1	9.0	21	5	63	1.10	0	14	0.67	–	–	–
35	1	16.0	51	5	111	1.35	2	48	0.94	–	–	–
35	1	32.0	17	5	153	1.49	0	13	0.76	–	–	–
35	1	46.0	53	5	201	1.60	1	51	0.96	–	–	–
35	1	94.0	12	5	187	1.57	0	12	1.00	–	–	–
35	2	5.0	22	10	276	1.44	4	19	0.83	–	–	–
35	2	9.0	15	16	250	1.19	4	14	0.91	–	–	–
35	2	16.0	26	13	562	1.64	5	26	1.00	–	–	–
35	2	32.0	33	10	275	1.44	5	31	0.93	–	–	–
35	2	46.0	20	13	465	1.55	4	19	0.94	–	–	–
35	2	94.0	12	12	397	1.52	3	11	0.89	–	–	–
35	3	5.0	16	13	346	1.43	5	14	0.82	–	–	–
35	3	18.0	29	5	343	1.84	3	27	0.92	–	–	–
35	3	66.0	16	11	495	1.65	3	16	1.00	–	–	–
44	4	7.5	189	–	318	–	–	–	–	189	165	0.87
44	4	15.5	401	–	325	–	–	–	–	401	356	0.89
44	5	6.0	43	–	126	–	–	–	–	43	31	0.72
44	5	10.5	263	–	147	–	–	–	–	263	184	0.70
44	6	19.5	189	–	73	–	–	–	–	189	140	0.74
44	6	33.5	401	–	171	–	–	–	–	401	326	0.81
45	7	7.0	ng	–	88	–	–	–	–	–	–	–
45	7	20.0	ng	–	144	–	–	–	–	–	–	–
45	8	7.0	ng	–	60	–	–	–	–	–	–	–
45	8	20.0	ng	–	87	–	–	–	–	–	–	–
29	9	7.0	48	12	71	0.77	9	39	0.77	48	37	0.77
29	9	20.0	41	14	96	0.84	12	36	0.83	41	31	0.76
29	10	7.0	81	48	129	0.43	49	75	0.81	81	34	0.42
29	10	20.0	88	46	170	0.57	53	81	0.80	88	48	0.55
29	10	60.0	16	22	89	0.61	4	15	0.92	16	10	0.62
29	11	7.0	26	17	63	0.57	35	106	0.78	26	66	0.52
29	11	20.0	34	18	65	0.56	40	115	0.80	34	83	0.62
29	12	7.0	26	20	50	0.40	40	92	0.60	26	47	0.37
29	12	20.0	34	21	75	0.55	51	103	0.63	34	62	0.46
30	13	7.0	41	–	–	–	2	33	0.79	–	–	–
30	13	20.0	39	–	–	–	2	36	0.92	–	–	–
30	14	7.0	92	–	–	–	32	78	0.77	–	–	–
30	14	20.0	126	–	–	–	45	100	0.68	–	–	–
30	15	7.0	92	–	–	–	24	62	0.56	–	–	–
30	15	20.0	126	–	–	–	45	90	0.56	–	–	–
31[a]	16	2.3	42	5	39	0.89	0	28	0.67	–	–	–
31[a]	16	7.0	161	5	39	0.89	0	106	0.66	–	–	–
31[a]	16	20.0	152	5	56	1.05	0	121	0.80	–	–	–
31[a]	17	2.3	4	5	80	1.20	0	4	1.00	–	–	–
31[a]	17	7.0	49	5	55	1.04	0	36	0.73	–	–	–
31[a]	17	20.0	58	5	80	1.20	0	45	0.78	–	–	–
31[a]	18	2.3	9	5	86	1.24	0	7	0.78	–	–	–
31[a]	18	7.0	109	5	36	0.86	0	60	0.55	–	–	–
31[a]	18	20.0	111	5	44	0.95	0	64	0.58	–	–	–
46	19	7.0	18	15	84	0.75	3	15	0.80	18	12	0.67
46	19	60.0	29	20	170	0.93	8	29	1.00	29	21	0.72
47	20	7.0	40	8	125	1.19	0	34	0.85	40	31	0.78
47	20	10.0	46	9	172	1.28	0	42	0.91	46	40	0.87
47	21	7.0	50	6	20	0.52	–	–	–	50	28	0.56
47	21	15.0	53	5	31	0.79	–	–	–	53	36	0.68
33	22	5.0	25	17	87	0.71	8	18	0.59	25	13	0.52
33	22	10.0	24	17	138	0.91	4	17	0.65	24	16	0.67
33	22	20.0	24	10	153	1.18	2	21	0.86	24	21	0.88
33	22	40.0	23	9	322	1.55	2	22	0.95	23	22	0.96
48	23	7.5	10	6	42	0.85	–	–	–	10	8	0.80
48	23	15.0	29	6	52	0.94	–	–	–	29	20	0.69
48	23	20.0	20	6	120	1.30	–	–	–	20	12	0.60
48	24	7.5	10	9	158	1.24	–	–	–	10	10	1.00
48	24	15.0	29	6	95	1.20	–	–	–	29	21	0.72
48	24	20.0	20	6	91	1.18	–	–	–	20	15	0.75
25	25	6.0	32	15	103	6.87	–	–	–	32	30	0.94
25	25	12.0	31	14	88	6.29	–	–	–	31	26	0.84
25	25	24.0	32	12	86	7.17	–	–	–	32	31	0.97
25	26	6.0	32	30	104	3.47	–	–	–	31	22	0.71
25	26	12.0	31	29	125	4.31	–	–	–	30	23	0.77
25	26	24.0	32	22	123	5.59	–	–	–	29	27	0.93
49	27	10.0	47	20	900	1.65	18	45	0.93	–	–	–
49	27	15.0	49	20	930	1.67	19	47	0.93	–	–	–

Ref.	Stratification group	Dose (μg HA)	N1	Pre GMT	Post GMT	MFI	Pre prot	Post prot	PR	N2	N Resp	RR
49	28	10.0	47	10	240	1.38	14	46	0.97	..	..	..
49	28	15.0	49	10	200	1.30	13	47	0.94	..	..	..
50	29	15.0	25	25	66	0.42	12	19	0.54	..	..	..
50	29	60.0	25	19	77	0.61	8	22	0.82	..	..	..
51	30	15.0	25	19	43	0.35	–	..	..	25	9	0.36
51	30	30.0	23	14	44	0.50	–	–	–	23	10	0.43
51	30	45.0	24	22	70	0.50	–	..	..	24	12	0.50
51	31	15.0	25	13	38	0.47	–	–	..	25	10	0.40
51	31	30.0	23	11	50	0.66	..	–	–	23	12	0.52
51	31	45.0	24	18	76	0.63	–	..	–	24	13	0.54
51	32	15.0	25	18	43	0.38	..	..	..	25	6	0.24
51	32	30.0	23	20	50	0.40	..	..	–	23	7	0.30
51	32	45.0	24	19	54	0.45	–	..	..	24	9	0.38
51	33	15.0	24	13	28	0.33	–	..	..	24	9	0.38
51	33	30.0	26	11	32	0.46	..	–	–	26	13	0.50
51	33	45.0	25	22	27	0.09	..	..	..	25	5	0.20
51	34	15.0	24	21	62	0.47	–	–	–	24	10	0.42
51	34	30.0	26	19	60	0.50	..	–	–	26	14	0.54
51	34	45.0	25	24	58	0.38	–	..	..	25	10	0.40
51	35	15.0	24	16	28	0.24	..	–	–	24	4	0.17
51	35	30.0	26	18	31	0.24	–	..	..	26	6	0.23
51	35	45.0	25	14	22	0.20	..	..	..	25	4	0.16
12	36	10.0	45	10	318	2.10	0	41	0.91	45	43	0.96
12	36	15.0	39	11	622	2.15	0	38	0.97	39	39	1.00
12	37	10.0	42	8	490	1.78	0	37	0.88	42	36	0.86
12	37	15.0	34	9	407	1.64	0	29	0.85	34	30	0.88
12	38	10.0	46	11	202	2.02	0	42	0.91	46	45	0.98
12	38	15.0	36	10	741	1.88	0	34	0.94	36	35	0.97
52	39	15.0	42	18	78	0.64	15	34	0.70	42	21	0.50
52	39	60.0	87	27	94	0.54	42	69	0.60	87	44	0.51
53	40	7.5	35	5	19	0.58	..	..	..	..	..	..
53	40	15.0	70	4	18	0.65	–	–	–	..	..	..
53	40	30.0	35	5	27	0.73	..	..	..	..	..	..
53	41	7.5	35	8	53	0.82	..	..	..	..	..	..
53	41	15.0	70	8	70	0.94	–	..	..	..	..	..
53	41	30.0	35	11	105	0.98	–	–	..	..	..	..
53	42	7.5	35	6	36	0.78	–	–	..	..	..	..
53	42	15.0	35	6	30	0.70	..	..	..	..	..	..
53	42	30.0	35	6	37	0.79	..	..	..	..	..	..
53	42	45.0	35	6	45	0.88	–	–	–	..	..	..
54[a]	43	7.0	603	9	104	1.06	–	–	–	525	408	0.78
54[a]	43	14.0	534	10	157	1.21	–	..	..	468	367	0.78
54[a]	44	7.0	603	9	108	1.09	..	..	..	509	426	0.84
54[a]	44	14.0	534	9	128	1.13	–	..	..	447	362	0.81
55	45	15.0	25	549	1783	0.51	0	12	0.48	25	17	0.68
55	45	30.0	26	446	2702	0.78	0	19	0.73	26	20	0.77
55	46	15.0	25	64	362	0.75	0	5	0.20	25	12	0.48
55	46	30.0	26	97	832	0.93	0	10	0.38	26	14	0.54
55	47	10.0	25	294	676	0.36	1	4	0.13	25	5	0.20
55	47	20.0	26	194	832	0.63	0	8	0.31	26	16	0.62
56	48	1.5	10	7	32	0.66	..	–	..	..	..	..
56	48	3.0	10	11	73	0.82	–	..	..	..	..	..
56	48	15.0	9	14	153	1.04	–	..	..	–	..	..
56	49	1.5	10	22	47	0.33	..	..	..	..	..	..
56	49	3.0	10	19	104	0.74	–	..	–	..	..	..
56	49	15.0	9	26	176	0.83	..	–	–	..	..	..
56	50	1.5	10	8	17	0.33	–	..	–	..	..	–
56	50	3.0	10	9	23	0.41	–	–	–	–	..	..
56	50	15.0	9	9	38	0.63	..	–	–	..	..	..

N1, size of dose group for calculation of GMT and PR; Pre GMT/Post GMT, absolute pre-/postvaccination geometric mean titre; MFI, logarithmated meanfold increase: log(Post GMT/Pre GMT); Pre prot/Post prot, number of subjects protected before/after vaccination; PR, protection rate: (Post prot-Pre prot)/(N1-pre prot); N2, size of dose group for calculation of RR; Nresp, number of responders (subjects with $\geqslant$4-fold titre rise); RR, response rate (Nresp/N2); –, missing value; ng, not given

[a]Possibly not randomized