



## Evaluation of serological trials submitted for annual re-licensure of influenza vaccines to regulatory authorities between 1992 and 2002

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### ABSTRACT

**Background:** As part of the regulatory requirements, serological evaluation of trivalent inactivated influenza vaccines must be performed before annual re-licensure in the European Union. These studies are typically set up as uncontrolled, open label trials including 2 groups of at least 50 healthy adults and healthy elderly.

**Methods:** The serological data submitted to the Dutch Medicines Evaluation Board (MEB) for annual re-licensure purposes between 1992 and 2002, were analysed with respect to their ability to assess the immunogenic properties of the vaccines. The trials in this meta-analysis were selected by strictly applying the inclusion and exclusion criteria described in the Committee of Human Medicinal Products (CHMP) Note for Guidance on harmonisation of requirements for influenza vaccines. To select the final dataset additional exclusion criteria were defined: age outside the inclusion criterion of the trial, incomplete demographics, co-morbid conditions, antibody determination by SRH assay, incomplete dataset and sample size smaller than 50 subjects.

**Results:** Out of 51 trials retrieved from the archives, 48 age-defined trials including 2510 adults and 2008 elderly fulfilled all the in- and exclusion criteria. A large proportion of vaccinees already met the threshold for seroprotection at baseline. Post-vaccination, the serological response was shown to be age dependent. Previous influenza vaccinations significantly affected pre-vaccination but not post-vaccination titres.

**Conclusions:** The annual update trials performed for regulatory purposes have serious methodological limitations, which affect their ability to identify influenza vaccines with low immunogenicity. To establish clinical (protective) efficacy different trials and different assessment criteria are needed.

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### 1. Introduction

Many countries recommend annual influenza vaccination for the elderly and individuals with specified high risk conditions, with the objective to induce protection against influenza infection [1]. Annually, the World Health Organisation issues an updated recommendation for the vaccine composition, based upon the expected circulating strains. As, in general, the strain composition changes each year, influenza vaccines are re-licensed annually. Ideally, clinical vaccine efficacy would be established in experimental field trials [2], but for an annual re-licensure procedure, this approach is unrealistic.

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Within the European Union, marketing authorisation holders of influenza vaccines are required to provide clinical immunogenicity data to support the annual re-licensure procedure, in addition to pre-clinical requirements addressing Good Manufacturing Practice, toxicity (e.g., endotoxin content), and antigenic content (i.e., potency as determined by the single radial immunodiffusion assay). The “Note for Guidance on Harmonisation of requirements for influenza vaccines” (CHMP/BWP/214/96) [3] describes the requirements and criteria for serological annual update trials (the so-called “CHMP criteria”). This procedure can only be followed if a first licensure, evaluating quality, immunogenicity and safety of the vaccine has been issued.

The rationale of the CHMP criteria is to assess the immunogenicity of annual influenza vaccines (in the case of new components) according to three pre-specified serological criteria as a proxy for clinical protection: increase in geometric mean antibody titre, proportion of subjects with a predefined response to vaccina-

tion and the proportion of subjects seroprotected post-vaccination. Although these criteria are objective and are age specific, they do not account for factors that may affect the pre- and post-vaccination titres or, more important, may contribute to protection. For instance, individuals are exposed, by infection or vaccination, to many influenza virus (sub) types and strains during their lifetime, which results in a wide range of pre-vaccination titres in study subjects [4]. Also, age and health status may affect humoral responses to influenza viral antigens [5–12], although immune function may be preserved in those reaching advanced age in overall good health [13]. Such population characteristics can influence the haemagglutinin inhibiting (HI) antibody response induced by vaccination, independently of the immunogenicity of the vaccine. Vaccine-unrelated population characteristics may seriously hamper the interpretation of an immunogenicity trial and should be controlled for.

The annual re-licensure trials attempt to control for these confounding factors through age stratification and a requirement of good general health of the trial population. To address the variability in pre-vaccination titres, the three different serological criteria, seroprotection rate, titre increase, and response rate are used (see Section 2). However, it is not clear to what extent these measures result in a more reliable assessment of the vaccines' immunogenicity [4].

The objective of this study is to describe the serological criteria and to explore the influence of population characteristics such as pre-vaccination titre, age and vaccination history on post-vaccination titres in a meta-analysis.

## 2. Methods

### 2.1. Data collection

We derived serological data from 51 immunogenicity trials for eight different trivalent inactivated split virus and subunit influenza vaccines that were submitted to the Dutch Medicines Evaluation Board (MEB) as the clinical part of the annual re-licensure dossier during the period 1992/1993 to 2002/2003. These data are stored on microfiche in the archives of the MEB.

The marketing authorisation holders of inactivated influenza vaccines in the Netherlands approved the use of their serological data for this study, provided that all product identifiers were removed and no direct between-product comparisons were published.

### 2.2. Study design

According to the CHMP Note for Guidance on harmonisation of requirements for influenza vaccines [3], the immunogenicity of an influenza vaccine should be studied in an open label trial in at least 50 healthy adults aged 18–60 years and in at least 50 healthy elderly of 61 years or older. Blood samples are to be taken before and approximately 3 weeks after one dose and tested (in 2-fold dilutions) for anti-haemagglutinin antibody by either the haemagglutination inhibition (HI) assay or the single radial haemolysis (SRH) assay.

### 2.3. CHMP criteria

Three criteria are applied for evaluation of the immune response. First, the seroprotection rate (i.e., proportion of subjects with a post-vaccination titre  $\geq 40$  for the HI assay). For individuals aged 18–60 years seroprotection should be achieved post-vaccination in at least 70% of the vaccinees, in individuals >60 years in at least 60%. Second, the mean geometric increase, also called mean fold increase (MFI, i.e., the quotient of post- and

pre-vaccination geometric mean titres) should be at least 2.5 in individuals aged 18–60 years and  $\geq 2$  in individuals >60 years. Finally, the seroresponse rate (i.e., proportion of previously seronegative subjects exceeding a post-vaccination titre of 40, and proportion of previously seropositive subjects with a  $\geq 4$ -fold increase in pre- and post-vaccination sera) should be achieved in at least 40% of the vaccinees aged 18–60 years and in  $\geq 30\%$  in individuals >60 years. For each virus strain and each age class, at least one out of 3 criteria should be met.

### 2.4. Inclusion of dataset for statistical analyses

All trials derived from the archives were included in the first dataset. For inclusion in the final dataset, documentation of the subjects' age, general health state and the complete set of pre- and post-vaccination titres were mandatory. Six exclusion criteria were applied, three for the subjects and three for the trials. Subjects were excluded from the dataset when the age was outside the inclusion criterion of the trial. This means that from trials in adults, subjects <18 or >60 years-of-age were excluded, and from trials in elderly, subjects <61 years were excluded. Subjects with no recorded age were also excluded. Furthermore subjects with recorded co-morbid conditions qualifying for inclusion in the seasonal vaccination program were excluded, since inclusion in the trial requested being healthy. For the trials we only regarded antibody titres determined by the HI assay, SRH results were excluded. Also studies with incomplete dataset were excluded. Finally trials which covered both adult and elderly subjects, were divided into the two age categories (18–60, or  $\geq 61$  years-of-age). A trial, which, after applying these selections, included less than 50 subjects, was removed.

The archives contained 51 trials including 7126 subjects. A number of studies covered both age groups and were each separated into two age-defined trials (18–60, and >60 years). This resulted in a total of 71 strictly age-defined trials. Twenty-three (23) trials and 2608 subjects were removed as they met the exclusion criteria of this study. Of the remaining 48 age-defined trials (26 in adults and 22 in elderly), including 4518 vaccinees (2510 adults, 2008 elderly), gender was reported in 4407 (55.5% female). Thus these 48 trials comprise selected study populations from the original trials derived from archives and outcomes may thus diverge from the original evaluations submitted to the regulatory authorities for the purpose of annual re-licensure.

### 2.5. Statistical methods

For database management and calculations, Microsoft® Excel 2002 and SPSS for Windows 10.0.1 1999 were used. Where appropriate, post-vaccination GMTs were adjusted for pre-vaccination GMTs by linear regression as described in Beyer et al. [4]. For comparisons between continuous sample statistics (e.g., GMT-values of subjects with negative versus positive vaccination history), the ratio was used as the effect measure. For comparisons between binominal statistics (e.g., seroprotection rates), the absolute rate difference was used. Ratios from different trials were combined by the inverse variance-weighted method [14], and rate differences by the meta-analysis method of DerSimonian and Laird for binominal data [15]. Interval estimates were given as 95% confidence intervals (CI<sub>95%</sub>).

## 3. Results

### 3.1. Age distribution of the subjects analysed

Fig. 1 shows the age distribution of the subjects included in the analyses. The median age for adults was 36 years and for elderly

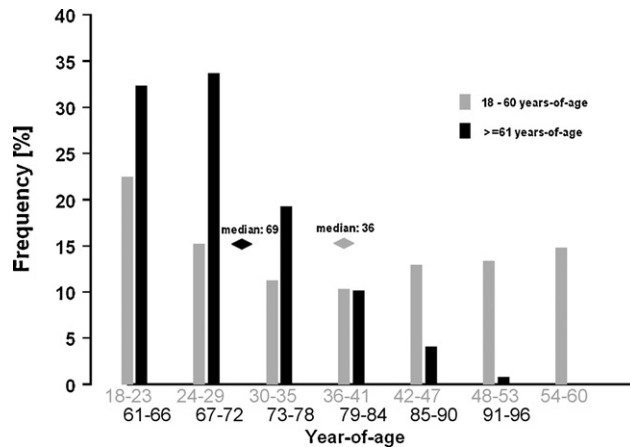


Fig. 1. Age distribution for adult (18–60 years) and elderly (>60 years) study populations.

69 years. Especially in the elderly population the majority of subjects was relatively young. This was strengthened by the observed decrease in median age of subjects included in the trials. In elderly median age decreased from 71 years in 1992 to 66 years in 2002 and in adults from 48 to 25 years (Fig. 2).

### 3.2. CHMP criteria in 48 age-defined trials

Table 1 presents the outcomes of the 48 age-defined trials. Single CHMP criteria were occasionally not met, most frequently the response rate (i.e., seroconversion or  $\geq 4$ -fold increase in HI titre). In most trials the seroprotection criterion (PR) was well above the threshold for each of the strains and age categories. In adults, the PR criterion of 70% was reached in all 26 trials for A/H1N1, in all but one for B strain and in 23 trials for A/H3N2. In elderly the PR criterion of 60% was not reached in one out of 22 trials for A/H1N1 and A/H3N2 and in 3 trials for B strain. The respective median post-vaccination GMTs were 161.8 for H1N1 (range 29.0–774.4), 149.8 for H2N3 (31.5–640.2) and 217.0 for B strain (range 16.0–794.0). The response rate (RR) and mean fold increase in GMTs (MFI) did less often meet the predefined criteria. However, only three times, a vaccine did not meet any the three CHMP criteria for one of the strains. In all instances this was in the elderly trial, while it did reach at least one of the criteria in the corresponding adult trial: trial 32 (1994, corresponding adult trial: 8), trial 36 (1996, corresponding adult trial: 17), and trial 47 (1997, corresponding adult trial: 25).

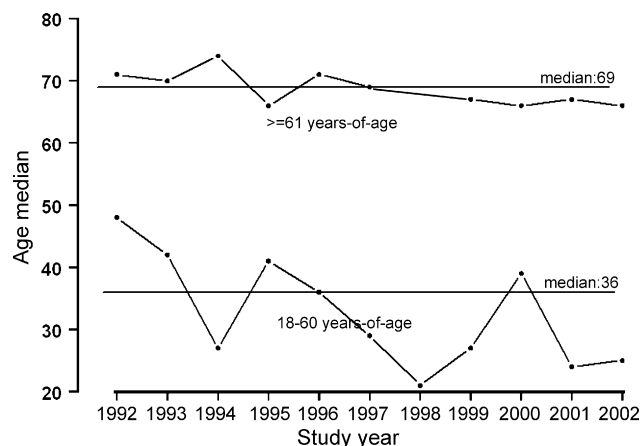


Fig. 2. Median age of the adult (18–60 years) and elderly (>60 years) study populations over the study period 1992–2002.

### 3.3. Baseline serostatus and the influence on the seroresponse

Table 2 shows the proportion of subjects seroprotected at baseline. Before vaccination at most 40% of the adults and 21% of the elderly were seronegative for at least one of the strains. In fact for the B strain in the elderly population the seroprotection criterion of 60% was almost reached prior to vaccination (59.6%). The relation between the baseline GMT and post-vaccination GMT is further exemplified in Fig. 3a–c in which pre- and post-vaccination seroprotection rates are shown for all individual trials, sorted by age class and pre-vaccination protection rates. A substantial number of trials had high pre-vaccination seroprotection rates, as shown in the black bars (illustrated as proportion of titres  $\geq 40$ ) especially in the elderly population.

Of the trials that failed to reach the post-vaccination threshold, virtually all had a low pre-vaccination GMT (data not shown). Although the combined pre-vaccination seroprotection rates were higher in elderly than in adults, the combined post-vaccination seroprotection rates were slightly lower in the elderly (see also Table 1). When, per trial, linear regression was performed with pre-vaccination titres as independent variable and post-vaccination titres as dependent variable, pre-vaccination titres were a significant predictor of post-vaccination titres in 70% of the trials.

### 3.4. The influence of age on pre- and post-vaccination titres in adults and elderly

To study to whether age may influence pre- and post-vaccination the 2 age categories were further broken down into 5-year age bands. This is illustrated in Fig. 4 where the age-specific pre- and post-vaccination GMT-values (black and grey bars), and the post-vaccination GMT-values corrected for pre-vaccination titres (black lines) are shown. Pre-vaccination GMT-titres were higher in the elderly than in the adults, but did not show a clear trend over the age bands. However, post-vaccination GMT-titres decreased with increasing age in adults and elderly for all three (sub)types (up to 4-fold for the A-H1N1 subtype in adults). This pattern was even clearer when post-vaccination titres were corrected for pre-vaccination titres. In terms of GMT increase, persons of  $\geq 80$  years responded poorly to vaccination.

### 3.5. Vaccination history and response to vaccination

Vaccination history (influenza vaccination in the year(s) before the trial) was recorded in only 22 trials. Although pre-vaccination seroprotection rate was higher in those with a recorded vaccination in the previous year(s) as compared to those with no history of vaccination, no difference in post-vaccination seroprotection rates was observed (1.1%; CI<sub>95%</sub>: 1.7%, 4.0%). Similar patterns were found for pre- and post-vaccination GMT (combined by the inverse variance-weighted method) for all 3 strains (data not shown).

## 4. Discussion

The present study intended to evaluate the value of annual serological trials of inactivated influenza vaccines for annual re-licensure. A requirement for such trials is unique to the EU regulatory system. The design of these trials is described in a regulatory guidance document [3] and the data are analysed by three predefined criteria, seroprotection rate, mean fold increase in antibody titre and response rate (the so-called “CHMP criteria”) in at least 50 healthy adults aged 18–60 years and at least 50 healthy elderly over 60 years of age.

To obtain re-licensure each strain needs to fulfil at least one of these criteria post-vaccination. Although the guidance document

**Table 1**  
Compliance of the trials with the CHMP criteria.

Age class	Trial	N	A-H3N2				A-H1N1				B			
			PR [%]	MFI	RR [%]	Req. met	PR [%]	MFI	RR [%]	Req. met	PR [%]	MFI	RR [%]	Req. met
CHMP criterion			≥70%	≥2.5	≥40%		≥70%	≥2.5	≥40%		≥70%	≥2.5	≥40%	
18–60 years	1	92	91.3	4.4	37.0 <sup>a</sup>	Yes	85.9	10.6	63.0	Yes	52.2	5.2	43.5	Yes
	2	60	98.3	24.3	85.0	Yes	100.0	40.1	93.3	Yes	100.0	14.3	73.3	Yes
	3	67	98.5	14.4	74.6	Yes	92.5	82.7	74.6	Yes	74.6	8.2	41.8	Yes
	4	58	100.0	14.5	74.1	Yes	96.6	42.4	93.1	Yes	77.6	11.8	67.2	Yes
	5	233	84.5	9.6	68.7	Yes	90.6	6.6	60.1	Yes	95.3	5.6	66.1	Yes
	6	115	84.3	11.1	76.5	Yes	88.7	3.2	40.9	Yes	100.0	4.2	54.8	Yes
	7 <sup>b</sup>	55	76.4	3.4	32.7	Yes	94.5	2.3	20.0	Yes				
	8	70	88.6	7.8	75.7	Yes	98.6	12.6	81.4	Yes	98.6	11.0	87.1	Yes
	9	52	69.2	5.6	55.8	Yes	88.5	3.5	53.8	Yes	100.0	3.2	50.0	Yes
	10	60	56.7	8.8	50.0	Yes	85.0	17.2	78.3	Yes	95.0	21.3	81.7	Yes
	11	57	80.7	8.4	63.2	Yes	96.5	9.8	59.6	Yes	96.5	6.6	59.6	Yes
	12	84	66.7	5.3	47.6	Yes	76.2	2.7	25.0	Yes	96.4	4.4	56.0	Yes
	13	70	70.0	4.9	48.6	Yes	81.4	2.6	25.7	Yes	98.6	3.8	45.7	Yes
	14	624	71.6	10.0	64.4	Yes	90.7	13.9	76.4	Yes	95.0	12.8	84.0	Yes
	15	70	78.6	6.3	67.1	Yes	81.4	7.5	62.9	Yes	71.4	5.7	60.0	Yes
	16	72	100.0	4.7	40.3	Yes	100.0	2.0	20.8	Yes	100.0	3.8	38.9	Yes
	17	117	88.9	4.4	47.0	Yes	73.5	3.0	30.8	Yes	88.0	1.8	16.2	Yes
	18	51	100	2.4	35.3	Yes	100.0	11.1	74.5	Yes	98.0	6.3	68.6	Yes
	19	81	95.1	13.8	70.4	Yes	91.4	22.6	67.9	Yes	71.6	8.2	46.9	Yes
	20	65	98.5	11.3	66.2	Yes	89.2	54.2	83.1	Yes	87.7	7.3	55.4	Yes
	21	61	95.1	10.2	78.7	Yes	88.5	9.8	72.1	Yes	98.4	8.5	73.8	Yes
	22	64	100.0	4.8	50.	Yes	92.2	29.6	70.3	Yes	82.8	7.0	57.8	Yes
	23	59	98.3	8.0	67.8	Yes	98.3	24.4	83.1	Yes	98.3	13.0	84.7	Yes
	24	57	98.2	10.9	71.9	Yes	100.0	13.9	77.2	Yes	100.0	8.9	68.4	Yes
	25	60	96.7	3.1	28.3	Yes	100.0	7.0	53.3	Yes	66.7	3.2	31.7	Yes
	26	56	100.0	19.1	73.2	Yes	100.0	10.8	66.1	Yes	98.2	3.2	39.3	Yes
CHMP criterion			≥60%	≥2	≥30%		≥60%	≥2	≥30%		≥60%	≥2	≥30%	
>60 years	27	66	63.6	2.0	12.1	Yes	68.2	2.5	22.7	Yes	28.8	2.8	25.8	Yes
	28	56	94.6	9.3	58.9	Yes	94.6	12.3	69.6	Yes	96.4	6.3	62.5	Yes
	29	180	82.8	6.7	63.9	Yes	77.8	3.6	43.9	Yes	92.2	5.3	61.7	Yes
	30	102	80.4	10.6	69.6	Yes	77.5	2.6	31.4	Yes	100.0	3.4	49.0	Yes
	31 <sup>b</sup>	51	62.7	3.1	35.3	Yes	86.3	2.3	21.6	Yes				
	32	78	42.3	1.9	21.8	No	60.3	1.6	16.7	Yes	65.4	2.0	19.2	Yes
	33	50	66.	4.8	50.0	Yes	76.0	2.6	24.0	Yes	100.0	2.9	32.0	Yes
	34	57	64.9	9.1	57.9	Yes	73.7	14.3	68.4	Yes	89.5	23.5	73.7	Yes
	35	66	93.9	5.2	53.0	Yes	86.4	3.7	37.9	Yes	93.9	6.4	53.0	Yes
	36	109	76.1	2.2	25.7	Yes	54.1	1.5	8.3	No	96.3	1.4	8.3	Yes
	37	100	88.	4.5	53.0	Yes	99.0	1.9	21.0	Yes	74.0	2.6	26.0	Yes
	38	55	100.0	1.4	9.1	Yes	100.0	2.0	18.2	Yes	94.5	1.9	10.9	Yes
	39	442	99.8	3.4	50.2	Yes	98.4	2.6	33.0	Yes	97.5	3.2	44.8	Yes
	40	56	98.2	8.8	28.6	Yes	96.4	12.3	17.9	Yes	96.4	6.6	35.7	Yes
	41	74	74.3	3.4	28.4	Yes	62.2	3.7	33.8	Yes	47.3	2.6	18.9	Yes
	42	105	87.6	5.4	54.3	Yes	42.9	3.2	21.0	Yes	94.3	2.9	33.3	Yes
	43	57	87.7	6.0	61.4	Yes	82.5	7.9	61.4	Yes	100.0	6.3	61.4	Yes
	44	75	93.3	7.0	57.3	Yes	57.3	3.9	37.3	Yes	92.0	5.2	50.7	Yes
	45	55	94.5	6.1	56.4	Yes	94.5	8.3	58.2	Yes	94.5	7.9	60.0	Yes
	46	53	98.1	6.1	60.4	Yes	98.1	5.9	50.9	Yes	100.0	5.1	50.9	Yes
	47	63	93.7	3.3	28.6	Yes	100.0	3.9	42.9	Yes	41.3	1.9	11.1	No
	48	58	89.7	10.8	67.2	Yes	87.9	7.0	50.0	Yes	89.7	6.3	44.8	Yes

Abbreviations: PR=Protection rate, MFI=mean fold increase, RR=response rate; Req. met: predefined CHMP requirement met (i.e., at least one of the assessment criteria fulfilled for the specific strain).

<sup>a</sup> Marked entry: trial result not meet the CHMP criterion.

<sup>b</sup> Bivalent vaccine without B component.

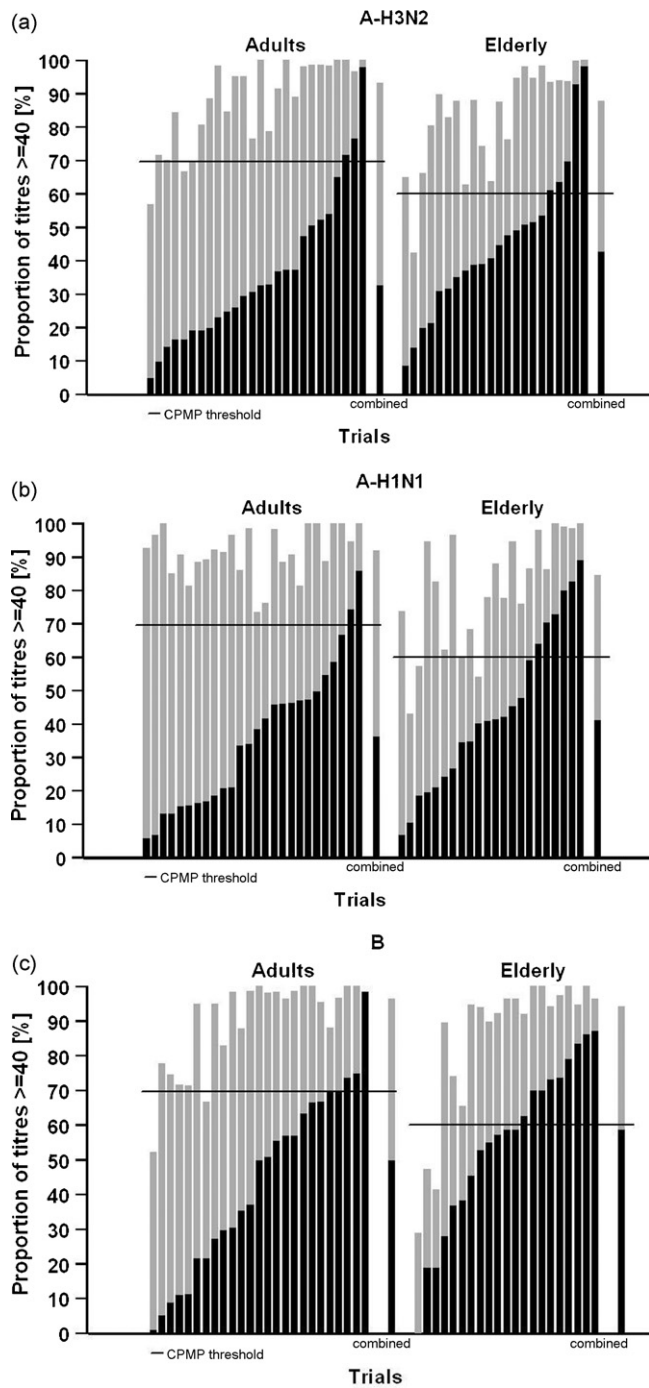
defined the in- and exclusion criteria, review of the study population indicated these are generally not fully adhered to. However, for the present analyses these in- and exclusion criteria were strictly applied to the trials and the participants in the trials, excluding

subjects outside the predefined age range, with co-morbidity, or with incomplete data and trials analysed by serial radial haemolysis (SRH) assay, or with less than 50 evaluable subjects. This resulted in 48 age-defined trials and a more homogenous selection of the

**Table 2**  
Baseline serostatus.

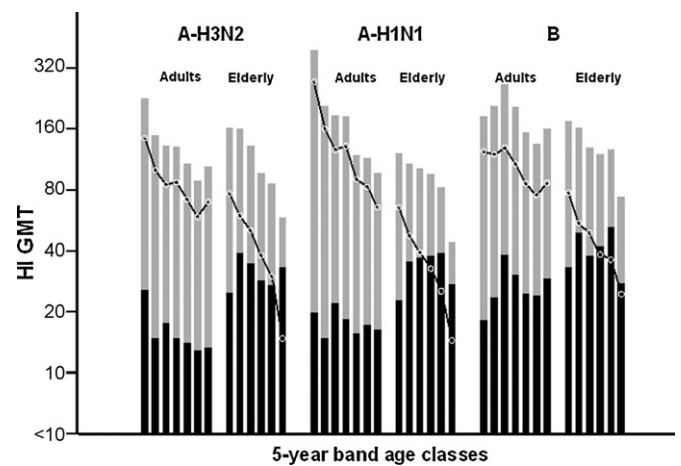
Pre-vaccination titre	No. (%)			
	Adults		Elderly	
	Seronegative (<10)	Seroprotected (≥40)	Seronegative (<10)	Seroprotected (≥40)
A/H3N2	996 (39.7)	729 (29.0)	399 (19.9)	1066 (53.1)
A/H1N1	101 (40.4)	812 (32.4)	426 (21.2)	1019 (50.7)
B	646 (26.3)	1031 (42.0)	265 (13.5)	1166 (59.6)

Seronegative (<10) indicates an HI-titre < 10 before vaccination. Seroprotected (≥40) indicates an HI-titre ≥ 40 before vaccination.



**Fig. 3.** (a–c) Pre- and post-vaccination protection rates (defined as HI-titre  $\geq 40$ ) for the individual trials and the combined ratio throughout the trials, stratified for age class (a) A-H3N2 strain, (b) A-H1N1 strain, and (c) B strain. The trials are ranked in order of increasing pre-vaccination seroprotection rate. X-axis: Combined means average seroprotection rate in the adult (left column) and elderly (right column) trials. Y-axis: Proportion of titres  $\geq 40$  (%) means the seroprotection rate (PR, see also Table 1). Dark grey columns: Pre-vaccination seroprotection rate. Light grey columns: Post-vaccination seroprotection rate. CHMP threshold: For subjects 18–60 years  $\geq 70\%$ , for elderly  $\geq 60\%$ .

original trial population. All 26 trials in adults and 19/22 trials in elderly fulfilled at least one criterion for all 3 strains. The 3 vaccines that did not meet any of the criteria for one of the 3 strains, failed in the elderly population on 3 different strains and concerned 3 different products. The cause for failure is unknown. Stratified analyses by age indicated a higher pre-vaccination titre with increasing age, but a lower response on vaccination. And whereas the limited data



**Fig. 4.** Geometric mean titres within 5-year band age classes. HI: Haemagglutinin inhibition; GMT: geometric mean titre. Dark grey columns: Pre-vaccination GMT. Light grey columns: Post-vaccination GMT. White circles: Post-vaccination GMT corrected for pre-vaccination titres by linear regression.

on vaccine history did suggest an impact on pre-vaccination titre, it did not on post-vaccination titre. This is in accordance with earlier observations [16]. However, for a single trial it does not exclude unwanted large variation by its strong positive influence on the pre-vaccination state. In this respect it may well be that in the original datasets in which the selection criteria were not applied, at least one of the criteria was met, especially since in all 3 cases one of the criteria only marginally failed the criterion threshold. For the purpose of annual re-licensure it is thus possible that different conclusions were drawn, however, because of blinding of the original product identifiers this cannot be checked. Over the period 1992–2002 no vaccine was refused because of failing to reach the assessment criteria.

The CHMP guideline gives the post-vaccination seroprotection rate a prominent role as serological surrogate marker for clinical protection. The 2 other parameters, the seroresponse rate and the mean fold increase in antibody titre, were added to the assessment criteria to control for pre-vaccination titre. The two age classes with different thresholds with respect to the serological parameters were introduced to address the decline of antibody induction with increasing age, and the inclusion of only healthy individuals should preclude an impact of underlying disease on immune responsiveness. However, it is doubtful whether these additional measures are sufficient to control for remaining confounding factors. First, baseline serostatus plays an important role in the response to vaccination. A high pre-vaccination titre requires only a small amount, or no, newly induced antibody to reach the required post-vaccination antibody titres. This precludes a reliable assessment of the antibody-inducing potency of a vaccine using the CHMP criteria. Correction of increase in geometric mean titre or response rate by pre-vaccination titre has been shown to be insufficient since these parameters remain dependent on pre-vaccination titre [4]. Also in the present study pre-vaccination titre was shown to be predictive for post-vaccination seroprotection. Of all the trials that failed to reach the post-vaccination seroprotection threshold, virtually all had low pre-vaccination GMTs. Second, the CHMP inclusion criterion of being healthy may not exclude unapparent pathological conditions. Our data were not detailed enough to assess this possibility. In addition not all trials adhered to this inclusion criterion. We adjusted for health status by excluding every individual with recorded co-morbidity (473 subjects, 6.7% of the original dataset), but co-morbidity of individual subjects was recorded in a minority of the trials, and in none of the trials conducted prior to 1995. Finally stratification in two age groups showed to be insufficient to con-

trol for the age dependency of the antibody response. In our study it was shown that within the predefined age categories increasing age did not have a marked influence on pre-vaccination titre, but did have a strong negative impact on post-vaccination titre.

Immunogenicity of influenza vaccines is determined each year in open label uncontrolled trials of limited size. Due to the lack of a comparator group and lack of random treatment assignment the immunogenic properties of the vaccines cannot be assessed unbiased. The main limitation of these small scale uncontrolled immunogenicity trials, is however, to control for (remaining) confounding factors. Stratification and restrictions are only partly successful in this. There are several population characteristics that can be controlled for, such as age, gender and health status to a certain extent. However, there are also many that cannot be controlled for, but all are correlated. For example, pre-vaccination titre, comorbidity and previous vaccinations may themselves be dependent on age, and again pre-vaccination titres on previous vaccinations, resulting in a complex pattern of interactions. A limitation of the present meta-analysis is that we could not account for variability in the HI assays which is known to be strongly influenced by protocol, laboratory and investigator.

It should be noted that the EU is the only region in which these clinical update trials are presently requested for annual re-licensure. Other regulatory authorities, like FDA only require assessment of the potency of the annually produced influenza vaccines by *in vitro* tests using a standardised SRH assay, which is also required in the CHMP NfG [3] and, which in several studies is validated for protection [17–19].

Ideally, each influenza vaccine should prove its quality, efficacy and safety in a sufficiently large dataset before first licensure. The subsequent annual update trials are a clinical model to address consistency of quality of production, a purpose for which they were initially developed and which may be valid. The value of CHMP criteria is in having a simple clinical tool to address seroresponsiveness to a seasonal influenza vaccine. This requires a more or less homogenous population, with minimal interference of disturbing population characteristics. For influenza vaccines this means a relatively naïve healthy individual, e.g., a healthy young adult with no vaccination history. The presently conducted annual update trials do not fulfil this requirement.

A drawback of the CHMP criteria is the ease of use. Although the assessment criteria are defined for annual re-licensure only, they are also used to substantiate efficacy claims for newly developed unadjuvanted seasonal vaccines [20], and adjuvanted seasonal [21] and (pre)pandemic vaccines [22] disregarding the different immunology conferred by the adjuvant and the population factors relevant for the response with pandemic strains. The need to define additional endpoints as surrogate parameters was also recently expressed in a joint WHO regulatory meeting [23]. Although for seasonal vaccines the correlates (i.e., CHMP criteria) applied for regulatory purposes were considered valid, the need to include endpoints that reflect a broad range of immune responses was emphasized. This is particularly relevant in support of the development of effective (new) influenza vaccines in special circumstances (i.e., a pandemic) and special populations (e.g., children).

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