Aluminium adjuvants used in vaccines versus placebo or no intervention (Protocol)

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Aluminium adjuvants used in vaccines versus placebo or no intervention (Protocol)

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Aluminium adjuvants used in vaccines versus placebo or no intervention

Snezana Djurisic1, Janus C Jakobsen2, Sesilje B Petersen3, Mette Kenfelt4, Christian Gluud2

1Copenhagen Trial Unit, Centre for Clinical Intervention Research, Department 7812, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. 2The Cochrane Hepato-Biliary Group, Copenhagen Trial Unit, Centre for Clinical Intervention Research, Department 7812, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. 3Department of Occupational and Environmental Medicine, Bispebjerg Hospital, Copenhagen, Denmark. 4Birkerød, Denmark

Contact address: Snezana Djurisic, Copenhagen Trial Unit, Centre for Clinical Intervention Research, Department 7812, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. snezana.djurisic@ctu.dk, sdjurisic@gmail.com.

Editorial group: Cochrane Hepato-Biliary Group.
Publication status and date: Edited (no change to conclusions), published in Issue 10, 2017.

Citation: Djurisic S, Jakobsen JC, Petersen SB, Kenfelt M, Gluud C. Aluminium adjuvants used in vaccines versus placebo or no intervention. Cochrane Database of Systematic Reviews 2017, Issue 9. Art. No.: CD012805. DOI: 10.1002/14651858.CD012805.

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To assess the benefits and harms of aluminium adjuvants used in vaccines versus placebo or no intervention, taking into consideration the type of the vaccine, and the type, size, and concentration of the aluminium adjuvant.

BACKGROUND

Description of the condition

The effectiveness of vaccinations has been proven repeatedly since the first introduction of the cowpox vaccine in the 18th century (Delany 2014; Whitney 2014). In fact, vaccination is considered one of the major triumphs of modern medicine (Delany 2014; Whitney 2014). Vaccination prevents infectious diseases (Delany 2014; Whitney 2014), and the worldwide eradication of the highly contagious and deadly smallpox and the restriction of diseases such as polio, measles, and tetanus can largely be ascribed to the numerous successful mass vaccination programmes launched since the 1960s (Delany 2014; Whitney 2014). In the late 1940s and 1950s, prior to public vaccinations, the poliovirus caused infantile paralysis associated with high mortality in hundreds of thousands of children (Global Polio Eradication Initiative 2016; WHO 2016c). The measles virus was responsible for post-infectious encephalomyelitis in 1 per 1000 infected individuals, leaving most with permanent impairment of the central nervous system (Miller 1964; CDC 2016). Today, polio is nearly eradicated (WHO 2016c), and global measles death has decreased by 79%, with an estimated 17.1 million deaths prevented from 2000 to 2014 (WHO 2016).

Current routine vaccine programmes recommended by the World Health Organization (WHO) include vaccines against Bacillus Calmette-Guérin (BCG); hepatitis; polio; diphtheria, tetanus, and pertussis (DTP); haemophilus influenza type B; pneumococcal bacteria; rotavirus; measles; rubella; and human papilloma virus (HPV) (WHO 2017). Additional programmes are proposed for certain regions, high-risk populations, and for fighting pathogens with certain characteristics (e.g. Bacillus anthracis causing anthrax).
Vaccination mimics infection in the body leading to activation of a potent immune response (Coffman 2010; Kool 2012; O’Hagan 2012; Oleszycka 2014). The vaccines are two types: traditional vaccines which contain vaccine antigens and excipients (substance formulated alongside the active vaccine ingredient) and do not use adjuvants (substance that aid the immune response to an antigen); and vaccines of a newer generation which typically require the addition of adjuvants to the vaccine antigens and the excipient (Coffman 2010; O’Hagan 2012). Vaccine antigens may comprise whole attenuated pathogens, pathogen components, virus-like particles, or genetic material of the pathogen (Strugnell 2011). Like other medicinal products, vaccines undergo preclinical testing for safety, for the ability to induce cancer, for the ability to induce immune response, and for overall efficacy before they are licensed. However, rare adverse events or adverse events with delayed onset are not easily detected during the relatively short duration of most preclinical and clinical phase studies, and as proven over the years, safety surveillance in the general population post marketing is essential (Ward 2000; Chen 2005). As an example, the childhood measles-mumps-rubella (MMR) vaccine was introduced in the late 1960s as a mixture of three live attenuated viruses, administered via injection (Olfit 2007). Over time, doubts about its safety were raised when serious cases of fever seizures, meningitis, and allergic reactions were reported (Kimura 1996; Dourado 2000; Ward 2000). In Japan, a nationwide surveillance programme launched in the early 1990s screened more than 38,000 children vaccinated with four different Urabe-containing MMR vaccines (Kimura 1996). Serious adverse events included convulsions and aseptic meningitis, and the incidence was shown to be linked to different vaccine strains of mumps virus (Kimura 1996). During the same time period, Brazil experienced a mass outbreak of aseptic meningitis following a Urabe-containing MMR vaccine with an estimated risk of 1 in 14,000 doses (Dourado 2000). As another example, the DTP vaccine was licensed in the late 1940s as a preparation of three different antigen components (trivalent vaccine) adsorbed to aluminium salt. It was suspected to cause acute encephalopathy and chronic nervous system dysfunction (Cowan 1993). Reports, prepared by the Institute of Medicine (IOM) in the US, concluded that the evidence was insufficient to indicate a causal relationship between the DTP vaccine and acute or permanent neurologic damage (Cowan 1993). In 2000, the WHO published a critical bulletin on vaccine safety, including an overview of serious vaccine-associated adverse events for which causality had been established or was highly likely (Ward 2000). Among several vaccine-specific serious adverse events, they found a causal relationship between vaccines against DTP hepatitis, MMR, and polio and disease dissemination, severe allergic reactions, or death (Ward 2000).

Vaccine toxicity may originate from a plethora of factors, including the vaccine components (e.g. the antigen itself, the adjuvant, or the excipients), interaction between different vaccine components, vaccine manufacture, overall vaccine composition, route of administration, dose, and number of vaccinations (Kocourkova 2016). One of the latest programmes added to the mass vaccination portfolio is the public HPV vaccination programme launched in the US a decade ago (WHO 2014). Human papilloma virus causes cervical cancer; the second most common cancer form in women (WHO 2014), and thus, the aim of the HPV vaccination is to prevent development of cervical cancer. More than 60 countries included the HPV vaccine in their routine vaccinations after its market approval (Bruni 2016). In recent years, concerns were raised about adverse events possibly related to the HPV vaccines. Since the US approval of Gardasil® in 2006 and up until 2012, a total of 21,265 adverse events was reported to the national Vaccine Adverse Event Reporting System (VAERS) (Tomijenovic 2012). HPV vaccines have been blamed for giving rise to more reported adverse events than other types of vaccines (Tomijenovic 2012). In the European Union, the European Medical Agency (EMA) has received similar reports, but found no scientific evidence for an association (EMA 2015). Several observational studies also failed to identify associations with clinical diagnoses (Klein 2012; Arnheim-Dahlstrom 2013; Donegan 2013; Grimaldi-Bensouda 2014; Scheller 2015), but reasons to oppose these findings have been put forward (Brinth 2015; Brinth 2015a; Dyer 2015; Gotszsche 2016; Gotszsche 2016a). The symptoms reported following HPV vaccination are varied and include headache, orthostatic intolerance, fatigue, cognitive dysfunction, blurred vision, feeling bloated, abdominal pain, light sensitivity, and involuntary muscle activity (Brinth 2015; Brinth 2015a). Despite the consistency in reported symptoms, they do not fit into a well-defined category of diseases or diagnoses, but rather present themselves as a constellation of nonspecific symptoms (Brinth 2015; Brinth 2015a). Consequently, the observational studies, which based their results on registered diagnoses, may have excluded an important fraction of eligible participants with unclear adverse symptoms, as most young girls that claim to suffer from adverse events following HPV vaccination receive no clinical diagnosis, and are therefore unlikely to appear in medical registers. Moreover, the randomised clinical trials on HPV vaccines, which formed the basis for the safety assessment, have been blamed for not using true placebo (e.g. placebo not containing adjuvants) as the control intervention (Exley 2011).

**Description of the intervention**

Adjuvants are added to vaccines to enhance the ability to provoke an immune response of weak antigens and improve the overall potency of the vaccine (O’Hagan 2009; Coffman 2010). Adjuvants may also pose other benefits, such as reducing the frequency of vaccination and the dose of antigen per vaccine, and some may provide cross-clade immunity (i.e. immunity against different clades of viruses or different clades of bacteria descending from different ancestors) or improve the stability of the final vaccine formu-
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Aluminium is the third most abundant element in the earth’s crust, but the metal has no known biological or physiological role (Reinke 2003). It is absorbed into the blood through the gastrointestinal tract, and rapidly eliminated by the kidneys and bile (Reinke 2003). While aluminium is considered safe and regularly ingested with food and water, it is toxic in high concentrations (Kisnerienė 2015). The toxicity, however, not only depends on the concentration, but on the chemical form and the environment as well (Kisnerienė 2015). In the blood, aluminium is bound to transferrin with high affinity, where it competes with iron at the binding site (Kisnerienė 2015). Aluminium also affects cellular processes and physiological functions (Kisnerienė 2015). For instance, aluminium competes with magnesium for membrane transporters; disturbs calcium metabolism; increases oxidative stress; binds to the phosphate groups of nucleoside di- and triphosphates; and also binds to metal-binding organic compounds (amino acids) and membrane lipids (Kisnerienė 2015).

In high concentrations, aluminium is predominantly accumulated in bone and brain tissue (Yokel 2000; Malluche 2002). In animal and human studies, it has been shown to act as a powerful neuro-logical toxicant and provoke toxic effects in foetuses and embryos if exposed during pregnancy (Reinke 2003). This is supported by recent data indicating that aluminium is able to cross the blood-brain barrier by directly affecting the cerebral blood vessels (Chen 2008; Sharma 2010).

Despite its unclear biological role, aluminium seems to have an impact on the immune system, which has rendered it useful as a vaccine adjuvant (Tritto 2009; Kool 2012). Aluminium binds antigens with high affinity (antigen adsorption) and was originally thought to exert its function by forming a depot, which allows for a high antigen concentration at the site of injection, and a continuous desorption and dispersion of antigens from the aluminium particles (Kool 2012). Nowadays, aluminium is believed to exert its adjuvantic effects by stimulating Th2-type responses and antibody production through B cells activation (Grun 1989; Awate 2013), by activating the complement system, and by recruiting immune cells to the site of injection (Ramanathan 1979; Goto 1997; Awate 2013). At the injection site, aluminium promotes antigen uptake by specialised antigen-presenting immune cells, termed dendritic cells, and dendritic cell maturation (Mannhalter 1985; Morefield 2005; Kool 2008).

One important aspect of adjuvants is the size of the particles, which seems to have a considerable influence on the immune response. Aluminium hydroxide adjuvant is comprised of particles with a calculated dimension of 100 nm, while aluminium phosphate particles are around 50 nm (Hem 2007). In an aqueous (water) solution, particles of both aluminium salts aggregate to form 1 to 20 µm sized particulates (Hem 2007). This size is also known as microscale-size. Aluminium hydroxide and aluminium phosphate can be produced in nanoscale-size ≤ 200 nm, but so far, only amorphous (non-crystalline solid) aluminium hydroxyphosphate sulfate is commercially produced on nanometre-scale, and represents one of the latest marketed commercial aluminium adjuvants.

How the intervention might work

Aluminium is the third most abundant element in the earth’s crust, but the metal has no known biological or physiological role (Reinke 2003). It is absorbed into the blood through the gastrointestinal tract, and rapidly eliminated by the kidneys and bile (Reinke 2003). While aluminium is considered safe and regularly ingested with food and water, it is toxic in high concentrations (Kisnerienė 2015). The toxicity, however, not only depends on the concentration, but on the chemical form and the environment as well (Kisnerienė 2015). In the blood, aluminium is bound to transferrin with high affinity, where it competes with iron at the binding site (Kisnerienė 2015). Aluminium also affects cellular processes and physiological functions (Kisnerienė 2015). For instance, aluminium competes with magnesium for membrane transporters; disturbs calcium metabolism; increases oxidative stress; binds to the phosphate groups of nucleoside di-and triphosphates; and also binds to metal-binding organic compounds (amino acids) and membrane lipids (Kisnerienė 2015).

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Different insoluble aluminium salts have been used as vaccine adjuvants since 1926 (Glenny 1926). Aluminium potassium sulphate was the first used. However, because of poor reproducibility, it has been almost completely replaced by aluminium hydroxide and aluminium phosphate, as they can be prepared in a more standardised way, and capture antigens by direct adsorption (Marrack 2009). Aluminium has been the standard adjuvant in vaccines such as those against diphtheria, tetanus, and pertussis, haemophilus influenza type B, pneumococcus conjugates, hepatitis A, and hepatitis B (Tritto 2009). More recently, it was co-formulated with vaccines against HPV in the form of AS04 (containing aluminium hydroxide) and amorphous aluminium hydroxyphosphate sulfate. Amorphous aluminium hydroxyphosphate sulfate is commercially produced in nanoscale-size, and represents one of the latest marketed aluminium adjuvants.

The mechanism of action of aluminium, like for most adjuvants, is poorly understood, and widespread beliefs change according to continuously new insights into immunology and physiochemical properties of aluminium (see How the intervention might work) (Carter 2010; Tomljenovic 2011). Despite our incomplete understanding of its effects, the repeated use of aluminium in vaccines is justified by its apparent safety profile, ease of preparation, stability, potent immunostimulatory ability (O’Hagan 2009; Tritto 2009; Mbow 2010), and importantly, due to the lack of suitable alternatives.
tire, shape, and surface charge have also been demonstrated to greatly affect uptake by dendritic cells (Thiele 2001; Foged 2005; Bartneck 2012; Son 2013).

### Why it is important to do this review

One previous attempt to assess the potential toxic effects of aluminium adjuvant with a systematic review was undertaken in 2004 by Jefferson and colleagues (Jefferson 2004). The systematic review covered existing evidence of adverse events after exposure to the aluminium-containing DTP vaccine, but it did not assess benefits (Jefferson 2004). The authors included three randomised trials, four semi-randomised trials, and one cohort study, and they were unable to demonstrate that aluminium adjuvant was responsible for any serious or long-lasting adverse events (Jefferson 2004). The authors advised the ending of future research despite concluding that their finding was based on poor-quality evidence (Jefferson 2004).

More than 10 years has passed since the systematic review by Jefferson and colleagues, new adjuvants are being introduced continuously, and FDA and WHO do not require genotoxicity or cardiotoxicity studies of new aluminium adjuvants (WHO 2014a; FDA 2015). Lately, symptoms following HPV vaccination have been suspected of being caused by the addition of aluminium adjuvant (Tomljenovic 2011; Lee 2012; Poddighe 2014; Brinth 2015a; Gruber 2015; Martinez-Lavin 2015). A recent animal study by Inbar and colleagues managed to spark further controversy by demonstrating behavioral abnormalities in mice administered the aluminium-containing HPV vaccine Gardasil (Inbar 2016a). Compared to previous animal studies on HPV vaccines, the authors included two control groups: one where mice were administered aluminium adjuvant alone and another with placebo without adjuvant (Inbar 2016a). Inbar and colleagues concluded that Gardasil via both its aluminium adjuvant and HPV antigens can trigger neuro-inflammation and autoimmune reactions, leading to behavioural changes in mice (Inbar 2016a). Upon submission to a peer-reviewed journal, the paper was accepted with revisions, and published. However, it was soon withdrawn by the editor (Inbar 2016), only to be published in a competing journal shortly thereafter (Inbar 2016a). The initial withdrawal was allegedly due to “unsound scientific results”; an assertion which was not supported by the final publisher.

The theory that aluminium adjuvant is responsible for symptoms following HPV vaccination is impossible to refute or prove based on the current data. Aluminium adjuvant has been administered to both experimental and control group in the vast majority of randomised clinical trials on HPV vaccines, thus masking its potentially harmful effects (Exley 2011). Clinical trials designed to administer vaccine adjuvants to the experimental group as well as the placebo group do, de facto, not compare an intervention against a true placebo, and therefore, do not adequately assess safety (Exley 2011). Indeed, aluminium adjuvants, new or old, should be evaluated for benefits and harms on their own merits.

Aluminium is the most frequently used adjuvant, introduced in vaccination programmes worldwide (Tritto 2009). While the consequences of adding aluminium to vaccines have been discussed broadly, no systematic review has been conducted to assess the effects of aluminium adjuvants across vaccines. The effects of aluminium adjuvants remain to be properly assessed using Cochrane methodology to determine whether they are beneficial, or causally linked to the numerous adverse events reported following immunisation.

### Objectives

To assess the benefits and harms of aluminium adjuvants used in vaccines versus placebo or no intervention, taking into consideration the type of the vaccine, and the type, size, and concentration of the aluminium adjuvant.

### Methods

#### Criteria for considering studies for this review

**Types of studies**

We will search for randomised clinical trials irrespective of publication type, publication status, and language of publication. We will not specifically search for observational studies (quasi-randomised studies; cohort studies; and patient series), but we may provide a narrative account of such data if we identify valid observational studies during our literature search. We are aware that this approach may be a weakness of our review, making us focus more on short-term benefits and harms in randomised clinical trials with the risk of overlooking late and very rare adverse effects in observational studies.

**Types of participants**

We will include all trial participants regardless of sex, age, ethnicity, diagnosis, comorbidity, and country of residence.

**Types of interventions**

We plan to include trials comparing:

- any type of vaccine including any type of aluminium adjuvant (including, but not limited to, aluminium potassium sulphate; aluminium hydroxide; aluminium phosphate; or
aluminium hydroxyphosphate sulfate) versus the same vaccine but without the aluminium adjuvant;

- any aluminium adjuvant versus placebo or no intervention.

We will accept any co-intervention if planned to be delivered equally to both intervention groups.

**Types of outcome measures**

**Primary outcomes**

- Proportion of participants with one or more serious adverse events. We will define a serious adverse event as any untoward medical occurrence that results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, or results in persistent or significant disability or incapacity (ICH-GCP 1997).

- All-cause mortality (as reported by trialists or measured by administrative data).

- Proportion of participants with disease (as defined per individual trial).

We will use the trial results reported at maximum follow-up. If the trialists report results at multiple time points, we will also assess the results reported at the time point closest to three years.

**Secondary outcomes**

- Health-related quality of life (as measured by interviews or self-report using any standardised continuous scale).

- Non-serious adverse events (defined as any adverse event not classified as a serious adverse event). We will analyse each adverse event separately.

**Exploratory outcomes**

- Serological response (as defined by trialists, e.g. measured with ELISA, agglutination, precipitation, complement-fixation, fluorescent antibodies, chemiluminescence, or similar).

We will use the trial results reported at maximum follow-up to achieve maximum precision and power.

**Search methods for identification of studies**

**Electronic searches**

We will search Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library, MEDLINE Ovid, Embase Ovid, BIOSIS (Web of Science), Lilacs (Bireme), Science Citation Expanded Web of Science (Web of Science), and Conference Proceedings Citation Index - Science (Web of Science) (Royle 2003). Appendix 1 gives the preliminary search strategies with the expected time spans of the searches.

In addition, we will search the Chinese Biomedical Literature Database (CBM), China Network Knowledge Information (CNKI), Chinese Science Journal Database (VIP), and Wanfang Database.

**Searching other resources**

We will also search Google Scholar, The Turning Research into Practice (TRIP) Database, ClinicalTrial.gov (www.clinicaltrials.gov/), European Medicines Agency (EMA) (www.ema.europa.eu/ema/), WHO International Clinical Trial Registry Platform (www.who.int/ictrp), The Food and Drug Administration (FDA) (www.fda.gov), and pharmaceutical company sources for ongoing or unpublished trials.

We will review bibliographic references of identified randomised clinical trials and review articles to identify randomised clinical trials missed during the electronic searches. We will consider unpublished and grey literature trials, if identified.

**Data collection and analysis**

We will perform the review following the recommendations of Cochrane (Higgins 2011). We will perform the analyses using Review Manager 5 (RevMan 2014), STATA 14 (STATA 14), and Trial Sequential Analysis version 0.9.5.10 Beta (Thorlund 2011; TSA 2011). We will present a table describing the types of adverse events (serious or non-serious) reported in each trial.

**Selection of studies**

Two review authors (SD and SBP) will independently screen titles and abstracts for inclusion of potentially eligible trials. We will code included studies as ‘retrieve’ (eligible or potentially eligible/unclear) or ‘do not retrieve’. Following any disagreements, we will ask a third author to arbitrate (JCJ or CG). The selected review author pair will collect full-text trial reports/publications, and independently screen the full-texts and identify trials for inclusion. We will report reasons for exclusion of the ineligible studies. We will solve any disagreement through discussion, or, if required, by consulting a third person (JCJ or CG). We will identify and exclude duplicates, and collate multiple reports of the same trial. We will record the selection process in sufficient detail to complete a PRISMA flow diagram and ‘Characteristics of excluded studies’ table.

**Data extraction and management**

The review authors, working in pairs, will independently extract and validate data using data extraction forms designed for the purpose. If a trial is identified as relevant by one author but not by
the other, the authors will discuss the reasoning behind their assessment. If an agreement is not reached between the two authors, JCJ or CG will serve as arbitrators.

**Assessment of risk of bias in included studies**

Methodological studies indicate that trials with unclear or inadequate methodological quality may be associated with risk of bias (systematic error) when compared to trials using adequate methodology (Schulz 1995; Moher 1998; Kjaergard 2001; Gluud 2006; Wood 2008; Higgins 2011; Hrobjartsson 2012; Savović 2012; Savović 2012a; Hrobjartsson 2013; Hrobjartsson 2014; Lundh 2017). Such bias may lead to overestimation of intervention benefits and underestimation of harms.

The selected review author pair (SD and SBP) will independently assess the risk of bias of each included trial according to the recommendations in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We will use the below definitions in the assessment of bias risk (Schulz 1995; Moher 1998; Kjaergard 2001; Gluud 2006; Wood 2008; Higgins 2011; Hrobjartsson 2012; Savović 2012; Savović 2012a; Hrobjartsson 2013; Hrobjartsson 2014; Lundh 2017).

**Allocation sequence generation**

- Low risk of bias: sequence generation was achieved using computer random number generation or a random number table. Drawing lots, tossing a coin, shuffling cards, and throwing dice were adequate if performed by an independent person not otherwise involved in the trial.
- Unclear risk of bias: the method of sequence generation was not specified.
- High risk of bias: the sequence generation method was not random or only quasi-randomised. We will only use these studies for the assessment of harms and not of benefits.

**Allocation concealment**

- Low risk of bias: the allocation sequence was described as unknown to the investigators. Hence, the participants’ allocations could not have been foreseen in advance of, or during, enrolment. Allocation was controlled by a central and independent randomisation unit, an on-site locked computer, identical looking numbered sealed opaque envelopes, drug bottles or containers prepared by an independent pharmacist, or an independent investigator.
- Unclear risk of bias: it was unclear if the allocation was hidden or if the block size was relatively small and fixed so that intervention allocations may have been foreseen in advance of, or during, enrolment.
- High risk of bias: the allocation sequence was likely to be known to the investigators who assigned the participants.

**Blinding of participants and treatment providers**

- Low risk of bias: it was described that both participants and treatment providers were blinded, or the extent of blinding was appropriately in a intention-to-treat analysis using proper methods (e.g. multiple imputations). Generally, the trial is judged to be at a low risk of bias due to incomplete outcome data if dropouts are less than 5%. However, the 5% cut-off is not definitive.
- Unclear risk of bias: there was insufficient information to assess whether missing data were likely to induce bias on the results.
- High risk of bias: the results were likely to be biased due to missing data either because the pattern of dropouts could be described as being different in the two intervention groups or the trial used improper methods in dealing with the missing data (e.g. last observation carried forward).

**Incomplete outcome data**

- Low risk of bias: missing data were unlikely to make intervention effects depart from plausible values. This could either be: 1) there were no dropouts or withdrawals; or 2) the numbers and reasons for the withdrawals and dropouts for all outcomes were clearly stated and could be described as being similar in both groups, and the trial handled missing data appropriately in an intention-to-treat analysis using proper methods (e.g. multiple imputations). Generally, the trial is judged to be at a low risk of bias due to incomplete outcome data if dropouts are less than 5%. However, the 5% cut-off is not definitive.
- Unclear risk of bias: it was not mentioned if the outcome assessors were blinded, or the extent of blinding was insufficiently described.
- High risk of bias: no blinding or incomplete blinding of outcome assessors was performed.

**Selective outcome reporting**

- Low risk of bias: a protocol was published before randomisation began and all outcome results were reported adequately.
- Unclear risk of bias: no protocol was published.
- High risk of bias: the outcomes in the protocol were not reported on.

**Vested interest bias**

- Low risk of bias: it was described that the trial was not sponsored by any pharmaceutical company, any person, or any group with a financial or other interest in a certain result of the trial.
- Unclear risk of bias: it was unclear how the trial was sponsored.
- High risk of bias: the trial was sponsored by a pharmaceutical company, a person, or a group with a certain financial or other interest in a given result of the trial.
Other bias

- Low risk of bias: the trial appeared to be free of other bias domains that could put it at risk of bias.
- Unclear risk of bias: the trial may or may not have been free of other domains that could put it at risk of bias.
- High risk of bias: there were other factors in the trial that could put it at risk of bias.

Overall risk of bias

- Low risk of bias: the outcome result will be classified as at overall 'low risk of bias' only if all of the bias domains described in the above paragraphs are classified as at low risk of bias.
- High risk of bias: the outcome result will be classified as at 'high risk of bias' if any of the bias risk domains described above are classified as at 'unclear' or 'high risk of bias'.

We will assess the domains 'Blinding of outcome assessment', 'Incomplete outcome data', and 'Selective outcome reporting' for each outcome. This will enable us to assess the bias risk for each outcome result in addition to each trial. We will base our primary conclusions as well as our presentation in the 'Summary of Findings' table on the results of our primary outcomes with low risk of bias.

Measures of treatment effect

Dichotomous outcomes

We will calculate risk ratios (RR) with 95% confidence interval (CI) for dichotomous outcomes, as well as the Trial Sequential Analysis-adjusted CI (see below).

Continuous outcomes

We will calculate the mean difference (MD) with 95% CI and Trial Sequential Analysis-adjusted CI for continuous outcomes. If various scales assessing comparable symptoms have been used, we will calculate the standardised mean difference (SMD) with 95% CI for continuous outcomes. Such data can then be calculated back to MD for a preferred scale, if needed.

Unit of analysis issues

We will include data from studies where participants are individually randomised to one of two or more intervention groups. We will collect and analyse single measurements for each outcome from each participant.

Dealing with missing data

We will contact investigators or study sponsors to obtain any missing data.

If standard deviations (SD) are not reported, we will calculate them using data from the trial, if possible. We will not impute missing values for any outcomes in our primary analysis. In our sensitivity analysis for dichotomous and continuous outcomes, we will impute data (see Sensitivity analysis).

Assessment of heterogeneity

We will first visually investigate forest plots to assess the risk of statistical heterogeneity. We will also assess the presence of statistical heterogeneity using the Chi² test (threshold P < 0.1) and measure the quantities of heterogeneity using the I² statistic (Higgins 2002; Higgins 2003).

Assessment of reporting biases

We will assess reporting bias using funnel plots where ten or more trials per comparison are included. Symmetry or asymmetry of each funnel plot will enable assessment of the risk of bias. For dichotomous outcomes, we will assess asymmetry using the Harbord test (Harbord 2006). For continuous outcomes, we will apply the regression asymmetry test (Egger 1997).

Data synthesis

Meta-analysis

We will conduct this systematic review according to the following recommendations stated in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins 2011), according to Keus and colleagues (Keus 2010), and according to the eight-step procedure for validation of meta-analytic results in systematic reviews as suggested by Jakobsen and colleagues (Jakobsen 2014). We will meta-analyse data using the statistical software Review Manager 5.3 (RevMan 2014).

Trial Sequential Analysis

Cumulative meta-analyses are at risk of producing random errors due to sparse data and multiple testing of accumulating data (Pogue 1997; Brok 2008; Wettreslev 2008; Brok 2009; Thorlund 2009; Higgins 2011; Wettreslev 2017); therefore, Trial Sequential Analysis (TSA 2011) can be applied to control this risk (Thorlund 2011). The required information size (that is the number of participants and number of trials needed in a meta-analysis to detect or reject a certain intervention effect) can be calculated in order to control random errors (Wettreslev 2008; Wettreslev 2009; Wettreslev 2017). The required information size takes into account the event proportion in the control group, the assumption
of a plausible relative risk (RR) reduction, and the heterogeneity of the meta-analysis (Wetterslev 2008; Wetterslev 2009; Turner 2013; Wetterslev 2017). Trial Sequential Analysis enables testing for significance to be conducted each time a new trial is included in the meta-analysis. On the basis of the required information size, trial sequential monitoring boundaries can be constructed. This enables one to determine the statistical inference concerning cumulative meta-analysis that has not yet reached the required information size (Wetterslev 2008; Wetterslev 2017).

If the trial sequential monitoring boundary is crossed before reaching the calculated information size, we may conclude that sufficient evidence is collected to validly assess benefit or harm, and that inclusion of additional trial data may be redundant. In contrast, if the boundaries for benefit or harm are not crossed, we may conclude that further trials are necessary before a certain intervention effect can be evaluated. Trial Sequential Analysis also allows for assessment of the sufficiency of evidence for a postulated intervention effect. A lack of effect is evident if the cumulative Z-score crosses the trial sequential monitoring boundaries for “futility” (the ability of a systematic review of clinical trials to reject a certain postulated intervention effect).

We will make relatively conservative estimations of the anticipated intervention effect to control the risks of random error (Jakobsen 2014). Large anticipated intervention effects lead to small required information sizes, and the thresholds for significance will be less strict after the information size has been reached (Jakobsen 2014). We will analyse all primary and secondary outcomes using Trial Sequential Analysis. These analyses will allow us to calculate the Trial Sequential Analysis-adjusted CIs based on the following assumptions:

### Primary outcomes

We will estimate the diversity-adjusted required information size (Wetterslev 2009; Wetterslev 2017) based on the proportion of participants with an outcome in the control group. We will use an alpha of 2.5%, a beta of 10%, and the diversity suggested by the trials in the meta-analysis (Jakobsen 2014).

As anticipated intervention effects for the primary outcomes in the Trial Sequential Analysis, we will use the following:

- **Serious adverse events**: a relative risk reduction of 20% and the observed proportion of serious adverse events in the control group.
- **All-cause mortality**: a relative risk reduction of 20% and the observed incidence of mortality in the control group.
- **Disease**: a relative risk reduction of 20% and the observed proportion of participants with disease in the control group.

### Secondary outcomes

We will estimate the diversity-adjusted required information size (Wetterslev 2009; Wetterslev 2017) based on the proportion of participants with an outcome in the control group when analysing dichotomous outcomes, and we will use the observed SD when analysing continuous outcomes. We will use an alpha of 2.5%, a beta of 10%, and the diversity suggested by the trials in the meta-analysis (Jakobsen 2014).

As anticipated intervention effects for the secondary outcomes in the Trial Sequential Analysis we will use the following relative risk reductions or increases:

- **Quality of life**: observed SD divided by 2.
- **Non-serious adverse events**: a relative risk reduction of 20%.

### Exploratory outcomes

As anticipated intervention effects for the exploratory outcomes in the Trial Sequential Analysis, we will use the following relative risk reductions or increases:

- **Serological response**: a relative risk reduction of 20% and the observed proportion of participants with no serological response in the control group.

We will include particle size (nano-size or micro-size as described by trialist or manufacturer) as a covariate in meta-regression to assess whether particle size influences the effect of aluminium adjuvant administration on outcomes.

### Assessment of significance

Intervention effects will be assessed with both random-effects (DerSimonian 1986) and fixed-effect meta-analyses (DeMets 1987). The more conservative point estimate of the two, comprised by the estimate closest to zero effect, will be chosen for assessment of significance (Jakobsen 2014). If the two estimates are comparable, the estimate with the widest confidence interval will be used. For analysis of three primary outcomes, a P value less than P < 0.025 will be considered significant (Jakobsen 2014) because this will secure a family-wise error rate (FWER) below 0.05. An eight-step procedure will be applied to assess if the results from the meta-analyses have passed the thresholds for significance (Jakobsen 2014).

A table describing all types of serious adverse events will be presented for each trial.

### Subgroup analysis and investigation of heterogeneity

We will perform the following subgroup analyses:

A: The effect of aluminium adjuvant administration in trials with high risk of bias compared to low risk of bias.

B: The effect of aluminium adjuvant administration between trials where the intervention groups were treated with different aluminium salts (aluminium hydroxide, aluminium phosphate, or amorphous aluminium hydroxyphosphate sulfate).
C: The effect of aluminium adjuvant administration between trials where the intervention groups were treated with different vaccines (including, but not limited to, vaccines against BCG, HPV, hepatitis A and B, HIV, anthrax, DTP, polio, influenza, rotavirus, and MMR).

D: Comparison of the effect of aluminium adjuvant administration between trials on newborns, children, adolescents, adults or elderly (or similar terms) as described by trialists.

E: The effects nanoparticle aluminium adjuvant compared to microparticle aluminium adjuvant regardless of type of aluminium salt.

F: The effects of aluminium adjuvant administration between trials with different maximal follow-up periods:
   - short-term (1-30 days after last administration);
   - medium-term (1-12 months after last administration); and
   - long term (more than 1 year after last administration).

G: The effect of total administration of aluminium between trials.

H: The effect of aluminium adjuvant administration between trials including healthy participants and trials including participants with any specific diagnosis.

**Sensitivity analysis**

A: To assess the potential impact of the missing data for dichotomous outcomes, we will perform the following analyses:
   - ‘Best-worst-case’ scenario: it will be assumed that all participants lost to follow-up in the experimental group survived and did not have a serious adverse event; and all those with missing outcomes in the control group had a serious adverse event and did not survive.
   - ‘Worst-best-case’ scenario: it will be assumed that all participants lost to follow-up in the experimental group did not survive and had a serious adverse event; and all those with missing outcomes in the control group survived and had no serious adverse event.

We will present results from both scenarios.

B: We will address missing data for continuous outcomes by calculating a ‘beneficial’ and a ‘harmful’ outcome. We will base the ‘beneficial’ outcome on the group mean minus 2 SDs (and 1 SD), and the ‘harmful’ outcome on the group mean minus 2 SDs (and 1 SD) (Jakobsen 2014).

C: Potential impact of missing SDs for continuous outcomes will be assessed with the following sensitivity analyses:
   - Where SDs are missing and not possible to calculate, we will impute SDs from trials with similar populations and low risk of bias. If no such trials can be found, we will impute SDs from trials with a similar population. As a final option, we will impute SDs from all trials.

**Summary of findings**

We will use the GRADE system (Guyatt 2008) to assess the quality of the body of evidence associated with each of the primary outcomes. We will construct the ‘Summary of findings’ (SoF) tables using the GRADEpro software (gradepro.org/). This GRADE system appraises the quality of a body of evidence based on the extent to which one can be confident that an estimate of effect or association reflects the item being assessed. The quality measure of a body of evidence considers within-trial risk of bias, the directness of the evidence, heterogeneity of the data, precision of effect estimates (Jakobsen 2014), and risk of publication bias. Our primary SoF tables and conclusions will be based on the results of trials with a low risk of bias in all bias risk domains (Schulz 1995; Moher 1998; Kjaergard 2001; Gluud 2006; Wood 2008; Savović 2012; Savović 2012a; Lundh 2017).

**Acknowledgements**

Cochrane Review Group funding acknowledgement: The Danish State is the largest single funder of The Cochrane Hepato-Biliary Group through its investment in the Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital, Denmark. Disclaimer: The views and opinions expressed in this review are those of the authors and do not necessarily reflect those of the Danish State or the Copenhagen Trial Unit.

Peer reviewers: Jagdish K. Zade, India, and Sachin Thakkar, US.

Contact Editor: Kurinchi Gurusamy, UK.

Sign-off Editor: Kurinchi S Gurusamy, UK.
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Awate 2013

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Brinth 2015

Brinth 2015a

Brok 2008

Brok 2009

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Chen 2008

Coffman 2010

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Delany 2014

DeMets 1987

DerSimonian 1986

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Aluminium adjuvants used in vaccines versus placebo or no intervention (Protocol)

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**Kjaergard 2001**


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**Kocourkova 2016**


**Kool 2008**


**Kool 2012**


**Lee 2012**


**Li 2014**


**Lindh 2017**


**Malluche 2002**


**Mannhalter 1985**


**Marrack 2009**


**Martinez-Lavin 2015**


Aluminium adjuvants used in vaccines versus placebo or no intervention (Protocol)

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## Appendix 1. Preliminary search strategies

<table>
<thead>
<tr>
<th>Database</th>
<th>Time span</th>
<th>Search strategy</th>
</tr>
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| Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library | Latest issue                | #1 MeSH descriptor: [Adjuvants, Immunologic] explode all trees  
#2 (immunologic* or alum* or vaccine*) and adjuvant*  
#3 #1 or #2  
#4 MeSH descriptor: [Vaccines] explode all trees  
#5 vaccin*  
#6 #4 or #5  
#7 #3 and #6 |
| MEDLINE Ovid                                  | 1946 to the date of search. | 1. exp Adjuvants, Immunologic/  
2. ((immunologic* or alum* or vaccine*) and adjuvant*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]  
3. 1 or 2  
4. exp Vaccines/  
5. vaccin*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]  
6. 4 or 5  
7. 3 and 6  
8. (random* or blind* or placebo* or meta-analysis*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]  
9. 7 and 8 |
| Embase Ovid                                   | 1974 to the date of search. | 1. exp immunological adjuvant/  
2. ((immunologic* or alum* or vaccine*) and adjuvant*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]  
3. 1 or 2  
4. exp vaccine/  
5. vaccin*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]  
6. 4 or 5 |
7. 3 and 6
8. (random* or blind* or placebo* or meta-analys*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word]

| BIOSIS (Web of Science) | 1969 to the date of search. | #5 #4 AND #3
| | | #4 TS=(random* or blind* or placebo* or meta-analys*)
| | | #3 #2 AND #1
| | | #2 TS=vaccin*
| | | #1 TS=((immunologic* or alum* or vaccine*) and adjuvan*)
| LILACS (Bireme) | 1982 to the date of search. | (immunologic$ or alum$ or vaccine$) and adjuvan$ [Words] and vaccin$ [Words]
| Science Citation Index Expanded (Web of Science) | 1900 to the date of search. | #5 #4 AND #3
| | | #4 TS=(random* or blind* or placebo* or meta-analys*)
| | | #3 #2 AND #1
| | | #2 TS=vaccin*
| | | #1 TS=((immunologic* or alum* or vaccine*) and adjuvan*)
| Conference Proceedings Citation Index - Science (Web of Science) | 1990 to the date of search. | #5 #4 AND #3
| | | #4 TS=(random* or blind* or placebo* or meta-analys*)
| | | #3 #2 AND #1
| | | #2 TS=vaccin*
| | | #1 TS=((immunologic* or alum* or vaccine*) and adjuvan*)

**WHAT'S NEW**

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<td>16 October 2017</td>
<td>Amended</td>
<td>We increased the clarity of a few sentences in the protocol text</td>
</tr>
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CONTRIBUTIONS OF AUTHORS
MK and SBP presented and drafted the idea for the systematic review. SD, JCJ, and CG drafted the protocol.

DECLARATIONS OF INTEREST
MK is co-founder of HPV update.dk.

SOURCES OF SUPPORT
Internal sources
• Copenhagen Trial Unit, Denmark.

External sources
• No sources of support supplied