APPENDIX 1: REVIEW PROTOCOL

(Version 2 September 2013)

1 Background

Over the past two decades, a number of publications have claimed that several vaccines routinely administered to infants may have "non-specific" effects on mortality unrelated to prevention of illness and deaths caused by the specific diseases against which the vaccines have been formulated. For example, some authors have suggested that receipt of both BCG vaccine and measles vaccine is associated with a reduced risk of death (e.g. all cause mortality), while receipt of DTP vaccine is associated with an increased risk of death, at least among female infants. The vast majority of the evidence reporting these effects was generated using observational study designs (i.e. not randomized clinical trials) that are afflicted by the risk of bias, and as a result, poorly-controlled or uncontrolled confounding and various types of selection and information bias have been suggested as alternative explanations for these findings.

The possible implications of any such non-specific vaccine effects for the any adjustments to the infant immunization schedule remain unclear. Some authors have suggested that if such effects are document with robust evidence, the infant immunization schedule might need to be adjusted. However, prior WHO led reviews of this subject, including several assessments by WHO's Global Advisory Committee on Vaccine Safety, have concluded that any such effects remain unproven and therefore not a justification for altering the current infant immunization schedule.

As part of its continue appraisal of cross-cutting issues that could be relevant to inform global immunization policy, the WHO's Strategic Advisory Group of Experts (SAGE) has requested the WHO Secretariat to lead a systematic review of the evidence concerning the possible non-specific effects of vaccines included in the routine infant immunization schedule. This evidence will inform discussions on the topic during the SAGE meetings in 2014.

Preparatory to the discussions at the SAGE in 2014, it is necessary to systematically assemble, and to critically appraise the available evidence.

Overall, our aim is to:

1. determine if the current evidence is sufficiently compelling to warrant further scientific investigation, and if so,
2. to define the path towards obtaining unequivocal evidence on these issues that would support future robust, evidence-based adjustments in immunization policies, if warranted.

2 Objectives

1. To systematically identify, assemble, and review all published and grey literature concerning epidemiological studies addressing "non-specific" effects of BCG, measles and, DTP-containing vaccines on: (i) survival/deaths from causes other than those conditions that the vaccine is designed to prevent and, (ii) on all-cause mortality in children under five years of age.

2. To critically appraise the evidence using existing guidelines[1] .

3 Methods

We will identify and critically appraise the available evidence that addresses the effect of BCG, DTP and measles-containing vaccines on: (i) survival/all-cause mortality/deaths from causes other than those conditions that the vaccine is designed to prevent and; (ii) on all-cause mortality/deaths by all causes/survival in children less than five years of age in all settings.

We will provide an evidence profile that summarizes the findings for each study question.

[1] We will develop a risk of bias assessment form for observational studies specifically for this review drawing on a new tool under development within the Cochrane Collaboration. We will also include any key elements reported in Fine P et al (2009) and Farringdon P et al (2009) and, WHO Strategic Advisory Group of Experts (SAGE) Guidance for the development of evidence-based vaccine related recommendations. (http://www.who.int/entity/immunization/sage/Guidelines_development_recommendations.pdf)

3.1 Study Questions

The primary questions to be addressed by this review are as follows:

A. Is the administration of BCG in infancy associated with an effect on survival /deaths from causes other than those conditions that the vaccine is designed to prevent in children up to five years of age?

B. Is the administration of DTP in infancy associated with an effect on survival/deaths from causes other than those conditions that the vaccine is designed to prevent in children up to five years of age?

C. Is the administration of Measles in infancy associated with an effect on survival/ deaths from causes other than those conditions that the vaccine is designed to prevent in children up to five years of age?

The secondary questions to be addressed by this review are as follows:

A. Is administration or non-administration of BCG vaccine in infancy associated with an effect on all-cause mortality/deaths from all causes in children up to five years of age?

B. Is administration or non- administration of DTP-containing vaccine in infancy associated with an effect on all-cause mortality/deaths from all causes in children up to five years of age?

C. Is administration or non- administration of measles-containing vaccine in infancy associated with an effect on all-cause mortality/deaths from all causes in children up to five years of age?

For each question we will also assess if the effect is modified by gender, number of doses, age at vaccination, sequence/order in which vaccines are given and/or prior, or co-administration of vitamin A.

For each question we will assess the effect at 1 year of age in addition to at five years of age. These analyses were added by consensus of the Co-investigators in July 2013.

STUDY QUESTIONS	**VACCINES**		
	BCG	**Measles**	**DTP**
PRIMARY QUESTION Is the administration of vaccine X in infancy associated with an effect on survival/deaths from causes other than those conditions that the vaccine is designed to prevent in children up to five years of age?	+	+	+
SECONDARY QUESTION Is administration or non- administration of vaccine X given in infancy associated with an effect on all-cause mortality/deaths from all causes in children up to five years of age?	+	+	+
Is there a difference of the effect:			
between boys and girls	+	+	+
by number doses and age dose is received?	+	+	+
by sequence/order in which vaccines are given?	+	+	+
by prior, or co-administration of vitamin A and/ or other vaccines?	+	+	+

3.2 Inclusion criteria

Studies done in any country and published in any language will be included

3.2.1 Types of studies

We plan to include the following study designs:

- RCT or quasi-randomized controlled trials
- Observational epidemiological studies
    - case-control studies and,
    - prospective, historical and ambi-directional cohort studies,
        - When two or more exposure cohorts are described by study investigators to be part of the same study, we will consider this to be a cohort study which could be considered for inclusion.

3.2.2 Types of participants

We will include studies containing data relating to the vaccination of children up to 5 years.

3.2.3 Types of interventions

We will include studies reporting on vaccination with BCG, DTP and measles-containing vaccine in children up to five years of age.

3.2.4 Types of outcomes measures

Studies reporting survival/all-cause mortality/deaths from causes other than those conditions that the vaccine is designed to prevent and studies reporting death from all causes (e.g. all-cause mortality, child survival) will be eligible for inclusion.

P	**Population**	**Children up to five years of age**
I	**Intervention**	Vaccination with BCG, DTP or standard measles containing vaccines
C	**Comparator(s)**	No vaccination (BCG, DTP or measles) or simultaneous administration of other vaccine or order of vaccine administration
O	**Outcome(s)**	(i) survival /deaths from causes other than those conditions that the vaccine is designed to prevent (ii) death from all causes (e.g. all-cause mortality, child survival)

3.3 Exclusion criteria

We will exclude ecological studies, uncontrolled studies (i.e. case reports, case series studies and studies in which all children receive the same vaccine(s)), studies including only individuals with the outcome of interest in the analyses ("case only" studies) and, self-controlled case series studies because these studies provide less reliable data for assessing non-specific effects of vaccine on mortality. Additionally, we will exclude animal or laboratory studies,

3.4 Search Strategy

The search strategy will be developed jointly by two Reviewers (Ximena Riveros (XRL) and Ana Maria Henao-Restrepo (AMHR) and, a Senior Librarian (Tomas Allen (TA) with expertise in systematic searches. The Senior Librarian (TA) will conduct the systematic search using the agreed criteria.

Element	Description
Sampling strategy	Comprehensive strategy to identify all articles on: (i) effect of vaccines on survival/deaths from causes other than those conditions that the vaccine is designed to prevent and, (ii) effect of vaccines on or all-cause mortality
Type of studies	No restrictions, all study types included
Approaches	Electronic search in various databases plus: • Grey literature • Hand searches • Contact lead authors in the field
Range of years (start date and end date)	No restrictions From the beginning of each candidate database to December 15, 2012.
Limits	No limits
Inclusions and exclusions	No inclusions or exclusions applied
Terms used	See Annex 1
Electronic sources	See below

- **Electronic search in various databases**: We will search PubMed, Embase (in Embase.com), the Cochrane Library, African Index Medicus (AIM), the Indian Medlars Centre (IndMed), Latin American and Caribbean Health Sciences (LILACs), Current Controlled Trials metaRegister of Controlled Trials (mRCT, active and archived registers), UK Clinical Trials Gateway (UKCTG), US Food and Drug Administration (FDA), European Public Assessment Report (EPAR), listings of the European Medicines Agency (EMEA), WHO International Clinical Trials Registry Platform Search Portal (includes: ClinicalTrials.gov, International Standard Randomized Controlled Trial Number Register (ISRCTN), and clinical trial registries of Australia, China, Germany, India, Iran, The Netherlands, New Zealand, Sri Lanka), GSK Clinical Study Register, and Clinicalstudyresults.org (includes Wyeth trial listings).
- **Grey Literature**: unpublished study reports, articles submitted for publication, conference proceedings (e.g. EMBASE and Scopus) and posters, dissertations and theses.
- **Manual searches**: We will examine bibliographies of relevant previous reviews and the reference lists of all articles found to identify studies not identified through the databases listed above. We will hand search content pages of the INDEPTH network and the OPTIIMUNIZE Initiative together with list of publications mentioned in this websites.
- **Contact with lead authors**: We will also contact some authors (e.g. C S Benn and F Shann) to ask for copies of any reports (published or unpublished) that relate to the subject and that might fit our selection criteria.

There will be no restrictions on language, study design (except as noted above), length of follow-up, and report characteristics (e.g. date of publication or listing, publication status). Full text of all articles identified will be sought, using internet downloads, interlibrary loans, and contacting of authors. We will use terms relating to:

- BCG, standard titer measles vaccine DTP containing vaccines, or names of licensed BCG, DTP and measles containing vaccines AND vaccination, immunization or vaccine AND
- all-cause mortality, mortality, child mortality, infant mortality or death, cause of deaths, child deaths, infant deaths.

Search terms will be adapted as required for each database. See Annex 1 for details on full electronic search strategy example for EMBASE and Medline.

3.5 Selection of Eligible Studies

We will use DistillerSR, an online application designed specifically for the screening and data extraction phases of a systematic review. Paul Zhang (PZ), Michelle Beam (MB), Emi Han (EH) and Emma Smith (ES) will conduct the screening under the supervision of AR and KS.

For each step of the selection of potentially eligible articles we will develop and pilot test forms for screening using predefined data fields.

Steps	Procedure
1. Systematic search	After completing the systematic search we (KS, XRL and AMHR) will cross check that all key articles were included in the search (e.g. hand search in reference lists of key articles, list on the web of INDEPTH's network, OPTIMUNIZE and, C S Benn and F Shann suggested publications)
2. Check for duplicates	We will compile all search results and identify any duplicates (KS, XL and AMHR).
3. Title screening	Two Reviewers (XRL and AMHR) will conduct title screening with the aim to exclude studies that obviously do not include information on the outcome of interest. Disagreements between reviewers will be resolved by consensus after reviewing the abstracts for the given articles. The reviewers will have three response options for excluding records or promoting them to the next stage of the winnowing process: yes, no and, maybe.
4. Abstract screening	Abstract screening form will be pilot tested by applying it to 30 selected studies. The articles ID and outcome of the pilot will be documented and filed. The outcomes of the pilot will be discussed by all reviewers and, an adjusted final form will be developed. Each abstract of each potentially eligible article will be examined by two reviewers (e.g. PZ, AM, EH, ES organized in pairs of two) using predefined data fields and lists of studies considered eligible for inclusion will be compared. Studies identified by both reviewers as being potentially eligible for inclusion will be included in the next step of the review. Where there are discrepancies, the reasons for these will be discussed by four Senior Reviewers (AR, KS, AMH, XL) who will adjudicate to make a final decision about eligibility after reviewing the full texts of the articles.
5. Pdf screening	Pdf screening form will be pilot tested by applying it to 10 selected studies. The articles ID and outcome of the pilot will be documented and filed. The outcomes of the pilot will be discussed by all reviewers and, an adjusted final form will be developed. Each pdf file of each potentially eligible article will be examined by a pair of two reviewers (PZ, AM, EH, ES) using predefined data fields and lists of studies considered eligible for inclusion will be compared. Studies identified by both reviewers as being potentially eligible for inclusion will be included in the next step of the review. Where there are discrepancies, the reasons for these will be discussed and a decision about inclusion reached by consensus. If there is no agreement, the four Senior Reviewers (AR, KS, AMH, XL) will adjudicate to make a final decision about eligibility. Both the reasons for the disagreements and the outcome will be documented.

3.6 Data Extraction Forms

We will develop a data extraction sheet using predefined data fields for extracting consistent data from eligible articles. The sheet will also include variables that will permit us to assess the risk of bias of each individual study[2] .

[2] Using the Cochrane Handbook guidelines for RCTs and we will develop a risk of bias assessment form for observational studies specifically for this review drawing on a new tool under development within the Cochrane

We will extract data in three ways. First, we will collect **Study-level data** relating to the whole study, including information about study design, demographic/participant characteristics and methods that are expected to apply to the whole study, such as randomization methods, methods of collecting outcome data and (for non-randomized studies) methods for collecting vaccination status.

Second, we will collect **Group-level data** for each group of children for whom 1-year or 5-year mortality is reported. This will allow us:

(i) to characterize the groups being compared (e.g. sequence of vaccines, ages at vaccinations, vitamin A, gender);

(ii) to collect information to inform risk of bias assessments (co-interventions and other departures from intended schedules, missing data, selective reporting of a subset of children); and

(iii) to collect results for possible unadjusted comparisons of groups, recognizing that in non-randomized studies these comparisons may be substantially affected by confounding (e.g. frailty bias).

Third, we will collect **Comparison-level data** for all comparisons of 1-year or 5-year mortality that are made across groups. Where these have been adjusted for baseline differences, information about these adjustments will be collected to inform the risk of bias assessments. Further information about any non-comparability of the groups being compared will also be collected to inform the risk of bias assessments.

The second and third types of data will be collected in the same way for randomized trials and non-randomized studies. One pragmatic reason for this is that observational comparisons are frequently made within randomized trials (e.g. an observational comparison of BCG vs no BCG among children randomized to different DTP schedules). For all comparisons, a detailed assessment of risk of bias will be undertaken. For randomized comparisons, it will be clear that the comparison is randomized, so that confounding and selection bias may be expected to be minimal or absent.

We will pilot test the data extraction forms to ensure ease of use and ability to capture all relevant data. The pilot test will include 10% randomly selected potentially eligible articles. It will be conducted by four selected independent epidemiologists not involved in the review who will use it independently.

We will develop the forms in an electronic format using web-based systematic review software called Distiller SR (http://systematic-review.net/).

3.7 Data Extraction

All relevant data will be extracted from articles meeting inclusion criteria using the structured electronic data extraction form and entered into a database. For all studies, data will be extracted by one reviewer. Data will be 100% checked for accuracy and completeness by one senior reviewer. Where there are discrepancies, the reasons for these will be discussed and a decision about inclusion reached by consensus among the co-principal investigators. If there is no agreement, one co-principal investigator (A. Reingold) will adjudicate to make a final decision. All disagreements and the outcome will be documented.

Some of the included studies might be described in more than one publication because in some cases, additional analyses may have been conducted after completion of a study or re-analyses were reported. Where methods of study design are described in additional publications, we will use all reports to inform our data extraction. Where additional analyses were conducted, we will include the analysis that provided the most information and avoid duplication of results.

To ensure that all relevant data have been located from all studies, tables with eligible studies will be generated and shared with leading authors of eligible articles to ask if they can provide any missing information.

3.8 Assessment of risk of bias of included studies.

We will develop a risk of bias assessment tool specifically for this review, drawing on a new tool under development within the Cochrane Collaboration and informed by two articles on methodological issues in the design and analysis of observational studies of non-specific effects of vaccines (Fine P et al 2009 and Farrington P et al 2009). The tool will include separate assessments for randomized (or quasi-randomized) trials, cohort studies and case-control studies.

Collaboration. We will also include any key elements reported in Fine P et al (2009) and Farringdon P et al (2009) and, will use the SAGE Guidelines for review of evidence

In randomized trials and quasi-randomized trials, for each outcome, we will classify results as at low, moderate, serious or critical risk of bias, based on domain-specific assessments of risk of bias done using the Cochrane Collaboration's existing "Risk of Bias" tool. If such assessments cannot be made due to lack of information, we will instead classify the study accordingly.

In developing this risk of bias assessment for observational studies we will consider inclusion of items addressing issues of (i) confounding and selection bias (including confounders measured and addressed, use of matching, methods of adjustment and issues of timing of vaccination); (ii) performance bias (including any considerations of co-intervention); (iii) missing data (including missing vaccination status – leading to survival bias – and missing mortality data); (iv) detection bias (for cohort studies, including consideration of subjective assessment of causes of death) or recall bias (for case-control studies, including misclassification of vaccination status); and (v) selective reporting bias. Most of these assessments are aided considerably by the specification of a 'target randomized trial', representing a hypothetical trial which would estimate the same parameter being targeted by the observational study. For each outcome in each observational study, we will classify results as at low, moderate, serious, or critical risk of bias. If such assessments cannot be made due to lack of information, we will instead classify the study accordingly. We will also document any inclusion and exclusion criteria, methods for ascertainment of vaccination status and measures to deal with missing data.

An *a priori* minimum set of confounders to designate a rate ratio as adjusted will be agreed based on consideration (blind to study results) of a tabulation of all the confounders used in each study, stratified by study design. Using existing literature we have identified potential confounding factors for a child being vaccinated. These are:

- Age of child
- Socioeconomic status (includes poverty, education, health insurance, urban/rural)
- Distance from vaccination centre
- Nutritional status
- Child's health
- Birth weight
- Hygiene conditions

The following important co-interventions have also been identified to inform assessments of the risk of so-called 'performance bias':

- Malaria interventions
- De-worming
- Micronutrient supplements
- Breast feeding
- Hygiene programmes

Two reviewers will independently evaluate study quality and differences will be resolved by discussions with a third reviewer. The overall results will be reported using a table designed for this review.

3.9 Data Analysis

3.9.1 Analysis of single studies

We will check the data for each study to identify possible data entry problems.

We will determine from the **Group-level data** which groups within each study allow, in principle, a comparison of vaccine vs no vaccine. We will then examine the **Comparison-level data** to determine whether we have available results for the in-principle comparisons. Discrepancies between the in-principle comparisons and the available comparisons will be tabulated and may inform risk of bias assessments (e.g. due to selective reporting or missing data).

For each study, we will use the best available Comparison-level data or Group-level data to derive the rate ratio (RR) for vaccinated compared with unvaccinated individuals, with 95% confidence interval (CI) . If only hazard ratios are available for a study, we will use these instead. If only 2×2 data (rather than person-years) are available we will estimate risk ratios. We will assume that these approximate to rate ratios provided that overall mortality risk is low.

For case-control studies we will derive odds ratios: we will assume that these approximate to rate ratios in the general population. Where possible, we will compare published estimates with those directly calculated from raw data. Where data are available for two or more time periods we will plot RRs and 95% CIs over time.

Descriptive tables - We will produce descriptive tables summarizing information about study design, risk of bias, and results of all included studies. Data will be presented by vaccine and by epidemiological or mortality setting, as appropriate.

3.9.2 Meta-analysis

Where studies are considered substantively similar enough for meta-analysis to be appropriate, both fixed- and random-effects analyses will be carried out using the **metareg** command for Stata. All meta-analyses will initially be stratified according to study design (RCT or quasi-randomized controlled trials, cohort studies, case-control studies) and by type of vaccine. Meta-analyses of crude and adjusted rate ratios will be derived separately for each observational study design. Analysis will be on the log rate ratio scale, while results will be displayed both as rate ratios and as vaccine efficacy (=1-RR) if this is appropriate.

Fixed- and random-effects summary estimates will be displayed with estimates from the individual studies in forest plots. Differences between fixed- and random-effects estimates suggest that there are differences between RRs estimated from smaller and larger studies: such differences will be examined using funnel plots and Harbord's test for funnel plot asymmetry.

Heterogeneity (differences between the true vaccine effects in the different studies) will be quantified by estimating the between-study variance τ^2. To illustrate the meaning of this quantity, the table below shows the ratio of the effect (for example, risk ratio or rate ratio) at the 90th centile of the distribution to the effect in a study at the 10th centile, based on the usual normal distribution assumption used in random-effects meta-analyses.

Variance τ^2	Standard deviation τ	Ratio of effect in study at 90th centile to study at 10th centile
0.02	0.141	1.44
0.05	0.224	1.77
0.1	0.316	2.25
0.2	0.447	3.15
0.4	0.632	5.06

In all analyses, τ^2 will be estimated by restricted maximum-likelihood. Inconsistency in findings across studies will also be quantified using the I^2 statistic, which measures the percentage of observed variation that can be attributed to true differences between the studies[i]

Studies assessed as being at critical risk of bias will be excluded from analyses and described in the final report. However, if the direction of the bias is predicted to be in such that a qualitative conclusion can be drawn, we will report this. For example, if all anticipated biases imply that the estimated risk ratio is too large, yet the estimated risk ratio is still convincingly less than 1, then a qualitative conclusion can be drawn.

3.9.3 Variation in efficacy according to characteristics of individuals and studies

We will conduct both univariable and multivariable meta-regression analyses: results from multivariable analyses will be interpreted with caution because the number of studies is typically small compared with the number of study characteristics of interest.

The following potential effect modifiers are to be considered in the meta-regression. They are divided into pre-specified **Research question covariates** (gender, number of doses, age at vaccination, vitamin A administration) and **Other covariates** (study design, nature of the population, risk of bias assessments, previous vaccines).

The **Other covariates** will be examined first in a series of single variable and multivariable models. They will be included in the main analyses only if they appear to explain variation (based on joint consideration of the proportion of variance (heterogeneity) explained, magnitude of estimated coefficient and statistical significance).

The **Research question covariates** will be addressed in a series of single variable and multivariable models, each including the **Other covariates** that are determined to be important.

Research question covariates	
Number of doses	(Typical) number of doses of the vaccine [not relevant for BCG]
Gender	Proportion of females across both groups
Age at vaccination	(Typical) age at administration of vaccine
Co-administration of vit A	Co-administration with vaccine only / Prior administration for all or most children / No administration / Other
Other covariates	
Study design	RCT / cohort / case control. If findings are consistent, we will consider combining across designs
Risk of bias (RoB) assessment	*See below.*
Nature of comparator	No vaccine / Other
Nature of population	General / Low birth weight / Twins / Other/ HIV [list may be extended]
Previous vaccines	For the whole population: None/ BCG / DTP1 / DTP 1 and 2/ DTP 1, 2 and 3. We will combine the last three groups if data are sparce.

3.9.4 Risk of bias

1. We will conduct overall risk of bias assessments, dividing studies in the first instance into those assessed at low or moderate risk of bias vs those assessed at serious risk of bias vs those with no information to enable assessment. If a sizeable number of studies including assessments of a specific direction of the likely bias, we will separate the serious risk of bias category according to the anticipated direction. Corresponding dummy variables will be included in a meta-regression analysis without the other covariates, to determine whether there is evidence that risk of bias assessments are correlated with relative risks. The power to detect statistical differences may be low, so we will take care not to over-interpret findings.

2. Domains of bias with the most concern are confounding (e.g. frailty bias), departure from intended intervention (e.g. co-interventions, including other vaccines), outcome measurement (e.g. retrospective collection of mortality information) and selective reporting. We will examine these four domains in meta-regression analyses, dividing studies in the first instance into those assessed at low or moderate risk of bias vs those assessed at serious risk of bias vs those with no information to enable assessment. If a sizeable number of studies including assessments of a specific direction of the likely bias, we will separate the serious risk of bias category according to the anticipated direction. Corresponding dummy variables for the four domains will be included in a meta-regression analysis without the other covariates, to determine whether there is evidence that risk of bias assessments are correlated with relative risks. We will look at the domains separately if the number of studies is small. The power to detect statistical differences may be low, so we will take care not to over-interpret findings.

3. Ideally we would restrict the main analyses to studies at low to moderate risk of bias and studies with an anticipated direction of bias that allow qualitative conclusions to be drawn. The number of studies with these characteristics may however be small, and we will be guided by the findings from the preceding analyses. For tests of null hypotheses, we will nevertheless seek to perform an analysis restricted to such studies. Quantification of effect sizes is unlikely to be reasonable if studies with serious risk of bias are included, even if the direction of bias allows for a qualitative conclusion to be drawn. For estimation of effect sizes, we will include all studies for which we conclude that biases are not seriously impacting on relative risks, taking care to avoid interpreting absence of evidence of bias as evidence of its absence.

3.9.5 Impact of sequence and schedule of vaccination

To examine the effect of vaccine sequence and schedule, we will focus an analysis on the relationship between risk of mortality and the amount of time during which the child's most recent vaccine was (i) BCG, rather than DTP or measles; (i) DTP, rather than BCG or measles; or (i) measles, rather than BCG or DTP. The rationale for this approach the hypothesis that receipt of live attenuated vaccines (e.g. BCG and measles-containing vaccines) is associated with a

subsequent decreased risk of mortality and receipt of inactivated vaccines (e.g. DTP) is associated with a subsequent increased risk of mortality. The effect of each of these vaccines on mortality risk is proposed to last until the time a vaccine of the other type is given (i.e. the decreased risk of mortality following BCG vaccine ends on receipt of DTP vaccine, and the increased risk of mortality following DTP vaccine ends on receipt of a measles-containing vaccine).

For this analysis we intend to include all distinct **vaccination sequence groups** identified from the **Group-level** data, where we define a **vaccination sequence group** to be a group of children defined by a different sequence or schedule of vaccines.

For the analysis, we will distinguish between the **vaccination sequence groups** using the proportionate decrease in time during which each vaccine was the most recent vaccine within that group compared with a chosen reference group. The reference group within each study will be the group with the largest proportion of time during which the vaccine was the most recent vaccine, a proportion that should be strictly bigger than 0% and strictly smaller than 100%. We will also include covariates to represent the actual vaccine sequence. If we have sufficient numbers of studies, the different categories will be No vaccine / BCG only / DTP only / Measles only / BCG+DTP / DTP+measles / DTP+BCG+measles / Other. Otherwise, we will collapse categories, guided by input from a content expert.

Since there may be several groups per study, we anticipate a multivariate meta-regression analysis, allowing each study to contribute several comparisons of sequences or schedules. The dependent variable will be the estimated (log) relative risk of mortality for each vaccination sequence group relative to the chosen reference group.

3.9.6 Detailed examination of effect modification

We will explore effect modification by gender and vitamin A status based on contrasts of subgroups. From each study we will, where available, extract or compute an estimate of interaction between effect modifier and vaccination status. We will then follow the strategies described for the main analysis, focusing on this interaction term (representing a difference in (log) relative risks) rather than the main effect (which represents a (log) relative risk).

Case-control studies - Factors that may bias estimates from case-control studies include whether a matched design has been ignored in the analysis (giving "crude" estimates from studies that have a matched design), and whether the controls were sampled from the same population as the cases. As sensitivity analyses we will report analyses restricted to studies assessed as at low, and low or unclear, risk of bias if this is feasible.

Assessment of the strength of conclusions - We will use the GRADE approach, as modified by WHO Strategic Advisory Group of Experts (SAGE)[3], to assess the evidence in support of various hypothesized associations between various vaccines and (i) survival/all-cause mortality/deaths from causes other than those conditions that the vaccine is designed to prevent and/or; (ii) on all-cause mortality/deaths by all causes/survival in children less than five years of age in all settings. Results will be summarized in GRADE tables.

4 Report

A report summarizing the findings and conclusions will be written and provided to WHO's Department of Immunization Vaccines and Biologicals using the internationally accepted guidelines for systematic reviews (http://www.prisma-statement.org/).

Overall, the report will include:

- a summary of the evidence (tables, forest plots figures) as described above
- critical appraisal of the evidence (methodological considerations, risk of bias and GRADE tables) as described above.

In addition, a verbal report of the findings will be presented at a meeting of SAGE in Geneva, Switzerland during 2014.

[3] Guidance for the development of evidence-based vaccine related recommendations. (http://www.who.int/entity/immunization/sage/Guidelines_development_recommendations.pdf)

5 References

1. Shann F. The non-specific effects of vaccines. Arch Dis Child 2010;95:662-7

2. Aaby P, Samb B, Simondon F, Seck AM, Knudsen K and Whittle H. Non-specific beneficial effect of measles immunisation: analysis of mortality studies from developing countries. Bmj 1995;311:481-5

3. Mathurin KS, Martens GW, Kornfeld H and Welsh RM. CD4 T-cell-mediated heterologous immunity between mycobacteria and poxviruses. J Virol 2009;83:3528-39

4. Ota MO, Vekemans J, Schlegel-Haueter SE, et al. Influence of Mycobacterium bovis bacillus Calmette-Guerin on antibody and cytokine responses to human neonatal vaccination. J Immunol 2002;168:919-25

5. Welsh RM, Che JW, Brehm MA and Selin LK. Heterologous immunity between viruses. Immunol Rev 2010;235:244-66

6. Netea MG, Quintin J and van der Meer JW. Trained immunity: a memory for innate host defense. Cell Host Microbe 2011;9:355-61

7. Anderson EJ, Webb EL, Mawa PA, et al. The influence of BCG vaccine strain on mycobacteria-specific and non-specific immune responses in a prospective cohort of infants in Uganda. Vaccine 2012;30:2083-9

8. Aaby P, Roth A, Ravn H, et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J Infect Dis 2011;204:245-52

9. Kagina BM, Abel B, Bowmaker M, et al. Delaying BCG vaccination from birth to 10 weeks of age may result in an enhanced memory CD4 T cell response. Vaccine 2009;27:5488-95

10. Kristensen I, Aaby P and Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. Bmj 2000;321:1435-8

11. Elguero E, Simondon KB, Vaugelade J, Marra A and Simondon F. Non-specific effects of vaccination on child survival? A prospective study in Senegal. Trop Med Int Health 2005;10:956-60

12. Lehmann D, Vail J, Firth MJ, de Klerk NH and Alpers MP. Benefits of routine immunizations on childhood survival in Tari, Southern Highlands Province, Papua New Guinea. Int J Epidemiol 2005;34:138-48

13. Moulton LH, Rahmathullah L, Halsey NA, Thulasiraj RD, Katz J and Tielsch JM. Evaluation of non-specific effects of infant immunizations on early infant mortality in a southern Indian population. Trop Med Int Health 2005;10:947-55

14. Vaugelade J, Pinchinat S, Guiella G, Elguero E and Simondon F. Non-specific effects of vaccination on child survival: prospective cohort study in Burkina Faso. BMJ 2004;329:1309

15. Chan GJ, Moulton LH, Becker S, Munoz A and Black RE. Non-specific effects of diphtheria tetanus pertussis vaccination on child mortality in Cebu, The Philippines. Int J Epidemiol 2007;36:1022-9

16. Breiman RF, Streatfield PK, Phelan M, Shifa N, Rashid M and Yunus M. Effect of infant immunisation on childhood mortality in rural Bangladesh: analysis of health and demographic surveillance data. Lancet 2004;364:2204-11

17. Farrington CP, Firth MJ, Moulton LH, Ravn H, Andersen PK and Evans S. Epidemiological studies of the non-specific effects of vaccines: II--methodological issues in the design and analysis of cohort studies. Trop Med Int Health 2009;14:977-85

18. Aaby P, Garly ML, Nielsen J, et al. Increased female-male mortality ratio associated with inactivated polio and diphtheria-tetanus-pertussis vaccines: Observations from vaccination trials in Guinea-Bissau. Pediatr Infect Dis J 2007;26:247-52

19. Aaby P, Jensen H, Gomes J, Fernandes M and Lisse IM. The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. Int J Epidemiol 2004;33:374-80

20. Aaby P, Ravn H, Roth A, et al. Early diphtheria-tetanus-pertussis vaccination associated with higher female mortality and no difference in male mortality in a cohort of low birthweight children: an observational study within a randomised trial. Arch Dis Child 2012

21. Agergaard J, Nante E, Poulstrup G, et al. Diphtheria-tetanus-pertussis vaccine administered simultaneously with measles vaccine is associated with increased morbidity and poor growth in girls. A randomised trial from Guinea-Bissau. Vaccine 2011;29:487-500

22. Aaby P, Benn C, Nielsen J, Lisse IM, Rodrigues A and Ravn H. Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. BMJ Open 2012;2

Abbreviations Used in this Protocol

BCG: Bacille Calmette-Guerin Vaccine

DTP: Diphtheria-Tetanus-Pertussis Vaccine

RCT: Randomized controlled trial

Protocol Annex 1 – DRAFT

Basic search strategy - not yet combined the various concepts.

Mortality-all cause mortality

(not included yet)

CHILD Concept

'child'/exp OR 'child' OR 'children'/exp OR 'children' OR 'youth'/exp OR 'youth' OR youth* OR newborn* OR 'newborn'/exp OR 'newborn' OR 'new born' OR 'childhood disease'/exp OR 'childhood disease' OR 'baby'/exp OR 'baby' OR babies OR 'infant'/exp OR 'infant' OR infant* OR childhood* OR toddler* OR kid OR kids OR 'young patient' OR boy* OR girl* OR 'young age' OR pediatr* OR paediatr* OR 'child death'/exp OR 'child death' OR 'child health'/exp OR 'child health' OR 'child care'/exp OR 'child care' OR 'childhood mortality'/exp OR 'childhood mortality' OR 'child hospitalization'/exp OR 'child hospitalization' OR 'pediatric hospital'/exp OR 'pediatric hospital' OR child*

BCG VACCINES

'BCG vaccine'/exp OR 'antituberculosis vaccine' OR ' b.c.g.' OR ' b.c.g. vaccine' OR ' bacillus Calmette Guerin' OR ' bacillus calmette guerin vaccine' OR ' BCG' OR ' bcg cell wall vaccine' OR ' bcg copenhagen 1331' OR ' BCG live' OR ' bcg test' OR ' calgevax' OR ' calmette guerin bacillus' OR ' calmette vaccine' OR ' calmette s vaccine' OR 'calmettes vaccine' OR ' immucyst' OR ' immun bcg pasteur' OR ' monovax' OR ' mva 85a' OR ' mva85a' OR ' mycobacterium bcg' OR ' mycobax' OR ' onco tice' OR ' oncotice' OR ' pacis' OR ' pastimmun' OR ' theracys intravesical' OR ' tice bcg' OR ' tice bcg vaccine' OR ' ticebcg' OR ' tubercle bacilli vaccine' OR ' tuberculosis vaccine' OR ' tuberculosis vaccines'

MEASLES VACCINE

'measles vaccine'/exp OR 'anti measles vaccin' OR ' attenuated live measles vaccine' OR ' attenuated live rubeola virus vaccine' OR ' attenuated measles vaccine' OR ' attenuvax' OR ' cam-kovac' OR ' diplovax' OR ' edmonston zagreb vaccine' OR ' fibroblast grown measles vaccine' OR ' formalin killed measles vaccine' OR ' hyperimmune measles serum' OR ' killed measles vaccine' OR ' killed measles virus vaccine' OR ' lirugen' OR ' lirugen measles' OR ' live attenuated measles vaccine' OR ' live attenuated measles vaccine moraten strain' OR ' live attenuated measles vaccine schwarz strain' OR ' live attenuated measles virus vaccine' OR ' live distemper vaccine' OR ' live distemper virus vaccine' OR ' live measles vaccine' OR ' live rubeola virus vaccine' OR ' m-vac' OR ' measle vaccine' OR ' measles killed vaccine' OR ' measles killed virus vaccine' OR ' measles live vaccine' OR ' measles vaccine, chick embryo fibroblast grown' OR ' measles vaccine, human diploid cell grown' OR ' measles vaccine, tween ether' OR ' measles virus vaccine' OR ' measles virus vaccine live' OR ' mevilin-l' OR ' morbilli vaccine' OR ' morbilvax' OR ' rimevax' OR ' rouvax' OR ' rubeola vaccine' OR ' rubeovax' OR ' tween ether measles vaccine' OR ' vaccine, measles' OR ' vaccine, rubeola' OR ' jeryl lynn moraten vaccine' OR ' mm vax' OR ' mmvax' OR ' mumps measles vaccine' OR ' rimparix' OR ' urabe am 9 rimparix' OR ' urabe am 9 schwarz vaccine' OR 'chickenpox measles mumps rubella vaccine'/exp OR 'measles mumps rubella varicella vaccine' OR ' measles plus mumps plus rubella plus varicella vaccine live ' OR ' proquad'

DIPTHERIA VACCINE

'diphtheria pertussis tetanus vaccine'/exp OR 'absorbed pertussis diphtheria tetanus vaccine' OR ' acel immune' OR ' acel imune' OR ' acelluvax dpt' OR ' acelluvax DTP' OR ' adacel' OR ' adsorbed dt coq' OR ' anatoxal di te per berna' OR ' boostrix' OR ' certiva' OR ' covaxis' OR ' d.t. coq' OR ' daptacel' OR ' dif per tet all' OR ' diphtheria-tetanus-acellular pertussis vaccines' OR ' diphtheria-tetanus-pertussis vaccine' OR ' diphtheria plus pertussis plus tetanus' OR ' diphtheria tetanus acellular pertussis vaccines' OR ' diphtheria tetanus pertussis trivaccine' OR ' diphtheria tetanus pertussis vaccine' OR ' diphtheria tetanus whooping cough vaccine' OR ' diteper anatoxal berna vaccine' OR ' dpt' OR ' DPT vaccine' OR ' DTAP vaccine' OR ' DTP vaccine' OR ' infanrix' OR ' neodiftepertus' OR ' p.d.t. vax purified' OR ' pertugen' OR ' pertussis diphtheria tetanus vaccine' OR ' tetanus diphtheria pertussis vaccine' OR ' tri immunol' OR ' triacelluvax' OR ' triaxis' OR

‘ tripacel’ OR ‘ tripedia’ OR ‘ triplo vaccine’ OR ‘ tripvac’ OR ‘ tritanrix’ OR ‘ trivax’ OR ‘ vaccine, pertussis diphtheria tetanus’ OR ‘*DTP vaccine’ OR ‘Infanrix’*

SEX DIFFERENCES

'sex difference'/exp OR ‘sex differences’ OR ‘ dimorphism, sex’ OR ‘ factor, sex’ OR ‘ gender difference’ OR ‘ gender differences’ ‘ sex dimorphism’ OR ‘ sex factor’ OR ‘ sex factors’ OR ‘ sexual difference’ OR ‘ sexual dimorphism’ OR ‘ sexual size dimorphism’ OR ‘evolution, sex’ OR ‘ sex characteristics’ OR ‘ sex development’ OR ‘ sex evolution’ OR ‘advanced puberty’ OR ‘ pseudopuberty’ OR ‘ pubescence’ OR ‘sex differentiation’/exp OR ‘sex related factors’ OR ‘sex related factor’ OR ‘sexual development’/exp OR 'male'/exp AND 'female'/exp AND 'gender'/exp OR 'boy'/exp AND 'girl'/exp AND 'gender'/exp

('Vitamin A')

'retinol'/exp OR ‘3, 7 dimethyl 9 (2, 6, 6 trimethyl 1 cyclohexen 1 yl) 2, 4, 6, 8 nonatetraen 1 ol’ OR ‘ 3, 7 dimethyl 9 (2, 6, 6 trimethyl 1 cyclohexenyl) 2, 4, 6, 8 nonatetraen 1 ol’ OR ‘ a 313’ OR ‘ a fil’ OR ‘ a mulsal’ OR ‘ a mulsin’ OR ‘ a mulsine’ OR ‘ a sol’ OR ‘ a vi pel’ OR ‘ a vit’ OR ‘ a vitadit’ OR ‘ a vitamin’ OR ‘ a vitan’ OR ‘ a313’ OR ‘ acon’ OR ‘ acrisina’ OR ‘ acrisine’ OR ‘ actifral a’ OR ‘ adatone’ OR ‘ afaxin’ OR ‘ afaxine’ OR ‘ afilina’ OR ‘ afiline’ OR ‘ agiolan’ OR ‘ alcovit a’ OR ‘ alfa monovite’ OR ‘ alfa sir’ OR ‘ alfaergin’ OR ‘ alfaergine’ OR ‘ alfamin’ OR ‘ alfamine’ OR ‘ alfamonovit’ OR ‘ alfasir’ OR ‘ alfasole’ OR ‘ alfasterolo’ OR ‘ alfatar’ OR ‘ alfavena’ OR ‘ alfavene’ OR ‘ alfavitina’ OR ‘ alfavitine’ OR ‘ alfene’ OR ‘ all trans retinol’ OR ‘ alphalin’ OR ‘ alphaline’ OR ‘ alphasterol’ OR ‘ amulsal’ OR ‘ amulsin’ OR ‘ amulsine’ OR ‘ amulvit’ OR ‘ anatola’ OR ‘ anatole’ OR ‘ anavit’ OR ‘ anti infective vitamin’ OR ‘ antixerophthalmic vitamin’ OR ‘ aoral’ OR ‘ apexol’ OR ‘ apostavit’ OR ‘ aquasol a’ OR ‘ arcavit A’ OR ‘ asol’ OR ‘ asteril’ OR ‘ atav’ OR ‘ aterapion’ OR ‘ avibon’ OR ‘ avibon theraplix’ OR ‘ avimin’ OR ‘ avimine’ OR ‘ avipel’ OR ‘ avipur’ OR ‘ avit’ OR ‘ avitabiol’ OR ‘ avitadit’ OR ‘ avital’ OR ‘ avitaminum kolin’ OR ‘ avitan’ OR ‘ avitana’ OR ‘ avitane’ OR ‘ avite’ OR ‘ avitil’ OR ‘ avitina’ OR ‘ avitol’ OR ‘ avogina’ OR ‘ avogine’ OR ‘ avoleum’ OR ‘ axerodina’ OR ‘ axerodine’ OR ‘ axerol’ OR ‘ axerophthol’ OR ‘ axerophthylium’ OR ‘ bentavit a’ OR ‘ bentavite a’ OR ‘ bio tan’ OR ‘ biosterol’ OR ‘ biotan’ OR ‘ chivibit a’ OR ‘ cytobiase’ OR ‘ dagravit a’ OR ‘ davitamon a’ OR ‘ difvitamin a’ OR ‘ dohyfral a’ OR ‘ elageno a’ OR ‘ endo a’ OR ‘ envit a’ OR ‘ epiteliol’ OR ‘ evitol zambeletti’ OR ‘ fletase’ OR ‘ gadeol’ OR ‘ gadol’ OR ‘ halivitan’ OR ‘ halivitane’ OR ‘ homagenets aoral’ OR ‘ hydrosol’ OR ‘ ido a’ OR ‘ ido a 50’ OR ‘ idratene’ OR ‘ inovitan a’ OR ‘ lord factor’ OR ‘ meditalfa’ OR ‘ mulsal a’ OR ‘ multamine’ OR ‘ nio a let’ OR ‘ oleovit a’ OR ‘ oleovitamin a’ OR ‘ ophthalamin’ OR ‘ panvita’ OR ‘ plivit a’ OR ‘ prepalin’ OR ‘ prepaline’ OR ‘ preparato a’ OR ‘ primavit’ OR ‘ quotivit’ OR ‘ retinol alcohol’ OR ‘ retinyl alcohol’ OR ‘ ro-a-vit’ OR ‘ ro a vit’ OR ‘ super a’ OR ‘ testavol’ OR ‘ ucemine a’ OR ‘ vaconex’ OR ‘ vaflol’ OR ‘ veroftal’ OR ‘ vi alpha’ OR ‘ vi dom a’ OR ‘ viadenin’ OR ‘ vialpha’ OR ‘ viatate’ OR ‘ vidoma’ OR ‘ vitadone’ OR ‘ vitadral’ OR ‘ vitalen a’ OR ‘ vitalfa’ OR ‘ vitama’ OR ‘ vitamin A’ OR ‘ vitamin a alcohol’ OR ‘ vitamin a1’ OR ‘ vitaplex a’ OR ‘ vitapur a’ OR ‘ vitasan a’ OR ‘ vitavel a’ OR ‘ vitpex’ OR ‘ vogan’ OR ‘ vogan neu’ OR ‘ wandervit a’ OR ‘ xerophthol’ OR ‘vitamin A’

[i] Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analysis. BMJ 2003; 327: 557-560.