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**TITLE:**

A step by step guide for conducting a systematic review and meta-analysis with simulation data

**ABSTRACT:**

**BACKGROUND:** The massive abundance of studies relating to tropical medicine and health has increased strikingly over the last few decades. In the field of tropical medicine and health, a well-conducted systematic review and meta-analysis (SR/MA) is considered a feasible solution for keeping clinicians abreast of current evidence-based medicine. Understanding of SR/MA steps is of paramount importance for its conduction. It is not easy to be done as there are obstacles that could face the researcher. To solve those hindrances, this methodology study aimed to provide a step-by-step approach mainly for beginners and junior researchers, in the field of tropical medicine and other health care fields, on how to properly conduct a SR/MA, in which all the steps here depicts our experience and expertise combined with the already well-known and accepted international guidance. We suggest that all steps of SR/MA should be done independently by 2–3 reviewers' discussion, to ensure data quality and accuracy. **CONCLUSION:** SR/MA steps include the development of research question, forming criteria, search strategy, searching databases, protocol registration, title, abstract, full-text screening, manual searching, extracting data, quality assessment, data checking, statistical analysis, double data checking, and manuscript writing.

**ELECTRONIC SUPPLEMENTARY MATERIAL:** The online version of this article (10.1186/s41182-019-0165-6) contains supplementary material, which is available to authorized users.

**Introduction:**

The amount of studies published in the biomedical literature, especially tropical medicine and health, has increased strikingly over the last few decades. This massive abundance of literature makes clinical medicine increasingly complex, and knowledge from various researches is often needed to inform a particular clinical decision. However, available studies are often heterogeneous with regard to their design, operational quality, and subjects under study and may handle the research question in a different way, which adds to the complexity of evidence and conclusion synthesis [1].

Systematic review and meta-analyses (SR/MAs) have a high level of evidence as represented by the evidence-based pyramid. Therefore, a well-conducted SR/MA is considered a feasible solution in keeping health clinicians ahead regarding contemporary evidence-based medicine. Differing from a systematic review, unsystematic narrative review tends to be descriptive, in which the authors select frequently articles based on their point of view which leads to its poor quality. A systematic review, on the other hand, is defined as a review using a systematic method to summarize evidence on questions with a detailed and comprehensive plan of study. Furthermore, despite the increasing guidelines for effectively conducting a systematic review, we found that basic steps often start from framing question, then identifying relevant work which consists of criteria development and search for articles, appraise the quality of included studies, summarize the evidence, and interpret the results [2, 3]. However, those simple steps are not easy to be reached in reality. There are many troubles that a researcher could be struggled with which has no detailed indication.

Conducting a SR/MA in tropical medicine and health may be difficult especially for young researchers; therefore, understanding of its essential steps is crucial. It is not easy to be done as there are obstacles that could face the researcher. To solve those hindrances, we recommend a flow diagram (Fig. 1) which illustrates a detailed and step-by-step the stages for SR/MA studies. This methodology study aimed to provide a step-by-step approach mainly for beginners and junior researchers, in the field of tropical medicine and other health care fields, on how to properly and succinctly conduct a SR/MA; all the steps here depicts our experience and expertise combined with the already well known and accepted international guidance.

**Research question and objectives :::** Detailed steps for conducting any systematic review and meta-analysis :::

**Methods and results:**

Like other study designs, the research question of SR/MA should be feasible, interesting, novel, ethical, and relevant. Therefore, a clear, logical, and well-defined research question should be formulated. Usually, two common tools are used: PICO or SPIDER. PICO (Population,

Intervention, Comparison, Outcome) is used mostly in quantitative evidence synthesis. Authors demonstrated that PICO holds more sensitivity than the more specific SPIDER approach [6]. SPIDER (Sample, Phenomenon of Interest, Design, Evaluation, Research type) was proposed as a method for qualitative and mixed methods search.

We here recommend a combined approach of using either one or both the SPIDER and PICO tools to retrieve a comprehensive search depending on time and resources limitations. When we apply this to our assumed research topic, being of qualitative nature, the use of SPIDER approach is more valid.

PICO is usually used for systematic review and meta-analysis of clinical trial study. For the observational study (without intervention or comparator), in many tropical and epidemiological questions, it is usually enough to use P (Patient) and O (outcome) only to formulate a research question. We must indicate clearly the population (P), then intervention (I) or exposure. Next, it is necessary to compare (C) the indicated intervention with other interventions, i.e., placebo. Finally, we need to clarify which are our relevant outcomes.

To facilitate comprehension, we choose the Ebola virus disease (EVD) as an example. Currently, the vaccine for EVD is being developed and under phase I, II, and III clinical trials; we want to know whether this vaccine is safe and can induce sufficient immunogenicity to the subjects. An example of a research question for SR/MA based on PICO for this issue is as follows: How is the safety and immunogenicity of Ebola vaccine in human? (P: healthy subjects (human), I: vaccination, C: placebo, O: safety or adverse effects)

Preliminary research and idea validation ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

We recommend a preliminary search to identify relevant articles, ensure the validity of the proposed idea, avoid duplication of previously addressed questions, and assure that we have enough articles for conducting its analysis. Moreover, themes should focus on relevant and important health-care issues, consider global needs and values, reflect the current science, and be consistent with the adopted review methods. Gaining familiarity with a deep understanding of the study field through relevant videos and discussions is of paramount importance for better retrieval of results. If we ignore this step, our study could be canceled whenever we find out a similar study published before. This means we are wasting our time to deal with a problem that has been tackled for a long time.

To do this, we can start by doing a simple search in PubMed or Google Scholar with search terms Ebola AND vaccine. While doing this step, we identify a systematic review and meta-analysis of determinant factors influencing antibody response from vaccination of Ebola vaccine in non-human primate and human [7], which is a relevant paper to read to get a deeper insight and identify gaps for better formulation of our research question or purpose. We can still conduct systematic review and meta-analysis of Ebola vaccine because we evaluate safety as a different outcome and different population (only human).

Inclusion and exclusion criteria ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

Eligibility criteria are based on the PICO approach, study design, and date. Exclusion criteria mostly are unrelated, duplicated, unavailable full texts, or abstract-only papers. These exclusions should be stated in advance to refrain the researcher from bias. The inclusion criteria would be articles with the target patients, investigated interventions, or the comparison between two studied interventions. Briefly, it would be articles which contain information answering our research question. But the most important is that it should be clear and sufficient information, including positive or negative, to answer the question.

For the topic we have chosen, we can make inclusion criteria: (1) any clinical trial evaluating the safety of Ebola vaccine and (2) no restriction regarding country, patient age, race, gender, publication language, and date. Exclusion criteria are as follows: (1) study of Ebola vaccine in non-human subjects or in vitro studies; (2) study with data not reliably extracted, duplicate, or overlapping data; (3) abstract-only papers as preceding papers, conference, editorial, and author response theses and books; (4) articles without available full text available; and (5) case reports, case series, and systematic review studies. The PRISMA flow diagram template that is used in SR/MA studies can be found in Fig. 2.

Search strategy ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

A standard search strategy is used in PubMed, then later it is modified according to each specific database to get the best relevant results. The basic search strategy is built based on the research question formulation (i.e., PICO or PICOS). Search strategies are constructed to include free-text terms (e.g., in the title and abstract) and any appropriate subject indexing (e.g., MeSH) expected to retrieve eligible studies, with the help of an expert in the review topic field or an information specialist. Additionally, we advise not to use terms for the Outcomes as their inclusion might hinder the database being searched to retrieve eligible studies because the used outcome is not mentioned obviously in the articles.

The improvement of the search term is made while doing a trial search and looking for another relevant term within each concept from retrieved papers. To search for a clinical trial, we can use these descriptors in PubMed: “clinical trial”[Publication Type] OR “clinical trials as topic”[MeSH terms] OR “clinical trial”[All Fields]. After some rounds of trial and refinement of search term, we formulate the final search term for PubMed as follows: (ebola OR ebola virus OR ebola virus disease OR EVD) AND (vaccine OR vaccination OR vaccinated OR immunization) AND (“clinical trial”[Publication Type] OR “clinical trials as topic”[MeSH Terms] OR “clinical trial”[All Fields]). Because the study for this topic is limited, we do not include outcome term (safety and immunogenicity) in the search term to capture more studies.

Search databases, import all results to a library, and exporting to an excel sheet ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:  
According to the AMSTAR guidelines, at least two databases have to be searched in the SR/MA [8], but as you increase the number of searched databases, you get much yield and more accurate and comprehensive results. The ordering of the databases depends mostly on the review questions; being in a study of clinical trials, you will rely mostly on Cochrane, mRCTs, or International Clinical Trials Registry Platform (ICTRP). Here, we propose 12 databases (PubMed, Scopus, Web of Science, EMBASE, GHL, VHL, Cochrane, Google Scholar, Clinical trials.gov, mRCTs, POPLINE, and SIGLE), which help to cover almost all published articles in tropical medicine and other health-related fields. Among those databases, POPLINE focuses on reproductive health. Researchers should consider to choose relevant database according to the research topic. Some databases do not support the use of Boolean or quotation; otherwise, there are some databases that have special searching way. Therefore, we need to modify the initial search terms for each database to get appreciated results; therefore, manipulation guides for each online database searches are presented in Additional file 5: Table S2. The detailed search strategy for each database is found in Additional file 5: Table S3. The search term that we created in PubMed needs customization based on a specific characteristic of the database. An example for Google Scholar advanced search for our topic is as follows: With all of the words: ebola virus With at least one of the words: vaccine vaccination vaccinated immunization Where my words occur: in the title of the article With all of the words: EVD With at least one of the words: vaccine vaccination vaccinated immunization Where my words occur: in the title of the article Finally, all records are collected into one Endnote library in order to delete duplicates and then to export into an excel sheet. Using remove duplicating function with two options is mandatory. All references which have (1) the same title and author, and published in the same year, and (2) the same title and author, and published in the same journal, would be deleted. References remaining after this step should be exported to an excel file with essential information for screening. These could be the authors' names, publication year, journal, DOI, URL link, and abstract.

Protocol writing and registration ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

Protocol registration at an early stage guarantees transparency in the research process and protects from duplication problems. Besides, it is considered a documented proof of team plan of action, research question, eligibility criteria, intervention/exposure, quality assessment, and pre-analysis plan. It is recommended that researchers send it to the principal investigator (PI) to revise it, then upload it to registry sites. There are many registry sites available for SR/MA like those proposed by Cochrane and Campbell collaborations; however, we recommend registering the protocol into PROSPERO as it is easier. The layout of a protocol template, according to PROSPERO, can be found in Additional file 5: File S1.

Title and abstract screening ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

Decisions to select retrieved articles for further assessment are based on eligibility criteria, to minimize the chance of including non-relevant articles. According to the Cochrane guidance, two reviewers are a must to do this step, but as for beginners and junior researchers, this might be tiresome; thus, we propose based on our experience that at least three reviewers should work independently to reduce the chance of error, particularly in teams with a large number of authors to add more scrutiny and ensure proper conduct. Mostly, the quality with three reviewers would be better than two, as two only would have different opinions from each other, so they cannot decide, while the third opinion is crucial. And here are some examples of systematic reviews which we conducted following the same strategy (by a different group of researchers in our research group) and published successfully, and they feature relevant ideas to tropical medicine and disease [9–11].

In this step, duplications will be removed manually whenever the reviewers find them out. When there is a doubt about an article decision, the team should be inclusive rather than exclusive, until the main leader or PI makes a decision after discussion and consensus. All excluded records should be given exclusion reasons.

Full text downloading and screening ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

Many search engines provide links for free to access full-text articles. In case not found, we can search in some research websites as ResearchGate, which offer an option of direct full-text request from authors. Additionally, exploring archives of wanted journals, or contacting PI to purchase it if available. Similarly, 2–3 reviewers work independently to decide about included full texts according to eligibility criteria, with reporting exclusion reasons of articles. In case any disagreement has occurred, the final decision has to be made by discussion.

Manual search ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

One has to exhaust all possibilities to reduce bias by performing an explicit hand-searching for retrieval of reports that may have been dropped from first search [12]. We apply five methods to make manual searching: searching references from included studies/reviews, contacting authors and experts, and looking at related articles/cited articles in PubMed and Google Scholar.

We describe here three consecutive methods to increase and refine the yield of manual searching: firstly, searching reference lists of included articles; secondly, performing what is known as citation tracking in which the reviewers track all the articles that cite each one of the included articles, and this might involve electronic searching of databases; and thirdly, similar to the citation tracking, we follow all “related to” or “similar” articles. Each of the abovementioned methods can be performed by 2–3 independent reviewers, and all the possible relevant article must undergo further scrutiny against the inclusion criteria, after following the same records yielded from electronic databases, i.e., title/abstract and full-text screening.

We propose an independent reviewing by assigning each member of the teams a “tag” and a distinct method, to compile all the results at the end for comparison of differences and discussion and to maximize the retrieval and minimize the bias. Similarly, the number of included articles has to be stated before addition to the overall included records.

Data extraction and quality assessment ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

This step entitles data collection from included full-texts in a structured extraction excel sheet, which is previously pilot-tested for extraction using some random studies. We recommend extracting both adjusted and non-adjusted data because it gives the most allowed confounding factor to be used in the analysis by pooling them later [13]. The process of extraction should be executed by 2–3 independent reviewers. Mostly, the sheet is classified into the study and patient characteristics, outcomes, and quality assessment (QA) tool.

Data presented in graphs should be extracted by software tools such as Web plot digitizer [14]. Most of the equations that can be used in extraction prior to analysis and estimation of standard deviation (SD) from other variables is found inside Additional file 5: File S2 with their references as Hozo et al. [15], Xiang et al. [16], and Rijkom et al. [17]. A variety of tools are available for the QA, depending on the design: ROB-2 Cochrane tool for randomized controlled trials [18] which is presented as Additional file 1: Figure S1 and Additional file 2: Figure S2—from a previous published article data—[19], NIH tool for observational and cross-sectional studies [20], ROBINS-I tool for non-randomize trials [21], QUADAS-2 tool for diagnostic studies, QUIPS tool for

prognostic studies, CARE tool for case reports, and ToxRtool for in vivo and in vitro studies. We recommend that 2–3 reviewers independently assess the quality of the studies and add to the data extraction form before the inclusion into the analysis to reduce the risk of bias. In the NIH tool for observational studies—cohort and cross-sectional—as in this EBOLA case, to evaluate the risk of bias, reviewers should rate each of the 14 items into dichotomous variables: yes, no, or not applicable. An overall score is calculated by adding all the items scores as yes equals one, while no and NA equals zero. A score will be given for every paper to classify them as poor, fair, or good conducted studies, where a score from 0–5 was considered poor, 6–9 as fair, and 10–14 as good.

In the EBOLA case example above, authors can extract the following information: name of authors, country of patients, year of publication, study design (case report, cohort study, or clinical trial or RCT), sample size, the infected point of time after EBOLA infection, follow-up interval after vaccination time, efficacy, safety, adverse effects after vaccinations, and QA sheet (Additional file 6: Data S1).

Data checking ::: Detailed steps for conducting any systematic review and meta-analysis :::

Methods and results:

Due to the expected human error and bias, we recommend a data checking step, in which every included article is compared with its counterpart in an extraction sheet by evidence photos, to detect mistakes in data. We advise assigning articles to 2–3 independent reviewers, ideally not the ones who performed the extraction of those articles. When resources are limited, each reviewer is assigned a different article than the one he extracted in the previous stage.

Statistical analysis ::: Detailed steps for conducting any systematic review and meta-analysis :::

Methods and results:

Investigators use different methods for combining and summarizing findings of included studies. Before analysis, there is an important step called cleaning of data in the extraction sheet, where the analyst organizes extraction sheet data in a form that can be read by analytical software. The analysis consists of 2 types namely qualitative and quantitative analysis. Qualitative analysis mostly describes data in SR studies, while quantitative analysis consists of two main types: MA and network meta-analysis (NMA). Subgroup, sensitivity, cumulative analyses, and meta-regression are appropriate for testing whether the results are consistent or not and investigating the effect of certain confounders on the outcome and finding the best predictors. Publication bias should be assessed to investigate the presence of missing studies which can affect the summary. To illustrate basic meta-analysis, we provide an imaginary data for the research question about Ebola vaccine safety (in terms of adverse events, 14 days after injection) and immunogenicity (Ebola virus antibodies rise in geometric mean titer, 6 months after injection). Assuming that from searching and data extraction, we decided to do an analysis to evaluate Ebola vaccine “A” safety and immunogenicity. Other Ebola vaccines were not meta-analyzed because of the limited number of studies (instead, it will be included for narrative review). The imaginary data for vaccine safety meta-analysis can be accessed in Additional file 7: Data S2. To do the meta-analysis, we can use free software, such as RevMan [22] or R package meta [23]. In this example, we will use the R package meta. The tutorial of meta package can be accessed through “General Package for Meta-Analysis” tutorial pdf [23]. The R codes and its guidance for meta-analysis done can be found in Additional file 5: File S3.

For the analysis, we assume that the study is heterogenous in nature; therefore, we choose a random effect model. We did an analysis on the safety of Ebola vaccine A. From the data table, we can see some adverse events occurring after intramuscular injection of vaccine A to the subject of the study. Suppose that we include six studies that fulfill our inclusion criteria. We can do a meta-analysis for each of the adverse events extracted from the studies, for example, arthralgia, from the results of random effect meta-analysis using the R meta package.

From the results shown in Additional file 3: Figure S3, we can see that the odds ratio (OR) of arthralgia is 1.06 (0.79; 1.42),  $p$  value = 0.71, which means that there is no association between the intramuscular injection of Ebola vaccine A and arthralgia, as the OR is almost one, and besides, the  $P$  value is insignificant as it is  $> 0.05$ .

In the meta-analysis, we can also visualize the results in a forest plot. It is shown in Fig. 3 an example of a forest plot from the simulated analysis.

From the forest plot, we can see six studies (A to F) and their respective OR (95% CI). The green box represents the effect size (in this case, OR) of each study. The bigger the box means the study weighted more (i.e., bigger sample size). The blue diamond shape represents the pooled OR

of the six studies. We can see the blue diamond cross the vertical line  $OR = 1$ , which indicates no significance for the association as the diamond almost equalized in both sides. We can confirm this also from the 95% confidence interval that includes one and the  $p$  value  $> 0.05$ .

For heterogeneity, we see that  $I^2 = 0\%$ , which means no heterogeneity is detected; the study is relatively homogenous (it is rare in the real study). To evaluate publication bias related to the meta-analysis of adverse events of arthralgia, we can use the metabias function from the R meta package (Additional file 4: Figure S4) and visualization using a funnel plot. The results of publication bias are demonstrated in Fig. 4. We see that the  $p$  value associated with this test is 0.74, indicating symmetry of the funnel plot. We can confirm it by looking at the funnel plot. Looking at the funnel plot, the number of studies at the left and right side of the funnel plot is the same; therefore, the plot is symmetry, indicating no publication bias detected.

Sensitivity analysis is a procedure used to discover how different values of an independent variable will influence the significance of a particular dependent variable by removing one study from MA. If all included study  $p$  values are  $< 0.05$ , hence, removing any study will not change the significant association. It is only performed when there is a significant association, so if the  $p$  value of MA done is 0.7—more than one—the sensitivity analysis is not needed for this case study example. If there are 2 studies with  $p$  value  $> 0.05$ , removing any of the two studies will result in a loss of the significance.

Double data checking ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

For more assurance on the quality of results, the analyzed data should be rechecked from full-text data by evidence photos, to allow an obvious check for the PI of the study.

Manuscript writing, revision, and submission to a journal ::: Detailed steps for conducting any systematic review and meta-analysis ::: Methods and results:

Writing based on four scientific sections: introduction, methods, results, and discussion, mostly with a conclusion. Performing a characteristic table for study and patient characteristics is a mandatory step which can be found as a template in Additional file 5: Table S3.

After finishing the manuscript writing, characteristics table, and PRISMA flow diagram, the team should send it to the PI to revise it well and reply to his comments and, finally, choose a suitable journal for the manuscript which fits with considerable impact factor and fitting field. We need to pay attention by reading the author guidelines of journals before submitting the manuscript.

Discussion:

The role of evidence-based medicine in biomedical research is rapidly growing. SR/MAs are also increasing in the medical literature. This paper has sought to provide a comprehensive approach to enable reviewers to produce high-quality SR/MAs. We hope that readers could gain general knowledge about how to conduct a SR/MA and have the confidence to perform one, although this kind of study requires complex steps compared to narrative reviews.

Having the basic steps for conduction of MA, there are many advanced steps that are applied for certain specific purposes. One of these steps is meta-regression which is performed to investigate the association of any confounder and the results of the MA. Furthermore, there are other types rather than the standard MA like NMA and MA. In NMA, we investigate the difference between several comparisons when there were not enough data to enable standard meta-analysis. It uses both direct and indirect comparisons to conclude what is the best between the competitors. On the other hand, mega MA or MA of patients tend to summarize the results of independent studies by using its individual subject data. As a more detailed analysis can be done, it is useful in conducting repeated measure analysis and time-to-event analysis. Moreover, it can perform analysis of variance and multiple regression analysis; however, it requires homogenous dataset and it is time-consuming in conduct [24].

Conclusions:

Systematic review/meta-analysis steps include development of research question and its validation, forming criteria, search strategy, searching databases, importing all results to a library and exporting to an excel sheet, protocol writing and registration, title and abstract screening, full-text screening, manual searching, extracting data and assessing its quality, data checking, conducting statistical analysis, double data checking, manuscript writing, revising, and submitting to a journal.