Phase III Clinical Studies for Vaccine Evaluation

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Introduction

The following chapter addresses how a researcher or a practitioner in charge of quality in a clinical trial may respond to the efficacy and safety of a candidate vaccine. Design par excellence is a randomized, double-blind, controlled trial where one group of the study receives the candidate vaccine while the other group receives the placebo as the control group. Several methodological considerations are discussed: how to structure a null hypothesis, how to select the study population and the importance and methods of randomization to avoid biases.

Since it is very difficult for an experiment to include the entire target population of the study, the sample size is explained and the important role of the researcher in defining the magnitude of the effect expected through the candidate vaccine, its significance and potency. The measurement, analysis and interpretation of study results, through intention to treat (ITT) or per-protocol analysis (PPA) methodologies are presented. Since one of the cornerstones of clinical study design is the ethical aspect of research conducted in human beings, a special section is included to explain the general principles behind research and the importance of informed consent. Finally, guidelines on how to design clinical studies and assess their quality are presented.

Overview

Based on the assignment to exposure, epidemiological studies fall into two types: observational and experimental studies. The former includes descriptive and analytical studies; the latter are conducted to verify a hypothesis and allow for the establishment of comparisons amongst various groups.[1]

The study factor in experimental studies is controlled by the research team and the studies are intended to evaluate the safety, efficacy, and optimal dose for one or more drugs, medical or technical devices for diagnostic, therapeutic, or prophylactic purposes, based on eligibility criteria, whose effects may demonstrate favorable or unfavorable effects for individuals. This design implies that the ethical requirements of research in human beings fulfill a key role in their execution.

Within experimental studies, the most important design is the randomized clinical trial (RCT).

The clinical study phases range between I and IV. Phase I is the first stage in an experimental study in human beings where the dose, the immunogenicity, and the administration path are assessed. Phase I is conducted in healthy individuals and requires about 100 individuals. Phase II is also conducted in volunteers, about 300 or 500 individuals, to study the immune response and product safety.

Phase III clinical studies share some common characteristics:

1. They are prospective;
2. They are closed: they use blinding techniques;
3. The researcher uses a research hypothesis with a clearly defined goal,
4. The outcome of a trial to demonstrate the protective efficacy of a vaccine depends on the case definition and the specificity for case detection and confirmation methods;[2]
5. They are designed to assess the efficacy and the safety of the intervention;
6. They are controlled and randomized;
7. They are the last phase of clinical research before the product is registered by the Regulatory Authority and authorized for entry into the market; therefore, the conditions of the study should attempt to replicate the conditions for regular use of the candidate medicine or product;
8. They use an estimated sample size to determine if there are statistically significant differences between the therapy and the placebo.[3]

Phase IV studies are conducted upon approval for distribution or marketing by national Pharmaceutical Regulatory Authorities (PRAs). Once a vaccine has been registered and is in use, pharmacovigilance is key to permanently maintaining information about its safety in the population. These studies are also conducted when the intention is to establish a new clinical indication.

Methodological Considerations

A clinical trial is initiated to answer a question on the efficacy and safety of a vaccine and it requires careful planning, ongoing monitoring of its execution, and follow-up of individuals to ensure no biases are present and the results are valid.

The research question is the most important step for research design and development. It should be:

- **Feasible:** adequate number of individuals, technical expertise, affordable in terms of time and financing.
- **Interesting:** it conveys the effect and safety of the vaccine to be used afterwards to solve a public health problem.
- **Novel (Original):** it confirms or refutes previous findings; it provides new results.
- **Ethical:** the benefits outweigh the damages and the main principles of research in human beings are respected.
- **Relevant:** to scientific knowledge, future research paths, or clinical and health policies.

Next, the meaning of each of the above-mentioned characteristics of Phase III studies shall be explained.

1. **Study hypothesis and purpose:** to detect through research whether the candidate vaccine is more effective than placebo. The null hypothesis will indicate that the effect of the candidate vaccine is similar to the placebo.
2. **Study population:** is selected from the population used to define the inclusion and exclusion criteria. The inclusion criteria define the population to be included in the study. Upon the understanding and signature of informed consent by the study participant, or their legal representative if the participant is under age, studies on preventive vaccines should be conducted in a healthy population of the same age group, sex and place of origin for which the vaccine will be recommended. Exclusion criteria are not the negation of the inclusion criteria: in general, experimental studies exclude individuals with an underlying pathology, pregnant women, vulnerable populations, and populations that would be unable to attend periodic checkups, or have contraindications to vaccine administration such as a history of allergy to some of the vaccine components.
3. **Controlled and randomized studies:** refer to the conditions which need to be controlled in every aspect, such as selection of individuals, storage, and medicine administration, registration of all variables and parameters that could impact the study, and measurement of results. They must have at least two groups or research arms: the study group (candidate vaccine) and a control group (placebo, the best available therapy shall be administered; it may be a vaccine with demonstrated efficacy).[4] Randomization in both groups is intended to distribute every participant at random in one or the other arm to obtain a balanced distribution of the demographic characteristics of the study population. Randomization is intended to attain as much homogeneity as possible in both groups to avoid selection biases since the only difference should be the intervention being studied.

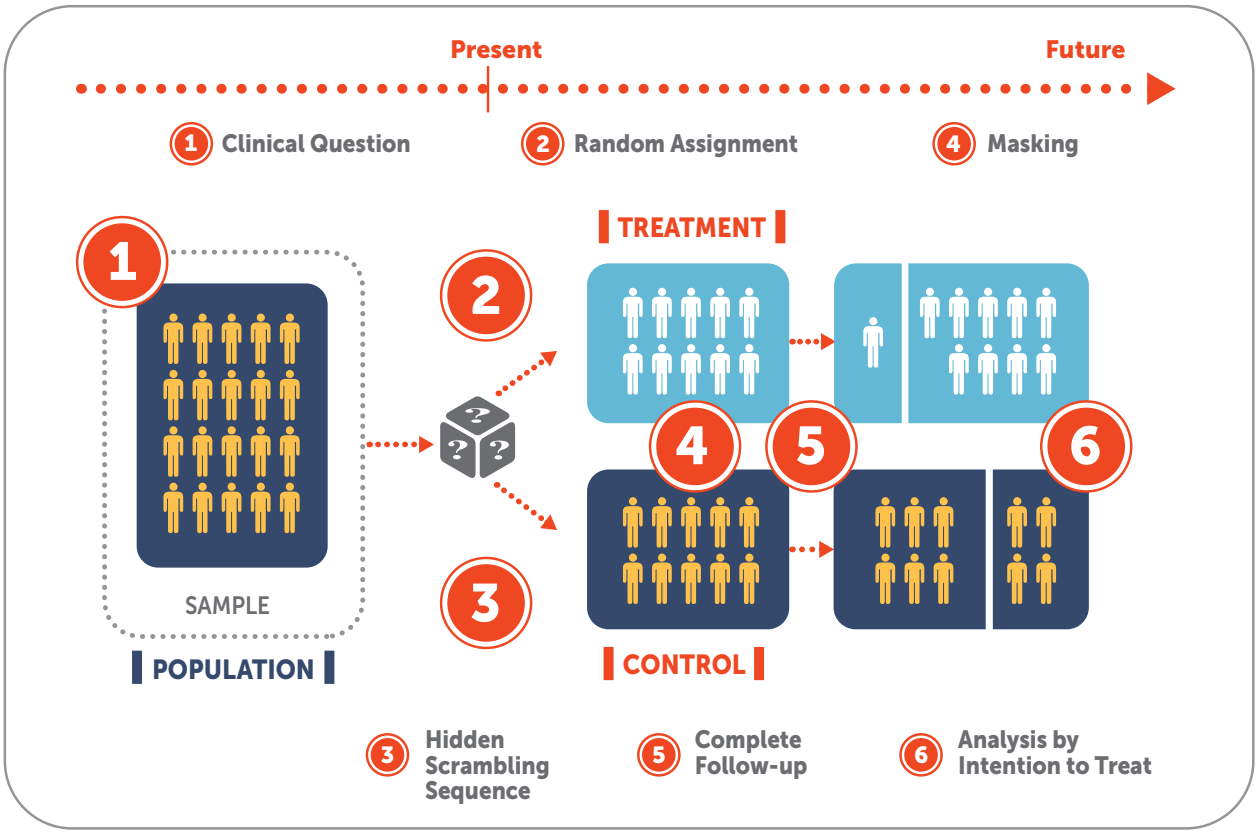
   Statistical randomization techniques include:

   - *Simple randomization:* it is the most straightforward way to randomize the intervention; uses as a basic tool the table or series of random numbers to avoid any type of bias; whenever possible, it should be computer generated and the person in charge should not be a member of the recruitment and follow-up team. With small samples, the use of this method may lead to imbalances in the number of individuals assigned to each group. Another drawback that must be taken into account is that sometimes repeated sequences of the same intervention may occur.[5]
   - *Block randomization:* this method counters the drawbacks under simple randomization. It comprises a series of blocks of cells which include an equal number of intervention options; the number of blocks to use depends on the number of patients to be assigned to an intervention, thus: number of blocks = number of patients/number of cells per block. The use number for each block is determined by the random number table and the assignment is done one patient at a time following the order obtained. The drawback with this method is that it does not balance the potentially modifiable variables of the effect or confounders.
   - *Cluster randomization:* is a simple or block randomization method in which the allocation unit is the group, rather than the individual. For this method it is important to estimate the measure of intracluster correlation (ρ) to determine the degree of response similarity amongst the group members; a positive ρ indicates that the variation in the observations amongst the different groups exceeds the variation within them.

4. **Blinding:** is a procedure used to prevent study participants from knowing the treatment they are receiving, to avoid the bias of the observer, and therefore, prevent them from impacting the answer. Types of blinding include:

    - *Single blind:* the participants are unaware of the intervention each individual receives.
    - *Double blind:* both the participants and researchers are unaware of the intervention.
    - *Triple blind:* in addition to the participants and researchers, other individuals are unaware of the treatment received by each individual, such as the statistician or those who assess the outcomes.

Figure 1. Phase III Clinical Study Design



Source: Molina Arias M, Ochoa Sangrador C. Ensayo clínico (III). Aleatorización. Enmascaramiento. *Evid Pediatr.* 2015;11:15.

5. **Sample size calculation:** is intended to determine the desired effect (answer to the research question) with a level of statistical significance and adequate power.[6,7] Based on the hypothesis, the research should issue findings on:

   - The scope of the candidate vaccine impact, i.e., how much the incidence of disease will diminish as compared to the control group or how much the number of deaths will decrease (depending on the outcome of the study). It is necessary to have baseline information, derived from epidemiological surveillance or through official morbidity or mortality registries, to estimate incidence in the population prior to the study.
   - The level of significance refers to the type I (α) error (Table 1); it is the error made when stating that the difference between the results obtained in the experimental group and the control group is significant at random. In general, this error is specified as 0.05, or there is a 5% probability of rejecting the null hypothesis when it is true, i.e., there is no difference between both treatment groups.
   - Another way to understand the size of the type I error is by interpreting its complement (1−α) as the level of evidence reached to reject the null hypothesis. In other words, this complement is the level of certainty with which the null hypothesis is rejected.
   - Power is derived from the difference of 1−β; rejecting the null hypothesis when it is false. Its complement is the type II β error, i.e., the probability of affirming there are no differences between the study groups when that is not the case. It is the capacity to detect a minimum difference of clinical significance, rejecting the null hypothesis when it is false.

Table 1. Type I and Type II Errors

	H_0 is True	**H_0 is False**
DO NOT REJECT H_0	Correct decision Confidence level Probability p=1−α	Type II error Probability p=β *H_0 is not rejected even though it is false (–)*
REJECT H_0	Type I error Level of significance Probability p= α *H_0 is rejected even though it is true*	Correct decision Test capacity Probability p=1−β

Source: Adapted from Biostatistics 2013.[8]

Table 2 suggests that as long as the researcher intends to obtain high power, error II is the lowest. In studies to determine vaccine efficacy, the working power is 90%.

Table 2. Statistical Power and Errors

Power	**Type II Error**	**Interpretation**
1.0	0.0	If there are differences between the group that received the candidate vaccine and the control group, it will be detected 100% of the time.
0.8	0.2	If the vaccine has an impact, it will be detected 80% of the time.
0.5	0.5	If the vaccine has an impact, it will be detected 50% of the time.

Sample size is important since it should be representative of the target population for the vaccination, and therefore, the results derived from the research may be extrapolated (external validity). There are various ways to estimate sample size depending on the outcome variable to be measured. One of them is estimation based on proportion difference:

Formula 1. Estimating Sample Size

$$N = \frac{(p1*q1) + (p2*q2) * f(\alpha, \beta)}{(p2 - p1)^2}$$

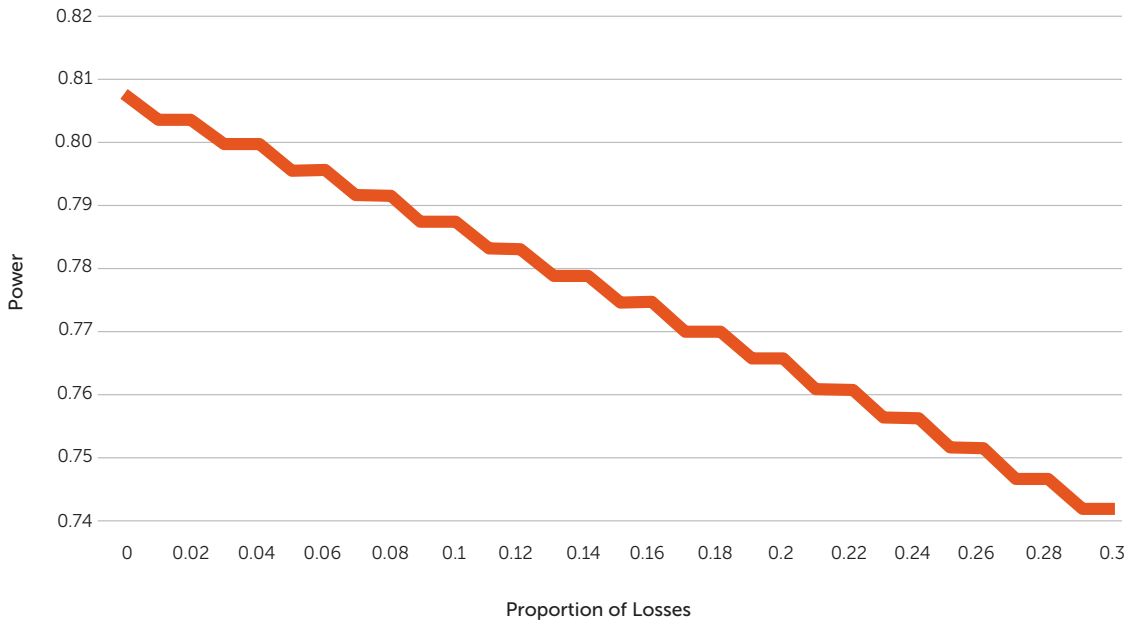
Note that p1 is the expected incidence in the experiment group and p2 is the incidence in the control group.

Follow-up begins once the study is initiated and individuals are randomized to an arm. The protocol should include strategies for adherence to the study since dropouts may result in a significant study bias and the loss of sample potency, more so if the loss is not random or homogenous in both groups.

Follow-up should take as long as necessary considering the natural history of the disease and the available background information on vaccine safety. The protocol should present follow-up strategies to avoid losses.

The loss should not be greater than 10% in population-based studies. To compensate for a 10% loss, the sample should be increased by 23%. If the loss is close to 20%, the sample should be increased by 56% as a number that will be added to the sample size.

Graph 1. Power Versus Losses



By accepting to lose no more than 5% of the initial power (80%), the acceptable loss ranges between 10% and 20% (Simulation by G. Cavada and M. Teresa Valenzuela). Therefore, a loss of up to 20% is considered acceptable with the assumption that losses need to be random with respect to the treatment arms.

Result Analysis

The first analysis should be a description that characterizes both populations, the candidate vaccine recipients and the placebo recipients, to guarantee similarity between the groups. Next, the results are analyzed to determine whether the null hypothesis should be rejected or not.

To that end, two types of analysis are conducted:

1. **Intention to treat:** the randomized individuals are analyzed based on the originally allocated treatment. If individuals are excluded upon randomization, biases may be introduced. Additionally, the individuals who left the study and their rationale for leaving shall be recorded; their exclusion from the analysis restricts generalization of the results.
2. **Per-protocol analysis:** the individuals are analyzed based on treatment completion, regardless of the original allocation.

How to measure the efficacy of the candidate vaccine versus the control group vaccine:

Using the incidence rate or incidence density of the disease in both groups, the subject years are estimated by adding the years each individual was free of the disease since enrollment until sickness within the follow-up period, and also the follow-up period for those who became sick. Based on this information, the incidence rate in both groups is established and the rate reduction percentage is determined.

The relative risk (RR) expresses the strength of association between vaccination and the decrease of cases of disease. As the RR value decreases below 1, the higher the vaccine efficacy.[9]

Formula 2. Calculating Vaccine Efficacy

$$\text{Vaccine efficacy} = \frac{\text{Incidence rate in the control group} - \text{Incidence rate in the vaccinated group}}{\text{Incidence rate in the control group}} \times 100$$

Formula 3. Calculating Relative Risk

$$\text{Relative risk} = \frac{\text{Risk for individuals exposed to the candidate vaccine}}{\text{Risk for individuals not exposed (control)}}$$

If the vaccine affords protection, the relative risk value obtained will be below 1.

Vaccine efficacy = 1–RR
The values obtained are specific and the 95% confidence intervals (95% CI) are estimated to have an accurate measure of the estimate. Values between the lower and upper bounds of the 95% CI include the specific value 95% of the time.

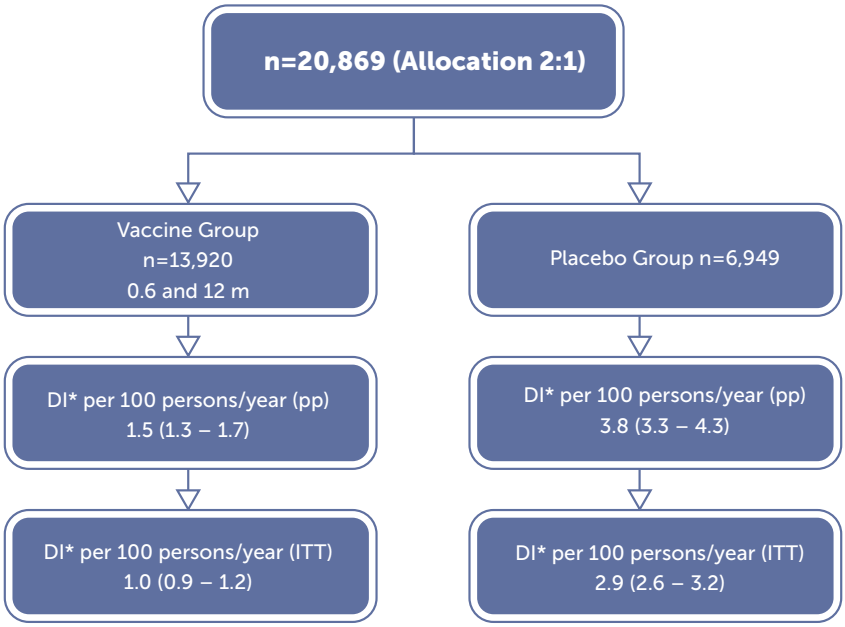
If the CI included one (1), the results would agree with the null hypothesis, i.e., there is no difference between the groups under study, and this means that the difference between the groups does not have statistical significance at a 0.05 α value.[10] . As an example, the candidate vaccine efficacy results expressed as RR are 0.62 (95% CI: 0.4–1.3); despite the RR value below 1, i.e., protective, the upper bound for 95% CI exceeds 1, therefore there is insufficient evidence to conclude that the findings are *statistically significant*.

Below follows an example of a Phase III trial:

Example 1. Efficacy of the Tetravalent Dengue Vaccine in Latin America[11]

20,869

Healthy children (9-16 years) are randomized to receive the vaccine or the placebo (sodium chloride at 0.9%) in 3 doses at 0, 6 and 12 months. Outcome: to measure the efficacy of the tetravalent vaccine against virologically confirmed dengue.



$$RR_{ITT} = \frac{1.0}{2.9} = 0.34$$

$$RR_{PP} = \frac{\text{incidence in vaccinated individuals}}{\text{incidence in unvaccinated individuals}} = \frac{1.5}{3.8} = 0.39$$

$$\text{Efficacy}_{PP} = \frac{\text{Unvaccinated incidence} - \text{Vaccinated incidence}}{\text{Unvaccinated incidence}} = \frac{3.8 - 1.5}{3.8} = 60.5\% \sim 61\% \ [\text{CI } 95\% \ (52.0–68.0)]$$

Vaccine efficacy in the PP group: 1 – RR = 61%
Lastly, the 95% CI needs to be estimated for the specific value as a determinant of the specific value accuracy.
Values between the lower and upper limits of the confidence interval include the specific value 95% of the times.

Source: Adapted from NEJM 2015; 372:113
Notes: DI: Density of incidence or incidence rate; PP: Per-protocol analysis, and ITT: Intention-to-treat analysis.

Ethical Considerations

When planning a clinical study, key components to consider are the essential ethical principles to be complied with, including:

1. Respect for people
2. Principle of beneficence
3. Principle of justice

Respect for people implies acknowledging human autonomy for deciding their voluntary participation in a study and the protection of individuals with diminished autonomy.

The principle of beneficence refers to avoiding harm and maximizing the potential benefits while minimizing the potential damages.[12]

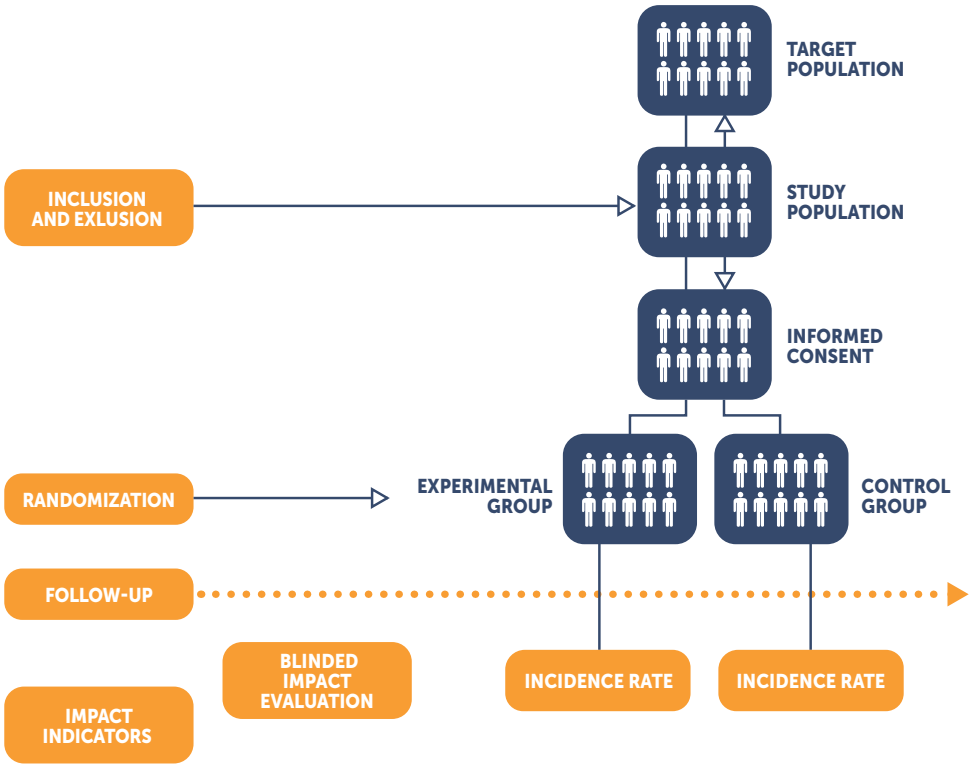
The principle of justice implies that to conduct a study, more vulnerable individuals cannot be systematically chosen, for example, individuals who are deprived of freedom and the elderly, nor can they be manipulated into entering a research investigation.

The application of the general principles to conduct a research study requires every participant to freely submit a written informed consent. This document shall contain clear, straightforward and easy to understand information on the study objectives, the potential risks and benefits expected, the vaccines to be received, the number of visits to be held, and the type and number of samples to be taken.

The informed consent shall be put in writing and explained to every study participant or their legal representative in a language that is adapted to their level of understanding, in a calm manner, with enough time to make sure it was understood and that the person had a chance to ask all the questions deemed necessary.

The individual or their legal representative shall accept participation voluntarily, without pressure or undue influence.[13]

Figure 2. Summary of Phase III Clinical Study Processes



Assessment of Clinical Trials

To develop a Phase III controlled clinical trial,[6] the following guidelines (CONSORT) should be followed:

1. **Title and summary:** must contain the randomized patient selection method.
2. **Scientific background and study justification.**
3. **Methods:** participants, interventions, objectives, results, sample size, randomization, blinding and statistical methods.
4. **Results:** flow diagram of participants, recruitment, baseline data, numbers analyzed, results and estimation, supplementary analyses and adverse events.
5. **Discussion:** interpretation, generalization and global evidence.

Furthermore, it is highly advisable to critically review the validity of the results of a clinical trial. To assess a clinical trial, three main questions need to be considered:

1. Are the results of the study valid?
2. What are the results?
3. Are the results useful?

For more information, the Critical Appraisals Skills Programme (CASP) offers a checklist to appraise a Randomized Controlled Trial: http://www.casp-uk.net/casp-tools-checklists.

References

1. Grimes DA, Schulz KF. An overview of clinical research: the lay of the land. *The Lancet* 2002;359(9300):57–61.

2. Halloran ME, Struchiner CJ, Longini IM. Study Designs for Evaluating Different Efficacy and Effectiveness Aspects of Vaccines. *Am J Epidemiol* 1997;146(10):789–803.

3. García AG, Juan LG. *El Ensayo Clínico en España*. Farmaindustria; 2001. http://www.farmaindustria.es/idc/groups/public/documents/publicaciones/farma_1031.pdf. Accessed January 7, 2016.

4. Madhi SA, Dangor Z, Heath PT, et al. Considerations for a phase-III trial to evaluate a group B Streptococcus polysaccharide-protein conjugate vaccine in pregnant women for the prevention of early- and late-onset invasive disease in young-infants. *Vaccine* 2013;31:D52-D57. doi:10.1016/j.vaccine.2013.02.029.

5. Lazcano-Ponce E, Salazar-Martínez E, Gutiérrez-Castrellón P, Angeles-Llerenas A, Hernández-Garduño A, Viramontes JL. Ensayos clínicos aleatorizados: variantes, métodos de aleatorización, análisis, consideraciones éticas y regulación. *Salud Pública México* 2004;46(6):559–584.

6. Cobos-Carbó, Albert. Ensayos clínicos aleatorizados. *Med Clin (Barc)* 124:21–27.

7. Soldevila N, Salleras L. Cálculo del tamaño de la muestra en los estudios epidemiológicos de evaluación de la efectividad vacunal. *Vacunas* 2011;12(3):102–105.

8. *Biostatistics: A Foundation for Analysis in the Health Sciences*. 10 Edition. Wiley; 2013.

9. Ruiz Morales Álvaro. *Epidemiología clínica: investigación clínica aplicada*. Bogotá, Col.; Madrid, España: Mdìca Panamericana; 2004.

10. Arístegui Fernández J. *Vacunaciones en el niño: de la teoría a la práctica*. [Madrid: Ciclo; 2004.

11. Villar L, Dayan GH, Arredondo-García JL, et al. Efficacy of a Tetravalent Dengue Vaccine in Children in Latin America. *N Engl J Med* 2015;372(2):113–123. doi:10.1056/NEJMoa1411037.

12. White A, Madhi SA. Ethical considerations for designing GBS maternal vaccine efficacy trials in low-middle income countries. *Vaccine* 2015;33(47):6396–6400. doi:10.1016/j.vaccine.2015.07.108.

13. López PP, Mena CR. Legislación vigente y Ética en Investigación Clínica. *Rev Int Cienc Podol* 2012;6(2):81–93.

14. Valenzuela MT, Urquidi C, Martínez C. Manual para la Estandarización de Estudios Clínicos en Chile: desde la concepción hasta la publicación. Available in: http://ensayosclinicos.ispch.gob.cl/.