Mediation Analyses for the Effect of Antibodies in Vaccination

MICHAEL P. FAY*, DEAN A. Follmann

Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, Rockville, Maryland, USA
mfay@niaid.nih.gov

June 7, 2023

Abstract

We review standard mediation assumptions as they apply to identifying antibody effects in a randomized vaccine trial and propose new study designs to allow identification of an estimand that was previously unidentifiable. For these mediation analyses, we partition the total ratio effect (one minus the vaccine effect) from a randomized vaccine trial into indirect (effects through antibodies) and direct effects (other effects). Identifying $\lambda$, the proportion of the total effect due to an indirect effect, depends on a cross-world quantity, the potential outcome among vaccinated individuals with antibody levels as if given placebo, or vice versa. We review assumptions for identifying $\lambda$ and show that there are two versions of $\lambda$, unless the effect of adding antibodies to the placebo arm is equal in magnitude to the effect of subtracting antibodies from the vaccine arm. We focus on the case when individuals in the placebo arm are unlikely to have the needed antibodies. In that case, if a standard assumption (given confounders, potential mediators and potential outcomes are independent) is true, only one version of $\lambda$ is identifiable, and if not neither is identifiable. We propose alternatives for identifying the other version of $\lambda$, using experimental design to identify a formerly cross-world quantity. Two alternative experimental designs use a three arm trial with the extra arm being passive immunization (administering monoclonal antibodies), with or without closeout vaccination. Another alternative is to combine information from a placebo-controlled vaccine trial with a placebo-controlled passive immunization trial.

Keywords: Controlled vaccine efficacy, Correlates of protection, Identifiability, Indirect effect, Mediation assumptions, Sequential ignorability.

1 Introduction

Our goal is identifying the proportion of the vaccine effect from a randomized placebo-controlled vaccine trial that is due to the antibodies induced by the vaccine, say $\lambda$. In the mediation literature, the total effect is partitioned into an indirect effect that acts through the mediator (e.g., the vaccine effect that acts through the antibody response) and a direct effect that acts directly on the outcome (e.g., the rest of the vaccine effect). Mediation analyses typically make certain positivity and independence assumptions in order to identify indirect and direct effects (see VanderWeele [2] or Section 3.1). This paper explores those assumptions in detail, specifically focusing on the application to this antibody/vaccine problem. For ease of exposition, unless stated otherwise, the term antibodies refers to only the particular type of antibody induced by the experimental vaccine. Also for simplicity, we focus on the placebo-controlled vaccine trial, but many of the same issues may apply when another type of control arm is used instead of placebo (e.g., an arm that is given a control vaccine for another disease). Our focus is on the case when individuals in the placebo (or other control) arm do not have detectable levels of the antibody of interest, which is often the case for new or rare diseases.

The antibody/vaccine mediation problem is important as seen by its application to COVID-19 vaccination. Large placebo-controlled vaccine trials showed impressive vaccine efficacies during the stages of the pandemic before the omnicron variant was prevalent. It would be useful to be able to evaluate modifications of the vaccines (e.g., dose changes, adjuvant changes, updating the antigen to current variants) under new populations and new exposures without having to re-run such large trials.

* To whom correspondence should be addressed.

1 Unfortunately, in the vaccine literature, the term indirect vaccine effect is used to describe another issue: the protective vaccine effect for a non-vaccinated individual due to nearby vaccinated individuals being less likely to be infectious, see e.g., Halloran et al. [1], Section 2.8. This paper is not about that type of indirect vaccine effect.
Instead, we could use antibodies to help predict vaccine efficacy. Gilbert et al. [3] comprehensively explored the effects of antibodies on the vaccine efficacy of the Moderna (mRNA-1273) COVID-19 vaccine, including a mediation analysis using methods described in Benkeser et al. [4].

Whereas Benkeser et al. [4] focuses on details of estimation and only briefly mentions standard mediation assumptions, in this paper we focus on identifiability and more explicitly discuss the implications of the standard assumptions to the vaccine/antibody mediation problem. The major difficulty of this identifiability problem is two cross-world quantities: the potential outcome of a vaccinee but with mediator values as if the vaccinee had gotten placebo, or vice versa. These two quantities lead to two versions of indirect effects, which we call the subtracting indirect effect (due to subtracting the antibody effect from the vaccinees) or the adding indirect effect (due to adding antibody effect to the placebo participants). (In the mediation literature these are called the total indirect effect and pure indirect effect, respectively [5].) We explore the typical mediation assumptions except without the often untenable assumption that the placebo participants have detectable antibodies specific to the infectious agent. We show that the subtracting indirect effect and $\lambda_s$ (its version of $\lambda$) are identifiable, but the adding indirect effect and $\lambda_a$ (its version of $\lambda$) are not. We further show that a key assumption for identifying the subtracting indirect effect is that the potential outcomes are independent of the potential mediator responses conditional on confounders. We show that in the simple case without confounders, the identification of $\lambda_a$ by that assumption is equivalent to identifying a correlation of two potential random variables as 0 by assuming their independence.

Some major contributions of our paper are new ways to identify the adding indirect effect by supplementing the randomized placebo-controlled vaccine trial with extra experimental information. This can be done by adding a randomized passive immunization arm to the randomized placebo-controlled vaccine trial, where passive immunization is done by infusing (or injecting) individuals with monoclonal antibodies. We discuss identifiability in that three arm trial, with and without a closeout vaccination in the passive immunization arm. Alternatively, we can combine the placebo controlled vaccine trial with a second randomized trial of passive immunization versus placebo to identify the adding indirect effect. We first identify controlled vaccine effects from the placebo versus vaccine trial and then the controlled protective effects (which act like adding indirect effects) from the placebo versus passive immunization trial. The latter effect does not require the conditional independence assumption between potential outcomes and mediators, since that independence can be met by randomization to antibody values in the passive immunization arm.

Because this paper is partially about explaining mediation analysis to vaccine researchers and vaccine trials to mediation experts, we necessarily give extensive background on the causal effects, vaccine biology, and mediation definitions (Section 2). Section 3 details typical mediation assumptions applied to this problem giving explicit identifiability results. The binary mediator problem is addressed in Section 4 to give some intuition about the assumptions. We describe identifying the adding indirect effect by supplementing the main placebo controlled vaccine trial with a passive immunization arm or experiment in Section 4.4 (binary mediator case) and Section 5 (general mediator case).

## 2 Background

### 2.1 Causal Vaccine Effects

Vaccines work by exposing the vaccinee to an antigen, a protein on an outward facing part of the infectious agent. The adaptive immune system of the vaccinee then produces activated antigen-specific B cells and helper T cells. Those cells are part of the germinal center that creates more antigen-specific B cells, which in turn develop into either antibody-producing plasma cells or other types of cells such as memory B cells. The plasma cells create antibodies specific to that antigen that travel to other areas of the body through the blood. These antigen-specific antibodies are usually detected from assays done on blood samples, and those assays are of different types (e.g., an ELISA where the antibodies bind to proteins in a plate, or a functional assay that measures how much the antibody neutralizes the agent). In this paper, we will often refer to the mediator as “antibodies”, and a more precise description would be the measured level of those antigen-specific antibodies collected at a specific study time from a blood sample using one assay. Although the antibodies measured in blood are one product of the vaccination, some of those antigen-specific antibodies may be in other parts of the body (e.g., mucosal surfaces) and will not be detected by a blood sample and hence would not be measured as the mediator. Further, there are many other responses to the vaccine that may lead to other mechanisms that may be helpful in protecting the vaccinee from developing disease in response to a future exposure from the infectious agent (for more details, see e.g., 6).

Vaccination is a way of preparing the immune system to be ready for future exposures. Vaccination is often especially
helpful for individuals that have not previously been exposed, since those that have previously been exposed and survived will
often have developed natural immunity. The gold standard for estimating vaccine efficacy is a controlled randomized clinical
trial. Prior to the availability of approved vaccines (e.g., for HIV, or in the early stages of the COVID-19 pandemic), the primary
efficacy analysis from vaccine randomized trials typically exclude individuals that already have antibodies protective against
the infectious agent of interest, since that population does not have the greatest need of vaccination. Further, because it takes
time for the vaccinee to develop antibodies, vaccine trials also typically exclude individuals from the primary efficacy analysis
data set that became diseased before the time when the peak antibody level is expected to be reached, usually 1 to 4 weeks after
the last dose of the vaccination [see e.g., 7 Table 1].

2.2 Vaccine Efficacy Using Potential Outcomes

We introduce formal causal notation, first for vaccine efficacy, then for mediation. Because the paper will focus on identifiability
not estimation, we do not need subscripts to differentiate individuals in the population. For example, we let $A = 1$ if the $i$th
participant is randomized to vaccine, and $A = 0$ if they are randomized to placebo, and no $i$ subscript is needed. Thus, $A$
represents the allocated arm of a typical (i.e., randomly selected) individual. Let $Y = 1$ if the typical participant gets the event
(e.g., has confirmed disease) during the time they are at risk during the study, and $Y = 0$ if they do not. Write the potential
outcomes for the typical participant as both $Y_0$, the outcome if they were randomized to placebo, and $Y_1$, the outcome if they
were randomized to vaccine. Although we can only observe one of the two potential outcomes per individual, we can compare
the expected response in both arms, because by randomization each individual has a positive and known probability of being in
either of the arms.

Vaccine efficacy (VE) is defined as 1 minus a ratio effect (e.g., incidence ratio, hazard ratio, odds ratio) [see e.g., 1, Table 2.2],
and in this paper we define it as, $VE = 1 - E(Y_1)/E(Y_0)$. For vaccine mediation, sometimes odds ratios are used [see e.g., 8, 9]. Fortunately, when only a small percentage of the placebos have the event, there is little difference between
the different ratios [10]. The VE estimand we use is reasonable in a randomized trial because the exposure processes are
approximately balanced between the two arms [11]. For example, although exposure may change by geographic area, the
distributions of geographic areas by arm are balanced by randomization. Similarly, although exposure will vary from individual
to individual because of differential calendar times joining the study or censoring times leaving the study, those differences will
be approximately equally distributed in the two arms as long as the rate of infection is not large, and the study entry times and
censoring times are independent of the potential outcomes.

Unlike a difference effect (e.g., $E(Y_1) - E(Y_0)$), a ratio effect is relatively invariant to differences in exposure, so it is
useful for a vaccine trial since we can rarely predict well how many participants will be exposed during the course of the trial.
To see this invariance, we reexpress the potential outcomes. Let $Y^*_a$ be the indicator of whether the typical participant would
get the event when they were in arm $a$ if they were exposed ($Y^*_a = 1$ is yes, $Y^*_a = 0$ is no). Let $Z = 1$ represent if they were
exposed during the study (1=yes, 0=no), so that $Y_a = Z Y^*_a$. If we assume the exposure is independent of whether they would
be protected if exposed (which is a reasonable assumption in a blinded randomized trial), then

$$VE = 1 - \frac{E(Y_1)}{E(Y_0)} = 1 - \frac{E(Z Y^*_1)}{E(Z Y^*_0)} = 1 - \frac{E(Z)E(Y^*_1)}{E(Z)E(Y^*_0)} = 1 - \frac{E(Y^*_1)}{E(Y^*_0)}.$$  (1)

The vaccine efficacy does not depend on the exposure rate, but only on the differential in the potential to be protected given
exposed, which is the scientific effect of interest.

2.3 Defining Direct and Indirect Effects

Now consider mediation. Let $M_a$ be the potential mediator for a typical individual when $A = a$. In our example $M_1$ is
the antibody level (e.g., IC50 titer at day 29 after vaccination) if randomized to vaccine, and $M_0$ that level if randomized to
placebo. Just as for the outcomes, we can only observe one of the potential antibody levels for each individual. To explicitly
denote the effect of the antibody on the outcome, we now write the outcome for the typical individual with $A = a$ as $Y_a M_a$.
More generally, we let $Y_{am}$ be a potential outcome variable where $A$ is set to $a$ and $M$ is set to $m$ (and $m$ does not need to equal
$M_a$). This notation implies that only the values of $a$ and $m$ matter in the response, not the manner in which those values are
assigned (e.g., if the mediator response for individual $i$ is $m$, it does not matter that the mediator value of $m$ occurred naturally
in response to a vaccine or placebo (i.e., $M_a = m$), or was it set to $m$ by some other external intervention). This is known as
the consistency assumption [12], and we will assume that for now, but revisit it later. Under consistency, the observed response, $Y$, is $Y = Y_{AMa}$ and the observed mediator, $M$, is $M = M_A$. In this notation,

$$VE = 1 - \theta_T = 1 - \frac{E[Y_{1Ma}]}{E[Y_{0Ma}]}$$

(2)

where $\theta_T$ represents the total ratio effect.

Typically mediation effects are defined as differences [see e.g., 13], but for vaccines we define mediation effects in terms of ratio effects. We partition the total ratio effect ($\theta_T$) into the product of an indirect ratio effect ($\theta_I$) and a direct ratio effect ($\theta_D$). Let $\theta_I = \theta_I^a$ and $\theta_D = \theta_D^{(1-\lambda)}$, giving $\theta_T = \theta_I \theta_D$. Thus, on the log scale $\log(\theta_T) = \log(\theta_I) + \log(\theta_D)$, indirect and direct effects are additive, and $\lambda$ is the proportion of the total log-ratio effect due to indirect log-ratio effects. By algebra, $\lambda = \frac{\log(\theta_I)}{\log(\theta_T)}$. The proportion mediated effect was defined this way in Gilbert et al. [3] (see e.g., Table S9), although its explicit form and motivation was not given.

An indirect effect is the effect of changing only the mediator while holding the rest of the effect of vaccine constant. There are two main ways to define an indirect ratio effect. The first way is $\theta_{Is} = \frac{E[Y_{0M_a}]}{E[Y_{0M_0}]}$, which measures the effect on the placebo arm (denominator) of changing the mediator to the value it would have after vaccination but without actually vaccinating participants (numerator). The ‘a’ subscript denotes “adding” antibody to unvaccinated participants at the value they would have gotten if vaccinated (i.e., $M_1$). The associated direct effect is part of the total effect that does not go through the mediator, $\theta_{Ds} \equiv \frac{\theta_T}{\theta_{Is}} = \frac{E[Y_{1M_1}]}{E[Y_{0M_1}]}$. Sections 4.4 and 5 focus on identification of $\theta_{Is}$. The second way to define an indirect effect is $\theta_{Is} = \frac{E[Y_{1M_0}]}{E[Y_{1M_0}]}$, which measures the effect on the vaccine arm (numerator) of changing the mediator to the value it would have if the individual had been in the placebo arm (denominator). The ‘s’ subscript denotes “subtracting” the extra antibodies in the vaccinated participants, so that the antibody levels are what would have been seen in the placebo arm (i.e., $M_0$). The associated direct effect is $\theta_{Ds} \equiv \frac{\theta_T}{\theta_{Is}} = \frac{E[Y_{0M_0}]}{E[Y_{0M_0}]}$. The mediation analysis in Gilbert et al. [3] estimated $\theta_{Is}$ and its associated $\lambda$ value. Figure 1 shows how the total ratio effect can be partitioned these two ways,

$$\theta_T = \theta_{Is}\theta_{Ds} = \theta_{Is}\theta_{Ds}$$

(3)

Let $\lambda_s$ be $\lambda$ under the first partition, and $\lambda_s$ be its value under the second.

[Figure 1 about here.]

Robins and Greenland [5] used different terminology: $\theta_{Is}$ is the pure indirect effect, $\theta_{Ds}$ is the total direct effect, $\theta_{Is}$ is the total indirect effect, and $\theta_{Ds}$ is the pure direct effect. Alternatively to equation 3, we can partition the total effect into 3 parts: pure indirect effect, the pure direct effect and an interaction effect (say, $\xi$), $\theta_T = \theta_{Is}\theta_{Ds}\xi$, where $\xi = \frac{E[Y_{1M_1}]}{E[Y_{0M_0}]}$. If $\xi = 1$ then there is no interaction, $\theta_{Is} = \theta_{Is}$ and $\theta_{Ds} = \theta_{Ds}$, the dotted quadrilateral in Figure 1 is a parallelogram, and all ways of partitioning the direct and indirect ratio effects are the same. There are many other ways to define interaction [see e.g., 2, Section 7.6].

3 Mediation Analysis Using the Sequential Ignorability Assumptions

3.1 Sequential Ignorability Assumptions

In estimating direct or indirect effects, the difficult parameters to identify are either $E[Y_{0Ma}]$ (for identifying $\theta_{Is}$) or $E[Y_{1M_0}]$ (for identifying $\theta_{Is}$), because we observe no participant with those kinds of responses. Those responses are called “cross-world” quantities because the intervention is in one world (e.g., where the participant was allocated to arm $A = a$) but the mediator is in another world (e.g., where the participant has mediator value as if allocated to arm $a'$, $M_{a'}$, where $a \neq a'$). Thus, in order to identify $E[Y_{1M_0}]$ or $E[Y_{0M_1}]$ (and hence, to identify $\lambda_s$ and $\lambda_s$, respectively) we need to make some assumptions that are not fully testable from the study data. Pearl [14] showed that using a set of assumptions, we can identify additive direct and indirect effects using a simple equation that has become known as the mediation formula [see e.g., 15]. In this paper, since we deal with ratio effects, we focus on the piece of the mediation formula that estimates $E[Y_{1M_0}]$ or $E[Y_{0M_1}]$ (equation 4 in
Changing the timing of the antibody measurement has a downside in that it may be less associated with the response, since the original timing is typically designed to be at peak antibody levels. Often the “sequential ignorability assumptions” refer to not just $SI_1$ and $SI_2$, but also to the positivity assumptions, which are implicitly assumed or listed as one positivity assumption \cite{16}. In this paper it will be important to separate $SI_1$ and $SI_2$ (which we call the sequential ignorability assumptions) and the different positivity assumptions. Later, as suggested by a referee, we explore modifications to the supports in the positivity assumptions.

Let $X$ be a vector of baseline covariates. The sequential ignorability assumptions are:

1. $SI_1$: \{\$Y_{am}, M_{a'}\} \perp A | X = x$, and
2. $SI_2$: \$Y_{am} \perp M_{a'} | A = a, X = x$, for all $a, a'$ and $m$, assuming the following positivity assumptions,

- $PosA$: $Pr[A = a | X = x] > 0$ for all $a, x$,
- $PosM_0$: $Pr[M_0 = m | A = 0, X = x] > 0$ for all $x$ and $m \in S_M$, and
- $PosM_1$: $Pr[M_1 = m | A = 1, X = x] > 0$ for all $x$ and $m \in S_M$.

where $S_M$ is the support for $M$.

Often the “sequential ignorability assumptions” refer to not just $SI_1$ and $SI_2$, but also to the positivity assumptions, which are implicitly assumed or listed as one positivity assumption \cite{16}. In this paper it will be important to separate $SI_1$ and $SI_2$ (which we call the sequential ignorability assumptions) and the different positivity assumptions. Later, as suggested by a referee, we explore modifications to the supports in the positivity assumptions. Under the positivity assumptions listed above and the sequential ignorability assumptions (and consistency), the cross-world conditional expected potential outcomes are given by

\[
E[Y_{AM,a} | X = x] = \sum_m E[Y | A = a, M = m, X = x] Pr[M = m | A = a', X = x]
\]

[see e.g., \cite{2} p. 465].

### 3.2 Application to the Vaccine/Antibody Mediation Analysis

Now consider identifying $\lambda_s$ or $\lambda_a$ from a randomized placebo-controlled vaccine trial. First, the assumptions $SI_1$ and $PosA$ are met for any randomized trial for any set of baseline variables $X$. In this section, we discuss the harder assumptions to justify for our scenario, which are consistency, $SI_2$, $PosM_0$, and $PosM_1$.

Let the support of $M_0$ and $M_1$ be $S_M = \{0^*, S_{detect}\}$, where $M = 0^*$ represents $M$ below the limit of detection, and $M > 0^*$ means $M \in S_{detect}$, and $S_{detect}$ is the set of possible detectable antibody values. Consider the case of a new pathogen for which it is possible that some individuals will have $M_0 = 0^*$. Then, a violation of $PosM_1$ occurs when the vaccine induces antibodies for all individuals with $X = x$, so that $Pr[M_1 = 0^* | A = 1, X = x] = 0$. A solution is to change the time of antibody measurement to sooner after vaccination so that there are some participants with $M_1 = 0^*$ within each $x$ and we can assume that $PosM_1$ is not violated, and then the resulting $\lambda_s$ estimate can be used as a lower bound for the originally defined $\lambda_s$ (i.e., the one using the original time of antibody measurement) under a reasonable monotonicity assumption [see e.g., \cite{3}]. Changing the timing of the antibody measurement has a downside in that it may be less associated with the response, $Y_{AM,A}$, since the original timing is typically designed to be at peak antibody levels.

Another problem, and a more common issue with vaccine trials, is violation of $PosM_0$ so that the analysis data set will have $Pr[M_0 > 0^* | A = 0, X = x] = 0$ for some $x$. In many cases that probability is 0 for all $x$. For example, we could have all $M_0 = 0^*$ for a new infectious agent, because no one would have been previously exposed. Another example is if we exclude from the study those who have antibody detectable at baseline as well as those exposed to the infectious agent before the time of measurement of the antibody (i.e., study time when $M$ is measured). Consider equation \cite{4} with $a = 0$ and $a' = 1$, giving the cross-world mean representing adding antibody to the unvaccinated,

$$E[Y_{0M_1} | X = x] = \sum_m E[Y | A = 0, M = m, X = x] Pr[M = m | A = 1, X = x].$$ (5)
The expression $E[Y|A = 0, M_0 = m, X = x]$ for $m > 0^*$ in equation 5 is not identifiable when $Pr[M_0 > 0^*|A = 0, X = x] = 0$, because in that case we do not observe $Y$ given $A = 0$ and $M_0 = m$ for any $m > 0^*$ on any individual. When $a = 1$ and $a' = 0$, both sequential ignorability assumptions hold, and all positivity assumptions hold except for allowing $Pr[M_0 > 0^*|A = 0, X = x] = 0$ (i.e., no detectable antibody in the placebo arm), then equation 4 can still be used (see Supplement Section S2) to give

$$E[Y_{1,M_0}|X = x] = E[Y|A = 1, M = 0^*, X = x].$$ (6)

The right-hand side of equation 6 is just the disease rate among vaccinated individuals with undetectable antibody among study participants with baseline $X = x$. Using equation 6, we can identify $E[Y_{1,M_0}]$ as

$$E[Y_{1,M_0}] = \sum_x E[Y|A = 1, M = 0^*, X = x] Pr[X = x],$$ (7)

where the summation is over the possible values of $X$, and $E[Y|A = 1, M = 0^*, X = x]$ is estimated with the sample mean of $Y$ among the vaccinated with undetectable antibody responses and $X = x$.

Consider the consistency assumption 12 in this antibody/vaccine mediation scenario. Suppose we define the antibody mediator as the amount of antibody measured at a particular time post vaccination, and an indirect effect is through that antibody mediator and the rest of the total effect is a direct effect. Consider the plasma cells that produce antibodies which give the antibody mediated vaccine effects. After the antibody mediator is measured, the plasma cells (especially if they are long-lived plasma cells [see 6]) may play a part in protection by producing more antigen-specific antibodies in the future. By the strict definition, those latter antibodies would be seen as direct effects. If we wish to apply the mediation analysis results to predict the proportion of total vaccine effect due to that antibody mediator from a similar vaccine, then the consistency assumption may approximately hold because the second similar vaccine will likely also produce plasma cells that remain after the antibody is measured. In contrast, if we wish to apply the mediation results from the original vaccine to predict the proportion of the total effect due to antibodies in the case when the intervention is infusion with monoclonal antibodies, then the consistency assumption will likely be violated (i.e., the two mediation analyses will be estimating different effects). It is likely that an individual’s outcome under monoclonal antibodies will be different than if they had an antibody response due to a vaccine, because the vaccine will induce plasma cells which may continue to make antibodies, and hence have a larger direct effect. This violation may not be a problem if the amount of antibodies detected produces overwhelming protection and additional antibodies would not affect risk much. Even if the consistency assumption was violated, the mediation analysis might be useful to estimate bounds on an indirect effect if we could assume that the vaccine induced antibodies are at least as effective as the addition of externally produced monoclonal antibodies.

Next, consider the $SI_{2}$ assumption. For this we once again simplify the immunology. Suppose we can partition the antigen-specific antibody response into two parts: the creation of the antibodies, and the subsequent protective function of the antibodies. Some protective functions of the antibodies are [6 Table 2.1]: (i) binding to toxins produced by the infectious agent to stop their deleterious effect, (ii) stopping replication of a virus by binding to the virus and preventing the virus from entering cells of the host, (iii) opsonizing, where the antibodies mark pathogens outside cells so that other immune cells (e.g., macrophages or neutrophils) may clear them, (iv) activating other processes (e.g., the complement cascade). The $SI_{2}$ assumption (i.e., within each arm, conditional on baseline covariates, $X$, the potential antibody response, $M_{a'}$, is independent on the potential outcomes for the study, $Y_{am}$, for all $a, a'$, and $m$), implies that within levels of $X$, the processes that create antibodies are independent from the processes that lead to their protective functions. Because the body has evolved to have a useful immune system, it is possible that there would be a dependence between those two processes; however, there may not be a high dependence (e.g., Goel et al. 17 showed that antibody level post-boost did not have any substantial correlation with post-boost memory B-cells, the latter of which are suspected to be related to protection). If there is such a dependence, in order to meet assumption $SI_{2}$, we would need to find baseline covariates, $X$, such that conditional on $X$, the potential antibody response vector is independent of the potential outcome vector. For example, suppose the baseline covariates can be used to classify individuals into $k$ immune classes, where some classes have healthier immune systems than others. If within each class we can assume $Y_{am} \perp M_{a'}$, then $SI_{2}$ would be met. Consider an example with only 2 classes, healthy and immunocompromised, and suppose $X$ could be used to classify each individual into those two types. Further suppose that within both the healthy and the immunocompromised types the process for creating antibodies is independent from the protective processes of the antibodies. Under those suppositions, $SI_{2}$ would be met.

In summary, we have detailed the main assumptions used in most, if not all, vaccine mediation analyses up to this point, including a recent important vaccine mediation analysis [see e.g., 3, 4]. We have explicitly shown that when $PosM_0$ fails such
that \( Pr[M_0 > 0 | A = 0] = 0 \) then we can still identify the subtracting indirect effect (i.e., total indirect effect), as long as the randomization is valid and the \( SI_2 \) and \( PosM_1 \) assumptions are met. We have focused on binary potential outcomes instead of time-to-event outcomes to avoid extra complications such as the timing of the outcomes and censoring, since those extra complications are peripheral to our main focus on identifiability.

## 4 Binary Mediators and Responses

From the previous section we showed that a key difficult assumption is \( SI_2 \). In this Section, we study the simple case when the mediators are binary to gain intuition about how \( SI_2 \) is affecting the identifiability of the estimators of \( \lambda \). In this section, let \( M_A = 1 \) if \( m \geq \tau \), and \( M_A = 0 \) if \( m < \tau \), where \( \tau \) is some specified threshold. The threshold \( \tau \), need not be the limit of detection, it could be some positive value of \( m \) related to the antibody activity. To keep the exposition simple, we will ignore baseline covariates, but it is straightforward (although notationally cumbersome) to apply the models of this section separately to each level of the baseline covariates if the analogous conditional independence assumptions are appropriate.

### 4.1 Base Model

To start we gather together some assumptions that appear reasonable for the placebo-controlled randomized vaccine trial. As a shorthand, we call this set of assumptions the “base model”. Robins and Greenland [5] studied the mediator problem with binary mediators and binary potential outcomes. Under this model there are \( 2^6 = 64 \) different types of possible vectors of potential mediator responses and potential outcomes (2 levels for the mediator, and 4 levels for the paired outcomes). Robins and Greenland [5] made three assumptions that reduce the 64 types to 18, which we apply and translate to our vaccine/antibody example:

\[
RG_1: \quad \text{Vaccination cannot block antibodies that would have been present under the placebo arm. This means we never have types where } M_1 = 0 \text{ and } M_0 = 1.
\]

\[
RG_2: \quad \text{Vaccination cannot cause the disease. This means we never have types where } Y_{1m} = 1 \text{ and } Y_{0m} = 0.
\]

\[
RG_3: \quad \text{Antibodies cannot cause the disease. This means we never have types where } Y_{a1} = 1 \text{ and } Y_{a0} = 0 \text{ for any } a.
\]

These assumptions are often called monotonicity assumptions [see e.g., [13]]. These seem reasonable assumptions for many vaccines, although there is one known case of a violation of \( RG_2 \) (a vaccine for Dengue virus for one type enhancing the probability of disease in another type [see e.g., [19]]). Additionally, \( RG_2 \) excludes cases where vaccination increases the risk of disease. For example, some individuals may change their behaviour to increase their risk of infection if they suspect they have been vaccinated and believe that the vaccination works. If that behaviour change results in a disease case, that would count as the vaccination causing the disease. Because of this, in vaccine trials double-blinding is especially important for justifying \( RG_2 \). Related to that, the reactogenicity of the vaccine may unintentionally unblind some participants. To alleviate that problem, sometimes the control arm uses a vaccine for a different disease than that of the experimental vaccine.

For the vaccine example, because we exclude individuals that had antibodies at baseline, and because we exclude anyone with detectable disease before the antibody is measured, it is very unlikely that anyone left in the analysis data set in the placebo arm will have a positive antibody response. So we assume that we never have types where \( M_0 = 1 \). This reduces the types of interest to 12.

These types are listed in Table 1 with each type described in the last column. The first column gives the notation for the proportion of the population with potential outcomes \( Y = [Y_{11}, Y_{10}, Y_{01}, Y_{00}] \) and potential mediators \( M = [M_1, M_0] \), which is \( \pi_Y \). For example, \( \pi_{[10]}^{[1010]} \) is the proportion with \( M = [10] \) (i.e., \( M_1 = 1 \) and \( M_0 = 0 \)) and \( Y = [0101] \) (i.e., \( Y_{11} = 0, Y_{10} = 1, Y_{01} = 0 \) and \( Y_{00} = 1 \)). For a person with that type, \( Y_{10} \) and \( Y_{01} \) are cross-world potential outcomes.

Under a randomized trial, \( A \) is independent of both the potential outcome vector (i.e., \( A \perp Y \)) and the potential mediator response vector (i.e., \( A \perp M \)). Altogether, the assumptions for the base model described in this section are \( RG_1, RG_2, RG_3 \), \( Pr[M_0 = 1] = 0 \), \( A \perp Y \), and \( A \perp M \). We feel that often these assumptions will be quite reasonable.

To find identifiable parameters, we first re-express the 12 proportions of Table 1 as Table 2 which lists the 12 proportions in a \( 4 \times 2 \) table with observable margins, and a \( 4 \times 6 \) table with one of the observable columns partitioned into 5 sub-columns. The 6
observable (and hence identifiable) margins are denoted with $\phi_{amy}$ parameters, where the $amy$ subscripts represent respectively, $A$ ($v =$vaccine, $p =$placebo), $M_A$ ($a =$antibody response positive, $n =$no antibody response), and $Y_{AM_A}$ ($f =$failure [had disease], $s =$success [did not have disease]). For example, $\phi_{paf}$ is the proportion of the population that if randomized to vaccine would produce antibodies and have an event (i.e., would fail). Recall, in the base model $Pr[M_0 = 1] = 0$ so $\phi_{pas} = 0$ and $\phi_{pa0} = 0$ and are not listed in Table 2.

To be identifiable under the base model is to be able to express a parameter in terms of the $\phi$ parameters. From Table 2 we see that both $E(Y_{1M_1})$ and $E(Y_{0M_0})$ are identifiable,

$$E[Y_{1M_1}] = \phi_{vaf} + \phi_{vnf}$$
$$E[Y_{0M_0}] = \phi_{pnf}.$$

On the other hand, the cross-world expectations are not identifiable without further assumptions, so are given with a combination of identifiable and other parameters (see Tables 1 and 2).

$$E[Y_{1M_0}] = \phi_{vaf} + \phi_{vnf} + \pi_{0101} + \pi_{1011} = E[Y_{1M_1}] + \pi_{0101} + \pi_{1011}$$
$$E[Y_{0M_0}] = \phi_{vaf} + \phi_{vnf} + \pi_{0001} + \pi_{1011} + \pi_{1101} = E[Y_{1M_1}] + \pi_{0001} + \pi_{1011} + \pi_{1101},$$

where $\pi_{ij..} = \pi_{ij01} + \pi_{ij11}$, and the “.” denotes summations over an index. From equation 8 we see that to identify $E[Y_{1M_0}]$, we need to be able to identify $\pi_{1001} + \pi_{1011}$. By inspection of the definition of the $\phi$ parameters in Table 2 we see that no combination of the $\phi$ parameters are able to identify $\pi_{1001} + \pi_{1011}$ (see third row, second column). Therefore, $E[Y_{1M_0}]$ is not identifiable from the base model. Further, under the base model the proportion of the total effect due to an indirect effect, $\lambda$, is not identifiable. We formally show this in Theorem 1.

**Theorem 1** Any $\lambda_s \in [0, 1]$ is compatible with the base model. Similarly, any $\lambda_a \in [0, 1]$ is compatible with the base model.

The proof is in Supplement Section S3. Theorem 1 shows that the base model is inadequate to get any information about $\lambda$ (either $\lambda_s$ or $\lambda_a$) from the data; more assumptions are needed when $\lambda$ is the interest.

### 4.2 Model with Potential Mediators Independent of Potential Outcomes

We previously discussed in Section 3 the sequential ignorability assumptions, and how they may be applied even when $Pr[M_0 = 1] = 0$ to estimate $E[Y_{1M_0}]$ (see equation 7 which additionally adjusts for baseline covariates). The base model essentially already includes $SI_1$, and now we explore adding $SI_2$ to the base model. In Section 3.2 we discussed why it is difficult to justify $SI_2$ in the vaccine/antibody mediation analysis; nevertheless, because the sequentially ignorability assumptions are common and allow some identifiability, we discuss them here. In this section (Section 4) we have simplified the exposition, leaving off the baseline variables, and write the independence expression in $SI_2$ without explicitly conditioning on $A = a, X = x$ as $Y_{am} \perp M_a$; it means that each mediator potential response is independent of each potential outcome. Let $M_2$ represent the model defined as the base model with the added assumption from $SI_2$ that $Y_{am} \perp M_a$.

**Theorem 2** Under $M_2$, then

(i) $\pi_{0000}, \pi_{0011}, \pi_{1001}, \pi_{1011}$ are identifiable, with $\pi_{0000} = \phi_{pns}\phi_{vn}, \pi_{0011} = \phi_{vns} - \phi_{pns}\phi_{vn}, \pi_{1001} = \phi_{vnf}, \pi_{1011} = \phi_{pns}\phi_{va}, \pi_{0101} = \phi_{vns} - \phi_{pns}\phi_{va},$ and $\pi_{1101} = \phi_{vaf},$ and $\pi_{1111} = \phi_{vnf},$ and $\phi_{va} = 1 - \phi_{vn} - \phi_{vaf} + \phi_{vas}$.

(ii) $E[Y_{0M_0}]$ is not identifiable, and

(iii) $E[Y_{1M_0}]$ is identifiable and equal to $\frac{\phi_{vaf}}{\phi_{vn}}$.

The proof of Theorem 2 is in Supplement Section S4. First, note that Theorem 2 (i) allows us to test for certain violations of the assumptions, because $\pi_{0000}$ and $\pi_{0011}$ must be positive, or rewriting

$$\pi_{0000} \geq 0 \iff \frac{\phi_{vns}}{\phi_{vn}} \geq \phi_{pns} \iff \frac{\phi_{vnf}}{\phi_{vn}} \leq \phi_{pnf}$$
$$\pi_{0011} \geq 0 \iff \frac{\phi_{vas}}{\phi_{va}} \geq \phi_{pns} \iff \frac{\phi_{vaf}}{\phi_{va}} \leq \phi_{pnf}.$$
Second, Theorem 2(ii) shows that \(E[Y_{0M_1}]\) is not identifiable, which may seem strange because the usual positivity and sequential ignorability assumptions show identifiability (see equation 5b); however, recall that \(P_r[M_0 = 1] = 0\) from the base model, which violates the \(PosM_0\) positivity assumption (see Section 5.1). A consequence is that \(\lambda_s\) is not identifiable. Finally, plugging in the value of \(E[Y_{1M_0}]\) from Theorem 2(iii), we get:

\[
\lambda_s = \frac{\log \left( \frac{\phi_{nf\theta_{vn}}}{\phi_{vnf}} \right)}{\log \left( \frac{\phi_{nf}}{\phi_{pf}} \right)},
\]

(10)

Thus, assuming \(Y_{am} \perp M_{a'}\) with the base model determines \(\lambda_s\). This is a red flag, because we do not want the parameter of interest to be identified entirely by an assumption that is not strongly justified by the subject matter. Below we express different consequences of \(SI_2\) to better critique its plausibility.

**Theorem 3** The following three statements are equivalent:

**Statement A:** Under the base model, \(E(Y_{1M_0})\) ranges from \(E(Y_{1M_1})\) to \(E(Y_{0M_0})\), and if we additionally assume \(Y_{am} \perp M_{a'}\), then \(E(Y_{1M_0}) = \phi_{vnf}/\phi_{ef}\).

**Statement B:** Under the base model, \(\lambda_s\) ranges from 0 to 1, and if we additionally assume \(Y_{am} \perp M_{a'}\), then \(\lambda_s\) is given by equation 10.

**Statement C:** Under the base model, \(\rho \equiv \text{Corr}(Y_{1M_0}, M_1)\) ranges from \(\rho_{min}\) to \(\rho_{max}\), and if we additionally assume \(Y_{am} \perp M_{a'}\) (which implies the assumption that \(Y_{1M_0} \perp M_1\)), then \(\rho = 0\), where

\[
\rho_{min} = \frac{\{\phi_{pf} - \phi_{vnf}\}}{\sqrt{(1 - \phi_{vn})\phi_{vn}\phi_{pnf}\{1 - \phi_{pf}\}}},
\]

\[
\rho_{max} = \frac{\{\phi_{nf} - \phi_{vnf}\}}{\sqrt{(1 - \phi_{vn})\phi_{vn}\phi_{ef}\{1 - \phi_{nf}\}}},
\]

\(\phi_{vn} = \phi_{vnf} + \phi_{vnf}, \text{ and } \phi_{nf} = \phi_{vnf} + \phi_{vnf}\).

The theorem is proven in Supplement Section S5. Statement A restates Theorem 2(iii) by rewriting the conditions implied by the base model that require \(\theta_T \leq \theta_s \leq 1\), i.e., that the vaccine cannot cause the event and the subtracting indirect effect cannot be harmful and is not as extreme as the total ratio effect. The usual approach to solving this problem is Statement B, which is just expressing \(\lambda_s\) as a function of \(E(Y_{1M_1}), E(Y_{1M_0}), \) and \(E(Y_{0M_0})\). Another approach is Statement C, which is expressing \(\rho = \text{Corr}(Y_{1M_0}, M_1)\) as a function of \(E(Y_{1M_0})\) and some identifiable parameters, and the range for \(\rho\) required by the base model just plugs in \(E(Y_{1M_1})\) and \(E(Y_{0M_0})\) into the expression for \(\rho\). The main point of Theorem 3 is that the usual approach to solving this problem (Statement B) is like trying to identify a correlation, and assuming independence of the two random variables to conclude that the correlation is 0 (Statement C). Statement C seems like assuming what we are trying to identify because independence and correlation are inherently linked, but our objective is estimating \(\lambda_s\) which is really \(\lambda_s(\rho)\), a complex function of \(\rho\). The function \(\lambda_s(\rho)\) may be steep or relatively flat for \(\rho's\) of interest, and the steepness depends on a given dataset. Also, \(\lambda_s(0)\) depends on identifiable parameters specific to each setting (see equation 10).

The models of this section ignore adjustments for baseline covariates. If those baseline covariates are measured and control for all of the differences between the antibody production processes and the antibody protection process, then \(SI_2\) may hold and equation 10 is justified. Those are the assumptions used in the mediation analysis for the Moderna (mRNA-1273) COVID-19 vaccine [3,4]. If only some of those baseline covariates are used but are assumed to be all of them, the resulting estimator may be improved. A problem is that it is often difficult to measure all variables needed to control for these processes and to know that \(SI_2\) approximately holds.
4.3 Example

In Table 3, we show the results of a made-up randomized placebo-controlled vaccine trial with 10,000 participants in each arm. To keep it simple, we assume there are no baseline variables measured. We estimate VE as 90% since \( \hat{\theta}_T = \frac{E[Y_{1,M}(1)]}{E[Y_{0,M}(0)]} = \frac{10/10000}{10/10000} = 0.10 \). If the sequential ignorability assumptions are met and \( Pr[M_0 = 1|A = 0] = 0 \), then we can use equation 6 with only 1 level of \( X \) (which is equivalent to the result of Theorem 2), to get, \( \hat{E}[Y_{1,M_0}] = \hat{E}[Y_{1,M_1}|M_1 = 0] = 0.4\% \). This gives us \( \hat{\theta}_T = \frac{E[Y_{1,M}(1)]}{E[Y_{1,M}(0)]} = 0.1\% \) and \( \hat{\theta}_D = \frac{E[Y_{1,M}(0)]}{E[Y_{0,M}(0)]} = 0.4\% = 0.40 \), and \( \hat{\lambda}_s = \frac{\log(\hat{\theta}_D)}{\log(\hat{\theta}_T)} = 0.6021 \), so that under model \( M_2 \) we estimate that about 60.21% of the total ratio effect is mediated through the antibodies.

4.4 Three Arm Trial

Of the models studied, only model \( M_2 \) achieves identifiability of \( \lambda_s \), and no model gives identifiability of \( \lambda_a \). Thus, we consider an experimental solution. Consider trial with three arms: vaccine, placebo, and passive immunization, where passive immunization is infusing (or injecting) participants with antibodies made externally, such as monoclonal antibodies. We start with the assumptions of the base model of Section 4.1 and add the third arm. We make a consistency assumption about the antibodies, assuming that the antibody effects from infusion of monoclonal antibodies will be the same as if those antibodies occurred due to vaccination.

We treat that intervention (\( A = 2 \)) as if it is a placebo with \( M = 1 \), so we observe \( Y_{01} \) as responses from this arm (see Table 4). We label the \( \phi \) parameters as before except that the arm index is now one of three: ‘v’, ‘p’, or ‘i’. Although we can observe \( Y_{01} \), we do not observe \( M_1 \) in the arm with the infused mediator. Thus, we can only estimate \( \phi_{1f} = \phi_{1af} + \phi_{1nf} \) and \( \phi_{is} = \phi_{ias} + \phi_{ins} \). Table 4 gives \( \phi_{1f} \) and \( \phi_{is} \) in terms of \( \pi \) parameters, and using arguments similar to those used in Supplement Section 3, those extra \( \phi \) parameters do not lead to identification of \( E[Y_{1,M_0}] \) (see equation 8) or \( E[Y_{0,M_1}] \) (see equation 9).

Table 4 about here.

Suppose that in addition to the base model assumptions, we can perfectly predict from baseline covariates which individuals will have \( M_1 = 1 \). Then in the passive immunization arm the expected proportion of failures among those predicted to have \( M_1 = 1 \) will be identified as \( Pr[Y_{01} = 1 \text{ and } M_1 = 1] = \phi_{1af} = \pi_{0011}^{10} + \pi_{0111}^{10} + \phi_{1af} \), while in the placebo arm the expected proportion of failure among those predicted to have \( M_1 = 0 \) will be identified as \( Pr[Y_{00} = 1 \text{ and } M_1 = 0] = \phi_{enf} + \pi_{0011}^{30} \).

Thus, we identify \( E[Y_{0,M_1}] \) from the 3 arm trial, \( E[Y_{0,M_1}] = \sum_{m=0}^{1} Pr[Y_{0m} = 1 \text{ and } M_1 = m] \), allowing us to identify \( \theta_{ts} \) and \( \lambda_a \). What makes this identification possible is that there are only 2 levels for \( M_j \) and all individuals randomized to placebo get one level, while all those randomized to passive immunization get the other level. Identification with more than two levels for \( M_j \) is not as straightforward.

5 Nonbinary Mediators and adding Passive Immunity Experiments

In this section, we again allow a more general antibody response such that the support of \( M_1, S_{M_1}, \) contains both undetectable (i.e., \( M_1 = 0^* \)) or any of a set of detectable antibody levels (\( M_1 > 0^* \)). We continue to assume (similar to the base model of the binary case) that \( Pr[M_0 = 0^*] = 1 \). We explore identification of \( \lambda_a \) from several different experimental designs, all of which allow three types of intervention, vaccine, placebo, and passive immunization.

5.1 Three Arm Trial

Consider a randomized vaccine trial with three arms: placebo, vaccine, and passive immunization. We denote the three arms as \( p, v, \) and \( i \), with the associated values of \( A \) equal to 0, 1 and 2, and similarly for antibody potential responses (\( M_0, M_1, \) and \( M_2 \)) and potential outcomes (\( Y_{0m}, Y_{1m}, \) and \( Y_{2m} \)).

We start by reviewing some results in Gilbert et al. [20], who studied controlled vaccine efficacy from a placebo controlled randomized vaccine trial, and also considered the case when \( Pr[M_0 = 0^*] = 1 \). Gilbert et al. [20] defined the controlled vaccine efficacy, which in our notation (i.e., when \( Y_{arm} \) is binary) is \( CV E(m_1, m_0) = 1 - \frac{E[Y_{1m_1}]}{E[Y_{0m_0}]} \). When \( Pr[M_0 = 0^*] = 1 \),
we write
\[\text{CVE}(m, 0^*) \equiv \text{CVE}(m) = 1 - \frac{E[Y_{1m}]}{E[Y_{00}]} = 1 - \theta_C(m),\]
where \(Y_{00}\) is the placebo response when \(m = 0^*\). The \(\text{CVE}(m)\) used \(E(Y_{1m})\), the expected value of the potential response \(Y_{am}\) where \(A\) is set to \(a\) and \(M\) is set of \(m\). To identify \(E(Y_{1m})\) from a random sample where individuals are vaccinated and each individual has their natural mediator value, we need to make some strong assumptions. Let \(X\) be baseline covariates such that the sequential ignorability assumptions, \(SI_1\) and \(SI_2\) hold, and assume the following positivity assumption holds,

\[\text{PosM1}(S_{M_1}): \Pr[M_1 = m|A = 1, X = x] > 0 \text{ for all } x \text{ and } m \in S_{M_1}.\]

Then
\[1 - \theta_C(m) = \text{CVE}(m) = 1 - \frac{\sum_{(m,x)} E[Y_{1m}|X = x, M_1 = m] \Pr[M_1 = m \text{ and } X = x]}{E[Y_{00}]},\]
where the summation is over the support of \(X\) and \(M_1\) combined, and we assume discrete support for ease of exposition. Then \(\theta_C(m)\) is identifiable for each \(m \in S_{M_1}\). See Gilbert et al. [20] for details of identification results, inferential methods, and sensitivity analyses.

We can define an analogous controlled protective efficacy by comparing the passive immunization arm to placebo,
\[\text{CPE}(m) = 1 - \frac{E[Y_{2m}]}{E[Y_{00}]} = 1 - \theta_{I_a}(m),\]
where we use the notation \(\theta_{I_a}(m) = 1 - \text{CPE}(m)\) because here \(Y_{2m}\) (potential outcome from passive immunization where \(M\) is set to \(m\)) is acting like \(Y_{0m}\) (potential outcome from placebo recipient where \(M\) is set to \(m\)). For the passive immunization arm, we can randomly assign individuals to \(M_2\) with a known distribution with support \(S_{M_1}\). In other words, we ensure by design that \(\Pr[M_2 = m|A = 2] > 0 \text{ for each } m \in S_{M_1} \text{ and } M_2 \perp Y_{am}\) instead of assuming sequential ignorability and positivity.

Since \(\theta_C(m)\) and \(\theta_{I_a}(m)\) are acting like a total effect and an adding indirect effect at \(m\), we can define the proportion of the controlled ratio effect at \(m\) due to the controlled indirect ratio effect at \(m\) as
\[\lambda_a(m) = \frac{\log \{\theta_{I_a}(m)\}}{\log \{\theta_C(m)\}}.\]
These are useful estimands themselves, but they can also be used to identify \(\theta_T\) and \(\theta_{I_a}\). Because the distribution of \(M_1\) is identifiable from the vaccine arm, and because the denominator of the ratios in equations [11] and [12] do not depend on \(m\), we get
\[\theta_T = \sum_m \theta_C(m) \Pr[M_1 = m]\]
and
\[\theta_{I_a} = \frac{E[Y_{0M_a}]}{E[Y_{0M_0}]} = \sum_m \theta_{I_a}(m) \Pr[M_1 = m],\]
and \(\lambda_a = \log(\theta_{I_a})/\log(\theta_T)\), where the summations are over \(S_{M_1}\).

For this section, we have identified \(\lambda_a\) using the controlled vaccine efficacy and its analog for passive immunization, controlled protective efficacy. Since this is a randomized trial, both \(SI_1\) and \(PosA\) are met for both CVE and CPE, but CVE additionally requires the \(SI_2\) and \(PosM1(S_{M_1})\) as untestable assumptions, while the CPE can meet those later assumptions by design.
5.2 Three Arm Trial with Closeout Vaccination

Follmann [21] considered closeout vaccination, where individuals in the placebo arm are vaccinated at the end of the response follow-up period, to identify $M_1$ in the placebo arm without using baseline variables. We modify that idea for the three arm trial.

To start, as in the previous section, we assume $E(Y_{0M_1}) = E(Y_{2M_1})$. We rewrite $E(Y_{2M_1})$ as

\[
E[Y_{2M_1}] = \sum_m Pr(Y_{2m} = 1|M_1 = m) \times Pr(M_1 = m)
\]

\[
= \sum_m \frac{Pr(M_1 = m)Pr(Y_{2m} = 1)}{Pr(M_1 = m)} \times Pr(Y_{2m} = 1)
\]

\[
= \sum_m Pr(M_1 = m)Pr(Y_{2m} = 1)Pr(Y_{2m} = 1)
\]

\[
= \sum_m \{Pr[M_1 = m] - Pr[M_1 = m|Y_{2m} = 0]Pr(Y_{2m} = 0)\},
\]

(16)

where the second step uses Bayes theorem and the last step uses

\[
P(M_1 = m) = P(M_1 = m|Y_{2m} = 1)P(Y_{2m} = 1) + P(M_1 = m|Y_{2m} = 0)P(Y_{2m} = 0).
\]

As in the previous section, we independently draw $M_2$ from a known distribution with support $S_{M_1}$, such that $M_2$ is independent of all potential mediators and outcomes, and $Pr[M_2 = m|A = 2] > 0$ for each $m \in S_{M_1}$. Then by independence and consistency,

\[
Pr[Y_{2m} = 0] = Pr[Y_{2m} = 0|M_2 = m] = Pr[Y|A = 2, M_2 = m],
\]

and $Pr[Y_{2m} = 0]$ is identifiable. Also, $Pr[M_1 = m]$ is identifiable from the vaccine arm. The only remaining piece to identify in equation (16) is $Pr(M_1 = m|Y_{2m} = 0)$, which we identify using closeout vaccination in the passive immunization arm.

At the end of the response follow-up period, we implement a closeout vaccination on individuals in the passive immunization arm with $Y_{2M_2} = 0$. We do not vaccinate individuals with $Y_{2M_2} = 1$ because the antibody response after the getting the disease and vaccination cannot substitute for $M_1$. We assume that on the closeout vaccinated individuals the antibody response after closeout vaccination would equal $M_1$, the value they would have gotten if vaccinated at the start of the trial. This may be a tenuous assumption if the response is disease and there are asymptomatic infections that have $Y_{2M_2} = 0$, since asymptomatic infections may affect a subsequent immune response to vaccination. We do not assume that $M_2 = M_1$. Instead, we use the independence and positivity assumptions on $M_2$ previously mentioned to get

\[
Pr[M_1 = m|Y_{2m} = 0] = Pr[M_1 = m|Y_{2m} = 0, M_2 = M_1],
\]

so that $Pr[M_1 = m|Y_{2m} = 0]$ is identifiable. Thus, by equation (16) we can identify $E[Y_{2M_1}]$, and hence also identify $\theta_{I_d}$.

In summary, a three arm trial with closeout vaccination of the passive immunization arm allows identification of $\theta_{I_d}$ and $\lambda_a$ without the use of any baseline covariates, without assuming $SI_2$, and allowing $Pr[M_0 = 0^*] = 1$.

5.3 Two Randomized Trials

In this section, we consider combining two randomized trials, a vaccine vs. placebo (VP) trial and a passive immunization vs. placebo (IP) trial. This requires more care because there will be differences between the trials, such as distributions for exposure, baseline covariates, potential mediator responses, and potential outcomes. To emphasize these differences, we use superscripts VP and IP to differentiate the two trials in the variables.

Suppose that using the VP trial, we can identify baseline predictors of $M_1$, such that for each $m \in S_{M_1}$, there is a predictor set of baseline variables, $S_{a}(m) = \{x: \text{if } X = x \text{ then } M_1 = m\}$. Further, assume that $S_{a}(m)$ can identify participants in the VP trial with $M_1 = m$. Then we design the IP trial using a quota sampling approach so that we chose the participants for the IP trial from the pool of available volunteers such that the baseline distribution of $X$ is similar between the two trials. For all
participants in the IP trial with \( X \in S_2(m) \), we set \( M_2 = m \). If the sampling is done well enough, then the distribution of \( M_1 \) will match the distribution of \( M_2 \) and 

\[
\hat{\theta}_{I_a} = \frac{E[Y_{VPM}^{IP}]}{E[Y_{VPM}^{IP}]} \approx \frac{E[Y_{2mM_2}^{IP}]}{E[Y_{0MM_0}^{IP}]}.
\]

(17)

In expression 17 the approximation may be reasonable because even though we will have different exposure distributions in the two trials, the exposure effects should cancel out as in equation 1. Then we modify equation 12 to standardize using the distribution of the \( m \) levels of \( X \) and make the same sequential ignorability and positivity assumptions as in Section 5.1. For the IP trial, we assume that within two trials, the exposure effects should cancel out as in equation 1.

In expression 17 the approximation may be reasonable because even though we will have different exposure distributions in the two trials, the exposure effects should cancel out as in equation 1. Then we modify equation 12 to standardize using the distribution of \( X \) from the VP trial.

\[
CV E(m) = 1 - \sum_x \frac{E[Y_{1mV}^{VP} X^{VP} = x]}{E[Y_{mV}^{VP}]} Pr[X^{VP} = x] = 1 - \theta_C(m),
\]

(18)

and make the same sequential ignorability and positivity assumptions as in Section 5.1. For the IP trial, we assume that within levels of \( X \), the study populations between the trials are comparable and capture all the differences between the trials except the exposure effects, which we assume cancel out by equation 1. Then we modify equation 12 to standardize using the distribution of \( X \) from the VP trial.

\[
CPE(m) = 1 - \sum_x \frac{E[Y_{2mV}^{IP} X^{IP} = x]}{E[Y_{00M}^{IP} X^{IP}]} Pr[X^{IP} = x] = 1 - \theta_{I_a}(m),
\]

(19)

where now we must standardize in both the numerator and denominator. As in Section 5.1 we can impose the independence of the \( m \) and potential outcomes in the \( A = 2 \) arm by randomization in the design, not by assumption. In summary, we are controlling for differences in \( X \) between the trials by standardization, and controlling for differences in exposure by equation 1. We again define \( \lambda_a(m) \) as in equation 13 and because the denominators still do not depend on \( m \), we can get \( \theta_T \) and \( \theta_{I_a} \) by equations 14 and 15 respectively.

Consider making inferences using equations 18 and 19. In Table 5 and Figure 2 we give a made-up example assuming the covariate adjustments of equations 18 and 19 have already been made. In this example, there are 3 levels of the Ab mediator \((m = 0,1,2)\). At \( m = 0 \) the value of \( \hat{\theta}_{I_a}(0) = 100\% \) so that \( \lambda_a(0) = 0 \). The overall effects are estimated by weighted averages, so that using equation 14 we get \( \hat{\theta}_T \) and using equation 15 we get \( \hat{\theta}_{I_a} \). The overall \( \lambda_a \) is then estimated by \( \lambda_a = \log(\hat{\theta}_{I_a})/\log(\hat{\theta}_T) \). Compare \( \hat{\theta}_{I_a} = 45.4\% \) and \( \lambda_a = 45.4\% \) from Table 5 to \( \hat{\theta}_T = 0.09/0.38 = 23.7\% \) and \( \lambda = \log(0.38)/\log(0.09) = 40.2\% \) calculated using assumption \( SI_a \) and equation 6 which gives \( E[Y_{1MM}] = 38\% \). So if \( SI_a \) is true and the sample size is large enough that we can ignore variability of the estimators, then \( \hat{\theta}_{I_a} < \hat{\theta}_T \) and there is an interaction (since \( \hat{\theta}_{I_a} \neq \hat{\theta}_T \)).

[Figure 2 and Table 5 about here]

6 Discussion

We have explored the assumptions related to identifying the proportion of the total ratio effect mediated through the antibodies in a vaccine versus placebo (VP) trial. We have shown that because the placebo arm will likely not have positive antibody values, from the VP trial we can only identify one of the two ways to define that proportion \( \lambda_a \), and that requires an independence assumption between potential mediators and potential outcomes. We discussed supplementing the VP trial with extra experimental information: either adding to the VP trial a third arm with a passive immunization, or combining the VP trial with a passive immunization versus placebo (IP) trial. These newly proposed experimentally supplemented trials allow us to identify the proportion of the total ratio effect due to adding antibodies to the placebo arm, \( \lambda_a \).

We note several differences between the traditional (i.e., VP trial alone) and non-traditional (i.e., VP trial with supplemental experimental passive immunization) mediation analyses. First, the traditional mediation analysis is estimating \( \lambda_a \), while the non-traditional one is estimating \( \lambda \). If we can make the simplifying assumption that the size of the effect of adding antibodies to participants in the placebo arm is the same as that of subtracting antibodies from participants in the vaccine arm, then \( \lambda = \lambda_a = \lambda \), and the proposed non-traditional supplemental experiments provide a way to estimate \( \lambda \) in a different way. For the passive immunization arm, we do not need to assume the antibodies are independent of the potential outcomes because we can impose that independence by actively randomizing participants to their antibody values. In practice, we can do both
mediation analyses and can compare estimates of both $\lambda_s$ and $\lambda_a$. If they are similar, then it may be reasonable to assume no interaction such that $\lambda_s = \lambda_a$ and they are two estimators of the same parameter, $\lambda$.

Even if we cannot assume $\lambda_s = \lambda_a$, an advantage of estimating $\lambda_a$ using the non-traditional mediation analyses is that the cross-world parameter $E(Y_{0M_1})$ is estimated experimentally (see e.g., Section 5.2). In contrast, the cross-world parameter $E(Y_{1M_0})$ used in traditional mediation analyses for estimating $\lambda_s$ requires making necessary and often difficult independence assumptions, which ideally should be accompanied with sensitivity analyses (see similar sensitivity analyses for controlled vaccine efficacy in [20]). In practice, some thought is required to accept the consistency assumptions with respect to the vaccine-induced antibodies having an equal effect as monoclonal antibodies. It could be the the monoclonal antibodies are made from a different virus strain that the vaccine-induced ones, and the monoclonal antibodies may have different immunological characteristics. Further, the decay pharmacokinetics are likely to be different between the two types of antibodies so timing of antibody measurements is important for these types of studies.

In this paper, we have focused on vaccine studies where very few or no placebo participants would produce antibodies. We have also focused almost exclusively on examining assumptions and identifiability. We explored different study designs to identify these mediation effects. One design in particular (a three arm randomized trial with closeout vaccination, see Section 5.2) is feasible and does not require as severe assumptions as the others. There is much room for future work in spelling out the details of that design and the other proposed study designs, including examining how the designs may need to be modified for feasibility reasons. Future work could explore these issues when the some placebo participants have existing antibodies due to natural exposure. Other work is needed to explore details of estimators (e.g., [4]), and this paper could be complementary to some of that work, since the estimator work typically does not have the space to fully discuss the implications of all their assumptions.

7 Supplementary Material

See Supplementary Material for proofs and extra mathematical details.

Acknowledgements

We thank Peter Gilbert and three anonymous reviewers for insightful comments that appreciably improved the article.

References

[1] M Elizabeth Halloran, Ira M Longini, Claudio J Struchiner, and Ira M Longini. Design and analysis of vaccine studies, volume 18. Springer, 2010.

[2] Tyler VanderWeele. Explanation in causal inference: methods for mediation and interaction. Oxford University Press, 2015.

[3] Peter B Gilbert, David C Montefiori, Adrian B McDermott, Youyi Fong, David Benkeser, Weiping Deng, Honghong Zhou, Christopher R Houchens, Karen Martins, Lakshmi Jayashankar, et al. Immune correlates analysis of the mrna-1273 covid-19 vaccine efficacy clinical trial. Science, 375:43–50, 2022.

[4] D. Benkeser, I. Diaz, and J. Ran. Inference for natural mediation effects under case-cohort sampling with applications in identifying covid-19 vaccine correlates of protection. arXiv:2103.02643v1, 2021.

[5] James M Robins and Sander Greenland. Identifiability and exchangeability for direct and indirect effects. Epidemiology, pages 143–155, 1992.

[6] C.A. Seigrist and P.H. Lambert. Chapter 2: How vaccines work. In BR Bloom and PH Lambert, editors, The Vaccine Book, pages 33–42. Elsevier, New York, 2016.

[7] Rekha R Rapaka, Elizabeth A Hammershaimb, and Kathleen M Neuzil. Are some covid-19 vaccines better than others? interpreting and comparing estimates of efficacy in vaccine trials. Clinical Infectious Diseases, 74(2):352–358, 2022.
[8] Benjamin J Cowling, Wey Wen Lim, Ranawaka APM Perera, Vicky J Fang, Gabriel M Leung, JS Malik Peiris, and Eric J Tchetgen Tchetgen. Influenza hemagglutination-inhibition antibody titer as a mediator of vaccine-induced protection for influenza b. *Clinical Infectious Diseases*, 68(10):1713–1717, 2019.

[9] Quynh C Nguyen, Theresa L Osypuk, Nicole M Schmidt, M Maria Glymour, and Eric J Tchetgen Tchetgen. Practical guidance for conducting mediation analysis with multiple mediators using inverse odds ratio weighting. *American journal of epidemiology*, 181(5):349–356, 2015.

[10] Michael G Hudgens, Peter B Gilbert, and Steven G Self. Endpoints in vaccine trials. *Statistical methods in medical research*, 13(2):89–114, 2004.

[11] Stephen Senn. The design and analysis of vaccine trials for covid-19 for the purpose of estimating efficacy. *Pharmaceutical Statistics*, 21(4):790–807, 2022.

[12] Stephen R Cole and Constantine E Frangakis. The consistency statement in causal inference: a definition or an assumption? *Epidemiology*, 20(1):3–5, 2009.

[13] Danella M Hafeman and Sharon Schwartz. Opening the black box: a motivation for the assessment of mediation. *International journal of epidemiology*, 38(3):838–845, 2009.

[14] Judea Pearl. Direct and indirect effects. In *Proceedings of the Seventeenth Conference on Uncertainty in Artificial Intelligence*, pages 411–420. Morgan Kaufmann, San Francisco, 2001.

[15] Pearl Judea. The mediation formula: A guide to the assessment of causal pathways in nonlinear models. In C. Berzuini, P. Dawid, and L. Bernardinelli, editors, *Causality: Statistical Perspectives and Applications*, pages 151–179. Wiley, 2012.

[16] Kosuke Imai, Luke Keele, and Teppei Yamamoto. Identification, inference and sensitivity analysis for causal mediation effects. *Statistical science*, 25(1):51–71, 2010.

[17] Rishi R Goel, Sokratis A Apostolidis, Mark M Painter, Divij Mathew, Ajinkya Pattekar, Oliva Kuthuru, Sigrid Gouma, Philip Hicks, Wenzhao Meng, Aaron M Rosenfeld, et al. Distinct antibody and memory b cell responses in sars-cov-2 naive and recovered individuals after mrna vaccination. *Science immunology*, 6(58):eabi6950, 2021.

[18] Danella M Hafeman and Tyler J VanderWeele. Alternative assumptions for the identification of direct and indirect effects. *Epidemiology*, pages 753–764, 2011.

[19] Rahul Shukla, Viswanathan Ramasamy, Rajgokul K Shanmugam, Richa Ahuja, and Navin Khanna. Antibody-dependent enhancement: a challenge for developing a safe dengue vaccine. *Frontiers in Cellular and Infection Microbiology*, 10: Article 572681, 2020.

[20] Peter B Gilbert, Youyi Fong, Avi Kenny, and Marco Carone. A controlled effects approach to assessing immune correlates of protection. *Biostatistics*, DOI:10.1093/biostatistics/kxac24, 2022.

[21] Dean Follmann. Augmented designs to assess immune response in vaccine trials. *Biometrics*, 62(4):1161–1169, 2006.

[22] Ashley I Naimi, Jay S Kaufman, and Richard F MacLehose. Mediation misgivings: ambiguous clinical and public health interpretations of natural direct and indirect effects. *International journal of epidemiology*, 43(5):1656–1661, 2014.

[23] Tyler J VanderWeele. Mediation analysis: a practitioner’s guide. *Annual review of public health*, 37:17–32, 2016.
Figure 1: Graphic of different ways of partitioning the total effect (TE) into an indirect effect (IE) and direct effect (DE). The vertical axis is on the log scale for ratio effects, and on the arithmetic scale for difference effects. Moving southeast from $E[Y_{0M_0}]$ to $E[Y_{0M_1}]$ corresponds to adding in antibodies, while moving northwest from $E[Y_{1M_1}]$ to $E[Y_{1M_0}]$ corresponds to subtracting out antibodies. The Robins and Greenland [5] terminology is pure indirect effect ($IE_a$), total direct effect ($DE_a$), total indirect effect ($IE_s$), and pure direct effect ($DE_s$).
Figure 2: Example for combining two trials, a vaccine vs. placebo (VP) trial and a passive immunization vs. placebo (IP) trial. In panel (a) we plot the controlled vaccine efficacy ($CVE(m) = 1 - \theta_C(m)$) for the VP trial and the controlled protective efficacy ($CPE(m) = 1 - \theta_I(m)$) for the IP trial, while in panel (b) we plot the same data in terms of ratio effects ($\theta_C(m)$ for the VP trial, and $\theta_I(m)$ for the IP trial) but on a log scale. The efficacies (i.e., vertical axes) are in percent.
Table 1: The 12 types left after excluding types based on assumptions $RG_1, RG_2$ and $RG_3$, and those with $M_0 = 1$. Values in gray boxes are not observable in the randomized trial (i.e., are cross-world counterfactuals). The notation for the proportion of the population for each type is $\pi_Y$, where $M = [M_1, M_0]$ and $Y = [Y_{11}, Y_{10}, Y_{01}, Y_{00}]$. In the description, the “either” or “both” refer to the antibody (Ab) and/or the direct effect, prot.=protected and resp.=responder.

| $\pi_{Y_{11}Y_{10}Y_{01}Y_{00}}^{M_1M_0}$ | $M_1$ | $M_0$ | $Y_{11}$ | $Y_{10}$ | $Y_{01}$ | $Y_{00}$ | $Y_{0M_1}$ | $Y_{1M_1}$ | $Y_{0M_0}$ | $Y_{1M_0}$ | $Y_{1M_1}$ | $Y_{0M_1}$ | $Y_{0M_0}$ | Type Description |
|-------------------------------------|-------|-------|---------|---------|---------|---------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------------|
| $\pi_{0000}^{0000}$                | 0     | 0     | 0       | 0       | 0       | 0       | 0         | 0         | 0         | 0         | 0         | 0         | 0         | Ab nonresp. uninfectable |
| $\pi_{0001}^{0001}$                | 0     | 0     | 0       | 0       | 0       | 1       | 0         | 0         | 1         | 1         | 1         | 1         | 1         | Ab nonresp. either alone prot. |
| $\pi_{0010}^{0010}$                | 0     | 0     | 0       | 0       | 1       | 0       | 1         | 1         | 0         | 1         | 1         | 1         | 1         | Ab nonresp. direct alone prot. |
| $\pi_{0100}^{0100}$                | 0     | 0     | 0       | 1       | 0       | 0       | 1         | 1         | 1         | 1         | 1         | 1         | 1         | Ab nonresp. Ab alone prot. |
| $\pi_{0110}^{0110}$                | 0     | 0     | 0       | 1       | 1       | 1       | 1         | 1         | 1         | 1         | 1         | 1         | 1         | Ab nonresp. need both |
| $\pi_{1000}^{1000}$                | 0     | 0     | 1       | 1       | 1       | 1       | 1         | 1         | 1         | 1         | 1         | 1         | 1         | Ab nonresp. totally doomed |
| $\pi_{1001}^{1001}$                | 1     | 0     | 0       | 0       | 0       | 0       | 0         | 0         | 0         | 0         | 0         | 0         | 0         | Ab resp. uninfectable |
| $\pi_{1010}^{1010}$                | 0     | 1     | 0       | 0       | 0       | 0       | 0         | 0         | 0         | 0         | 0         | 0         | 0         | Ab resp. either alone prot. |
| $\pi_{1011}^{1011}$                | 1     | 0     | 0       | 1       | 0       | 1       | 0         | 1         | 1         | 1         | 1         | 1         | 1         | Ab resp. direct alone prot. |
| $\pi_{1100}^{1100}$                | 1     | 0     | 0       | 1       | 1       | 1       | 1         | 1         | 1         | 1         | 1         | 1         | 1         | Ab resp. Ab alone prot. |
| $\pi_{1101}^{1101}$                | 0     | 1     | 1       | 1       | 1       | 1       | 1         | 1         | 1         | 1         | 1         | 1         | 1         | Ab resp. need both |
| $\pi_{1110}^{1110}$                | 1     | 0     | 1       | 1       | 1       | 1       | 1         | 1         | 1         | 1         | 1         | 1         | 1         | Ab resp. totally doomed |

Table 2: The proportions of the twelve types of responses in the base model, and the marginal combinations of them. Let $M = [M_1, M_0]$ and $Y = [Y_{11}, Y_{10}, Y_{01}, Y_{00}]$. The proportions are written as $\pi_Y$. For example, $\pi_{10}^{10}$ is the proportion of the population with potential mediators equal to $M_1 = 1$ and $M_0 = 0$, and potential outcomes equal to $Y_{11} = 0, Y_{10} = 1, Y_{01} = 0$ and $Y_{00} = 1$. We only observe six marginals from the table (4 from the treatment arm $[A = 1]$, and 2 from the control arm $[A = 0]$), so those combinations of types are identifiable, as well as certain functions of them (e.g., the sum of the marginals for the first two rows is $Pr[M_1 = 0]$, which is identifiable). The observable marginals as written as $\phi_{amy}$, where the $amy$ subscripts refer to respectively, $A (a = v$ is vaccine arm, $a = p$ is placebo arm), $M_A (m = a$ is detectable antibodies, $m = n$ is no detectable antibodies), and $Y_{AMA}$ ($y = f$ is failure, $y = s$ is success).

| $A = 0, M_0 = 0, Y_{00} = 0$ | $A = 0, M_0 = 0, Y_{00} = 1$ | observable marginal |
|-----------------------------|-----------------------------|---------------------|
| $[0000]$                    | $[0001]$                    | $[0011]$            |
| $[0101]$                    | $[0111]$                    | $[1111]$            |
| $\pi_{0000}^{0000}$        | $\pi_{0001}^{0001}$        | $\pi_{0010}^{0010}$ |
| $\pi_{0001}^{0001}$        | $\pi_{0011}^{0011}$        | $\pi_{0101}^{0101}$ |
| $\pi_{0010}^{0010}$        | $\pi_{0011}^{0011}$        | $\pi_{0111}^{0111}$ |
| $\pi_{0100}^{0100}$        | $\pi_{0101}^{0101}$        | $\pi_{0110}^{0110}$ |
| $\pi_{1000}^{1000}$        | $\pi_{1001}^{1001}$        | $\pi_{1010}^{1010}$ |
| $\phi_{pn,s}$              | $\phi_{pn,f}$              | $\phi_{vns}$      |
| $\phi_{vn,f}$              | $\phi_{vas}$               | $\phi_{vaf}$      |

18
Table 3: Made-up results from a randomized placebo-controlled vaccine trial with 10,000 participants in each arm.

| $a$ | $m(a)$ | $y(a,m(a))$ | Number of Participants | $\phi$ parameter | Conditional Event Proportions |
|-----|--------|-------------|------------------------|------------------|-------------------------------|
| 1   | 1      | 1           | 2                      | $\phi_{va} = 0.02\%$ | $\hat{E}[Y_{1M_1}|M_1 = 1] = 2/8000 = 0.025\%$ |
| 1   | 1      | 0           | 7998                   | $\phi_{va} = 79.98\%$ |
| 1   | 0      | 1           | 8                      | $\phi_{vn} = 0.08\%$ | $\hat{E}[Y_{1M_1}|M_1 = 0] = 8/2000 = 0.4\%$ |
| 1   | 0      | 0           | 1992                   | $\phi_{vn} = 19.92\%$ |
| 0   | 1      | 1           | 0                      | $\phi_{ps} = 0$ |
| 0   | 1      | 0           | 0                      | $\phi_{ps} = 0$ |
| 0   | 0      | 1           | 100                    | $\phi_{pn} = 1\%$ | $\hat{E}[Y_{0M_0}|M_0 = 0] = 100/10000 = 1\%$ |
| 0   | 0      | 0           | 9900                   | $\phi_{pn} = 99\%$ |

Table 4: Ten strata (4 strata in the vaccine arm, 4 in the placebo arm, and 2 in the passive immunization arm), where the 5th and 6th rows represent strata that are not observed when $M_0 = 0$ always (as in the base model). The last 6 columns represent the observed values $(A, M_A, Y_{A|M_A})$ put in the appropriate column, with ‘·’ representing unobserved potential outcomes.

| description                  | $\phi$ | $\pi$ | $A$ | $M_A$ | $Y_{A|M_A}$ | $M_1$ | $M_0$ | $Y_{11}$ | $Y_{10}$ | $Y_{01}$ | $Y_{00}$ |
|------------------------------|--------|-------|-----|-------|-------------|-------|-------|-----------|----------|----------|---------|
| vaccine/Abs/failure         | $\phi_{va}$ | $\pi_{11}$ | 1   | 1     | 1           | 1     | 1     | 0         | 0        | 0        | 0       |
| vaccine/Abs/success         | $\phi_{vs}$ | $\pi_{10} + \pi_{01}$ | 1   | 1     | 0           | 1     | 0     | 0         | 0        | 0        | 0       |
| vaccine/no Abs/failure      | $\phi_{vn}$ | $\pi_{01}$ | 1   | 0     | 1           | 0     | 1     | 0         | 0        | 0        | 0       |
| vaccine/no Abs/success      | $\phi_{vn}$ | $\pi_{00} + \pi_{10}$ | 1   | 0     | 0           | 0     | 0     | 0         | 0        | 0        | 0       |
| placebo/Abs/failure         | $\phi_{pf}$ | 0      | 0   | 1     | 1           | 1     | 1     | 0         | 0        | 0        | 0       |
| placebo/no Abs/failure      | $\phi_{pf}$ | $\pi_{00} + \pi_{10} + \pi_{01} + \pi_{11}$ | 0   | 0     | 1           | 0     | 0     | 0         | 1        | 1        | 1       |
| placebo/no Abs/success      | $\phi_{pn}$ | $\pi_{00} + \pi_{10} + \pi_{01} + \pi_{11}$ | 0   | 0     | 0           | 0     | 0     | 0         | 0        | 0        | 0       |
| passive Imm/failure         | $\phi_{if}$ | $\pi_{00} + \pi_{10} + \pi_{01} + \pi_{11}$ | 2   | 1     | 1           | 0     | 0     | 1         | 0        | 0        | 0       |
| passive Imm/success         | $\phi_{is}$ | $\pi_{00} + \pi_{10} + \pi_{01} + \pi_{11}$ | 2   | 0     | 0           | 0     | 0     | 0         | 0        | 0        | 0       |
Table 5: Example for combining two trials, a vaccine vs. placebo (VP) trial and a passive immunization vs. placebo (IP) trial. All values except Ab level are in percent.

| m (Ab level) | CVE(m) | CPE(m) | θ(m) | μ(m) | λ(m) | P(M₁ = m) |
|--------------|--------|--------|------|------|------|------------|
| 0            | 62.0   | 0.0    | 38.0 | 100.0| 0.0  | 10         |
| 1            | 92.0   | 60.0   | 8.0  | 40.0 | 36.3 | 40         |
| 2            | 96.0   | 85.0   | 4.0  | 15.0 | 58.9 | 50         |
| Overall      | 91.0   | 66.5   | 9.0  | 33.5 | 45.4 | 100        |
Supplementary Materials for:
Mediation Analyses for the Effect of Antibodies in Vaccination
Michael P. Fay and Dean A. Follmann

S1 Pearl Assumptions

We restate standard assumptions introduced by Pearl [14] used to identify direct or indirect effects, replacing “exposure” with “vaccine” for our example. These 4 assumptions are listed in many publications; we choose the wording from [22] for the first 3, and from [23] for the last one.

| Text Mathematical |
|-----------------------------------|
| A1 “No uncontrolled [vaccine]-outcome confounding” | $Y_{am} \perp \perp A|C$ |
| A2 “No uncontrolled mediator-outcome confounding” | $Y_{am} \perp \perp M|A, C$ |
| A3 “No uncontrolled [vaccine]-mediator confounding” | $M_{a} \perp \perp A|C$ |
| A4 “No mediator-outcome confounding that is itself affected by the [vaccine]” | $Y_{am} \perp \perp M_{a'}|C$ |

In the mathematical notation, the lack of a subscript denotes that the independence expression holds for all individuals. The value $C$ denotes a vector of confounding variables (including possibly variables measured after baseline [i.e., after vaccination]). Lower case letters indicate that the independence assumption holds if the values were set to a specific allowable constant. For example, $Y_{am} \perp \perp A|C$ means that the potential outcome for individual $i$ given $A_i = a$ and their mediator was set to $m$ (i.e., $Y_{am}^{(i)}$) is independent of $A_i$ given $C_i$ for all $a \in \{0, 1\}, m \in [0, \infty)$, and $i \in \{1, \ldots, n\}$. Typically, the positivity assumptions are implicitly assumed.

S2 Proof of Equation 4 When $Pr[M_0 > 0^*|X = a, X = x] = 0$

We loosely follow the proof of VanderWeele [2, p. 465], except we focus on the binary mediator and outcome case with $a = 1$ and $a' = 0$ and we do not require that $Pr[M_0 > 0^*|A = 0, X] > 0$. In the following, we generalize the $PosM_0$ and $PosM_1$ assumptions as

$PosM_0(S_{M_0|x})$: $Pr[M_0 = m|A = 0, X = x] > 0$ for all $x$ and $m \in S_{M_0|x}$, and

$PosM_1(S_{M_1|x})$: $Pr[M_1 = m|A = 1, X = x] > 0$ for all $x$ and $m \in S_{M_1|x}$,

where the supports for the mediator random variables may not be equal, and may change based on $x$. We consider the case when $S_{M_0|x} = \{0^*\}$ and $0^* \in S_{M_1|x}$ for $X = x$. In the following, all summations are
over the support $S_{M|x}$:

$$E[Y_{1M_0}|X = x] = \sum_m E[Y_{1m}|M_0 = m, X = x] \Pr[M_0 = m|A = 0, X = x] \quad \text{(by iterated expectations)}$$

$$= \sum_m E[Y_{1m}|A = 1, M_0 = m, X = x] \Pr[M_0 = m|A = 0, X = x] \quad \text{(by } SI_1 \text{)}$$

$$= \sum_m E[Y_{1m}|A = 1, X = x] \Pr[M_0 = m|A = 0, X = x] \quad \text{(by } SI_2 \text{)}$$

$$= \sum_m E[Y_{1m}|A = 1, M_1 = m, X = x] \Pr[M_0 = m|A = 0, X = x] \quad \text{(by } SI_2 \text{)}$$

$$= \sum_m E[Y|A = 1, M = m, X = x] \Pr[M = m|A = 0, X = x] \quad \text{(by consistency)}$$

$$= E[Y|A = 1, M = 0^*, X = x] \quad \text{(when } Pr[M > 0^*|A = 0, X = x] = 0 )$$

\section*{S3 Proof of Theorem 1}

We show the proof for $\lambda_s$. The proof for $\lambda_a$ is analogous and is not shown.

For $\pi$ parameters, let ‘$\tau$’ denote summation over an index, so that the sums for the 5 columns under $A = 0, M_0 = 0, Y_{00} = 1$ for each of the first 3 rows of the Table 2 are

$$\pi_{00}^{00} = \pi_{0000}^{00} + \pi_{0011}^{00} \quad \text{(S1)}$$

$$\pi_{10}^{00} = \pi_{0101}^{00} + \pi_{0011}^{00} + \pi_{1111}^{00} \quad \text{(S2)}$$

$$\pi_{01}^{10} = \pi_{0011}^{10} + \pi_{1011}^{10} + \pi_{0111}^{10} \quad \text{(S3)}$$

The issue is that we cannot differentiate among the terms in each of the sums on the right-hand side of the above equations. Suppose we could differentiate the sum in equation \textbf{S3} such that if we knew $\tau_s$, then

$$\pi_{0101}^{10} + \pi_{1011}^{10} = \tau_s (\pi_{001}^{00} + \pi_{011}^{00}), \text{ so that for any } \tau_s \in [0, 1],$$

$$\pi_{001}^{00} + \pi_{011}^{10} + \pi_{1011}^{10} = (1 - \tau_s) (\pi_{001}^{00} + \pi_{011}^{10}).$$

Since $E[Y_{1M_0}] = E[Y_{1M_1}] + \pi_{0101}^{10} + \pi_{0111}^{10}$, when $\tau_s = 0$ then

$$E[Y_{1M_0}] = E[Y_{1M_1}],$$

$$\Rightarrow \quad \theta_I_s = \frac{E[Y_{1M_1}]}{E[Y_{1M_0}]} = 1$$

$$\Rightarrow \quad \lambda_s = \frac{\log(\theta_I_s)}{\log(\theta_T)} = 0.$$ 

Since $E[Y_{1M_0}] = E[Y_{0M_0}] - (\pi_{001}^{00} + \pi_{011}^{10} + \pi_{0011}^{10})$, when $\tau_s = 1$ then

$$E[Y_{1M_0}] = E[Y_{0M_0}],$$

$$\Rightarrow \quad \theta_I_s = \frac{E[Y_{1M_1}]}{E[Y_{0M_0}]} = \theta_T$$

$$\Rightarrow \quad \lambda_s = \frac{\log(\theta_I_s)}{\log(\theta_T)} = 1.$$
Now we just need to show that for any values of the $\phi$ parameters (i.e., identifiable parameters) under the base model, that for any value of $\tau_s \in [0, 1]$ the $\phi$ parameters do not change. By Table 2 we have:

$$
\phi_{va f} = \pi_{1111}^{10} \\
\phi_{va s} = \pi_{00}^{00} \\
\phi_{va s} = \pi_{1100}^{10} + \pi_{1000}^{00} + \pi_{0000}^{00} \\
\phi_{va s} = \pi_{1111}^{10} + \pi_{1100}^{10} + \pi_{1000}^{00} + \pi_{0000}^{00} \\
\phi_{va s} = \pi_{0000}^{00} + \pi_{0000}^{10} \\
\phi_{va s} = \pi_{1111}^{10} + \pi_{1100}^{10} + \pi_{1000}^{00} + \pi_{0000}^{00} \\
$$

If $\tau_s = 0$ then $\pi_{0100}^{10} + \pi_{0111}^{10} = 0$, and we can just let $\pi_{0100}^{10} = \pi_{0001}^{10} + \pi_{0011}^{10}$, which does not change the $\phi$ parameters. If $\tau_s = 1$ then $\pi_{0001}^{10} + \pi_{0011}^{10} = 0$, and it is straightforward to allow $\pi_{0001}^{10} + \pi_{0011}^{10} = 0$, but setting $\pi_{0001}^{10}$ (equation S1) to 0 can also be accomplished without changing the $\phi$ parameters. If $\pi_{0001}^{10} = 0$ then $\pi_{0000}^{00} = \phi_{vns}$, so that $\pi_{0000}^{10} = \phi_{vns} - \phi_{vns}$, and $\pi_{0001}^{10} = \phi_{vns} - \phi_{vns} + \phi_{vns} = \phi_{vns} - \phi_{vns}$, and neither of $\pi_{0001}^{10}$ and $\pi_{0011}^{10}$ will change; therefore, we can set $\pi_{0001}^{10} = 0$ without changing any $\phi$ parameter. Because the bounds $\tau_s = 0$ and $\tau_s = 1$ do not change the $\phi$ parameters, by continuity, all $\tau_s \in (0, 1)$ also do not change the $\phi$ parameters.

**S4 Proof of Theorem 2**

Consider first statement (i). Let $[\pi_{0000}, \pi_{0001}, \pi_{0011}, \pi_{0101}, \pi_{0111}, \pi_{1111}]$ be the marginal probability for the 6 possible potential outcomes, $Y = [Y_{11}, Y_{10}, Y_{01}, Y_{00}]$, from Table 2, which sum to 1. Let $\phi_{vn} = \phi_{vn f} + \phi_{vns}$ be $Pr[M_1 = 0]$, and let $\phi_{va} = \phi_{va f} + \phi_{vas}$ be $Pr[M_1 = 1] = 1 - Pr[M_1 = 0]$. Under $Y_{am} \perp M_{a'}$ for all $a, a', m$, we have:

$$
\phi_{vn} = \begin{bmatrix}
\pi_{0000} \\
\pi_{0001} \\
\pi_{0011} \\
\pi_{0101} \\
\pi_{0111} \\
\pi_{1111}
\end{bmatrix} = \begin{bmatrix}
\pi_{0000}^{00} \\
\pi_{0001}^{00} \\
\pi_{0011}^{00} \\
\pi_{0101}^{00} \\
\pi_{0111}^{00} \\
\pi_{1111}^{00}
\end{bmatrix}
$$

(S4)

and

$$
\phi_{va} = \begin{bmatrix}
\pi_{0000} \\
\pi_{0001} \\
\pi_{0011} \\
\pi_{0101} \\
\pi_{0111} \\
\pi_{1111}
\end{bmatrix} = \begin{bmatrix}
\pi_{0000}^{10} \\
\pi_{0001}^{10} \\
\pi_{0011}^{10} \\
\pi_{0101}^{10} \\
\pi_{0111}^{10} \\
\pi_{1111}^{10}
\end{bmatrix}
$$

(S5)

Using the first rows of equations S4 and S5 and the fact that $\phi_{pns} = \pi_{0000}^{10} + \pi_{0000}^{00}$, we get

$$
\phi_{pns} = \pi_{0000}^{00} \phi_{vn} + \pi_{0000}^{00} \phi_{va} = \pi_{0000}^{00}
\Rightarrow \pi_{0000}^{00} = \phi_{pns} \phi_{vn}
\Rightarrow \pi_{0000}^{00} = \phi_{pns} \phi_{va}
$$
Then since $\phi_{vns} = \pi_{0000}^{00} + \pi_{00-1}^{00}$, we have

$$\pi_{00-1}^{00} = \phi_{vns} - \phi_{pms} \phi_{vn},$$

(S6)

and since $\phi_{vas} = \pi_{0000}^{10} + \pi_{00-1}^{10}$, we have

$$\pi_{00-1}^{10} = \phi_{vas} - \phi_{pms} \phi_{va}.$$  

(S7)

From Tables 1 and 2 we get $\pi_{11}^{00} = \phi_{vns}$ and $\pi_{11}^{10} = \phi_{vas}$, and this completes the proof of statement (i). For statement (iii), we can similarly use the last rows of equations S4 and S5 to solve for $\pi_{1111}^{00}$. We have $\pi_{1111} = \pi_{1111}^{00}/\phi_{vn} = \pi_{1111}^{10}/\phi_{va} = \phi_{vaf} / \phi_{va}$ giving $\pi_{1111}^{00} = \phi_{vaf} \phi_{vn} / \phi_{va}$. Then

$$\pi_{0101}^{00} + \pi_{0111}^{00} = \pi_{01-1}^{00} - \pi_{1111}^{00} = \phi_{vn} - \phi_{vaf} \phi_{vn} / \phi_{va}.$$  

Then using the fourth and fifth rows of equations S4 and S5 we get $\pi_{0101} + \pi_{0111} = (\pi_{0101}^{00} + \pi_{0111}^{00}) / \phi_{va}$ giving

$$\pi_{0101}^{10} + \pi_{0111}^{10} = \frac{\phi_{va}}{\phi_{vn}} (\phi_{vaf} - \phi_{vaf} \phi_{vn} / \phi_{va})$$

$$= \frac{\phi_{va} \phi_{vfn} - \phi_{vaf}}{\phi_{vn}}.$$  

Thus,

$$E[Y_{1M_0}] = E[Y_{1M_1}] + \pi_{0101}^{10} + \pi_{0111}^{10}$$

$$= \phi_{vaf} + \phi_{vfn} + \frac{\phi_{va} \phi_{vfn}}{\phi_{vn}} - \phi_{vaf}$$

$$= \phi_{vfn} \left(1 + \frac{\phi_{va}}{\phi_{vn}}\right) - \phi_{vfn} \frac{\phi_{vfn}}{\phi_{vn}}.$$  

where in the last line we use $\phi_{va} + \phi_{vn} = 1$. This proves statement (iii).

To show statement (ii), that $E[Y_{0M_1}]$ is not identifiable under $M_2$, we note that $E[Y_{0M_1}] = E[Y_{0M_0}] - (\pi_{0001}^{10} + \pi_{0011}^{10})$. We have shown that $\pi_{0101}^{10} + \pi_{0111}^{10}$ are identifiable, and $\pi_{00-1}^{10}$ is identifiable; therefore, $\pi_{0001}^{10} + \pi_{0011}^{10}$ is identifiable. In order to differentiate between $\pi_{0001}^{10}$ and $\pi_{0011}^{10}$, we would need to be able to either (i) differentiate the potential outcome vectors between individuals with $Y = [0001], M = [10]$ and individuals with $Y = [0011], M = [10]$, or (ii) differentiate the potential outcome vectors between with $Y = [0001], M = [00]$ and individuals with $Y = [0011], M = [00]$, which have the same potential mediator response vectors as in (i), but different values of the potential mediator responses. From Table 2 there is no way to differentiate between individuals with $Y = [0001]$ and individuals with $Y = [0011]$, regardless of the value of $M$, and hence $E[Y_{0M_1}]$ is not identifiable, and statement (ii) is proved.

S5 Proof of Theorem 3

The conditions of Statement A can be found by writing the expectations in terms of the $\pi$ parameters (see Table 2),

$$E(Y_{1M_1}) = \pi_{11-1}^{00} + \pi_{1111}^{00} \leq E(Y_{1M_0}) = E(Y_{1M_1}) + \pi_{0101}^{10} + \pi_{0111}^{10} \leq E(Y_{0M_0}) = E(Y_{1M_0}) + \pi_{00-1}^{10} + \pi_{0011}^{10} + \pi_{0011}^{10},$$

4
where \( \pi_{0,1}^{00} = \sigma_{0101}^{00} + \sigma_{0100}^{00} + \sigma_{0111}^{00} = \phi_{enf} \). The result in Statement A is Theorem 2(iii).

Statement B immediately follows from writing

\[
\lambda_s = \frac{\log \{E(Y_{1M_1})\} - \log \{E(Y_{1M_0})\}}{\log \{E(Y_{1M_1})\} - \log \{E(Y_{0M_0})\}}.
\]

For Statement C, we first note that \( Y_{am} \perp M' \) implies that \( M_1 \perp Y_{10} \), and since \( M_0 = 0 \) for all individuals in the base model, \( Y_{1M_0} = Y_{10} \) (see also Table 1). By definition, the correlation between \( M_1 \) and \( Y_{1M_0} \) is

\[
\text{Corr} (M_1, Y_{1M_0}) = \frac{E(M_1 Y_{1M_0}) - E(M_1) E(Y_{1M_0})}{\sqrt{\text{Var}(M_1) \text{Var}(Y_{1M_0})}}
\]

where the last step uses \( Y_{1M_0} = Y_{10} \) since \( Pr[M_0 = 0] = 1 \), and

\[
E(M_1 Y_{1M_0}) - E(M_1) E(Y_{1M_0}) = \frac{Pr[Y_{10} = 1 \& M_1 = 1] Pr[M_1 = 1]}{Pr[M_1 = 1]} - E(M_1) E(Y_{10})
\]

which is a function of \( E(Y_{1M_0}) \) and identifiable parameters. Substituting \( \phi_{enf} \) for \( 1 - E(M_1) \), we get

\[\text{Corr} (M_1, Y_{1M_0}) = \frac{\left\{ E(Y_{1M_0}) - \frac{\phi_{enf}}{\phi_{en}} \right\} \phi_{en}}{\sqrt{(1 - \phi_{en}) \phi_{en} E(Y_{1M_0})} \{1 - E(Y_{1M_0})\}}\]  \hspace{1cm} (S9)

Substituting \( E[Y_{1M_0}] = \frac{\phi_{enf}}{\phi_{en}} \) gives \( \text{Corr} (M_1, Y_{1M_0}) = 0 \). From Section S3

\[\phi_{ef} = E(Y_{1M_1}) \leq E(Y_{1M_0}) \leq E(Y_{0M_0}) = \phi_{pmf}.\]

Substituting the minimum and maximum values for \( E(Y_{1M_0}) \) in terms of \( \phi \) parameters gives \( \phi_{min} \) and \( \phi_{max} \), respectively.