



**Karolinska  
Institutet**

## **5.2 Negative controls**

# "Negative controls" for detecting bias

## Idea:

choose negative controls so effect of interest would be impossible by the hypothesized mechanism

Logic discussed in *Lipschitz et al, Epi, 2010*

## Negative controls for exposure or for outcome:

Extensive DAGS in *Arnold et al. Epidemiol 2016*.

# Negative controls in laboratory assays

- Routine practice for laboratories
- For example, saline in one of the test tubes(wells)
- Other examples?

## Negative controls can be used to assess:

- Confounding bias
- Selection bias
- Measurement error bias

# General idea of a negative control

Leave out an essential ingredient (in exposure)

.... **negative exposure** (like placebo in RCT)

OR

Checking for a response that would be unexpected or impossible under the proposed hypothesised mechanism.

... **negative outcome**

## Negative control ideas used to address the controversy re flu vaccination in 2006\*

Found that after careful adjustment, lower risk of flu before vaccination  
(essential ingredient omitted: vaccine!!)

AND

Found protective effect of vaccine for hospitalization and injury (negative outcomes)

\* Jackson et al, Int Jour Epi 2006

# Negative controls for confounding bias

Commonly used to check for confounding by unmeasured lifestyle factors:

family studies to investigate genetic effects,  
(spouse as negative control)

perinatal epidemiology studies of maternal exposures:

paternal exposure

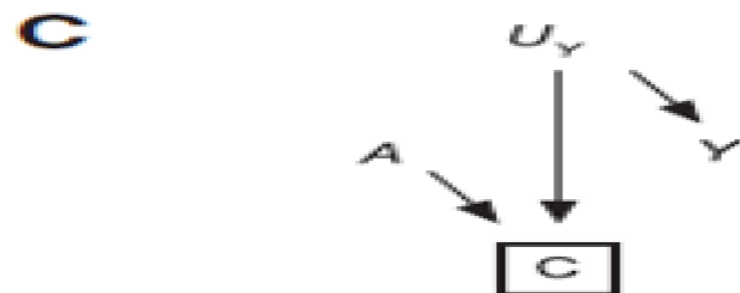
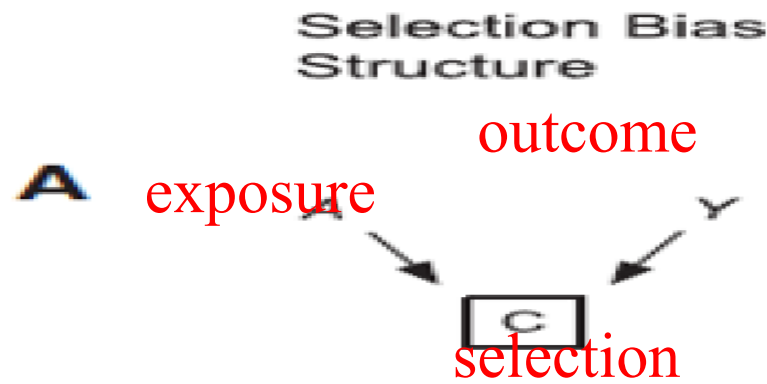
mother prior to, or after, the studied pregnancy

maternal exposure in a different pregnancy

# Negative controls for detecting selection bias



# Figure 1 from Arnold Epidemiology 2016



Bias introduced by selection that depends on

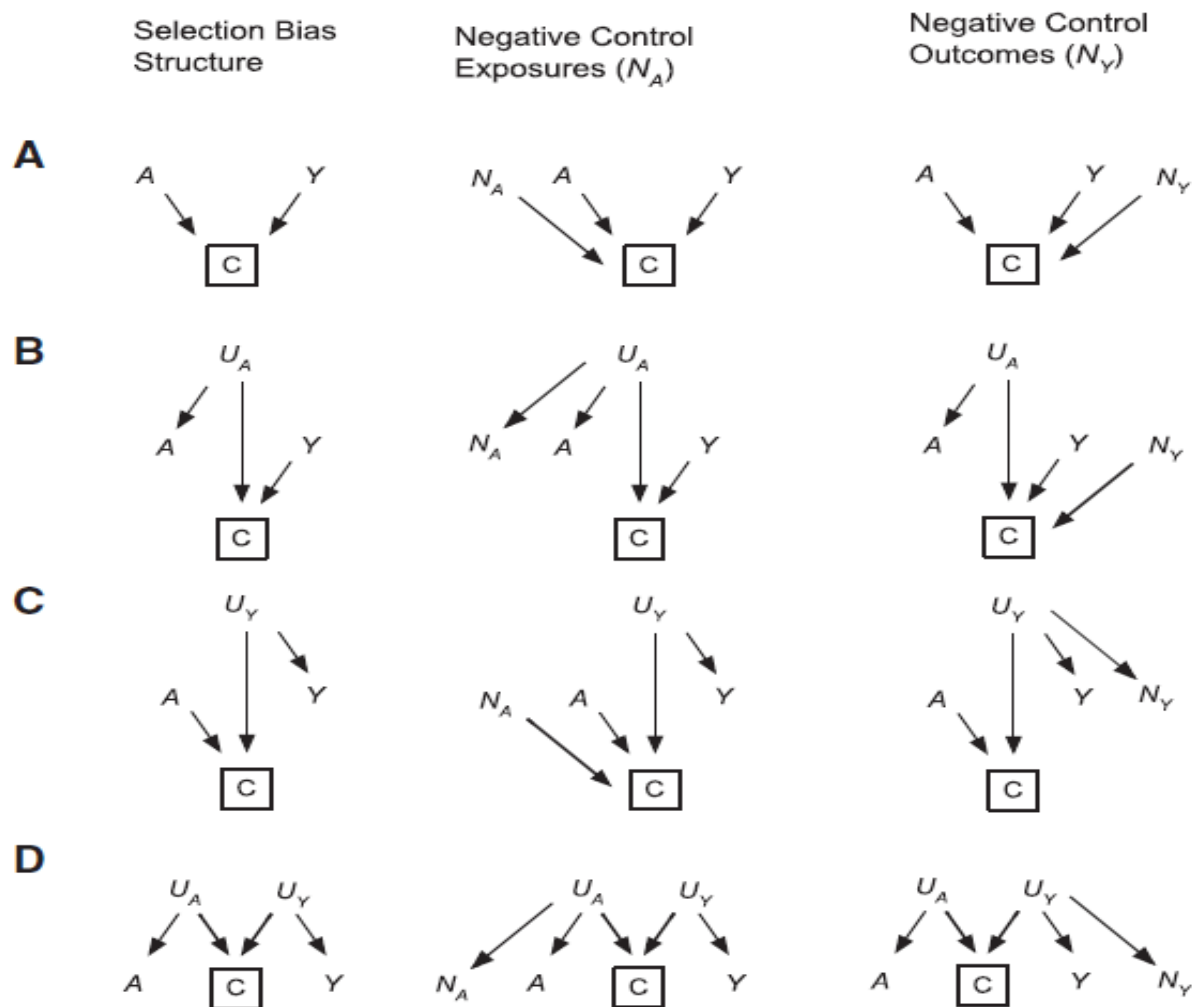
exposure and outcome

outcome and a cause of exposure

exposure and a cause of outcome

a cause of exposure and outcome

# Choice of negative controls:



**FIGURE 1.** Simplified causal diagrams of selection bias for exposure  $A$  and outcome  $Y$  along with negative control exposures ( $N_A$ ) and outcomes ( $N_Y$ ). In all four structures, selection bias results from conditioning on  $C$ , a common descendant of (A) exposure  $A$  and outcome  $Y$ , (B) cause of exposure  $U_A$  and outcome  $Y$ , (C) exposure  $A$  and cause of outcome  $U_Y$ , or (D) cause of exposure  $U_A$  and cause of outcome  $U_Y$ .

# Examples of published studies\*

(type of bias on causal diagram)

Study	Exposure	Outcome	Potential bias	Negative control
Ivers et al. Lancet Glob Health, 2015	Oral cholera vaccine	Diarrhea stool positive for cholera	Selective enrollment cases vs. Controls (1A)	Noncholera diarrhea
De Groot et al Eur J Epi 2014	Use of ACE inhibitors, statins, PPI	Community acquired pneumonia	Selective enrollment among hospitalized patients (1B)	Selective serotonin reuptake inhibitors

\* *Arnold et al. Negative Controls to Detect Selection Bias and Measurement Bias in Epidemiologic Studies. Epi Sept 2016.*

# Measurement error bias

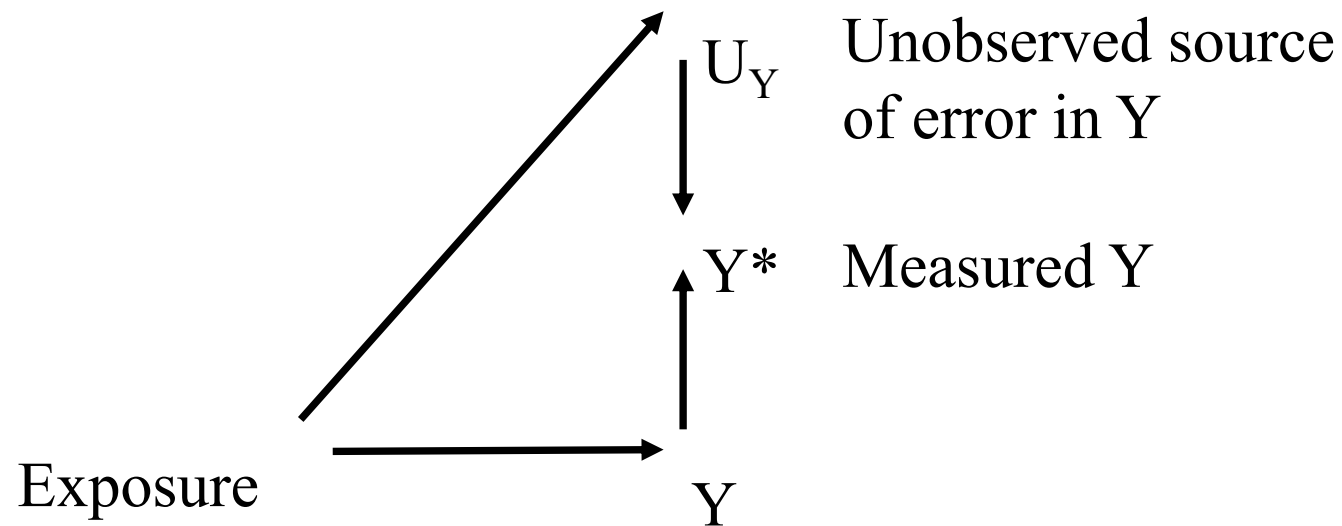
Where there are (unmeasured) sources of error in the measured value for outcome or exposure

”non-differential”: source of error in exposure not associated with outcome or vice versa

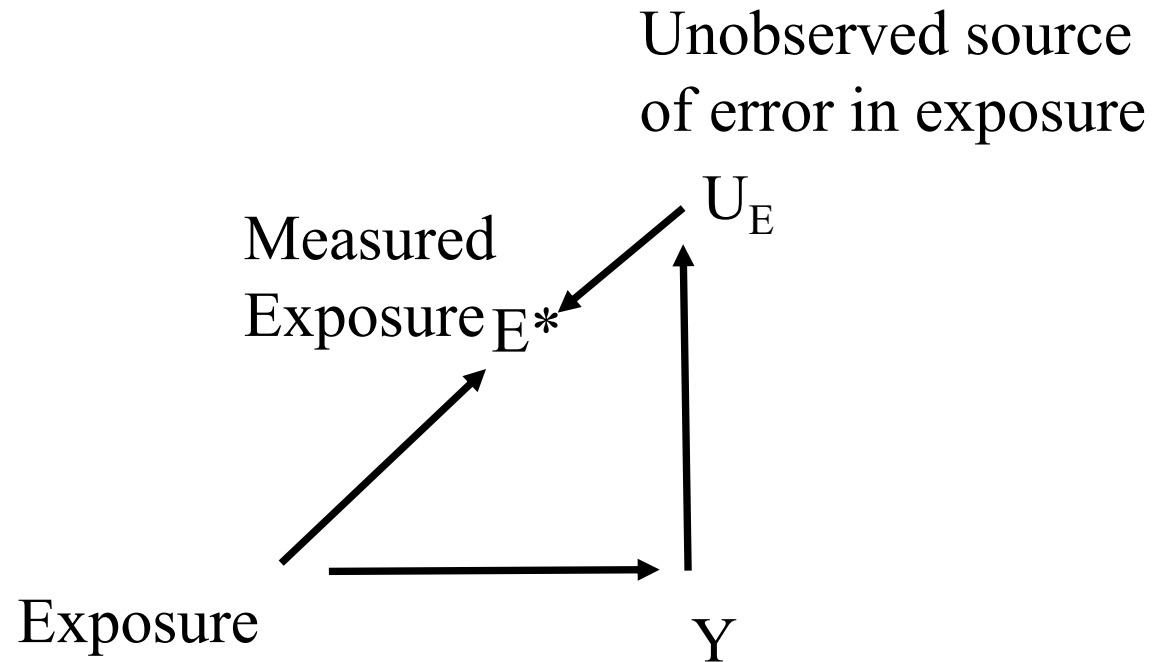
# Examples of measurement error

- Using symptoms as outcome, when a specific infection is of interest (e.g. diarrheal disease in children)
- Using prescriptions as exposure when consumption of the medication is the exposure of interest
- Using area of residence when individual's exposure to pollution (or social environment, ....) is of interest
- ... others?

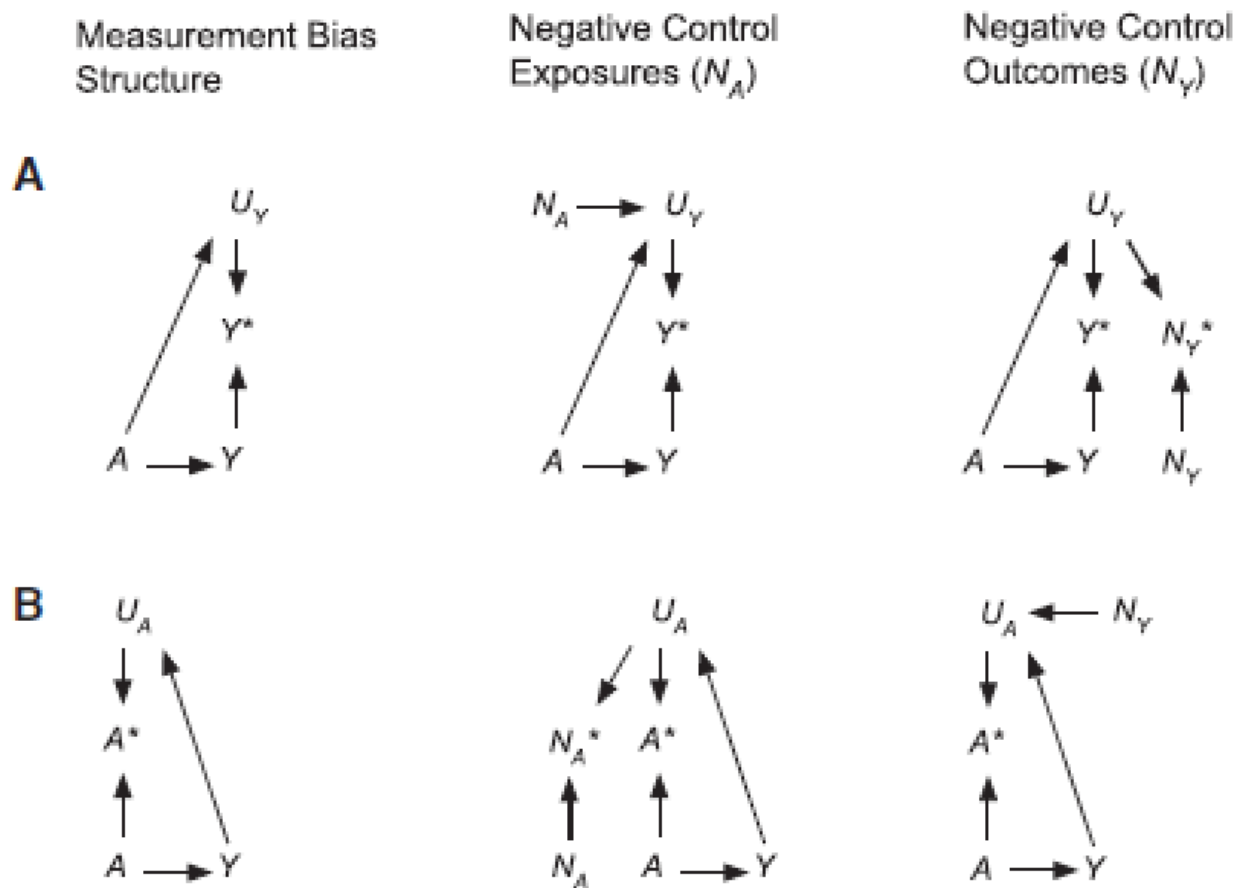
# Measurement error in outcome



# Measurement error in exposure



# Figure 2 from Arnold Epidemiology 2016





# Examples from Arnold\*

Study	Exposure	Outcome	Potential bias	Negative control
. Colford et al. Prospective cohort	Swimmer exposure to <i>Enterococcus</i> levels in water	Reported Diarrhea	Differential reporting error (2A)	<i>Enterococcus</i> levels assigned to nonswimmers
Zaadstra et al. Mult Scler, 2008	Viral infections in early childhood	Multiple sclerosis	More accurate exposure recall among cases (2B)	Broken arm, concussion, tonsillectomy

\* Arnold et al. *Negative Controls to Detect Selection Bias and Measurement Bias in Epidemiologic Studies*. Epi Sept 2016.

# Numerous example of negative controls in perinatal epidemiology



*International Journal of Epidemiology*, 2018, 587–596

doi: 10.1093/ije/dyx213

Advance Access Publication Date: 27 October 2017

Original article



---

## Methods

# Negative control exposure studies in the presence of measurement error: implications for attempted effect estimate calibration

Eleanor Sanderson,\* Corrie Macdonald-Wallis and George Davey Smith

---

# Extract from Sanderson\* paper

*International Journal of Epidemiology*, 2018, Vol. 47, No. 2

589

**Table 1.** Selected examples of studies which have used negative control exposure methods

Exposure	Negative control exposure	Outcome(s)
Maternal smoking	Paternal smoking	Offspring outcomes: Inattention/hyperactivity <sup>15,20</sup> Obesity/adiposity <sup>16,22–24</sup> Blood pressure <sup>17</sup> Gestational diabetes <sup>21</sup> ADHD symptoms <sup>19</sup> Cognitive development <sup>18</sup> Offspring psychotic symptoms <sup>46</sup> Offspring vascular function <sup>54</sup>
Maternal psychosocial stress	Paternal psychosocial stress	Offspring respiratory outcomes <sup>39</sup> Offspring psychotic symptoms <sup>46</sup> Offspring ADHD symptoms <sup>40</sup>
Maternal smoking during pregnancy	Maternal smoking after pregnancy	Offspring BMI/adiposity <sup>26–33</sup> Offspring cognitive and psychomotor development <sup>55</sup>
Maternal alcohol consumption during pregnancy	Maternal alcohol consumption before pregnancy	Risk of schizophrenia in the offspring <sup>56</sup>
Maternal BMI/obesity	Paternal BMI	Autism spectrum disorders <sup>37</sup> Language development delays <sup>38</sup>
Length of pre-birth inter-pregnancy interval	Length of post-birth inter-pregnancy interval	
Folic acid supplements in pregnancy	Other supplements in pregnancy	

*Sanderson et al Int J Epi 2018 Negative control exposure studies in the presence of measurement error.*

# Negative self-control (same principles)

Recent example:

Northrup et al.

Inference of naturally acquired immunity using a self-matched negative-control design.

*Epidemiology*, 32:168–178, 2021.

**Outcome:** rotavirus-positive diarrhoea

**Exposure:** previous rotavirus infection

**Negative control outcome:** non-rotavirus diarrhoea

# Negative self-control exposure

Many of earlier examples from perinatal epidemiology were self-control exposures  
(same woman, different pregnancy/time)

## Recent Example:

Magen-Molho et al. Air pollution and autism spectrum disorder in israel: A negative control analysis. Epidemiology, 2021.  
(case-control study of ASD)

Exposure: air pollution in the pre-pregnancy, pregnancy and postnatal periods

**self-control negative exposure:** pollution levels measured 28-36 months after delivery.

# Negative self-control exposure vs. exposure-crossover design?

- Standard design
- Different purpose
- Additional analysis

# For your own research area:

identify a study where:

- There is potential bias due to the sampling design from the underlying cohort
- A suitable “negative control” group is available for assessing such bias.