# Test-negative design: An efficient method to assess typhoid conjugate vaccine effectiveness

**Matt Laurens, MD, MPH**
Center for Vaccine Development and Global Health
University of Maryland School of Medicine

**On behalf of the TyVAC Malawi Team**

**For more details see Lancet Global Health 2023; 11:e136-44.**


TyVAC Typhoid Vaccine Acceleration Consortium
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# Background

- As typhoid conjugate vaccines (TCVs) are introduced in low-income and middle-income countries to prevent typhoid illness in children via national immunisation programmes, post-introduction monitoring is important to understand how they perform under real-world conditions.
- **Test-negative design** (TND) has previously been efficiently used to evaluate post-introduction vaccine effectiveness for other vaccines (e.g., influenza, rotavirus, and COVID-19)
  - Cases and controls selected based on diagnostic testing
  - Reduce confounding due to health care seeking behavior
- TND has not been formally evaluated for TCVs
  - Low disease incidence
  - Low blood culture sensitivity
  - Vaccine misclassification



Thompson et al. N Engl J Med 2021; 385: 1355–71.
Dean et al. N Engl J Med 2021; 385: 1431–33.
Chua et al. Epidemiology 2020; 31: 43–64.

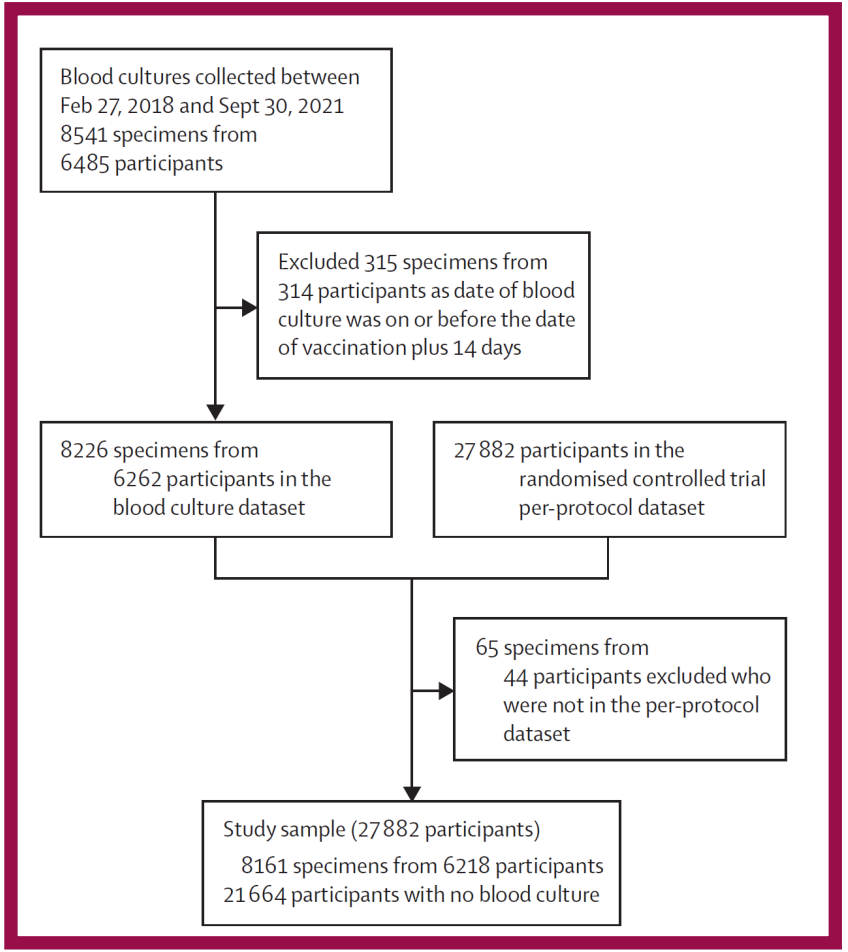
# Objective

- Evaluated the appropriateness of the TND as a method to assess typhoid Vi polysaccharide-tetanus toxoid conjugate vaccine (Vi-TT), using blood culture surveillance data from a RCT of Vi-TT in Malawi
  - Verify the core assumption of the TND: Vi-TT has no effect on nontyphoid fever
  - Compare vaccine effectiveness derived by TND with RCT efficacy results
  - Assess the effect of vaccine misclassification, blood culture *S.* Typhi positivity rate, and blood culture sensitivity to detect typhoid fever on vaccine effectiveness estimation in TND



# Study participants

- Malawi RCT per-protocol sample: 27,882 Malawian children in Blantyre aged 9 months to 12 years randomly assigned (1:1) to receive a single dose of Vi-TT or MenA from 2/21/2018 to 9/27/2018
- **TND sample**: Had at least one blood culture sample collection at least 14 days after vaccination
- Blood culture results
  - Test-positive: Positive for *S.* Typhi
  - Test-negative
    - Negative for *S.* Typhi but positive for another pathogen
    - Negative for any pathogen
    - Positive for contaminants






Patel et al. N Engl J Med 2021; 385: 1104–15.

# TND approaches

- Because some participants had more than one blood culture specimen collected, three TND samples were selected:
  - **Method A**: *Participant-based* analysis *without censoring* for *S.* Typhi
    - Cases: participants with an episode of *S.* Typhi
    - Controls: participants with an episode of non- *S.* Typhi illness, *including* those who may have tested positive for *S.* Typhi at another time point
  - **Method B**: *Participant-based* analysis *with censoring* for *S.* Typhi
    - Cases: same as Method A
    - Controls: participants with an episode of non- *S.* Typhi illness, *excluding* those who ever had a test that was *S.* Typhi positive during the study period
  - **Method C**: *Specimen-based* analysis:
    - Cases: *S.* Typhi positive specimens
    - Controls: *S.* Typhi negative specimens
- Vaccine Effectiveness = (1-Odds Ratio [OR]) × 100%, where OR = relative odds of Vi-TT vaccination in cases vs. controls



De Serres et al. Euro Surveill 2013; 18: 20585.

# Influence of vaccine against blood culture-confirmed *S.* Typhi and non- *S.* Typhi illnesses

| | Participant-based analysis | | | | | Specimen-based analysis* | |
|---|---|---|---|---|---|---|---|
| | Total | Test-positive for typhoid | Test-negative with no censoring† | Test-negative with censoring‡ | No blood culture | Test-positive specimens | Test-negative specimens |
| Vi-TT | 13 945 | 16/97 (16·5%) | 3087/6166 (50·1%) | 3077/6121 (50·3%) | 10 852 | 17/101 (16·8%) | 4092/8060 (50·8%) |
| MenA | 13 937 | 81/97 (83·5%) | 3079/6166 (49·9%) | 3044/6121 (49·7%) | 10 812 | 84/101 (83·2%) | 3968/8060 (49·2%) |
| VE against typhoid§ (95% CI), p value | .. | 80·4% (66·4 to 88·5)¶, p<0·0001 | 80·3% (66·2 to 88·5)‖, p<0·0001 | 80·5% (66·5 to 88·6)**, p<0·0001 | .. | .. | 80·4% (66·9 to 88·4)††, p<0·0001 |
| VE against non-typhoid‡‡ (95% CI), p value | .. | .. | −0·4% (−4·9 to 3·9)‖, p=0·87 | −1·0% (−5·6 to 3·3)**, p=0·65 | .. | .. | −2·5% (−6·4 to 1·3)††, p=0·20 |

RCT vaccine efficacy

Data are n or n/N (%), unless otherwise indicated. MenA=meningococcal capsular group A conjugate vaccine. VE=vaccine efficacy in the randomised controlled trial or vaccine effectiveness in the test-negative design. Vi-TT=typhoid Vi polysaccharide-tetanus toxoid-conjugate vaccine. *Cases are typhoid-positive specimens and controls are typhoid-negative specimens. †Controls include participants with an episode of non-typhoid illness, without censoring for typhoid (ie, controls might have tested positive for typhoid at another timepoint). ‡Controls include participants with an episode of non-typhoid illness, with censoring for typhoid (ie, controls exclude participants who ever had a test that was typhoid-positive during the study period). §VE=(1−odds ratio)×100%, using the test-negative design sample only. ¶VE=(1−incidence rate ratio)×100%. ‖Test-negative design A. **Test-negative design B. ††Test-negative design C. ‡‡VE=(1−risk ratio)×100%, using the whole randomised controlled trial.

For all three methods

- TND vaccine effectiveness estimates were almost identical to the RCT vaccine efficacy estimate
- Receipt of Vi-TT did not affect the risk of non-typhoid fever (core assumption of the TND)

# Effect of overall vaccine misclassification rate (p1 + p2) on vaccine effectiveness estimation by TND specimen-based analysis

| | Cases vaccinated by Vi-TT | Controls vaccinated by Vi-TT | VE against typhoid* (95% CI) |
|---|---|---|---|
| 0% vaccine misclassification—gold standard | 17/101 (16·8%) | 4092/8060 (50·8%) | 80·4% (66·9 to 88·4) |
| 5% vaccine misclassification | | | |
| Misclassifying vaccinated as unvaccinated, both groups† | 12/101 (11·9%) | 3689/8060 (45·8%) | 84·0% (70·8 to 91·3) |
| Differential misclassification, lowest possible VE‡ | 22/101 (21·8%) | 3689/8060 (45·8%) | 67·0% (47·0 to 79·5) |
| Differential misclassification, highest possible VE§ | 12/101 (11·9%) | 4495/8060 (55·8%) | 89·3% (80·4 to 94·2) |
| 10% vaccine misclassification | | | |
| Misclassifying vaccinated as unvaccinated, both groups† | 7/101 (6·9%) | 3286/8060 (40·8%) | 89·2% (76·7 to 95·0) |
| Differential misclassification, lowest possible VE‡ | 27/101 (26·7%) | 3286/8060 (40·8%) | 47·0% (17·5 to 66·0) |
| Differential misclassification, highest possible VE§ | 7/101 (6·9%) | 4898/8060 (60·8%) | 95·2% (89·6 to 97·8) |
| 15% vaccine misclassification | | | |
| Misclassifying vaccinated as unvaccinated, both groups† | 2/101 (2·0%) | 2883/8060 (35·8%) | 96·4% (85·3 to 99·1) |
| Differential misclassification, lowest possible VE‡ | 32/101 (31·7%) | 2883/8060 (35·8%) | 16·9% (–27·0 to 45·4) |
| Differential misclassification, highest possible VE§ | 2/101 (2·0%) | 5301/8060 (65·8%) | 98·9% (95·7 to 99·7) |

p1=probability of misclassifying vaccinated as unvaccinated.
p2=probability of misclassifying unvaccinated as vaccinated.

†Only misclassifying vaccinated as unvaccinated for both cases and controls due to the loss of vaccination cards, that is, p1 + p2=p1, hence p2=0 among both cases and controls.

‡p1=0 among cases (misclassifying unvaccinated as vaccinated among cases) and p2=0 among controls (misclassifying vaccinated as unvaccinated among controls), resulting in the lowest possible VE.

§p2=0 among cases (misclassifying vaccinated as unvaccinated among cases) and p1=0 among controls (misclassifying unvaccinated as vaccinated among controls), resulting in the highest possible VE.

VE estimations were not reliable when misclassification of vaccination status exceeded 10%.

# Effect of blood culture test sensitivity on vaccine effectiveness estimation by TND specimen-based analysis, stratified by blood culture positivity rate

| | Adjusted blood culture typhoid-positive* | Adjusted cases vaccinated* | Adjusted controls vaccinated* | Adjusted VE against typhoid*† (95% CI) |
|---|---|---|---|---|
| **Observed blood culture typhoid positivity 101/8161 (1·2%)** | | | | |
| 100% BCS | 101/8161 (1·2%) | 17/101 (16·8%) | 4092/8060 (50·8%) | 80·4% (66·9–88·4) |
| 80% BCS | 126/8161 (1·5%) | 21/126 (16·7%) | 4088/8035 (50·9%) | 80·7% (69·1–87·9) |
| 50% BCS | 202/8161 (2·5%) | 34/202 (16·8%) | 4075/7959 (51·2%) | 80·7% (72·0–86·7) |
| 30% BCS | 337/8161 (4·1%) | 57/337 (16·9%) | 4052/7824 (51·8%) | 81·0% (74·7–85·8) |
| **Observed blood culture typhoid positivity 408/8161 (5·0%)** | | | | |
| 100% BCS | 408/8161 (5·0%) | 69/408 (16·9%) | 4040/7753 (52·1%) | 81·3% (75·7–85·6) |
| 80% BCS | 510/8161 (6·2%) | 86/510 (16·9%) | 4023/7651 (52·6%) | 81·7% (76·8–85·6) |
| 50% BCS | 816/8161 (10·0%) | 137/816 (16·8%) | 3972/7345 (54·1%) | 82·9% (79·3–85·8) |
| 30% BCS | 1360/8161 (16·7%) | 228/1360 (16·8%) | 3881/6801 (57·1%) | 84·8% (82·4–87·0) |
| **Observed blood culture typhoid positivity 816/8161 (10·0%)** | | | | |
| 100% BCS | 816/8161 (10·0%) | 137/816 (16·8%) | 3972/7345 (54·1%) | 82·9% (79·3–85·8) |
| 80% BCS | 1020/8161 (12·5%) | 171/1020 (16·8%) | 3938/7141 (55·1%) | 83·6% (80·6–86·2) |
| 50% BCS | 1632/8161 (20·0%) | 274/1632 (16·8%) | 3835/6529 (58·7%) | 85·8% (83·7–87·7) |
| 30% BCS | 2720/8161 (33·3%) | 457/2720 (16·8%) | 3652/5441 (67·1%) | 90·1% (88·9–91·2) |

When the blood culture positivity rate is low, blood culture sensitivity (BCS) is less critical for VE point estimation but influences the precision (width of 95% CI) of the estimation.

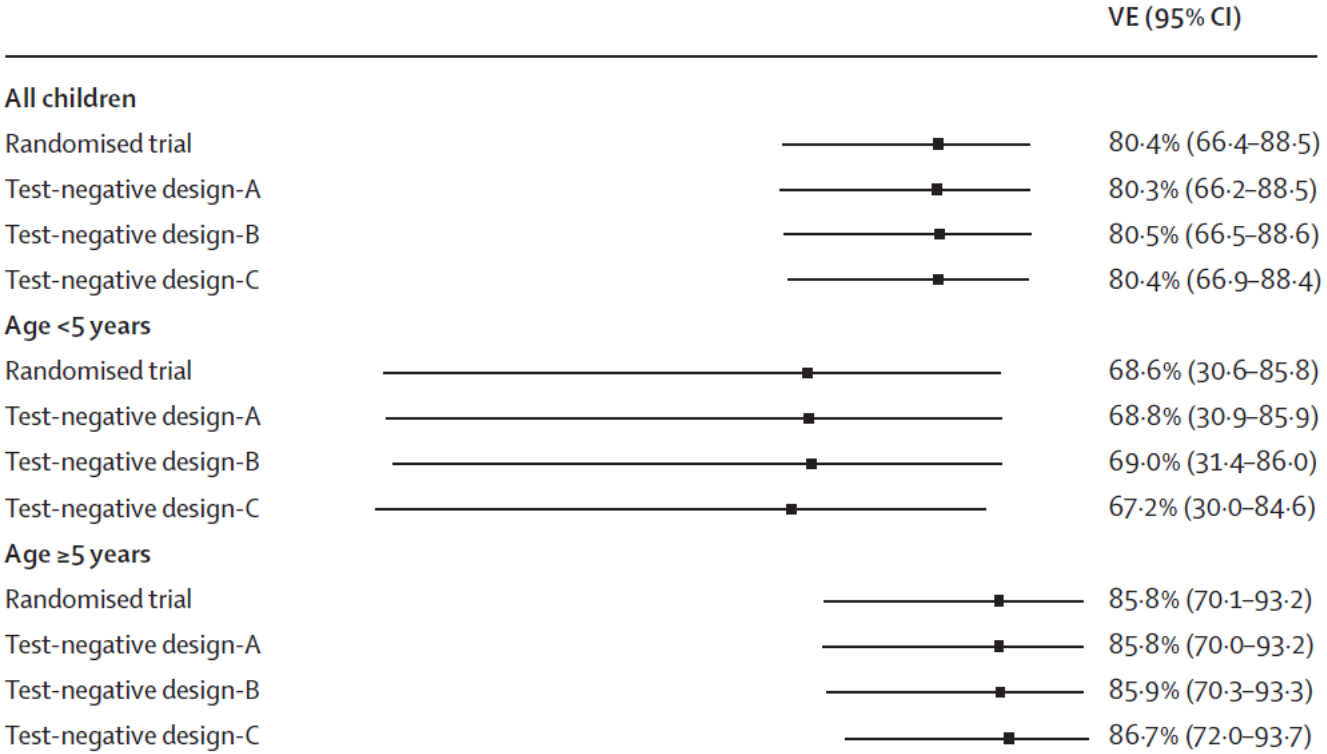
- When BCS decreased from 100% to 30%, the adjusted point estimates of VE increased only slightly, but the width of the 95% CIs became much narrower.

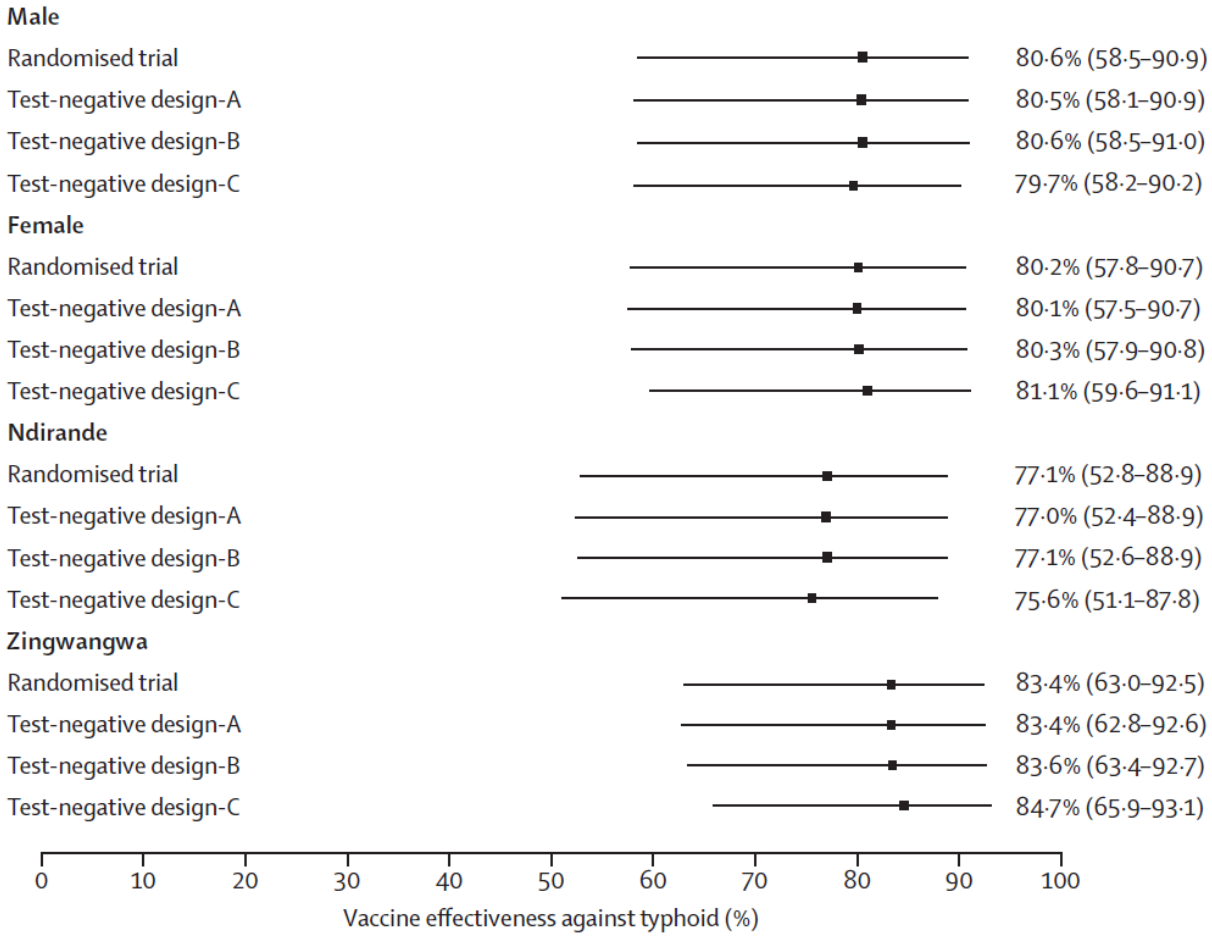
# Conclusions

- This study validated the TND core assumption that TCV has no effect on febrile illnesses that are not caused by *S.* Typhi.
- This study showed that, even with suboptimal blood culture sensitivity, TND can produce accurate and precise estimates of vaccine effectiveness compared with RCT vaccine efficacy results in a Malawian pediatric population when
  - The misclassification of vaccination status is <10%
  - The proportion of blood cultures that are typhoid positive is <10%
- These results suggest that TND is well-suited for post-introduction assessments of TCV effectiveness in low-income settings due to its efficiency, convenience, and low cost.

# Thanks to the MLW TyVAC team

# Effect of vaccine on blood culture-confirmed typhoid overall and by subgroups




Male
Randomised trial
Test-negative design-A
Test-negative design-B
Test-negative design-C
Female
Randomised trial
Test-negative design-A
Test-negative design-B
Test-negative design-C
Ndirande
Randomised trial
Test-negative design-A
Test-negative design-B
Test-negative design-C
Zingwangwa
Randomised trial
Test-negative design-A
Test-negative design-B
Test-negative design-C
80·6% (58·5–90·9)
80·5% (58·1–90·9)
80·6% (58·5–91·0)
79·7% (58·2–90·2)
80·2% (57·8–90·7)
80·1% (57·5–90·7)
80·3% (57·9–90·8)
81·1% (59·6–91·1)
77·1% (52·8–88·9)
77·0% (52·4–88·9)
77·1% (52·6–88·9)
75·6% (51·1–87·8)
83·4% (63·0–92·5)
83·4% (62·8–92·6)
83·6% (63·4–92·7)
84·7% (65·9–93·1)
0
10
20
30
40
50
60
70
80
90
100
Vaccine effectiveness against typhoid (%)