Adapted Newcastle–Ottawa Risk-of-Bias Tool

Domains, signalling questions, and scoring guide

#	Domain	Signalling questions (answer Yes / Partial / No)	Scoring rule
1	Case selection / Sampling frame	1. Was the source population clearly enumerated (birth-cohort, census list, HDSS)? 2. Were all eligible cases recruited consecutively or randomly?	Low = "Yes" to both. Moderate = "Yes" to Q1 but "Partial"/"No" to Q2. High = "No" to Q1 (e.g., convenience or special-subgroup enrolment).
2	Control representativeness	1. Were controls drawn from the same geographic catchment as cases? 2. For susceptibility outcomes, were controls parasite-negative? 3. For severity outcomes, were controls community-based rather than hospital out-patients?	Low = "Yes" to all applicable questions. Moderate = "Yes" to Q1 but at least one "Partial". High = "No" to Q1 or controls are hospitalised with malaria.
3	Exposure ascertainment (genotyping)	1. Was genotyping done by validated PCR (or sequencing) methods? 2. Were ≥ 5 % of samples duplicated with ≥ 99 % concordance? 3. Were < 2 % genotypes missing?	Low = "Yes" to all three. Moderate = One "Partial". High = Any "No".
4	Confounding control	1. Did analyses adjust for age and sex? 2. Did they adjust for bed-net use or socio-economic status? 3. Did they adjust for at least one other RBC polymorphism (e.g., α-thalassaemia)?	Low = "Yes" to all three. Moderate = "Yes" to Q1 but "Partial/No" to Q2 or Q3. High = "No" to Q1 or unadjusted analyses.

5	Precision	1. Were ≥ 30 individuals carrying the dual-variant combination? 2. Was the 95 % CI width < ±30 % of the point estimate?	Low = "Yes" to either Q1 or Q2. Moderate = 10–29 dual carriers *and* CI width 30–60 %. High = < 10 dual carriers *or* CI width > 60 % or infinite OR.
6	Outcome measurement	1. Were WHO malaria criteria used? 2. Were laboratory indices measured with accredited equipment? 3. Was follow-up ≥ 90 % complete (for cohort studies)?	Low = "Yes" to all applicable questions. Moderate = One "Partial". High = Any "No".
7	Missing data / Exclusions	1. Were < 5 % of participants excluded after enrolment? 2. Were exclusions balanced across genotype groups? 3. Were reasons for exclusion stated?	Low = "Yes" to all. Moderate = 5–10 % missing *or* unclear balance. High = > 10 % missing *or* selective exclusions.

How to use the tool

1. Answer each signalling question for a study with *Yes*, *Partial* (some but not all criteria met), or *No*.
2. Assign a domain rating using the rule in the right-hand column.
3. Overall study risk
    - Serious (high) = ≥ 2 domains rated High or one High plus a High Precision judgement.
    - Moderate = one High or three Moderate domains.
    - Low = all domains Low or one Moderate.
4. GRADE downgrades: –1 level for overall Moderate risk; –2 levels for overall Serious risk.