Title	Serological Protection Following Routine Two-Dose Measles Vaccination Among Children Aged 1–5 Years in LMICs
Registration Platform	This protocol will be registered in the International Prospective Register of Systematic Reviews (PROSPERO). Any important amendments made after registration will be documented with date, rationale, and description of changes.
Amendments	Any protocol amendments will be documented with date, rationale, and description
Background and Rationale	Measles remains a major cause of vaccine-preventable morbidity and mortality, particularly in low- and middle-income countries (LMICs). Although administrative coverage of measles-containing vaccine (MCV) has improved, outbreaks and immunity gaps persist. Coverage indicators measure program performance but do not directly measure population immunity. Serological evaluation (measles-specific IgG) provides a biological indicator of protection and can reveal immunity gaps that may arise due to factors such as delayed vaccination, missed opportunities, cold-chain challenges, nutritional deficits, or immune vulnerability. Existing evidence is fragmented across settings and laboratory methods, and there is limited synthesis focused specifically on routine two-dose measles vaccination among children aged 1–5 years in LMICs. A pooled estimate of serological protection and an evaluation of determinants can support measles elimination strategies and strengthen routine immunization systems.
Objectives	**Primary objective**
	To estimate the **pooled serological protection rate** (measles-specific IgG seroprevalence meeting protective thresholds) among **children aged 1–5 years** in **LMICs** following **routine two-dose** measles-containing vaccination.
	Secondary objectives
	1. To identify **biological factors** associated with reduced serological protection (e.g., undernutrition, HIV exposure/infection, anemia, immune vulnerability).
	2. To evaluate **programmatic determinants** of reduced seroprotection (e.g., delayed vaccination, missed opportunities, service delivery platform, stock-outs, outreach delivery, health worker training, QI approaches such as RED-QI where reported).
	3. To assess **sociodemographic determinants** (e.g., urban/rural setting, maternal education, socioeconomic status where reported).
	4. To quantify heterogeneity and explore sources of variability across studies (assay type, protection cutoff, country/region, study setting).
	5. To conduct sensitivity analyses including wider pediatric age ranges (when 1–5 data are not extractable) and mixed routine/campaign contexts (where appropriate).
Review Question (PICO)	
Population:	Children aged 1–5 years in LMICs who have received **two doses** of measles-containing vaccine through routine immunization services.

Exposure:	Routine two-dose measles vaccination delivered within health systems (programmatic context).
Comparator:	Differences across settings/subgroups (e.g., facility vs outreach delivery, higher vs lower program performance, differing biological/sociodemographic strata) where reported.
Outcome:	Serological protection against measles (protective measles-specific IgG antibody levels). Secondary outcome: failure to achieve seroprotection
Methods	
Reporting standard	The protocol follows **PRISMA-P** principles, and the review will be reported according to **PRISMA 2020**. Meta-analysis methods will follow the Cochrane Handbook where applicable.
Eligibility Criteria	
	Inclusion criteria
	1. Population: Children aged **1–5 years**, residing in LMICs (World Bank classification at time of study or closest available).
	Studies including broader ages will be included if **extractable subgroup data** for 1–5 years are available.
	2. Exposure: Two doses of measles-containing vaccine delivered **routinely** through national immunization programs (EPI schedule).
	3. Outcomes: Laboratory-measured measles serology with defined thresholds for seroprotection (e.g., ELISA, PRNT; protective IgG cutoff as defined by authors).
	4. Study types: Cross-sectional serosurveys, cohort studies, immunogenicity studies, and randomized trials reporting relevant serological outcomes post-two-dose routine vaccination.
	5. Setting: Community or health-facility settings in LMICs.
	Exclusion criteria (primary analysis)
	Single-dose measles vaccination only.
	Campaign-only vaccination populations where routine two-dose exposure cannot be separated.
	Studies without measurable serological outcomes for measles.
	Case reports/very small case series (<10), editorials, commentaries, narrative reviews, protocols, modeling-only studies.
	High-income country-only studies.
	Sensitivity inclusion: Studies in older children (e.g., 6–18 years) may be included in sensitivity analyses if they inform waning/durability but will not be merged into the primary 1–5 pooled estimate.
Information Sources	Databases will include:
	PubMed/MEDLINE
	Embase

	Scopus
	Web of Science
	Cochrane Library
	Additional methods:
	Hand-searching reference lists of included studies
	Citation tracking of key included papers
Search Strategy	A comprehensive search strategy will combine terms related to:
	Measles (measles virus)
	Vaccination / measles-containing vaccine / MMR / MCV
	Two-dose schedules / second dose
	Serology / IgG / seroprevalence / antibody / immunity / neutralizing antibodies
	Children / preschool
	LMICs (and country names where needed)
	Search strategies will be adapted per database using MeSH/Emtree and keywords. The full strategy will be included as an appendix in the final manuscript.
Study Selection	1. Titles/abstracts will be screened independently by two reviewers.
	2. Full texts of potentially eligible studies will be reviewed independently.
	3. Disagreements will be resolved by discussion; if unresolved, a third reviewer will arbitrate.
	4. A PRISMA flow diagram will document inclusion/exclusion at each stage.
Data Extraction	**Extraction process**
	Two reviewers will extract data independently using a piloted extraction sheet.
	Discrepancies will be resolved by consensus.
	When data are unclear, authors will be contacted (if feasible).
	Data items (core domains)
	A) Study information
	Author, year, country, setting, design, sampling, study period, sample size, funding, ethics
	B) Population data
	Age range, mean/median age, sex distribution, inclusion/exclusion criteria, special populations (HIV-exposed, undernourished)
	C) Vaccination data (your standard fields)
	Dose_1_Age_Months
	Dose_2_Age_Months

	Two_Dose_Confirmed
	Verification_Method
	Routine_or_Campaign
	Time_Since_Dose2_Months
	Vaccine_Type
	Cold_Chain_Issue_Reported
	D) Serology outcomes
	Assay type (ELISA/PRNT/etc.)
	Definition of protective threshold
	Seropositive n / total N
	Antibody titers if available
	Failure to seroprotect (n/N)
	E) Biological factors
	Nutritional status (stunting, wasting, undernutrition)
	HIV status/exposure, anemia, comorbidities
	Other immune vulnerability markers (where reported)
	F) Programmatic factors (your standard fields)
	Delayed_Vaccination_%
	Missed_Opportunities_%
	Stockout_Reported (Yes/No)
	Outreach_Service (Yes/No)
	Facility_Based (%)
	Health_Worker_Training (Yes/No)
	RED_QI_Intervention (Yes/No)
	G) Sociodemographic factors
	Urban/rural, maternal education, SES/wealth, access indicators, region (if available)
Risk of Bias Assessment	For observational (cross-sectional/cohort): **Newcastle–Ottawa Scale (NOS)** adapted for seroprevalence studies.
	For randomized trials: **Cochrane Risk of Bias 2 (RoB 2)**.
	Two reviewers will assess risk of bias independently with consensus resolution.
	Risk of bias will be incorporated in sensitivity analyses (excluding high-risk studies).
Effect Measures	**Primary effect**
	Proportion seroprotected (seropositive / total tested) after routine two-dose vaccination.

	Transformation
	Because proportions can be near 0 or 1, meta-analysis will use a variance-stabilizing transformation (e.g., **logit** or **Freeman–Tukey double arcsine**) depending on distribution and software.
Data Synthesis and Meta-analysis	**Primary synthesis**
	A **random-effects meta-analysis** will be conducted to estimate pooled seroprotection.
	Heterogeneity will be evaluated using:
	I^2 statistic
	τ^2 (between-study variance)
	Visual inspection of forest plots
	Subgroup analyses (planned)
	WHO region / geographic region
	Study setting: community vs facility vs mixed
	Laboratory assay type: ELISA vs PRNT vs others
	Seroprotection cutoff categories (if varied)
	Time since second dose (e.g., ≤12 months vs >12 months, or as reported)
	Risk of bias (low/moderate vs high)
	Meta-regression (if sufficient studies)
	If ≥10 studies per covariate:
	Explore association between seroprotection and:
	delayed vaccination,
	outreach delivery,
	undernutrition prevalence,
	HIV exposure/infection prevalence,
	time since dose 2,
	assay type.
Sensitivity analyses	Exclude high risk of bias studies
	Routine-only vs mixed routine/campaign contexts
	Restrict to studies with verified vaccination records (cards/registry)
	Restrict to the strict 1–5-year age group only (excluding wider ages)
Publication Bias	If ≥10 studies:
	Funnel plots
	Egger's test (or comparable test appropriate for proportions)

Certainty of Evidence	Overall certainty will be assessed using **GRADE**, adapted for prevalence/observational evidence, considering:
	risk of bias,
	inconsistency,
	indirectness,
	imprecision,
	publication bias.
Ethics and Dissemination	Ethical approval is **not required** because the review uses published data only. Findings will be disseminated through:
	peer-reviewed publication,
	conference presentations,
	policy briefs (optional) for immunization program stakeholders.
Timeline (suggested)	Screening and selection: 2–3weeks
	Data extraction & RoB: 1–3 weeks
	Meta-analysis & writing: 2–3 weeks
Author Contributions (template)	**Imtiaz Hussain, Haider Abbas, Ahmed Khan, Sara Qurban and Yusra Altaf:** Conceptualization, protocol drafting, screening, data extraction, analysis, manuscript writing.
	Co-authors/supervisors: Methodology review, arbitration, interpretation, critical revisions.
Conflicts of Interest	All authors will declare any potential conflicts of interest. No commercial funding will influence study design, analysis, or reporting.
Data Management and Software	Screening: Rayyan
	Data extraction: Excel
	Meta-analysis: RaVmen/R
	Figures: PRISMA flow diagram + forest plots