SOP: IgG IFA for *Rickettsia typhi* (typhus)

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1. Purpose

To outline the standardized procedure for the detection of **IgG antibodies against *Rickettsia typhi* in human serum using the Indirect Immunofluorescent Assay (IFA)**, following the manufacturer's instructions and as adapted to the ZAFI project.

2. Scope

This SOP applies to all laboratory personnel performing and interpreting the results of the *Rickettsia typhi* IFA test antibodies at ZAFI project laboratories in Uganda, Kenya, and Ethiopia.

3. Definitions

- **IFA:** Indirect Immunofluorescent Assay
- **IgG:** Immunoglobulin G
- **PBS:** Phosphate Buffered Saline
- **QC:** Quality Control
- **FITC:** Fluorescein Isothiocyanate

4. Responsibilities

Role	Responsibility
Laboratory technician	Perform laboratory testing according to this SOP; handle and process samples appropriately; adhere to biosafety and quality control procedures; document results accurately in logbooks and KOBO tools; report deviations or issues to the laboratory lead/supervisor.
Laboratory lead/supervisor	Oversee laboratory testing; ensure staff competency and adherence to quality and biosafety standards; review and validate results; escalate issues as appropriate.
Site PI	Ensure relevant staff are trained on this SOP.

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5. Materials and Equipment

Item	
Supplied in the Kit:	**Additional Materials (not supplied):**
Rickettsia typhi IFA slides (10 slides × 10 wells)	Calibrated micropipettes and sterile tips
PBS powder (pH 7.2)	Humid incubation chamber
Positive control serum (IgG)	Thermostatically controlled incubator (37 °C)
Negative control serum	Fluorescence microscope (400× magnification) with appropriate filters
FITC-labelled anti-human IgG conjugate	Distilled or deionized water
Mounting medium (buffered glycerol)	Coverslips (24 × 60 mm)
	Timer
	PPE: lab coat, gloves, eye protection

6. Safety Precautions

- Treat all human specimens as potentially infectious
- Wear appropriate PPE at all times
- Avoid contact with reagents containing sodium azide and Evans blue
- Dispose of waste according to the SOP on Biohazard Waste Disposal
- Slides contain inactivated antigen but must still be handled with care

7. Procedure

7.1 Preparation

a) Bring all samples and reagents to room temperature before use and allow the slides to reach room temperature before opening.

b) Reconstitute PBS by dissolving the contents of one vial in 1 litre of distilled water. Mix thoroughly until fully dissolved
   - Store reconstituted PBS at **2–8 °C** (maximum 4 months; never beyond expiry date)

7.2 Sample Dilution

Initial screening is performed on 1/40 and 1/80 dilutions:

a) Prepare **1/40 dilution** of sample serum by adding 10 µL sample + 390 µL PBS

b) Prepare **1/80 dilution** by two-fold dilution of 1/40 dilution using 50ul PBS and 50ul 1/40dilution from above.

NOTE: DO NOT DILUTE CONTROL SERA (VIRCELL RICKETTSIA TYPHI IgG Positive Control and VIRCELL RICKETTSIA TYPHI NEGATIVE CONTROL)

7.3 Slide Preparation and First Incubation

a) Apply **20 µL** of diluted samples (1/40 and 1/80) into two slide wells
b) Add **20 µL** of positive and negative controls to separate wells
c) Incubate slides in a humid chamber at **37 °C for 30 minutes**

7.4 Washing

a) Rinse slides briefly with a gentle stream of PBS (do not direct into wells)
b) Immerse slides in PBS for **10 minutes**
c) Briefly dip slides in distilled water
d) Allow slides to **air-dry completely**

7.5 Conjugate Incubation

a) Add **20 µL** of FITC-labelled anti-human IgG conjugate to each well (No dilution required)
b) Incubate in a humid chamber at **37 °C for 30 minutes**
c) Rinse slides briefly with a gentle stream of PBS (do not direct into wells)
d) Immerse slides in PBS for **10 minutes**
e) Briefly dip slides in distilled water

7.6 Mounting and Reading

a) Add a small drop of mounting medium to each well
b) Carefully place a coverslip without trapping air bubbles
c) Read slides immediately using a fluorescence microscope at **400× magnification**
d) If not read immediately, store slides at **2–8 °C in the dark** for up to **24 hours**

7.7 Further Dilutions of Positive Screens

If the screening dilutions are positive, further analyse with increasing dilutions up to 1/640.

a) Prepare two-fold dilutions up to 1/640 starting from the already prepared 1/80 dilution.
    i. Add 50ul PBS and 50ul of the 1/80 diluted sample to prepare a 1/160 dilution.
    ii. Add 50ul PBS and 50ul of the 1/160 dilution to prepare a 1/320 dilution
    iii. Add 50ul PBS and 50ul of the 1/320 dilution to prepare a 1/640 dilution
b) Analyse according to Section 7.6

8. Interpretation of Results

Observation	**Interpretation**
Apple-green fluorescence with cocco-bacillary morphology	Positive
No specific fluorescence	Negative

- The **antibody titre** is the highest dilution at which specific fluorescence is observed
- Titres **≥1/40** are generally considered positive
- A significant rise in titre between paired samples confirms recent infection
- The reaction is negative when no fluorescence can be observed.

9. Quality Control

- Include **positive and negative controls** in every run
- Results are valid only if:
    - Positive control shows characteristic fluorescence
    - Negative control shows no fluorescence
- Failed QC invalidates the run and requires repeat testing

10. Documentation

- Record assay details in the **IFA Run Log**, including date, operator, kit lot number, sample IDs, dilutions, final titres and interpretation
- Enter validated results into dedicated **KOBO form**
- Maintain **IFA QC logs** for review.
- Archive raw data printouts and electronic files securely.

11. Related templates

- IFA Run Log
- IFA QC Log

12. Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*
Date: *15 April 2026*

Annex

IFA Slide Layout Template

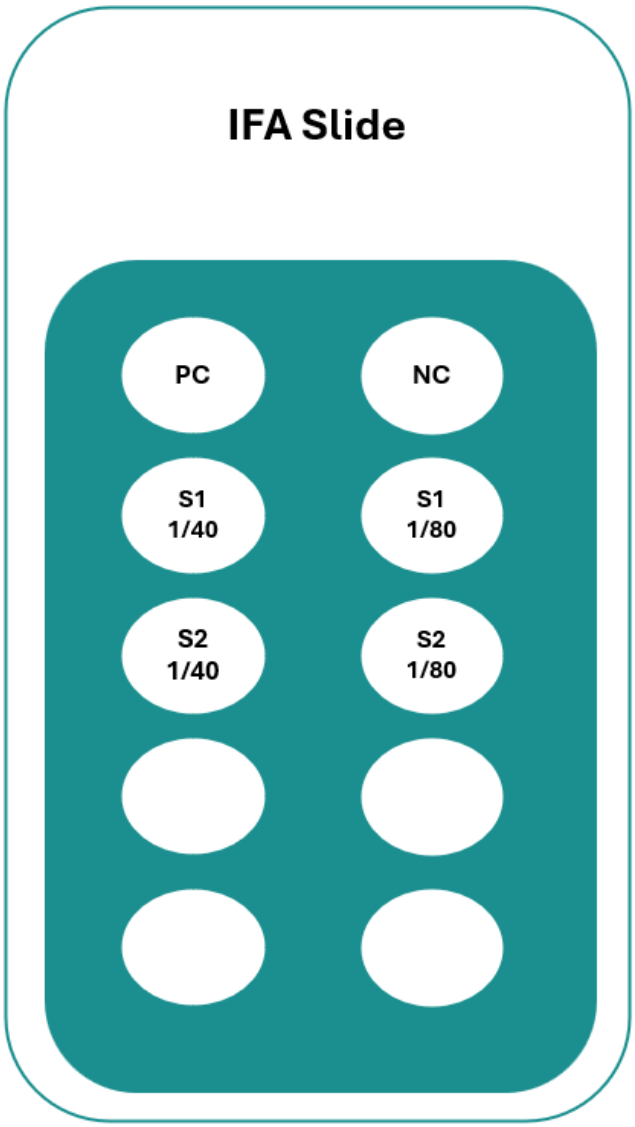

Figure 1 Diagram showing an example slide layout. PC = Positive Control, NC= Negative Control. The two dilutions are plated next to each other. S1 and S2 are names used to demonstrate two different samples, please use the sample numbers and keep a note of the well positions of each sample.