



SOP: IgG IFA for *Rickettsia conorii* (spotted fever)

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1. Purpose

To describe the standardized procedure for the detection of **IgG antibodies against *Rickettsia conorii* 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 conorii* IFA test at ZAFI project laboratories in Uganda, Kenya, and Ethiopia.

3. Abbreviations

- **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.

5. Materials and Equipment

Item	
Supplied in the Kit:	Additional Materials (not supplied):
<i>Rickettsia conorii</i> 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

- Handle all human specimens as potentially infectious
- Wear appropriate PPE at all times
- Avoid skin and eye 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 the 1/40 dilution (above) using 50ul PBS and 50ul 1/40 dilution

NOTE: Do not dilute control sera (VIRCELL RICKETTSIA IgG POSITIVE CONTROL and VIRCELL RICKETTSIA NEGATIVE CONTROL)

7.3 Slide Preparation and First Incubation

- a) Allow all reagents and slides to reach room temperature before use
- b) Apply 20 μL of diluted samples (1/40 and 1/80) into separate wells
- c) Add 20 μL of positive and negative controls to separate wells
- d) 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 (avoid directing liquid 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
- b) Incubate in a humid chamber at 37 °C for 30 minutes
- c) Rinse slides briefly with a gentle stream of PBS (avoid directing liquid into wells)
- d) Immerse slides in PBS for 10 minutes
- e) Briefly dip slides in distilled water
- f) Allow slides to air-dry completely

7.6 Mounting and Reading

- a) Add a small drop of mounting medium to each well
- b) Carefully apply a coverslip without trapping air bubbles
- c) Read slides immediately using a fluorescence microscope at 400 \times 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 samples 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

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

- The **antibody titre** is the highest dilution showing specific fluorescence
- Titres $\geq 1/40$ are generally considered positive

- Demonstration of seroconversion or a significant rise in titre 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

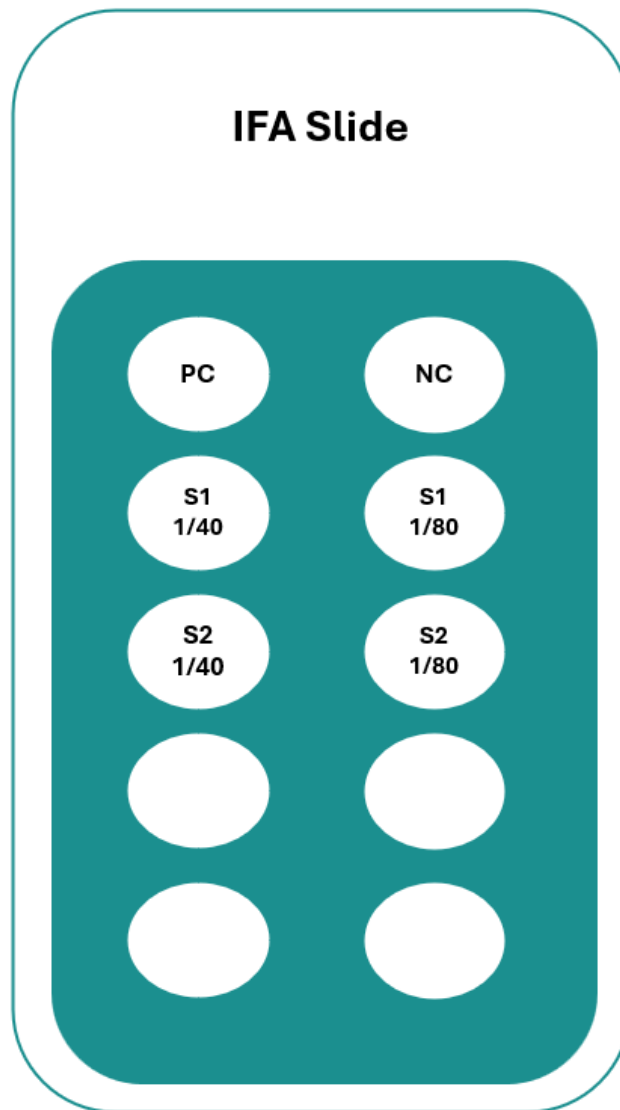


Figure 1 Diagram showing an example slide layout. PC = Positive Control, NC= Negative Control. The two dilutions are plated next to each other. S1 and S2 are names used to demonstrate two different samples, please use the sample numbers and keep a note of the well positions of each sample.