QIMR Berghofer
Medical Research Institute

Development of CRISPR-based technologies in schistosomes

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CRISPR-based technologies

1. The development of CRISPR-Cas9, a method for genome editing, was awarded the Nobel Prize in 2020. However, there has been limited development in helminth research.

2. Schistosomiasis: No human/animal vaccine is available and praziquantel (PZQ) is the only drug for treatment.

3. The challenge for researchers in mining the genomes is the lack of suitable tools to effectively characterise schistosome gene products as potential drug/vaccine/diagnosis targets and to translate them into urgently needed interventions.

4. Three CRISPR-based technologies we have developed in schistosomes.

   ➢CRISPR/Cas9 gene editing system

   ➢CRISPR interference and activation (CRISPRi/a) for gene functional studies

   ➢CRISPR/Cas13 diagnostic platform

Firstly established in helminths

RESEARCH ARTICLE

THE FASEB JOURNAL

CRISPR/Cas9-mediated genome editing of *Schistosoma mansoni* acetylcholinesterase

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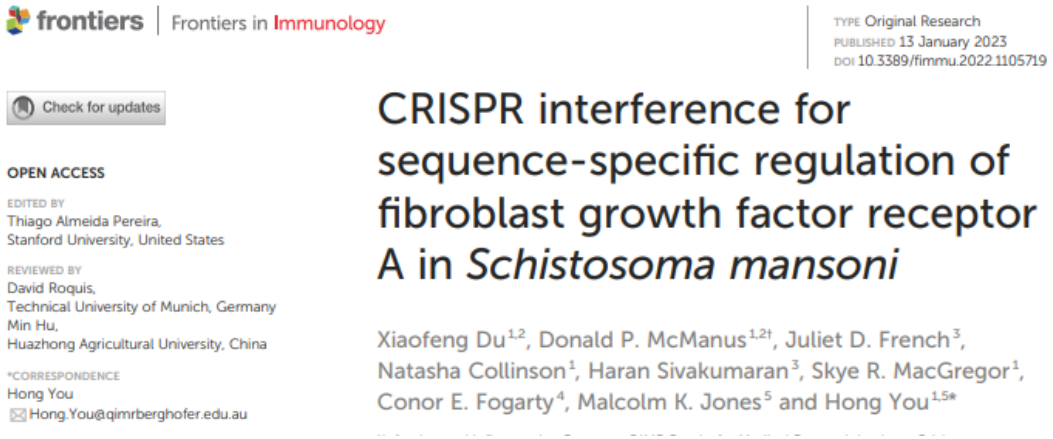

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CRISPR interference for sequence-specific regulation of fibroblast growth factor receptor A in *Schistosoma mansoni*

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Development of CRISPR/Cas13a-based assays for the diagnosis of Schistosomiasis

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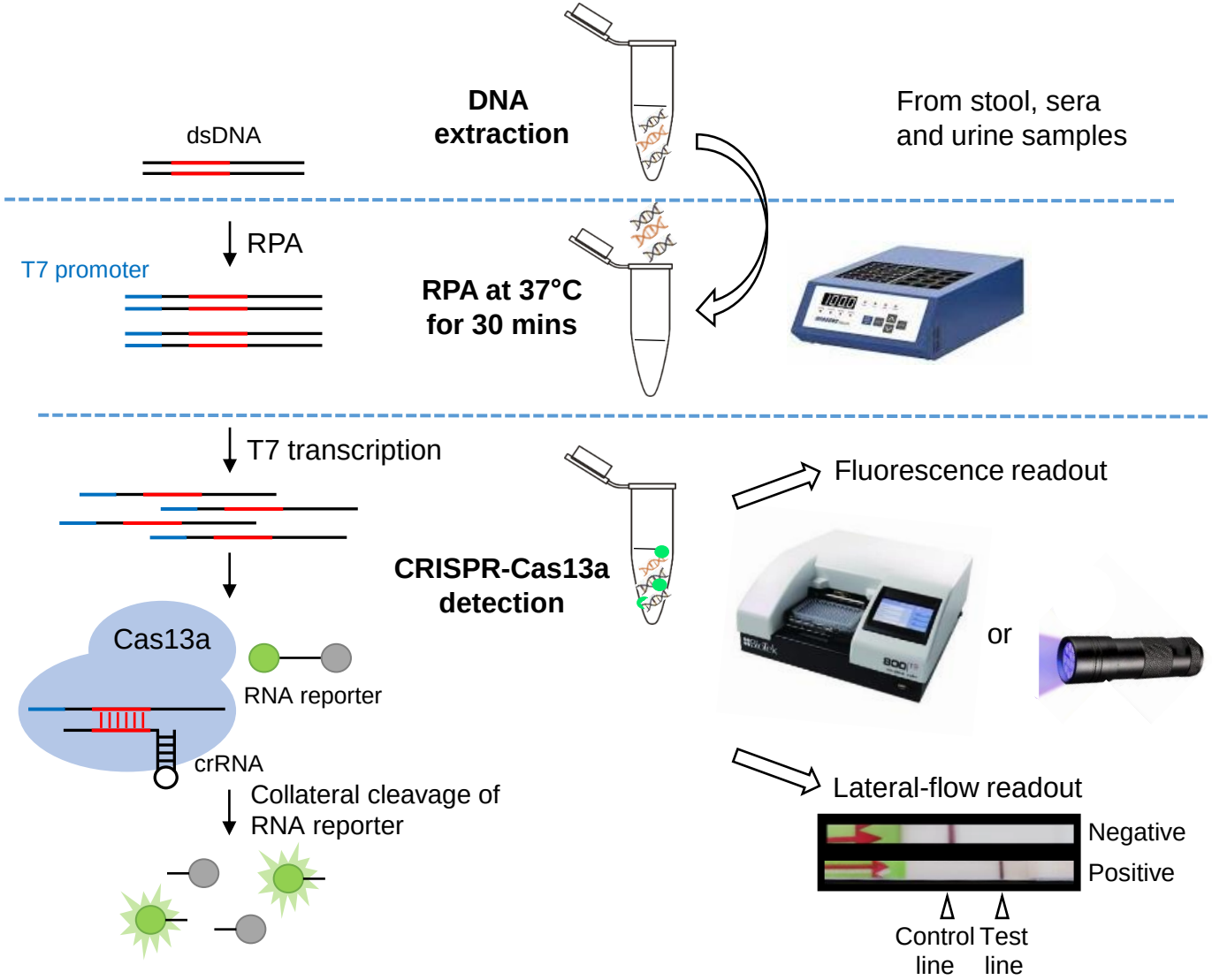
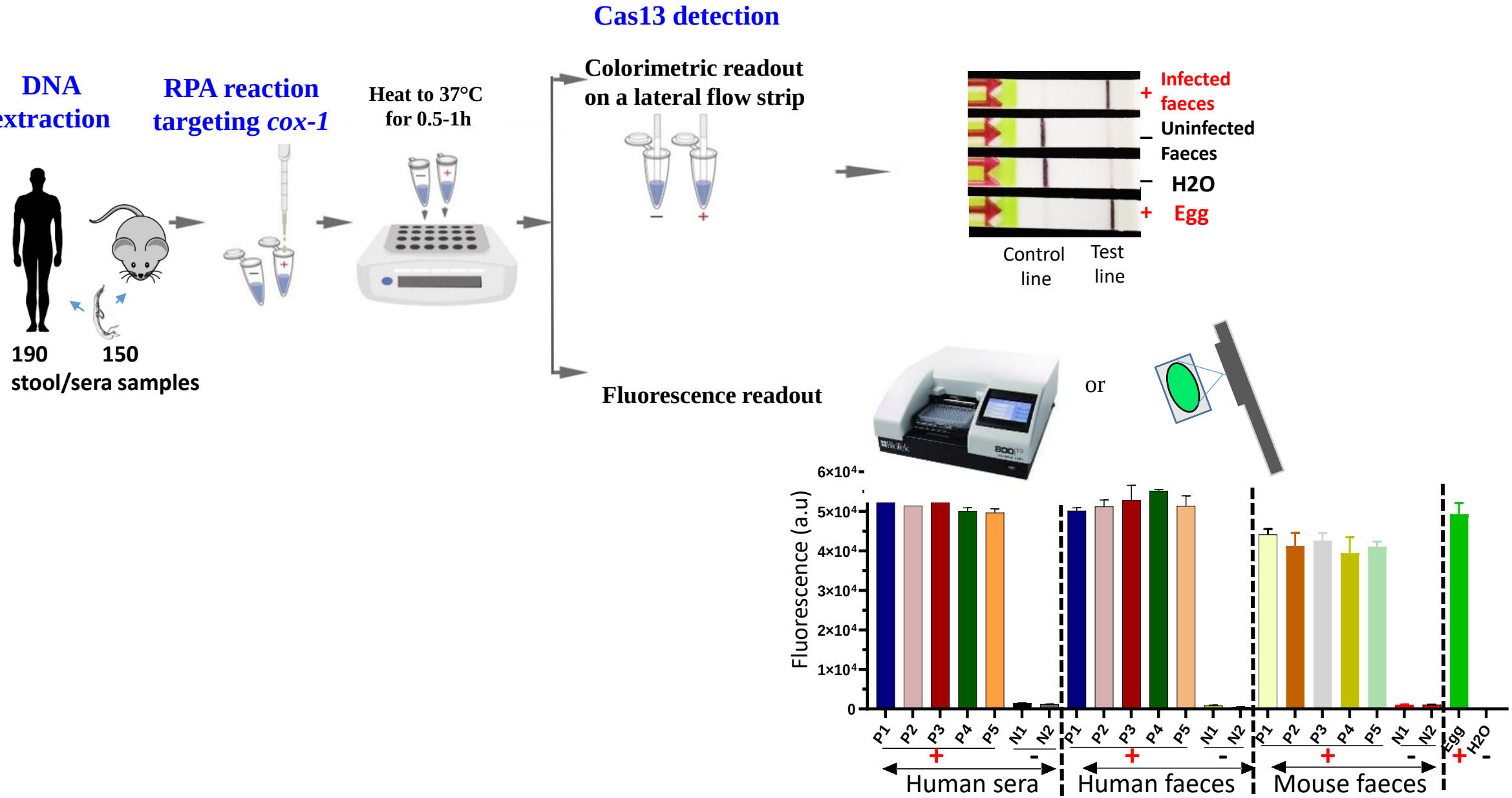
A highly sensitive and field-friendly point-of-care diagnosis for schistosomiasis

CRISPR-Cas13 platform (SHERLOCK)

- **Accurate**
- **Fast**
- **Affordable**
- **Field-friendly, without the need for specialized equipment/expertise**
- **Point of care (POC)**

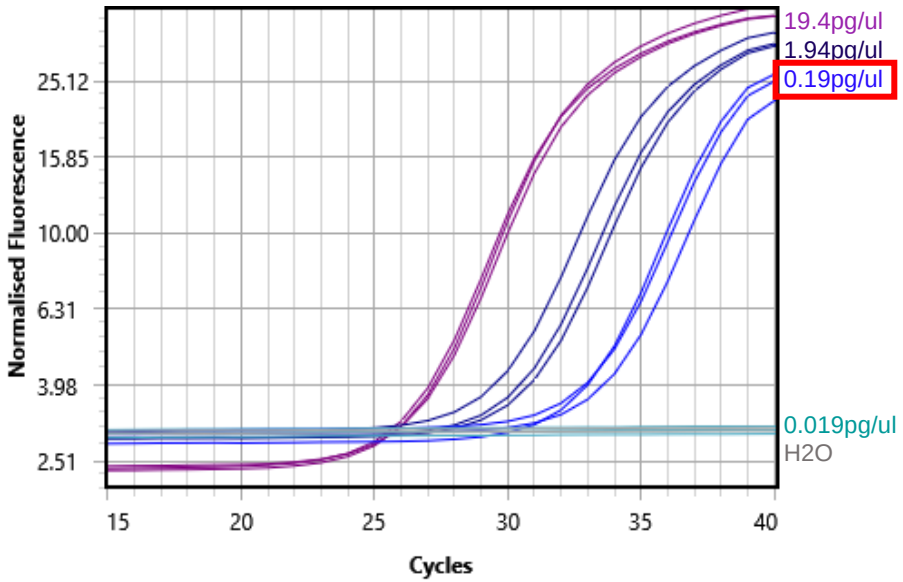


SHERLOCK assay for detection of schistosomiasis

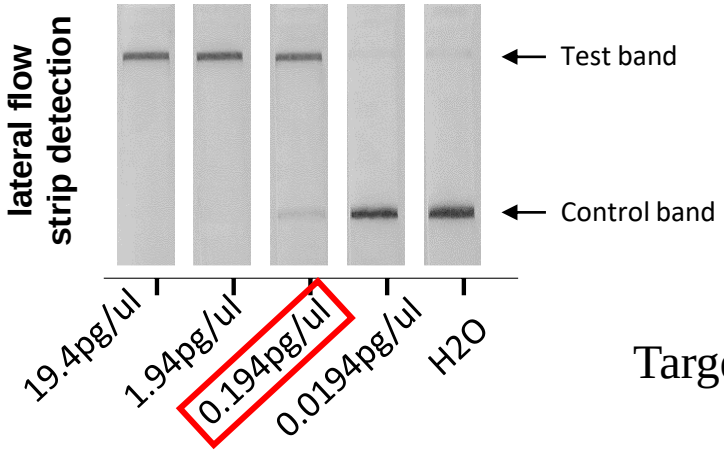


SHERLOCK assays for detection of *S. japonicum* targeting *Sjcox1*

Real time PCR

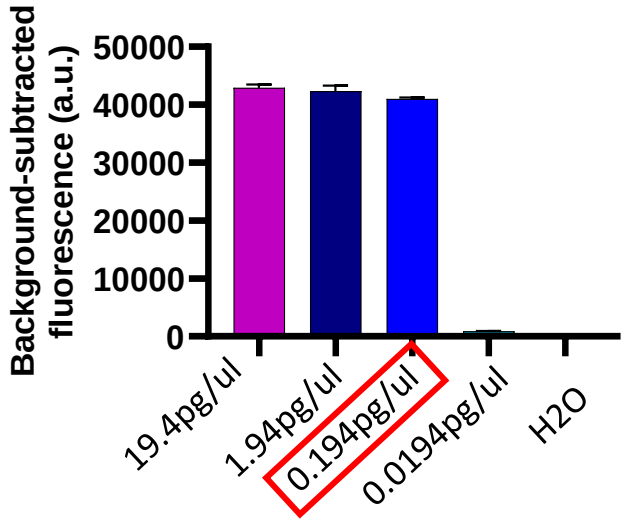


Lateral flow strip detection

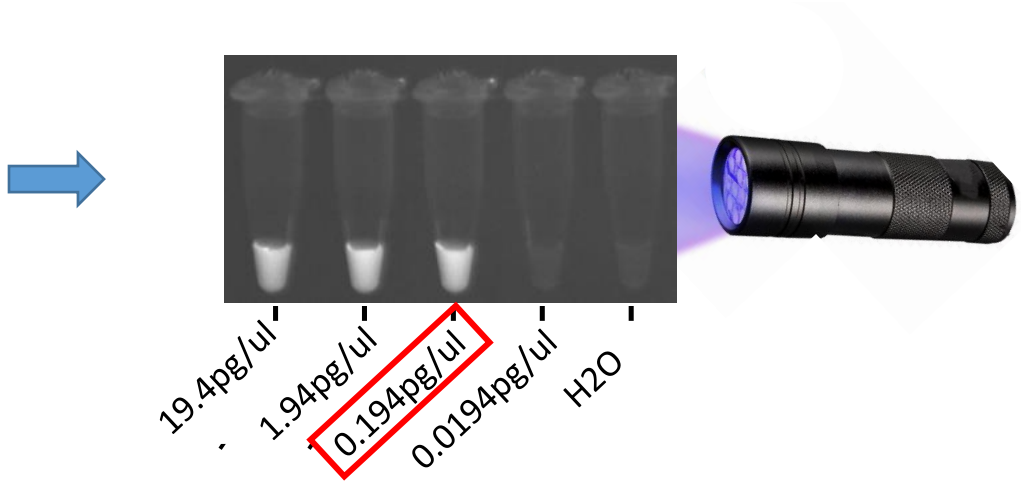


Targeting *Sjcox-1*

Fluorescence readout

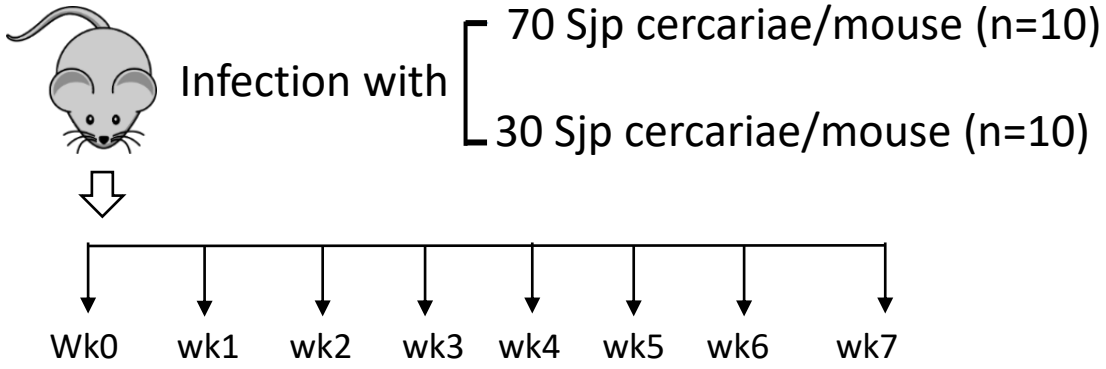


Fluorescence detected under UV light

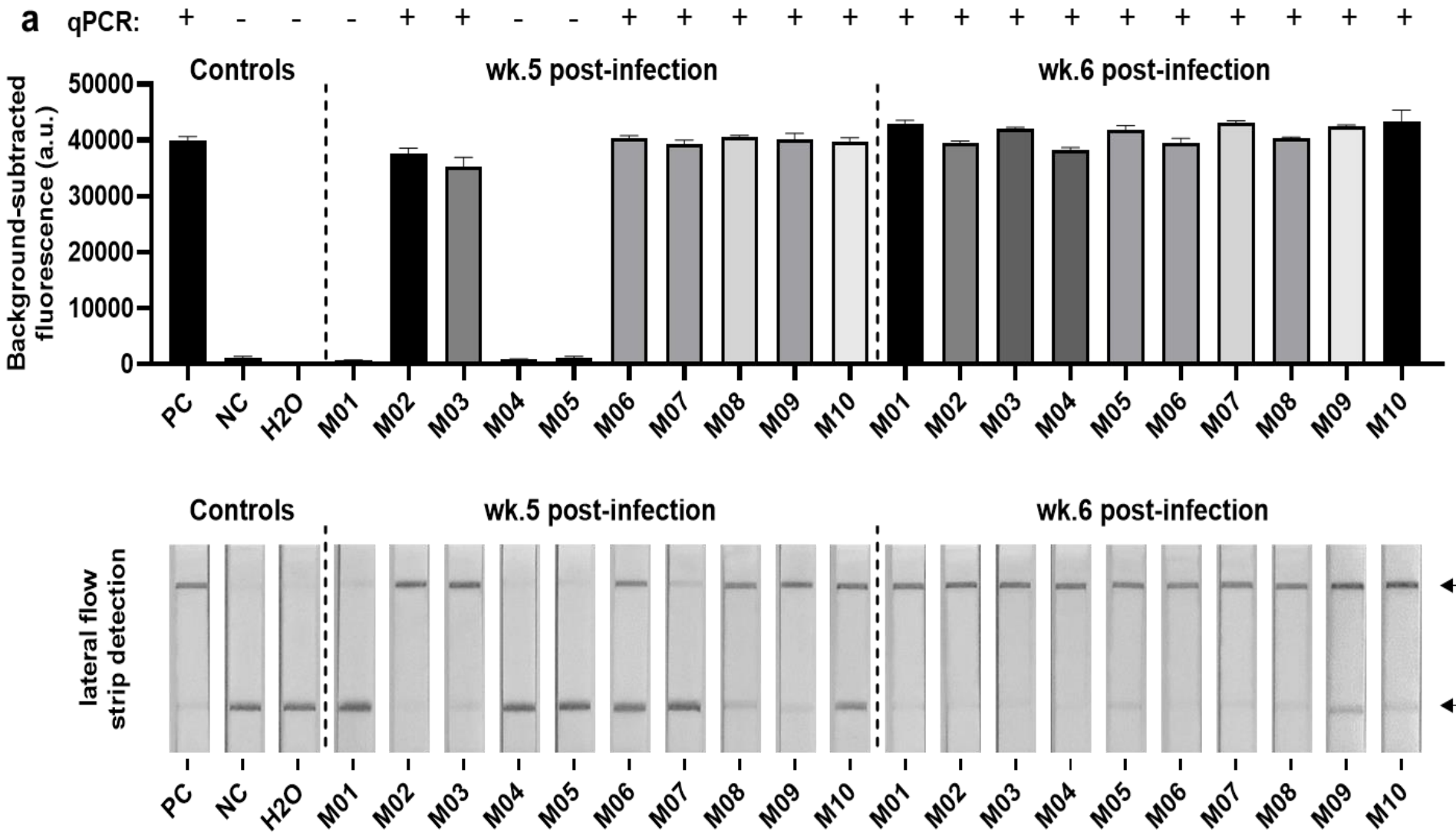


Collection of stool samples from mice infected with *S. japonicum* cercariae

-used for SHERLOCK (via lateral flow and fluorescence) and qPCR assays



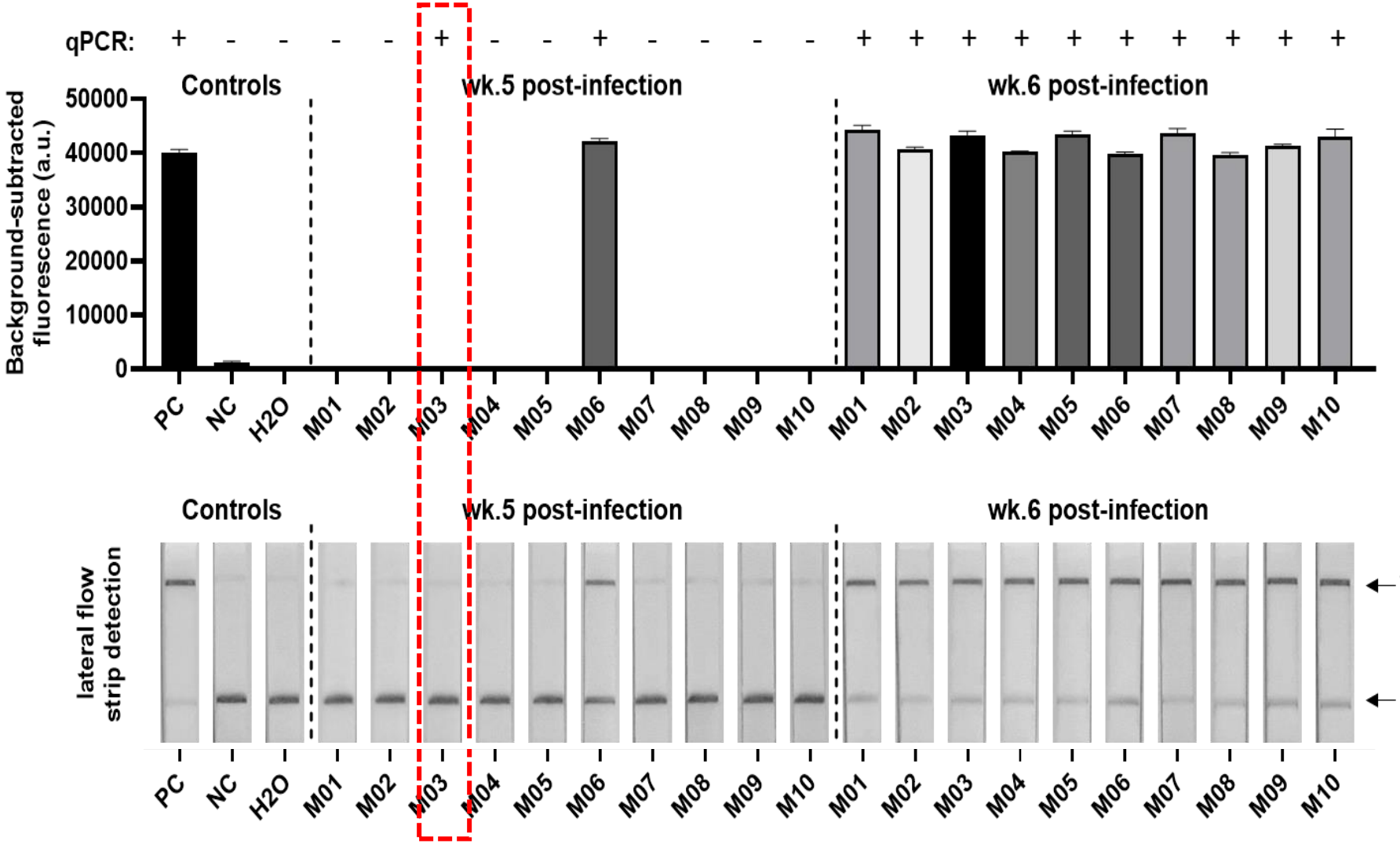
Stool samples collected from mice infected with 70 *S. japonicum* cercariae (17/17)



Fluorescence

Lateral flow

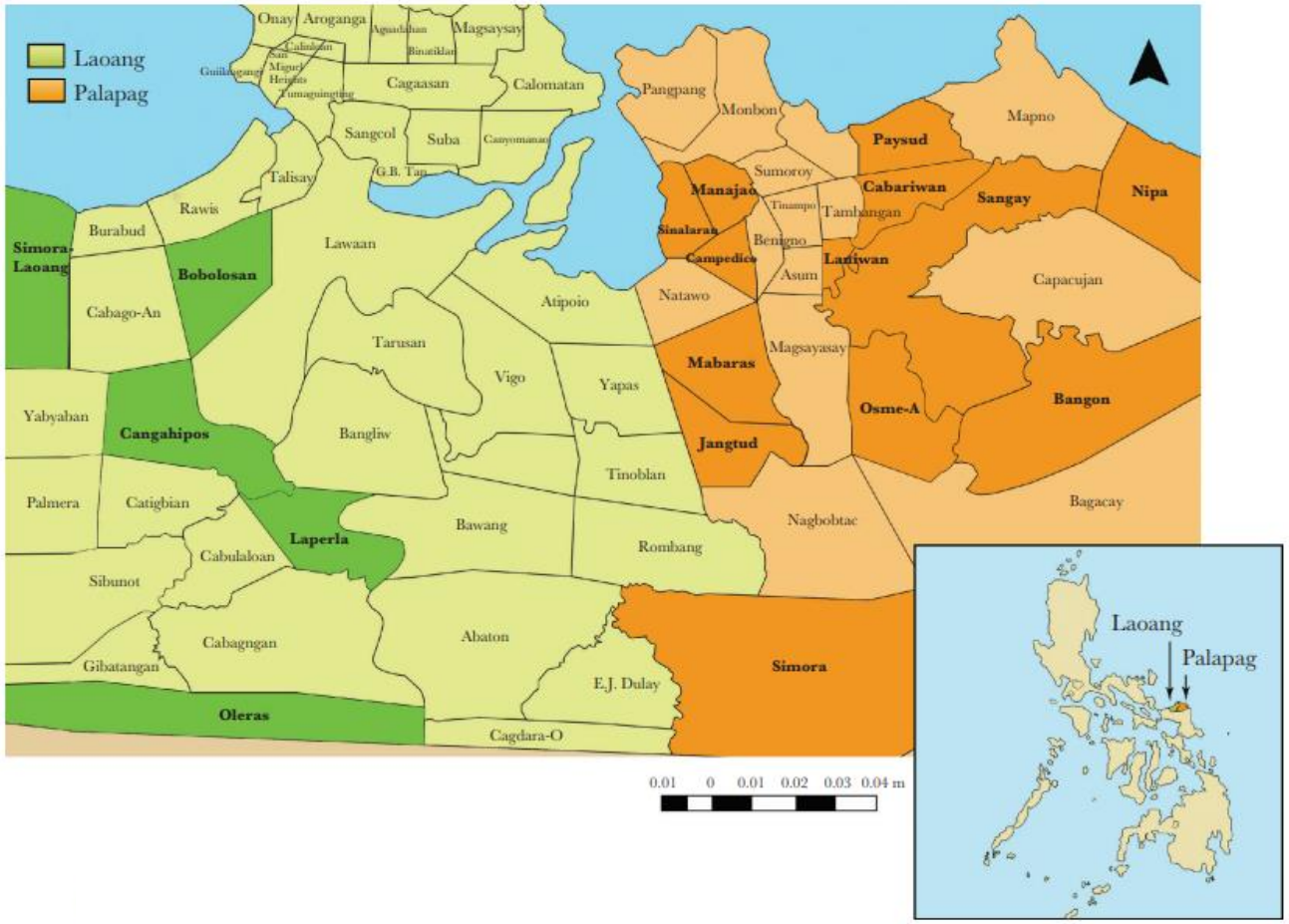
Stool samples collected from mice infected with 30 cercariae (11/12=92%)

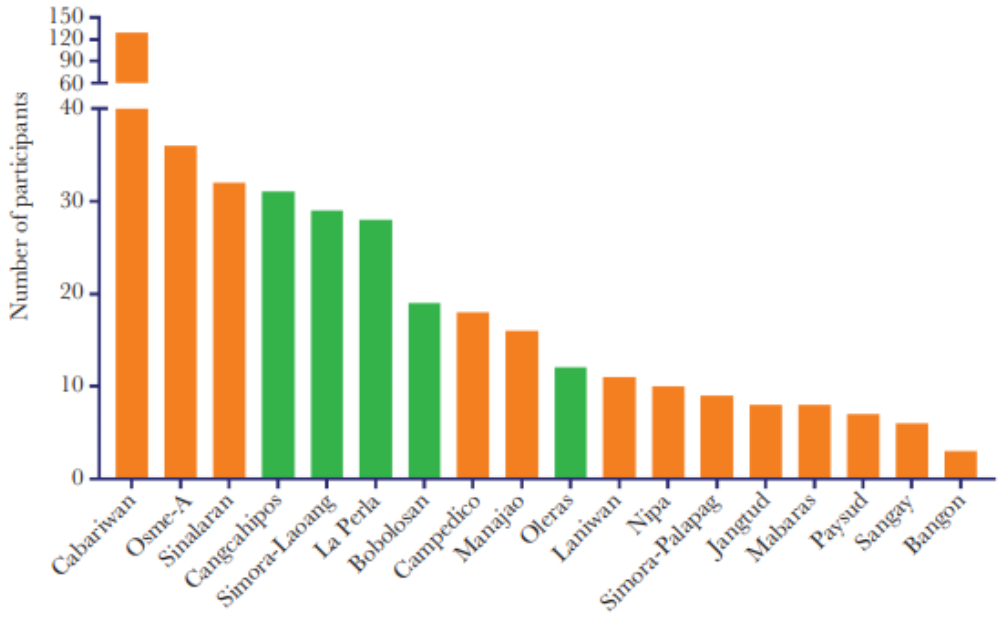


Fluorescence

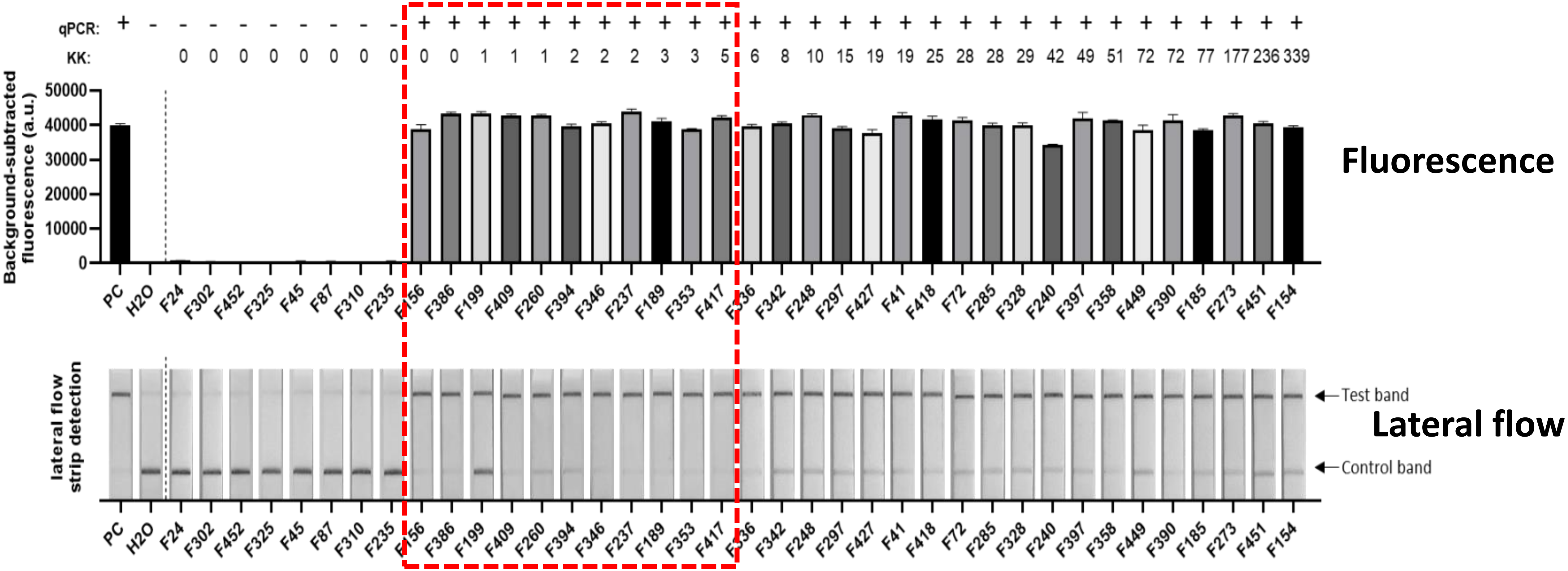
Lateral flow

Clinical samples collected from *S. japonicum*-endemic areas in the Philippines -18 villages in Palapag and Laoang

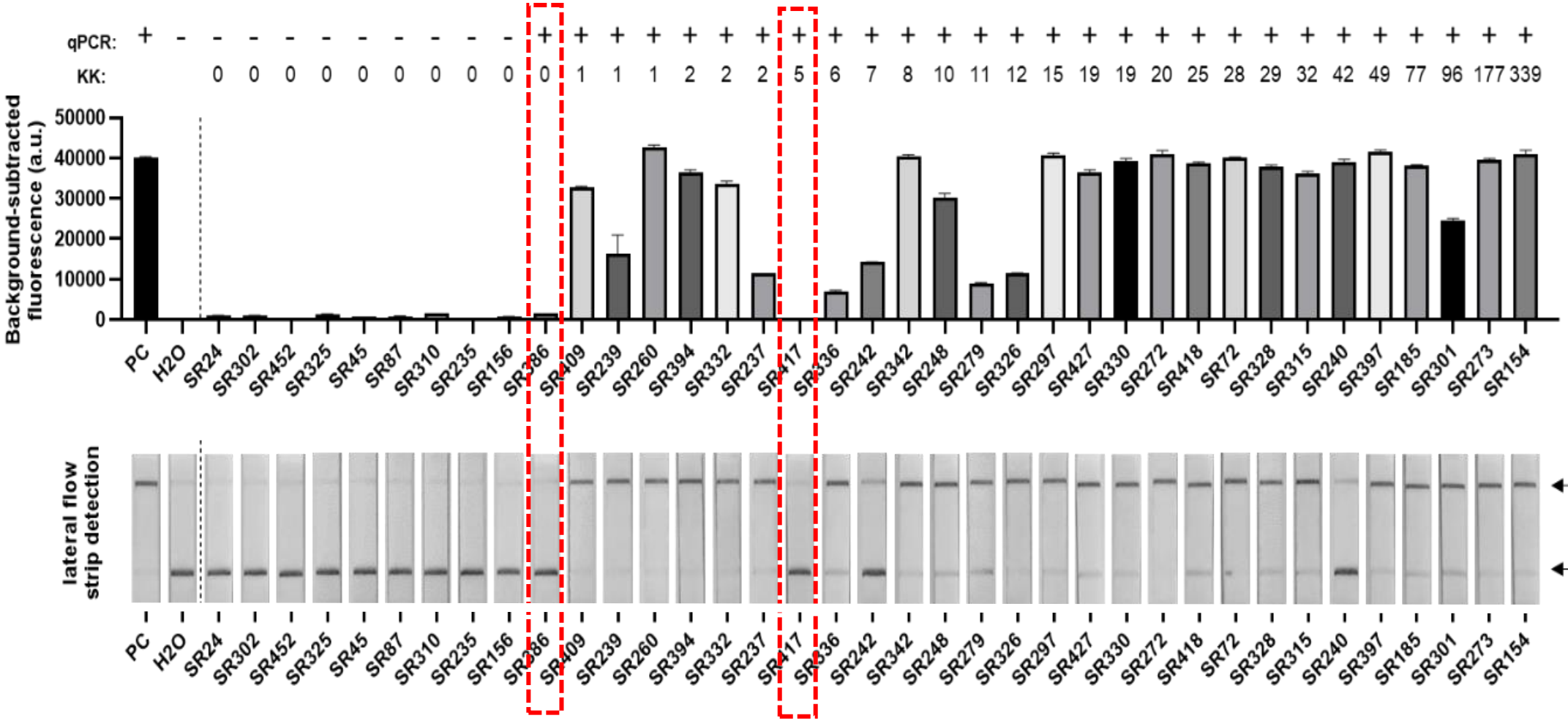




Human stool samples collected from the Philippines (30/30)



Human sera samples collected from the Philippines (26/28=93%)

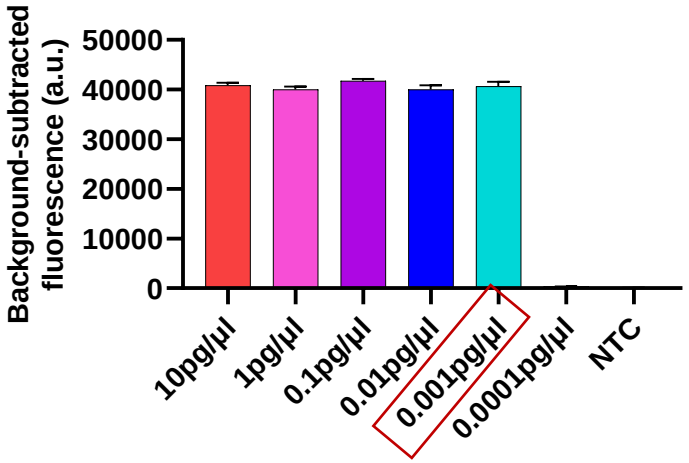


Fluorescence

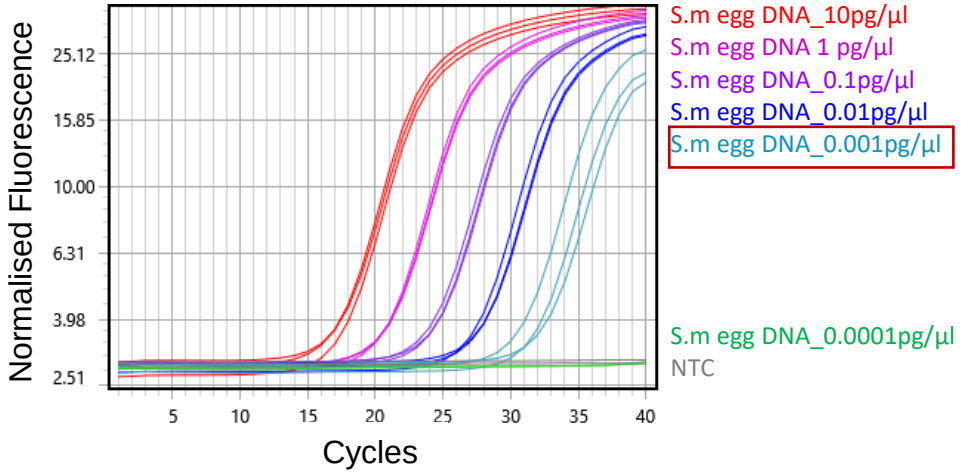
Lateral flow

SHERLOCK assays for detection of *S. mansoni* targeting *Sm1-7*

Similar sensitivity detected by SHERLOCK assay to qPCR

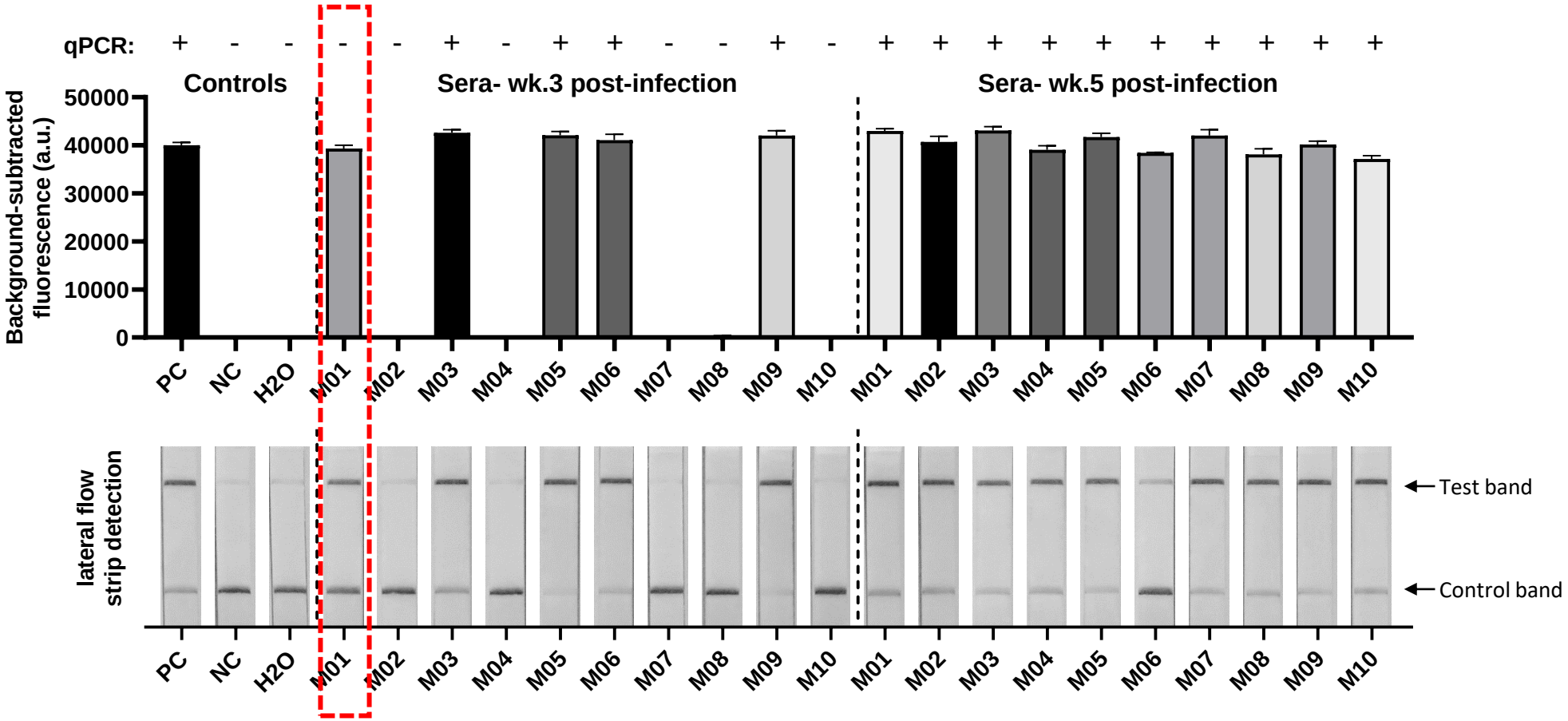


SHERLOCK



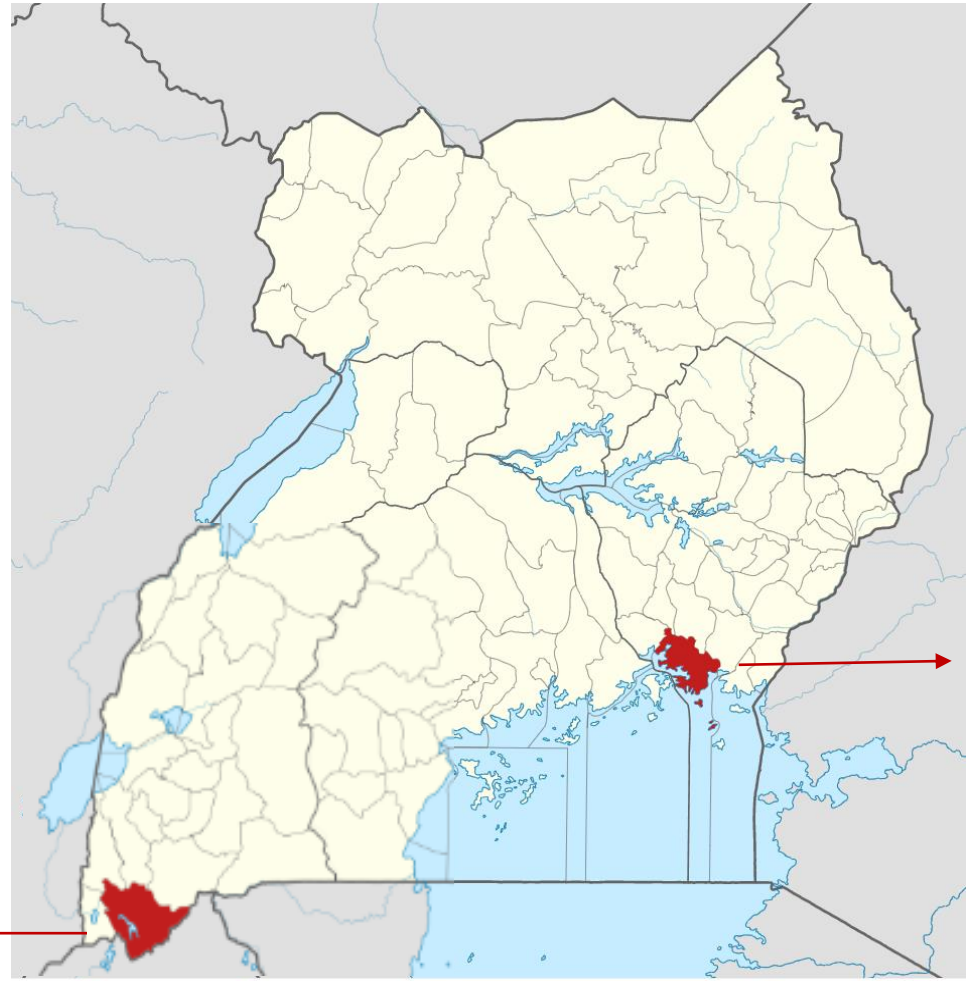
qPCR

Early detection of *S. mansoni* infection in mouse sera by SHERLOCK assays



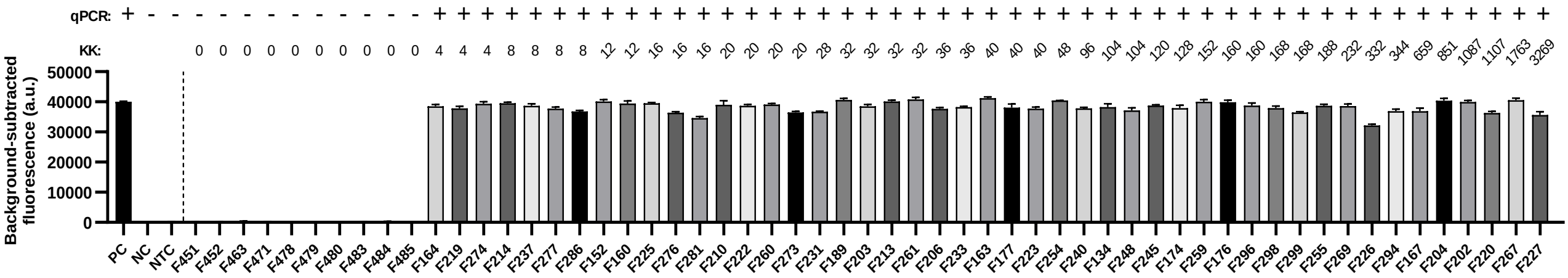
Stool/sera samples collected human in Uganda (57/57)

-Fluorescence-based SHERLCOK assays

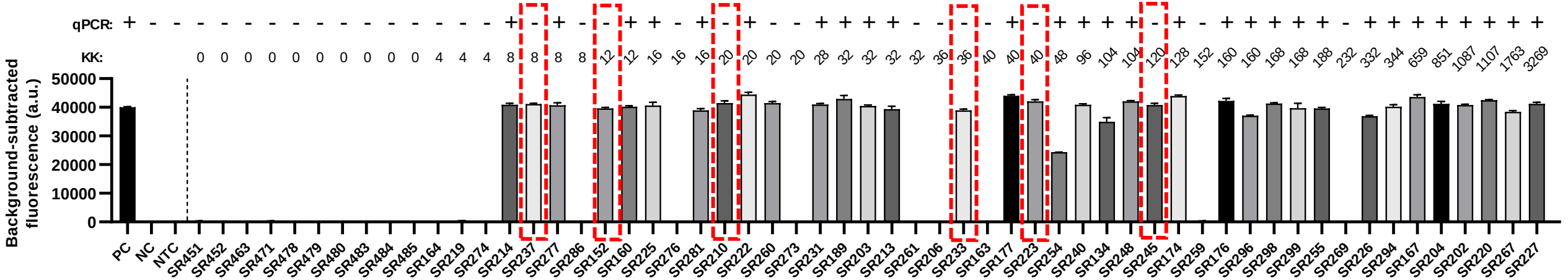


Stool/sera samples collected human in Uganda (57/57)
-Fluorescence-based SHERLCOK assays

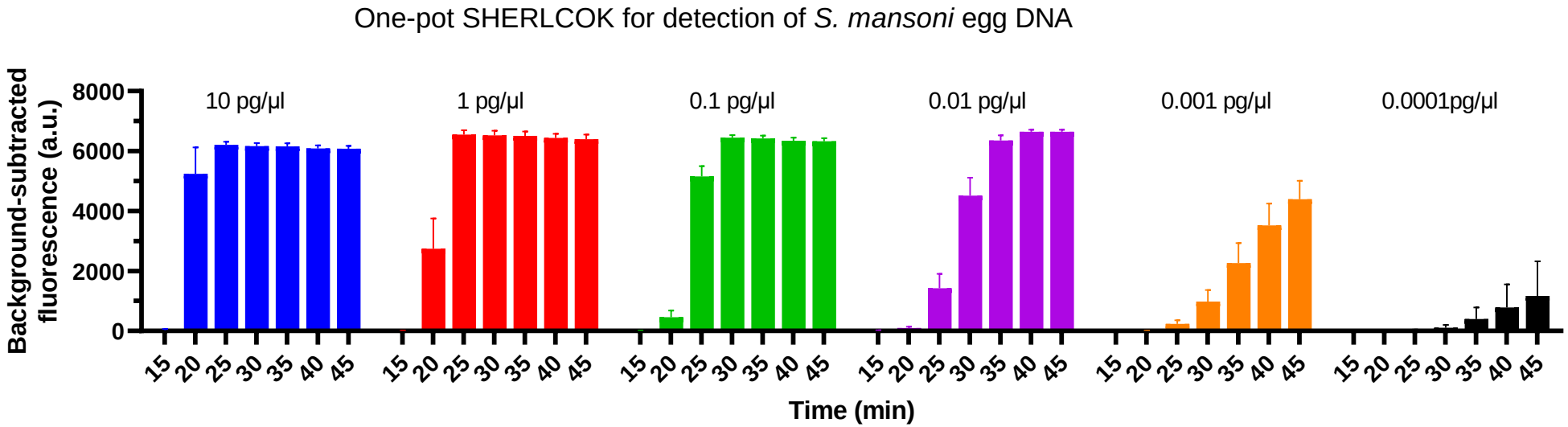
Stool

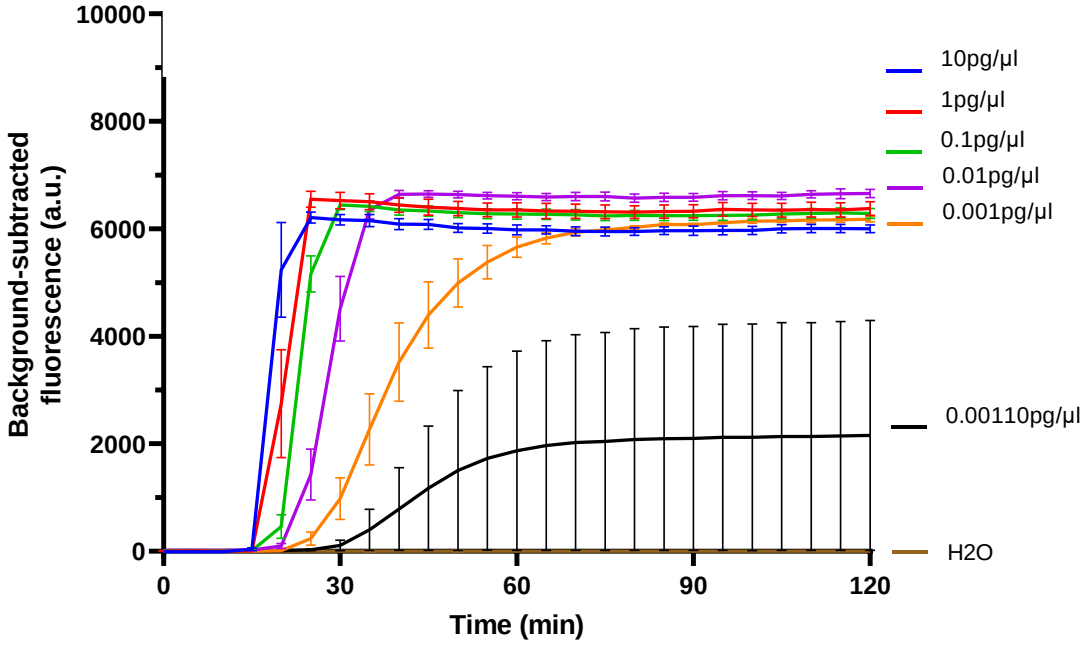


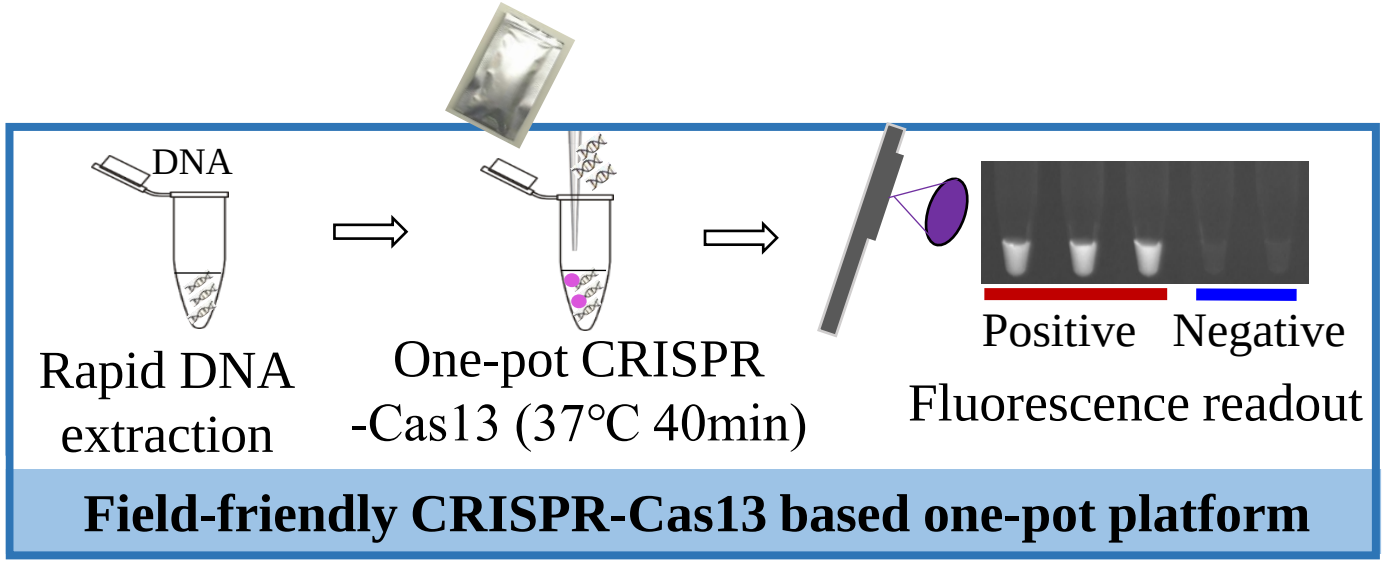
Sera



Simplify /shorten the process by using a one-pot SHELOCK reaction







Summary

1. Our SHERLOCK diagnostic tool exhibits a similar level of sensitivity (92-100%) as qPCR assay which is currently the most sensitive approach for the diagnosis of helminthic infections, but is substantially cheaper, more field-friendly, without the need for specialized equipment/expertise.

2. SHERLOCK can provide accurate, affordable and portable POC diagnosis for schistosomiasis.

3. More strategies:

- Target other interest genes by using multiple types of samples;
- SHERLOCK for improved diagnosis of other helminth infections
  - ✓ Hookworm (*Necator americanus*, *Ancylostoma ceylanicum*)
  - ✓ Strongyloides (*Strongyloides ratti*, *S. stercoralis*)
- CRISPR-Cas12/13 for detection of helminth co-infection.

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AID Australian Infectious Diseases Research Centre

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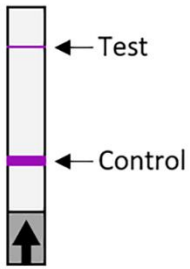
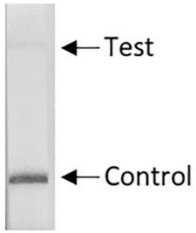
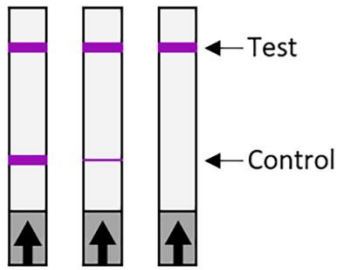
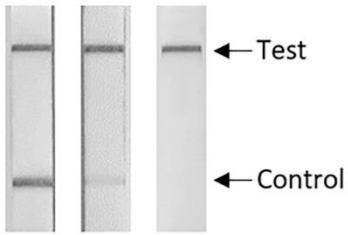
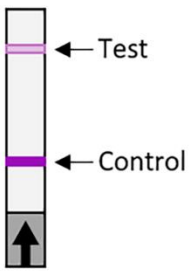
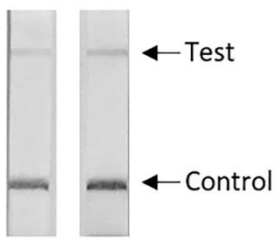
Lateral Flow Result	Diagram	Examples	Description
Negative (-)			Negative strips have a strong band at the control line. A very faint signal at the test line occurs due to non-specific reporter cleavage or incomplete T-line elimination (see Milenia Biotec website for details, https://www.milenia-biotec.com/en/tips-lateral-flow-readouts-crispr-cas-strategies/).
Positive (+)			Strips indicating a positive result have a strong band at the test line. The control band may be very faint or absent due to near complete digestion of the reporter molecule by Cas13a.
Ambiguous (+/-)			Strips with a test band that is still faint but higher intensity than negative control strips can be difficult to interpret by eye. These have been given an ambiguous result classification (+/-).