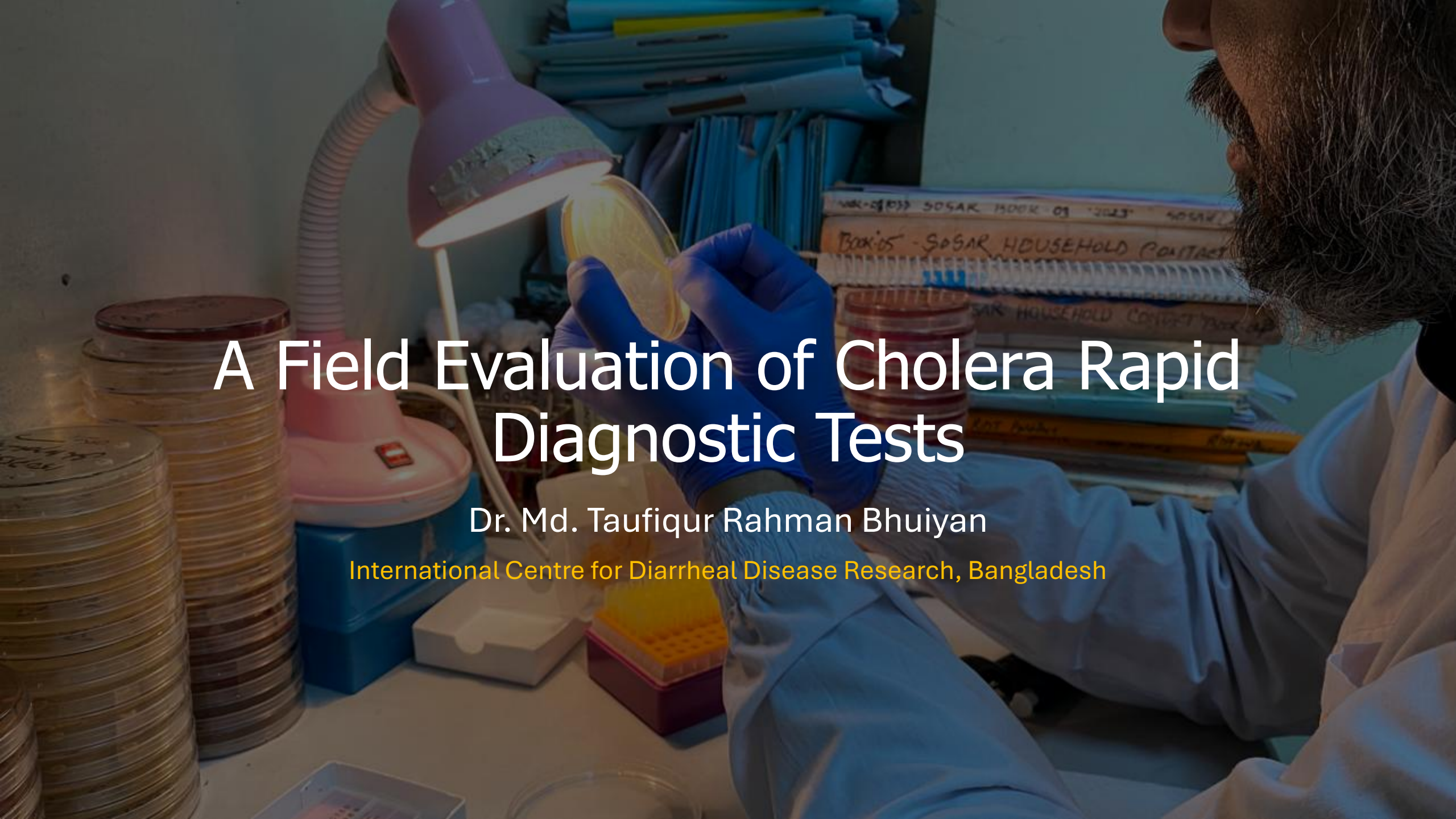
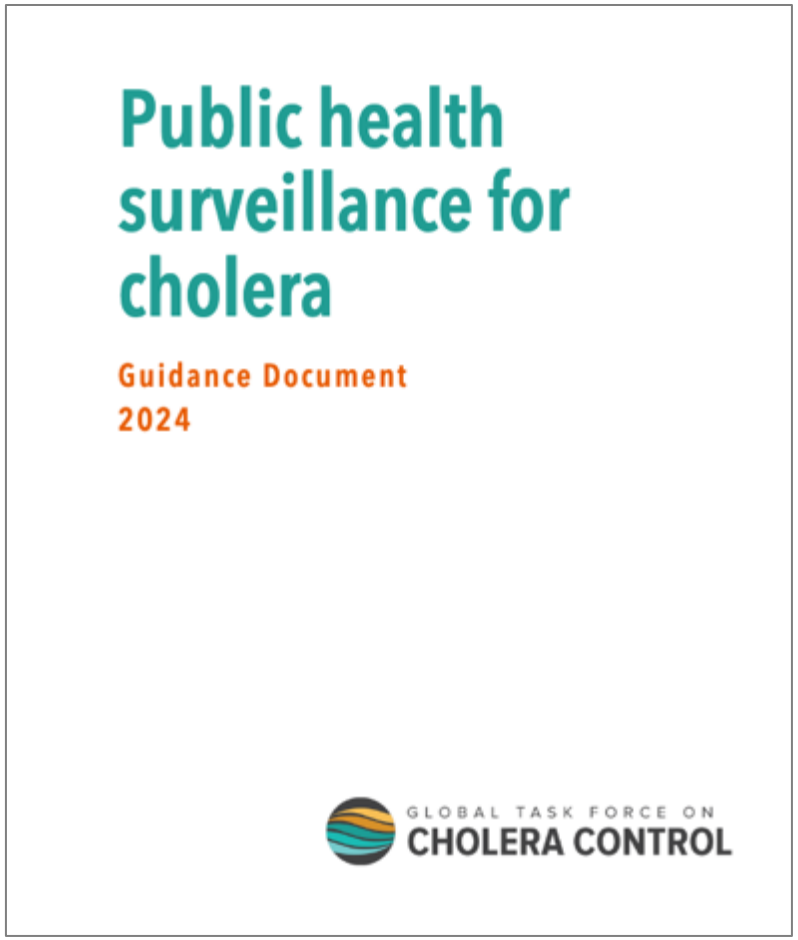


A Field Evaluation of Cholera Rapid Diagnostic Tests

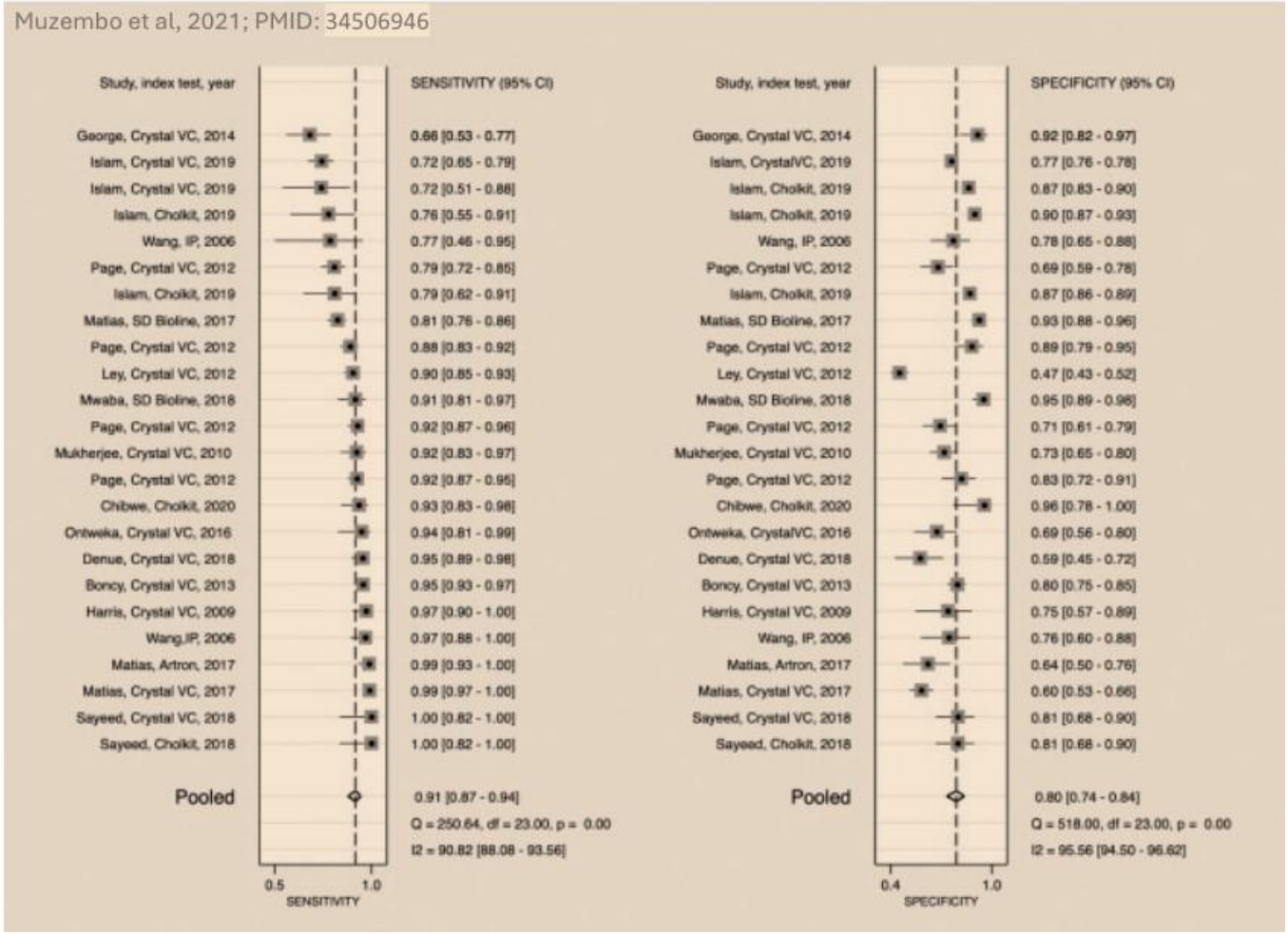
Dr. Md. Taufiqur Rahman Bhuiyan

International Centre for Diarrheal Disease Research, Bangladesh

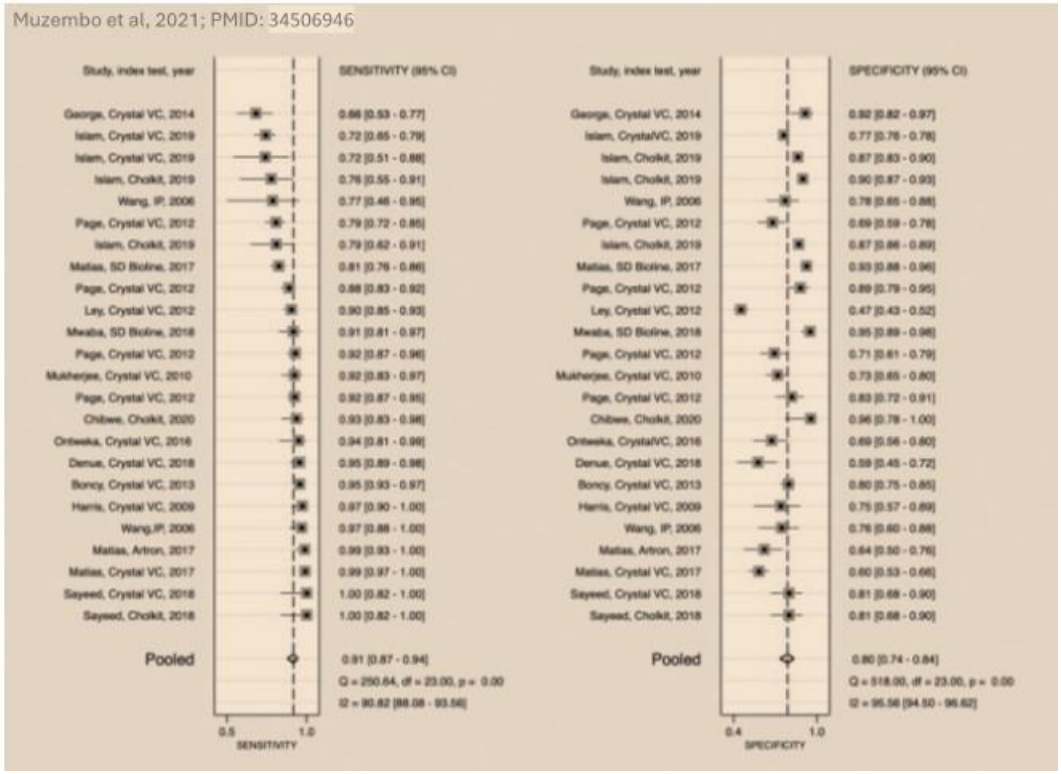
Surveillance with RDTs is now a possibility



But the test performance of RDTs is unknown



RDT clinical sensitivity and specificity is highly variable



Variability may be attributable to differences in:

- Test type/manufacturer
- Pre-treatment of stool
- Sample type (stool/rectal swab)
- Reader errors
- Phage and antibiotics
- Age groups
- Gold standard used

Primary Objective

To estimate the **sensitivity** and **specificity** of commercially available lateral flow RDTs for *V. cholerae* compared to PCR as the reference standard among medically attended symptomatic suspected cholera cases *(under optimal testing conditions)*

Secondary Objectives

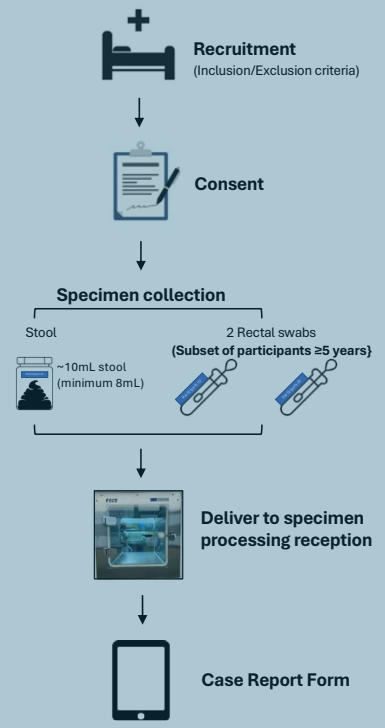
1. To estimate how test performance varies as a function of (a) gold standard, (b) enrichment, (c) sample type, (d) antibiotic use, (e) disease severity, and (f) age and season
2. To quantify the color intensity of the RDT control and test bands through an image processing algorithm and compare to technician classification
3. To quantify the loss in PCR test sensitivity over time from dry filter paper samples left in ambient conditions
4. To compare the use of RDTs for testing suspected colonies to slide agglutination

Study Design

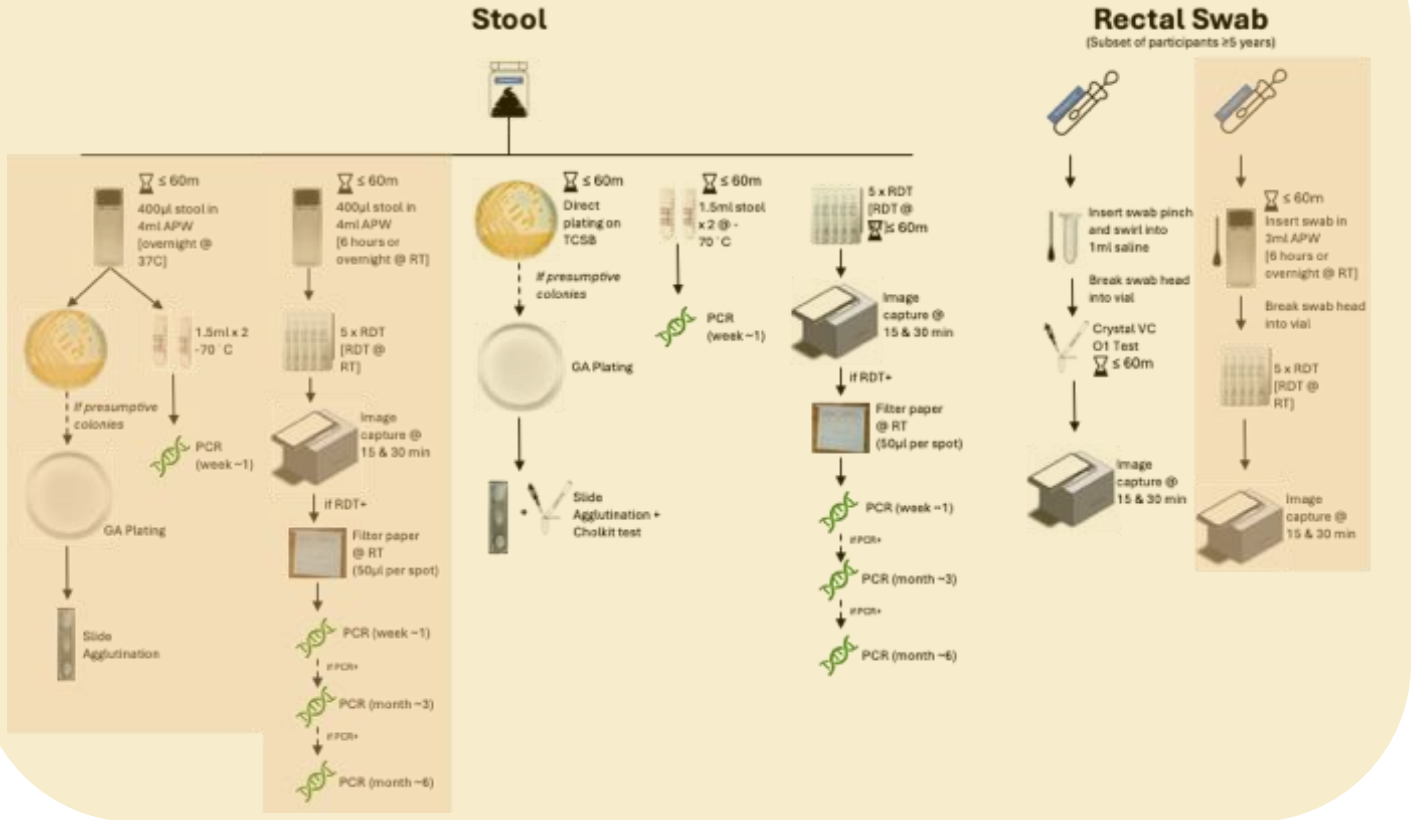
- *Setting*: 'Ideal' setting of icddr,b Dhaka hospital to ensure rapid and high-quality processing of samples
- *Sample population*: patients seeking care at icddr,b hospital with acute watery diarrhea
- *Sample size*: stratified sample by age group (1-4 and 5+) and season (pre-monsoon, monsoon, post-monsoon and winter) with **3,292 AWD cases** being recruited
- *Tests:* 5 commercially available lateral flow RDTs - - *Crystal VC O1, Crystal VC O1/O139, Artron, Abbott, Cholkit*

Study Design

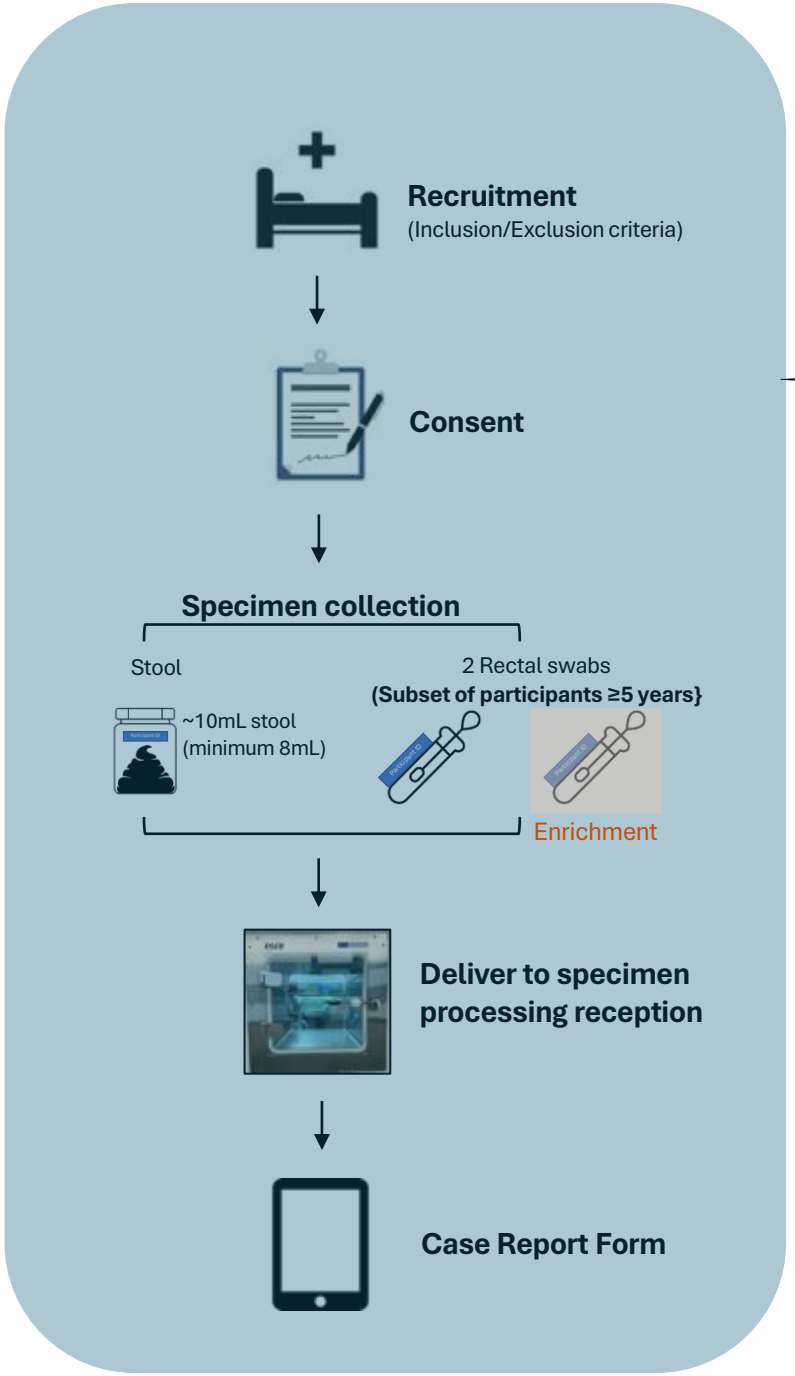
Clinical



Laboratory



Clinical



Inclusion criteria

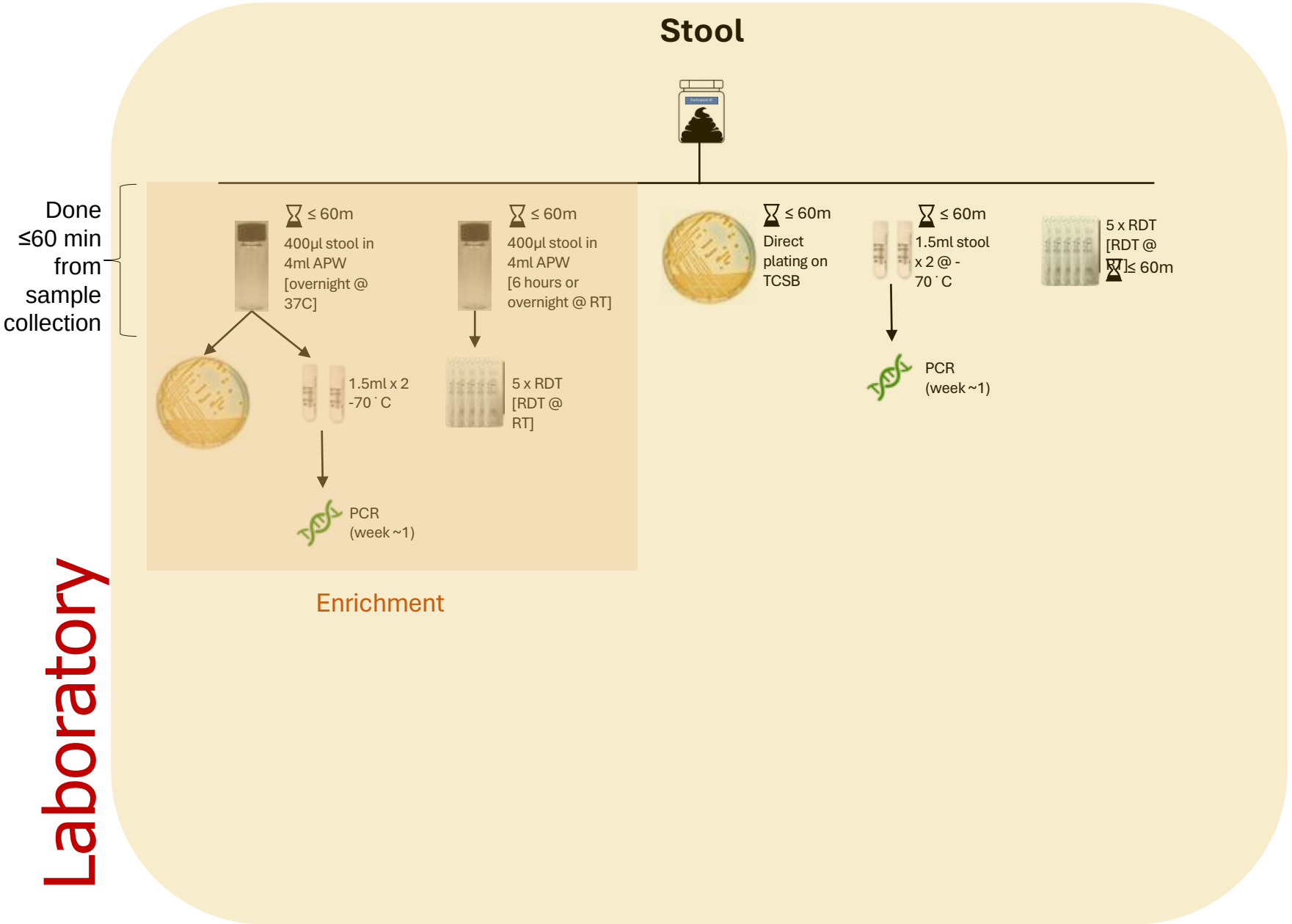
- AWD (≥3 or more loose stools within 24-hour period) and symptoms in last 24 hours (having lasted <8 days before hospital visit)
- Non-bloody stool
- Purged stool in 3 hours before study enrollment
- Willing and able to provide a stool sample and rectal swab

Exclusion criteria

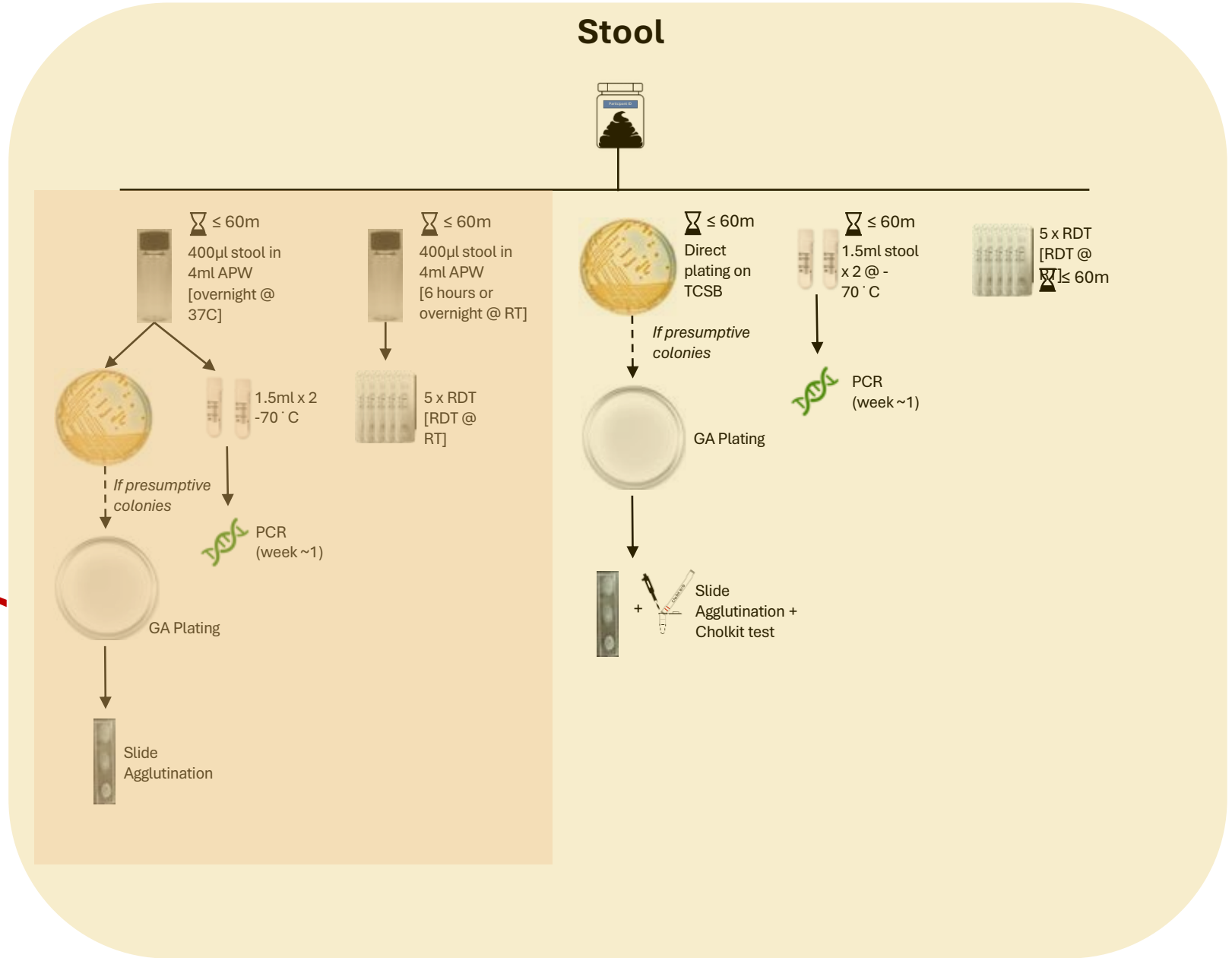
- Administered antibiotics for treatment after entering icddr,b

Laboratory

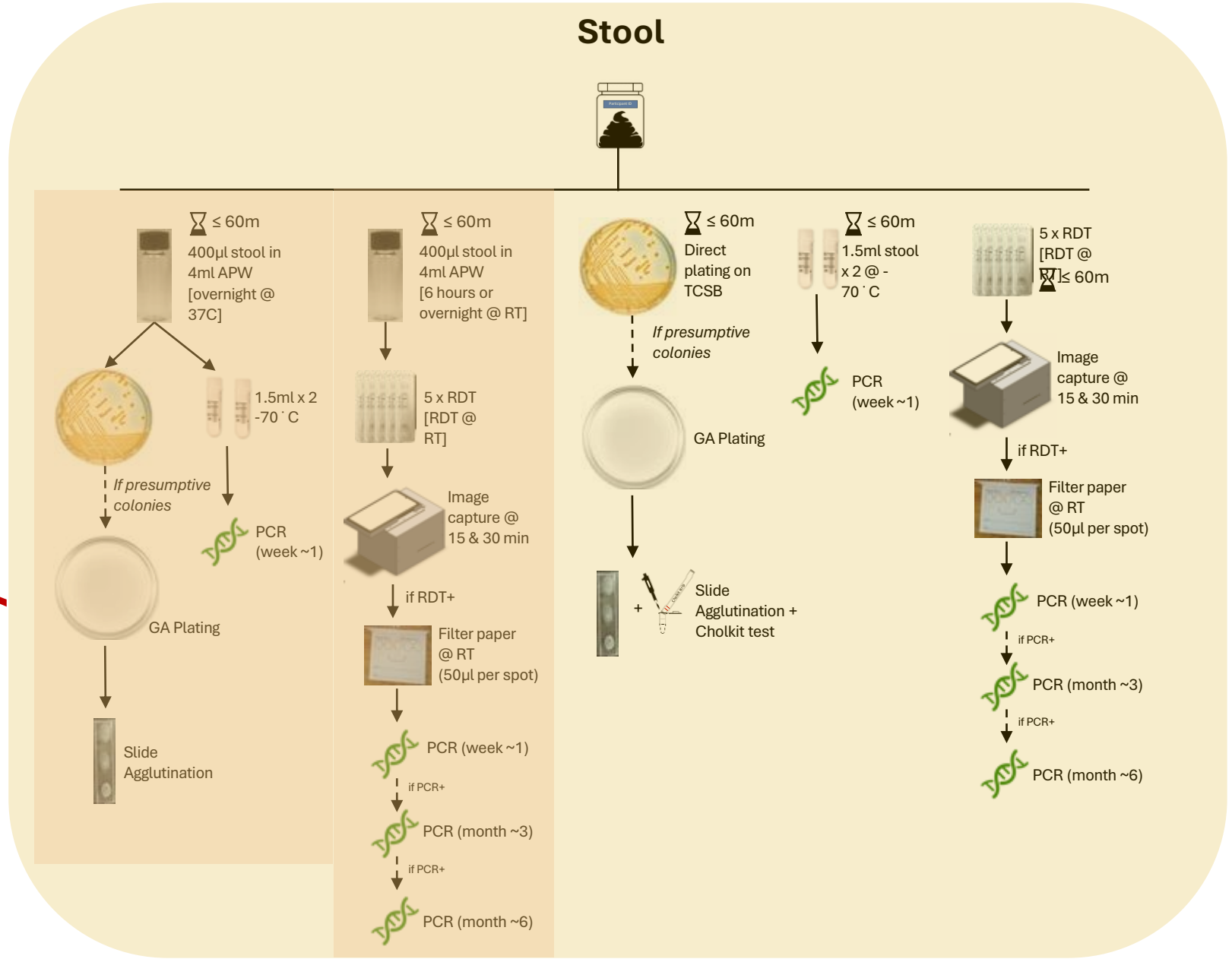
Stool


Stool
Done ≤60 min from sample collection
≤ 60m
400µl stool in 4ml APW [overnight @ 37C]
1.5ml x 2 -70 ˚ C
PCR (week ~1)
≤ 60m
400µl stool in 4ml APW [6 hours or overnight @ RT]
5 x RDT [RDT @ RT]
≤ 60m
Direct plating on TCSB
≤ 60m
1.5ml stool x 2 @ -70 ˚ C
PCR (week ~1)
5 x RDT [RDT @ RT]≤ 60m
Enrichment
Laboratory

Laboratory


Stool
≤ 60m
400µl stool in 4ml APW [overnight @ 37C]
1.5ml x 2 -70 ˚ C
If presumptive colonies
PCR (week ~1)
GA Plating
Slide Agglutination
≤ 60m
400µl stool in 4ml APW [6 hours or overnight @ RT]
5 x RDT [RDT @ RT]
≤ 60m
Direct plating on TCSB
If presumptive colonies
GA Plating
Slide Agglutination + Cholkit test
≤ 60m
1.5ml stool x 2 @ -70 ˚ C
PCR (week ~1)
5 x RDT [RDT @ RT]≤ 60m

Laboratory


Stool
≤ 60m
400µl stool in 4ml APW [overnight @ 37C]
1.5ml x 2 -70 ˚ C
If presumptive colonies
PCR (week ~1)
GA Plating
Slide Agglutination
≤ 60m
400µl stool in 4ml APW [6 hours or overnight @ RT]
5 x RDT [RDT @ RT]
Image capture @ 15 & 30 min
if RDT+
Filter paper @ RT (50µl per spot)
PCR (week ~1)
if PCR+
PCR (month ~3)
if PCR+
PCR (month ~6)
≤ 60m
Direct plating on TCSB
If presumptive colonies
GA Plating
+
Slide Agglutination + Cholkit test
≤ 60m
1.5ml stool x 2 @ -70 ˚ C
PCR (week ~1)
5 x RDT [RDT @ RT] ≤ 60m
Image capture @ 15 & 30 min
if RDT+
Filter paper @ RT (50µl per spot)
PCR (week ~1)
if PCR+
PCR (month ~3)
if PCR+
PCR (month ~6)

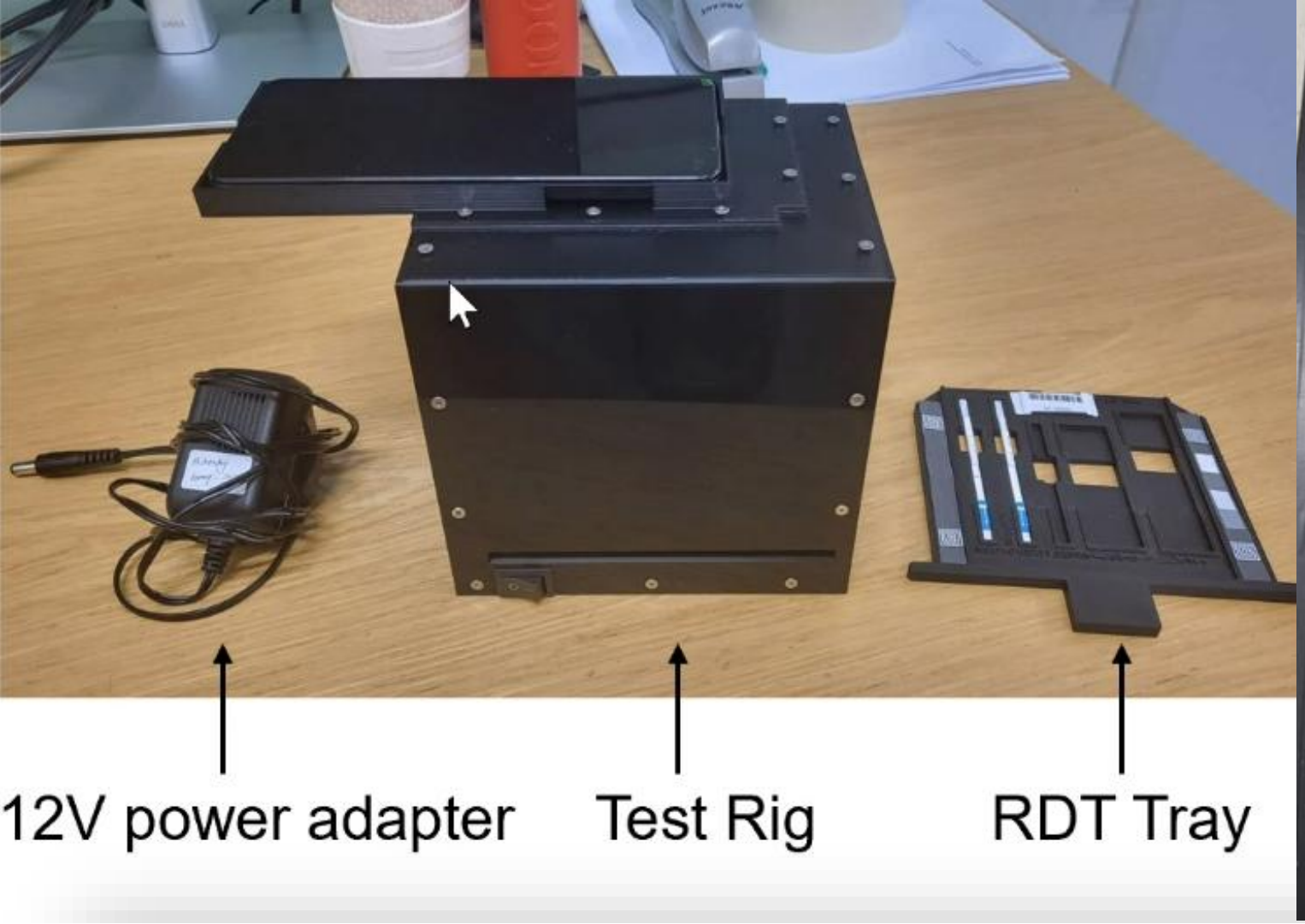


Image capture rig

Laboratory

Stool

≤ 60m
400µl stool in 4ml APW [overnight @ 37C]

1.5ml x 2 -70 ˚ C

If presumptive colonies

PCR (week ~1)

GA Plating

Slide Agglutination

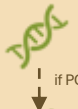
≤ 60m
400µl stool in 4ml APW [6 hours or overnight @ RT]

5 x RDT [RDT @ RT]

Image capture @ 15 & 30 min

if RDT+

Filter paper @ RT (50µl per spot)



PCR (week ~1)

if PCR+

PCR (month ~3)

if PCR+

PCR (month ~6)

≤ 60m
Direct plating on TCSB

If presumptive colonies

GA Plating

Slide Agglutination + Cholkit test

≤ 60m
1.5ml stool x 2 @ -70 ˚ C

PCR (week ~1)

5 x RDT [RDT @ RT] ≤ 60m

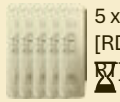
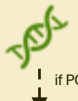


Image capture @ 15 & 30 min

if RDT+

Filter paper @ RT (50µl per spot)



PCR (week ~1)

if PCR+

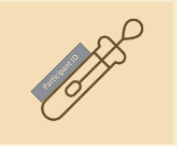
PCR (month ~3)

if PCR+

PCR (month ~6)

Rectal Swab

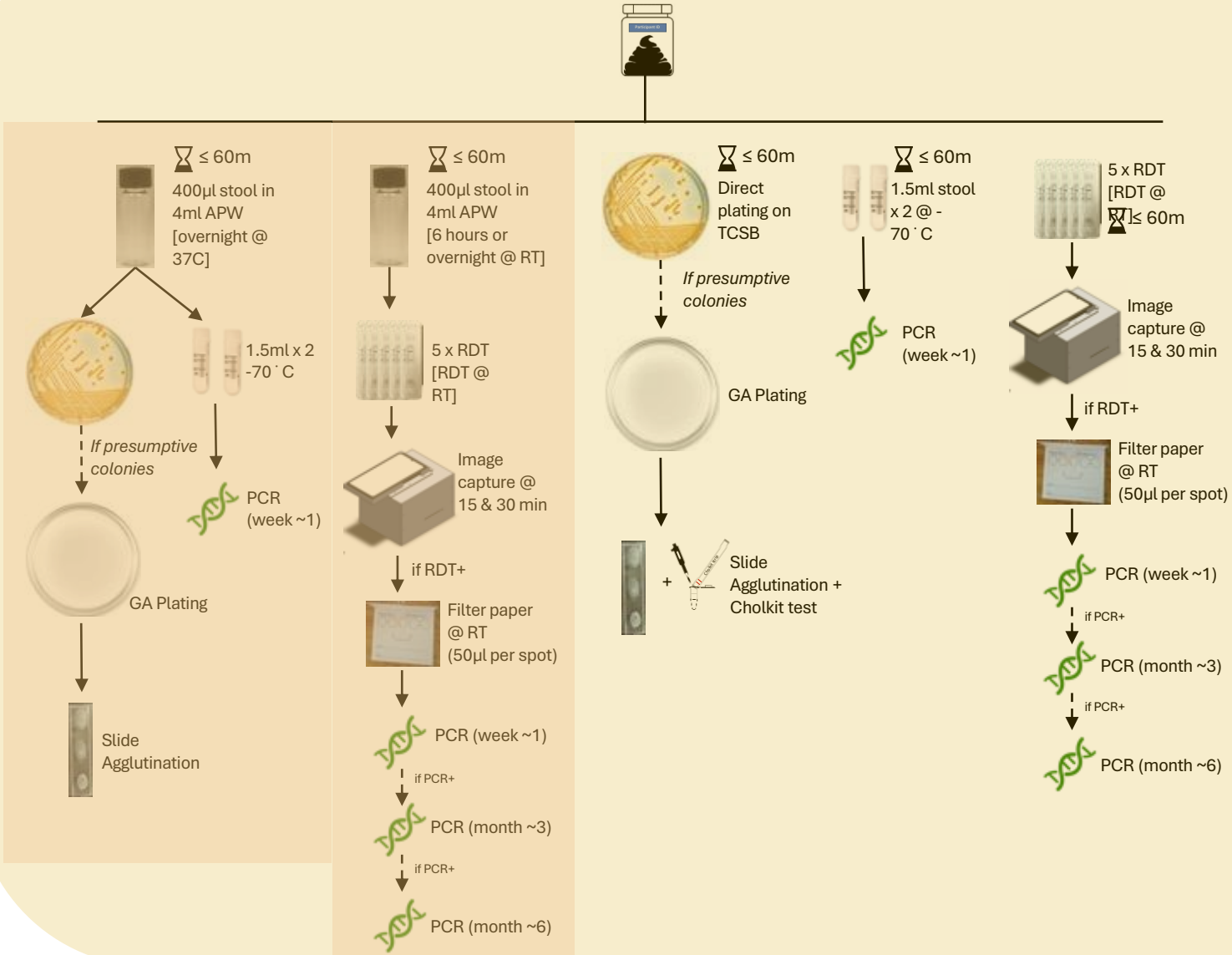
(Subset of participants ≥5 years)



Enrichment

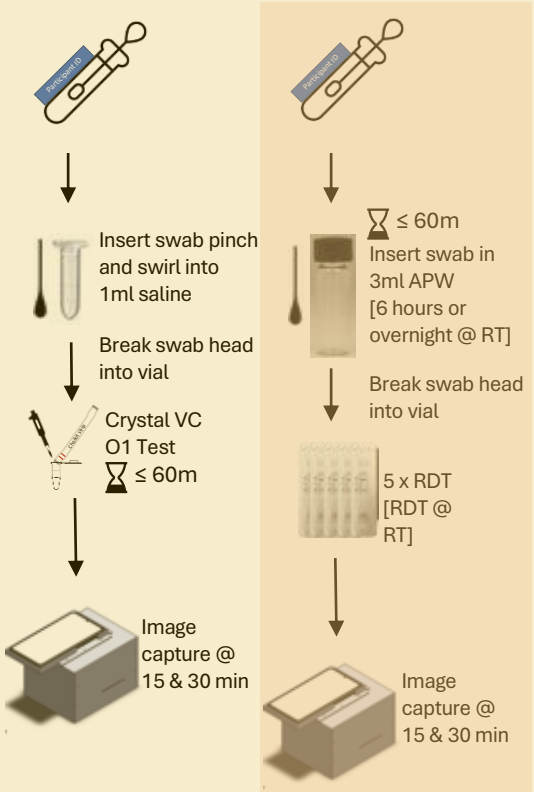
Laboratory

Stool



Rectal Swab

(Subset of participants ≥5 years)

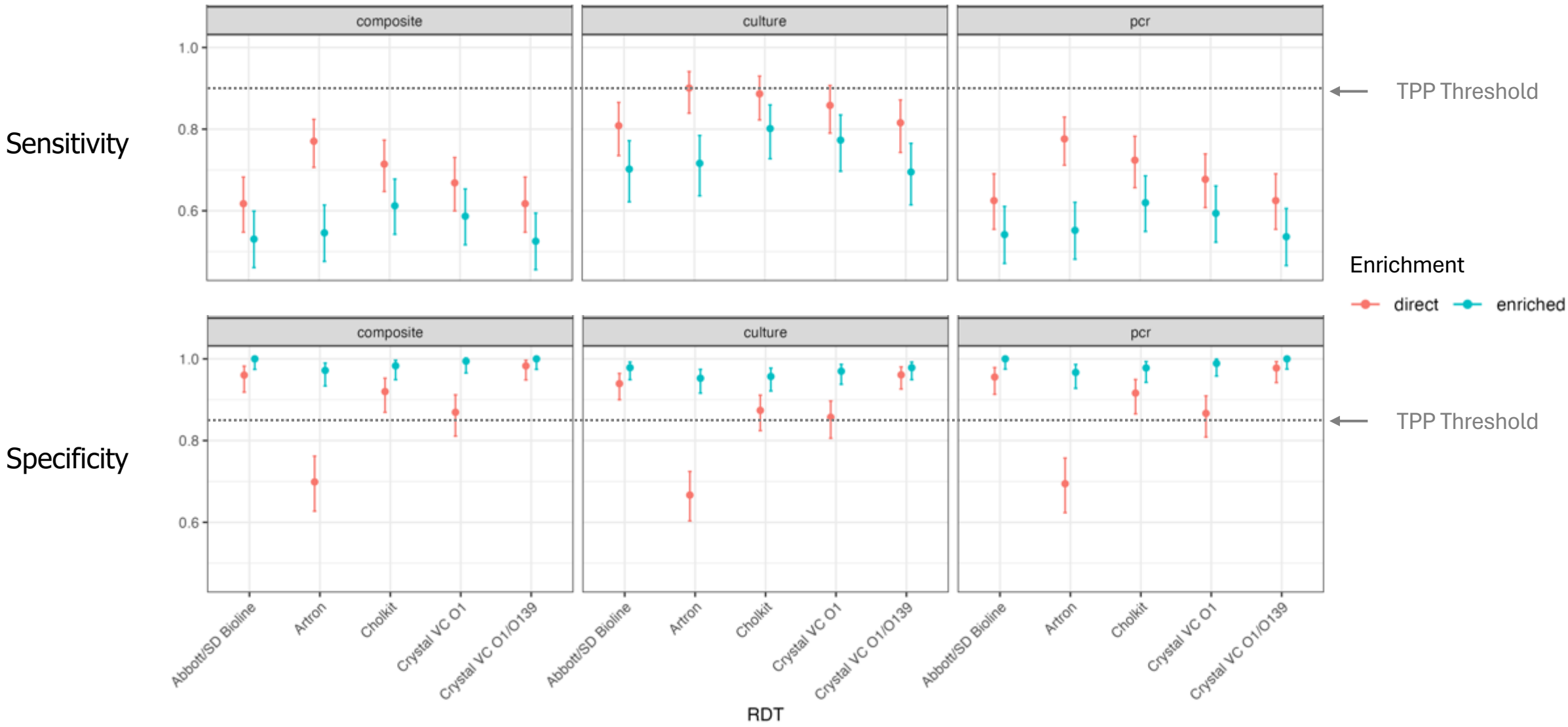


Interim results

Enrollment to date

	Winter (November – February)	Pre-monsoon (March – June)	Monsoon (July – August)	Post-monsoon (September – October)	Total
1-4 years target	0 suspected cases (0 culture confirmed)	1891 suspected cases (104 culture confirmed)	0 suspected cases (0 culture confirmed)	0 suspected cases (0 culture confirmed)	1891 suspected cases (104 culture confirmed)
1-4 years actual	**0 suspected (0 culture confirmed)**	**102 suspected cases (20 confirmed)**			**102 suspected cases (20 confirmed)**
≥5 years target	296 suspected cases (52 culture confirmed)	206 suspected cases (52 culture confirmed)	532 suspected cases (52 culture confirmed)	367 suspected cases (52 culture confirmed)	1401 suspected cases (208 culture confirmed)
≥5 years actual	**98 suspected cases (32 culture confirmed)**	**199 suspected cases (95 culture confirmed)**			**297 suspected cases (127 culture confirmed)**

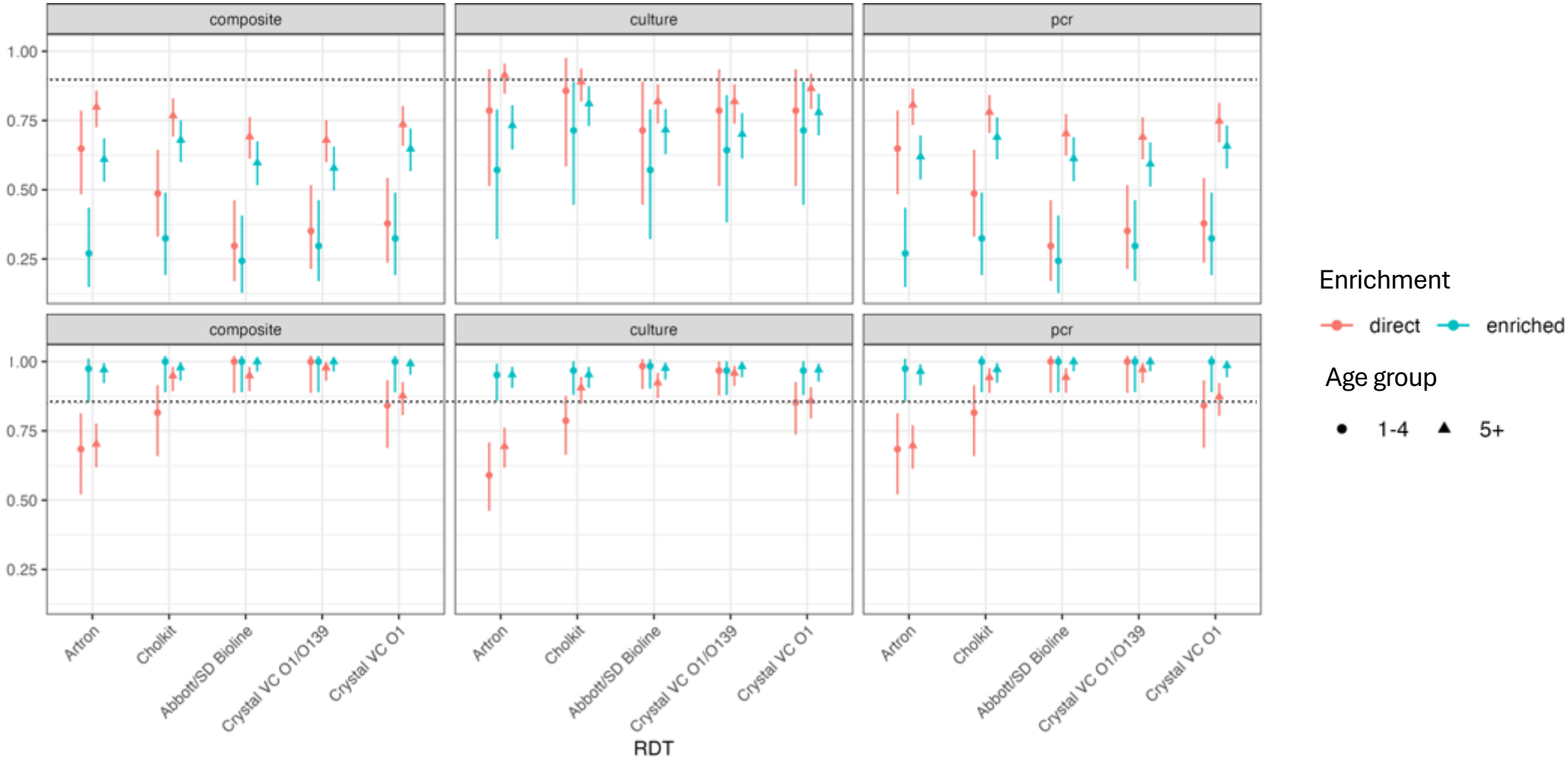
RDT test performance (all ages)



RDT test performance (by age group)

Sensitivity

Specificity



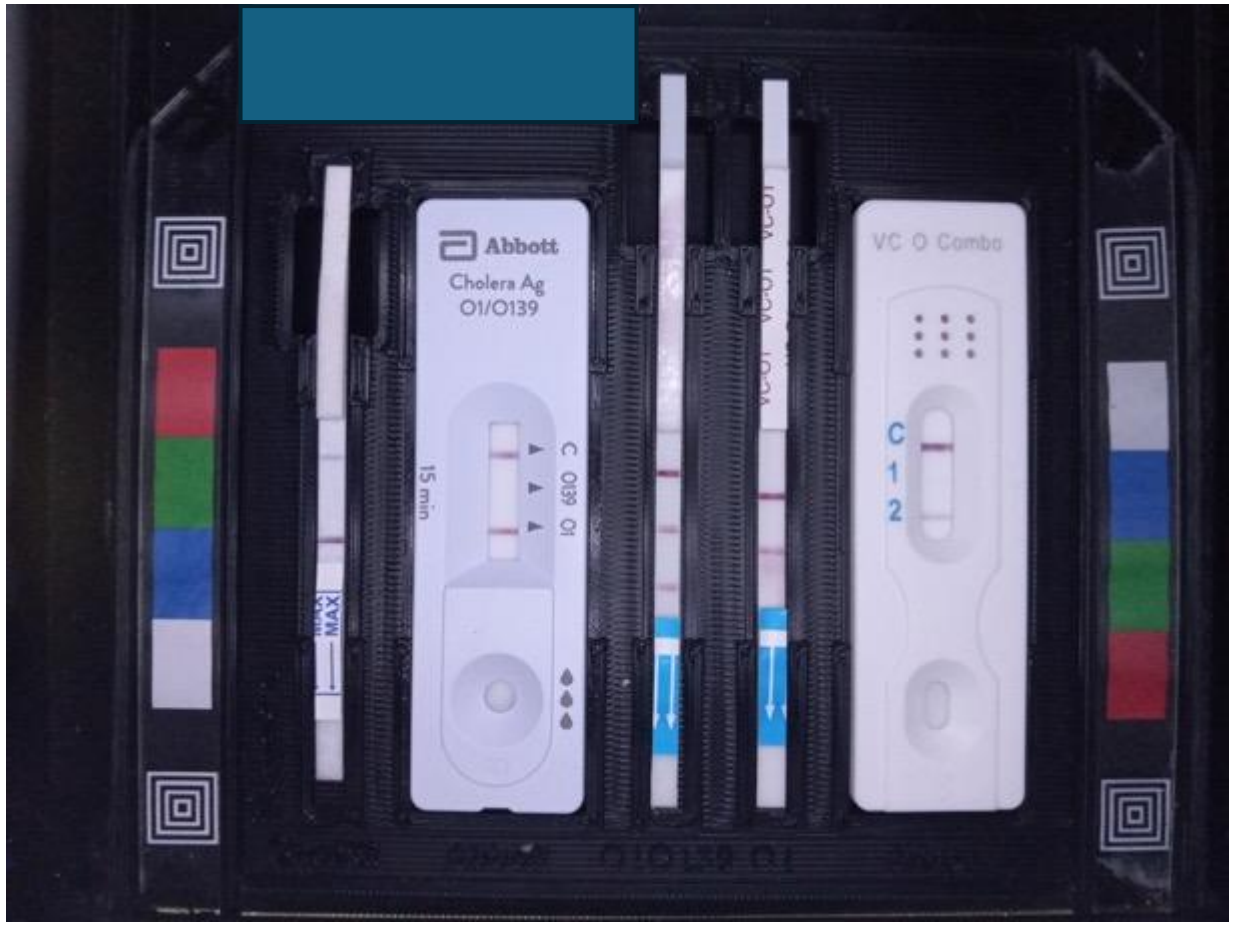
Observation of false positive VC O139 results

	Direct	Enriched
Abbott/SD	1.3% (5/372)	0.0% (0/382)
Crystal VC O1/O139	11.0% (42/372)	1.3% (5/382)

*compared to PCR and culture

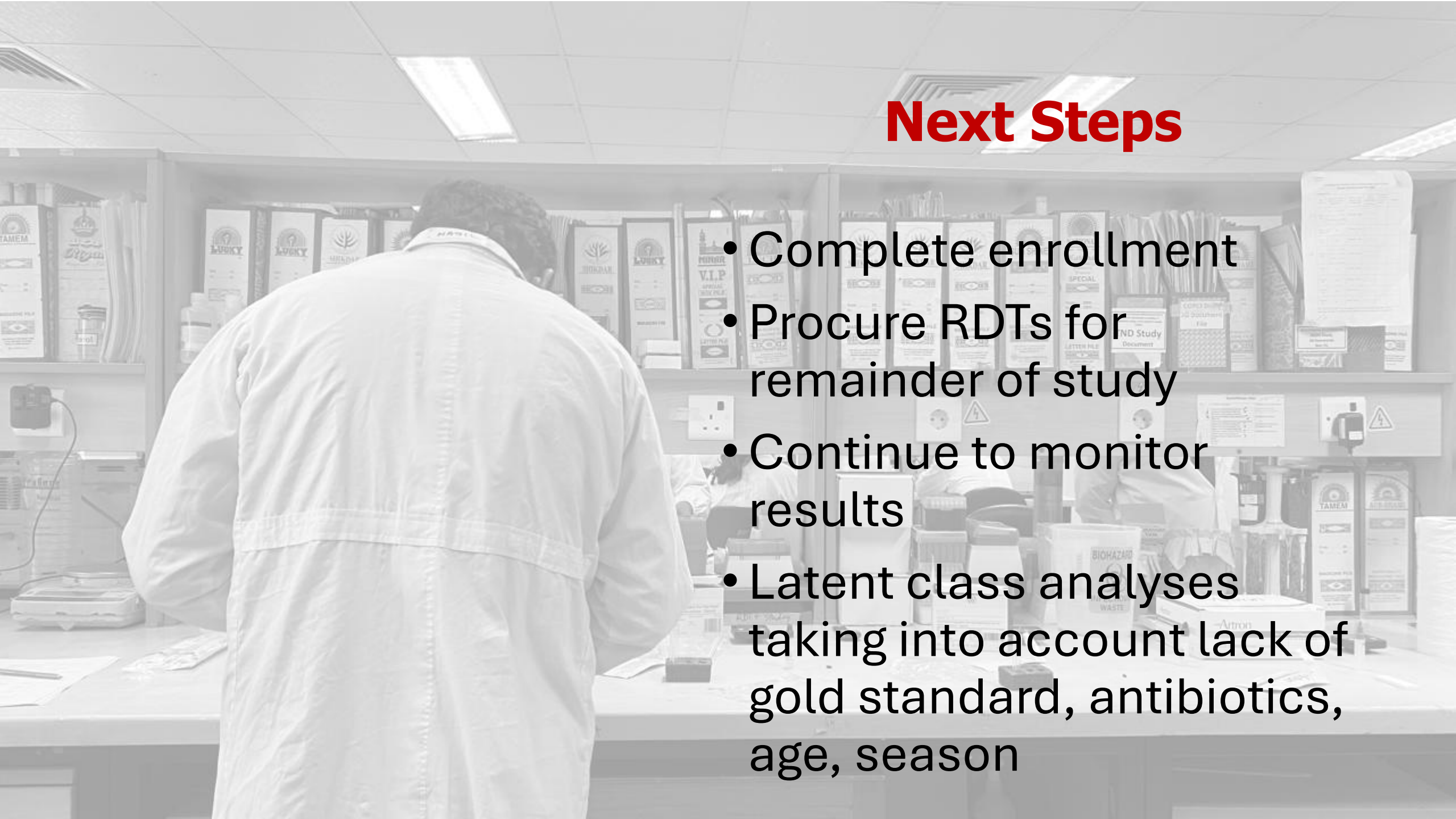
Standardized photos provide more nuanced insights into test performance


Abbott
Cholera Ag
O1/O139
15 min
C O139 O1
VC O Combo
C
1
2


Abbott
Cholera Ag
O1/O139
15 min
C O139 O1
MAX
VC O Combo
C
1
2

Interim Conclusions (interpret with caution)

- Sensitivity
  - Three tests, when used directly on stool, demonstrate performance consistent with TPP minimum of 90% sensitivity using culture as gold standard
  - No tests, direct or enriched, when compared to PCR meet TPP sensitivity minimum
- Specificity
  - All tests but one, when used directly or with enrichment, meet or exceed the TPP 80% specificity threshold.
- Age
  - Sensitivity of all tests appears reduced in young children 1-4 years old compared to others with larger effects when PCR is used
- Enrichment
  - Enrichment decreases sensitivity of all tests consistently
  - Enrichment increases specificity though variable across tests



Next Steps

- Complete enrollment
- Procure RDTs for remainder of study
- Continue to monitor results
- Latent class analyses taking into account lack of gold standard, antibiotics, age, season

Acknowledgements

icddr,b
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