RLDT - A rapid and simple molecular diagnostic assay for cholera, applicable to endemic countries

SUBHRA CHAKRABORTY, Ph. D, MPH

Associate Professor

Johns Hopkins Bloomberg School of Public Health

JOHNS HOPKINS
BLOOMBERG SCHOOL
of PUBLIC HEALTH

Diagnostics of Cholera

Simple, rapid, sensitive and specific diagnostic test for cholera facilitates

- Rapid outbreak responses (Treatment, OCV, WASH)
- Reliable surveillance data to guide long-term policies and interventions.

Current Diagnostics of Cholera

- **Clinical diagnostics** - Africhol 2018 - case definition by WHO and CDC - sensitivity 92.7%, specificity 8.1%

- **Microbiological stool culture** - current recognized gold standard, requires 2-3 days, needs lab facility and trained personal.

- **PCR** - not widely used, several methods, no standard, validated technique, need to procure reagents, competent laboratory support, technical skills, time consuming.

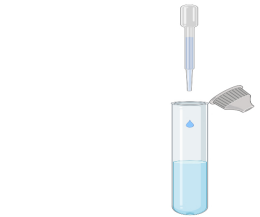
- **RDTs** – Several studies and systematic reviews of the evaluation of RDTs reported the performance characteristics did not meet the expected minimal performance recommended by the GTFCC.

RLDT

- Rapid Loop-mediated Isothermal Amplification based Diagnostic Test (RLDT)

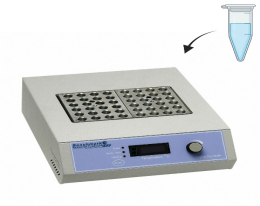
- Detects cholera *ctxA* and O1*rfb* directly from stool in <1 hour.

RLDT

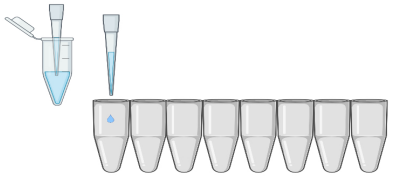


Step 1. Add sample into SPT.

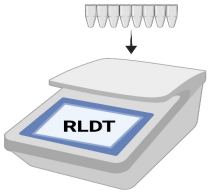
Step 2. Mix the sample with lysis buffer and add to lysis tube.



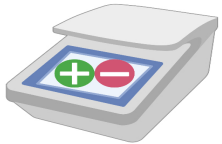
Step 3. Heat lysis.



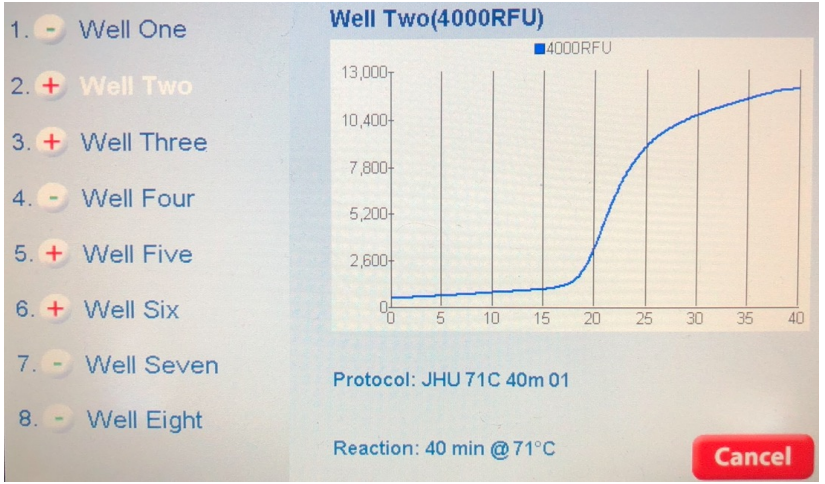
Step 4. Transfer lysate to LRTs.

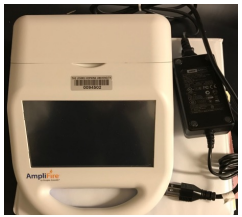


Step 5. Place LRTs into the reader.



Step 6. Read results positive/negative.





Chakraborty et al 2023 PLOS NTD

Johns Hopkins Bloomberg School of Public Health

Performance Specification tests of cholera RLDT

- Analytical sensitivity
- Analytical specificity
- Sensitivity, range
- Repeatability
- Reproducibility
- Stability
- Matrix inhibition

RLDT can be performed from fresh stool, frozen stool, dried stool on filter paper, rectal swab, and from environmental and drinking water.

Lowest Detection Limit is $4x10^4$ CFU/gm of stool

LOD: The lowest concentration at which the target could be detected in all 10 runs

Chakraborty et al 2023 PLOS NTD

Advantages of RLDT

- **Simple** - RLDT is performed directly from the stool with minimum treatment
  Hands on time – 5 minutes ; O1 and *ctxA* both detected
- **Rapid** - <1 hour from stool to result.
- **Sensitive** - LOD: ~10^4CFU/gm of stool
- **Specific** - Six primers are used for detecting each target.
- **Dry formulations** - Stable in ambient temperature, avoids maintaining a cold chain, and is mostly electricity-free.
- **No need to add any reagents -** LRTs are already filled with dry reagents and primers.
- **All reagents and plastics required are provided in the RLDT kit**
- **Easy reading results** - Results are read as +/- using a battery-operated handheld reader.
- **Minimum equipment -** Only requires a heat block and a reader.
- **Since rapid** – can perform downstream applications
- **Semiquantitative**

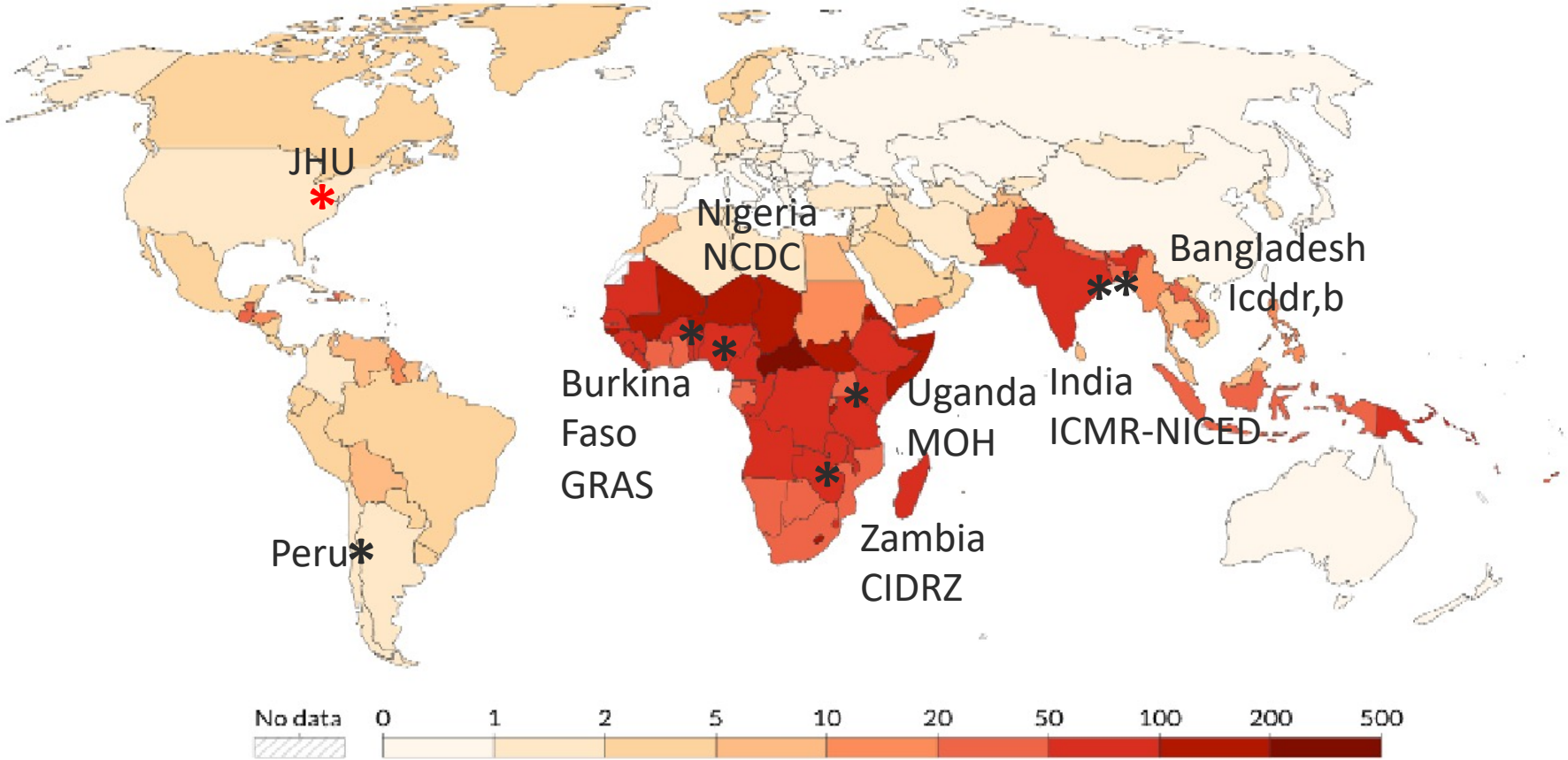
RLDT for Detection of Other Pathogens

- *Shigella spp*
- *Enterotoxigenic E. coli*
- *Campylobacter spp*
- *Salmonella typhi (from blood and stool)*
- Norovirus
- Pediatric tuberculosis

Once a site is trained and has the reader, can perform detection of all these pathogens.

Field Evaluation of RLDT

Field Evaluation of RLDT



RLDT Implemented for surveillance and Epidemiology

- Zambia (CIDRZ) : Funding EDCTP: 2000 children
- Burkina Faso (GRAS) : EDCTP: 700 children
- Mirzapur, Bangladesh (icddr,b): NIH: 1600 children
- Dhaka, Bangladesh (icddr,b): NIH: 400 children

Field Evaluation of Cholera RLDT

Field Evaluation of Cholera RLDT

Funding: Wellcome Trust

PI: Subhra Chakraborty

Trained the sites, the sites performed RLDT

- **Uganda: NHLDS, Francis Ongole (PI)**
- **Bangladesh: icddr,b, Firdousi Qadri (PI)**, Kamrul Islam, Dr. Ashraful Islam Khan, Dr. Md. Taufiqur Rahman Bhuiyan
- **Nigeria: NCDC, Tochi Okwor (PI),** Chinedu Okoroafor, Zaayanatu Nuru

Total Samples: N=665

Funding: National Institute of Health (NIH), NIAID

India: NICED (Shanta Dutta, Asish Mukhopadhaya, Goutam Chowdhury)

Implementation of Cholera RLDT

Phase I:

A Lab facility of the country

Nigeria, Uganda and Bangladesh

Implementation of RLDT in two health facilities in each country.

JHU has trained the central labs in RLDT in each country, and the central labs then trained the health facilities in their country – **Decentralization approach.**

Phase II:

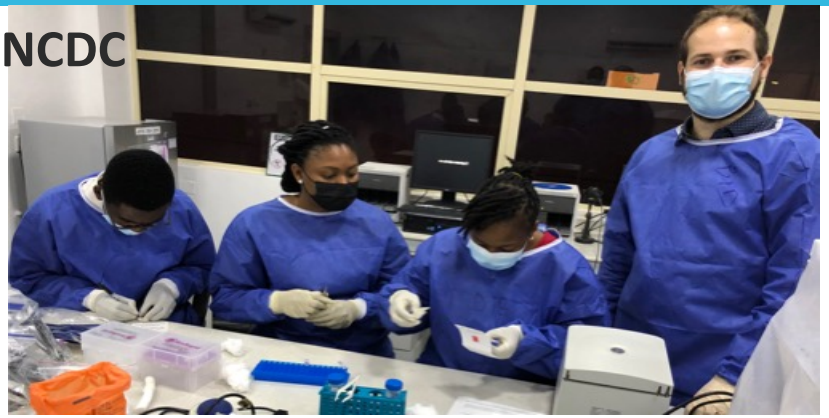
Health Facility 1 **Health Facility 2**

NIGERIA: GH Toro and State Specialist Hospital Bauchi
UGANDA: Nakivale HC and Kyangwali HC
BANGLADESH: Naraynganj HC and icddr,b hospital

Funding: Wellcome Trust

Training

NCDC



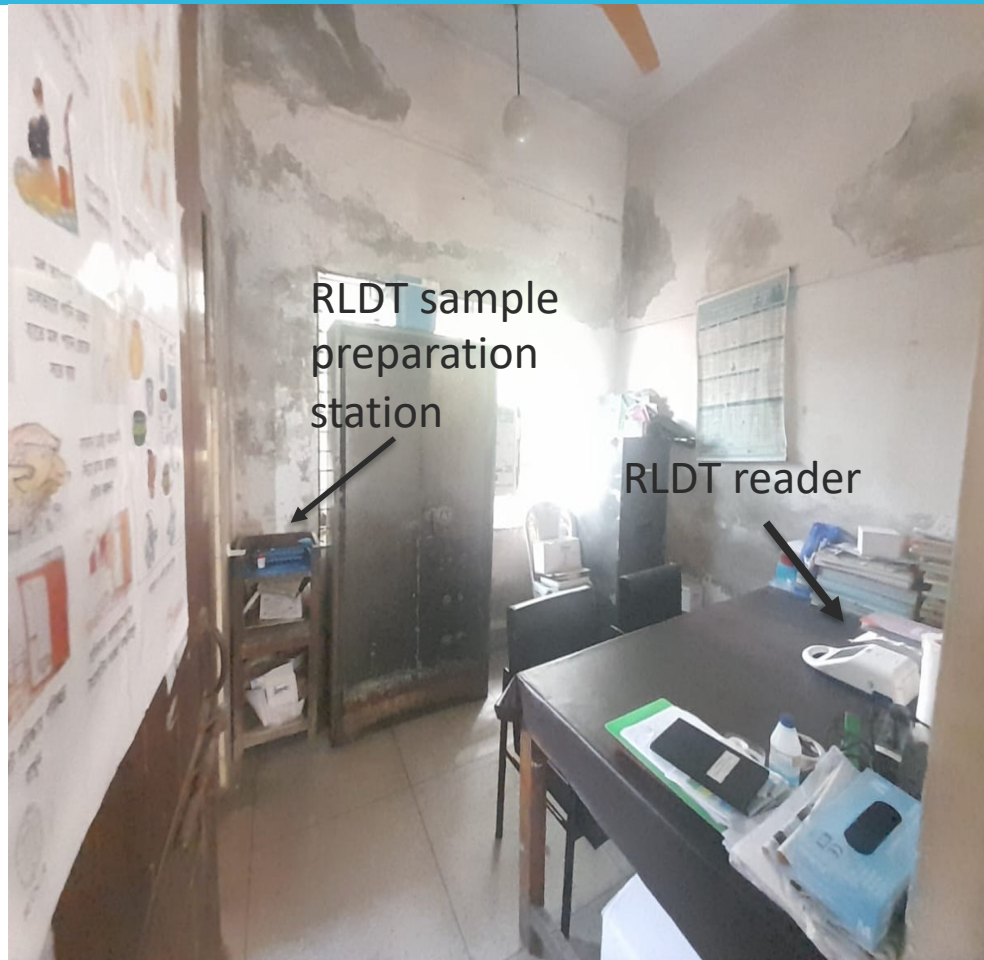
Toro

Bauchi

Bauchi

JOHNS HOPKINS
BLOOMBERG SCHOOL
of PUBLIC HEALTH

Implementation of Cholera RLDT in rural health facilities



Summary

- RLDT is a simple assay, can be applied to the endemic countries.
- RLDT is more sensitive than RDTs and culture.
- RLDT exhibited excellent and sufficient sensitivity and specificity for detection of cholera.
- RLDT could be implemented at the primary health care facilities.
- RLDT warrants broader application and evaluation as a culture-independent simple and rapid diagnostic test.
- RLDT is ready for large scale production.

PCR

Laborious process, many steps and equipment, maintaining cold chain for the reagents, time consuming, results reading depends on the end users.

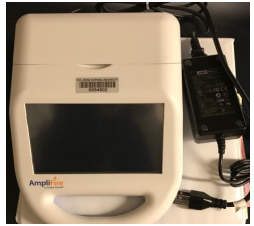
QPCR

Lateral flow RDT assays have variable sensitivity/specificity

Quality of Culture varies

RLDT

Heat Block



Battery operated, hand-held RLDT reader

Simple and rapid (<1hr) process, two steps, hands on time 5min, only heat block and reader needed, no cold chain needed, the reader reads the results as +/-

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Acknowledgement

Johns Hopkins Bloomberg School of Public Health

Chakraborty Lab
Mirza Velagic
Sean Connor
YiYi Zhou
Jessica Brubaker
Fatema Johura
Xueyan Zhang

Prof. David Sack

icddr,b
CIDRZ for a healthy Zambia 20 years
ICMR INDIAN COUNCIL OF MEDICAL RESEARCH | NICED NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES
GROUPE DE RECHERCHE GRAS ACTION EN SANTE
MINISTRY OF HEALTH REPUBLIC OF UGANDA
NCDC
wellcome
NIH National Institute of Allergy and Infectious Diseases
PATH
EDCTP
BactiVac
UKRI Medical Research Council

Thank You

schakr11@jhu.edu