External Quality Assurance in the Tropics

The LAMP4Yaws Project

Christina Ries, Simone Lüert, Michael Marks and Sascha Knauf
Institute of International Animal Health/One Health
Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health
Greifswald - Isle of Riems

1

Friedrich-Loeffler-Institut

- Independent higher federal authority under the Ministry of Food and Agriculture
- Research contract embedded in animal health law (TierGesG)
  - Diagnosis, prevention, control incl. treatment of animal diseases
- Health and welfare of food producing animals – from the honey bee to cattle
- Protection of humans against zoonoses

2

Greifswald Island Riems

3

FLI Key Figures

- 5 locations in Germany
- 12 institutes
- 89 Laboratories
- 163 stables
- 850 staff members
- 10,500 animals (~250 large animals)
- up to BSL4

4

BSL 4 – Laboratory and Stable Unit for Large Animals

5

National Reference Laboratories for more than 80 notifiable and reportable animal diseases

VIRUSES (thereof zoonoses)
Avian Influenza
Crimean-Congo Hemorrhagic Fever
Ebola Virus Infection
Equine Encephalomyelitis (all forms)
Hantavirus
Japanese Encephalitis
Monkeypox
Nipah/Hendra Virus Infection
Rabies
Rift Valley Fever
West-Nile-Virus
African Horse Sickness (AHS)
African Swine Fever (ASF)
Aujeszky's Disease (AD)
Bee diseases: 3 notifiable diseases
Bluetongue Disease (BTV)
Bov. Herpesvirus Type 1 (all forms)
Bovine Viral Diarrhea (BVD)
Crustacean diseases (including Taura Syndrom, Whitespot, Yellowhead)
Fish diseases: 5 diseases
(ISA, EHN, VHS, IHN, KHV)
Enzootic Bovine Leucosis
Epizootic Hemorrhagic Disease (EHD)
Equine Infectious Anemia (EIA)
Equine Viral Arteritis (EVA)
Foot- and Mouth-Disease (FMD)
Infectious Laryngotracheitis (ILT)
Mollusc diseases (incl. Infections with Bonamia excitosia, Bonamia ostreae, Marteilia refringens, Microcytos mackini, Perkinsus marinus)
Swine Vesicular Disease (SVD)
Maedi-Visna (MV) & CAE
Lumpy Skin Disease (LSD)
Newcastle Disease
Peste des Petits Ruminants
Rinderpest
Schmallenberg Virus Infections
Sheep and Goat Pox
Swine Fever (CSF)
Vesicular Stomatitis

PARASITES (thereof zoonoses)
Echinococcosis
Toxoplasmosis
Bovine Trichomoniasis
Dourine

BACTERIA (thereof zoonoses)
Anthrax
Bovine Salmonellosis
Bovine Tuberculosis (M. bovis & M. caprae)
Brucellosis
Campylobacteriosis (thermophilic Campylobacter)
Chlamydial Infections of Birds and Sheep
Glanders
Q Fever
Tularemia
Verotoxin-forming Escherichia coli (VTEC)
Blackleg
Bovine Genital Campylobacteriosis (BGC)
Contagious Bovine Pleuropneumonia
Contagious Equine Metritis (CEM)
Infectious Epididymitis (Brucella ovis)
Paratuberculosis

PRIONS (zoonoses)
Transmissible Spongiform Encephalopathies (TSEs) (incl. BSE, scrapie)

6

FLI and International Organisations

OIE Collaborating Centre for Zoonoses in Europe

Reference laboratories for Avian Influenza (fowl), Bee diseases, Bovine Herpesvirus Type 1 Infections, Bovine, Porcine, Ovine and Caprine Brucellosis, Chlamydial Infections of Birds and Sheep, Koi Herpesvirus Disease, Newcastle Disease, Glanders, Rabies

WHO Collaborating Centre

for research and surveillance of rabies

FAO Reference Centres

for Animal influenza & Newcastle Disease

for Classical Swine Fever

for Emerging Zoonotic Pathogens and High Biosecurity/Biocontaiment Facilities

EU Reference Centre for Animal Welfare

7

FLI on the forefront of One Health

1. Institute of Bacterial Infections and Zoonoses (IBIZ)
2. Institute of Epidemiology (IfE)
3. Institute of Immunology (IfI)
4. Institute of Infectology (IMED)
5. Institute of Molecular Pathogenesis (IMP)
6. Institute of Molecular Virology and Cell Biology (IMVZ)
7. Institute of Farm Animal Genetics (ING)
8. Institute of Novel and Emerging Infectious Diseases (INNT)
9. Institute of Animal Nutrition (ITE)
10. Institute of Animal Welfare and Animal Husbandry (ITT)
11. Institute of Diagnostic Virology (IVD)

8

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9. Institute of Animal Nutrition (ITE)
10. Institute of Animal Welfare and Animal Husbandry (ITT)
11. Institute of Diagnostic Virology (IVD)
12. **Institute of International Animal Health / One Health (IITG)** [since 2020]

Our goal is to fight animal infectious diseases and in particular zoonoses where they emerge

- Sustainable partnerships and capacity building
- International collaborative applied and basic research
- International policy counselling
- Consulting with e.g., FAO, OIE and WHO

9

Mandate

§27 (8) Tiergesundheitsgesetz (German Animal Health Law)

- Strengthening German commitment and visibility in infectious disease prevention abroad
- Capacity building and technical support for international partners

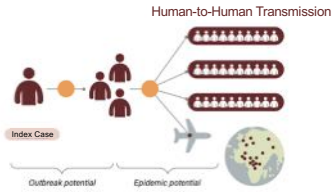
10

One Health, Neglected Tropical Diseases, and External Quality Assurance. How does this go together?

11

Disease X

Outbreak investigation

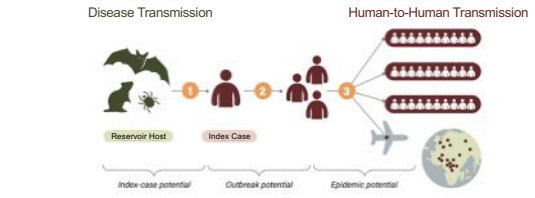


Africa CDC. 2020. modified

12

Disease X

Outbreak investigation



Africa CDC. 2020. modified

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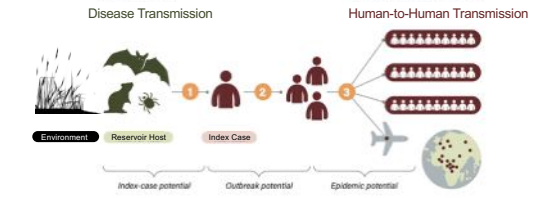
Disease X

Outbreak investigation

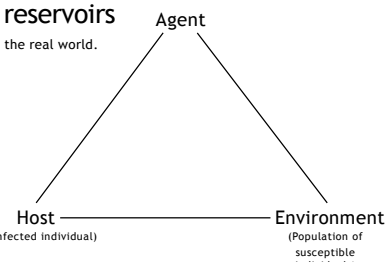


Africa CDC. 2020. modified

14

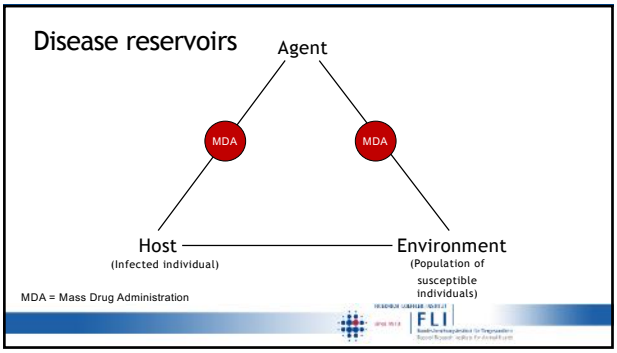
Disease reservoirs

Example from the real world.



15

Disease reservoirs



16

Disease reservoirs

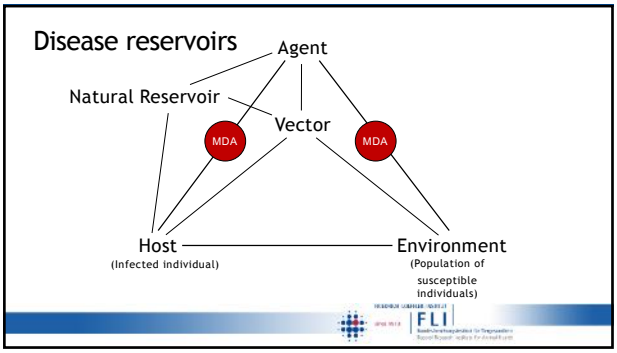
e.g. Smallpox

17

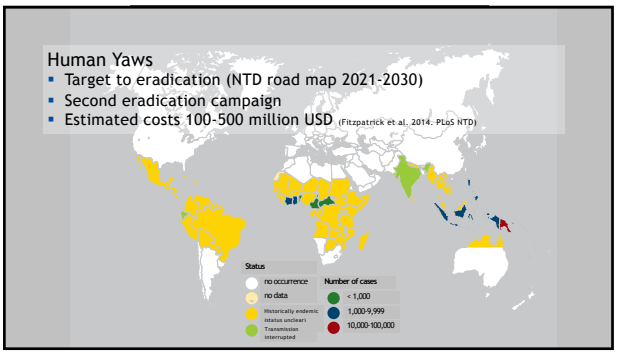
Disease reservoirs

e.g. Malaria

18


Disease reservoirs
Agent
Natural Reservoir
Vector
MDA
MDA
Host
(Infected individual)
Environment
(Population of susceptible individuals)

19


Human Yaws
Target to eradication (NTD road map 2021-2030)
Second eradication campaign
Estimated costs 100-500 million USD (Fitzpatrick et al. 2014. PLoS NTD)
Status
no occurrence
no data
Historically endemic (status unclear)
Transmission interrupted
Number of cases
< 1,000
1,000-9,999
10,000-100,000

20

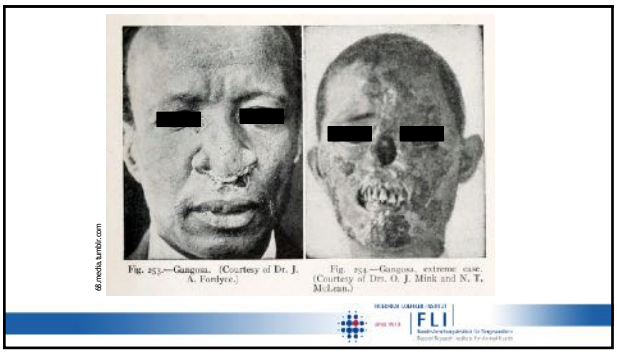


Fig. 253.—Gangosa. (Courtesy of Dr. J. A. Fordyce.)
Fig. 254.—Gangosa, extreme case. (Courtesy of Drs. O. J. Mink and N. T. McLean.)

21

Papio anubis at Tarangire National Park, Tanzania

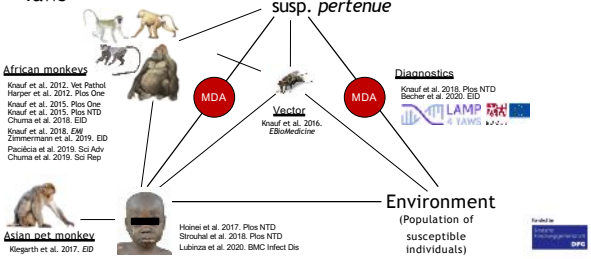
CORRESPONDENCE Open Access

Nonhuman primates across sub-Saharan Africa are infected with the yaws bacterium *Treponema pallidum* subsp. *pertenue*

SCIENTIFIC REPORTS

OPEN Strain diversity of *Treponema pallidum* subsp. *pertenue* suggests rare interspecies transmission in African nonhuman primates

22



23

Yaws

- Spirochete *Treponema pallidum* subsp. *pertenue*

subsp. *pallidum* (syphilis) | subsp. *pertenue* (yaws) | subsp. *endemicum* (bejel)

- Chronic infection of the skin, bone and cartilage
- Poor access to water and sanitation are known predisposing factors

24

Why Do We Need Diagnostic Tests for Yaws?

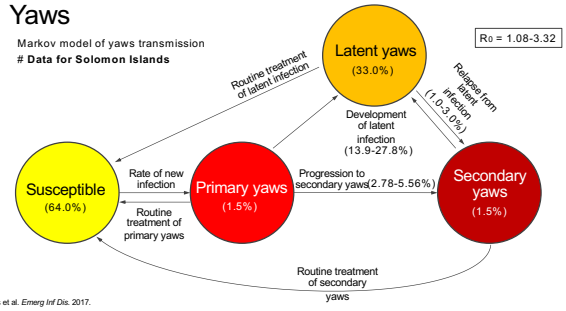
- Many skin diseases can look like yaws (*Treponema pallidum* vs. *Haemophilus ducreyi)*
- It can be difficult to tell the difference by observation



25

Yaws

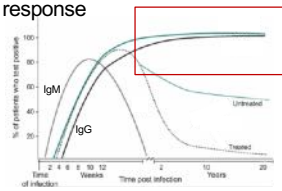
Markov model of yaws transmission
 # Data for Solomon Islands



Marks et al. *Emerg Inf Dis.* 2017.

26

Treponema pallidum elicits a strong antibody response

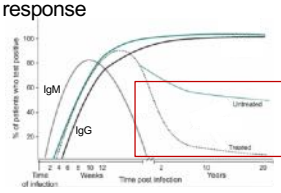


Treponemal tests
- Antibody against *T. pallidum* OMPs
- High specificity
- Lifetime seropositivity

Nontreponemal tests
- Antibody against cardiolipids
- Lower specificity
- Treatment control

27

Treponema pallidum elicits a strong antibody response



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cp.neurology.org

28

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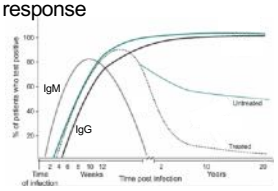

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ESPLINE TP (Fujirebio Inc.)

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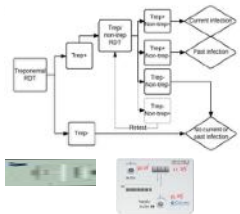
29

Challenges in Yaws Diagnosis



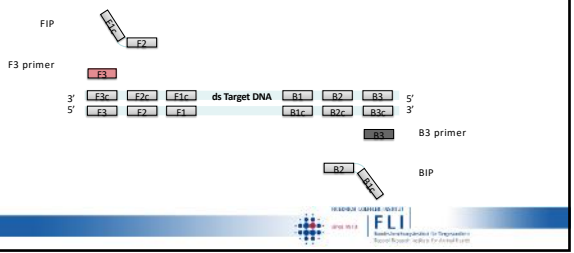
WHO yaws eradication approach
- Mass treatment at the district/sub-district level with azithromycin followed by active-case finding
- Usage of 2-stage RDT screening strategy

- Lack of discrimination between *TP* subsp.
- Low specificity of non-treponemal tests
- Treponemal test are life-long positive
- Problems in early infection detection
- Yaws-like ulcers (*e.g.*, *Haemophilus ducreyi*)
- Antimicrobial resistant strains

30

Loop-Mediated Isothermal Amplification

Primer binding sites on the target DNA

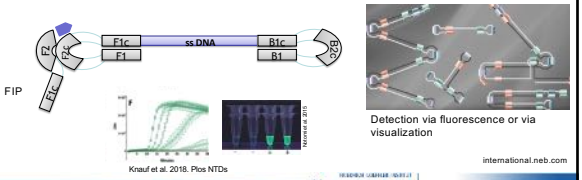


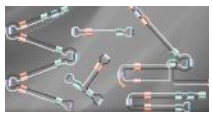
31

Loop-Mediated Isothermal Amplification

Additional loop primers can enhance amplification efficacy

Start template for chain reaction and exponential amplification





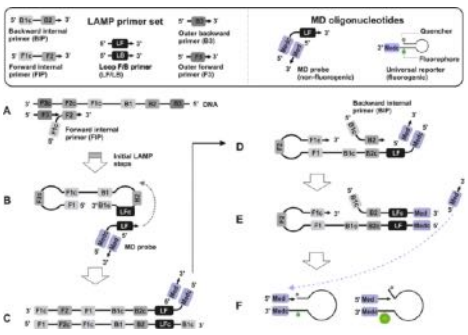
Detection via fluorescence or via visualization

Knauf et al. 2018. Plos NTDs

international.neb.com

32

Multiplex: Mediator displacement LAMP



Becker et al. 2018. Analytic Chem

33

TPHD-LAMP

- Mediator displacement LAMP – *Treponema pallidum* (*pol A* gene) and *Hemophilus ducreyi* (16S rRNA gene) amplification
- Limit of Detection (LoD) similar to qPCR (Becherer et al, 2020. EID)
- High analytical sensitivity and specificity of the single plex assay
  - *T. pallidum:* 84.7% sensitivity/95.7% specificity
  - *H. ducreyi:* 91.6% sensitivity/84.8% specificity
  (Becherer et al, 2020. EID)
- Isothermal and rapid (64°C for 45-60 minutes)

mast-group.com

34

TPHD-LAMP

Advantages
- Sensitive, specific and fast
- No need for sophisticated thermal management
  - Cheaper laboratory equipment
  - Point-of-care/point-of-need tests
- Adaptability to include azithromycin resistance detection

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35

TPHD-LAMP

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- Sensitive, specific and fast
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  - Cheaper laboratory equipment
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- Adaptability to include azithromycin resistance detection

Disadvantages
- Not high-throughput
- Oligos required

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36

LAMP 4 YAWS

Clinical Evaluation of a Loop-Mediated Isothermal Amplification Test for *Treponema pallidum pertenue*

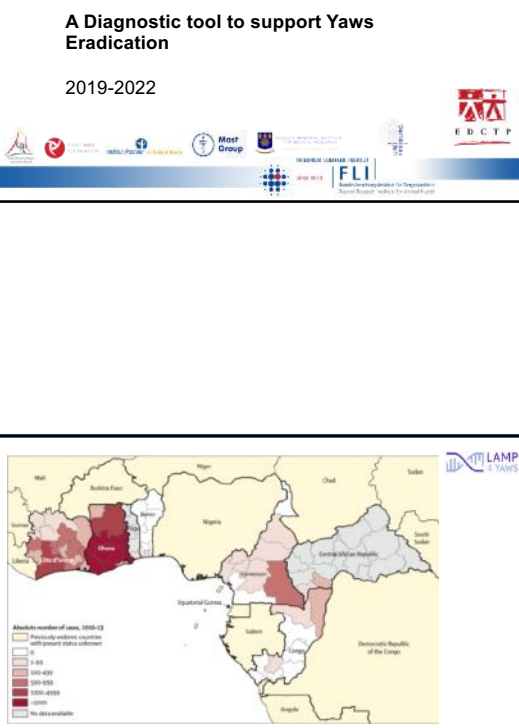
A Diagnostic tool to support Yaws Eradication

2019-2022

EDCTP

FLI

37



Mitjà et al. 2015. Lancet Global Health

LAMP 4 YAWS

FLI

38

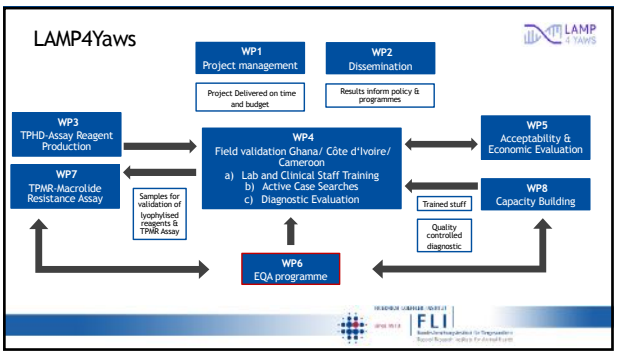
LAMP4Yaws

- Validation of a TPHD-LAMP assay at the district level
- Capacity Building
- External quality assurance programme
- Ease detection of macrolide resistance (LAMP-based)
- Outreach and dissemination of the project's primary and final outcomes

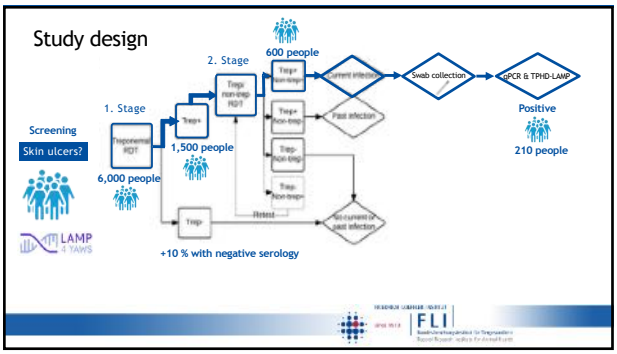
LAMP 4 YAWS

FLI

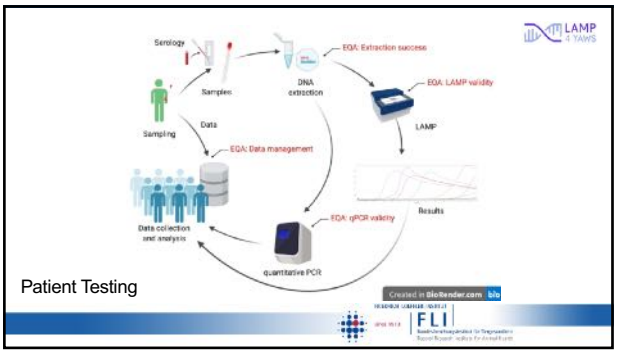
39


LAMP4Yaws
WP1
Project management
WP2
Dissemination
Project Delivered on time and budget
Results inform policy & programmes
WP3
TPHD-Assay Reagent Production
WP4
Field validation Ghana/ Côte d'Ivoire/ Cameroon
a) Lab and Clinical Staff Training
b) Active Case Searches
c) Diagnostic Evaluation
WP5
Acceptability & Economic Evaluation
WP7
TPMR-Macrolide Resistance Assay
Samples for validation of lyophylised reagents & TPMR Assay
WP8
Capacity Building
Trained stuff
Quality controlled diagnostic
WP6
EQA programme

40


Study design
600 people
2. Stage
Swab collection
qPCR & TPHD-LAMP
Positive
210 people
1. Stage
Screening
Skin ulcers?
1,500 people
6,000 people
Past infection
+10 % with negative serology

41


Serology
Samples
Sampling
Data
DNA extraction
EQA: Extraction success
EQA: LAMP validity
LAMP
EQA: Data management
Results
Data collection and analysis
EQA: qPCR validity
quantitative PCR
Patient Testing

42

High quality laboratory work is the backbone of any disease control, elimination or eradication

43

Problem Statement

Many NTD laboratories believe that they could act as a reference laboratory, but only a fraction of a study reporting institutes applied national and international standards or were enrolled in a EQA/PT program. (Dean et al. 2018)

Training in 'Good Laboratory Practice` paired with internal and external quality control is the only way to achieve this goal and to provide objective evidence for a laboratory's credibility of reporting valid test results within a given health program.

44

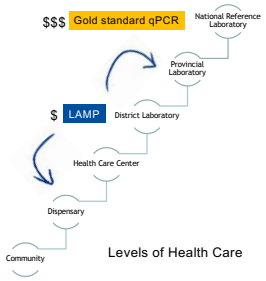
External Quality Assurance

- Comparison on the level of
  - Peers (inter-laboratory)
  - Reference laboratories
  - Testing sites

45

External Quality Assurance

- Comparison on the level of
  - Peers (inter-laboratory)
  - Reference laboratories
  - Testing sites



46

External Quality Assurance

E	On-site monitoring	Regular
Q	Organisation of proficiency test rounds	Bi-annual
A	Retesting of a subset of samples (LAMP4Yaws 20% pos/5% neg)	Yearly

47

External Quality Assurance

Expected outcome

- Comparison of performance and results among different test sites
- Early warning for systemic problems
- Objective evidence of testing quality
- Identification
  - areas that need improvement
  - Training needs

Hazard Analysis Critical Control Point (HACCP)

48

External Quality Assurance in Low Income Settings

Recognise the needs for

- Significant resource limitations
- Sample shipping (quality assurance of PT items)
- Unexperienced laboratory workers
- Weak communication infrastructure

49

External Quality Assurance

Situational analysis - risk assessment

- Existing national disease programs
- Laboratory quality management measures
- Extent and effectiveness of internal quality (process) control procedures
- Supervisory and mentoring networks
- Supply chain management system
- Type of laboratory/testing sites (clinical or public health) and their location (zonal regional/provincial, district, primary community)
- Infrastructure in the laboratories (power supply, water supply, distilled water supply, drainage, waste disposal)
- Calibrators, Proficiency test items and their supply
- Internet and mobile communication
- Existing infrastructure for transport of proficiency test items
- Number of tests performed per year, laboratory techniques, frequency of their use
- Technical and medical professionals and their levels of training, experience and qualifications

50

PT Design

Whole organism or synthetic gene ?

polA gene of *T. pallidum* | 16SrRNA gene of *H. ducreyi*

- Contamination risk high with plasmids
- Distinguishable from wild type
- Stability (can be lyophylized for shipping)

51

PT Design

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polA gene of *T. pallidum* | 16SrRNA gene of *H. ducreyi*

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- Stability (can be lyophylized for shipping)

Transformation of plasmid DNA using *E.coli*

- Sustainable supply for NRF (TP is extremely difficult to culture!)

52

PT Design

Plasmid Map

5' Restriction Site: NONE
3' Restriction Site: NONE
Cloning: via Type IIS restriction enzymes
(Type IIS sites not present in final plasmid)

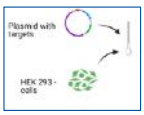
MCS of pEX-A128

eurofins

53

PT Design

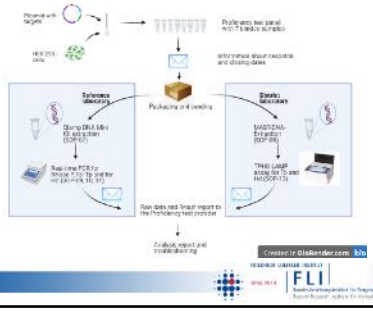
	1	2	3	4	5	6	7
T. pallidum (plasmid/µl)	10E6	10E5	10E2	0	10E4	0	10E2
H. ducreyi (plasmid/µl)	10E6	10E2	1006	0	0	10E4	10E1
HEK293 (eukaryotic cell)	10E6	10E5	1006	1006	1006	10E5	1006



- HEK293 human cells (background) to test extraction success
- TP/HD copy number checked by ddPCR
- Duplicates for reproducibility
- Blinded
- Can be send dry at environmental temperature

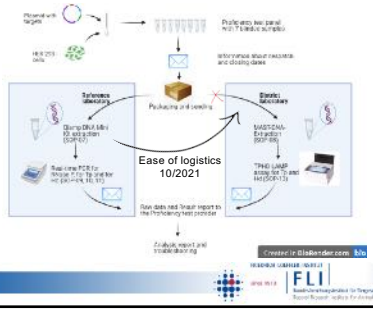
54

PT Design



55

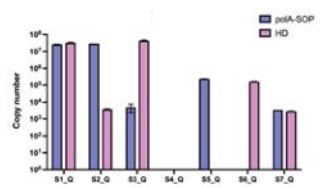
PT Design



56

PT Design

Batch testing
qPCR



HEK	all swabs contained 10e6 cells						
TP	10e9	10e9	10e5	0	10e7	0	10e5
HD	10e9	10e5	10e9	0	0	10e7	10e5
ng DNA/µl	35	26	23	101	79	98	25

Cutoff C_{T50}

57

PT Design

Plasmid stability testing	Panels	Swabs
-80°C (1, 3 & 6 months)	3	21
60% humidity		
room temperature (1, 3 & 6 months)	3	21
37°C (1, 3, 6 months)	3	21
90% humidity		
room temperature (1, 3 & 6 months)	3	21
37°C (1, 3 & 6 months)	3	21
Sum	15	105

For the qPCR (need to be determined)

For the LAMP (Becherer et al, 2020)

fishersci.de
mast-group.com

58

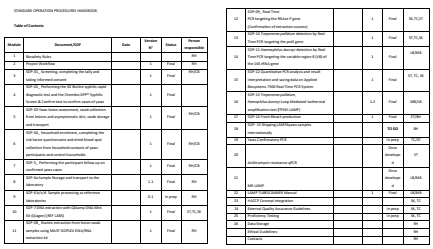
External Quality Assurance vs. Standard Operation Protocols

EQA guarantees the implementation of internal quality control, PT and quality improvement

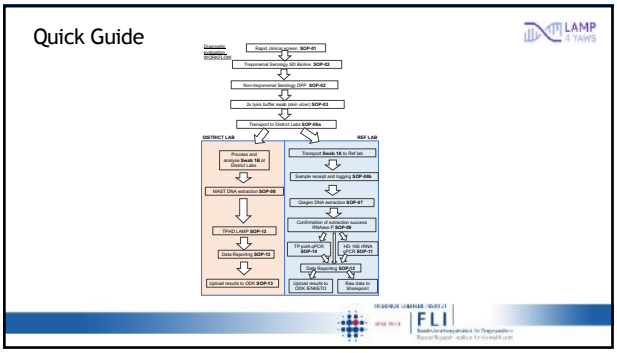
SOP is a instalment important towards quality assurance and reproducibility

59

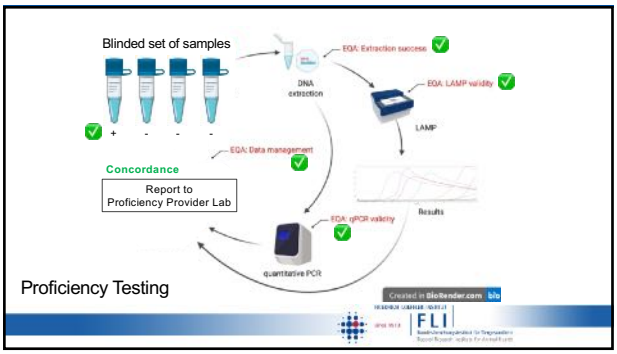
SOPs



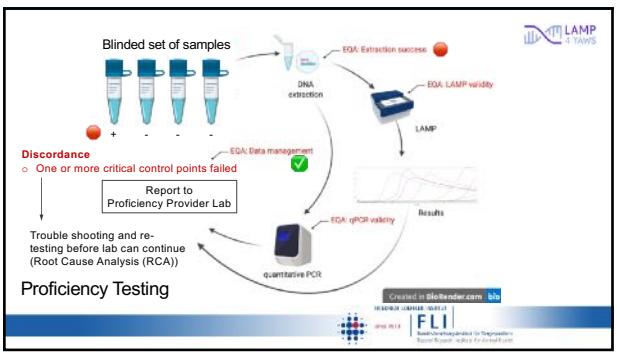
60


Quick Guide
LAMP 4 YAWS
DISTRICT LAB
REF LAB
FLI

61


Blinded set of samples
DNA extraction
EQA: Extraction success
EQA: LAMP validity
LAMP
EQA: Data management
Concordance
Report to Proficiency Provider Lab
Results
EQA: qPCR validity
quantitative PCR
Proficiency Testing
Created in BioRender.com
FLI

62


LAMP 4 YAWS
Blinded set of samples
DNA extraction
EQA: Extraction success
EQA: LAMP validity
LAMP
EQA: Data management
Discordance
One or more critical control points failed
Report to Proficiency Provider Lab
Trouble shooting and re-testing before lab can continue (Root Cause Analysis (RCA))
Results
EQA: qPCR validity
quantitative PCR
Proficiency Testing
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63

EQA Summary

- EQA standard mean of quality assurance in medical laboratory diagnostics (HACCP)
- Allows objective comparison of test results between
  - Peer group of laboratories
  - Reference laboratory
  - Testing sites

EQA and proficiency testing (PT) is a prerequisite to guarantee correct, standardized and comparable diagnostic tests within and across health care programs.

64

Conclusion

- EQA programmes are an essential need for NTD programmes (currently only the Buruli ulcer programme includes EQA)
- More EQA programmes on the district level in Africa needed (LAMP4Yaws EQA work in progress)
- Intersectoral programmes required to adequately address the One Health approach (bridging human and animal health, zoonoses)
- Combine multiple programmes to save resources

65

London School of Hygiene &Tropical Medicine
Michael Marks
Becca L Handley
Emma Harding-Esch

Fundación Privada de Lucha contra el sida
Oriol Mitjà
Camila González Beiras

Centre Pasteur Cameroun
Serges Tchatchouang
Sara Eyangoh
Patrick Awonda
Jean P. Ndzomo-Ngono

Noguchi Memorial Institute for Medical Research, University of Ghana
Laud Antony Basing
Ivy Amanor
Daniel Kojo Arhinful
Kennedy Kwasi Addo

Mast Diagnostica GmbH
Mohammed Bakheit
Sieghard Frischmann
Emelie Landmann

Institut Pasteur de Côte d'Ivoire
Solange Ngazoa Kakou
Kouadio Aboh Hugues
Aboubacar Sylla
Mireille S. Kouamé-Sina
Adingra Tano

Albert-Ludwigs-Universität Freiburg
Lisa Becherer
Nadine Borst
Tamara Haerpfer

Institut Pasteur de Madagascar
Tania Crucitti

Friedrich-Loeffler-Institut
Sascha Knauf
Simone Lueert
Christina Ries

National Yaws, Leishmaniasis, Leprosy and Buruli ulcer Control Programme, Ministry of Public Health, Yaounde, Cameroon,
Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, West Region, Cameroon
Earnest Njih-Tabah

66


Never Stop Exploring.
Welcome to the One Health Community.
PD Sascha Knauf, PhD habil.
Institute of International Animal Health/One Health
Friedrich-Loeffler-Institut
sascha.knauf@fli.de
@knauf_lab
FLI

67