External Quality Assurance in the Tropics
The LAMP4Yaws Project

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Friedrich-Loeffler-Institut
Greifswald - Isle of Riems

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

Greifswald Island Riems
FLI Key Figures
- 5 locations in Germany
- 12 institutes
- 89 laboratories
- 163 stables
- 850 staff members
- up to BSL4

BSL 4 - Laboratory and Stable Unit for Large Animals

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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reference Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian Influenza</td>
<td>Geelong</td>
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<tr>
<td>Crimean-Congo Hemorrhagic Fever</td>
<td>Winnipeg</td>
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<tr>
<td>Ebola Virus</td>
<td>Isle of Riems</td>
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<tr>
<td>Equine Encephalomyelitis</td>
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<tr>
<td>Hantavirus</td>
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<td>Japanese Encephalitis</td>
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<td>Monkeypox</td>
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<td>Nipah/Hendra Virus Infection</td>
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<td>Rabies</td>
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<td>Rift Valley Fever</td>
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<td>West-Nile - Virus</td>
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<td>African Horse Sickness (AHS)</td>
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<td>African Swine Fever (ASF)</td>
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<td>Aujeszky's Disease (AD)</td>
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<td>Bee diseases: 3 notifiable diseases</td>
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<td>Bluetongue Disease (BTV)</td>
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<td>Bov. Herpesvirus Type 1 (all forms)</td>
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<tr>
<td>Bovine Viral Diarrhea (BVD)</td>
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<td>Crustacean diseases (including Taura Syndrome, Whitespot, Yellowhead)</td>
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<td>Fish diseases: 5 diseases (ISA, EHN, VHS, IHN, KHV)</td>
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<td>Enzootic Bovine Leucosis</td>
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<td>Infectious Laryngotracheitis (ILT)</td>
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<td>Mollusc diseases (incl. Infections with Bonamia excitosia, Bonamia ostreae, Marteilia refringens, Microcytos mackini, Perkinsus marinus)</td>
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<tr>
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<td>Maedi-Visna (MV) &amp; CAE</td>
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<td>Rinderpest</td>
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<td>Schmallenberg Virus Infections</td>
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<td>Sheep and Goat Pox</td>
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<td>Swine Fever (CSF)</td>
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<td>Vesicular Stomatitis</td>
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VIRUSES (thereof zoonoses)

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<tr>
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<tr>
<td>Toxoplasmosis</td>
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<tr>
<td>Bovine Trichomoniasis</td>
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<tr>
<td>Dourine</td>
<td>WinnPEG</td>
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<tr>
<td>Anthrax</td>
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<tr>
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</tr>
<tr>
<td>Bovine Tuberculosis (M. bovis &amp; M. caprae)</td>
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<td>Glanders</td>
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<tr>
<td>Q Fever</td>
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<tr>
<td>Tularemia</td>
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<td>Verotoxin-forming Escherichia coli (VTEC)</td>
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PRIONS (thereof zoonoses)

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<tr>
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<td>(incl. BSE, scrapie)</td>
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FLI and International Organisations
OIE Collaborating Centre for Zoonoses in Europe
WHO Collaborating Centre
FAO Reference Centres
EU Reference Centre for Animal Welfare

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)
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
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

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

Disease X
Outbreak investigation

Human-to-Human Transmission
Disease reservoirs

Agent

Host

Infected individual

Environment

Population of susceptible individuals

MDA = Mass Drug Administration

E.g. Smallpox

E.g. Malaria
Disease reservoirs

Agent

Natural Reservoir

Host (susceptible individuals)

Vector

Environment (population of susceptible individuals)

1. Disease reservoirs

2. Human Yaws
   - Target to eradication (NTD road map 2021-2030)
   - Second eradication campaign
   - Estimated costs 100-500 million USD

3. Estimated costs: 100-500 million USD

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19. Human Yaws
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    - Estimated costs 100-500 million USD

20. Human Yaws
    - Target to eradication (NTD road map 2021-2030)
    - Second eradication campaign
    - Estimated costs 100-500 million USD

21. Human Yaws
    - Target to eradication (NTD road map 2021-2030)
    - Second eradication campaign
    - Estimated costs 100-500 million USD
Papio anubis at Tarangire National Park, Tanzania

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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

Yaws

Markov model of yaws transmission

Susceptible

Primary yaws

Secondary yaws

Latent yaws

Rate of new infection

Routine treatment of primary yaws

Development of latent infection

Progression to secondary yaws

Routine treatment of secondary yaws

Markov et al. Emerg Inf Dis. 2017

Treponema pallidum elicits a strong antibody response

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

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

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Treponema pallidum elicits a strong antibody response

- **Treponemal tests**
  - Antibody against *T. pallidum* C5P
  - High specificity
  - Lifetime seropositivity

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

ESPLINE TP (Fujirebio Inc.)

Challenges in Yaws Diagnosis

- Whole 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 sub-p.
- Low specificity of nontreponemal tests
- Treponemal test are life-long positive
- Problems in early infection detection
- New disease strains (e.g., *Haemophilus ducreyi*)
- Antimicrobial resistant strains
Loop-Mediated Isothermal Amplification

Primer binding sites on the target DNA

Additional loop primers can enhance amplification efficacy

Start template for chain reaction and exponential amplification

Detection via fluorescence or via visualization

Multiplex: Mediator displacement LAMP

Detection via fluorescence or via visualization
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: 94.7% sensitivity/95.7% specificity
- H. ducreyi: 91.6% sensitivity/84.8% specificity

Isothermal and rapid (84 °C for 45-60 minutes)

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

Disadvantages
- Not high-throughput
- Oligos required
Clinical Evaluation of a Loop-Mediated Isothermal Amplification Test for Treponema pallidum pertenue

A Diagnostic tool to support Yaws Eradication

2019-2022

LAMP4Yaws

- Validation of a TPHO-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
High quality laboratory work is the backbone of any disease control, elimination or eradication.

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/P 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.

External Quality Assurance

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

Gold standard qPCR
Comparison on the level of:
- Peers (inter-laboratory)
- Reference laboratories
- Testing sites

On-site monitoring
- Regular

Organisation of proficiency test rounds
- Bi-annual

Retesting of a subset of samples (LAMP Yaws 20% pos/5% neg)
- Yearly

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)
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

Situational analysis - risk assessment

Existing national disease programs
Supply chain management system
Type of laboratory testing items (clinical or public health) and their locations (national, regional, provincial, district, primary community)
Existing infrastructure for transport of proficiency test items
Technical and medical professionals and their levels of training, experience and qualifications

PT Design

Whole organism or synthetic gene?

- plasmid gene of T. pallidum or M. ducreyi
- Contamination risk high with plasmids
- Distinguishable from wild type
- Stability (can be lyophilized for shipping)
Whole organism or synthetic gene?

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

Transformation of plasmid DNA using E.coli

- Sustainable supply for MEF
- TP is extremely difficult to culture!

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

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
External Quality Assurance vs. Standard Operation Protocols

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

**SOP** is an instalment important towards quality assurance and reproducibility

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## SOPs

<table>
<thead>
<tr>
<th>Sample Storage</th>
<th>Performance Monitoring</th>
<th>Analytical Validation</th>
<th>Quality Assurance</th>
<th>Biosafety Rules</th>
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<tbody>
<tr>
<td>Room temperature (1, 3 &amp; 6 months)</td>
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<tr>
<td>90% humidity</td>
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<td>37°C (1, 3 &amp; 6 months)</td>
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<tr>
<td>60% humidity</td>
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<th>1, 3, 6 months</th>
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**Total:** Sum 15 105

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## External Quality Assurance

- **Monitoring:**
  - Performance Monitoring
  - Analytical Validation
  - Quality Assurance
  - Biosafety Rules

- **Sample Storage:**
  - Room temperature (1, 3 & 6 months)
  - 90% humidity
  - 37°C (1, 3 & 6 months)
  - 60% humidity
  - 37°C (1, 3 & 6 months)

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**Total:** Sum 15 105

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**External Quality Assurance**

- **EQA** guarantees the implementation of internal quality control, PT and quality improvement
- **SOP** is an instalment important towards quality assurance and reproducibility
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.

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
Never Stop Exploring.
Welcome to the One Health Community.

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