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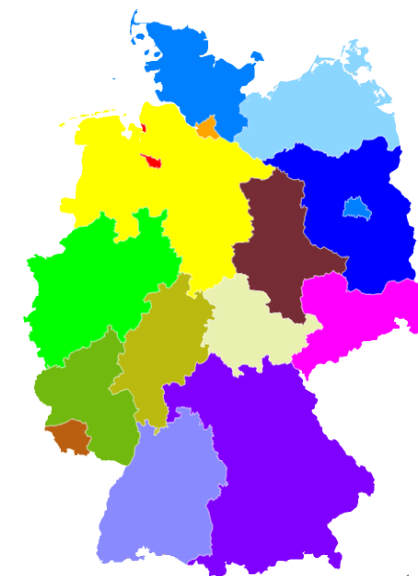
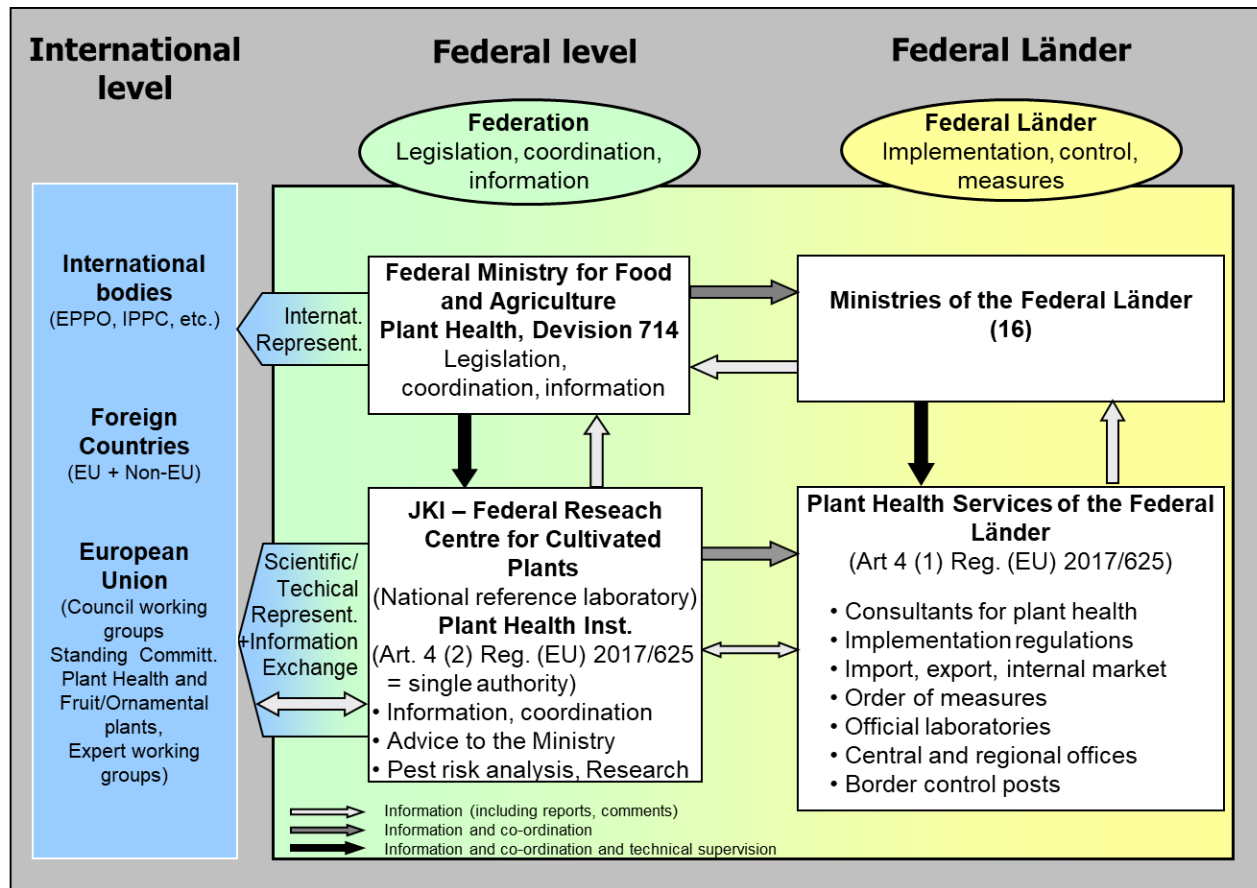


PT organization at national level: Challenges and lessons learned

S. König

Why does the JKI align PT?

1. Structure of Plant Protection and Plant Health in Germany



In short: 16 Federal Länder in Germany have the responsibility as a NPPO in Germany and implement plant health regulations and diagnostics (surveys, admission controls...), 13 officially designated laboratories (OL) – JKI coordinates and harmonises these processes

Why does the JKI align PT?



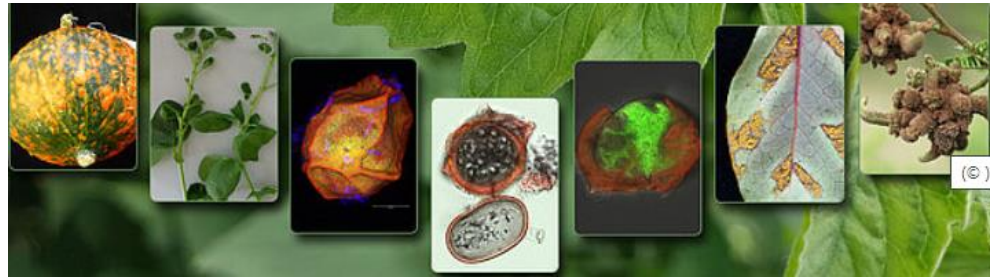
2. Statutory tasks of the NRL to facilitate diagnostic labs and in checking the competence of the national officially designated laboratories (OL)

- The JKI carries out the task of a German national reference laboratory and was therefore designated by the Federal Ministry in a national regulation (Pflanzenschadorganismenreferenzlaborzuweisungsverordnung – PflSchadORZV)

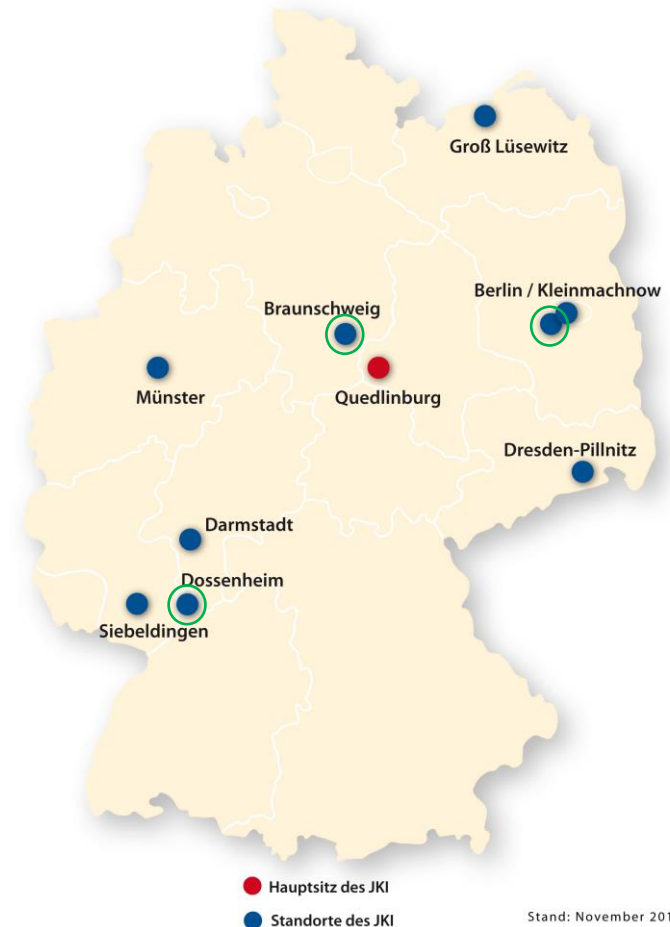
Art. 101 (EU) 2017/625 – NRL=JKI fulfils tasks for the coordination of the officially designated laboratories of the MS:

- Verification of test results from the designated Federal Länder diagnostic laboratories in terms of a confirmatory diagnosis
- Harmonization, improvement and development of new testing methods (includes ILC for such methods, the attempt to introduce new methods into EPPO protocols, briefing/teaching of laboratory staff)
- Provides the possibility for participation at proficiency tests focussed on „key quarantine organisms“ from implementing regulations of the EU and risk based surveys of priority and union-quarantine pest organisms (UQPO) – mostly in parallel to PT organised by the EURL corresponding for the different pest organism groups

JKI as NRL Structure, Organisms and Activities



- Commission implementing regulation 2019/2072 EU App. II (a+b) currently comprises 395 organisms regulated as quarantine pest organisms
- JKI: 14 different NRL on 3 locations in Germany = structure of the NRL is not identical to that of the EURL, which is why there are often several NRLs in a single organism group
- Spectrum of target organisms per single NRL broken down by groups of organisms, cultivated plants/habitats as there are (i) orchards/vineyards, (ii) forestry and (iii) remaining agricultural habitats - with multiple subdivisions of large groups (arthropods, fungi)
- In the most cases, the OL are offered proficiency tests for the same regulated organisms that the EURL also offers to check the competence of the NRL - but not necessarily to the same extent and matrix



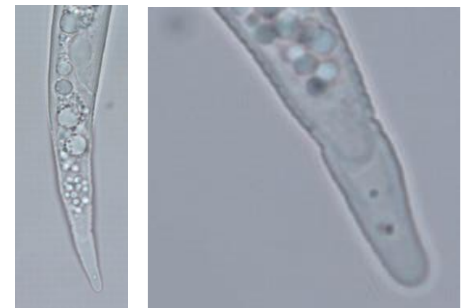
PT-participants and selection of Organisms

DE: 13 OL - Not all Plant-Protection-Services of the 16 federal states have their own diagnostic laboratory (Bremen, Saarland not, Berlin to a limited extent, not accredited) so that laboratories from neighboring states have been named in administrative assistance

Occasionally, diagnostic laboratories outside of Germany are also asked to participate in our PT

Organisms:

- Priority quarantine pests (e.g. ALB, *Bursaphelenchus xylophilus*, *Xylella fastidiosa*)
- Union quarantine pests with annual survey obligation (PCN, *Synchytrium endobioticum*, Rs, Cs, *Epitrix* spp.)
- Pest organisms with relevance in the annual survey program (e.g. *Meloidogyne chitwoodi* and *M. fallax*; in DE the program is focused on a small number of annually changing crops to bundle resources)



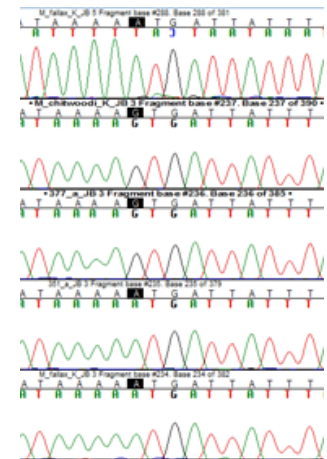
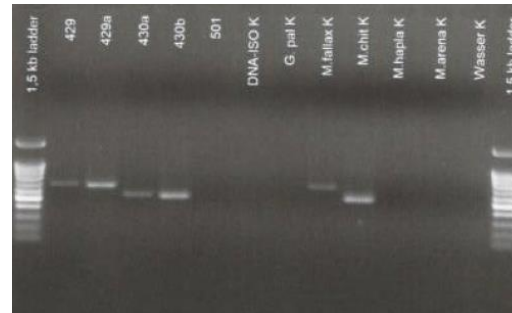
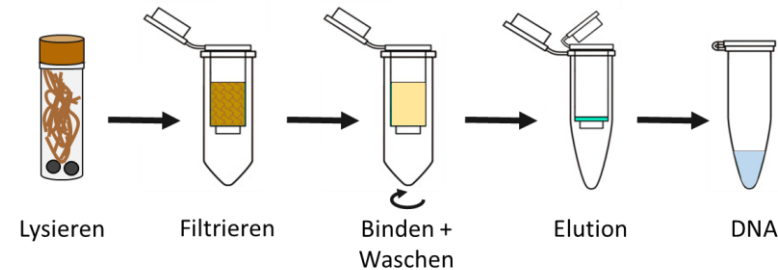


- JKI has no legal authority to force Federal Länder laboratories to participate in proficiency testing
- Based on diagnostic problems identified in PT there is the need for definition of appropriate corrective measures to meet the requirements of the accreditation standard ISO EN 17025:2017 (Pt. 7.7.3 regarding monitoring activities to improve lab-internal testing activities; e.g. repetition of the PT using a 2nd panel of samples, teaching the lab-staff at JKI...)
- Information of NPPO regarding non-conformity in PT (was not necessary until now 😊)



PT and methods selection

- Method selection is left up to the laboratories as comparable in PT organized by the EURLs
- Information on the methods that can be used is presented to the laboratory diagnosticians of the Federal Länder within the framework of annual meetings of working groups for the different groups of organisms
- In this circles responsible scientists from NRL-JKI and diagnostic experts of the OL benefiting from the exchange of experiences to strengths and weaknesses of individual methods (e.g. for emerging pests...)
- Current information from the EURL WS is also passed on in this way



PT in AGQN – the NRL for nematology

- NRL Nematology consists of a single technical assistant and the test laboratory manager (myself)
- Approved quarantine station under supervision of the NPPO in Lower Saxony
- 2 proficiency tests offered alternately every 2 years
- Organisms to be detected in PT Potato cyst nematodes and *Meloidogyne chitwoodi*/*M. fallax*,
- For root gall nematodes, specifications of PM 7/122(2) for sample preparation regarding homogeneity and storage/transport stability relevant
- Our laboratory participates in these PT itself = test of sample composition approach according to 4-eyes principle, TA prepares samples; lab manager decides for preparation scheme, panel composition and randomization
- Preceding to the PT:
 - (i) 6-8 L of pure sample matrix is tested to be free from target species & look alike organisms
 - (ii) testing of used populations
 - (iii) stability & homogeneity testing



PT in AGQN – Detection & identification of PCN



PT has been organized eight times **since 2010**

In agreement with the participants the PT is performed in spring and summer to avoid temporal overlaps with PCN surveys in autumn

PT consists of two parts:

- (i) Detection of **PCN** cysts = cysts w/o vulval cone in soil
- (ii) Species-identification of PCN (and look alike species without vulval cone)

Part I:

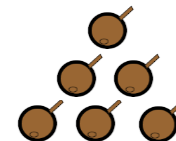
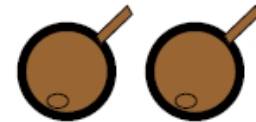
- In total 24 samples with 250 ml soil each
- Soil matrix provided by participants on a biennial basis („home and away“)
- Use of heat-treated cysts (*G. rostochiensis*)

ca. 1/3 of samples „healthy“

ca. 1/3 of samples at the **Limit of Detection** infected with **2** cysts

ca. 1/3 samples 3-12 PCN cysts for proof of recovery rate (e.g. 2x3; 3x5; 1x7; 2x10)

Part II: 10 samples with three viable cysts without vulval cone



PT in AGQN – PCN - evaluation

| Assigned value Participant result | + | - |
|--------------------------------------|---------------------|---------------------|
| + | TP = True Positive | FP = False Positive |
| - | FN = False Negative | TN = True Negative |



Qualitative performance criteria should be achieved by 100 perc.

Specificity: Agreement between the result obtained and the reference result, for samples whose reference value is negative

Sensitivity: Agreement between obtained result and reference result, for samples whose reference value is positive

Accuracy: Agreement between achieved result and reference result or reference value

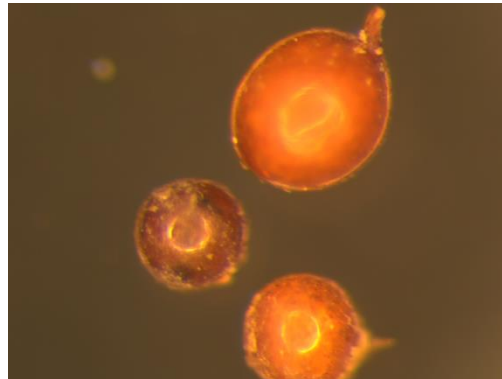
The only quantitative parameter of the **recovery rate** **does not have to be fulfilled 100 percent** and is used exclusively to find errors due to loss or carryover of cysts

Certification: The PT certificate confirms only the participation, results are transmitted individually to the participants in terms of data protection and summarized in anonymous form

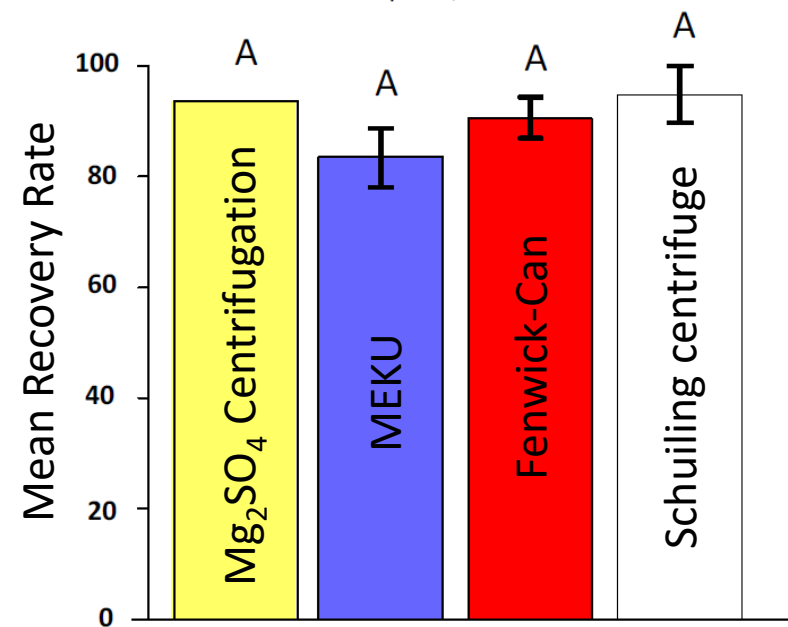
Identified problems:

- Extraction of nematodes from the matrix
- Although we observed differences - no extraction method can be named as absolutely reliable - but always laboratory experience is decisive
- Species identification never led to PT failure since 2010
- Changing soil types increase competence and reliability of laboratories also in dealing with unexpected non-target organisms (nematode cyst vs. AMF spore)
- Remedy is the insertion of an EtOH separation of the cysts from the organic fraction after density separation = methodological improvements

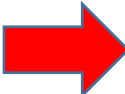
Two smaller **AMF spores** (one with subtending hyphae) – compared to a larger **PCN** cysts with **neck region**



Kruskal-Wallis-Test
p = 0,43



Corrective actions

- PT Globodera performed 8x since 2010
-  Some participants already failed 3x in extraction
- Corrective action required = retraining of 3 laboratories 2022
- Especially equipment handling, settings of rinsing times on MEKU
- Internal “small” PT of participants on site in BS
- Certification of performed service to attest participants proof of successful performance of corrective action.



PT in AGQN – Root gall nematodes on potato



- Detection and identification of *Meloidogyne chitwoodi* and *M. fallax* is currently performed the first time
- According to the PT from EURL nematode extraction and species identification is performed together from the same samples
- Sample matrix coarse sand
- after multiplication on susceptible potato cultivars in the greenhouse, the extracted nematodes are mostly dripped onto the sand in the juvenile stage and mixed together
- One sample panel consists of 11 samples:
 - ca. $\frac{1}{4}$ = healthy samples
 - ca. $\frac{1}{4}$ = related species (look alike) e.g. *M. hapla*
 - ca. $\frac{1}{4}$ = each of the target organisms
 - 1 balancing sample („lure sample“)

Goal: Adapt sample matrix in the future to the needs in practice with highest possible commutability = prepare naturally infected tubers in the future





- In the past, the soil matrix was provided by JKI and too much organic material was inside, which resulted in 50% of the participants performing unsuccessful
- Since then, the standard soil of another participating Federal Land has been used every year for sample preparation (“home and away principle”).
- In several cases nematode cysts were found in the 5-7 l of pre-tested matrix during pre-testing (but so far exclusively *Heterodera* sp.)
- **Reconciliation to the detection limit - optimal and practice-oriented LOD of 2 cysts**
- Despite the fact that in pre-tests and also in parallel in the PT we have successfully examined the samples according to the centrifugation method, many of the participants do not seem to achieve any migration of *Meloidogyne* from the sand by means of Baermann funnels.



Not sorted for relevance

- The OL of the Federal Länder and invited non-German laboratories are in principle grateful for the offer of proficiency testing
- Although the spatial conditions at the JKI are limited, offers for follow-up training in small groups should be made as close as possible to the time of PT
- Access to test material should be plannable (optimal are own collections of target organisms and possibilities of confusion - or contact to colleagues who have them in stock)
- Practical implementation conditions (e.g. LOD), relevant test materials (manipulated matrix)
- Consider commutability of sample material (as close as possible to “natural conditions”)
- Regular exchange with participants





- Requirement: detection limit for the performance evaluation of the labs should be one of the important goals of PT organized for advanced participants not for such of new organisms where participants have no experience
- LOD should be nevertheless solvable
- Consider suggestions from the participants (e.g. determination of nematode viability is important in practice for taking action in infested field sites or not)
- Not to keep PT static but continuously develop it with regard to practical requirements
- Take up methods from research (e.g. optimizing determination of PCN cyst/egg viability)
- The aim is not to frustrate the participating colleagues but to support them in their very extensive task management
- Problems in diagnosis must be identified and solved in the foreseeable future if possible
- Certification with evaluation of successful/unsuccessful participation in the certificate directly is no longer practiced.



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