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Naktuinbouw Laboratories

Marcel Toonen
Head of Laboratories

Locations

Roelofarendsveen

Horst

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2. Laboratories

Film Laboratories

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Naktuinbouw Laboratories

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    graph TD
      A["Naktuinbouw Laboratories  
Marcel Toonen"] --- B["Application manager  
Quality coordinator  
Relation manager"]
      A --- C["Research & Development  
9 scientists"]
      B --- D["Health testing & Seed analysis  
administration  
virology  
bacteriology  
nematology/mycology  
seed analysis"]
      B --- E["Diagnostics"]
      B --- F["Test Centre Horst"]
    
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Scope

- Testing of seed and plant material for plant diseases
- Diagnostic analysis of symptomatic plant material
- Quality testing for seeds
- Molecular marker research for variety identification and pathogen identification
- Production and maintenance of virus free material
 - strawberry
 - fruit crops


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Clients

- Companies & other organizations
 - voluntary testing
- Dept. of Inspections
- Plant Protection organisation (NVWA)
- Other inspection services

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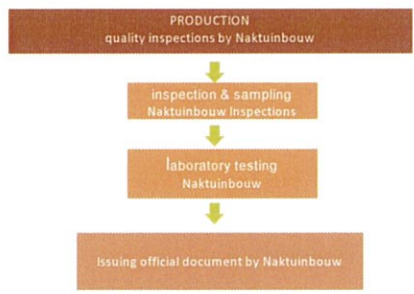
Quality assurance



- ISO 9001
- ISO 17025
 - Cmm, PSTVd, pospiviroids in leaves, Plum Pox Virus (L549)
- ISTA accredited laboratory

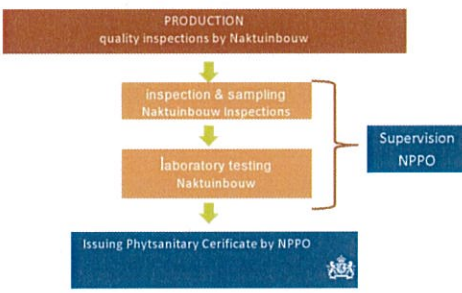
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Official testing




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Official testing export



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Quality assurance



- Under supervision of Plant Protection Service
 - 2008/61/EG
- routine testing for quarantine organisms
 - ca. 40 quarantaine organisms
- audit on technique
 - serological (ELISA)
 - molecular (PCR)
 - biotesting

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Activities Laboratories



- Pathogens
 - Bacteria
 - Fungi
 - Viruses
 - Viroids
 - Phytoplasma's
 - Nematodes
- Plants
 - Seed analysis
 - Molecular identification

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Activities Laboratories




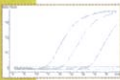
	2011	2012	2013	2014
Bacteria	12.549	14.595	10.775	10.196
Fungi	5.247	6.563	5.904	5.790
Virus	337.172	286.708	256.003	248.336
Nematodes	4.865	4.906	4.053	5.033

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Methods






- Plating on (semi) specific growth media
- Serological
 - ELISA
 - lateral flow devices (diagnostics)
 - immunofluorescence microscopy
 - agglutination
- Molecular
 - (bio-)PCR, RT-PCR
 - real-time (RT-)PCR
 - AFLP/SSR

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Methods


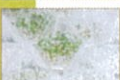





Bioassays

- Virus
 - Herbaceous indicator plants
 - Grafting
 - *Prunus serulata*, GF305, 'Sam',
 - UC5, UC12, EMC
- Bacteria
- Virus

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Seed analysis

- Germinating rate
- Purity
- Tetrazolium test
- Moisture content determination
- Usable Plant Test
- ISTA certificates

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Seed analysis

	2011	2012	2013	2014
Germination	7.315	8.343	7.397	7.717
Purity	4.110	4.399	4.018	4.132
Others	701	1.033	955	740

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Test Centre Horst

- Making material virus free (woody crops / strawberry plants)
- Maintenance (mother plants / strawberry plants)
- Biotests
 - fruit crops
 - strawberry
 - ornamentals

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Diagnostics

- Finding the cause of abnormalities, symptoms or damage in plant material
- Combination of practical expertise, experience of diagnostic experts and researchers
- Scope: floricultural, arboricultural and vegetable crops and flower bulbs

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Research & Development



- Knowledge centre
 - expertise on plant pathogens and their interaction
- Development and validation of diagnostic tests
 - quarantine pathogens (Plant Protection Organisation)
 - other plant pathogens
- Identification of varieties
 - molecular markers
 - AFLP/SSR
 - Next Generation Sequencing
- Proficiency testing
 - quality assurance of laboratories

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Reference collections




- Bacterial collection (~4000 isolates):
 - *C. michiganensis* subsp. *michiganensis* (~600 isolates)
 - *X. campestris* pv. *campestris* (~500 isolates)
 - *X. fragariae* (> 150 isolates)
 - *X. axonopodis* pv. *poinsetticola* (> 40 isolates)
 - *X. axonopodis* pv. *pruni* (~200 isolates)
- Fungal collection (> 800 isolates)
 - *Fusarium* (> 300)
 - *F. foetens* (> 40)
 - *F. oxysporum* f.sp. *asparagi*, *chrysanthemi*, *cyclaminis*, etc.
- Viruses, viroids, phytoplasma's (> 100 species)
 - Ornamentals
 - Arboriculture / fruit

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Protocol development




- Scientific developments
 - collaboration (Wageningen UR, UvA, FERA, ISHI, etc.)
 - TESTA
 - scientific literature
- Optimalization and standarization
- Define the protocol
- Validation



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Valdiation



- Dutch guideline for the validation of detection and identification methods for plant pathogens and pests
 - Detection limit
 - Measuring range
 - Analytical specificity
 - Selectivity
 - Reproducibility / Repeatability
 - Robustness
- Based on:
 - NEN 7777: Standardisation of the validation process
 - PM 7/98: Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity (EPPD)
 - Explanatory document Microbiology (RVA-T2)

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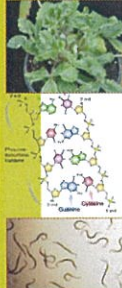
Collaboration



- International Seed Health Initiative (ISHI)
- European Plant Protection Organisation (EPPO)
- International Seed Testing Organisation (ISTA)
- International Union for the Protection of New Varieties of Plants (UPOV)
- Netherlands Food and Consumer Product Safety Authority (NVWA)
- International projects

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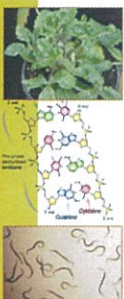
R&D projects



- Virus
 - blueberry (Vaccinium)
 - nepowiviruses in strawberry
 - RT-PCR's for detection of viruses in Rubus
 - VImproved sampling for apple proliferation
 - Internationale harmonisation detection PSTVd/TCDVd in tomato seeds
- Detection *Agrobacterium tumefaciens* in soil
- Identification and sampling *Xanthomonas axonopodis* pv. *poinsetticola*
- Detection of *Phytophthora ramorum* by real-time PCR
- Molecular and bioassay for the detection of CGMMV
- Detection of *Pseudomonas syringae* pv. *actinidiae* in kiwi
- Detection of *Xanthomonas arboricola* pv. *pruni* in pruni

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R&D projects



- direct PCR
 - Detection *Pseudomonas syringae* pv. *maculicola* (Psm) in radish and cabbage (TESTA)
 - Detection *Acidovorax citrulli* in cucurbits (TESTA)
 - Detection Cmm in tomato seed
- DNA based
 - IDC-Virus diagnostic in perennials and flower bulbs
 - sRNA in soft fruit
- Reference material
 - internal controls
- Stable Isotope authentication
 - determine origin

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NAL proficiency tests




- Develop and run proficiency test
 - Experimental set-up
 - acquire or produce contaminated seeds
 - distribution and communication by NAL
 - statistical analysis of results
- Requirements
 - Realistic samples
 - Statistically sound (where possible)


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Variety identification

- Molecular testing combined with morphological testing
- AFLP



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