Practical requirements for plant authentication methods

Workshop Geel, Belgium, september 2009

Tamara Peelen

Chemist/ DNA analyses

Dutch Customs Laboratory

Amsterdam, The Netherlands

t.peelen@belastingdienst.nl

Douane Laboratorium

Belastingdienst **Douane**

Introduction (Dutch Customs lab)

Douane Laboratorium

Belastingdienst **Douane**

25% of EU cargo via Dutch territory

Douane Laboratorium

Belastingdienst **Douane**

Number of employees

Tax and customs administration
30,000 employees

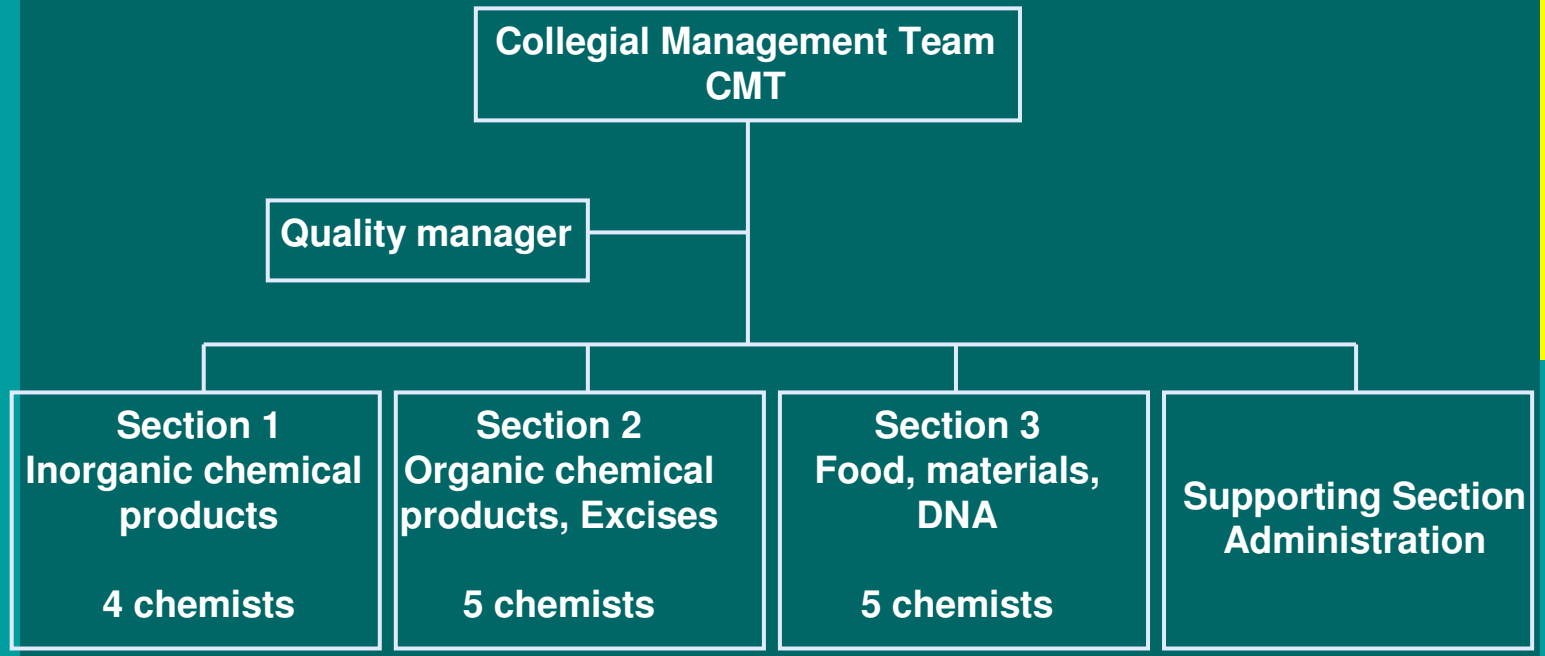
Customs administration
5,500 employees

Dutch Customs Laboratory
60 FTE

Douane Laboratorium

Belastingdienst **Douane**

Organisation of Laboratory



Douane Laboratorium

Belastingdienst **Douane**

Tasks

- Analyse samples for classification in the Combined Nomenclature

- Analyse samples for non-fiscal customs tasks (Safety, Health, Economics, Environment) in cooperation with other ministeries

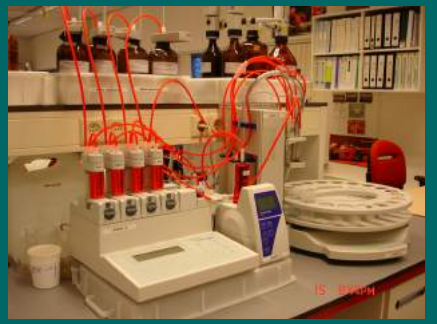
- Analyse samples on location (Mobile lab)
- Sample transport and storage
- Helpdesk (safety and sampling)
- Customs courses

Douane Laboratorium

Belastingdienst **Douane**

Analysis techniques

- XRF XRD ICP C-analysis
- FTIR Thermal analysis DSC
- LCMS GCMS
- GC HPLC
- Destillation Density
- Kjeldahl UV-VIS NIR
- DNA techniques
- Electrophoresis
- Wet chemical analyses (fat, starch, …)



Douane Laboratorium

Samples

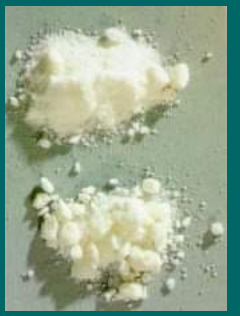
Ca. 18,000 samples/ year











Douane Laboratorium

Belasting

Identification of varieties: Requirements in the lab (1)

- Samples
Sample supply/expectations

- Equipment
PCR machine, UV workstation, Sequencer, real-time PCR, separate rooms, ….

- Knowledge/ method
Familiarity with ID-methods
Specific knowledge varieties

Douane Laboratorium

Belastingdienst **Douane**

Identification of varieties: Requirements in the lab (2)

- Trained personel

- Capacity (impact expected number of samples)

- Budget (equipment, maintenance, chemicals)

Douane Laboratorium

Belastingdienst **Douane**

Identification of varieties: Requirements of analysis

Quality:	(Sub)Sampling (!) Protocols/SOP Controls References/ standards (!!!) Validation Ringtest/ sample exchange
Time:	Shipments stopped

Douane Laboratorium

Belastingdienst **Douane**

Identification of varieties: Example of Basmati rice

EC regulation 1549/2004 (972/2006, 1234/2007)

Nine Basmati varieties free of import duty

Import certificate must state:
-origin of Basmati rice
-variety (listed in regulation?)

Testing of Basmati samples:
-country of origin
-memberstate of EU

Douane Laboratorium

Belastingdienst **Douane**

Identification of varieties: Dutch Customs lab

- Samples: expected, based on 2004
- Equipment: OK, other DNA analyses
- Trained personel: technician, myself
- Budget: OK
- Knowledge/method: experience MS analysis
  no knowledge varieties
  and specific method Basmati

Douane Laboratorium

Belastingdienst **Douane**

Identification of varieties: Microsatellite Analysis

February 2005: visit to UK

Method developed by the University of Wales

8 STR-markers (di- or trinucleotides)
Each Basmati variety specific haplotype

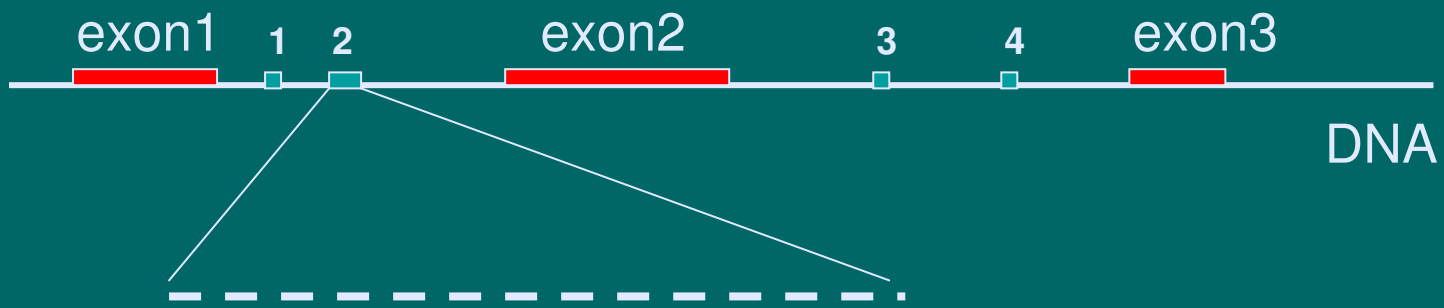
Bulk analysis
Single grain analysis } both qualitative

Mixed? ⟶ Additional quantitative test

Douane Laboratorium

Belastingdienst **Douane**

Microsatellite Analysis: principle



Microsatellite (repeat)

Position	1	2	3	4
Variety A	8	4	17	5
Variety B	8	4	15	3
Variety C	5	4	15	5

Douane Laboratorium

Belastingdienst **Douane**

Microsatellite Analysis: haplotypes

	201	44	252	212	263	110	282	339
Varieties listed in Com. Reg. 1549/04								
Basmati 370	162	127	252	134	177	170	149	200
Dehra Dun (type 3)	162	127	254	134	181	170	149	200
Basmati 217	162	127	254	134	177	170	149	200
Ranbir	162	127	244	134	177	170	149	200
Taraori (HBC_19)	162	131	264	134	181	170	149	200
Basmati 386	162	131	258	134	181	170	149	200
Kernel	162	131	260	134	181	170	149	200
Pusa basmati	162	131	254	134	181	170	149	200
Super Basmati	162	127	260	134	181	170	149	204
Other approved Basmati varieties								
Basmati 198	162	127	254	152	177	170	149	200
Basmait 385	162	131	252	152	181	170	149	200
Kasturi	162	121	254	132	220	170	149	166
Haryana Basmati	162	121	238	152	177	150	149	166
Mahi sugandha	176	121	256	152	177	156	155	166
Punjab Basmati	162	127	256	152	177	156	155	200
Non-Approved varieties								
Basmati 2000	162	131	254	152	181	170	149	204
Shaheen Basmati	162	131	254	152	181	170	157	200
Sherbati	178	121	238	130	202	174	160	166
Mugad Suganhda	178	121	238	132	202	176	157	166
Pak 386	178	121	238	130	220	174	155	166
Superfine	178	131	238	132	202	174	157	166
Pusa Sugandha	162	121	238	132	181	170	157	178
Yamini	162	127	260	134	181	170	149	200

Douane Laboratorium

Belastingdienst **Douane**

Microsatellite Analysis: DNA isolation

Grind 20 g Basmati rice,
sample 1 g for isolation

Grind 1 grain (tissue lyser)

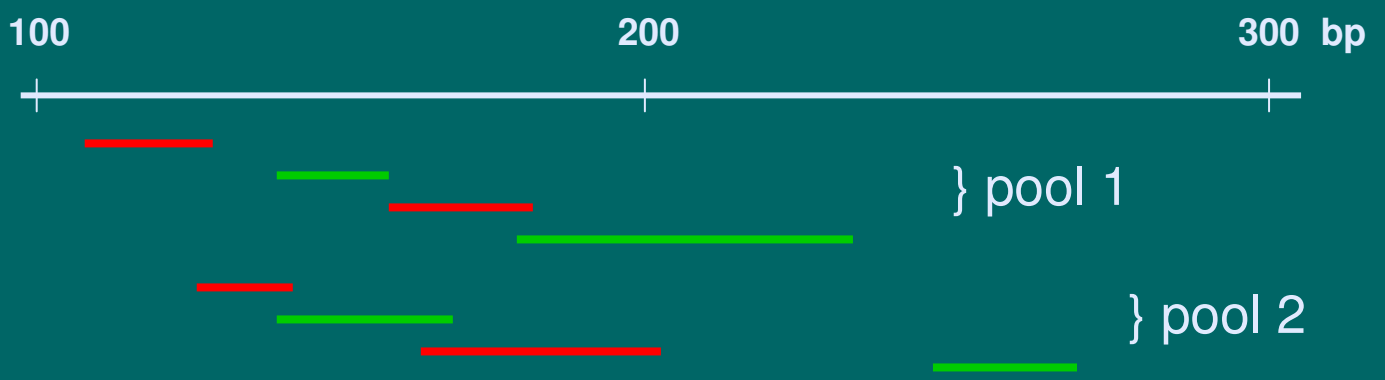
Extract DNA:
- kit (nucleon phytopure, Tepnel)
- in-house method (CTAB/Wizard)

Optimise for quality, safety, time, costs

Douane Laboratorium

Belastingdienst **Douane**

Microsatellite Analysis: set up



Basmati: 8 microsatellite markers

4 fragments labelled in red, separate PCR's
4 fragments labelled in green, separate PCR's

2 pools analysed separately on sequencer (2 capillairies)

Douane Laboratorium

Belastingdienst **Douane**

Microsatellite Analysis: challenges

Bulk (duplicate): 2 x 8 = 16 signals (or more)
8 Single grains : 8 x 8 = 64 marker signals
80

Start-up:
- Optimise pools for similar signal intensities
- Link results to given allele table
- Scoring alleles takes lot of time: automatically

Although requirements are met, and a protocol is given, it takes time to implement an analysis.

Ringtest: samples correctly identified (reproducible).

Douane Laboratorium

Microsatellite Analysis: summary

Advantage
- Method provided (ring tested)
- Precise identification
- Automatic allele calling
- Screening (marker subset)

Disadvantage
- PCR failures
- Some markers difficult to score
- Interpretation problems (bulk)
- Quantification dependent on adulterant
- Time consuming
- Strange alleles/ new varieties?

Douane Laboratorium

Other example: collaboration with national herbarium

Rauvolfia serpentina (root)
(endangered species, CITES annex B)

Herbarium:
impressive collection of dried plants
experience with variety identification
(DNA lab and microscopy)

Customs lab:
contra lab sequencing varieties : consensus
screening alkaloids using LCMS

Douane Laboratorium

Belastingdienst **Douane**

Variety authentication: Conclusions

Collaboration is needed between research institutions, the JRC and (customs) laboratories to come to qualitatively good analyses that are practical to use.

References/ standards should be available.

Not all member states will have imports of the same kind of goods and also the amount of certain goods varies throughout the EU. It should be considered that for more complicated analyses, samples can be send to member states with experience in that specific analysis.

Douane Laboratorium

Conclusions, continued

Development of analyses takes time:
Authors of EC regulations should realize that before a regulation becomes valid, methods will be developed and provided beforehand, to ensure uniformity in testing throughout the EU.

If applicable, levels of permissible admixtures should be included in the regulation to avoid country to country (or lab to lab) differences.

Douane Laboratorium

Belastingdienst **Douane**

Acknowledgements

John Gorham
Katherine Steele
(University of Wales, UK)
Mark Woolfe
(Food Standards Agency, UK)

Bastiaan Ebbelaar
Arwin van der Rhee
(Technicians Dutch Customs Laboratory)

Douane Laboratorium

Belastingdienst **Douane**

Douane Laboratorium

Belastingdienst **Douane**