

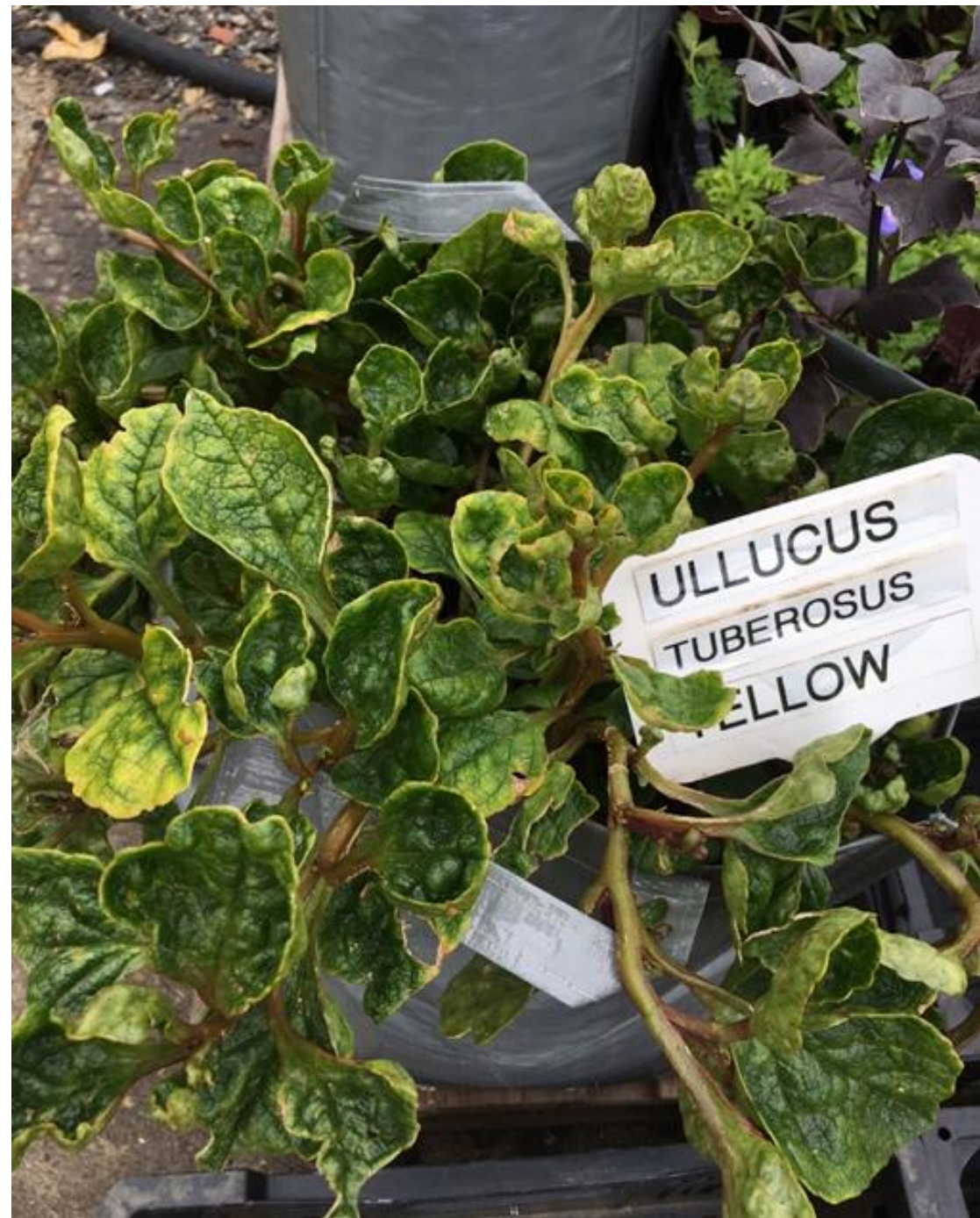


Original thinking... applied

# Reporting HTS results and follow up actions

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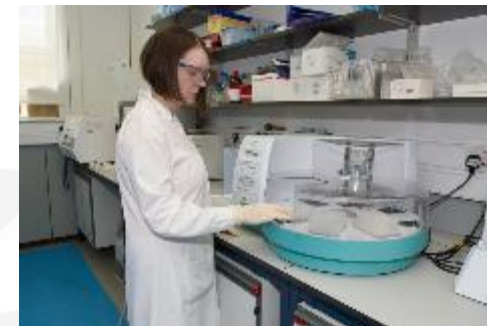
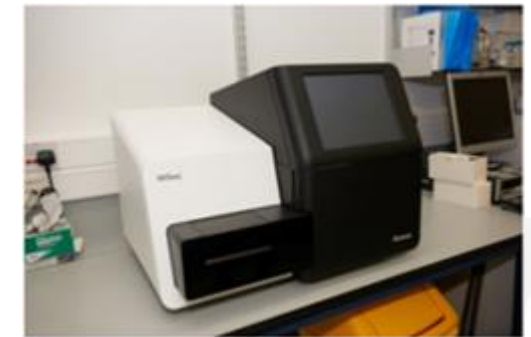
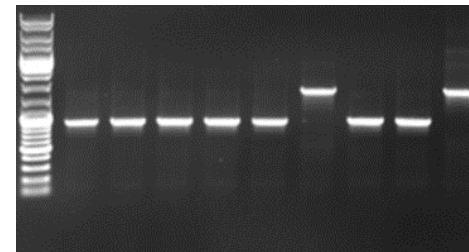
ⓘ Start presenting to display the joining instructions on this slide.



# Long road of diagnostic development



Source <http://wellcomeimages.org>



# Reporting and actions...

- We've just found a "thing"
  - Do I need to confirm?
  - Is it regulated?
  - Is it novel?
  - Is it pr...



These are general diagnostics questions but HTS has brought them into focus

- Do you need all this data?
  - How much uncertainty can you live with?



# Don't just take my word for it...

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## EPPO STANDARD ON DIAGNOSTICS

### **PM 7/151 (1) Considerations for the use of high throughput sequencing in plant health diagnostics**

#### **Section 6: Confirmation, biological interpretation and reporting**

- Confirmation of the detection and identification of the pest(s)
  - Critical cases?
  - Previously uncharacterised pests
- Interpretation of the biological relevance of the identified targets
  - Plant pathogen or “by-catch”?
  - What information do I need to support my decisions?
- Reporting
  - Report everything? (and to whom? e.g. pathogens relevant to human and animal health...)



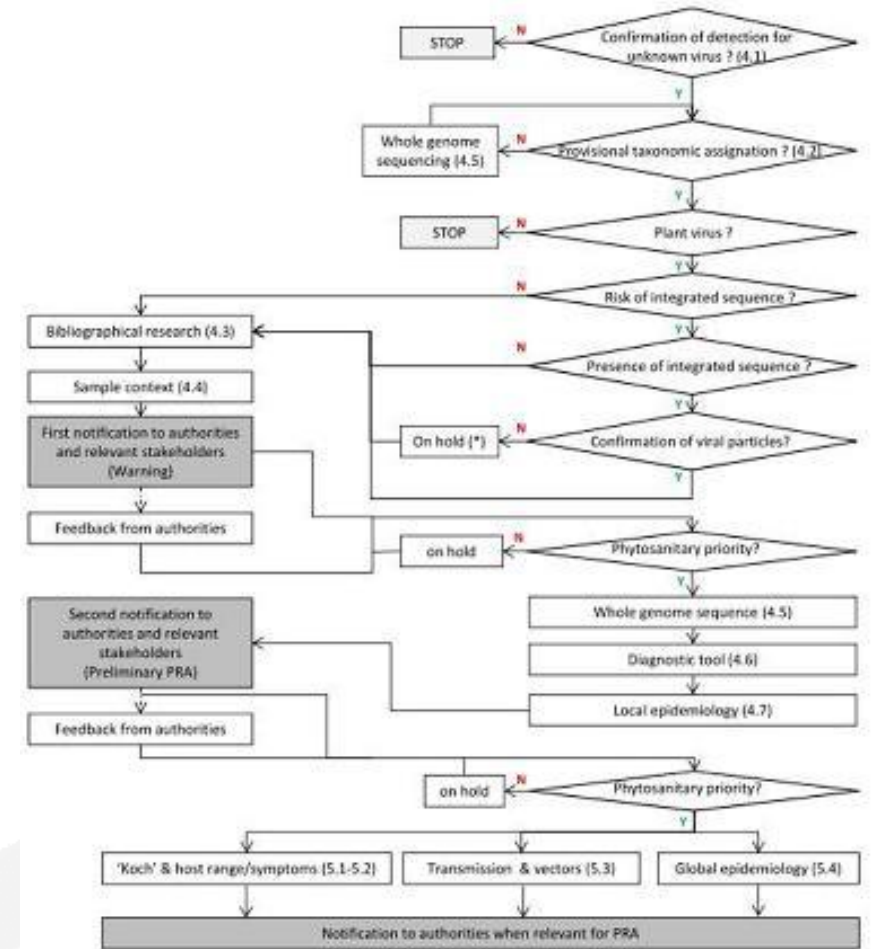
# Early experience: Guiding risk managers

Sample no	FERA no	Sample	BlastN	BlastX	Result	Possible finding (to confirm)
1	2021021661	Crocoshia (Potyvirus first finding UK/host)	lots of Pseudomonas, Genome Ornithogalum mosaic virus (98%ID), genome Bean yellow mosaic virus (88%ID), fragments Avian avulavirus (AAV)	Genome Ornithogalum mosaic virus, genome Bean yellow mosaic virus, 2.8kb novel mitovirus		NDR
2	2021025184	Sambucus nigra	fragments of pepper cryptic virus 1 (80%ID, 809n), Soybean leaf-associated mitovirus 2 (92%ID, 299 n) AAV- need to check if that is linker contamination?	<b>genome novel Caulimovirus (35% CaMV), more in root</b>		
3	2021024333	Vitis	Grapevine leafroll-associated virus 1 (partial genome), grapevine leafroll-associated 3 (61 fragments), grapevine rupestris stem pitting associated virus (12x 200-1k), grapevine virus A (37 frag), possible grapevine virus D, grapevine virus F (7 frag), grapevine fleck virus (67 frag)	Same viruses as BlastN, Don't think there is anything extra but worth a closer look to check when confirming		GRLaV 1+3 ELISA-negative, map grapevine
4	2021024111	Nerine virus X	genome NVX and lots of fragments (fragment other Potex), fragment Cowpea chlorotic mottle virus (87%ID), AAV	NVX		Nerine viruses to send off
5	2021024111	Agapanthus X	genome NVX, genome Chenopodium quinoa mitovirus 1 (100%ID)	genome NVX, genome Chenopodium quinoa mitovirus 1		Nerine viruses to send off
6	2021023845	Crataegus	AAV	no virus		
7	2021022960	Acer palmatum	AAV fragment Hubei tombus like virus 8	<b>large fragments &gt;5kb, 45% ID to Citrus blight associated pararetrovirus (root)</b>		
8	2021023744	Camellia japonica	no virus, plenty of reads, lots of Camellia	no virus		
9	2021022416	Quercus	fragments <i>Siphoviridae</i> (bacterial virus), large fragment Partitivirus 2k, AAV	<b>large fragments Partitivirus (root)</b>		Data sharing- compare oak partitivirus sequences
10	2021020919	Capsicum annuum	9 fragments NVX, fragment Chenopodium quinoa mitovirus, fragment herpes virus, 17 fragments AAV	<b>large fragments (7kb) tobacco vein clearing virus (80%ID) / also IDs as tobacco??</b>		
11	2021021507	Liriodendron	11 fragments AAV	no virus		
12	2021021213/2021021215	Vitis vinifera ssp. Vinifera	Not many contigs (700) BUT lots of grape, single fragment genome Candidatus Phytoplasma ziziphi (80%ID, not convincing, 300n), 35 fragments Grapevine rupestris stem pitting virus (98%ID), fragment NVX (292n), fragment lily latent virus (82%, 272n)	2 fragments Grapevine rupestris stem pitting virus		Confirm phyto- both tested negative and carla by m
13	2021021168	Vitis vinifera ssp. Vinifera	Not many contigs (961) BUT lots of grape, 10 fragments Grapevine rupestris stem pitting-associated virus (97%ID, 10x200-579), 1 fragment Grapevine rupestris vein feathering virus (90%ID, 220n)	No virus		
14	2021020452	Quercus	lots of Quercus, single fragment Pear alphapartitivirus (82%ID), single fragment NVX	single fragment partitivirus		
15	2021018136	Solanum macrocarpon (tamarillo fruit ring virus)	4 fragments Begomo virus (possibly tomato leaf curl 2kb, 87%ID- Aimee thinks Novel), Genome possible novel potyvirus ( related to African eggplant mosaic virus 75%ID over second half of genome- not novel, whole genome tamarillo fruit ring virus), 4 fragments NVX, lily latent virus	nothing not already detected by BLASTN		NDR on TFRV, begomo needs checking though
16	2021025057	Capsicum	large fragment Ranunculus white mottle virus (97%ID our fragment 50% bigger than what is on genbank) 2 fragments Pepper cryptic virus 1 (100%ID), 2 fragments Pepper cryptic virus 2 (100%ID), 1 fragment NVX	agrees with BLASTN also <b>large fragments Tobacco vein clearing virus/Tobacco</b>		To confirm ophiovirus Possible NDR
17	2021020472/2021020473	Vitis vinifera ssp. Vinifera	Not many contigs (254) BUT lots of grape. 1 fragment Grapevine leafroll-associated virus 1 (96%ID, 204n), 4 fragments Arabis mosaic virus (89%ID), 3 fragments Grapevine rupestris stem pitting-associated virus (99%ID, 3x200n)	no virus		confirm GRLaV-1 and ArMV by ELISA
18	2021019899	Vitis vinifera ssp. Vinifera	lots of grape, 6 fragments Grapevine Pinot gris virus (99%, 7x 207-726nD), 13 fragments of Grapevine rupestris stem pitting-associated virus (93-98%ID, ~200-300n)	no virus		
19	2021019328	Quercus robur	lots of quercus, AAV	no virus		
20	2021021455	Saponaria officinalis	most of the genome ?? Arabis mosaic virus (>90%ID), AAV, NVX	as per BlastN also <b>2 x1kb fragments in root, weak (32% ID) to Acyrthosiphon pisum virus</b> , also 1kb weak 50%ID) to Moriarty virus (unclassified Narnaviridae-fleas)		confirm ArMV
21	2021024913	Sambucus (bulk of two)	genome of Elderberry carlavirus B or related (81%ID), 2 fragments Arabis mosaic virus (92%ID), 2 fragments Genomoviridae (related to Geminiviruses (95%ID), AAV	as per BLASTN, also <b>1kb Lulavirus (Grapevine associated cogu-like virus 2 related 67%ID)</b>		Confirm carla and ArMV, NDR on carla? Genomoviridae for RCA
22	2021024601	Chrysanthemum	lots of Chrysanthemum, 4 fragments Carnation mottle virus (98%, 250-400n), 12 fragments short region homology to Diuris virus A (not convinced), 4 fragments Tobacco mosaic virus (100%ID, 3x200n)	BlastX diuris virus A individually, no virus	carnation mottle virus, tested negative by ELISA (1403 ELISA 21)	
23		HC Control (White Burley)	AAV	no virus		
24		negative	single fragments bacterial ( <i>Siphoviridae</i> ) and paramecium viruses	no virus		

Sample ID	Host	BlastN (de novo assembly)	BlastX
		<ul style="list-style-type: none"> <li>4 fragments Begomo virus (possibly tomato leaf curl 2kb, 87%ID- Novel),</li> <li>Genome possible new begomovirus (related to African begomovirus)</li> </ul>	
Import sample : Reported to Defra for interest, New host report for potyviruses in prep			
2021018136	<b>Solanum macrocarpon (tamarillo fruit ring virus)</b>	<ul style="list-style-type: none"> <li>Whole genome tamarillo fruit ring virus</li> <li>4 fragments NVX,</li> <li>lily latent virus</li> </ul>	<ul style="list-style-type: none"> <li>nothing not already detected by BLASTN</li> </ul>
		<ul style="list-style-type: none"> <li>large fragment Ranunculus white mottle virus (97%ID our fragment 50% bigger than what is on genbank)</li> </ul>	
UK glasshouse sample : Reported to Defra for action, New host/country report in prep			
2021025057	<b>Capsicum</b>	<ul style="list-style-type: none"> <li>2 fragments Pepper cryptic virus 2 (100%ID),</li> <li>1 fragment NVX</li> <li>fragments <b>Sinboviridae</b> (bacterial virus)</li> </ul>	<ul style="list-style-type: none"> <li>STN PLUS large fragments Tobacco vein clearing virus</li> </ul>
		UK field sample : No plant health interest	
2021022416	<b>Quercus</b>	<ul style="list-style-type: none"> <li>Avian avulavirus</li> </ul>	<ul style="list-style-type: none"> <li>Partitivirus (root)</li> </ul>
		<ul style="list-style-type: none"> <li>9 fragments NVX,</li> </ul>	<ul style="list-style-type: none"> <li>large fragments (7kb) clearing virus</li> </ul>
UK glasshouse sample : No plant health interest			
2021020919	<b>Capsicum annuum</b>	<ul style="list-style-type: none"> <li>17 fragments AAV</li> <li>genome NVX and lots of fragments (fragments other Potex)</li> </ul>	<ul style="list-style-type: none"> <li>clearing virus as tobacco??</li> </ul>
		Historical Isolate – Euphresco VirusCurate (manuscript in review)	
2021024111	<b>Nerine virus X</b>	<ul style="list-style-type: none"> <li>Avian avulavirus</li> </ul>	<ul style="list-style-type: none"> <li>genome NVX</li> <li>genome NVX</li> </ul>
		Historical Isolate – Euphresco VirusCurate (manuscript in review)	
2021024111	<b>Agapanthus X</b>	<ul style="list-style-type: none"> <li>genome Chenopodium quinoa mitovirus 1 (100%ID)</li> </ul>	<ul style="list-style-type: none"> <li>Chenopodium quinoa mitovirus 1</li> </ul>

# HTS reporting for Plant Health

- Plant health regulations are based on species lists
- Essential questions in PRA are:
  - Presence
  - Incidence
  - Distribution
  - Impact
- How to handle increase in novel pathogens?
  - Framework proposed by Massart et al, 2017
    - Revised version in review
- Implications
  - Time
  - Cost
  - Skills and Resources
  - How to prioritise...?

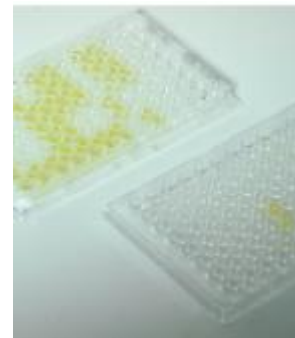




# Using HTS to confirm virus ID (Fox et al (2019) Plant Path)



Summer 2017: NPPO notified of crop of *U. tuberosus* being grown for seed without certification

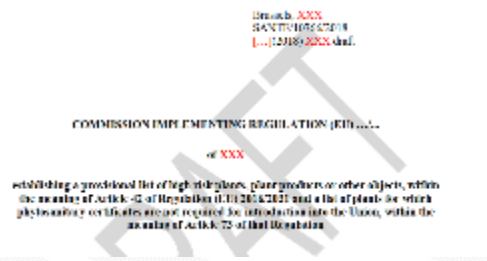
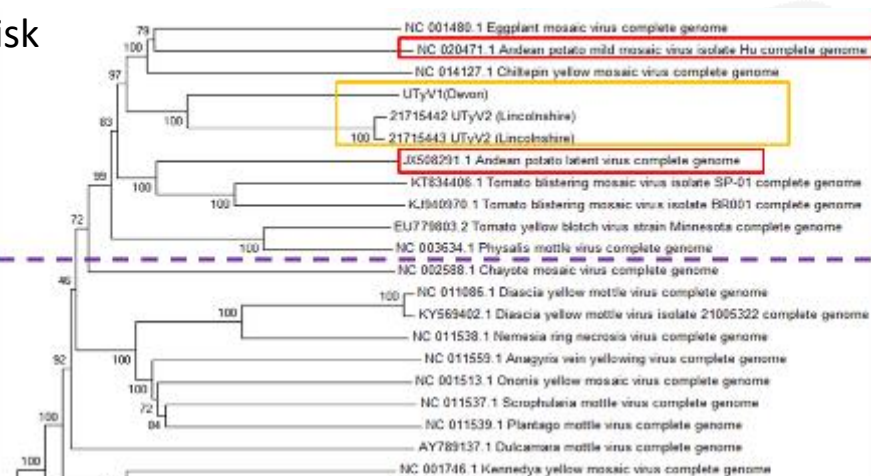


Initial screen suggests presence of quarantine viruses but this could not be confirmed



Genetic sequencing revealed 6 viruses new to science and non-native pathogens.

Two novel viruses considered to be high risk

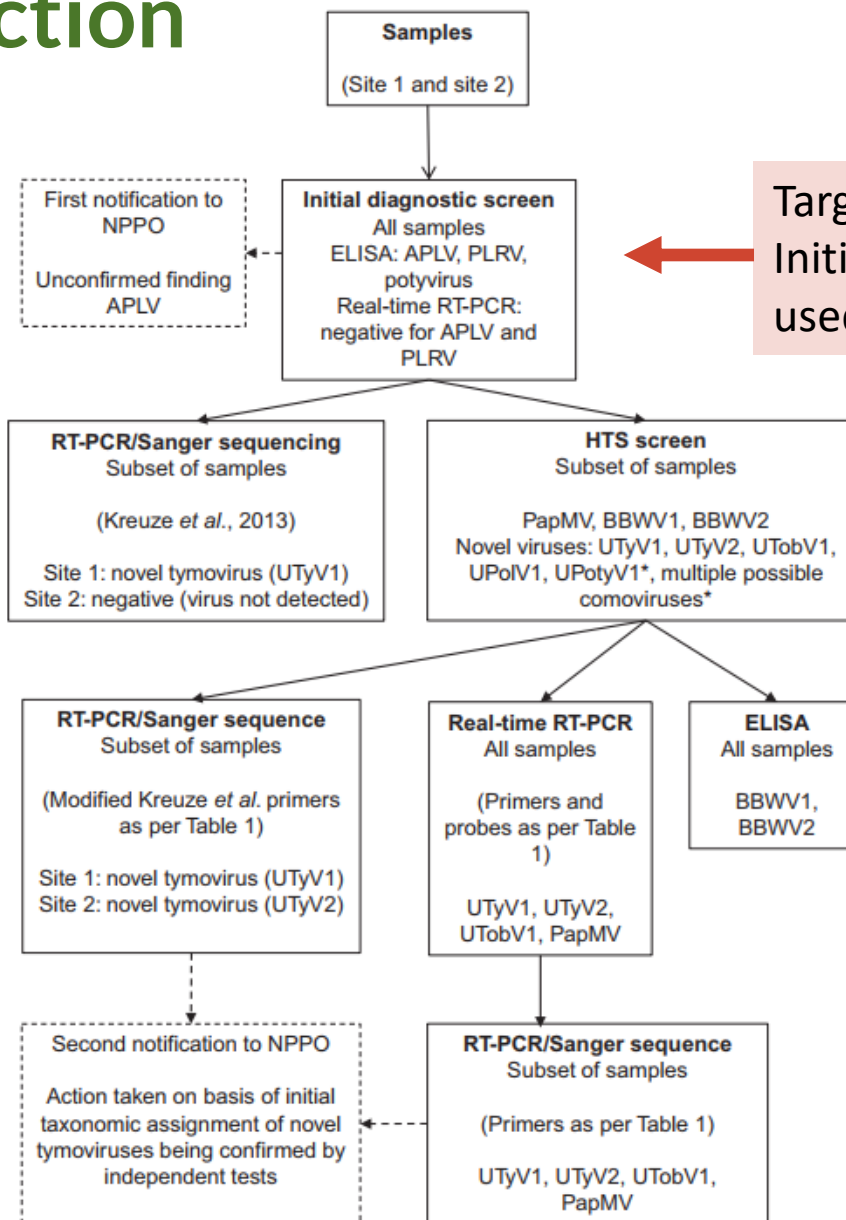


2019: ulluco included in European legislation as a high-risk plant species

# Ullucus – taking action on novel viruses

NPPO notified due to APLV (1A1 pest)

Conventional approaches could not confirm APLV, and suggested a novel virus



Targeted screen for quarantine pests. Initial agreement that HTS would not be used due to risk of novel findings.

HTS confirms two APLV-like tymoviruses plus+++

Tymoviruses, contact transmitted viruses and new host records confirmed

Partial sequences match HTS

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**How important is it to confirm a HTS diagnosis with a secondary method?**

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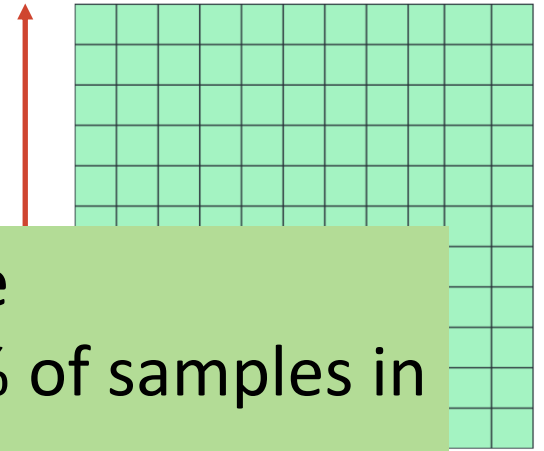


# Is confirmation necessary?

*Confirm the presence in \*critical cases\* with a secondary method, preferably using a different biological principle...*

- Approach generally taken for both regulatory reporting and publication (e.g. Disease Notes)
- What does this mean in practice... e.g.
  - ELISA → PCR
  - ELISA → qPCR
  - Bioassay → ELISA or PCR or qPCR
  - qPCR → PCR
  - PCR → Sanger sequencing
  - qPCR → qPCR (different primers?)
- Building risk assessment data and confirmation into the workflow?
- Can sample context plus test validation for HTS allow adequate confidence in the result for action?
- Where not possible or considered not needed – Document this course of action.
- See also PM7/151, Section 6.1

# HTS for crop survey (Fowkes et al (2021) *Viruses*)



- Turnip yellows virus detected in UK peas for the first time
  - Most prevalent virus, in 70-100% of fields, up to 100% of samples in some fields
  - No “visible symptoms”
  - Estimated yield loss of ~40%
- First UK records of:
  - Pea necrotic yellow dwarf virus (widespread in Europe)
  - Soybean dwarf virus (no evidence of impact)
  - Cabbage cytorhabdovirus (no evidence of impact)

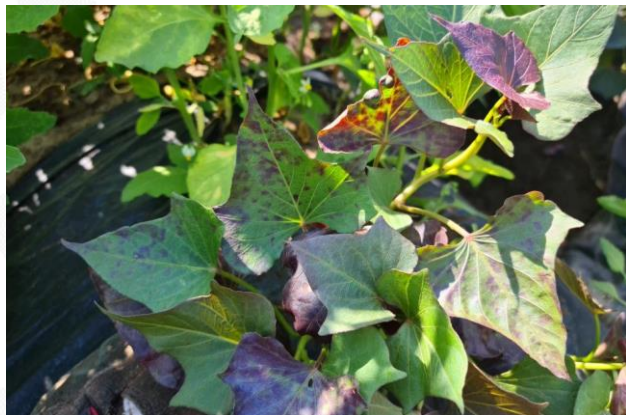
➤ **No plant health action**

# Sweet potato viruses in The Netherlands – action without confirmation?

2022 NPPO-NL  
Official survey in Sweet  
potato (*Ipomoea batatas*)

Validated HTS test;  
ISO 17025 accreditation

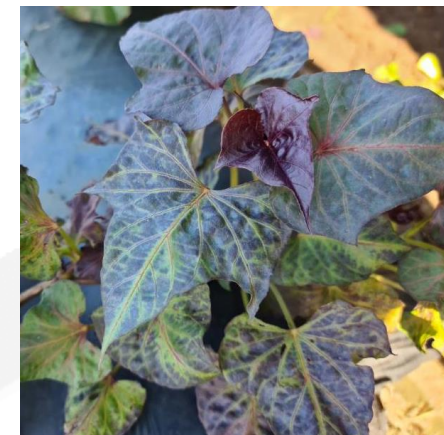
4 different species detected  
1 listed as EU-Quarantine  
(Nearly) complete genome  
sequences



**Sweet potato chlorotic stunt virus**  
(Quarantine)



**Sweet potato chlorotic stunt virus** (Quarantine)  
Sweet potato leaf curl virus (non Q)  
Sweet potato feathery mottle virus (non Q)



Sweet potato virus G + (non Q)



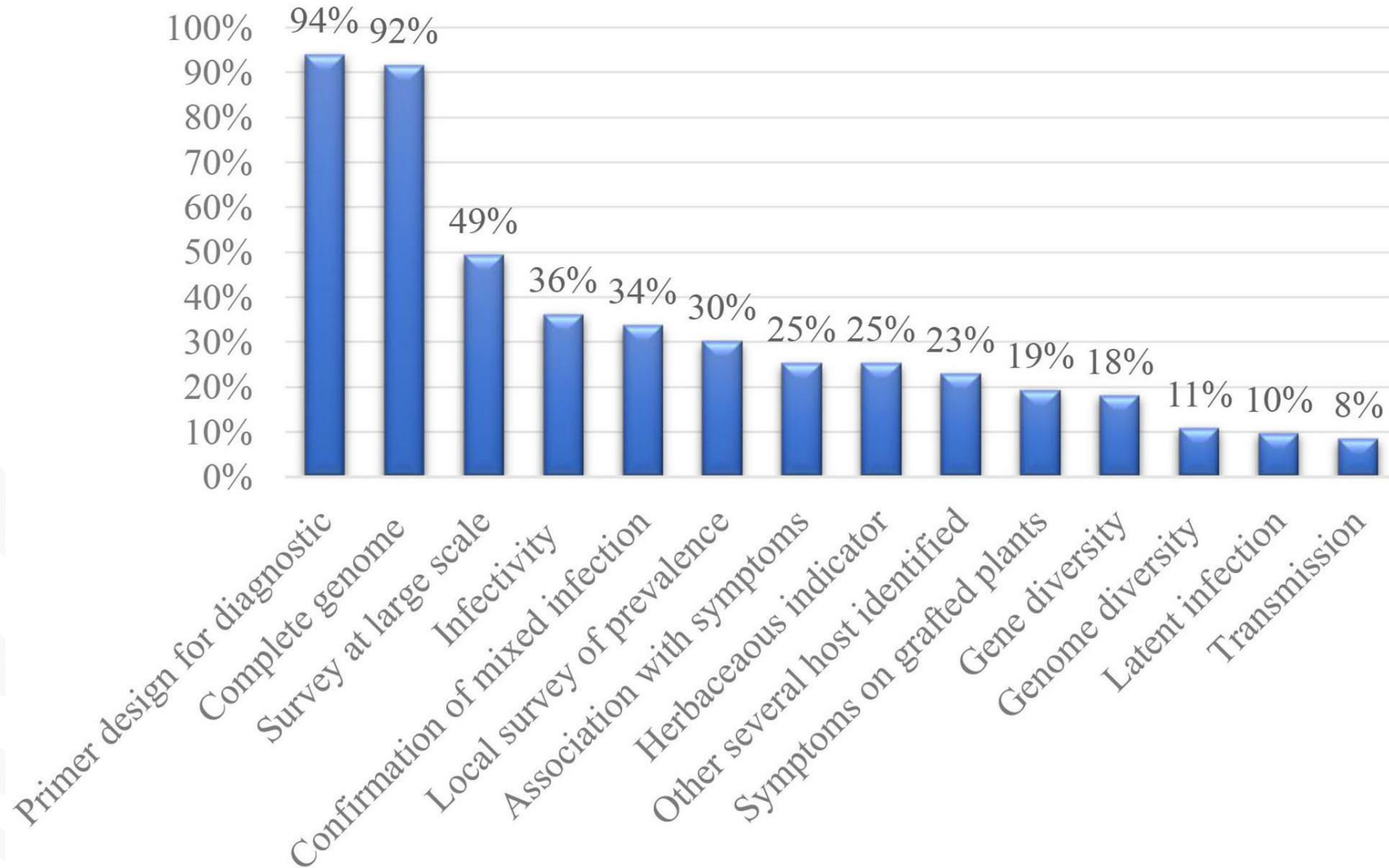
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**Which of these are the most important factors for interpreting risk from a novel virus?**

ⓘ Start presenting to display the poll results on this slide.

# The emerging biological desert...



# “In the sample” or “on the sample”

- Highly sensitive molecular testing can pick up both infection “in” the plant and environmental contamination “on” the plant...
- This may not matter for reporting, but will matter for action!
- Context?
  - Is this surveillance for presence ?
    - Suggests it is present even if not in the plant...
  - Is it on unexpected hosts?
    - Further survey needed?
    - Biological confirmation?

Glasshouse 1

Glasshouse 2

People...

	real-time PCR
	Ct Ave
Top of light	35.36
Fan	22.09
Under Gutter	20.86
Behind whiteboard	33.55
Top of Cable tray	20.25
Bee Box 1	22.91
Inside panel door	23.39
socket lid	25.57
Pheromone trap	31.63
Roof structure G2	17.36
Hive G2	16.98
Top light G2	25.33
Fan G2	17.54
Under gutter	16.76
Tyvek Suit	16.23
Grower Phone	31.18
Grower Glove	19.80
Neg - H2O	40
Pos - ToBRFV+	11.21
Bee box 2 - PEG	n/a
NFT Water	
Substrate Water	

**These are all general diagnostics questions...  
(but HTS has brought them into focus)**



# Reporting and actions...

- We've just found a "thing"
  - Do I need to confirm?
  - Is it regulated?
  - Is it novel?
  - Is it present?
  - If present, is it widespread?
  - Will it impact this crop, other crops or the environment?
- **Do you have these data?**
- Do you need all this data?
  - How much uncertainty can you live with?



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**What do I need to think about when reporting and acting on HTS outcomes?**

ⓘ Start presenting to display the poll results on this slide.