

NAK Services B.V. Randweg 14 Postbus 1115 8300 BC Emmeloord Tel.: +31(0)527 635400 Website: www.nak.nl

Website: www.nak.nl E-mail: nakservices@nak.nl

Rabobank 1344.52.976 IBAN NL13RABO0134452976

BIC: RABONL2U KvK-nr. 53704274

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To our relations

Subject: virus and bacterial diagnostics in potatoes

Date : 18 July 2018 **Character** : 2018/NS/210

About NAK Services Ltd.

We analyse your potato tubers on virus, bacteria and/or nematodes in combination or separately. We offer you several high end examination methods for your seed potatoes. Hereby we work according EN-ISO 17025 norm and quality standards.

Virus

We analyse potato tubers on different viruses with the ELISA- or PCR-technique.

Virus examination	ELISA	Real-time PCR
Determined potato	PVA, PVM, PVS, PVX, PVY,	Standard: PVY, PVA, PVX and PLRV
virus	PVV, PLRV (potato leaf roll	(potato leaf roll virus)
	virus), PMTV (potato mop top	Ontional DVC may 4 virus
	virus) and TSWV (Tomato	Optional: PVS; max. 4 virus combinations in the PCR test
	spotted wilt virus)	combinations in the PCR test
	PVX can be examined separately	TRV (tobacco rattle virus), PMTV
	from PVS, but can also be joined	(potato mop top virus) can also be
	in one ELISA reaction. ELISA:	tested in a PCR test. PMTV and TRV
	combination is cheaper since	are tested on tubers with PMTV and/or
	costs are only charged for one	TRV symptoms
	virus	
Required time for	6 - 9 weeks. Only available in the	< 2 weeks
examination	period 15 August - 15 December	(Priority: within 1 week)
	The required time of an everying time	The DCD test is done directly on tuber
	The required time for examination	The PCR test is done directly on tuber
	depends on the weather	material which saves time compared
	conditions because the samples	to ELISA test
Sample size and	grow in a greenhouse < 110 potato tubers delivered in	200 tubers delivered in mesh bag
packaging	mesh bag	200 tubers delivered in mesh bag
Collecting	96 plants will be tested	50 tubers collected directly taken from
Conceing	4 tubers/plants collected	the tuber (more dependable test)
	Max. 24 reactions per sample	4 reactions per sample
Retesting	On request within two weeks	Retesting is not necessary since
Recesting	after receiving results in case of	germination is no part of the test
	low germination	gommation is no part of the test
	10 vv germination	



Erwinia: bacterial blackleg/stem rot test

We examine potato tubers on the presence of bacteria which causes blackleg/stem rot.

This test will detect (latent) infections of *Pectobacterium atrosepticum (Pa)*, *Dickeya* spp. (Dic), *Pectobacterium paramentieri (Pp)* and *Pectobacterium carotovorum spp brasiliensis (Pcb)*.

	Erwinia
Required time for examination	3 weeks (priority < 2 weeks)
Sample size packaging	200 potato tubers in a meshbag
Collecting	50 tubers collected (4 reactions per sample)
Examination method	Real-time PCR

Meloidogyne chitwoodi and fallax

Meloidogyne chitwoodi (Mc) and M. fallax (Mf) are both quarantine nematodes that can cause severe damage to their host plants, of which Solanum tuberosum (potato) is the most important. Identification to species level is based on molecular method using the real-time PCR.

	Meloidogyne chitwoodi and fallax
Required time for examination	< 4 weeks. Priority < 2 weeks
Sample size packaging	200 potato tubers in a polypropbag
Collecting	100 tubers collected (2 reactions per sample)
Examination method	Real-time PCR (multi-plex)

Ringrot and brownrot test

We offer tests for latent infections of bacterial ring rot caused by *Clavibacter michiganensis* subsp. sepedonicus (*Cms*) and/or brownrot caused by *Ralstonia solanacearum* (*Rsol*).

	Ringrot and brownrot examination
Required time for examination	< 2 weeks; The results of negative samples will
	then become available.
	In case of a suspect sample we will perform
	confirmation tests. The complete diagnosis of
	positive samples for Cms or Rsol will be
	performed within 15 weeks after completing the
	fast screening test
Sample size packaging	200 tubers in a polypropbag
Collecting	200 potato tubers collected (1 reaction per
	sample)
Examination method	IF-method (first screening test).
	When a sample gives a positive reaction in the
	first screening, the sample will be send to Dutch
	NPPO where additional tests will be carried out
	(second screening)



Combination of tests

We advise you to combine the various tests on the same potato sample of 200 tubers. Each analysis will be invoiced separately. In case of an combination of an Erwinia test with a virus test you will pay

€ 75,00 for the Erwinia test instead of € 165,00. *Meloidogyne* examination on 200 tubers will be € 125,00 instead of € 165,00.

All of the following examinations can be performed on one single potato sample of 200 tubers (see annex):

- Virus PCR (standard combination PVY, PVA, PVX and PLRV), on request PVS can also be tested
- Erwinia (Black leg tests)
- Meloidogyne chitwoodi and M. fallax
- Brownrot and ringrot.

Sample labelling

- All your samples should be labelled with unique NAK Services labels which are available on request. Send your request by e-mail to nak@nak.nl
- You should attach one label on the outside of the sample bag and one inside the sample bag
- The samples with GMO modified material should be marked with special GMO labels.
 Contact us before sending GMO samples.

Test results

You receive the results as an Excel- and/or PDF-file, per e-mail.

We present the amount of positive reactions instead of percentage and spread. If you want the percentage of diseased plants and spread, you can use the <u>seedcalc8 program</u>.

Packaging and logistics

Please send your samples in sample bags closed with a ty-rap or string.

We are able to provide any transport and labelling material necessary for transporting your samples to our laboratory (use euro-pallets). Your samples must be transported in a temperature between 10°C and 25°C.

Samples are only accepted on weekdays from 8.00 - 17.00 hour (except on Dutch public holidays). Before sending your samples, please inform us via nak@nak.nl one week beforehand, before sending us your samples. Please provide us with the following details:

- 1) expected date of delivery
- 2) the amount of samples
- 3) description which tests needs to be performed on your samples.

<u>Delivery address:</u> NAK Services Ltd., Randweg 14, 8304 AS Emmeloord, The Netherlands.

Shipment non EU-countries

For shipments and import license from the Dutch Plant Protection Service is needed, which will be sent by NAK Services. Therefore you have to inform NAK Services about the exact number of samples you want to send per shipment, so we can prepare the documents.



Advice and support

Specific questions regarding tests, samples, analytical issues and logistics can be forwarded to our Customer service department via \$\mathbb{\alpha}\$ 0031 (0)527-635400 or nak@nak.nl

The NAK standard terms and conditions for the supply and services apply to our activities.

With kind regards,

M.A.M. Puylaert Account manager NAK Services Ltd.

Attachments: - Overview test methods

- Price list seed potato testing.