



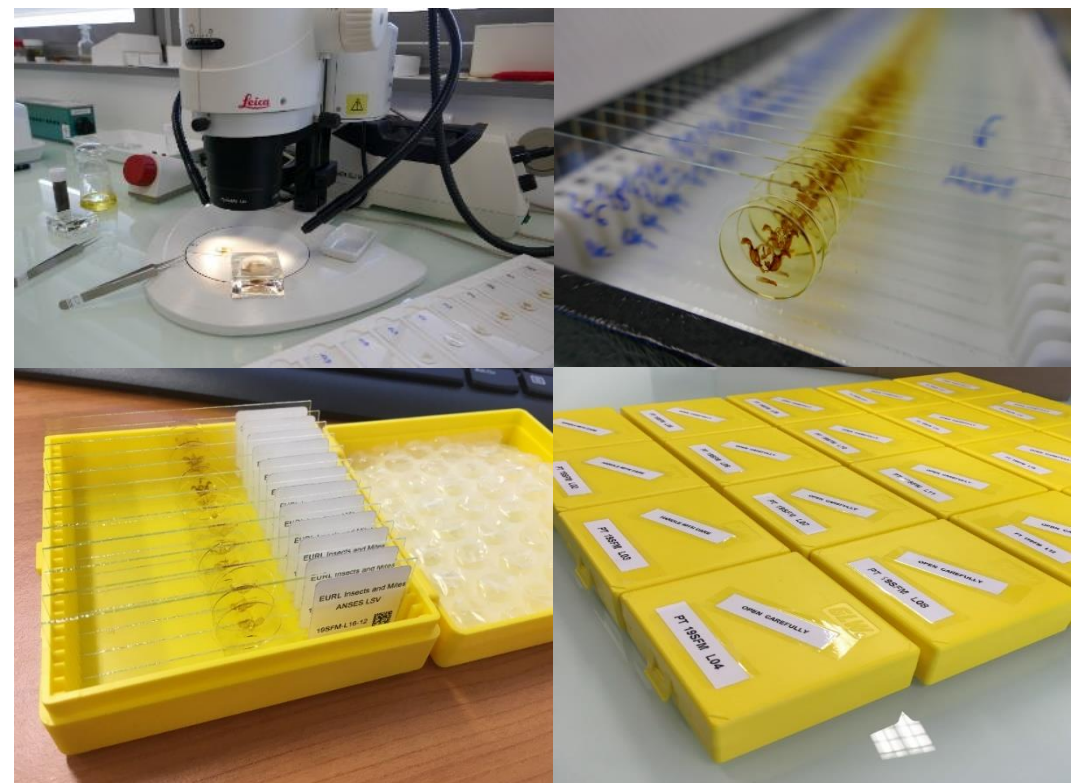
PT on *Spodoptera frugiperda* (2019)

# PT organisation for National Reference Laboratories: Challenges for morphological tests in entomology

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# PT on morphological identification

- Scope: **assess proficiency** of participants to identify the target organism by **morphological analysis** → evaluating accuracy of results (sensitivity and specificity)
- **Diagnostic protocol (EPPO, IPPC)**: recommended by organiser or any other equivalent morphological diagnostic tool
- **Qualitative results**: positive/negative
- Frequency: **one PT/ year**
- Kind of PT samples:
  - Development stage (adult and/or larva)
  - Preparation (specimens in EtOH, slide-mounted specimens, slide-mounted genitalia) → **no** need for **stability check**
- Thorough preparation and validation of PT samples!
  - **Homogeneity and validation of assigned value is exhaustive** → each sample is checked for
    - ✓ presence of all diagnostic characters (targets)
    - ✓ absence of at least one diagnostic character of the target (non-targets)
    - ✓ assigned value (POS/NEG) is confirmed (all)



PT on *Spodoptera frugiperda* (2019)



# PTs, but on which species?

<i>Acidiella kagoshimensis</i>	<i>Bactrocera</i> spp. (except <i>B. oleae</i> )	<i>Diabrotica undec.howardi</i>	<i>Homalodisca insolita</i>	<i>Macugonalia leucomelas</i>	<i>Pissodes nitidus</i>	<i>Strauzia</i> spp. (except <i>S. longipennis</i> )
<i>Acidoxantha bombacis</i>	<i>Bactrocera zonata</i>	<i>Diabrotica undec.undecimpunctata</i>	<i>Homalodisca vitripennis</i>	<i>Margarodes capensis</i>	<i>Pissodes punctatus</i>	<i>Taomyia marshalli</i>
<i>Acleris gloverana</i>	<i>Bemisia tabaci</i>	<i>Diabrotica virgifera zeae</i>	<i>Insizwa oblita</i>	<i>Margarodes greeni</i>	<i>Pissodes strobi</i>	<i>Tapajosa rubromarginata</i>
<i>Acleris issikii</i>	<i>Bemisia tabaci</i> (pop. non eu)	<i>Diaphorina citri</i>	<i>Ips amittinus</i>	<i>Margarodes prieskaensis</i>	<i>Pissodes terminalis</i>	<i>Tecia solanivora</i>
<i>Acleris minuta</i>	<i>Bistrispinaria fortis</i>	<i>Dilobopterus costalimai</i>	<i>Ips cembrae</i>	<i>Margarodes trimeni</i>	<i>Pissodes yunnanensis</i>	<i>Tephritis leavittensis</i>
<i>Acleris nishidai</i>	<i>Bistrispinaria magniceps</i>	<i>Dimargarodes meridionalis</i>	<i>Ips duplicatus</i>	<i>Margarodes vitis</i>	<i>Pissodes zitacuarensis</i>	<i>Tephritis luteipes</i>
<i>Acleris nivisellana</i>	<i>Bothrogonia ferruginea</i>	<i>Dioxyna chilensis</i>	<i>Ips sexdentatus</i>	<i>Margarodes vredendalensis</i>	<i>Pityophthorus juglandis</i>	<i>Tephritis ovatipennis</i>
<i>Acleris robinsoniana</i>	<i>Bucephalagonia xanthopis</i>	<i>Dirioxa pornia</i>	<i>Ips typographus</i>	<i>Marriottella exquisita</i>	<i>Plesiommata corniculata</i>	<i>Tephritis pura</i>
<i>Acleris semipurpurana</i>	<i>Callistomyia flavilabris</i>	<i>Draeculacephala minerva</i>	<i>Keiferia lycopersicella</i>	<i>Massicus raddei</i>	<i>Plesiommata mollicella</i>	<i>Thaumatotibia leucotreta</i>
<i>Acleris senescens</i>	<i>Campiglossa albiceps</i>	<i>Draeculacephala</i> spp.	<i>Leptinotarsa decemlineata</i>	<i>Molomea consolidata</i>	<i>Polygraphus proximus</i>	<i>Thaumatopoea pityocampa</i>
<i>Acleris variana</i>	<i>Campiglossa californica</i>	<i>Dryocosmus kuriphilus</i>	<i>Lepyronia quadrangularis</i>	<i>Monacrostichus citricola</i>	<i>Pomacea</i>	<i>Thaumatopoea processionea</i>
<i>Acrobasis pirivorella</i>	<i>Campiglossa duplex</i>	<i>Eotetranychus lewisi</i>	<i>Leucinodes orbonalis</i>	<i>Monochamus</i> (pop. non eu.)	<i>Poophilus costalis</i>	<i>Thrips palmi</i>
<i>Acroceratitis distincta</i>	<i>Campiglossa reticulata</i>	<i>Euleia separata</i>	<i>Leucinodes pseudorbonalis</i>	<i>Myndus crudus</i>	<i>Popillia japonica</i>	<i>Toxoptera citricidus</i>
<i>Acrogonia citrina</i>	<i>Campiglossa snowi</i>	<i>Eumargarodes laingi</i>	<i>Liriomyza bryoniae</i>	<i>Naupactus leucoloma</i>	<i>Porphyrophora tritici</i>	<i>Toxotrypana curvicauda</i>
<i>Acrogonia virescens</i>	<i>Carpomya incompleta</i>	<i>Euphranta camelliae</i>	<i>Liriomyza huidobrensis</i>	<i>Neaspilota alba</i>	<i>Premnatypus</i>	<i>Toxotrypana recurvicauda</i>
<i>Adrama</i> spp.	<i>Carpomya pardalina</i>	<i>Euphranta canadensis</i>	<i>Liriomyza sativae</i>	<i>Neaspilota reticulata</i>	<i>Procecidochares</i> spp.	<i>Trioza erytreae</i>
<i>Agrilus anxius</i>	<i>Carposina sasakii</i>	<i>Euphranta cassia</i>	<i>Liriomyza trifolii</i>	<i>Nemoriomyza maculosa</i>	<i>Prodioplosis longifila</i>	<i>Trirachys sartus</i>
<i>Agrilus planipennis</i>	<i>Cephalcia lariciphila</i>	<i>Euphranta japonica</i>	<i>Listronotus bonariensis</i>	<i>Neokolla hyperoglyphica</i>	<i>Pseudopityophthorus minutissimus</i>	<i>Trupanea bisetosa</i>
<i>Aleurocanthus citripertus</i>	<i>Ceratitidis</i> spp. (except <i>C. capitata</i> )	<i>Euphranta oshimensis</i>	<i>Lopholeucaspis japonica</i>	<i>Neokolla severini</i>	<i>Pseudopityophthorus pruinosus</i>	<i>Trupanea femoralis</i>
<i>Aleurocanthus spriferus</i>	<i>Ceratotheripoides claratris</i>	<i>Eurhizococcus brasiliensis</i>	<i>Lycorma delicatula</i>	<i>Neoleucomis elegans</i>	<i>Pteronotus confinis</i>	<i>Trupanea wheeleri</i>
<i>Aleurocanthus woglumi</i>	<i>Chloridea virescens</i>	<i>Eurhizococcus colombianus</i>	<i>Macugonalia cavifrons</i>	<i>Oncophanes persimilis</i>	<i>Pteronotus persimilis</i>	<i>Trypanocentra nigrithorax</i>
<i>Anastrepha ludens</i>	<i>Choristoneura carnana</i>	<i>Eurosta solidaginis</i>	<i>Homalodisca insolita</i>	<i>Oncophanes persimilis</i>	<i>Resseliella citrifrugis</i>	<i>Trypeta flaveola</i>
<i>Anastrepha</i> spp.	<i>Choristoneura conflictana</i>	<i>Eutreta</i> spp.	<i>Homalodisca insolita</i>	<i>Oncometopia facialis</i>	<i>Rhagoletis pomonella</i>	<i>Unaspis citri</i>
<i>Anoplophora chinensis</i>	<i>Choristoneura fumiferana</i>	<i>Euwallacea fornicatus</i>	<i>Homalodisca insolita</i>	<i>Oncometopia nigricans</i>	<i>Rhagoletis</i> spp. (with exceptions)	<i>Urophora christophi</i>
<i>Anoplophora glabripennis</i>	<i>Choristoneura lambertiana</i>	<i>Exomala orientalis</i>	<i>Homalodisca insolita</i>	<i>Oncometopia orbona</i>	<i>Rhigopsidius tucumanus</i>	<i>Xanthaciura insecta</i>
<i>Anthonomus bisignifer</i>	<i>Choristoneura orae</i>	<i>Ferraria viridula</i>	<i>Ips amittinus</i>	<i>Oragua discoidula</i>	<i>Rhynchophorus ferrugineus</i>	<i>Xyphon flaviceps</i>
<i>Anthonomus eugenii</i>	<i>Choristoneura parallela</i>	<i>Fingeriana dubia</i>	<i>Ips cembrae</i>	<i>Pagaronia confusa</i>	<i>Rhynchophorus palmarum</i>	<i>Xyphon fulgidum</i>
<i>Anthonomus grandis grandis</i>	<i>Choristoneura pinus</i>	<i>Friscanus friscanus</i>	<i>Ips duplicatus</i>	<i>Pagaronia furcata</i>	<i>Rioxoptilona dunlopi</i>	<i>Xyphon triguttata</i>
<i>Anthonomus quadrigibbus</i>	<i>Choristoneura retiniana</i>	<i>Gastrozona nigrifemur</i>	<i>Ips sexdentatus</i>	<i>Pagaronia tredecimpunctata</i>	<i>Ripersiella hibisci</i>	<i>Zacerata asparagi</i>
<i>Anthonomus signatus</i>	<i>Choristoneura rosaceana</i>	<i>Gilpinia hercyniae</i>	<i>Ips typographus</i>	<i>Pagaronia trinota</i>	<i>Saperda candida</i>	<i>Zeugodacus</i> spp.
<i>Aphrophora angulata</i>	<i>Clasteroptera achatina</i>	<i>Goedenia stenoparia</i>	<i>Keiferia lycopersicella</i>	<i>Paracantha trinotata</i>	<i>Scirtothrips aurantii</i>	<i>Zonosemata electa</i>
<i>Aphrophora permutata</i>	<i>Clasteroptera brunnea</i>	<i>Gonipterus scutellatus</i>	<i>Leptinotarsa decemlineata</i>	<i>Parastenopa limata</i>	<i>Scirtothrips citri</i>	
<i>Apriona cinerea</i>	<i>Conotrachelus nenuphar</i>	<i>Graphocephala atropunctata</i>	<i>Lepyronia quadrangularis</i>	<i>Paratephritis fukaii</i>	<i>Scirtothrips dorsalis</i>	
<i>Apriona germari</i>	<i>Craspedoxantha marginalis</i>	<i>Graphocephala confluens</i>	<i>Leucinodes orbonalis</i>	<i>Paratephritis takeuchii</i>	<i>Scolytinae</i> (species non eu.)	
<i>Apriona rugicollis</i>	<i>Cuerna costalis</i>	<i>Graphocephala versuta</i>	<i>Leucinodes pseudorbonalis</i>	<i>Paraterellia varipennis</i>	<i>Sibovia sagata</i>	
<i>Aromia bungii</i>	<i>Cuerna occidentalis</i>	<i>Grapholita inopinata</i>	<i>Liriomyza bryoniae</i>	<i>Parathona gratiosa</i>	<i>Sonesimia grossa</i>	
<i>Arrhenodes minutus</i>	<i>Cyphonia clavigera</i>	<i>Grapholita packardii</i>	<i>Liriomyza sativae</i>	<i>Paysandisia archon</i>	<i>Sphaeniscus binoculatus</i>	
<i>Aschistonyx eppoi</i>	<i>Dacus</i> spp.	<i>Grapholita prunivora</i>	<i>Liriomyza trifolii</i>	<i>Philophylla fossata</i>	<i>Sphenella nigricornis</i>	
<i>Asimoneura pantomelas</i>	<i>Daktulosphaira vitifoliae</i>	<i>Gymnocarena</i> spp.	<i>Listronotus bonariensis</i>	<i>Phyrdenus muricensis</i>	<i>Spodoptera eridania</i>	
<i>Austrotephritis protrusa</i>	<i>Dechacoona missionum</i>	<i>Helicoverpa zea</i>	<i>Lopholeucaspis japonica</i>	<i>Pissodes cibriani</i>	<i>Spodoptera frugiperda</i>	
<i>Bactericera cockerelli</i>	<i>Dendroctonus micans</i>	<i>Helochara delta</i>	<i>Lycorma delicatula</i>	<i>Pissodes fasciatus</i>	<i>Spodoptera litura</i>	
<i>Bactrocera dorsalis</i>	<i>Dendrolimus sibiricus</i>	<i>Hishimonus phycitis</i>	<i>Macugonalia cavifrons</i>	<i>Pissodes nemorensis</i>	<i>Spodoptera ornithogalli</i>	
<i>Bactrocera latifrons</i>	<i>Diabrotica barberi</i>	<i>Homalodisca ignorata</i>			<i>Sternochetus mangiferae</i>	

Final count: 266 taxa



Regulation (EU) 2021/2285  
(RNQP excluded)  
&  
Regulation (EU) 2022/1941

# PTs, but on which species?

- Number of EU quarantine insects and mites has increased over the years
- Regulation (EU) 2021/2285 and (EU) 2022/1941: **266 quarantine taxa** (250 species, 15 genera, 1 family) belonging to 8 orders and 40 families
- “Priority to EU priority pests!” → **16 species** as from Regulation (EU) 2019/1702
- **Criteria of choice** :
  - Actual threat (range expansion ongoing)
  - Presence on the EU territory with limited distribution
  - Availability of official diagnostic protocols
  - Availability of suitable biological material (non-targets as critical factor)
- “**Two PTs in one**” formula when possible (*Bactrocera dorsalis* and *B. zonata*) → optimization of resources



PT on *Spodoptera frugiperda* (2019) © MATT BERTONE 2014



PT on *Popillia japonica* (2021)



PT on *Bactrocera* spp. (2023)

# A matter of quantity and quality

- PT requires availability of **high quality specimens in large number**
    - 12 samples per panel
    - 26 participant NRLs (+ EFTA + Third Countries)
    - + Spare panels
- ➔
- ~360 samples  
(130 target & 230 non-target)

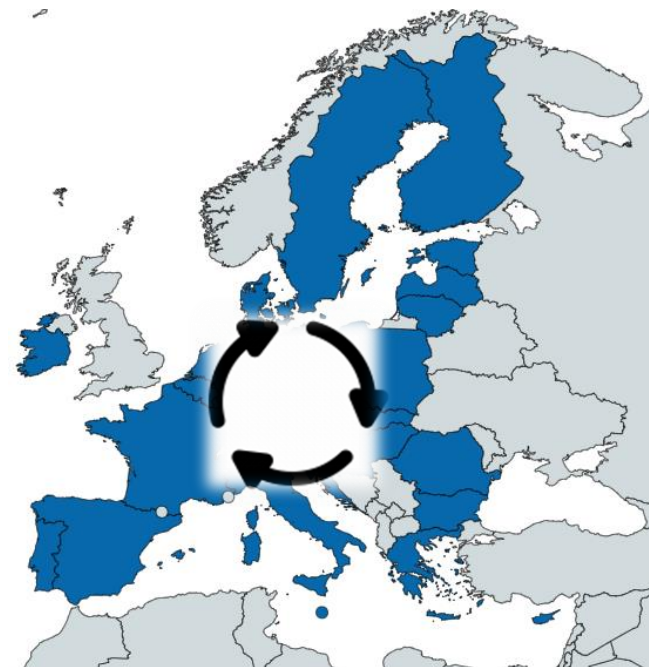


Availability of enough specimens at EURL?  
Quality of available specimens? Homogeneity?

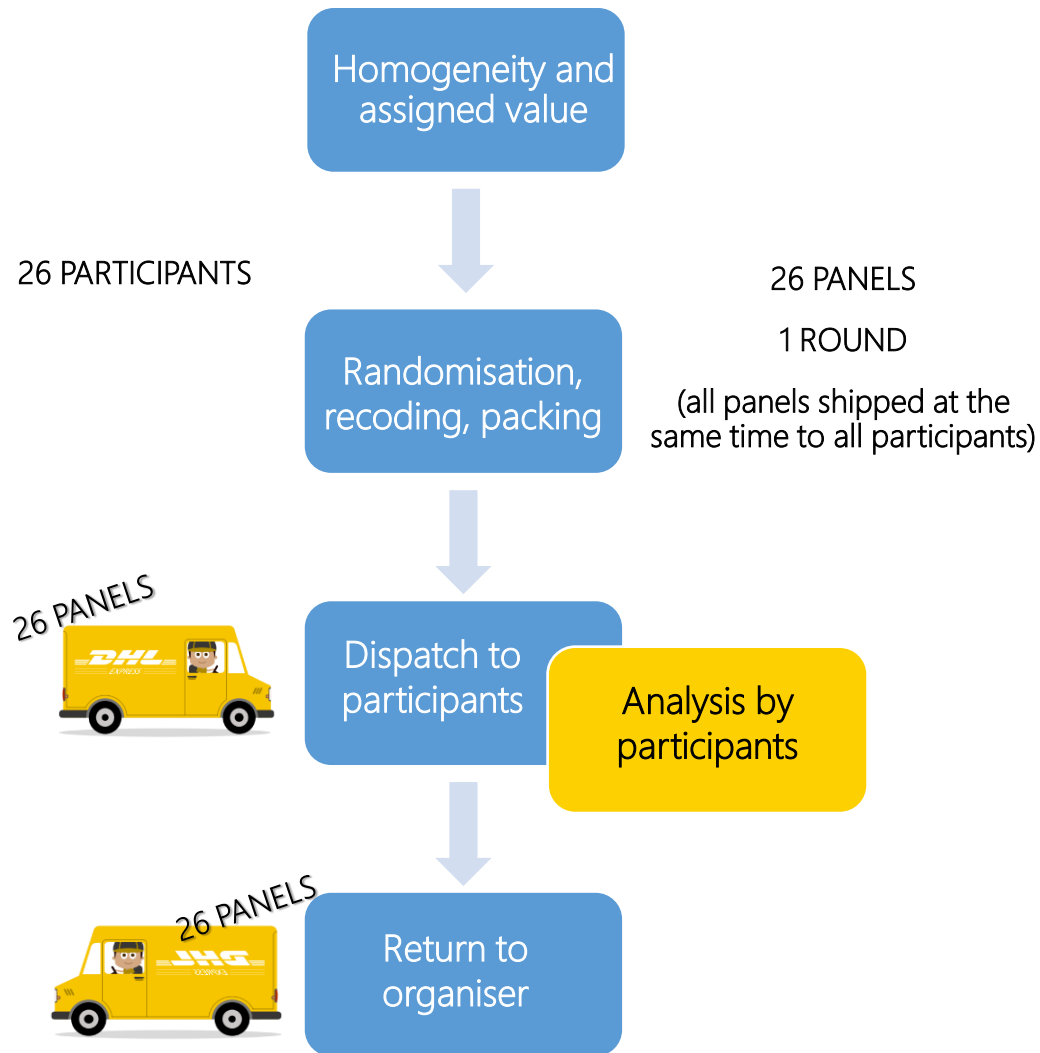
- **Strategy:**
  - specimens available in the collections of the two consortium partners (Anses et Ages)
  - constant effort in obtaining specimens from rearing centres and research institutes

➢ If still not enough? ➔

PT format based on fewer panels  
being circulated among  
participants



# The classic format

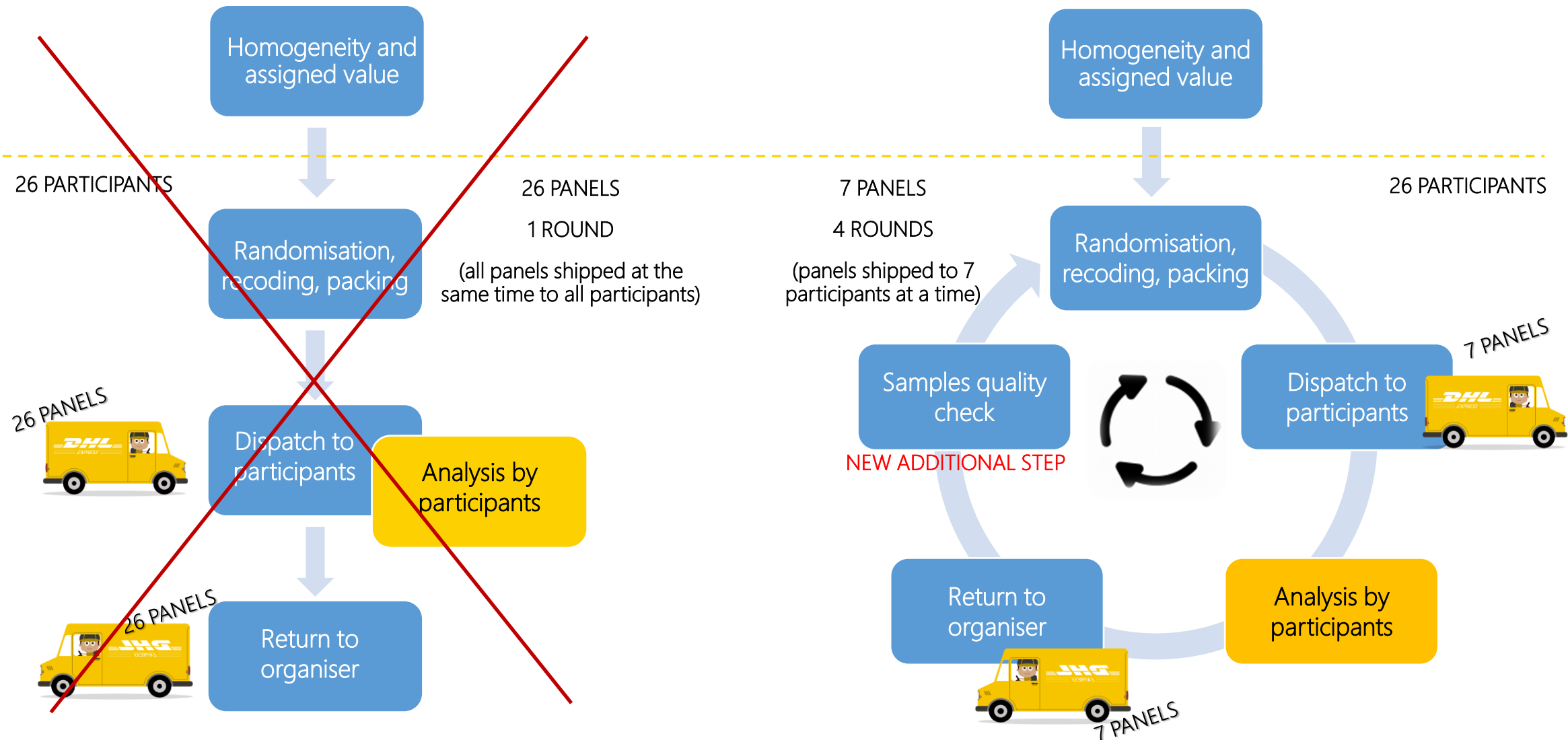


- Biological material is available in sufficient quantity and quality → one single PT round
- All panels shipped at the same time, all participants perform the analysis at the same time
- Return to organiser → PT samples can be re-used in future



PT on *Popillia japonica* (2021)

# A new format: sequential PT



# A new format: sequential PT

A **limited number** of panels is sent **sequentially** to a corresponding number of participants at a time → several dispatch rounds

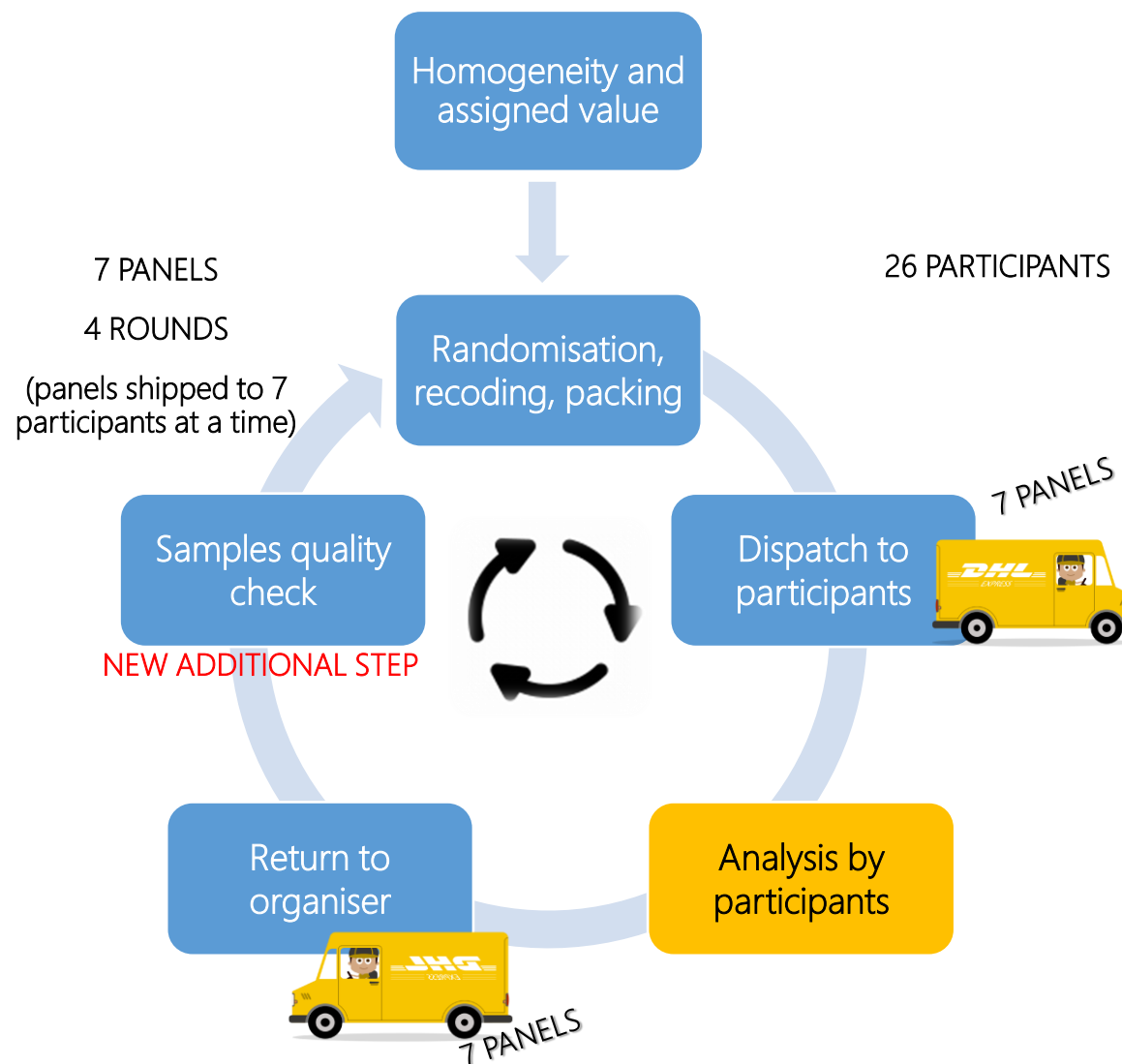
## Pros..

- ✓ reduced number of PT samples
- ✓ high quality samples
- ✓ better adequacy of non-target samples

## ..& cons

- **NEW ADDITIONAL STEP:** sample quality check after each round of analysis
- extended time frame (delayed final report)
- higher risk of damaged samples due to repeated manipulation by different operators
- single spare samples to replace damaged ones (in addition to spare panels in case of shipment issues)

Participants are invited to **respect of deadlines** and handle samples **with extreme care** → potential impact on PT schedule





# Are there alternatives to PTs?

- Large number of quarantine pests on which to assess NRLs proficiency makes it complicated to increase frequency of PTs on a given pest. As a consequence:
  - NRLs need to find other means to maintain Accreditation (ISO 17025, § 7.7 Ensuring the validity of results) → internal means of control
  - Non-conforming results: it takes a long time before a NRL can rectify them
  
- Exploring possible alternatives to PTs/ additional services :
  - **comparison of results** on characterised samples available as
    - reference materials (RM)
    - panels from previous PT sessions
  - **bilateral testing**: the EURL performs the test in parallel with the laboratory to be evaluated using samples encountered in routine analyses, provided either by the
    - EURL
    - laboratory under evaluation



**Non-conforming results**

EURL supports NRLs in cause analysis and follow up actions

- Reference material
- Targeted, hands-on training sessions

# Conclusions

Organisation of PTs for morphological test in entomology can be challenging

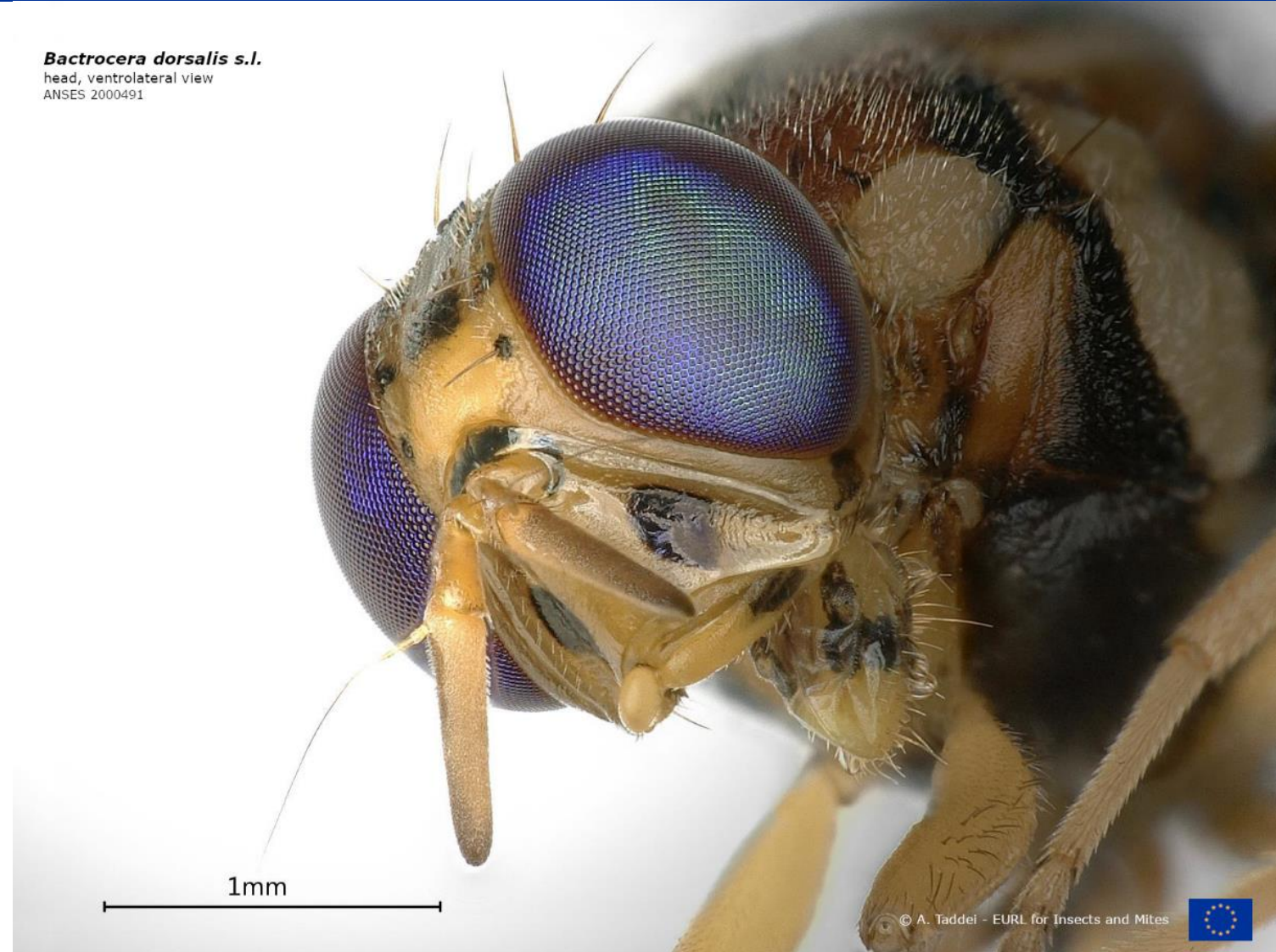
- **Large number** of quarantine pests (266 taxa, of these 16 are priority pests) → low frequency of organisation of the same PT
  - possible negative impact on maintaining ISO 17025 accreditation for NRLs
  - long time before non-conforming results are rectified
- “Two PTs in one”, combining two target organisms in the same PT (congeneric, similar species)
- Availability of suitable (quality-quantity) biological material as critical factor
  - **Sequential PT**: limited number of panels is sent sequentially to a corresponding number of participants at a time → several dispatch rounds
    - fewer samples needed, better adequacy of non-target...
    - ..but delayed report, higher risk of samples damage, additional quality check step
- Exhaustive homogeneity check and validation of assigned value (PT samples checked one by one)
- Return of panels to organiser → valuable material for future PTs!



PT on *Thaumatotibia leucotreta* (2022)

# Thank you!

***Bactrocera dorsalis s.l.***  
head, ventrolateral view  
ANSES 2000491



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*The consortium between ANSES (France) and AGES (Austria)  
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