

Survey on diagnostic tests for ruminant helminth infections and anthelmintic resistance across COMBAR members' labs, and their Technology Readiness Level

A questionnaire on diagnostic tests used in the COMBAR members' labs was developed and made available online (from 26th September 2019 to 22nd April 2021) at the link <https://survey.zohopublic.eu/zs/D9B86u>.

The survey consisted of 11 questions listed on page 5.

A total of 37 researchers (out of 98 contacted) from 33 institutions (23 universities and 10 research centres) of 24 countries (Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Spain, Switzerland, Tunisia, Turkey, United Kingdom) participated in the survey. In three institutions more than one researcher responded to the survey.

Most of the interviewed researchers focussed their studies on helminths and AR in sheep (89.2%), followed by goats (59.5%) and cattle (48.7%). In 43.2% of cases, researchers studied two/three ruminant species contemporaneously.

Moreover, most researchers (75.7%) were interested in both gastrointestinal nematodes (GIN) and liver flukes (*Fasciola hepatica*).

Coprological techniques and serological methods were the approaches most widely used for the diagnosis of GIN and *F. hepatica* infection in all ruminant species. In particular, McMaster (71.4%) and Mini-FLOTAC (40%) were the FEC methods most used to detect GIN infections, whereas sedimentation (56.7%) was the most used technique to detect *F. hepatica* infections.

As for the immunological techniques, the commercial ELISA kits (60%) were more used than the home-made ELISAs (40%) to detect GIN infections (e.g. bulk milk ELISA for *Ostertagia*), as well as for the diagnosis of *F. hepatica* (71.4%).

As for the DNA based techniques, the Real-time PCR (50%) and end-point PCR and/or nested-PCR (50%) were the most used approaches for GIN detection, while the PCR-end point and/or the nested-PCR (77.8%) and the Loop-mediated isothermal Amplification (LAMP) (44.4%) were the molecular techniques most used for the diagnosis of *F. hepatica*.

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All the data collected in this survey are reported in the Annex 1. Links to dynamic maps of institutions that use the different diagnostic techniques are available under the static maps reported in the Annex 1.

A complete list of the diagnostics (tools, distributors, producers, prices, and targets) used in the COMBAR members' labs is reported on page 25.

The Technology Readiness Levels (TRLs) of the most used techniques for the diagnosis of GIN and *F. hepatica*, as well as for the detection of anthelmintic resistance are reported in the Annex 2.

Noteworthy, also through the activities of this COST project techniques such as the Mini-FLOTAC method have been developed to TRL 9 level.

It appears remarkable that according to this survey still copromicroscopic techniques are by far the most often used methods for the direct detection of infections with helminth parasites in ruminants. This is certainly a major difference to other fields of infectious diseases such as bacteriology or virology, where molecular and proteomic approaches are being used in routine diagnostics to a much greater level. In addition to economics/costs also the comparatively high practicability and precision of copromicroscopic techniques might be regarded as reasons for their still high popularity. Concerning the molecular techniques for the identification of intestinal helminths in ruminants novel next-generation-sequencing approaches that have recently been described, are currently being established in several COMBAR labs. These as well as the already established molecular tools require further increase concerning TRL. However, proteomic tools such as Matrix-Assisted-Laser-Desorption-Ionisation/Time-Of-Flight assays have thus far not been employed for the identification and differentiation of helminth infections in ruminants.

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Annex 1- Outcome of the survey

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WG1

Survey on Diagnostic Tools used in COMBAR members' labs

26th September 2019- 22nd April 2021

Aim: To create a list of harmonized, validated and newly introduced diagnostic tests across European COMBAR labs

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Survey questionnaire

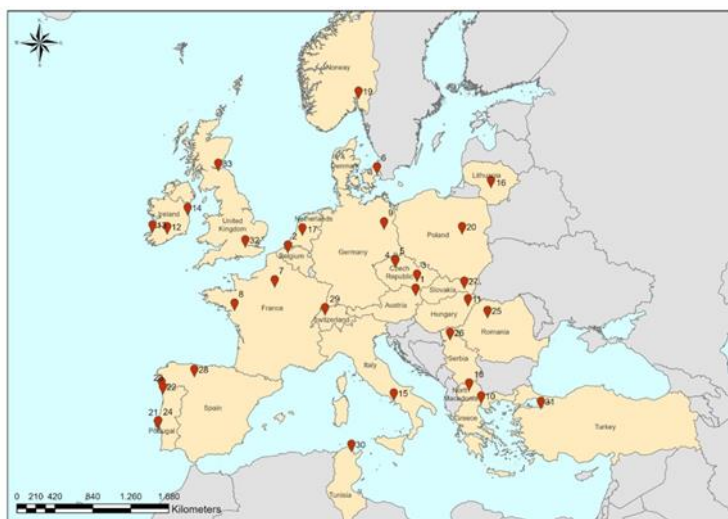


11 Questions:

1. Main ruminant species studied
2. Type of samples (faeces, blood, serum, milk, etc.) used to detect gastrointestinal nematode (GIN) infection
3. Type of samples (faeces, blood, serum, milk, etc.) used to detect *Fasciola hepatica* infection
4. Technique used to detect GIN infection
5. Technique used to detect *Fasciola hepatica* infection
6. Indicate the FEC technique used to detect GIN eggs
7. Indicate the FEC technique used to detect *Fasciola hepatica* eggs
8. Indicate the immunological technique used to detect GIN infection, if it is a home made or a commercial kit
9. Indicate the immunological technique used to detect *Fasciola hepatica* infection, if it is a home made or a commercial kit
10. Specify the DNA-based technique to detect GIN infection
11. Specify the DNA-based technique to detect *Fasciola hepatica* infection

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33 Labs – 24 Countries

Link to dynamic map: <https://arcg.is/0vPHyf>

| ID | Institution | Country | Survey responders |
|----|---|-----------------|-------------------|
| 1 | Institut für Parasitologie und Zoologie | Austria | 1 |
| 2 | Ghent University | Belgium | 1 |
| 3 | Masaryk University | Czech Republic | 1 |
| 4 | Czech University of Life Sciences Prague | Czech Republic | 2 |
| 5 | Charles University (Faculty of Pharmacy) | Czech Republic | 1 |
| 6 | University of Copenhagen | Denmark | 1 |
| 7 | Agence Nationale de Sécurité Sanitaire Alimentaire (ANSES) | France | 1 |
| 8 | Institute National de la Recherche Agronomique (INRA Oniris) | France | 1 |
| 9 | FU Berlin - Institute of Parasitology and Tropical Veterinary Medicine | Germany | 1 |
| 10 | Veterinary Research Institute Hellenic Agricultural Organization (ELGO-DIMITRA) | Greece | 1 |
| 11 | University of Debrecen | Hungary | 1 |
| 12 | Agriculture and Food Development Authority (Teagasc) | Ireland | 1 |
| 13 | Department of Agriculture, Food and the Marine | Ireland | 1 |
| 14 | University College of Dublin | Ireland | 1 |
| 15 | University of Naples Federico II | Italy | 1 |
| 16 | Lithuanian University of Health Sciences (Veterinary Academy) | Lithuania | 1 |
| 17 | Utrecht University | Netherlands | 1 |
| 18 | Local Action Group Agro Lider | North Macedonia | 1 |
| 19 | Norwegian University of Life Sciences (NMBU) | Norway | 3 |
| 20 | Warsaw University of Life Sciences | Poland | 1 |
| 21 | Instituto de Higiene e Medicina Tropical | Portugal | 1 |
| 22 | Escola Superior Agrária - Instituto Politécnico de Viana do Castelo | Portugal | 1 |
| 23 | Instituto Nacional de Investigação Agrária e Veterinária | Portugal | 1 |
| 24 | Lusofona University of Lisbon | Portugal | 1 |
| 25 | University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca | Romania | 1 |
| 26 | Faculty of Agriculture, University of Novi Sad | Serbia | 1 |
| 27 | Institute of Parasitology | Slovakia | 1 |
| 28 | Universidad de León (Institute of Mountain Livestock) | Spain | 1 |
| 29 | Institute of Parasitology | Switzerland | 1 |
| 30 | Ecole Nationale de Médecine Vétérinaire (ENMV) | Tunisia | 1 |
| 31 | Bursa Uludag University | Turkey | 1 |
| 32 | Animal and Plant Health Agency (APHA) | United Kingdom | 1 |
| 33 | Moredun Research Institute | United Kingdom | 2 |

Figure 1. Map and list of COMBAR labs that participated at the questionnaire survey on diagnostic tests.

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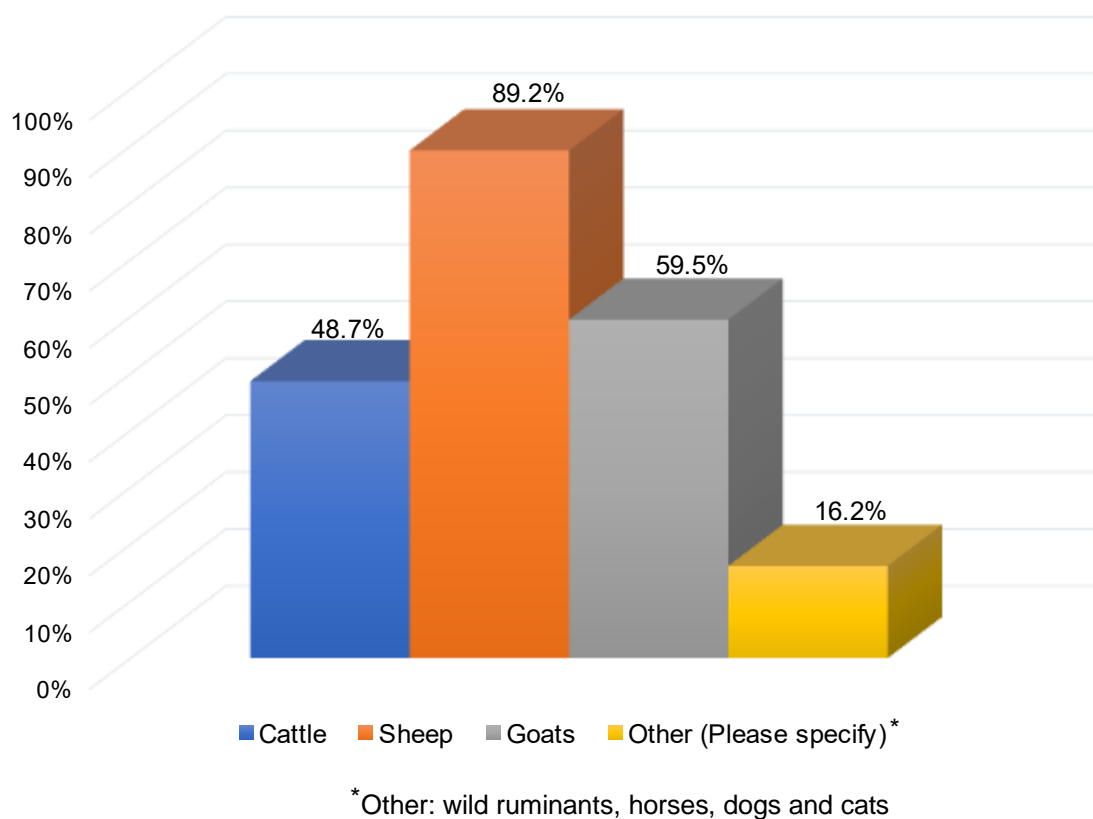


Figure 2. Main ruminant species studied in the COMBAR labs.

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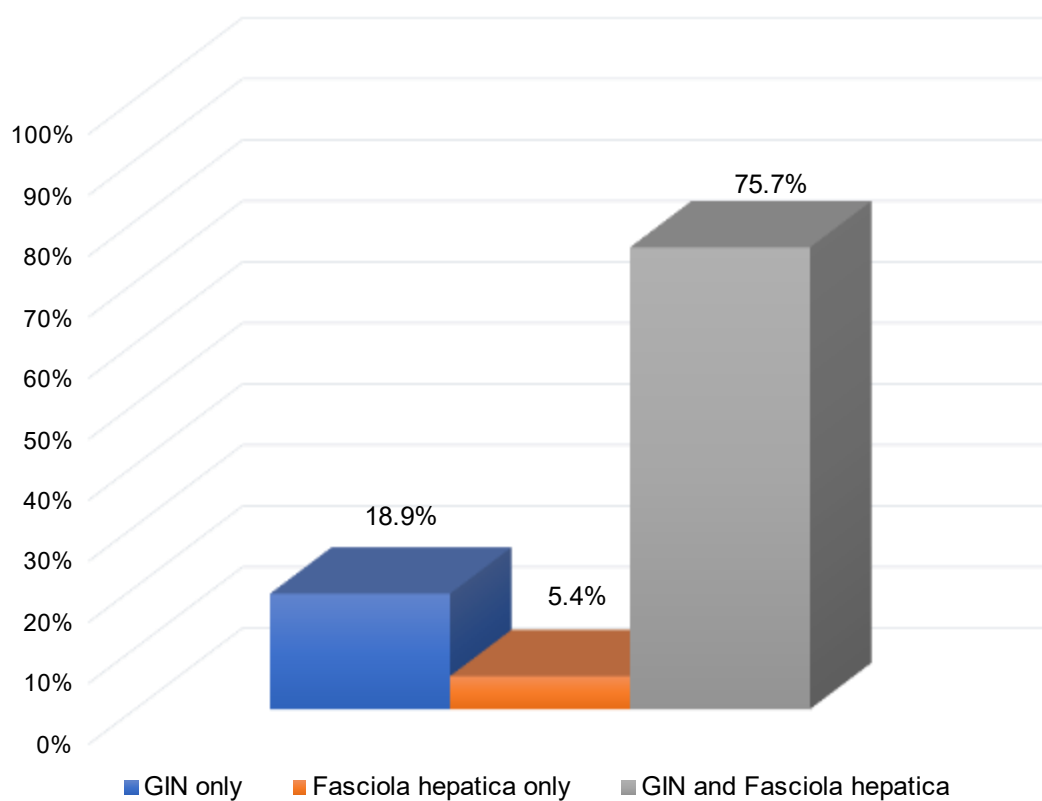
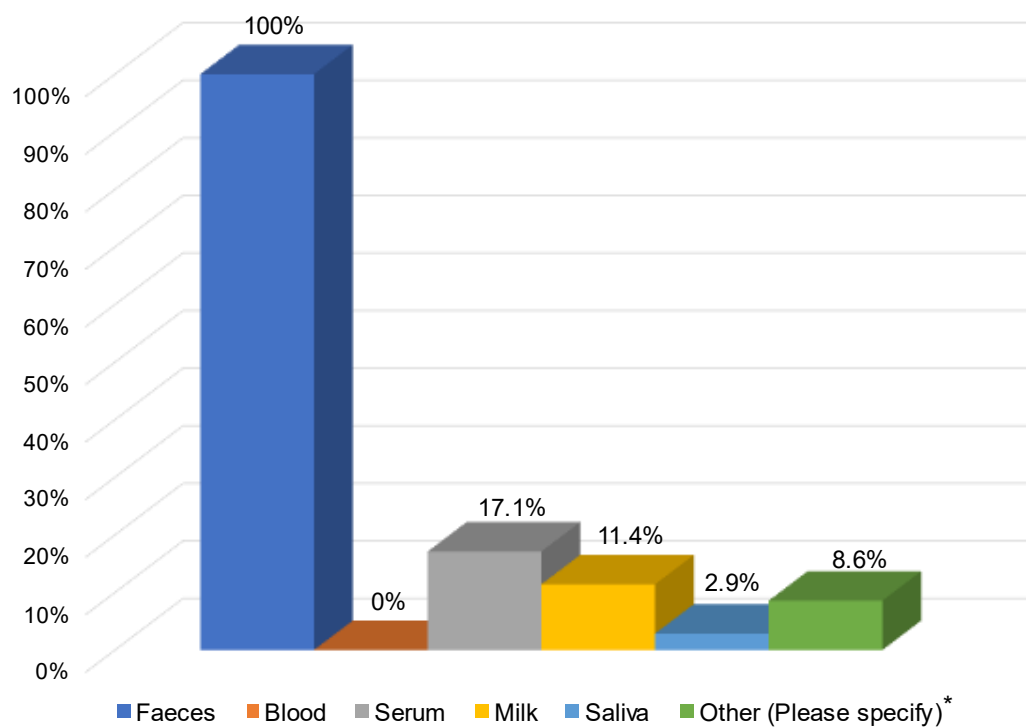


Figure 3. Main parasites diagnosed in the COMBAR labs.

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*Other: necropsy - nasal secretions

Figure 4. Type of matrices (faeces, blood, serum, milk etc.) used for the diagnosis of GIN in the COMBAR labs.

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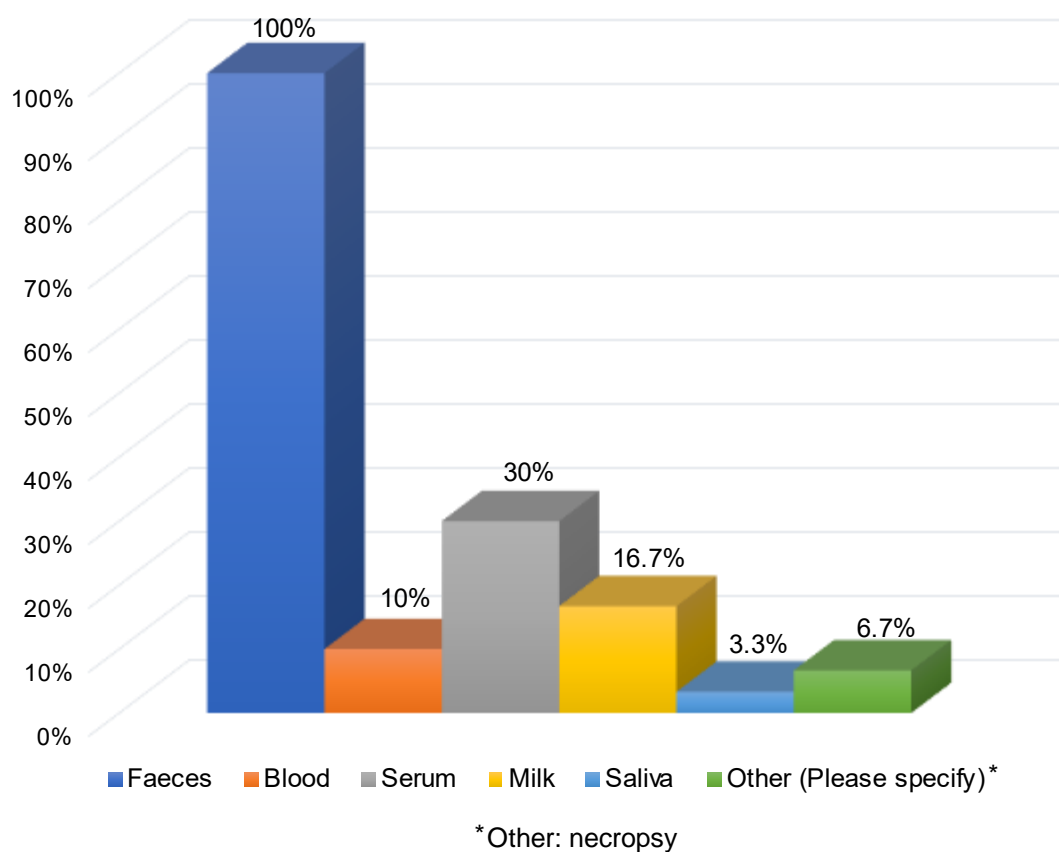


Figure 5. Type of matrices (faeces, blood, serum, milk etc.) used for the diagnosis of *Fasciola hepatica* in the COMBAR labs.

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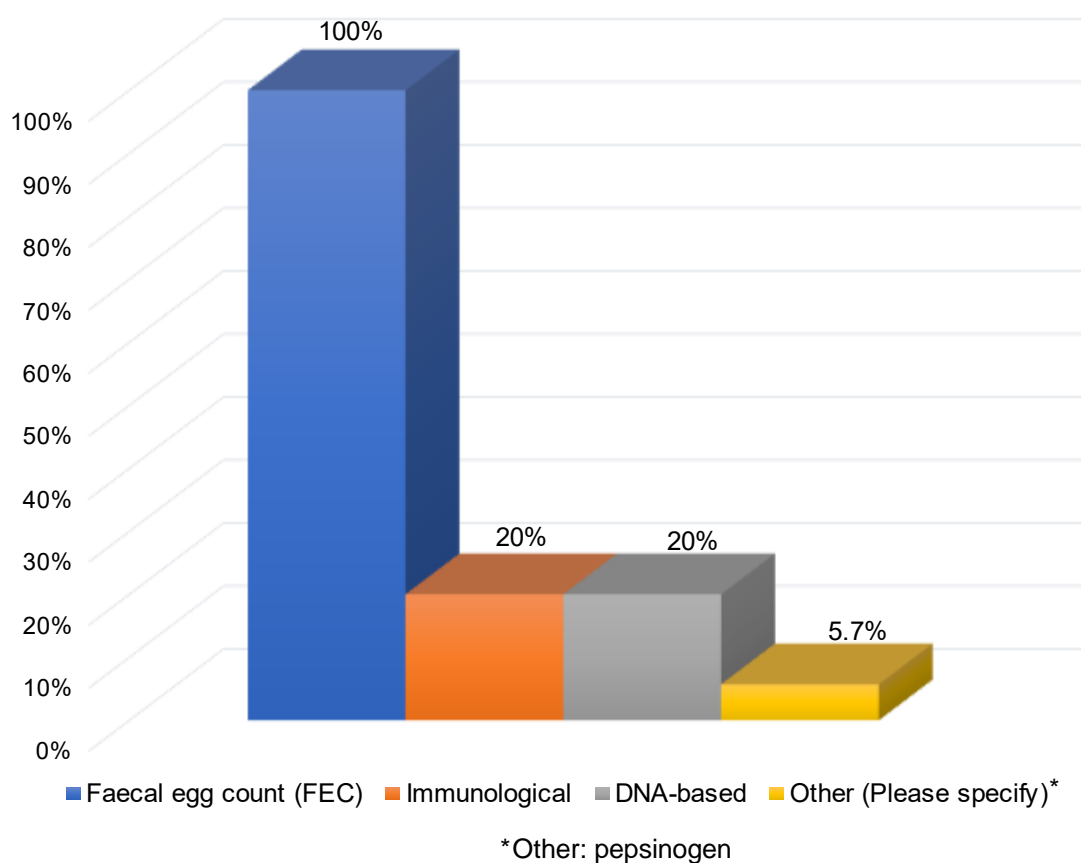


Figure 6. Techniques used to detect GIN infection in the COMBAR labs.

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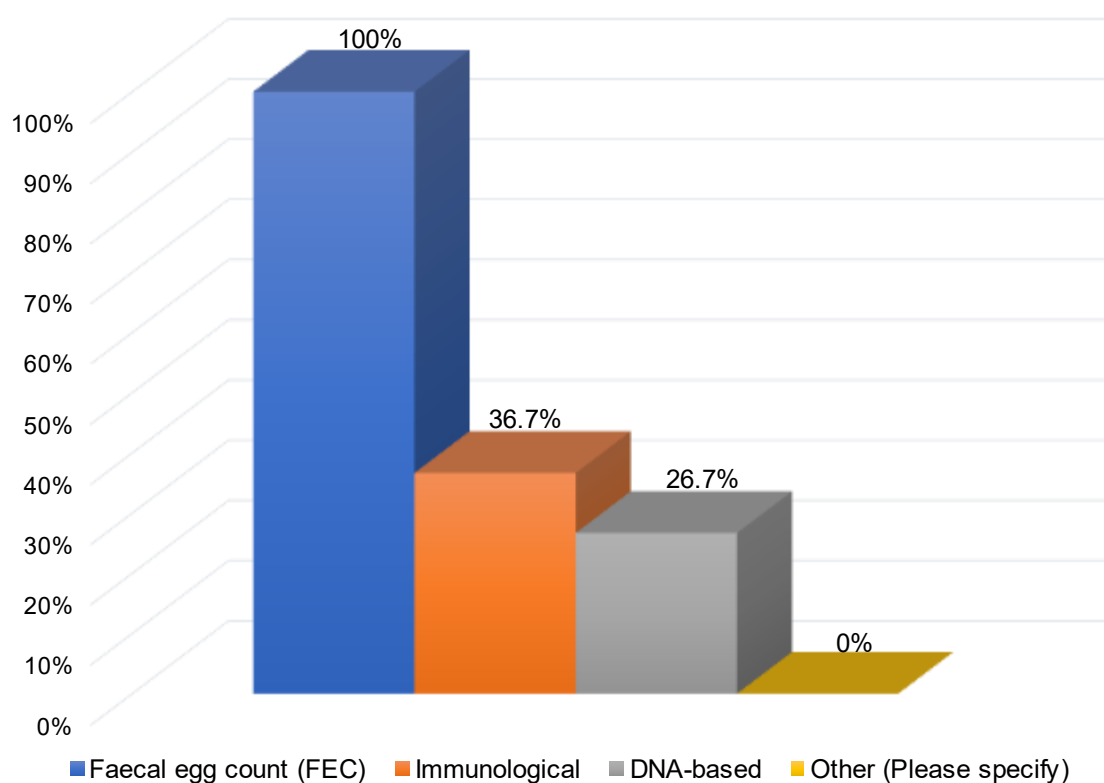
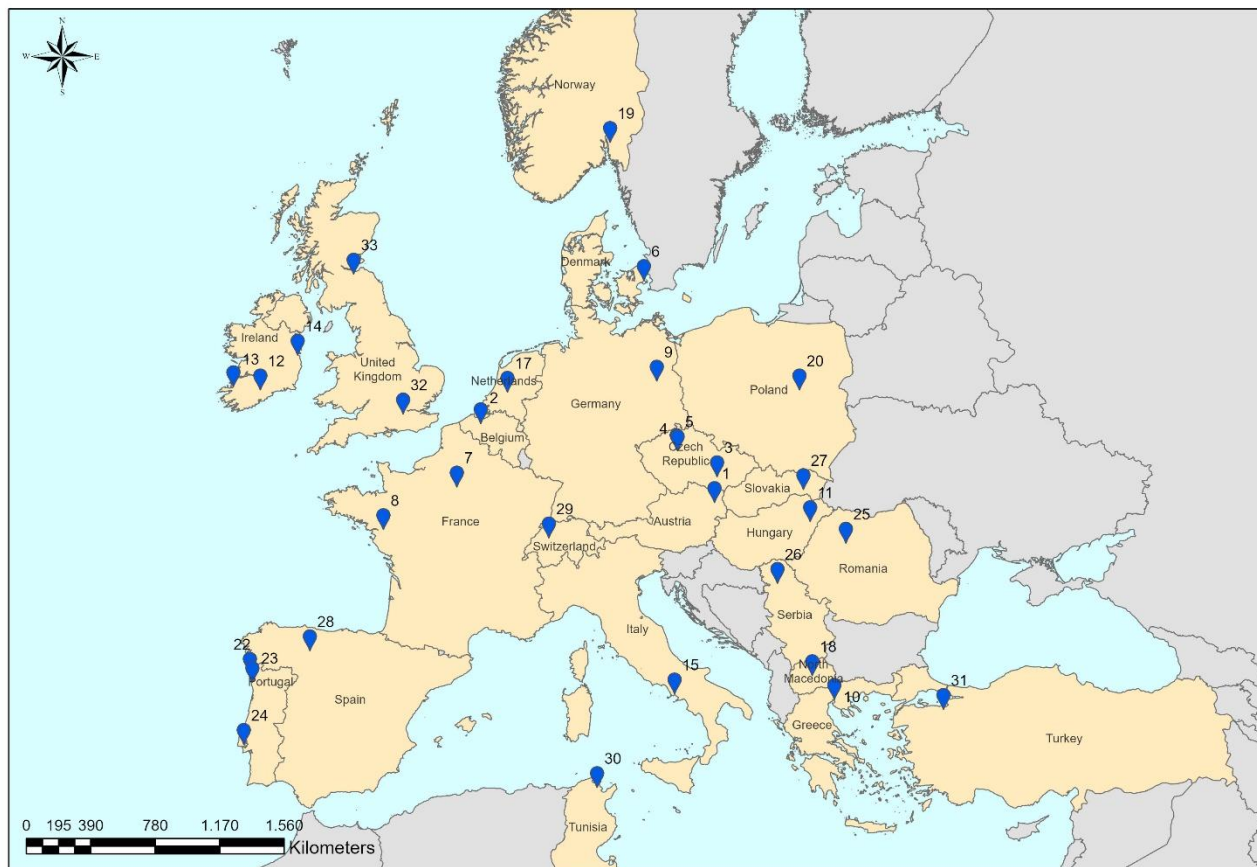


Figure 7. Techniques used to detect *Fasciola hepatica* infection in the COMBAR labs.

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31 Labs – 23 Countries

Link to dynamic map: <https://arcg.is/0OnTrC>

Figure 8. COMBAR labs where FEC techniques are used for diagnosis of GIN.

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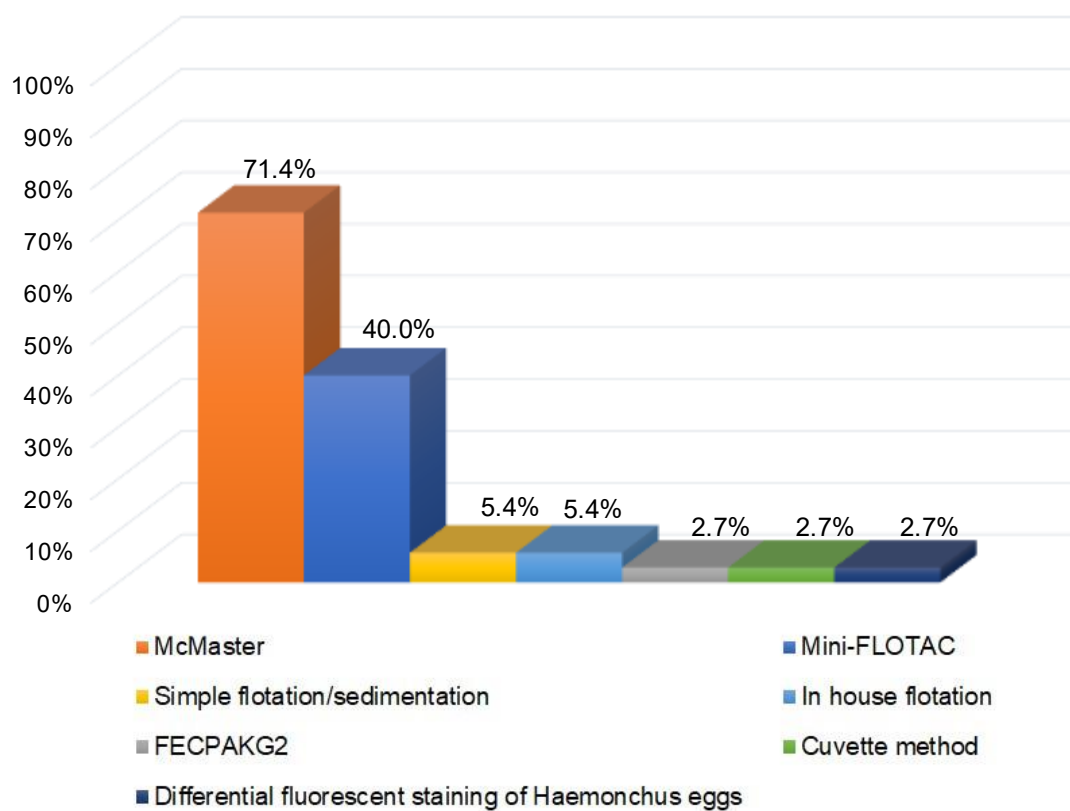
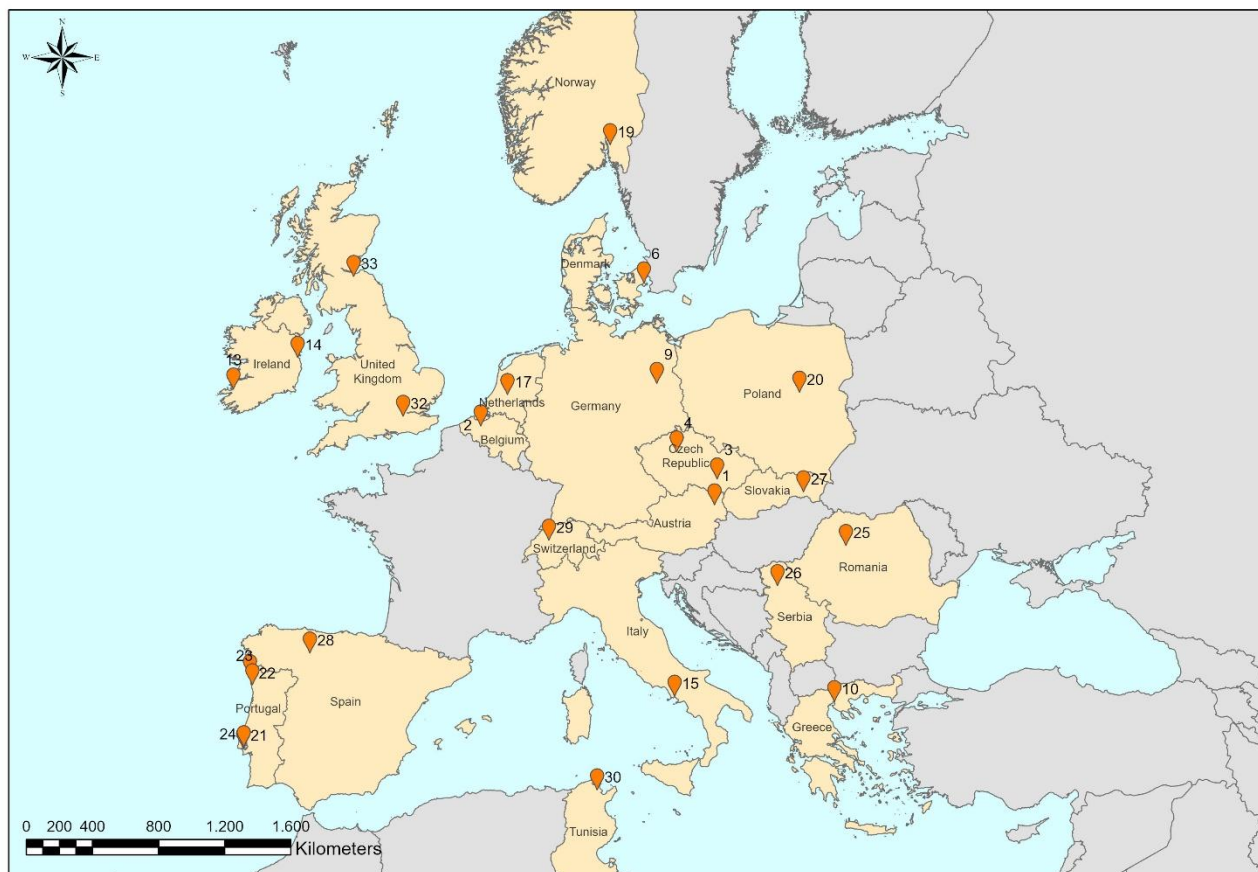


Figure 9. FEC techniques used for diagnosis of GIN eggs in the COMBAR labs.

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25 Labs – 19 Countries

Link to dynamic map: <https://arcg.is/0OnTrC>

Figure 10. COMBAR labs where FEC techniques are used for diagnosis of *Fasciola hepatica*.

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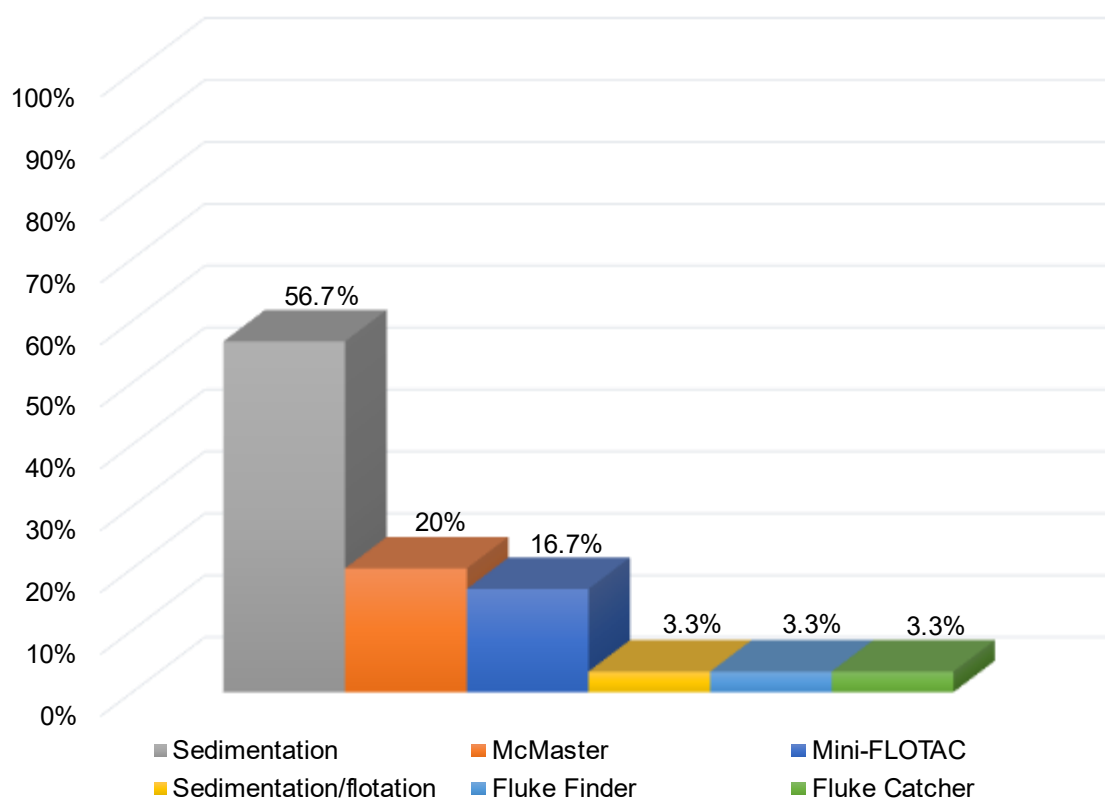
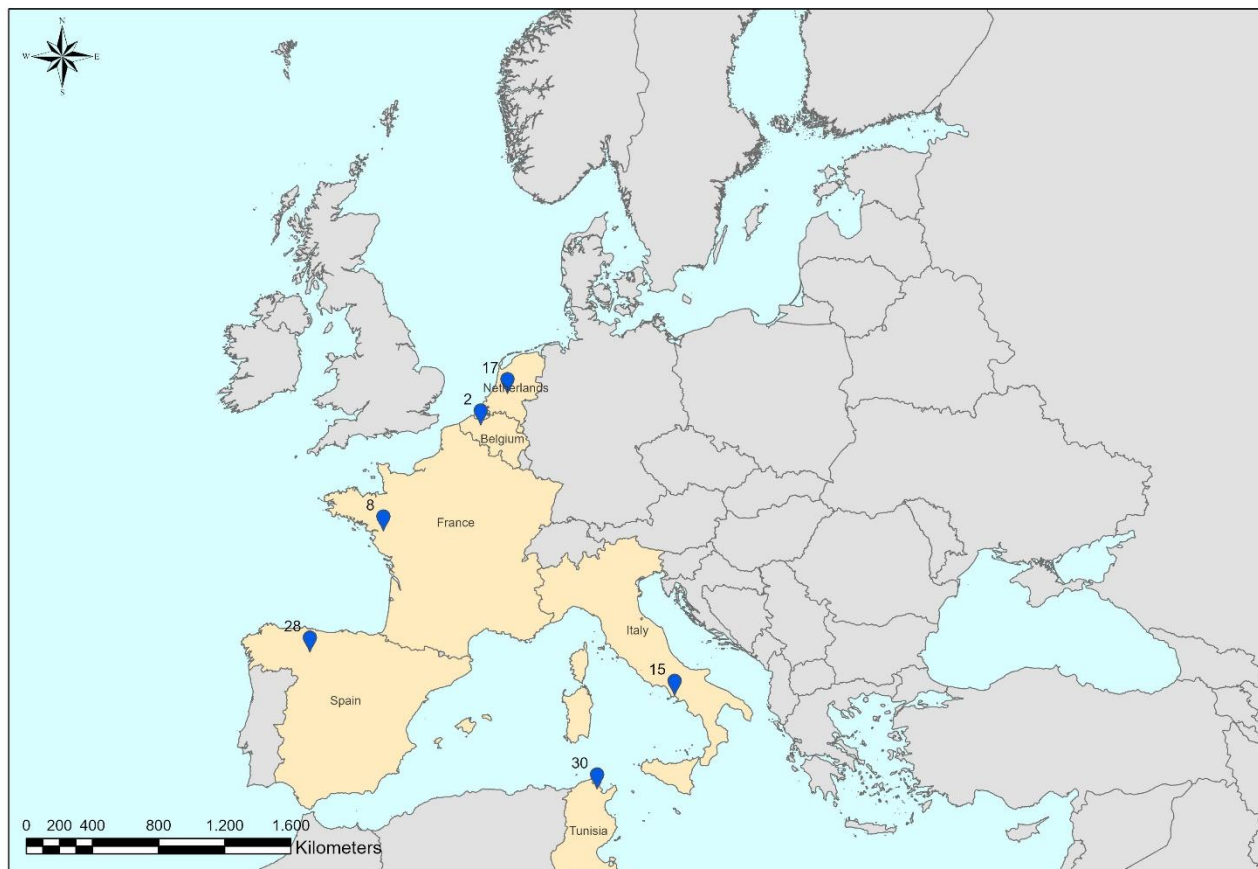


Figure 11. FEC techniques used for diagnosis of *Fasciola hepatica* eggs in the COMBAR labs.

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6 Labs – 6 Countries

Link to dynamic map: <https://arcg.is/0fr84G>

Figure 12. COMBAR labs where immunological techniques are used for diagnosis of GIN infection.

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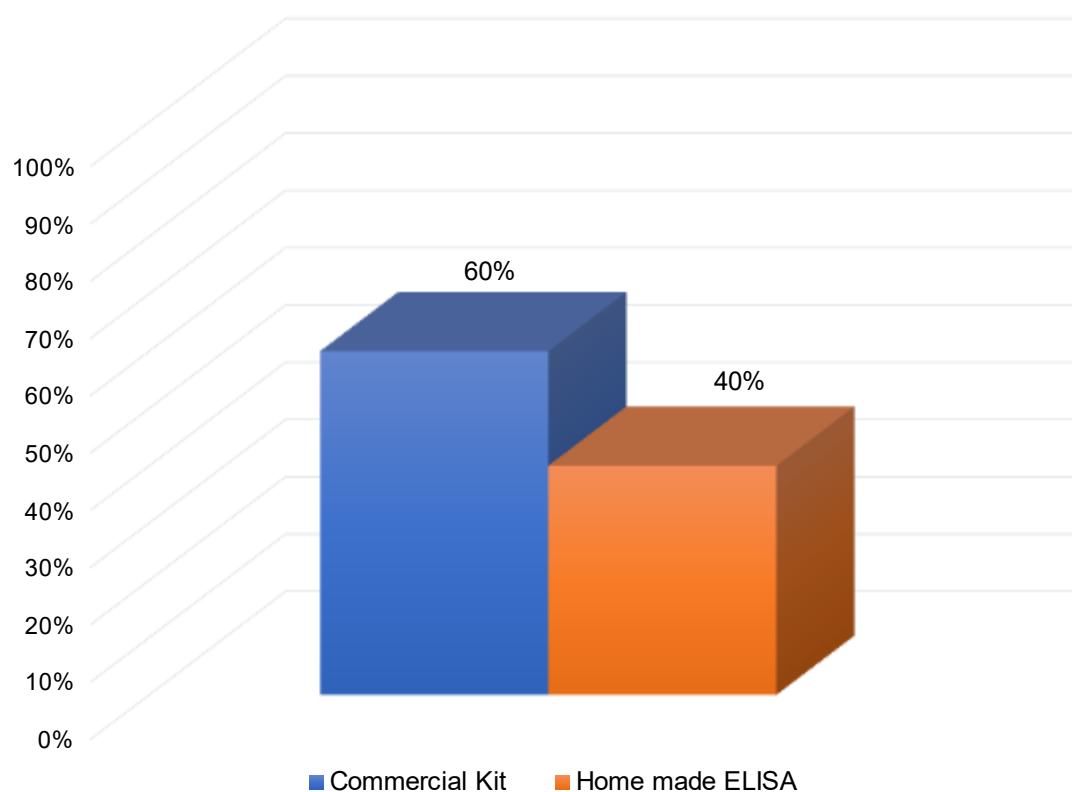
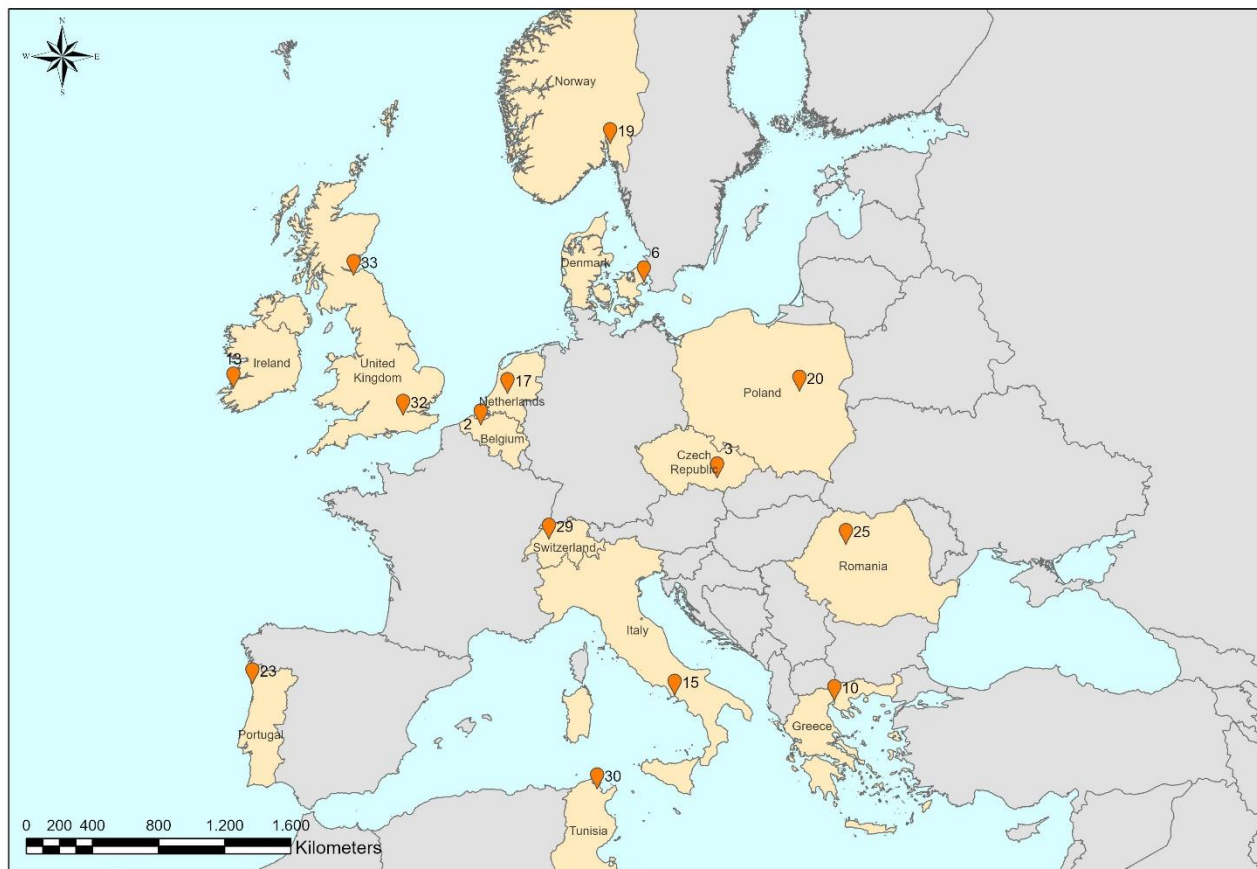


Figure 13. Immunological techniques used to detect GIN infection in the COMBAR labs.

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15 Labs – 14 Countries

Link to dynamic map: <https://arcg.is/0fr84G>

Figure 14. COMBAR labs where immunological techniques are used for diagnosis of *Fasciola hepatica* infection.

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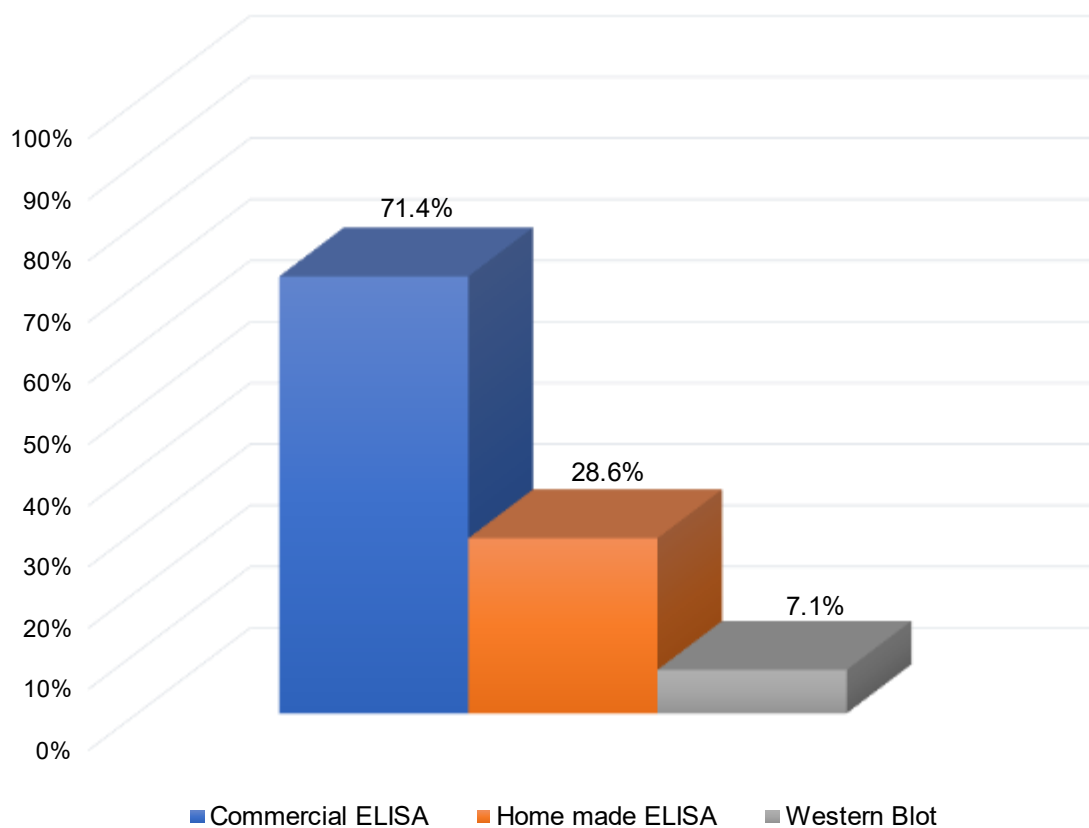
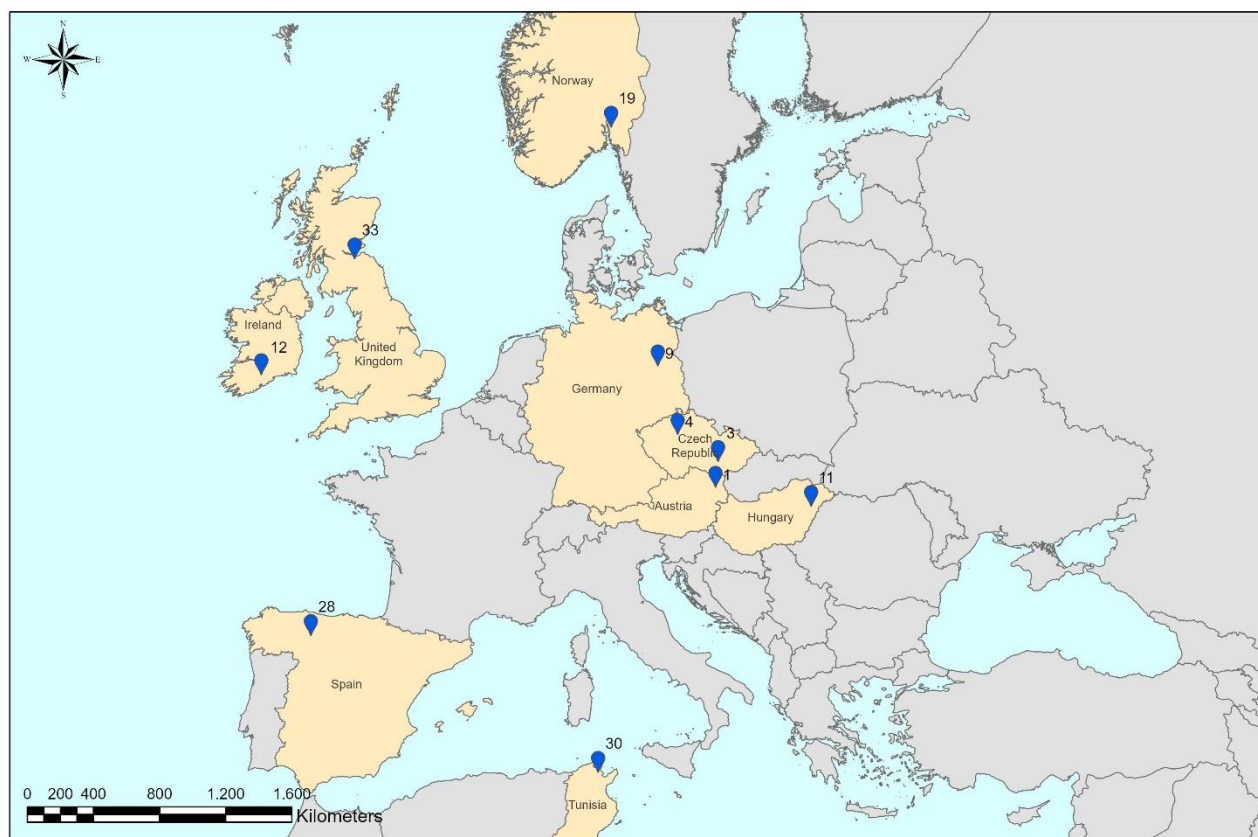


Figure 15. Immunological techniques used to detect *Fasciola hepatica* infection in the COMBAR labs.

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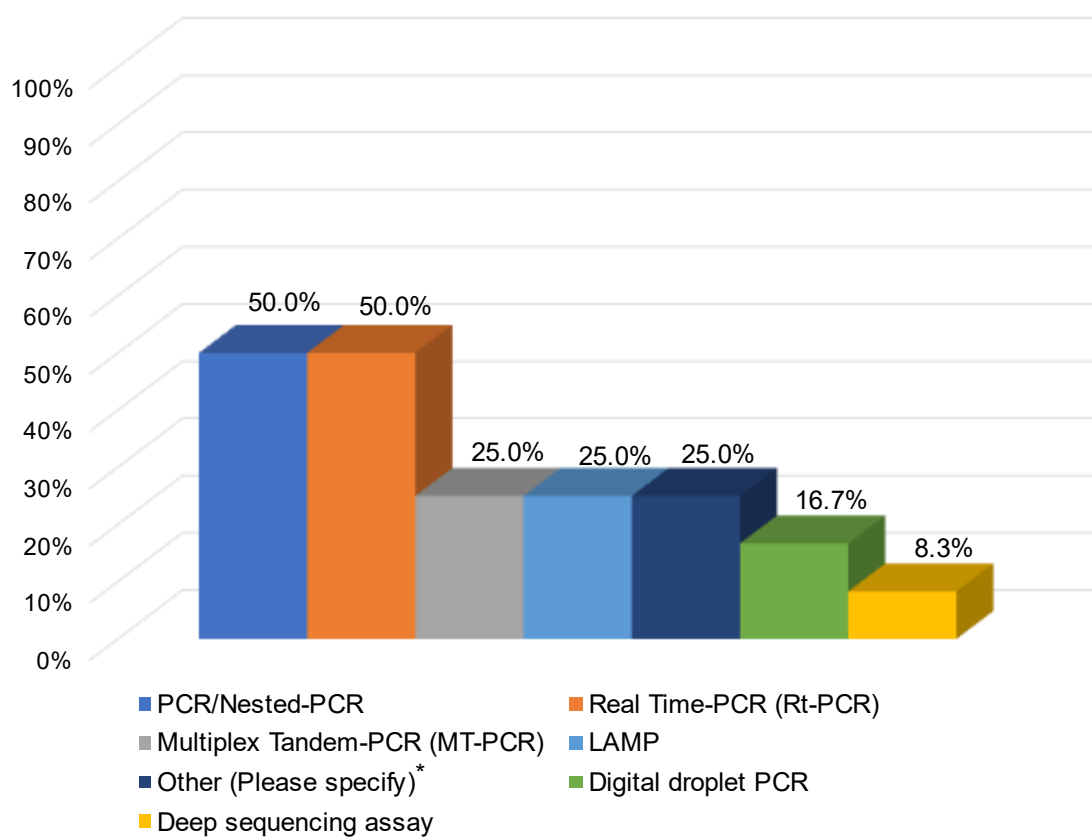
10 Labs – 9 Countries

Link to dynamic map: <https://arcg.is/0eOrPP>

Figure 16. COMBAR labs where DNA-based techniques are used for diagnosis of GIN infection.

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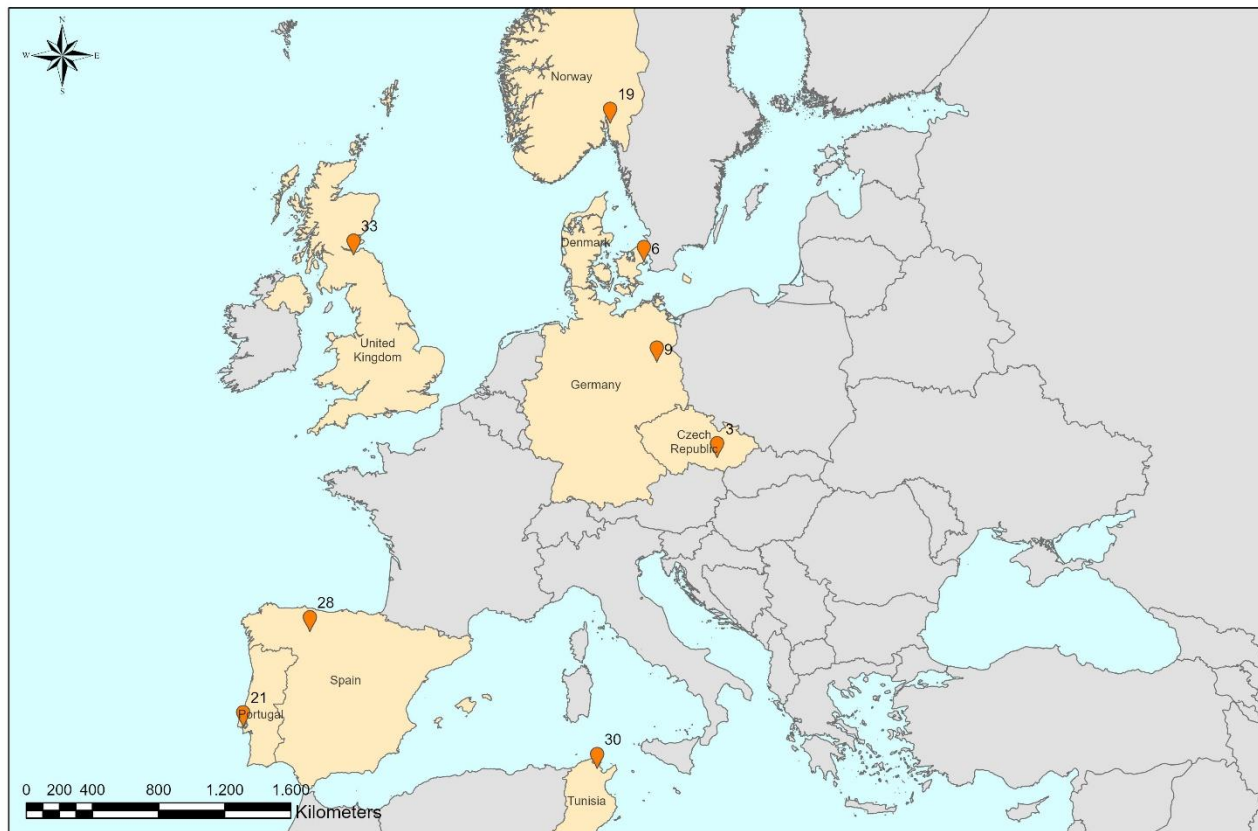


*Other: pyrosequencing, RFLP

Figure 17. DNA-based techniques used to detect GIN infection in the COMBAR labs.

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8 Labs – 8 Countries

Link to dynamic map: <https://arcg.is/0eOrPP>

Figure 18. COMBAR labs where DNA-based techniques are used for diagnosis of *Fasciola hepatica* infection.

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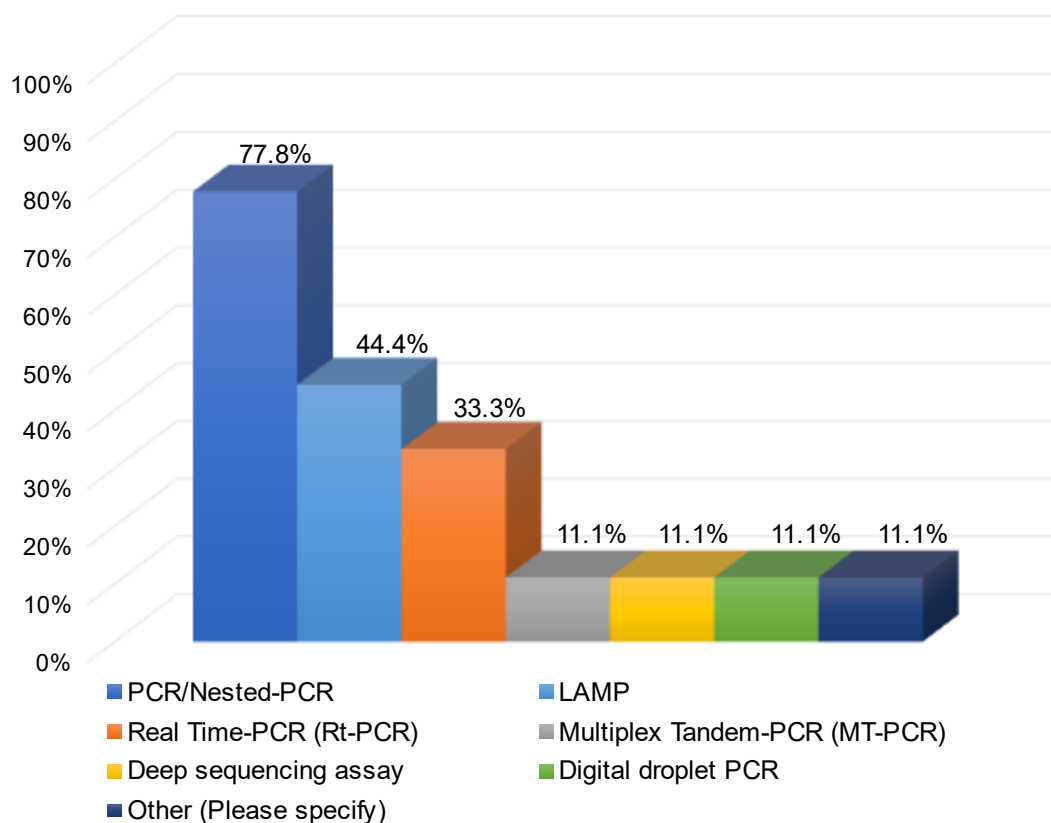


Figure 19. DNA-based techniques used to detect *Fasciola hepatica* infection in the COMBAR labs.

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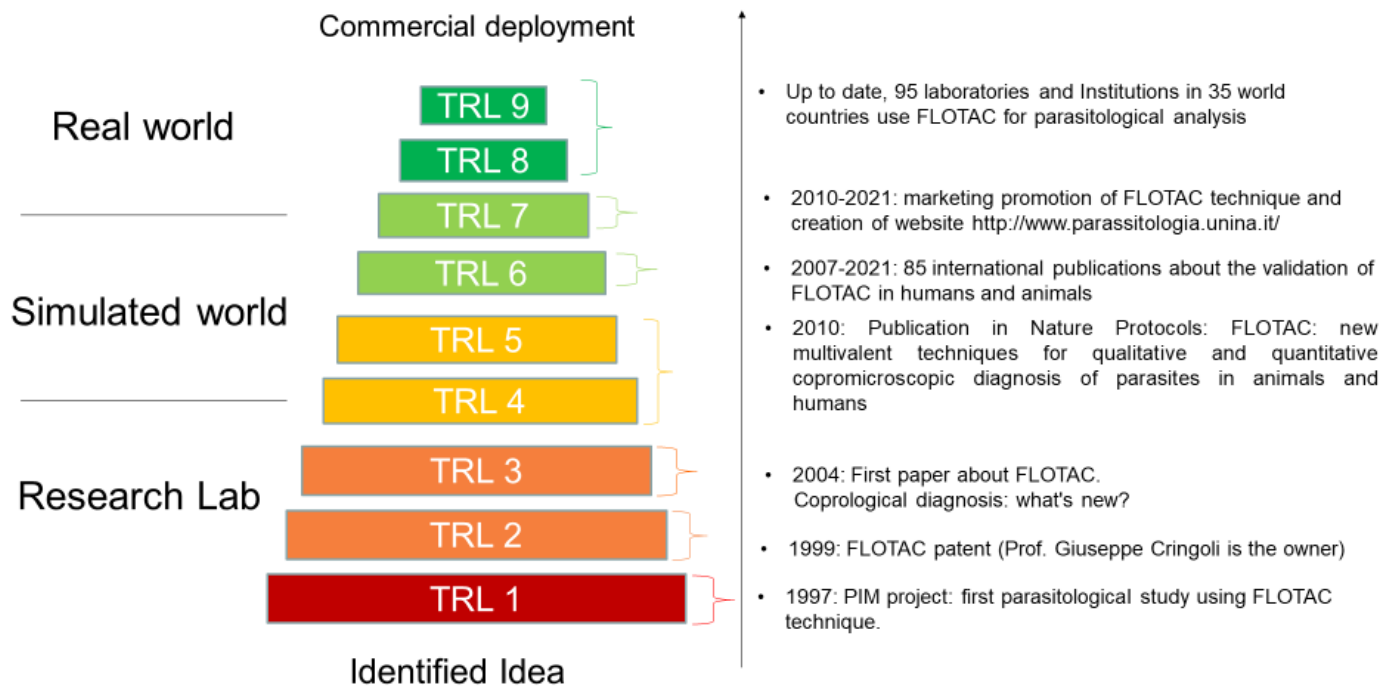
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| Tool | Distributor | Producer | Price | TARGET |
|--------------------------------------|----------------------------------|----------------------------------|--|------------------|
| Simple flotation | | | | All |
| Sedimentation | | | | All |
| McMaster (acrylic) | Vetlab supplies | Vetlab supplies | £39.50 (ex VAT) | All |
| | Focal Point (www.mcmaster.co.za) | Focal Point (www.mcmaster.co.za) | 30 \$ | All |
| | Eggzamin | Eggzamin | 17.95\$ | All |
| | Chalex | Chalex | 15\$ (opaque lines)-20\$ (green lines) | All |
| McMaster (glass) | Mariefeld Superior | Mariefeld Superior | £119.00 (ex VAT) | All |
| FECPAK | Techion Group LTD | Techion Group LTD | 600 € | All |
| FLOTAC Kit | University of Naples Federico II | Ideal Plastik Sud srl | 250 € | All |
| Mini-FLOTAC | University of Naples Federico II | Ideal Plastik Sud srl | 15 € | All |
| Fill-FLOTAC | University of Naples Federico II | Ideal Plastik Sud srl | 10 € | All |
| Fluorescent stains - GIN eggs | | | / | Livestock |
| Cuvette method (Jackson, F., 1974) | | | | All |
| Flukefinder | Flukefinder | Flukefinder | \$149.00 | Livestock |
| FlukeCatcher | Provinos | Provinos | 95 € | Livestock |
| ELISA-Fasciola hepatica | IDEXX | IDEXX | 550 € | Cattle and sheep |
| ELISA- SVANOVIR Ostertagia Ostertagi | SVANOVA | SVANOVA | 583,20 € | Cattle |
| ELISA - SVANOVIR Fasciola hepatica | SVANOVA | SVANOVA | 583,20 € | Cattle |
| BioX C-Elisa Fasciola | Listarfish | BioX Diagnostic S.A. | 486,00 € | Livestock |
| ISOLATE Fecal DNA Kit | Bioline | Bioline | / | All |
| QIAamp DNA Stool Mini Kit | Qiagen | Qiagen | 240 € | All |
| Quick-DNA Fecal/Soil Microbe Kits | Zymo Research | Zymo Research | / | All |
| Stool DNA Isolation Kit | Biochain | Biochain | / | All |
| Genomic DNA Extraction Kit - Stool | Cepharm Life Sciences, Inc. | Cepharm Life Sciences, Inc. | / | All |
| PCR end-point/nested/multiplex | | | / | All |
| Rt-PCR | | | / | All |
| MT-PCR | AusDiagnostics | AusDiagnostics | 1000€ x 96 samples | All |
| Droplet-PCR | Biorad | Biorad | / | All |
| LAMP | | | / | All |

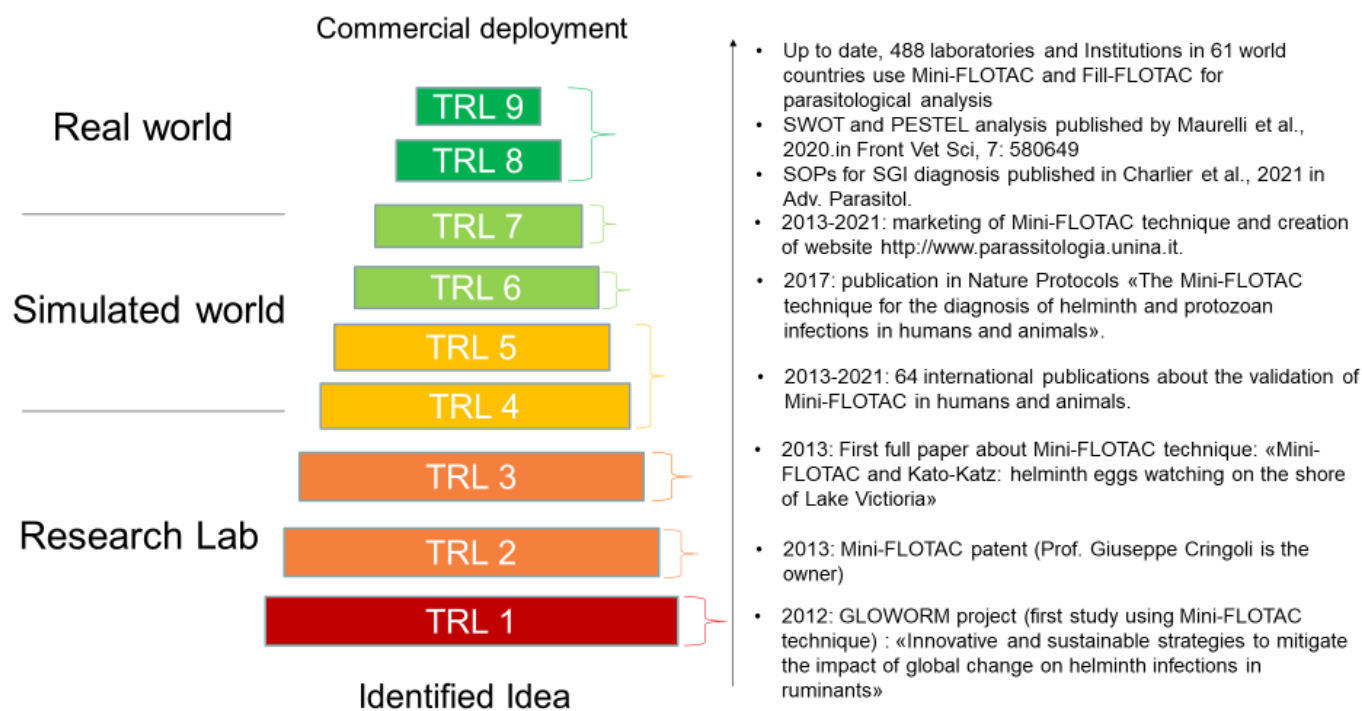
Figure 20. List of diagnostics used in the COMBAR labs.

Annex 2- Technology Readiness Levels (TRLs)

FLOTAC



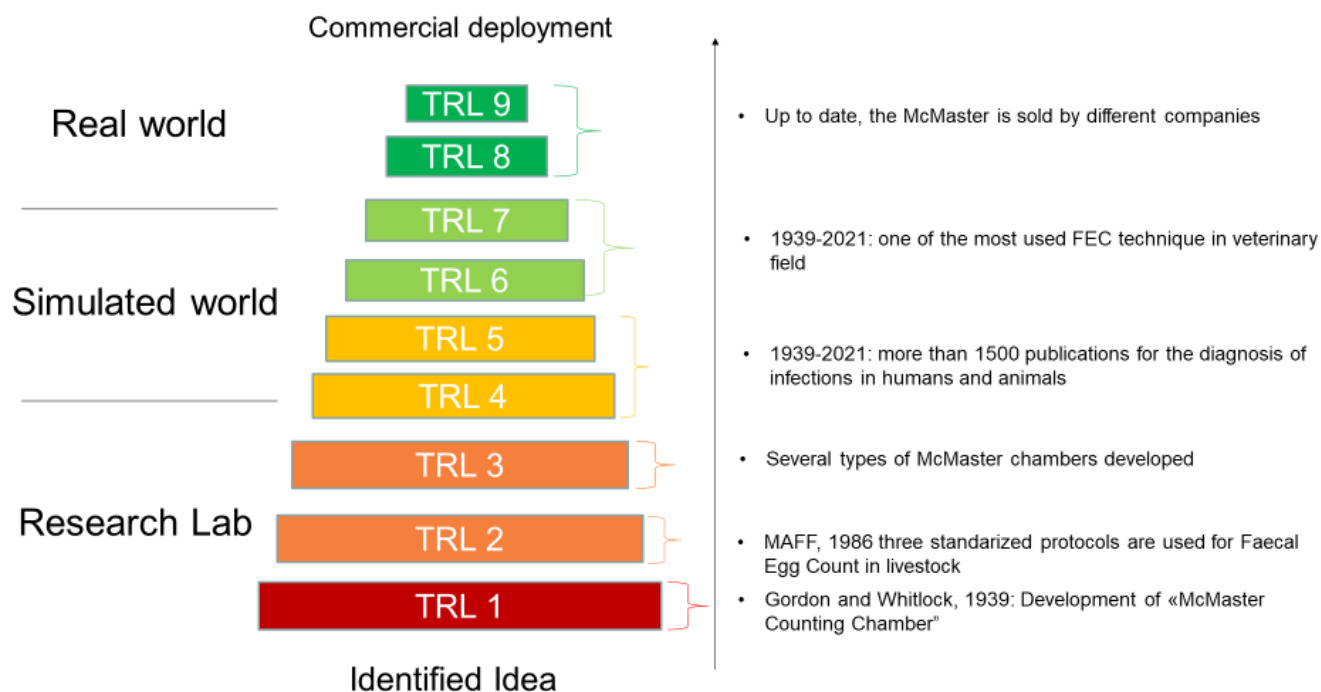
Mini-FLOTAC



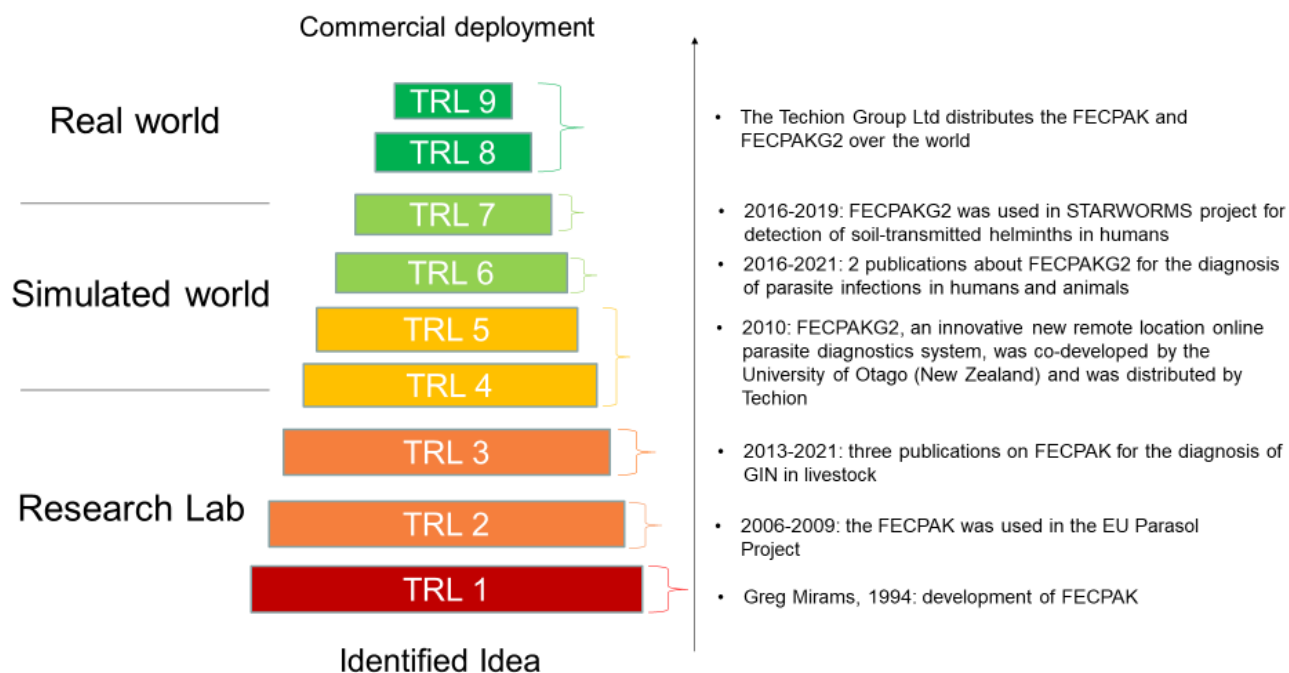
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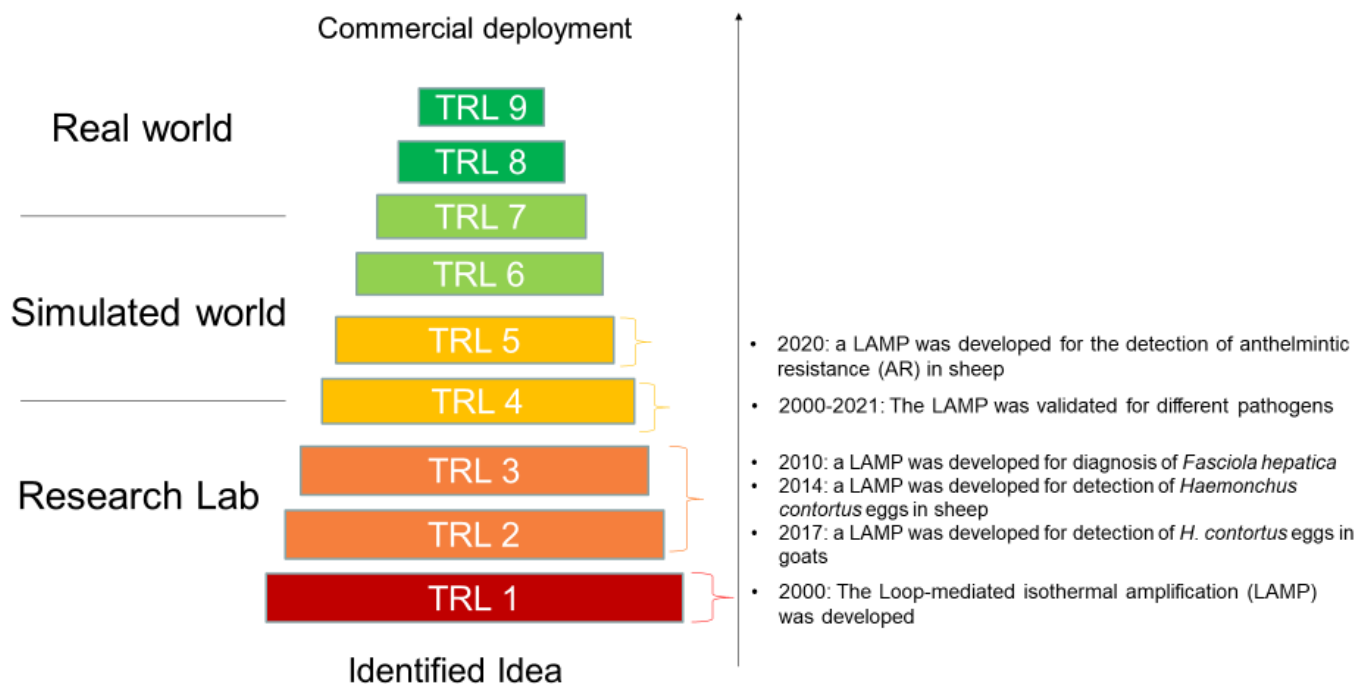
McMaster



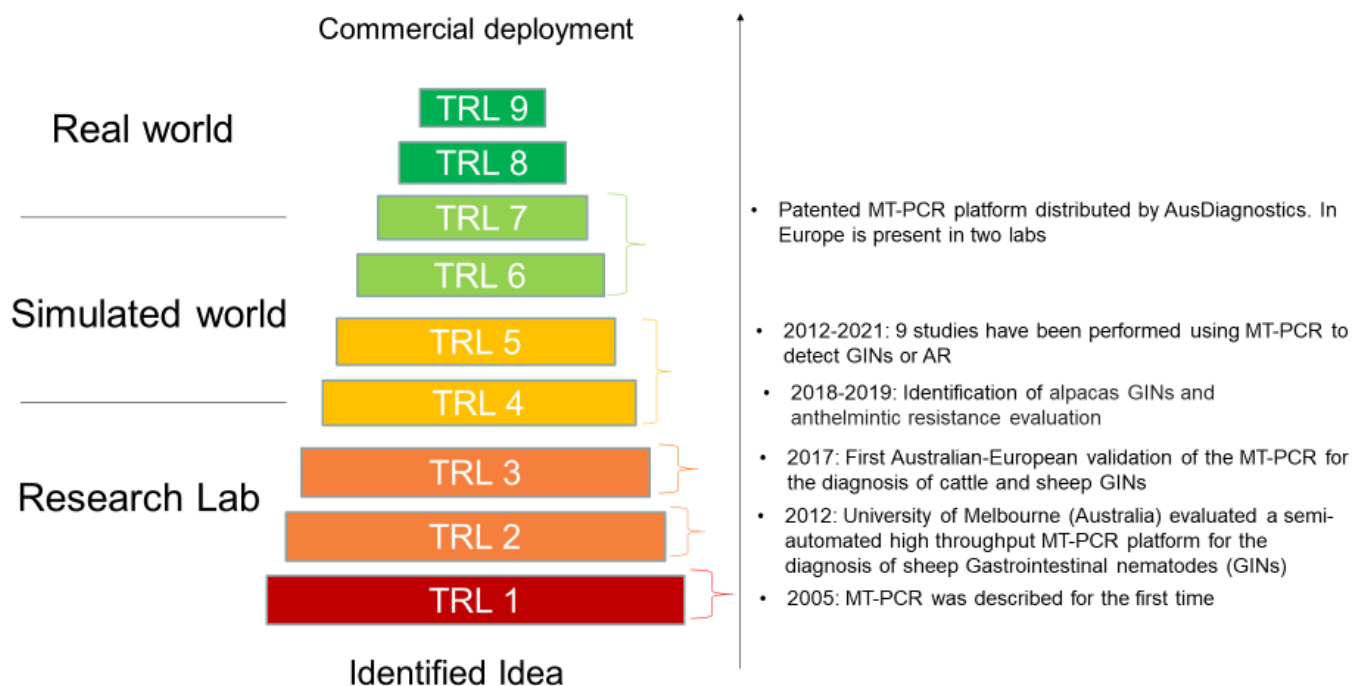
FECPAK/FECPAKG2



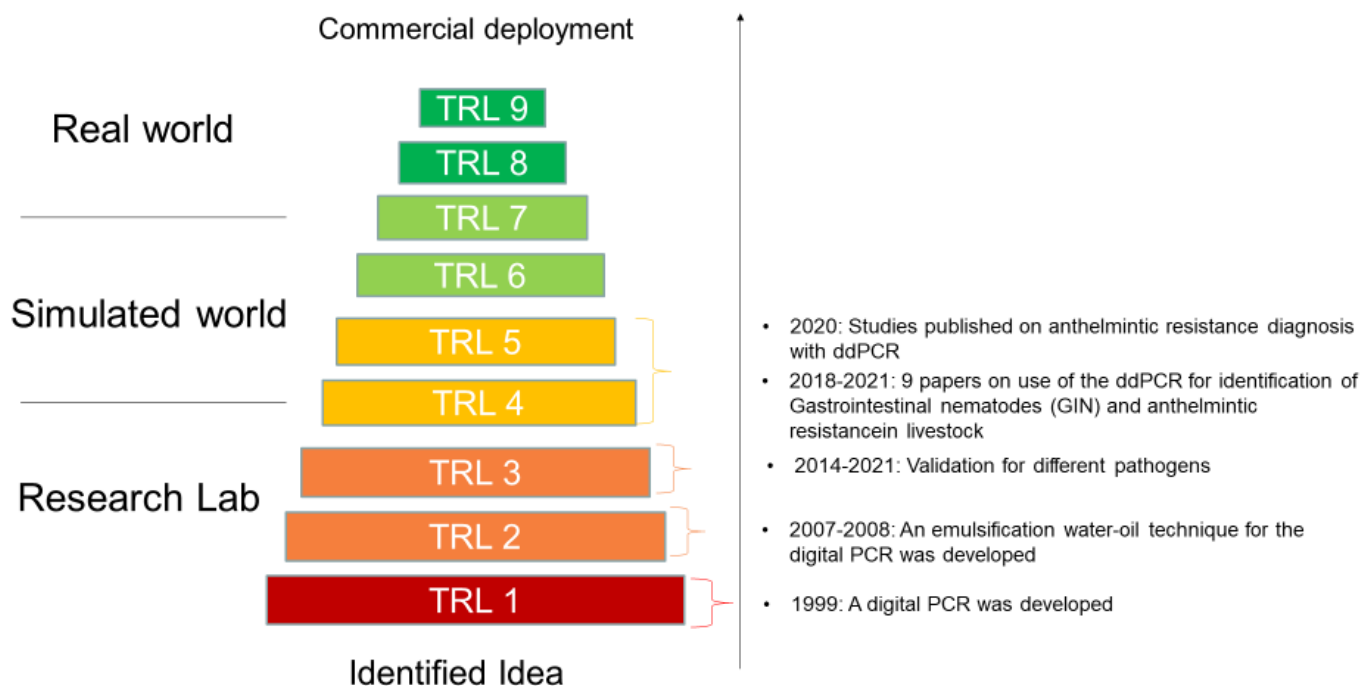
Loop-mediated isothermal Amplification (LAMP)



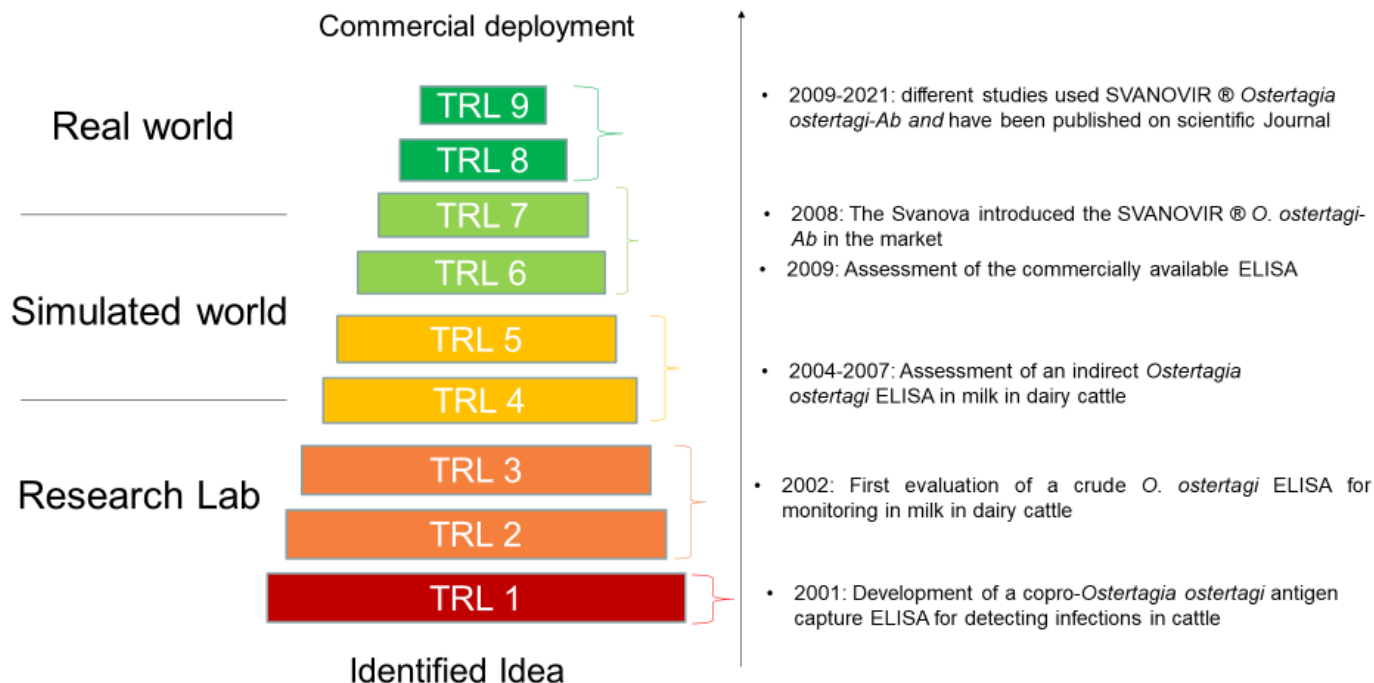
Multiplex Tandem-PCR (MT-PCR)



DROPLET DIGITAL PCR (ddPCR)



SVANOVIR ® *O. ostertagi*-Ab



SVANOVIR ® *Fasciola hepatica*-Ab

