ANALYSIS OF ALTERNATIVES

and

SOCIO-ECONOMIC ANALYSIS

Legal name of applicant(s):	IDEXX Laboratories Limited
Submitted by:	IDEXX Laboratories Limited
Date:	21st of October 2021
Substance:	4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated [covering well-defined substances and UVCB substances, polymers and homologues] 4-Nonylphenol, branched and linear, ethoxylated [substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof]
Use title:	Use of 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated and use of 4-Nonylphenol, branched and linear, ethoxylated in *in vitro* diagnostic veterinary products (SNAP tests and ELISA Plate tests) as an ingredient in the wash solutions, sample diluents, control solutions, conjugate solutions, SNAP wash solutions, tissue soaking buffers and detection solutions
Use number:	Use #1

CONTENTS

DECLARATION 12
1. SUMMARY 13
2. AIMS AND SCOPE OF THE ANALYSIS 16
2.1. Aims of Analysis 16
2.2. Applicant 16
2.2.1. IDEXX's acquisition history 18
2.3. Supply Chain 18
2.4. Scope of the analysis 20
2.4.1. Geographical scope 20
2.4.2. Temporal scope 20
3. CONSULTATIONS 22
3.1. SNAP tests survey 23
3.2. ELISA plate tests survey 24
3.3. User of both IDEXX SNAP tests and ELISA plate tests 24
4. APPLIED FOR "USE" SCENARIO 25
4.1. Market and business trends including the use of the substance 25
4.1.1. Market trend 25
4.1.2. Business trend 26
4.1.3. Competition landscape 26
4.2. Analysis of the substance function(s) and technical requirement(s) for the product(s) 27
4.2.1. IDEXX's *in vitro* diagnostic products 27
4.2.2. ELISA technology in plate tests 34
4.2.3. ELISA technology in SNAP tests 35
4.2.4. Description of the technical function provided by the Annex XIV substance 36
4.2.5. Description of the product(s) resulting from the use of the Annex XIV substance 38
4.2.5.1. ELISA plate tests 39
4.2.5.2. SNAP test kits 39
4.2.6. Description of the technical requirements that must be achieved by the products(s) made with the substance 41
4.2.7. Annual tonnage 48
4.3. Remaining risk of the "applied for use" scenario 48
4.3.1. Characterisation of Use 48
4.3.1.1. Environmental Risk 48
4.3.1.2. Exposure and risks for the environment and man via the environment 48
4.3.1.3. Release assessment 49
4.4. Human health and environmental impacts of the applied for use scenario 49
4.4.1. Human health impacts 49
4.4.2. Impact on the environment 49

5. SELECTION OF THE "NON-USE" SCENARIO 50
5.1. Efforts made to identify alternatives 50
5.1.1. Research and development 50
5.1.2. Data searches 51
5.2. Identification of known alternatives 51
5.3. Assessment of shortlisted alternatives 53
5.3.1. Tergitol 15-S-40 53
5.3.1.1. Substance ID and properties of Tergitol 15-S-40 53
5.3.1.2. Technical feasibility of Tergitol 15-S-40 54
5.3.1.3. Economic feasibility and economic impacts of Tergitol 15-S-40 55
5.3.1.4. Availability of Tergitol 15-S-40 56
5.3.1.5. Reduction of overall risk due to the transition to Tergitol 15-S-40 56
5.3.1.6. Conclusions on Tergitol 15-S-40 56
5.3.2. Tergitol 15-S-9 57
5.3.2.1. Substance ID and properties of Tergitol 15-S-9 57
5.3.2.2. Technical feasibility of Tergitol 15-S-9 57
5.3.2.3. Economic feasibility and economic impacts of Tergitol 15-S-9 58
5.3.2.4. Availability of Tergitol 15-S-9 58
5.3.2.5. Reduction of overall risk due to transition to Tergitol 15-S-9 58
5.3.2.6. Conclusions on Tergitol 15-S-9 58
5.3.3. SNAP wash solutions with a concentration of <0.1 % Triton X-100 59
5.4. The most likely non-use scenario 59
5.4.4. IDEXX UK 60
5.4.5. End-users 60
6. IMPACTS OF GRANTING AUTHORISATION 62
6.1. Human Health and/or Environmental Impacts 62
6.2. Economic impacts 62
6.3. Social impacts 64
6.4. Wider economic impacts 65
6.5. Combined assessment of impacts 67
6.5.1. Comparison of impacts 67
6.5.2. Distributional impacts 68
6.6. Uncertainty analysis 68
6.7. Information for the length of the review period 68
6.8. Substitution effort taken by the applicant if an authorisation is granted 70
7. CONCLUSIONS 71
REFERENCES 72
APPENDICES 74
Appendix 1 Consultations 74

Appendix 2. Test procedure for SNAP test kits 79
Appendix 3. Typical test procedure for ELISA plate assays 80
Appendix 4. Comparison of alternative assays and IDEXX SNAP tests .. 81
Appendix 5. License and other permit requirements by countries 90

TABLES

Table 1. End-users in the UK 19
Table 2. Summary of the surveyed DUs grouped by business sector 22
Table 3. The list of products covered by this application along with a short description and country of origin (COO). 4-tert-OPnEO or 4-NPnEO is contained in (a) sample diluents, (b) controls, (c) conjugate solutions, (d) SNAP wash solutions, I wash solution, (f) tissue soaking buffer or (g) detection solution. 29
Table 4. IDEXX uses three different substances to prevent NSB in their products 37
Table 5. European National surveillance/eradication program examples performance requirements for several of IDEXX's products. The TSE requirements are relevant for the HerdChek BSE-scrapie Ag kit. 43
Table 6. Current product requirement for non-USDA licensed kits 47
Table 7. Annual tonnage of Triton in products supplied to the UK (6 % annual growth rate) 48
Table 8. The physicochemical properties of Triton X-100 and Triton X-405 in comparison to their respective alternatives. 53
Table 9. The substance identity and classification information of Tergitol 15-S-40. The information originates from the manufacturer, DOW. 54
Table 10. The substance identity and classification information of Tergitol 15-S-9. The information originate from the SDS of the manufacturer, DOW. 57
TABLE 11. Summary of impacts of the non-use scenarios 62
TABLE 12. Conclusion of benefits and risks 67
Table 13. Distributional impacts 68
Table 14. The long review period criteria set by the Socio-Economic Committee*. 69
Table 15. The detailed survey results of the online questionnaire for the DUs using SNAP tests. The results include all UK respondents (n = 32) 74
Table 16. The results of the email survey involving three university laboratories situated in Denmark, France and UK. The respondents are IDEXX SNAP tests users. 75
Table 17. The detailed survey results for the DUs using ELISA plate tests. The results include all UK respondents (UK n = 7). 76
Table 18. The detailed survey results of the UK DU using both SNAP tests and ELISA plate tests 77
Table 19. Comparison of IDEXX SNAP assay and colloidal gold lateral-flow assay 81
Table 20. The results of a comparative study assessing the performance of IDEXX SNAP Giardia Test and three alternative in-vitro diagnostic tests in detecting *Giardia*-specific antigens. 176 samples were tested in total. 83
Table 21. The results of a comparative study assessing the performance of IDEXX SNAP Giardia Test and three alternative in-vitro diagnostic tests in detecting *Giardia*-specific antigens. 177 samples were tested in total. 83

Table 22. The results of a comparative study assessing the performance of IDEXX Feline Triple Test and two alternative in-vitro diagnostic tests in detecting FeLV-specific antigens. 137 samples were tested in total. 84
Table 23. The results of a comparative study assessing the performance of IDEXX SNAP Feline Triple Test and two alternative in-vitro diagnostic tests in detecting FeLV-specific antigens. 185 samples were tested with the ELISA assay ViraCHECK FeLV (Zoetis) for the presence or absence of the FeLV. The same samples were tested with the three rapid assays and the results compared. 84
Table 24. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus Test and one alternative in-vitro diagnostic test in detecting antibodies specific to *A. phagocytophilum* and *A. platys*. 85
Table 25. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus Test and one alternative in-vitro diagnostic test in detecting antibodies specific to *E. canis, E. ewingii* and *E. chaffeensis*. 85
Table 26. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus Test and one alternative in-vitro diagnostic test in detecting several antibodies. 844 samples were tested on each assay. Each result was read independently by three readers. 86
Table 27. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx and one alternative in-vitro diagnostic test in detecting antibodies specific to E. canis and A. platys. 86
Table 28. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus and two alternative in-vitro diagnostic test in detecting E. Canis specific antibodies. 104 positive and 163 negative samples were tested. Positive and negative predictive values (PPV and NPV) were calculated for a representative population with 5% prevalence rate for E. canis. 87
Table 29. The results of a comparative study assessing the performance of IDEXX SNAP FIV/FeLV Combo Test in detecting FeLV-specific antigens and FIV-specific antibodies. 87
Table 30. Correlation of normals and abnormals. The SNAP cPL correctly interpreted 100% of the Consistent with Pancreatitis samples while one Normal sample was called as Abnormal and two Elevated samples as Normal. n = 70. 88
Table 31. The results of a comparative study assessing the performance of IDEXX SNAP Leishmania and one alternative in-vitro diagnostic test in detecting antibodies specific to Leishmania infantum.[23] 89

FIGURES

Figure 1. Supply chain.....20
Figure 2. The principle of an indirect ELISA. The sample antibody is "sandwiched" between the pre-coated antigen and an enzyme-labelled antibody. Pre-coated antigens are represented in grey, sample antibodies in red, conjugates in yellow-blue and substrates in green.....34
Figure 3. The principle of a direct ELISA. The sample antigen is "sandwiched" between two antibodies. Antigens are represented in grey, pre-coated antibodies in red, conjugates in yellow-blue and substrates in green.....35
FIGURE 4. 1) Conjugates are added to a sample containing antigens. The antigens and conjugates bind to each other. 2) The sample is poured onto an ELISA plate that is pre-coated with similar antigens as sample antigens. The unbound conjugates bind to the immobilized antigens. 3) The plate is washed. The sample antigen-conjugate complexes are eliminated from the plate. The substrate is added for colour development. The higher the number of antigens present in the sample, the lighter the colour produced.....35
Figure 5. The mechanism of a SNAP test. 1) Conjugate and target antigen binding step. 2) Wash step. 3) Colour development step.....36
Figure 6. (A) The generic structure of 4-tert-OPnEO. (B) The generic structure of 4-NPnEO.....37
Figure 7. On the left, the non-specific binding of the conjugate to the surface of the assay leads to a falsely high signal. On the right, the use of blocking agent, such as Triton X-100, saturates unoccupied binding sites eliminating NSB.....38
Figure 8. Typical content of an IDEXX ELISA diagnostic kit. Components from left to right: substrate, wash concentrate, positive and negative controls, conjugate, sample diluent and coated plates.....39
Figure 9. On the left, the components of SNAP test kit for whole blood, serum, plasma and milk samples. The typical content of snap test kit intended for faecal samples is presented on the right.....40
Figure 10. The SNAP 4DX plus is an example of a qualitative SNAP test that is used to test the presence of four different antigens/antibodies in canine whole blood, serum or plasma sample.....40
Figure 11. Semi-quantitative SNAP tests are *in-vitro* assays used to determine semi-quantitatively the level of a marker substance in the sample by comparing the intensity of two spots.....41
Figure 12. Five replicates of the same product. Canine whole blood was used in all five SNAP devices. The spot in the top left corner is the positive control spot. The analyte spots are masked by the dark colour of the background and thus, they cannot be seen.....55
Figure 13. Non-use scenario.....60
Figure 14. A known heartworm antigen-positive haemolysed sample was tested with two assays. (A) Colloidal gold lateral flow assay (B) SNAP 4Dx Plus Elisa.....82

Figure 15. A diagram illustrating the percent positive agreement column of Snap Test, Speed Duo Test and Antigen Test 84
Figure 16. Diagram illustrating the percentage of *E. canis* positive samples diagnosed correctly by IDEXX SNAP 4Dx and Uranotest EC-AP. The Uranotest EC-AP missed a significant number of positive samples. More so in the titer < 400, which is critical for early detection of diseases.[23] 87
Figure 17. The three groups are normal, elevated and consistent with pancreatitis based on the measured serum concentration of cPLI in ug/L.[26] 88
Figure 18. Repeated testing of 3 different samples 3 times per day for 5 days. %CV for Spec cPL was 8.2, 5.4, 3.9 % for samples 1-3, respectively. %CV for VCheck cPL for the same 3 samples were 33.4, 25.4, and 17.0 %.[26] 89

LIST OF ABBREVIATIONS

ANSES	French Agency for Food, Environmental and Occupational Health & Safety
AoA	Analysis of Alternatives
BLV	Bovine Leukemia Virus
BSE	Bovine Spongiform Encephalopathy
BVDV	Bovine Viral Diarrhea Virus
CAG	Companion Animal Group
CAS	Chemical Abstract Service
CAEV	Caprine arthritis encephalitis virus
CH	Switzerland
CIRAD	Agricultural Research for Development
COFRAC	French Accreditation Committee
COO	Country of Origin
cPL	Canine Pancreatic Lipase
CSR	Chemical Safety Report
DE	Germany
DEREA	Food and drug administration export reform and enhancement act of 1996
DIVA	Differentiating Infected from Vaccinated Animals
DU	Downstream User
EC	European Commission
ECHA	European Chemicals Agency
EDTA	Ethylenediamine tetraacetic acid
EEA	European Economic Area
EFSA	European Food Safety Agency
ELISA	Enzyme-Linked Immunosorbent Assay
EO	Etylene oxide

ES	Spain
EU	European Union
EUR	Euro
EURL	European Union Reference Laboratories
EUSES	The European Union System for the Evaluation of Substances
FMD	Falsified Medicines Directive
FR	France
HLB	Hydrophilic-lipophilic Balance
IBR	Infectious Bovine Rhinotracheitis
IBV	Infectious Bronchitis virus
IT	Italy
IVD	In Vitro Diagnostics
JMAFF	Japanese Ministry of Agriculture, Forestry, and Fisheries
LPD	Livestock, Poultry and Dairy
M	Million
NDV	Virulent Newcastle disease
Nonidet	Nonidet P-40 Substitute (CAS 9016-45-9)
NSB	Non-Specific Binding
OECD	Organisation for Economic Co-operation and Development
OIE	Office International des Epizooties/World Organisation for Animal Health
OPS	Operations
PEC	Predicted Environmental Concentrations
PNEC	Predicted No Effect Concentration
PPE	Personal Protective Equipment
PRRS	Porcine Reproductive and Respiratory Syndrome

PRV	Pseudorabies Virus
QA	Quality Assurance
RA	Regulatory Affairs
R&D	Research and Development
RCR	Risk Characterization Ratios
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REO	Avian Reovirus
RMM	Risk Management Measures
SEA	Socio-Economic Analysis
SEAC	Committee for Socio-Economic Analysis
STP	Sewage Treatment Plan
SVHC	Substance with Very High Concern
Triton substances	Triton X-100/IGEPAL CA-630 (CAS# 9002-93-1) and Triton X-405/IGEPAL CA-720 (CAS# 9036-19-5)
TSE	Transmissible spongiform encephalopathies
UK	The United Kingdom
USA	The United States of America
USD	US Dollar
USDA	The United States Department of Agriculture
UVCB	Chemical Substances of Unknown or Variable Composition

DECLARATION

The Applicant, IDEXX Laboratories Limited, is aware of the fact that evidence might be requested by the UK HSE to support information provided in this document.

Also, we request that the information blanked out in the "public version" of the Analysis of Alternatives and Socio-economic analysis is not disclosed. We hereby declare that, to the best of our knowledge as of today 10/21/2021 the information is not publicly available, and in accordance with the due measures of protection that we have implemented, a member of the public should not be able to obtain access to this information without our consent or that of the third party whose commercial interests are at stake.

Signature: 

Date, Place: Westbrook Maine, USA
10/21/2021

1. SUMMARY

This application covers the use of 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated and use of 4-Nonylphenol, branched and linear, ethoxylated. The use covered in this document is the downstream use of the solutions and products manufactured by the applicants. The downstream use takes place in UK by the applicant's customers, which are veterinary clinics, reference laboratories, universities, governmental laboratories or private livestock and milk laboratories.

The use is defined as use of 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated (hereafter referred to as Triton X-100/IGEPAL CA-630, Triton X-405/IGEPAL CA720 or 4-tert-OPnEO) and use of 4-Nonylphenol, branched and linear, ethoxylated (hereafter referred to as Nonidet P-40 Substitute or 4-NPnEO) in *in vitro* diagnostic veterinary products (SNAP tests and ELISA Plate tests) as an ingredient in the wash solutions, sample diluents, control solutions, conjugate solutions, SNAP wash solutions, tissue soaking buffers and detection solutions. In the present case, IDEXX uses 4-tert-OPnEO and 4-NPnEO in their in-vitro diagnostic kits to prevent the non-specific binding of undesired macromolecules, such as conjugates and sample impurities, to the bottom of the wells in ELISA plate tests and to the assay's matrix in SNAP tests.

The parent company, IDEXX GLOBAL, is a global leader of in vitro diagnostics with the aim of enhancing the health and well-being of pets, people and livestock. With their extensive portfolio, IDEXX serve tens of thousands of customers in more than 175 countries providing them with elegant solutions for monitoring animal health and water and milk quality. IDEXX develops, manufactures, and distributes products and provide services primarily for the companion animal veterinary, livestock and poultry, dairy and water testing markets.

IDEXX's IVD products are used for the detection or quantitative measurement of a wide variety of antigens and antibodies linked with infectious diseases. They contribute to disease prevalence monitoring (including emerging diseases), control of disease outbreaks, animal health movements, food safety and fighting against zoonoses (infectious diseases transmitted by animals to humans). By allowing the early detection of diseases in animals, the spread of diseases can be controlled. The benefits to animal/human health include less infected animals, less euthanised animals, lower risk of animal-to-human transmission of zoonotic diseases and timely treatment of diseases. While some animals are tested individually, several tests and programs use pooled serum and milk samples, as well as tank milk samples, with milk from 100 or more cows. Poultry tests for between 5-10 animals can usually represent epidemiological units of 500 to several thousand chickens.

The diseases tested by IDEXX's IVD products are critical in terms of animal/human health and economic impact. For example:

- Avian Influenza: A highly contagious viral disease affecting food producing birds, pet birds and wild birds. Highly pathogenic strains can be associated with high mortality rates among poultry. Some strains of the avian influenza virus may also be transmitted to humans (e.g. the well-known H5N1 and H7N9). Outbreaks of avian influenza are considered a global public health concern.

- Classical Swine Fever: A contagious viral disease affecting domestic and wild swine. Affected swine may present no symptom thus, testing is required to detect the presence of the virus. In case of infection, no treatment is attempted. Affected swine must be culled.

- Bluetongue: A viral disease affecting domestic and wild ruminants, primarily sheep. Symptoms may include weight loss, disruption in wool growth and death. In endemic areas, the presence of the virus is actively monitored by testing herds.

- Canine Leishmaniasis: A potentially fatal zoonotic disease transmitted by sand flies. One-third of infected dogs will experience swollen lymph nodes, an enlarged spleen, and will progress to kidney failure.

- BVDV: The most costly bovine viral disease. Eradication programs in several countries in Europe ongoing (Ireland, Belgium, Germany) or starting (France) or planned (Spain, The Netherlands, etc.). The bovine industry loses up to 100 EUR per animal if virus is circulating in the herd. Testing is crucial as persistently infected animals cannot be identified otherwise. IDEXX tests are the most widely used in these programs.

- Paratuberculosis: A non-curable disease in bovines which causes diarrhea, weight loss and much reduced performance and ends usually fatal. Testing ensures to identify animals as early as possible and helps apply cost saving management programs in dairy and beef herds.

The Applicant conducted several surveys to assess how their DUs would react in case IDEXX's SNAP and ELISA plate tests were not available and to assess how DUs manage their wastes. Answers were received from both the CAG and LPD market sectors, which are the Applicant's main market sectors. IDEXX's DUs rely on the Applicant to provide in vitro diagnostics free from 4-tert-OPnEO and 4-NPnEO.

By applying specific criteria, the applicant was able to identify five alternatives for Triton X-100 and three were found for Triton X-405. The physicochemical properties of the identified alternatives were then compared with their Triton counterpart. The alternative with the closest physicochemical properties was selected by IDEXX to undergo feasibility testing. In the case of Triton X-100, the most comparable alternative was Tergitol 15-S-9 whereas it was Tergitol 15-S-40 (70 %) for Triton X-405. Based on the initial tests performed by IDEXX, Tergitol 15-S-9 was the best candidate, of the three alternatives in consideration (Tergitol 15-S-9, Tergitol 15-S-40 and SNAP wash formulations containing 0.1 % of Triton X-100). However, as issues with spot colour development were observed during the tests, Tergitol 15-S-9 is not considered feasible at the present time. A significant amount of R&D work remains in order to fully determine its applicability as a replacement for Triton X-100/IGEPAL CA-630 in all of IDEXX's SNAP products. In terms of economic feasibility, Tergitol 15-S-9 is considered feasible despite the fact that IDEXX will have to bear the reformulation costs amounting to an estimated [40-70 M USD] #BM USD ([35-62 M EUR] #BM EUR). In addition, the alternative is available in sufficient quantities from multiple suppliers and based on the manufacturer's self-classification, it is less hazardous to the environment than Triton X-100/IGEPAL CA-630. Similar testing has not yet been carried out on ELISA plates however, the applicant will test the same alternatives for ELISA

plates and SNAP tests. Due to the similarities between the Triton substances and Nonidet, it is the Applicant's goal to substitute them with the same alternative substance.

In the non-use scenario IDEXX is not able to sell products containing 4-tert-OPnEO and 4-NPnEO to the UK market since their use is banned in the UK. Consequently, IDEXX's product and service portfolio in UK decreases and less people is needed to maintain the operations in the UK. IDEXX UK will lay-off [55-70] % of their UK based personnel, primarily from the Windsor Berkshire commercial office. These lay-offs are the main socio-economic impact of the non-use scenario. The negative economic impacts from losing the UK market, such as profit losses, are out the scope since the profits are generated in IDEXX productions facilities located outside of the UK. The production facilities in Montpellier, Bern and Westbrook continue producing, but with lesser quantity, and selling products containing 4-tert-OPnEO and 4-NPnEO to the European and global market. They will likely encounter profit losses and possible lay-offs. However, these impacts are out of the scope of this application.

There is no benefit for society in the non-use scenario since the emissions are already now 0 kg and cannot be reduced. The main cost for society from the non-use scenario are societal cost of 13.8 M GBP. The conclusion from the non-use scenario is that the releases to the environment would be reduced by 0 with societal cost of 13.8 M GBP to the society. The applicant deems the societal cost to be disproportionate to the risk.

In order to substitute 4-tert-OPnEO and 4-NPnEO from their products, IDEXX must reformulate the 60 products covered by this authorisation application. These products are manufactured in three different sites, which are located in Montpellier (FR), Westbrook (USA) and Bern (CH). The practical work for the substitution of 4-tert-OPnEO and 4-NPnEO in these products covered is starting at all sites in H2 2021 and is expected to last 18-23 years depending on the site. As the Applicant is aware that a review period of more than 12 years should not be considered for non-threshold substances for which the risks cannot be quantified, IDEXX is requesting for a review period of 12 years. The Applicant will apply for a review of the authorisation in order to finish the reformulation of all products.

2. AIMS AND SCOPE OF THE ANALYSIS

2.1. Aims of Analysis

The scope of this use is the downstream use of 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated and use of 4-Nonylphenol, branched and linear, ethoxylated in *in vitro* diagnostic veterinary products (SNAP tests and ELISA Plate tests) as an ingredient in the wash solutions, sample diluents, control solutions, conjugate solutions, SNAP wash solutions, tissue soaking buffers and detection solutions.

Given the uncertainty surrounding possible exemption from the need for authorisation covered by Article 56(3) of the UK REACH Regulation (Scientific Research and Development) for the *in vitro* diagnostics sector, IDEXX is preparing this application for Authorisation covering one use as a risk management measure to ensure continued supply of their products to their supply chain.

The substances covered by Authorisation list entries 42 and 43 are substances which, through their degradation, have endocrine disrupting properties for which there is scientific evidence of probable serious effects to the environment. As such, they give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of UK REACH.

The aim of this report is to: 1) prove that there are no suitable alternative substances or technologies implementable before the Sunset Date for the applied use, 2) demonstrate that the socio-economic benefits of the continued use of 4-tert-OPnEO and 4-NPnEO outweigh the risks to the environment, and 3) demonstrate disproportionality for the applicant in the case of non-use of the substance.

This Analysis of Alternatives (AoA) and Socio-economic Analysis (SEA) report has been prepared for IDEXX as the applicant, addressing the downstream use of 4-tert-OPnEO and 4-NPnEO in *in vitro* diagnostic veterinary products (SNAP tests and ELISA Plate tests) as an ingredient in the wash solutions, sample diluents, control solutions, conjugate solutions, SNAP wash solutions, tissue soaking buffers and detection solutions.

2.2. Applicant

IDEXX is a global leader of *in vitro* diagnostics with the aim of enhancing the health and well-being of pets, people and livestock. With their extensive portfolio, IDEXX serve tens of thousands customers in more than 175 countries providing them with elegant and effective solutions for monitoring animal health and water and milk quality.

At the time of its incorporation, in 1983, IDEXX was known as AgriTech Systems, inc. The company, which started with as little as five employees, has grown into a multinational corporation headquartered in Westbrook, Maine (USA) that employs more than 8 500 employees worldwide. With three sites in the UK – a commercial office in Windsor Berkshire, a reference laboratory in Wetherby and a small manufacturing site in Newmarket – IDEXX has a solid presence in the UK.

IDEXX's business is divided into several segments such as IDEXX Livestock and Poultry and IDEXX Small Animal Health. The former focuses on providing diagnostic tests to monitor the health of ruminants, poultry and swine as well as test the quality of milk

whereas the latter is committed to provide solutions to monitor the health of companion animals. The applicant produces hundreds of products for dozens of diseases and their annual turnover is approximately 2,707 M USD in 2020.

IDEXX develops, manufactures, and distributes products and provide services primarily for the companion animal, livestock and poultry, dairy and water testing markets. They also sell a line of portable electrolytes and blood gas analyzers for the human point-of-care medical diagnostics market. IDEXX's primary products and services are:

1. Point-of-care veterinary diagnostic products, comprising instruments, consumables, and rapid assay test kits;
2. Veterinary reference laboratory diagnostic and consulting services;
3. Practice management softwares and diagnostic imaging systems and services used by veterinarians;
4. Biological materials testing, laboratory diagnostic instruments and services used by the biomedical research community;
5. Diagnostic, health-monitoring products for livestock, poultry and dairy;
6. Products that test water for certain microbiological contaminants; and
7. Point-of-care electrolytes, blood gas analyzers and SARS-COVID diagnostic test kits used in the human point-of-care medical diagnostics market.

From the aforementioned list, products and services mentioned in bullets 1, 2, 4 and 5 are dependent on the future authorisation of 4-tert-OPnEO and 4-NPnEO.

The categories above describe products that can be produced and sold by different operating lines of business or business segments. IDEXX operates primarily through three business segments: diagnostic and information technology-based products and services for the veterinary market, which are referred to as the Companion Animal Group ("CAG"); water quality products ("Water"); and diagnostic products and services for livestock and poultry health and to ensure the quality and safety of milk and food, and improve bovine reproductive efficiency, which are referred to as Livestock, Poultry and Dairy ("LPD"). CAG, Water and LPD accounts for 88 %, 5 % and 5 % of IDEXX's total revenue respectively.

CAG and LPD business segments are impacted by the possible ban on 4-tert-OPnEO and 4-NPnEO.

IDEXX's research and development expenses, which consist of salaries, employee benefits, materials and external consulting and development costs, were 141.2 M USD or 5.2 % of consolidated revenue in 2020.

IDEXX invests more than 4 times in R&D Investments than their largest competitor in veterinary diagnostic space (>100 M USD vs <20 M USD in 2018). Any impact to their manufacturing in Montpellier would impact future discoveries for emerging disease. However, on the UK this impacts only indirectly. IDEXX is often the frontrunner in developing diagnostic kits for emerging disease, such as African swine fever and

contagious bovine pleuro-pneumonia, manufacturing kits IDEXX CPBB (Contagious Bovine Pleuropneumonia) and PCR ASFV.

2.2.1. **IDEXX's acquisition history**

IDEXX is a company that has consistently grown since its incorporation. During this growth, IDEXX has acquired multiple companies. This is the case of IDEXX France (Montpellier), which was acquired in 2007 and IDEXX Switzerland (Bern), which was acquired in 2004. By acquiring these two sites, IDEXX also inherited the product lines of these sites. This is the reason why IDEXX is using multiple 4-tert-OPnEOs and 4-NPnEOs for the same purpose in their IVD kits. The reasons are only historical as the 4-tert-OPnEOs and 4-NPnEOs have the same function in all the ELISA plate assays and SNAP tests.

Through these acquisitions, IDEXX product portfolio has expanded. This can be seen in the high number of products that are covered by this application. Moreover, this is the reason why two products, the IDEXX PRV/ADV gB and IDEXX PRV/ADV GI kits, are manufactured both in Bern and Westbrook.

2.3. **Supply Chain**

IDEXX markets, sells, and services products worldwide through their marketing, customer service, sales, and technical service groups, as well as through independent distributors and other resellers. IDEXX maintains sales offices outside the U.S. in all major regions including Africa, Asia Pacific, Canada, Europe, Middle East, and Latin America. Generally, IDEXX selects the appropriate distribution channel based on the type of product, technical service requirements, number and concentration of customers, regulatory requirements, and other factors. In the UK, IDEXX sells their companion animal diagnostic products, veterinary diagnostics and consulting services, and LPD products through direct sales force. The majority products are imported into the UK directly from the Hoofdorp logistics center which warehouses the products produced or imported into Europe.

Many of the instruments that IDEXX sells are manufactured by third parties. IDEXX relies on third parties in its supply chain to supply them, and their direct suppliers, with certain important components, raw materials and consumables used in or with IDEXX's products. In some cases, these third parties are sole or single source suppliers.

Formulation/manufacture use takes place in Montpellier France. Other formulation/manufacture sites are located in Bern, Switzerland and Westbrook, US. Triton products are produced in all of these three sites and then supplied to the UK end-users from all of these three sites. Most of the traffic in Europe goes via IDEXX logistics center in Hoofddorp, Netherlands. End-users are performing tests with IDEXX products in companion animal, livestock and poultry, and dairy and water testing markets. All end-users are professionally trained personnel, including veterinarians, laboratory technicians, scientists, and IDEXX reference laboratory staff.

In the UK IDEXX has three sites: Windsor Berkshire Commercial office, Wetherby Reference laboratory and Newmarket manufacturing site. The commercial office employs roughly 100 people (commercial, finance, HR, Field Sales Reps, Veterinary Diagnostic Consultants, Call center etc.). The reference laboratory employs over 210 people. This location also incorporates a warehouse for stock pertaining to supporting the lab, and

office spaces. Newmarket manufacturing site is a small site for Water testing products, including an R&D laboratory, warehouse, shipping and sales, administrative offices. It employs approximately 30 people. The Newmarket site is out of the scope of the application since they are manufacturing products which are not related to 4-tert-OPnEO and 4-NPnEO and no impacts are foreseen on the site.

Overall, IDEXX has almost 2.5 thousand end-users in the UK. A vast majority of the end-users are veterinary clinics. The following table outlines the number of each type of end-users in the UK.

TABLE 1. END-USERS IN THE UK

End-user type	Number
Veterinary clinics	2,370
Reference laboratories	22
Universities	3
Farmers	1
Other	31
Total	2,427

Figure 1 outlines the 4-tert-OPnEO and 4-NPnEO and UK related supply chain. It illustrates every step from raw material supplier to the end-user. Operators marked with orange color are in the scope of the application and operators marked with blue color are not in the scope of the application.

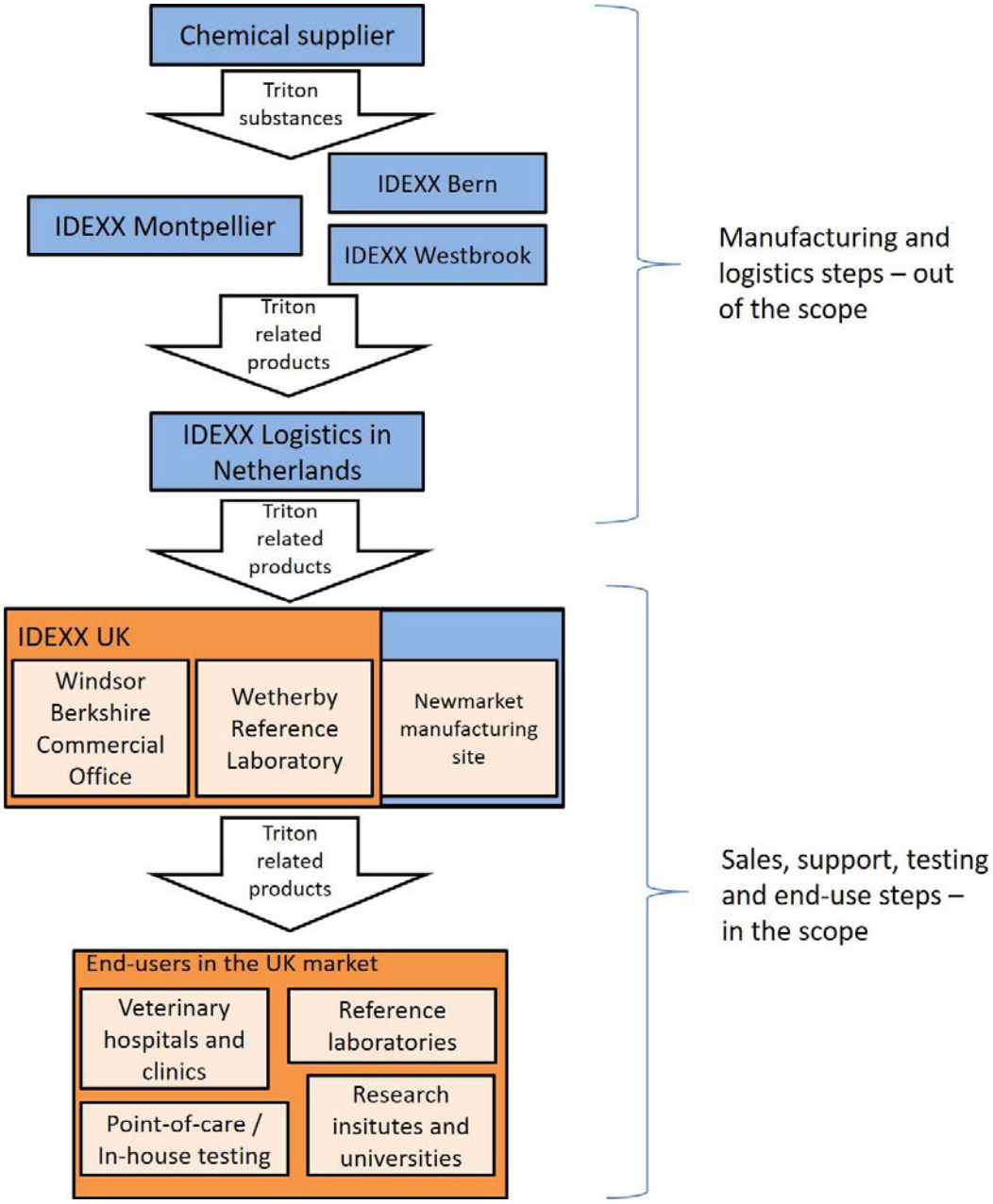


FIGURE 1. Supply chain

2.4. Scope of the analysis

2.4.1. Geographical scope

Geographical scope will depend on where the impacts of the non-use scenario are felt. As the end-use of products containing 4-tert-OPnEO and 4-NPnEO would be banned in the UK and the related IDEXX's UK operators would encounter lay-offs, the geographical scope of the analysis is the UK.

2.4.2. Temporal scope

It is expected that phasing out 4-tert-OPnEO and 4-NPnEO from all of their SNAP and ELISA products would take IDEXX 18-23 years. The 18-23 years take into account the

research of a suitable alternative and also the subsequent verification, stability testing, validation and global regulatory approval. Nevertheless, the Applicant is aware that a review period of more than 12 years should not be considered for non-threshold substances for which the risks cannot be quantified, as further explained in Section 6.7. As this is the case for 4-tert-OPnEO and 4-NPnEO, the Applicant is requesting for a review period of 12 years and will apply for a review of the authorisation in order to finish the reformulation of all products.

For that reason, the proposed temporal scope of 12 years is based on the review period applied for.

Currently, there are no qualified alternatives for the 4-tert-OPnEOs and 4-NPnEOs present in the SNAP tests' wash solutions and the controls, sample diluents and wash concentrate of the ELISA plate tests. Nevertheless, the applicant has developed a realistic plan for substituting the 4-tert-OPnEOs and 4-NPnEOs from their products. The substitution plan is presented in a separate report submitted with this application.

3. CONSULTATIONS

The Applicant conducted several surveys to assess how their DUs would react in case IDEXX's SNAP and ELISA plate tests were not available and to assess how DUs manage their wastes. We discuss briefly the results of the survey regarding the impacts of the non-availability of IDEXX's SNAP and ELISA plate tests in the next sections. For more detailed results, please consult Appendix 1 of this document. The survey results regarding waste management are discussed in the CSR provided with this application.

In total, 41 of IDEXX's UK DUs answered the surveys conducted by the Applicant. Answers were received from both the CAG and LPD market sectors (Table 2), which are the Applicant's main market sectors. The DUs in question were veterinary clinics, reference laboratories, universities, governmental laboratories or private livestock and milk laboratories using SNAP tests, ELISA plate tests or both.

TABLE 2. Summary of the surveyed DUs grouped by business sector

	SNAP tests	**ELISA plate tests**	**Users of both**	**Total**
CAG UK DUs	33		1	34
LPD UK DUs		7		7
No. of answers				41

Justification of DU selection for the survey:

The Applicant's goal for the downstream user survey was to establish a representative response by surveying typical IDEXX customers who use the Applicant's products consistently.

For CAG, IDEXX focused on a variety of clinics from small, medium and large volume users to ensure that the data collected was representative of most customers. The waste management options available to a smaller clinic may be different from what is possible at a larger clinic. Similarly, region-specific waste regulations may vary, and by collecting data across multiple regions within a country, the survey captured a broad customer base. The survey was conducted by a 3rd party research organization, with a goal of 50 customers for the UK. The survey was administered in an online format with the link to the survey sent by email with a description of the purpose.

For LPD, IDEXX surveyed a variety of customers including government national programs, private livestock labs, independent reference labs, veterinary clinics, and private milk labs. The goal was to survey the representative customers in UK by phone.

Based on this and due to the high number of answers collected and the variety of DUs surveyed, the results are considered representative of IDEXX UK customer base.

3.1. **SNAP tests survey**

The SNAP tests survey was conducted between January 2019 and March 2019 through an online questionnaire sent to IDEXX's DUs from the CAG business sector. The target audience of this survey were veterinarians situated in the UK who spend 70 % of their time treating cats and dogs. More specifically, the questionnaire was addressed to the person responsible or involved in the decision-making process about purchasing diagnostic tests.

The combined results from the UK show that 69 % are more likely to outsource testing to a reference laboratory should IDEXX SNAP tests not be available. However, it is not known to the Applicant whether the alternatives and the reference laboratories envisaged by the DUs contain or use 4-tert-OPnEOs and 4-NPnEOs. Thus, these alternatives may also be affected by the ban on 4-tert-OPnEO and 4-NPnEO.

Regarding the economic impact associated with the non-availability of IDEXX SNAP tests, 63 % of UK DUs consider that it will have a moderate or major economic impact on their practice. 41 % of the UK DUs estimated that implementing their chosen alternative would have a somewhat negative impact on them whereas 47 % estimated that implementing their chosen alternative would not have a positive nor negative cost impact on them.

75 % of UK respondents estimates that not having IDEXX SNAP tests would negatively impact on efficiently and accurately supplying results to pet owners. In addition, 84 % of the respondents claimed that the lack of SNAP tests will have a somewhat negative or very negative impact on animal care. Lastly, there is a clear agreement among the surveyed DUs that accuracy is paramount when selecting rapid assays.

SNAP email survey:

In addition to the online questionnaire, IDEXX surveyed directly by email three university laboratories in the CAG sector. The survey took place at the beginning of 2019 and involved DUs situated in Denmark, France and UK. The number of employees trained to use IDEXX SNAP tests in each laboratory were 3, 6 and 12.

The three DUs surveyed were of the opinion that accuracy is very/extremely important when evaluating veterinary rapid test. Should IDEXX SNAP tests not be available, two DUs answered they would use an alternative rapid test while the remaining DU would change to a comparable plate test. In this case as well, it is not known whether these alternatives use substances falling under entries 42 and 43 of Annex XIV, in which case they would be affected by the ban on Triton X-100 and related substances.

One of the respondents reported a somewhat negative cost impact for their business to implement the alternative while the remaining two DUs expected neither a positive nor negative cost impact.

From the economic side, one of the respondents declared that the non-availability of IDEXX SNAP tests would have a moderate economic impact on their laboratory whereas the remaining two expected it would not have an economic impact on them. On the other hand, two DUs indicated that the absence of IDEXX SNAP tests would have a somewhat negative impact on their ability to efficiently and accurately supplying results to pet owners or veterinarians while the remaining laboratory expected neither a positive nor negative impact.

Lastly, one respondent indicated that the non-availability of IDEXX SNAP tests will have a somewhat negative impact on animal care while the remaining two expected neither a positive nor negative impact.

3.2. **ELISA plate tests survey**

The ELISA plate tests survey was conducted by phone between the end of 2018 and the beginning of 2019 and was aimed at IDEXX's LPD customers. The survey was conducted in two rounds. During the first round, the answers of 24 DUs were gathered while 21 DUs responded to the second round of the survey. In total, 45 DUs were interviewed during the course of the survey, out of which 7 were from the UK.

The target audience in the UK were governmental laboratories and private livestock and milk laboratories. The majority of respondents were either laboratory managers, general managers or CEOs.

Based on the results of the survey, only one of the contacted UK DUs would outsource to a reference laboratory in case the IDEXX ELISA plate tests would not be available. The rest would preferably use an alternative ELISA test. One of the UK respondents indicated that some of IDEXX's plate tests do not have alternatives available. Once again, it is not known whether the alternatives envisaged by the DUs contain 4-tert-OPnEO or 4-NPnEO and whether their ban would change their answer. All the surveyed UK DUs responded that implementing their chosen alternative would have a negative cost impact on their laboratory.

71 % of the UK DUs estimated that not having ELISA plate tests would have a moderate or major economic impact to their laboratory. The same number of respondents considered the non-availability of the IDEXX plate tests will have a negative impact on efficiently and accurately supplying results while 43 % estimated that it would have a very negative impact on animal health. Lastly, all respondents agreed that accuracy is very or extremely important when evaluating ELISA tests.

3.3. **User of both IDEXX SNAP tests and ELISA plate tests**

One reference laboratory located in the UK uses both IDEXX SNAP tests and ELISA plate tests. This is a small sized DU with 10 employees working in the laboratory where IDEXX's *in vitro* diagnostics are used.

In the event, IDEXX SNAP and ELISA assays were not available, it would have a major impact on the surveyed DU. They indicated they would have to stop offering the tests completely as the tests they use are not available in other formats. In the DUs opinion, such scenario would have a very negative impact on efficiently and accurately supplying results to their clients and it would also have a very negative impact on animal care. More importantly, this change would have an important economic impact on the DU as they expect an 11-19 % decrease in lab personnel should the IDEXX ELISA and SNAP tests not be available to them.

4. APPLIED FOR "USE" SCENARIO

4.1. Market and business trends including the use of the substance

4.1.1. Market trend

The veterinary diagnostics market is expected to reach USD 10.550 billion by 2026 from an estimated USD 5.985 billion in 2020, at a CAGR of 10.5 %. The factors driving the market growth include increased growing companion animal population, animal healthcare expenditure, rising incidence of transboundary and zoonotic diseases, and the growing number of veterinary practitioners as well as disposable income in the developing regions. Currently, the lack of skilled veterinarians and diagnostic infrastructure, especially in developing countries, is one of the major factors limiting the uptake of advanced diagnostic solutions among veterinarians. The high cost of advanced diagnostic tests is another major barrier to its widespread adoption.[1,2]

In 2020, the immunodiagnostics segment occupies the highest share in the global veterinary diagnostics market. The large share of immunodiagnostics segment can primarily be attributed to the widespread popularity of immunodiagnostics in disease diagnosis as well as in screening disease progression and observing patients' responses to therapy. In addition, the low cost, low procedural complexity, and greater adoption of immunodiagnostics due to ease of training are further driving the growth of this market segment.[1]

Based on product, the global veterinary diagnostics market is segmented into instruments and consumables. In 2020, the consumables segment accounted for the largest share of the global veterinary diagnostics market. The large share of the consumables segment can be attributed to the rising prevalence of zoonotic diseases, growing animal population, increasing awareness on animal healthcare, and increasing veterinary expenditure.[1]

In 2020, the companion animals segment accounted for the largest share of the global veterinary diagnostics market. This can be attributed to the rising number of companion animals across the globe, the willingness of owners to spend more on their pets, the rising adoption of pet insurance, and the availability of cheaper and easy-to-use POC diagnostic tests for companion animals.[1]

Veterinary reference laboratories are the major end users in the veterinary diagnostics market in 2020. The large share of the veterinary reference laboratories segment can be attributed to the increasing number of veterinary diagnostic reference laboratories, high test volumes at reference laboratories, and the increasing demand for veterinary diagnostic testing for infectious diseases in small and large animals. Rising awareness among pet owners regarding routine and preventive care is further expected to propel market growth.[1]

The global veterinary diagnostics market is segmented into North America, Europe, Asia Pacific, Latin America, Middle East, and Africa. North America is the largest regional market

[1] https://www.marketsandmarkets.com/Market-Reports/veterinary-diagnostics-market-26017452.html

[2] https://www.businesswire.com/news/home/20210409005326/en/Worldwide-Veterinary-Diagnostics-Industry-to-2026---Increasing-Pet-Ownership-and-Animal-Health-Expenditure-is-Driving-Growth---ResearchAndMarkets.com

for veterinary diagnostics market. The growth in the veterinary diagnostics market of North America is characterized by the increasing population of companion and food-producing animals, rising meat and dairy product consumption, the availability of technologically advanced veterinary reference laboratories, rising veterinary healthcare expenditure, and growth in pet insurance coverage.[1]

The key players in the global veterinary diagnostics market are IDEXX Laboratories, Inc. (US), Thermo Fisher Scientific Inc. (US), Zoetis Inc. (US), NEOGEN Corporation (US), Bio-Rad Laboratories Inc. (US), bioMérieux SA (France), Virbac (France), Heska Corporation (US), Agrolabo S.p.A. (Italy), INDICAL BIOSCIENCE GmbH (Germany), Randox Laboratories Ltd. (Ireland), IDvet (France), Biopanda Reagents (UK), Bionote, Inc. (South Korea), BioChek (Netherlands), Fassisi GmbH (Germany), Biogal Galed Labs (Israel), Alvedia (France), SKYER, Inc. (South Korea), and Shenzhen Bioeasy Biotechnology Co., Ltd. (China).[1]

4.1.2. **Business trend**

UK Sales contributes approx. 3.3 % IDEXX total revenue. In 2018 the UK sales revenue was 87.8 M USD, in 2019 91.0 M EUR and in 2020 90.2 M EUR. The profit information is not disclosed in this application, since it is not relevant as the products are manufactured and thus the related profit generated out of the UK.

IDEXX foresees their business growing in the future in the UK. Sales of ELISA tests for LPD segment are anticipating a 6 % annual growth rate. Main driver in the LPD segment is milk pregnancy tests with 18 % annual growth rate. Sales of the CAG segment is expecting a 6% annual growth rate, with particular growth in the bovine area. In addition, the UK is a significant market for advancing veterinary care and diagnostic testing.

Competition landscape is described below in Section 4.1.3.

4.1.3. **Competition landscape**

IDEXX competes with many companies ranging from large human and animal health and medical diagnostics companies to small businesses focused on animal health. IDEXX's companion animal veterinary diagnostic products and services compete with both reference laboratory service and in-clinic product providers. IDEXX's competitors vary in different markets. In some markets, academic institutions, governmental agencies, and other public and private research organizations conduct research activities and may commercialize products or services which could compete with IDEXX products, on their own or through joint ventures.

Competitive factors in different business areas are detailed below:

- **Companion animal diagnostic offerings.** IDEXX competes primarily on the basis of ease of use and speed of results of products diagnostic accuracy, product quality, breadth of product line and services, unique product innovations, fully integrated technology, information management capability, availability of medical consultation, effectiveness of sales and distribution channels, quality of technical and customer service and pricing relative to the value of products and services in comparison with competitive products and services. IDEXX's major competitors in most geographic locations in North America are Antech Diagnostics, a unit of VCA

Inc., a division of Mars, Incorporated; Zoetis Inc. (including its wholly-owned subsidiary Abaxis, Inc.); Heska Corporation; Samsung Electronics Co., Ltd., and FUJIFILM North America Corporation. IDEXX also competes in certain international markets with Zoetis, Fujifilm Holdings Corporation, Samsung Electronics, Arkray, Inc., Heska, Mindray and BioNote, Inc.

- **Water, livestock, poultry, and dairy testing products.** IDEXX competes primarily on the basis of the ease of use, speed, accuracy, product quality and other performance characteristics of products and services (including unique tests), the breadth of product line and services, the effectiveness of sales and distribution channels, the quality of technical and customer service, ability to receive regulatory approvals from governing agencies and pricing relative to the value of products in comparison with competitive products and services. IDEXX's competitors include highly focused smaller companies and multibillion-dollar companies with small livestock and poultry diagnostics and water testing solution franchises.

- **Veterinary Software, Services and Diagnostic Imaging Systems.** IDEXX competes primarily on the basis of functionality, connectivity to equipment and other systems, performance characteristics, effectiveness of implementation, training process and customer service, information handling capabilities, advances in technologies and pricing relative to the value of products and services. IDEXX sells these products primarily in North America and Europe. IDEXX's largest competitor in North America and the U.K. is Covetrus, Inc., which offers several systems and leverages its animal health distribution business in sales and service. IDEXX also compete with numerous focused smaller companies throughout the markets in which they offer veterinary software, including those offering cloud-based solutions. IDEXX's competitors in the diagnostic imaging systems market include Sound-Eklin, Antech Diagnostics, FUJIFILM, and Heska

- **Electrolyte, blood gas analyzers and SARS-COVID diagnostic test kits for the human point-of-care medical diagnostics market.** IDEXX competes primarily on the basis of the ease of use, menu, convenience, international distribution and service, instrument reliability, and pricing relative to the value of products. IDEXX competes primarily with large human medical diagnostics companies such as Radiometer A/S, Siemens Medical Solutions Diagnostics, Instrumentation Laboratory Company, Abbott Diagnostics, a division of Abbott Laboratories and Roche Diagnostics Corporation. IDEXX also compete with a number of companies around the world that produce human COVID-19 testing.

4.2. **Analysis of the substance function(s) and technical requirement(s) for the product(s)**

4.2.1. **IDEXX's *in vitro* diagnostic products**

The products marketed by IDEXX in the UK can be divided in two main categories: the SNAP tests and the ELISA plate tests. Both type use ELISA technology to provide rapid and reliable diagnostics. All the SNAP products are manufactured at IDEXX's US facilities

in Westbrook, Maine whereas the ELISA plate tests originate from three sites in total, namely, Montpellier (France), Bern (Switzerland) and Westbrook (USA).

In total, IDEXX has 58 unique products (60 if the duplicates are counted) that are covered by this application (Table 3). 33 products are manufactured in Westbrook, USA, 13 in Bern, Switzerland (CH) and 14 are produced in Montpellier, France (FR). The IDEXX PRV/ADV gB and IDEXX PRV/ADV GI kits are manufactured both in Bern and Westbrook.

The products impacted by this Application contains 4-tert-OPnEO or 4-NPnEO in the sample diluents, controls, conjugate solutions, SNAP sample and/or conjugate wash solutions, wash solutions, tissue soaking buffers and detection solutions. The products are used by large veterinary laboratories, veterinary clinics, veterinarians and farmers for the detection or quantitative measurement of various antigens and antibodies linked with infectious diseases. In addition, IDEXX has a small range of veterinary products dedicated for the detection of pregnancy-associated proteins. The type of sample that can be tested with each assay varies from product to product. Compatible samples may be serum, plasma, tissue, faeces, whole blood, meat juice and milk samples from a wide array of species, such as bovine, canine, feline, swine, ovine, caprine, avian and equine.

Certain tests are used for disease detection, others are used for vaccine monitoring where the objective is disease control rather than eradication. In special cases, the use of DIVA tests (Differentiating Infected from Vaccinated Animals) is able to differentiate naturally infected animals from vaccinated animals. IDEXX has three DIVA tests concerned by this application: the IDEXX APP-ApxIV, IDEXX IBR gE Ab and IDEXX PRV/ADV gI Ab.

TABLE 3. The list of products covered by this application along with a short description and country of origin (COO). 4-tert-OPnEO or 4-NPnEO is contained in (a) sample diluents, (b) controls, (c) conjugate solutions, (d) SNAP wash solutions, I wash solution, (f) tissue soaking buffer or (g) detection solution.

Trade name	Intended use	CAS No.	Detergent Conc. (%)	COO
IDEXX ALV Ab	Indirect ELISA plate assay used to detect antibodies specific to the ALV subgroups A and B in chicken serum.	9036-19-5	0.10[(a)(b)]	USA
IDEXX ALV-J Ab	Indirect ELISA plate assay used to detect antibodies specific to the ALV subgroup J in chicken serum.	9036-19-5	1.00[(a)(b)]	USA
Bovine pregnancy	Capture ELISA plate assay used to detect pregnancy-associated glycoproteins in serum and EDTA plasma of cattle, serum of sheep and goat, EDTA plasma of water buffalo and bison as a marker for pregnancy.	9002-93-1	1.00[(a)(b)]	CH
Canine Cardiopet Plus	Direct ELISA plate assay for the quantitative measurement of NTproBNP from canine EDTA plasma and serum as a marker substance for heart failure.	9036-19-5	1.00[I]	USA
Feline Cardiopet proBNP	Direct ELISA plate assay for the quantitative measurement of NTproBNP from feline EDTA plasma and serum as a marker substance for heart failure.	9036-19-5	1.00[I]	USA
HerdChek BSE-scrapie Ag	Indirect ELISA plate assay used to detect the abnormal conformer of the prion protein (PrPSc) in bovine, caprine and ovine post-mortem tissues (obex, spleen and lymph node samples).	9036-19-5	5.00[(a)(b)(c)]	USA
HerdChek CWD Ag	Indirect ELISA plate assay used to detect the abnormal conformer of the prion protein (PrPSc) in post-mortem white-tailed and mule deer retropharyngeal lymph node tissue.	9036-19-5	5.00[(a)(b)] 1.00 [I]	USA
IDEXX IBV Ab	Indirect ELISA plate assay used to detect antibodies specific to the infectious bronchitis virus from chicken serum samples.	9036-19-5	0.10[(a)(b)]	USA
IDEXX AI MultiS-Screen Ab	Competitive ELISA plate assay used to detect antibodies specific to avian influenza in serum samples from multiple species (chicken, turkey, duck, goose and others).	9036-19-5	1.00[(a)(b)]	USA
IDEXX APP-ApxIV Ab	ELISA plate assay used to detect antibodies specific to Actinobacillus pleuropneumoniae, which is the causative pathogen for swine pleuropneumonia, in serum and plasma of swine.	9036-19-5	0.22[(a)(b)]	CH
IDEXX APV Ab	Indirect ELISA plate assay to detect antibodies specific to the avian pneumovirus in chicken and turkey serum.	9036-19-5	0.11[(a)(b)]	CH
IDEXX BVDV Ag/Serum Plus	ELISA plate assay used to detect the antigens specific to the bovine viral diarrhea virus in bovine serum, plasma, whole blood and ear-notch tissue samples.	9036-19-5, 9016-45-9	1.00[(a)(b)] 0.52[(g)]	CH
IDEXX BVDV Total Ab	Indirect ELISA plate assay used to detect antibodies specific to the bovine viral diarrhea	9036-19-5	0.11[(a)(b)]	CH

	virus in bovine serum, plasma and milk samples.		0.50[I]	
IDEXX CSFV Ab	Competitive ELISA plate assay used to detect the antibodies specific to the classical swine fever virus in swine serum and plasma samples.	9036-19-5, 9016-45-9	2.00[(a)(b)]	CH
IDEXX CSFV Ag Serum	Indirect ELISA plate assay used to detect the Erns proteins of the classical swine fever virus in swine serum and plasma samples.	9036-19-5, 9016-45-9	2.08[(f)]	CH
IDEXX IBR gE Ab	Competitive ELISA plate assay that can detect antibodies specific to the infectious bovine rhinotracheitis in bovine serum, plasma and milk sample. It is used to differentiate between naturally infected cattle from vaccinated cattle.	9036-19-5, 9016-45-9	1.00[(a)(b)]	CH
IDEXX *M. Bovis* Ab	Indirect ELISA plate assay that can detect *Mycobacterium bovis* antibodies in cattle serum and plasma samples.	9036-19-5	1.00[(a)(b)]	USA
IDEXX *M. Hyo* Ab	Indirect ELISA plate assay used to detect antibodies specific mycoplasma hyopneumoniae in swine serum and plasma samples.	9036-19-5	0.20[(a)(b)]	USA
IDEXX Milk Pregnancy	ELISA plate assay to detect pregnancy-associated glycoproteins in cow and goat milk samples.	9002-93-1	1.00[(a)(b)]	CH
IDEXX PRV/ADV gB Ab	Competitive ELISA plate assay used to detect antibodies specific to the gB antigen of the pseudorabies virus in swine serum and plasma samples.	9002-93-1, 9036-19-5	1.07[(a)(b)]	CH, USA
IDEXX PRV/ADV gI Ab	ELISA plate assay used to detect antibodies specific to the gI antigen of the pseudorabies virus in swine serum samples. The test differentiates infected from vaccinated animals.	9002-93-1, 9036-19-5	1.07[(a)(b)]	CH, USA
IDEXX Rapid Visual Pregnancy	Indirect ELISA plate assay used to detect early pregnancy-associated glycoproteins in whole blood (EDTA), plasma (EDTA) and serum of cattle, serum of goats, whole blood (EDTA) and serum of sheep and whole blood (EDTA) of water buffalo.	9036-19-5	1.00[(a)(b)]	CH
IDEXX SNAP BVDV Ag	SNAP test used to detect antigens specific to the bovine viral diarrhea virus from serum and ear-notch tissue samples.	9036-19-5	1.00[(d)]	USA
IDEXX Swine *Salmonella* Ab	ELISA plate assay used to detect antibodies specific to several *salmonella* serogroups (B, C1 and D) in serum, plasma and meat juice samples.	9036-19-5	0.20[(a)(b)]	CH
Lyme Quant C6 Antibody Kit	ELISA plate assay for the quantitative measurement of C6 antibodies specific to *Borrelia burgdorferi* in canine serum.	9036-19-5	0.10[(a)(b)]	USA
IDEXX NDV Ab	Indirect ELISA plate assay used to detect antibodies specific to the Newcastle disease virus in chicken serum.	9036-19-5	0.10[(a)(b)]	USA

IDEXX *Neospora* Ab	ELISA plate assay used to detect antibodies specifc to *Neospora caninum* in serum and plasma samples of bovine, caprine and ovine.	9036-19-5	0.25[(a)(b)]	USA
IDEXX BSE Non-biohazard pos control material	BSE positive calibration sample that is sold separately from the IDEXX HerdChek BSE-Scrapie kit.	9036-19-5	0.15[(a)(b)]	USA
IDEXX REO Ab	Indirect ELISA plate assay used to detect antibodies specific to the avian reovirus in chicken serum.	9036-19-5	0.10[(a)(b)]	USA
SNAP 4Dx Plus Test	SNAP test used to detect *Dirofilaria immitis* antigens, antibodies to *Anaplasma phagocytophilum*, antibodies to *Anaplasma platys*, antibodies to *Borrelia burgdorferi*, antibodies to *Ehrlichia canis* and antibodies to *Ehrlichia ewingii* in canine serum, plasma and whole blood samples.	9036-19-5	1.00[(d)]	USA
SNAP cPL Test	SNAP test for the determination of pancreas-specific lipase levels in canine serum.	9036-19-5	1.00[(d)]	USA
SNAP Feline Heartworm Test	SNAP test for the semi-quantitative detection of Dirofilaria immitis antigen in feline whole blood.	9036-19-5	1.00[(d)]	USA
SNAP Feline proBNP Test	SNAP test for the measurement of circulating NTproBNP in feline serum and EDTA plasma.	9036-19-5	1.00[(d)]	USA
SNAP Feline Triple Test	SNAP test for the detection of *Dirofilaria immitis* antigens, antigens to feline leukemia virus and antibodies to feline immunodeficiency virus in feline serum, plasma and whole blood.	9036-19-5	1.00[(d)]	USA
SNAP FeLV Antigen Test	SNAP test for the detection of feline leukemia virus antigens in feline serum, plasma and whole blood.	9036-19-5	1.00[(d)]	USA
SNAP FIV/FeLV Combo Plus Test	SNAP test for the detection of feline leukemia virus antigens and antibodies to feline immunodeficiency virus in feline serum, plasma and whole blood.	9036-19-5	1.00[(d)]	USA
SNAP FIV/FeLV Combo Test	SNAP test for the detection of feline leukemia virus antigens and antibodies to feline immunodeficiency virus in feline serum, plasma and whole blood.	9036-19-5	1.00[(d)]	USA
SNAP Foal IgG Test	SNAP test for the semi-quantitative detection of immunoglobulin G in equine serum, plasma and whole blood.	9036-19-5	1.00[(d)]	USA
SNAP fPL Test	SNAP test for the determination of pancreas-specific lipase levels in feline serum.	9036-19-5	1.00[(d)]	USA
SNAP Giardia Test	SNAP test for the detection of *Giardia* antigens in canine and feline feces.	9036-19-5	1.00[(d)]	USA
SNAP Heartworm RT Test	SNAP test for the semi-quantitative detection of *Dirofilaria immitis* antigens in canine and feline whole blood, serum and plasma.	9036-19-5	1.00[(d)]	USA

SNAP Leishmania Test	SNAP test for the detection of Canine leishmaniasis in canine whole blood samples.	9036-19-5	1.00[(d)]	USA
SNAP Lepto Test	SNAP test for the detection of anti-*Leptospira* antibodies to the serovars Grippotyphosa, Canicola, Pomona, and Icterohaemorrhagiae in canine serum.	9036-19-5	1.00[(d)]	USA
SNAP Parvo Test	SNAP test for the detection of canine parvovirus antigens in canine feces.	9036-19-5	1.00[(d)]	USA
IDEXX Bluetongue Competition Ab	Competitive ELISA plate assay used to detect antibodies specific to the bluetongue virus in sheep, goat and cattle serum.	9036-19-5	2.00[(a)(b)]	FR
IDEXX Brucellosis Ovine/Caprine Serum Ab	ELISA plate assay used to detect antibodies specific to the bacteria Brucella abortus (found in cattle) and Brucella melitensis (found in sheep and goats) from animal serum.	9036-19-5	2.00[(a)(b)]	FR
IDEXX BVDV P80 Ab	Blocking ELISA plate assay used to detect antibodies specific to bovine viral diarrhea virus from bovine serum and plasma as well as bovine milk samples. It can also be used to detect specific antibodies directed to the border disease virus from ovine serum.	9036-19-5	2.00[(a)(b)]	FR
IDEXX Fasciolosis Verification	ELISA plate assay used to detect the level of *Fasciola hepatica* antibodies in bovine and ovine serum samples as well as bovine milk samples.	9036-19-5	2.00[(a)(b)]	FR
IDEXX IBR Individual Ab	ELISA plate assay used to detect antibodies specific to the Bovine Herpesvirus-1 from individual bovine serum samples.	9036-19-5	2.00[(a)(b)]	FR
IDEXX IBR Pool Ab	ELISA plate assay used to detect antibodies specific to the Bovine Herpesvirus-1 from pool bovine serum samples and tank milk samples.	9036-19-5	2.00[(a)(b)]	FR
IDEXX Leukosis Serum Screening Ab	ELISA plate assay used to detect antibodies specific to the Bovine Leukemia virus from individual and pool bovine serum samples.	9036-19-5	2.00[(a)(b)]	FR
IDEXX MAP Ab	Indirect ELISA plate assay used to detect *Mycobacterium avium* subsp. *Paratuberculosis* antibodies from bovine milk, serum and plasma samples.	9036-19-5	2.00[(a)(b)]	FR
IDEXX Paratuberculosis Screening Ab	Indirect ELISA plate assay used to detect *Mycobacterium avium* subsp. *Paratuberculosis* antibodies from bovine milk, serum and plasma samples as well as serum and plasma of sheep and goats.	9036-19-5	2.00[(a)(b)]	FR
IDEXX Paratuberculosis Verification Ab	ELISA plate assay used to detect *Mycobacterium avium* subsp. *Paratuberculosis* antibodies from bovine milk, serum and plasma samples as well as serum and plasma of sheep and goats.	9036-19-5	2.00[(a)(b)]	FR
IDEXX PI-3 Ab	ELISA plate assay used to detect antibodies specific to the parainfluenza type 3 virus from individual bovine serum samples.	9036-19-5	2.00[(a)(b)]	FR

IDEXX RSV IgG Ab	ELISA plate assay used to detect immunoglobulin G antibodies specific to the bovine respiratory syncytial virus from individual bovine serum samples.	9036-19-5	2.00[(a)(b)]	FR
IDEXX RSV IgM Ab	ELISA plate assay used to detect immunoglobulin M antibodies specific to the bovine respiratory syncytial virus in individual bovine serum samples.	9036-19-5	2.00[(a)(b)]	FR
IDEXX Trivalent Ab	ELISA plate assay used to detect antibodies specific to the bovine respiratory syncytial virus, parainfluenza virus type 3 and adenovirus from individual bovine serum samples.	9036-19-5	2.00[(a)(b)]	FR

4.2.2. **ELISA technology in plate tests**

IDEXX's in vitro diagnostic kits use ELISA (enzyme-linked immunosorbent assay) technology to ensure rapid and reliable diagnostics. The technology takes advantage of antigen-antibody interactions, and more importantly, the specificity of these interactions to detect and quantify the amount of antigens or antibodies of interest in a patient's sample. Quantification is achieved through the addition of a chromogenic substrate, which will produce colour of varying intensity depending on the quantity of target antibodies or antigens present. Analytical instruments, such as spectrophotometers, are typically used to read the results.

Several formats of ELISA exist: direct (sometimes called antigen-capture), indirect and competitive (or blocking) ELISA. Regardless of the format, all ELISA assays include the same basic steps: a target antigen or antibody binding step, a conjugate binding step, one or two wash steps and a colour development step.

In an indirect ELISA, the antibodies present in the sample bind to the corresponding antigens pre-coated to the test. Any unbound components are removed with a wash solution before an enzyme-labelled antibody, called a conjugate, is added. The conjugates will target the sample antibodies such that the sample antibodies will be "sandwiched" between a conjugate and a pre-coated antigen. The test is washed again to remove any unbound materials. Lastly, a chromogenic substrate is added causing colour to develop as it reacts with the enzymes of the conjugate. The intensity of the colour increases proportionally with the amount of bound antibody in the test. Therefore, no colour will develop if the sample does not contain the target antibody. Conversely, a high concentration of antibody of interest will cause a strong colour development in the test (Figure 2).

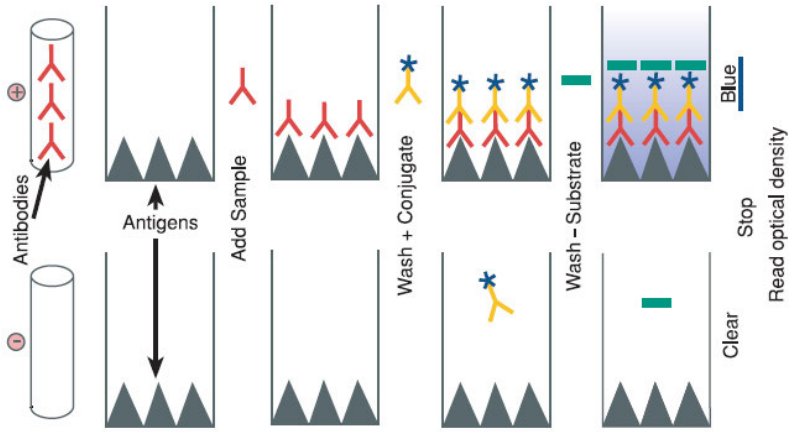


FIGURE 2. The principle of an indirect ELISA. The sample antibody is "sandwiched" between the pre-coated antigen and an enzyme-labelled antibody. Pre-coated antigens are represented in grey, sample antibodies in red, conjugates in yellow-blue and substrates in green.[3]

Several of IDEXX's ELISA plate tests use the indirect ELISA format whereas others are designed around the direct format (Figure 3). In a direct ELISA, any antigens present in the sample will be "sandwiched" between the pre-coated antibodies and the conjugates.

[3] IDEXX Laboratories Inc., 2013

Once again, a chromogenic substrate is used for colour development. The larger the amount of antigens present in the patient's sample, the more intense the colour will be.

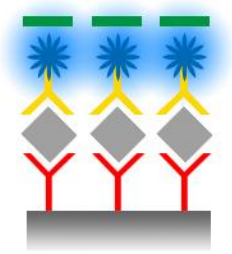


FIGURE 3. The principle of a direct ELISA. The sample antigen is "sandwiched" between two antibodies. Antigens are represented in grey, pre-coated antibodies in red, conjugates in yellow-blue and substrates in green

In some cases, the target antigen is so small in size that it is not detectable through direct ELISA assay as two antibodies cannot properly bind to the antigen. In such circumstances, the competitive ELISA format is preferred. In this format, the target antigen (or antibody) competes with the pre-coated reference antigen (or antibody) for binding to a limited amount of enzyme labeled antibodies (or antigens). In FIGURE 4, an example procedure is illustrated. First, a limited amount of enzyme labeled antibodies are added to the sample. The antigens present in the sample will bind to the antibodies after which the sample solution is added to an ELISA plate that has been pre-coated with the same antigens as the sample antigens. Any unbound enzyme labeled antibodies will bind to the reference antigens whereas the sample antigen-enzyme labeled antibody complexes will be removed during the wash step. Consequently, when the substrate is added to the plate, the intensity of the signal will be inversely proportional with the amount of antigen present in the sample.

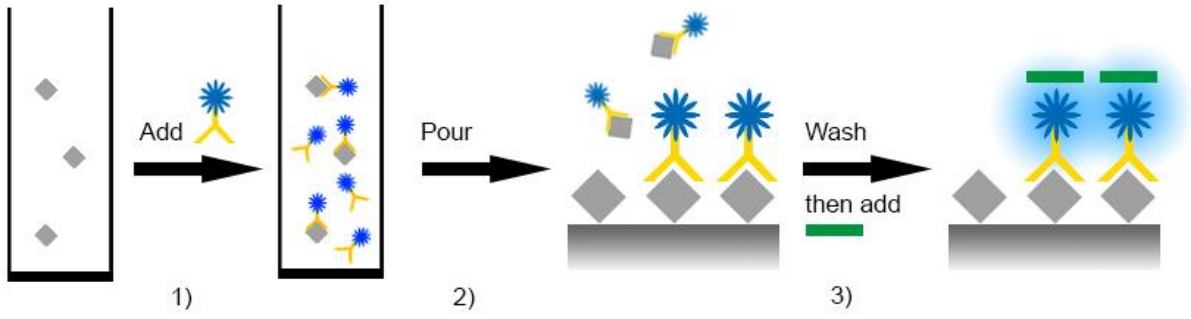


FIGURE 4. 1) Conjugates are added to a sample containing antigens. The antigens and conjugates bind to each other. 2) The sample is poured onto an ELISA plate that is pre-coated with similar antigens as sample antigens. The unbound conjugates bind to the immobilized antigens. 3) The plate is washed. The sample antigen-conjugate complexes are eliminated from the plate. The substrate is added for colour development. The higher the number of antigens present in the sample, the lighter the colour produced.

4.2.3. **ELISA technology in SNAP tests**

SNAP tests were developed by IDEXX scientists in the early 1990s, when the first heartworm antigen assay was marketed. They combine ELISA technology with well-known diagnostic markers to deliver reference-laboratory quality results in a short amount of

time. The same basic steps of an ELISA test are incorporated in the SNAP tests however, unlike with typical ELISA plate tests, the majority of the steps occur inside the plastic casing of the device. This allows for the use of SNAP tests outside of laboratories e.g. SNAP tests compatible with whole blood samples are often use in the field.

In a SNAP assay, the sample and the conjugate, are combined before being introduced into the device. They subsequently flow through the test's matrix and bind with the spots of pre-coated antigen-specific antibodies (step 1 in Figure 5). The SNAP test is activated by pressing on the activator, which pierces the reagent reservoirs (V = 0.4 ml) of the substrate and the wash solution, and reverses the flow of liquid through the matrix. The wash solution, which may contain Triton substances, cleans any unbound material and debris from the matrix to produce a clean background (step 2 in Figure 5). The substrate flows through the cleaned matrix and reacts with the conjugate to produce blue coloured spots (step 3 in Figure 5). All excess liquid in the device are soaked up by the absorbent pad; thus, any solution present in the SNAP assay remains trapped within the device and are not released during the normal use of the device. If the SNAP assay contains Triton substances, they are a component in the SNAP wash solutions, which are present in the assay in only minimal volumes (0.4 ml).

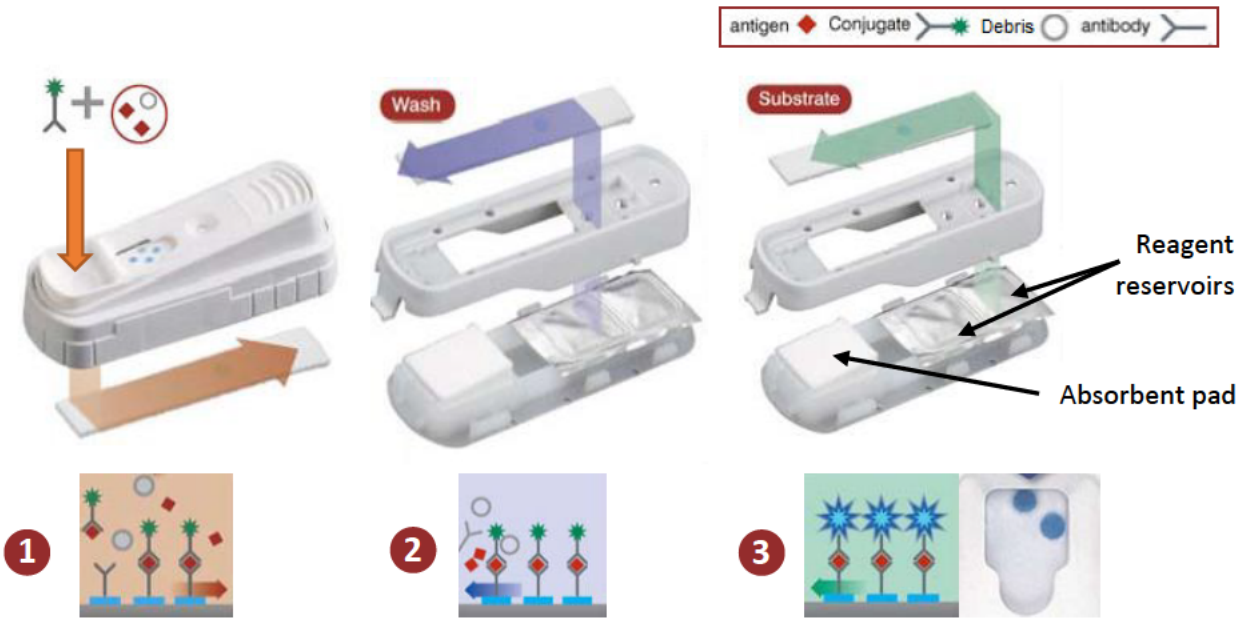


FIGURE 5. The mechanism of a SNAP test. 1) Conjugate and target antigen binding step. 2) Wash step. 3) Colour development step.

4.2.4. **Description of the technical function provided by the Annex XIV substance**

As seen in the previous section, one of ELISA's key feature lies in the ability of the test's surface to immobilize biomolecules that in turn act as "anchors" for other biomolecules. However, ELISA is prone to non-specific binding (NSB) where other unwanted macromolecules, such as the conjugate or proteins originating from the sample bind to unoccupied spaces on the surface of the assay. Non-specific binding has a detrimental effect on the quality of the assay. It results in high backgrounds and leads to a reduction

in specificity and sensitivity of the assay and, in some cases, may even cause false positive results.[4] Therefore, it is crucial to minimize NSB. This is typically achieved by saturating unoccupied binding sites with a blocking reagent, which is a substance, usually a protein or a detergent, used specifically to reduce NSB but has no active role in the ELISA-specific reactions.[5] Bovine serum albumin, non-fat dry milk, fish gelatin and non-ionic detergents such as Triton X-100 are examples of blocking agents commonly used in the immunoassay industry.

In the present case, IDEXX uses three different detergents to prevent NSB. These substances are summarized in Table 4. The first two (CAS No. 9002-93-1 and 9036-19-5) are 4-tert-OPnEO and fall under Entry 42 of REACH Annex XIV whereas the last one (CAS No. 9016-45-9) is a 4-NPnEO and falls under Entry 43 of the same annex.

TABLE 4. IDEXX uses three different substances to prevent NSB in their products

EC number	CAS number	Trade name	IUPAC name	Description
618-344-0	9002-93-1	Triton X-100/IGEPAL CA-630	4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated	Covering well-defined substances and UVCB substances, polymers and homologues. Entry 42 of Annex XIV of REACH. (4-tert-OPnEO, OPnEO)
618-541-1	9036-19-5	Triton X-405, IGEPAL® CA-720	4-(1,1,3,3-Tetramethylbutyl) phenol, ethoxylated	Covering well-defined substances and UVCB substances, polymers and homologues. Entry 42 of Annex XIV of REACH (4-tert-OPnEO, OPnEO)
500-024-6	9016-45-9	Nonidet® P 40 Substitute	4-Nonylphenol, branched and linear, ethoxylated	Substances with a linear and/or branched alkyl chain with a carbon number of 9 covalently bound in position 4 to phenol, ethoxylated covering UVCB- and well-defined substances, polymers and homologues, which include any of the individual isomers and/or combinations thereof. Entry 43 of Annex XIV of REACH. (4-NPnEO, NPnEO)

4-tert-OPnEO and 4-NPnEO are very similar in structures as illustrated in Figure 6. The main difference between them arise from the alkyl chain.

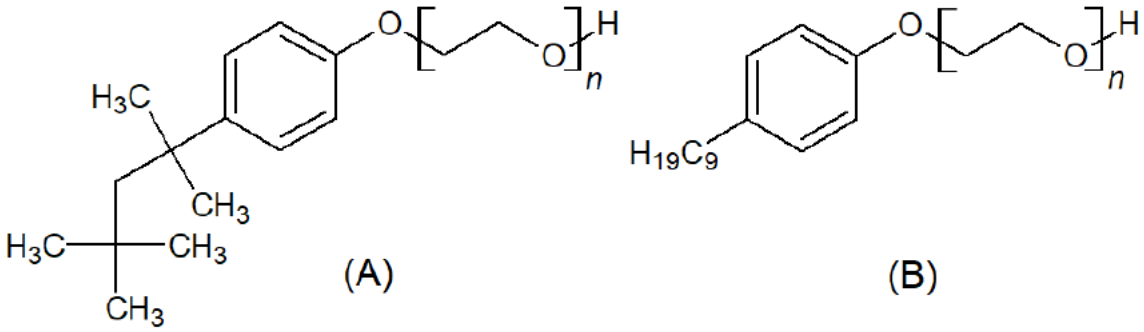


FIGURE 6. (A) The generic structure of 4-tert-OPnEO. (B) The generic structure of 4-NPnEO.

As a consequence of this structural similarity, the Triton substances and Nonidet have the same function in IDEXX's IVD products. More precisely, the Applicant uses 4-tert-OPnEO

[4] Güven, Duus, Lydolph, Jørgensen, Laursen, Houen, 2013

[5] Gibbs, 2014

and 4-NPnEO in their *in-vitro* diagnostic kits to prevent the non-specific binding of undesired macromolecules, such as conjugates and sample impurities, to the bottom of the wells in ELISA plate tests and to the assay's matrix in SNAP tests (Figure 7). As the colour signal arising from the assays is proportional to the amount of substrates that react with the conjugates, non-specifically binded conjugates leads to falsely high signal. This is unwanted as it can lead to false positive results, which in worst cases can result in a healthy animal being euthanised.

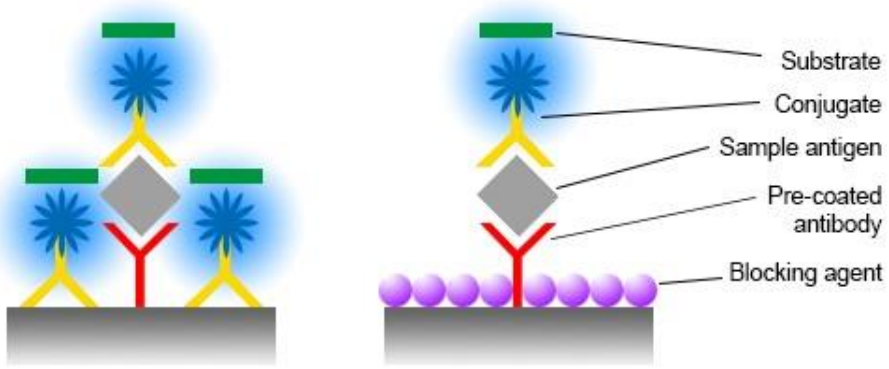


Figure 7. On the left, the non-specific binding of the conjugate to the surface of the assay leads to a falsely high signal. On the right, the use of blocking agent, such as Triton X-100, saturates unoccupied binding sites eliminating NSB.

Preventing NSB is the main function of 4-tert-OPnEO and 4-NPnEO in the different components of ELISA and SNAP test kits. However, they have also other functions in the solutions. They ensure optimum protein conformation and stability such that specific interaction between target antigen and antibody can occur. They are also effective in removing impurities and unwanted material that may be present in the wells of the ELISA assay and matrix of SNAP tests.

It should be noted that among the reasons behind the use of several different 4-tert-OPnEOs and 4-NPnEOs to achieve the same function, i.e. preventing NSB in *in vitro* diagnostic kits, one is historical. When IDEXX acquired the facility situated in Bern, Switzerland, they inherited the product lines of the site, which used IGEPAL CA720, IGEPAL CA-630 and Nonidet as detergents. Additionally, each assay is formulated individually to achieve the technical requirements; thereby a reason why the applicant has a variety of components each with unique concentrations. While it is an internal goal of the applicant to streamline their product lines in order to reduce the number of different reagents used in their products over the next decade or more, the primary goal of the substitution of 4-tert-OPnEO and 4-NPnEO will be to replace them with a non-hazardous alternative substance, while maintaining current assay requirements.

4.2.5. **Description of the product(s) resulting from the use of the Annex XIV substance**

This use covers the downstream use of two types of IVDs: SNAP tests and ELISA plate tests.

4.2.5.1. ELISA plate tests

IDEXX manufactured the first commercial livestock ELISA for infectious bursal disease (IBD) in 1985. Since that time, IDEXX has developed a wide array of ELISAs for the detection of various diseases in ruminants, equine, swine, cervids and poultry. Typically, an ELISA plate kit contains several coated plates, a bottle of sample diluent, a bottle of conjugate, a bottle of substrate, several bottles of controls and a bottle of wash concentrate (Figure 8). Some kits contain fewer components as each test is designed for a specific disease. The components of different kits or lots cannot be mixed as each component is carefully manufactured and specifically optimized to work as a unit. A typical test procedure for ELISA plates is described in Appendix 3 of this document.

FIGURE 8. Typical content of an IDEXX ELISA diagnostic kit. Components from left to right: substrate, wash concentrate, positive and negative controls, conjugate, sample diluent and coated plates.

The ELISA assays covered by this application are typically used by large veterinary laboratories to detect a wide variety of predefined infectious diseases in bovines, ovine and caprine. The samples to be tested are typically serum or milk samples. Depending on the test, the assay can be used on pool or individual samples. Individual samples originate from a single animal whereas pool samples are the samples of multiple animals which are combined in order to screen the health of a particular herd.

4.2.5.2. SNAP test kits

IDEXX's SNAP tests are compact plastic devices that encase a sample wash solution, a substrate solution and a matrix pre-coated with a layer of antigen-specific antibodies or antibody-specific antigens. Each test is designed to detect the diagnostic markers for one or multiple diseases or semi-quantitatively measure the level of a specific enzyme. As IDEXX's SNAP tests are compatible with several types of samples, namely, whole blood, serum, plasma, milk and faecal samples, the devices can be used to test a wide array of diseases and other marker substances, such as lipases, peptides or antibiotics.

SNAP tests are easy-to-use *in vitro* diagnostics that deliver reliable, accurate results within minutes. A typical SNAP test kit for whole blood, serum, plasma and milk samples is composed of a bottle of conjugate, a disposable sample tube, a pipette and the diagnostic device itself. For faecal samples, the kit contains only the SNAP device and a swab tube

fitted with a reservoir for the conjugate (Figure 9). Triton substances are present in the wash solution encased within the device.

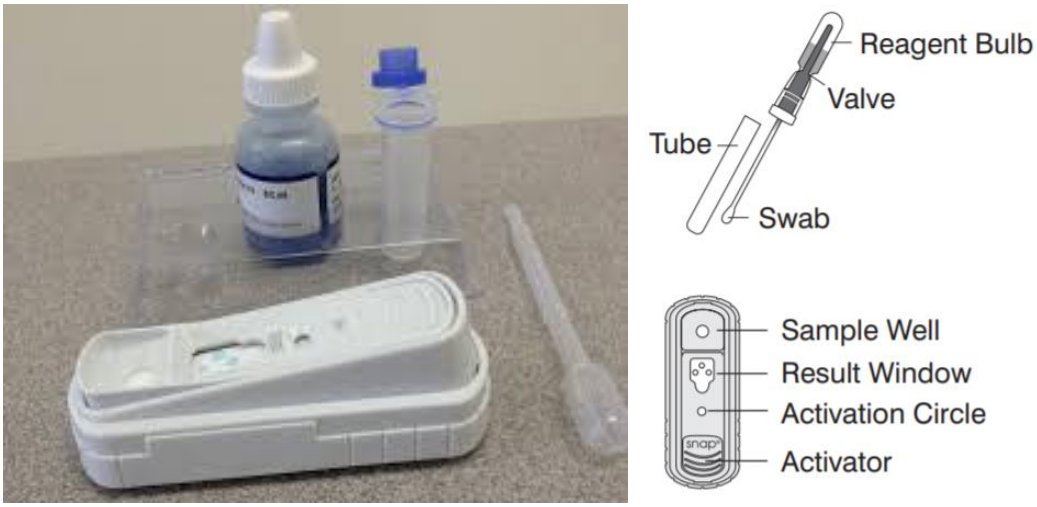


FIGURE 9. On the left, the components of SNAP test kit for whole blood, serum, plasma and milk samples. The typical content of snap test kit intended for faecal samples is presented on the right.

Appendix 2 of this document gives a short description of the five easy steps required to use a SNAP test.

IDEXX proposes two types of SNAP tests to their customers, semi-quantitative and qualitative SNAP tests. The qualitative SNAP tests, such as the SNAP 4DX plus pictured below (Figure 10), relies on the development of coloured spots to determine the presence of specific antibodies and antigens in the sample. Any colour development, even faint, in the sample spot indicates a positive result. Some SNAP tests may have multiple sample spots, each spot being indicative of the presence of a specific antibody or antigen.

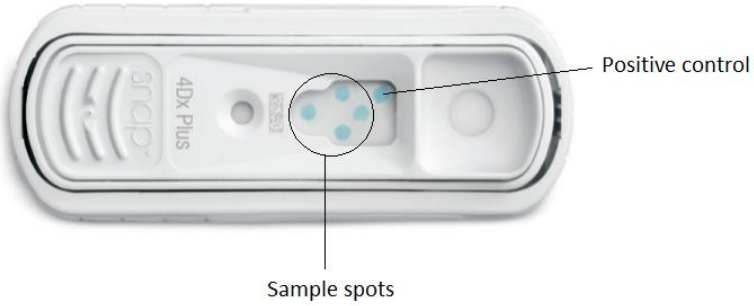


FIGURE 10. The SNAP 4DX plus is an example of a qualitative SNAP test that is used to test the presence of four different antigens/antibodies in canine whole blood, serum or plasma sample.

Semi-quantitative SNAP tests are used to determine the level of a marker substance, such as pancreas-specific lipase, NT-proB-type Natriuretic Peptide or an antibiotic, in the sample (Figure 11). They rely on the comparative intensity between the reference spot (left spot on the device) and the spot arising from the sample (right spot on the device). More precisely, the level of the marker substance is considered normal if the sample spot is non-visible or lighter than the reference spot. In contrast, the marker substance's level in

the sample is abnormal if the sample spot is the same color or darker than the reference spot.

FIGURE 11. Semi-quantitative SNAP tests are *in-vitro* assays used to determine semi-quantitatively the level of a marker substance in the sample by comparing the intensity of two spots.

All the SNAP tests used by DUs in the UK are produced in IDEXX's manufacturing plant of Westbrook, USA. They are designed for the companion animal veterinary and livestock market sectors.

4.2.6. **Description of the technical requirements that must be achieved by the products(s) made with the substance**

The accuracy of the results given by a diagnostic test is paramount as demonstrated by the survey conducted by IDEXX (please see Chapter 3). Sensitivity and specificity are characteristics that are typically used to measure the accuracy of a diagnostic tests results.

The sensitivity of a diagnostic test corresponds to the likelihood that the test will correctly diagnose a positive animal as positive. In other words, a diagnostic test with high sensitivity will detect even small concentrations of target analyte in the sample. On the other hand, a test with low sensitivity will falsely diagnose an infected animal as healthy, in which case the animal will not receive the treatment it needs, and the disease may spread to other animals.

The specificity of a diagnostic test corresponds to the test's ability to correctly diagnose a negative animal as free from a given disease. A test with low specificity may diagnose a healthy animal as infectious, in which case the animal may be unnecessarily given a treatment or even euthanized.

Broadly, the applicant must meet certain regulatory, product licensing, national animal health competent authority requirements or commercial tender procedures, which may further define or narrow the product requirements. IDEXX manufactures 'universal' products sold worldwide and compliance with product marketing requirements will extend to third party countries where the tests are licensed: among others United States, China, Brazil, India and Japan. In other words, all kits reformulated due to the substitution of 4-tert-OPnEO and 4-NPnEO with an alternative detergent will need to comply with the regulatory requirements of all the countries where they are marketed as IDEXX's products are 'universal'.

Given the inherent level of performance variability at the batch level, due to the nature of biological materials, and at the downstream user laboratory level, where conditions can vary significantly, internal performance specifications for product release must be set significantly tighter than official requirements to pass the performance criteria worldwide. Major formulation changes impacting the test's key biological active ingredients are therefore even more challenging.

Conducting validation work and presenting it to agencies for evaluation and approval is required for the assay's initial approval and every time the assay is modified thereafter. Regulatory submissions must be repeated in each country where a product is subject to a marketing authorization. The detailed region requirements are presented below.

Animal Health Agency requirements (EU Only):

As *in vitro* diagnostics are heavily regulated, there are a series of requirements that the assays must fulfil in order to be marketed. Firstly, veterinary IVDs in the EU need to be certified by the relevant Animal Health agencies.

The OIE (Office International des Epizooties / World Organisation for Animal Health) has developed good practices for the development of immunological assays. These guidelines are setting standards for Animal Health agencies with a mandate to evaluate and approve veterinary IVDs in the EU. In addition, the OIE has implemented since 2012 a certification procedure that include specific assay validation principles. In the procedure, four stages of validation have been defined:

- Stage 1 validation – Analytical characteristics
- Stage 2 validation – Diagnostic characteristics
- Stage 3 validation – Reproducibility
- Stage 4 validation – Applications

During the validation procedure, the IVD kits' "fitness for purpose" is assessed. The concept of "fitness for purpose" indicates the purpose of the test and it is an important criterion of the validation procedure. The purpose of the test can, for instance, be one of the following.

1. To demonstrate population 'freedom' from infection (prevalence apparently zero)
    a) 'free' with and/or without vaccination,
    b) historical 'freedom',
    c) re-establishment of 'freedom' following outbreaks;
2. To demonstrate freedom from infection or agent in individual animals or products for trade purposes;
3. To demonstrate efficiency of eradication policies;
4. To confirm diagnosis of clinical cases;
5. To estimate prevalence of infection to facilitate risk analysis (surveys, classification of herd health status, implementation of disease control measures);
6. To determine immune status in individual animals or populations (post-vaccination).

Fitness for any of the above purposes is demonstrated by assessing and verifying the following test criteria/performance parameters:

- Assay cut-off (interpretation) determination
- Analytical sensitivity (detectability, inclusivity)
- Analytical specificity (exclusivity, cross-reactivity)
- Diagnostic Sensitivity
- Diagnostic Specificity
- Repeatability
- Reproducibility
- Robustness
- Stability

Further information on OIE certification procedure can be found on the OIE Website.

Specific performance expectation values are defined for these criteria by several Animal Health Agencies (ANSES in France, SCIENSANO in Belgium, etc...) and the European Commission (Table 5).

TABLE 5. European National surveillance/eradication program examples performance requirements for several of IDEXX's products. The TSE requirements are relevant for the HerdChek BSE-scrapie Ag kit.

Tests Minimum Performance requirements – European National surveillance/eradication program examples							
	Belgium	**Belgium**	**Belgium**	**France**	**France**	**EU**	**EU**
CRITERIA	**IBR gE Tank milk ELISA**	**IBR Ab ELISA**	**Bluetongue Ab ELISA**	**BVDV AB ELISA**	**IBR Ab ELISA**	**TSE**	**Brucellosis**
Diagnostic Sens.	≥95 %	≥95 %	≥99 %	Ind. Sera ≥99.5 %, pool sera =100 %, tank milk = 100 %	see Detectability	≥ 98 % (200 samples)	-
Diagnostic Spec.	≥95 %	≥95 %	≥99 %	Ind. Sera ≥99. 3%, pool sera ≥97 %, tank milk ≥ 99 %	Ind. Sera ≥99.3 %, pool sera ≥97 %, tank milk ≥ 99 %	≥ 99.95 % (10.000 samples)	-
Repeatability	≤10 %	≤10 %	≤10 %	≤10 %	≤12 %	Based on duplicate testing of 200 reference samples	-
Reproducibility (intra-lab)	≤15 %	≤15 %	≤15 %	≤15 %	≤15 %		-
Cut-off determination	RoC analysis	RoC analysis	RoC analysis	RoC analysis	RoC analysis	RoC analysis	-
Analytical sens. (detectability)	Belgium ref. weak pos. material must be found positive		CIRAD ref. sample 1/16	ANSES NED ref. sample	EU1, EU2, Ref46, LFI Ref. samples	Using Scrapie a-typical TSE strains	COMMISSION DECISION of 10 December 2008 amending Annex C to Council Directive 64/432/EEC and Decision 2004/226/EC as regards diagnostic tests for bovine brucellosis, Annex C, 2.2
Analytical specificity	BHV-2	BHV-2	EHDV, FMD, BHV-1, BVDV, BHV-4	FCO, BHV-1, BHV-4, Schmallenberg	-	-	-

Robustness	Demonstrate consistency of results using extreme test parameter tolerance values (time, temperature…)					-	-
Stability	Real time stability over product shelf-life					-	-
Other	-	Target: BHV-1	Target: Bluetongue Virus Serotype 8 (BLT-8)	-	Target: BHV-1	Target: detection of the abnormal conformer of the prion protein (PrPSc) in postmortem brain (obex preferred)	[OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018, Section 3.1.4] Brucella abortus strain 99 (Weybridge) (S99)3 or B. abortus strain 1119-3 (USDA) (S1119-3)4 should be used

The list of diseases in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals containing standardizing provisions applying to IDEXX product is extensive:

- Aujeszky (PRV)
- Avian Infectious Bronchitis
- Avian Influenza
- Bluetongue
- BLV
- Border disease
- Bovine tuberculosis
- Brucella ovis
- Brucellosis
- BSE
- BVDV
- CAEV/MVV
- Classical swine fever
- Contagious equine metritis
- Equine Infectious anaemia
- IBR
- Infectious Bursal disease
- Newcastle Disease
- Paratuberculosis
- PRRS
- Salmonella
- Scrapie
- Swine influenza

For the listed diseases, the OIE may have specific requirements the kit manufacturers have to follow. These requirements can be manufacturing or test protocol prescriptions as well as minimum expected performance for the kit. This limits the Applicant in term of possible adjustments they can make during reformulation. Some of the OIE requirements are also listed under EU Animal Health decisions regarding diagnostics characteristics and performance, for instance, COMMISSION DECISION of 15 December 2009 amending Annex D to Council Directive 64/432/EEC as regards diagnostic tests for enzootic bovine leucosis and COMMISSION DECISION of 10 December 2008 amending Annex C to Council Directive 64/432/EEC and Decision 2004/226/EC as regards diagnostic tests for bovine brucellosis to name a few.

The validation of an IVD kit is a lengthy process. Regulatory approval timelines typically range:

- Initial evaluation: from 3 months to 24 months per kit
- Product change: from 2 months to 12 months per kit (if stability is not impacted, otherwise the timeline will extend until real time stability data for 3 batches is completed, which typically lasts 12-24 months (corresponds to product shelf life)).

On top of the assay's initial assessment, national Animal Health agencies may also require batch to batch evaluation prior to placing a product on the market. This is typically the case for national surveillance / eradication program diseases.

IDEXX Montpellier holds approximately 150 marketing authorizations for ELISA plate tests containing 4-tert-OPnEO. Replacing the 4-tert-OPnEO with an alternative will require all these marketing authorizations to be renewed. The total number of marketing authorizations held by IDEXX USA and IDEXX Switzerland for the products covered by is even higher, with marketing authorizations in the hundreds.

The European TSE program devised by the EU Commission Health & Consumer protection Directorate-General and the European Food Safety Agency (EFSA) include very specific provisions on how to submit any changes to approved TSE rapid diagnostics, such as the IDEXX HerdChek BSE-scrapie Ag kit: only minor changes would be examined by the EU-TSE Reference Laboratory while changes impacting the kit antibody capture system or sample preparation would require full assessment by EFSA, including testing large quantity of positive samples which have become very difficult to gather, notwithstanding the complex biosafety conditions necessary to handle such material. In the case of the HerdChek BSE-scrapie Ag kit, IDEXX assumes that substituting 4-tert-OPnEO from the sample diluents, controls and conjugate solutions would impact the capture and detection system, in which case a full assessment will be required by EFSA.

Customer requirements:

In addition to the requirements set by Animal Health agencies, IDEXX also has to fulfil requirement set by their customers. The customers that are ISO 17025 certified require IDEXX to provide them with up-to-date validation data detailing product performance such that they can adopt the same validation methods in their laboratory. Major product changes that have a performance impact, such as a change in detergent, would oblige IDEXX to renew their validation reports while IDEXX customers would have to re-evaluate their methods.

For example, guidance documents (LAB-GTA-27) issued by French COFRAC on ISO 17025 implementation requires the same level of validation by the manufacturer for all accredited methods than diagnostic tests controlled by the national animal health agency ANSES.

Other requirements:

A significant part of IDEXX production animal testing in Europe (around 45 %) is driven by governmental eradication or surveillance programs which contribute to disease prevalence monitoring (including emerging diseases), control of disease outbreaks, animal health and movements, food safety and fighting against zoonoses which may be transmitted to humans.

A number of these programs are decided and funded at the EU level on a yearly basis. For instance, the 2019 Approved veterinary programmes[6] include:

- African swine fever*
- Avian influenza*
- Bluetongue*

[6] See https://ec.europa.eu/food/funding/animal-health/national-veterinary-programmes_en

- Bovine brucellosis*
- Bovine tuberculosis*
- Classical swine fever*
- Lumpy skin disease
- Rabies
- Salmonella*
- Sheep and goat brucellosis*
- TSE*

(*) IDEXX diagnostics contributes to most of these programmes.

Additional programmes are decided and funded at EU Member states level by ministry of agriculture and/or producer organizations for which IDEXX is also a major contributor:

- IBR
- BVDV
- Paratuberculosis

The selection and adoption of appropriate diagnostics is carried out through tender procedures for most of these programs: multiple factors such as diagnostics performance, registration status, manufacturer quality assurance system (including environmental, ethical engagement), production capacity, customer service/support, cost… are considered during the selection process. Tenders are frequently concluded for 2/3-year periods. The inability of a diagnostics manufacturer to fulfil its tender commitments (temporary back-order situation or product no longer available) will result in fines.

IDEXX is a trusted partner for many animal health institutions across Europe and contributes to the overall EU animal health programmes and policies: the reduction of diagnostics availability and capacity to update existing and develop new tests to support these programmes would have negative impacts.

Private testing, which is focusing mostly on diseases of economical relevance, plays a key role in the production efficacy of the food sector: IDEXX offers not only diagnostic solutions to most of the animal production chain but also provides services to help animal producers manage more efficiently vaccination, reduce the use of antibiotics, re-introduction of animals in herds after treatments, optimize reproduction cycles, etc… The reduction of diagnostics availability in this area would also limit the information tools used by producers to control diseases and improve productivity.

<u>USDA Licensed for Infectious Disease Assays (North America):</u>

For the validation of all infectious CAG and LPD products marketed in the North America, IDEXX must follow specific requirements defined by the USDA. This affects most SNAP products and some ELISA plate products covered by this application. The requirements are described in the Veterinary Services Memorandum No 800.73, which states that assay validation studies should be conducted to demonstrate the kit's diagnostic accuracy, analytical sensitivity, analytical specificity and ruggedness. Diagnostic sensitivity and specificity should be assessed separately for each proposed sample type (e.g. blood, faeces, etc…) and host species. In addition, an inter-laboratory comparison study should be conducted.

- Diagnostic sensitivity and specificity are determined by running a defined sample set on both the experimental kit and a reference kit.
- Analytical sensitivity or limit of detection are determined by conducting a study to establish the relationship between analyte concentration and the percentage of samples classified as positive for a given concentration. If the concentration of the analyte in the sample cannot be determined, a dilution can be used in place of concentration.
- Analytical specificity demonstrates a lack of cross-reactivity of the kit by testing for analytes that are similar to, but different from, the intended analyte.
- Ruggedness is evaluated by observing the effect of changes in incubation time, incubation temperature, or other critical test conditions on the final test results.
- Confirmation of dating occurs by conducting a real-time stability study.

For non-USDA licensed kits, the requirements are defined by medically determined sensitivity and specificity to develop an assay that provides a relevant result to aid in diagnosis. Table 6 represents current product requirements that would need to be maintained during any reformulation efforts, and source of requirements.

TABLE 6. Current product requirement for non-USDA licensed kits

Product	SNAP Lepto	SNAP Giardia	Parvo	Combo Plus	Leishmania	cPL	fPL	Fe proBNP
Requirement source (licensure, notification)	USDA	USDA	USDA	DEREA (Export only)	DEREA (Export only)	Medically defined	Medically defined	Medically defined
Diagnostic Sens.	73.3% vs MAT*	94.9% vs Immunofluorescence microscopy 96.2% vs ELISA plate	97.1% vs USDA reference test	92.3% FeLV 100% FIV	96.3% vs IFA*	95.8% vs Spec cPL*	87% vs clinical assessment	99.5% agreement when <100 pmol/L
Diagnostic Spec.	82.1% vs MAT*	99.3% vs Immunofluorescence microscopy 100% vs ELISA plate	98.7% vs USDA reference test	97.3% FeLV 99.6% FIV	99.2% vs IFA*	95.6% vs Spec cPL*	100% vs clinical assessment	95% agreement when >270 pmol/L
Analytical sensitivity/ specificity	No cross-reactivity to Lyme Ab	No cross-reactivity to other parasitic infections	No cross-reactivity with parvovirus vaccinations	No cross-reactivity between analytes on device		96% overall agreement to Spec cPL	>95% overall agreement to Spec fPL	>90% overall agreement to Fe Cardiopet proBNP Test
Stability	12 months at 2-8°C	12 months at 4°C	9 months at 2-25°C	12 months at 2-8°C	9 months at 2-7°C	9 months at 2-7°C	12 months at 2-8°C	12 months at 2-8°C
Cut-off	Must meet specifications without changing current diagnostic cut-off values							
Repeatability	Typical repeatability is determined through replicate testing of 5 samples run 10 times. (USDA requires a plan to demonstrate reproducibility. Product specific plans must be approved prior to testing.)							
Reproducibility	Typical Reproducibility is determined through intra- and inter-laboratory comparison. At least 20 panel member samples are run by 3 operators internally and sent to 3 participating labs for evaluation. (USDA requires a plan to demonstrate							

	repeatability. Product specific plans must be approved prior to testing.)

*Reference assays are considered Gold Standard for comparison. Reformulated product performance will need to match existing product performance.

Other Region Requirements:

See Appendix 5 for a table of countries/regions that require licenses or other permits.

4.2.7. **Annual tonnage**

The volume of Triton X-100 or IGEPAL CA-630 used by the Applicant in the products concerned by Use 3 is expected to be 0.002 tons (<1 t/y) per year in 2021. For Triton X-405/IGEPAL CA-720 and Nonidet P-40 Substitute, the volumes in 2021 are expected to be 0.0053 (<1 t/y) and 0.0010 tons/year (<1 t/y), respectively. Total annual tonnage in products supplied to the UK is expected to be 0.0065 tons of Triton substances and Nonidet in 2021. Tonnage is projected to increase with 6 % annual growth rate. Annual tonnage and the forecast of the Triton substances and Nonidet supplied to the EEA are outlined below.

TABLE 7. Annual tonnage of Triton in products supplied to the UK (6 % annual growth rate)

Year	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2023-34
Triton X-100 / IGEPAL CA-630	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0004	0.0004	0.0034
Triton X-405 / IGEPAL CA-720	0.0053	0.0056	0.0060	0.0063	0.0067	0.0071	0.0075	0.0080	0.0084	0.0089	0.0095	0.0101	0.0107	0.0113	0.1004
Nonidet P-40	0.0010	0.0011	0.0011	0.0012	0.0013	0.0013	0.0014	0.0015	0.0016	0.0017	0.0018	0.0019	0.0020	0.0021	0.0188
Total	0.0065	0.0069	0.0073	0.0077	0.0082	0.0087	0.0092	0.0097	0.0103	0.0109	0.0116	0.0123	0.0130	0.0138	0.1226

For the review period applied for (2021-32) total tonnage of Triton substances and Nonidet supplied to the EEA is estimated to be 0.1226 tons.

It is to be noted that the tonnage forecast presented above is the worst-case scenario. Tonnage is not foreseen to top these expectations.

4.3. **Remaining risk of the "applied for use" scenario**

4.3.1. **Characterisation of Use**

Please refer to Chapter 9.0.1 in the CSR of this application where characterisation of use is given in detail.

4.3.1.1. Environmental Risk

As outlined in the CSR of this application, users collect the waste streams containing traces of the detergents and dispose of them according to national and local regulations. The insert supplied with the kits informs users to follow the waste disposal instructions given in the Safety Data Sheets (SDS). The SDS provided with the kits instructs users to dispose of residual waste as per local regulations.

4.3.1.2. Exposure and risks for the environment and man via the environment

There are no emissions to the environment as downstream users are instructed to collect all waste streams for adequate disposal. As such no exposure or risk calculations are required. Please refer to CSR of this application for more detailed information.

4.3.1.3. Release assessment

In Table 7 the trend of Triton substances usage is presented. For the review period applied for the total downstream use of the substances is 0.1226 tons. However, as explained earlier 0 % of that is released to the environment as downstream users are instructed to collect all waste streams for adequate disposal.

4.4. Human health and environmental impacts of the applied for use scenario

4.4.1. Human health impacts

Human health impacts are not relevant for the proposed identification of the substance as an SVHC in accordance with article 57 (f). In summary, 4-tert-octylphenol does not represent a substance with ED properties of strong potency for the mammalian system. Therefore this endpoint is considered not to warrant further consideration.

4.4.2. Impact on the environment

As explained 0 % of the substances are released in the environment and there is no impact on the environment.

5. SELECTION OF THE "NON-USE" SCENARIO

5.1. Efforts made to identify alternatives

5.1.1. Research and development

As *in vitro* diagnostic kits contain ready to use solutions and devices, the DUs rely on the Applicant to provide IVD kits that do not contain 4-tert-OPnEO and 4-NPnEO. The kits are manufactured to work as a whole thus, the DUs cannot simply buy an alternative solution (e.g. sample diluent or control) that do not contain Annex XIV substances as they will not be compatible with the kits. In SNAP tests, it is impossible for the DUs to change the wash solution as it is encased in the device itself.

In case IDEXX's SNAP and ELISA plate tests were not available, the Applicant's DUs would use an alternative in vitro diagnostic test or outsource the tests to a reference laboratory, as demonstrated by IDEXX's survey. However, 95 % of the DUs surveyed expect this change to have an economic impact on their business. This is particularly true for certain livestock, poultry and dairy laboratories that are accredited through agencies that require their own revalidation of kit changes. It could take up to a year for the DUs to go through the required revalidation, time during which they cannot use the affected kits.

In addition to economic impacts, 74 % of IDEXX's DUs consider that without IDEXX assays they would not be able to supply results to pet and livestock owners as efficiently and accurately. As seen in the DU survey, accuracy is a crucial factor when evaluating SNAP tests or ELISA plate tests. This is how IDEXX differentiate themselves from their competitors. IDEXX *in vitro* diagnostics have typically higher sensitivity and specificity in comparison to available alternatives. This has been demonstrated by IDEXX reference laboratories and third parties through a series of tests aimed at assessing the performance of alternative rapid tests in comparison to several of IDEXX SNAP tests. The results of these studies are presented in Appendix 4 and have been published in a series of white papers available on the Applicant's website and/or in scientific journals. Similar comparative studies have not been published on IDEXX's ELISA plate tests.

In addition, a number of IDEXX kits have ready to use conjugates which makes them easier and faster to use than alternate in vitro diagnostic kits where the conjugate needs to be diluted separately.

A number of IDEXX in vitro diagnostics also have unique properties and unique applications. For instance, the IDEXX APP-ApxIV Ab is a unique DIVA test (Differentiating Infected from Vaccinated Animals) used on swine nucleus herds that provide genetics to the European swine industry. With this test, vaccinated animals can be differentiated from infected animals as the test can differentiate the antibody originating from the naturally occurring disease from the antibody created by vaccination. Without the DIVA test, it would be impossible to differentiate an infected animal from a vaccinated animal, which is crucial for this disease as many herds infected with APP do not present any clinical evidence of the disease. This allows this highly contagious disease to spread between herds and cause important economic losses to swine owners. In addition to limiting the spread of the disease, IDEXX APP-ApxIV Ab allows for a quick diagnostic and treatment of the disease as APP progresses rapidly and is associated with high morbidity and mortality.

In the absence of treatment, the disease can progress very rapidly and death can occur within a few hours.

Another example of a unique test is the IDEXX BSE-Scrapie test, which is a state-of-the-art test approved by the EU (Regulation (EC) No 956/2010 amending Annex X to Regulation (EC) No 999/2001) used for the detection of BSE and Scrapie-related PrP^{Sc} in cattle and small ruminants. The test uses proprietary technology that allows detection of abnormal prions without the use of Proteinase K, unlike alternative tests. Replacing IDEXX BSE-Scrapie test with an alternative would require the DUs to purchase additional equipment such as Proteinase K robots and centrifuges. Such equipment requires space that may be difficult to find in a laboratory setting. In addition, the use of IDEXX BSE-Scrapie test is associated with less waste and fewer disposables in comparison to alternative tests. This translates to reduced operational costs for the DUs.

In conclusion, IDEXX's DUs rely on the Applicant to provide in vitro diagnostics free from 4-tert-OPnEO and 4-NPnEO. In case IDEXX SNAP tests and ELISA plate tests were not available, the DUs would either opt for an alternative in vitro diagnostic kit or outsource the tests to a reference laboratory. However, it is not known whether the alternatives contain 4-tert-OPnEO and 4-NPnEO. Changing to an alternative in vitro diagnostic may have an important economic impact on the DUs and finding an appropriate and equivalent replacement for IDEXX's products is not an easy task. As discussed above, IDEXX products are more accurate and easier to use in comparison to other IVD products on the market. Some tests are unique, and they do not have alternatives or the alternatives have less accuracy or other constraints, such as requiring equipment. As expressed by the DUs in the survey, the absence of IDEXX in vitro diagnostic kits will have a negative impact on animal care and on the DUs ability to provide accurate results in an efficient way. Therefore, IDEXX has developed a substitution plan in order to ensure a non-interrupted supply of their assays for their DUs, please refer to the separate report submitted with this application for more detail.

In an effort to phase out 4-tert-OPnEO and 4-NPnEO from their SNAP tests and ELISA plate tests, the Applicant has already started testing alternatives to these substances. The tests are still ongoing, as the first series of tests did not yield positive results. The results are reported and discussed in the next sections.

Ideally, the Applicant is looking to find an alternative detergent that is applicable for all SNAP and ELISA plate tests. However, they do acknowledge that this might not be possible. It is most likely that one solution will work for similar products. If a one-to-one substitution will not be achievable, IDEXX will have to evaluate the feasibility of the alternative on a case by case basis and reformulate the entire product line.

5.1.2. **Data searches**

In an aim to find alternatives suitable to replace the substances of very high concern (SVHC) present in the different solutions covered by this Application, IDEXX has performed a literature search and questioned Merck regarding potential alternative detergents.

5.2. **Identification of known alternatives**

IDEXX has set certain requirements that the alternative should fulfil in order to be selected for further testing. The alternative substance should not:

- be classified as CMR or SVHC.
- present a greater exposure/safety risk as current substances.
- represent a higher compliance risk.
- represent a higher supply chain risk.

In addition, the alternative substance should provide the same technical functionalities to prevent non-specific binding:

- Ensure optimum protein conformation and stability.
- Prevent non-specific binding of samples and controls.
- Be as effective to remove non-target material on the sample spots/wells of the tests.

The effectiveness of alternative substances for each of the functions above may not be easily predicted and is highly dependent on the specific type of purified proteins used on each diagnostic test.

Many ingredients of animal origin used in diagnostics such as sera, foetal bovine serum, milk, albumin etc., can be considered as UVCB substances and IDEXX's experience with past formulation changes shows that the delicate balance between competing affinity processes in antibody or antigen capture systems is easily upset and that unwanted side effects are frequently observed (e.g., stability issues, precipitation, viscosity problems, colour variations, field issues related to the variety of laboratory equipment and consumables in laboratories, field sample treatments etc.). Further optimization or changes may be necessary to counter the undesirable effects.

The alternative should also offer similar operational benefits:

- Substance stability and robustness under existing standard laboratory conditions and with existing formulations.
- No incompatibilities with other reagent formulations which are part of the test kits.
- Same formulation is suitable across a broad variety of tests in technical manufacturing and in sample diluents and controls (generic reagents shared by multiple tests).
- Be effective at low concentration.

<u>Three substances, one alternative:</u>

Due to historical reasons, several detergents i.e. Triton X-100/IGEPAL CA-630, Triton X-405/IGEPAL CA720 and Nonidet are used to achieve the same function. These detergents, which are very similar in structure, all prevent NSB in both ELISA plate tests and SNAP tests. As they have the same function and they are used in a similar manner, IDEXX is seeking for a single alternative to replace all three substances in their products.

IDEXX started their search for an alternative by identifying any possible alternatives to Triton X-100/IGEPAL CA-630 and Triton X-405/IGEPAL CA720 based on chemical supplier data. In total, five alternatives were identified for Triton X-100 and three were found for Triton X-405. The physicochemical properties of the identified alternatives were then compared with their Triton counterpart (Table 8). The alternative with the closest physicochemical properties was selected by IDEXX to undergo feasibility testing. In the

case of Triton X-100, the most comparable alternative was Tergitol 15-S-9 whereas it was Tergitol 15-S-40 (70 %) for Triton X-405.

TABLE 8. The physicochemical properties of Triton X-100 and Triton X-405 in comparison to their respective alternatives.[7]

Product name	Moles of EO	Cloud point (1%) [°C]	HLB	Pour Point [°C]	Readily Biodegradable (OECD 301F)
Triton X-100	**9.5**	**66**	**13.4**	**1**	**No**
Ecosurf EH-9	Proprietary	61	12.5	12	Yes
Ecosurf EH-9 (90 %)	Proprietary	61	12.5	-5	Yes
Ecosurf SA-9	Proprietary	57	11.1	4	Yes
Tergitol 15-S-9	9	60	13.3	9	Yes
Tergitol TMN-100X (90 %)	9	65	14	-6	No
Triton X-405 (70 %)	**35**	**> 100**	**17.6**	**-6**	**No**
Tergitol 15-S-30	31	>100	17.4	39	Yes
Tergitol 15-S-40	41	>100	18	43	Yes
Tergitol 15-S-40 (70 %)	41	>100	18	5	Yes

In the next chapters, the results of the tests carried out by IDEXX on Tergitol 15-S-9 and Tergitol 15-S-40 on SNAP tests are discussed in further detail. Comparable tests have not yet been carried out on ELISA plate tests however, the same alternative will be considered for ELISA plates and SNAP tests. This is due to the fact that the function of the Annex XIV substance is the same in both type of products. Similarly, the same alternative used to substitute Triton substances is expected to be applicable to substitute Nonidet as well should a feasible alternative be found.

In addition to the two aforementioned alternatives, IDEXX also tested a more diluted version of their SNAP wash formulation where the concentration of Triton X-100 was reduced to 0.1 %. The aim of this test was to demonstrate whether a SNAP wash formulation of <0.1 % could be adopted in their SNAP tests without compromising the performance of the test. In this case, the results are considered applicable only to SNAP tests and not to ELISA plate tests.

5.3. **Assessment of shortlisted alternatives**

5.3.1. **Tergitol 15-S-40**

5.3.1.1. Substance ID and properties of Tergitol 15-S-40

[7] Merck, 2018

Tergitol 15-S-40 is a secondary alcohol ethoxylate manufactured by DOW. In line with the amphiphilic nature characteristic to surfactants, Tergitol 15-S-40 is composed of a hydrophobic secondary alcohol moiety and a hydrophilic polyoxyethylene chain. Tergitol 15-S-40 has a relatively long polyoxyethylene chain with an average ethoxylation degree of 41 moles of EO. Unlike Tergitol 15-S-9, which is only supplied as a neat solution, Tergitol 15-S-40 is also marketed as a 70 % aqueous solution.

Further details on the surfactant's identity and classification are given in Table 9 below.

TABLE 9. The substance identity and classification information of Tergitol 15-S-40. The information originates from the manufacturer, DOW.

	Tergitol 15-S-40
Chemical name	Alcohols, C11-15-secondary, ethoxylated
EC number	614-295-4
CAS number	68131-40-8
Molecular formula	$C_{12\text{-}14}H_{25\text{-}29}O[CH_2CH_2O]_xH$, x=41
Hazard information	Not classified
Surfactant type	Non-ionic

5.3.1.2. Technical feasibility of Tergitol 15-S-40

The technical feasibility of Tergitol 15-S-40 was tested in practice by IDEXX on three different SNAP test products. The aim of the tests was to determine the performance of a Tergitol 15-S-40 based wash solution.

The performance of the Tergitol 15-S-40 wash solution was found to be lower than the original wash solution in all of the three products tested. This was particularly true for one of the products, where the alternative wash solution gave unacceptable results (Figure 12). In this test, an intense blue background was observed, masking the colour development of the analyte spot. This rendered the SNAP test unusable as the analyte spot cannot be seen and its colour intensity cannot be determined.

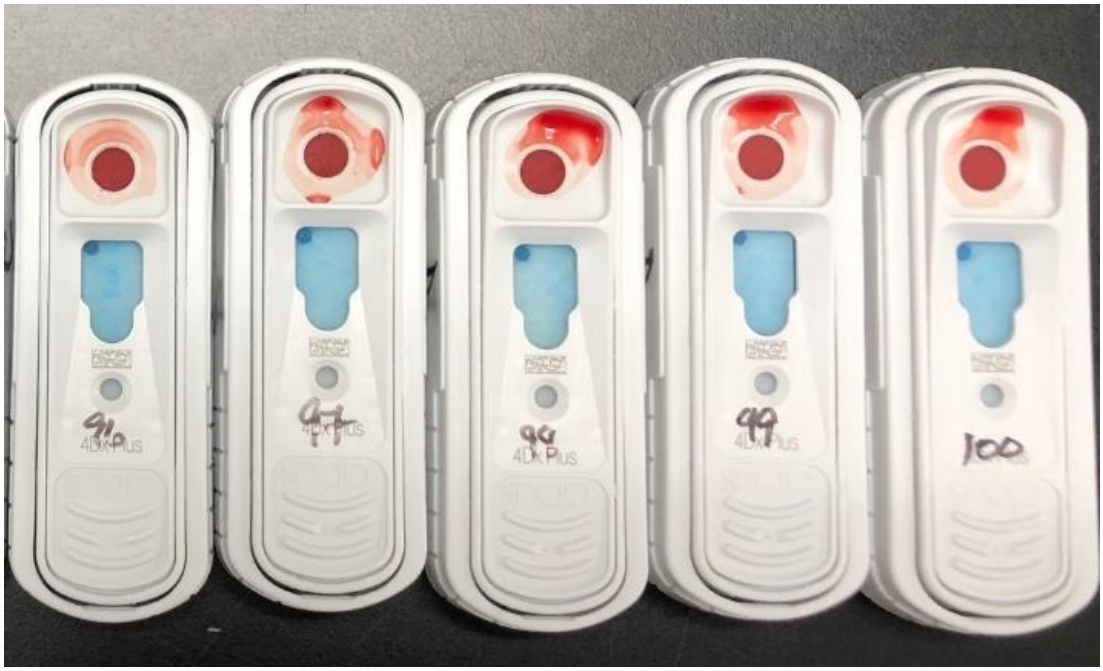


FIGURE 12. Five replicates of the same product. Canine whole blood was used in all five SNAP devices. The spot in the top left corner is the positive control spot. The analyte spots are masked by the dark colour of the background and thus, they cannot be seen.

The intense blue background colour observed means that Tergitol 15-S-40 failed to prevent NSB in the conditions the test was carried out. More precisely, the conjugates bound non-specifically to the whole SNAP's matrix instead of binding specifically to the target analyte immobilized on the matrix. This resulted in the entire background turning blue instead of the specific analyte spots.

Based on the test results, it can be concluded that Tergitol 15-S-40 is not a feasible alternative under the present test conditions. However, the test conditions warrant further investigation, for example, different concentrations of Tergitol 15-S-40 may alleviate the high background colour.

5.3.1.3. Economic feasibility and economic impacts of Tergitol 15-S-40

Overall, surfactants are relatively inexpensive substances, thus, the substitution of 4-tert-OPnEO or 4-NPnEO with another surfactant will not change the cost of raw materials significantly. In addition, as the volume of surfactant in the SNAP wash solutions is low, the associated costs of changing to an alternative reagent will be minor for the Applicant.

Assuming technical feasibility, as Tergitol 15-S-40 can be directly introduced in the process in place of the 4-tert-OPnEOs or 4-NPnEOs, the change in process cost will be negligible. As the SNAP tests will function in the same way with the alternative surfactant, the substitution of 4-tert-OPnEO and 4-NPnEO will not generate additional need for maintenance or service of the SNAP PRO* analysers, in case one is used by the DU.

Replacing 4-tert-OPnEO and 4-NPnEO with an environmentally friendly surfactant will, in theory, reduce the waste management costs by removing the need for hazardous waste collection. However, some regions may require used IVD assays to be discarded as biohazardous waste due to the fact that they are used to tests biological samples, such as

blood or faecal samples, even though they would not include 4-tert-OPnEO or 4-NPnEO. As a result, the waste management cost savings will be dependent on local regulations for each region.

For the Applicant, by far the largest economic impact from substituting 4-tert-OPnEO and 4-NPnEO will arise from the reformulation costs IDEXX will face. Reformulation costs cover material and labour costs for research and development, as well as validation costs, trial and launch costs and worldwide regulatory approval costs including the associated labour costs. It is estimated that the reformulation costs will amount to approximately [40-70 M USD] #B M USD ([35-62 M EUR] #B M EUR) in total for all products covered by this application.

Although, the manufacturing plants of Westbrook, Montpellier and Bern are out of scope of this application, any cost incurred by the American, French and Swiss branch of IDEXX will have an impact on the global IDEXX group, to which IDEXX's UK entities belong to. It should be noted that the reformulation costs have to be bore by IDEXX alone as these cost cannot be transferred to the DUs.

In conclusion, the substitution of 4-tert-OPnEOs and 4-NPnEOs with Tergitol 15-S-40 will not lead to significant material costs for IDEXX. The most significant cost for IDEXX will arise from the reformulation costs to find and implement a new alternative reagent. The impact of reformulating will, most likely, have no economic impacts on the DUs as the reformulation costs will not be transferred to them. In contrast, as seen in the DU survey, all of the ELISA plate users foresee a negative cost impact of implementing an alternative to IDEXX products. For SNAP users, this number was only 41 %. In the light of these arguments, the reformulation to Tergitol 15-S-40 is considered to be economically feasible.

5.3.1.4. Availability of Tergitol 15-S-40

IDEXX currently does not use Tergitol 15-S-40 in any of their products however, it is readily available for purchase from numerous suppliers. Therefore, it will not be an issue for IDEXX to acquire the alternative surfactant in sufficient quantities.

It can be concluded that Tergitol 15-S-40 is feasible in terms of availability however, its technical feasibility has to be first demonstrated before it can be used in IDEXX's products.

5.3.1.5. Reduction of overall risk due to the transition to Tergitol 15-S-40

Tergitol 15-S-40 has been self-classified by its manufacturer, DOW, as non-hazardous. It is unlikely that the surfactant will ever be classified as a Substance of Very High Concern (SVHC). Therefore, it can be concluded that the transition to Tergitol 15-S-40 will lead to a reduction of environmental and human health risk.

5.3.1.6. Conclusions on Tergitol 15-S-40

Tergitol 15-S-40 was found inadequate under the test conditions used by IDEXX in their initial analysis. The alternative wash solution caused the development of an abnormally intense background colour, which masked the analyte spots thus, making the interpretation of the SNAP test results impossible. Consequently, Tergitol 15-S-40 is not considered technically feasible at the moment however, a considerable amount of work is still needed before a definitive conclusion can be drawn.

Economically, Tergitol 15-S-40 is considered feasible although IDEXX will have to face significant reformulation costs of approximately [40-70 M USD] #B M USD ([35-62 M EUR] #B M EUR) in total for all products covered by this application. These costs cannot be transferred to the DUs.

In terms of availability, Tergitol 15-S-40 is sold by several suppliers therefore, it is expected to be available in sufficient quantities and purities. In addition, there are no identified risks with the use of substance, as it is self-classified as non-hazardous by the manufacturer.

5.3.2. **Tergitol 15-S-9**

5.3.2.1. Substance ID and properties of Tergitol 15-S-9

Similarly to Tergitol 15-S-40, Tergitol 15-S-9 is also a secondary alcohol ethoxylate manufactured by DOW. It also has a hydrophobic secondary alcohol moiety and a hydrophilic polyoxyethylene chain. The only structural difference between Tergitol 15-S-9 and Tergitol 15-S-40 is the ethoxylation degree. Tergitol 15-S-9 has a medium-sized polyoxyethylene chain with an average ethoxylation degree of 9 moles of EO.

Further information on the surfactant's substance identity and classification are given in Table 10 below.

TABLE 10. The substance identity and classification information of Tergitol 15-S-9. The information originate from the SDS of the manufacturer, DOW.

	Tergitol 15-S-9
Chemical name	Alcohols, C11-15-secondary, ethoxylated
EC number	614-295-4
CAS number	68131-40-8
Molecular formula	$C_{12-14}H_{25-29}O[CH_2CH_2O]_xH$, x=9
Hazard information	Acute Tox. 4, H302 Acute Tox. 4, H332 Skin Irrit. 2, H315 Eye Dam. 1, H318
Surfactant type	Non-ionic

5.3.2.2. Technical feasibility of Tergitol 15-S-9

The technical feasibility of Tergitol 15-S-9 was tested in practice by IDEXX on three different SNAP test product. The aim of the tests was to determine the performance of a Tergitol 15-S-9 based wash solution in comparison with the Triton X-100 solution currently in use.

The alternative wash solution did not achieve equivalent performance in any of the three SNAP test products tested. All the SNAP tests containing the Tergitol 15-S-9 wash solution showed varying color development of the reference spot and light spot streaking was observed in some of the tests.

In addition, the intensity of the control spot was lower with the alternate surfactant, which significantly compromises IDEXX's analyzers ability to interpret correctly. In fact, the resulting control spot intensity was so low when using Tergitol 15-S-9, the test would have been interpreted as false negative or failed run. With this kit (SNAP cPL), the optimization of each lot is challenging to balance. Because it is a semi-quantitave assay, any changes with colour intensity causes the window of optimization to shift. The test compares the patient spot intensity to the control spot intensity and if there is misalignment, then the test loses sensitivity or specificity. It is usually difficult to determine which.

The signal variation will particularly impact cut-off samples i.e. samples where the pancreas-specific lipase levels are at the limit of the normal/abnormal diagnostic bins (see Figure 17 for more information on diagnostic bins). IDEXX's product accuracy at the cut-off differentiates the Applicant from their competitors and is therefore critical for their products.

From the preliminary tests performed by IDEXX, it can be concluded that the alternative wash solution prepared with Tergitol-15-S-9 impacts the sensitivity and specificity of the SNAP tests. Furthermore, the products would no longer meet neither regulatory nor customer requirements. More precisely, the test's sensitivity, specificity, repeatability and reproducibility would not fulfil the level of performance set by the "gold standard" method, which is a regulatory requirement, whereas customers require a certain level of performance for the tests that is determined based on reference laboratory methods or by comparing the performance to competitor's product performance.

5.3.2.3. Economic feasibility and economic impacts of Tergitol 15-S-9

The economic feasibility and economic impacts of Tergitol 15-S-9 are expected to be similar to the ones for Tergitol 15-S-40 therefore, please refer to chapter 5.3.1.3 for additional information.

5.3.2.4. Availability of Tergitol 15-S-9

The availability of Tergitol 15-S-9 is expected to be similar to Tergitol 15-S-9 therefore, please refer to chapter 5.3.1.4 for additional information.

5.3.2.5. Reduction of overall risk due to transition to Tergitol 15-S-9

In comparison to the 4-tert-OPnEOs and 4-NPnEOs used by IDEXX, Tergitol 15-S-9 is less hazardous to the environment. The only hazards arising from the substance are human hazards, for which the associated risks can be limited with appropriate RMM and PPE.

In conclusion, Tergitol 15-S-9 is less hazardous than 4-tert-OPnEOs or 4-NPnEOs and consequently, it could potentially be used as an alternative surfactant if demonstrated technically feasible.

5.3.2.6. Conclusions on Tergitol 15-S-9

Based on the initial tests performed by IDEXX, Tergitol 15-S-9 was a better candidate in comparison to Tergitol 15-S-40. However, as issues with spot colour development were observed during the tests, Tergitol 15-S-9 is not considered feasible at the present time. A significant amount of R&D work remains in order to fully determine its applicability as a replacement for 4-tert-OPnEOs and 4-NPnEOs in all of IDEXX's products.

In terms of economic feasibility, Tergitol 15-S-9 is considered feasible despite the fact that IDEXX will have to bear the reformulation costs amounting to an estimated [40-70 M USD] #B M USD ([35-62 M EUR] #B M EUR) for all the products covered by this application. In addition, the alternative is available in sufficient quantities from multiple suppliers and based on the manufacturer's self-classification, it is less hazardous for the environment than 4-tert-OPnEO and 4-NPnEO.

5.3.3. **SNAP wash solutions with a concentration of <0.1 % Triton X-100**

In their preliminary tests, IDEXX explored the possibility of reformulating their Triton X-100 based wash solution to a more dilute version. For reasons of practicality, IDEXX made a 10-fold dilution of their original 1 % Triton X-100 wash solution to yield a 0.1 % Triton X-100 solution. In case good results were achieved with the 0.1 % Triton X-100 solution, IDEXX would have conducted additional testing with a Triton X-100 solution that goes below the 0.1 % concentration threshold.

In the tests, the dilute Triton X-100 wash solutions showed similar performance than IDEXX's original wash solutions however, spot streaking was observed. Spot streaking is unwanted as it compromises the spot intensity determination. This is particularly true in cases where the SNAP test is automatically interpreted with the help of a SNAP PRO* analyser, where reading the results based on colour intensity is critical. In addition, identification of the control or reference spot is used to align the read window of the device in the SNAP PRO* analyser and streaking can cause inaccurate judgement of the control or reference spot within the device, resulting in a misaligned reading.

In conclusion, additional tests are required in order to fully assess the technical feasibility of wash solutions containing <0.1 % of Triton X-100. Presently, spot streaking is an issue that compromises the reliability of the SNAP tests and cannot be overlooked. In terms of economic impacts, more dilute wash solutions will lead to a reduction in reagent costs however, the reformulation costs will still be significant.

5.4. **The most likely non-use scenario**

Depending on the supply chain roles the non-use scenario will differ. This is shown schematically below in Figure 13 for the supply chain operators in the scope of the application and an elaboration of the details of these scenarios provided in the following sections.

As mentioned in Chapter 2, only Windsor Berkshire Commercial Office, Wetherby Reference Laboratory and end-users are in the cope of this application. The IDEXX sites outside of the UK would face profit losses and unemployment in the non-use scenario due to losing one of the main markets. However, no further analysis is done in the analysis since these operators are out of scope.

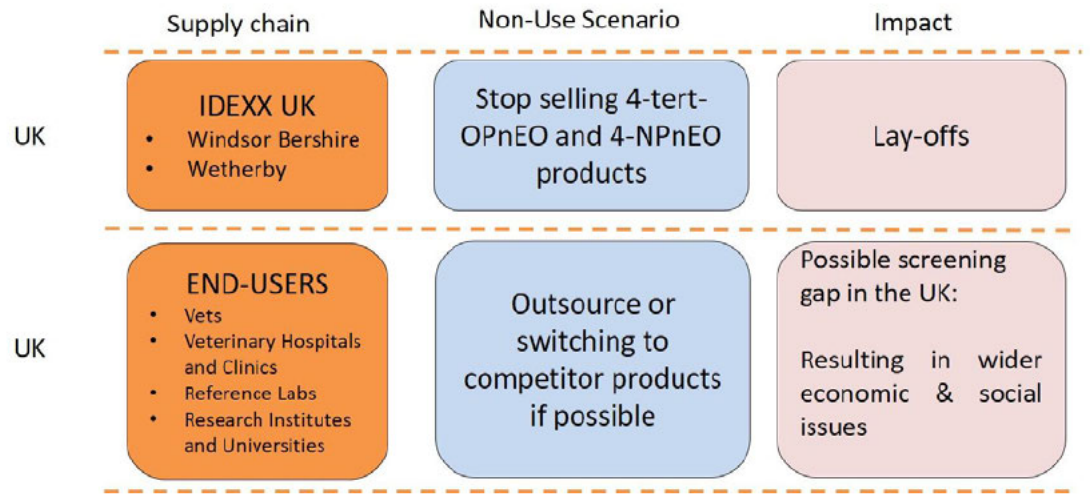


FIGURE 13. Non-use scenario

5.4.4. **IDEXX UK**

In the non-use scenario IDEXX can't sell its production which is related to Triton substances to the UK anymore. This results in profit losses and lay-offs. As mentioned in Chapter 4.1.2 the profit losses are out of the scope and thus excluded from the analysis. To counter the diminished market and decreased demand IDEXX will have to lay-off [55-70] % of its personnel from its commercial office and reference laboratory in the UK.

5.4.5. **End-users**

IDEXX surveyed its downstream users to define the non-use scenario and the related impacts in the UK. When surveying the end-users, they were divided in two separate groups: 1) Users of Snap tests; and 2) Users of Elisa plates.

1) Snap test survey
    a. 32 respondents in total
    b. Average number of Staff members is 20
    c. Non-use scenario: If IDEXX Snap tests were not available, what would you do?
        i. 31 % of the end-users would use an alternative rapid test
        ii. 69 % of the end-users would outsource testing to a reference laboratory

2) Elisa plate survey
    a. 7 respondents in total
    b. Average number of Staff members is 7
    c. Non-use scenario: If IDEXX Elisa plate tests were not available, what would you do?
        i. 43 % of the end-users would use an alternative Elisa test
        ii. 14 % of the end-users would outsource testing to a reference laboratory
        iii. 43 % of the end-users would do something else

According to the results of the downstream user survey the end-users would either outsource or use alternative test methods in the non-use scenario.

6. IMPACTS OF GRANTING AUTHORISATION

The main impacts on the operators in the scope are summarized in the following table.

TABLE 11. Summary of impacts of the non-use scenarios

Actor	The supply chain member's non-use scenario	Impacts of the non-use scenarios	Analysis method and notes
IDEXX UK	Unable to sell Triton related products to the UK.	[55-70]#B % personnel lay-offs in the commercial office and reference lab.	Quantitative
End-users in the UK	Outsourcing or switching to competitors' products	Possible screening gap	Qualitative

6.1. Human Health and/or Environmental Impacts

Cost effectiveness analysis

In the cost effectiveness analysis emissions reductions are compared to compliance cost of a policy measure. Societal cost from unemployment, which are calculated in Section 6.3 are taken into account in the calculation below.

Downstream use

- Triton substances and Nonidet releases to the environment: 0
- Equivalent 4-tert-OP releases to the environment: 0.
- Societal cost: 13.8 M GBP

In the non-use scenario Triton substances and Nonidet releases to the environment would not be further reduced as downstream users are already instructed to collect all waste streams for adequate treatment but the societal cost from unemployment would be 13.8 M GBP. This is considered disproportionate by the applicant.

6.2. Economic impacts

There will be no quantifiable economic impacts, such as profit losses, in the scope of the application. However, economic impacts on the end-users are described qualitatively. As explained in Section 5.4.5 end-users were divided in two groups: 1) Users of Snap tests; and 2) Users of Elisa plates.

1) Snap test users

Snap test users reported the following economic impacts in the non-use scenario.

1) Cost impact to laboratory to implement the alternative:

Very negative	Somewhat negative	Neither positive or negative	Somewhat positive	Very positive
0 %	41 %	47 %	9 %	%

41 % of the users of snap test foresee a negative cost impact when implementing the selected alternative.

2) Impact on supplying results efficiently and accurately if snap tests were not available:

Very negative	Somewhat negative	Neither positive or negative	Somewhat positive	Very positive
6 %	69 %	19 %	3 %	3 %

75 % of the users of snap test foresee negative effects on supplying results efficiently and accurately without IDEXX tests.

3) Economic impact of not having snap tests available (e.g. lost revenue from lack of testing options):

No economic impact	Minor economic impact	Moderate economic impact	Major economic impact
6 %	31 %	56 %	6 %

94 % of the users of Elisa plate test foresee negative economic impact if IDEXX Snap tests were not available.

2) Elisa plate users

Elisa plate users reported the following economic impacts in the non-use scenario.

1) Cost impact to laboratory to implement the alternative:

Very negative	Somewhat negative	Neither positive or negative	Somewhat positive	Very positive
43 %	57 %	0 %	0 %	0 %

100 % of the users of Elisa plate test foresee a negative cost impact when implementing the selected alternative.

2) Impact on supplying results efficiently and accurately if Elisa tests were not available:

Very negative	Somewhat negative	Neither positive or negative	Somewhat positive	Very positive
57 %	14 %	29 %	0 %	0 %

71 % of the users of Elisa plate test foresee negative effects on supplying results efficiently and accurately without Elisa tests. None of the respondents foresee positive effects.

3) Economic impact of not having Elisa tests available (e.g. lost revenue from lack of testing options):

No economic impact	Minor economic impact	Moderate economic impact	Major economic impact
0 %	43 %	29 %	29 %

100 % of the users of Elisa plate test foresee a negative economic impact if IDEXX Elisa tests were not available.

Both end-user groups (Snap & Elisa) are going to encounter negative economic impacts in the non-use scenario. 94 % of Snap users foresee a negative economic impact. Similarly, 100% of Elisa users foresee a negative economic impact.

6.3. **Social impacts**

IDEXX UK

IDEXX Windsor Berkshire currently (2021) employs 100 people and IDEXX Wetherby employs 210 people. In the non-use scenario [55-70] #B %, [150-200] #B employees ([50-70] #B at Windsor Berkshire and [100-150] #B at Wetherby), of them would be laid off in 2023, when products containing Triton substances can no longer be sold in the UK. Average annual gross salary at IDEXX Windsor Berkshire is [40,000-50,000] #B GBP and at IDEXX Wetherby [30,000-40,000] #B GBP. Overall salary losses are as follows:

- Windsor Berkshire: Annual gross salary for [50-70] #B lost jobs is [40,000-50,000] #B GBP * [50-70] #B = 3.12 M GBP.
- Wetherby: Annual gross salary for [100-150] #B lost jobs is [30,000-40,000] #B GBP * [100-150] #B = 4.32 M GBP.
- Total: 7.44 M GBP

The main economic consequence of unemployment is considered to be the reduced spending power due to salary loss. To achieve the most realistic economic impact, taxes and household saving and investment of workers have to be deducted from the salary.

To capture all the welfare costs associated with unemployment SEAC[8] and Dubourg[9] have proposed default values for one job lost. In the UK the value is 2.09 times the annual pre-displacement wages for a job. Furthermore, in the UK the employer tax rate is 11 %9. So the welfare cost to society equates to:

(1 – 0.11) * 7.44 M GBP * 2.09 = 13.8 M GBP

To conclude, the social impact of the non-use scenario is [150-220] #B lost jobs in the UK. This equates to welfare costs of 13.8 M EUR to society.

End-users

In the end-user survey it was asked how animal health care would be impacted in the non-use scenario. As explained in Section 5.4.5 end-users were divided in two groups: 1) Users of Snap tests; and 2) Users of Elisa plates.

1) Snap test users

Snap test users reported the following social impacts in the non-use scenario.

1) Impact on animal care:

Very negative	Somewhat negative	Neither positive or negative	Somewhat positive	Very positive
16 %	69 %	16 %	0 %	0 %

[8] https://echa.europa.eu/documents/10162/13555/seac_unemployment_evaluation_en.pdf/af3a487e-65e5-49bb-84a3-2c1bcbc35d25

[9] https://echa.europa.eu/documents/10162/13555/unemployment_report_en.pdf/e0e5b4c2-66e9-4bb8-b125-29a460720554

85 % of the users of snap test foresee a negative impact on animal care in the non-use scenario.

2) *Elisa test users*

Elisa test users reported the following social impacts in the non-use scenario.

1) Impact on animal care:

Very negative	Somewhat negative	Neither positive or negative	Somewhat positive	Very positive
43 %	0 %	57 %	0 %	0 %

43 % of the users of Elisa test foresee a negative impact on animal care in the non-use scenario. 58 % of the users of Elisa test don't foresee a negative nor positive impact on animal care. None of the respondents foresee a positive impact.

According to the results of the survey made, quality of animal health care could decrease in the non-use scenario.

6.4. **Wider economic impacts**

Food and Agriculture Organization of the United Nations (FAO) has prepared a study called "Economic Analysis of Animal Diseases" in 2016.[10] The study is focused on transboundary animal diseases and shows how important it is to screen animals. Main findings of the study are presented in the next paragraphs.

Transboundary animal diseases (TADs) result in several kinds of economic impact. They cause livestock production losses, which may be very high if the disease in question spreads very rapidly, and particularly if it causes high levels of mortality. They can also result in considerable disruption to trade, causing particular concern in countries where export is an important source of revenue for the livestock sector. The prevention and control of TADs add to the cost of livestock production and to the national veterinary budget. Zoonotic TADs (those that can infect humans and cause human disease) cause economic impacts from human sickness and costs to public health systems. Governments spend scarce resources controlling outbreaks of TADs and applying prevention measures; farmers must deal with the impacts in their livestock production systems, and consumers experience the effects of local or widespread market disruptions caused by TADs.10

There are four main sources of impact of TADs. The first three are experienced within the livestock sector, namely:

- Disease effects: the mortality and loss of production caused by clinical or subclinical disease. When livestock are affected by a TAD, clinical or subclinical disease may result in the loss of animals as productive assets or may reduce their productivity.10
- Market disruption: as a result of consumer fears, or supply shortage causing market shocks, or as a consequence of restrictions on international trade in livestock and livestock products that are applied because of TADs. If consumers

[10] http://www.fao.org/3/a-i5512e.pdf

fear that animal products or exposure in markets will make them ill, this can lead to a sharp fall in consumption of certain livestock products when an outbreak of a TAD is announced. The fall in demand results in a fall in prices and loss of revenue for producers until consumer confidence is restored.10

- Control measures: the costs and benefits of measures applied by farmers, governments and industry to prevent or control disease outbreaks. Prevention and control measures for TADs aim to reduce the negative impacts of disease losses and market disruption discussed previously. Because of the externalities associated with TADs, governments as well as farmers invest in prevention and control. However, control initiatives have their own costs, and these contribute to the total impact of TADs.10

In addition to effects within the livestock sector, there is also a fourth source of impact:

- Effects beyond the livestock sector: these may include impacts on human health, the public health system, tourism and wildlife.10

Transboundary animal diseases can have direct and indirect impacts on human health. Direct impacts occur when humans are infected by zoonotic TADs (those that are naturally transmitted between vertebrate animals and humans) and become ill. Indirect effects can occur if the presence of TADs severely disrupts the food supply or the ability of poor families to access food. Zoonotic TADs can have economic impacts if they cause mortality in people, or through illness prevent them from doing the things that they would normally do or oblige them to require medical treatment. Brucellosis, certain strains of Influenza rabies, West Nile fever, BSE and Rift Valley fever are all examples of zoonotic TADs. The first two have economic impacts within the livestock sector and in human health. The last three are primarily diseases of humans, with wildlife and/or domestic animals involved in transmission; neither the disease nor the control process has any notable economic effect in livestock. The economic impact of zoonotic diseases on human health includes the value (or number) of human lives lost; the value of lost productivity through illness; and the cost of treating sick individuals, either privately or through the public health system.10

The presence of a zoonotic TAD or the measures taken to control a TAD may have impacts on tourism, if tourists are discouraged from visiting an infected area or access to the countryside is restricted by control measures, this results in loss of revenue for the tourist industry. If a zoonotic disease in livestock resulted in an epidemic of human disease, this could cause very widespread disruption of businesses and the operation of public sector services. Transboundary animal disease prevention and control can potentially lead to impacts on wildlife and biodiversity if, for example, they require wild animals to be culled to remove a potential disease reservoir, or result in an expansion of feed and forage production that encroaches on forest and grassland used by wild animals.10

As explained in FAO's study, transboundary animal diseases have far reaching effects. Unscreened animals might spread diseases which have negative impacts on productivity, markets, human health, the public health system, tourism and wildlife.

6.5. **Combined assessment of impacts**

6.5.1. **Comparison of impacts**

In Table 12 below the qualitative and quantitative impacts of the in-use and non-use scenarios are compared. The impacts are monetized when possible; the tables also list the qualitative impacts as losses or benefits.

TABLE 12. Conclusion of benefits and risks

Impact category	**Stakeholder group**	**Difference between the in-use and non-use scenario**	**Values Quantified impact in total**
Human health impacts	Workers and citizens in the UK	Not applicable	Not applicable
Environmental Impacts	UK	No difference	Emission tons: 0
Social impacts	Workers in the UK	The welfare cost due to the unemployment of [150-220] #B people in the UK.	[150-220] #B lost jobs in the UK, in monetary terms 13.8 M GBP
Economic impacts	End-users	Negative economic impact foreseen by end-users	Not quantified
Wider economic impact	Animals & Society	Negative impact on animal care. Possible issues in disease testing which could increase transboundary diseases. Unscreened animals might spread diseases which have negative impacts on productivity, markets, human health, the public health system, tourism and wildlife.	Not quantified
Cost Effectiveness Analysis	Applicant	Societal cost (M GBD) per 1 kg of 4-tert-OPnEO and 4-NPnEO emissions reduced	13.8 M GBP vs 0 kg
Summary No benefits since4-tert-OPnEO and 4-NPnEO emissions are zero.			

[150-220] #B direct lost jobs (monetized 13.8 M EUR); negative impact on animal care and potential negative wider impacts originated from decreased level of testing animal diseases.

6.5.2. **Distributional impacts**

To support the socio-economic analysis, a qualitative assessment of the distributional impacts of the continued use of 4-tert-OPnEO and 4-NPnEO compared to the non-use scenario are presented briefly below in Table 13. It is foreseen that only manufacturers of competitive products might experience a positive impact in the non-use scenario if their products do not contain SVHCs. or if they have obtained an authorisation on the downstream use in the UK. All other stakeholders or socio-economic groups would suffer in the non-use of the substance in the UK.

TABLE 13. Distributional impacts

Distributional Analysis		Impact of non-use
UK	Suppliers	n.a
	Applicant	--
	Manufacturers (competitors)	+
	End-users	-
	Animals	-
	Public	-
	Area in general	-
non-UK	Suppliers	n.a
	Applicant	--
	Manufacturers (competitors)	n.a
	End-users	n.a
	Animals	n.a
	Public	n.a
	Area in general	n.a
Socio-economic group	Highly skilled	-
	Skilled/semi-skilled	-
	Manual/non-skilled	-

6.6. **Uncertainty analysis**

Since the monetised benefit of the continued use is relatively high, 13.8 M GBD, and there is no release to the environment coming from the use, an uncertainty analysis is unnecessary.

6.7. **Information for the length of the review period**

Presently, the Applicant does not have an alternative to the 4-tert-OPnEO and 4-NPnEO-based surfactants that are used in the products covered by this application. Furthermore, IDEXX does not expect to have an alternative available such that it would be possible to reformulate the affected products before the Sunset Date. Nonetheless, the Applicant has devised a plan for the reformulation of the components containing 4-tert-OPnEO and 4-NPnEO from the products covered by this application.

The practical work for the substitution of 4-tert-OPnEO and 4-NPnEO in the products covered by this authorisation application is starting at all sites in H2 2021 and is expected to last 18-23 years depending on the site. The Applicant is aware that a review period of more than 12 years should not be considered for non-threshold substances for which the risks cannot be quantified, as indicated in the CARACAL paper CA/101/2017.[11] As this is the case for 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated and 4-Nonylphenol, branched and linear, ethoxylated, the Applicant is requesting for a review period of 12 years and will apply for a review of the authorisation in order to finish the reformulation of all products.

The following table lists the criteria the Socio-Economic Committee has set for applications requesting a long review period and how the Applicant's situation reflects these criteria.

TABLE 14. The long review period criteria set by the Socio-Economic Committee*.

Criterion	Situation for the applicant
The applicant's investment cycle is demonstrably very long (i.e. the production is capital intensive) making it technically and economically meaningful to substitute only when a major investment or refurbishment takes place.	The Applicant does not foresee any major investments to their facilities related to the reformulation in the near future.
The costs of using the alternatives are very high and very unlikely to change in the next decade as technical progress (as demonstrated in the application) is unlikely to bring any change. For example, this could be the case where a substance is used in very low tonnages for an essential use and the costs for developing an alternative are not justified by the commercial value.	The combined volumes of Triton X-100/IGEPAL CA-630, Triton X-405/IGEPAL CA-720 and Nonidet P-40 Substitute dispatched by IDEXX in the UK in 2021 was approximately 6.5 kg. If these detergents were to be removed from the solutions provided in the ELISA plate and SNAP tests kits, the assays would not function. For the assays that use these detergents, 4-tert-OPnEOs and 4-NPnEOs are essential and the assays cannot function without them: the background noise signal is so high that it masks an accurate result.
The applicant can demonstrate that research and development efforts already made, or just started, did not lead to the development of an alternative that could be available within the normal review period.	As the IVD kits are produced to work as a whole, substituting the detergent used in the different components of ELISA and SNAP tests with another can have important consequences on the function and performance of the assays. Therefore, extensive testing is required in order to assess the technical feasibility of any possible alternative, possibly even on a case by case basis for each disease type separately. As illustrated in IDEXX's substitution plan, it will take years to assess the technical feasibility of alternatives for all products, which is why a long review period is requested.
The possible alternatives would require specific legislative measures under the relevant legislative area in order to ensure safety of use (including acquiring the necessary certificates for using the alternative).	Any changes made to an *in vitro* diagnostic kit component leads to the revalidation of the entire product. As IDEXX is USDA licensed, they are required to meet certain product and data specifications and a review must be conducted before any product changes can be made. In addition, regulatory approval is necessary before a product can be placed on the market. As IDEXX manufactures "global" products, regulatory approval needs to be sought for every country where the product will be

[11] CARACAL, 2017

	marketed (USA, EU, Japan, China, etc…). This is a very time-consuming process. In addition, some products must meet EU directive requirements.
The remaining risks are low and the socio-economic benefits are high, and there is clear evidence that this situation is not likely to change in the next decade.	Ceasing the supply of ELISA and SNAP test kits containing 4-tert-OPnEO and 4-NPnEO in their various components would lead to a reduction of 0 kg of released 4-tert-OPnEO and 4-NPnEO. This would lead to a cost of 13.8 M GBP. This is considered disproportionate by the Applicant.

*https://echa.europa.eu/documents/10162/13580/seac_rac_review_period_authorisation_en.pdf

6.8. Substitution effort taken by the applicant if an authorisation is granted

As the DUs purchase ready-to-use kits, they rely on the Applicant to provide products that do not contain 4-tert-OPnEO and 4-NPnEO. Consequently, it is the applicant's responsibility to substitute them from their products. To this end, the applicant has devised a substitution plan for the reformulation of the products manufactured in Montpellier, Bern and Westbrook. Please refer to the separate substitution plan report submitted with this authorisation application for a detailed description of the substitution plan.

7. CONCLUSIONS

The aim of this report was to: 1) prove that there are currently no suitable alternative substances or technologies implementable for the applied use, 2) demonstrate that the socio-economic benefits of the continued use of 4-tert-OPnEO and 4-NPnEO outweigh the risks to the environment, and 3) demonstrate disproportionality for the applicant in the case of non-use of the substance.

By applying specific criteria, the applicant was able to identify five alternatives to Triton X-100 and three to Triton X-405. The alternative with the closest physicochemical properties was selected by IDEXX to undergo feasibility testing. In the case of Triton X-100, the most comparable alternative was Tergitol 15-S-9 whereas it was Tergitol 15-S-40 (70 %) for Triton X-405.

Based on the initial tests performed by IDEXX, Tergitol 15-S-9 was the best candidate in comparison to Tergitol 15-S-40 and SNAP wash formulations containing 0.1 % of Triton X-100. However, as issues with spot colour development were observed during the tests, Tergitol 15-S-9 is not considered feasible at the present time. A significant amount of R&D work remains in order to fully determine its applicability as a replacement for Triton X-100/IGEPAL CA-630 in all of IDEXX's SNAP products.

In terms of economic feasibility, Tergitol 15-S-9 is considered feasible despite the fact that IDEXX will have to bear the reformulation costs amounting to an estimated [40-70 M USD] #B [redacted] ([35-62 M EUR] #B [redacted] In addition, the alternative is available in sufficient quantities from multiple suppliers and based on the manufacturer's self-classification, it is less hazardous to the environment than Triton X-100/IGEPAL CA-630. Similar testing has not yet been carried out on ELISA plates however, the same alternative will apply to ELISA plates and SNAP tests. Due to the similarities between 4-tert-OPnEOs and 4-NPnEOs, it is expected that they can be substituted by the same alternative substance.

In the non-use scenario IDEXX is not able to sell products containing 4-tert-OPnEO or 4-NPnEO to the UK market since use of those products is banned in the UK. IDEXX UK will stop and selling Triton substances and Nonidet products to the UK market, and consequently encounter lay-offs. There would be no benefits for society from the non-use scenario since the releases of the hazardous substance to the environment are already zero. The main cost for society from the non-use scenario are welfare losses from related to lay-offs of 13.8 M GBP in the UK. This is considered disproportional.

REFERENCES

Bewsey, H., Liu, J., Rodon Vernet, J., Chandrashekar, R. (2017), Evaluation of Rapid Diagnostic Test Kits for Canine Vector-Borne Diseases, ECVIM, (ISCAID-P-14)

Bowman, D. D., Liotta, J. L., Mizhquiri-Barbecho, J. S., Rishniw, M., Simpson, K. (2017), Performance of Four In-Clinic Tests for Giardia Antigen Compared with Direct Immunofluorescence Assay, J. Vet. Intern Med., 31(5):1590.

CARACAL. (2017): REACH Authorisation – Criteria for longer review periods, Doc. ID. CA/101/2017

Drexel, J., Liu, J., Beall, M., O'Connor, T., Chandrashekar, R., Lappin, M. (2018), Results of In-clinic Rapid Tests for FeLV Antigen Can Vary Significantly, WSAVA.

Gibbs, J. (2014), Effective Blocking Procedures, ELISA Technical bulletin – No.3.

Guven, E., Duus, K., Lydolph, M. C., Jørgensen, C. S., Laursen, I., Houen, G. (2014), Non-specific binding in solid phase immunoassays for autoantibodies correlates with inflammation markers, Journal of Immunological Methods, 403, 26-36.

IDEXX Laboratories Inc. (2008), IDEXX SNAP cPL Test – Reference Laboratory Accuracy Pet-side.

IDEXX Laboratories Inc. (2016), The SNAP Giardia Test provides sensitive and specific detection of Giardia antigen in dogs.

IDEXX Laboratories Inc. (2015), The SNAP® Feline Triple® Test provides sensitive and specific detection of FeLV infection in cats.

IDEXX Laboratories Inc. (2016), IDEXX SNAP® 4Dx® Plus Test provides sensitive and specific detection of tick-borne diseases – Poor performance of Abaxis® VetScan® Canine Anaplasma Rapid Test has clinically relevant implications.

IDEXX Laboratories Inc. (2016), The SNAP® 4Dx® Plus Test provides sensitive and specific detection of tick-borne diseases – Abaxis® VetScan® Canine Ehrlichia Rapid Test demonstrates poor sensitivity compared to reference methods.

Levy, J. K., Crawford, P. C., & Tucker, S. J. (2017), Performance of 4 Point-of-Care Screening Tests for Feline Leukemia Virus and Feline Immunodeficiency Virus. *Journal of veterinary internal medicine*, *31*(2), 521–526. Doi:10.1111/jvim.14648.

Liu, J., Drexel, J., Andrews, B., Eberts, M., Breitschwerdt, E., Ramaswamy, C. (2018), Comparative Evaluation of 2 In-Clinic Assays for Vector-Borne Disease Testing in Dogs, Topics in Companion Animal Medicine, 33(4), 114-118.

Merck (2018), REACH Update – Inclusion of Alkylphenol ethoxylates in Annex XIV

O'Connor, T. P. (2015), SNAP Assay Technology, Topics in Companion Animal Medicine, 30(4), 132-138.

Steiner, J. M., Liu J., Drexel J., Chandrashekar R. (2018), Partial analytical validation of a new in-clinic cPLI test (Vcheck cPL), WSAVA.

Webpages:

IDEXX Laboratories:
https ://www.idexx.com/en/veterinary/understanding-test-sensitivity-and-specificity/

EU Commission – National Veterinary Programmes :
https ://ec.europa.eu/food/funding/animal-health/national-veterinary-programmes_en

APPENDICES

Appendix 1 Consultations

SNAP test survey results

TABLE 15. The detailed survey results of the online questionnaire for the DUs using SNAP tests. The results include all UK respondents (n = 32)

	UK	%
When evaluating veterinary rapid tests, how important is accuracy to you?		
Extremely important	24	75
Very important	7	22
Moderately important	1	3
Slightly important	0	0
Not at all important	0	0
Total	32	
Which alternative would your clinic pursue if IDEXX SNAP tests were not available?		
Use an alternative rapid test	10	31
Outsource testing to a reference laboratory	22	69
Diagnose without a rapid test	0	0
Other	0	0
Total	32	
If IDEXX SNAP tests were not available, what would be the impact on efficiently and accurately supplying results to pet owners?		
Very positive	1	3
Somewhat positive	1	3
Neither positive nor negative	6	19
Somewhat negative	22	69
Very negative	2	6
Total	32	
How would you describe the economic impact of not having IDEXX SNAP tests available in your practice?		
No economic impact	2	6
Minor economic impact	10	31
Moderate economic impact	18	56
Major economic impact	2	6
Total	32	
What would be the impact on animal care if DEXX SNAP tests were not available?		
Very positive	0	0

Somewhat positive	0	0
Neither positive nor negative	5	16
Somewhat negative	22	69
Very negative	5	16
Total	32	
How would you describe the cost impact to your clinic to implement the alternative you have selected?		
Very positive	1	3
Somewhat positive	3	9
Neither positive nor negative	15	47
Somewhat negative	13	41
Very negative	0	0
Total	32	

TABLE 16. The results of the email survey involving three university laboratories situated in Denmark, France and UK. The respondents are IDEXX SNAP tests users.

	DK, FR, UK
When evaluating veterinary rapid tests, how important is accuracy to you?	
Extremely important	1
Very important	2
Moderately important	0
Slightly important	0
Not at all important	0
Which alternative would your clinic pursue if IDEXX SNAP tests were not available?	
Use an alternative rapid test	2
Outsource testing to another reference laboratory	0
Use a comparable plate test	1
Other	0
If IDEXX SNAP tests were not available, what would be the impact on efficiently and accurately supplying results to pet owners or veterinarians?	
Very positive	0
Somewhat positive	0
Neither positive nor negative	1
Somewhat negative	2
Very negative	0
How would you describe the economic impact of not having IDEXX SNAP tests available in your lab?	
No economic impact	2
Minor economic impact	0

Moderate economic impact	1
Major economic impact	0
What would be the impact on animal care if IDEXX SNAP tests were not available?	
Very positive	0
Somewhat positive	0
Neither positive nor negative	2
Somewhat negative	1
Very negative	0
How would you describe the cost impact to your lab to implement the alternative you have selected?	
Very positive	0
Somewhat positive	0
Neither positive nor negative	2
Somewhat negative	1
Very negative	0

ELISA plate tests survey results:

TABLE 17. The detailed survey results for the DUs using ELISA plate tests. The results include all UK respondents (UK n = 7).

	UK	%
When evaluating ELISA tests, how important is accuracy to you?		
Extremely important	6	86
Very important	1	14
Moderately important	0	0
Slightly important	0	0
Not at all important	0	0
Total	7	
Which alternative would your laboratory pursue if IDEXX ELISA tests were not available?		
Use an alternative ELISA test	3	43
Outsource testing to a reference laboratory	1	14
Diagnose without using ELISA tests	0	0
Other	3	43
Total	7	
If IDEXX ELISA tests were not available, what would be the impact on efficiently and accurately supplying results to pet owners?		
Very positive	0	0
Somewhat positive	0	0

Neither positive nor negative	2	29
Somewhat negative	1	14
Very negative	4	57
Total	7	
How would you describe the economic impact of not having IDEXX ELISA tests available in your laboratory?		
No economic impact	0	0
Minor economic impact	3	43
Moderate economic impact	2	29
Major economic impact	2	29
Total	7	
What would be the impact on animal health if IDEXX ELISA tests were not available?		
Very positive	0	0
Somewhat positive	0	0
Neither positive nor negative	4	57
Somewhat negative	0	0
Very negative	3	43
Total	7	
How would you describe the cost impact to your laboratory to implement the alternative you have selected?		
Very positive	0	0
Somewhat positive	0	0
Neither positive nor negative	0	0
Somewhat negative	4	57
Very negative	3	43
Total	7	

Survey results for the DU using both SNAP tests and ELISA plate tests:

TABLE 18. The detailed survey results of the UK DU using both SNAP tests and ELISA plate tests

Please indicate the level of impact not having these tests would have on your laboratory	
SNAP tests	Major impact
ELISA plate tests	Major impact
Based on your answer(s) above, please briefly explain your reasoning for deciding on this level of impact to your lab	
Many ELISA and SNAP tests are not available in other formats and therefore we would not be able to offer those tests to customers.	

Which action would your lab be most likely to take if SNAP tests or ELISA plate tests were not available?
Stop offering the test completely
When considering your answers above, how do you think this would impact your laboratory in terms of lab personnel change
An 11-19 % decrease in lab personnel
Impact on efficiency of performing analysis and supplying results to veterinarians
Very negative
Potential impact on animal care
Very negative
Costs related to implementing the actions/solutions
Very negative
Time to implement replacement option
Very negative

Appendix 2. Test procedure for SNAP test kits

Whole blood/serum/plasma and milk samples

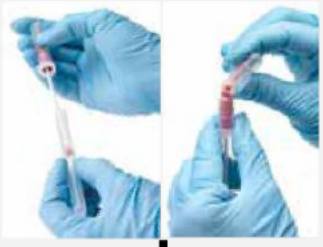
Step 1. Dispense sample and conjugate into the sample tube.

Step 2. Gently invert the sample tube 4-5 times to mix.

Step 3. Pour the sample into the sample well of the SNAP device.

Faecal samples

Step 1. Swab sample and place swap into tube. Break seal and release conjugate.

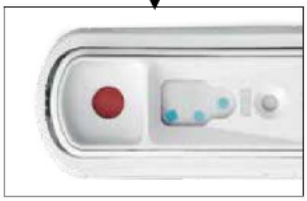


Step 2. Squeeze and release bulb 3 times to mix sample and conjugate.

Step 3. Squeeze bulb to dispense 5 drops into the well of a SNAP device.

Step 4. When colour appears in the activation circle, press firmly to activate. You will hear a distinct "snap"

Step 5. Read the results after the appropriate time has passed



Alternative step 4 and 5. Use the SNAP PRO* Analyzer to automatically activate the SNAP test and interpret the results

Appendix 3. Typical test procedure for ELISA plate assays

Step 1. Dilute the sample with sample diluent in test tubes. Dilute controls in the same way.

Step 2. Add the diluted sample to the pre-coated plate. (In some assays the sample is added undiluted)

Step 3. Incubate the plate.

Step 4. Dilute the wash concentrate tenfold with distilled water. Wash the plate with the diluted wash solution manually or using an automated plate washer.

Step 5. Add the conjugate to the plate wells

Step 6. Incubate the plate and repeat step 4.

Step 7. Add the substrate to the plate wells

Step 8. Measure the colour development in each well with a spectrophotometer

Appendix 4. Comparison of alternative assays and IDEXX SNAP tests

A. Comparison of IDEXX SNAP assays to colloidal gold lateral-flow assays:

IDEXX SNAP tests have several advantages over other assays present on the market, such as lateral-flow immunoassays that use colloidal gold or coloured latex particles instead of enzyme conjugates. Due to the reverse directional flow and the wash step that eliminates non-specific binding, SNAP tests have increased sensitivity and specificity. In addition, the enzymatic reaction step amplifies the signal and the distinct blue colour is easy to observe against the white colour of the matrix. The differences between colloidal gold lateral-flow assay and IDEXX SNAP tests are summarized in Table 19.[12]

TABLE 19. Comparison of IDEXX SNAP assay and colloidal gold lateral-flow assay

Assay step	Colloidal gold lateral-flow assay	SNAP assay
Wash Step	No wash step	Wash step removes unbound sample components and unreacted conjugate before addition of substrate
Flow orientation	Unidirectional flow	Bidirectional flow of sample and wash or substrate provides a second chance of binding and eliminates non-specific colour development
Signal generation mechanism	Accumulation of gold particles	Enzymatic signal amplification
Colour of results	Results may be difficult to interpret especially with whole blood samples	Distinct blue dot enhances ability to read results

The differences between the two assays are well illustrated when testing haemolysed samples (Figure 14). The red background colouration of the flow matrix on the colloidal gold lateral-flow assay makes it difficult to interpret the results as the test line is hardly visible. In contrast, there is no background colour on the SNAP test and the results are easy to interpret due to the high contrast between the white matrix and the blue coloured spots.

[12] O'Connor, 2015

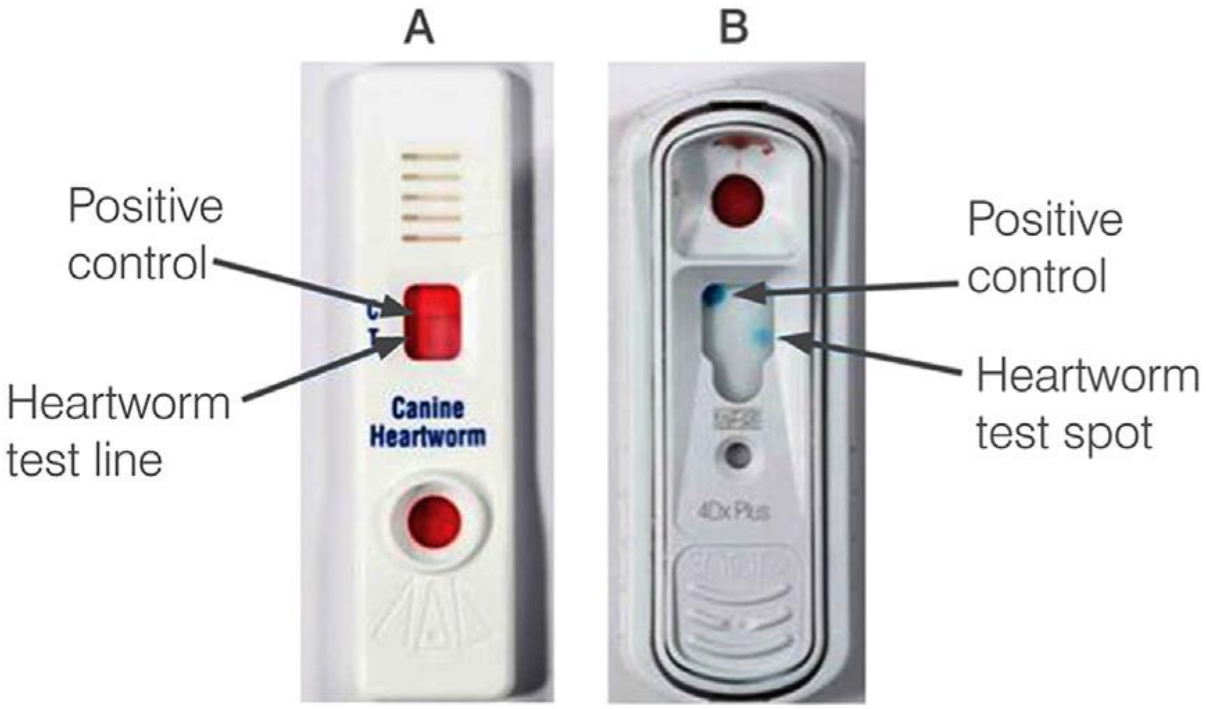


FIGURE 14. A known heartworm antigen-positive haemolysed sample was tested with two assays. (A) Colloidal gold lateral flow assay (B) SNAP 4Dx Plus Elisa.

B. Comparison of IDEXX SNAP assays to other rapid assays:

Below are presented the results of comparative studies where the sensitivity and specificity of alternative in-vitro diagnostic tests were compared to the performance of IDEXX SNAP tests. Sensitivity and specificity are typically reported as percentages. In practice, a 90 % specificity corresponds to 1 out of 10 negative animals testing false-positive based on the following probability theory equation.[13]

$$1 - \text{specificity} = 1 - 0.90 = 0.1 \text{ or } 10\ \%$$

On the other hand, a sensitivity of 85 % means that out of 100 infected animals, the *in-vitro* diagnostic test will misdiagnose 15 animal as healthy. Once again, it is calculated based on the following probability theory equation.[13]

$$1 - \text{sensitivity} = 1 - 0.85 = 0.15 \text{ or } 15\ \%$$

IDEXX SNAP Giardia Test:

TABLE 20. The results of a comparative study assessing the performance of IDEXX SNAP Giardia Test and three alternative in-vitro diagnostic tests in detecting *Giardia*-specific antigens. 176 samples were tested in total.[14]

In-vitro diagnostic test	Sensitivity	Specificity
SNAP Giardia Test	89.2 %	100 %
VetScan Canine Giardia Rapid Test	71.0 %	83.1 %
Witness Giardia Test	63.7 %	86.8 %
Anigen Rapid CPV/CCV/Giardia Test	78.5 %	70.1 %

Table 21. The results of a comparative study assessing the performance of IDEXX SNAP Giardia Test and three alternative in-vitro diagnostic tests in detecting *Giardia*-specific antigens. 177 samples were tested in total.[15]

In-vitro diagnostic test	Sensitivity	Specificity
SNAP Giardia Test	87.1 %	93.4 %
Anigen Rapid CPV/CCV/Giardia Test	80.2 %	80.3 %
Witness Giardia Test	73.3 %	71.1 %
VetScan Canine Giardia Rapid Test	70 %	85.5 %

[13] https://www.idexx.com/en/veterinary/understanding-test-sensitivity-and-specificity/
[14] The SNAP Giardia Test provides sensitive and specific detection of Giardia antigen in dogs, 2016
15 Bowman, 2017

IDEXX SNAP Feline Triple Test:

TABLE 22. The results of a comparative study assessing the performance of IDEXX Feline Triple Test and two alternative in-vitro diagnostic tests in detecting FeLV-specific antigens. 137 samples were tested in total.[16]

In-vitro diagnostic test	Sensitivity	Specificity
SNAP Feline Triple Test	96.6 %	≥ 98 %
VetScan Feline FeLV/FIV Rapid Test	71.3 %	
Witness FeLV-FIV Test	80.5 %	

TABLE 23. The results of a comparative study assessing the performance of IDEXX SNAP Feline Triple Test and two alternative in-vitro diagnostic tests in detecting FeLV-specific antigens. 185 samples were tested with the ELISA assay ViraCHECK FeLV (Zoetis) for the presence or absence of the FeLV. The same samples were tested with the three rapid assays and the results compared.[17]

In-vitro diagnostic test	Percent positive agreement [a]	Percent negative agreement [b]
SNAP Feline Triple Test	97.6 %	100 %
Speed Duo FeLV/FIV Test	51.2 %	99 %
Anigen Rapid FIV/FeLV Test	66.7 %	97 %

[a] represents the proportion of tests where the FeLV-specific antigen was detected by both the reference ELISA assay and the rapid assay of interest. [b] represents the proportion of tests where both the reference ELISA assay and the rapid assay of interest yielded negative results.

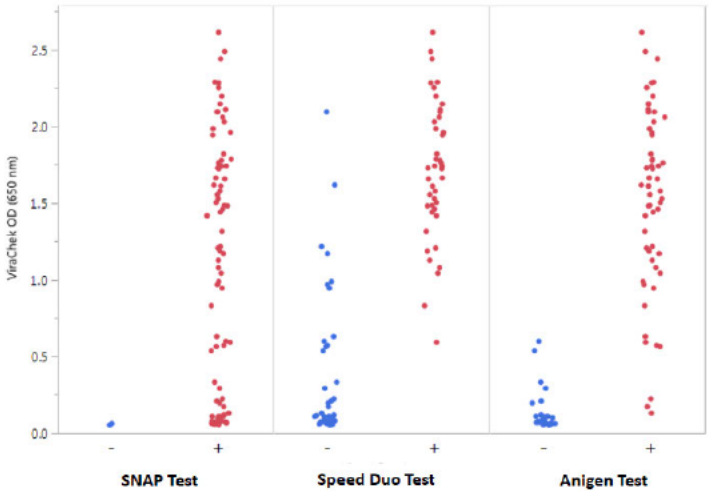


FIGURE 15. A diagram illustrating the percent positive agreement column of Snap Test, Speed Duo Test and Antigen Test

16 The SNAP® Feline Triple® Test provides sensitive and specific detection of FeLV infection in cats, 2015
17 Drexel, 2018

The red dots represent samples that tested positive both with the reference ELISA assay and the rapid assay of interest. The blue dots represent false negative.

IDEXX SNAP 4Dx Plus Test:

The data presented below originate from two separate studies.

TABLE 24. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus Test and one alternative in-vitro diagnostic test in detecting antibodies specific to *A. phagocytophilum* and *A. platys*.[18]

Organism	Reference test (pos/neg)	VetScan Canine Anaplasma Rapid Test		SNAP 4Dx Plus Test	
		Sensitivity	Specificity	Sensitivity	Specificity
A. phagocytophilum	IFA (87/50)	29.9 %	88.0 %	92.0 %	98.0 %
A. platys	ELISA (47/50)	68.1 %	86.0 %	89.4 %	96.0 %

TABLE 25. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus Test and one alternative in-vitro diagnostic test in detecting antibodies specific to *E. canis, E. ewingii* and *E. chaffeensis*.[19]

Organism	Number of samples	Reference test	VetScan Canine Ehrlichia Rapid Test	SNAP 4Dx Plus Test
			Sensitivity	Sensitivity
E. canis	30	IFA	93 %	100 %
E. ewingii	52	ELISA	60 %	92 %
E. chaffeensis	29	ELISA	41 %	69 %

[18] The SNAP® 4Dx® Plus Test provides sensitive and specific detection of tick-borne diseases, 2016
[19] The SNAP® 4Dx® Plus Test provides sensitive and specific detection of tick-borne diseases, 2016

TABLE 26. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus Test and one alternative in-vitro diagnostic test in detecting several antibodies. 844 samples were tested on each assay. Each result was read independently by three readers.[20]

	Sensitivity		Specificity	
Organism	SNAP 4Dx Plus	VetScan FLEX4	SNAP 4Dx Plus	VetScan FLEX4
A. phagocytophilum	84.5 %	12.7 %	98.9 %	98.9 %
A. platys	83.3 %	33.3 %	98.7 %	96.2 %
B. burgdorferi	95.5 %	40.9 %	100 %	100 %
E. canis	97.1 %	61.4 %	100 %	100 %
E. ewingii	98.2 %	59.3 %	100 %	98.2 %
E. chaffeensis	64.3 %	35.7 %	100 %	98.2 %
D. immitis	94.1 %	88.2 %	100 %	100 %

TABLE 27. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx and one alternative in-vitro diagnostic test in detecting antibodies specific to E. canis and A. platys.[21]

	Sensitivity		Specificity		
Organism	SNAP 4Dx Plus	Uranotest EC-AP	SNAP 4Dx Plus	Uranotest EC-AP	Reference test (sample size)
E. canis	96.2 %	65.4 %	100 %	100 %	IFA & ELISA (52+/48-)
A. platys	83.0 %	48.9 %	98.1 %	100 %	ELISA (47+/53-)

[20] Liu, 2018
[21] Bewsey, H., Liu, J., Rodon Vernet, J., Chandrashekar, R., 2017

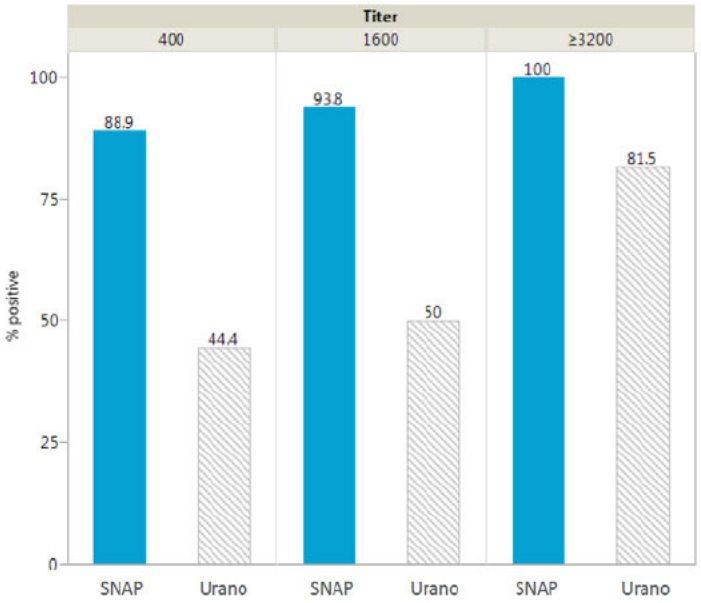


FIGURE 16. Diagram illustrating the percentage of *E. canis* positive samples diagnosed correctly by IDEXX SNAP 4Dx and Uranotest EC-AP. The Uranotest EC-AP missed a significant number of positive samples. More so in the titer < 400, which is critical for early detection of diseases.[21]

TABLE 28. The results of a comparative study assessing the performance of IDEXX SNAP 4Dx Plus and two alternative in-vitro diagnostic test in detecting E. Canis specific antibodies. 104 positive and 163 negative samples were tested. Positive and negative predictive values (PPV and NPV) were calculated for a representative population with 5% prevalence rate for E. canis.

In-vitro diagnostic test	Sensitivity	Specificity	PPV	NPV
SNAP 4Dx Plus	95.2 %	100 %	100 %	99.7 %
Speed Duo Leish K/Ehrli	84.6 %	84.7 %	22.5 %	99.1 %
FASTest EHRLICHIA canis	82.2 %	85.9 %	23.5 %	98.9 %

IDEXX SNAP FIV/FeLV Combo Test:

TABLE 29. The results of a comparative study assessing the performance of IDEXX SNAP FIV/FeLV Combo Test in detecting FeLV-specific antigens and FIV-specific antibodies.[22]

	SNAP FIV/FeLV Combo Test		Witness FeLV-FIV Test		Anigen Rapid FIV/FeLV Test		VetScan Feline FeLV/FIV Rapid Test	
Disease	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
FIV	97.9 %	99.0 %	94.7 %	100 %	96.8 %	99.0 %	91.5 %	99.0 %
FeLV	100 %	100 %	89.0 %	95.5 %	91.8 %	95.5 %	85.6 %	85.7 %

[22] Levy, 2017

IDEXX SNAP cPL Test:

The IDEXX SNAP cPL test was developed based on the Spec cPL test, due to market demands requiring a point of care version of the Spec cPL Test. The correlation between the two assays was determined in a study. 70 samples were tested with both assays and the optical density of the results were compared. The results are presented below.[23]

TABLE 30. Correlation of normals and abnormals. The SNAP cPL correctly interpreted 100% of the Consistent with Pancreatitis samples while one Normal sample was called as Abnormal and two Elevated samples as Normal. n = 70.

Total Spec cPL		Total SNAP cPL		Correlation SNAP cPL/Spec cPL
Normal	24	Normal	23	95.8 %
Elevated and Consistent with Pancreatitis	46	Abnormal	44	95.6 %

In addition, a readability study was conducted where the results displayed by the IDEXX Spec cPL and the IDEXX SNAP cPL were interpreted visually by veterinary professionals. Each result was interpreted twice by each 14 veterinary professional and 20 samples were tested in total. In addition, the study was conducted twice giving an n > 1000. The results showed a 95 % correlation on both sensitivity and specificity measures between the Spec cPL and the SNAP cPL. Thus, it can be concluded that the SNAP test is almost as performant as the Spec cPL in terms of accuracy and reliability.

No comparative studies were carried out on the IDEXX SNAP cPL test however, a study was conducted on the Spec cPL Test, which has similar performance as discussed above.

42 clinical samples were tested using IDEXX's Spec cPL Test (sensitivity: 82-94 %, specificity: 96 %) and BioNote's VCheck cPL.[24] The results given by the assays are based on the serum concentration of pancreatic lipase immunoreactivity (cPLI) present in the samples and they can be divided in three groups, known as diagnostic bins, as followed:

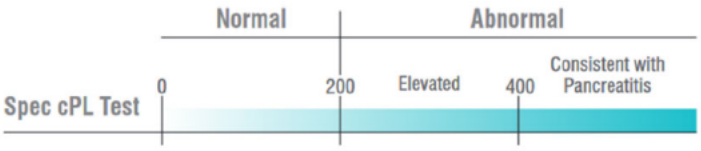


FIGURE 17. The three groups are normal, elevated and consistent with pancreatitis based on the measured serum concentration of cPLI in ug/L.[24]

In comparison to IDEXX Spec cPL Test, the VCheck cPL yielded lower cPLI values for 34 out of 42 samples and 7 samples changed diagnostic bins. Intra-assay variability was 14-36 % (mean 23 %), and inter-assay variability was 17-56 % (mean 35 %).[24]

[23] IDEXX SNAP cPL Test – Reference Laboratory Accuracy Pet-side, 2008

[24] Steiner, 2018

In addition, repeated testing was conducted on three different samples three times a day for five days. As illustrated in Figure 18, variability was observed in the results provided by the VCheck cPL assay. In some cases, the results even changed diagnostic bins.[24]

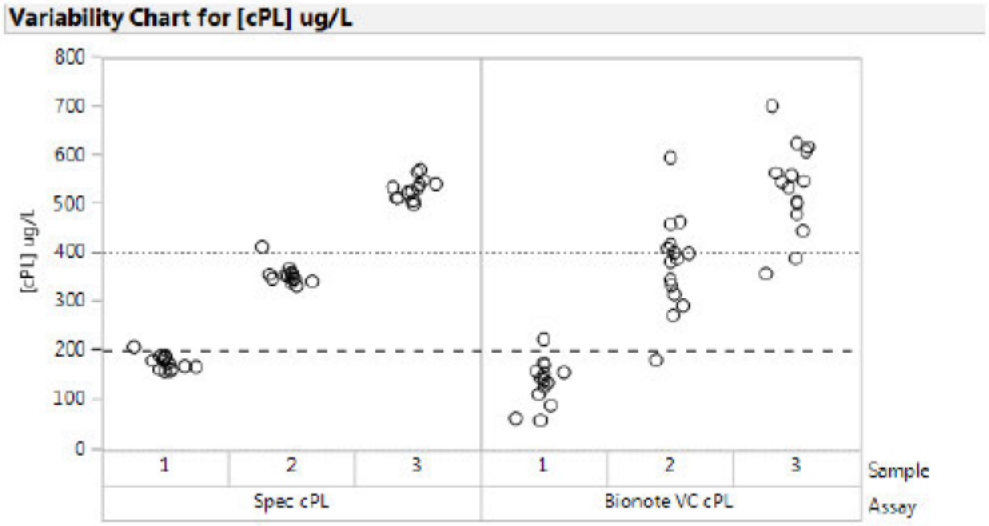


FIGURE 18. Repeated testing of 3 different samples 3 times per day for 5 days. %CV for Spec cPL was 8.2, 5.4, 3.9 % for samples 1-3, respectively. %CV for VCheck cPL for the same 3 samples were 33.4, 25.4, and 17.0 %.[24]

Based on the results of this study, the VCheck cPL assay lacked linearity, precision, reproducibility, and accuracy in comparison to IDEXX's Spec cPL Test.

Due to market demands, IDEXX developed a point of care version of the Spec cPL Test, the SNAP cPL Test. It uses the same biological reagents as the Spec cPL Test.

SNAP Leishmania:

TABLE 31. The results of a comparative study assessing the performance of IDEXX SNAP Leishmania and one alternative in-vitro diagnostic test in detecting antibodies specific to Leishmania infantum.[21]

Sensitivity		Specificity		Reference test (sample size)
SNAP Leishmania	Uranotest Leishmania	SNAP Leishmania	Uranotest Leishmania	
90.3 %	67.7 %	100 %	100 %	ELISA (31+/48-)

Appendix 5. License and other permit requirements by countries

Legend:

- LC = license
- IP = Import Permit
- LC-PG = License for Program Diseases
- FSC = Free Sales Certificate
- MR = Mutual Recognition with (country/disease)
- n/a = Not applicable

Country	Region	Infectious		Non-infectious	
		LPD	CAG	LPD	CAG
Belgium	European Union	LC-PG	n/a	n/a	n/a
Bulgaria	European Union	LC-PG	n/a	n/a	n/a
Croatia	European Union	TSE only	n/a	n/a	n/a
Czech Republic	European Union	LC	LC	LC	LC
France	European Union	LC-PG	n/a	n/a	n/a
Germany	European Union	LC-PG	n/a	n/a	n/a
Ireland	European Union	MR-DE (BVDV)	n/a	n/a	n/a
Italy	European Union	LC-PG	n/a	n/a	n/a
Netherlands	European Union	LC-PG	n/a	n/a	n/a
Poland	European Union	LC	n/a	LC	n/a
Portugal	European Union	LC (Rapid tests only)	n/a	n/a	n/a
Romania	European Union	LC-PG	n/a	n/a	n/a
Russia	Europe	IP	IP	IP	IP
Serbia	Europe	LC	LC	LC	LC
Slovakia	European Union	LC	LC	LC	n/a
Spain	European Union	LC	LC	LC	LC
Switzerland	Europe	LC-PG	n/a	n/a	n/a
Ukraine	Europe	LC/IP	LC/IP	n/a	n/a
Cyprus	European Union	n/a	n/a	n/a	n/a
Morocco	Africa	IP	IP	IP	IP

South Africa	Africa	IP	IP	IP	IP
Egypt	Africa	IP	IP	IP	IP
China	Asia	LC	n/a	LC	n/a
Indonesia	Asia	LC	n/a	LC	n/a
Japan	Asia	LC	n/a	LC	n/a
Malaysia	Asia	LC	n/a	FSC	n/a
Philippines	Asia	LC	n/a	FSC	n/a
South Korea	Asia	LC	n/a	LC	n/a
Thailand	Asia	FSC	FSC	FSC	FSC
Cambodia	Asia	IP	n/a	IP	n/a
India	Asia	IP	IP	IP	n/a
Kazakhstan	Middle East	LC-PG	n/a	n/a	n/a
Turkey	Middle East	LC	n/a	LC	n/a
Canada	North America	LC/IP	n/a	n/a	n/a
United States	North America	LC	n/a	n/a	n/a
Australia	Oceania	IP	IP	IP	IP
New Zealand	Oceania	IP	IP	IP	IP
Argentina	LAO	LC	n/a	n/a	n/a
Brazil	LAO	LC	n/a	n/a	n/a
Bolivia	LAO	LC	n/a	LC	n/a
Colombia	LAO	LC	n/a	n/a	n/a
Costa Rica	LAO	LC	n/a	LC	n/a
Ecuador	LAO	LC	n/a	n/a	n/a
El Salvador	LAO	LC	n/a	n/a	n/a
Guatemala	LAO	LC	n/a	n/a	LC
Panama	LAO	LC	n/a	n/a	LC
Paraguay	LAO	LC	n/a	n/a	LC

Peru	LAO	LC	n/a	LC	n/a
Venezuela	LAO	LC	n/a	n/a	LC
Honduras	LAO	LC	n/a	LC	n/a
Mexico	LAO	LC	n/a	n/a	n/a
Puerto Rico	LAO	MR-USA	n/a	n/a	n/a