JRC Scientific and Technical Reports

Community Reference Laboratory For Feed Additives
Annual Report
Authorisation Activities 2008

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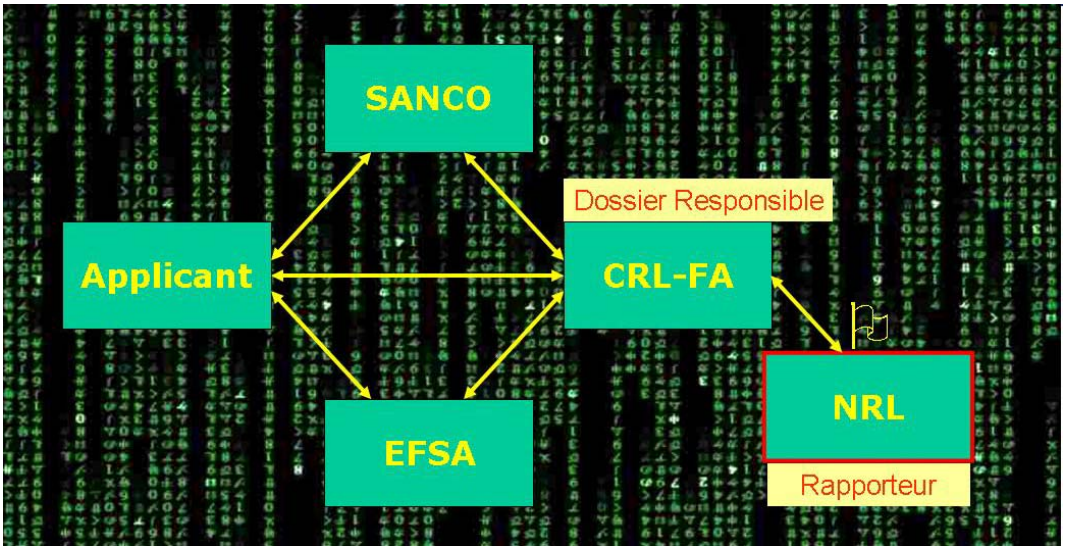

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CRL Feed Additives

The mission of the JRC-IRMM is to promote a common and reliable European measurement system in support of EU policies.

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FOREWORD

The team of the Community Reference Laboratory for Feed Additives (CRL-FA) is very proud to present its annual report 2008. With this report we would like to show the various activities conducted by the CRL-FA in 2008.

As a matter of fact, the evaluation of analytical methods submitted by the applicants makes up the primary activity of the CRL team. In 2008 we evaluated in close cooperation with the national reference laboratories analytical methods related to 27 dossiers. The various feed additives of these dossiers are quite different, not only in terms of the analytical methodology involved but also regarding the complexity of the evaluation. For instance, the determination of a product for which the applicant submitted exclusively a single-laboratory validated method is often more difficult compared to a situation where standard methods are available. In addition, there are also applications in which the feed additive contains several active substances, thus requiring the CRL evaluating different analytical methods for the same product.

Since June 2008 applicants need to prepare their dossiers according to the new Regulation (EC) No 429/2008, which also strengthens the role of analytical methods. An important and new aspect is that the analytical methods need to comply with specific criteria in order to guarantee their suitability for official control purposes. Furthermore applicants have to demonstrate that their proposed methods can be successfully transferred to a second laboratory, which is especially important in cases where exclusively single-laboratory validation data are available. To assist the applicants in conducting a corresponding experimental study, the CRL established a guidance document available from the CRL's web site.

We are now approaching quickly the 2010 deadline, by which dossiers for all notified products need to be submitted to ensure that they remain on the market. In consequence the number of submitted dossiers could increase significantly. In order to cope with this enormous burden in 2010, our team supported the Commission in drafting a Regulation to amend Regulation (EC) No 378/2005 to allow for more flexibility regarding the grouping of specific products and the use of analytical methods that allow for the simultaneous detection of various products. We expect that this new option will further improve the efficiency of dossier evaluation.

Christoph von Holst
Operating Manager
CRL – FA

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Main Activities of the CRL-FA Authorisation in 2008 *(by S. Yasar)*

In addition to the scientific evaluations of analytical methods the CRL-FA organised in 2008 a workshop and various expert meetings on specific analytical methodologies, established a number of web tools, maintained a database on methods of analysis, and a sample bank of feed additives and drafted several strategic documents related to the evaluation procedure of the dossiers.

Evaluation of Dossiers

In 2008, a total of 26 dossiers were evaluated and the corresponding reports were submitted to EFSA on due time (Table 1). Furthermore, the CRL drafted an opinion related to MRLs (cf. Panaferd-AX), upon request from SANCO. All *Executive Summaries* are presented in Annex I.

The dossier distribution - related to the different active substances - covers a variety of categories and functional groups, as presented hereafter:

Category	Functional group	count
Coccidiostats	Coccidiostats	4
Nutritional	Amino acids	1
Sensory	Colourants	1
Zootechnical	Enzymes	6
	Microorganisms	14
	Substances favourably affect environment	1
	Total	***27***

Overview of dossiers evaluated in 2008

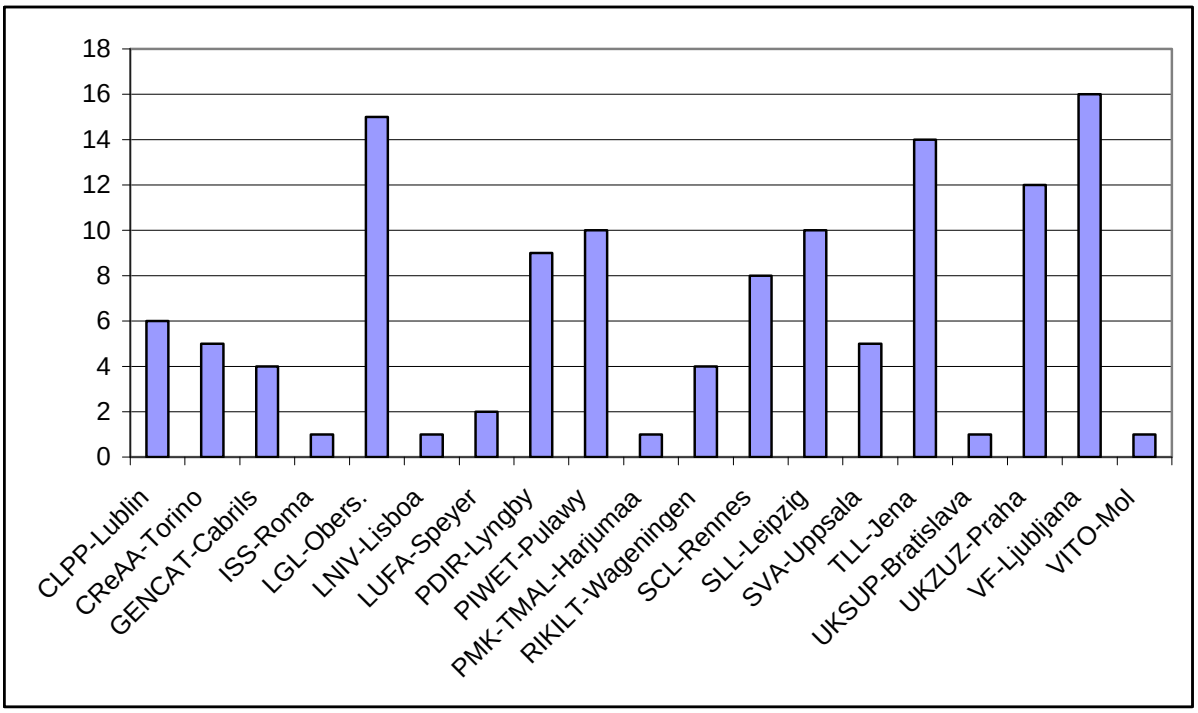
FAD Dossiers	Product/Additive Name	Active Substance	Rapporteur	Date of report
FAD-2006-0029	Probiotic_Lactina	L.(acidophilus, helveticus, bulgaricus, lactis), S.thermophilus, E.faecium	CRL-FA	24/01/2008
Report on MRLs	Panaferd-AX	Canthaxanthin	CRL-FA	18/03/2008
FAD-2007-0015	L-valine Feed Grade	L-valine	CRL-FA	06/03/2008
FAD-2007-0016	Maxiban	Nicarbazin, Narasin	CRL-FA	26/03/2008
FAD-2007-0019	Ronozyme P5000CT & L	6-phytase	PDIR-LYNGBY	05/06/2008
FAD-2007-0020	Econase XT L&P	Endo-1,4-beta-xylanase	CRL-FA	15/02/2008
FAD-2007-0022	MycoCell	S. cerevisiae	CRL-FA	10/06/2008
FAD-2007-0027	Ronozyme NP-CT/L	6-phytase	CRL-FA	05/03/2008
FAD-2007-0028	Bioplus 2B	Bac. Subtilis, Bac. Licheniformis	CRL-FA	26/06/2008
FAD-2007-0029	Levucell SC_LAMB	S. cerevisiae	CRL-FA	17/04/2008
FAD-2007-0030	Elancoban	Monensin sodium	CRL-FA	13/05/2008
FAD-2007-0034	Advastat	Acarbose	CRL-FA	09/04/2008
FAD-2007-0035	Cycostat 66G	Robenidine hydrochloride	ISS-Roma	23/07/2008
FAD-2007-0043	Ecobiol®	B. amyloliquefaciens	CRL-FA	10/07/2008
FAD-2007-0044	Natugrain TS and TS L	Endo-1,4-beta-(glucanase & xylanase)	CRL-FA	04/08/2008
FAD-2007-0046	Bactocell PA	P. acidilactici	CRL-FA	07/07/2008
FAD-2007-0048	Yea-Sacc 1026	S. cerevisiae	CRL-FA	18/09/2008
FAD-2007-0049	BIOSAF® Sc47	S. cerevisiae	CRL-FA	14/07/2008
FAD-2008-0001	Avatec® 150G	Lasalocid Sodium	CRL-FA	22/09/2008
FAD-2008-0003	Phyzyme XP_10000 TPT & L	6-Phytase	CRL-FA	24/10/2008
FAD-2008-0005	Selsaf	Selenium enriched yeast	CRL-FA	12/12/2008
FAD-2008-006	Biosprint	S. cerevisiae	CRL-FA	06/11/2008
FAD-2008-0007	Bonvital	E. faecium	CRL-FA	15/10/2008
FAD-2008-0009	Toyocerin®	B. cereus var. toyoi	CRL-FA	20/10/2008
FAD-2008-0012	Miya-Gold S	C. butyricum Miyairi	CRL-FA	10/10/2008
FAD-2008-0014	MLB	L. acidophilus	CRL-FA	09/10/2008
FAD-2008-0015	Bactocell PA 10	P. acidilactici	CRL-FA	04/11/2008

In 2008, two dossiers were drafted by the following "Rapporteur-NRLs": ISS-Rome and PDIR-Lyngby. Furthermore, all reports were thoroughly reviewed and commented by the NRLs listed hereafter (depending on their field of expertise), thus demonstrating their enthusiastic and professional involvement to the CRL-FA network. The CRL wishes to acknowledge the contribution of the following NRLs:

CLPP-Lublin, PL
CReAA-Torino, IT
GENCAT-Cabrils, ES
ISS-Roma, IT
LGL-Oberschleißheim, DE
LNIV-Lisboa, PT
LUFA-Speyer, DE
PDIR-Lyngby, DK
PIWET-Pulawy, PL
PMK-TMAL-Harjumaa, EE
RIKILT-Wageningen, NL
SCL-Rennes, FR
SLL-Leipzig, DE
SVA-Uppsala, SE
TLL-Jena, DE
UKSUP-Bratislava, SK
UKZUZ-Praha, CZ
VF-Ljubljana, SI
VITO-Mol, BE

Unlike previous years, the NRLs contributed significantly in 2008 to the peer review process. Furthermore, it is foreseen to outsource more reports to NRL rapporteurs. In order to increase the number of experienced and recognised evaluators, a series of training seminars was started by the CRL. The first event was organised in November 2008 as described later.

Overview of the NRL comments during the 2008 review process



CRL-FA Workshop 2008 - Executive summary *(by C. von Holst)*

The 8th Workshop of the CRL-FA network was organized at the IRMM on April, 16 - 17 2008. A total of forty-one participants attended the event, including representatives from 27 National Reference Laboratories, EFSA.

The 2007 activities of the CRL-FA were reviewed and the important aspects of the dossier evaluations were discussed. Participants were updated about the "New Guidelines", the "Guidance for Applicants" and the "Validation/Verification draft document". FEFANA presented the "Current Activities of the Feed Additives Authorisation Consortia".

The second day was devoted to scientific lectures such as, the "CEN methods for trace elements by ICP-AES", the "Carry-over of coccidiostats in non-targeted animal feeds" by Professor C. Van Peteghem (from the University of Gent, BE), and "the Current methodology for residues of coccidiostats in animal tissues and animal products" by Dr. W. Radek (from the Community Reference Laboratory for Residue Testing – Berlin, DE).

The closing presentation by Christoph von Holst highlighted the importance of getting ready for the high number of dossiers expected by 2010. The CRL committed to organise training sessions for the representatives of the NRLs, in order to ensure that dossiers are properly evaluated in a harmonised and effective way.

The evaluation forms collected indicated the high satisfaction of the participants and identified the three most appreciated topics: - review of the dossier evaluation, - carry-over and – method validation/verification.

CRL Training Seminars *(by P. Robouch)*

The CRL developed a training material and started a series of training seminars in order to increase the number of experienced Rapporteurs drafting the CRL Evaluation Reports on analytical methods submitted in connection with the application for authorisation as feed additives according to Regulation (EC) 1831/2003.

The following menu was considered for the two days seminars:

* Starter: "Typical" difficulties encountered during a "first" dossier evaluation are presented. Some of them relate to the Commission Administrative jargon and procedures, not trivial to all National Administrations. Others concern the challenge of translating the contract before acceptance by the National Institution or the challenge in finding the right path through the many Commission decisions and regulations.

* Main Course: The evaluation process is then thoroughly explained. This includes an overview of the legislative requirements relevant to the Feed Additive Authorisation. It stresses also the main topics to be considered and presents the common pitfalls to be avoided. Finally an "ideal" report is presented. Many issues need to be considered and well understood (i.e. cascade approach; active agent vs. feed additive; provisional requirements; etc.).

* Dessert: After these academic presentations it is time for some hands-on practice. Two Feed Additives (FAD) applications are to be evaluated. For the first case participants perform the evaluation of a dossier (later disclosed as already "authorised") with the support of the Training moderator. The conclusions elaborated by the participants are compared to the CRL's evaluation report and discussed. The second case relates to a "new/ongoing" dossier. Participants need to (a) evaluate whether the applicant provided all the relevant information, (b) draw the recommendations to be put forward and (c) draft the initial report.

The first Training Seminar of CRL Rapporteurs was organised at the IRMM on 24-26 November 2008, dedicated to the evaluation of dossier type (4a), with enzyme active agents. Seven participants representing the NRLs listed above - sorted by country code- attended the event with great enthusiasm and commitment

- *AGES, Wien (AT)*
- *FLVVT-FAVV, Tervuren (BE)*
- *LGL, Oberschleißheim (DE)*
- *TLL, Jena (DE)*
- *PDIR, Lyngby (DK)*
- *SCL, Rennes (FR)*
- *CLPP, Lublin (PL)*

Three other training seminars are foreseen in 2009.

Verification? Validation? *(by P. Robouch)*

The validation procedure of method of analysis as requested in the feed additive regulations may be unclear to applicants.

> … *Any person seeking an authorisation for a feed additive or for a new use of a feed additive shall submit an* ***application*** *in accordance with Article 7*… [Regulation (EC) No 1831/2003, Art. 4].
> … *An application for an authorisation as provided for in Article 4 shall be sent to the Commission. The Commission shall without delay inform the Member States and forward the application to the European Food Safety Authority*… [Regulation (EC) No 1831/2003, Art. 7.1].
> … *At the time of application, the applicant shall send the following particulars and documents directly to the Authority: … a description…… of the* ***method of analysis*** *of the additive in feed according to its intended use and, where appropriate, of the method of analysis for the determination of the level of residues of the feed additive, or its metabolites, in food …* [Regulation (EC) No 1831/2003, Art. 7.3.c].
> … *The methods of analysis shall be submitted in the* ***standard layout*** *as recommended by ISO (i.e. ISO 78-2). According to Regulation (EC) No 1831/2003 and Regulation (EC) No 378/2005, methods of analysis included in this section shall be* ***evaluated by the CRL****.*
> *… The CRL shall submit to the Authority an evaluation report indicating whether these methods are suitable to be used for official controls of the feed additive that is the object of the application. The CRL evaluation shall focus on the methods specified in sections 2.6.1 and 2.6.2…* [Regulation (EC) No 429/2008, Annex II,2.6].
> *…Detailed characterisation of the qualitative and, where applicable,* ***quantitative analytical method****(s) for determining compliance with maximum or minimum proposed levels of the active substance/agent(s) in the additive, premixtures, feedingstuffs and, when appropriate, water, shall be provided* … [Regulation (EC) No 429/2008, Annex II,2.6.1].
> *…These methods shall meet the same requirements as those for methods of analysis used for official control purpose laid down in Article 11 of Regulation (EC) No 882/2004 In particular they shall meet at least one of the following requirements:*
> *- comply with relevant Community rules (e.g. Community methods of analysis) where they exist;*
> *- comply with internationally recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted, or those agreed in national legislation (e.g. CEN Standard methods);*
> *- are fit for the intended purpose, developed in accordance with scientific protocols and validated in a ring test in accordance with an internationally recognised protocol on collaborative trials (e.g. ISO 5725 or IUPAC); or*
> *- are validated in-house according to* ***international harmonised guidelines*** *for the* ***in-house validation*** *of methods of analysis*[(1)] *with respect to the characterising parameters mentioned in 2.6.1.2…* [Regulation (EC) No 429/2008, Annex II,2.6.1.1];
> *… The detailed characterisation of the method(s) shall include the* ***appropriate characteristics*** *set out in Annex III of Regulation (EC) No 882/2004...* [Regulation (EC) No 429/2008, Annex II,2.6.1.2];
> … ***Performance characteristics*** *of in-house validated methods shall be* ***verified*** *by testing the method in a second, accredited and independent laboratory…* [Regulation (EC) No 429/2008, Annex II,2.6.1.3].

The sequence of legal requirements presented above is sketched hereafter.

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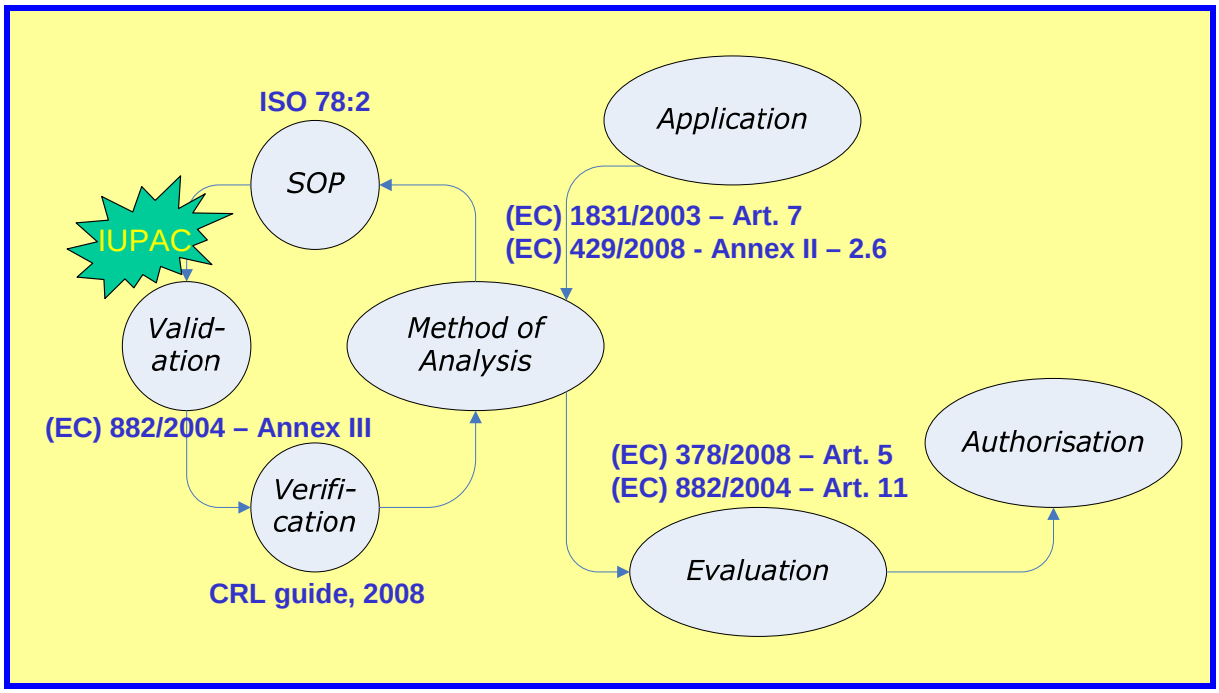
(1) M. Thompson et al.: Harmonized Guidelines For Single Laboratory Validation Of Methods Of Analysis (IUPAC Technical Report) Pure Appl. Chem., Vol. 74, No. 5, pp. 835-855, 2002.



As the single-laboratory validation study does not ensure whether the method is successfully transferable to another laboratory, Regulation (EC) 429/2008 requires the verification of performance characteristics of single-laboratory validated methods by an accredited and independent laboratory. The good agreement between validation and verification results shall support the transferability of the method to an official control laboratory. The CRL has published in July 2008 the CRL-FA Technical Guide: Protocol for verification studies of single-laboratory/in-house validated methods. The Verification Form is presented in Annex II.

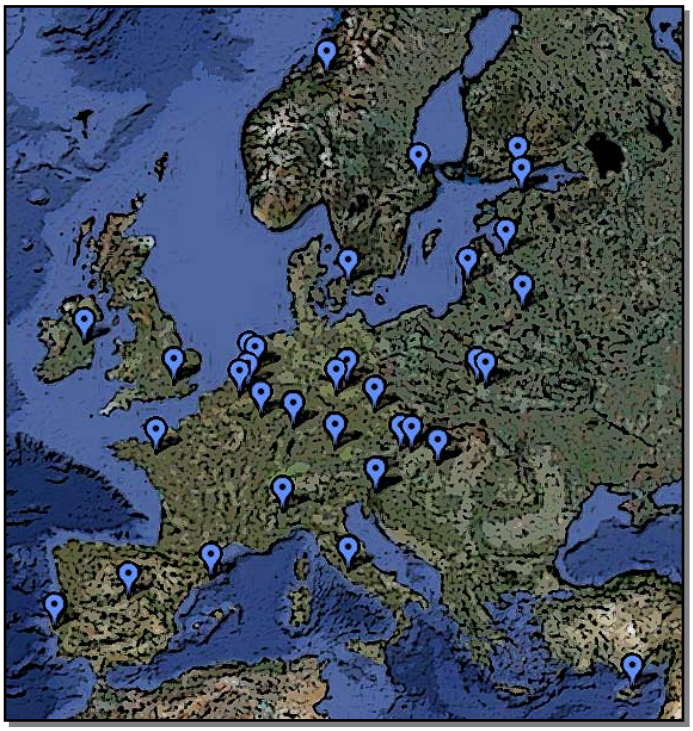
Acknowledgment

We sincerely thank our colleagues within the Institute for their strong support and interest in the CRL-FA activities, both with regards to secretarial support, review of reports and development of tailor made systems.

We are also very grateful to all experts from the NRLs for contributing to the evaluation of the dossiers and to the discussions in the workshops and working groups which was indispensable for the successful operation of the evaluation procedure. The List of NRLs follows.

The CRL-FA Authorisation group

The CRL-FA Network Map



The list of NRLs of the CRL-FA network

Country	National Reference Laboratory
	- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren. BE - Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol. BE
	- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha. CZ
	- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby. DK
	- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim. DE - Landwirtschaftliches Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer. DE - Sächsische Landesanstalt für Landwirtschaft. Fachbereich 8 – Landwirtschaftliches Untersuchungswesen, Leipzig. DE - Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen, Jena. DE
	- Põllumajandusuuringute Keskus (PMK). Jääkide ja saasteainete labor, Saku, Harjumaa. EE - Põllumajandusuuringute Keskus (PMK), Taimse materjali labor, Saku, Harjumaa. EE
	- Laboratorio Arbitral Agroalimentario, Ministerio de Agricultura, Pesca y Alimentación, Madrid. ES - Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Cabrils. ES
	- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes. FR
	- The State Laboratory, Kildare. IE

Country	National Reference Laboratory
	- Istituto Superiore di Sanità. Dipartimento di Sanità alimentare ed animale, Roma. IT - Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino. IT
	- Feedingstuffs Analytical Laboratory, Department of Agriculture, Nicosia. CY
	- Valsts veterinārmedicīnas diagnostikas centrs (VVMDC), Riga. LV
	- Nacionalinis maisto ir veterinarijos rizikos vertinimo institutas, Vilnius - Klaipėdos apskrities VMVT laboratorija, Klaipėda. LT
	- Laboratoire de Contrôle et d'essais – ASTA, Ettelbruck. LU
	- Mezőgazdasági Szakigazgatási Hivatal Központ, Élelmiszer- és Takarmány-biztonsági Igazgatóság, Takarmányvizsgáló Nemzeti Referencia Laboratórium, Budapest. HU
	- RIKILT- Instituut voor Voedselveiligheid, Wageningen. NL - Rijkinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven. NL
	- LabNett AS, Agricultural Chemistry Laboratory, Stjørdal. NO
	- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien. AT
	- Instytut Zootechniki w Krakowie. Krajowe Laboratorium Pasz, Lublin. PL - Państwowy Instytut Weterynaryjny, Pulawy. PL
	- Instituto Nacional dos Recursos Biológicos, I.P./Laboratório Nacional de Investigação Veterinária (INRB,IP/LNIV), Lisboa. PT
	- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana - Kmetijski inštitut Slovenije, Ljubljana. SL
	- Skúšobné laboratórium - Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava. SK
	- Elintarviketurvallisuusvirasto/Livsmedelssäkerhetsverket (Evira), Helsinki/Helsingfors. FI
	- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala
	- The Laboratory of the Government Chemist, Teddington

CRL – FA / Authorisation

Objectives

Since November 2004 feed additives have to be authorised according to the procedure laid down in Regulation (EC) No 1831/2003. The procedure is based on a strict separation between scientific assessment of the feed additive - which falls under the responsibility of the European Food Safety Authority (EFSA), having the role of risk assessor - and the actual authorisation for placing the product on the market which is granted by the European Commission in its role as risk manager.

The assessment of the feed additive also includes a close evaluation of the analytical methods that are proposed by the applicant in order to determine the active substance in various matrices such as premixtures or animal feed. This evaluation and some other tasks that are described later on in this report are entrusted to the Community Reference Laboratory which - according to Regulation (EC) No 1831/2003 - is the European Commission's Joint Research Centre (JRC). Within the JRC the Food Safety and Quality Unit of the Institute for Reference Materials and Measurements (IRMM) has taken up the task to establish the CRL-FA.

Moreover, since the 1st of January 2006, Regulation (EC) No 882/2004 on official food and feed controls assigned additional tasks to the CRL-FA.

CRL
Feed Additives

Tasks

The main task of the CRL-FA is the evaluation of the analytical methods submitted by the applicant, in order to establish whether these methods are suitable for the intended purpose. Analytical methods are for instance required to determine the active substance of the feed additive in animal feed and – if applicable – residues in animal tissue. As specified in Article 6.1 of Regulation (EC) No 378/2005 the CRL-FA is assisted by a consortium of National Reference Laboratories (NRLs) which contribute to the evaluation procedure with their expertise on specific analytical methodology. The appointed laboratories are listed in the Annex.

Analytical methods are evaluated in a stepwise manner in which the CRL-FA and a rapporteur laboratory, which belongs to the consortium of NRLs and which the CRL-FA selects individually for each dossier, conduct a documentary evaluation of the protocol of the methods and the corresponding validation report. Based on this evaluation the rapporteur laboratory and the CRL-FA write a report, which is afterwards sent to EFSA. In the case that the submitted methods are considered suitable for official control a favourable opinion is given to EFSA, without performing experiments. In agreement with Regulation (EC) No 378/2005, as amended by Regulation (EC) No 850/2007, the CRL-FA charges the applicant 6000€ for each application. More details on the evaluation procedure are given later on in this report. If necessary, the CRL-FA may also test the method in its own or a NRL laboratory, or it may organise an inter-laboratory comparison study to validate it.

In addition, the CRL-FA maintains a bank of reference samples of feed additives authorised since 2005.

In agreement with Regulation (EC) No 1831/2003 and Regulation (EC) No 378/2005 the CRL-FA responsibilities also include other tasks, namely

- disseminating analytical methods;
- providing scientific and technical assistance to the Commission, especially in cases of dispute;
- coordinating the consortium of National Reference Laboratories.

Last but not least, the CRL-FA aims to contribute to the mission of IRMM which is to promote a common and reliable European measurement system in support of EU policies.

Official Food and Feed Control Regulation

Since 1st January 2006 and in accordance to Article 32 of Regulation (EC) No 882/2004, the CRL-FA is also responsible for:

- Providing national reference laboratories (NRLs) with details of analytical methods, including reference methods;
- Coordinating the application of the above mentioned methods by the NRLs, in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols, when available;
- Coordinating practical arrangements needed to apply new analytical methods and informing the NRLs of advances in this field;
- Conducting initial and further training courses for the benefit of staff from NRLs and of experts from developing countries;
- Providing scientific and technical assistance to the Commission, especially in cases where Member States contest the result of analysis
- Collaborating with laboratories responsible for analysing feed and food in third countries.

In accordance with Article 12, sampling and analysis in the context of official control are carried out by official laboratories designated by competent authorities in each Member State.

CRL-FA website

The CRL-FA website, available since early 2005, is regularly updated.

Under the section "public pages" information is presented on the activities of the CRL-FA and the composition of the consortium of NRLs. In addition support is given to applicants seeking feed additive authorisations.

In the network pages assistance is given to the NRLs on the procedures and activities in which the consortium is involved. A list of the reference samples stored at the CRL-FA is also available for the consortium via the network pages.

www.irmm.jrc.be/html/CRLs/crl_feed_additives/index.htm

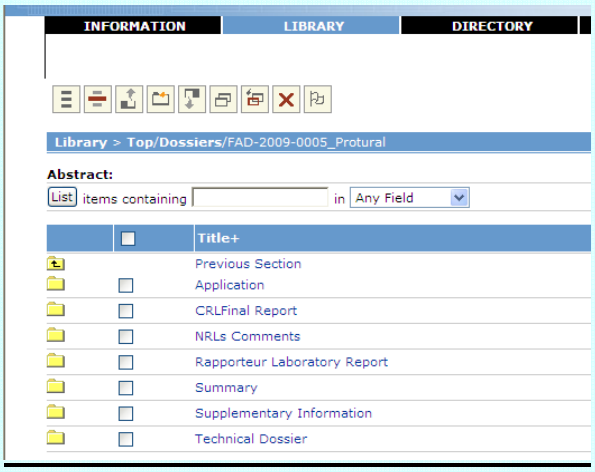
CRL-FA Dossiers Tracking System

A browsable interface to search the database is made available to the NRLs, EFSA and DG for Health and Consumer (DG SANCO) via the Network pages of the CRL-FA website. Two different kinds of search can be performed: (1) samples, (2) dossiers.

https://irmm.jrc.ec.europa.eu/crldossiercat/dossier/search.do
https://irmm.jrc.ec.europa.eu/crlsamplecat/sample/search.do

CIRCA

The Communication and Information Resource Centre Administrator (CIRCA) enabled the CRL-FA and the consortium of NRLs to maintain a secure space on the Internet where they can share documents information, and they can participate in a discussion forum. More than 50 users (consortium members, EFSA officers, DG for Health and Consumer Protection administrators and the CR-FA team itself) share information and documents on a daily basis, allowing for effective and fast communication. The system is managed by the CRL-FA. In the near future, CIRCA will be replaced by a more powerful system, to handle efficiently the increasing number of incoming documents.



https://circa.europa.eu/

FEEDACAM, the method database

The revision process of the FEEDACAM database was continued in 2008, in collaboration with the Informatics Unit of the IRMM. It is expected that the finalised database will be available in 2009.

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Annex I

CRL-FA Evaluation Reports
Executive Summaries

FAD number	Product/Additive Name
FAD-2006-0029	Probiotic_Lactina
MRLs	Canthaxanthin
FAD-2007-0015	L-valine Feed Grade
FAD-2007-0016	Maxiban
FAD-2007-0019	Ronozyme P5000CT&P20000 L
FAD-2007-0020	Econase XT L&P
FAD-2007-0022	MycoCell
FAD-2007-0027	Ronozyme NP-CT/Ronozyme NP-L
FAD-2007-0028	Bioplus 2B
FAD-2007-0029	Levucell SC_LAMB
FAD-2007-0030	Elancoban
FAD-2007-0034	Advastat
FAD-2007-0035	Cycostat 66G
FAD-2007-0043	Ecobiol®
FAD-2007-0044	Natugrain TS and TS L
FAD-2007-0046	Bactocell PA or Fermaid PA
FAD-2007-0048	Yea-Sacc 1026
FAD-2007-0049	BIOSAF® Sc47
FAD-2008-0001	Avatec® 150G
FAD-2008-0003	Phyzyme XP 10000 TPT and L
FAD-2008-0005	Selsaf
FAD-2008-006	Biosprint
FAD-2008-0007	Bonvital
FAD-2008-0009	Toyocerin®
FAD-2008-0012	Miya-Gold S
FAD-2008-0014	MLB
FAD-2008-0015	Bactocell PA 10 or Fermaid PA 10

Full reports available on the CRL-FA website
http://irmm.jrc.ec.europa.eu/html/CRLs/crl_feed_additives/authorisation/evaluation_reports/index.htm

FAD-2006-0029	EFSA-Q-2006-135
Product Name	Probiotic LACTINA
Active substance	Lactobacillus acidophilus NBIMCC 8242, Lactobacillus helveticus NBIMCC 8268, Lactobacillus bulgaricus NBIMCC 8244, Lactobacillus lactis NBIMCC 8250, Enterococcus faecium NBIMCC 8270, Streptococcus thermophilus NBIMCC 8253,
Rapporteur	Renata Leuschner CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive Probiotic LACTINA® under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Probiotic LACTINA® for chickens for fattening, piglets and pigs is requested. Probiotic LACTINA® consists of a minimum of 5 x 10^9 of viable cells (colony-forming units, c.f.u.) of lactic acid bacteria (LAB) per gram which comprise six strains as active agents, *Lactobacillus acidophilus* NBIMCC 8242, *Lactobacillus helveticus* NBIMCC 8269, *Lactobacillus bulgaricus* NBIMCC 8244, *Lactobacillus lactis* NBIMCC 8250, *Streptococcus thermophilus* NBIMCC 8253, *Enterococcus faecium* NBIMCC 8270. The feed additive is intended to be mixed into complete feedingstuffs at final concentrations of 5 x 10^8 to 9 x 10^9 c.f.u./kg for chickens for fattening, of 5 x 10^9 to 1 x 10^{10} c.f.u./kg for piglets and of 9 x 10^8 to 5 x 10^9 c.f.u./kg for pigs.

For the determination of the active agents (LAB), in the *feed additive*, identification and control methods for lactic acid bacteria monocultures in accordance to International Dairy Federation Standard Methods IDF 146:1991 and IDF 149A:1997 are used by the applicant. For enumeration of the active agents de Man, Rogosa, Sharp (MRS) agar is used whereby for *Streptococcus thermohilus* M17 agar is suggested. The incubation temperature used is 37 ºC. These methods are considered appropriate. ISO 4833 is used for the enumeration of the active agents in *premixtures* and *feedingstuffs*.

For official controls of the active agents (LAB) in the *feed additive, premixtures* and *feedingstuffs* a spread plate method using MRS agar is suggested by the CRL-FA. The enumeration method was validated in a collaborative study [Food Microbiol., (2003), 20, 57-66]. The method's performance characteristics of the enumeration method using MRS, acidified MRS or MRS supplemented with triphenyl tetrazolium chloride (TTC) agar and an incubation temperature of 37 ºC revealed standard deviations for repeatability (s_r) and reproducibility (s_R) of around 0.10 – 0.26 $\log_{10}$ and 0.18 – 0.39 $\log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively.

The limit of quantification (LOQ) of this method is 100 colony forming units (c.f.u) per gram (g) feed additive or premixture and 10000 c.f.u./g feedingstuff. These performance characteristics are considered acceptable.

For identification of the active agents, methods suitable for the purpose of analysis were used by the applicant. For official controls pulsed-field gel electrophoresis (PFGE) is recommended in principle, however - as the concentrations for individual strains in the product are not provided - it may not be applicable.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

MRLs	DG SANCO_Letter (D2008/420101, 11/02/2008)
Product Name	
Active substance	Canthaxanthin
Rapporteur	Christoph von Holst CRL-FA, European Commission

The evaluation of analytical methods for the determination of canthaxanthin in food matrices in feed showed that suitable methods are available for egg yolk, fish tissue and feed, whereas a validated analytical protocol for the matrix "poultry tissue" is missing. It is assumed that the currently available methods for the determination of the target analyte in egg yolk and fish tissue can also be applied to the analysis of poultry tissue. However, experimental data to support this assumption are not available.

FAD-2007-0015	EFSA-Q-2007-103
Product Name	L-valine Feed Grade
Active substance	L-valine
Rapporteur	Giuseppe Simone CRL-FA, European Commission

In the current application authorisation is sought for L-valine Feed Grade under the category 'nutritional additives', functional group 'amino acids, their salts and analogues', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use L-valine Feed Grade for supplementing feed for all animal species. The product is a crystalline powder with a minimum content of 95 % L-valine. The feed additive is intended to be included into feedingstuffs at a final concentration up to 2500 to 3000 mg of total L-valine/ kg complete feedingstuffs, depending on the concentration of L-valine already present in the feed components.

For the determination of the active substance (L-valine) in the feed additive, premixtures, and feedingstuffs the applicant proposes the official Community and fully ring-trial validated method for determination of amino acids [Commission Directive 98/64/EC]. The method is applicable for both the determination of free (synthetic and natural) and the determination of total (peptide-bound and free) amino acids, using an amino acid analyser or High Pressure Liquid Chromatography (HPLC) equipment combined with post column derivatisation using ninhydrin and photometric detection at 570 nm. The same method is adopted by ISO and described in the ISO standard 13903:2005 [Animal feedingstuffs – determination of amino acids content], which additionally reports the results from a second intercomparison study performed on different premixtures and feeds [Llames & Fontaine, J. of AOAC Int., Vol. 77, No. 6, 1994]. Performance characteristics for the target analyte (L-valine) include the relative standard deviation for repeatability (RSDr) ranging from 1.71 to 3.80 % and relative standard deviation for reproducibility (RSDR) ranging from 8.83 to 16.05 %, depending on the matrix. The method is considered suitable for official controls. It is not suitable to differentiate between the salts or D- and L-forms of amino acids, or between naturally occuring and added L-valine.

Alternatively, for the determination of the active substance (L-valine) in the feed additive and premixtures for official controls, the CRL considers validated methods based on the same technique suitable, such as the method 4.11.6 of the Association of German Agricultural Analytical and Research Institutes (VDLUFA) [Methodenbuch III, 5. Erg. 2004, VDLUFA – Verlag, Darmstadt] and the similar AOAC Method 999.13 [Fontaine and Eudaimon, J. of AOAC Int., Vol. 83, No. 4, 2000]. These methods have been validated for the quantitative determination of three free (non protein bound) amino acids (lysine, methionine and threonine) at an individual concentration of more than 10 % in feed grade amino acid commercial products. The methods are therefore also considered applicable for the quantification of L-valine.

Further testing or validation by the CRL is not considered necessary.

FAD-2007-0016	EFSA-Q- 2007-111
Product Name	Maxiban
Active substance	Narasin, Nicarbazin
Rapporteur	Giuseppe Simone CRL-FA, European Commission

Maxiban®G160 is a product already authorised as feed additive by Regulation (EC) No 2430/1999, amended by Council Regulation (EC) No 1756/2002 under the category 'coccidiostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active agents of Maxiban®G160 are narasin and nicarbazin and the authorised inclusion level is 80 to 100 mg/ kg complete feedingstuffs

In the current application a modification of the terms of authorisation is sought for Maxiban®G160 according to Article 13(3) of Regulation (EC) No 1831/2003. Specifically, establishment of Maximum Residues Limits (MRLs) for nicarbazin (marker residue 4,4'-dinitrocarbanilide (DNC)) is sought. The proposed limit is 750 micrograms/kg liver of chickens for fattening

For the quantification of DNC in chicken liver the applicant proposed a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method. Mass spectrometry detection of DNC is based on one precursor ion and one transition (300.8 m/z > 136.9 m/z). The CRL evaluated the method performance profile based on information provided in the dossier (first validation study) and on additional information provided by the applicant upon request (supplementary data). The following method performance characteristics were obtained from the first validation study: the limit of quantification (LOQ) and the limit of detection (LOD) were 50 and 4.06 micrograms/kg liver, respectively. Mean recovery, determined under repeatability conditions ranged from 85.9 to 104 %, with a coefficient of variation (CV) ranging between 3.2 and 10.8 %, depending on the fortification level. Mean recovery determined under intermediate precision conditions (different time and analyst) ranged between 93.8 and 101 %, with a CV ranging between 6.6 and 11.4 %, depending on the fortification level. Linearity was demonstrated over the range 50-750 micrograms/kg liver (target tissue). The specificity of the method for DNC was demonstrated by analysis of aliquots of each matrix without the addition of DNC and by addition of a number of commonly used ionophores coccidiostats (Narasin, Lasalocid, Salinomycin, Maduramicin, Monensin and Semduramicin) and other coccidiostats (Decoquinate, Diclazuril, Halofuginone and Robenidine). No interferences at the retention time of DNC were observed.

Supplementary data from a second in-house validation study covering a range which at least includes one-half and twice the proposed MRL were submitted by the applicant upon request of the CRL. Linearity was demonstrated over the range 50-1500 micrograms /kg liver. Mean recovery, determined under repeatability conditions ranged from 92.3 to 98.6 %, with a coefficient of variation (CV) ranging between 4.6 and 9.1 %, depending on the fortification level. Mean recovery determined under intermediate precision conditions (different time and analyst) at a fortification level of 1500 micrograms/kg was 99.4 %, with a CV 16.9 %,.

The supplementary data submitted showed acceptable performance characteristics in terms of sensitivity, precision, and trueness. However, the application of the method in the frame of official control is limited because the proposed protocol does not allow for the unequivocal identification of nicarbazin in the case of a suspected non-compliant result, i.e. when the analytical results indicate exceeding the proposed MRL. This is due the fact that the protocol uses one precursor ion and one transition thereby obtaining 2.5 identification points whereas at least three identification points would be required for identification of these substances according to Commission Decision 2002/657/EC. The proposed method can only be considered suitable as a quantitative method to determine residues of nicarbazin in target tissue samples at or around the MRL value. The CRL assumes that this method could slightly be modified by measuring a second transition in order to fulfil the criteria of the Commission Decision. However, validation data for this modification have not been provided.

Another LC-MS/MS method has been identified and is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety. The method was successfully in-house validated in accordance with the requirements of Commission Decision 2002/657/EC with acceptable performance characteristics. The CRL therefore recommends this method for official control purposes in the frame of this authorisation.

Further testing or validation is not considered necessary.

FAD-2007-0019	**EFSA-Q-2007-132**
Product Name	Ronozyme P5000CT&P20000 L
Active substance	6-phytase (EC 3.1.3.26)
Rapporteur	Annette Plöger Danish Plant Directorate Lyngby, Denmark

In the current application authorisation is sought for Ronozyme® P under the category "zootechnical additives", the functional groups 4(a) and 4(c), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use this product as a digestibility enhancer for pigs and poultry, and as a substance which favourably affects the environment.

The active agent of Ronozyme® P is 6-phytase, produced by a microorganism Aspergillus oryzae (DSM 14223). The activity of 6-phytase is expressed in FYT (phytase) units. According to the applicant, one FYT unit is the quantity of enzyme which liberates 1 micromole of inorganic phosphate per minute from sodium phytate under specific conditions (pH 5.5 and 37oC). The product is intended to be placed on the market as solid formulation Ronozyme® P5000 (CT) containing at least 5000 FYT/g and as liquid form Ronozyme® P20000 (L) containing at least 20000 FYT/g of the product. The product is intended to be incorporated into premixtures and/or complete feedingstuffs to obtain enzyme activity levels of minimum 250 FYT/kg (poultry and pigs except sows) and 750 FYT/kg (sows) of complete feedingstuffs.

For the determination of the activity of Ronozyme® P in the feed additive and premixtures, the applicant proposes single-laboratory validated colorimetric methods, based on the release of inorganic phosphate during the hydrolysis of sodium phytate at pH 5.5 and 37oC by the 6-phytase. The released phosphate forms with molybdate and vanadate ions a coloured complex that is measured at 405 or 415 nm and quantified against a phosphate standard curve. The content of endogenous phosphate - present in the samples and not related to the phytase activity - is measured in a separate analysis and subtracted from the response of the enzymatic activity measurement.

For the analysis of the enzyme activity in the feed additive, the applicant submitted two protocols, which differ in terms of the equipment used - robot versus conventional instruments. Since both methods show similar performance characteristics, the CRL recommends for official control the use of the method requiring conventional instruments, which are easily available in official feed laboratories.

The method for the determination of the enzyme activity in premixtures is similar to the corresponding method for the analysis of feedingstuffs, and shows values for relative standard deviation for repeatability (RSDr) between 1.2 to 5.1% and for intermediate precision (within-laboratory RSDR) between 2.4 to 4.1%. The values for the percentage recovery rate were between 95 and 99%. Based on the obtained method performance characteristics the method is considered suitable for official control purposes.

For the quantification of 6-phytase activity in feedingstuffs the applicant proposes a method which is identical to the harmonised method developed on behalf of the European Association of Feed Additive Manufacturers (FEFANA). This method is currently under evaluation to become standards of the European Committee for Standardisation (CEN) and International Organisation for standardisation (ISO). It is based on the same principle as the method for the determination of the phytase activity in the feed additive. The method has been fully ring trial validated on various feed samples that contained different phytase products including Ronozyme® P,

covering a phytase activity from 750 to 1500 FYT/kg. The obtained precision of the method was 10 % for the RSDr and 12% for RSDR. These precision data have been calculated from the pooled results of all enzyme products included in the study and therefore apply irrespective of the specific phytase to be analysed. Therefore, the CRL recommends this method for official controls to determine the activity of 6-phytase in feedingstuffs at the target activity levels.

Further testing or validation is not considered necessary.

FAD-2007-0020	**EFSA-Q-2007-120**
Product Name	Econase XT L & P
Active substance	Endo-1,4-β-xylanase (EC 3.2.1.8)
Rapporteur	Sulhattin Yasar CRL-FA, European Commission

The current application authorisation is sought for Econase XT L & P under the category 'zootechnical additives', group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Econase XT L&P as a digestibility enhancer for chickens for fattening/reared for laying; turkey for fattening/reared for breeding; and for piglets (weaned). The product is intended to be marketed as solid (Econase XT P) and as liquid (Econase XT L) formulations.

The active agent of Econase XT L&P is endo-1,4-β-xylanase produced by a strain of Trichoderma reesei (CBS 114044). The enzymatic activity is expressed in xylanase unit (BXU) where 1 BXU is the amount of endo-1,4-β-xylanase that liberates 1 nmol xylose from birch xylan per second at pH 5.3 and 50oC. The solid product (Econase XT P) has a target activity of 4 000 000 BXU/g. It is intended to be mixed into premixtures and/or feedingstuffs to provide an enzyme activity range of 6 000 to 24 000 BXU/kg feedingstuffs. The liquid product (Econase XT L) has an enzyme activity of 400 000 BXU/g, and is sprayed directly onto the post-pelleted feed to obtain an enzyme activity range of 6 000 to 24 000 BXU/kg feedingstuffs.

An absolute colorimetric method based on the formation of reducing sugar reacted with dinitrosalysilic acid (DNS) is in-house validated for the determination of the activity of endo-1,4-β-xylanase in the feed additive and premixtures. The following performance characteristics were obtained for liquid and powder feed additives and for turkey and broiler premixtures: recovery greater than 85% and relative standard deviations for repeatability and intermediate precision ranging from 2.0 to 6.0% and 4.0 to 11.0%, respectively. However, low recovery rate (60%) and high relative standard deviations for repeatability and intermediate precision (ca. 24%) were reported for the piglet premixture.

An analytical method based on the measurement of the rate of release of water soluble dyed fragments by endo-1,4-ß-xylanase from the dye cross-linked wheat arabinoxylan in a form of "Xylazyme AX tablet" is in-house validated for the determination of the activity of endo-1,4-β-xylanase in the feedingstuffs. The following performance characteristics were obtained for turkey and broiler feedingstuffs: recovery of 104% and relative standard deviations for repeatability and intermediate precision ranging from 4 to 7% and 5 to 7%, respectively. Insufficient experimental data was provided to establish the validity of analytical method for the determination of active substance (xylanase) in the piglet feedingstuffs.

Based on acceptable performance characteristics, the two proposed methods are considered suitable for determination of xylanase activity - in feed additives, premixtures and feedingstuffs for turkeys and broilers (not for piglets) - for official control purposes in the frame of authorisation.

Further testing or validation for the methods determining xylanase activity - in feed additives, premixtures and feedingstuffs for turkeys and broilers is not considered necessary.

FAD-2007-0022	EFSA-Q-2007-165
Product Name	MycoCell
Active substance	Saccharomyces cerevisiae NCYC R404
Rapporteur	Renata Leuschner CRL-FA, European Commission

In the current application authorisation is sought for MycoCell under the category 'zootechnical additives', functional groups 'digestibility enhancers', 'gut flora stabilisers', 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of MycoCell for dairy cows is requested. MycoCell is provided in two forms, MycoCell concentrate and MycoCell farm packs which contain at least 5 x 109 and 2 x 108 to 2 x 109 c.f.u. viable cells of the yeast strain Saccharomyces cerevisiae NCYC R404 as the active agent per gram, respectively. The feed additive may be effectively used in any feed for dairy cows at a recommended minimum dose of 1 x 1010 c.f.u. per day. For the determination of the active agent (Saccharomyces cerevisiae NCYC R404) in the MycoCell concentrate and farm packs, a pour plate method for enumeration is proposed which is considered appropriate for the intended purpose.

For the quantification of the active agent S. cerevisiae NCYC R404 in premixtures and feedingstuffs, the CRL-FA proposes a ring-trial validated method. The method's performance characteristics are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.17 – 0.36 log10 and 0.55 – 0.60 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively [System. Appl. Microbiol. 2003, 26, 147-153]. The method has a limit of quantification (LOQ) of 10 x 105 c.f.u./kg. The CRL considers the method suitable for official control purposes, if the target level is expressed in terms of c.f.u. per kg feedingstuffs not in terms of c.f.u. per day as specified in the proposed register entry. A PCR method for strain identification which performed appropriately in a ring-trial validation study [System. Appl. Microbiol. 2004, 27, 492-500] is recommended for official controls for the field of application sought.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required by the CRL.

FAD-2007- 0027	EFSA-Q- 2007-133
Product Name	Ronozyme®NP
Active substance	6-phytase (EC 3.1.3.26)
Rapporteur	Christoph von Holst CRL-FA, European Commission

In the current application authorisation is sought for Ronozyme®NP under the category 'zootechnical additives' and the functional groups 4(a) and 4(c), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Ronozyme®NP as a digestibility enhancer for chickens for fattening and as a substance which favourably affects the environment.

The active agent of Ronozyme®NP is 6-phytase, produced by a strain of Aspergillus oryzae (DSM 17594). Enzymatic activity is expressed in FYT (phytase) units. One FYT unit is defined as the amount of enzyme that liberates one □mol of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37oC. The additive is intended to be marketed as a solid formulation Ronozyme NP (CT) containing 10,000 FYT/g and as liquid formulation Ronozyme NP (L) containing 20,000 FYT/g. The products are intended to be mixed into premixtures and/or feedingstuffs to obtain a recommended enzyme activity level rainging from 1500 to 3000 FYT/kg in feedingstuffs.

For the determination of the activity of 6-phytase in feed additive, premixtures and feedingstuffs, the applicant proposes colorimetric methods, based on the release of

inorganic phosphate during the hydrolysis of sodium phytate at pH 5.5 and 37oC by the enzyme phytase. The released phosphate forms with molybdate and vanadate ions a coloured complex that is measured on a spectrophotometer at 415 nm and quantified against the phosphate standard curve. The phosphate content which is present in the samples and which is not related to the phytase activity (endogenous phosphat) is measured in a separate analysis and subtracted from the response of the enzymatic activity measurement.

For the determination of the enzyme activity in the feed additive the applicant submitted two very similar protocols, which mainly differ in terms of the equipment utilised (robot versus conventional instruments). Since both methods deliver very similar results, the CRL recommends the use of the method requiring conventional instruments, which are easier available in official feed laboratories. The method has been single-laboratory validated obtaining values for the relative standard deviation for repeatability (RSDr) ranging from 0.5 to 1.4%. The relative standard deviation for intermediate precision (within-laboratory RSDR) varied from 1.4 to 2.6%.

The method for the determination of the enzyme activity in premixtures, is similar to the corresponding method for the analysis of feedingstuffs, and shows values for RSDr between 1.2 to 5.1% and for the within-laboratory RSDR between 2.4 to 4.1 %. The values for the percentage recovery rate were between 95 and 99%.

For the determination of the enzyme activity in feedingstuffs the applicant proposed a method which is identical with the harmonised method developed on behalf of the European Association of Feed Additive Manufacturers (FEFANA). This method is applicable to the analysis of different phytase products and has been validated through an interlaboratory study. The limit of quantification is 50 FYT/kg feedingstuffs and the obtained values for the recovery rate are close to 100 %. Precision data for the method were taken from the interlaboratory study, obtaining 10 % for the RSDr and 12% for the relative standard deviation for reproducibility. These precision data have been calculated from the pooled results of all enzyme products included in the study and therefore apply irrespective of the specific phytase to be analysed. This method is currently under evaluation to become a standard of the European Committee for Standardisation (CEN).

Based on acceptable performance characteristics, the proposed methods are considered suitable for determination of phytase's activity in feed additive, premixtures and feedingstuffs for official control purposes in the frame of authorisation.

Further testing or validation is not considered necessary.

FAD-2007-0028	**EFSA-Q-2007-166**
Product Name	BioPlus 2B® for rabbits
Active substance	Bacillus subtilis DSM 5750 and Bacillus licheniformis DSM 5749
Rapporteur	Renata Leuschner CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive BioPlus® 2B under the category 'zootechnical additives', functional group 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. The active agent in the additive are viable cells of two microorganism strains, Bacillus subtilis DSM 5750 and Bacillus licheniformis DSM 5749. The additive contains equivalent numbers of both strains and a minimum total concentration of 1.6 x 109 colony forming units (c.f.u.) per gram additive of each of the strains. Specifically, authorisation is sought to use BioPlus® 2B for rabbits for fattening. The conditions of use are proposed with a recommended total dosage of 1.28 x 109 c.f.u./kg complete feedingstuffs including both strains.

For the quantification of the active agents (Bacillus subtilis DSM 5750 and Bacillus licheniformis DSM 5749) of BioPlus® 2B in the feed additive, premixtures and feedingstuffs the applicant uses tryptose blood agar. This is appropriate for the intended purpose.

For the quantitative determination of the colony forming units of the active agents for official controls in the feed additive, premixtures and feedingstuffs, a spread plate enumeration method is recommended which has been ring-

trial validated using premixtures and feedingstuff samples (J. AOAC Int. 2003. 86, 568-575). The method is similar to that used by the applicant using tryptone soya agar. The method's performance characteristics are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.07 – 0.28 log10 and 0.32 – 0.58 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limit of quantification (LOQ) for the method is around 2 to 3 x 106 c.f.u./kg sample which is well below the minimum anticipated target level of application in feedingstuffs.

The identity of the bacterial strains, Bacillus subtilis DSM 5750 and Bacillus licheniformis DSM 5749, was analysed by pulsed-field gel electrophoresis (PFGE) which showed a sufficient degree of differentiation. PFGE is a generally recognised standard methodology for microbial identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2007-0029	**EFSA-Q-2007-139**
Product Name	Levucell® for lambs
Active substance	Saccharomyces cerevisiae CNCM I-1077
Rapporteur	Renata Leuschner CRL-FA, European Commission

In the current application authorisation is sought for LEVUCELL® SC under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of LEVUCELL® SC for lambs in its two forms LEVUCELL® SC20 and LEVUCELL® SC 10ME is requested. LEVUCELL® SC20 (non-coated form) contains at least 20 x 109 of viable cells (c.f.u., colony forming units) of Saccharomyces cerevisiae CNCM I-1077 as active agent per gram and LEVUCELL® SC10ME (coated form) contains at least 10 x 109 c.f.u./g, respectively. The feed additive is intended to be mixed into complete feedingstuffs for lambs at a final concentration of 3 to 7.3 x 109 c.f.u./kg.

For the determination of the active agent (Saccharomyces cerevisiae CNCM I-1077) in the feed additive LEVUCELL® SC, a pour plate method for both forms of the additive and a polymerase chain reaction (PCR) method for identification are proposed which are considered appropriate for the intended purpose. The method was shown to be applicable for the coated and uncoated form of the active agent in the feed additive.

For official controls of the active agent S. cerevisiae CNCM I-1077 in the feed additive, premixtures and feedingstuffs, a similar ring-trial validated pour plate method that is applicable for the feed additive is proposed. The method's performance characteristics are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.17 – 0.36 log10 and 0.55 – 0.60 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively [System. Appl. Microbiol. 2003, 26, 147-153]. The method was shown to be equally applicable for the coated and uncoated form of the active agent in the feed additive, premixtures and is therefore expected to perform similarly in feedingstuffs with regards to the coating. The pour plate method has a limit of quantification (LOQ) of 105 c.f.u./kg sample.

A PCR method for strain identification which performed appropriately in a ring-trial validation study [System. Appl. Microbiol. 2004, 27, 492-500] is recommended for official controls for the field of application sought.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required by the CRL.

FAD-2007-0030	EFSA-Q- 2007-140
Product Name	Elancoban® G100/g200
Active substance	Monensin sodium
Rapporteur	Giuseppe Simone CRL-FA, European Commission

Elancoban®G100/G200 is a product already authorised as feed additive by Regulation (EC) No 1356/2004, amended by Regulation (EC) No 108/2007 under the category 'coccidiostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active agent of Elancoban®G100/G200 is monensin sodium and the authorised inclusion level is ranging from 60 to 125 mg active substance/ kg complete feedingstuffs, depending on the target animal species.

In the current application a modification of the terms of authorisation is sought for Elancoban®G100/G200 according to Article 13(3) of Regulation (EC) No 1831/2003. Specifically, a reduction of the withdrawal time from 3 days to 1 day is sought.

For the determination of the active substance (monensin) in the feed additive an isocratic High Performance Liquid Chromatography (HPLC) method with post-column derivatisation and Ultraviolet (UV) detection is proposed by the applicant. The method is also used to demonstrate the purity of monensin, which mainly consists of monensin A. The performance characteristics are considered acceptable, and the method is considered suitable for official control.

For the determination of the monensin in premixtures and feedingstuffs the applicant proposes a HPLC method, which is based on the same principle as mentioned above. The limit of quantification (LOQ) of the method for the determination of monensin is 4 mg /kg.

The method has also been validated by conducting an interlaboratory study (Coleman et al.,1997) performed on various feed matrices including poultry feedingstuff. Acceptable precision data were obtained, since the repeatability relative standard deviation (RSDr) ranged from 6.1 to 12 % for poultry feed and the reproducibility relative standard deviation (RSDR) ranged from 2.8 to 3.4 % for premixtures and from 8.6 to 15 % for poultry feed. This method is also adopted as AOAC Official method (AOAC Official Method 997.04) and is considered suitable for the intended purpose.

For official control the CRL recommends the ISO standard 14183:2005 which is a multi-analyte method, since it allows for the simultaneous determination of monensin, narasin and salinomycin in feedingstuffs. This method is based on the same principle as the method proposed by the applicant.
Since monensin belongs to group B of Annex I of Council Directive 96/23/EC, analytical methods for the determination of residues in chicken and turkey tissues target matrices for official control have to comply with the criteria specified in Commission Decision 2002/657/EC. A method based on liquid chromatography coupled to low resolution tandem mass spectrometry (LC-MS/MS) is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety. The method was successfully in-house validated in accordance with the requirements of Commission Decision 2002/657/EC with acceptable performance characteristics and is recommended by the CRL for Feed Additives for official control.

Further testing or validation is not considered necessary.

FAD-2007-0034	EFSA-Q-2007-172
Product Name	Advastat
Active substance	Acarbose
Rapporteur	Christoph *von* Holst CRL-FA, European Commission

The current application authorisation is sought for Advastat under the category 'zootechnical additives', group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Advastat as a digestibility enhancer for cattle for fattening and dairy cows for milk production.

The product Advastat contains the active agent acarbose at a concentration of 10 % (w/w %). The intended use of Advastat is to be added to the cereal concentrate part of the ration, obtaining a final concentration of acarbose in the cereal concentrate ranging from 50 to 200 mg/kg. The cereal concentrate containing Advastat may be fed separately or may be incorporated into a total mixed ration. Maximum inclusion levels of acarbose in feed are proposed, which are 70 mg/kg for cattle for fattening and 35 mg/kg for dairy cows for milk production.

For the determination of acarbose in the feed additive the applicant proposed a method based on reversed phase high performance liquid chromatography (HPLC) coupled to Ultraviolet (UV) detection. The target analyte is quantified against standard solutions of acarbose diluted into the mobile phase. Validation experiments were carried at 8 %, 10 % and 12 % of acarbose in the feed additive, obtaining an average value for the recovery of 99 % and relative standard deviations for repeatability ranging between 0.4 and 2.5%. For the determination of the intermediate precision, experiments conducted at acarbose concentration of 10 % yielded a value of 1.1 % for the relative standard deviation for reproducibility.

For the determination of acarbose in cereal concentrate and in feedingstuffs the applicant proposed an identical method based on reversed phase HPLC coupled to a triple quadrupole mass spectrometer (MS/MS). Acarbose is quantified against matrix matched standard obtained from the dilution of a known amount of acarbose into a feed matrix, which does not contain acarbose (blank matrix). The method was validated on various feed matrices containing acarbose at different concentrations ranging from 35 to 1500 mg/kg. Acceptable performance characteristics were obtained, since the percentage recovery rate varied from 95 to 108% and the precision, expressed as percentage relative standard deviation, varied between 2.8 and 5.4%. Sufficient sensitivity of the method was demonstrated, since the lowest calibration level corresponding to acarbose concentration in the matrix of 10 mg/kg is well below the maximum levels in feedingstuffs.

The proposed method for the determination of acarbose in feedingstuffs is only applicable when matrix matched blank feed samples are available. Within the frame of official control this is – however – not always the case. Upon request from the CRL, additional experiments using the "standard addition technique" were conducted by the applicant, since this technique does not require the use of matrix matched blank feed samples. Milled wheat samples containing about 520 mg acarbose/kg were analysed and the results revealed the following method performance characteristics: The precision, expressed as percentage relative standard deviation was 11 % and the percentage rate of recovery was about 125 %.

The CRL considers both methods, i.e. the method based on matrix matched calibration and the method based on the standard addition technique, suitable for official control purposes within the frame of the authorisation. However, when matrix matched blank test samples are not available, only the "standard addition technique" can be applied.

No further testing or validation is required.

FAD-2007- 0035	EFSA-Q- 2007-180
Product Name	Cycostat 66G
Active substance	robenidine hydrochloride
Rapporteur	Cinzia Civitareale Istituto Superiore di Sanità (ISS), Italy

Cycostat 66G is a coccidiostat already authorised as feed additive for use in rabbits for breeding by Commission Regulation (EC) No 2430/1999 and for use in rabbits for fattening, chickens for fattening and turkeys by Commission Regulation (EC) No 1800/2004.

The active agent of Cycostat 66G is robenidine hydrochloride. The authorised inclusion level is ranging from 30 to 66 mg active substance/kg complete feedingstuffs, depending on the species or category of animal.

In the current application a modification of the conditions of authorisation is sought for Cycostat 66G according to Article 13(3) of Regulation (EC) No 1831/2003. Specifically Maximum Residue Limits (MRLs) in chickens for fattening and turkeys are proposed. The provisional MRLs proposed for chickens for fattening are (1) 200 □g kg-1 in muscle, (2) 2000 □g kg-1 in skin/fat, (3) 1250 □g kg-1 in liver and (4) 750 □g kg-1 in kidney. For turkeys the MRLs proposed are (1) 200 □g kg-1 in muscle, (2) 1000 □g kg-1 in skin/fat, (3) 1000 □g kg-1 in liver and (4) 200 □g kg-1 in kidney.

Since robenidine hydrochloride belongs to group B of Annex I to Council Directive 96/23/EC, the confirmatory methods for the detection of residues in target matrices that are suitable for official control have to comply with the criteria specified in Commission Decision 2002/657/EC.

For the determination of the residues of robenidine in tissues of all target species the applicant proposed a High Pressure Liquid Chromatography (HPLC) method with single wavelength Ultraviolet (UV) detection adjusted at 317 nm. A limit of quantification (LOQ) of 100 □g kg-1 has been established for all tissues and animal species which is well below the proposed MRLs. Also acceptable values for the precision and accuracy have been obtained and therefore the method is considered suitable for quantification of robenidine in target tissues at concentrations below or at the proposed MRLs. However, the method does not comply with the required criteria for the confirmation of the presence of robenidine in the case of exceeding the proposed MRLs, since Commission Decision 2002/657/EC requires that for LC/UV, two different chromatographic systems or a second, independent detection method are used.

For confirmatory purposes a method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been developed and is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety, Berlin. This method was successfully in–house validated in accordance with the requirements of Commission Decision 2002/657/EC. The decision limit (CCα) for robenidine was 2.79 μg kg-1 and the detection capability (CCβ) was 4.64 μg kg-1.

Another confirmatory method for detection of robenidine in muscle validated according Commission Decision 2002/657/EC is available and published in literature (Dubois M. et al., (2004). Journal of Chromatography B, 813: 181-189). Also this method is based on liquid chromatography coupled to low resolution tandem mass spectrometry (LC-MS/MS). The CCα was 0.2 μg kg-1 and the CCβ was 0.5 μg kg-1.

The CRL concludes that both LC-MS/MS method can be applied for confirmatory purposes of robenidine in animal tissue.

Further testing or validation is not considered necessary.

FAD-2007-0043	EFSA-Q-2007-190
Product Name	Ecobiol
Active substance	*Bacillus amyloliquefaciens* CECT 5940
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the product Ecobiol® under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. The active agent is Bacillus amyloliquefaciens CECT 5940. The preparations Ecobiol® and Ecobiol® plus contain a minimum total concentration of 1 x 109 or 1 x 1010 colony forming units (c.f.u.) per gram premixture of Bacillus amyloliquefaciens CECT 5940, respectively. Specifically, authorisation is sought to use the preparations Ecobiol® and Ecobiol® for chickens for fattening. The conditions of use are proposed with a recommended final dosage of 1 x 109 c.f.u./kg complete feedingstuffs.

For the quantification of the active agents (Bacillus amyloliquefaciens CECT 5940) of Ecobiol® and Ecobiol® plus in the product, premixtures and feedingstuffs the applicant uses trypticase soya agar (TSA). This is appropriate for the intended purpose.

For the quantitative determination of the colony forming units of the active agents for official controls in the product, premixtures and feedingstuffs, a spread plate enumeration method is recommended which has been ring-trial validated using premixtures and feedingstuff samples (J. AOAC Int. 2003. 86, 568-575). The method is similar to that used by the applicant using tryptone soya agar (TSA). The performance characteristics of the method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.07 – 0.28 log10 and 0.32 – 0.58 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limit of quantification (LOQ) for the method is 10 x 106 c.f.u./kg sample which is well below the minimum anticipated target level of application in feedingstuffs.

The identity of the bacterial strains, Bacillus amyloliquefaciens CECT 5940, was analysed by polymerase chain (PCR) methodology. For official controls in the frame of the authorisation pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2007-0044	EFSA-Q-2008-0013
Product Name	Natugrain®TS and Natugrain®TS L
Active substance	Endo-1,4-β-xylanase (E.C. 3.2.1.8) Endo-1,4-β-glucanase (E.C. 3.2.1.4)
Rapporteur	Sulhattin Yasar, CRL-FA, European Commission

In the current application authorisation is sought for Natugrain®TS and Natugrain®TS L, under the category 'zootechnical additives' and the functional group 4(a), according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought to use Natugrain®TS and Natugrain®TS L as a digestibility enhancer for piglets (weaned), laying hens, chicken and turkeys for fattening and ducks.

The two active agents of Natugrain®TS and Natugrain®TS L are (1) thermostable endo-1,4-β-xylanase, produced by a strain of Aspergillus niger-CBS 109.713, and (2) thermostable endo-1,4-β-glucanase, produced by a strain of Aspergillus niger-DSM 18404. The additive is intended to be marketed as powder (Natugrain®TS) and as liquid formulation (Natugrain®TS L). Both formulations contain an endo-1,4-β-xylanase activity of 5600 TXU/g product and an endo-

1,4-β-glucanase activity of 2500 TGU/g product. They are intended to be mixed into premixtures and/or feedingstuffs to obtain a minimum endo-1,4-β-xylanase activity level of 280 up to a maximum recommended of 840 TXU per kg feedingstuffs and a minimum endo-1,4-β-glucanase activity level of 125 to a maximum recommended of 375 TGU per kg feedingstuffs. Enzymatic activity of endo-1,4-β-xylanase is expressed in thermostable xylanase units (TXU). One TXU is defined as the amount of enzyme that liberates 5 µmol of reducing sugars (xylose equivalents) from wheat arabinoxylan per minute at pH = 3.5 and 40oC. Enzymatic activity of endo-1,4-β-glucanase is expressed in thermostable glucanase units (TGU). One TGU is defined as the amount of enzyme that liberates 1 µmol of reducing sugars (glucose equivalents) from barley betaglucan per minute at pH = 3.5 and 40oC.

For the determination of the activity of endo-1,4-β-xylanase in the feed additive, premixtures and feedingstuffs, the applicant proposes an in-house validated viscosimetric method. Endo-1,4-β-xylanase catalyses the hydrolysis of glycosidic bonds in the substrate wheat arabinoxylan to yield xylose and reduces consequently the viscosity of sample solution. The decrease in viscosity of sample solution, expressed in terms of a drop time, is a measure for the endo-1,4-β-xylanase activity and is determined using a falling ball viscosimeter at pH = 3.5 and 55oC. The quantification is performed using an endo-1,4-β-xylanase standard curve based on reference enzyme provided by the applicant. The method performance characteristics, determined for the feed additive, premixtures and feedingstuffs matrices are: - a relative standard deviation for repeatability (RSDr) ranging from 2.4 to 5.7%; - a relative intermediate precision (RSDR) ranging from 3.4 to 11.8%; - a recovery rate ranging from 82 to 115%; - a limit of detection (LOD) of 11 TXU/kg feedingstuffs; - a limit of quantification (LOQ) of 36 TXU/kg feedingstuffs.

For the determination of the activity of endo-1,4-β-glucanase in the feed additive, premixtures and feedingstuffs, the applicant proposes an in-house validated viscosimetric method. Endo-1,4-β-glucanase catalyses the hydrolysis of glycosidic bonds in the substrate barley betaglucan to yield glucose and reduces consequently the viscosity of sample solution. The decrease in viscosity of sample solution, expressed in terms of a drop time, is a measure for the endo-1,4-β-glucanase activity and is determined using a falling ball viscosimeter at pH = 3.5 and 40oC. The quantification is performed using an endo-1,4-β-glucanase standard curve based on reference enzyme provided by the applicant. The method performance characteristics, determined for the feed additive, premixtures and feedingstuffs matrices, are: -a RSDr ranging from 4.1 to 10.4%; - a RSDR ranging from 7.5 to 12.3%; - the recovery rate ranging from 88 to 117%; - a LOD of 16 TGU/kg feedingstuffs; - a LOQ of 49 TGU/kg feedingstuffs.

Based on acceptable performance characteristics, both methods are considered to be suitable for official control purposes in the frame of authorisation.

Further testing or validation is not considered necessary.

FAD-2007-0046	**EFSA-Q-2007-205**
Product Name	Bactocell PA or Fermaid PA
Active substance	Pediococcus acidilactici CNCM MA 18/5M
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for Bactocell PA or Fermaid PA under the category 'zootechnical additives', functional group 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of this product for fish is requested. The product contains Pediococcus acidilactici CNCM MA 18/5M as active agent at a concentration of 1010 viable cells c.f.u. (colony forming units) per gram. The feed additive can be effectively used in any feed for fish at a target dose of 1 to 3 x 109 c.f.u./kg complete feedingstuffs.

For the determination of the active agent (Pediococcus acidilactici CNCM MA 18/5M) in the feed additive, premixtures and feedingstuffs, the applicant proposes a ring-trial validated method. The method's performance characteristics are standard deviations for repeatability (sr) and

reproducibility (sR) of around 0.13 – 0.17 log10 and 0.20 – 0.26 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively [J. AOAC 2003, 86, 791-801]. This method is recommended for official controls. The spread plate method has a limit of quantification (LOQ) of 10 x 106 c.f.u./kg.

For identification of the active agents, methods suitable for the purpose of analysis were used by the applicant. For official controls pulsed-field gel electrophoresis (PFGE) is recommended for the field of application sought.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required by the CRL.

FAD-2007-0048	EFSA-Q-2008-009
Product Name	Yea-Sacc^{1026}
Active substance	Saccharomyces cerevisiae CBS 493.94
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive Yea-Sacc1026® under the category 'zootechnical additives', functional groups 'digestibility enhancers and gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of YEA-SACC1026® for horses is requested. YEA-SACC1026® contains a minimum of 1 x 109 viable cells (c.f.u., colony-forming units) of Saccharomyces cerevisiae CBS 493.94 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 4.0 x 108 to 2.5 x 1010 c.f.u./kg.

For the determination of the active agent, Saccharomyces cerevisiae CBS 493.94, in the feed additive, a pour plate method based on ISO 7954 and a molecular identification method (polymerase chain reaction (PCR)) are proposed by the applicant, which are considered appropriate. For the determination of the active agent S. cerevisiae CBS 493.94 in premixtures and feedingstuffs the same methods are proposed by the applicant. The enumeration method was validated in a collaborative study [System. Appl. Microbiol. 2003. 26, 147-153]. The method's performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.17 – 0.36 log10 and 0.55 – 0.60 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are 1000 colony forming units (c.f.u) per gram (g) feed additive or premixture and 106 c.f.u./kg feedingstuff. These performance characteristics are considered acceptable. This method is recommended for official controls of the active agent expressed in c.f.u. in the feed additive, premixtures and feedingstuffs.

The PCR method for strain identification was ring trial validated and demonstrated a high level of correct identification between laboratories [System. Appl. Microbiol. 2004. 27, 492-500]. It is therefore considered appropriate for official controls. On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2007-0049	EFSA-Q-2008-010
Product Name	Biosaf® Sc47
Active substance	Saccharomyces cerevisiae NCYC Sc47
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive BIOSAF® Sc47 under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of BIOSAF® Sc47 for dairy buffalos is requested. BIOSAF® Sc47 contains a

minimum of 5 x 109 of viable cells (c.f.u., colony-forming units) of Saccharomyces cerevisiae NCYC Sc47 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 5.0 x 108 to 1.4 x 109 c.f.u./kg.

For the determination of the active agent, a strain of Saccharomyces cerevisiae NCYC Sc47, in the feed additive, a pour plate method and a molecular identification method (polymerase chain reaction (PCR)) are proposed by the applicant, which are considered appropriate. For the determination of the active agent S. cerevisiae NCYC Sc47 in feedingstuffs a similar pour plate method for enumeration and the same molecular PCR method for identification of the strain are proposed by the applicant. This enumeration method was validated in a collaborative study [System. Appl. Microbiol. 2003. 26, 147-153]. The method's performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.17 – 0.36 log10 and 0.55 – 0.60 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are 1000 colony forming units (c.f.u) per gram (g) feed additive or premixture and 106 c.f.u./kg feedingstuff. These performance characteristics are considered acceptable. This method is recommended for official controls of the active agent in the feed additive, premixtures and feedingstuffs.

The PCR method for strain identification was ring trial validated and demonstrated a high level of correct identification between laboratories [System. Appl. Microbiol. 2004. 27, 492-500]. It is therefore considered appropriate for official controls.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2008-0001	**EFSA-Q-2008-80**
Product Name	Avatec 150 G (Lasalocid A sodium 15g/100g)
Active substance	Lasalocid A sodium
Rapporteur	Christoph von Holst, CRL-FA, European Commission

Avatec 150 G (Lasalocid A sodium 15g/100g) is a product already authorised as feed additive by Regulation (EC) No 2037/2005, under the category 'coccidiostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active substance of *Avatec 150 G* is lasalocid A sodium and the proposed inclusion level of this compound in complete feedingstuffs is 75 mg/kg for the minimum content and 120 mg/kg for the maximum content.

In the current application submitted according to Article 4(1) of Regulation (EC) No 1831/2003 the extension of the use of *Avatec 150 G* for other animals, namely pheasants, partridges, quails, guinea fowl, ducks, geese is sought.

For the determination of the active substance (lasalocid A sodium) in the *feed additive* the applicant proposed a reverse-phase High Performance Liquid Chromatography (HPLC) method equipped with Ultraviolet (UV) detection measuring at 304 nm. The performance characteristics estimated on different formulations are considered acceptable, since the obtained percentage relative intermediate standard deviations were below 1.5 % and the percentage relative recovery rates were close to 100 %. The method is considered suitable for official control.

For the determination of lasalocid A sodium in *premixtures* and *feedingstuffs* the applicant proposes a HPLC method, which is very similar to the method utilised for the determination of the active substance in the feed additive. The method has been single-laboratory validation, showing an acceptable performance profile. For official control the CRL recommends the community method for the determination of lasalocid A sodium published in Commission Directive 1999/76/EC. The method is based on reverse-phase HPLC coupled to spectrofluorimetry applying an excitation wavelength of 310 nm and measuring at 419 nm. The method has been fully ring trial validated for premixtures and feeds at concentrations that are very close to the target level of lasalocid A of this application. The obtained values for the percentage relative standard deviation for *repeatability* varied between 2.12 and 5.37 %

and for the percentage relative standard deviation for *reproducibility* varied between 5.03 and 10.7 %, depending on the matrix and concentration level included in the study.

Further testing or validation is not considered necessary.

FAD-2008-0003	EFSA-Q-2008-272
Product Name	Phyzyme XP 10000 TPT and Phyzyme XP 10000 L
Active substance	6-Phytase (EC 3.1.3.26)
Rapporteur	Sulhattin Yasar/Christoph von Holst, CRL-FA, European Commission

In the current application authorisation is sought for Phyzyme XP 10000 TPT and Phyzyme XP 10000 L under the category zootechnical additives, groups 4.a and 4.c, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Phyzyme XP 10000 L and Phyzyme XP 10000 TPT as a digestibility enhancer and as a substance that favourably affects the environment for chickens for fattening, laying hens, ducks for fattening, turkeys for fattening, piglets, pigs for fattening and sows. The product is intended to be marketed as liquid (Phyzyme XP 10000 L) and as dry (Phyzyme XP 10000 TPT) form.

The active substance of Phyzyme XP 10000 TPT and Phyzyme XP 10000 L is 6-Phytase (EC 3.1.3.26) produced by Schizosaccharomyces pombe (ATCC 5233). The enzymatic activity is expressed in FTU, where 1 FTU is the amount of enzyme which liberates one µmol of inorganic phosphate from sodium phytate per minute at pH 5.5 and 37°C. The liquid and the solid form of the product have an minimum enzymatic activity of 10000 FTU/g and the product is intended to be mixed into compound feedingstuffs to obtain a phytase activity range of 150 – 1000 FTU/kg depending on the target species or category of animal.

For the determination of the phytase activity in the feed additive a spectrophotometric method is proposed. The method is based on the principle that phytase releases inorganic phosphate from a sodium phytate substrate, which in the presence of a molybdate/vanadate reagent forms a yellow complex. The yellow complex is measured with a spectrophotometer and the inorganic phosphate is quantified against a phosphate standard curve. The applicant method was in-house validated, obtaining 7.7% for the percentage relative standard deviation for repeatability (RSDr). The CRL considers this method suitable for the intended purpose.

For the determination of the phytase activity in premixtures the applicant proposes a method which is based on the dilution of the premixture sample into blank feed matrix and applying the corresponding method for the determination of the phytase activity in feedingstuffs. The CRL confirms the principle validity of such an approach. However, since a precise protocol of this method and corresponding validation data are missing, the suitability of the method for official control cannot be evaluated.

For the determination of the phytase in feedingstuffs a harmonised method is available, which is based on the same principle as the applicant's method for the determination of the phytase activity in the feed additive. The harmonised method is currently under evaluation to become a standard of the European Committee for Standardisation (CEN) and has been validated in an interlaboratory study which was performed on feedingstuffs fortified with different phytase products including a feed additive that contained the specific enzyme of the present application (Gizzi et al., J. of AOAC International, 91, 259-267, (2008)). The obtained values of the precision for the various products could be pooled, obtaining 10% for the RSDr and 12% for the relative standard deviation for reproducibility (RSDR). Based on the acceptable method performance profile the CRL recommends this method for official control of 6-phytase in the feedingstuffs.

No further testing or validation is required.

FAD-2008-0005	EFSA-Q-2008-381
Product Name	Selsaf
Active substance	Selenium enriched yeast Saccharomyces caerevisiae CNCM I-3399)
Rapporteur	Piotr Robouch, CRL-FA, European Commission

In the current application authorisation is sought for Selsaf under the category/functional group (3/b), nutritional additives/compounds of trace elements, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Selsaf as a source of selenium for all animal species. Selsaf is an inactivated Selenium enriched yeast (Saccharomyces cerevisiae CNCM I-3399) product, containing high levels of the essential trace element selenium. The inactivated and dried Selenium enriched yeast product is blended with non viable dehydrated yeast (Saccharomyces cerevisiae CNCM I-3399) to adjust the selenium content. The final product is an inactivated whole cell yeast containing minimum 2000 mg/kg of total selenium with a maximum of 2% of residual inorganic selenium. At least 60% of the total organic selenium is in the form of seleno-methionine. Selsaf is added to the feedingstuffs to obtain a concentration of total Se up to 0.5 mg/kg.

The active substance is measured as total selenium regardless of its chemical form, i.e. independently of whether it is present as organically-bound or inorganic Se.

For the determination of the active substance in Selsaf either flame atomic absorption spectrometry (FAAS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) methods are proposed by the applicant. Since both methods are based on well known principles, they are considered suitable for the determination of selenium in the feed additive.

For the determination of the active substance (total selenium) in premixtures and feedingstuffs the same two methods (FAAS and ICP-AES) are proposed. Since information on a complete validation study performed on the target feed was not provided, the suitability of these methods for official control purposes cannot be evaluated.

However, for official control regarding the determination of the active substance in premixtures and feedingstuffs, the CRL recommends an analytical method that has been ring-trial validated in the relevant matrices at the relevant concentrations of the active substance. The method and the results from the related inter-laboratory study are presented in the method collection of the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, Germany). The method for the determination of selenium by hydride generation atomic absorption spectrometry (HGAAS) after microwave digestion – based on the extraction with 65% nitric acid and 30% H2O2. The following method performance characteristics are reported: - a reproducibility relative standard deviation (RSDR) of 7.3 % for a pre-mixture containing 112 mg/kg of Se; RSDR = 7.4 % for a feedingstuffs containing 0.48 mg/kg of Se and the limits of quantification are clearly below the legal limit of 0.5 mg Se /kg feed and therefore acceptable for the purpose of analysis. This VDLUFA method is currently being adopted as a CEN standard.

No further testing or validation is required.

FAD-2008-0006	EFSA-Q-2008-302
Product Name	Biosprint®
Active substance	Saccharomyces cerevisiae BCCM/MUCL 39885
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive Biosprint® under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Biosprint® for sows is requested. Biosprint® in one of its three commercialised forms: powder, spherical

or oval granulated contains a minimum of 1 x 109 viable cells (c.f.u., colony-forming units) of Saccharomyces cerevisiae BCCM/MUCL 39885 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 6.4 x 109 to 1.9 x 1010 c.f.u./kg.

For the determination of the active agent, Saccharomyces cerevisiae BCCM/MUCL 39885, in the feed additive, a pour plate method based on ISO 7954 and a molecular identification method (polymerase chain reaction (PCR)) are proposed by the applicant, which are considered appropriate. For the determination of the active agent S. cerevisiae BCCM/MUCL 39885 in premixtures and feedingstuffs the same methods are proposed by the applicant. The enumeration method was validated in a collaborative study [System. Appl. Microbiol. 2003. 26, 147-153]. The performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.17 – 0.36 log10 and 0.55 – 0.60 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are around 1000 colony forming units (c.f.u) per gram (g) feed additive or premixture and around 106 c.f.u./kg feedingstuff. These performance characteristics are considered acceptable. This method is recommended for official controls of the active agent expressed in c.f.u. in the feed additive, premixtures and feedingstuffs.

The PCR method for strain identification was ring trial validated and demonstrated a high level of correct identification between laboratories [System. Appl. Microbiol. 2004. 27, 492-500]. It is therefore considered appropriate for official controls.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2008-0007	**EFSA-Q-2008-289**
Product Name	Bonvital
Active substance	Enterococcus faecium DSM 7134
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive Bonvital under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. The active agent in the additive is Enterococcus faecium DSM 7134. The additive is available in two forms (powder or granules (micro-encapsulated)) both of which contain a minimum concentration of 1 x 1010 colony forming units (c.f.u.) per gram. Specifically, authorisation is sought to use Bonvital for chickens for fattening. The conditions of use are proposed with a recommended dosage of 0.2 to 2.0 x 109 c.f.u./kg.

For the quantification of the active agent (Enterococcus faecium DSM 7134) of Bonvital in the feed additive, premixtures and feedingstuffs, an appropriate pour plate method using a selective enterococci agar was proposed by the applicant. The method was in-house validated and was shown to be transferable to three external laboratories. The method precision data resulting from the in-house and between-laboratory trials were acceptable for the intended purpose.

For official controls regarding the quantitative determination of the colony forming units of the active agent in the feed additive, premixtures and feedingstuffs, a spread plate enumeration method is recommended which has been fully ring-trial validated (J. Appl. Microbiol. 2002, 93, 781-786).

The method's performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.12 – 0.20 log10 and 0.23 – 0.41 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are around 104 colony forming units (c.f.u.) per gram (g) feed additive or premixture and around 107 c.f.u./kg feedingstuff.

The identity of the bacterial strain, Enterococcus faecium DSM 7134, was analysed by a range of techniques including biochemistry, protein-fingerprinting and molecular methods such as polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (PFGE). PFGE is a generally recognised standard methodology for microbial

identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2008-0009	EFSA-Q-2008-287
Product Name	Toyocerin
Active substance	*Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive Toyocerin® under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Toyocerin® for rabbit breeding does from service until weaning is requested. Toyocerin® contains a minimum of 1 x 1010 colony forming units (c.f.u.) of viable spores of Bacillus cereus var. toyoi NCIMB 40112/CNCM I-1012 per gram (g). The feed additive is intended to be mixed into complete feedingstuffs at a final concentration of 0.2 to 1 x 109 c.f.u./kg.

For the quantification of the active agent (Bacillus cereus var. toyoi NCIMB 40112/CNCM I-1012) of Toyocerin® in the feed additive, premixtures and feedingstuffs appropriate enumeration methods were proposed by the applicant. Analysis data confirmed an appropriate method performance in a second laboratory.

For official controls regarding the quantitative determination of colony forming units of the active agent in the feed additive, premixtures and feedingstuffs, another surface plate count enumeration method is recommended which includes a heat-treatment and uses subsequently a non-selective agar. This method has been fully ring-trial validated (J.AOAC Int. 2003, 86, 568-575). The method's performance characteristics revealed standard deviations for repeatability (sr) and reproducibility (sR) of around 0.07 – 0.09 log10 and 0.35 – 0.32 log10 calculated from the base 10 logarithms of the measured c.f.u./g premixture or feedingstuff, respectively. The limits of quantification (LOQ) of this method are around 104 c.f.u./g feed additive or premixture and around 107 c.f.u./kg feedingstuff which is well below the minimum anticipated target level of application.

The identity of the bacterial strain, Bacillus cereus var. toyoi NCIMB 40112/CNCM I-1012, was analysed by a range of techniques including biochemistry, phage-typing, molecular methods and pyrolysis mass spectrometry. Pulsed field gel electrophoresis (PFGE) is a generally recognised standard methodology for microbial identification and is therefore considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2008-0012	EFSA-Q-2008-303
Product Name	Miya-Gold® S
Active substance	Clostridium butyricum MIYAIRI 588
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the microbial product Miya-Gold® S as a feed additive under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Miya-Gold® S for chickens for fattening is requested. Miya-Gold® S contains 5.0 x 108 viable cells (c.f.u., colony-forming units) of Clostridium butyricum MIYAIRI 588 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at final concentrations of 5 x 108 c.f.u./kg.

For the quantification of the active agent, Clostridium butyricum MIYAIRI 588, in the feed additive and premixtures an enumeration method using iron sulfite agar as described in ISO Standard 15213 (2003) is proposed by the applicant. For feedingstuffs the applicant proposes a selective CMB588 agar (Microbiol. Immunol. 1997, 41(9), 665-671). A three-laboratory validation study demonstrated satisfactory performance of the iron sulfit agar and selective CMB588 agar using samples of the additive, premixtures and feedingstuffs. The method performance characteristics were standard deviations for repeatability (sr) and reproducibility (sR) of below 0.10 log10 and of between 0.09 – 0.31 log10 calculated from the base 10 logarithms of the measured c.f.u./g, respectively. Presumptive colonies of Clostridium butyricum MIYAIRI 588 shall be confirmed microscopically, for absence of growth under aerobic conditions and for formation of butyric acid on plate count agar.

For official controls of the active agent Clostridium butyricum MIYAIRI 588 in the feed additive and premixtures iron sulfite agar according to ISO 15213 and in feedingstuffs the selective CMB588 agar (Microbiol. Immunol. 1997, 41(9), 665-671) are recommended followed by confirmation of presumptive Clostridium butyricum MIYAIRI 588 colonies on plate count agar. The CRL-FA recommends using for analysis of premixtures 20 g samples for feedingstuffs 50 g.

The limits of quantification (LOQ) of the spread plate methods are around 104 colony forming units (c.f.u) per gram (g) feed additive or premixture and around 107 c.f.u./kg feedingstuff which is well below the anticipated target concentrations

The applicant applied a wide range of biochemical and molecular techniques for strain identification of Clostridium butyricum MIYAIRI 588 including pulsed field gel electrophoresis (PFGE) [Jpn. Pharmacol. Ther. 2000, 28(12), 999-1004]. PFGE is widely applied for microbial strain identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2008-0014	**EFSA-Q-2008-337**
Product Name	MLB
Active substance	Lactobacillus acidophilus **DSM** 13241
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for the microbial feed additive MLB under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of MLB for dogs and cats is requested. MLB contains a minimum of 1.0 x 1011 viable cells (c.f.u., colony-forming units) of Lactobacillus acidophilus DSM 13241 (as active agent) per gram. The feed additive is intended to be mixed into complete feedingstuffs at final concentrations of 6 x 109 to 1 x 1011 c.f.u./kg for dogs and 3 x 109 to 2.5 x 1010 c.f.u./kg for cats, respectively.

For the enumeration of the active agent, Lactobacillus acidophilus DSM 13241, in the feed additive, premixtures and feedingstuffs a plate count method using MRS agar is proposed by the applicant. For official controls of the active agent Lactobacillus acidophilus DSM 13241 in the feed additive, premixtures and feedingstuffs a similar spread plate enumeration method using MRS agar is recommended. This enumeration method was validated in a collaborative study [Food Microbiol. 20 (2003) 57-66]. The method's performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.10 – 0.26 log10 and 0.18 – 0.39 log10 calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are 104 colony forming units (c.f.u) per gram (g) feed additive or premixture and 107 c.f.u./kg feedingstuff.

The applicant applies pulsed field gel electrophoresis (PFGE) for strain identification of Lactobacillus acidophilus DSM 13241. PFGE is a widely recognised methodology for microbial strain identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

FAD-2008-0015	EFSA-Q-2008-421
Product Name	Bactocell PA 10 or Fermaid PA 10
Active substance	Pediococcus acidilactici CNCM MA 18/5M
Rapporteur	Renata Leuschner, CRL-FA, European Commission

In the current application authorisation is sought for Bactocell PA 10 or Fermaid PA 10 under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. Specifically, the use of Bactocell PA 10 for shrimps is requested. Bactocell concentrate consists of 1010 viable cells c.f.u. (colony forming units) per gram of Pediococcus acidilactici CNCM MA 18/5M as active agent. The feed additive can be effectively used in any feed for shrimps at a recommended dose of 1 x 109 to 1 x 1010 c.f.u./kg complete feedingstuffs.

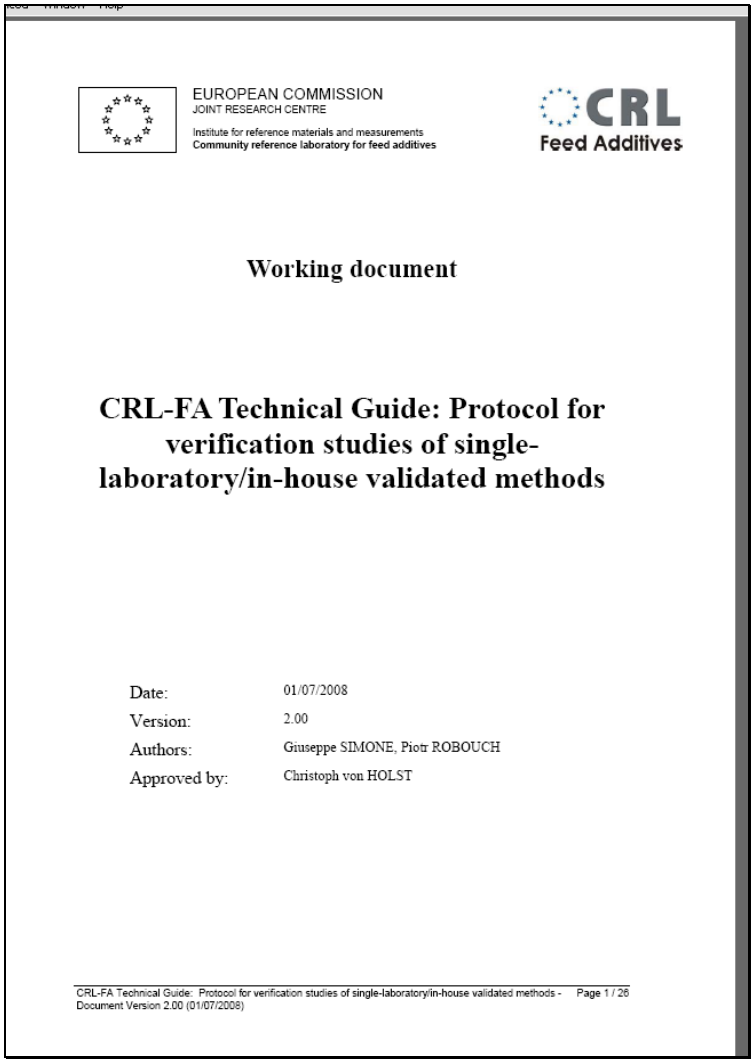
For the determination of the active agent (Pediococcus acidilactici CNCM MA 18/5M) in the feed additive, premixtures and feedingstuffs, a ring-trial validated method is proposed. The method's performance characteristics are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.13 – 0.17 log10 and 0.20 – 0.26 log10 calculated from the base 10 logarithms of the measured c.f.u./g, respectively [J. AOAC 2003, 86, 791-801]. This method is recommended for official controls to determine colony forming units in the frame of the authorisation. The spread plate method has a limit of quantification (LOQ) of around 107 c.f.u./kg which is well below the anticipated concentrations of the active agent in feedingstuffs.

For identification of the active agents, methods suitable for the purpose of analysis were used by the applicant. For official controls pulsed-field gel electrophoresis (PFGE) is recommended for the field of application sought [J. Microbiol. Methods 2006, 64, 120-125].

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required by the CRL.

Annex II

CRL-FA Verification Protocol Form



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for reference materials and measurements
Community reference laboratory for feed additives

CRL Feed Additives

Working document

CRL-FA Technical Guide: Protocol for verification studies of single-laboratory/in-house validated methods

Date:	01/07/2008
Version:	2.00
Authors:	Giuseppe SIMONE, Piotr ROBOUCH
Approved by:	Christoph von HOLST

CRL-FA Technical Guide: Protocol for verification studies of single-laboratory/in-house validated methods - Page 1 / 26
Document Version 2.00 (01/07/2008)

Full document available from CRL website
http://irmm.jrc.ec.europa.eu/html/CRLs/crl_feed_additives/authorisation/guidance_for_applicants/index.htm

ANNEX III: VERIFICATION STUDY REPORT

⚐ One report for each analyte, to be compiled by the Independent Expert Laboratory

Section 1

1.1. Laboratory Identification

Company / Institute	
Department	
Laboratory / Group	

1.2. Experience in the field, *related to the method(s) under investigation*

- Your laboratory carries this type of analyses
  ☐ Often ☐ Seldom ☐ Never
- Accreditation: ☐ Yes ☐ No ☐ Pending
  - according to/compliant with: *(specify standard)*
  - specify scope of accreditation :

1.3. List of samples provided by the Applicant

	Description, *specify analyte, matrix (specify major constituents)*	**Amount delivered & units**
Standard(s) for Calibration	▪ . ▪ .	▪ . ▪ .
Blank(s), if applicable	▪ . ▪ .	▪ . ▪ .
Known Samples	▪ . ▪ .	▪ . ▪ .
Blind Samples	▪ . ▪ .	▪ . ▪ .

Sample Delivery Date	
Storage conditions used (*short description)*[11]	
Date (s) of Measurement campaign	

[11] *Specify relevant information, such as temperature, humidity, darkness/light, etc.*

Verification Study Report approved by:

Name	
Function	
Date	
Signature	

Send the completed Report to the Applicant. Thank you

Section 2 (⚐ One for each **method**)

2.1. Scope: Verification of

Title of the Method

for the determination of (specify analyte)

in the following matrices

- ☐ **Feed Additive**
- ☐ **Premix** for *(specify species)*
- ☐ **Feedingstuffs** for *(specify species)*
- ☐ **Water**
- ☐ **Target tissues/animal products** *(specify tissues/product)*

2.2. Review of the Operating Procedure (OP)

(list of comments discussed with the Applicant)

Num	Describe problem	Modification suggested
1		
2		
3		
…		

2.3. Overall evaluation of each method

Is the Operating Procedure clear & understandable?	☐ Yes ☐ No ☐ Could improve (cf. Section 2.2.)
Is the Operating Procedure easy /practical?	☐ Yes ☐ No
Do your results confirm the in-house validate characteristics?	☐ Yes ☐ No ☐ Not quite
Would you implement this method in your laboratory?	☐ Yes ☐ No Explain why?
Do you have knowledge of similar methods fit for the purpose?	☐ Yes ☐ No If Yes, please provide reference:

Section 3 (☞ One for each **matrix**)

3.1. Calibration *(when applicable)*

☞ Provide one set of calibration **for each** matrix.

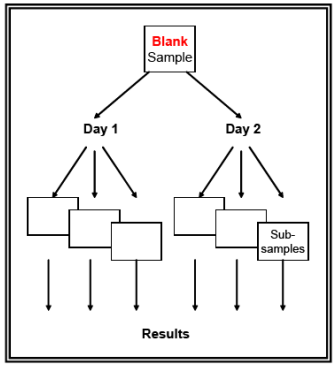
Method	
Analyte	
Matrix	

Calibration date (Day 1)	
Standard for calibration	
Calibration Equation & correlation coeff.	
Calibration Graph (*insert Graph*➔)	

Calibration date (Day 2)	
Calibration Equation & correlation coeff.	
Calibration Graph (*insert Graph*➔)	

Comments - describe experimental problems encountered (if any)

3.2. Blank *(when applicable)*



Method	
Analyte	
Matrix	

	Date	Sample ID	Sample intake[12]	Result (*) or less than value
Day 1				
Day 2				
		Units:		

(*) Provide (when possible) 2 significant digits *(i.e. 0,12 or 1,2 or 12 or 120)*

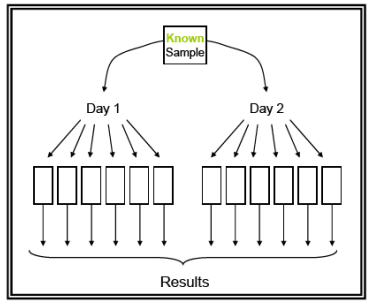
Estimates of Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD	
LOQ	
Units:	

Comments
- Explain / specify how LOD and LOQ were calculated
- describe experimental problems encountered (if any)

[12] Amount of sample used for the analysis

3.3. "Known" samples



Method	
Analyte	
Matrix	
Expected content, unit	

	Date	Sample ID	Sample intake[13]	Results (a)
Day 1				
Day 2				
		Units:		

(a) Provide (when possible) 3 significant digits *(i.e. 0,123 or 1,23 or 12,3 or 123)*

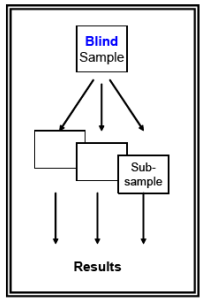
[13] Amount of sample used for the analysis

Estimates of relative standard deviations for repeatability (RSD_r) and intermediate precision (RSD_R); and Recovery

RSD_r (%)	
RSD_R (%)	
Recovery	

Comments
- Specify calculation of RSD_r, RSD_R and Recovery rate
- describe experimental problems encountered (if any)

3.4. "Blind"/*unknown* samples



Method	
Analyte	
Matrix	

Measurement Date	Sample ID	Sample intake[14]	Results (*)
	Units:		

(*) Provide (when possible) 3 significant digits *(i.e. 0,123 or 1,23 or 12,3 or 123)*

Computed mean, standard deviation and RSD%

Mean (#)	
Repeatability Standard Deviation (#)	
RSD%	
(#) Units:	

Comments
- describe experimental problems encountered (if any)

[14] Amount of sample used for the analysis

ANNEX IV: VERIFICATION REPORT

One report for each analyte, to be compiled by the Applicant

Section 1

1.1. Introduction:

One/Several method(s) has/have been in-house validated:

- ☐ Method 1 (short descriptor) for the determination of (specify active substance) in the **Feed Additive**.
- ☐ Method 2 *(short descriptor)* for the determination of *(specify active substance)* in **Premix**.
- ☐ Method 3 *(short descriptor)* for the determination of *(specify active substance)* in **Feedingstuffs**
- ☐ Method 4 (short descriptor) for the determination of (specify active substance) in the **Water**.
- ☐ Method 5 (short descriptor) for the determination of (specify active substance) in the **target tissues/animal products** (specify tissue/product).

(adapt accordingly - add or remove)

The following independent expert laboratory (denoted here after as Lab.2) was selected to confirm the outcome of the validation study(ies):

Company / Institute	
Department	
Laboratory / Group	

This report:
a) presents the comments made by Lab.2 concerning the Operating Procedure document and the consequent corrections implemented;
b) (if required) provides additional experimental evidence resulting from a major modification in the experimental protocol (see previous point);
c) compares the performance characteristics submitted by Lab.2 to those obtained during the in-house validation study;
d) draws conclusions about the successful verification study.

1.2. Review of suggested modifications for the Operating Procedure(s):

OP Method #	Comment #	Modification Suggested *by Lab.2*	Reply/Justification *by Applicant*	Category (*)
1	1			
1	2			
1	3			
…				

(*) E: editorial; m: minor; M: major/critical

☐ All modifications are implemented accordingly. The final operating procedure is included in Enclosure .

☐ At least one "Major/critical" modification was implemented; the following additional experimental data are submitted to complement the in-house validation study. See Enclosure .

Section 2 (⚐ One for each **matrix**)

2.1. Comparison of performance characteristics

Method	
Analyte	
Matrix	

	Applicant	Lab.2	Significance test used	Acceptable? Yes or No
LOD			---	
LOQ			---	
RSD_r %			---	
RSD_R %(*) intermediate precision			Compare with Target (*)	
Concentration **Known** Sample		---	---	
Recovery (%)			t-test	
Concentration **Blind** sample (X)			z-score[15]	

(*) *Target derived from - Legislation or - the Horvitz equation or - an expert opinion; Target to be specified in the comments.*

Comments

Conclusion

Successful Verification Study: ☐ Yes ☐ No

[15] z-score defined as: $(X_{App.} - X_{Lab.2})/(RSD_R * X_{App.})$. The result is considered *satisfactory* when $|z| \leq 2$.

Verification Report Approved by:

Name	
Function	
Date	
Signature	

Notes

European Commission

EUR 24008 EN – Joint Research Centre – Institute for Reference Materials and Measurements
Title: Community Reference Laboratory for Feed Additives - Annual Report 2008, Authorisation Activities
Author(s): C. von Holst, S. Yasar, G. Simone, J. Petrova, R. Leuschner, S. Staes, M. De Smet and P. Robouch (Editor)
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Abstract
In 2008 the CRL-FA Authorisation submitted a total of 27 evaluation reports to EFSA and organised the annual workshop attended by 27 National Reference Laboratories. This report provides a detailed overview of the yearly activities, thus including a major deliverable of the year 2008: The CRL-FA Guide: *"Protocol for verification studies of single laboratory / in-house validated methods"*.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

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