





#### **COURSE PROGRAM**

Academic Year: 2019/2020

Identificación y características de la asignatura								
Code	502238			5,25				
Course name (English)	Técnicas Rápidas de Análisis Microbiológico							
Course name (Spanish)	Rapid Microbiological Analysis Techniques							
Degree programs	DEGREE IN SCIENCE AND FOOD TECHNOLOGY							
Faculty/School	School of Agricultural Engineering							
Semester	Second (8°)		Type of course		Optional			
Module	Optic	nal	าลไ					
Matter	Rapid Microbiological Analysis Techniques							
				Lec	turer/s			
Name			Office		E-mail	Web pag		
Mª José Benito Bernáldez		Edific	-720 cio Valle Jerte	mjben	ito@unex.es	http://www.unex.es/investigacion/gru /camiali		
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Subject Area			Nutrition and Bromatology					
Department	Α	Animal Production and Food Science						
Coordinating Lecturer (If more than one) Ma José Benito Bernáldez					7			
				Compe	etencies*			
			Ва	sic Co	mpetencies			
CR1 Students h	ovo do	mon	ctrotod	that th	1011 POSSOSS 01	nd understand know	uladaa in an	

CB1 - Students have demonstrated that they possess and understand knowledge in an area of study that is based on general secondary education, and is often found at a level that, while supported by advanced textbooks, also includes some aspects that involve knowledge from the cutting edge of their field of study.

<sup>\*</sup> The sections concerning competencies, course outline, educational activities, teaching methodologies, learning outcomes and assessment systems must conform to that included in the ANECA verified document of the degree program.







- CB2 Students know how to apply their knowledge to their work or vocation in a professional manner and possess the skills that are usually demonstrated by making and defending arguments and solving problems within their field of study.
- CB3 Students have the ability to gather and interpret relevant data (usually within their area of study) to make judgments that include reflection on relevant social, scientific or ethical issues.
- CB4 Students are able to convey information, ideas, problems and solutions to both specialised and non-specialised audiences.
- CB5 Students have developed the learning skills necessary to undertake further studies with a high degree of autonomy.

#### **General Competencies**

- CG1 In the area of process and product quality management and control capacity to establish quality control procedures and manuals; implement and manage quality systems; analyse and report on food, raw materials, ingredients, additives; evaluate and improve the quality of methods of analysis applied to food control.
- CG2 In the field of food safety acquire knowledge to assess the hygienic, sanitary and toxicological risk of a process, food, ingredient, packaging; identify the possible causes of food deterioration and establish traceability mechanisms.
- CG6 In the field of collective catering, to know how to manage collective catering services; to propose suitable feeding programmes for the different groups; to ensure the quality and food safety of the food managed; to provide adequate training for the personnel involved.
- CG8 In the field of legal, scientific and technical advice, to be able to study and interpret administrative reports and files relating to a product, in order to be able to respond reasonably to the question posed; to know the legislation in force; to defend before the administration the need to modify regulations relating to any product.

#### **Transversal Competencies**

- CT1 Skills in ICT at a basic level.
- CT2 Knowledge of a foreign language (English).
- CT3 Provide knowledge and teaching-learning methodologies at different levels; collect and analyse existing information.
- CT4 Capacity for effective and efficient problem solving, demonstrating principles of originality and self-direction.
- CT5 Capacity for critical reasoning, analysis and synthesis.
- CT6 Effective and efficient management capacity with entrepreneurial spirit, initiative, creativity, organization, planning, control, decision making and negotiation.
- CT7 Capacity for autonomous learning and concern for knowledge and lifelong learning.
- CT8 Knowledge of the principles and methods of scientific and technical research.
- CT9 Ability to work in a team.







CT10 - Permanent concern for quality and the environment, occupational risk prevention and social and corporate responsibility.

CT11 - Working appropriately in a biological laboratory with biological material including safety, handling, disposal of biological and chemical waste and annotated record of activities.

#### **Specific Competencies**

CECSA1: Identify and establish the possible causes of food spoilage.

CECSA2: To know and evaluate the hygienic-sanitary and toxicological hazards in food and their effects on the health of the consumer.

CECSA3 - Capacity to know, understand and promote safety and quality in the food chain, from the production of raw materials to consumption.

#### **Contents**

#### Course outline\*

Know the fundamentals and applications of rapid and automated techniques. Importance of the rapid techniques application for the detection of microorganisms. Microbiological analysis of food using specific fast and accurate techniques. Characterization and identification of microorganisms using nucleic acid techniques. Detection of microorganisms or their products by physical-chemical and immunological methods. Uses of bioassays and related methods. This subject will be taught in English.

#### Course syllabus

Denomination of Lesson 1: GENERAL ASPECTS

Contents of Lesson 1:

1.1. INTRODUCTION. Systems, methods, importance of new detection techniques. General concepts.

# Denomination of Lesson 2: **DETECTION TECHNIQUES OF MICROORGANISMS OR THEIR PRODUCTS IN FOODS BY MOLECULAR BIOLOGY METHODS**Contents of Lesson 2:

- 2.1. NUCLEIC ACIDS. Physical and chemical structure of DNA. Renaturalization. Structure of RNA.
- 2.2. RECOMBINANT DNA TECHNOLOGY I. DNA isolation: extraction. Visualization of DNA. DNA fragmentation: restriction enzymes. Union of DNA molecules.
- 2.3. RECOMBINANT DNA TECHNOLOGY II. Nature of vectors: plasmids and phage vectors. Genes synthesized in the laboratory: complementary DNA. Cloning: stages.
- 2.4. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS I. Hybridization: Definition of probe. Labelling of the probe. Advantages of the probes. Sensitivity and specificity. Fragment hybridization techniques: Southern Blotting and Northern Blotting.
- 2.5. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS II. Sequencing, visualization and types. DNA digestion with restriction enzymes (REN). Amplification of DNA fragments by the polymerase chain reaction: limitations and efficiency; uses and applications. Study of C + G values. Complementarity of DNA.
- 2.6. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS III. Studies of genetic polymorphisms I. Karyotyping. Restriction Fragment Analysis (RFLPs), rDNA study.







Study of non-ribosomal DNA and RNA (RT-PCR).

2.7. TECHNIQUES USED FOR THE STUDY OF NUCLEIC ACIDS VI. Studies of genetic polymorphisms II. DNA fingerprinting or fingerprinting, Random PCR or RAPD, PCR fingerprinting or PCR fingerprinting, polymorphisms of amplified DNA fragments or AFLP.

# Denomination of Lesson 3: TECHNIQUES FOR DETECTING MICROORGANISMS OR THEIR PRODUCTS IN FOODS BY PHYSICAL, CHEMICAL AND IMMUNE METHODS

Contents of lesson 3:

- 3.1. PHYSICAL METHODS: Impedance, microcalorimetry and flow cytometry. Turbidimetry.
- 3.2. CHEMICAL METHODS: determination of adenosine triphosphate (ATP), direct Epifluorescence (DEFT). Radiometry. Fluorogenic and chromogenic substrates. API Galleries. Thermostable nuclease. Limulus lysate for endotoxin screening (LAL).
- 3.3. CHROMATOGRAPHIC METHODS: classification of chromatographic methods. Analysis and detection.
- 3.4. IMMUNOLOGICAL METHODS I: Precipitation in A) liquid medium: quantitative and qualitative and in B) solid medium: double immunodiffusion, radial immunodiffusion and immunoelectrophoresis. Agglutination: agglutination in port, sero-agglutination in tube and direct hemagglutination in microplate.
- 3.5. IMMUNOLOGICAL METHODS II: Immunofuorescence: direct and indirect. Radioimmunoassay: solid phase; Direct and indirect. ELISA: Fundaments and types. Direct ELISA. Indirect ELISA. Double ELISA antibody sandwich. Indirect ELISA double antibody sandwich. ELISA competition.
- 3.7. BIOSENSORS. Definition. Components of a biosensor. Characteristics of biosensors. Future of biosensors.

# Denomination of lesson 4: TECHNIQUES FOR DETECTING MICROORGANISMS OR THEIR PRODUCTS IN FOOD THROUGH BIOASSAYS AND RELATED METHODS

Contents of Lesson 4:

- 4.1. TESTS IN LIVE ANIMALS. Lethality in mouse, lactating mouse, rabbit and mouse diarrhea, kitten test, skin tests in rabbit and guinea pig.
- 4.2. MODELS REQUIRING SURGICAL TECHNIQUES. Ligament bowel ligation techniques. RITARD method
- 4.3. SYSTEMS OF CELL CULTURES. Human cells of mucous epithelium. Guinea pig intestinal cells. Vero cells

Competencies acquired: CB1, CB4, CB5, CG1, CG2, CG6, CT3, CT7, CT8,

CT10, CECSA1, CECSA2, CECSA3 Learning outcomes: 1,2,3,5,6,7,8,9,10

#### **Practical sessions**

Denomination of lesson: Practical session 1.

Contents of Lesson: Identification of microbial toxins by nucleic acid techniques (PCR). Bacterial DNA extraction, real-time PCR Competencies acquired: CB2, CB3, CG1, CG2, CT8, CT9, CT11, CECSA1, CECSA2, CECSA3

Learning outcomes: 1, 2, 3, 5, 6, 7, 8, 9







Denomination of lesson: Practical session 2.

Contents of Lesson: Identification of different microorganisms by DNA profiles. Competencies acquired: CB2, CB3, CG1, CG2, CT8, CT9, CT11, CECSA1,

CECSA2, CECSA3

Learning outcomes: 1,2,3,5,6,7,8,9

Denomination of lesson: Practical session 3.

Contents of Lesson: Identification of microorganisms using immunological techniques test agglutination kit, ELISA, TECRA UNIQUE and VIDAS. **Competencies acquired:** 

CB2, CB3, CG1, CG2, CT8, CT9, CT11, CECSA1, CECSA2, CECSA3

Learning outcomes: 1,2,3,5,6,7,8,9

Denomination of lesson: Practical session 4.

Contents of Lesson: Use of other rapid methods for detection of microorganisms index

indicators such as Chromogenic agar, SIMPLATE (coliforms and E. coll).

Competencies acquired: CB2, CB3, CG1, CG2, CT8, CT9, CT11, CECSA1,

CECSA2, CECSA3

Learning outcomes: 1,2,3,5,6,7,8,9

Denomination of lesson: Practical session 5.

Contents of Lesson: Rapid biochemical methods: API GALLERIES. Competencies acquired: CB2, CB3, CG1, CG2, CT8, CT9, CT11, CECSA1, CECSA2, CECSA3

Learning outcomes: 1,2,3,5,6,7,8,9

Denomination of lesson: Practical session 6.

Contents of Lesson: Physical-chemical methods (Flow cytometry - technique used to detect and measure physical and chemical characteristics of a population of cells). Competencies acquired: CB2, CB3, CG1, CG2, CT8, CT9, CT11, CECSA1,

CECSA2, CECSA3

Learning outcomes: 1,2,3,5,6,7,8,9

# **SEMINAR ACTIVITIES**

# Denomination of the lesson: Rapid technique for the microbiological analysis of food

Activity content: Each student will perform different searches of rapid techniques used for the detection and identification of microorganisms. The works will be presented in power point with the following sections: Basis of the method, food and microorganisms detected, method development, total time for detection, sensitivity and specificity.

Type and place: Seminar (A-25, A32)

Competencies acquired: CB1, CB2, CB3, CB4, CB5, CG2, CT1, CT2, CT3, CT4,

CT5, CT6, CT7, CT9, CECSA1, CECSA2, CECSA3

Learning outcomes: 1,2,3,5,6,7,8,9, 10

Materials and tools to be used: Computers, databases of scientific literature

Educational activities *									
Student workload in hours by lesson		Student workload in hours by lesson	Student workload in hours by lesson			Student workload in hours by lesson	Student workload in hours by lesson		
Lesson	Total	GG	PCH	LAB	ORD	SEM	TP	EP	
Lesson 1	18,5	2,5					1	15	







Lesson 2	28	8			20
Lesson 3	26,5	5		1,5	20
Lesson 4	11	3			8
CAMPO O LABORATORIO					
1	11		6		5
2	11		5	1	5
3	11		6		5
4	11		5	1	5
5	10		5		5
6	10		5		5
Assessment  **	2	2			
TOTAL	150	20,5	32	4,5	93

L: Lectures (100 students)

HI: Hospital internships (7 students)

LAB: Laboratory or field practices (15 students)

COM: Computer room or language laboratory practices (30 students)

SEM: Problem classes or seminars or case studies (40 students)

SGT: Scheduled group tutorials (educational monitoring, ECTS type tutorials) PS: Personal study, individual or group work and reading of bibliography

#### Teaching Methodologies\*

- 1. Lectures and discussion of theoretical contents
- 2. Development of problems
- 3. Laboratory practices, pilot plants and field
- 6. Development and presentation of seminars
- 7. Use of the virtual classroom
- 9. Study of the subject
- 10. Search and management of scientific literature
- 11. Exams

#### Learning outcomes \*

- 1. Know the Fundamentals and Applications of Rapid and Automated Techniques.
- 2. Know the fundamentals of microorganism counts in food.
- 3. Know the relevance of the rapid techniques to detect personal hygiene, products and processes.
- 4. Manage food safety through rapid detection of pathogens and their toxins.
- 5. Evaluate, control and manage food quality through the application of automated techniques.
- 6. Improve quality systems.
- 7. Controlling and assessing food risks. Analysing and assessing food risks.
- 8. Analyse food using specific techniques.
- 9. Controlling and optimizing processes.
- 10. Advise the food industry scientifically and technically.

#### Assessment systems \*

<sup>\*\*</sup> Indicate the total number of evaluation hours of this subject.







It will be evaluated:

#### Practical knowledge

The learning of the practical part of the subject will be evaluated continuously, through attendance control to the practical sessions and their participation in them. A questionnaire will be answered at the end of the practical sessions, which will also be evaluated. Likewise, its use will be evaluated through the accomplishment of a practical work. The final exam will also evaluate the practical part of the subject through short questions related to the practices performed (fundamentals, implementation procedure, etc.).

# **Seminars or tutored works ECTS**

The seminars will be evaluated through the execution of monographic works that will be presented throughout the course in a large group. It will be evaluated continuously, through attendance control to ECTS tutorials and their participation in them. The knowledge of the seminars in the final exam will also be evaluated through a questionnaire that will consist of short questions.

#### Theoretical knowledge

Weekly exams will be made consisting of quiz questions and short intermixed questions. Test-type questions will only have a true answer; Those questions answered incorrectly will subtract ½ from the value of the question, that is, two wrong answers cancel a successful one. The short questions will deal with definitions, basic concepts of the subject, etc., and will be scored, if correctly answered, as a test question. To pass the theoretical part it is necessary to obtain a grade of 5 points or higher in this exam.

A final exam will be done in June-July (official dates) which will consist of quiz questions and short quizzes intermixed. Test-type questions will only have one true answer; Those questions answered incorrectly will subtract ½ from the value of the question, that is, two wrong answers cancel a successful one. The short questions will deal with definitions, basic concepts of the subject, etc., and will be scored, if correctly answered, as a test question. To pass the theoretical part it is necessary to obtain a grade of 5 points or higher in this exam.

Each part will represent a percentage of the final grade:

- Theoretical knowledge 60%
- Seminar: preparation and presentation 20%
- Laboratory: assistance, knowledge and presentation 20%

The examinations, grades and periods of claim of the exams will be exposed in the corresponding planks and through the virtual classroom of the subject in time and form as established by the regulations approved by the Board of Government and published by Resolution 9/03/2012, DOE No. 59 of March 26, amended by Resolution 27/11/2012, DOE n° 242, of December 17 and Resolution 17/03/2014, DOE 62, of March 31, and RESOLUTION of November 25, 2016, DOE N° 236 of December 12, 2016.

#### SINGLE EVALUATION

- 1. In the first three weeks of the semester, the student who accepts this type of evaluation must notify the subject coordinator in writing of the intention to take part in this type of evaluation\*.
- 2. There will be an exam corresponding to the practical contents and the part of







seminars, both tests can be oral or written, in which case they will follow the same criteria of overcoming each part that for the continuous evaluation.

3. To pass the subject will be necessary to pass the test related to the practical knowledge. For this, a practical exam will be carried out, which can also include both oral and written test on the practical contents. A minimum of five points will need to be achieved in the examination of skills.

Each part will represent a percentage of the final grade:

- Theoretical knowledge 60%
- Seminars: preparation and presentation 20%
- Laboratory work: assistance and knowledge 20%

\*In order to choose this evaluation system, the students must fill out, sign and return to the EIA office, through registration, the application form available on the EIA website (Secretariat, administrative procedures), in the first three weeks of the semester.

#### Bibliography (basic and complementary)

#### **BASIC**

- -CASARETT, L.J., AMDUR, M.O., KLAASSEN, C.D. (1995). Casarett and Doull's Toxicology: The basic science of poison. McGraw-Hill,
- -DOYLE, M.P. (2000). Microbiología de los alimentos: fundamentos y fronteras. Acribia. Zaragoza
- -LINDNER, E. (1995). Toxicología de los Alimentos. 2a ed. Acribia. Zaragoza.
- -FREIFELDER, D. (1988). Fundamentos de biología molecular. Acribia S. A. Zaragoza.
- -FRAZIER, W.C. y WESTHOFF, D.C. (1996). Microbiología.de los Alimentos. 4aEd. Acribia. Zaragoza.
- -GRUENWEDEL, D.W. y WHITAKER, J. R. (1984). Food Analysis. Principles and Techniques. Volumen 3. Marcel Dekker, Inc. New York and Basel.
- -HAYES, P.R. (1993) Microbiología e Higiene de los Alimentos. Acribia. Zaragoza.
- -ICMSF. Microorganismos de los Alimentos. Ecología microbiana de los productos alimentarios (2001): Acribia. Zaragoza.
- -JAY, J. (2002) Microbiología Moderna de los Alimentos. 4a ed. Acribia. Zaragoza.
- -MORTIMER, S.E. y WALLACE, C. (1996) HACCP: Enfoque práctico. Acribia. Zaragoza.
- -NELSON, W.H. (1985). Instrumental methods for rapid microbiological analysis. VCH Publishers.
- -PASCUAL ANDERSON M.R. (2000) Microbiología Alimentaria: Metodología Analítica para Alimentos y Bebidas. Díaz de Santos. Madrid.
- -STANNARD, C.J., PETIT, S.B. Y SKINNER, F.A. (1989). Rapid microbiological methods for foods, beverages y pharmaceuticals. Blackwell scientific publications.
- -WALKER, J.M. Y GINGOLD, E.B. (1997). Biología molecular y Biotecnología. 2ª edición. Acribia S. A. Zaragoza.

#### **COMPLEMENTARY:**

- http://www.dce.ksu.edu/dce/cl/rapidmethods/
- http://www.rapidmethod.com
- Aguas: http://www.ua.es/es/servicios/juridico/aguas.htm
- HACCP: http://www.calidadalimentaria.com
- HACCP: http://www.juridicas.es







- Seguridad Alimentaria:

http://www.aesa.msc.es/aesa/web/AesaPageServer?idcontent=92&idpage=58

- FDA, métodos rápidos de análisis: http://www.cfsan.fda.gov/~ebam/bam-a1.html
- Journal of Rapid Methods & Automation in Microbiology:

http://www.blackwellpublishing.com/journal.asp?ref=1060-3999

- AOAC: http://www.aoac.org/testkits/microbiologykits.htm y http://www.aoac.org/pubs/microcompendium.htm

#### Other resources and complementary educational materials

Prior to the explanation of the lesson. It will be provided with a summary of the lesson in which the main content to be taught is included. These contents can be shown in PowerPoint, Word format or any of them transformed into pdf. For your disposal will be deposited within each thematic block in the moodle for which it will be necessary to briefly explain its use and its way of being registered in the first weeks of class.

For this purpose, extension material, both bibliographical and other documentation (eg web pages) may be used to develop other transverse or specific degree qualifications. All this on the moodle virtual campus platform.

Virtual classroom of the subject in the virtual campus of the Uex.

(http://campusvirtual.unex.es/portal/)