
	PROCESO PARA EL DESARROLLO DE LAS ENSEÑANZAS DE LA ESCUELA DE INGENIERÍAS AGRARIAS	 Escuela de Ingenierías Agrarias
	CÓDIGO: P/CL009_D002	

PLAN DOCENTE RAPID MICROBIOLOGICAL ANALYSIS TECHNIQUES

Academic course: 2017-2018

Identification and characteristics of the subject			
Code	502238		Créditos ECTS 6
Denomination (español)	Técnicas Rápidas de Análisis Microbiológico		
Denomination (english)	Rapid Microbiological Analysis Techniques		
Degree	DEGREE IN SCIENCE AND FOOD TECHNOLOGY		
Center	School of Agricultural Engineering		
Semester	Second (8º)	Carácter	Optional
Module	Optional		
Materia	Rapid Microbiological Analysis Techniques		
Profesor/s			
Name	Office	email	web
María José Benito Bernáldez	D-720 Edificio Valle del Jerte	mjbenito@unex.es	http://www.unex.es/investigacion/grupos/camiali
Santiago Ruiz Moyano Seco de Herrera	D-717 Edificio Valle del Jerte	srmsh@unex.es	http://www.unex.es/investigacion/grupos/camiali
Area of Knowledge	Nutrition and Bromatology		
Department	Animal Production and Food Science		
Coordinating Professor	María José Benito Bernáldez		
Themes and content			
Brief description of the content			
<p>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 techniques, fast and accurate. 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.</p>			
Theme of the subject			
<p>Denomination of Theme 1: GENERAL ASPECTS. Contents of Theme 1: 1.1. INTRODUCTION. Systems, methods, importance of new detection techniques. General concepts.</p>			
<p>Denomination of Theme 2: DETECTION TECHNIQUES OF MICROORGANISMS OR THEIR PRODUCTS IN FOODS BY MOLECULAR BIOLOGY METHODS Contents of Theme 2: 2.1. NUCLEIC ACIDS. Physical and chemical structure of DNA. Renaturalization. Structure of RNA.</p>			

- 2.2. RECOMBINANT DNA TECHNOLOGY I. DNA extraction: 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. Marking 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 Theme 3: **TECHNIQUES FOR DETECTING MICROORGANISMS OR THEIR PRODUCTS IN FOODS BY PHYSICAL, CHEMICAL AND IMMUNE METHODS**

Contents of theme 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. A) in liquid medium: quantitative and qualitative. B) in 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: Immunofluorescence: direct and indirect. Radioimmunoassay: solid phase; Direct and indirect. ELISA: Fundamentals 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 theme 4: **TECHNIQUES FOR DETECTING MICROORGANISMS OR THEIR PRODUCTS IN FOOD THROUGH BIOASSAYS AND RELATED METHODS**

Contents of Theme 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

Practical sessions

Denomination of theme: Practical session 1. Contents of Theme: Identification of microbial toxins by nucleic acid techniques (PCR). Bacterial DNA extraction, real-time PCR
Denomination of theme: Practical session 2. Contents of Theme: Visualization of toxins of a protein nature by polyacrylamide gel electrophoresis. Identification of different microorganisms by protein profiles in polyacrylamide gel electrophoresis.
Denomination of theme: Practical session 3. Contents of Theme: Identification of microorganisms using immunological techniques ELISA, TECRA UNIQUE and VIDAS.
Denomination of theme: Practical session 4. Contents of Theme: Use of other rapid methods for detection of microorganisms index indicators such as VIP (E.coli EHEC), SIMPLATE (coliforms and E. coli).
Denomination of theme: Practical session 5. Contents of Theme: Rapid biochemical methods: API GALLERIES.
Denomination of theme: Practical session 6. Contents of Theme: Physical-chemical methods (Chromatographies).

SEMINAR ACTIVITIES

Denomination of the theme: 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: Fundamentals of the method, food and microorganisms detected, method development, total time for detection, sensitivity and specificity.

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

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

Activities

Student work hours by subject		PresentTial		Monitoring activity	No presentTial
Theme	Total	GG	SL	TP	EP
1	18,5	2,5		1	15
2	28	8			20
3	26,5	5		1,5	20
4	11	3			8
LABORATORY					
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
Evaluation	2	2			
Total	150	20,5	32	4,5	93

GG: Large Group (100 students).

SL: Seminar / Laboratory (hospital clinical practices = 7 students, laboratory or field practices = 15, room computer or language laboratory practices = 30, classes problems or seminars or practical cases = 40).TP: Tutorías Programadas (seguimiento docente, tipo tutorías ECTS).

EP: Personal study, individual or group work, and bibliography reading.

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

Evaluation

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.). This part will be obligatory to pass the subject. To pass this part it is necessary to obtain a score equal to or higher than 5 points.

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. This part will be obligatory to pass the subject. To pass this part it is necessary to obtain a score equal to or higher than 5 points in each one of the parts.

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 $\frac{1}{2}$ 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 $\frac{1}{2}$ 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 nº 242, of December 17 and Resolution 17/03/2014, DOE 62, of March 31, and RESOLUTION of November 25, 2016, DOE Nº 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%

Bibliography and other resources

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 complementary resources and teaching materials

Prior to the explanation of the theme It will be provided with a summary of the theme in which the main content to be taught is included. These contents can be 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/>)

Horario de tutorías

Scheduled Tutorials: The days when indicated by the teacher on the school website.
<http://www.unex.es/conoce-la-uex/centros/eia/centro/profesores>

Tutorials of free access: the days in which this is indicated by the teacher in the web of the school. <http://www.unex.es/conoce-la-uex/centros/eia/centro/profesores>

Recomendations

The general recommendations for a better use of the subject by the students are:

- Attend and participate in the classroom and practical classes of the subject.
- Frequently use the virtual classroom and other web resources (forums, blogs, etc.)
- Attend tutoring sessions scheduled by the teacher to follow the course.
- Use the bibliography recommended by the teacher.