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XI Workshop "Métodos rápidos y automatización en microbiología alimentaria"

El XI Workshop sobre Métodos rápidos y automatización en microbiología alimentaria (MRAMA) se celebró del 20 al 23 de noviembre de 2012 en la Universidad Autónoma de Barcelona (UAB). Este evento anual está organizado por el Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA) y por el Departamento de Ciencia Animal y de los Alimentos de la UAB, y se encargan de dirigirlo los Drs. Marta Capellas Puig y Josep Yuste Puigvert, profesores de Ciencia y Tecnología de los Alimentos.

El objetivo del Workshop MRAMA es ampliar y difundir los conocimientos teóricos y prácticos sobre métodos innovadores para detectar, contar, aislar y caracterizar rápidamente los microorganismos, y sus metabolitos, habituales en los alimentos y el agua. En esta edición reunió a 224 participantes, procedentes de diversos colectivos nacionales e internacionales: laboratorios, asesorías y consultorías; industrias de los ámbitos agroalimentario y afines; profesores y estudiantes de la UAB y otras universidades; otros centros de investigación y diversas administraciones.

Además de la habitual ponencia principal a cargo del profesor Dr. Daniel Y. C. Fung, de la *Kansas State University* (KSU), que repasó las últimas novedades en el ámbito de los métodos rápidos y miniaturizados y la automatización, el Workshop contó con otros conferenciantes de renombre:

- La Dra. Cécile Lahellec, directora honoraria de investigación de la Agence Française de Sécurité Sanitaire des Aliments (AFSSA), en Alfort (Francia), se encargó de la ponencia inaugural, que mostró el enfoque multidisciplinar de la microbiología de los alimentos.
- El Dr. Armand Sánchez Bonastre, director del Servicio Veterinario de Genética Molecular de la UAB, habló sobre la técnica de la reacción en cadena de la polimerasa (PCR) para detectar e identificar microorganismos.
- El Dr. Ferran Ribas Soler, presidente de la Comisión de Normalización y Validación de la Sociedad Española de Microbiología (SEM), informó sobre la nueva norma ISO 9308-2 para enumerar *Escherichia coli* y bacterias coliformes en agua.
- Fernando M. Rubio, de Abraxis LLC, en Warminster (Pensilvania, EE UU), expuso el desarrollo y la aplicación de los ensayos por aglutinación del látex para *E. coli* verotoxigénicas no-O157.
- Jon Basagoiti Azpitarte, consultor y auditor de Imagining Management Systems, explicó su experiencia en gestión de la calidad y la inocuidad de los alimentos e hizo especial hincapié en el reconocimiento del comportamiento de los peligros alimentarios en los procesos industriales para su control efectivo.
- Alfredo Corujo Fernández, de Nutreco Servicios, habló sobre un nuevo sistema molecular para detectar patógenos en productos avícolas.
- Por último, el Dr. José Juan Rodríguez Jerez, investigador principal del grupo de investigación Biorisc de la UAB, se centró en los métodos rápidos para controlar online la contaminación ambiental en las industrias alimentarias. También se presentaron los ensayos interlaboratorios con estándares de microorganismos y la secuenciación masiva para la caracterización rápida de los patógenos.

Además, hubo una mesa redonda con el Dr. Fung, otros ponentes y profesionales de empresas de microbiología y laboratorios de análisis. También se realizaron unas sesiones prácticas en el laboratorio, en las que se trabajó con algunos equipos y los productos más innovadores del campo de los métodos rápidos y la automatización. Por último, se organizaron tres talleres:

- Uso de los recursos para microbiología predictiva disponibles en internet, a cargo de Montse Vila Brugalla (Servicio de Control alimentario de mercados centrales de la Agencia de Salud Pública de Barcelona).
- Uso de un método rápido y no instrumental para identificar las seis *E. coli* verotoxigénicas no-O157 más frecuentes, a cargo de Abraxis.

• No conformidades típicas en las auditorías de seguridad alimentaria (IFS, BRC y FSSC22000), a cargo de SGS ICS Ibérica.

Además, expusieron sus novedades diversas empresas de microbiología, que patrocinaron el XI Workshop: 3M España; Becton Dickinson; bioMérieux – AES CHEMUNEX España; Bioser (distribuidor de Bio-Rad Laboratories y Microgen Bioproducts); BIOTECON Diagnostics Gomensoro; IDEXX Laboratorios; IMICROQ; Itram Higiene; IUL; Life Technologies; MicroPlanet Laboratorios (distribuidor de BioControl Systems y LIOFILCHEM); Merck Millipore (división de Merck); Nirco (distribuidor de Neogen Europe); Oxoid (parte de Thermo Fisher Scientific Inc) y Sigma-Aldrich Química.

También colaboraron con el Workshop MRAMA: ACONSA (Asesoría y Consultoría Sanitaria); Tiselab; BIPEA (Bureau Interprofessionnel d'Etudes Analytiques); la Associació Catalana de Ciències de l'Alimentació (ACCA); EyPASA – Revista Alimentaria (publicación oficial del Workshop); la SEM; la Asociación de Consultores y Formadores de España en Seguridad Alimentaria (ACOFESAL); la Sociedad Española de Seguridad Alimentaria (SESAL); la Agencia de Salud Pública de Barcelona y la Sociedad Española de Químicos Cosméticos (SEQC).

El XII Workshop MRAMA se celebrará del 19 al 22 de noviembre de 2013.

A continuación, incluimos una de las ponencias que se impartieron durante el Workshop y en números posteriores publicaremos otras ponencias.

Microbiología rápida para... ¿decisiones adecuadas?

Jon Basagoiti | Imagining Management Systems

Esta presentación y mi trayectoria profesional

La propuesta de esta presentación nace de una combinación de tres factores: una frustración, una ilusión y la posibilidad de compartir ambas en un foro especializado.

A mediados de los ochenta y durante seis años, realicé miles de análisis de materias primas, de productos, de procesos de limpieza, etc., en un pequeño laboratorio de una industria alimentaria. La empresa fue innovadora en el ámbito mundial en la década de los sesenta, pero para entonces había dejado de serlo seguramente por el fracaso del proceso de innovación en el que participó: la fabricación de cerveza por medio de la fermentación en continuo, que ha sido un camino que ha acabado en vía muerta por mostrarse incapaz de competir en calidad con los métodos de fermentación en discontinuo.

Cuando apuntaba el resultado de un análisis de producto acabado en el libro de registro, el producto ya estaba en el mercado. Cuando apuntaba el resultado de un producto interme-

dio o de un proceso de limpieza, hacíamos una estadística: "esta vez ha ido bien", "esta vez no ha ido bien, a ver cómo sale la próxima". No explotábamos con eficacia los datos disponibles para intentar mejorar la siguiente ocasión en la que desarrollábamos el mismo proceso. Los datos erráticos de los análisis no eran más que el resultado de una variabilidad cuyos orígenes no conocíamos, y, por lo tanto, no podíamos controlar. Muy poco a poco, con el análisis de los datos conseguimos que la variabilidad de nuestros procesos disminuyera algo. Estábamos en ese lento proceso de disminución de la variabilidad cuando la empresa cerró.

Mi ilusión de que mi trabajo como microbiólogo industrial fuera capaz de mejorar los procesos y los productos y que esto redundara en beneficio de los consumidores y de los resultados de la empresa se iba viendo frustrada con el lento avance de la mejora.

La microbiología rápida

Las técnicas de microbiología rápida permiten acelerar la obtención de los resultados de la muestra tomada con una precisión cada vez mayor. Estas técnicas pueden variar en el tiempo de respuesta, en la tecnología, en el coste de cada análisis, etc., pero, en cualquier caso, permiten acelerar el proceso de decisión sobre la muestra tomada. Qué mejor muestra de estas técnicas que este Workshop (figura 1).

Desde primeros de los años noventa y en mi trayectoria profesional posterior como auditor de sistemas de calidad y seguridad alimentaria y como formador en este campo, me he ido encontrando con algunas empresas que emplean las herramientas de la microbiología rápida.

En estas actividades, también me he encontrado con mucha variabilidad: desde empresas que utilizan de manera masiva herramientas de la microbiología rápida hasta las que emplean las herramientas de la microbiología tradicional, pasando por las que emplean poco la microbiología.

En la decisión de los gestores de las empresas sobre qué técnicas emplear y con qué intensidad utilizarlas influyen muchos factores, entre ellos los siguientes: la historia previa de la empresa, el tipo de productos elaborados, el uso del producto por el cliente o por el consumidor, la conciencia del riesgo incurrido, la capacidad para interpretar y dar sentido a los resultados disponibles, etc.

Uno de los grandes obstáculos de la microbiología, a mi juicio, sigue siendo la interpretación adecuada de los resultados, resultados que se ven condicionados por todos los factores de incertidumbre que influyen en ellos.

La incertidumbre en los resultados microbiológicos

La norma ISO/TS 19036:2006, Microbiología de los alimentos y piensos para alimentación animal –Guía para la estimación de la incertidumbre de medida de las determinaciones cuantitativas, indica que "el muestreo [la toma de la(s) unidad(es) de muestra que se van a ensayar del lote que se controla] introduce una parte significativa (si no la mayor) del error total, pero no es parte de la incertidumbre relacionada con el error de medición". Esta norma asocia al laboratorio microbiológico el concepto *black-box* o *caja negra*, que expresa que las mismas muestras pueden tener resultados distintos por las fuentes de variabilidad internas que tiene el propio laboratorio. La norma también indica que esas fuentes de variabilidad son independientes de la propia variabilidad de las muestras tomadas del lote.

Es evidente que la industria alimentaria es en sí misma una fuente de variabilidad mucho mayor que el laboratorio microbiológico. Y que los factores que inciden en la variabilidad son más en número y mayores en variabilidad que la aportada por el laboratorio.

En una escala de variabilidad de los resultados microbiológicos, el orden de los elementos, de menor a mayor, que aportan variabilidad sería el siguiente:

– Variabilidad interna del laboratorio microbiológico.
– Variabilidad del muestreo.
– Variabilidad del lote de producto del que se toma la muestra.
– Variabilidad de todos los factores que inciden en la fabricación de ese lote, desde las materias primas a todos los factores que pueden incidir.

Estos factores que aportan incertidumbre durante la producción han sido descritos en el Codex Alimentarius y han sido acogidos por la legislación alimentaria y por las normas de inocuidad alimentaria actualmente en vigor.

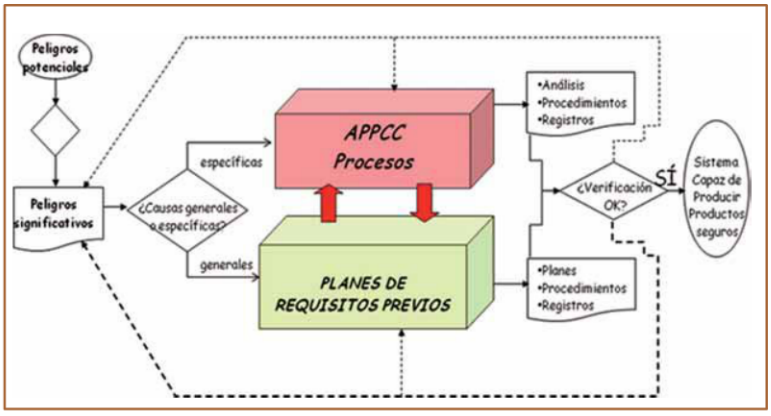

Figura 1.-

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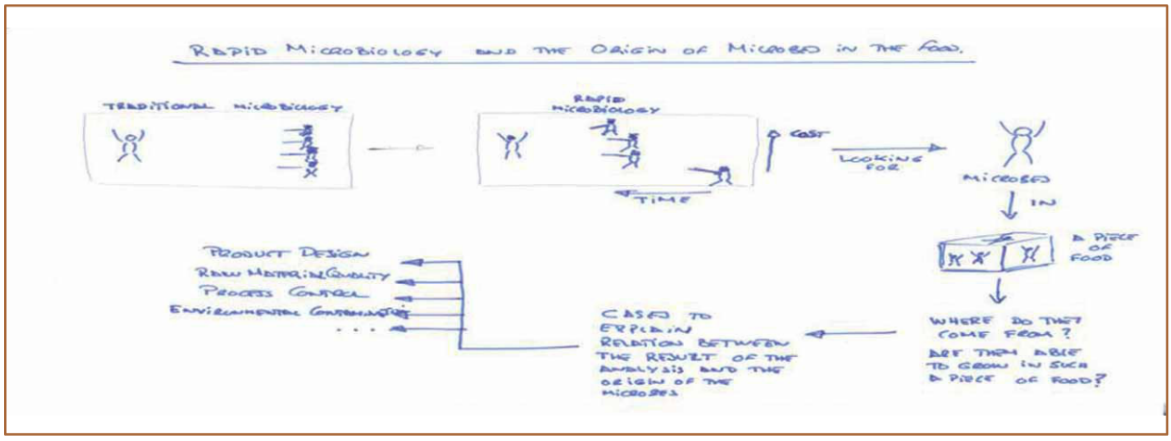

Figura 2.-

La legislación y los actuales sistemas de seguridad alimentaria

La legislación alimentaria y los actuales sistemas de inocuidad de los alimentos están basados en los contenidos del documento del *Codex Alimentarius: Principios Generales de Higiene de los Alimentos (CAC/RCP* 1-1969, descargable en la dirección: http://www.codexalimentarius.org/standards/list-of-standards/en/?no_cache=1) y en su Anexo: *Sistema de Análisis de Peligros y de Puntos Críticos de Control (HACCP) –Directrices para su Aplicación.*

Todos los contenidos de inocuidad alimentaria del Reglamento (CE) 852/2004, relativo a la higiene de los alimentos; y de las normas ISO 22000:2005, BRC v6 e IFS v6 están basados en este documento del *Codex Alimentarius*. En las últimas versiones de las normas BRC e IFS se van introduciendo elementos que profundizan en aspectos como la gestión de alérgenos o amplían el campo hacia conceptos como food defence. Pero estas normas apenas aportan ninguna innovación, ya que todas las posibles fuentes de incertidumbre asociada a los peligros alimentarios han sido descritas por el *Codex Alimentarius*.

En un rápido vistazo a este documento, se puede comprobar que en sus 24 primeras páginas se identifican todas las fuentes comunes de acceso de los peligros alimentarios, los microorganismos entre ellos, a los alimentos. Están incluidos los requisitos para los conocidos como prerrequisitos o requisitos previos, que cada empresa debe desarrollar como procedimientos o planes. La empresa podría acogerse a alguna de las numerosas guías sectoriales disponibles para desarrollar sus propios planes o procedimientos de requisitos previos. No es casual que a estos requisitos que están situados al principio del documento se les llame requisitos previos.

En las siguientes 10 páginas del documento del *Codex Alimentarius*, se trata de proteger al consumidor de los peligros que podrían llegar a los alimentos con las materias primas y de los peligros que se generan durante el propio proceso de producción. Es para estos dos tipos de peligros o de origen de los peligros para los que se definen los famosos siete principios del *Codex Alimentarius*, y se propone una guía, voluntaria, de cómo desarrollar estos principios para que la protección al consumidor sea efectiva.

De estas dos maneras —peligros que se pueden controlar con los requisitos previos y peligros que provienen de las materias primas o que se producen durante los propios procesos de producción—, se proponen distintas estrategias para luchar contra los distintos orígenes de los peligros (figura 2).

La legislación y todas las normas voluntarias de certificación de los sistemas de inocuidad de los alimentos siguen esta filosofía, aunque sí hay que decir que no está tan clara la estructura en estas normas como lo está en el documento del *Codex Alimentarius* o en el propio Reglamento (CE) 852/2004, que, a mi juicio, es el documento que más claramente incluye los requisitos de inocuidad de los alimentos. Creo que si se leen el artículo 4 (y su Anexo II) y el artículo 5 de este Reglamento, desde el espíritu con el que están escritos, cualquier profesional del sector podría llegar a esta conclusión.

Las normas BRC, IFS e ISO 22000:2005 tienen los mismos requisitos, pero en otro orden. Este orden proviene de la redacción del documento del que se adoptaron los requisitos, la Directiva 93/43. El orden de los requisitos en esta Directiva, trasladado directamente a estas normas, complica su lectura y oculta o desdibuja la estructura del sistema propuesto por el documento original del *Codex Alimentarius*, que no ha tenido modificaciones sustanciales desde 1997, antes de la primera edición de cualquiera de estas normas.

Para el adecuado control de los peligros alimentarios y sus diversas fuentes de acceso a los alimentos, cada empresa tiene que identificar la incidencia de cada una de ellos y desarrollar procedimientos o procesos que den respuesta proporcionada a los riesgos en los que se incurre.

Reubicación de los análisis motivada por la legislación de higiene de los productos alimenticios

La legislación de higiene de los productos alimenticios, derivada del Reglamento (CE) 852/2004, ha propiciado que muchas empresas alimentarias hayan hecho cambios en sus planes analíticos, reubicando su función y produciendo cambios en los microorganismos analizados y en los volúmenes y frecuencias de análisis. En muchas ocasiones se ha pasado de un tipo de análisis que validaba un lote o un grupo de lotes de productos o validaba un procedimiento de limpieza, a un tipo de análisis que valida algunos planes de requisitos previos y que valida un plan de análisis de peligros y puntos de control crítico (APPCC).

Muchas empresas consideran que con un APPCC validado la necesidad de realizar análisis es mucho menor, y que con algunos resultados satisfactorios tienen suficiente información para afirmar que los alimentos que elaboran son seguros. Pero para que esta afirmación sea cierta es necesario que el análisis de peligros realizado sea representativo de la realidad de la empresa.

La propia redacción de la legislación a este respecto no debería dejar dudas, ya que indica que se debe "detectar cualquier peligro que deba evitarse, eliminarse o reducirse a niveles aceptables" (Reglamento 852/2004, Artículo 5.2.a). Lo que supone que los peligros ya están, o que podrían estar, en la empresa, en sus instalaciones, o que pueden introducirse con las materias primas. Es función de la empresa detectarlos.

Una de las limitaciones que encuentro con frecuencia es que, cuando en un sistema de inocuidad de los alimentos se intentan detectar los peligros, en muchas ocasiones me cuesta encontrar los peligros con nombres y apellidos. No se suelen encontrar sistemas que hayan identificado peligros como *Bacillus cereus, Salmonella spp, Listeria monocytogenes*, etc.

Cada microorganismo tiene unas condiciones de acceso, supervivencia, crecimiento y diseminación específicas, por lo que debería ser tratado de manera individual o asociado al grupo que se comporte de una manera muy similar.

Para profundizar en los peligros microbiológicos que aparecen con frecuencia en los alimentos, se puede acceder al portal de sistema de alerta rápida de alimentos y piensos en la dirección https://webgate.ec.europa.eu/rasff-window/portal/.

Con los resultados analíticos disponibles se toman decisiones sobre la higiene de los alimentos fabricados y su aptitud para el consumo. La cuestión que se plantea en esta presentación es la siguiente: ¿se están tomando las decisiones adecuadas?

Casos de intoxicaciones alimentarias en la actualidad

Se puede afirmar, sin temor a equivocarse, que la situación actual de la higiene alimentaria es la mejor en toda la historia y que han sido numerosos los factores que han influido en que la situación actual sea esta. Sin embargo, hay datos que indican que se tiene que seguir mejorando.

A pesar de que todas las empresas (operadores) alimentarias europeas están obligadas a desarrollar sus sistemas de seguridad alimentaria para cumplir con los requisitos del Reglamento (CE) 852/2004, y muchas empresas han abordado procesos de certificación de sistemas de inocuidad de los alimentos siguiendo alguna de estas normas voluntarias, siguen siendo numerosos los casos de intoxicaciones alimentarias que se producen. En la actualidad se observan numerosos casos aislados, para los que no se encuentra una causa que los haya desencadenado.

La sistemática empleada para tomar los datos de las intoxicaciones alimentarias no facilita la clasificación de casos para identificar cuáles proceden directamente de alimentos elaborados por un operador alimentario y cuáles proceden de casos domésti-

En la decisión de los gestores de las empresas sobre qué técnicas emplear y con qué intensidad utilizarlas influyen muchos factores: la historia previa de la empresa, el tipo de productos elaborados, el uso del producto por el cliente o por el consumidor, la conciencia del riesgo incurrido, la capacidad para interpretar y dar sentido a los resultados disponibles, etc.

cos. No conozco de qué manera se pueden identificar los orígenes de estos casos.

Además, las intoxicaciones alimentarias están subdetectadas. Datos de la Autoridad de Seguridad de los Alimentos de Irlanda indican que su sistema de salud detecta aproximadamente el 10% de los casos que se producen. La extrapolación de sus datos indica que cada persona del país padece un día al año una intoxicación alimentaria. No es necesario profundizar en este momento en los costes personales, sociales o económicos de estas situaciones.

No son raros los resultados de análisis microbiológicos que superan los límites establecidos. En numerosas ocasiones no se pueden identificar los motivos por los que estos resultados se salen de los parámetros especificados y deseados. Los sistemas de inocuidad de los alimentos desarrollados por las empresas deben, de manera activa, identificar los orígenes de esos microorganismos en la muestra. La función de estos sistemas es la de anticiparse y evitar que eso ocurra; pero una vez que ha ocurrido un resultado anómalo no previsto, se deben poner en marcha los elementos de verificación y mejora de estos sistemas para la identificación indudable del origen de este resultado.

A veces es muy difícil encontrar la causa real de un resultado fuera de especificación, y la disposición de un número limitado de análisis dificulta esta identificación. La disposición de otros resultados que sí cumplen la especificación tranquiliza al sistema y a las personas que lo gestionan.

La alternancia de muchos resultados dentro de especificación y algunos fuera de especificación desorienta a la empresa que los recibe, pero no siempre le hace pensar que, tal vez, la variabilidad de sus procesos es tal que todos los días pueden estar produciendo un cierto número de productos fuera de especificación. Si ese volumen de productos que están fuera de especificación llega a consumidores que pueden verse afectados habrá un pequeño número de casos de intoxicación, cuyo origen es difícilmente detectable por los mecanismos de investigación epidemiológica implantados en la actualidad.

Para profundizar en las incidencias, se puede acceder a datos de infecciones digestivas en la página de la OMS: http://data.euro.who.int/hfadb/.

> Las técnicas de microbiología rápida permiten disponer de datos antes de que el producto se haya expedido al mercado y reaccionar en el caso de no conformidad

¿Qué decisiones tomamos a partir de los resultados microbiológicos?

No vamos a fijarnos en esta ocasión en los microoperadores alimentarios, como el pequeño comercio alimentario o el establecimiento de restauración, donde la posibilidad de contaminación cruzada puede ser frecuente, pues observamos a menudo casos como el de la pequeña carnicería que vende un pollo troceado y a continuación lonchea un producto cárnico cocido sin aplicar medidas higiénicas apropiadas. Con los datos analíticos conocidos en boletines epidemiológicos y los casos de la bibliografía, no se puede descartar que esta sea una vía de acceso de microorganismos patógenos al frigorífico y a la boca del consumidor.

Considerando exclusivamente los operadores industriales, aun cuando sean pequeños, cualquier resultado microbiológico anómalo tiene una o varias causas. Las técnicas de microbiología rápida permiten disponer de datos antes de que el producto se haya expedido al mercado y reaccionar en el caso de no conformidad, sin que tenga una repercusión negativa en la salud de los consumidores.

Pero los datos que se obtienen de los análisis microbiológicos tienen que servir para identificar el *microbioma* de la empresa, es decir, los microorganismos que residen en el establecimiento alimentario, y especialmente los patógenos. Los datos microbiológicos tienen que servir para:

– Identificar las vías de acceso de los microorganismos a las instalaciones del establecimiento.

– Identificar las zonas donde se alojan y desde las que se diseminan a las superficies de contacto con los alimentos y a los propios alimentos.

– Identificar las vías de diseminación desde las zonas de alojamiento a los alimentos.

La finalidad de los datos: identificar la realidad, reconocer lo que está sucediendo

Durante esta presentación vamos a ver casos reales (anónimos) que plantean que en la práctica diaria de las empresas alimentarias se dan distintas situaciones que requerirían:

– La realización de un análisis de peligros más preciso.

– La selección de análisis microbiológicos o de alérgenos que representen adecuadamente las situaciones que están produciéndose en la realidad.

– Aprovechar la información derivada de los análisis para asegurarse de que los alimentos fabricados son seguros para el consumidor.

– Aprovechar las posibilidades de la microbiología rápida para acelerar este proceso de identificación de la realidad y la toma de decisiones.

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XI Workshop Métodos rápidos y automatización en microbiología alimentaria (MRAMA)

A continuación, incluimos algunas de las ponencias que se impartieron durante el XI Workshop MRAMA celebrado en la Universidad Autónoma de Barcelona (UAB). Este evento anual está organizado por el Centre Especial de Recerca Planta de Tecnologia dels Aliments (CERPTA) y por el Departamento de Ciencia Animal y de los Alimentos de la UAB.
En cuanto a la edición de este año, el XII Workshop MRAMA se celebrará del 26 al 29 de noviembre de 2013.

Food microbiology: a multifaceted approach

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What is Food microbiology?

The term "Food microbiology" includes two words: Food and Microbiology, i.e microbes in foods; it is very easy to understand it may be very hard to have even a basic knowledge of that field if we think to the variety of foods and also the multiplicity of microbes susceptible to contaminate the foods and, finally, the multiplicity of incidences of microbes in foods.

What is **food**? "Officially"? According to the European Regulation EC n° 178/2002, 28 January 2002, giving the general prescriptions on which EFSA has been based, a food is "every substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be ingested by humans".

"Food" includes drink, chewing-gum and any substance, including water, intentionally incorporated into the food during its manufacture, preparation or treatment. It includes water after the point of compliance as defined in Article 6 of Directive 98/83/EC and without prejudice to the requirements of Directives 80/778/EEC and 98/83/EC.

"Food" shall not include:

(a) Feed;

(b) Live animals unless they are prepared for placing on the market for the human consumption;

(c) Plants prior to harvesting;

(d) Medicinal products within the meaning of Council Directives 65/65/EEC(21) and 92/73/EEC(22).

(e) Cosmetics within the meaning of Council Directives 89/622/EEC(24);

(f) Tobacco and tobacco products within the meaning of Council Directive 89/622/EEC(24);

(g) Narcotic or psychotropic substances within the meaning of the United Nations Single Convention on Narcotic Drugs, 1961, and the United Nations Convention on Psychotropic Substances, 1971;

(h) Residues and contaminants.

Looking at the multiplicity of types of foods, the multiplicity of combinations food-microbe, we understand immediately that it is far more complex than a few centuries ago when bread and meat were the main foods for humans.

Microbes, including bacteria, appeared on earth a very long time, about 3 billion years ago. We all know the world of microbes is a fascinating one: it is a real world with a lot of individuals having special ways of living, important possibilities of adaptation and also genetic changes. The existence of microbes is known especially from the 17th century, due to the development of optical devices. From that time, different approaches of microbiology have been developed in different fields and their knowledge has been improved.

In fact, if we summarize, three groups are usually recognized among a lot of microbes, and especially bacteria, present in the environment and in foods:

- The first group concerns microorganisms implicated in Food safety.
- The second group concerns microorganisms leading to Food spoilage.
- The third group concerns microorganisms used for biotechnologies, fermentations…

Whichever the type of contaminant and the type of food, we can make some general comments:

- The microbes are placed in the food environment and their possibilities

of growth or inhibition depends on different factors: disponibility in oxygen, pH, activity of water, but also nutrient agents, inhibitors…

And the food itself may also be placed in a special environment and at different temperatures which may facilitate or reduce the possibilities of growth of a given microorganism according to its nature itself. Food technology "plays" with the food and microbes susceptible to find there some possibilities of growth.

Important changes have been observed somewhat recently: during the past decades, the conditions of food production have been submitted to tremendous changes, leading to more and more complexity and giving to microorganisms new opportunities to emerge. I will tell a few words about food production, then about changes in the landscape of food microbes…

• The contamination of food we eat nowadays can be considered as the consequence of what happens at different steps of the food chain in a context which differs considerably from one century ago: for example, nobody can ignore the intensification of all types of productions; but this intensification has enhanced the risk of microbial contamination and their transmission from one animal to another; following the rearing stage, the conditions of slaughtering lead to a contamination of the meat through faeces found on animal skin and we must not forget the contamination of the environment of processing and further-processing plants.

• While the food chain was changing and beginning more and more complex, new microorganisms have emerged, and, when they emerge, those pathogens are unknown dangers; we can mention that the new situation will necessitate new duties for the laboratories (in terms of methods, but they concern also the scientific and technical team, equipment...). We must also add that the types of foods we eat are very different of that we ate 50 years ago, but there are also large changes concerning the origin (different types of meats or vegetables…), come from different parts of the world); moreover, different meals are prepared in a central place, then distributed around… That explains the contamination of a lot of people from a unique preparation.

Those changes have led progressively to large outbreaks which have been known from the consumers: from 1992, we have had to face different problems of Food Safety. As an example, we can remember the problems of *Listeria monocytogenes* when the responsibility in different outbreaks have been discovered; then, other foodborne diseases have taken place in the public debate.

The result of those food crisis, which were better and better investigated due to new techniques and new technologies have led, in Europe, to large changes in the apprehension of Food Safety: I want to speak of the creation of Food Safety agencies in quite all European countries and, in 2002, the creation of the European Food Safety Authority. All problems are now investigated according to a protocol of Risk Analysis. In order to set up a good Risk assessment necessitates to have a lot of good and representative data –and that is very difficult in microbiology; in that context, the improvement of methods as well as the introduction of predictive microbiology can help but realistic quantitative risk analysis are only a few at the moment… as microbes are alive and can grow and multiply in very different conditions; a lot of analytical problems are not solved, in spite of many improvements; virulence characterization is always very difficult to appreciate and may vary even for strains belonging to a same species; we have also to consider a lot of factors which may influence the growth or, simply, the survival of microorganisms (pH, temperature, water activity, oxygen, nature of the food and available substrates...); then, we must not forget the individual susceptibility… A quantitative evaluation of microbial risk will always be very difficult to set up…

We have to remember that we have a multiplicity of combinations food-microbe; each time that looks like a new situation. The way in which we consider Food microbiology has completely changed from the past decades, changes we were not expecting… and which lead to a lot of connections with different fields of Microbiology.

Connections of Food Microbiology with other fields of Microbiology

The final contamination of a product depends on the possibilities of contamination at different stages of production. From the concept "from farm to fork", well known from everybody nowadays, we can immediately undersee the connections with different fields of microbiology.

If you don't mind, I would like to take an example from my personal experience, as I think it is very significative. A long time ago, around 1970, as I was working in Ploufragan (Brittany), in a laboratory devoted to poultry and eggs, the Ministry of Agriculture asked our Institute to participate in the setting up of standards for poultry further-processed products, as there was a tremendous development of turkey roasts and other products whose processing was quite unknown; the authorities were very much concerned by the possible incidence on human health. That is the reason why we began analyzing different products as turkey roasts and looked at the usual bacteria, including pathogens as *Salmonella*.

From the first observations, we were somewhat anxious: 10% of the turkey roasts were contaminated by *Salmonella* (most of the strains were *Salmonella saint-paul*); at that time, some official measures had to be taken against the industrials considered as responsible on the contamination of their production; the difficulty was that we had been analyzing those products in a purpose of applied research… that was the reason why we decided, in order to help industrials to improve their

production, to look at the possible origin of those *Salmonella*. And that was the beginning of the story I shall summarize.

We began by the processing plants and we found the same type of *Salmonella* on carcasses but also on some live birds entering the processing plant. So, we decided to look at the contamination of poultry farms, analyzing birds, but also the possible ways of those microorganisms to enter the poultry farm (chicks, feed, water, air, soil, farmers...). If we want to summarize, we must say that different sources were important to consider; the first one was chicks –and that is the reason why we decided to investigate hatcheries and breeders farms and we found the same serovar...; the second was the contamination of the environment and of what we called "resident" *Salmonella*; the third one is feed which surely contributes to the contamination of breeders, of chicks and birds of the poultry farm, but the direct relationship is always difficult to establish.

The reason why I give that example is that it shows immediately the connections of Food microbiology with different other fields of microbiology. The first connection, which is the reason why we were interested in that problem is **Medical microbiology**; presently, no controverse is observed, considering different outbreaks from poultry and eggs...

The second connection is **Veterinary microbiology** and, if you allow me, I would like to share with you a short story: In March 1980, I was giving a talk to a group of people (many of them were veterinarians) and I was explaining the problem of *Salmonella* on poultry may become a public health problem... immediately, I received a lot of complains: I had no right to say such a thing as I could not be sure the type of *Salmonella saint-paul* found on breeders was identical to that found on turkey roasts... That was partially true as molecular biology techniques were not used at that time; there was only a high probability (but later, we knew that was true). That shows, too, the connections with **Bacterial Taxonomy,** which is also another branch of Microbiology.

If we consider the importance of the environment in the final contamination of products, we see immediately the connections with the **Microbiology of the Environment** and with **Industrial Microbiology**; moreover, we must not forget the important connections with **Biotechnologies**.

I can add that, due to the new aspects of Food Safety, Food Microbiology is also closely connected with mathematics (**predictive microbiology**).

To improve the knowledge in all those fields, it is necessary to get quick results from a large number of samples; for obvious reasons, rapid methods appeared at first in Medical microbiology, then later in Food microbiology which is the subject of the present symposium. Without giving too many details, I would like now to say a few general data about the techniques used in Food microbiology and their evolution.

Techniques used for detection and identification of microorganisms in foods

The first real developments in Food microbiology can be observed after the second World war only: as nowadays, the evaluation of Food Safety always began by a visual inspection and microbiology was used as a help to the decision.

The microbiological techniques used, which, in France, were taught from the early fifties by R.Buttiaux (who was a physician), in the Pasteur Institute in Lille (F) derived from the discoveries of Louis Pasteur. Those techniques are the conventional ones used at reference methods even if, of course, a lot of improvements have been afforded, new media have appeared of the market, a lot of specifications have been given in the purpose of accreditation, new technologies may be introduced in some cases (it has to be mentioned that, in France, the first regulation giving microbiological criteria, published 21st December 1979, gave criteria and the related microbiological conventional methods). Quite at the same period, due to the necessity of examining a sufficient number of samples, representing as closer as possible the population, many scientists began to think to alternative methods which may be used in laboratories in order to control and maintain Food Safety in most countries.

The first scientist to propose practical solutions is somebody who, providentially, is among us today, and I like to tell you a story I repeat each year: I was participating in a symposium in Kiel(D) in 1974. I can't forget it... I did not present anything on that day; I was just learning and listening at the different papers when, suddenly, we had a very interesting and unusual presentation: the speaker, a young American Chinese was quite "dancing", showing beautiful slides: the miniaturized methods used to detect or enumerate microorganisms from poultry was the subject of his presentation: he was using microplates instead of tubes and a micro-inoculator instead of platina loops… He had a very simple principle "THINK SMALL". As I was working on poultry meat microbiology and was very much interested by rapid methods in order to study a sufficient number of strains to get reliable results, I asked the speaker, at the end of his talk, if he would be so kind as to send me more informations. That was the beginning of a long story in Science and Friendship. Dr. Fung visited our Institute in 1976 and, from that time, miniaturized methods were introduced in our laboratory and used to identify a lot of strains to trace *Salmonella*, then different types of pathogenic microorganisms "from farm to fork"; we were so enthusiastic with those methods that we presented the technique as well as the results during different meetings in different laboratories: we wanted those methods be adopted by the scientific community… and were somewhat successful. In fact,

Dr. Fung is really the "father" of all the commercial methods of identification which appeared on the market from the seventies and I must say I have been very lucky to stay in his laboratory during two months in 1980.
Simultaneously, different scientists around the world began to think to new techniques derived from different branches of Science; in fact, the microbiological controls taking place in industrial laboratories, they had to find a way in order to:
- Get a rapid answer in order to cancel any abnormality in processing food and, by that way, avoid to put on the market some products which would not fill regulatory standards or standards proper to the concerned industry.
- Low cost in order to allow a high number of samples to be examined.
All that being realized, keeping in mind the results have to be accepted by the scientific community.
In that context, many efforts have been proposed quite early, in order to set up methods based on the following principle: the microbial population is evaluated by detecting a signal related with the activity of microorganisms, most often an enzymatic or the concentration of a molecule (coenzyme, metabolite) or a change appearing in the medium (pH or Redox potential impedance, heat production variation) in connection with that activity. Radiometric methods were also proposed.
Then, a lot of methods based on immunology have been developed but it seems that, during the last decade, the most spectacular developments have concerned the introduction and use of methods based on molecular biology, including now DNA chips, which means a fantastic technological evolution, for the characterization of strains. During the week, you will be lucky to learn a lot about the evolutions but one of the difficulties, of course, has been to make the commercial techniques accepted for official controls. That is the reason why I would like to share with you an example of the difficulties encountered to introduce new technologies in regulations. Due to the evolution of techniques proposed, one important problem discussed during many ISO/CEN (I'll say a few words on those organizations in the last part) meetings has been the introduction of new technologies in Food microbiological methods and, to finalize the project, it took a very long time, many hours of discussions…
Finally, an important resolution was taken during the joined meeting held in Parma (It) in April 2004.
• Each time a standard method is being revised, the possibility of using new technologies, including PCR, must be examined by comparing results with those obtained when using the official conventional method.
• For a given microorganism, in order to complete the existing method, the development of standardized methods based on new technologies can be proposed when the purpose to be obtained (for example pathogenicity level) makes it necessary.
• When new technologies, including PCR, are used as alternative methods, they must be validated against the reference method.
Those sentences look probably as quite simple, but I am sure you cannot imagine the number of hours of discussions which were necessary to obtain a consensus: that is "international cooperation" we shall discuss in the last part of my talk.
And that is a new facet of Food Microbiology, i.e Standardisation, and, consequently, relations with Regulations.
Concerning new facets in Food microbiological techniques, I think we can consider Food microbiology as a tree which has roots in different disciplines (biochemistry, immunology, molecular biology…) and that, of course, necessitates a good Scientific cooperation I shall examine briefly in the last part of my presentation.

Food Microbiology and Scientific cooperation

You know probably that Louis Pasteur said: "It is a characteristic of Science that it always open new horizons". Personally, when I began to work, I was far to imagine all the new horizons appearing through new facets. Those facets appeared step by step... I remember that, in 1981, Dr. Fung kindly invited me to co-chair a session of the meeting on "Rapid methods and automation in microbiology" in Washington DC and the title of this session was something like "Rapid methods in Food microbiology: new horizons".
New horizons appearing through new facets, that is so true in our fascinating field!
Let me have a look to different words I used previously:
- The first word is "**Food**". I gave you the "official" definition and we saw that we must not forget that microbes may grow (or not) in the food environment; you also know, of course, that Food Technology is a major field in the context of Food Safety. Most of you, I am sure, know that Josep and Marta have been involved, for a long time now, in the effects of high pressure on the microbiological quality of foods; Food science and Food technology take an important place in many different other Universities: in KSU (KS), of course, but also in Wageningen (NL) and probably, the most important organization in that field is the Institute of Food Technologists (concerning IFT, I must say that Dr. Fung who won a lot of awards, especially from IFT, gave me the possibility to participate in one of the annual meetings (probably in 1986. in New Orleans (LA) and I am very grateful as it is possible, in that type of meeting, to meet a lot of scientists working in Food technology and connected fields… I hope many of you will be as lucky as to participate in such meetings.
- The second word I used is "**microbe**". You surely know there is a national society in quite each country, each of them being involved in the different

branches we have examined, speaking of the connections with the different fields of Microbiology; all the national societies for microbiology are in liaison through the International Union of Microbiological Societies (IUMS) which is one of the 29 Scientific Unions of the International Council of Science.

"The objectives of the Union are to promote the study of microbiological sciences internationally, initiate, facilitate and coordinate research and other scientific activities which involve international cooperation, ensure the discussion and dissemination of the results of international conferences, symposia and meetings and assist the publication of their reports, represent microbiological sciences in ICSU and maintain contact with other international organizations"

Then, after having given some comments concerning the connections of Food microbiology with other sectors of that discipline, I gave a short overview of the evolution of techniques and of difficulties encountered in some cases to make them officially accepted. Of course, the different societies or associations we have mentioned are concerned by techniques but, I would like to focus a few minutes in those which are especially involved in harmonization: ISO (International Standards Organization) and CEN (Comité Européen de Normalisation).

• ISO (International Standards Organization) was created in October 1946; its seat is located in Geneva (CH); the creation results from the fusion of two organizations: ISA, which was the International Federation of National Associations of Standardization, founded in New-York in 1926, and UNSC, i.e Committee for coordination of standardization of United Nations, created in 1944.

The first national Assembly was held in 1949 in the great amphitheater in Sorbonne (Paris). ISO is composed of 247 committees. All standards are obtained by consensus; however, they are not mandatory... The technical committee in charge of Food microbiology is TC34 / SC9; the actual president is Bertrand Lombard (F); the meetings are held in a different country each year; for example, in 2009, the meeting was held in Valencia (Spain); In June 2012, the meeting was held in Brussels (B); as usual, it was a joined ISO/CEN meeting.

• CEN, Comité Européen de Normalisation ("European Committee for Standardization") has been created in 1961 in order to harmonize the standards elaborated in Europe; that means that the standards are mandatory in all countries of EC (at the contrary, the standards which are elaborated by ISO are facultative, which means a great difference). All members are members of ISO as well.

The seat of CEN is located in Brussels (B). In the beginnings, it was created by the national organisms for standardization from France, Germany and Benelux countries. Nowadays, the full members are the 27 countries of EU and the three contries of AELE (Association Européenne de Libre Echange) which own such an organism (Switzerland, Norway and Iceland). CEN elaborates technical standards in favor of international trade.

CEN/TC275/WG6 was created in 1993 and I was nominated as the convenor; nowadays, from July 2005, Alexandre Leclercq from Institut Pasteur in Paris, is in charge of the group.

From the beginnings, one main principle has been followed during the work of this group, i.e the Vienna Agreement; this agreement requires that, as often as possible, ISO methods are taken In order to avoid any overlap, there is also an agreement between different groups of CEN that only one group is in charge of a particular method (the "Vienna agreement"). For example, TC302 in charge of milk and dairy products analysis may choose one specific technique. In this case, it requests TC 275/WG6 to refer to this specific technique in the standard method. The necessity of taking into account the experience of other groups around the world, for example AOAC and IDF (International Dairy Federation), has also been emphasized from the beginning and, presently, the basis for a good cooperation has been set up.

In order to maintain a good international cooperation, one part of the meeting concerns the liaison with other organizations: i.e. International Dairy Federation (IDF) Codex Committee on Food Hygiene, AOAC (Association of Official Analytical Chemists), WHO (World'Health Organisation), IUMS (International Union of Microbiological Societies). The necessity of getting an international consensus, especially with Codex Alimentarius, is always kept in mind; I precise a short information about that organization: "The Codex Alimentarius Commission, established by FAO in 1963 develops harmonized international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair trade practices in the food trade. The Commission promotes coordination of all food standards work undertaken by international governmental and non- governmental organisation".

Finally, I would like to mention that Food microbiologists may find a lot of useful informations from different other organisations. I want to speak of EFSA (European Food Safety Authority, whose seat is in Parma (It)) and the different national agencies (FDA and USDA in US).

Conclusion

It was, of course, impossible to give an exhaustive overview of all the horizons opened by Food microbiology and that was not my purpose. I only wanted to make you show that the field in which we are involved is really an "opened" world, a wonderful world opening anytime new horizons through new facets.

Proficiency Testing with Microorganism Standards

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Today's Situation in Microbiology Control

We are living in a world with more and more regulation. But the microbiology is still a quality control parameter with some open questions. Microbial safety is a major issue in water plants, food and beverage industry but there is a high pressure on the costs (higher volumes).

Today we have following situation:

- Diverse recommendations & regulations.
- Wide range of methods.
- Trend to more standardization.
- Non regulated or new steps/situations/samples.

Organisations like ISO, AFNOR, UKAS, ASTM and ILAC propose guidelines and support on this way the standardization also in the microbiology quality control.

The problems in a microbiology QC lab are broad and compared to chemical analyis there are more open questions to unknown and uncontrolled variables. It is sometimes difficult to decide if results obtained are correct or if we are faced to faults or a natural phenomenon.

Here are some examples of problems:

- Big deviations in analysis.
- Discrepancies between labs.
- Difficult to compare (parameters, methods and results).
- Confusion of finding the best suited methods.
- Validations need lot of time.
- Human faults (handling, calculation, reporting).
- Equipment and culture media failures.

It is no question the today trends go into the right direction with standardization and the knowledge about microbiology quality control increased over the last 20 years. The methods are more accurate and labs control themselves more.

Following needs are important to get more reliable results in the microbiology quality control:

- Trend to ISO, UKAS, EU Regulation 388/2012, FDA, other National Accreditation bodies → standardization of methods.
- Good and stable performance of test (qualitative and quantitative).
- Standards (reference strains, certified reference material).
- Proficiency testing.

Microorganism Standards

One of the major and basic tools to improve accuracy of testing are standards. In case of a microbiology lab that means microorganism standards (or control strain) but not only (also standards for calibration of equipment like balances or incubators). Reference cultures are needed for testing performance of media and tests but also for the validation of method and to confirm the competency of the lab. It is also possible to use reference strains which are derivatives of national or international reference cultures as long as it can be approved that the relevant properties for the application are still exist. According ISO 11133-1 it is possible to culture reference strains one time to get reference stock cultures which are then controlled for purity and biochemical tests. They should be stored in a freezer or as freeze-dried form in small aliquots, defrosted cultures should not be frozen again. Working strains cultures are then preferred made out of a stock cultures and should not be subcultured again.

What is important for a Microorganism Standard:

- Specific defined organisms (reference strains from organizations like ATCC, NCTC, DSMZ...).

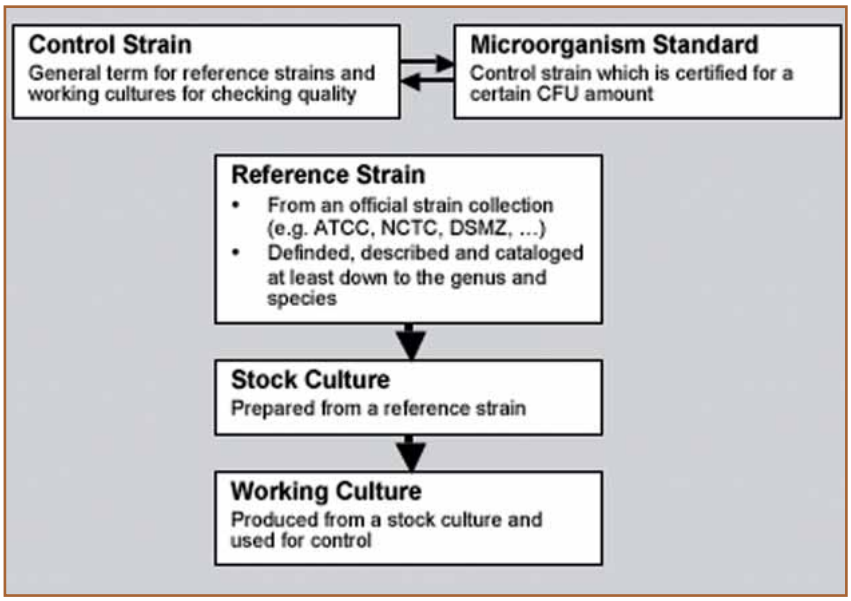

Figure 1.- Control strains.

3.4 Where control media are used for comparative evaluation of performance, they should be prepared independently of the media under test and should be demonstrated to be suitable for control use, in that they are shown to provide consistency of appropriate performance. Conformance with ISO/IEC 17025:2005 necessitates control strains (i.e. reference materials) being traceable to certified materials, where possible. Using cultures obtained from a recognised national culture collection or from a reference materials producer accredited to ISO Guide 34:2000 (PD6532-5:2000) would provide a suitable level of assurance. In-house maintenance of control cultures must guard against contamination and deterioration. Guidance on the preservation and handling of control strains may be found in DD ENV ISO 11133-1:2009. If microbiological certified reference materials are used, they should comply with the definition for CRMs given in ISO Guide 30:1992 (PD 6532-1:1993) and need to contain an appropriate assigned number of organisms.

Figure 2.- Official guidelines from UKAS (Source: UKAS LAB 31 2nd Edition).

Figure 4.- Vitroids™ discs.

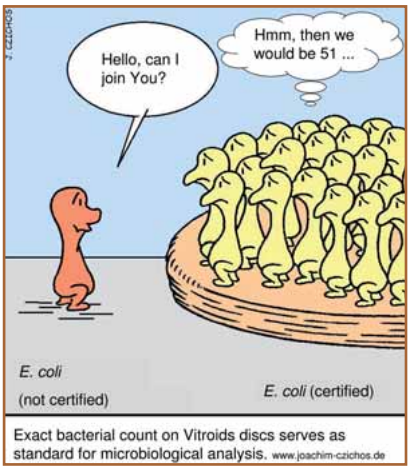

Figure 3.- Vitroids™ cartoon for visualisation.

• Certified Reference Material produced under ISO guide 34 and certified acc. ISO 17025.
• Highly reproducible.

Vitroids™ a possible solution for Microorganism Standards

An easy to use form of microorganism standards are for example the Vitroids™ which are certified reference material. They are made out of reliable reference strains from ATCC and NCTC and produced under ISO guide 34 and the CFU value is certified under ISO 17025. The organisms are in a disc and in this form they can be controlled in numbers and stability. The possible range is 30 to 109 CFU per disc and the reproducibility is 3% at levels of 100 CFU.

The discs are easy to use since they can be placed directly in water, diluent, broth, or even on agar plates. The Vitroids™ contain highly viable bacteria and when placed in contact with media, they dissolve rapidly and start to grow without a lag-phase. The viability of the CFU in a disc is stable for at least one year (for most organisms, more than two years) when kept under refrigeration (-20°C). It is also not a problem if the product is transported for short time at ambient temperature. Each disc is packed in an individual tube with some desiccant and the tubes are then packed in mylar foil. Each package comes with a comprehensive certificate of analysis reporting the CFU and standard deviation.

All of the above mentioned features help microbiologist to have reliable results, save a lot of time (labor, documentation), and lower the costs.

Overview feature and benefits:

• Standards in concentrations of 30-50,000 -CFU per disc.
• Produced acc. ISO Guide 34.
• CFU certified acc. ISO 17025.
• Delivered with detailed certificate of origin.
• Reference strains from ATCC, NCTC, etc.
• Minimum 1 year shelf life at -20°C (usually 2 years).
• No lag-phase.
• Amazingly little standard deviation (e.g. 100 CFU+/- 3%).
• No maintenance of stock and working cultures.
• Dilutions of control strains → no or minimal.
• Recovery time not needed, organisms are ready to grow.
• Pre-enrichment step not necessary
• More reliability (controlled by ISO certified process).
• Easy to use.
• Save costs and time.

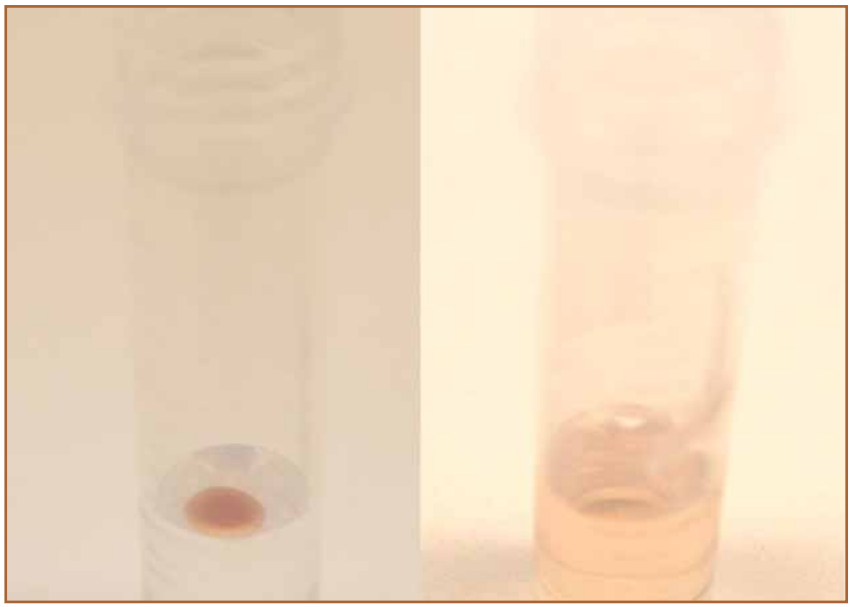

Figure 6.- A Vitroid™ just put into buffer (left) and after 10 minutes it is completely dissolved (right).

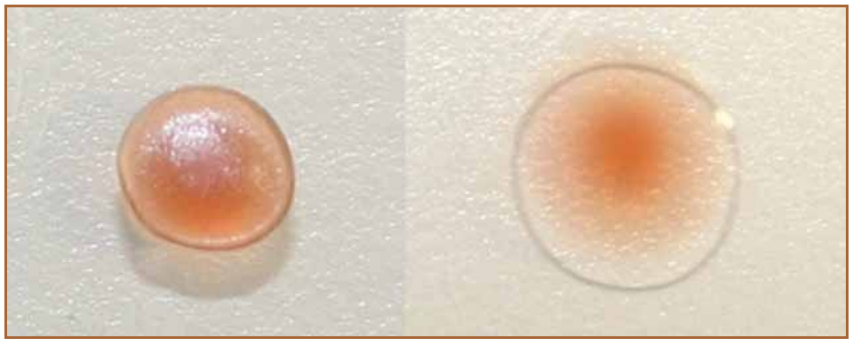

Figure 7.- A single Vitroids™ disc on an agar plate. After about ten minutes on a plate it is rehydrated automatically and forms a droplet (no water addition is needed). The drop can be spread with a loop.

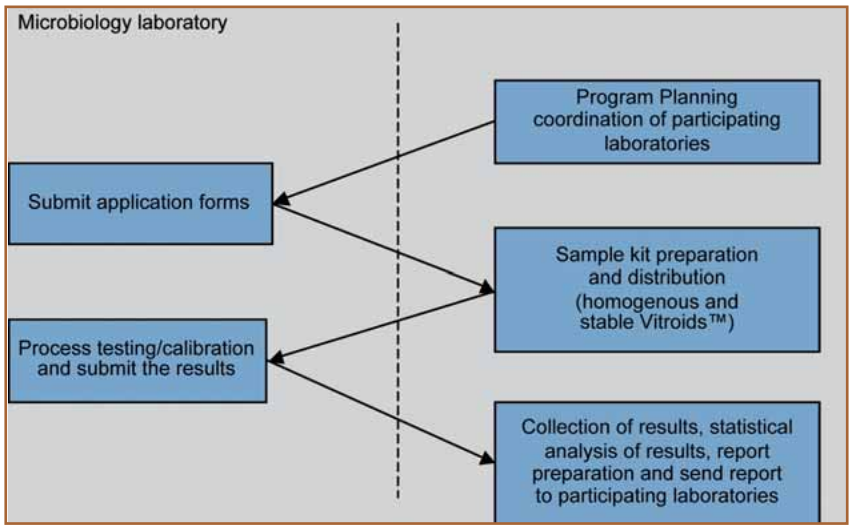

Figure 8.- PT flow chart.

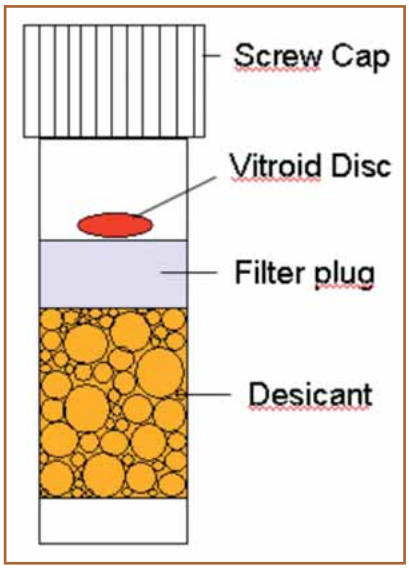

Figure 5.- Packaging of the Vitroids™.

Preparation:
Rehydrate the disc with a common phosphate buffer, or place the disc onto a solid or into a liquid medium. The rehydration process takes approximately 10 minutes. On solid media, the disc forms a droplet that can be spread with a loop. Liquid media may simply be shaken to dissolve the disc. The discs can be rehydrated in as little as 100uL of water or added into larger volumes, e.g. 100mL, for general water testing methods (MF, MTF, Quanti-Tray, etc). It is also possible to add the disc to the media for pour plate techniques.

Proficiency testing (PT) and ISO 17025

PT is an effective tool in helping laboratories to assure themselves that they report correct results.
Principle: Samples of known, but undisclosed, content goes through the routine procedures.
Result: Independent external assessment of performance. Assure the results of the testing laboratory.
Philosophy of ISO/IEC 17025: The same sample at different times, from different analysis and from many different laboratories should reflect an agreeable result.

Table 1.- Available range of Vitroids™

Vitroids™ Test Strains	Origin	Strain #	CFU	Cat #
Aspergillus niger	ATCC	16404™	80	RQC15003
Bacillus subtilis	ATCC	6633™	80	RQC16003
Bacillus subtilis	ATCC	6633™	10000	RQC02258
Candida albicans	ATCC	10231™	80	RQC14003
Clostridium perfringens	NCTC	10240	30	RQC02351
Clostridium perfringens	NCTC	10240	500	RQC20106
Clostridium sporogenes	ATCC	19404™	80	RQC19003
Enterobacter aerogenes	ATCC	13048™	50	RQC01652
Enterobacter aerogenes	ATCC	13048™	200	RQC01655
Enterobacter aerogenes	ATCC	13048™	1000	RQC01657
Enterococcus cloacae	ATCC	35030™	50	RQC21102
Enterococcus faecalis	ATCC	19433™	50	RQC01772
Enterococcus faecalis	ATCC	19433™	200	RQC01774
Enterococcus faecalis	ATCC	19433™	500	RQC01775
Enterococcus faecalis	ATCC	19433™	1000	RQC01777
Escherichia coli	ATCC	11775™	50	RQC01702
Escherichia coli	ATCC	11775™	200	RQC01705
Escherichia coli	ATCC	11775™	1000	RQC01707
Escherichia coli	ATCC	8739™	80	RQC11003
Heterotrophic Organisms			100	RQC02504
Legionella bozemanii	NCTC	11368	50000	RQC02908
Legionella pneumophila	NCTC	12821	50000	RQC02008
Listeria monocytogenes	ATCC	19115™	30	RQC01901
Pseudomonas aeruginosa	ATCC	9027™	30	RQC02202
Pseudomonas aeruginosa	ATCC	9027™	50	RQC12002
Pseudomonas aeruginosa	ATCC	9027™	100	RQC02204
Pseudomonas aeruginosa	ATCC	9027™	200	RQC12005
Pseudomonas aeruginosa	ATCC	9027™	1000	RQC12007
Salmonella enterica subsp. Enterica serovar Abony	NCTC	6017	80	RQC18003
Salmonella enterica subsp. Enterica serovar Typhimurium	ATCC	14028™	50	RQC17002
Salmonella goldcoast	NCTC	13175	30	RQC02301
Staphylococcus aureus susp. Aureus	ATCC	6538™	50	RQC13002
Vitroids™ Blank	-	-	0	RQC0001

ILAC – International Laboratory Accreditation Cooperation

"Proficiency testing is one of the important tools used by laboratories and Accreditation Bodies for monitoring test and calibration results and for verifying the effectiveness of the accreditation process. As such, it is an important element in establishing confidence in the competence of Signatories and their accredited laboratories covered by this Arrangement."

ILAC-P1 ILAC Mutual Recognition Arrangement: Requirements for the Evaluation of Accreditation Bodies.

P1 further mandates Accreditation Bodies (AB) "…to demonstrate the technical competence of its accredited laboratories by their satisfactory participation in Proficiency Testing Activity."

Below in the flow pad the process of a PT cycle is shown. It start as a program and the PT organization (e.g. Sigma-Aldrich) overtakes the coordination for the participating laboratories. The kits with for example different Vitroids with water as sample matrix are sent out to the labs. The labs doing their testing and submit the results to the PT organization. The PT organization collect all results and perform the statistically analysis of the results. Then a report is created and send back to the participating laboratories.

What can I do when I got wrong results?

- Check for all possible sources of error.
- Repeat the process with standards e.g. Vitroids™ or directly use a quality control kit which is similar to the PT kit (may also before the PT).
- Implement corrective actions to avoid future faults.
- Repeat PT (as confirmation).