

# A Current FDA Viewpoint on the Use of Alternative Microbiological Methods

Bryan S. Riley, Ph.D.  
Senior Review Microbiologist  
Office of Pharmaceutical Science

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Center for Drug Evaluation and Research

## Topics

- Process Analytical Technology (PAT)
- Validation of Alternative Methods
- Current FDA Activities



EDQM Microbiology Symposium  
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## Process Analytical Technology (PAT)

- Guidance  
PAT – A Framework for Innovative  
Pharmaceutical Development,  
Manufacturing and Quality  
Assurance (September 2004)

## PAT Guidance

“The Agency considers PAT to be a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality.”

## PAT Guidance

“It is important to note that the term analytical in PAT is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner.”

## PAT

- Process Understanding
- Process Control
- Increased Efficiency
- Reduced Risk
  - Reduced Regulatory Oversight?

## PAT Initiative

- Encourages Use of PAT

How?

## PAT Initiative

- “PATRIOT” = PAT Review and InspectiOn Team
  - CMC Reviewers (CDER & CVM)
  - Compliance Officers (CDER & CVM)
  - Field Investigators (ORA)

## PAT Initiative

- “PATRIOT” = PAT Review and InspectiOn Team
  - Formal PAT Training Program
  - Certification for PAT Teams
    - Including Microbiologists

## PAT Initiative

- PAT submissions - review/inspection by PAT Team
- Communication with FDA to discuss PAT applications
  - Meetings
    - Technical Issues
    - Regulatory Issues
  - Pre-operational site visits

## PAT Initiative

- For more information go to:  
[www.fda.gov/cder/ops/PAT.htm](http://www.fda.gov/cder/ops/PAT.htm)
- Questions or Comments regarding PAT initiative?  
– [pat@cder.fda.gov](mailto:pat@cder.fda.gov)

## Traditional Microbiology Methods

- Growth Based
  - Slow
- Simple
- Inexpensive
- Accepted

## Traditional Microbiology Methods

- Timely????

## Rapid Microbiology Methods

- Faster Results
  - Faster Corrective Action
  - Better Control of Manufacturing Processes
  - Faster product release

## Validation Guidance

- PDA Technical Report 33
  - Evaluation, Validation and Implementation of New Microbiological Testing Methods
- EP 5.1.6.
  - Alternative Methods for Control of Microbiological Quality
- USP Chapter <1223>
  - Validation of Alternative Microbiological Methods

## Validation

- Equivalent or Better
  - At least as good as current test
  - “The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.”  
(ICH Q2A Text on Validation of Analytical Procedures, 1995)

## Validation

- Equivalent or Better
  - Traditional Methods are not perfect
  - Timeliness

## Validation

- Specificity
  - Test Organisms
    - “Compendial Organisms”
    - Facility Isolates
    - Organisms that Challenge the Test Method

## Current FDA Activities

- FDA has Approved Applications for RMMs
- Inter-Center Cooperation for RMMs
- Meetings with Applicants


## Conclusion

- Rapid Microbiology Methods are Useful
- FDA Prepared for RMM Submissions
- Discussion w/ FDA Helpful

# Thank You

[bryan.riley@fda.hhs.gov](mailto:bryan.riley@fda.hhs.gov)

	
<h2>The perspective from regulatory authorities in Europe</h2> <p>Maria Arfwedson Medical Products Agency, Sweden Strasbourg, October 2-3, 2006</p>	
	

<h2>Outline of presentation</h2> <ul style="list-style-type: none"><li>• The new general chapter in Ph Eur<ul style="list-style-type: none"><li>▪ content</li><li>▪ view of EU competent authorities</li></ul></li><li>• Experience among EU regulatory authorities</li><li>• Actual example</li><li>• Conclusion</li><li>• Discussion</li></ul>	
	

## 5.1.6 Alternative methods for control of microbiological quality

- The aim of the new chapter is to *facilitate* the development and implementation of alternative microbiological methods by
  - providing basic principles and potential applications for a number of new methods
  - describing general and specific validation requirements
  - giving an example of a detailed validation protocol

## New methods – potential advantages

- offer more **rapid** results (real-time or near real-time)
  - ⇒ improve cost-effectiveness
- improve the **quality** of testing
- facilitate **automatization** of processes
- enable **proactive** corrective actions
- offer alternatives for **biologicals** e.g. cell therapy products and tissue engineering

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## New methods - limitations

- **extensive validation** work required
- **cost/analysis**
- **expensive** equipment
- further **identification** of microorganisms quantified in the test sample generally not possible
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## Validation section

”It is **critical** to understand the new method and to define what the procedure is intended to achieve”

- General requirements
  - equipment
  - data base
  - method
- Detailed protocol
  - example
- Specific requirements
  - primary validation (by supplier)
  - validation for the intended use (by user)
  - risk/benefit-analysis (by user)

## General view of regulatory authorities

- The progress in pharmaceutical manufacturing incl. the development of new technologies in order to increase process understanding and product quality is strongly supported
- The introduction of chapter 5.1.6 in Ph Eur is supported
- The regulatory concerns in connection with the implementation of alternative methods, e.g. site specific changes, is recognized

## General view of regulatory authorities on the new chapter

- **Informational** chapter
- Methods and validation requirements are not described in detail for the intended application
- Where compliance with Ph Eur is mandatory, a new method can not replace the standard method unless equivalence has been shown and approved by the competent authority



## Experience among EU member states

### • Questionnaire to QWP members – representing human and vet. competent authorities

1. Have your agency received a request for scientific advice regarding alternative microbiological methods?
  - If Yes, which areas of use have been discussed (chemical, biotech products, raw materials, equipment)?
2. Have your agency received an application concerning the implementation of an alternative/rapid microbiological method?
  - If Yes, how many?
  - If Yes, what was the outcome of the assessment?

## Questionnaire to QWP members

- 3. Have your agency performed an inspection in relation to the implementation of an alternative/rapid microbiological method?
  - If Yes, how many?
  - If Yes, what was the outcome of the inspection?
- 4. How many manufacturers in your MS have implemented an alternative method for control of microbial quality according to your knowledge?
  - If any, in which areas?
    - - finished product manufacturing of chemical product?
    - - finished product manufacturing of biotech product?
    - - control of purified water?
    - - control of WFI?
    - - control of other raw materials (chemical/biotech)?

## Input from QWP members (14 responses)

11/14 answered NO to all questions

- UK

- a few (< 20) applications for variation
- variations approved (following GMP inspection) for ATP bioluminescence for finished product testing

- Norway

- 1 type II variation approved, an alternative method for ipc. Release method remained unchanged

## Input from QWP members cont'd

- Sweden

- 1 scientific advice concerning a non-product specific change
- A few (< 5) type II variations concerning non-sterile products approved

- Conclusion: experience among EU competent authorities is very limited

## Actual example

- Request for scientific advice submitted to the MPA, Sweden
- Meeting at the MPA
- Issue raised at the CHMP QWP resulted in a proposed statement
- Proposed statement discussed and agreed on at the ad Hoc Inspectors meeting
- Statement published at the EMEA web site

([www.emea.eu.int/Inspections/QWPfaq](http://www.emea.eu.int/Inspections/QWPfaq))

## Problem statement

- Implementation of an alternative method for rapid control of Purified Water used as an excipient in the pharmaceutical production of non-sterile products
- "Chem Scan"- based on solid phase cytometry - is one of the methods described in the general chapter
- Both methods (Ph Eur 2.6.12 and Chem Scan) was intended to be used as alternative equivalent methods

## Problem statement

- the new method would be used in the production of a large number of non-sterile products
- the change was site-specific
- the extent of regulatory requirements (i.e. number of variations) was crucial for whether or not the company would continue development/validation activities

## QWP discussion

- Should we request the company to submit >100 applications for variation concerning the same change? *No, try to avoid many variations*
- Would a joint GMP inspection of the production site concerned, including the assessment of the validation report by one MS, be acceptable to all MS? *No*

## QWP discussion

- Would it be acceptable to submit one variation application including a list of all products concerned? *No, the current variation regulation does not give the option to including a large number of products in one single application*
- Would it be possible to give the new methods in chapter 5.1.6 the same status as the current agar methods? *No, not in the near future, methods generally described, applications are not specified, validation is not described in detail*

## Variations - PAT

- Current variation regulation is not suitable for site specific variations which may also apply to PAT related applications
- A revision of the regulation is needed
- Assessment of quality documentation and the GMP inspection process need to be more integrated in order to support the development of new technologies in pharmaceutical manufacturing

## Conclusion – Outcome of discussion at QWP/Inspectors group meetings

- Pharmaceutical manufacturers are required to use Ph Eur grade water in the manufacture of medicinal products
- The Ph Eur has introduced a new chapter making reference to the acceptability of alternative methods to replace the standard methods provided that appropriate validation has been demonstrated

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- The implementation might require specific review to ensure that
  - validation data are acceptable
  - the water continues to meet Ph Eur specifications
- If, as in the case of water, the change is not product specific it is suggested that the company request the supervisory authority to make a specific inspection of the site. The authority may decide to involve a quality assessor in the inspection

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## Conclusion – Outcome of discussion

- Since it is expected that the water will continue to meet Ph Eur specification, if tested, no change to dossier requirements would be involved and therefore no regulatory impact on the products concerned would be anticipated

## To summarise the perspective of EU regulatory authorities ...

- We support the development of new techniques
  - increase process understanding
  - develop cost-effective production
- We agree that a procedure for PAT related applications should be developed
- An EU PAT-team was formed in 2004 (4 inspectors, 6 assessors, EDQM observer)

# Use of blood culture method for the microbiological control of cellular products

Agence française  
de sécurité sanitaire  
des produits de santé



**Developing suitable tests: European regulatory perspectives**

Beatrice Panterne – Afssaps – DLC -Blood and cellular products control unit

## Bacteriological control: Regulation



### **Regulatory framework in France:**

**Decree from the 16/12/1998 relative to good practices applicable to human haematopoietic cells for therapeutic use.**

**A microbiological control is required but neither references to a quality standard nor to a control method exist.**

### **Regulation in Europe:**

**Guidelines for tissue engineering products to come**

**Technical drafts following the UE directive 2004-23**

**Points to consider for cell therapy products: CPMP/BWP/41450/98 and CPMP/BWP/1199/02**

## Implementation of an external quality control for haematopoietic products



- In France, since 1999, an external quality control is organized for centres preparing haematopoietic products
- Before organizing this network, and to better know the current practices, a questionnaire was sent to the centres.
- Few standardization for bacteriological methods noticed from this questionnaire.
- Creation of a working group with Afssaps members and experts from production sites to establish a bacteriological control of cellular products.

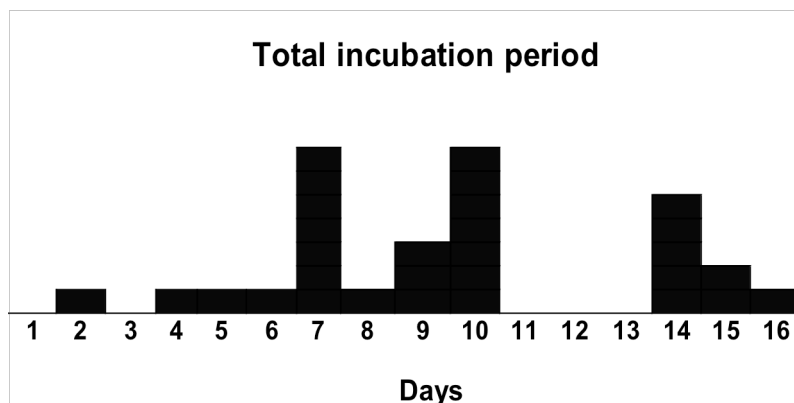
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## Incubation period used by laboratories before French guidelines



**Questionnaire in 1998:** 30 answers, wide diversity in the used methods, with techniques sometimes unsuitable.



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## Why do we need guidelines?



### Problems encountered by this investigation:

- At what step to collect the sample control?
- Sample volume?
- Control on sample from washing supernatant?
- Culture medium?
- Incubation conditions and duration?

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## Working group with experts and Afssaps members



- 1999-2000: survey of the literature, laboratory assays: phagocytosis, bacteriostatic effect of DMSO, method comparison, validation of blood culture method
- First comments circulated and decision to organize a collaborative study: distribution of voluntarily contaminated samples (various levels of contamination).
  - ⟨ Publication in April 2002 of national technical guidelines for the bacteriological control of haematopoietic products.
- Nowadays, at least, one collaborative study per year is organized by DLC for the bacteriological control of cells. Contribution to method validation using inter-laboratory comparison of results.

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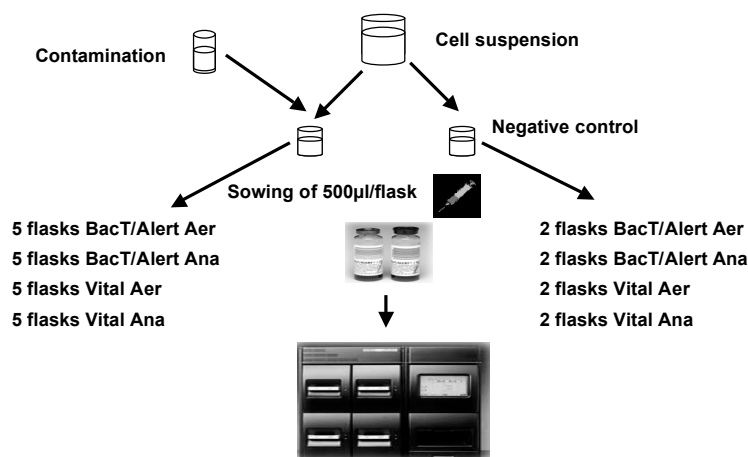
## Why do we use blood culture method?



- Experience gained by the hospital laboratories for the control of bone marrow
- Liquid medium: reduction of the effect of possible inhibiting substances with dilution.
- Presence of SPS: anticoagulant, phagocytosis inhibition
- Presence of a sedimentation agent (manual analysis): improvement of lecture
- Detection system (automated analysis): increase in the contamination detection
- Improvement detection of fastidious germs

REMIC reference frame in medical microbiology, Chapter 17, Société Française de Microbiologie

## Validation of the Bact Alert system (1)



## Validation of the Bact Alert system (2)



### Used microorganisms for the validation

**Aerobic bacteria:**

*Bacillus subtilis* (environment)  
*Corynebacterium jeikeium* (skin)  
*Enterococcus faecium* (environment and intestinal)  
*Escherichia coli* (environment and intestinal)  
*Pseudomonas aeruginosa* (ubiquitous)  
*Serratia marcescens* (environment and intestinal)  
*Staphylococcus aureus* (skin and infectious)  
*Streptococcus pneumoniae* (infectious)  
*Streptococcus pyogenes* (infectious)  
*Yersinia enterocolitica* (intestinal)

**Anaerobic bacteria :**

*Clostridium sporogenes* (environment)  
*Propionibacterium acnes* (derm)  
*Bacteroides fragilis* (intestinal)

**Fungi:** *Aspergillus niger* (environment)

**Yeast:** *Candida albicans* (donor, manipulator)

## Bact Alert Validation - Results



- **Aerobic bacteria:**

BacT/Alert: 100% of positive results as for the smallest contamination (46 UFC/ml)

Vital : 100 % of positive results from 76 UFC/ml

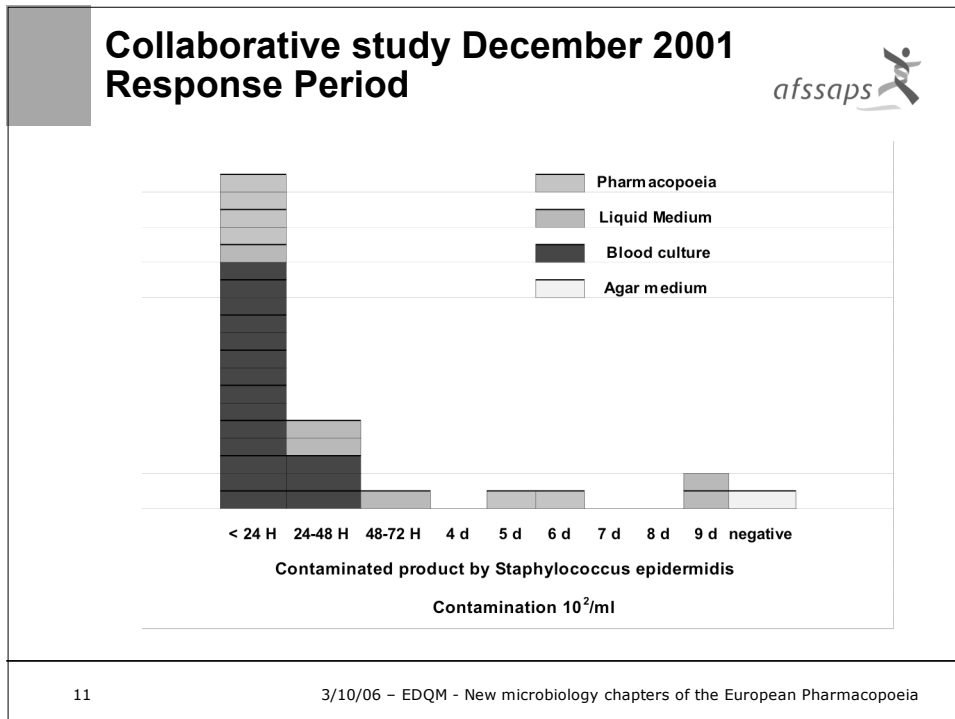
→ correlation of 97% between the two systems

No false positive

Detection time equivalent between the 2 systems

- **Anaerobic bacteria:**

a better detection with BacT/Alert for low contaminations (<50 UFC/ml)



### Collaborative study July 2002

Propionibacterium acnes 100 UFC/ml assay

	Positive	Negative
Blood culture medium (n=23)	82.6%	17.4%
Pharmacopoeia medium (n=7)	71.4%	28.6%
Agar medium (direct inoculation) (n=8)	25%	75%

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## Results of collaborative studies



Year	Percent of good results	Percent of blood culture users
2001	96 ( <i>Staphylococcus epidermidis</i> )	54
2002	100 ( <i>Enterobacter cloacae</i> ) 73 ( <i>Propionibacterium acnes</i> )	77
2003	60 ( <i>Propionibacterium acnes</i> ) 84 ( <i>Aspergillus niger</i> )	84
2004	100 ( <i>Yersinia enterocolitica</i> ) 100 ( <i>Streptococcus agalactiae</i> )	87
2005	100 ( <i>Candida glabrata</i> ) 93,5 ( <i>Acinetobacter sp</i> ) 96,7 ( <i>Bacteroides fragilis</i> )	90,6
2006	93 ( <i>Clostridium sporogenes</i> )	90,6

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## Collaborative study - May 2005 Comparison of blood culture methods




Mean time detection (H)	<i>Candida glabrata</i>	<i>Acinetobacter</i> 15 UFC/ml	<i>Acinetobacter</i> 150 UFC/ml	<i>Bacteroides fragilis</i>
<b>BactAlert</b> (n=13)	26 ± 12.8	30 ± 13.2	26 ± 11.8	49 ± 20
<b>Bactec</b> (n=9)	102 ± 12.2	18 ± 6.4	14 ± 6.7	18 ± 4.8
<b>Manual blood culture</b> (n=7)	39 ± 36.7	101 ± 141	85 ± 127.4	43 ± 38.9

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## Bacteriological control for dendritic cells




Average time detection of voluntary contaminations for dendritic cell samples (clinical grade)

	Blood culture system (n=4)	Pharmacopoeia medium (n=4)
<b>Staphylococcus aureus</b>	<b>20,3 ± 0,96 h</b>	<b>48,0 ± 0 h</b>
<b>Propionibacterium acnes</b>	<b>109 ± 3 h</b>	<b>162 ± 12 h</b>

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## Bacteriological control for chondrocytes



A study has been performed with a french producer of chondrocytes in 2004

- **Blood culture system (n=30)**
  - All contaminations were detected
  - Detection time significantly shorter (whereas inoculum was 2,5 times smaller)
- **Sterility assay §2.6.1 European Phar. (n=30)**
  - Rate of false negatives: 15%
  - Rate of false positives: 8,1%

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## Contaminations found within the external quality control



### Contamination rate:

16 bacterial contaminations (2,5%) detected among the controlled samples (n=630)

### Identified germs :

Staph. hominis, Staph. Warneri (2), Staph. epidermidis (5), Staph. capitis, Strep. mitis, Propionibacterium acnes (3), Acinetobacter johnsonii, Micrococcus sp., Enterobacter cloacae.

### Sensitivity threshold :

Around  $10^3$  micro-organisms per bag when 1% of the bag volume is tested.

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## Bacterial contaminations in haematopoietic products from French Cell Therapy Units



### • Contamination rate:

- 0.82% (44/5388) in 2004 and 0.92% (107/11593) in 2005

### • Most frequent germs:

- Staph. epidermidis (22.4%)
- Propionibacterium acnes (19.6%)
- Other negative-coagulase staphylococcus (12.2%)

### • Time detection > 7 days

- 17,8% of the found contaminations (19/107):
  - In 42% of these cases, the used technique was not an automated blood culture system
  - In 58%, it was a contamination by Propionibacterium acnes (very fastidious growing germ with no clinical relevance)

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## **Bacteriological control of cell therapy products**



**French guidelines published in April, 2002**

**Personnel  
Environment  
Sample  
Analysis  
Result  
Method validation**

**Size of the sample is probably the main problem due to the very small quantity of product available for the bacteriological control in most cases.**

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## **« Microbiological control of cellular products » (2.6.27)**



- **Proposal of a guideline to the European Pharmacopoeia on the basis of the french one**
- **Public inquiry in 2005 using the network of the OMCLs and regulatory agencies.**
- **Adoption in January 2006**
- **Dealing with the type of media, inoculum volume, technical validation (possibility of inter-laboratories comparison) and incubation time.**
  - 7 days only for automated blood culture system due to the higher sensitivity of the bacterial growth detection

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## European perspectives for the bacteriological control



- **Points to consider for evaluation:**

- Need a control at the producer level even if the result is known after the release and the injection to the patient. The results help to better monitor performances of the production facilities.
- Should the specification « products not contaminated » be mandatory considering the single and rare character of some products?
- Notion of benefit/risk: the challenge is to have the best suitable test to be sure to take the best decision even if a contamination has been found.
- Need a method as blood culture one to allow identification
- Clinical sequelae following infusion of a microbially contaminated progenitor cells are extremely rare (Kamble et al, Transfusion,2005)

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## General implementation of blood culture method for cell products



- **In Europe:**

- for haematopoietic cells, most of the bacteriological controls are made using a blood culture method.

- **In USA:**

- Comparison of automated culture systems with a CFR/USP-compliant method ... (Khuu et al, Cytotherapy, 2004)
- Roadmap to approval of automated sterility test method (Kielpinski et al, Cytotherapy,2005)

These data support the general use of automated culture systems for sterility testing of cell therapy product

- **At the Australian Red Cross Blood Services:**

- Validation of the bacteriological control for CSP according to 2.6.27

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**Head of Unit: G. Huyghe**

**C. Kahn**

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control of cell therapy  
products**

**French Cell Therapy Units (33)**