ABRASP Meeting

Sao Paulo, October 20th, 2011
Ultra-rapid Microbiology using ChemScan® RDI

&

Regulatory Requirements for Validation of the RMM
Solid Phase Cytometry
ChemScan® RDI

Content:

1. Principle of the Laser Scanning Cytometry Technology

2. Applications

3. An example of implementation of ChemScan® RDI
Principle of the Laser Scanning Cytometry Technology

ChemScan® RDI
Chemunex technology avoiding the need for cell growth
Fluorassure® Viability Markers

1. Accumulation of the viability substrate in the cell
   Membrane integrity

2. Activation of the viability substrate by the enzyme in the cytoplasm
   Enzyme activity
ChemScan analysis: A simple three step procedure

1. Filtration

2. Cell Labelling

3. Laser scanning
1. Sample Filtration

Large volumes can be tested using:
standard filtration units or ready to use FIFUs

0.4μm polyester track-etched membranes (= ChemFilter)
2. Analysis Protocol with FIFU

1. FILTRATION

Sample

Filtration

Pad saturated with activation solution

Activation

2. LABELLING

Labelling

Pad saturated with labelling solution

3. LASER SCANNING

ChemScan Analysis
3. Laser Scanning

3 ou 5 min scanning for the all membrane
Fluorescent cell labelling

Incident light

488 nm

Fluorescent Signal

Fluorescence Based Cell Labels
3. Scanning is fully overlapping
3. Data Processing

PMT Detector

Digital Signal Processor collects blocks of data

Data Processing

- Colour discrimination
- Light intensity
- Signal shape

Rejected Background

Results

© Copyright 2011 - All Rights Reserved AES Chemunex – September 2011
Signal Shape

Labelled Micro-organism

Autofluorescent Particle
Signal Discrimination

Data Map = Total count

Results Map
= labelled microorganisms

Rejected Background

- Autofluorescent Particles
- Membrane Fluorescence
- Electronic Noise
Results Display
Microscope Validation

1. Membrane holder

2. Automated Microscope Stage

3. Validation of the Scan map

- Microscope objective
- Monotized platform
Labelled Microorganisms

Bacillus

Candida

Mould
Direct viability labelling demonstrated with wide range of microorganisms

**Bacteria Gram -**

Achromobacter xylosoxydans  
Aeromonas hydrophila  
Agrobacterium radiobacter  
Alcaligenes eutrophus  
Alcaligenes faecalis  
Burkholderia cepacia  
Burkholderia diminuta  
Burkholderia pickettii  
Caulobacter sp.  
Cedecea lapagei  
Citrobacter diversus  
Citrobacter freundii  
Comamonas terrigena  
Edwardsiella hoshiniae  
Enterobacter aerogenes  
Enterobacter agglomerans  
Enterobacter cloacae  
Enterobacter gergoviae  
Enterobacter sakasakii  
Enterobacter intermedium  
Erwinia Sp.  
Escherichia coli  
Escherichia coli HB 101  
Escherichia coli 0126 :B16  
Flavobacterium Sp.  
Klebsiella oxytoca  
Klebsiella planticola  
Klebsiella planticola  
Klebsiella pneumoniae  
Klebsiella terrigena  
Kluyvera Sp.  
Moraxella sp.  
Pasteurella aerogenes  
Proteus mirabilis  
Pseudomonas diminuta  
Pseudomonas aeruginosa  
Pseudomonas alkanes  
Pseudomonas mesophila  
Pseudomonas putida  
Pseudomonas fluorescens  
Pseudomonas stutzeri  
Salmonella enterica  
Salmonella indiana  
Salmonella tymphimurium  
Salmonella eboni  
Salmonella sp.  
Salmonella virchow  
Serratia marcescens  
Shigella sonnei  
Xanthomonas maltophilia  
Yersinia enterocolitica  

**Bacteria Gram +**

Aerococcus viridans  
Bacillus anthracis  
Bacillus amyloliquefaciens  
Bacillus cereus  
Bacillus circulans  
Bacillus coagulans  
Bacillus globigii  
Bacillus lentus  
Bacillus licheniformis  
Bacillus megaterium  
Bacillus mycoide  
Bacillus pumilus  
Bacillus sphaericus  
Bacillus steatorrhophilus  
Bacillus subtilis  
Bacillus thuringiensis  
Bacteroides fragilis  
Bacteroides thetaiotaomicron  
Bacteroides vulgatus  
Clostridium acetobutylicum  
Clostridium bifermentans  
Clostridium butyricum  
Clostridium perfringens  
Clostridium sporogenes  
Clostridium tyrobutyricum  
Corynebacterium aquaticum  
Corynebacterium pseudoprophiltheriticum  
Enterococcus faecium  
Enterococcus faecalis  
Fusobacterium nucleatum  
Lactobacillus acidophilus  
Lactobacillus brevis  
Lactobacillus buchneri  
Lactobacillus bulgaricus  
Lactobacillus casei casei  
Lactobacillus casei  
Lactobacillus cellobiosus  
Lactobacillus curvatus  
Lactobacillus delbrueckii  
Lactobacillus fermentum  
Lactobacillus leichmannii  
Lactobacillus plantarum  
Lactobacillus lactis  
Lactobacillus sake  
Lactobacillus sp.  
Leuconostoc oenos  
Leuconostoc Sp.  
Listeria innocua  
Listeria monocytogenes  
Micrococcus luteus  
Mycobacterium bovis  
Mycobacterium parafortuitum  
Mycobacteriums megmatis  
Mycobacterium tuberculosis  
oerskovia sp.  
Pediococcus damnosus  
Pediococcus pentosaceus  
Porphyromonas canoris  
Porphyromonas gingivalis  
Propionibacterium acnes  
Staphylococcus aureus  
Staphylococcus epidermidis  
Staphylococcus hominis  
Staphylococcus warneri  
Staphylococcus xylosus  
Streptococcus faecalis  
Streptococcus salivarius  
Streptococcus thermophilus  
Streptococcus viridans  
Thiobacillus ferrooxidan
## Direct viability labelling demonstrated with wide range of microorganisms

### Yeast
- Acremonium kiliense
- Candida albicans
- Candida ciferii
- Candida colliculosa
- Candida famata
- Candida famata
- Candida fumentans
- Candida humicola
- Candida humicola
- Candida krusei
- Candida magnolia
- Candida parapsilosis
- Candida pelliculosa
- Candida tropicalis
- Cryptococcus albidus
- Debaryomyces hansenii
- Galactomyces geotrichum
- Geotrichum candidum
- Hansenulaspora uvarum
- Hansenula anomala
- Kloechera japonica
- Kloechera Apis apiculata
- Pichia anomala
- Pichia guilliermondii
- Pichia menbrena faciens
- Rhodotorula rubra
- Saccharomyces baillii
- Saccharomyces bisparus
- Saccharomyces cerevisiae
- Saccharomyces rosei

### Mould
- Acremonium Sp.
- Aspergillus versicolor
- Aspergillus versicolor
- Aspergillus flavus
- Aspergillus niger
- Basydiomycetes Sp.
- Byssochlamys Sp.
- Cladosporium cladosporioides
- Epicoccum nigrum ou altenaria
- Fusarium oxysporum
- Fusarium oxysporum
- Fusarium gramineatium roseum
- Humicola fuscoatra
- Mucor circinelloides
- Mucor plumbeus
- Mucor racemosus
- Mucor Sp.
- Neosartoea Sp.
- Penicillium decumbens
- Penicillium expansum
- Penicillium frequentans
- Penicillium roquefortii
- Rhizopus Sp.
- Rhodoturola rubra
- Rhizopusoligosporus
- Scopulariopsis candida
- Trichoderma Sp.
ChemScan® RDI Applications
Pharmaceutical applications

Exemple of applications:

**Sterility Test**
Sterility Test for filterable products

**Biourden**
TVC Bioburden for in-process
90 min
TVC Bioburden for raw material
90 min
TVC Bioburden for end-product
< 3 hours

**Environmental controls**
TVC Bioburden for pharmaceutical water
90 min
Air monitoring using Coriolis
< 3 hours
Surface monitoring using ChemSwab
< 3 hours

**Biotechnology**
Contaminations of cell cultures
< 2 hours
Control of fermentations
90 min

Time to results

Less than 4 hours
90 min
< 3 hours
90 min
< 3 hours
< 3 hours
< 2 hours
90 min
1. Pharmaceutical water testing

- Water Testing demands a high sensitivity & enumeration of microorganisms (quantitative results)

- Current growth based methods have limitations:
  - can lead to underestimation since certain micro-organisms, such as spores, stressed cells & fastidious cells are unable to grow on TSA agar
  - Incubation period between 48-72 hours leads to delay in detection of problems and intervention
  - retrospective to the use of water in the production process (2-14 days to result)
  - can be a compromise between speed & sensitivity
  - delay investigative & corrective activities
1. Pharmaceutical water testing

Applications evaluated:
- Characterisation of new and existing water systems
- Biofilm monitoring
- Routine water system trending

Acknowledgements:
- Gunter Gapp - Novartis
- Sylvie Guyomard – Sanofi Aventis
- Pascale Nabet – Sanofi Aventis
- Jean Scouvault - UCB Pharma

Ref: Evaluation of the applications of a system for real time microbial analysis of pharmaceutical water systems, EJPS; 1999, 4(4): 131-136
1. Pharmaceutical water testing

Case study:

- two purified water systems analysed:
  - 1\textsuperscript{st}: newly constructed system designed to minimise opportunities for biofilm formation
  - 2\textsuperscript{nd}: older system prone to biofilm formation and required regular attention

- both systems analysed with the ChemScan® RDI and using R2A plates incubated for 5 days at 32°C
1. Pharmaceutical water testing

Monitoring of ‘new’ water system

- all results in the range of 0-10 counts / ml
- good correlation between ChemScan & plate counts
1. Pharmaceutical water testing

Monitoring of ‘old’ water system

- counts higher than seen in the ‘new’ water system by both methods
- higher counts on ChemScan compared to plate count data
- related to the ability of ChemScan to detect stressed cells, spores and fastidious microorganisms not recovered by high nutrient plate count methods (e.g. TSA)
- plate count depends on the ability of the organism present to grow under the particular culture condition used
1. Pharmaceutical water testing

Conclusions:

ChemScan typically equivalent to plate count methods in water systems which are in control
- results within current action levels

System has substantially higher sensitivity for detection of stressed cells, spores and fastidious organisms
- sensitivity in 90 minutes at least as good as with low nutrient media incubated for extended periods (5-14 days)
- improved detection of biofilm formation
- opportunities for early detection of contamination not seen with current plate count methods (clear financial benefits)
- opportunity for routine real time monitoring of water systems without compromising sensitivity
2. Bioburden in Intermediates

Moving microbial controls from Product to Process:

- **Microbial quality of incoming raw materials**
- **Bioburden on bulk solutions**

Fast analysis of bulk products before filling

Faster turn-over of production tanks

Fast detection, localisation & correction of contamination
2. Bioburden in Intermediates

Pharmaceutical Products (examples):

- Analgesics
- Antibiotics
- Contact lens washing solutions
- Detergents and cleaning products
- Antiseptic solution
- Heparin
- Nasal solutions
- Peritoneal dialysis solution
- Shampoo
- Sugar solutions
- Vaccines
- Vitamins
3. Surface Monitoring using ChemSwab®

Quantitative rapid surface monitoring using ChemSwabs (Flocked Fiber swabs) and ChemScan® RDI rapid method
3. Surface Monitoring using ChemSwab®

**SWAB RECOVERY CAPACITY**

Recovery from flocked swabs up to **3 TIMES HIGHER** than traditional swabs (60% versus 20%).

**SWAB RELEASE CAPACITY**

Flocked swabs release **4 TIMES HIGHER** than traditional swabs, (90% versus 20%).

Ref: GSK Article in PDA Journal
3. Surface Monitoring using ChemSwab®

Comparative Study

**Material and Methods:**
- 50uL of the suspension on the swabs
- Filtrate solution on ChemFilter CB04 (with a funnel) also compatible with FIFU
- Rinse the Cone with a specific solution / filtrate the rinsing volume
- Analyze the sample with “Surface monitoring using ChemSwabs” protocol on ChemScan® RDI

**Tested strains:**
- Staphylococcus epidermidis SE
- Bacillus subtilis Spores BSS
- Escherichia coli EC
- Aspergillus brasiliensis spores ABS
- Candida albicans CA
- Burkholderia cepacia BC
### Comparative Results

(CFU = control plate, Scan = ChemSwabs + Scan RDI protocol (number of micro-organism detected)):

<table>
<thead>
<tr>
<th>Strains</th>
<th>EC</th>
<th>CA</th>
<th>ABS</th>
<th>BSS</th>
<th>SE</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scan</td>
<td>CFU</td>
<td>Scan</td>
<td>CFU</td>
<td>Scan</td>
<td>CFU</td>
</tr>
<tr>
<td>EC</td>
<td>91</td>
<td>81</td>
<td>158</td>
<td>103</td>
<td>79</td>
<td>66</td>
</tr>
<tr>
<td>CA</td>
<td>95</td>
<td>83</td>
<td>176</td>
<td>98</td>
<td>92</td>
<td>61</td>
</tr>
<tr>
<td>ABS</td>
<td>81</td>
<td>87</td>
<td>143</td>
<td>99</td>
<td>91</td>
<td>73</td>
</tr>
<tr>
<td>BSS</td>
<td>-</td>
<td>-</td>
<td>170</td>
<td>102</td>
<td>136</td>
<td>80</td>
</tr>
<tr>
<td>SE</td>
<td>91</td>
<td>84</td>
<td>162</td>
<td>101</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>BC</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>91</td>
<td>84</td>
<td>162</td>
<td>101</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>Correlation Scan/CFU</td>
<td>108%</td>
<td>160%</td>
<td>143%</td>
<td>169%</td>
<td>101%</td>
<td>95%</td>
</tr>
</tbody>
</table>
4. Air Monitoring using Coriolis®µ

Portable Air Sampler for bio-aerosol collection

Your results in few hours combining

Coriolis®µ

with ChemScan®RDI

Designed and developed by:

© Copyright 2011 – All Rights Reserved AES Chemunex – September 2011
4. Air Monitoring using Coriolis®μ

Operating principle of the technology

Before collection
Cone ready to use with the adapted collection liquid

During collection
1. Vortex beginning
Air and particles come into the cone forming the vortex
2. During the vortex
Aspirated particles are centrifuged with the liquid on the cone’s inner surface

After collection
Particles concentrated in the liquid
4. Air Monitoring using Coriolis®μ

Comparative Study

Material and Methods :

- 1,5m³ sampling: 5 minutes at 300 L/min with Coriolis® μ using collection liquid
- Filtrate solution on ChemFilter CB04 (tests with a funnel) also compatible with FIFU
- Rinse the Cone with a specific solution / filtrate the rinsing volume
- Analyze the sample with "Air monitoring using Coriolis" protocol on ChemScan RDI

Tested strains :

- Staphylococcus epidermidis SE
- Bacillus subtilis Spores BSS
- Escherichia coli EC
- Aspergillus brasiliensis spores ABS
- Candida albicans CA
- Burkholderia cepacia BC
- Micrococcus luteus ML
4. Air Monitoring using Coriolis®µ

Comparative results

(CFU = control plate, Scan = Coriolis + Scan RDI protocol (number of microorganism detected)):

<table>
<thead>
<tr>
<th>Strains</th>
<th>EC</th>
<th>CA</th>
<th>ABS</th>
<th>ML</th>
<th>BSS</th>
<th>SE</th>
<th>BC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Scan</td>
<td>CFU</td>
<td>Scan</td>
<td>CFU</td>
<td>Scan</td>
<td>CFU</td>
<td>Scan</td>
</tr>
<tr>
<td>EC</td>
<td>55</td>
<td>52</td>
<td>91</td>
<td>97</td>
<td>57</td>
<td>45</td>
<td>101</td>
</tr>
<tr>
<td>CA</td>
<td>64</td>
<td>56</td>
<td>69</td>
<td>78</td>
<td>45</td>
<td>46</td>
<td>113</td>
</tr>
<tr>
<td>ABS</td>
<td>68</td>
<td>55</td>
<td>94</td>
<td>82</td>
<td>72</td>
<td>57</td>
<td>88</td>
</tr>
<tr>
<td>ML</td>
<td>62</td>
<td>54</td>
<td>85</td>
<td>86</td>
<td>58</td>
<td>49</td>
<td>101</td>
</tr>
<tr>
<td>BSS</td>
<td>58</td>
<td>49</td>
<td>101</td>
<td>84</td>
<td>78</td>
<td>92</td>
<td>82</td>
</tr>
<tr>
<td>SE</td>
<td>62</td>
<td>72</td>
<td>82</td>
<td>41</td>
<td>62</td>
<td>69</td>
<td>62</td>
</tr>
<tr>
<td>BC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>62</td>
<td>54</td>
<td>85</td>
<td>86</td>
<td>58</td>
<td>49</td>
<td>101</td>
</tr>
<tr>
<td>Correlation Scan/CFU</td>
<td>115%</td>
<td>99%</td>
<td>118%</td>
<td>120%</td>
<td>85%</td>
<td>201%</td>
<td>85%</td>
</tr>
</tbody>
</table>
Evaluation of Coriolis® microbial air sampler coupled with RMM
Alternative solution for rapid airborne contamination control

Abstract

Environmental contamination control in cleanrooms, Berlin Technologies (France) has developed a technology dedicated to the monitoring of particles. The goal is to propose a sampling method compatible with Rapid Microbiological Methods (RMM) in order to get reliable specific results within impaction method.

A microbial air sampler has been validated according to ISO 14698-1 in terms of physical and biological efficiencies (HPA study - July 2008): the Coriolis® μ is as efficient as the traditional method and even better for high particles diameter results available on www.coriolis-air.com.

AES Chemunex has also validated a protocol coupling Coriolis® μ with ScanRD® (cytometry) and allows to get the results in only 3 hours (from step to results). This study aims at completing this data and at testing different RMM on the samples collected with Coriolis® μ.

Context

Pharmaceutical quality control of environments aims at ensuring the quality of products in case of cleanrooms production / health and safety of workers and exposed people.

Rapid & reliable measurements of microbial contamination depend on the choice of an adapted air sampler / a representative sample from sterilized environment / the limitation of losses due to a failure of the sampler to capture particles containing microorganisms or to due to loss of viable microorganisms during collection so that formation of visible colonies on agar surfaces will not occur.

Objective: realize the feasibility study of the Coriolis® μ air sampler coupled with RMM (Scan RD®) in order to implement it as a new solution for rapid investigation in case of contamination and for monitoring in production sites.

Material & methods

Sampling has been carried out in one of the Micro Lab using either traditional air sampler (impactor) or Coriolis® μ. Samples with the Coriolis® μ were further processed by filter-plate or ScanRD method (RMM).

SAMPLERS = impactor vs Coriolis® μ (Berlin Technologies)

The Coriolis® μ air sampler is based on a patented cyclonic technology: it concentrates airborne particlesolid culture media.

RD METHOD = ScanRD (AES CHEMUNEX)

RD® is based on cytometry and uses a double discrimination key: viability and cell membrane integrity.

Discussion

Table 1 - the impactor and Coriolis® μ give equivalent results on the air samples in the lab

<table>
<thead>
<tr>
<th>Samples</th>
<th>Impaction</th>
<th>Coriolis® μ with filter plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm</td>
<td>0</td>
<td>1 M</td>
</tr>
<tr>
<td>Biofilm</td>
<td>0</td>
<td>1 M</td>
</tr>
<tr>
<td>Countertop</td>
<td>6 (4 M + 2 B)</td>
<td>2 B</td>
</tr>
<tr>
<td>Countertop</td>
<td>4</td>
<td>2 B</td>
</tr>
<tr>
<td>Countertop</td>
<td>3 (2 M + 2 B)</td>
<td>2 B</td>
</tr>
<tr>
<td>Countertop</td>
<td>2</td>
<td>2 B</td>
</tr>
</tbody>
</table>

M = Mold - B = Bacteria

Table 2 - ScanRD analysis gives higher counts than the plate method does, especially for contaminated air samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Coriolis® μ + Scan RD</th>
<th>Coriolis® μ with filter plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm</td>
<td>0</td>
<td>1 M</td>
</tr>
<tr>
<td>Biofilm</td>
<td>0</td>
<td>1 M</td>
</tr>
<tr>
<td>Countertop</td>
<td>6 (4 M + 2 B)</td>
<td>2 B</td>
</tr>
<tr>
<td>Countertop</td>
<td>4</td>
<td>2 B</td>
</tr>
<tr>
<td>Countertop</td>
<td>3 (2 M + 2 B)</td>
<td>2 B</td>
</tr>
<tr>
<td>Countertop</td>
<td>2</td>
<td>2 B</td>
</tr>
</tbody>
</table>

M = Mold - B = Bacteria

These higher results are partly due to the viable but non-cultivable (VNC) microorganisms present in the air which cannot be detected by the traditional impaction method although they can be pathogenic.

Liquid sample → Access to alternative methods → Rapid results (RMM beyond cultivable flora)

The Coriolis® system is an easy to use portable air sampler that collects air samples into liquid media. This allows quick microbial detection when coupled with RMM technologies as demonstrated into this study.

The feasibility study conducted in Saint-Louis Micro Lab indicates that Coriolis® system collects airborne microorganisms comparable amount to the impaction system does. ScanRD can be used as Rapid Microbiology Method coupled with cell collection in order to give rapid results (around 3 hours from sampling to result) It is also shown here that the amount of microorganisms detected with the ScanRD is higher than with the traditional method as far as it is not based on culturability/viability; appropriate alert and action limits may thus need to be re-evaluated for ScanRD results. This couple of innovative equipments fits for the Environmental Monitoring application and could be implemented for investigation and routine monitoring in production sites and in critical areas and cleanroom environments.

Acknowledgement

Pfizer study Team: Dr Lin Chen (responsible of the study), S. Fenne

- Bertin Technologies: Alexandra Guerin, Quitterie Desjonqueres

- AES CHEMUNEX: P. Barthes

Keywords: biocontamination - airborne particles - microbial air

- Pfizer study Team: Dr Lin Chen (responsible of the study), S. Fenne

- Bertin Technologies: Alexandra Guerin, Quitterie Desjonqueres

- AES CHEMUNEX: P. Barthes

Keywords: biocontamination - airborne particles - microbial air
Conclusion: an exemple of implementation of the ChemScan® RDI
How useful is microbiological testing of end product?

Lot: 0.1% defectives → 10 samples analyzed: Probability of detection ~ 1%

Lot: 20% defectives → 10 samples analyzed: Probability of detection ~ 35%
How Can we move Forward?

- Improving sampling sensitivity
  - Air and gases
  - Surfaces and personnel

- Implementation of Rapid Micro Technology
  - Selecting the suitable for the purpose
  - Defining an appropriate strategy

- Moving microbial controls from Product to Process
  - Microbial quality of incoming materials: Raw material testing and In-process Bioburden
  - Environmental monitoring: Air and surface monitoring
  - Water for pharmaceutical use
Recent Case of implementation for PAT

- Non sterile nasal product

- Deployment of environmental monitoring, intermediates and waters data for **Real Time Release (RTR) of drug products** manufactured under conventional aseptic process

- 6 critical points from risk analysis
Recent Case of implementation for PAT

From which:

- **Process Water** → ChemScan® RDI - 90 min
- **Bulk Product** → ChemScan® RDI - 3 hours
- **Surface** → ChemScan® RDI - 3 hours
- **Air** → Other RMM - 24 hours
- **Filled Vials** → ChemScan® RDI - 3 hours

This manufacturing site receives FDA inspection and formal approval in July 08 to use ChemScan® RDI system as a part of a rapid microbiological in-process monitoring of a non sterile product **eliminating the need for end product microbial testing prior to release.**
ChemScan® RDI Validation
Alternative methods validation

3 standards on the validation of alternative methods:

• **European Pharmacopoeia**, chapter 5.1.6 “Alternative Methods for Control of Microbiological Quality”
  – Description of some alternative methods in which the ChemScan® RDI description (solid phase cytometry) and BactiFlow® ALS description (flow cytometry)

• **USP**, chapter 1223 “Validation of Alternative microbiological methods”

Validation steps

1. System Qualification
   - FAT (Factory Acceptance Test)

2. Qualification of the installation of the system and its main operational functions
   - Commissioning
   - IQ : Installation Qualification
   - OQ : Operational Qualification

3. Performance Qualification of the system
   - PQ1 : Performance Qualification 1

4. Performance Qualification in real conditions (with samples)
   - PQ2 : Performance Qualification 2
## Performance Qualification

<table>
<thead>
<tr>
<th>Validation Parameter</th>
<th>Qualitative Test</th>
<th>Quantitative Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>European Pharmacopeia</td>
<td>US Pharmacopeia</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Linearity</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Precision</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Assay Range</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Robustness / Ruggedness</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
Validation of the ChemScan® RDI
## Validation of the ChemScan® RDI with quantitative applications

- **Commissioning**

  1. **Installation Qualification**

  2. **Operational Qualification**

  3. **Performance Qualification 1**

  4. **Performance Qualification 2**

<table>
<thead>
<tr>
<th></th>
<th>Customer service</th>
<th>3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Or</td>
<td>Laboratory</td>
<td>1 week</td>
</tr>
<tr>
<td>Or</td>
<td>Or</td>
<td>Several weeks</td>
</tr>
<tr>
<td></td>
<td>Laboratory</td>
<td>5 – 6 weeks*</td>
</tr>
</tbody>
</table>

* With the recommended strains

**Depend on the laboratory**
Validation of the ChemScan® RDI with qualitative applications

- Commissioning

1. Installation Qualification
2. Operational Qualification
3. Performance Qualification 1
4. Performance Qualification 2

<table>
<thead>
<tr>
<th></th>
<th>Customer service</th>
<th>Or</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>3 days</td>
<td>1 week</td>
<td>Several weeks</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td>4 weeks*</td>
<td>Depend on the laboratory</td>
</tr>
</tbody>
</table>

* With the recommended strains
PERFORMANCE QUALIFICATION 1
ANALYTICAL PERFORMANCE PROTOCOLS AND REPORT
AES CHEMUNEX, France

(Chem)Scan®RDI UNIT
for TVC Bioburden, Fungi, Scan Bio II protocols

Document Reference: 201-D0225-11
Date of issue: 28 October 2008
Page: 1 of 54

Affix the self adhesive control label here

Protocol Approvals

<table>
<thead>
<tr>
<th>Name</th>
<th>Job Title</th>
<th>Company</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. Jost</td>
<td>Application Specialist</td>
<td>AES CHEMUNEX</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Approved By:

<table>
<thead>
<tr>
<th>Name</th>
<th>Job Title</th>
<th>Company</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.L. Drooqart</td>
<td>Scientific &amp; Technical Director</td>
<td>AES CHEMUNEX</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Report Approvals

<table>
<thead>
<tr>
<th>Name</th>
<th>Job Title</th>
<th>Company</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

TABLE OF CONTENTS

1. OBJECTIVES AND SCOPE ........................................... 3
2. DESCRIPTION ................................................................... 5
3. RESPONSIBILITIES ......................................................... 7
4. DATA COLLECTION AND ENTRY ......................................... 8
5. MATERIALS / EQUIPMENT ............................................... 9
6. OUTSTANDING ISSUES .................................................... 10
7. SAFETY PRECAUTIONS AND APPROVAL TO COMMENCE WORK .... 11
8. IDENTIFICATION OF PERSONNEL ....................................... 12
9. QUALIFICATION STRATEGY ............................................... 13
10. SOP VERIFICATION ...................................................... 16
11. SYSTEM PERFORMANCE MONITORING ................................ 17
12. RECOMMENDED METHODS FOR STANDARD MICRO-ORGANISM SAMPLE PREPARATION .................................................. 20
13. ANALYTICAL CHECKS .................................................... 31
14. PREVENTATIVE MAINTENANCE ......................................... 32
15. TRAINING ................................................................. 32
16. CHANGE CONTROL ....................................................... 33
17. LIST OF APPENDICES .................................................. 34
18. NON-COMFORMANCE LIST .............................................. 35
19. SUMMARY AND CONCLUSIONS ....................................... 37
20. DOCUMENT REVIEW AND APPROVALS ............................. 38
21. FINAL APPROVALS ...................................................... 39
22. REFERENCE DOCUMENTS ............................................... 40
23. KEY TO ABBREVIATIONS ............................................... 41
24. PROTOCOL REVISION HISTORY ...................................... 42
5. QUALIFICATION STRATEGY

5.1 Qualification source references

The qualification strategy as defined in the PQ1 has been compiled from the following key analytical references:

- US Pharmacopeia 31, <1223> Validation of Alternative microbiological methods
- PDA Technical Report 33, "The Evaluation, Validation and Implementation of New Microbiological Methods"
- European Pharmacopoeia 6th Edition, "5.1.6 Alternative Methods for Control of Microbiological Quality"

The key analytical parameters that need to be tested are for the PQ1 of a quantitative application:

- Accuracy: This is defined as the closeness of the test results obtained by the alternative method to the value obtained by the pharmacopoeial method. Accuracy must be demonstrated across the practical range of the test.
- Linearity: This is defined as the ability to produce results that are proportional to the concentration of micro-organisms present on the sample within a given range.
- Precision: This is defined as the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of homogeneous suspensions of micro-organisms under the prescribed conditions.
- Limit of quantification: This is defined as the lowest number of micro-organisms that can be accurately counted.
- Limit of Detection: This is defined as the lowest number of micro-organisms in a sample that can be detected under the stated experimental conditions.
- Assay Range: This is defined as the interval between the upper and lower levels of micro-organisms that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.
- Specificity: This is defined as the ability of the method to accurately detect a required range of micro-organisms that are present in the sample under test.
- Robustness (and Ruggedness): This is defined as the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its robustness under a variety of normal test conditions, such as different analysts, instruments, batches of reagents and laboratories.

Check 1: Determination of Accuracy and Linearity

OBJECTIVES

To compare the microbiological counts derived from testing samples with the (Chem)Scan® RDI unit with those from a traditional microbiological technique (a reference standard) to determine Accuracy and Linearity.

The studies should include all the organisms of relevance to the production process assayed as single pure cultures. The identity of the micro-organism should be proven by a defined method and reference made as to the location of this information.

The range of analysis should be determined according to the range of interest for the routine analysis. The highest concentration of the range should be higher than the acceptance limit and the lowest concentration of the range should correspond to the limit of quantification of the technology (in most of the cases, 5 cfu/filtered volume is acceptable).

The limit of detection of the technology will be calculated later on.

It is recommended to analyse five suspensions where concentrations are within the range of analysis, and repeat the assay 3 times.

ND: Assay the dilutions in 3 series of five singletons and perform the plate assays at the same time. This removes any bias that may be caused by the cultures multiplying between assays.

Two working sessions are then carried out under conditions of maximum variability (different reagents, different operators, different days, etc.).

As the reproducibility is referring to the "Use of microbiological method within the same laboratory over a short period of time using different analysts with the same equipment" (Source: PDA Technical Bulletin 33), it is recommended to change analysts for the different working sessions.

The following table shows an example for a range of interest between 5 and 1000 cfu/filtered volume:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Working day 1</th>
<th>Working day 2</th>
<th>Working day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>160 cfu</td>
<td>160 cfu</td>
<td>160 cfu</td>
</tr>
<tr>
<td>B</td>
<td>80 cfu</td>
<td>80 cfu</td>
<td>80 cfu</td>
</tr>
<tr>
<td>C</td>
<td>40 cfu</td>
<td>40 cfu</td>
<td>40 cfu</td>
</tr>
<tr>
<td>D</td>
<td>10 cfu</td>
<td>10 cfu</td>
<td>10 cfu</td>
</tr>
<tr>
<td>E</td>
<td>5 cfu</td>
<td>5 cfu</td>
<td>5 cfu</td>
</tr>
</tbody>
</table>

The same data will be used to determine the Linearity, Accuracy, Precision and Limit of Quantification of the alternative method.
Muito Obrigado!