ABRASP Meeting

Sao Paulo, October 20th, 2011



AES CHEMUNEX
The Rapid Microbiology Company

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The Rapid Microbiology Company

Ultra-rapid Microbiology using Chem*Scan*® *RDI*

&

Regulatory Requirements for Validation of the RMM

Solid Phase Cytometry ChemScan® RDI

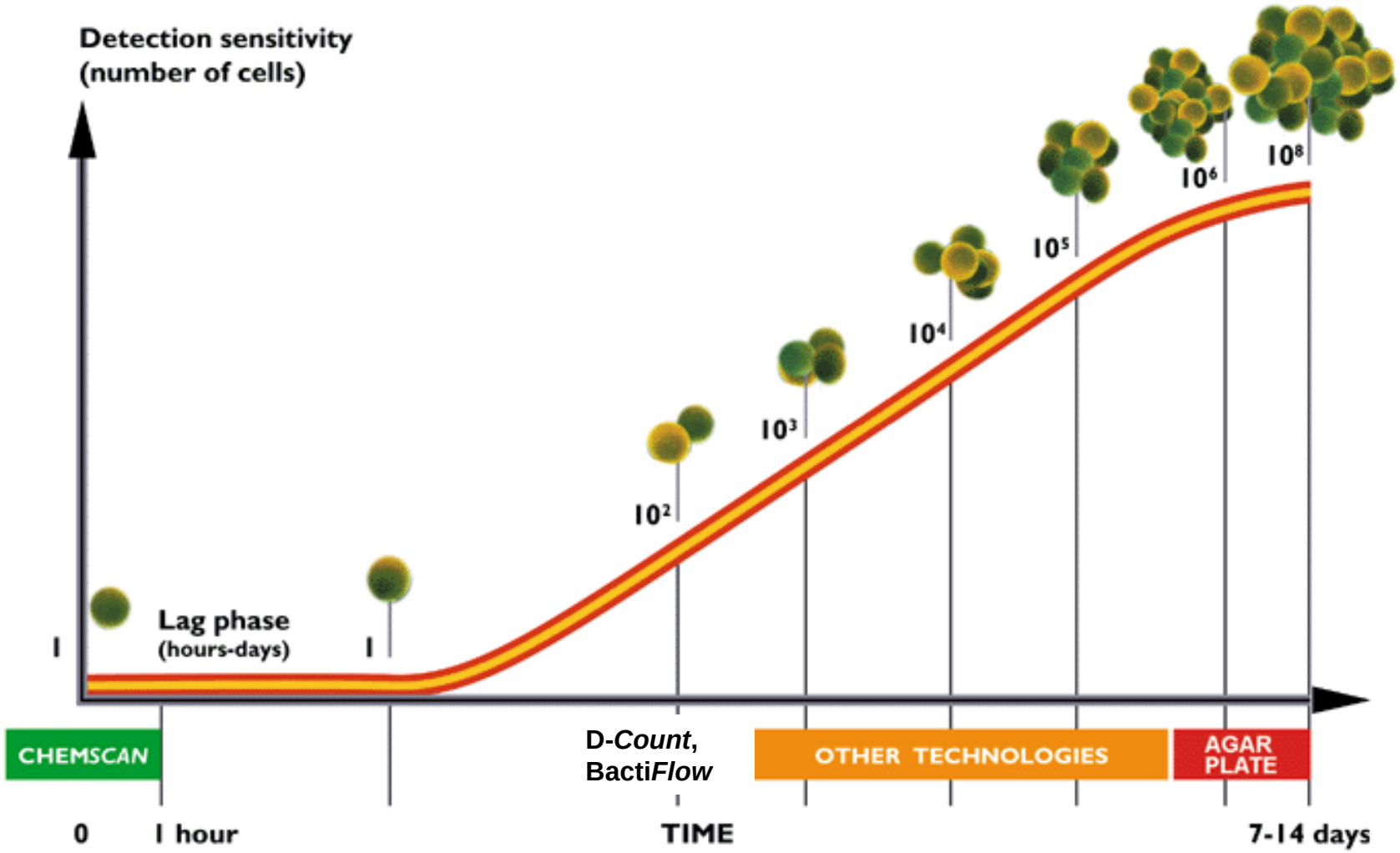
Content:

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

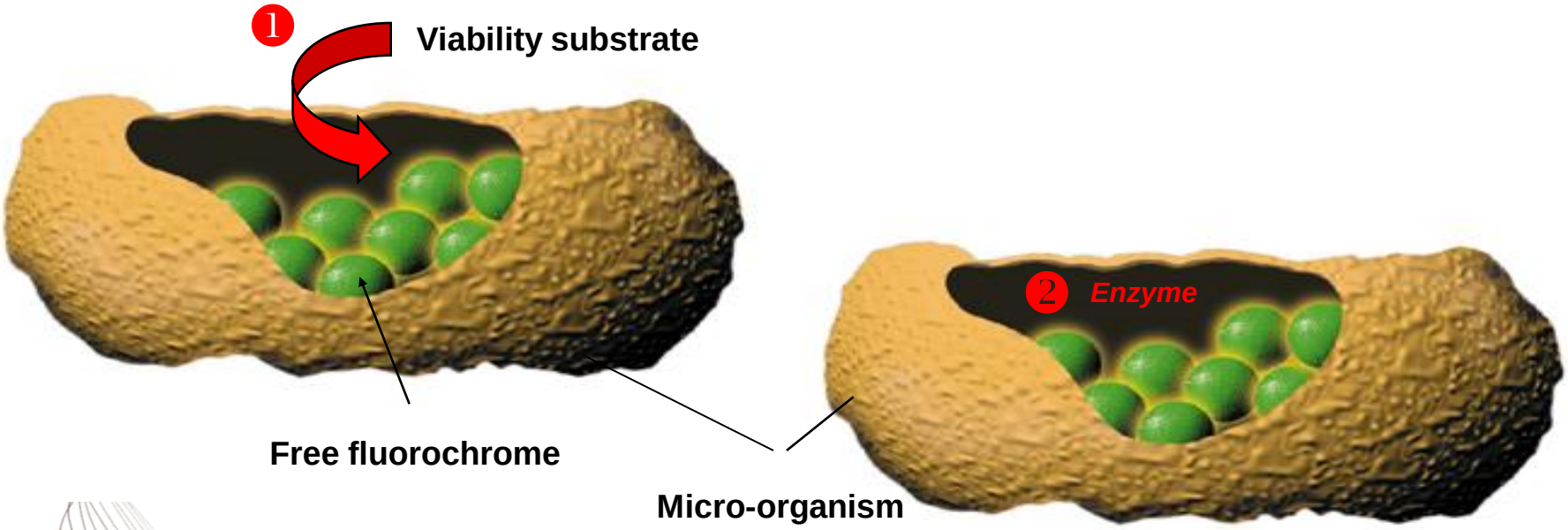
Principle of the Laser Scanning Cytometry Technology

Chem*Scan*® *RDI*

Chemunex technology avoiding the need for cell growth



Fluorassure® Viability Markers



❶ Accumulation of the viability substrate in the cell

Membrane integrity

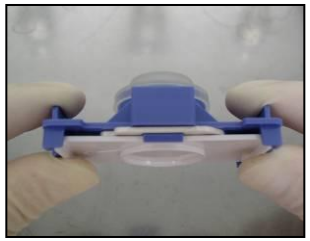
❷ Activation of the viability substrate by the enzyme in the cytoplasm

Enzyme activity

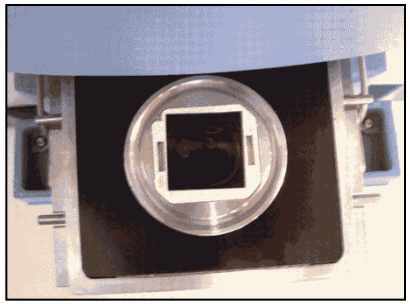
ChemScan analysis : A simple three step procedure

1. Filtration

2. Cell Labelling



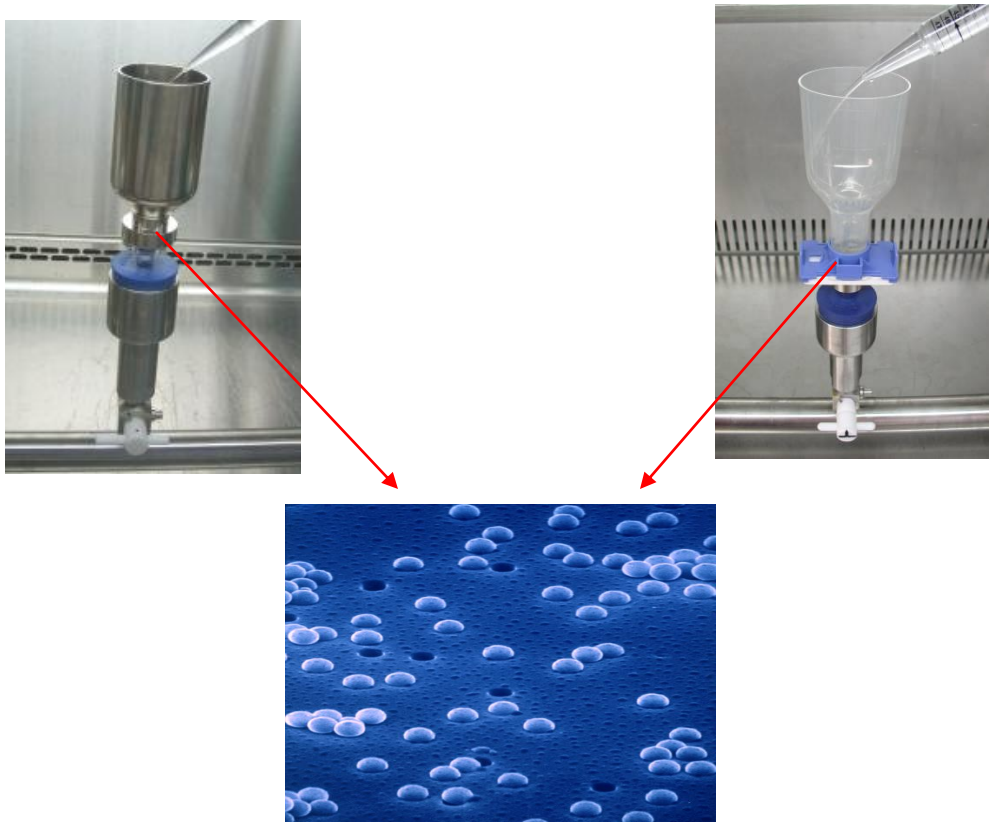
3. Laser scanning



1. Sample Filtration

Large volumes can be tested using :

standard filtration units or ready to use FIFUs



0.4µm polyester track-etched membranes (= ChemFilter)

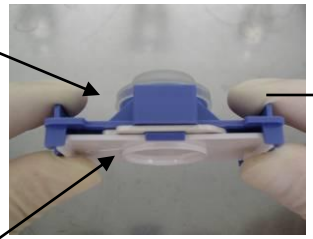
2. Analysis Protocol with FIFU

1. FILTRATION **2. LABELLING** **3. LASER SCANNING**

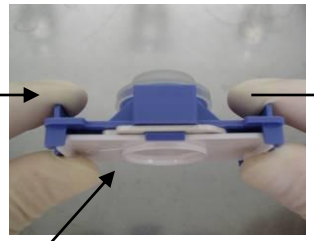
Sample

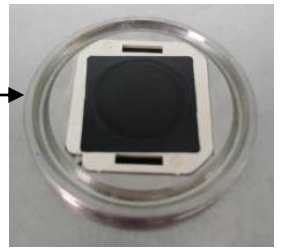
Filtration

Activation

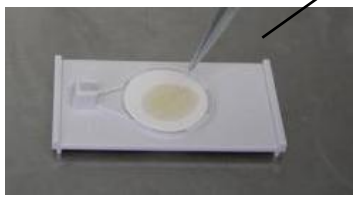


Labelling

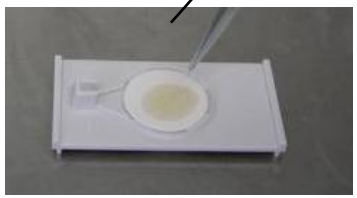




Chem *Scan* Analysis

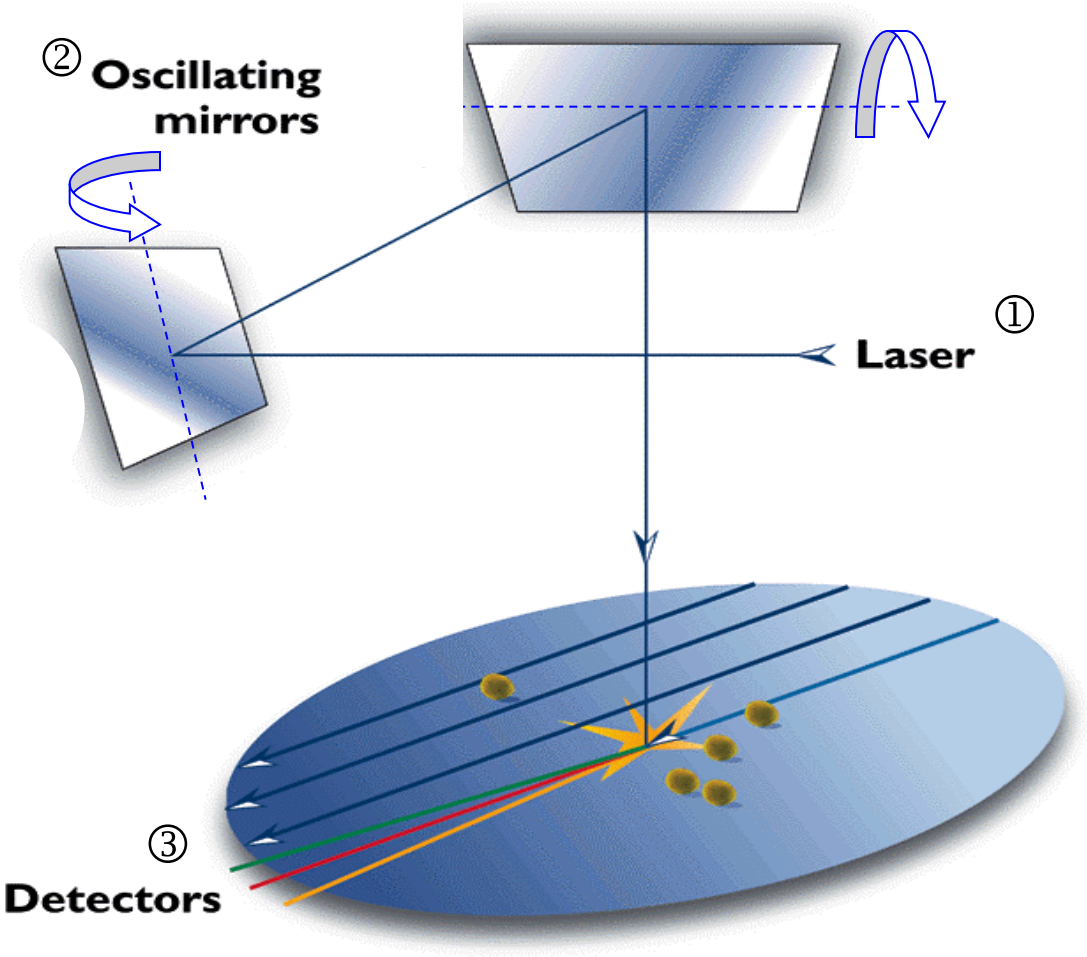


Pad saturated with activation solution



Pad saturated with labelling solution

3. Laser Scanning

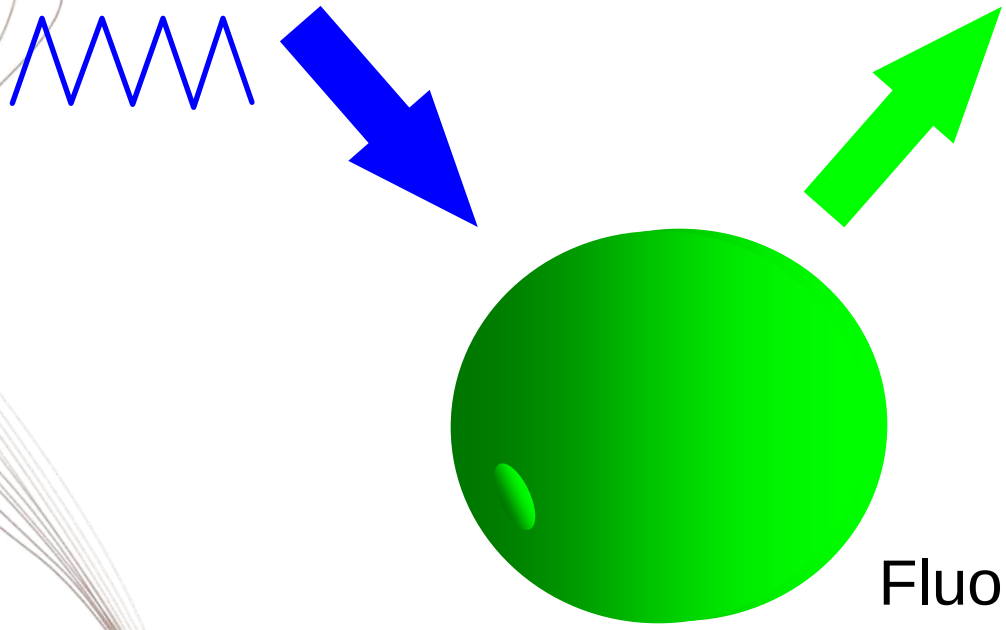


⇨ 3 ou 5 min scanning for the all membrane

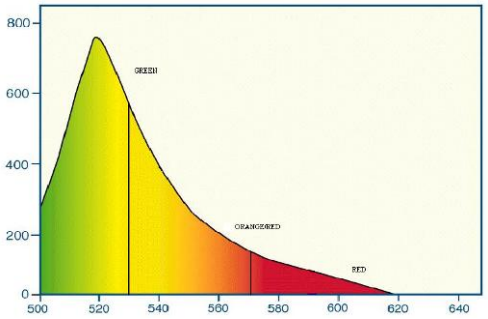
Fluorescent cell labelling

Incident light

488 nm

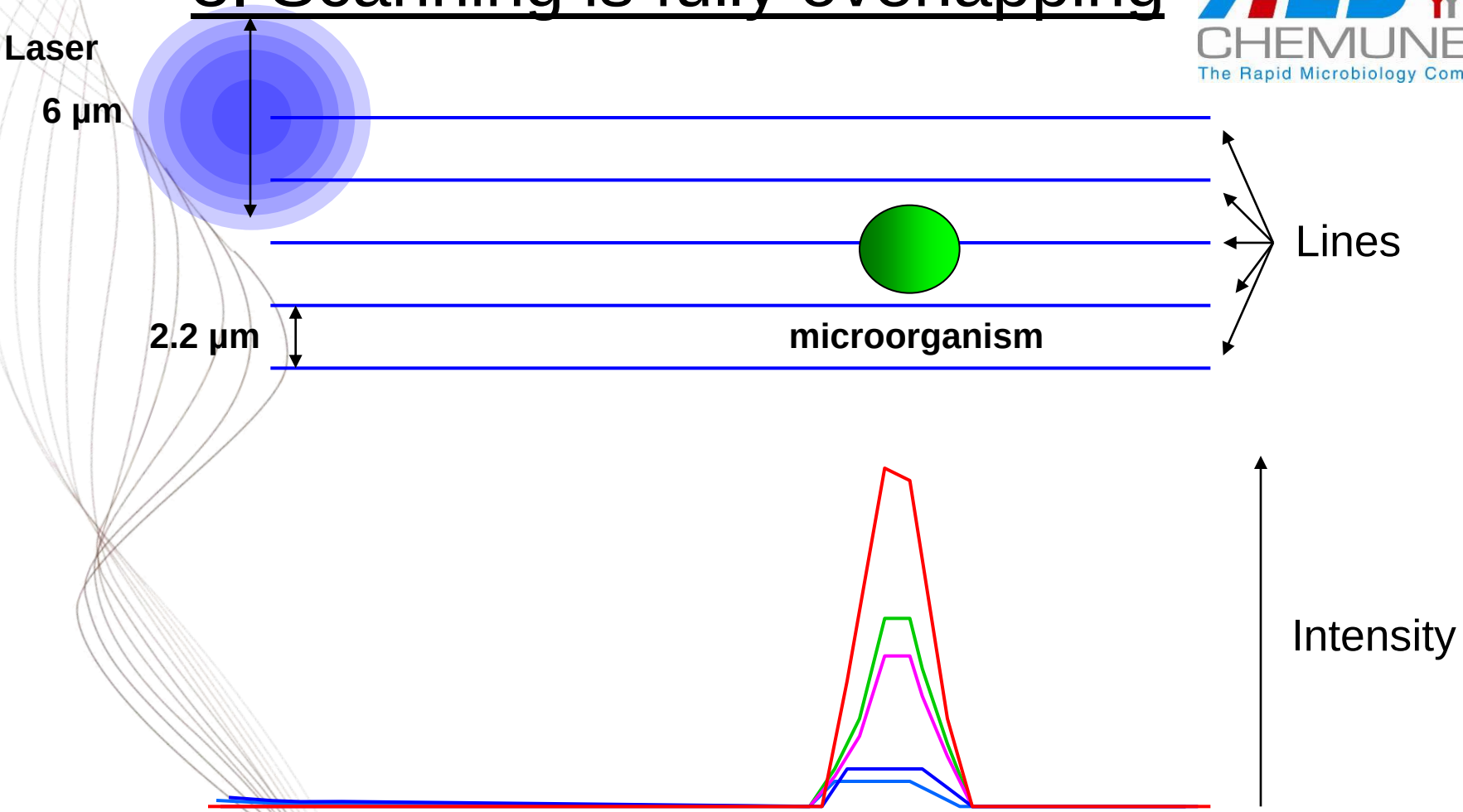


Fluorescent Signal

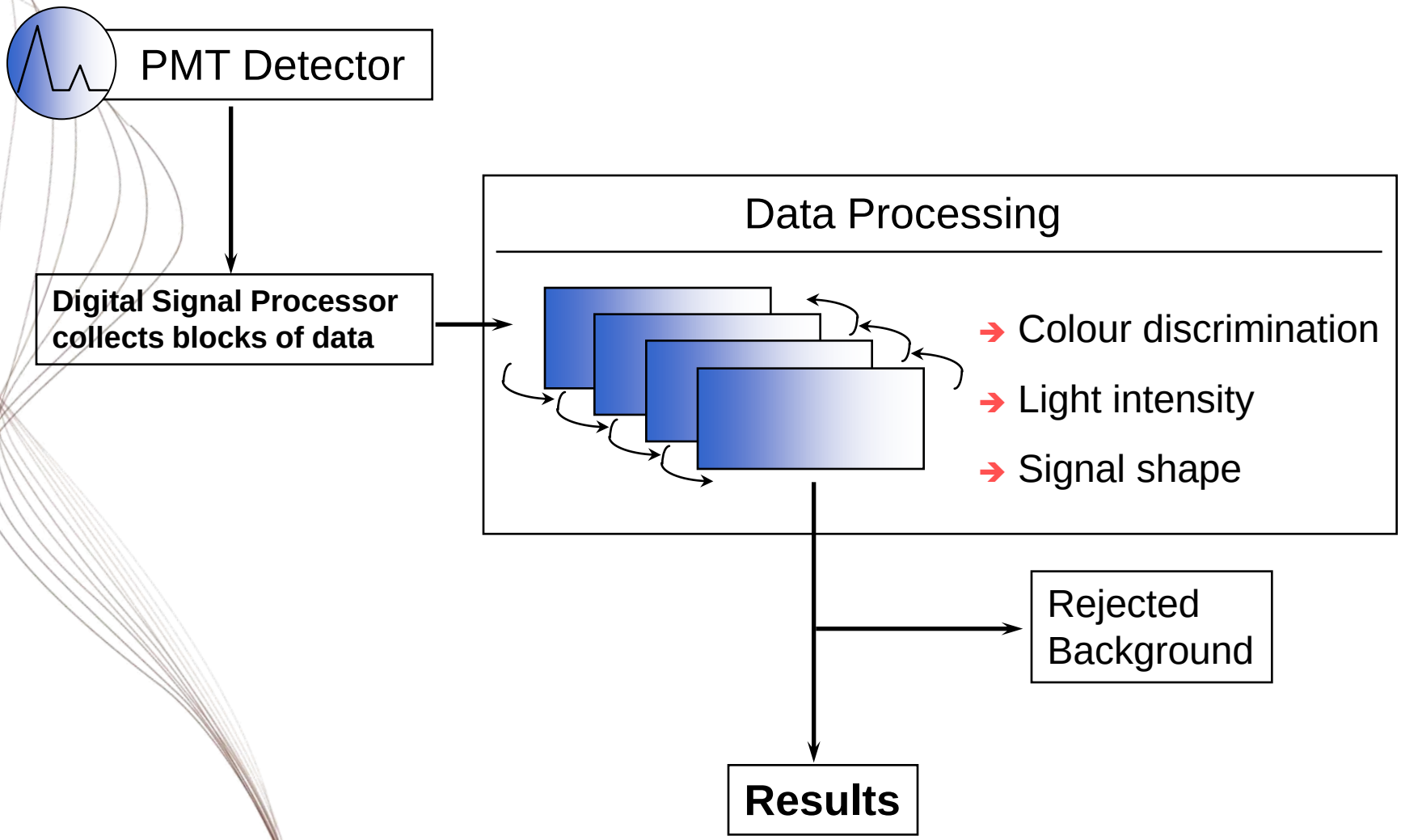


Fluorescence Based Cell Labels

3. Scanning is fully overlapping

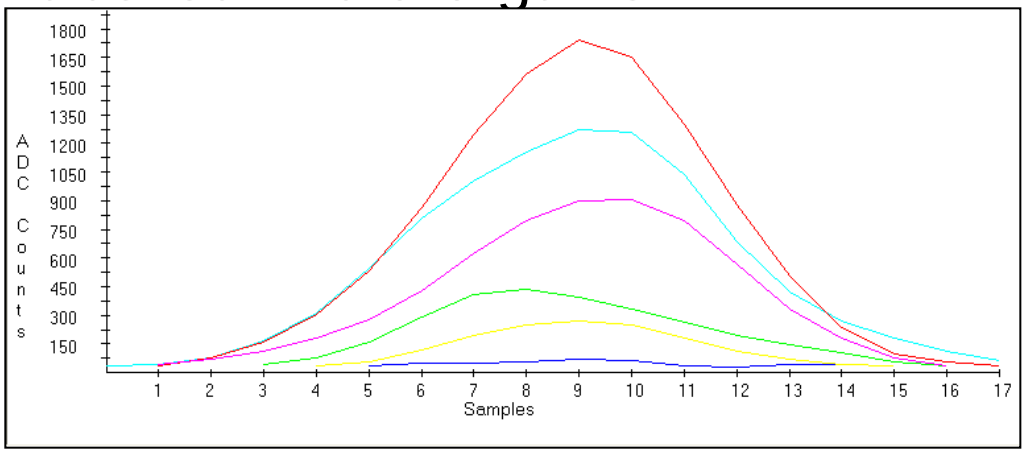


3. Data Processing

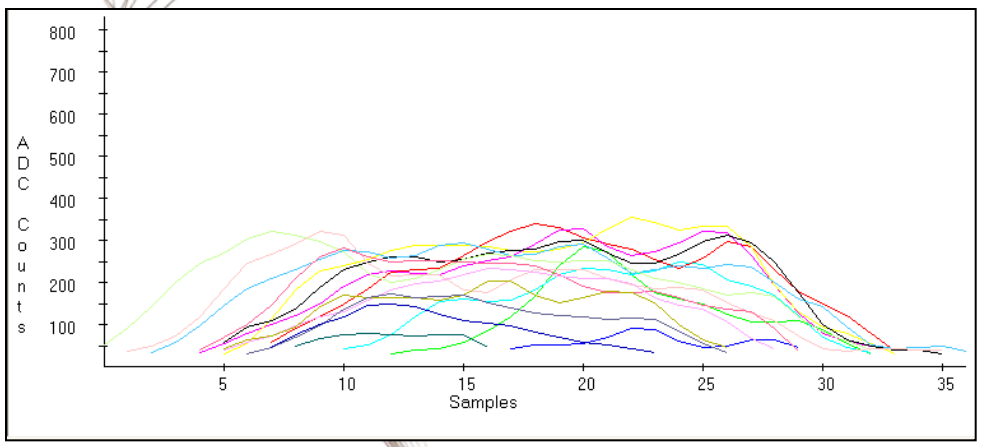


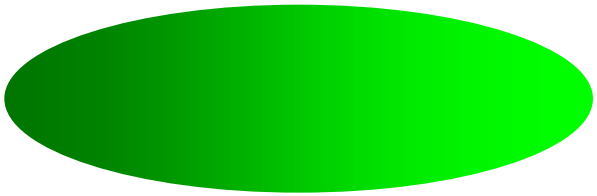
Signal Shape

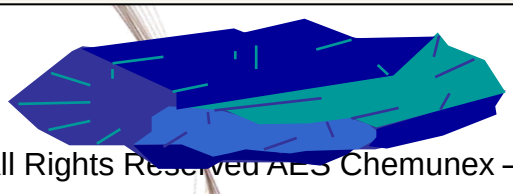
Labelled Micro-organism



Autofluorescent Particle

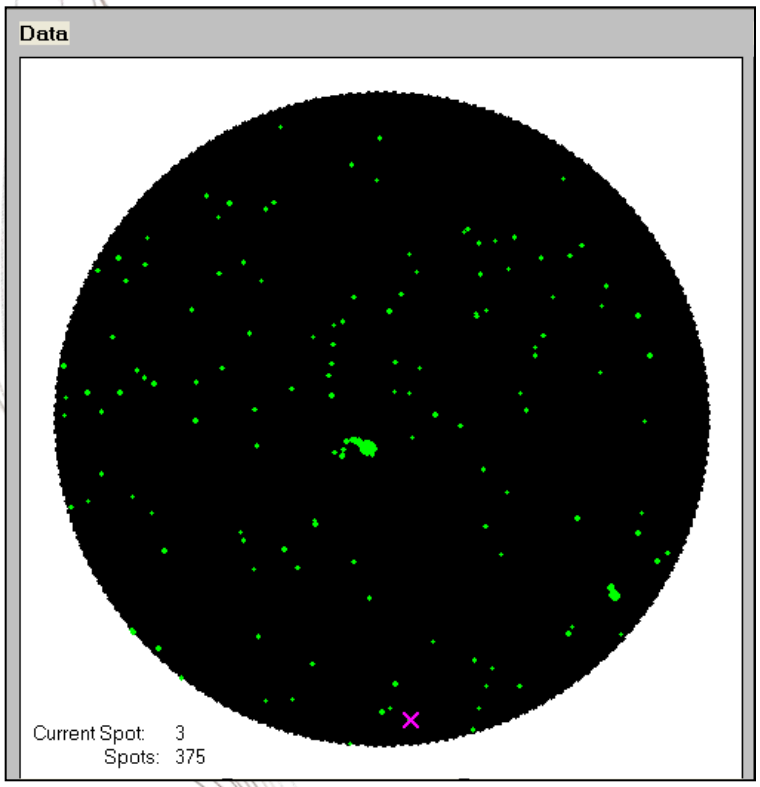




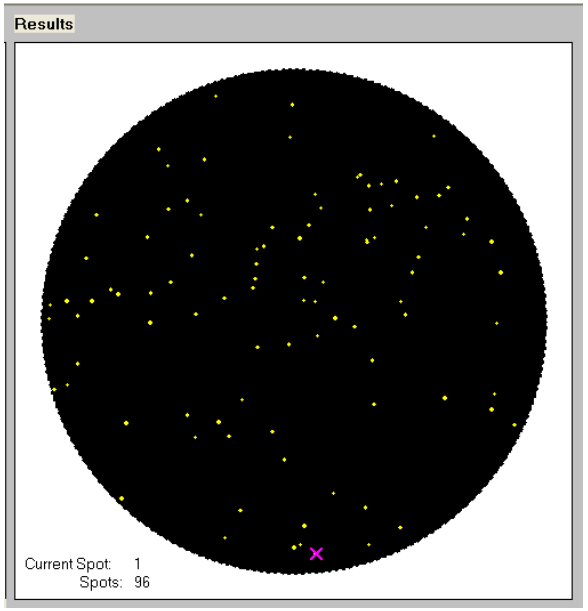


Signal Discrimination

Data Map = Total count



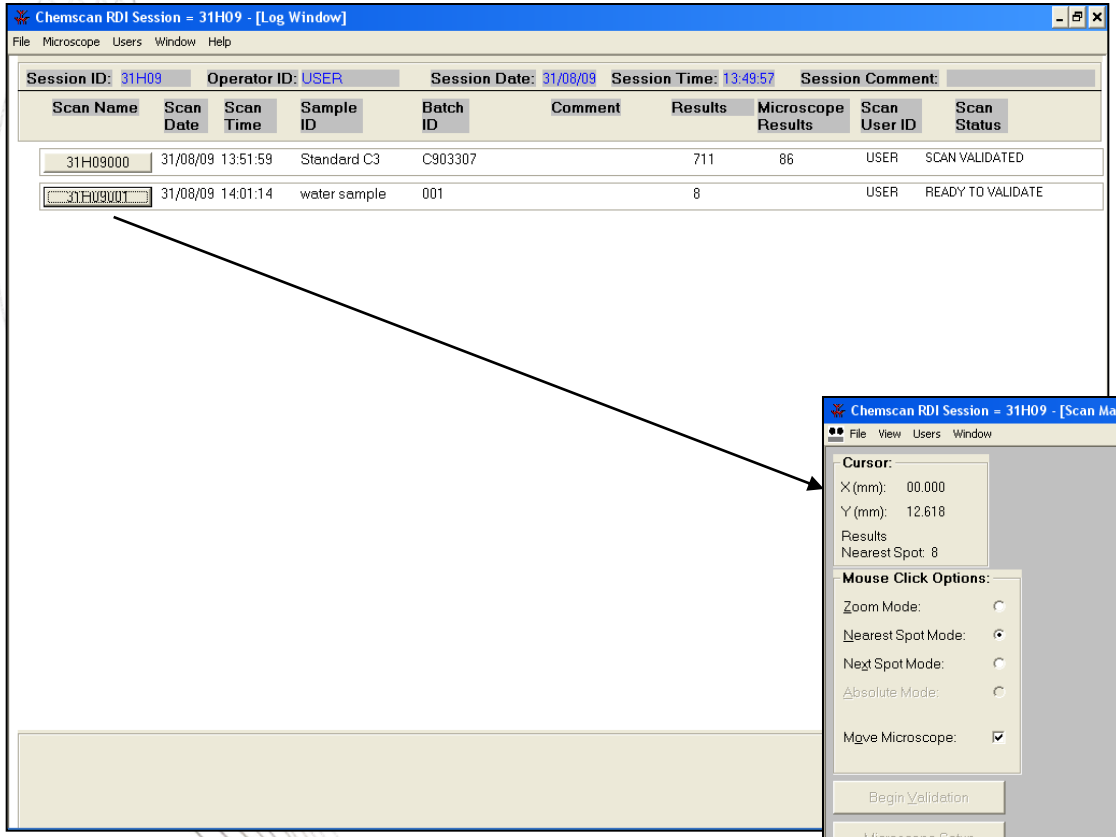
Results Map
= labelled microorganisms

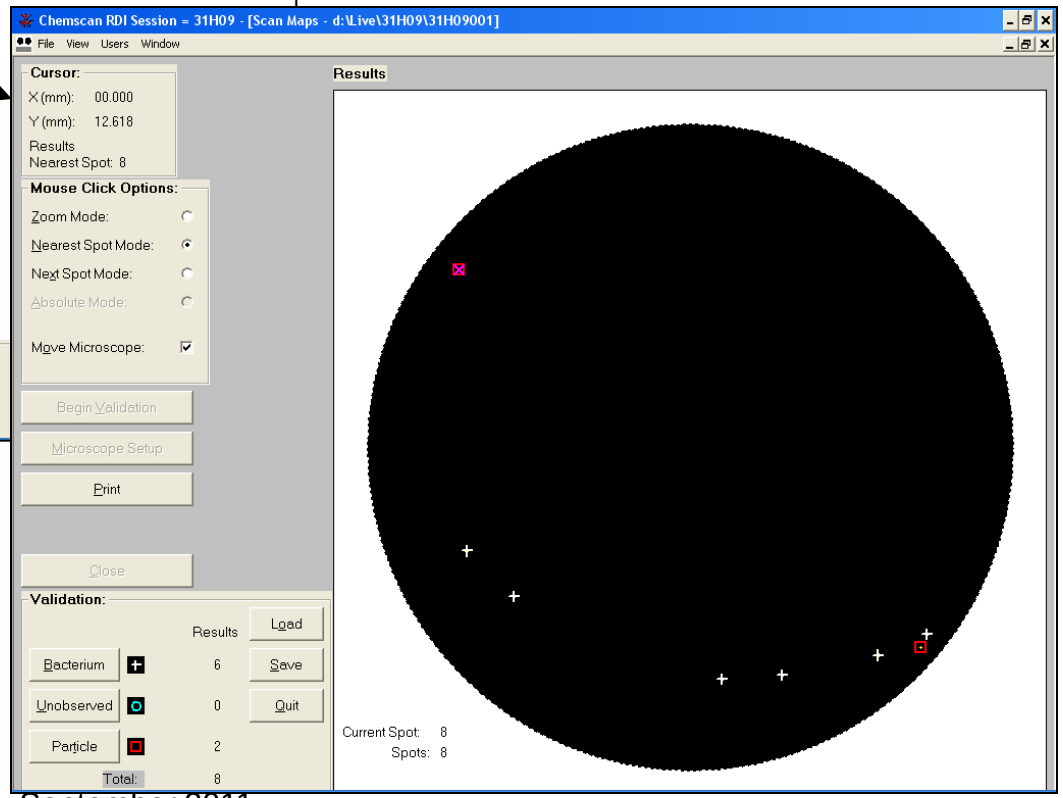


Rejected Background

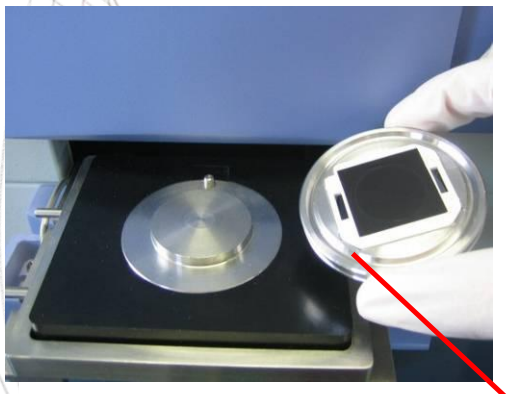
- Autofluorescent Particles
- Membrane Fluorescence
- Electronic Noise

Results Display





Microscope Validation

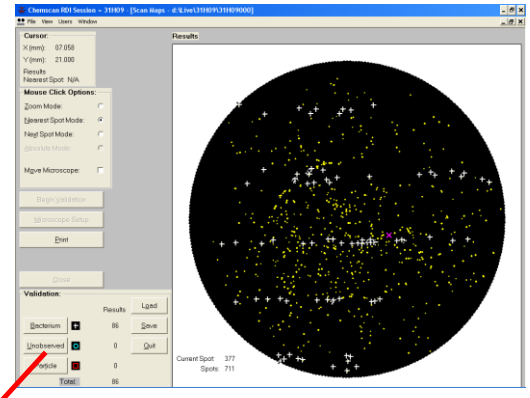


1. Membrane holder

Microscope objective

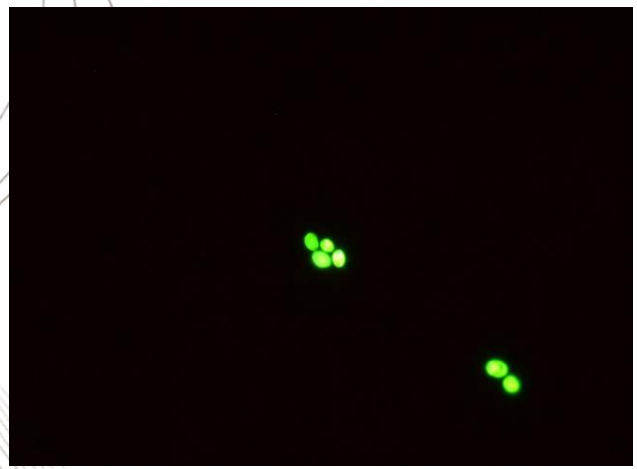
Monotized platform

2. Automated Microscope Stage

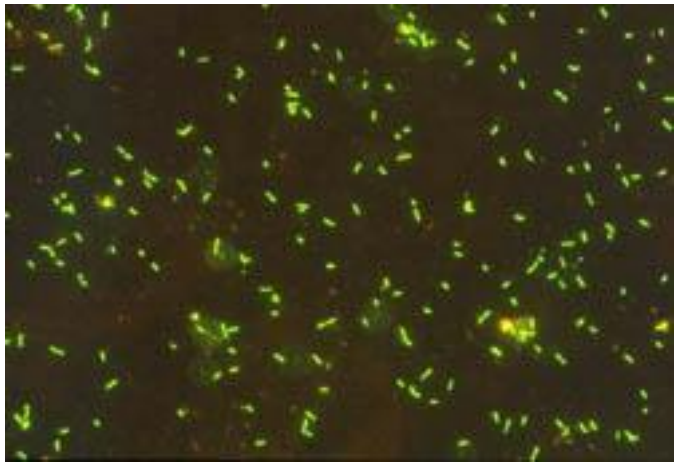


3. Validation of the Scan map

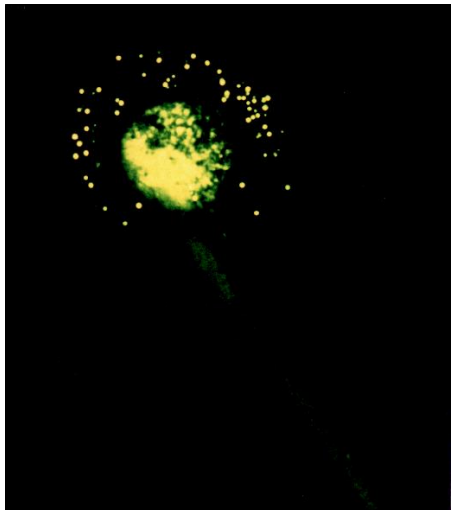
Labelled Microorganisms



Candida



Bacillus



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 hoshinae
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 alkagenèse
Pseudomonas mesophilica
Pseudomonas putida
Pseudomonas fluorescens
Pseudomonas stutzeri
Salmonellac holeraesuis
Salmonella indiana
Salmonella typhimurium
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 mycoides
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilus
Bacillus subtilis
Bacillus thuringiensis
Bacteroides fragilis
Bacteroides thetaiotamicron
Bacteroides vulgatus
Clostridium acetobutylicum
Clostridium bifermentans
Clostridium butyricum
Clostridium perfringens
Clostridium sporogenes
Clostridium tyrobutyricum
Corynebacterium aquaticum
Corynebacterium pseudodiphtheriticum
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 luxitaniae
Candida magnolia
Candida parapsilosis
Candida pelliculosa
Candida tropicalis
Cryptococcus albidus
Debaryomyces hanseni
Galactomyces geotrichum
Geotrichum candidum
Hansenulaspora uvarum
Hansenula anomala
Kloechera japonica
Kloechera Apis apiculata
Pichia anomala
Pichia guillermondii
Pichia menbrena faciens
Rhodotorula rubra
Saccharomyces bailli
Saccharomyces bisparus
Saccharomyces cerevisiae
Saccharomyces rosei
Torulopsis candida Torulopsisi
inconspicua Torulopsis maris
Torulospora delbrueckii
Zygosaccharomyces bailli
Zygosaccharomyces rouxii

Mould

Acremonium Sp.
Aspergillus versicolor
Aspergillus versicolor
Aspergillus fumigatus
Aspergillus niger
Basydiomycetes Sp.
Bassochlamis fulva
Byssochlamys Sp.
Cladosporium cladosporioides
Epicocum nigrum ou altenaria
Fusarium oxysporum
Fusarium oxysporum
Fusarium gramineatium roseum
Humicola fuscoatra
Mucor circinelloides
Mucor plumbeus
Mucor racemosus
Mucor Sp.
Neosartoeya 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:

	Time to results
Sterility Test	
Sterility Test for filterable products	Less than 4 hours
Biourden	
TVC Bioburden for in-process	90 min
TVC Bioburden for raw material	90 min
TVC Bioburden for end-product	< 3 hours
Enviromnental 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

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

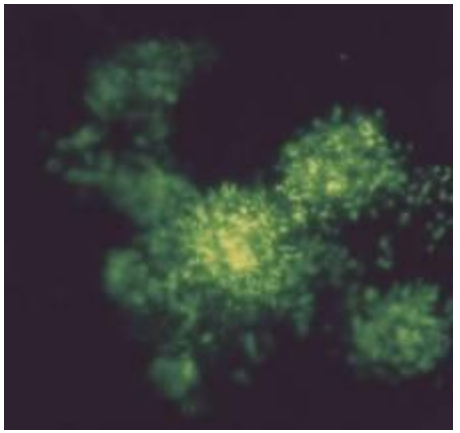
- Acknowledgements :
  - Gunter Gapp - Novartis
  - Sylvie Guyomard – Sanofi Aventis
  - Pascale Nabet – Sanofi Aventis
  - Jean Scouvart - UCB Pharma

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

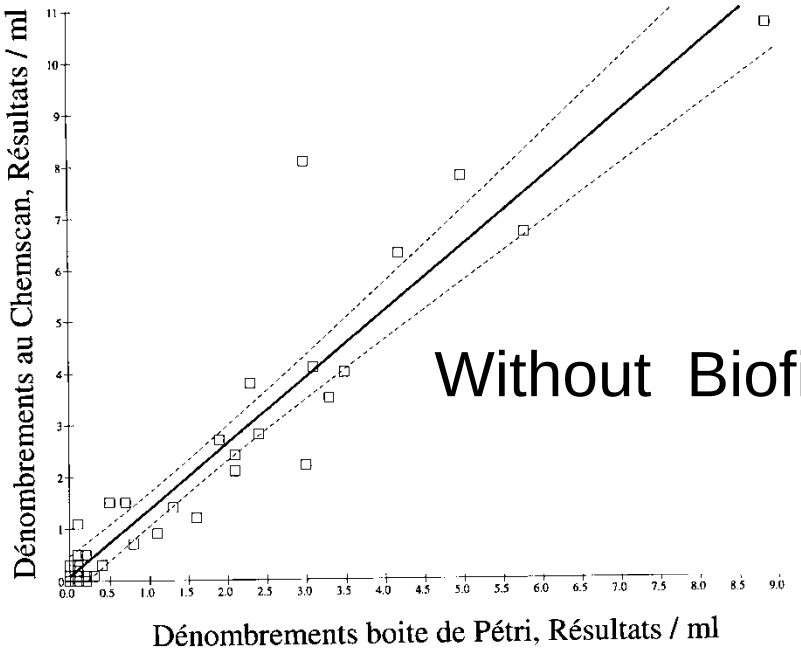
Case study :

- two purified water systems analysed :
  - 1rst: newly constructed system designed to minimise opportunities for biofilm formation
  - 2nd: older system prone to biofilm formation and required regular attention
- both systems analysed with the Chem*Scan*® *RDI* and using R2A plates incubated for 5 days at 32°C



1. Pharmaceutical water testing

Monitoring of ‘new’ water system



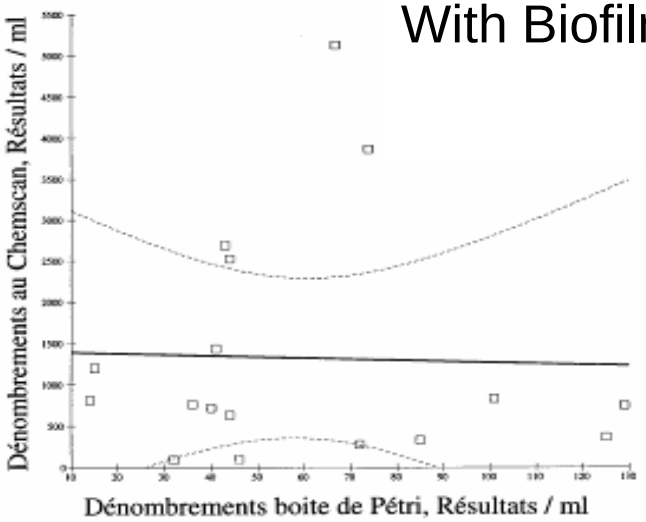
Without Biofilm

- 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

With Biofilm



- 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

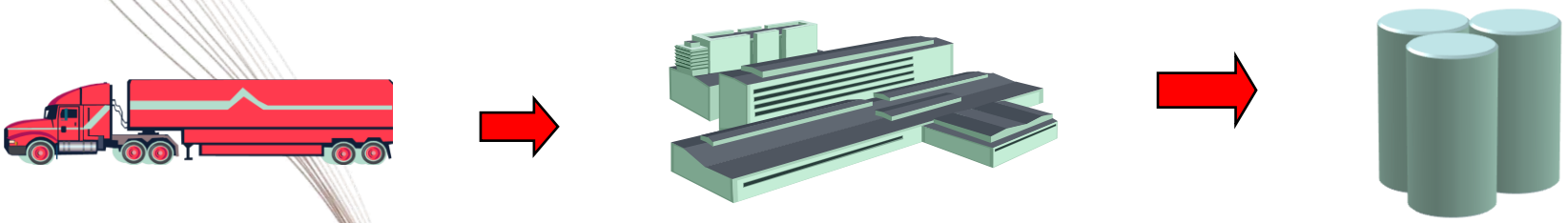
Moving microbial controls from Product to Process :

- **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



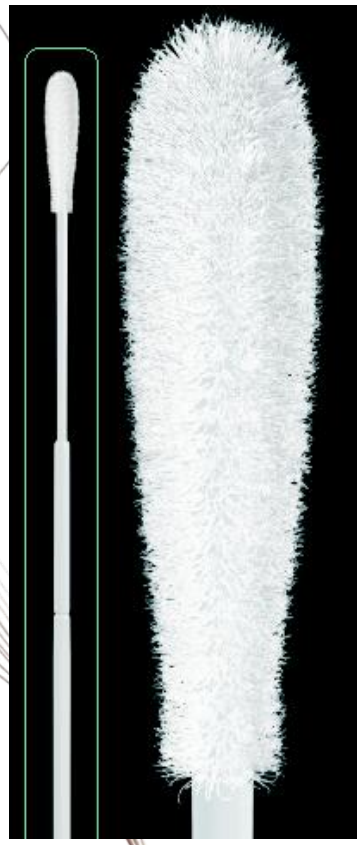
2. Bioburden in Intermediates

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 Chem*Scan*® *RDI* rapid method



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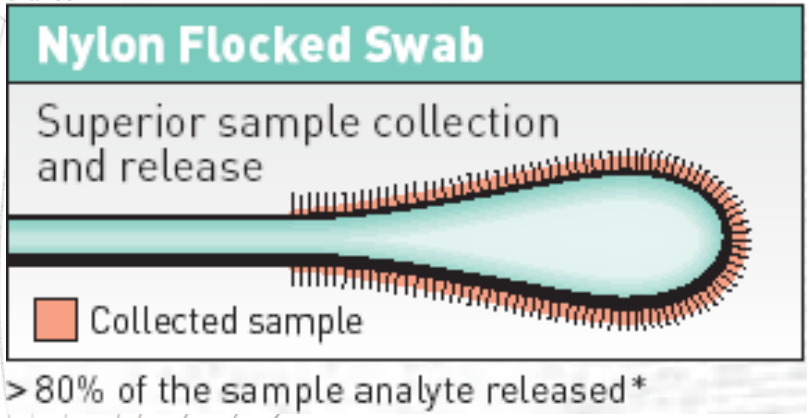
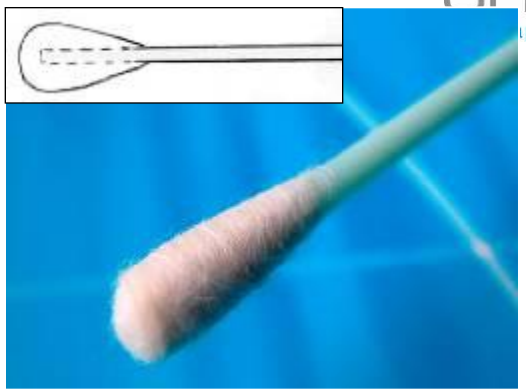
3. Surface Monitoring using ChemSwab®





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 Chem*Scan*® *RDI*

Tested strains :

Staphylococcus epidermidis SE
Bacillus subtilis Spores BSS
Escherichia coli EC
Aspergillus brasiliensis spores ABS
Candida albicans CA
Burkholderia cepacia BC

3. Surface Monitoring using ChemSwab®

Comparative Results

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

Strains	EC		CA		ABS		BSS		SE		BC	
	Scan	CFU	Scan	CFU	Scan	CFU	Scan	CFU	Scan	CFU	Scan	CFU
	91	81	158	103	79	66	141	91	158	156	105	103
	95	83	176	98	92	61	148	77	176	159	89	78
	81	87	143	99	91	73	172	86	143	155	68	92
	-	-	170	102	136	80	125	99	170	168	84	93
Mean	91	84	162	101	100	70	147	87	162	160	87	92
Correlation Scan/CFU	**108%**		**160%**		**143%**		**169%**		**101%**		**95%**	

4. Air Monitoring using Coriolis®µ

Portable Air Sampler for bio-aerosol collection

Your results in few hours combining

Coriolis®µ

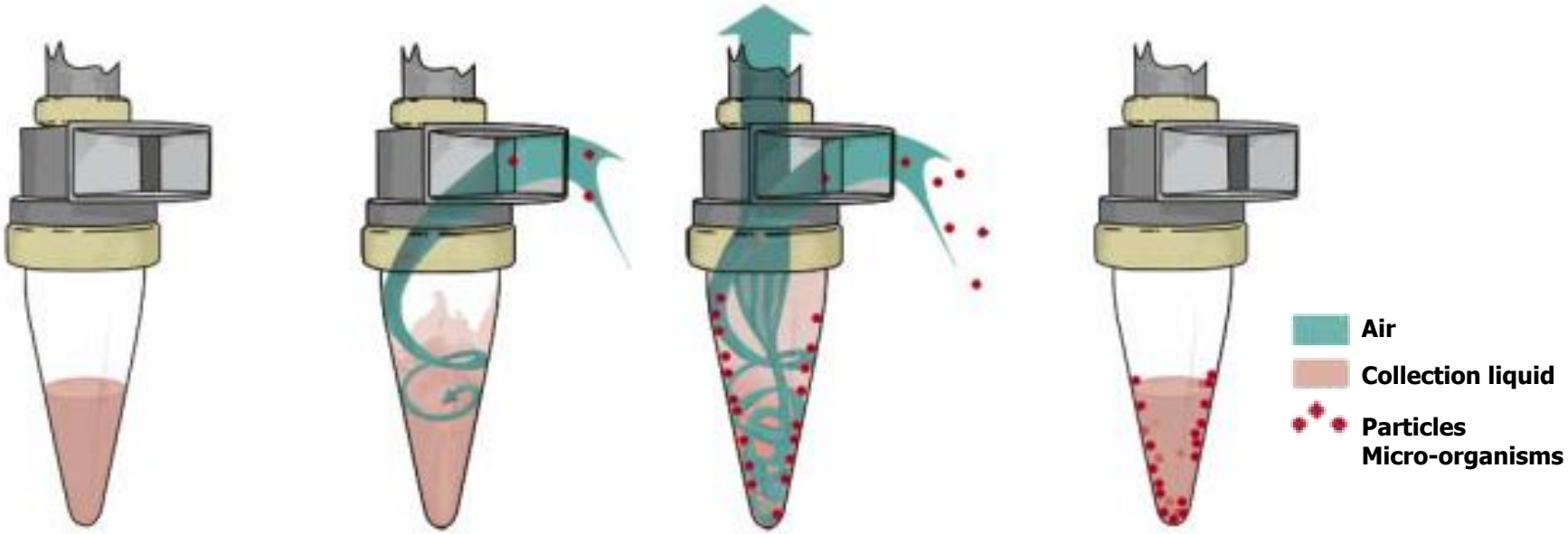
with ChemScan®RDI

Designed and developed by:

Designed and developed by:

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 :

- 1,5m^3 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 Chem*Scan* 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)) :

Strains	EC		CA		ABS		ML		BSS		SE		BC	
	Scan	CFU	Scan	CFU	Scan	CFU	Scan	CFU	Scan	CFU	Scan	CFU	Scan	CFU
	55	52	91	97	57	45	101	89	72	98	91	33	64	81
	64	56	69	78	45	46	113	85	69	91	74	48	59	67
	68	55	94	82	72	57	88	77	93	88	80	41	62	69
Mean	**62**	**54**	**85**	**86**	**58**	**49**	**101**	**84**	**78**	**92**	**82**	**41**	**62**	**72**
Correlation Scan/CFU	**115%**		**99%**		**118%**		**120%**		**85%**		**201%**		**85%**	

coriolis-airsampler.com
generation AIR SAMPLER
& reliable air control

Evaluation of Coriolis® microbial air sampler coupled with RMM
Alternative solution for rapid airborne contamination control

Pfizer

Abstract

nmental contamination control in cleanrooms, Bertin Technologies (France) has developed a technology dedicated to the monitoring of articles. The goal is to propose a sampling method compatible with Rapid Microbiological Methods (RMM) in order to get reliable & specific ackle the impact on pharmaceutical production of time-to results within impaction method.
bial air sampler has been validated according to ISO14698-1 in terms of physical and biological efficiencies (HPA study – July 2008) : the ow the Coriolis® μ is as efficient as the traditional method and even better for high particles diameter (results available on www.coriolis-r.com).
munex has also validate a protocol coupling Coriolis® μ with ScanRDI® (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 RMMs on the samples collected with Coriolis® μ.

Context

robiological quality control of environments aims at ensuring the **quality of products** in case of cleanrooms production / **health and safety** of workers and exposed people.
& reliable measurements of microbial contamination depend on the choice of an adapted air sampler / a representative sample from olled environment / the limitation of losses due to a failure of the sampler to capture particles containing micro-organisms or to due to n of viable micro-organisms during collection so that formation of visible colonies on agar surfaces will not occur.

ective : realize the feasibility study of the Coriolis® μ air sampler coupled with RMM (Scan RDI) in o implement it as a **new solution for rapid investigation** in case of contamination and for **e monitoring in production sites.**

Material & methods

mpling 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 ScanRDI method (RMM).

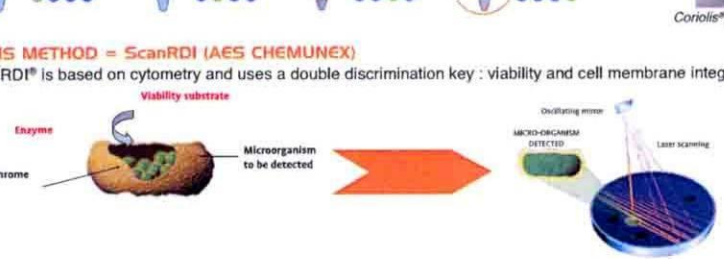
MPLERS = impactor vs Coriolis® μ (Bertin Technologies)

lis® μ air sampler is based on a patented cyclonic technology: it concentrates airborne particles id collection media.

Coriolis® μ, Bertin Technologies

S METHOD = ScanRDI (AES CHEMUNEX)

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



rds : biocontamination – airborne particles – microbial air

Results

Experiment A = comparison of Coriolis® μ and impactor (table 1)
- Different areas controlled, equipments placed side by side, 2 replicates
- Filtration of the whole sample from Coriolis® sampling (0,45μm filter) + filter placed on TSA agar plate
- Incubation of plates 20-25°C for 72-96h + 30-35°C for 48-72h

Experiment B = Coriolis® μ sampling and ScanRDI detection (table 2)
- Each liquid sample from Coriolis® splitted in A&B aliquots
  - A = filter plate method
  - B = filtered and analysed with ScanRDI

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

Samples	Impaction	Coriolis® + filter plate
Biohood	0	1 M
Biohood	0	0
Counter top 2	6 (4 M +2 B)	2 B
Counter top 2	4 (2 M + 2 B)	1 M
Counter top 3	6 (3 M + 3B)	8 (7 M + 1 B)
Counter top 3	2 B	6 (3 M + 3 B)

M = Mold – B = Bacteria

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

Samples	Coriolis® + Scan RDI	Coriolis® +filter pla
Biohood	0	0
Biohood+hands	1	0
Counter top 3	9	2 (1 M + 1
Counter top + walking	66	7 (3 M + 4
Counter top + hands	103	8 B

These higher results are partly due to the viable but non-c (VNC) microbials present in the air which can not be detec traditional impaction method allthough they can be path

Discussion

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 m 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 microorgar comparable amount to the impaction system does. ScanRDI can be used as Rapid Microbiology Method coupled with collection in order to give rapid results (around 3 hours from sampling to result). It is also shown here that the ar microorganisms detected with the ScanRDI is higher than with the traditional method as far as it is not based on cultivabili viability; appropriate alert and action limits may thus need to be re-evaluated for ScanRDI results. This couple of innovative fits for the Environmental Monitoring application and could be implemented **for investigation and routine monitoring** in pr sites and in critical areas and **cleanroom environments.**

Acknowledgement

- Pfizer study Team: Dr Lin Chen (responsible of the study), S. Fennel
- Bertin Technologies: Alexandra Guerin, Quitterie Desjonquères
- AES CHEMUNEX: Pierre Barbez

bertin

AES CHEMUNEX

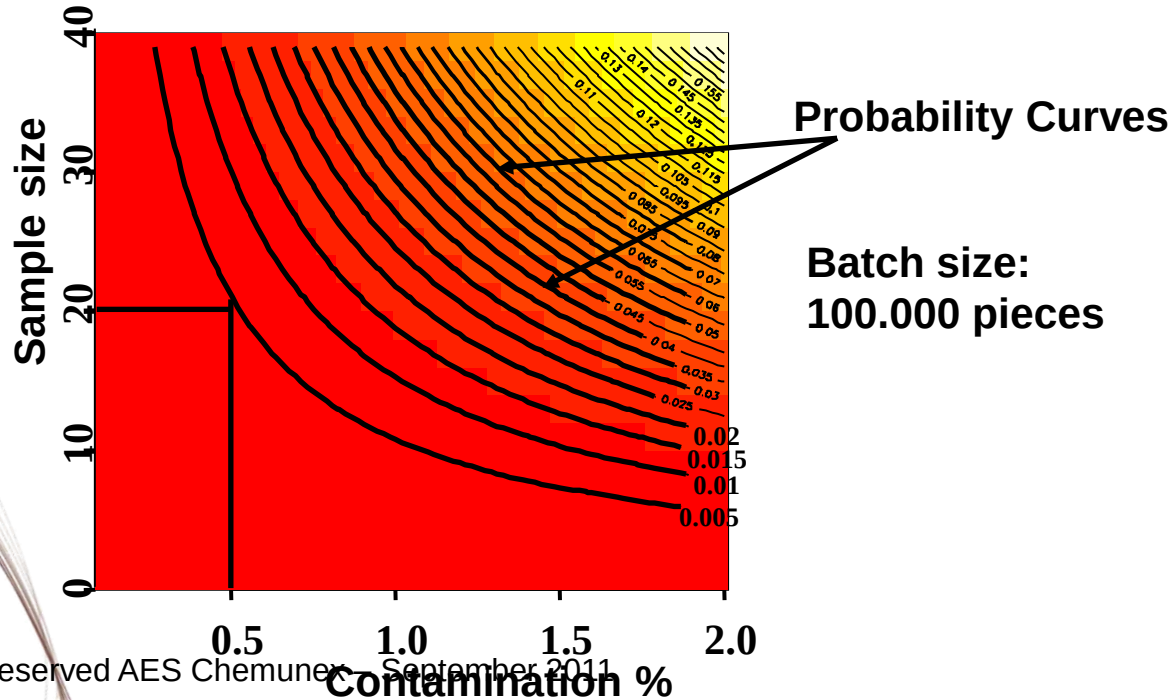
Conclusion : an exemple of implementation of the Chem*Scan*® *RDI*

How useful is microbiological testing?

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 → Chem*Scan*® *RDI* - 90 min
- Bulk Product → Chem*Scan*® *RDI* - 3 hours
- Surface → Chem*Scan*® *RDI* - 3 hours
- Air → Other RMM - 24 hours
- Filled Vials → Chem*Scan*® *RDI* - 3 hours

This manufacturing site receives FDA inspection and formal approval in July 08 to use Chem*Scan*® *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 Chem*Scan*® *RDI* description (solid phase cytometry) and Bacti*Flow*® *ALS* description (flow cytometry)
- USP, chapter 1223 “ Validation of Alternative microbiological methods”
- PDA, Technical Report 33, “The Evaluation, Validation and Implementation of New Microbiological Methods”

Validation steps

1. System Qualification
   - FAT (Factory Acceptance Test)

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

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

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

Performance Qualification

Validation Parameter	Qualitative Test		Quantitative Test	
	European Pharmacopeia	US Pharmacopoeia	European Pharmacopeia	US Pharmacopoeia
Accuracy	Yes	No	Yes	
Linearity	No		Yes	
Precision	Yes	No	Yes	
Limit of Detection	Yes	Yes	No	Yes
Limit of quantification	No		Yes	
Assay Range	No		Yes	
Specificity	Yes		Yes	
Robustness / Ruggedness	Yes		Yes	

Validation of the ChemScan® RDI

ChemScan® RDI

Validation of the Chem*Scan*® *RDI* with quantitative applications

- Commissioning

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

Customer service	3 days
Customer service Or Laboratory	1 week Or Several weeks
Laboratory	5 – 6 weeks*
	Depend on the laboratory

* With the recommanded strains

Validation of the Chem*Scan*® *RDI* with qualitative applications

- Commissioning

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

Customer service	3 days
Customer service	1 week
Or	Or
Laboratory	Several weeks
Laboratory	4 weeks*
	Depend on the laboratory

* With the recommanded 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:	200-D0225-11
Date of Issue:	28 October 2008
Page:	1 of 54

Affix the self adhesive control label here

Protocol Approvals

Authors:

Name	Job Title	Company	Signature:	Date:
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Approved By:

Name	Job Title	Company	Signature:	Date:
J-L. Drocourt	Scientific & Technical Director	AES CHEMUNEX		

Report Approvals

Name	Job Title	Company	Signature:	Date:

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9. QUALIFICATION STRATEGY

9.1 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 be 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 reliability 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* 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 determinated 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 case, 5 cfu / filtered volume is acceptable).
The limit of detection of the technology will be calculated later on.

It is recommended to analyze five suspensions where concentrations are within the range of analysis, and repeat the assay 3 times.
N.B: 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 other 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 160 cfu / filtered volume:

	Working day 1			Working day 2			Working day 3			
Dilution A	160 cfu	160 cfu	160 cfu	160 cfu	160 cfu	160 cfu	160 cfu	160 cfu	160 cfu	Dilution factor 2
Dilution B	80 cfu	80 cfu	80 cfu	80 cfu	80 cfu	80 cfu	80 cfu	80 cfu	80 cfu	Dilution factor 2
Dilution C	40 cfu	40 cfu	40 cfu	40 cfu	40 cfu	40 cfu	40 cfu	40 cfu	40 cfu	Dilution factor 4
Dilution D	10 cfu	10 cfu	10 cfu	10 cfu	10 cfu	10 cfu	10 cfu	10 cfu	10 cfu	Dilution factor 2
Dilution E	5 cfu	5 cfu	5 cfu	5 cfu	5 cfu	5 cfu	5 cfu	5 cfu	5 cfu	

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

CHEMUNEX
The Rapid Microbiology Company

Subsidiary of AES Laboratoire GROUPE

presents

Muito Obrigado !