

## **The ChemScan RDI<sup>®</sup>, a real time and ultrasensitive microbial analysis system.**

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In pharmaceutical, biopharmaceutical and drinking waters industries, quality control laboratories are under strong pressure to reduce the time for the microbiological controls due to increasing financial constraints and stricter regulatory standards.

The goal is to switch away from reliance on final product microbiological quality controls toward in process control at key points of the manufacturing process.

This Total Quality Management (TQM) approach in all industries can confer operational and financial benefits, such as faster time to market, increased yields and reduced spoilage.

The use of standard agar growth plates for detection and enumeration of viable microorganisms still represents the main analytical methodology used. With this type of analysis, the results are dependant upon incubation period, typically taking 3-5 days, resulting in a retrospective response to microbiological contamination.

In order to improve their ability to respond to potential problems and move towards real-time process control and TQM, manufacturers have looked towards new technologies to reduce the time for sample analysis and data production. Nevertheless, the main challenge is not to only produce a quicker method of analysis, but also to ensure that the sensitivity of detection is maximised, i.e the technology has the ability to detect very low levels of microorganisms, even down to a single cell.

In the forefront of rapid methods development have been such techniques as ATP bioluminescence, impedance, microscopy-based fluorescence, antibody labelling techniques and more recently, PCR-based technologies. These techniques represent a compromise on the requirements of laboratories users in term of speed vs sensitivity.

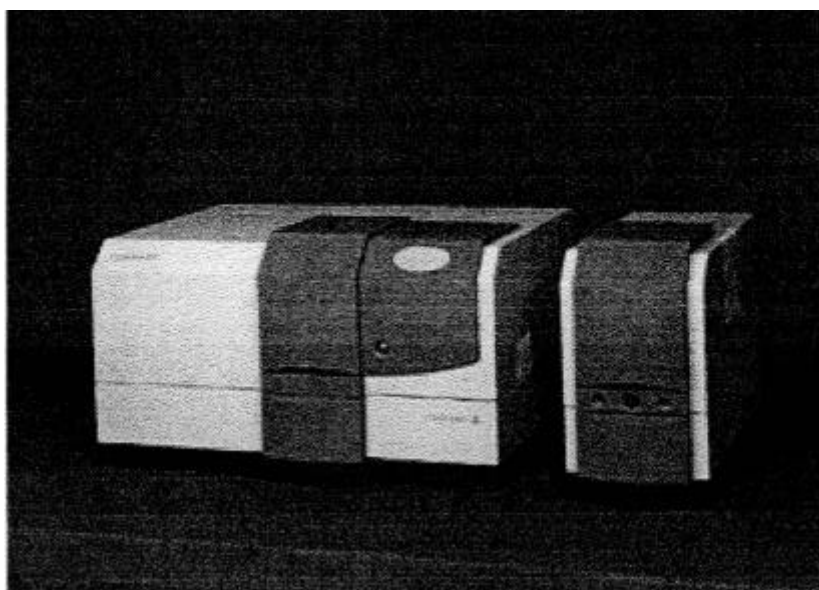
This is particularly true for methods adapted for the total counts, such impedance and ATP bioluminescence, since their relatively low sensitivity demands an incubation period to increase low levels of microorganisms to the detection level of the test. This significantly extends the total analysis time, especially if there is a prolonged lag phase, as can be the case with stressed organisms leading to a reduction in the effectiveness of the technology as a rapid microbial method for a real-time process control measures. These methods will therefore provide results retrospective to the production process (typically a few days).

In order to address the need for a truly rapid assay with a high level of sensitivity, Chemunex has developed the ChemScan RDI<sup>®</sup> (Figure 1) (BRAILSFORD. M 1997, MIGNON-GODEFROY. K et al 1997). This is based on direct fluorescent labelling of viable microorganisms, coupled with an ultra-sensitive laser scanning and counting system.

The high level of sensitivity of the system allows the direct detection of a single cell and eliminates the need for cell growth and multiplication.

Viable microorganisms are labelled using Fluorassure proprietary based reagents. These are based on non-fluorescent substrates that liberate free fluorochrome into the cytoplasm when enzymatically cleaved. In addition to esterase activity, viability staining by fluorescein derivatives

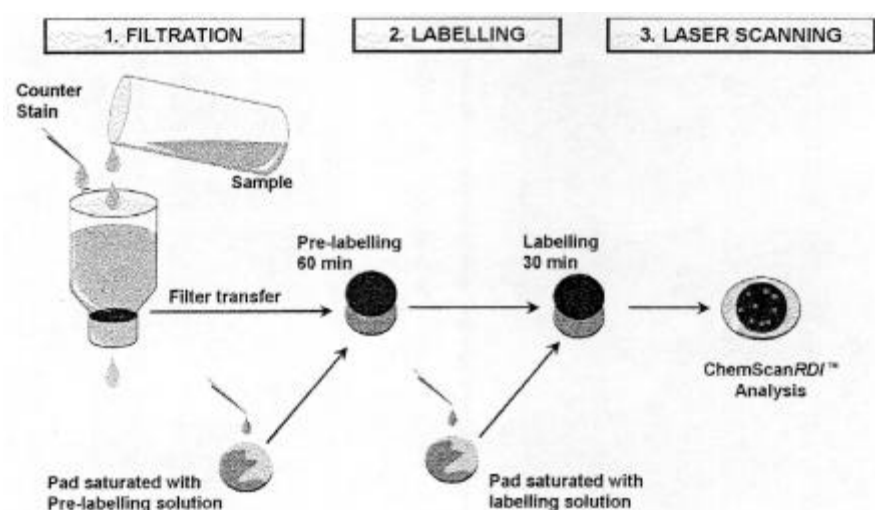
is based on the principle that only viable cells, which have an intact cell membrane, are able to retain and accumulate the fluorescent probe.



**Figure 1:** The ChemScan RDI<sup>®</sup> system

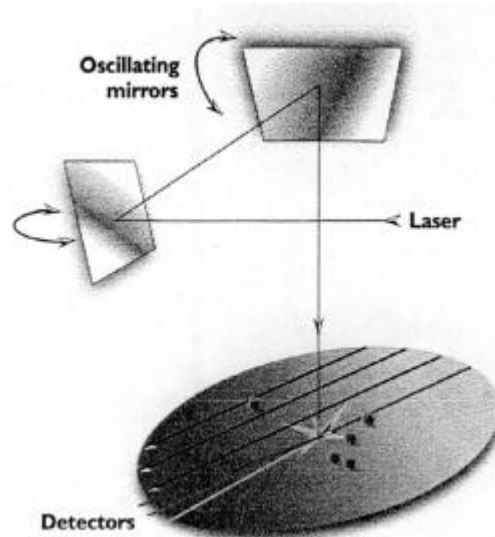
Since only viable cells (including vegetative forms, spores, anaerobe bacteria and moulds) (Table 1, see appendix A) have the ability to perform this cleavage, they can be easily detected by the laser scanning technology.

The samples are filtered through a 25mm diameter, 0.4 $\mu$ m pore size polyester track-edged membrane, which retains the microorganisms on its surface. This is transferred to absorbent pads saturated with an activation reagent for 60 minutes at 37°C, followed by Fluorassure Labelling Solution and labelled for 30 minutes at 30°C. (Figure 2).



**Figure2:** Direct fluorescent labelling protocol of viable microorganisms.

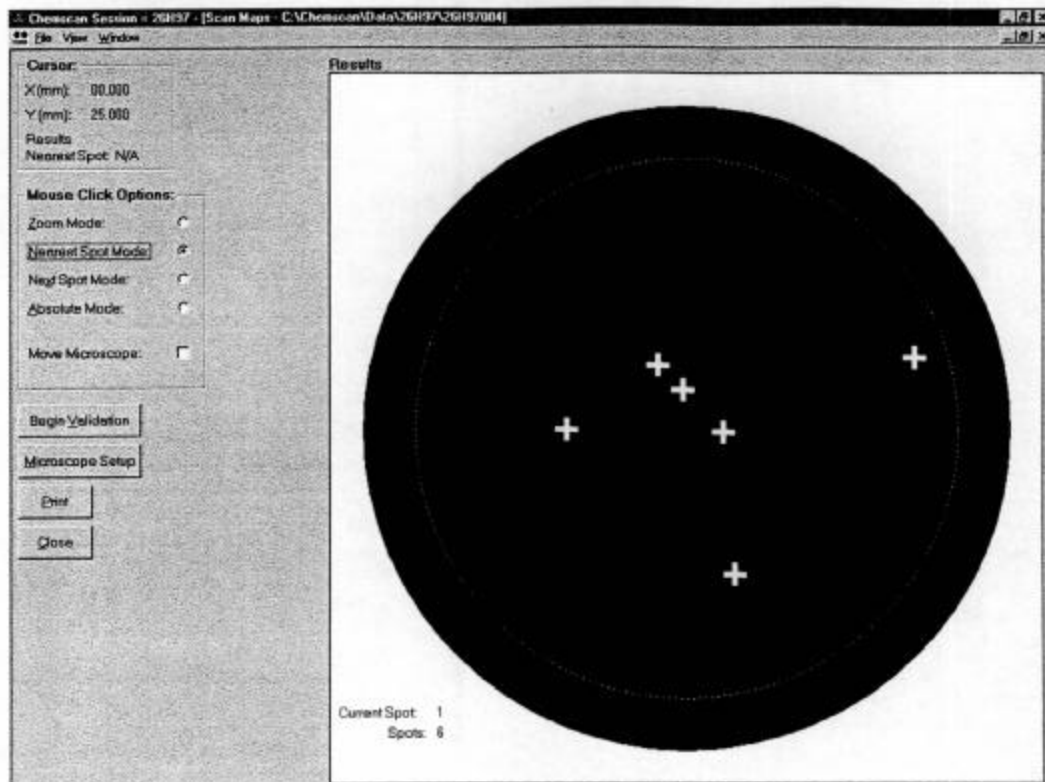
The membrane is subsequently transferred to the analyser where using a high speed 488nm Argon laser scanning system, the entire surface of the membrane is scanned and analysed in three minutes. The scanning pattern of the laser ensures that all microorganisms are detected on at least two consecutive scan lines. The laser spot which overlaps scan lines ensures that each section of the membrane is illuminated twice. The labelled cells fluoresce and two or three photomultiplier tubes detect the emitted fluorescence light in specific wavelength windows (Figure 3).



**Figure 3:** Principle of membrane laser scanning by ChemScan RDI® for microorganism detection.

The signals produced by the photomultipliers are processed through a number of discrimination parameters that enable the instrument to differentiate between valid signals (labeled bacteria) and background noise (electronic, optical and/or auto-fluorescent particles).

The results of the scan are displayed as a direct viable cell count. In addition, the data can be displayed in the form of a membrane scan map, identifying the position of every labeled cell on the membrane surface (Figure 4). The entire labelling process is non-destructive, which allows subsequent visual examination of the sample by microscopy, if required by use of an automated microscope with motorised stage.



**Figure 4:** Scan map displays the position of each detected microorganism by ChemScan RDI® allowing microscopic result confirmation.

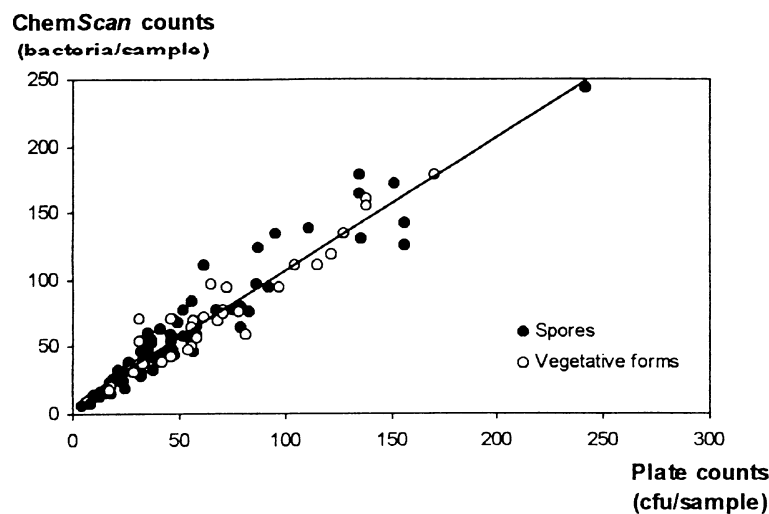
As no pre-incubation step is required, sample preparation and laser scanning takes a few hours to perform from sample collection.

In addition to its speed to result, the new system has a sensitivity that is at least equal to that of the culture plate: one microorganism in a sample.

The correlation between direct counts from ChemScan RDI<sup>®</sup> and results for colony forming units (cfu) on culture plates has been evaluated in pure cultures and process samples. Data presented for pure cultures of microorganisms have shown the correlation to be excellent (Figure 5) (NUNZI et al 1997). The good degree of correlation (slope = 9.992,  $r^2 = 0,915$ ) also was verified even at low cell concentration, down to 1-10 microorganisms per sample, a region in which it breaks down for many alternative techniques.

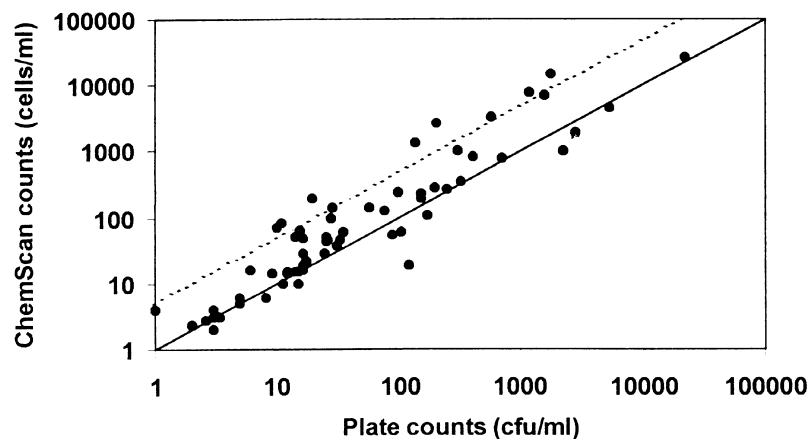
Comparative analysis of routine quality control samples at Hoechst Marion Roussel in Germany (WALLNER G et al 1997, HABERER. K et al 1998), has shown that the accuracy, precision, sensitivity and range of the ChemScan RDI<sup>®</sup> to be at least matched those of the plate count method when used for in-process water samples. The results indicated that the system had a detection limit of one viable cell per sample and a large detection range that has been shown by in-house studies to be 1-35,000 cells per filter. These data are in agreement with studies conducted by other European and US pharmaceutical companies (Figure 6).

These studies have shown the benefits associated with the reliability of the ChemScan RDI<sup>®</sup> to directly detect and count all viable microorganisms in a sample including spores, moulds, anaerobes species, stressed or fastidious organisms.



**Figure 5:** Comparison of plate counts versus ChemScan RDI<sup>®</sup> counts with microbial cultures.

This has been demonstrated by the capability of the instrument to detect a filter deterioration in a water system, not detected by the plate, before the contamination level rose above set parameters and cause process failure. This enabled immediate resolution of the process failure, which not have been possible with traditional culture plates techniques. The detection of cells missed by culture plates was confirmed by subsequent correlation to LAL endotoxin tests (ZEIDY. N et al 1996, GUYOMARD. S 1997).



**Figure 6:** Comparison of plate counts versus ChemScan RDI<sup>®</sup> counts for routine analysis of process water samples from 4 pharmaceutical production facilities.

In addition to provide Total Viable Counts (TVC), the ChemScan RDI<sup>®</sup> can also be used in conjunction with antibody, specific enzyme-based or nucleic acids probes to identify and enumerate specific species. The speed and sensitivity of ChemScan RDI<sup>®</sup> allows direct cell detection without the need for selective enrichment.

Reliable detection, within three hours, with ChemScan RDI<sup>®</sup> of *Cryptosporidium parvum* in water concentrates using specific fluorescent monoclonal antibodies has been recently demonstrated (FRICKER. C, 1998 a,b), this new application is in validation, at present time, for routine analysis of drinking waters.

Detection method, in less than 4 hours, of *Escherichia coli* in drinking waters using specific enzyme substrate has been recently developed, successfully with ChemScan RDI<sup>®</sup> (NELIS. H 1998).

Studies, carried out at Thames Water Utilities (Readings, England), at Institut Pasteur (Prof. P. Grimont, Paris) and at Chemunex have shown that it was possible to detect with the ChemScan RDI, directly on filter, bacteria by Fluorescent In Situ Hybridisation (FISH) in using eubacteria or specific nucleic acids probes. This new application which is being developed opens new avenues in the specific detection of pathogen or objectionable microorganisms in waters.

The ChemScan RDI<sup>®</sup> system provides a rapid and sensitive method for the detection and enumeration of microorganisms. The ChemScan RDI<sup>®</sup> microbial counts are not only comparable with those of standard plate count methods, but also appear to provide an enhanced detection sensitivity for vegetative cells and spores.

With its high speed and sensitivity the ChemScan RDI<sup>®</sup> is a new and powerful system for rapid and reliable control of the microbiological quality of pharmaceutical and drinking waters.

## References

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<b>Bacteria</b>	<b>Yeasts</b>	<b>Moulds</b>
<i>Acholeplasma laidlawii</i>	<i>Enterococcus faecium</i>	<i>Candida albicans</i>
<i>Actinomyces sp.</i>	<i>Escherichia coli</i>	<i>Candida krusei</i>
<i>Agrobacterium radiobacter</i>	<i>Lactobacillus brevis</i>	<i>Candida guilliermondii</i>
<i>Alcaligenes eutrophus</i>	<i>Mycobacterium bovis</i>	<i>Candida parapsilosis</i>
<i>Alcaligenes faecalis</i>	<i>Mycobacterium parafortuitum</i>	<i>Candida humicola</i>
<i>Bacillus anthracis</i>	<i>Mycobacterium tuberculosis</i>	<i>Cryptococcus neoformans</i>
<i>Bacillus cereus</i>	<i>Mycro</i>	<i>Geotrichum candidum</i>
<i>Bacillus megaterium</i>	<i>Ochrobactrum anthropii</i>	<i>Saccharomyces cerevisiae</i>
<i>Bacillus circulans</i>	<i>Pasteurella aerogenes</i>	
<i>Bacillus pumilis</i>	<i>Propionibacterium acnes</i>	<i>Aspergillus niger</i>
<i>Bacillus licheniformis</i>	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus versicolor</i>
<i>Bacillus sphaericus</i>	<i>Pseudomonas diminuta</i>	<i>Clodosporium herbarum</i>
<i>Bacillus stearothermophilus</i>	<i>Pseudomonas fluorescens</i>	<i>Epicoccum nigrum</i>
<i>Bacillus subtilis</i>	<i>Pseudomonas mesophilica</i>	<i>Fusarium graminecarium</i>
<i>Bacillus subtilis globigii</i>	<i>Salmonella eboni</i>	<i>Mucor hiemalis</i>
<i>Bacillus thermophilus</i>	<i>Salmonella typhimurium</i>	<i>Mucor racemosus</i>
<i>Bacillus thurigiensis</i>	<i>Serratia marcescens</i>	<i>Penicillium aurantiogriseum</i>
<i>Bacteroides fragilis</i>	<i>Staphylococcus aureus</i>	<i>Penicillium chrysogenum</i>
<i>Burkholderia cepacia</i>	<i>Staphylococcus epidermidis</i>	<i>Penicillium frequentens</i>
<i>Burkholderia picketii</i>	<i>Streptococcus lactis</i>	<i>Penicillium notatum</i>
<i>Caulobacter sp.</i>	<i>Streptococcus viridans</i>	<i>Penicillium roquefortii</i>
<i>Clostridium bifermentans</i>	<i>Sphaerotilus sp.</i>	
<i>Clostridium butyricum</i>	<i>Thiobacillus ferrooxidans</i>	
<i>Clostridium perfringens</i>	<i>Thiotrix sp.</i>	
<i>Clostridium sporogenes</i>	<i>Xanthomonas maltophilia</i>	
<i>Commamonas terrigena</i>		
<i>Corynebacterium acetoacidophilum</i>		
<i>Corynebacterium aquaticum</i>		
<i>Corynebacterium pseudodiphtheriticum</i>		
<i>Enterobacter cloacae</i>		