The New Automated Bacteria Analyzer BACSYS-40*i*

Atsushi NAKAYAMA[*1], Tsuyoshi ISHII[*1], Susanne JAEKEL[*2], and Ulrike BLASEIO[*2]

**1 Scientific Division, Sysmex Corporation, 4-4-4 Takatsukadai, Nishi-ku, Kobe 651-2271, Japan.*
**2 Sysmex Europe GmbH, Germany.*

This new compact analyzer has been developed for use in the microbiological screening of urine and other human body fluids. The remarkable feature of the analyzer is its ability to quantify both bacteria and leukocytes from a small volume sample within two minutes. The measurement is based on fluorescence flowcytometry using a proprietary fluorescence polymethine dye which is excited by a red semi-conductor laser. The device covers wide measurement ranges including clinically relevant low concentrations. Reproducibility, linearity, carryover and correlation studies revealed excellent results indicating that the BACSYS-40i can improve overall efficiency within the microbiology laboratory.

(Sysmex J Int 13 : 43-47, 2003)

Key Words BACSYS-40*i*, Microbiological Culture, Fluorescence Flowcytometry, Bacteria Screening

Received 23 May, 2003; Accepted 30 May, 2003

INTRODUCTION

Rapid and successful treatment of infection requires identification of the causative micro-organism as quickly as possible. This is of increasing concern for practice in European countries and North America, especially when faced with a DRG (diagnosis-related group) system, which significantly affects laboratory economy. Although many microbial testing products are available, the identification of bacteria normally takes several hours due to the need for prior incubation. This is increasingly viewed as a severe limitation and for this reason SYSMEX has developed BACSYS-40*i* (***Fig. 1***), a flowcytometric device capable of simultaneously counting bacteria and leukocytes, as a further marker of infection, from a small volume sample in 2 minutes. Initially intended for urine examination, the technology may be extended to other body fluids. BACSYS-40*i*, as an initial screening analyzer, can contribute to an efficient workflow in microbiology laboratories by providing bacteria and leukocyte counts as determinants of the need for microbiological culture.

Fig. 1 *The fully automated bacteria analyzer, BACSYS-40i*

CONCEPT OF DEVELOPMENT

BACSYS-40*i* was developed as a rapid screening device, to identify samples from patients with possible infections in an accurate and timely manner in order that appropriate further testing can be initiated. At the same time samples which proved negative in the screening process can be reported to clinicians with confidence.

SPECIFICATIONS

Technology:	fluorescence flowcytometry with semi-conductor laser
Fluorescence Stain:	proprietary polymethine dye[1)]
Throughput:	1 batch (5 samples)/10 min approximately 2 minutes per test
Display:	color touch screen
Specimen:	human urine
Sample Volume:	0.1 mL
Aspiration Volume:	0.05 mL
Quantitative Parameters:	BACT , WBC
Linearity Ranges:	BACT:1.0×10^3–1.0×10^7/mL WBC :1.0 – 5,000/μL
Data Storage:	numerical data with scattergrams for 150 samples
Quality Control:	12 data files Sysmex control material BACTCHECK
Options:	barcode reader, ticket printer, line printer, graphic printer
Dimensions:	380×590×450(W×D×H[mm])
Weight:	33 [kg]

TECHNOLOGY

Measurement process

A 50µL aliquot of the sample is aspirated from the special sample tube and pipetted into the reaction tube. Subsequently 340 µL of diluent solution and 10 µL of dye solution are automatically added. The mixture is incubated at 42 ºC for 30 seconds. A 400 µL aliquot of the reaction mixture is discharged into the flow cell. Here the cells pass a semi-conductor laser light beam and the forward scattered light signals and side fluorescence light signals of all particles are detected (***Fig. 2***). These signals are transmitted to a waveform processing unit where they are converted to forward scattered pulse intensity, fluorescence pulse intensity and forward scattered pulse width as well as the fluorescence pulse width. Bacteria and WBC counts are distinguished based on complex discrimination algorithms and are presented as numerical results as well as in graphic scattergram display (***Fig. 3***).

RESULT DISPLAY

The result display screen of BACSYS-40*i* (***Fig. 3***) shows the system status, the numerical result and corresponding scattergrams as well as flagging information. The display of high counts for quantitative parameters can be combined to a user-definable review mark ('+' highlighted in red). The low reliability mark ('*' highlighted in red) is added in case of values exceeding the linearity range or due to possible particle interference.

FLAGGING INFORMATION

Flag:	Display criteria
BACT+:	The bacteria count exceeds the upper linearity limit.
WBC+:	The WBC count exceeds the upper linearity limit.
Debris+:	Particles other than bacteria or WBC exceed limit.
EC+:	A high number of epithelial cells may affect the WBC count.
YLC/SPERM+:	A high number of yeasts cells or spermatozoa may affect the WBC count.

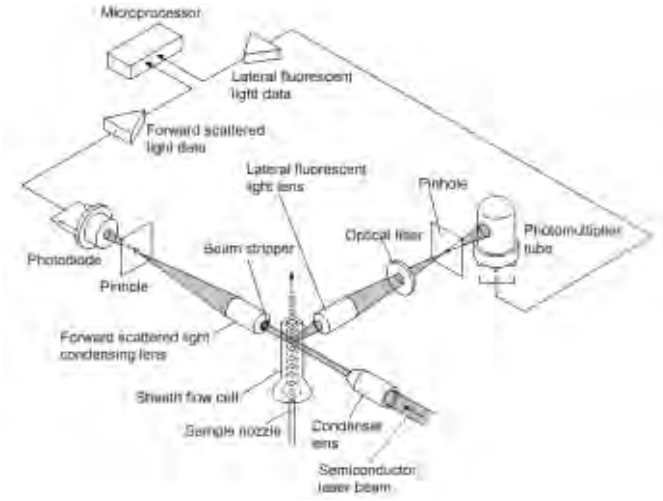

Fig. 2 *Optical unit of BACSYS-40i*

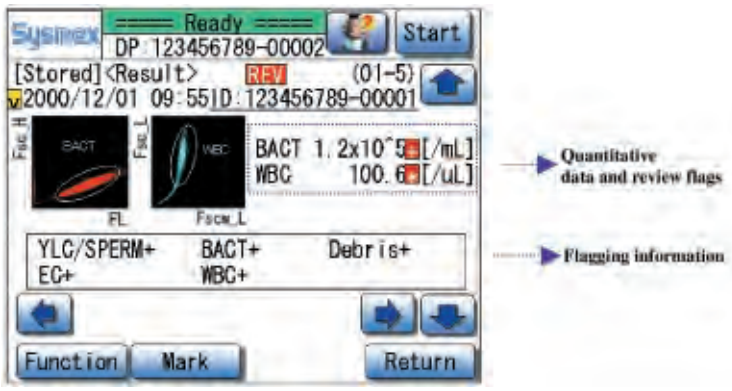

Fig. 3 *The result display screen of BACSYS-40i*

TECHNICAL EVALUATION

Reproducibility, carryover and comparison between microbiological culture and BACSYS-40*i* counts were performed. First evaluations were done with routine urine specimens and further studies with *E. coli* (strain NCTC 10538) as well as Pseudomonas aeruginosa (strain ATCC 15442) at the Institut für Umweltmedizin, Umwelttoxikologie und Hygiene of the University of Kiel, Germany.

Reproducibility

Samples containing 3 different concentration levels of two cultivated *E. coli* strains were used for the examination of within-run-reproducibility. Each sample was aliquotted into two tubes and the contents of each tube measured 5 times. Coefficients of variation (CV) amounted to 3.7% and 5.6% in the concentration ranges of approximately 10^5/mL and 10^6/mL (***Table 1***).

Linearity

Samples containing cultivated *E.coli* (strain NCTC 10538) in Casein-Pepton-Soy-Broth were serially diluted in 1:10 dilutions down to 1×10^3 bacteria/mL with liquid culture medium and each sample was analysed three times. The mean value of the three measurements was calculated.
The mean value of three repeat measurements was plotted against the predicted value at each dilution. The coefficient of determination was found to be R^2=0.9986 (***Fig. 4***).

Carryover

The method of Broughton, et al. was used, as recommended by the International Council for Standardization in Haematology (ICSH)[2]. Three urine specimens with high bacteria concentrations (>10^7/mL) were analysed, followed by a triple measurement of saline. Carryover was calculated according to the following formula:

$$\text{Carryover (\%)} = \frac{\text{B1-B3}}{\text{H3-B3}} \times 100$$

B = saline
H3 = third measurement of the highly concentrated urine sample

Carryover was found to be extremely low, ranging from 0.004% to 0.065% (***Table 2***).

Table 1 *Reproducibility study with E- coli*

ATCC11175			ATCC10538		
	BACT/mL			BACT/mL	
	Sample 1	Sample 2		Sample 1	Sample 2
1	8.9×10^4	4.1×10^6	1	8.7×10^4	5.4×10^5
2	9.2×10^4	4.1×10^6	2	8.9×10^4	5.3×10^5
3	9.0×10^4	4.1×10^6	3	8.4×10^4	5.3×10^5
4	8.1×10^4	4.3×10^6	4	8.0×10^4	5.3×10^5
5	8.4×10^4	4.4×10^6	5	8.4×10^4	5.2×10^5
6	8.8×10^4	4.1×10^6	6	7.3×10^4	5.7×10^5
7	8.2×10^4	4.2×10^6	7	7.9×10^4	5.7×10^5
8	8.0×10^4	4.4×10^6	8	7.7×10^4	5.8×10^5
9	7.8×10^4	4.4×10^6	9	8.3×10^4	5.9×10^5
10	8.3×10^4	4.5×10^6	10	8.5×10^4	6.2×10^5
Mean	8.5×10^4	4.3×10^6	Mean	8.21×10^4	4.3×10^5
SD	4.6×10^3	1.6×10^5	SD	4.8×10^3	3.2×10^4
CV%	5.4%	3.7%	CV%	5.6%	3.7%

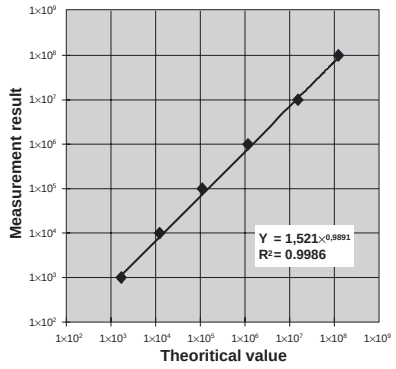

Fig. 4 *Linearity study*
A concentrated sample was serially diluted and measured in triplicate. The measurement results (x-axis) were plotted against the theoretical values (y-axis).

Table 2 *Carryover study of BACSYS-40i*

	Sample 1	Sample 2	Sample 3
H1	1.8×10^7	6.6×10^7	4.3×10^7
H2	1.8×10^7	6.6×10^7	4.3×10^7
H3	1.8×10^7	6.6×10^7	4.3×10^7
B1	1.5×10^3	2.5×10^3	5.0×10^2
B2	1.3×10^3	6.7×10^2	0
B3	3.3×10^2	3.3×10^2	3.3×10^2
Carryover%	0.0065%	0.0033%	0.0004%

Unit:BACT/mL

Accuracy

115 urine specimens were used for the comparison study with quantitative microbiological culture as suggested by the European Urinalysis Guidelines as the high quality level 3 method[3].
The samples were diluted 1:10 with filtered urine. 100µL of each dilution were spread onto CLED Agar medium plates (Cystine-Lactose-Electrolyte-Deficient) and then incubated for 24 hours at 35 °C. The colony count on a minimum of three plates was used to calculate the original concentration of the sample. Excellent correlation (R^2 = 0.95 – 0.98) was found in the range of 10^3 CFU*/mL-10^8 CFU*/mL (***Fig. 5***).
*CFU= Colony forming unit

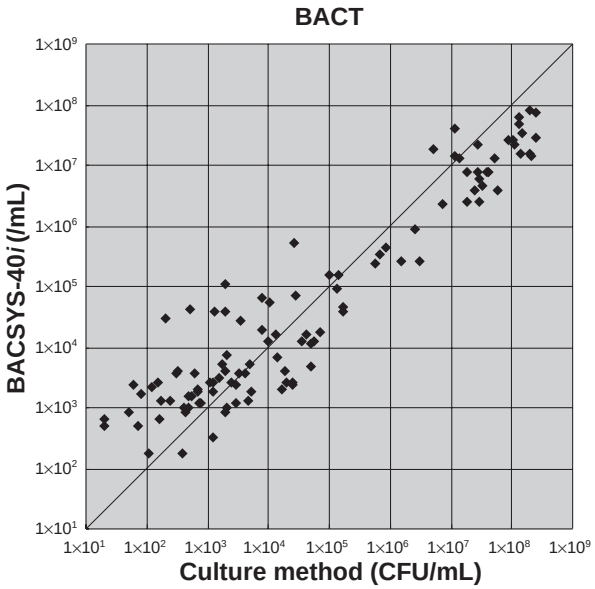

Fig. 5a *Correlation of BACSYS-40i to culture of routine urine specimens*

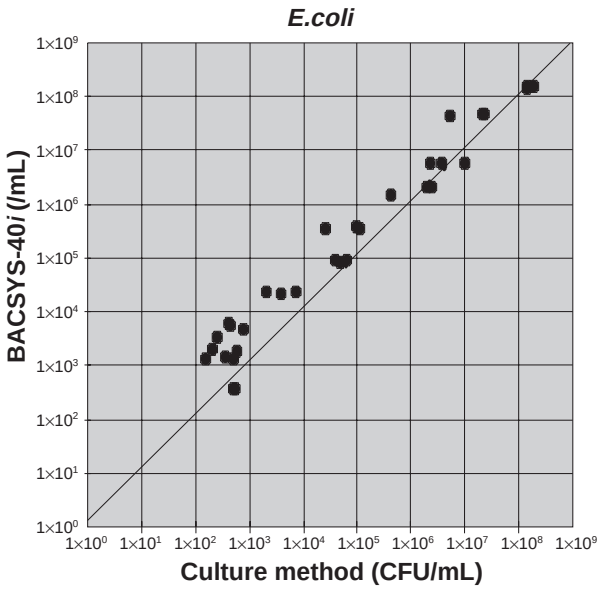

Fig. 5b *Correlation of BACSYS-40i to culture of E.coli (strain NCTC 10538)*

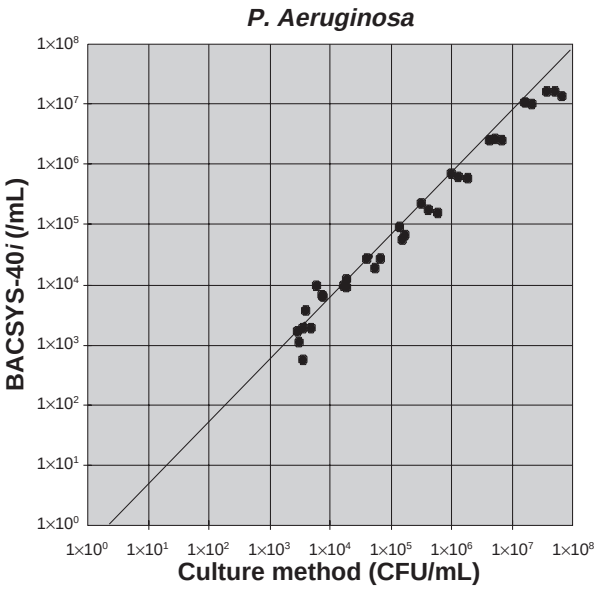

Fig. 5c *Correlation of BACSYS-40i to culture of Ps. Aeruginosa (strain ATCC 15442)*

DISCUSSION

In conventional clinical microbiology, the diagnosis of urinary tract infection is usually based on bacteria detection, species identification and susceptibility testing by classical culture techniques as routine methods. However, these examinations are time consuming and were often applied unnecessarily to negative samples. Furthermore it takes one to several days to obtain the final results. "Real-time reporting" of microbiological examination results has not yet been widely applied. Under most circumstances the microscopy count is not considered as a useful method for immediate bacteria enumeration, since it is time-consuming and strongly influenced by non-standardized factors.

As shown in this evaluation, the results from BACSYS-40*i* compare well with those from quantitative microbiological culture if the latter is performed under standardized conditions. The device allows the detection of bacteria precisely, accurately and quickly as an initial microbiological screening procedure. Positive samples can be selected for further analysis, such as inhibition tests, species identification and susceptibility testing. A workflow which includes the BACSYS-40*i* will therefore contribute to the overall efficiency by reducing turn-around-time and saving labour costs.

Additional benefit for the patient can be expected from decision criteria for urinary tract infection, being based not only on the bacteria count but also on the leukocyte count. Such improved diagnostic schemes will identify the inflammatory process as a meaningful indicator. Further studies are needed to prove the clinical benefit of such a multi-factor approach.

References

1) *Matsumoto H: The technology of reagents in the automated haematology analyzer Sysmex XE-2100 – red fluorescence reaction. Sysmex J Int, 9 (2): 179 –185, 1999.*
2) *ICSH: Guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting and cell matker applications. Clin Lab Haemat 16, 157-174, 1994.*
3) *Kouri T, et al.:European Urinalysis Guideline, The Scandinavian Journal of Clinical & Laboratory Investigation, 60, supplement 231, 2000*