BCB-blood culture system (Roche) detects *Brucella* bacteremia in less than 8 days

R. Auckenthaler, B. Hirschel and S. Shiro-Lente
Hôpital Cantonal Universitaire, CH-1211 Genève

From July 1982 to December 1984 all blood culture bottles from patients with fever of unknown origin, suspected endocarditis, or brucellosis were routinely incubated at 35 °C for 14 days using BCB (Roche) system. Aerobic bottles (AER) contained columbia broth and anaerobic bottles (ANAER) thioglycolate broth. Subcultures were performed using BCB slides for AER twice a day during the first 48 h and daily thereafter. ANAER was subcultured only if AER was positive or if broth became turbid. 63/71 submitted AER originated from 16 patients with at least two cultures positive for *Brucella* spp. with a mean detection time of 6.2 days (range 4–10 d). 8/71 submitted AER (5/7 patients with previous antibiotics) were negative. At least one positive culture was detected within 8 days in all cases. 13/39 submitted ANAER became positive only after venting and subculturing, with a mean detection time of 10.2 days (range 7–17 days).
98.4% of positive cultures were detectable within 8 days. In the BCB Roche system incubation more than 10 days does not seem to be necessary for detection of *Brucella* spp., if vented or subcultured properly.

Gas-liquid chromatography as a complement in the research of *Clostridium difficile* in stools of clinically relevant patients

S. Taillens, J. Barman and A. Gallusser
Institut de Microbiologie, CHUV, CH-1011 Lausanne

The research of *C. difficile* in stools of patients with antibiotic associated diarrhea or with pseudomembranous colitis is of routine in many laboratories. This research is mainly done using a selective agar with cycloserine and cefoxitin (CCFA). When the laboratory has an available source of cell cultures (human lung fibroblast cells) a specific cytotoxic effect in the stool extract can be looked after. In many cases results may be paradoxal and there is a need of a third way of establishing the presence of *C. difficile*. In a still undergoing work, we have looked for the presence of isocaproic acid (iC_6), a major metabolic product of *C. difficile* in the stool extracts. Among 61 clinically relevant cases there was a major agreement in 88.5% of the tests (negative for culture, toxin and iC_6 in 40 cases, positive for culture, toxin and iC_6 in 14 cases). There was a minor agreement in 5% of tests (twice iC_6 and toxin positive, cultures negative; once iC_6 and culture positive, toxin negative). The minor (1.5%) and the major (5%) disagreements will be discussed.
This rapid and easy technique has allowed an univocal orientation in 93.5% of cases and it is specially useful when discordant results between cytotoxicity and culture exist; it can be used as a reliable screening method.

Evaluation of the 5-h Cobas-Bact® identification rotor for Enterobacteriaceae. A preliminary report

A. Wenger, L. Tissières, F. Praplan, W. Kamm and J. Bille
Laboratoire de bactériologie médicale, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne

The Cobas-Bact®, a new automated system for rapid susceptibility testing of non-fastidious bacteria has now the ability of identifying 36 different species of Enterobacteriaceae within 5 h. It relies on 16 biochemical reactions and requires only spot indole as a preliminary test.
So far we have tested 190 different strains (both fresh clinical isolates and stock cultures) identified by API 20E. Among 138 strains of the most commonly encountered Enterobacteriaceae in our hospital bacteriology laboratory (*E. coli* (24), *K. pneumoniae* (10), *K. oxytoca* (5), *E. cloacae* (19), *E. aerogenes* (2), *H. alvei* (3), *S. marcescens* (6), *P. mirabilis* (9), *P. vulgaris* (7), *P. rettgeri* (6), *M. morganii* (8), *C. freundii* (15), *C. diversus* (13), and *Salmonella* sp. (11). Cobas-Bact® agreed with API 20E on 137 identifications (99%). However 16.7% of these (23/138) required additional tests as well as all the Shigella tested (10). One single isolate (*S. marcescens*) was not identified by Cobas-Bact®. When restricted to the most common strains of Enterobacteriaceae, the Cobas-Bact® instrument appears promising for their rapid identification though a sizeable portion of the strains still requires additional tests.

Hydrophobic grid membrane filters (HGMF)

H. Illi
Federal Office of Public Health, Dept of Food Control, P.O. Box 2644, CH-3001 Bern

On the surface of conventional membrane filters with a pore size of 0.45 µm a grid of hydrophobic material is printed on so that the filtration area is divided into 1800 individual growth compartments. The hydrophobic property of the grid lines determines the positions and limits the lateral growth of microbial colonies and produces a miniaturized most probable number system equivalent to a large number of tubes inoculated from a single dilution. HGMF provides a counting range of more than 3 log 10 cycles on a single membrane filter.

Comparison of virus detection by EIA and cell culture

W. Wunderli, P. Gowland, D. Germann, A. Bächler and K. Schopfer
Institute of Clinical Microbiology and Immunology, CH-9000 St. Gallen

Sarkkinen et al. (J. clin. Microbiol. *13* (1981) 258) have published an enzyme immunoassay (EIA) for the rapid detection of viral antigens in nasopharyngeal secretions (NPS). The following viral antigens can thus be found: RSV, Adeno, Parainfluenza 1, 2, 3, Influenza A and B. Although the results of EIA have been compared with immunofluorescence, a comparative study using the classical cell culture method has not yet been done systematically. Therefore, we analyzed more than 200 specimens of NPS from children with respiratory tract infections both by EIA and cell culture. From the freshly collected NPS an aliquot was put into transport medium and used for inoculation of various cell lines. Cells were observed for the development of cytopathic effects (CPE) for RSV and Adeno viruses. Parainfluenza and influenza viruses were detected by CPE and hemadsorption. The data so far obtained show a correlation of about 90%. A detailed comparison of the results will be presented.

Direct 5-h Cobas-Bact® susceptibility testing of gram stain confirmed positive urine samples

W. Kamm and J. Bille
Laboratoire de bactériologie médicale, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne

A rapid antimicrobial susceptibility test of positive urine samples with an automated instrument Cobas-Bact® was compared to conventional Kirby-Bauer agar diffusion disc method. 10 ml of gram stain confirmed positive urine samples were centrifuged at 2800 rpm for 15 min and 200 µl of the sediment suspended in saline were inoculated into Cobas-Bact® broth.
So far we tested the bacteria from 74 episodes of monomicrobial bacteriuria. In 3 cases (4.1%) not enough growth occured within 5 h. The 71 other organisms were: 61 gram-negative bacilli (48 *E. coli*, 12 other enterobacteriaceae, 1 *P. aeruginosa*), 10 gram-positive cocci (4 *S. aureus*, 2 coag. neg. sta., 3 enterococci and 1 *S. sanguis*). Overall, 516 antibiotic-organism tests were performed. Discrepancies (D) were found in 14.5% of the tests (75/516): 31 minor D (6%), 37 major D (7.2%) and 7 very major D (1.3%).

Full agreement was 85.5%, essential agreement 91.5%. Over half of the minor and major D concerned the pair *E. coli* – cephalothin.
Cobas-Bact® direct antimicrobial susceptibility test of positive urine samples seems to give accurate results within 5 h of detection by gram stain of monomicrobial bacteriuria.

Passive hemagglutination test for detection of antibodies to streptolysin O

A. Sziegoleit and S. Bhakdi
Institute of Medical Microbiology, Giessen University, Frankfurter Strasse 107, D-6300 Giessen, FRG

A passive hemagglutination test (PHT) for assaying antibodies to streptolysin O (SLO) is described. The test uses glutaraldehyde treated and SLO-sensitized sheep erythrocytes as reagent. In contrast to the antistreptolysin O (ASO) test, the PHT utilizes the membrane form of SLO as antigen. Recently it was shown that SLO, after binding to a cholesterol-containing membrane, self-associates to form curved, rod-shaped oligomers of up to 80 SLO monomers that are amphiphilic and that penetrate into the apolar domains of the membrane. During naturally occurring infections with SLO producing streptococci the membrane form of the toxin may represent the primary antigenic form of the toxin. To test the utility of the PHT, antibodies to SLO were determined in 636 human sera and the results compared with the titres of the ASO test. All sera with elevated titres in the ASO test agglutinated the sensitized erythrocytes in dilutions higher than 1:800. In addition however, the PHT recognized anti-SLO antibodies in high concentrations in some sera with normal ASO titres. These sera were mostly derived from patients suffering from streptococcal skin infections which are known to induce low levels of neutralizing anti-SLO antibodies. The PHT appears superior to the ASO test for detecting anti-SLO antibodies and is also far simple to perform.

Rapid susceptibility testing of *Nocardia asteroides* with an automated instrument

R. Auckenthaler, M. Michéa-Hamzehpour and J. C. Pechère
Département de Microbiologie, Faculté de Médecine, CH-1211 Genève

MIC determination for *Nocardia asteroides* (NA) by agar dilution takes 48 h and MIC performed with broth dilution is unreliable because of inhomogenous growth. We evaluated the Cobas-Bact instrument (CBR) capable of measuring automatically the optical density of a growing broth culture during centrifugation; comparatively with the standard agar dilution method (SAD), the MICs obtained by the CBR with 9 antibiotics on 6 clinical isolates of Na were equal in 16.6%, within a two-fold dilution in 52.6%, four-fold dilution in 20.3% and eight-fold in 10.5%. MICs in CBR were higher than MICs in SAD in 48%, and concerned mainly cephalosporins, quinolones and amikacin. In contrast, MICs and CBR were lower than in SAD in 35.3% and concerned mainly tetracyclines and netilmicine. With CBR, 11% of results were obtained in 6 h, and 100% within 12 h.
In conclusion, CBR can be used for the rapid susceptibility testing of Na and looks promising for the MIC determination of organisms with inhomogenous growth in broth cultures.

Use of rapid bacteriuria screening tests in urines transported with Urine C&S Transport Kits

R. Auckenthaler and H. Favre
Laboratoire central de Bactériologie et Laboratoire central des Examens Biologiques, Hôpital Cantonal Universitaire, CH-1211 Genève

Fresh urine (U) obtained by catheterization or mid-stream from 124 hospitalized adult patients were analyzed immediately and after 24 h of conservation at 20 °C in Urine C&S Transport Kit (UT, Becton Dickinson). Screening for bacteriuria and pyuria was performed with Bac-T-Screen (BTS, Marion Laboratories), BM-Nephur-Test and Leuco (LN, Boehringer), hemocytometer cell counts (WBC) and quantitative cultures of uncentrifuged U and UT. 62 U (50%), (56 UT = 45%) were culture positive with two or less pathogenic organisms, 33.9% (UT 35.7%) with predominant gram-negative rods (GNR), 14.5% (UT 14.3%) with gram-positive bacteria (GP) and 51.6% (UT 50.0%) with mixed GP-GNR. Agreement between BTS-U versus BTS-UT was obtained in 96.7%, between LN-U versus LN-UT in 95.2% for Leucocyte esterase and 79.8% for Nitrate respectively and WBC-U versus WBC-UT in 74.1% of Leucocyte counts over 8/mm^3 (±25%). 18 BTS-U (16 BTS-UT) could not be evaluated due to filter clogging.
Rapid screening methods such as Bac-T-Screen, leucocyte esterase and WBC can be used after one day conservation in Urine C&S Transport Kit.

Description and interest of the new API kit (API 20 EC) for identification of coliform enterobacteria

F. Gavini, D. Monget, J. M. Boeufgras and H. Leclerc
INSERM U.146, Domaine du CERTIA, Villeneuve-d'Ascq, France, API SYSTEM, La Balme-les-Grottes, Montalieu Vercieu, France

An identification system for β galactosidase positive enterobacteria, which group known species of coliform group ('fecal coliforms') and saprophytic species from environment ('non fecal coliforms') was developed in collaboration with API research laboratory (France).
It consists of 20 biochemical characters and identify 31 species. Interest of this kit is discussed in two fields: – water analysis control, with the differenciation of fecal and non fecal species – medical analysis, with the recent isolation of some of saprophytic species such as *Rahnella aquatilis* and *Klebsiella trevisanii.*

Gas chromatographic analysis of bacterial cells: a rapid and accurate identification of pathogenic Campylobacters

S. Taillens and M. Heitz
Institut de Microbiologie CHUV, CH-1011 Lausanne

Cells harvested from five Petri dishes with Müller Hinton Blood Agar were treated according to the method outlined by Moss et al., Appl. Microb. *28* (1974) 80. The final extract does or does not contain lactobacillic acid (C19:OΔ) depending on whether the bacteria were *Campylobacter jejuni* or *coli* on the hand or *Campylobacter fetus* ssp. *fetus* (*Campylobacter intestinalis*) on the other.
The method tested and verified by using 11 reference strains has been applied to 55 strains of *C. jejuni* and to 14 strains of *C. fetus* ssp. *fetus*. The clear results of the gas chromatography (100% of the strains of *C. jejuni* contained C19:OΔ, whereas none of the strains of *C. fetus* ssp. *fetus* contained it) has permitted us to simplify the bacteriological tests. We now establish the following characteristics: mobility, gram stain, oxidase, TTC, susceptibility to nalidixic acid, and gas chromatographic analysis used as a confirmation.

Identification of gram-negative rods with the Quantum II Microbiology System: probabilities and reproducibility

P. Kappeler, J. Zollinger-Iten and A. von Graevenitz
Department of Medical Microbiology, University of Zürich, CH-8028 Zürich

The Quantum II Microbiology System (Abbott Laboratories Diagnostic Division, North Chicago, Ill.) provides for the mechanized identification of Enterobacteriaceae (E) spp., a few spp. of nonfermenters, and *Aeromonas/Plesiomonas*. Reactions