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

of 20 lyophilized substrates are read with a dichromatic spectrophotometer and compared with a data base. Identifications with probabilities (PR) are printed after 4–5 h at 37 °C.
6 E strains (str.) were each tested three times in two different lots of cuvettes with sterile distilled water (SDW, pH 6.3) and SDW ad infundibilia (SDWI, pH 6.0). Different biocodes but identical sp. diagnoses at 97.6% PR were obtained. 594 Str. (479 E, 80 NF, 35 V), identified by CDC methods (E) or API 20 NE (NF, V) were tested for indentification. SDW (262 E+ (NF+V) str.) and SDWI (332 E+ (NF+V) str.) gave no significantly different results. At > 80–99% PR, 77% each of E and NF+V were diagnosed correctly; from < 50% upward, figures were 92.5% and 79%. 24 str. were misidentified, 16 only to species. 62.5% of E were diagnosed at 4 h; the rest and all NF+V at 5 h. Problems occurred with *P. mirabilis, S. liquefaciens, S. paratyphi A,* and *Yersinia* spp. and *E. agglomerans.*

Evaluation of the Cobas-Bact® automated antimicrobial susceptibility testing system

G. Dupuis
Division of Clinical Microbiology and Infectious Diseases, Valais Central Institute, CH-1950 Sion

The Cobas-Bact® is an analyzer for the microbiological laboratory capable of producing 5 h susceptibility tests and kinetic growth curves. The Cobas-Bact® system was evaluated for its efficacy by determining the susceptibilities of 2000 clinical, nonfastidious, falcultative and aerobic isolates compared with standard Kirby-Bauer disk diffusion. For gram-positive isolates, overall full and essential agreements between Cobas-Bact® and reference antibiograms were 90% and 95.5% respectively. For Enterobacteriaceae the overall full and essential agreements were 91% and 95.5% respectively. With *Pseudomonas* spp. the full and essential agreements were 90% and 96% respectively. Reproducibility studies gave essential agreement in 98% of cases. On the basis of this preliminary evaluation, it seems that the Cobas-Bact® can yield rapid, reasonably accurate and reproducible results when testing gram-positive cocci, Enterobacteriaceae and *Pseudomonas aeruginosa.*

A simple rapid technique to measure minimal bactericidal concentrations and combined antimicrobial action

I. Heinzer
Institute of Hygiene and Medical Microbiology, University of Bern, CH-3010 Bern

The methods used most frequently in vitro to measure the interaction of antibiotic combinations are 1) chessboard method (MIC and/or MBC), 2) killing curves and 3) diffusion tests, e.g. the cellophane transfer method of Chabbert. None of these is satisfactory. I have developed a new technique which combines many advantages of the known methods without their major disadvantages – especially the 'carry over' of antibiotics.
Chessboard titrations are done in tissue culture plates (Costar 3424, Mark II, 24 holes). After overnight incubation the plates are centrifuged in an oblique position of 45°. The supernatant is removed and the sediment may be washed several times to remove the antibiotics. After resuspension of the bacteria a double strength agar medium is added. After reincubation for another 24 h 99% or 99.9% bactericidal activity is calculated for each combination of antibiotics.

Evaluation of the Api 20 Strep system for species identification of viridans streptococci from blood cultures

R.G. Hafner
Institut für Hygiene und Medizinische Mikrobiologie, Universität Bern, Friedbühlstrasse 51, CH-3010 Bern

87 (100%) strains of Aerococci and viridans streptococci isolated from blood cultures in the years of 1983 and 1984 were classified according to a modification of Facklman's methods: 3 Aerococci, 10 *S. bovis* I, 3 *S. bovis* II, 15 *S. sanguis* I, 21 *S. sanguis* II, 12 *S. mitis*, 14 *S. milleri*, 4 *S. salivarius*, 2 *S. mutans*, 3 strains unclassified. Api system identifications: 28 (32%) strains after 4 h of incubation by the profile index. 68 (78%) strains after 24 h. 73 (84%) strains by additional testing: Optochin reaction and polysaccharid production. 74 (85%) strains by computer identification. 76 (87%) strains by computer identification and additional testing. 3 strains were misidentified. 8 (9%) strains remained unidentified, including the 3 unclassified strains by the reference method.
The Api system proves to be an accurate method for species identification of viridans streptococci. With additional testing and identification by the profile index the rate of identification was 84%.

Plasmids and Transposons

Replication of the tetracycline resistance plasmid pSC101

G. Churchward, Xia Guixan, T. Goebel, D. Manen, E. Othon-Izaurraulde and L. Caro
Université de Genève, Département de Biologie Moléculaire, 30, quai Ernest Ansermet, CH-1211 Genève 4

We have a series of mutations in the replication genes of the *E. coli* plasmid pSC101 caused either by insertion in vivo of the transposon Tn*1000*, or in vitro of a segment of DNA carrying an antibiotic resistance marker. These mutations have enabled us to map the origin of replication, a gene, *repA*, whose product is essential for replication and regions implicated in the regulation of replication. The RepA protein represses transcription of its own gene. We are currently isolating and analyzing mutations which affect replication control and using gene fusion techniques to map transcriptional units within the replication origin. We want to elucidate the molecular mechanisms which determine plasmid copy number.

Ω mutagenesis in gram-negative bacteria: a selectable DNA fragment which terminates transcription in a wide range of bacterial species

J. Frey, J. Deshusses and H.M. Krisch
Départements de Biochimie et Biologie Moléculaire, Faculté des Sciences, Université de Genève, CH-1211 Genève 4

A novel method for in vitro insertional mutagenesis of genes cloned in *E. coli* has been previously described (Prentki and Krisch, Gene *29* (1984) 303). It employed the Ω fragment, a 2.0 kb DNA segment consisting of the antibiotic resistance gene *aadA*$^+$ (Sm^r/Spc^r) flanked by small inverted repeats carrying transcription and translation termination signals and synthetic polylinkers. In *E. coli* the Ω fragment has been shown to terminate RNA and protein synthesis prematurately at the site of insertion. In this communication we demonstrate that Ω mutagenesis is equally effective in a wide range of gram-negative bacteria other than *E. coli.* To facilitate this analysis, we constructed in a broad host range vector, a hybrid plasmid which contains the entire *meta*-cleavage pathway operon of the *Pseudomonas putida* TOL plasmid pWWO. Since one of the downstream genes in the polycistronic mRNA encodes an easily assayable enzyme, the Ω fragment was inserted between this gene and the promoter. Comparison of the enzyme levels produced by the plasmid with and without the Ω insertion indicates that in all the strains examined Ω reduced transcription beyond the point of insertion at least 50-fold. We conclude that Ω mutagenesis is equally applicable as a method to study gene structure and function on these organisms.