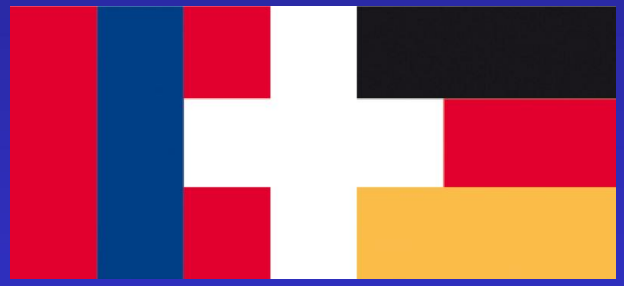
Medical Care Center Dr.Eberhard u. Partner in Dortmund / Germany

… let us have a look at

some aspects of microbiological diagnostics

MIKROBIOLOGY ŏ www.labmed.de / mikro@labmed.de



Medical Care Center
Dr. Eberhard & Partner Dortmund
Germany

Mongolia ŏ March 2014

accreditation since april 2003 (DIN EN ISO 15189; DACH)

Deutscher Akkreditierungs Rat
DAR

… the situation of laboratories in UB …

laboratories in general in Mongolia:

- **buildings / laboratory-rooms**
  - **not always modern room-arrangements: suboptimal workflow!**
    (but: often new laboratory-rooms planned or they are ready to move)

ŏ **companies / reliable and guaranteed delivery**
  - **not always sufficient technical support available: no short reaction time!**
    (modern technical equipment is mostly very sensitive!)

- **health care system / prescription of antibiotics**
  - **free availability of antibiotics and treatment without medical indication**
    (one cause for the increase of Multi-Drug-Resistance!)

- **costs**
  - **sometimes not effective use of equipment and testkits could be found**
    (donated equipment is available - substances and reagents are not)

- **skill-level of staff / motivation of employees**
  - **… very gratifying: cooperative, engaged, motivated staff!**
    (finding suitable solutions for changing and even suboptimal conditions)

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prevention of Hospital Aquired Infections (HAI)

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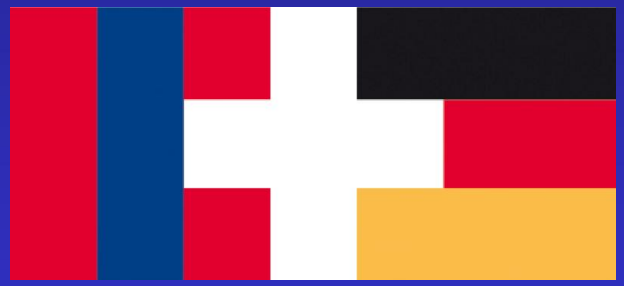

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successfully spreading bacteria!

- **preconditions for bacterial growth:**
  - **water**
  - **solved nutrients / chemical elements**
    (for metabolism, energy recovery, enzymatic activity etc.)

  ⇒ **irreversible increase of biomass**
  ⇒ **growth of bacteria-cells until cell division**

- **reproduction of bacteria**

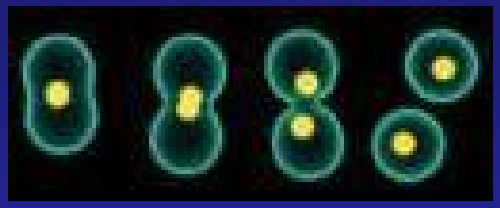
  ⇒ **cell division/segmentation**
  ⇒ **2 identical daughter cells !**

- **generation time: (duplication of quantity)**

fast/ colony forming units/CFU: < 1 day : E.coli
slow/ colony forming units/CFU: > 14 days : M.tuberculosis

ŏ fast spreading bacteria by cell division !

e.g. bacteria E.coli: duplication every 15 minutes *



1 4 8

> after 1 h: 16

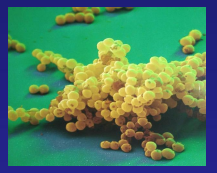
> after 2 h: 256

> after 5 h: 1.048.576 bacteria!

***... slower at low temperatures ...**

Multi-Drug-Resistance - a challenge

- **clinical/epidemiological relevant bacteria (e.g. wound)**

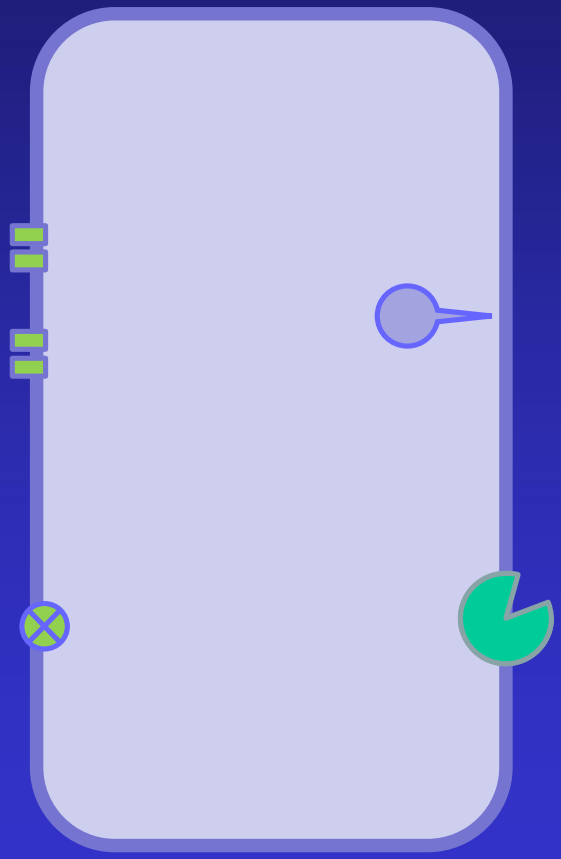


- **Staphylococcus aureus (CAVE: MRSA!)**
- **Enteroccus sp. (CAVE: VRE!)**
- **increasing: drug resistant Gram-Negative bacteria (GN)**
  - **Escherichia coli (CAVE: ESBL-pos.)**
  - **Klebsiella sp. (CAVE: ESBL-pos.)**
  - **Pseudomonas aeruginosa (CAVE: MDR)**
  - **Proteus sp.**
  - **Enterobacter cloacae etc.**

- **anaerobes (Clostridium perfringens, Bacteroides sp.)**

ŏ mechanisms of drug resistance

loss of porines/ changes in permeability



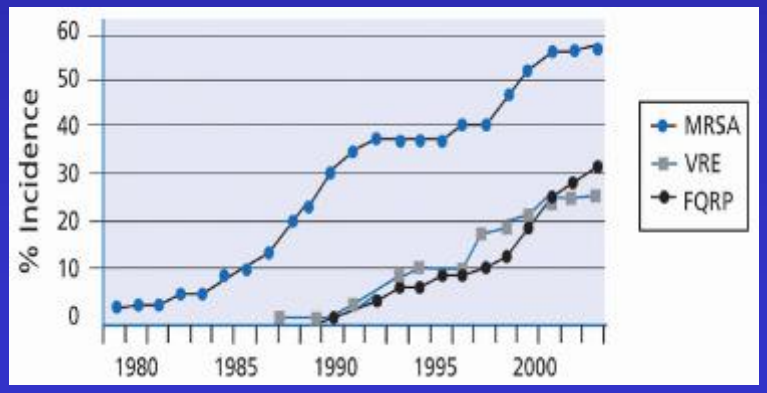
ß-laktamase/ enzyme

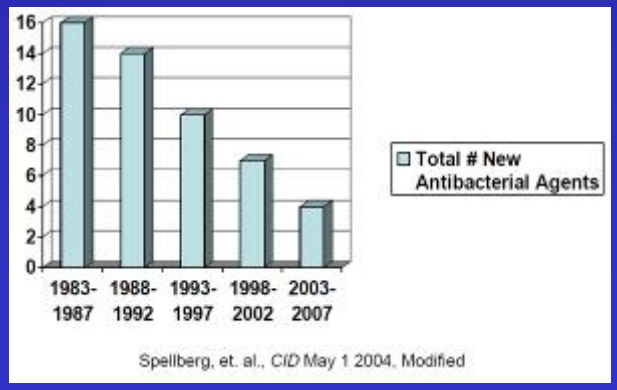
efflux-pump

change in bonding site

ŏ No ESKAPE ? problematic bacteria (IDSA / USA)

E nterococcus faecium	VRE
S taphyloc. aureus	MRSA
K lebsiella pneumoniae	ESBL
A cinetob. baumannii	Carbapenemases, MDR
P seudom. aeruginosa	Carbapenemases, MDR
E nterobacter cloacae	ESBL+AmpC, MDR





IDSA:

Bad bugs

–

No drugs

Robert-Koch-Institute / RKI

- **central institution for health protection**

  - supports the Federal Ministry of Health as a central scientific institution in the field of biomedicine

ROBERT KOCH INSTITUT

www.rki.de

Tasks of the Robert Koch Institute:

- developing concepts for the prevention of communicable diseases
(for early detection of infections and the prevention of their spread)

- draws up guidelines, recommendations, and other information

- determines criteria for nosocomial infections and pathogens
(with special resistances / multiple resistances - see IfSG § 23)

- publishes results of epidemiological evaluations
(e.g. in the Federal Health Gazette)

… this has an important impact on the role of laboratories / national reference centers in Germany!

monitoring Hospital Aquired Infections

- **hospitals**

  "How many hospital aquired infections do we have?"

  - establish screenings - all incoming patients (for a defined period)
  (to detect risk-patients - e.g. MRSA: colonisation?
  ... existing or real hospital aquired infection?)

  - duty to monitor development of hospital aquired infections!
  (§ 23 IfSG; reported to health autorities!)

- **laboratories / National Reference Centers**

  - offer statistics to the central institute of health protection (RKI)
  (bacteria, resistance etc.; actually in Germany: ARS-project)

  - offer statistics as a helpful service for the hospitals / doctors
  (data concerning every case or patient are available in the laboratory!)

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essential precondition: praeanalytics

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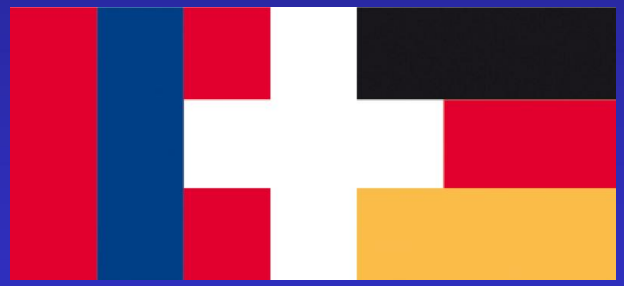

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collection and transport of specimen

a common trend in Germany ... and in Ulaanbaatar too:

- **not every hospital provides microbiological diagnostics**

  less specimen / throughput = more expensive diagnostics

- **fast + appropriate transport of specimen is important!**

- **suitable time:**

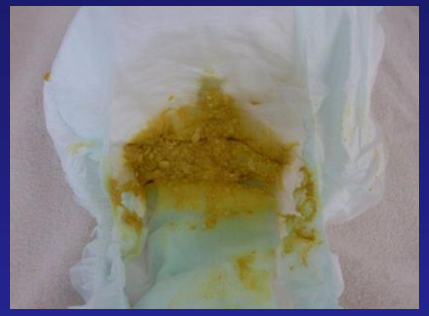
  - examination of microbiological specimen should start at the same day !
  (... if not possible, you have to follow special storing conditions)

We usually collect and transport specimen:
- 2 x a day for hospitals: before midday and in the afternoon
- 1 x a day for smaller medical centers / practitioners

(by our own car-fleet, 20 cars equipped with climatisated boxes)

- **"not-suitable" collection and transport of specimen ...**



baby's napkin...

- **better:**




microbiological diagnostics:
- containers MUST be always sterile!
- storage-conditions AFTER collecting the specimen:
... often room-temperature recommended !

Microbiology: Wounds

- **specimen - classic indicators of a local infection:**
  - **smear test of wound (...ideal: from the depth of the wound!)**
- **specimen - sepsis with fever:**
  - **if necessary: blood-cultures**
- **specimen - necrosis at the edge of wounds:**
  - **biopsy / tissue sample**

in sterile universal tubes / containers
(advantageous: adding 1 drop physiological sterile NaCl-solution to prevent dehydration!)

or:

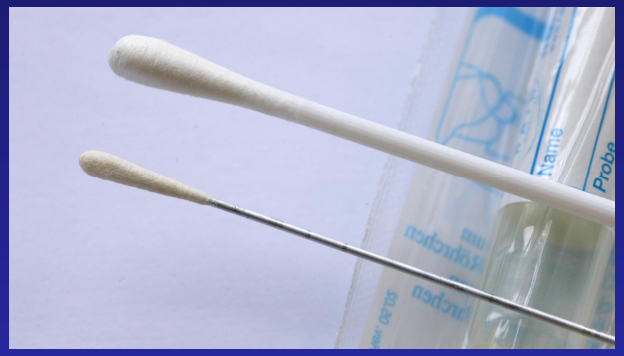
in bouillon
(dilution! usually done in the Lab:
bouillon after inoculation of culture media)

proper closing of tubes / containers !
NO FORMALIN !

... not this way!

swabs for smear tests:

- **thick inflexible swabs with transport-media (BLUE)**



  - **wounds • throat, tonsilla, nose • genital area, cervix ...**

- **thin, flexible swabs with transport media (ORANGE)**
  - **smear test of urethra ...**

for smear tests with swabs in general:

> put swabs into transport-media, close tube properly
> instant transport to Lab or interim storage at room-temperature

Microbiology: Sepsis / Fever

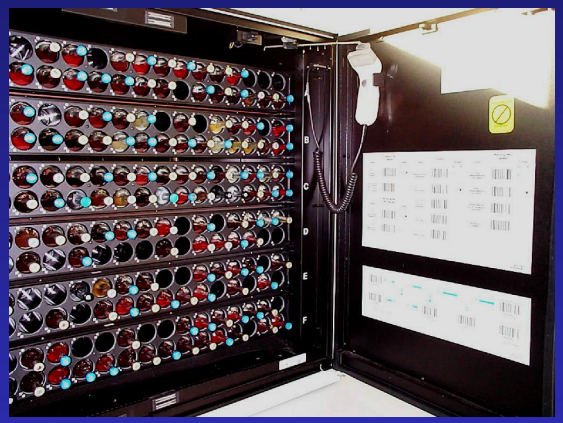
- **indication for blood cultures at:**
  - **SIRS, (serious) sepsis, septical shock / FUO**
  - **systemic involvement of local infections like:**
    cyclic infections
    (typhus, brucellosis etc.; symptoms not always like sepsis!)
    bacteremia / fungemia
    (subacute endokarditis, katheter-associated infections)

as well as serious cases of:

- **purulent meningitis**
- **ambulant acquired / respiration-associated pneumonia**
- **komplicated (!) Pyelonephritis**
- **infections of skin- / soft parts**

BACTEC-plus® blood culture System)

(fluorescence-technology / infrared-absorption-technology)



- **blood • liquor cerebrospinalis / puncture specimen:**
  - **2 blood culture bottles (set) for aerobic / anaerobic bacteria**
  - **bottles contain media for transport AND cultural growth !**

for inoculated blood cultures: *possibly fast transport to LAB !*

> 3-10 ml / bottle inoculation (paediatric media / bottle: 1-3 ml)

> otherwise interim storage at room temperature (max. 48 h!)

sterile Universal-tubes:

suitable for fluid primary sterile specimen like:

- **liquor • punctures • exudats (abscess) • biopsy (tissue)**
  - **aseptic conditions during collection of specimen !**
  - **1 ml minimum (3 ml incl. serological examinations)**
  - **proper closing of tubes !**

for primary sterile materials (native):

possibly fast transport to LAB; if necessary delivery service!

> otherwise interim storage at room temperature (max. 48 h!)

Microbiology: Respiratory Tract Infections:

ŏ collection of bronchial- / tracheal-secretion:

- if isotonic fluids are used before aspiration (NaCl-solution), this must be free of antibacterial additives !
- very suitable: primarily very few contamination by physiological bacterial flora of mouth and throat

• collection of bronchial lavage (BAL):

- anaesthetising gels might be antimicrobial / antibacterial !

• collection of sputum

- saliva / spittel from mouth is not suitable ! (physiological flora)
- perhaps inducing sputum-production:

(inhaling several times and holding breath for 3-5 seconds before coughing up; after deep inhaling once again the sputum can be given into the opened vial; repeating procedure)

Microbiology: Urinary Tract Infections (UTI):

ǒ indications für mikrobiological urine-diagnostics:

ǒ symptomatic Infektion / UTI (except uncomplicated cystitis: I)
- signs of hospital-acquired UTI
- ongoing symptoms during/after antibiotic therapy
- fever or sepsis of unknown origin

• interpretation of results (urine: midstream/dip slides):

< 10 ^{5} CFU / ml (colony forming units) :

1 path. species: susceptibility-testing; UTI possible (with symptoms, I urethritis, children, chronic UTI)

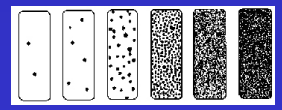
1 contaminant: NO susceptibility-testing; UTI doubtful

> 10 ^{5} CFU / ml :

1 path. species: susceptibility-testing; UTI very likely

2 path. species: UTI by dominating species very likely

1 contaminant: NO susceptibility-testing; UTI doubtful



sputum-/ urine-tubes

ŏ **bronchoscopic material/ tracheal-secretion • sputum • urine**

midstream urine : 5-10 ml necessary
(minimum 20 ml if suspicion: Mycobacteria)

katheter urine: if possible, a fresh layed catheter !

CAVE: use only sterile and tight closable transport vials !

for respiratory-secretions and urine (native):

> instant transport to Lab or interim storage cool at 4 °C

Microbiology: Diarrhoea

ŏ recurring watery diarrhoe during hospital treatment:

ŏ Salmonella/ Shigella, Yersinia, Campylobacter,

- bloody: EHEC;
Clostridium difficile (Toxin) ?

- antibiotic treatment: Clostridium difficile (Toxin) ?

- children < 3 J.: Rota-/Adeno-Virus;
EHEC/EPEC ?

- foreign country (-side): parasites

ŏ diarrhoe and vomiting - suspicion Noro-Virus!

ŏ Noro-Virus is highly infectious: Hospital Hygiene!

… do not wait for laboratory results to start hygiene-management!
Same symptoms? Diagnostic not necessary for every patient!

Cost efficiancy by ordering specific Lab diagnostics !

(e.g. control after treatment / situation after clinical symptoms)

stool-vials:

ǒ **sampling of stool specimen**

- ǒ **use a toilet (with paper-layer) or a clean bowl**
- **collect some stool with the spoon and put it into the vial (a portion like a bean / a hazelnut is enough !)**
- **liquid / watery stool: ca. 5 ml**

1 negative result will not exclude a gastrointestinal infection for shure (if necessary / ongoing clinical symptoms: control)

for stool specimen:

fast transport to LAB; physiological flora !

> instant transport to Lab or interim storage cool at 4 °C

Medizinisches Versorgungszentrum
Dr. Eberhard & Partner Dortmund

Laboratoriumsmedizin Dortmund
Mikrobiologie

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Stand April 2011

Kurzanleitung: Gewinnung von mikrobiologischem Untersuchungsmaterial Stand 05.2012

Diese Kurzanleitung soll allgemeine praktische Empfehlungen zu Probengewinnung, Transport und evtl. unumgänglicher längerer Lagerung geben. Angaben zur Proben-lagerung sind Empfehlungen unter Vorbehalt und können in Einzelfällen unter wissenschaftlicher Betrachtung suboptimal sein, erfahrungsgemäß gewährleisten Generalisierungen jedoch häufig ein höheres Maß an Anwendungssicherheit in Verbindung mit größtmöglicher Zuverlässigkeit bei der Diagnostik.
wichtig! Ein schneller Probentransport ist der längeren Lagerung (nur in Ausnahmefällen!) immer vorzuziehen!!
Entsprechend den Fortschritten der Wissenschaft und ihren Konsequenzen für die Praxis wird diese Kurzanleitung in Anlehnung an Hersteller-angaben und die Angaben in der MIQ (Qualitätsstandard in der mikrobiologischen Diagnostik; DGHM) aktualisiert.

BLUT:
Bei jeder Untersuchung sollte mindestens 1 Blutkultur-Set (2 BACTEC-plus®-Flaschen: aerob: blau/ anaerob: gelb; Vacutainer-kompatibel) angelegt werden. Nach gründlicher Hautdesinfektion Blut entnehmen und jede Flasche mit 8 - 10 ml beimpfen. Für geringes Blutvolumen (ca. 3 ml) stehen spezielle Medien (BACTEC-PEDS plus® für Pädiatrie; nur aerob/ rosa) zur Verfügung.
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten Blutkultur-Flaschen bei Raumtemperatur lagern!

LIQUOR CEREBROSPINALIS:
Probe unter streng aseptischen Bedingungen gewinnen und in ein steriles Röhrchen überführen (gut verschließen!). Parallel dazu sollte ein Teil der Probe in Blutkultur-Flaschen eingeimpft werden!
Probenlagerung: Probe möglichst schnell ins Labor; ggf. durch Boten! ansonsten bei Raumtemperatur lagern!
(ACHTUNG: Liquor in Blutkultur-Flaschen bei 37 °C bebrüten!)

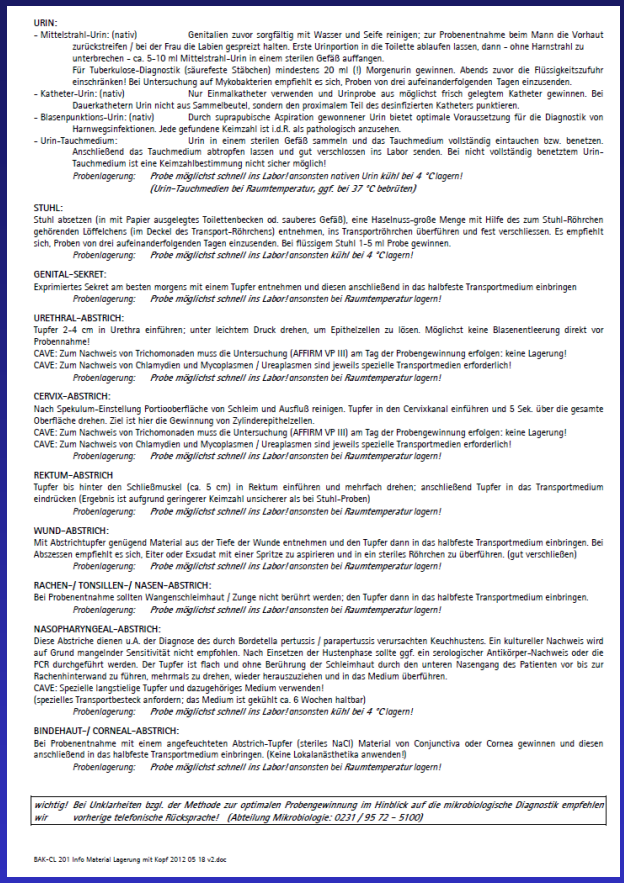
PLEURA-/ SYNOVIALFLÜSSIGKEIT:
Probe unter streng aseptischen Bedingungen gewinnen und in ein steriles Röhrchen überführen. (gut verschließen!). Bei Verdacht auf Anaerobier einen Teil der aspirierten Probe zusätzlich in Blutkultur-Flaschen einimpfen!
Probenlagerung: Probe möglichst schnell ins Labor; ggf. durch Boten! ansonsten bei Raumtemperatur lagern!
(Pleura-/Synovialflüssigkeit in Blutkultur-Flaschen bei Raumtemperatur lagern!)

SPUTUM:
Sputum als Sekret der Atemwege gelangt beim Husten in den Rachen und sieht i.d.R. eitrig aus. Die Sputumproduktion ist morgens leichter! (Speichel aus dem Mundbereich ist zur Untersuchung ungeeignet!).
Die Probenqualität wird durch mögliche Kontamination des Untersuchungsmaterials mit Mund- und Rachenflora entscheident beeinflußt. Es sollte vor dem Abhusten mehrmals tief eingeatmet und der Atem für jeweils ca. 3-5 Sek. angehalten werden; die Atemarbeit entfaltet die Lunge und regt die Sputumproduktion an. Probe nach erneutem tiefem Einatmen in das geöffnete Sputumgefäß abhusten. Dieser Vorgang ist möglichst zu wiederholen, um eine ausreichend große Proben-menge zu erhalten. Kann spontan kein Sputum aus der Tiefe produziert werden, lässt sich durch Inhalation von ca. 25 ml steriler hyperosmolarer NaCl-Lösung (3%) mittels Ultraschallvernebler die Sekretion in den Atemwegen anregen und auf diese Weise ein induziertes Sputum gewinnen.
Für die Untersuchung auf Mykobakterien empfiehlt es sich, Proben an drei aufeinanderfolgenden Tagen einzusenden.
(Achtung: nur dicht schließende, sterile Transportgefäße mit Schraubverschluß versenden!)
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten kühl bei 4 °C lagern!

BRONCHIAL- / TRACHEALSEKRET:
Bronchialsekret wird als über einen Arbeitskanal des Bronchoskops aspirierte Flüssigkeit gewonnen. Die vor der Aspiration ggf. instillierte isotone Flüssigkeit (z.B. NaCl-Lösung) darf keine antibakteriellen Zusätze enthalten! Bei beatmeten Patienten sollte möglichst unmittelbar nach Wechsel des Trachealtubus mit Hilfe eines sterilen Katheters Sekret aus tiefen Abschnitten des Bronchialbaumes aspiriert und in ein Sputumgefäß gegeben werden. Unter Sicht gewonnenes eitriges Material aus dem Infektionsherd besitzt eine hohe diagnostische Sensitivität und Spezifität. Bronchoskopisch gewonnenes Material und Trachealspirate sind besonders zur bakteriologischen Untersuchung geeignet, da sie primär kaum Kontamination durch Mund- und Rachenflora aufweisen.
Für die Untersuchung auf Mykobakterien empfiehlt es sich, Proben an drei aufeinanderfolgenden Tagen einzusenden.
(Achtung: nur dicht schließende, sterile Transportgefäße mit Schraubverschluß versenden!)
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten kühl bei 4 °C lagern!

BRONCHOALVEOLÄRE LAVAGE / BAL:
Zur Vermeidung von Kontaminationen sollten im Mund-Nasen-Rachenraum und der Trachea befindliche Sekretansammlungen vor Einführen des Bronchoskops abgesaugt und vor der Probengewinnung kein Sog angewandt werden. Anästhesierende Gele können antimikrobiell wirken!
Bei der BAL ist die Spitze des Bronchoskops in das Bronchuslumen einzuführen und mit der Spitze abzudichten. Nach Instillation von isotoner Flüssigkeit in das Lumen diese soweit wie möglich wieder aspirieren. Das erste Aspirat sollte verworfen werden (Ausnahme: Suche nach obligat pathogenen Erregern bei immunsupprimierten Patienten); die folgenden Aspirate entstammen eher der Lungenperipherie und sind in ein steriles Transportgefäß zu überführen. (Achtung: nur dicht schließende, sterile Transportgefäße mit Schraubverschluß versenden!)
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten kühl bei 4 °C lagern!

BAK-CL 201 Info Material Lagerung mit Kopf 2012 05 18 v2.doc



URIN:
- Mittelstrahl-Urin: (nativ) Genitalien zuvor sorgfältig mit Wasser und Seife reinigen; zur Probenentnahme beim Mann die Vorhaut zurückstreifen / bei der Frau die Labien gespreizt halten. Erste Urinportion in die Toilette ablaufen lassen, dann - ohne Harnstrahl zu unterbrechen - ca. 5-10 ml Mittelstrahl-Urin in einem sterilen Gefäß auffangen.
  Für Tuberkulose-Diagnostik (säurefeste Stäbchen) mindestens 20 ml (!) Morgenurin gewinnen. Abends zuvor die Flüssigkeitszufuhr einschränken! Bei Untersuchung auf Mykobakterien empfiehlt es sich, Proben von drei aufeinanderfolgenden Tagen einzusenden.
- Katheter-Urin: (nativ) Nur Einmalkatheter verwenden und Urinprobe aus möglichst frisch gelegtem Katheter gewinnen. Bei Dauerkathetern Urin nicht aus Sammelbeutel, sondern den proximalen Teil des desinfizierten Katheters punktieren.
- Blasenpunktions-Urin: (nativ) Durch suprapubische Aspiration gewonnener Urin bietet optimale Voraussetzung für die Diagnostik von Harnwegsinfektionen. Jede gefundene Keimzahl ist i.d.R. als pathologisch anzusehen.
- Urin-Tauchmedium: Urin in einem sterilen Gefäß sammeln und das Tauchmedium vollständig eintauchen bzw. benetzen. Anschließend das Tauchmedium abtropfen lassen und gut verschlossen ins Labor senden. Bei nicht vollständig benetztem Urin-Tauchmedium ist eine Keimzahlbestimmung nicht sicher möglich!

Probenlagerung: Probe möglichst schnell ins Labor! ansonsten nativen Urin kühl bei 4 °C lagern!
(Urin-Tauchmedien bei Raumtemperatur, ggf. bei 37 °C bebrüten)

STUHL:
Stuhl absetzen (in mit Papier ausgelegtes Toilettenbecken od. sauberes Gefäß), eine Haselnuss-große Menge mit Hilfe des zum Stuhl-Röhrchen gehörenden Löffelchens (im Deckel des Transport-Röhrchens) entnehmen, ins Transportröhrchen überführen und fest verschliessen. Es empfiehlt sich, Proben von drei aufeinanderfolgenden Tagen einzusenden. Bei flüssigem Stuhl 1-5 ml Probe gewinnen.
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten kühl bei 4 °C lagern!

GENITAL-SEKRET:
Exprimiertes Sekret am besten morgens mit einem Tupfer entnehmen und diesen anschließend in das halbfeste Transportmedium einbringen
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten bei Raumtemperatur lagern!

URETHRAL-ABSTRICH:
Tupfer 2-4 cm in Urethra einführen; unter leichtem Druck drehen, um Epithelzellen zu lösen. Möglichst keine Blasenentleerung direkt vor Probennahme!
CAVE: Zum Nachweis von Trichomonaden muss die Untersuchung (AFFIRM VP III) am Tag der Probengewinnung erfolgen: keine Lagerung!
CAVE: Zum Nachweis von Chlamydien und Mycoplasmen / Ureaplasmen sind jeweils spezielle Transportmedien erforderlich!
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten bei Raumtemperatur lagern!

CERVIX-ABSTRICH:
Nach Spekulum-Einstellung Portiooberfläche von Schleim und Ausfluß reinigen. Tupfer in den Cervixkanal einführen und 5 Sek. über die gesamte Oberfläche drehen. Ziel ist hier die Gewinnung von Zylinderepithelzellen.
CAVE: Zum Nachweis von Trichomonaden muss die Untersuchung (AFFIRM VP III) am Tag der Probengewinnung erfolgen: keine Lagerung!
CAVE: Zum Nachweis von Chlamydien und Mycoplasmen / Ureaplasmen sind jeweils spezielle Transportmedien erforderlich!
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten bei Raumtemperatur lagern!

REKTUM-ABSTRICH
Tupfer bis hinter den Schließmuskel (ca. 5 cm) in Rektum einführen und mehrfach drehen; anschließend Tupfer in das Transportmedium eindrücken (Ergebnis ist aufgrund geringerer Keimzahl unsicherer als bei Stuhl-Proben)
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten bei Raumtemperatur lagern!

WUND-ABSTRICH:
Mit Abstrichtupfer genügend Material aus der Tiefe der Wunde entnehmen und den Tupfer dann in das halbfeste Transportmedium einbringen. Bei Abszessen empfiehlt es sich, Eiter oder Exsudat mit einer Spritze zu aspirieren und in ein steriles Röhrchen zu überführen. (gut verschließen)
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten bei Raumtemperatur lagern!

RACHEN-/ TONSILLEN-/ NASEN-ABSTRICH:
Bei Probenentnahme sollten Wangenschleimhaut / Zunge nicht berührt werden; den Tupfer dann in das halbfeste Transportmedium einbringen.
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten bei Raumtemperatur lagern!

NASOPHARYNGEAL-ABSTRICH:
Diese Abstriche dienen u.A. der Diagnose des durch Bordetella pertussis / parapertussis verursachten Keuchhustens. Ein kultureller Nachweis wird auf Grund mangelnder Sensitivität nicht empfohlen. Nach Einsetzen der Hustenphase sollte ggf. ein serologischer Antikörper-Nachweis oder die PCR durchgeführt werden. Der Tupfer ist flach und ohne Berührung der Schleimhaut durch den unteren Nasengang des Patienten vor bis zur Rachenhinterwand zu führen, mehrmals zu drehen, wieder herauszuziehen und in das Medium überführen.
CAVE: Spezielle langstielige Tupfer und dazugehöriges Medium verwenden!
(spezielles Transportbesteck anfordern; das Medium ist gekühlt ca. 6 Wochen haltbar)
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten kühl bei 4 °C lagern!

BINDEHAUT-/ CORNEAL-ABSTRICH:
Bei Probenentnahme mit einem angefeuchteten Abstrich-Tupfer (steriles NaCl) Material von Conjunctiva oder Cornea gewinnen und diesen anschließend in das halbfeste Transportmedium einbringen. (Keine Lokalanästhetika anwenden!)
Probenlagerung: Probe möglichst schnell ins Labor! ansonsten bei Raumtemperatur lagern!

wichtig! Bei Unklarheiten bzgl. der Methode zur optimalen Probengewinnung im Hinblick auf die mikrobiologische Diagnostik empfehlen wir vorherige telefonische Rücksprache! (Abteilung Mikrobiologie: 0231 / 95 72 - 5100)

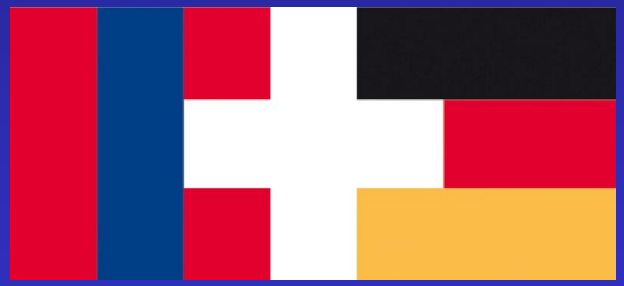
BAK-CL 201 Info Material Lagerung mit Kopf 2012 05 18 v2.doc

separate instruction manual available:
Sampling, Interim Storage and Transport of Specimen

Medical Care Center Dr.Eberhard u. Partner in Dortmund / Germany

essential precondition: culture media and equipment

MIKROBIOLOGY ŏ www.labmed.de / mikro@labmed.de



Medical Care Center
Dr. Eberhard & Partner Dortmund
Germany

bacterial cultures

ǒ **microbiological examination of bacteria:**

1. microscopy
2. culture, identification
3. susceptibility testing (resistance)

(identification is the most complex step in examination - microscopic, cultural, biochemical and serological methods etc.!)

• **main appearance of bacteria (microscopy)**

- coccus:

(Staphylo-/Strepto-/Pneumococcus, Neisseria meningitidis etc.)

- bacillus:

(Escherichia coli etc.)

ŏ nutrients needed by bacteria:

... broad range from "few basic nutrients" up to "very complex culture media" possible ... (modest: E. coli)
... if high requirements: additional substances are needed!

• influence on growth of bacteria:

- **temperature:**
  - **< 0 °C usually no groth**
  - **Bacillus sp. can resist high temperatures (> 100 °C)**
- **humidity / water:**
  - **bacteria need humid culture media**
  - **yeast need less ...**
- **pH-value:**
  - **usually around pH 7.0 for human pathogens (neutral)**
- **oxygen:**
  - **aerobic : growth needs available oxygen**
  - **anaerobic : growth only without oxygen (toxic!)**

ǒ inoculation of culture media

main target: single colonies !

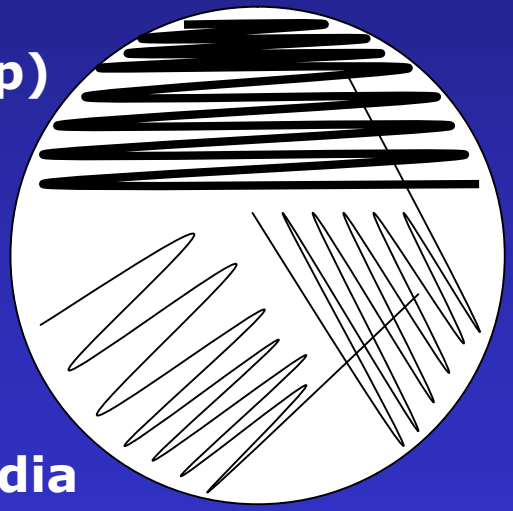
… only on semisolid culture media you will find single colonies for identification and susceptibilty-testing!

- **streaking of culture media: 3 sectors !**

1. sector:
streaking specimen (with swab / loop) in zigzag-line on ½ of the media

2. sector:
with new loop through 1. sector and again zigzag on ¼ of the media

3. sector:
mwith same loop through 2. sector and again zigzag on last ¼ of the media



… at the latest one can find single colonies in the 3. sector

▶ … and at least the result is ready to be printed

Abnahmedatum: 31.12.12 01:00
Klinische Angaben:
UNTERSUCHUNGSMATERIAL:
Nährmedium MRSA (NRR)
ANGEFORDERTE UNTERSUCHUNGEN:
Staphylococcus aureus: MRSA
(Methicillin-/Oxacillin-resistent)
NACHGEWIESENE KEIME:
1.Staphylococcus aureus
(mäßig viel)
BEMERKUNG:
C A V E ! MRSA: Methicillin/Oxacillin-resistenter Staphylococcus aureus!
mecA-Gen [PCR] : POSITIV
mecC/LGA251-Gen [PCR] : negativ
PVL-Gen (community-acquired/CA-MRSA) [PCR] : negativ
Sequenztyp ST398 (livestock-associated/LA-MRSA) [PCR] : negativ
casus vorab telefonisch mitgeteilt
HINWEIS zum Infektionsschutzgesetz:
Folgende hier nachgewiesenen Keime sind aufgrund ihrer Resistenz in der Liste der gemäß §23 IfSG zu erfassenden Erreger aufgeführt:
* Staphylococcus aureus
Unverbindliche Kodierempfehlung:
S. aureus als Erreger: B95.6!
zusätzlich zu kodieren:
Staphylococcus aureus mit Resistenz gegen Oxacillin: U80.0!
Keimträger von MRSA: Z22.3
ggf.: Isolierung wegen MRSA: Z29.0
Auch an OPS 8-987 denken (Krankenhaushygienische Komplexbehandlung)!

ANTIBIOGRAMME	**1.**
Ampi-/Amoxicillin	R
Mezlocillin	R
Piperacillin	R
Penicillin G	R
Amoxicillin+Clavulans.	R
Ampicil.+Sulbactam	R
Piperacil.+Tazobactam	R
Cefazolin	R
Cefuroxim	R
Cefotaxim/Ceftriaxon	R
Imipenem	R
Meropenem	R
Oxacillin	R
Gentamicin	S
Tobramycin	S
Fosfomycin	S
Ciprofloxacin	R
Levofloxacin	R
Cotrimoxazol	S
Tetracyclin	S
Erythromycin	S
Clindamycin	S
Vancomycin	S
Teicoplanin	S
Linezolid	S
Fusidinsäure	S
Rifampicin	S
Mupirocin	S

(S=sens. I=interm. R=resistent)

… including comments on important results: e.g. MRSA, VRE, other multi-drug-resistant bacteria …

… and is available for other ways of transmission to the sender (mail, fax, intranet, internet via VPN …) and is part of statistical evaluations

ŏ advantages of a paperless LIS:

- all doctors can see the actual examination level

  always and everywhere via LIS-network - even from home!

- all diagnostical steps are documented in the LIS-network

  until the results !

- contains a statistic-module

  is able to do a "case-management"; putting together all results of ONE patient ("revised statistics") to get convincing data

- provides monitoring for the senders / doctors / physicians

  of clinical / epidemiological relevant bacteria as a routine every six months ... or on demand.

monitoring epidemiological data

statistics for hospitals every six months ... or on demand:
list of all cases (Patients) with relevant bacteria (special drug resistance) including patients name, date of detection, clinic, ward etc.

Hybase 6 Statistik
MVZ Dortmund - Mikrobiologie
Labor Dr.Eberhard u. Partner
Balkenstr. 17-19
44137 Dortmund

separate Niederschrift gemäß §23 IfSG (Erreger mit besonderen Resistenzen)

01.09.2013 bis 15.09.2013

Einsender:
MUSTER-Krankenhaus

Stat.:
Station Muster

Staphylococcus aureus (Einzelresistenzen)

Nr.	Patient	Erstnachweis	GENTAMICIN	CIPROFLOXACIN	COTRIMOXAZOL	PENICILLING	ERYTHROMYCIN	CLINDAMYCIN	OXACILLIN	VANCOMYCIN	CEFOXITIN	RIFAMPICIN	FOSFOMYCIN	MUPIROCIN	TEICOPLANIN	LINEZOLID	TIGECYCLIN	DAPTOMYCIN	Bemerkung
1	Name / Vorname	Datum / Material / Station	S	R	S	R	R	R	R	S	-	S	-	S	S	S	-	-	Abstrich Unterschenkel rechts
2	Name / Vorname	Datum / Material / Station	S	R	S	R	S	S	R	S	-	S	S	S	S	S	-	-	Sekret Stoma n. Absaug
3	Name / Vorname	Datum / Material / Station	S	R	S	R	R	R	R	S	-	S	S	S	S	S	-	-	Abstrich Nasen-/Rachenraum
4	Name / Vorname	Datum / Material / Station	S	R	S	R	-	R	R	S	-	S	S	S	S	S	-	-	Abstrich Wunde

Enterococcus faecalis (Einzelresistenzen)

--- Kein Befund ---

Enterococcus faecium (Einzelresistenzen)

Nr.	Patient	Erstnachweis	AMPIC./AMOXIC.	VANCOMYCIN	IMIPENEM	PIPERAC.+TAZOB.	TEICOPLANIN	LINEZOLID	TIGECYCLIN	Bemerkung
1	Name / Vorname	Datum / Material / Station	R	R	R	R	S	S	-	Abstrich Leiste / Anal

Streptococcus pneumoniae (Pneumok.) (Einzelresistenzen)

--- Kein Befund ---

Escherichia coli (relevant gem. §23: Einzel- und Multiresistenzen)

list of all bacteria and the corresponding specimen / total frequency; revised > same patient and same specimen > counted only once

Hybase 6 Statistik
MVZ Dortmund - Mikrobiologie
Labor Dr.Eberhard u. Partner
Balkenstr. 17-19
44137 Dortmund

(Tel. Hr.Roßburg: 0231 / 9572-614)

Statistik - Verteilung

Erreger - Material

01.12.2011 bis 10.12.2011

Stat.:
Station Muster 05

Einsender:
Krankenhaus St.Muster

nur ein Erregernachweis pro Patient, im gleichen Material

Erreger-Gruppe / Materialien - Gruppe	Gesamt	Abstrich (Sonstige)	Abstrich Bauch -raum	Abstrich Cervix/ Uterus	Abstrich Fuß/ Diabetes	Abstrich Gelenk/ Knochen	Abstrich Körper-oberfläche	Abstrich obere Atemwege	Abstrich Organe	Abstrich untere Atemwege	Abstrich Urethra	Abstrich Wunde/ Eiter	Biopsat/ Excision	Katheter: venös	Kult aer Blut
Gesamt	555	18	3	4	2	1	48	93	2	3	2	27	1	12	
Anzahl ausgewerteter Materialien	335	10	2	2	1	1	21	46	1	1	1	16	1	11	
Materialien mit einem Erreger	178	7	1	0	0	1	6	8	0	0	0	8	1	10	
Materialien mit 2 Erregern	109	0	1	2	1	0	10	30	1	0	1	5	0	1	
Materialien mit 3 Erregern und mehr	48	3	0	0	0	0	5	8	0	1	0	3	0	0	
E1 Acinetobacter spp.	4							1							
E1 Bacteroides spp.	4	1					1					2			
E1 Campylobacter jejuni	1														
E1 Candida albicans	24	1					1	1				1		1	
E1 Candida glabrata	6						1								
E1 Candida spp. (non-albicans/-krusei/-glabrata)	4														
E1 Citrobacter spp.	1														
E1 Clostridium spp. (non-perfringens/-tetani)	9														
E1 Corynebacterium spp. (non-diphtheriae)	1						1								
E1 Coryneforme Bakterien	5						1								
E1 Enterobacter spp.	9	1					1	2							
E1 Enterococcus faecalis	8	2		1			1					1		1	
E1 Enterococcus faecium	11	1	1				1					1		1	

sensitive antibiotics for important bacteria / in parenthesis number of isolates; revised > same bacteria, same patient and same specimen > counted only once

Hybase 6 Statistik
MVZ Dortmund - Mikrobiologie
Labor Dr.Eberhard u. Partner
Balkenstr. 17-19
44137 Dortmund

Übersicht Erreger-Resistenzen
Resistenzen - Vergleich
sensibel

01.12.2011 bis 10.12.2011
Einsender:
Krankenhaus St.Muster

Stat.:
Station Muster 05

nur ein Erregernachweis pro Patient, im gleichen Material, Strategie: zeitlicher Verlauf

Interpretation: voll sensibel in [%] (Anza

Antibiotika / Erreger -Gruppen	E1 Enterobacter spp.	E1 Enterococcus faecalis	E1 Enterococcus faecium	E1 Enterococcus spp. (non-faecalis/-faeci	E1 Escherichia coli	E1 Klebsiella pneumoniae	E1 Proteus spp.
Ampic./ Amoxic.	0% (7)	100% (6)	10% (10)	100% (27)	53% (76)	0% (19)	33% (12)
Amoxic.+ Clavulans.	0% (7)	100% (6)	10% (10)	100% (27)	67% (76)	63% (19)	58% (12)
Mezlocillin	57% (7)	100% (6)	10% (10)	100% (27)	53% (75)	0% (19)	42% (12)
Piperacillin	57% (7)	100% (6)	10% (10)	100% (27)	54% (76)	0% (19)	42% (12)
Gentamicin	86% (7)	0% (6)	0% (10)	0% (27)	92% (73)	83% (18)	92% (12)
Tobramycin	86% (7)	0% (6)	0% (10)	0% (27)	88% (76)	84% (19)	92% (12)
Ciprofloxacin	86% (7)	0% (6)	0% (10)	0% (27)	74% (76)	74% (19)	83% (12)
Cotrimoxazol	86% (7)	0% (6)	0% (10)	0% (27)	68% (76)	79% (19)	42% (12)
Tetracyclin	86% (7)				59% (76)	79% (19)	0% (12)
Penicillin							
Erythromycin							
Clindamycin							
Oxacillin							
Vancomycin		100% (6)	80% (10)	100% (27)			
Imipenem	100% (7)	100% (6)	10% (10)	100% (27)	100% (76)	100% (19)	0% (12)
Ceftazidim	57% (7)				87% (75)	63% (19)	67% (12)
Ampic.+ Sulbactam	0% (7)	100% (6)	10% (10)	100% (27)	67% (76)	63% (19)	58% (12)
Piperac.+ Tazobactam	57% (7)	100% (6)	10% (10)	100% (27)	68% (75)	63% (19)	58% (12)
Cefotaxim/ Ceftriaxon	57% (7)	0% (6)	0% (10)	0% (27)	87% (76)	63% (19)	67% (12)

reachability Microbiological Laboratory

ŏ service of the microbiology-unit:

- regular working hours of laboratory (microbiology):

mo-fr	07:00 - 19:00 h
sat	07:00 - 15:00 h
sun	08:30 - 13:30 h

- 24 hours-service for urgent diagnostic (specimen-receipt) !
  ("on-call-service" ... e.g. ICU's: specimen announced by call)

- doctors in laboratory: advising on medical matters by telephone
  (interpretation of relevant / komplex results
  ... on-site-visits possible too ... e.g. ICU's)

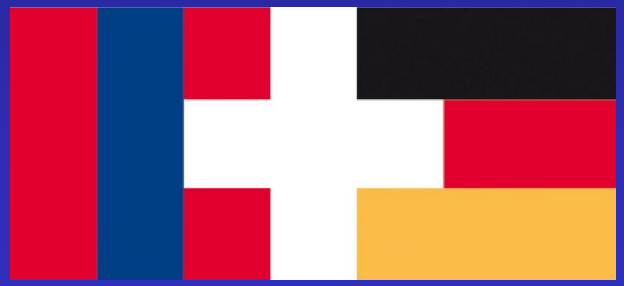
- cooperation with executive personnel in hygiene (hospitals)

- presentations in hospitals
  (optimized collection of specimen, suited specimen, statistics
  concerning the situation of antibiotic resistance, cost-controlling etc.)

Medical Care Center Dr.Eberhard u. Partner in Dortmund / Germany

modern microbiological diagnostics - including mass-spectrometry ?!

MIKROBIOLOGY ŏ www.labmed.de / mikro@labmed.de



Medical Care Center
Dr. Eberhard & Partner Dortmund
Germany

principle: microbiological examination

... methods currently used in our laboratory routine ...

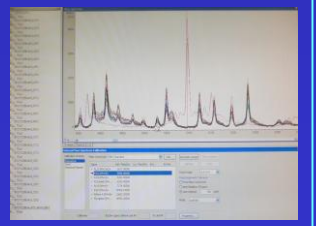
culture
streaking manually or by Innova (automated)




identification
VITEK 2/XL (biochem.) or MALDI-TOF (Mass-Spectrom.)





resistance
agar-diffusion or VITEK 2/XL (MIC)




The mainly used method for identification is the API-system - a reliable method:

- **API-method: ... easy, well experianced, mostly valid**
  - **interpretation by the staff (subjective)**
  - **some problems with detection of bacteria**
    (gram (+) bacilli, Streptococci etc.: not to find in database; discrimination between Streptococcus mitis and Streptococcus oralis often not significant etc.)



• automatisation: ... faster, more sensitive and specific

e.g. VITEK (BioMerieux), Phoenix (BectonDickinson) or MicroScan (Siemens):

- interpretation by an photometer (objective)

- revision of the results by an "Expert-Program"

(software which suggests plausibility-checked identifications of bacteria - even for susceptibility-testing!

... advantage concerning quality management)

The configuration of the used panels / cards must be well chosen; good experience we had with the panels:

gram (negative) bacteria:

routine: AST-N118

MDR: AST-N110

gram (positive) bacteria:

Staphylococci: AST-P580

Enterococci AST-P586

Staphylococci, Enterococci and Enterobacteriaceae are usually provided well.

... but also here you can find some problems with the discrimination of Streptococci (special cards available).

... Non-fermenters show some gaps

... anaerobic bacteria are sometimes better identified by API ...

… VITEK 2/XL-system is modern until now …

… but we are always looking for better methods!

"egg-laying-wool-milk-pig"

… in the future perhaps:
easy, reliable, sensitive, specific and inexpensive detection-method for bacteria
…within 5 minutes…!?

ŏ **theoretic advantages of mass-spectrometry-method in a medical microbiology laboratory:**

ŏ **method for identification of cultural grown microorganisms**

ŏ **significant faster than the biochemical identification methods**
- **biochemistry: hours / days**
- **MALDI-TOF-MS: few minutes (!)**

ŏ **in some cases more precise identification possible?**
- **based on identification of ribosomal proteins (!)**

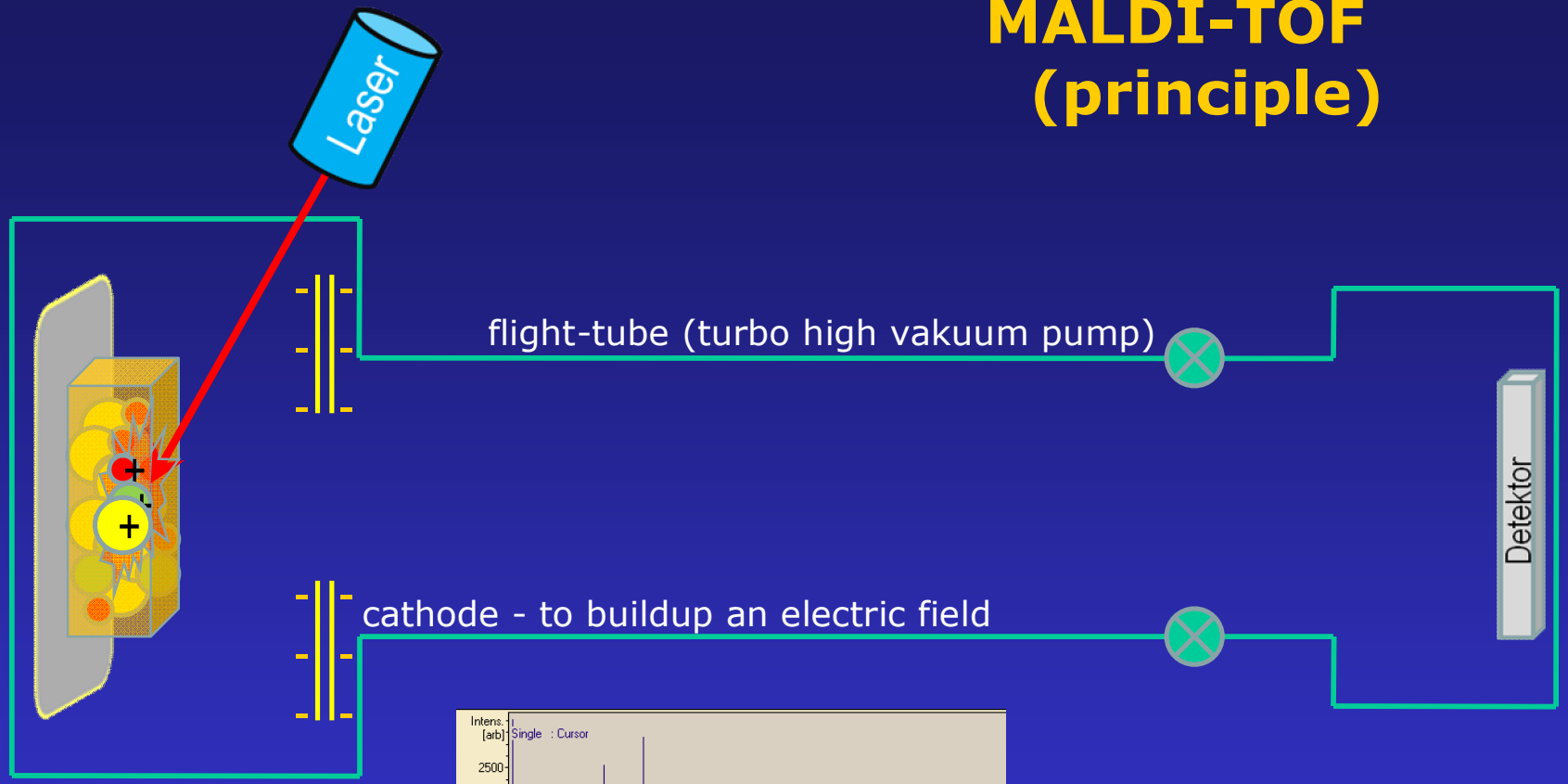
Ȝ **improvement of microbiological diagnostics?**

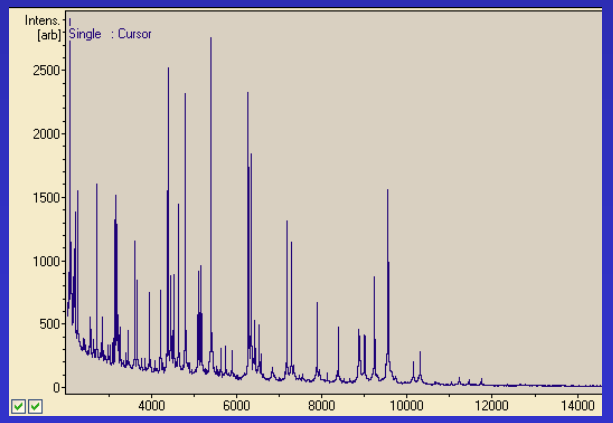
… faster and more precise !?

… what means „MALDI-TOF-MS“?

M atrix
A ssisted
L aser
D esorption /
I onisation
-
T ime
O f
F light
-
M ass
S pectrometry

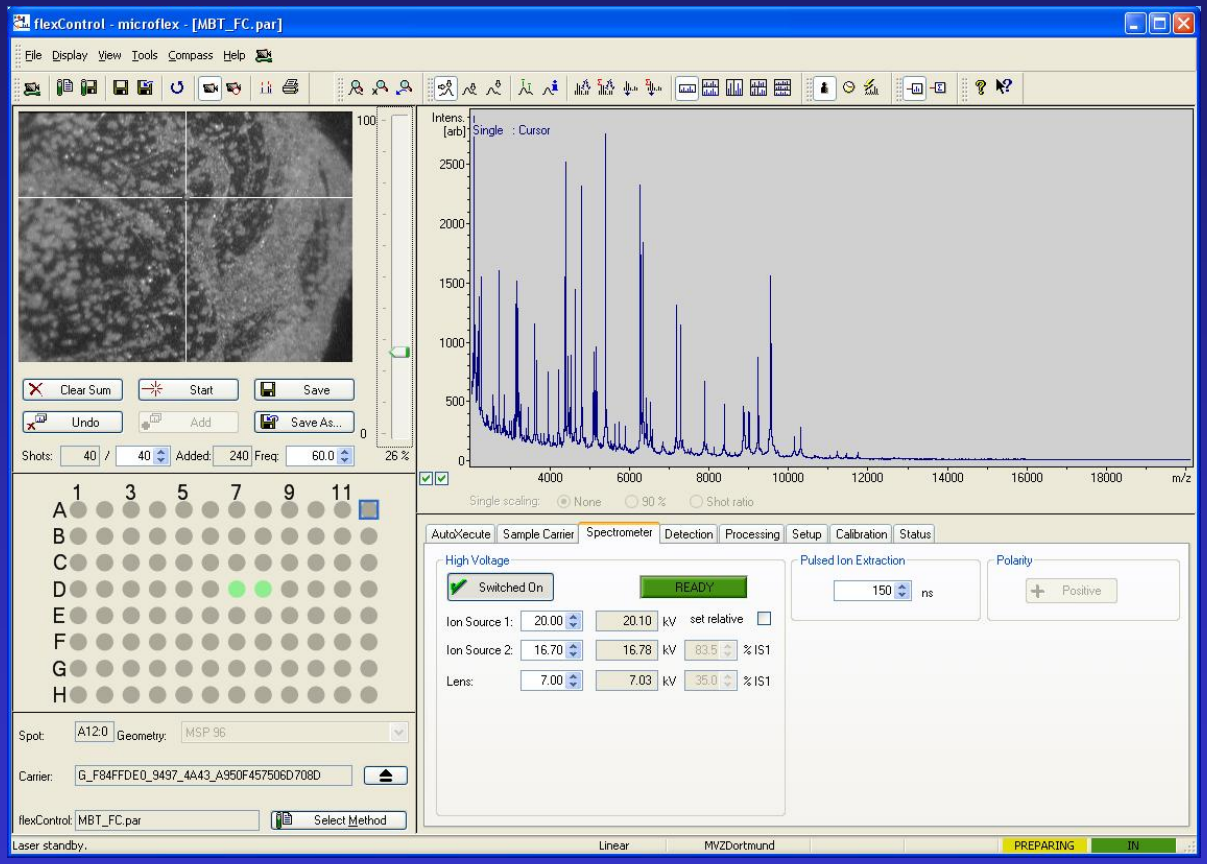
MALDI-TOF (principle)





specific spektrum of masses - compared to database ...

comparison with reference-spectres in data base (similarity / probability is calculated)



3 identifikation possible within minutes ...

reported results

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Klebsiella oxytoca ATCC 700324 THL	2.408	571
2 (+++)	Klebsiella oxytoca ESBL 30298 PFM	2.394	571
3	Klebsiella oxytoca DSM 5175T HAM	2.14	571
(+)			
8 (+)	Raoultella ornithinolytica DSM 7464T HAM	1.751	54291
9 (+)	Raoultella planticola DSM 3069T DSM	1.741	575
10 (-)	Enterobacter aerogenes 15282_1 CHB	1.659	548

Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

… pathogens found in specimen

MVZ Dr.Eberhard Dortmund in 2012: revised (same material same patient: 1 x)

in blood-cultures:

(blood/liquor)

bacteria (group)	positive	%
total 2012	**3875**	
Staphylococcus spp. (koag.(-))	1236	**32**
Escherichia coli	820	**21**
Staphylococcus aureus	484	**12**
Propionibacterium spp.	155	**4**
Streptococcus spp. (non-pneumoniae)	147	**4**
Enterococcus faecalis	141	**4**
Klebsiella pneumoniae	105	**3**
Streptococcus (ß-hämolys.)	98	**3**
Enterococcus faecium	82	**2**
Proteus spp.	61	**2**
Enterobacter spp.	60	**2**
Streptococcus pneumoniae	52	**1**
Klebsiella oxytoca	44	**1**
Candida albicans	40	**1**
Pseudomonas aeruginosa	33	**1**

in wounds:

bacteria (group)	positive	%
total 2012	**13949**	
Staphylococcus aureus	2976	**21**
Staphylococcus spp. (koag.(-))	2450	**18**
Enterococcus spp. (non-faecalis/-faecium)	1075	**8**
Escherichia coli	769	**6**
Streptococcus (ß-hämolys.)	705	**5**
Streptococcus spp. (non-pneumoniae)	697	**5**
Proteus spp.	577	**4**
Pseudomonas aeruginosa	536	**4**
Bacteroides spp.	528	**4**
Enterobacter spp.	347	**2**
Anaerococcus (Peptostreptoc.) spp.	210	**2**
Acinetobacter spp.	201	**1**
Klebsiella oxytoca	172	**1**
Prevotella spp.	157	**1**
Morganella spp.	115	**1**
Citrobacter spp.	111	**1**
Klebsiella pneumoniae	110	**1**
Candida albicans	109	**1**
Serratia spp.	106	**1**
Enterococcus faecium	82	**1**

in upper respiratory tract:

bacteria (group)	positive	%
total 2012	**13841**	
Staphylococcus aureus	3579	**26**
Candida albicans	1484	**11**
Streptococcus (ß-hämolys.)	1253	**9**
Staphylococcus spp. (koag.(-))	1212	**9**
Haemophilus parainfluenzae	1039	**8**
Haemophilus influenzae	1005	**7**
Streptococcus spp. (non-pneumoniae)	444	**3**
Moraxella catarrhalis	340	**2**
Streptococcus pneumoniae	337	**2**
Escherichia coli	313	**2**
Pseudomonas aeruginosa	281	**2**
Enterobacter spp.	258	**2**
Klebsiella pneumoniae	176	**1**
Candida glabrata	172	**1**
Klebsiella oxytoca	165	**1**
Haemophilus spp. (non-infl./-parainfl.)	135	**1**
Serratia spp.	133	**1**
Candida spp. (non-albicans/-krusei/-glabrata)	108	**1**
Aspergillus spp.	41	**0**

in lower respiratory tract:

bacteria (group)	positive	%
total 2012	**4249**	
Candida albicans	993	**23**
Staphylococcus aureus	525	**12**
Candida glabrata	412	**10**
Staphylococcus spp. (koag.(-))	292	**7**
Pseudomonas aeruginosa	228	**5**
Streptococcus (ß-hämolys.)	208	**5**
Escherichia coli	193	**5**
Streptococcus spp. (non-pneumoniae)	141	**3**
Haemophilus influenzae	114	**3**
Candida spp. (non-albicans/-krusei/-glabrata)	106	**2**
Klebsiella pneumoniae	100	**2**
Enterobacter spp.	90	**2**
Haemophilus parainfluenzae	82	**2**
Streptococcus pneumoniae	66	**2**
Serratia spp.	58	**1**
Stenotrophomonas (Xanth.) maltophilia	54	**1**
Klebsiella oxytoca	52	**1**
Moraxella catarrhalis	52	**1**
Aspergillus spp.	51	**1**

in urine:

bacteria (group)	positive	%
total 2012	**29384**	
Escherichia coli	9480	**32**
Enterococcus spp. (non-faecalis/-faecium)	5916	**20**
Staphylococcus spp. (koag.(-))	4565	**16**
Proteus spp.	1253	**4**
Klebsiella pneumoniae	1031	**4**
Streptococcus spp. (non-pneumoniae)	962	**3**
Streptococcus (ß-hämolys.)	822	**3**
Lactobacillus spp.	770	**3**
Pseudomonas aeruginosa	632	**2**
Staphylococcus aureus	606	**2**
Klebsiella oxytoca	382	**1**
Candida albicans	375	**1**
Enterobacter spp.	374	**1**
Citrobacter spp.	329	**1**
Candida glabrata	200	**1**
Morganella spp.	173	**1**
Enterococcus faecium	150	**1**
Serratia spp.	105	**0**
Acinetobacter spp.	98	**0**

susceptibility-testing of bacteria

- **agar diffusion**

mainly used method for susceptibility-testing in UB/Mongolia is agar-diffusion:

- **a valid and good evaluated technique**
- **... but also well known problems:**
  - **- measuring (diameter) and**
  - **- interpretation**
    - **(in vitro- vs. in vivo-effectiveness of antibiotics;**
    - **e.g. the Oxacillin-testing for the detection of MRSA)**

dispenser

• important rules - who is the „Expert"?

... it must be assured, that important rules are considered like (short excerpt):

- **Enterococcus faecium:**
  - **natural resistance ; always R at nearly all ß-lactam-antibiotics (Cephalosporines/Penicillines; even combinations with ß-lactam-inhibitors)**
- **Klebsiella pneumoniae:**
  - **natural resistance ; always R at Ampi-/Amoxicillin and Mezlo-/Piperacillin**
  - **Imipenem-R: Carbapenemases / Carbapenem-resistance !**
- **Proteus spp.:**
  - **natural resistance ; always R at Nitrofurantoin and Tetracyclin**
  - **Imipenem-R: NO Carbapenemases (poor target for Proteus spp.)**
- **Pseudomonas aeruginosa:**
  - **natural resistance ; always R at Ampi-/Amoxicillin (even combinations with ß-lactam-inhibitors) and most cephalosporines (e.g. Cefaclor, Cefazolin, Cefotaxim / Ceftriaxon) and Cotrimoxazol, Nitrofurantoin, Tetracyclin, Moxifloxacin, Trimethoprim, Tigecyclin ...**
  - **Imipenem-R: Carbapenemases/Carbapenem-resistance !**
- **Staphylococcus aureus:**
  - **if MRSA (Oxacillin-R): all ß-lactam-substances and Carbapenems are R**

• range of antibiotics, that should be tested

The measured values must always be revised (plausibility!) and sometimes adopted to the rules, because the communicated results should be:

advice for an effective antimicrobial treatment!

Sometimes not all relevant antibiotics are tested because of the lack of availability - perhaps because of the lack of money.

Very important first:

- **to set terms of susceptibility-testing**
  (which spectre of antibiotics should be tested for the bacteria-groups?)
- **to assure the availability of all these substances and then**
- **to establish this as a "standard"**
  (... ideally in all microbiological laboratories in UB (a challenge!)

Practical hint:
... for methods like agar-diffusion and also minimal-inhibitory-concentration (e.g. VITEK) the inoculation has to be done with a

- **defined amount of bacteria (NCCLS/CLSI, McFarland etc.) and**
- **only pure monocultures**

will give sufficient results.

• automatisation – the „Expert" is included !

... a more modern kind of susceptibility-testing of bacteria:

- usage of an automated system

like VITEK/BioMerieux® or Phoenix/BectonDickinson®

great advantage is:

- the included "Expert-Program"-software

adopted to hundreds of rules concerning the susceptibility-testing

... software verifies the measured values and gives some suggestions for changing the values:

- 3 values during the examination:

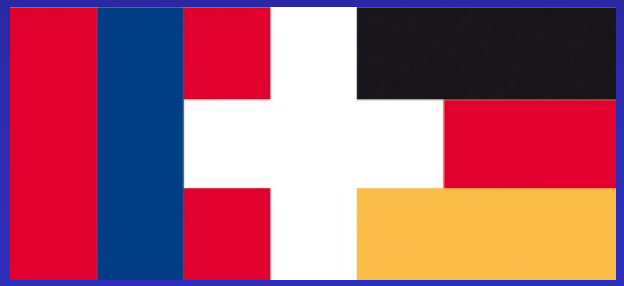
measured value ⇨ suggestion (Expert-Program) ⇨ result

To find a result for an effective antimicrobial treatment, this way is more comfortable and also better documented in the case of quality management.

Medical Care Center Dr.Eberhard u. Partner in Dortmund / Germany

bacterial resistance - not only a therapeutical challenge!

MIKROBIOLOGY ŏ www.labmed.de / mikro@labmed.de



Medical Care Center
Dr. Eberhard & Partner Dortmund
Germany

... some important pathogens

common annotations

- important: pure monoculture (!) - otherwise no reliable results
- statistical data (hospitals/practitioners): 01.01.2012 - 31.12.2012
  (data / situation can vary by different hospitals / regions)
- revised: same patient, same specimen, same bacteria > 1 case
- green: important antibiotics ... should be tested
- ineffective antibiotics tested? to provide reliability for identification!
- agardiffusion: measuring the diameter - ONE standard! (CLSI?)
- VITEK 2/XL panels used:

gram (+)	usually	AST-N118
	MDR	AST-N110
gram (-)	Staphylocossus	AST-P580
	Enterococcus	AST-P586

Acinetobacter spp.

- **owns a lot of natural resistances**

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	12	2	86	647	clin. relevant isloates: not efficiant
Amoxic.+ Clavulana.	26	2	72	501	clin. relevant isloates: not efficiant
Cefaclor	21	2	77	183	clin. relevant isloates: not efficiant
Gentamicin/Tobramycin	88	0	11	439	
Ciprofloxacin/Levofloxacin	81	3	16	695	effective; even ambulant patients
Cotrimoxazol	86	0	13	669	e.g. urinary-tract infections
Tetracyclin	84	3	13	660	oral;
Imipenem/Meropenem	88	0	12	464	i.v. at serious infections
Ceftazidim	51	17	32	428	i.v. at serious infections
Ampic.+ Sulbactam	51	2	47	371	like Amoxic.+Clavulana. - but i.v.
Piperac.+ Tazobactam	63	16	20	377	i.v. at serious infections
Cefixim/Cefpodoxim	25	4	71	150	3.gen.-cephalosp.: oral
Cefotaxim/ Ceftriaxon	9	6	85	372	3.gen.-cephalosp.: i.v.
Cefuroxim	7	2	91	358	clin. relevant isloates: not efficiant

Citrobacter spp.

- **fast development of resistances against cephalosporines (e.g. cefotaxim/ ceftriaxon) and other ß-lactam-antibiotics (except carbapenems)**

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	0	0	100	787	always R (natural resistance)
Amoxic.+ Clavulans.	51	1	48	766	oral
Ampic.+ Sulbactam	48	1	51	486	i.v.
Cefaclor/Cefazolin	44	1	55	125	1.gen.-cephalosp. (oral/i.v.)
Gentamicin/Tobramycin	96	0	4	520	aminoglycosides: combi at serious inf.
Ciprofloxacin/Levofloxacin	98	0	2	787	gyrase-inhibitor: wide spectre; oral/i.v.
Cotrimoxazol	97	0	3	787	urinary-tract infection
Nitrofurantoin	76	22	2	167	only urinary-tract-infections
Tetracyclin	92	5	3	751	
Imipenem/Meropenem	100	0	0	480	carbapenems: only i.v.
Ceftazidim	92	0	8	486	3b.gen.-cephalosp.: i.v.
Piperac.+ Tazobactam	90	0	10	483	wide spectre: only i.v.; serious inf.
Cefotaxim/ Ceftriaxon	91	0	9	488	3.gen.-cephalosp.: i.v.
Cefpodoxim/Cefixim	90	1	9	217	3.gen.-cephalosp.: oral
Cefuroxim	37	8	55	484	2.gen.-cehpalosp.: i.v.
Fosfomycin	100	0	0	125	urinary-tract inf. (not complicated)
Cefuroxim- Axetil	38	15	47	303	2.gen.-cehpalosp.: oral
Trimethoprim	96	0	4	113	urinary-tract-infection

Enterobacter spp.

like Citrobacter spp.:
ŏ fast development of resistances against cephalosporines (e.g. cefotaxim/ ceftriaxon) and other ß-lactam-antibiotics (except carbapenems)

• natural resistance (always R):
- Ampicillin/A.+Clav.
- Amoxicillin/A.+Sulb.
- Cefuroxim /C.-Axetil
- Cefaclor/Cefazolin

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.+Clavul.	0	0	100	1674	always R (natural resistance)
Mezlocillin	68	0	32	1100	
Piperacillin	70	0	30	1150	
Gentamicin/Tobramycin	92	0	8	1236	aminoglycosides: combi at serious inf.
Ciprofloxacin/Levofloxacin	94	1	5	1680	gyrase-inhibitor: wide spectre; oral/i.v.
Cotrimoxazol	91	0	9	1680	urinary-tract infection
Nitrofurantoin	31	45	24	182	only urinary-tract-infections
Tetracyclin	86	10	4	1600	
Imipenem/Meropenem	100	0	0	1137	only i.v.: serious infections
Ceftazidim	72	0	28	1159	3b.gen.-cephalosp.: i.v.; serious inf.
Piperac.+ Tazobactam	74	0	26	1124	wide spectre: only i.v.; serious inf.
Cefotaxim/ Ceftriaxon	71	0	29	1172	3.gen.-cephalosp.: i.v.
Fosfomycin	49	0	51	140	urinary-tract-inf. (not complicated)
Trimethoprim	85	0	15	128	urinary-tract-inf. (not complicated)

Enterococcus faecium

ŏ natural resistance (always R):
- nearly all ß-lactam-antibiotics: cephalosporines/ penicillines! (even combinations with ß-lactamase-inhibitors!)

• suitable:
- Vancomycin/Teicopl. and Linezolid

• Tigecycline:
treatment only at soft tissue infection - NOT at blood-steam-infections

CAVE:
usually within this species we can find VRE! (13 %!)

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	7	0	93	811	not suitable!
Amoxic.+ Clavulans.	7	0	93	809	not suitable!
Cefotaxim/ Ceftriaxon	0	0	100	777	not suitable!
Gentamicin/Tobramycin	0	0	100	779	only combi (endocarditis: +Van/Tei)
Ciprofloxacin/Cotrimoxazol	0	1	99	808	not suitable!
Vancomycin/Teicoplanin	88	0	12	797	important substance: initial therapy
Imipenem	6	0	94	780	not suitable!
Levofloxacin	0	15	85	811	not suitable!
Linezolid	100	0	0	298	important substance: initial therapy
Tigecyclin	100	0	0	161	

Enterococcus spp. (non-faecium)

- **natural resistance (always R):**
  - **all cephalosporines!**
  - **all aminoglycosides**

- **suitable:**
  - **Ampicillin/Amoxic. (even combinations with ß-lactamase-inhibitors … but usually not necessary)**

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	97	0	3	3888	important substance: initial therapy
Amoxic.+ Clavulans.	97	0	3	3886	in most cases not necessary
Cefaclor and others!	0	0	100	1182	always R (natural resistance)
Gentamicin/Tobramycin	0	0	100	2549	only as combi (endocarditis)
Ciprofloxacin/Cotrimoxazol	0	2	98	3882	not suitable!
Nitrofurantoin	96	3	1	1222	only urinary-tract-infections
Tetracyclin	8	0	92	118	
Vancomycin/Teicoplanin	98	0	2	2457	not necessary: only if VRE!
Imipenem	96	0	4	2498	only i.v.: serious infections
Fosfomycin	52	14	34	123	only as a combi (low grade inf.)
Levofloxacin	0	71	29	3883	not suitable!
Moxifloxacin	73	1	26	161	
Trimethoprim	0	0	100	145	not suitable!

Escherichia coli

ŏ resistance varies from "polysensitive" to "MDR"

treatment depends on susceptibility testing and seriousness of desease

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	50	1	49	13572	
Amoxic.+ Clavulans.	63	17	20	13559	
Mezlocillin/Piperacillin	48	0	52	8127	
Cefaclor	90	1	9	4065	
Gentamicin/Tobramycin	92	0	8	7694	
Ciprofloxacin/Levofloxacin	79	0	21	13572	
Cotrimoxazol	68	0	32	13563	CAVE: increasing resistance!
Nitrofurantoin	92	6	2	5026	only urinary-tract-infections
Tetracyclin	65	0	35	12998	
Imipenem	100	0	0	8618	... if not carbapenem-resistent ...
Ceftazidim	87	0	13	8282	
Piperac.+ Tazobactam	62	20	18	8185	
Cefixim	94	0	6	156	
Cefotaxim/ Ceftriaxon	84	0	16	8595	here: about 16 % ESBL
Meropenem	100	0	0	8334	
Cefpodoxim	92	0	8	5289	
Cefuroxim/ C.-Axetil	82	5	13	8208	i.v./oral
Fosfomycin	99	0	1	4383	urinary-tract inf. (not complicated)
Moxifloxacin	77	0	23	611	
Trimethoprim	67	0	33	4002	

Haemophilus influenzae

ŏ respiratory tract infection treatment with:
- Ampicillin/Amoxicillin (if sensitive)

almost always sensitive:
- green marked subst. (oral /i.v.: depending on seriousness of desease)

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	90	0	10	1268	
Amoxic.+ Clavulans.	99	0	1	1268	oral
Cefaclor	31	53	16	366	
Gentamicin/Tobramycin	82	16	2	352	
Ciprofloxacin	100	0	0	1267	
Cotrimoxazol	76	0	23	826	
Tetracyclin	32	32	36	1269	
Imipenem	100	0	0	239	
Ceftazidim	100	0	0	237	
Cefixim	100	0	0	1020	
Cefotaxim/ Ceftriaxon	100	0	0	237	
Cefazolin	3	54	43	127	
Meropenem	100	0	0	237	
Cefuroxim	96	3	1	236	
Levofloxacin	100	0	0	1267	
Cefuroxim- Axetil	98	2	0	1031	oral

Klebsiella oxytoca

ŏ natural resistance (always R):
- Ampi-/Amoxicillin
- Mezlo-/Piperacillin;

ŏ suitable
see above - in combination with ß-lactam-inhibitor

CAVE:
- ESBL:

ŏ Imipenem - R:
carbapenem-resistance !!
(3MRGN/4MRGN ?)

here:
not found

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	0	0	100	1129	always R (natural resistance)
Amoxic.+ Clavulans.	79	2	19	1124	
Cefaclor	81	0	19	140	
Gentamicin/Tobramycin	98	0	2	784	
Ciprofloxacin/Levofloxacin	90	0	10	1128	
Cotrimoxazol	94	0	6	1128	
Nitrofurantoin	66	25	8	185	
Tetracyclin	91	2	7	1075	
Imipenem	100	0	0	762	no carbapenem-resistence (3/4MRGN)
Ceftazidim	97	0	3	740	
Ampic.+ Sulbactam	77	2	21	741	
Piperac.+ Tazobactam	78	3	19	742	
Cefotaxim/ Ceftriaxon	93	2	5	743	...only 5 % ESBL
Meropenem	100	0	0	750	
Cefpodoxim	93	1	6	382	
Cefuroxim/ C-Axetil	79	3	18	740	i.v./oral
Fosfomycin	73	0	27	144	
Trimethoprim	90	0	10	126	

Klebsiella pneumoniae

ŏ natural resistance (always R):
- Ampi-/Amoxicillin
- Mezlo-/Piperacillin;

ŏ suitable
see above - in combination with ß-lactam-inhibitor

CAVE:
- ESBL: more isolates found here than in K. oxytoca

ŏ Imipenem - R:
carbapenem-resistance !! (3MRGN/4MRGN ?)

here:
not found

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	0	0	100	1950	always R (natural resistance)
Amoxic.+ Clavulans.	80	3	16	1950	
Cefaclor	54	33	13	381	
Gentamicin/Tobramycin	90	3	7	1278	
Ciprofloxacin/Levofloxacin	89	0	11	1950	
Cotrimoxazol	86	0	14	1949	
Nitrofurantoin	23	44	33	496	
Tetracyclin	81	5	15	1838	
Imipenem	100	0	0	1353	no carbapenem-resistence (3/4MRGN)
Ceftazidim	87	0	13	1302	
Ampic.+ Sulbactam	80	4	16	1280	
Piperac.+ Tazobactam	82	3	15	1284	
Cefotaxim/ Ceftriaxon	84	0	16	1352	... about 16 % ESBL !
Meropenem	100	0	0	1312	
Cefpodoxim	89	0	11	658	
Cefuroxim/ C.-Axetil	84	4	13	1280	i.v./oral
Fosfomycin	79	0	21	413	
Trimethoprim	83	0	17	362	

Proteus spp.

ŏ **treatment depending on localisation and seriousness of desease**

ŏ **Imipenem - R:**
NO (!) carbapenemase but "poor target" for Proteus spp.

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	62	0	38	2650	
Amoxic.+ Clavulans.	91	5	4	2637	
Mezlocillin/Piperacillin	73	0	27	1702	
Cefaclor	87	0	13	451	
Gentamicin/Tobramycin	90	0	10	1813	
Ciprofloxacin	85	0	15	2645	
Cotrimoxazol	67	0	33	2644	
Nitrofurantoin	0	0	100	610	always R (natural resistance)
Tetracyclin	0	0	100	2538	always R (natural resistance)
Imipenem	4	13	83	1669	not suitable! "poor target"
Ceftazidim	99	0	1	1701	
Ampic.+ Sulbactam	91	5	4	1707	
Piperac.+ Tazobactam	94	4	2	1698	
Cefotaxim/ Ceftriaxon	98	0	2	1710	...only about 2 % ESBL
Meropenem	100	0	0	1719	no carbapenem-resistence (3/4MRGN)
Cefpodoxim	98	0	2	938	
Cefuroxim/C.Axetil	86	1	13	1714	i.v./oral
Fosfomycin	84	0	16	477	urinary-tract inf. (not complicated)
Levofloxacin	85	0	15	2647	

Pseudomonas aeruginosa

ŏ natural resistance (always R):
- **Ampic./Amoxicillin (incl. +ß-lactam.-inhib.)**
- **most Cephalosporines (e.g. Cefaclor,Cefazolin, Cefotaxim/Ceftriaxon)**
- **Cotrimoxazol**
- **Nitrofurantoin**
- **Tetracyclin**
- **Moxifloxacin**
- **Trimethoprim**
- **Tigecyclin**

ŏ Imipenem-R:
carbapenem-resistence !! (3MRGN/4MRGN)

here: found !!!

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	0	0	100	2818	always R (natural resistance)
Mezlocillin	3	26	71	547	not suitable!
Piperacillin	77	14	9	1732	like Piperacillin+ß-lactamase-inhib.
Gentamicin	90	4	6	2175	as a combi-partner
Tobramycin	95	0	5	1745	as a combi-partner
Ciprofloxacin	76	11	13	2814	the only oral substance
Cotrimoxazol	0	0	100	2816	
Nitrofurantoin	0	0	100	270	
Tetracyclin	0	0	100	2712	
Imipenem/Meropenem	89	3	8	2705	
Ceftazidim	91	3	6	2722	3b.gen.-cephalosp.: i.v.; serious inf.
Ampic.+ Sulbactam	0	0	100	1726	always R (natural resistance)
Piperac.+ Tazobactam	77	14	9	1772	like Piperacillin
Colistin	99	0	1	98	many side-effects! lokal application?!
Fosfomycin	18	0	82	218	as a combi partner
Levofloxacin	75	1	24	2810	

Serratia spp.

like Citrobacter spp., Enterobacter spp.:
• fast development of resistances against cephalosporines (e.g. cefotaxim/ ceftriaxon) and other ß-lactam-antibiotics (except carbapenems)

• natural resistance (always R):
- Ampicillin/A.+Clav.
- Amoxicillin/A.+Sulb.
- Cefuroxim /C.-Axetil

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	0	0	100	602	always R (natural resistance)
Mezlocillin	87	1	12	355	
Piperacillin	88	1	11	364	
Gentamicin	97	0	3	399	aminoglycosides: combi at serious inf.
Tobramycin	85	13	2	341	
Ciprofloxacin/Levofloxacin	91	0	9	602	gyrase-inhibitor: wide spectre; oral/i.v.
Cotrimoxazol	97	0	3	600	urinary-tract infection
Tetracyclin	40	12	48	496	
Imipenem/Meropenem	99	1	0	312	only i.v.: serious infections
Ceftazidim	92	0	8	362	3b.gen.-cephalosp.: i.v.; serious inf.
Piperac.+ Tazobactam	91	0	9	363	wide spectre: only i.v.; serious inf.
Cefotaxim/ Ceftriaxon	90	0	10	365	3.gen.-cephalosp.: i.v.
Cefpodoxim	64	0	36	100	

Staphylococcus aureus (koagulase-pos)

ŏ first choice:
basic cephalosporines (Cefazolin, Cefuroxim), and Oxacillin

ŏ Oxacillin - R (MRSA):
all ß-lactam-substances and carbapenems are R!

ŏ in the case of MRSA:
Vancomycin/Teicoplanin (in combination with Rifampicin if necessary) or Linezolid

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	24	0	76	12247	same resistence like Penicillin
Mezlocillin/Piperacillin	18	0	82	8018	same resistence like Penicillin
Amoxic.+ Clavulans.	73	0	27	12241	
Amp.+ Sulb./Pip.+Taz.	58	0	42	8021	
Cefaclor	81	0	19	5104	for children
Gentamicin	97	0	3	8827	
Tobramycin	88	0	12	7934	
Ciprofloxacin/Levofloxacin	68	0	31	12230	
Cotrimoxazol	99	0	1	12227	
Tetracyclin	95	0	5	12234	
Penicillin	24	0	76	12245	77 % penicillinase-bilding; rarely S
Erythromycin	70	0	30	11539	
Clindamycin	71	0	29	11495	
Oxacillin	73	0	27	12220	27 % MRSA; most in hospitals
Vancomycin/Teicoplanin	100	0	0	8009	
Imipenem/Meropenem	58	0	42	8024	
Cefotaxim/ Ceftriaxon	58	0	42	8021	
Cefazolin/Cefuroxim	59	0	41	7309	same resistance like Oxacillin
Rifampicin	100	0	0	7615	combi with Vanco/Teico: endocarditis
Chloramphenicol	98	0	2	182	
Fusidinsäure	98	1	1	3597	
Fosfomycin	99	0	1	7979	combi with Vanco/Teico: endocarditis
Mupirocin	100	0	0	3249	nasal ointment
Cefuroxim- Axetil	81	0	19	5213	oral - ambulant patients
Linezolid	100	0	0	3368	

Staphylococcus spp. (koagulase-neg)

ŏ first choice:
basic cephalosporines (Cefazolin, Cefuroxim), and Oxacillin

ŏ Oxacillin - R (MRSE):
all ß-lactam-substances and carbapenems are R!

ŏ in the case of MRSE:
Vancomycin/Teicoplanin (in combination with Rifampicin if necessary: advantage e.g. for katheter infection with biofilm)
or Linezolid

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	16	0	84	5228	same resistence like Penicillin
Mezlocillin/Piperacillin	14	0	86	4109	same resistence like Penicillin
Amoxic.+ Clavulans.	45	0	55	5232	
Amp.+ Sulb./Pip.+Taz.	39	0	61	4113	
Cefaclor	70	0	30	1053	for children
Gentamicin	64	0	36	4450	
Tobramycin	59	0	41	4105	
Ciprofloxacin/Levofloxacin	58	1	41	5221	
Cotrimoxazol	82	0	18	5198	
Tetracyclin	57	0	43	5234	
Penicillin	16	0	84	5228	84 % penicillinase-bilding; rarely S
Erythromycin	36	0	64	4356	
Clindamycin	52	0	48	3943	
Oxacillin	45	0	55	5223	55 % MRSE; most in hospitals
Vancomycin/Teicoplanin	100	0	0	4196	
Imipenem/Meropenem	39	0	61	4112	
Cefotaxim/Ceftriaxon	39	0	61	4112	
Cefazolin/Cefuroxim	39	0	61	4086	same resistance like Oxacillin
Rifampicin	97	0	3	3635	combi with Vanco/Teico: endocarditis
Fusidinsäure	67	11	22	214	
Fosfomycin	48	0	52	4189	
Cefuroxim- Axetil	69	0	31	1131	oral - ambulant patients
Linezolid	100	0	0	166	

Streptococcus spp. (ß-haemolys.)

ð **first choice:**
Penicillines,
Cephalosporines
(here: no resistance detected)

ð **Gentamicin and Tobramycin.:**
only as a partner of combination;
e.g. endocarditis
(if not "high-level-resistant")

ð **Penicillin-allergy and high-level resistance:**
- **Erythromycin**
- **Clindamycin**
- **Moxifloxacin**
- **Vancomycin**

substance	S [%]	I [%]	R [%]	isolates [n]	
Ampic./ Amoxic.	100	0	0	3617	
Amoxic.+ Clavulans.	100	0	0	3612	
Cefaclor	100	0	0	1947	
Gentamicin/Tobramycin	0	0	100	1834	only combination: endocarditis
Cotrimoxazol	64	4	32	559	
Nitrofurantoin	97	0	3	325	
Penicillin	100	0	0	3619	
Erythromycin	83	0	17	3109	
Clindamycin	86	0	14	3031	
Vancomycin	100	0	0	356	
Imipenem	100	0	0	1569	
Ampic.+ Sulbactam	100	0	0	1612	
Piperac.+ Tazobactam	100	0	0	1560	
Cefotaxim/ Ceftriaxon	100	0	0	1620	
Cefazolin	100	0	0	1523	
Meropenem	100	0	0	1621	
Cefuroxim	100	0	0	1616	
Teicoplanin	100	0	0	296	
Cefuroxim- Axetil	100	0	0	1998	
Moxifloxacin	99	1	0	188	

conclusion:

effective cooperation of all participants is necessary !

- fast (!), effective diagnostic

- fast (!), effective treatment

- Paul Ehrlich (1854-1915):

"Frapper fort et frapper vite"
"Hit hard and fast"

(Address to the 17th International Congress of Medicine, 1913)

I was proud to have the opportunity to visit your laboratories ....

and your wonderful country!

And we all have to bear in mind....

… according to all medical issues it is useful to act as qualified, effective and innovative PARTNERS …

… working hand in hand, and …

… remembering hand disinfection!

(… the most important vehicle for microorganisms!)

Please support your hygiene-management !

Thanks for your attention!