

Bacteria Contaminations in the Cell Culture

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Cell Threats

- Contamination with other cell lines
- Yeast
- Fungi
- Viruses:
especially bovine Pestiviruses
 BVDV – Virus of Bovine Virus diarrhea
 CSFV – Virus of the classical swine
but also BDV (Borna Disease Virus)
- Bacteria
- Mycoplasma

Relevance of Bacteria Contaminations

- contamination due to impure techniques and source materials
- typically recognized via organoleptic test (turbidity, coloration of the media turns yellow)
- Approx. 6 % of the cultures are unrecognized contaminated (180 samples, 11 positive with Onar[®]EUB)
 - Caulobacter sp.*
 - Methylophilus sp.*
 - Staphylococcus epidermis*
 - Methylobacterium sp.*
- Not recognized due to antibiotics in the cell culture media
- **No cure possible ⇒ Destroy!**



Bacteria Contamination - Examples

- **Blood bottles**
- **Organ culture**
Incidence of bacterial and fungal contamination of donor corneas preserved by organ culture. Lagenah M, Bohnke M, Engelmann K, Winter R. *Cornea*. 1995 Jul;14(4):423-6.
- **Fermentation/cell culture**
Safety of biological products prepared from mammalian cell culture. *DNA. Dev Biol Stand.* 1998;93:136-8.
- **Tissue Engineering**
Microbial contamination of cellular products for hematolymphoid transplantation therapy: assessment of the problem and strategies to minimize the clinical impact. Lowder JN, Whelton P., *Cytotherapy*. 2003;5(5):377-90.
Endogenous microbial contamination of cultured autologous preparations in trials of cancer immunotherapy. Padley DJ, Greiner CW, Heddlesten TL, Hopkins MK, Maas ML, Gastineau DA. *Cytotherapy*. 2003;5(2):147-52.
- **R&D**
Cell culture contamination by mycobacteria. Buehring GC, Valesco M, Pan CY. *In Vitro Cell Dev Biol Anim.* 1995 Nov;31(10):735-7.

Bacteria Detection with Onar[®]EUB

One-step PCR with conventional gel evaluation

Needs EUB Polymerase or other DNA-free polymerases

Detection limit: 6 genomes/ μ l

Specific for more than 45 bacteria

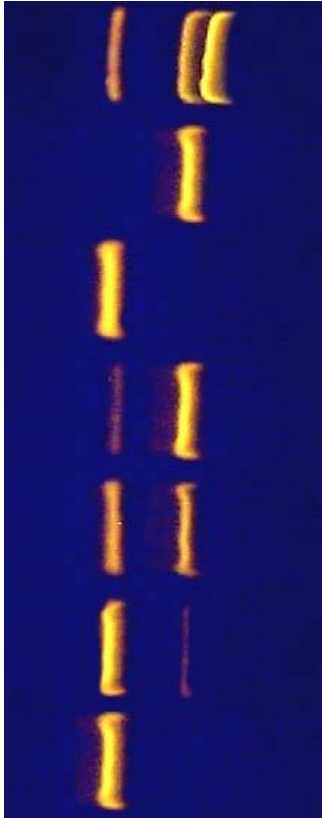
Includes positiv control and internal amplification control.

yes/no-result after 3 hours

Package sizes: 25, 50, 100 und 250 tests, aliquotes á 25 tests.

Aliquots of Master-Mix with hot-start Taq possible.

Analysis using Onar[®]EUB



1 kb DNA ladder

positive control amplification

internal control amplification

positive sample / strong contamination

positive sample / moderate contamination

positive sample / weak contamination

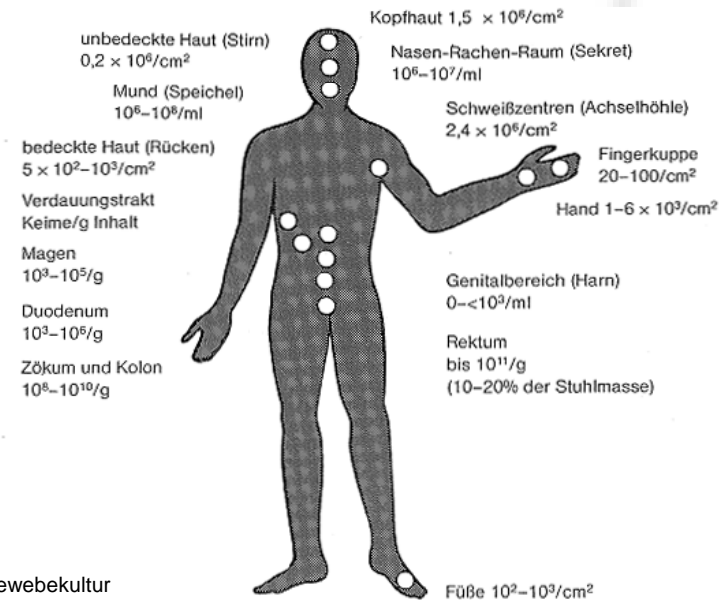
negative sample with internal control

Sources of Contamination

- Primary cultures from the original tissue (incidence approximately 4 %)
- Cross contamination
 - contaminated cultures
 - new cultures from unknown sources, also partly from cell banks
 - virus suspensions, antibody- solutions or other additions of contaminated cell cultures
- Direct contamination
 - laboratory instruments, media and reagents, which came into contact with contaminated cultures

Sources of Contamination (continued)

- **The User**
- direct entry while handling, usually from the oral flora
- droplet transfer
- lacking disinfection
- careless technical work



From: Toni Lindl, Zell-und Gewebekultur

Frequency of Testing

- New cell and virus cultures
- Each month for continuous cell lines; each week in cases of laboratory contamination
- Before each liquid nitrogen storage
- Upon modification of the cell characteristics
- In case of problems with result reproducibility

Procedure for Contaminated Cell Cultures/ Virus

- Isolate the culture immediately (incubator and if possible autoclave)
- Immediately lock and test cryo- conserves
- Isolate all cryo-conserved samples
- Inform all possible receipts
- Standard disinfection of the laboratory
- Initiate immediately treatment with irreplaceable cells

Recommendations for Improvement

- Regularly and sensitive testing (monthly); weekly testing in cases of laboratory contamination
- Operate free of standards antibiotics
- Never use contained cultures; reject immediately or treated
- Disinfect working surfaces and hands with alcoholic spray before and after working procedures, or with the change of the working material
- Quarantine new cells of any origin and integrate into the laboratory only after testing negative