

Regensburg's unique concept of a "Common LC Protocol"

C. pneumoniae L. pneumophila B. pertussis H. pylori H. pylori Clar-Res. EHEC

> one uniform thermocycle protocol fits to the majority of our PCR assays.

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Helicobacter spp.

© Zentrales EM-Labor Pathologie Uni Regensburg
 $\leq 0.3 \mu\text{m}$

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Evaluation of Diagnostic Protocols in Clinical Bacteriology

- H. pylori - Clarithromycin resistance

Hybridization probe / melting curve-based detection of mutations in the H. pylori 23S rDNA gene associated with macrolide resistance:

5' Primer 1 ...g G A A a... PCR amplicon 3' Primer 2

Fluorescein (F) LC-Red 640 (Red)

h x v₁ h x v₃

PCR design according to: Maeda et al., 1998, *Gut* 43:317-321.

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Evaluation of Diagnostic Protocols in Clinical Bacteriology

- H. pylori - Clarithromycin resistance

LC melting curve analysis performed with 30 cultured H. pylori isolates:

GAG (mt) GGA (mt) GAA (wt)

04.28.0537 is presumably a mixture of GGA and GAA

...g G A A a... (A)GG

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Medizin Schock

Es ist passiert +++ Erstes Bakterium völlig resistent gegen Antibiotika +++
 Es heißt Staphylococcus aureus +++ Es hat in Hongkong bereits eine Frau getötet
 +++ Ein Mikro-Biologe sagt: Es hat unsere letzte Verteidigung durchbrochen

Die größte Gefahr lauert in Kliniken

Info Antibiotika

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Evaluation of Diagnostic Protocols in Clinical Bacteriology

MRSA - Diagnostic laboratory workflow

Microbiology vs. PCR workup:

Microscopy (Gram stain) preparation of Blood agar + AB discs, Mannitol salt agar, Oxacillin mannitol bouillon

Coagulase Agglut. Test, Catalase product, MH Agar + AB discs, Oxacillin Screening Plate (DNase Agar)

Antibigram, MRSA / MSSA, Agglut. Test, Readout of AB discs & Oxa Screening Plate, Tubed Coagulase, Biochemical tests

MRSA MSSA, Readout of supplementary tests

meCA pos. neg. MRSA MSSA

simultaneous presence of meCA-positive CoNS and meCA-negative S. aureus prevents from direct PCR testing of samples for MRSA on day 0

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Evaluation of Diagnostic Protocols in Clinical Bacteriology

Bordetella pertussis - Diagnostic Workup

Microbiology & PCR workup:

- Microscopy** (Gram stain) → Gram + rods / cocci, low sensitivity and specificity
- Serology** (Specific IgG, IgA, IgM) → Serology often inconclusive, sensitivity < 50%
- DNA preparation & PCR for B. pertussis** (B. pertussis) → PCR highly sensitive and specific
- Biochemical testing** (e.g., urease / oxidase & susceptibility testing)
- Antibiogram**
- Species Identification**

Throat swab (day 0) → **Nasopharyngeal swab** (day 0)

Prerequisite:

- calcium alginate swab
- catarrhal stage (< day 14)

Diagnostic Culture (gold standard)

- modified Bordet-Gengou medium
- charcoal-horseblood agar (Sjogren-Lewy)
- BCYE agar

day 2-4 → when positive → sensitivity below 50%

day 4-10

U. Reusch/RMM01/2003 Bpert 09

Evaluation of Diagnostic Protocols in Clinical Bacteriology

- Bordetella pertussis -

Vers. 1.0 / 2.0

Selection of primer and HybProbe sequences:

Repetitive insertion sequence IS 481
(primers and HybProbes are located in specific regions)

```

>>>gt;gaagattcaatagttg; Primer BP-neu1 >
666937 1127 ..... Primer BP-1 >
M22031 12 ..... attcaatagatttctgcatgttcctccgaacggatttgagaactgaaacccca 1186
L26973 1127 ..... 71
666929 12 ..... 1186
X58488 237 ..... 71
U07800 237 ..... 178
666937 1187 ..... BP-HP-3 > F R BP-HP-4 > BP-HP-3 > 1246
M22031 72 ..... accccatctcactcaaggccggccgatgaaaccccatagatcaccatctacc 131
L26973 1187 ..... 1246
666929 72 ..... 131
X58488 167 ..... 108
U07800 177 ..... 318
666937 1247 ..... F R BP-HP-4 > < Primer BP-neu2: caaacacagcagcttcggc 1307
M22031 132 ..... cttcctagctcagctcaaatggtccagcattgatggccatcaagttgtgctgctaa 1307
L26973 1247 ..... 1307
666929 132 ..... 192
X58488 107 ..... 63
U07800 117 ..... 53
  
```

Ref.: Gray et al. (1990) J. Clin. Microbiol. 28:1982-1987.

U. Reusch/RMM01/2003

Evaluation of Diagnostic Protocols in Clinical Bacteriology

- Bordetella pertussis / parapertussis -

Vers. 1.0

Evaluation of the duplex PCR assay with a set of 30 nasopharyngeal specimens:

B. pertussis

IS481
[640 nm]
[F2]

B. parapertussis

IS1001
[705 nm]
[F3]

Patient → has a simultaneous infection with B. pertussis and B. parapertussis

U. Reusch/RMM01/2003

Evaluation of Diagnostic Protocols in Clinical Bacteriology

- Bordetella pertussis / parapertussis -

Vers. 1.0

Results of IS481- and IS1001-specific PCR versus results of diagnostic culture:

Culture result	No. of patients (n = 208)			
	B. pertussis PCR		B. parapertussis PCR	
	Positive	Negative	Positive	Negative
Positive ^{a)}	20	0	10	0
Negative ^{b)}	2	164	8	190

^{a)} Includes 2 patients whose samples were both positive by PCR and culture for B. pertussis and B. parapertussis.

^{b)} Includes 2 patients whose samples were both positive by PCR and negative by culture for B. pertussis and B. parapertussis.

U. Reusch/RMM01/2003

Evaluation of Diagnostic Protocols in Clinical Bacteriology

- Bordetella pertussis / parapertussis -

Target quantification in clinical specimens applying an external standard:

IS481
[640 nm]
[F2]

Linear Regression

Mean: 1.36
Standard Error: 0.001
Slope: 4.179
R: 0.999

IS481 copies	log10(Copies/mL)
9	10,000,000
10	1,000,000
11	100,000
12	10,000
13	1,000
14	100
15	10
16	1
17	0.1
18	0.01
19	negative control
20	Patien 090415
21	Patien 090415
22	Patien 017203

180,000 copies
3,390,000 copies
19,000 copies

U. Reusch/RMM01/2003

Evaluation of Diagnostic Protocols in Clinical Bacteriology

- Bordetella pertussis / parapertussis -

Vers. 2.8

Evaluation of the Multiplex Master with specimens of the QC-panel (INSTAND e.V.):

Target genes: h-DNA, Pert, Para

Triplex PCR Assay *

FastStart Multiplex DNA Master

β-globin [610 nm] | **IS481** [640 nm] | **IS1001** [705 nm]

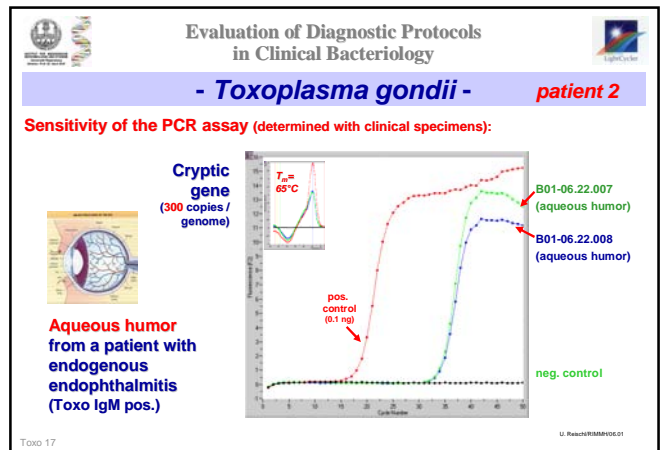
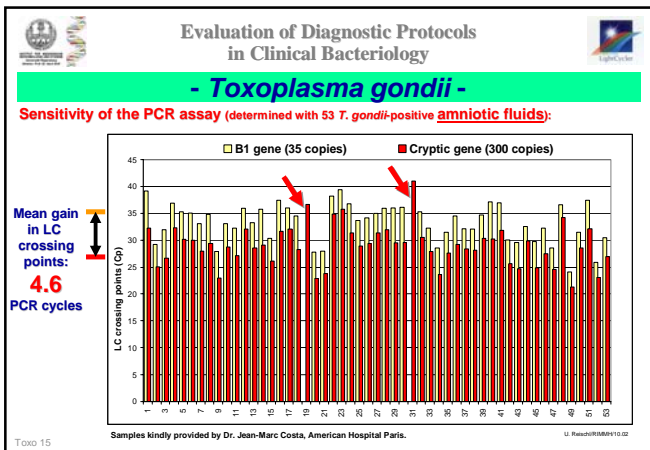
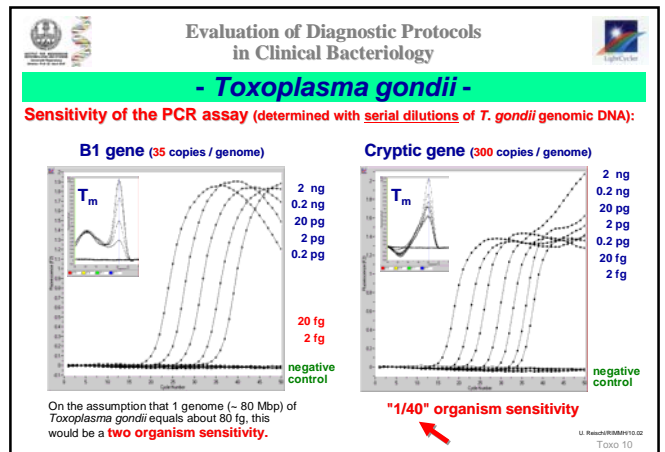
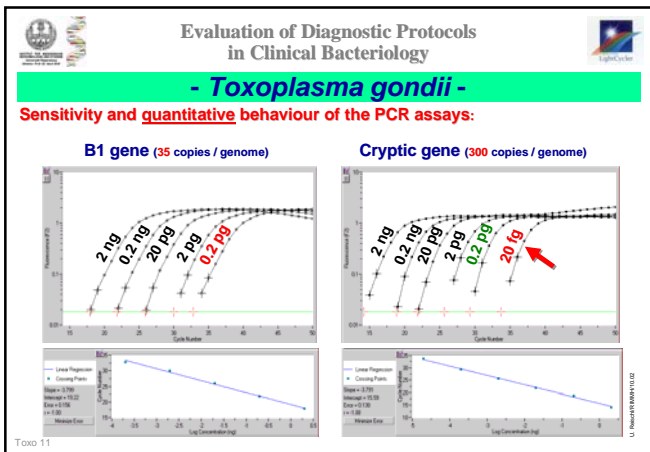
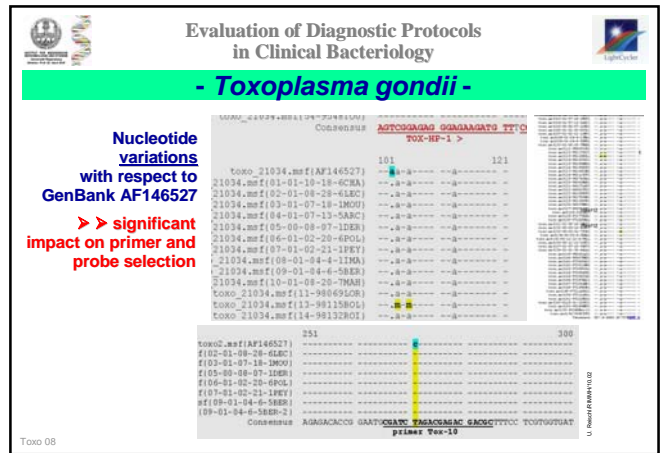
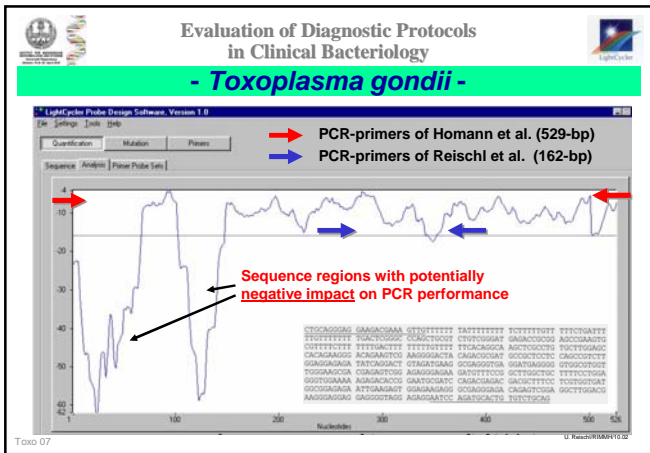
T_m = 60°C | **T_m = 66°C** | **T_m = 51°C** | **expected T_m = 60°C**

References:

Reusch, U., C. Grotzer, B. Engemann, W. J. Lindt, and H. Lohr (2003) Rapid detection and simultaneous differentiation of Bordetella pertussis and Bordetella parapertussis on clinical specimens by LightCycler PCR. In: Rapid Cycle Real-Time PCR: Methods and Applications (Reusch, U., Wilmes, C., and Cichewski, P., eds.), Springer-Verlag, Heidelberg, pp. 33-44.

MULTIplex-06

U. Reusch / RMM01/2.2003





Quality Control Program for Diagnostic PCR

status 03.2004

Spectrum of our QC panels:

Established since April 2003:



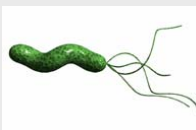
BACTERIAL GENOME DETECTION
PCR-/NAT *Chlamydia trachomatis*
RV 431, April 2003



BACTERIAL GENOME DETECTION
PCR-/NAT *Bordetella pertussis*
RV 432, April 2003



BACTERIAL GENOME DETECTION
PCR-/NAT *Helicobacter pylori*
RV 433, April 2003



BACTERIAL GENOME DETECTION
PCR-/NAT **EHEC / STEC**
RV 434, April 2003



BACTERIAL GENOME DETECTION
PCR-/NAT *Borrelia burgdorferi*
RV 435, April 2003



starting March 2004:

BACTERIAL GENOME DETECTION
PCR-/NAT *C. trachomatis* & *N. gonorrhoeae*
RV 430, March 2004

BACTERIAL GENOME DETECTION
PCR-/NAT *Legionella pneumophila*
RV 436, March 2004

BACTERIAL GENOME DETECTION
PCR-/NAT *Salmonella enterica*
RV 437, March 2004

BACTERIAL GENOME DETECTION
PCR-/NAT *Listeria spp.*
RV 438, March 2004

planned:

BACTERIAL GENOME DETECTION
PCR-/NAT **MRSA / cMRSA**
RV 439, March 2005



Qualitativer und quantitativer Nachweis erregerspezifischer Nukleinsäure von:

Bakterien / Pilze (broad-range)

- Anm. ②
- Bakterien spp.
- Pilz spp.

Respiratorische Infektionen

- Bordetella pertussis
- Bordetella parapertussis
- Chlamydia pneumoniae
- Chlamydia psittaci
- Haemophilus influenzae
- Legionella pneumophila
- Legionella spp.
- Moraxella catarrhalis
- Mycobact. tuberculosis (TB)
- Mycobacterium spp. ⑦
- Mycoplasma pneumoniae
- Nocardia spp.
- Pseudomonas aeruginosa
- Strept. pneumoniae
- Adenoviren
- Influenza Typ A Typ B

Gastrointestinale Infektionen

- E. coli (EHEC) (stx-1,-2, eae, hlyA)
- E. coli (ETEC)
- E. coli (EPEC)
- E. coli (EAEC)
- E. coli (EIEC)
- Salmonella spp.
- Helicobacter pylori
- Helicobacter spp.
- S. aureus (Enterotoxine A, B, C, D, E)
- Yersinia enterocolitica
- Campylobacter jejuni / C. coli
- Clostridium difficile Tox. A+B
- Adenoviren

Urogenitale Infektionen

- Chlamydia trachomatis
- Strept. agalactiae (B-Strep)
- Treponema pallidum
- Mycoplasma genitalium ④
- Mycoplasma hominis ④
- Neisseria gonorrhoeae
- Ureaplasma urealyticum ④

ZNS Infektionen

- Borrelia burgdorferi
- Cryptococcus spp.
- Mycobact. tuberculosis (TB)
- Neisseria meningitidis
- Staphylococcus aureus
- Toxoplasma gondii
- Treponema pallidum
- Tropheryma whippelii
- Adenoviren
- CMV
- Enteroviren
- HHV-6
- HSV-1,2
- JCV
- VZV

Molekulare Resistenztestung

- MRSA (S. aureus)
- Clarithromycin-Res. (H. pylori)
- Rifampicin-Res. (M. tuberculosis)
- Isoniazid-Res. (M. tuberculosis)
- Rifabutin-Res. (H. pylori)
- VRE (van A, B) (Enterokokken)
- ESBL
- HBV-Lamivudin-Resistenz
- HIV-Resistenztestung ⑥

Hepatitisviren

- HAV
- HBV qual. quant.
- HBV Genotypisierung
- HBsAg Sequenzierung
- HCV qual. quant. ⑤
- HCV Genotypisierung
- HDV
- HEV

Herpesviren

- CMV quantitativ
- EBV quantitativ
- HHV-6
- HSV-1,2
- VZV

HIV quantitativ ⑥

HPV Genotypisierung

Parvo B19 qual. quant.

- BKV quantitativ
- JCV quantitativ
- Enteroviren-Differenzierung

Pilze

- Aspergillus fumigatus
- Blastomyces dermatitidis
- Candida albicans
- Coccidioides immitis
- Cryptococcus spp.
- Histoplasma capsulatum
- Microsporium canis
- Paracoccidioides brasiliensis
- Trichophyton verrucosum
- Speziesidentifizierung von Kultur

SPEZIALDIAGNOSTIK ④

Bakterien

- Bacillus anthracis
- Bartonella henselae/quintana
- Borrelia burgdorferi
- Brucella spp.
- Corynebact. diphtheriae
- Coxiella burnetii (Q-Fieber)
- Enterococcus spp.
- Leptospira spp.
- Listeria monocytogenes
- Neisseria meningitidis
- Pseudomonas aeruginosa
- Staphylococcus aureus
- S. aureus (Toxic Shock Syndr. Tox.)
- S. aureus (Exfoliatives Toxin A, B)
- S. aureus (PV-Leukozidin, cMRSA)
- Strept. pyogenes (A-Strep)
- Tropheryma whippelii
- Treponema pallidum
- Yersinia pestis
- Speziesidentifizierung von Kultur

Protozoen

- Acanthamoeba spp.
- Cryptosporidium spp.
- Entamoeba histolyt. / E. dispar
- Pneumocystis carinii
- Plasmodium spp. quant.
- Plasmodium Speziesdiff.
- Toxoplasma gondii
- Acanthamoeba spp.

PCR-Assay Portfolio Regensburg

status **March 2004**



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