INDICAL BIOSCIENCE

Because milk matters – bactotype Mastitis Test System for rapid, flexible and **meaningful** Mastitis monitoring and control

Carsten Schroeder, Daland C. Herrmann, Christine Gaunitz, Marco Labitzke, Oliver Sasse, Claudia Engemann

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Fast facts

- Mastitis is an inflammation of the udder
- Mastitis is common in dairy herds
- Mastitis is causing important economic losses worldwide
- Mastitis can't be eradicated -- but can be reduced to low levels by good management of dairy cows
- Of the several causes of Mastitis **only microbial infection is important**
- Bacteria, fungi, yeasts and possibly viruses can cause udder infection but the **main agents are bacteria**
- **Most common pathogens are *S. aureus*, *S. agalactiae*, *S. dysgalactiae*, *S. uberis* and *E. coli***
- Other Mastitis pathogens can cause occasional herd outbreaks

www.fao.org/docrep/004/T0218E/T0218E04.htm#ch4.1

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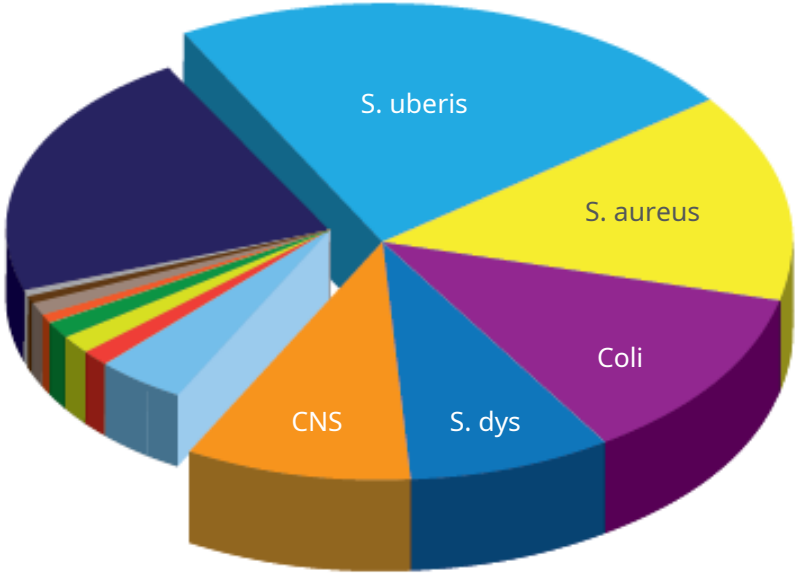
Mastitis pathogens

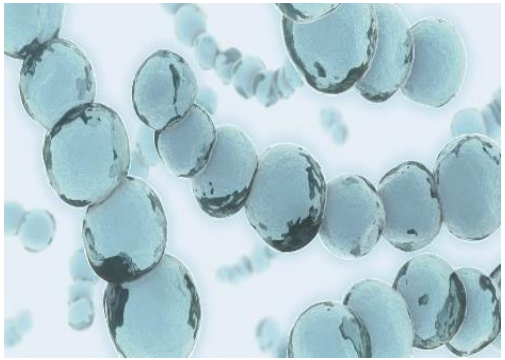
- Streptococcus uberis
- Staphylococcus aureus
- Coliforms
- Streptococcus dysgalactiae
- Coagulase-negative Staphylococci
- Enterococcus
- Trueperella pyogenes
- Aerococcus spp.
- Yeast
- Streptococcus agalactiae
- Prototheca
- Corynebacteriae
- Miscellaneous
- Unidentified – *Mycoplasma*?

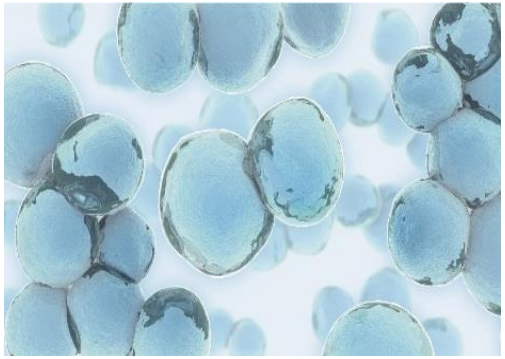
- 2010 study in collaboration with MBFG Wunsdorf and Boehringer Ingelheim
- Culture method used



www.vetion.de/neues/detail.cfm?main_id=821&StartRow=4

Mastitis pathogens





Contamination!

Can be managed by improved hygiene or vaccination. Antibiotics not (always) necessary.

Contagious

- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Mycoplasma* spp.

Infectious!

Requires strict disease control and antibiotic treatment and even culling.

Environmental

- *Streptococcus uberis*
- *Escherichia coli* (*E. coli*)
- *Klebsiella pneumoniae*
- *Trueperella pyogenes* (previously *Arcanobacterium pyogenes*)
- *Staphylococcus epidermidis*
- *Pseudomonas aeruginosa*
- *Klebsiella oxytoca*
- *Corynebacterium bovis*
- *Enterobacter aerogenes*
- *Pasteurella* spp.
- *Brucella melitensis*
- *Proteus* spp.
- *Prototheca zopfii* (achlorophyllic algae)
- *Prototheca wickerhamii* (achlorophyllic algae)

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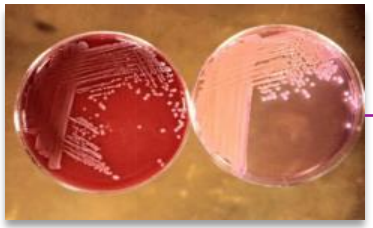
Detection methods



Bacterial culture	Real-time PCR (qPCR)
Broad range of detection	Fast, sensitive diagnostics
Semi-quantitiative analysis	Quantitative analysis (C_T value, curve)
Low costs for Agar plates	Multiplexing
Time and labour-intensive	Milk and pooled milk samples from disease control programs (e.g. *Brucellosis*) can be used
Not suitable for sodium acide stabilised milk samples	Detection of other pathogens from milk (BVDV) possible
Some pathogens are difficult to culture (e.g. *Mycoplasma*)	Detects bacteria which are difficult to grow (*Mycoplasma)*

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Rapid, flexible and meaningful Mastitis monitoring and control

Bovine Mastitis is the single most common and most costly disease of dairy cows.

Mastitis reduces milk production and raw milk quality, making it unsuitable for human consumption. Treating a cow herd for mastitis is costly, leads to milk withholding after treatment and may force producers to cull their dairy cows.

Reliably identifying pathogens that cause Mastitis is the cornerstone of targeted therapy strategies.

From surveillance to diagnostics, INDICAL's modular bactotype Mastitis Test System enables customers to implement different strategies for rapid and reliable Mastitis testing to meet their specific needs.

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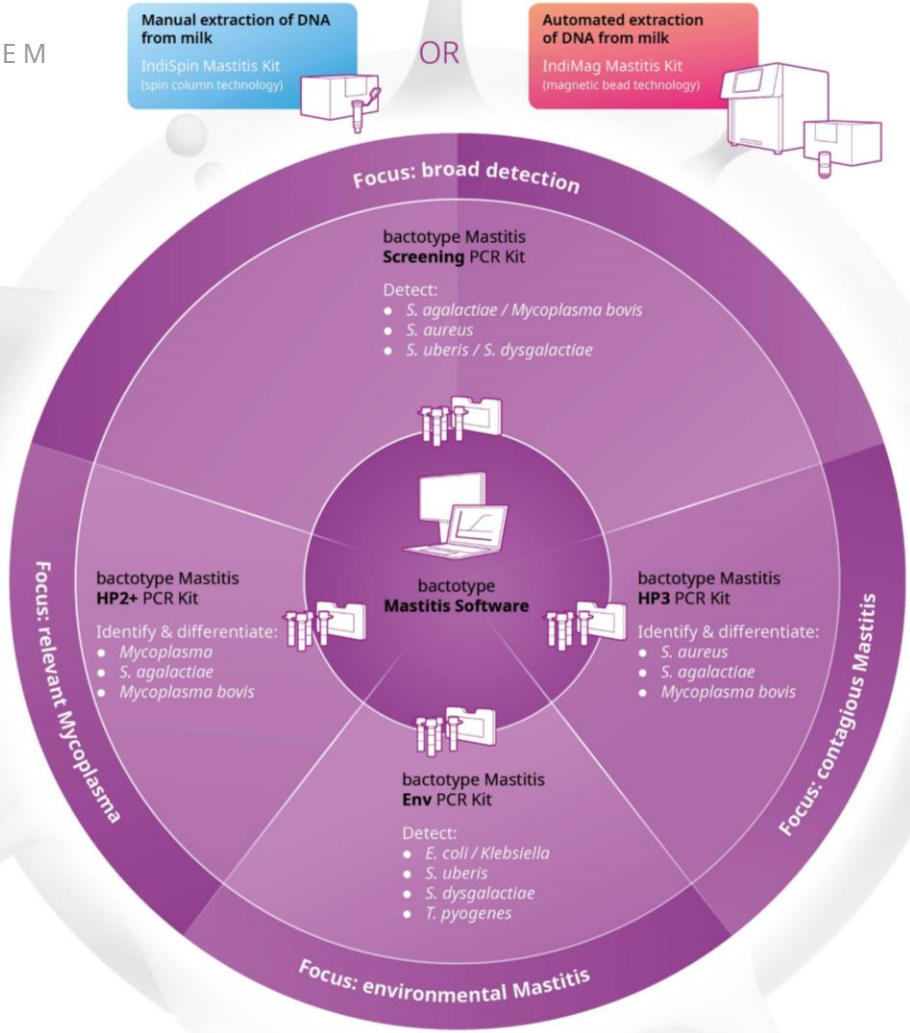
Tailored to your specific testing requirements



Contact our experts!

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DNA extraction from milk

Milk is a challenging sample type to extract DNA for PCR

Mastitis milk is even more challenging due to the wide range of sample conditions

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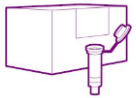
DNA extraction from milk



IndiField Mastitis Kit

For **manual DNA isolation**
(spin-column/silica-membrane)

2

Optimized chemical lysis and silica-membrane extraction

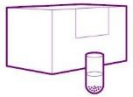
- 1:30 h (24 samples)
- 6 h (96 samples)

1

Homogenize

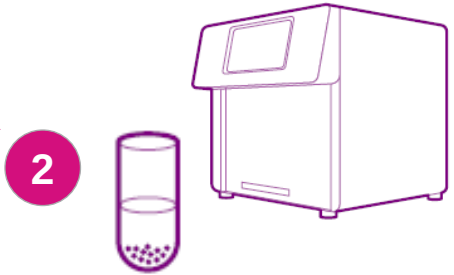
Pathogen Lysis Microtubes
+ TissueLyser II

Fast and efficient disruption of gram positive and gram negative bacteria.



IndiMag Mastitis Kit

For **automated DNA isolation**
(magnetic beads)



2

Optimized chemical lysis and magnetic bead extraction

- 1:30 h (24 samples)
- 3 h (96 samples)

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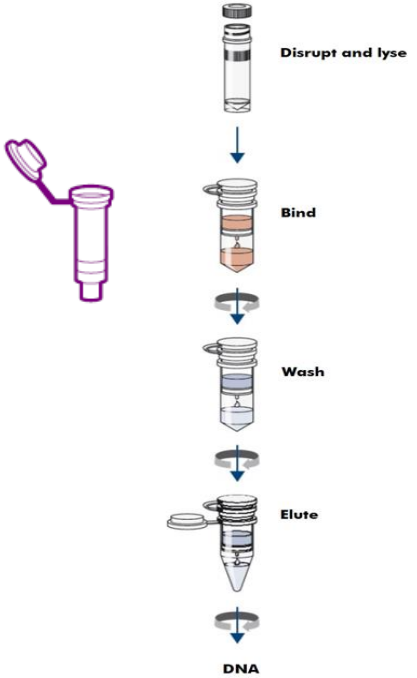
DNA extraction from milk



Pathogen Lysis Microtubes S

+

Buffer ML

+

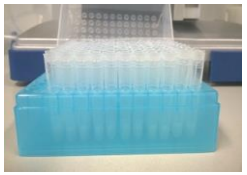
400 µl milk sample

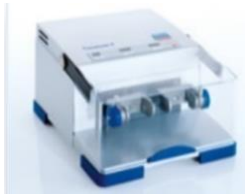
Homogenisation (TissueLyser II)

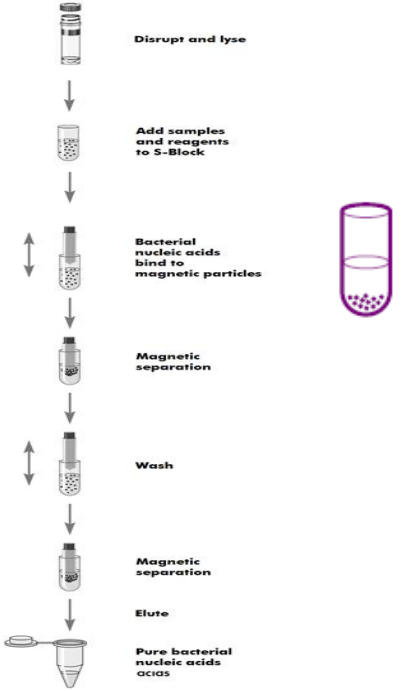
2 x 8 min (30 Hz)

+

Buffer MVL







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bactotype Mastitis qPCR assays

For screening/detection:

- **Four** ready-to-use Multiplex real-time PCR Kits For analysis of quarter milk, bulk tank and pool samples
- PCR Kit-specific Master Mix (including IC system)
- PCR-Kit-specific Positive Control
- **One** Protocol for all bactotype Mastitis PCR Kits:
  - 5 min 95°C
  - 10 s 95°C
  - 30 s 57°C

  } 40x
- qPCR protocol ~ 1 hour
- Total time <3 hours (96 samples)

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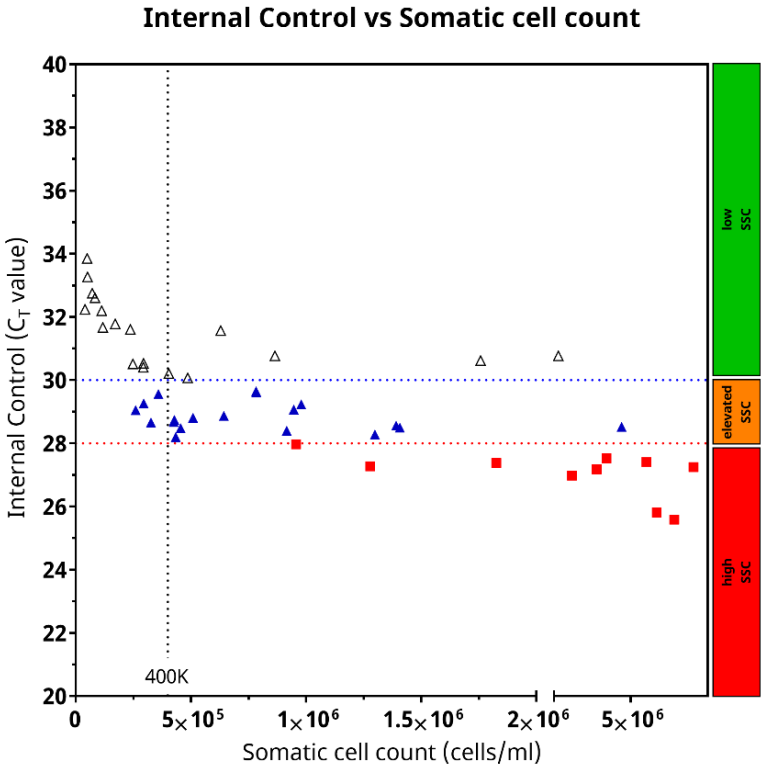
Internal PCR Control

Our bactotype Internal PCR Control is based on a house-keeping gene and monitors correct DNA extraction and amplification

- Serves as indicator for elevated somatic cell counts:
- Low C_T of the bactotype Internal PCR Control = High somatic cell count



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Bacterial culture (dilution series)

vs. Mastitis PCR (commercial competitor and INDICAL)

Mastitis Pathogen	**Commercial competitor PP** (magnetic beads extraction + detection)		**INDICAL** MagAttract magnetic bead extraction + bactotype Mastitis HP3 PCR Kit for detection	
	cfu/ml	C_T value	cfu/ml	C_T value
Staphylococcus aureus	900	36.91	180	37.21
Streptococcus agalactiae	122	36.42	122	36.21

bactotype Mastitis Test System equally sensitive detecting *S. agalactiae*
bactotype Mastitis Test System more sensitive detecting *S. aureus*

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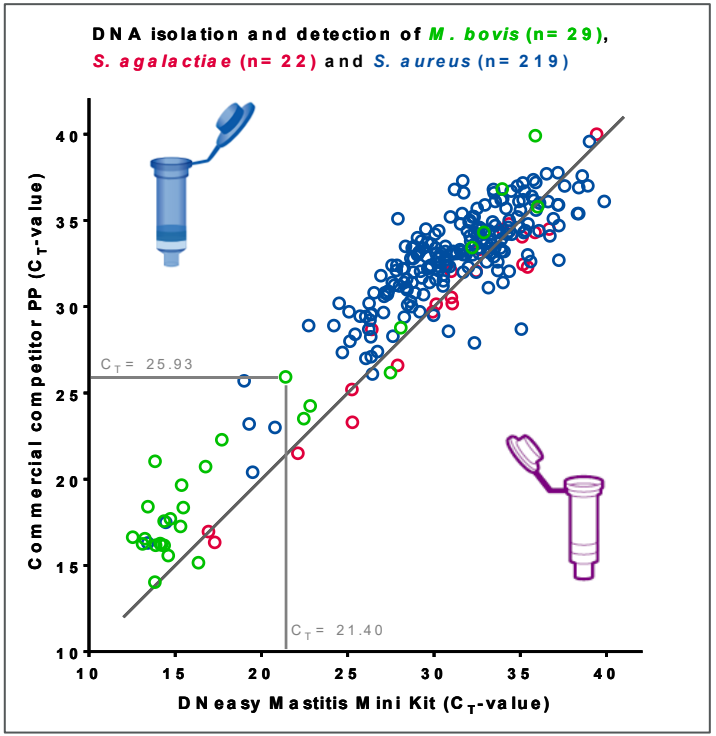
Sensitivity for contagious Mastitis vs. commercial PCR PP

DNeasy Mastitis Mini Kit (extraction) + bactotype Mastitis HP3 PCR Kit (detection)

- Results are equal (*S. agalactiae*) or better (*M. bovis, S. aureus*)

compared to commercial competitor PP



bactotype Mastitis HP3

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Case study

Rapid and reliable identification of *Mycoplasma bovis*

Procedure

In a field study, several animals in a herd of 600 dairy cows showed signs of clinical mastitis with unknown pathogenic cause.

60 milk pools (each of 10 animals) were generated, and DNA was extracted using the IndiMag Mastitis Kit. DNA samples were analyzed using the bactotype Mastitis HP2+ PCR Kit to detect *Mycoplasma*, *S. agalactiae* and *M. bovis* DNA. During the study, 12 animals succumbed to the infection.

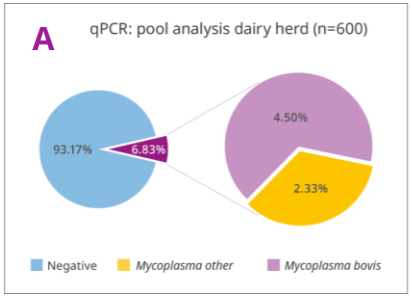
The remaining 588 milk samples were individually tested by bacterial culture analysis by an audited and qualified milk analysis laboratory.

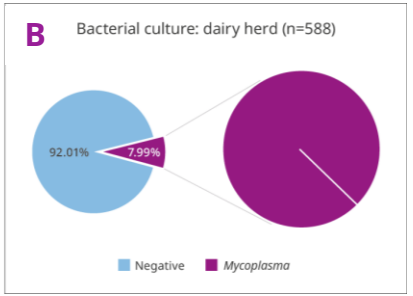
Note, bacterial culture can not distinguish between *Mycoplasma* and *M. bovis*.

Results:

At least 6.83 % of the herd tested *Mycoplasma* positive and at least 4.50 % tested *M. bovis* positive the by the bactotype Mastitis HP2+ PCR Kit (pool testing; Figure A)*.
7.99% of the herd tested *Mycoplasma* positive when testing individual cows using bacterial culture (Figure B).

Bacterial culture analysis confirm results obtained by the bactotype Mastitis HP2+ PCR Kit.





* To normalize results, one confirmed DNA positive milk pool was regarded as one *Mycoplasma* positive animal, even though more than one individual milk sample may have contributed to that positive pool.

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Testing pooled milk samples (*Mycoplasma bovis*) – positive animals contributing to the pool

SAMPLE	C_T (*M. bovis*)
Pool 16	**30.64**
16-01	-
16-02	35.55
16-03	37.65
16-04	-
16-05	-
16-06	26.37
16-07	-
16-08	-
16-09	-
16-10	-
Pool 59	**24.89**
59-01	-
59-02	-
59-03	27.15
59-04	21.96
59-05	-
59-06	-
59-07	-
59-08	-
59-09	-
59-10	27.03

Are pooled milk samples commonly used for monitoring programmes suitable for mastits surveillance?

SAMPLE	DILUTION	C_T (*M. bovis*)
Sample 59/ 04	**-**	**22.99**
	1:10	26.74
	1:20	28.11
	1:30	29.13
	1:40	28.91
	1:50	28.80
Sample 16/ 06	**-**	**28.77**
	1:10	33.79
	1:20	37.16
	1:30	34.43
	1:40	34.58
	1:50	35.14
Sample 16/ 02	**-**	**34.19**
	1:10	-
	1:20	-
	1:30	-
	1:40	-
	1:50	-

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Testing pooled milk samples (*S. aureus* and *S. agalactiae*)

SAMPLE	DILUTION	C_T (*Saureus*)
Sample 217	**-**	**25.18**
	1:10	28.23
	1:20	30.40
	1:30	30.00
	1:40	30.70
	1:50	32.24
Sample UL-2	**-**	**29.17**
	1:10	33.60
	1:20	33.66
	1:30	33.68
	1:40	33.71
	1:50	33.95
Sample UL-1	**-**	**33.13**
	1:10	35.28
	1:20	36.21
	1:30	36.04
	1:40	36.47
	1:50	38.21

SAMPLE	DILUTION	C_T (*Sagalactiae*)
Sample 172	**-**	**15.71**
	1:10	15.66
	1:20	15.99
	1:30	17.02
	1:40	16.66
	1:50	16.87
Sample 177	**-**	**26.41**
	1:10	30.46
	1:20	31.50
	1:30	32.14
	1:40	32.29
	1:50	32.14
Sample 176	**-**	**35.44**
	1:10	39.64
	1:20	36.88
	1:30	36.18
	1:40	36.59
	1:50	36.25

Positive results can be obtained from pooled milk samples (up to 50 animals) if at least one strongly positive animal (C_T <32 in the individual milk sample) contributed to that pool. Milk pools containing only **one weak positive** sample (C_T >34) may not be detected in a pool of 50 animals.

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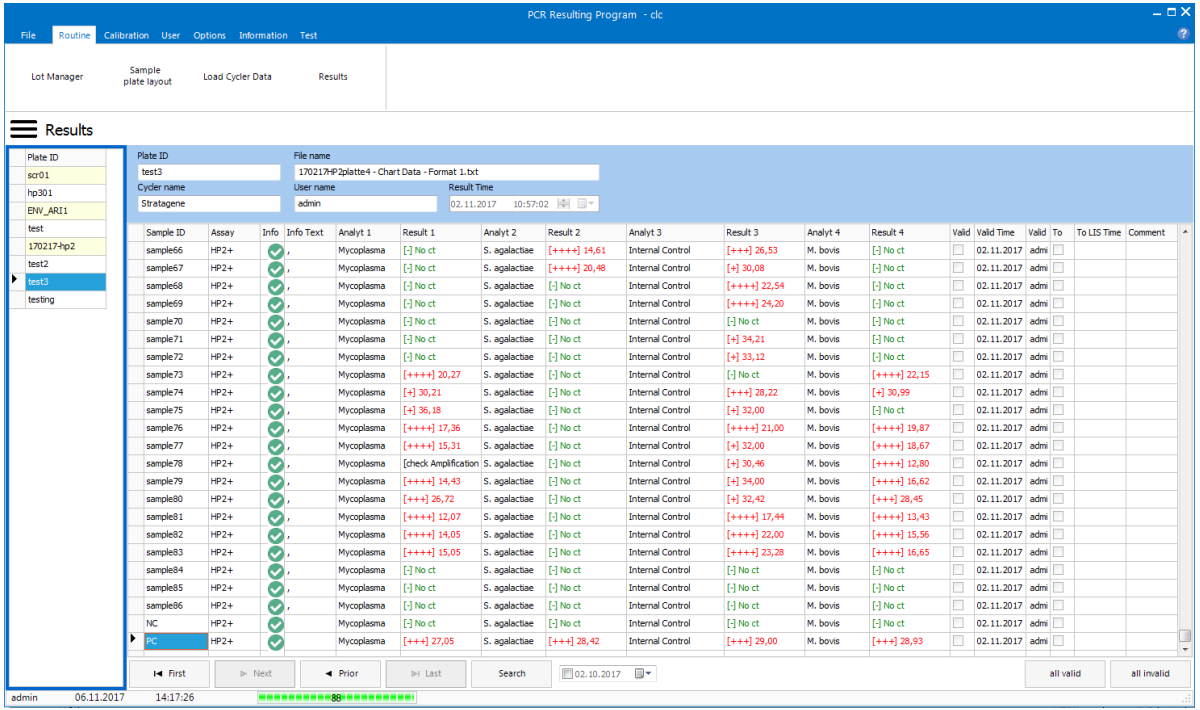
bactotype Mastitis Software



- Results: pathogen, Ct value and rough quantification (+, ++, +++) will be given
- Information to run and possible faults (e.g. Internal Control not detected → extraction failed etc) will also be shown
- Print out and export functions will be implemented

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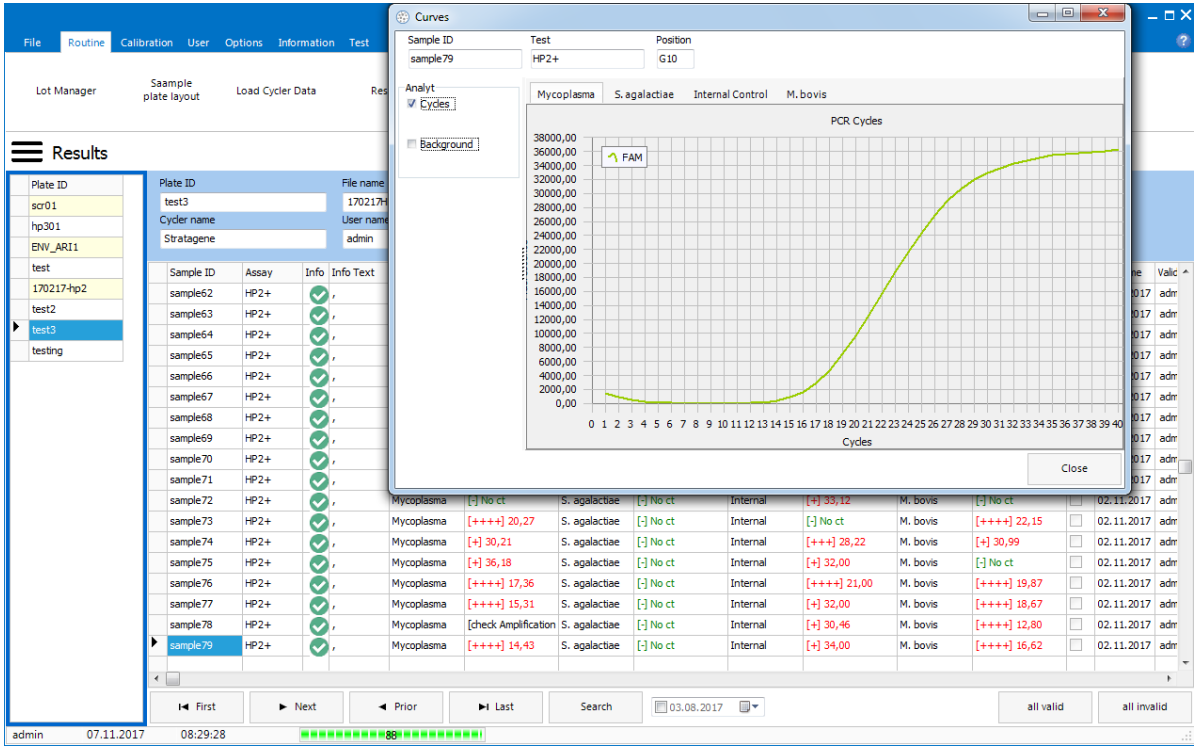
bactotype Mastitis Software



- Amplification curves can be viewed for each sample (pathogen and assay) to check for a specific highlighted fault or just to check the curves)

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Summary

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Mastitis reduces milk production and raw milk quality, making it unsuitable for human consumption. Treating a cow herd for mastitis is costly, leads to milk withholding after treatment and may force producers to cull their dairy cows.

Reliably identifying pathogens that cause Mastitis is the cornerstone of targeted therapy strategies.

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bactotype Mastitis Test System features and advantages

- **Highly sensitive, fast and easy to perform**
- **2.5 to 3 hours runtime** from DNA extraction to results
- **Identifies difficult to grow bacteria**, like *Mycoplasma*
- **Optimized sample prep kits** for manual AND automated extraction of bacterial DNA from milk samples without compromising on quality
- **Four assays – one protocol:** combine and run up to four ready-to-use multiplex qPCR kits for targeted identification of Mastitis-causing pathogens
- Individual milk samples and pools of up to 50 milk samples can be tested
- **Advanced Internal PCR Control** for monitoring correct DNA extraction and amplification;
- **can also be used as indicator of somatic cell count in milk samples**
- **Data analysis made easy** with the bactotype Mastitis Software
- **Open platform** designed, tested and validated to integrate with most common
- qPCR thermal cyclers (AriaMx, Mx3005P, ABI 7500, Bio-Rad CFX96)
- **Supply certainty**: High production capacity and safety stocks
- **Exceptional technical support by experienced veterinarians and molecular biologists**