DNA
DIAGNOSTIC

THE GLOBAL STANDARD
FOR LIVESTOCK DATA

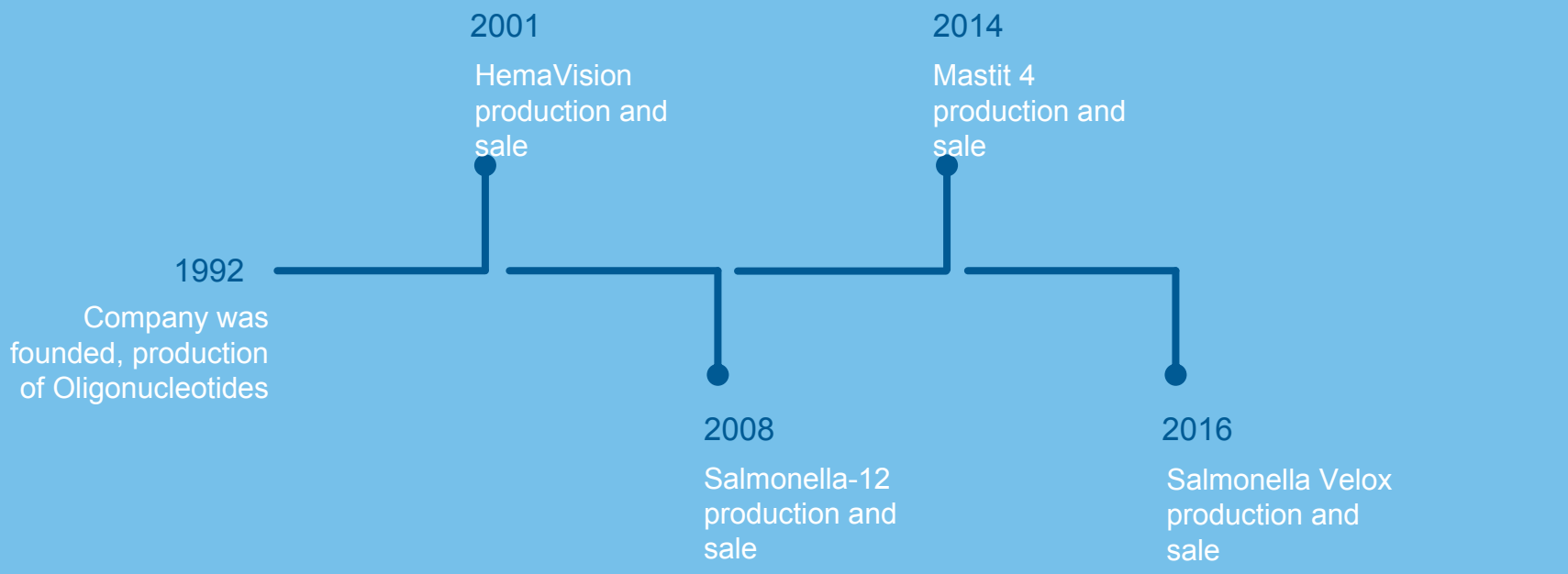
Mastit 4

Interpretation of results from milk samples tested for mastitis bacteria with Mastit 4 qPCR test from DNA Diagnostic

The 40th ICAR Biennial Session Puerto Varas, Chile, 24-28 october 2016

Jorgen Katholm

Timeline – company development



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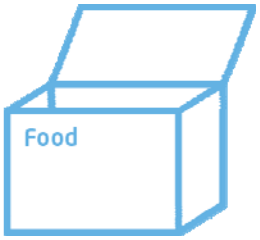
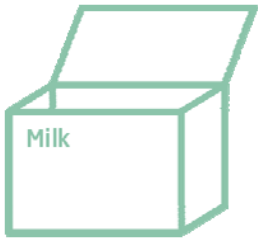
What we do



Salmonella Velox

PCR test kits for Salmonella screening in raw meat, poultry, fish, and seafood

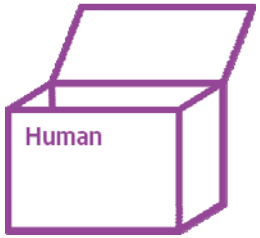


Mastit 4

PCR test kits for mastitis bacteria screening in milk

New !! TBC 4

PCR test kits for screening of total bacterial count in milk



HemaVision

IVD test kits for screening of chromosome translocations associated with leukemia

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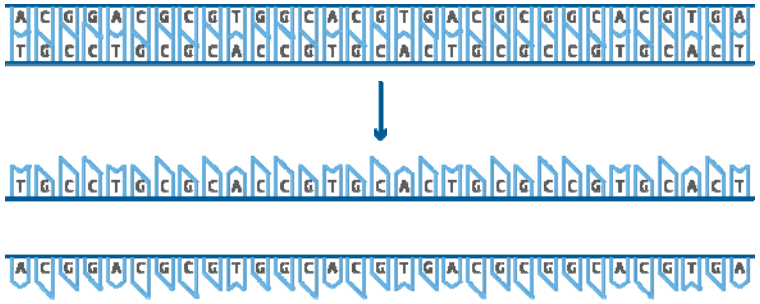
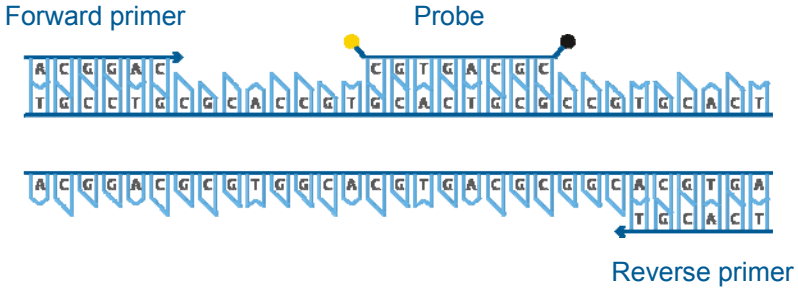
Principles of qPCR tests



Step 1

Denaturation



Step 2

Primers and probe

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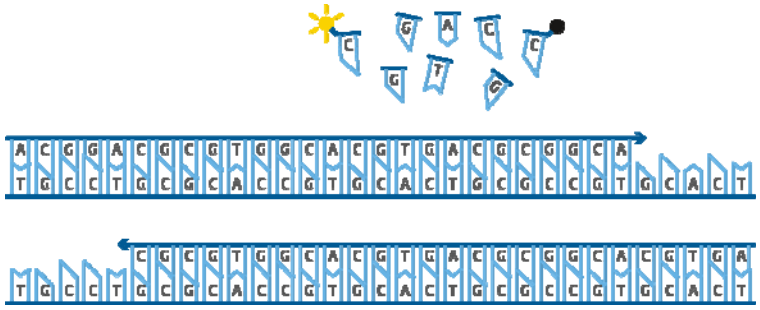
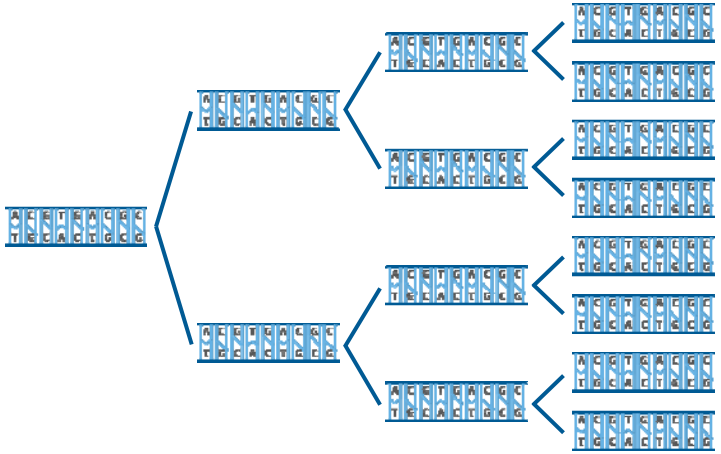
Principles of qPCR tests



Step 3

Polymerase



Step 4

Amplification
40 cycles resulting in 1 x 10^{12} copies

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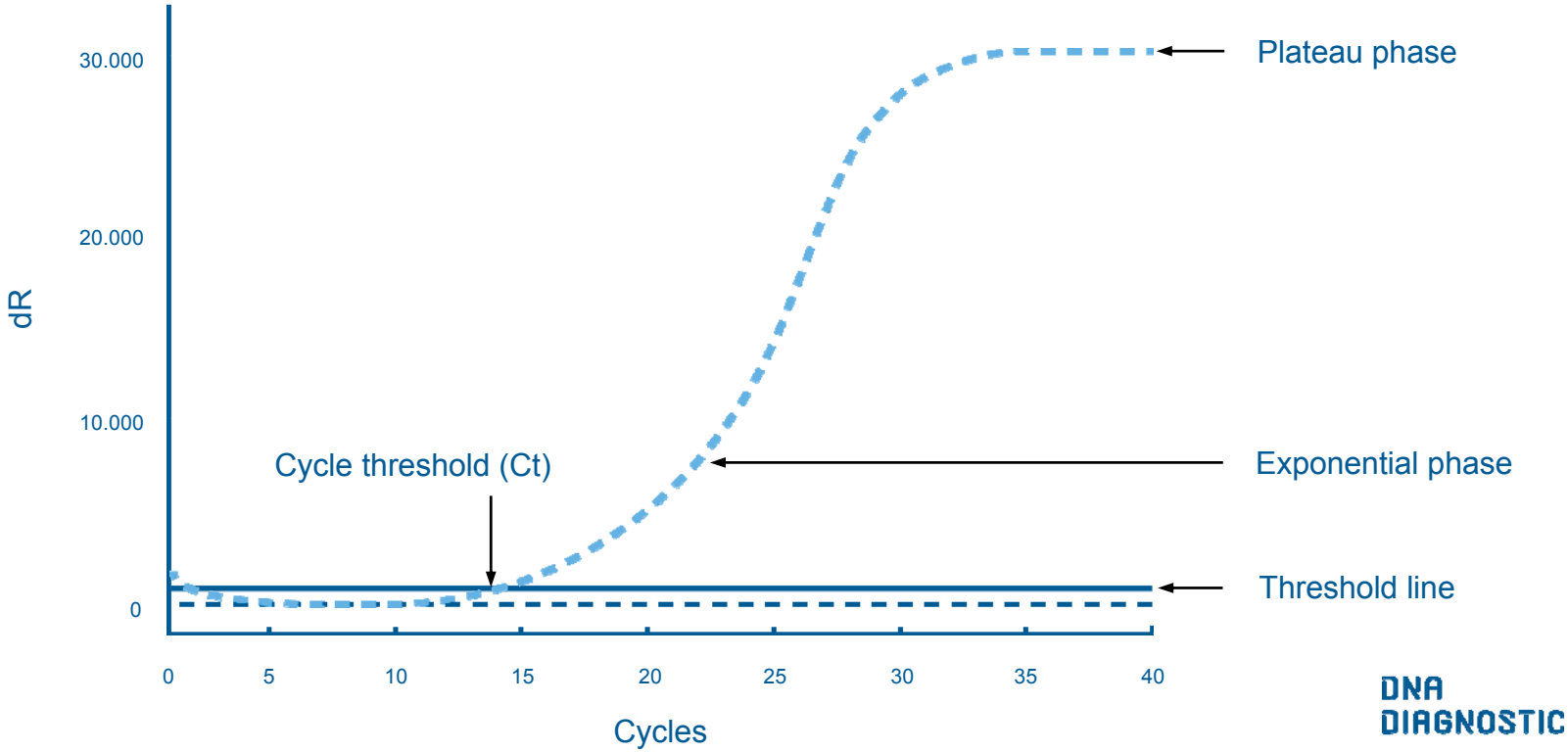
Amplification plot from qPCR



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Easy and fast workflow

2 x 96 samples

DNA extraction

0.5 ml samples are pelleted and directly lysed for DNA extraction

qPCR

Run Mastit 4 program

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Mastit 4

Target / Cat. No.*	M4A	M4B	M4C	M4D	M4E	M4F	M4BD	M4BDF
Staphylococcus aureus	+	+	+		+		+	+
Streptococcus agalactiae	+	+	+		+		+	+
Streptococcus dysgalactiae	+			+			+	+
Streptococcus uberis	+	+					+	+
Mycoplasma bovis		+	+		+		+	+
Mycoplasma species			+	+			+	+
β-Lactamase gene (blaZ gene)				+			+	+
Coagulase Negative Staphylococcus				+			+	+
Prototheca					+	+		+
E.coli						+		+
Klebsiella						+		+
Enterococcus and Lactococcus lactis						+		+

Advice to farmer after result of PCR test of Bulk Tank Milk

Mycoplasma bovis

Segregation of clinical cases, test, and culling

Staphylococcus aureus, Streptococcus agalactiae and Prototheca

5 point plan, good milking, post milk teat dipping, dry cow therapy, quick therapy of new cases, culling, milking with gloves

Streptococcus uberis, Enterococcus, E-coli, Klebsiella

Clean cows, foam before milking, fresh food for standing cows after milking, clean bedding, deep bedding with more space – specially in the fresh cow pen

Streptococcus dysgalactiae

Better skin quality of teats, conditioner in post milk teat dipping, virus infections at teats

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Mastit 4 – qPCR test

Bulk tank testing

- Screening for contagious pathogens — nr. of test in DK > 10,000
- Selection of herds for selective dry cow therapy

DHI samples

- High cell count cows - Prevalence of contagious mastitis
- Selective dry cow therapy — nr. of test in DK > 100,000
- Therapy - Wrong time in relation to pathogen - environmentals

Quarter samples aseptically

- Therapy of infection

Mastit 4 – qPCR test

Bulk tank testing

Screening for contagious pathogens

Selection of herds for selective dry cow therapy

nr. of test in DK > 10,000

DHI samples

High cell count cows - Prevalence of contagious mastitis

Selective dry cow therapy

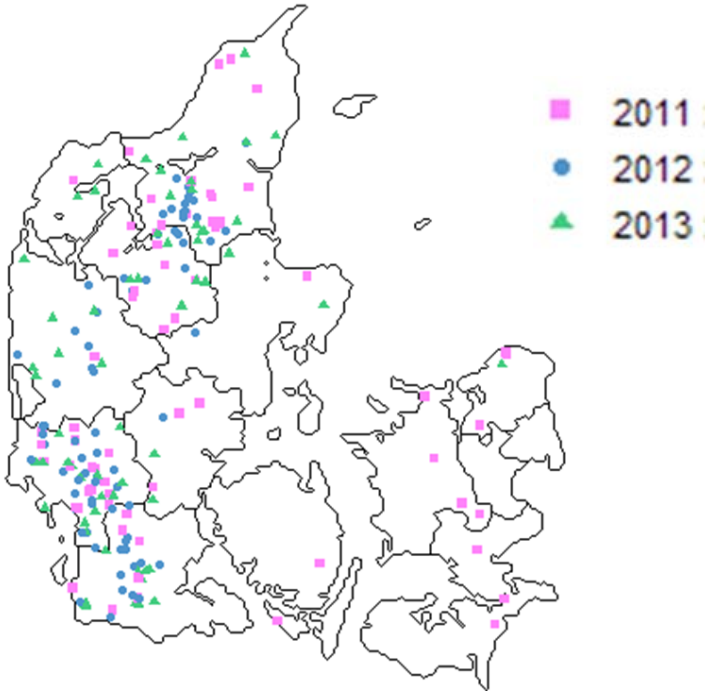
Therapy - Wrong time in relation to pathogen - environmentals

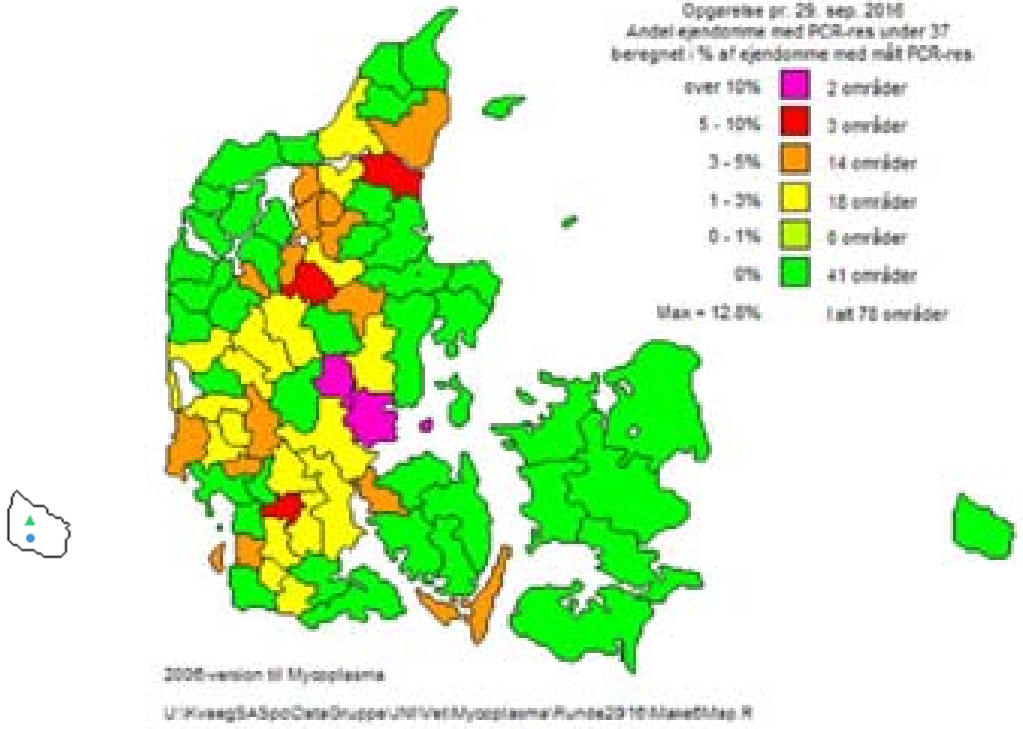
nr. of test in DK > 100,000

Quarter samples aseptically

Therapy of infection

Mycoplasma PCR from Danish herds
all herds tested once every year since 2011





Mastit 4 – qPCR test

Bulk tank testing

Screening for contagious pathogens nr. of test in DK > 10,000

Selection of herds for selective dry cow therapy

DHI samples

High cell count cows - Prevalence of contagious mastitis

Selective dry cow therapy nr. of test in DK > 100,000

Therapy - Wrong time in relation to pathogen - environmentals

Quarter samples aseptically

Therapy of infection

How to interpretate Ct values in DHI Contaminated samples ?

Contagious Pathogens - OK

But we do recommend retest of aseptic sample before culling

Environmental pathogens - clinical important Ct < 20 – 25

DHI samples positive for Enterococcus/Lactococcus

All but one negative in aseptically quarter samples

PK	Enter	25.17
Sample 01	Enter	No Ct
Sample 02	Enter	31.32
Sample 03	Enter	27.96
Sample 04	Enter	32.45
Sample 05	Enter	No Ct
Sample 06	Enter	29.34
Sample 07	Enter	30.93
Sample 08	Enter	27.31
Sample 09	Enter	No Ct
Sample 10	Enter	31.33
Sample 11	Enter	No Ct
Sample 12	Enter	No Ct
Sample 13	Enter	29.41
Sample 14	Enter	31.83
Sample 15	Enter	No Ct
Sample 16	Enter	29.65
Sample 17	Enter	No Ct
Sample 18	Enter	No Ct
Sample 19	Enter	31.84
Sample 20	Enter	28.67
Sample 21	Enter	28.42
Sample 22	Enter	32.88
Sample 23	Enter	33.42
Sample 24	Enter	31.98
Sample 25	Enter	No Ct
Sample 26	Enter	No Ct
Sample 27	Enter	31.22
Sample 28	Enter	30.29
Sample 29	Enter	30.58
Sample 30	Enter	32.28
Sample 31	Enter	30.93
Sample 32	Enter	30.01
Sample 33	Enter	No Ct
Sample 34	Enter	25.50
Sample 35	Enter	32.07
Sample 36	Enter	32.22

Mastit 4 – qPCR test – You always get Mycoplasma

Bulk tank testing

Screening for contagious pathogens

nr. of test in DK > 10,000

Selection of herds for selective dry cow therapy

DHI samples

High cell count cows - Prevalence of contagious mastitis

Selective dry cow therapy

nr. of test in DK > 100,000

Therapy - Wrong time in relation to pathogen - environmentals

Quarter samples aseptically

Therapy of infection

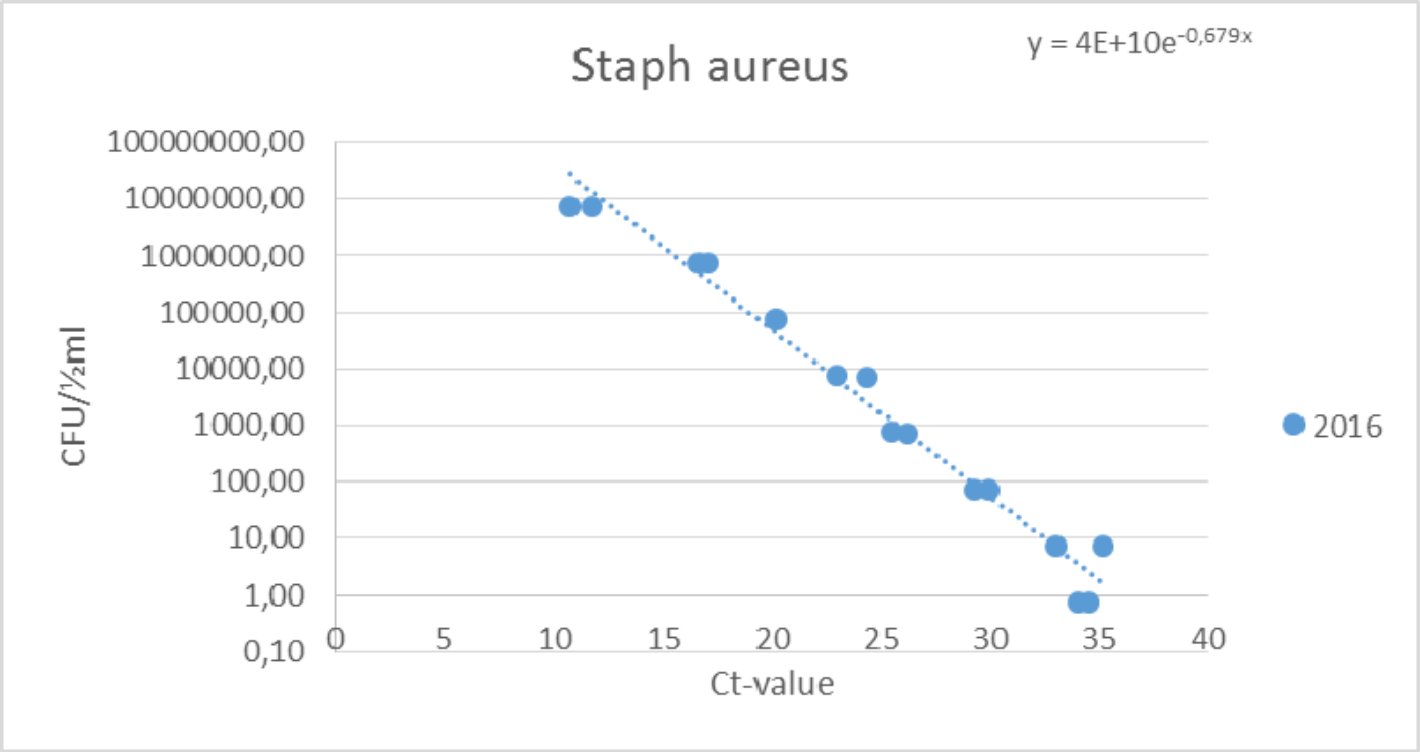
Mastitis therapy – Lactation – Test before treatment

Subclinical	Only treat after test	Staph, Streps and Ent
Mild	Only treat after test	Staph, Streps and Ent
Moderate	Only treat after test	Staph, Streps and Ent

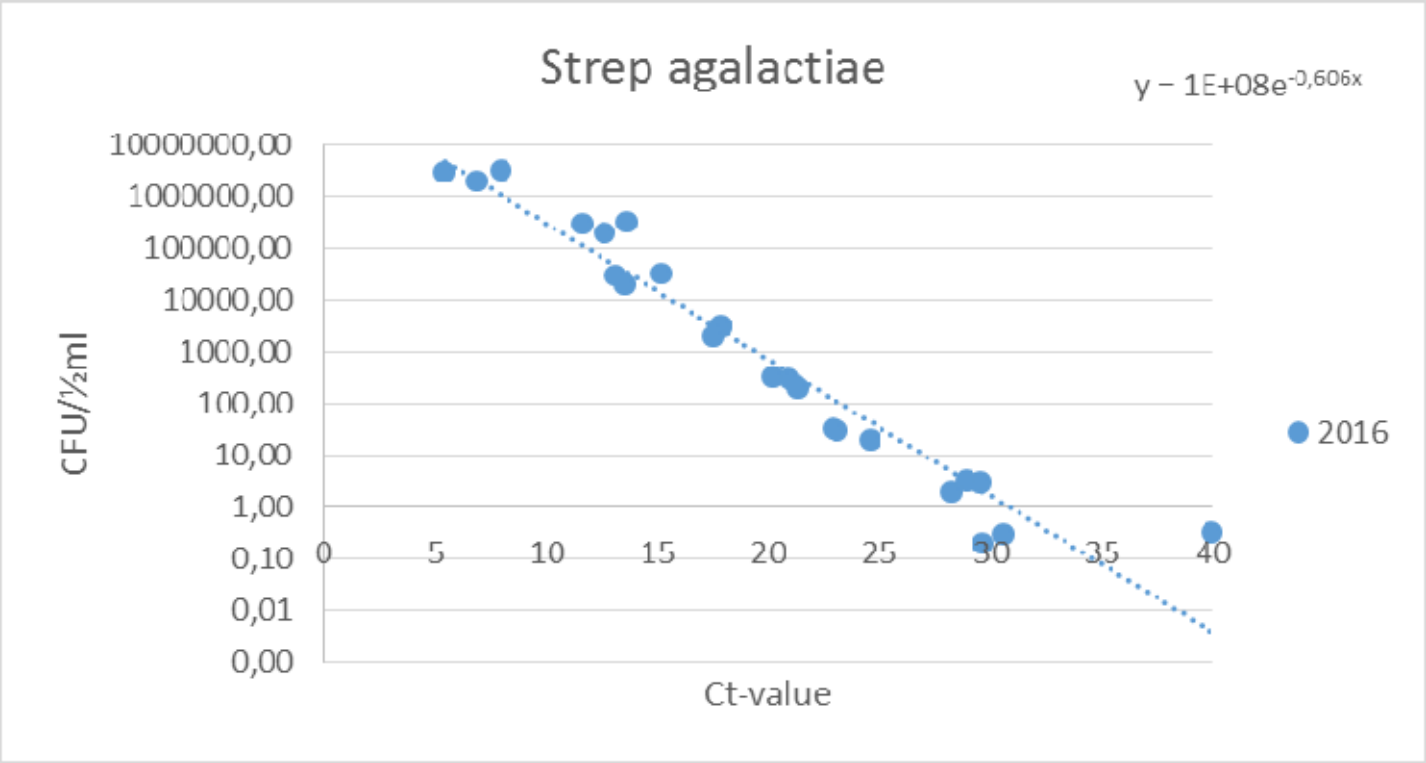
More than 60% not treated Nydam et al. 2015

Severe	Supportive therapy, painkiller, fluid	Antibiotic
		Staph, Streps and Klebsiella

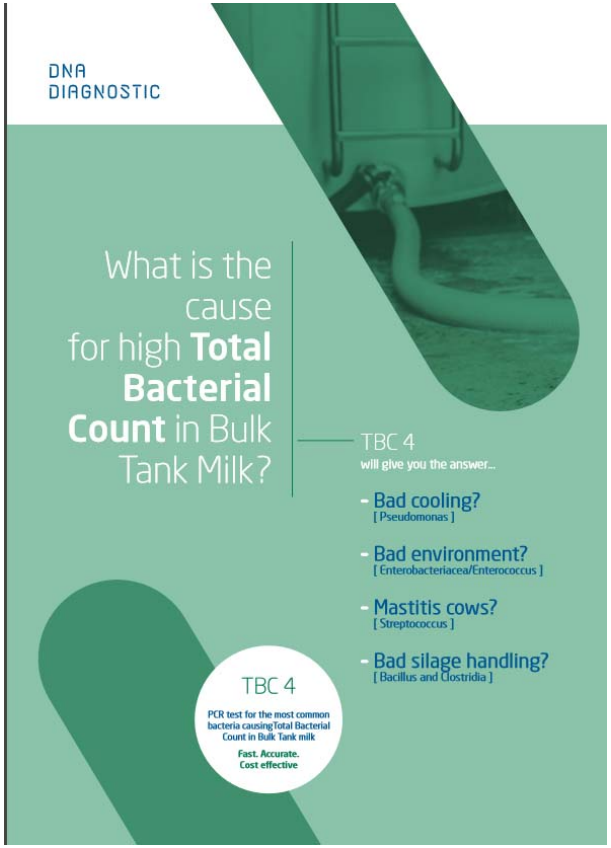
Correlation curve for Mastit 4 – Ct value and CFU Staphylococcus aureus – 2 isolates

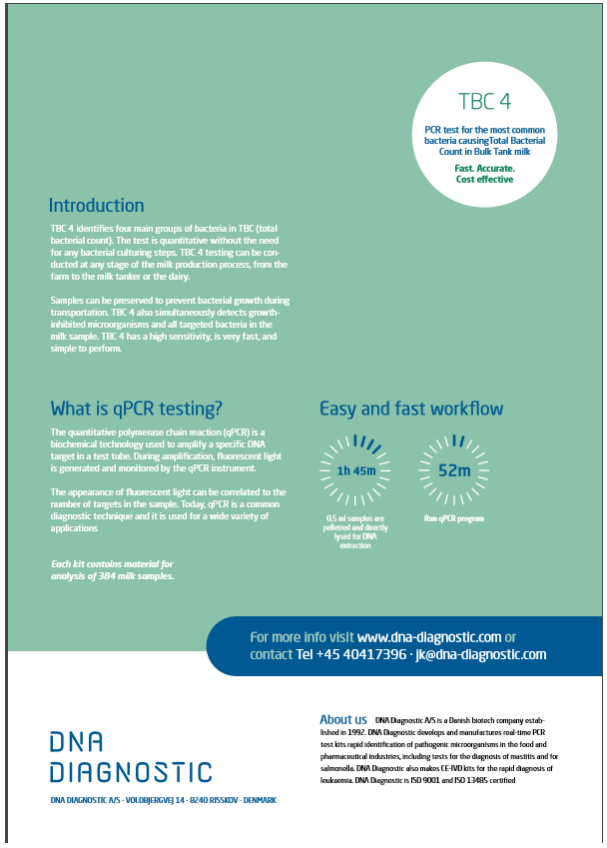


Correlation curve for Mastit 4 – Ct value and CFU Streptococcus agalactiae – 3 isolates



New qPCR test for high TBC counts – TBC 4





High TBC counts – TBC 4 indicate answer to the problem

- Bad cooling?
  [Pseudomonas]
- Bad environment?
  [Enterobacteriacea/Enterococcus]
- Mastitis cows?
  [Streptococcus]
- Bad silage handling?
  [Bacillus and Clostridia]

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Mastit 4
TBC 4

Good partners for better milk quality