

Comparison of Gen-Probe TMA, Abbott real-time PCR, and Roche PCR for the detection of wild-type and mutant plasmid strains of *Chlamydia trachomatis* in Sweden



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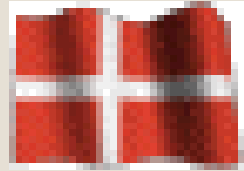


Department of Clinical Microbiology

C. trachomatis – the clinical perspective!



Agnete and the Merman
Sculpture/fountain by the City Hall in Aarhus



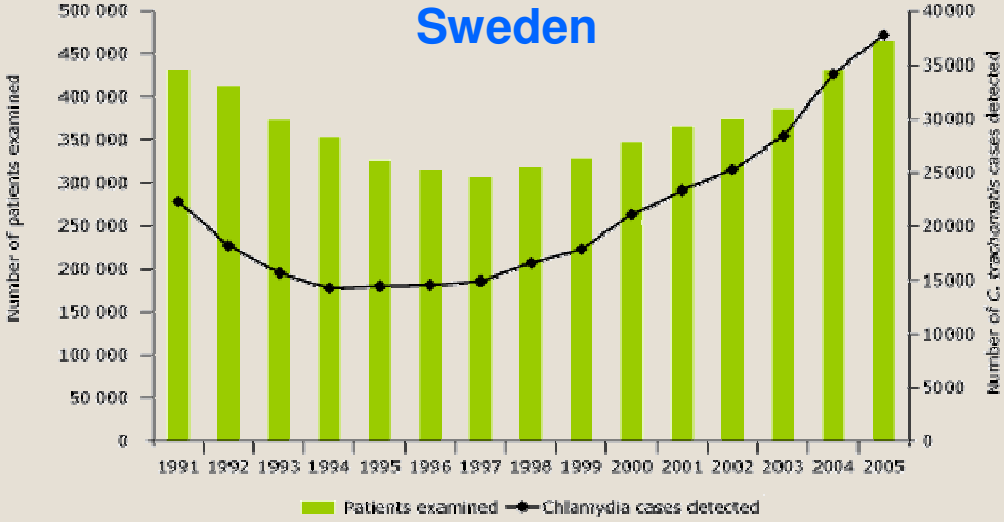
Hans Christian Andersen



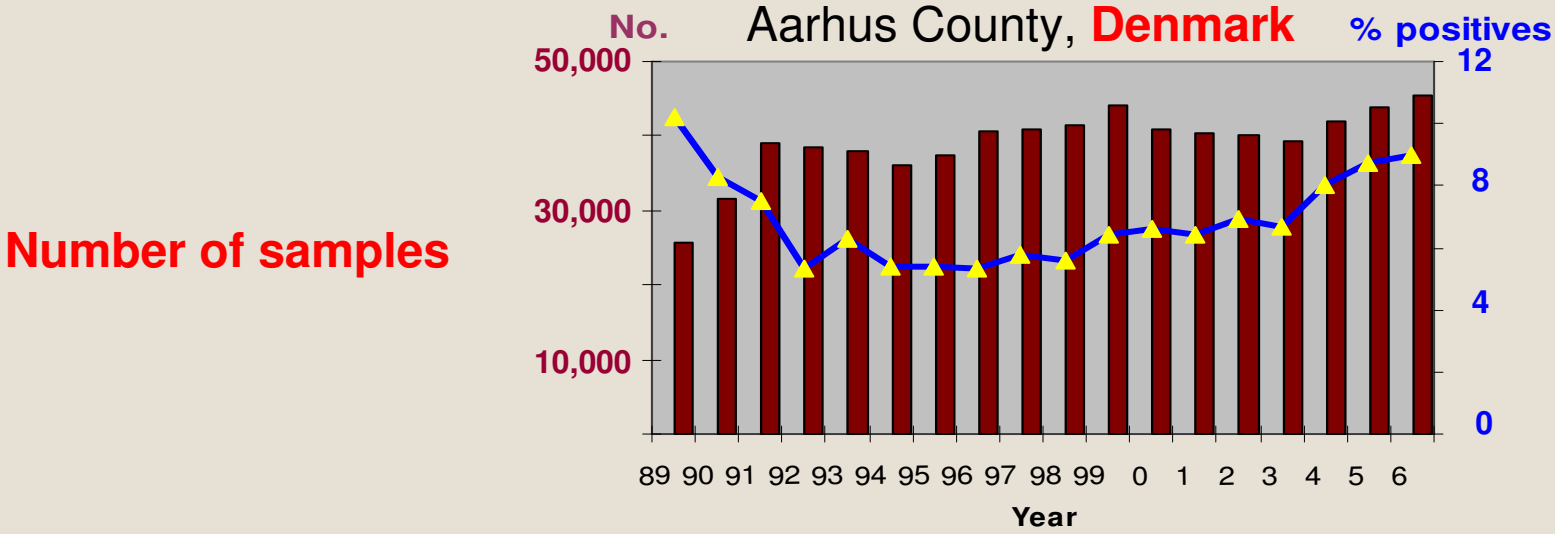
Jenny Lind



C. trachomatis – the clinical perspective in Scandinavia!



Number of patient cases



Number of samples

The Swedish mutant CT

..... From mid-September to October 2006, the county microbiology laboratory in Halmstad, Halland county, tested 1700 consecutive incoming specimens with a MOMP-specific PCR in parallel with Abbotts m2000 plasmid PCR. In 13% of all diagnosed *C. trachomatis* cases in Halland county during this period (24/186), we found a variant strain that was only positive in MOMP tests. Clinical data indicates no difference from infections with the wild type strains.....

T Ripa, P Nilsson

A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests.

Euro Surveill. 2006 Nov ;11 (11):E061109.2 17213548

The Swedish mutant CT

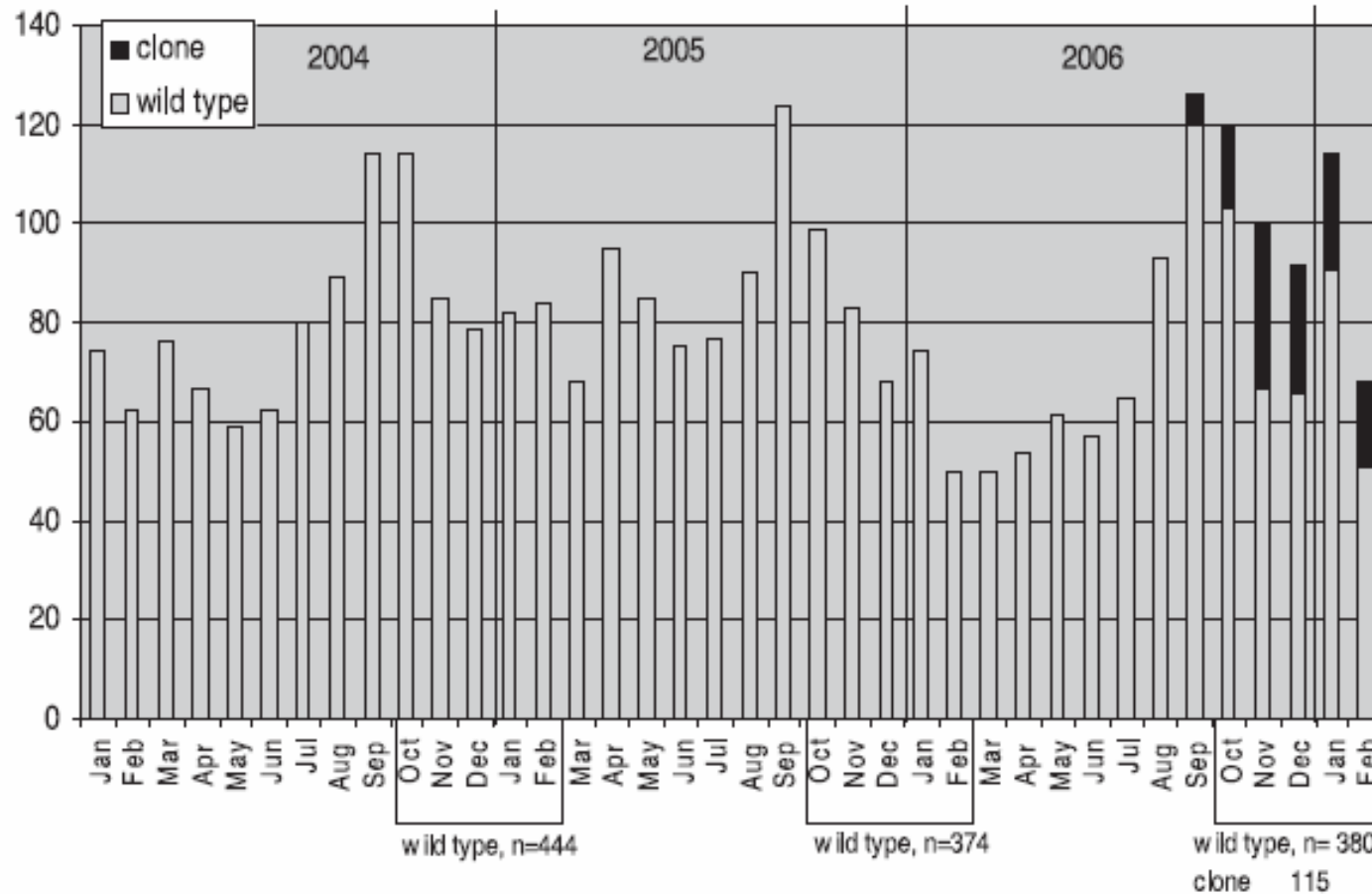
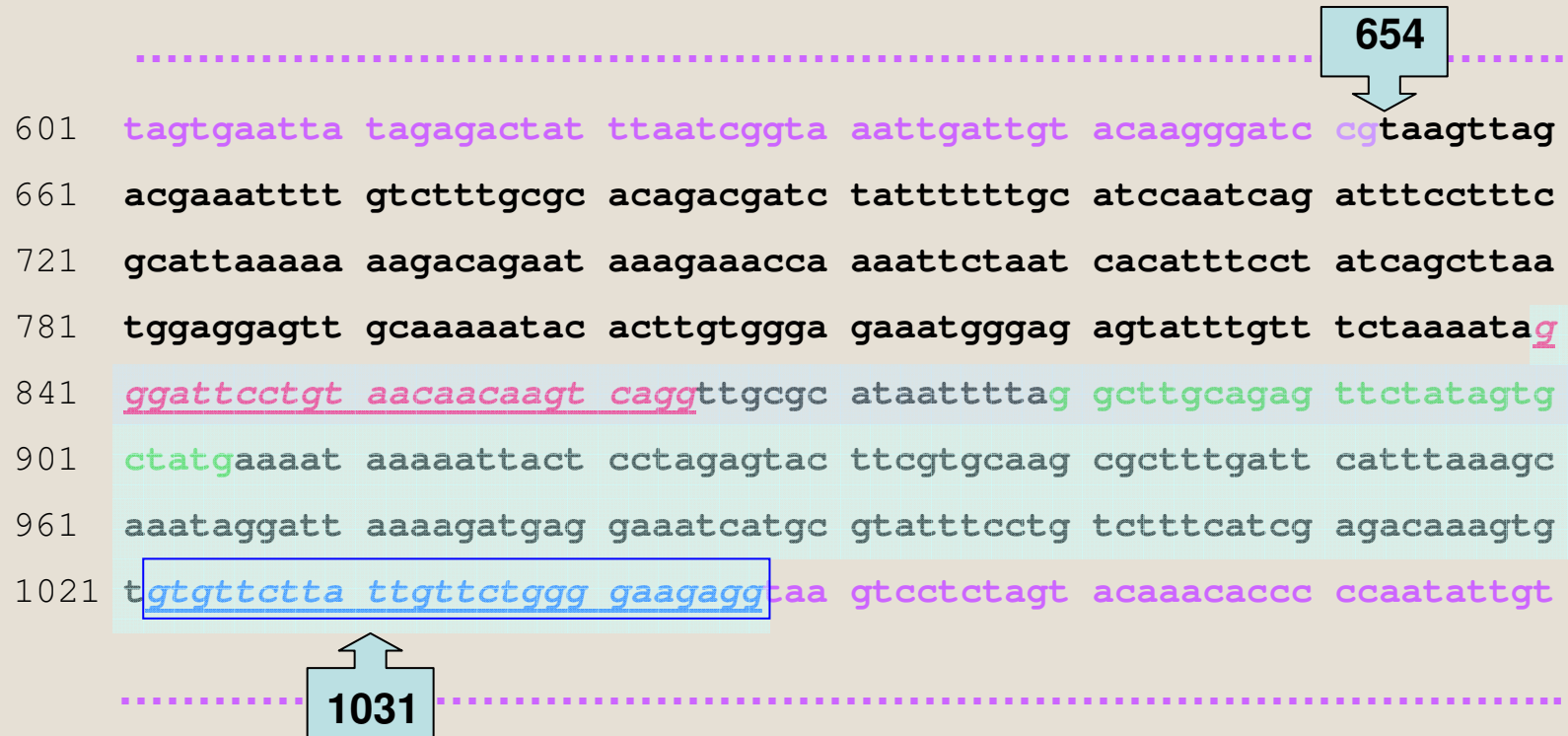


Fig. 1. Number of diagnosed *Chlamydia trachomatis* infections by PCR in Halland County monthly from January 2004 through February 2007. The 5-months period, October to February, is highlighted, giving the numbers of wild-type infections and the mutant-strain infections.

Ripa & Nilsson , Sex Transm Dis. 2007 May;34(5):255-6.

The Swedish mutant CT

(Deletion of 377-bp, from position 654 to 1031) *



Roche PCR amplicon

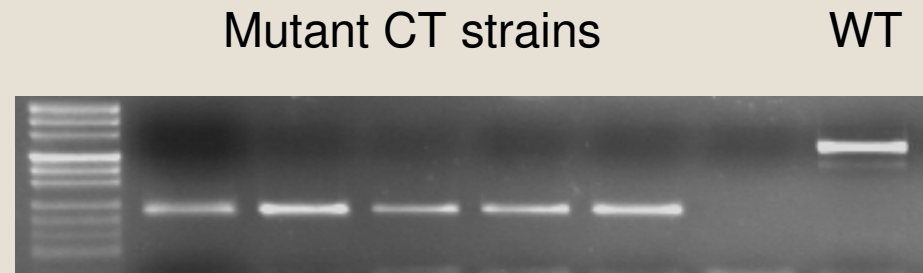
* Ripa & Nilsson , Sex Transm Dis. 2007 May;34(5):255-6.

Mutant PCR

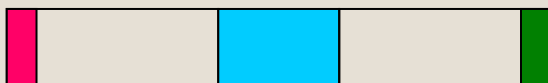
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601 tagtgaatta tagagactat ttaatcggta aattgattgt acaagggatc cgtaagttag
661 acgaaatttt gtctttgccc acagacgacg tattttttgc atccaatcag atttcctttc
721 gcattaaaaa aagacagaat aaagaaacca aaattcctaa cacatttctc atcagcttaa
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1081 gatataatta aaattatatt catattctgt tgccagaaaa aacactttta ggctatatta
1141 gagccaatct tctttgaagc gttgtcttct cgagaagatt tatcgtagc aaatatcatc
1201 tttgcggttg cgtgtcctgt gaccttcatt atgtcggagt ctgagcacc taggcgtttg
    
```

CT cryptic plasmid, **deleted sequence** marked



Mutant *C. trachomatis*



Wild-type *C. trachomatis*

PCR-products

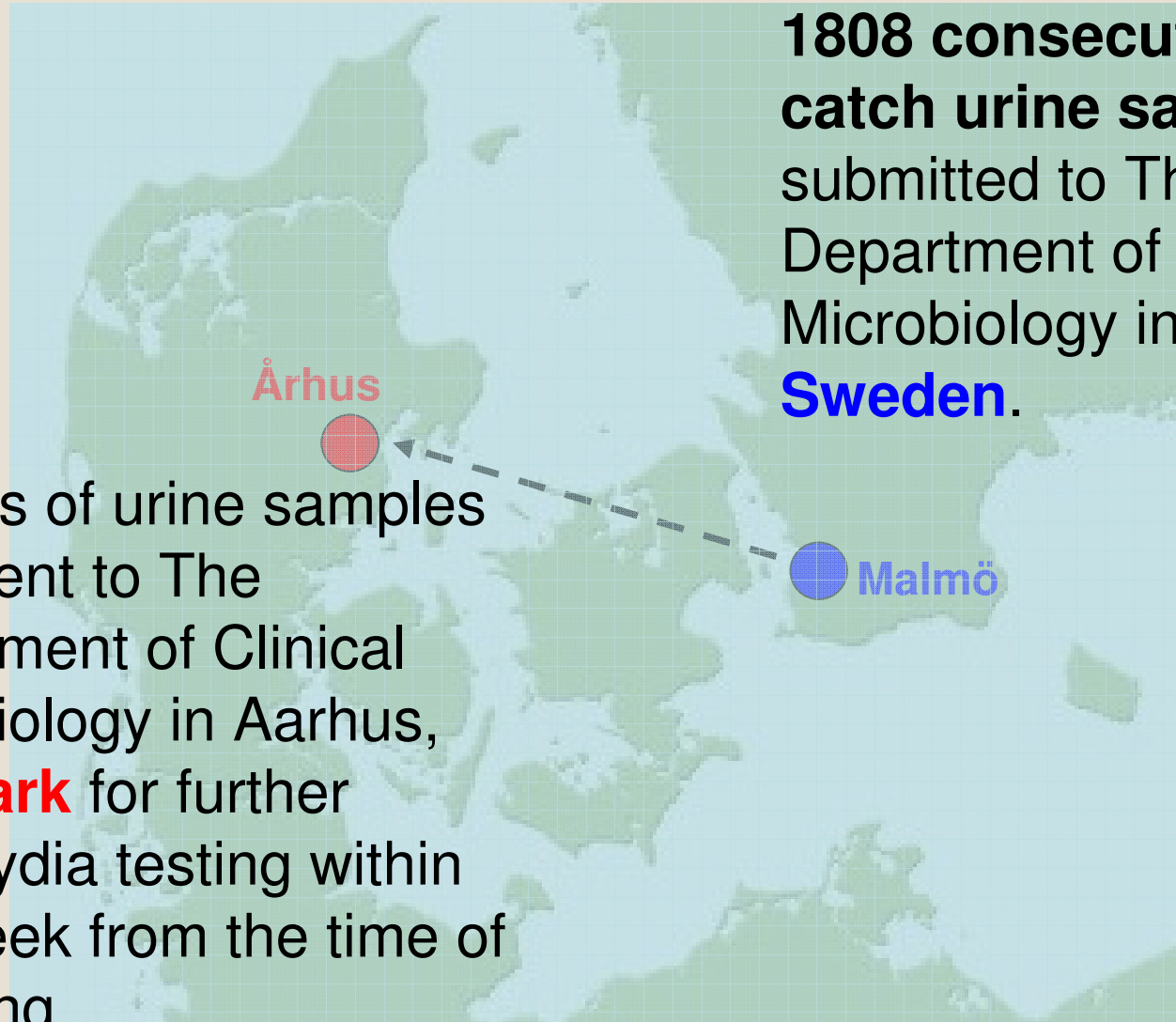
Aim of study

- ❖ To compare the clinical performance of the GEN-PROBE APTIMA Combo 2 assay (AC2) with the Roche Cobas Amplicor and the Abbott m2000 assay.
- ❖ To determine the clinical performance of the three assays with the Swedish mutant CT strains.

Material

1808 consecutive first catch urine samples submitted to The Department of Clinical Microbiology in Malmö, **Sweden**.

Aliquots of urine samples were sent to The Department of Clinical Microbiology in Aarhus, **Denmark** for further Chlamydia testing within one week from the time of sampling.



Material

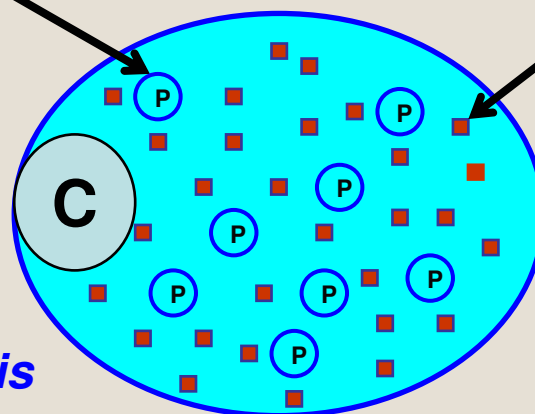
- **Female** urine samples: **68 %**
(average age of females: 25 years)
- **Male** urine samples: **32 %**
(average age of males: 26 years)

Methods

| Malmoe | | Aarhus | |
|--------------|-----------------|------------|-----------|
| m2000 | Abbott | AC2 | Gen-Probe |
| Mutant PCR | <i>In-house</i> | ACT | Gen-Probe |
| | | RCA | Roche |

The cryptic plasmid is the "target"

23S rRNA is the "target"



"Biological amplification"

1000 : 10 : 1

RNA : plasmid : chromosome

Chlamydia trachomatis

Definition of “true positives”

A **combined reference standard** was used that defines a sample as true positive if **at least 2 of the 4 commercial CT assays** employed were found positive.

Samples with discrepant test results (**AC2 +, m2000 -, and RCA -**) were examined with an in-house PCR for the presence of a plasmid with the 377-bp deletion.

Patterns of test results

| No. samples | True positive | m2000 Abbott | RCA Roche | AC2 Gen-Probe | ACT | Mutant PCR |
|-------------|---------------|--------------|-----------|---------------|-----|------------|
| 102 | + | + | + | + | + | ND |
| 10 | + | + | - | + | + | ND |
| 2 | + | - | + | + | + | ND |
| 5 | + | - | - | + | + | - |
| 44 | + | - | - | + | + | + |
| 2 | - | - | + | - | - | ND |
| 1 | - | + | - | - | - | ND |
| 1 | - | - | - | - | + | ND |
| 1645 | - | - | - | - | - | ND |
| 1808 | 163 | 113 | 106 | 163 | 164 | 44 |

Results

- Positive sample prevalence:

9 % (163/1808)

- Prevalence of mutant strains:

27 % (44/163)

GEN-PROBE APTIMA COMBO 2

| | AC2 + | AC2 - | |
|--------|------------|-------------|------|
| True + | 163 | 0 | 163 |
| True - | 0* | 1645 | 1645 |
| | 163 | 1645 | 1808 |

*5 initially positive samples were repeatedly negative by retest

All positive samples are routinely retested in our lab.

ROCHE COBAS AMPLICOR

| | RCA + | RCA - | |
|--------|------------|-------------|------|
| True + | 104 | 59* | 163 |
| True - | 2 | 1643 | 1645 |
| | 106 | 1702 | 1808 |

* 44 mutant strains

ABBOTT m2000

| | m2000 + | M2000 - | |
|--------|------------|-------------|------|
| True + | 112 | 51* | 163 |
| True - | 1 | 1644 | 1645 |
| | 113 | 1695 | 1808 |

* 44 mutant strains

GEN-PROBE APTIMA CT#

| | ACT + | ACT - | |
|--------|------------|-------------|------|
| True + | 163 | 0 | 163 |
| True - | 1* | 1644 | 1645 |
| | 164 | 1644 | 1808 |

Confirmatory assay targeting another target than AC2

* Repeatedly positive sample

All samples

| Assay | Sensitivity | | Specificity | |
|-------|-------------|---------|-------------|-----------|
| | % | No. | % | No. |
| AC2 | 100.0 | 163/163 | 100.0 | 1645/1645 |
| ACT | 100.0 | 163/163 | 99.9 | 1644/1645 |
| m2000 | 68.7 | 112/163 | 99.9 | 1644/1645 |
| RCA | 63.9 | 104/163 | 99.9 | 1643/1645 |

All samples but mutants

| Assay | Sensitivity | |
|-------|-------------|---------|
| | % | No. |
| AC2 | 100.0 | 119/119 |
| ACT | 100.0 | 119/119 |
| m2000 | 96.6 | 112/119 |
| RCA | 88.8 | 104/119 |

Conclusions

The **Gen-Probe Aptima Combo 2** assay for the detection of CT is highly sensitive and specific irrespectively of the presence of the Swedish mutant CT strain.

The **Roche Cobas Amplicor** plasmid PCR and the new **Abbott m2000** real-time PCR plasmid PCR cannot detect the mutant CT strain, and thus have a poor clinical sensitivity in an epidemiological situation with an increasing number of CT strains harbouring the plasmid mutant.

Aptima Combo 2 is more sensitive than **m2000** and **Cobas Amplicor** in detection of wild-type CT strains in urine samples.

Acknowledgements

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