# DETECTION OF *CHLAMYDIA TRACHOMATIS* BY MOLECULAR BIOLOGICAL METHODS IN LATVIA

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# Microbiology

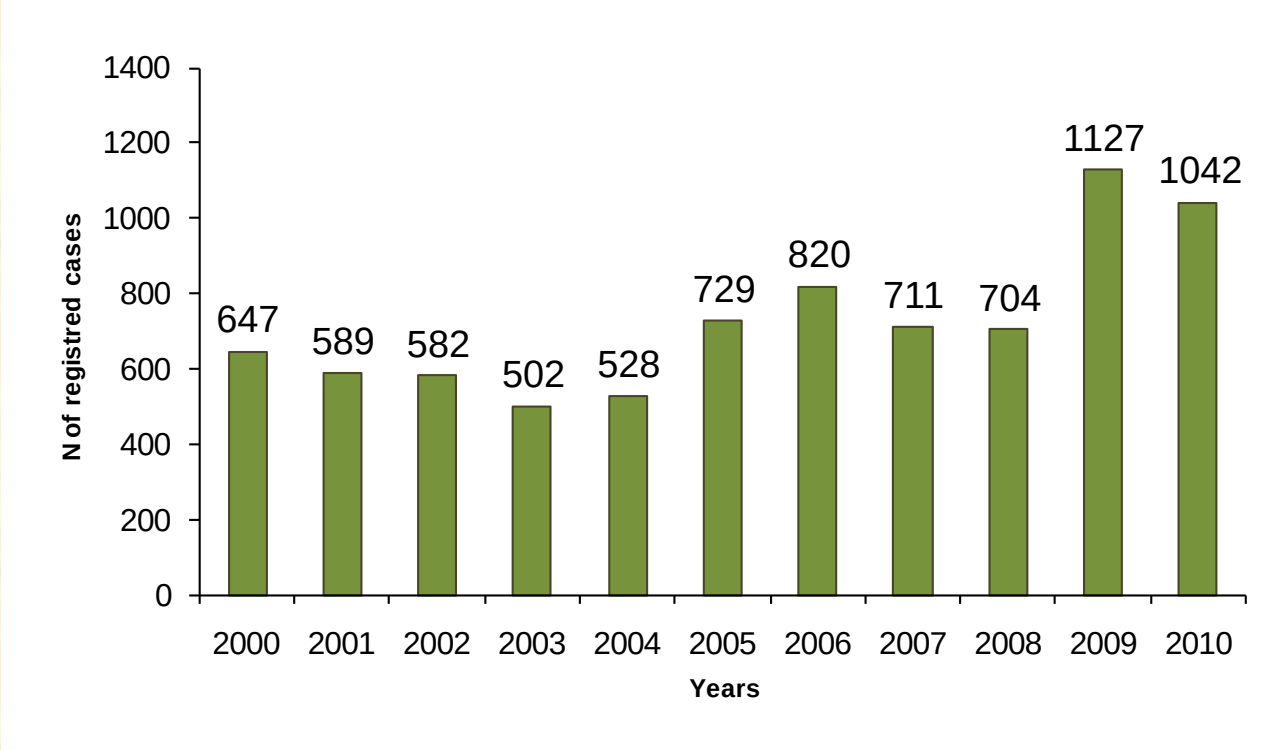
- ***Chlamydia trachomatis*** is obligate intracellular Gram negative bacteria
- Unique biphasic (dimorphic) developmental cycle within an intracellular cytoplasmic inclusion
- *C. trachomatis* serovars/genovars (based on antigenic diversities of major outer membrane protein [MOMP*]/ompA* gene)

| Serovar | Disease | Distribution |
|---|---|---|
| A B Ba C | Trachoma | Asia and Africa |
| D - K | ***Disease of eye and genitals****:* conjunctivitis, urethritis, cervicitis<br><br>***Respiratory System:*** Infant pneumonia | World wide |
| LGV1 LGV2 LGV3 | Lymphogranuloma venerium | World wide |

# Epidemiology of Chlamydia 1

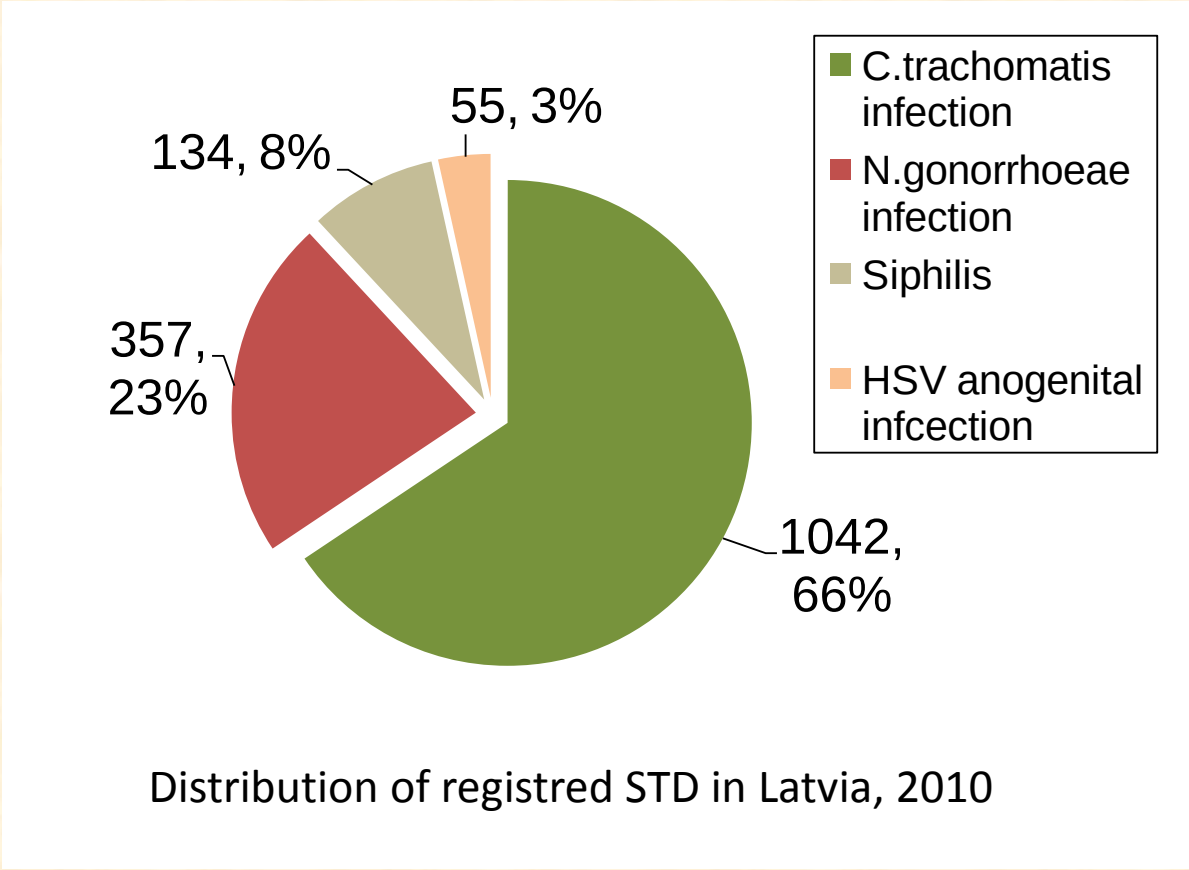
**Reporting of *Chlamydia trachomatis* infections in Latvia started in 1992**

The total number of reported cases of *C.trachomatis* infections in Latvia, 2000-2010



# Epidemiology of Chlamydia 2

Chlamydia is the most frequently reported sexually transmitted infection in Latvia




Distribution of registred STD in Latvia, 2010

# Diagnostic methods for detection of *C. trachomatis* used in Latvia

**Since early 1990s:**

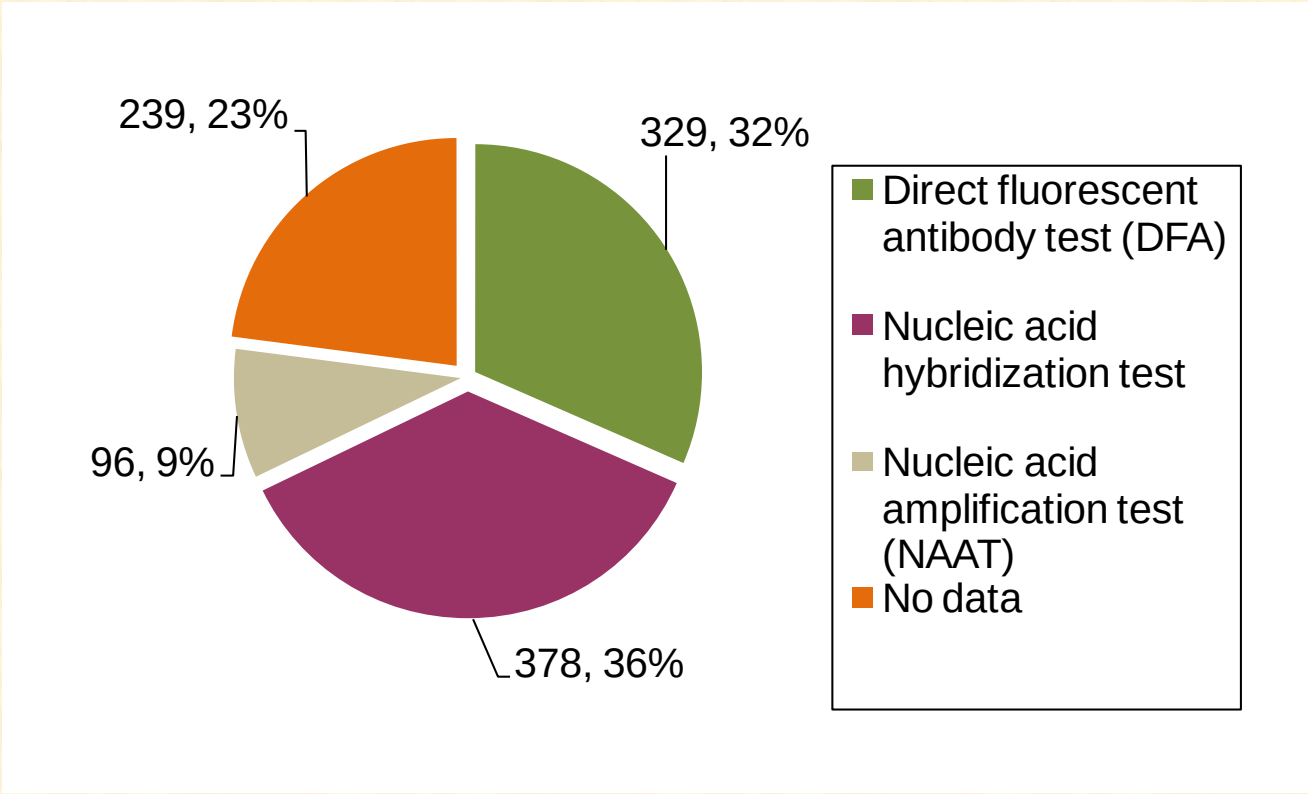
- **ELISA (EIA**) tests for antibodies detection
- **ELISA (EIA**) tests for antigen detection
  - MOMP-Major Outer Membrane Protein
  - LPS -lipopolysaccharide
- DFA (Direct fluorescent antibody) tests (MOMP)

**Since 1996s -molecular biological methods were used:**

- Nucleic acid hybridization test or DNA-probe test (Gen-Probe, PACE2)
- NAAT (nucleic acid amplification techniq) –Cobas Amplicor CT, Roche

Culture in viable cell lines was not performed

# Diagnostic methods for detection of *C.trachomatis* used in Latvia in 2010 (for 1042 registred cases)

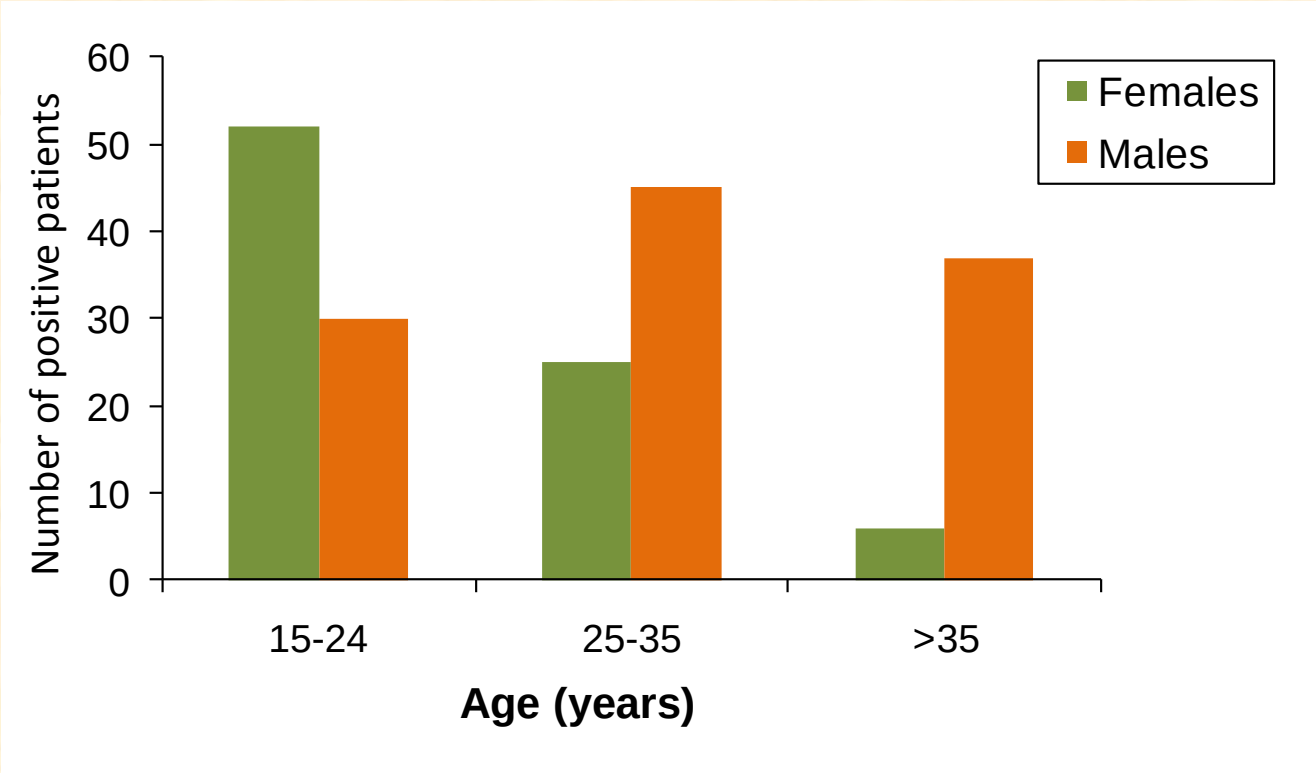


# Methods for confirmation of *Chlamydia* infection used in ICL, 2010

| Methods | Patient (n) | | | Positive for *C.trachomatis* | | |
|---|---|---|---|---|---|---|
| | male | female | total | male | female | total |
| DFA (MicroTrak Trinity Biotech) | 2065 | 1764 | 3829 | 112/2065<br>**5.4%** | 83/1764<br>**4.7%** | 195/3829<br>**5.1%** |
| Hibridization assay (Gene Probe, PACE2) | 15 | 111 | 126 | 1/15<br>**6.0 %** | 7/111<br>**6.3%** | 8/126<br>**6. 3%** |

Both methods were successful in EQA programme Labquality

# DFA positive for *C.trachomatis* male and female by age groups




Groups with highest rate of positive results were:

- **women 15 – 24 years old;**
- **men 25 – 35 years old.**

# Consideration for choose of NAAT method for detection of C.trachomatis in ICL

1. After STD department inclusion in ICL structure in 2010 method more suitable for large volume testing was required

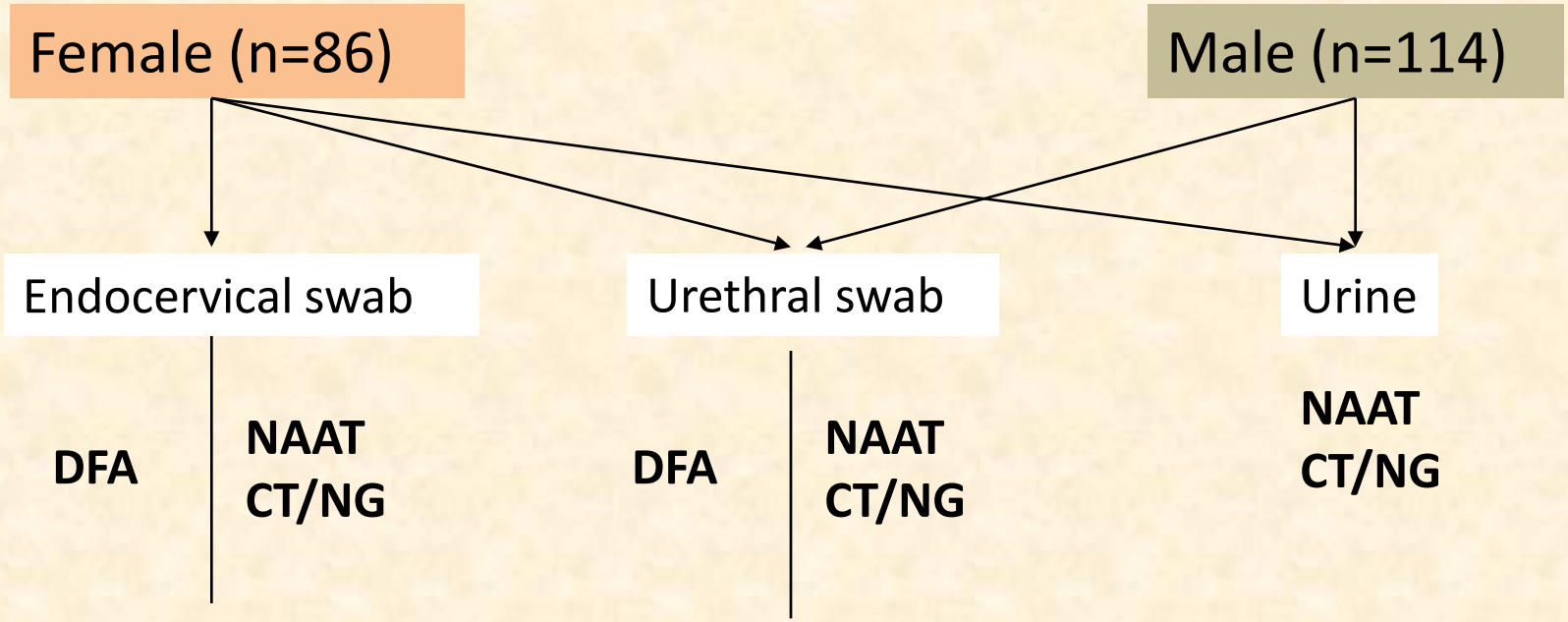
2. According to published data, NAAT are the most sensitive (90-95%) tests for genital Chlamydia, DFA have sensitivity 65-75% and depends on the skills of laboratory specialist

3. The prevalence of Swedish mutant (C.trachomatis with 377 bp deletion in cryptic plasmid) in Latvia is unknown. That is why we try two tests: Aptima Combo 2 Assay (Gene-Probe) with target rRNA and Cobas Amplicor CT (Roche) with target cryptic plasmid

4. Costs of tests

# Study design

**Patients: 200 symptomatic STD outpatients department attenders were included**




1. **DFA test (MicroTrak Trinity, Biotech)**
2. **NAAT tests:**

   - Gen-Probe, Aptima Combo 2, isothermal amplification (TC-TMA)

   -Roche, Cobas Amplicor CT/NG, PCR

# Comparison of DFA and NAAT tests for *C.trachomatis* testing

| Patients | Sample | DFA | | | | Gen-Probe Aptima Combo2 | | | Cobas Amplicor | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nr tested | Positive nr | Positive % | +/- | Nr tested | Positive nr | Positive % | Nr tested | Positive nr | Positive % |
| Male N=114 | Urethral swab | 114 | 12 | 10.5 | - | 114 | 14 | 12.3 | - | | |
| | Urine | - | - | | - | 114 | 15 | 13.1 | 45 | 6 | 13.3 |
| Female N=86 | Endocervical swab | 79 | 4 | 5.1 | 1 | 79 | 7 | 8.9 | - | | |
| | Urethral swab | 85 | 3 | 3.5 | 2 | 85 | 8 | 9.4 | - | | |
| | urine | - | - | | - | 85 | 8 | 9.4 | 34 | 3 | 8.8 |

# Comparison of NAAT tests with DFA: discordant results

| Gender | GEN-PROBE Aptima Combo2 | | | Cobas Amplicor CT | DFA | |
|---|---|---|---|---|---|---|
| | Urethral swab | Endocervical swab | Urine | Urine | Urethral swab | Endocervical swab |
| Female | negative | positive | positive | NA | **not detected** | **not detected** |
| | positive | positive | positive | positive | **not detected** | **not detected** |
| | positive | positive | positive | positive | **not detected** | **not detected** |
| | positive | positive | positive | positive | uncertain | uncertaine |
| | positive | positive | NA | NA | uncertain | detected |
| Male | **negative** | - | **positive** | **positive** | **not detected** | |
| | **negative** | - | **positive** | - | **not detected** | - |
| | **positive** | - | **positive** | **positive** | **not detected** | |

# Chlamydia infection diagnosed by DFA and NAAT tests

| | DFA | Gene-Probe Aptima Combo2* | Cobas Amplicor CT/NG* |
|---|---|---|---|
| **Male** | 10.5% (12/114) | 13.2% (15/114) | 13.3%(6/45) |
| **Female** | 4.7% (4 /86)<br>7.0 % ( 6/86) | 9.3% (8/86) | 8.8% (3/34) |
| **Total** | 8% (16/200)<br>9% (18/200) | 11.5% (23/200) | 11.4% (9/79) |

*Only in one male patient *N.gonorrhoeae* was detected

# Conclusions

- 474/1042 (45.5%) cases of *Chlamydia* infection registered in Latvia in 2010 were confirmed by molecular biological methods, including 9.4% by NAAT.
- Most commonly used tests were DFA (329/1042, 32 %) and DNA probe assays (378/1042, 36 %).
- Comparison of DFA and NAAT tests, performed in ICL, demonstrated higher sensitivity of NAAT: rate of positive results by DFA was 8%, by NAAT - 11.4%. In our study on limited number of specimen Aptima Combo (rRNA) and Cobas Amplicor (DNA) demonstrated same rate of positivity
- In all patients with positive by NAAT swab specimens, urine samples were positive too.
- Higher sensitivity of NAAT tests, simultaneous detection of

  *C. trachomatis* and *N. gonorrhoeae*, possibility to use for testing non invasive clinical samples, make it suitable for improvement of *C.trachomatis* diagnosis for large volume testing.

# Thank you for your attention!