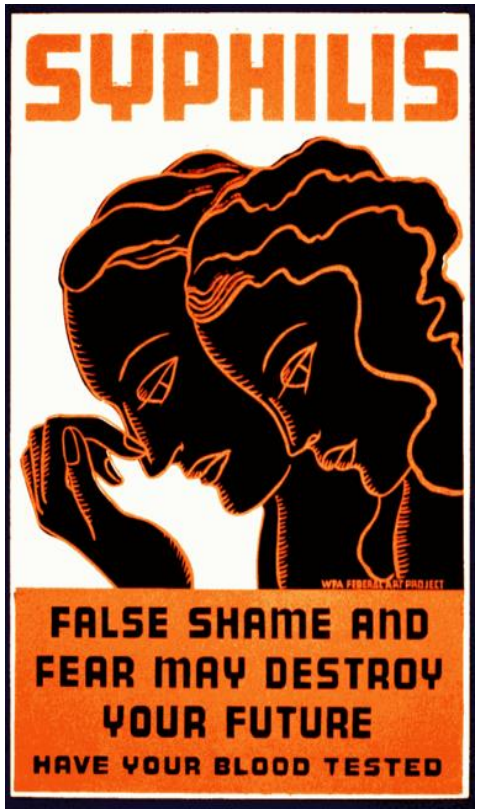
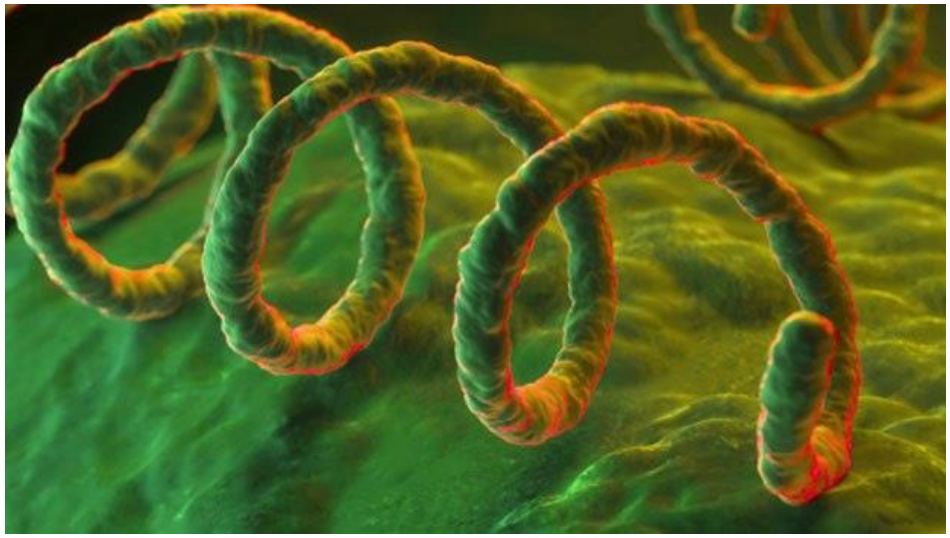
The Great Imitator: reverse testing algorithm in focus

1936





Kamran Kadkhoda, Ph.D, FCCM, D(ABMM), D(ABMLI)

Clinical Microbiologist, Cadham Provincial Public Health Laboratory

Assistant Professor, Dept. Medical Microbiology & Infectious Diseases; Dept. Immunology, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba

Introduction

- Syphilis has been known for centuries as the "Great imitator" → caused by a spirochete: *Treponema pallidum ssp. pallidum*
- Transmission typically happens during primary and secondary syphilis from highly infectious lesions (ID_{50}: 57 organisms)
- Congenital transmission: 1) trans-placentally; 2) during vaginal delivery
- Through blood transfusion and C/T/O transplantation: both rare in developed countries
- Syphilis paves the way for contracting HIV: behaviourally and biologically (like canary in the coal mine for HIV)

Epidemiology

- WHO estimates in 2008: 11 million incident cases and 36 million prevalent cases of venereal syphilis globally
  - ~1.36 million of them estimated to be pregnant
- Mainly in developing countries especially in sub-saharan Africa, SE Asia, as well as Eastern Europe, Russia, and China
- Huge drop in incident syphilis cases in 1940s after the introduction of penicillin with small peaks every decade

Epidemiology

- Drastic rise in incident syphilis cases during 2000-2010 especially among MSM:
  - *i*) risky sexual behaviour
  - *ii*) on-line dating
  - *iii*) HIV sero-sorting
  - *iv*) Availability of HAART and HIV PrEP misconception
  - *v*) condom fatigue

  - Similar trend in Canada with 481% rise during 2000- 2012 from 1.84 to 8.85/100,000 population
  - Incidence: 2016 in MB: 21 per 100,000 (RPR-pos)

- Increased male-to-female ratio
- Early prenatal screening and Rx → Decreased the number of congenital syphilis cases though with small fluctuations in North America

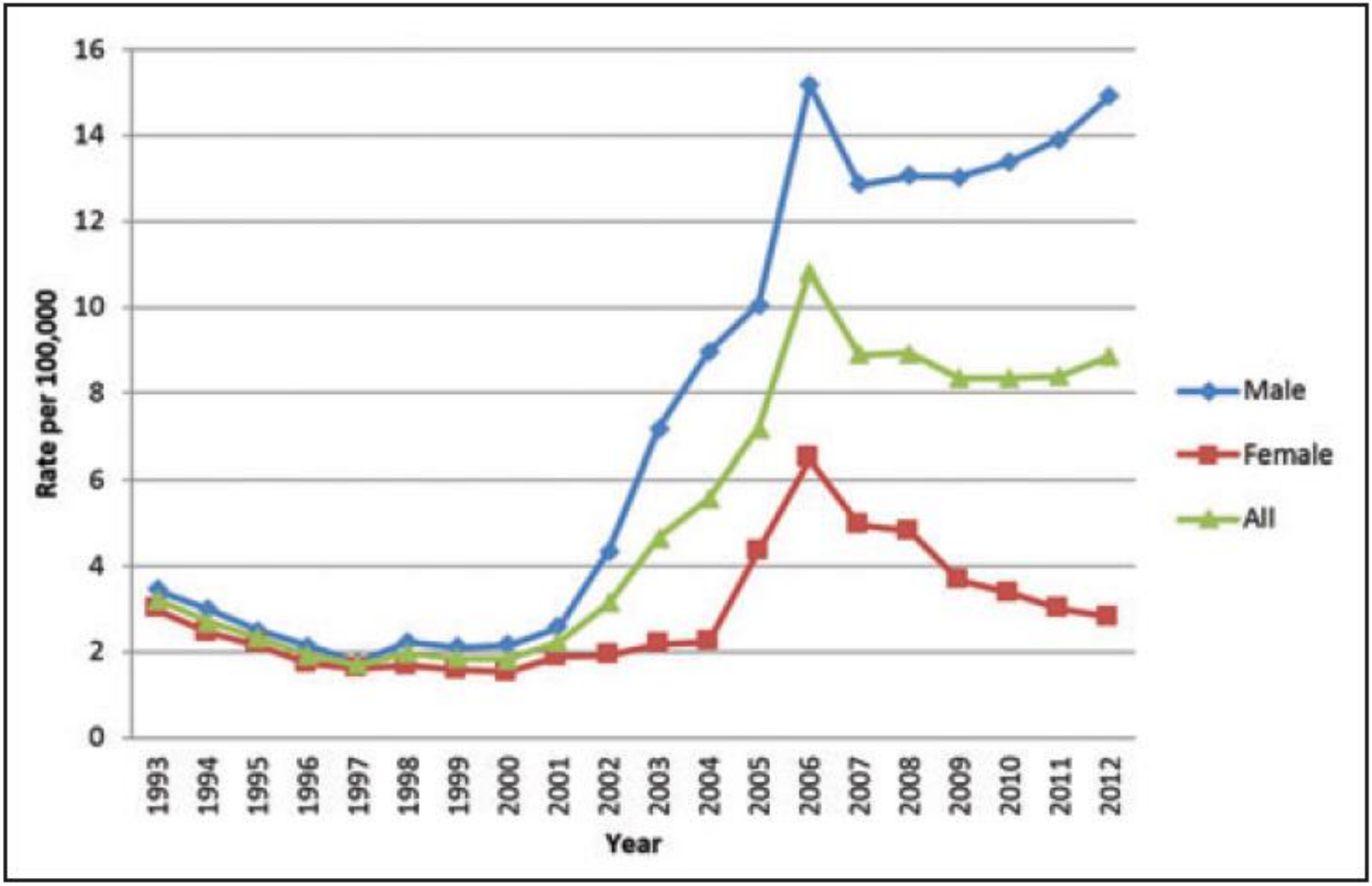


Figure 1) *Reported overall and sex-specific rates of infectious syphilis in Canada, 1993 to 2012 (9)* *CPHLN Syphilis task group; CJIDMM, Jan-Feb 2015*

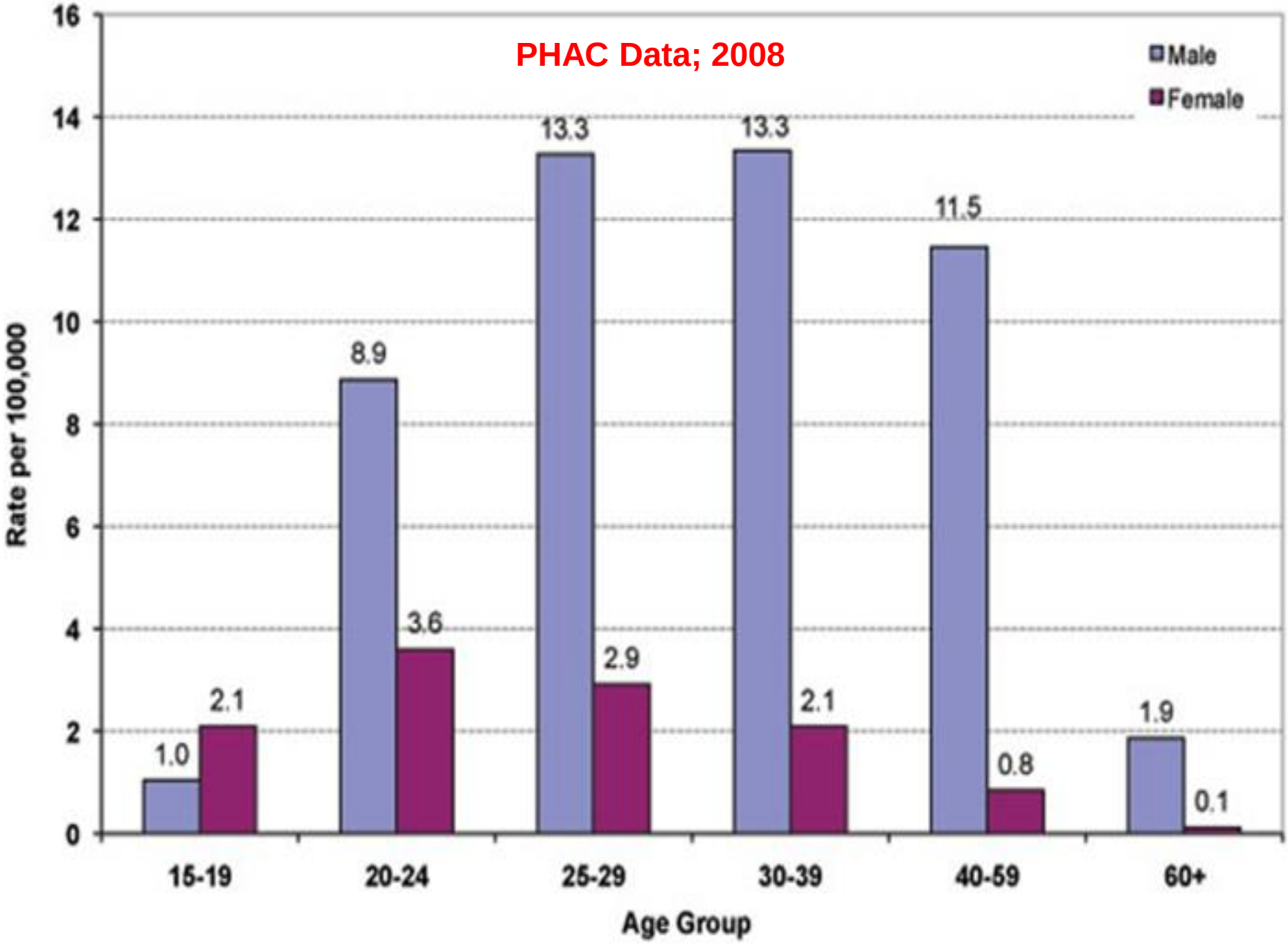

PHAC Data; 2008
Male
Female
Rate per 100,000
16
14
12
10
8
6
4
2
0
1.0
2.1
8.9
3.6
13.3
2.9
13.3
2.1
11.5
0.8
1.9
0.1
15-19
20-24
25-29
30-39
40-59
60+
Age Group

Figure 12: Reported Rates of Infectious Syphilis in Males by Age Group, 1999 to 2008, Canada

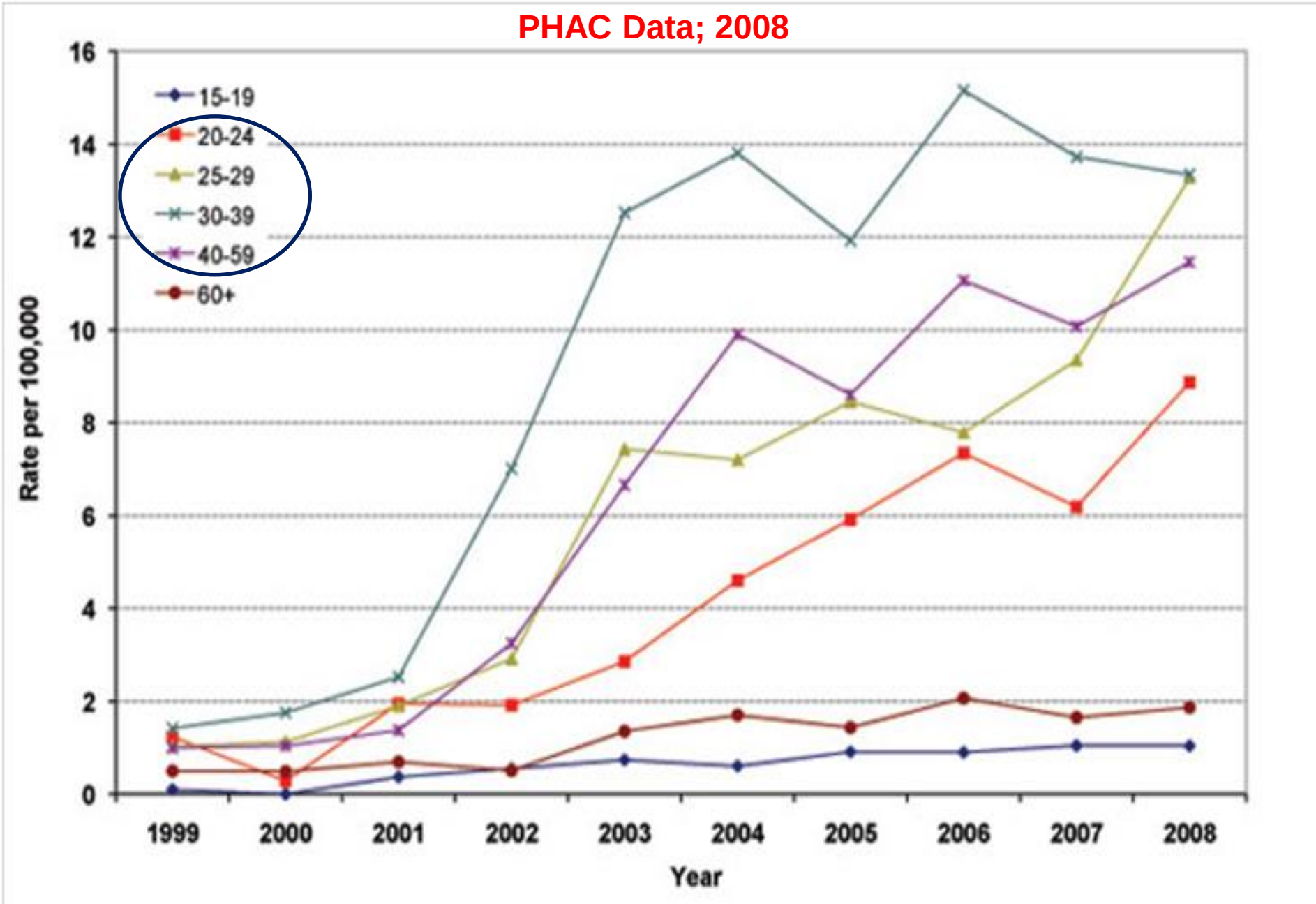
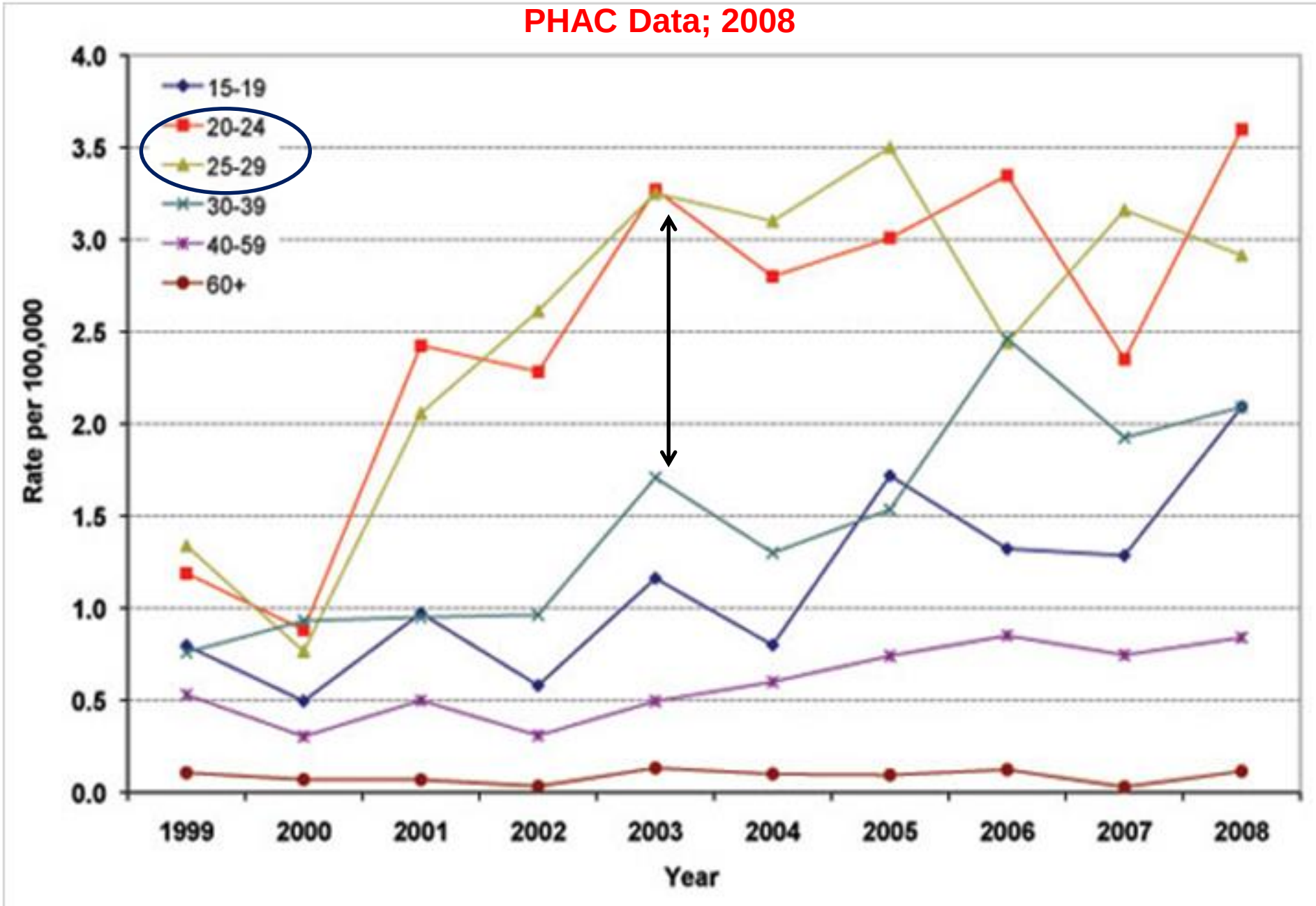
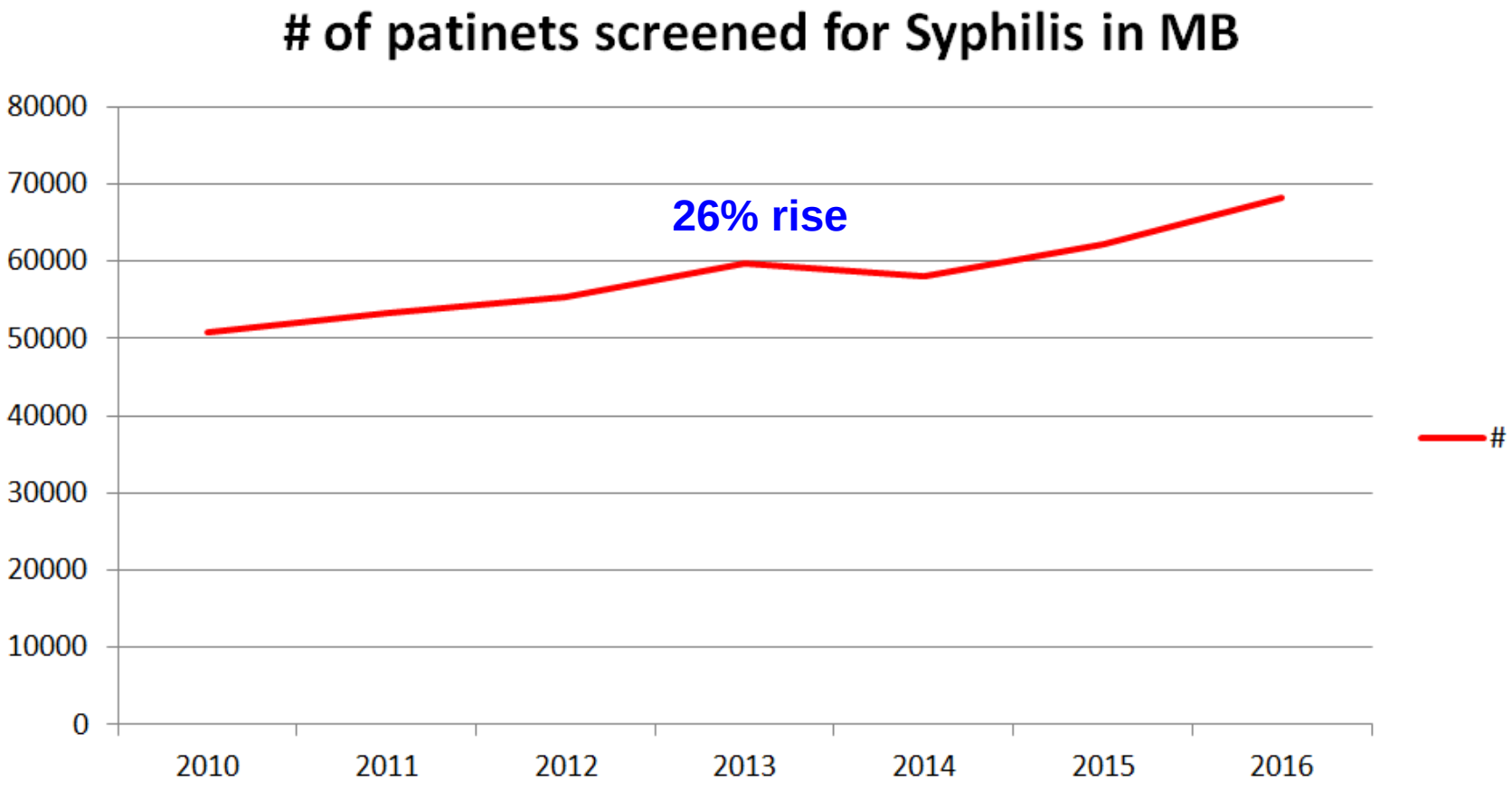


Figure 13: Reported Rates of Infectious Syphilis in Females by Age Group, 1999 to 2008, Canada



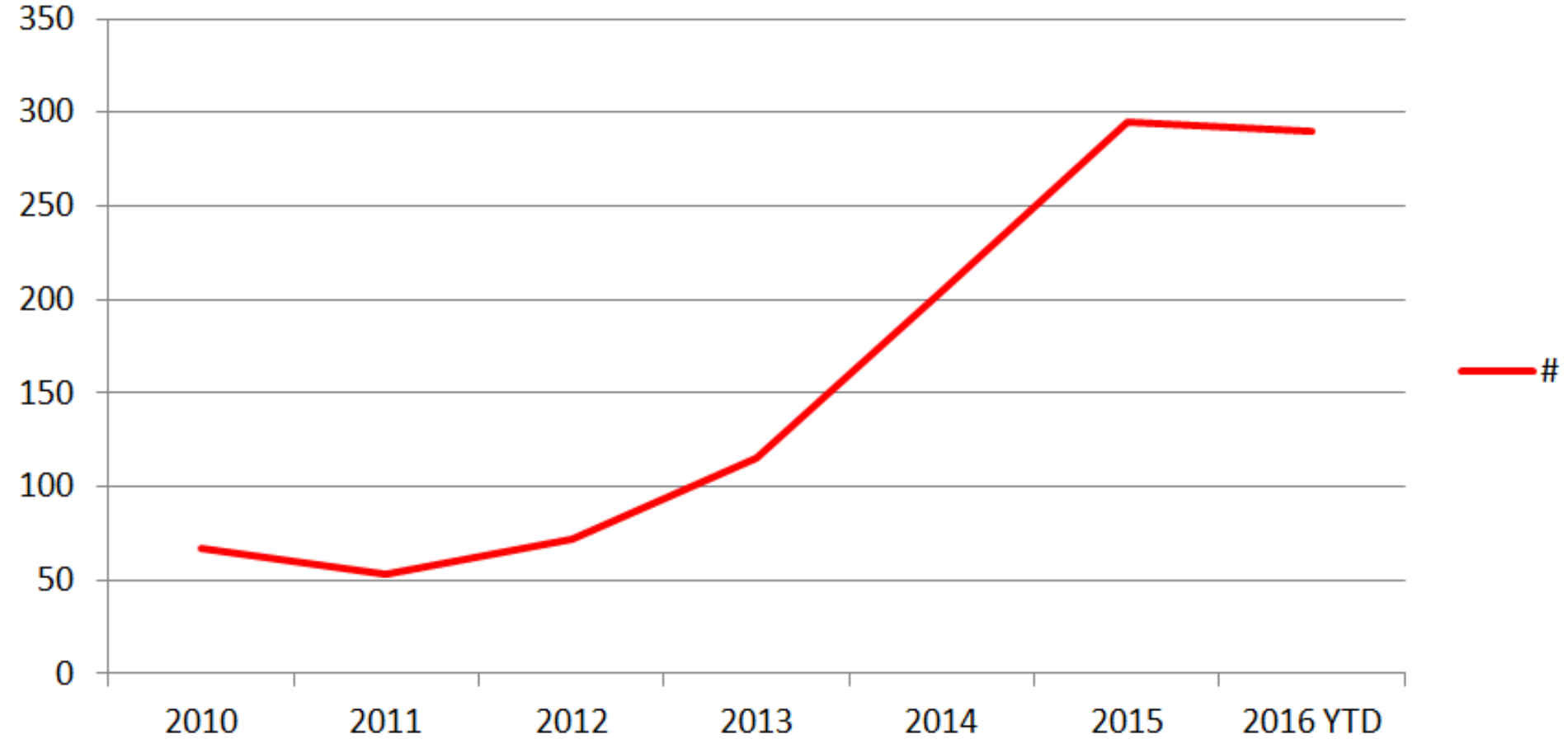
CPL Data



Manual testing not quite feasible with such high and increasing numbers

51% rise in numbers since 2000 !

of newly diagnosed syphilis cases of all stages at CPL since 2010 (77% rise)

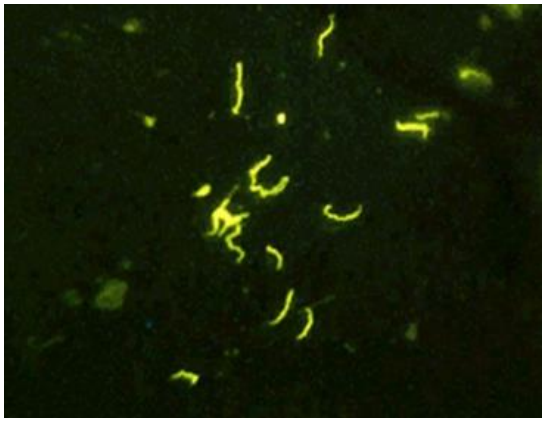


Laboratory Dx

- Direct detection:
  - *In vivo* propagation
  - Microscopy
  - PCR
- Serological detection:
  - Traditional (conventional) algorithm
  - Reverse-sequence algorithm

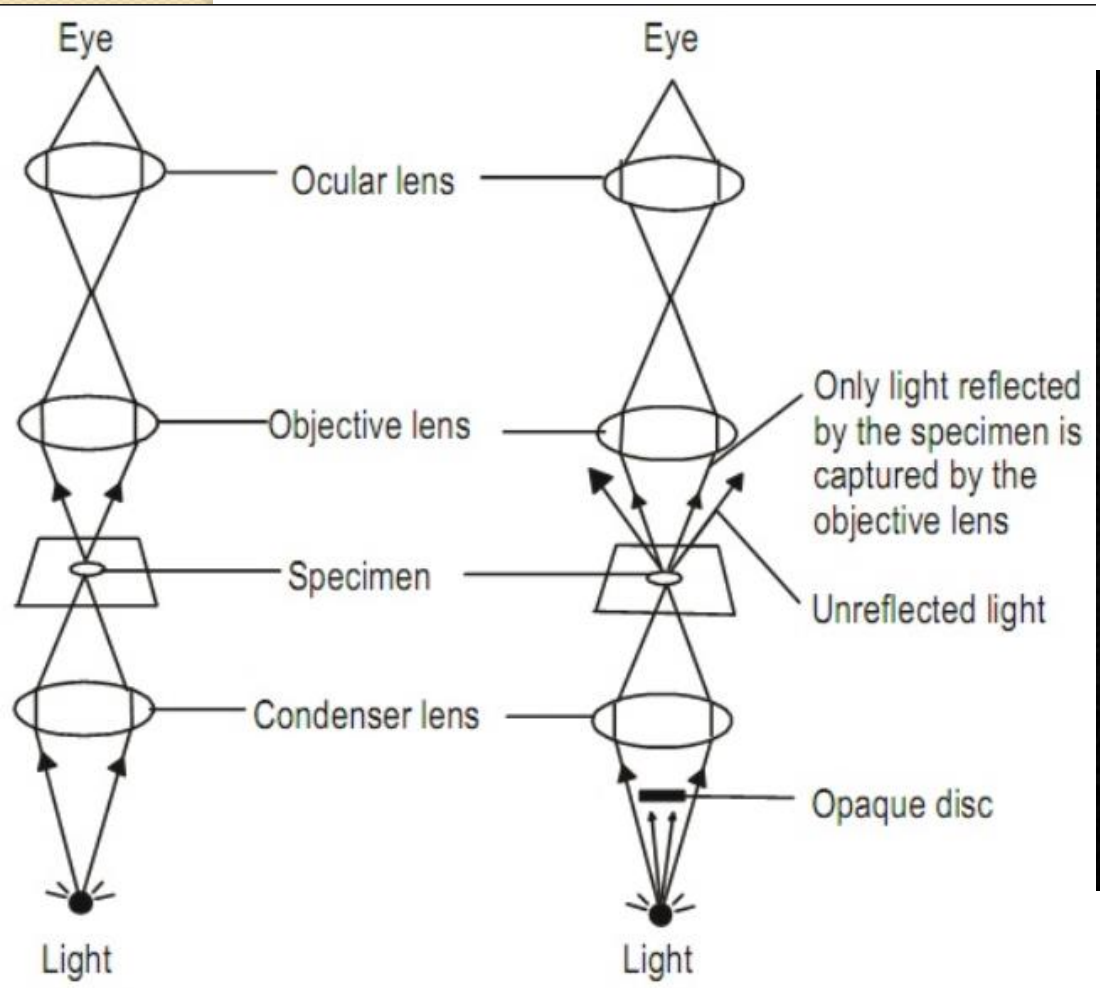
Direct Detection

- Isolation not feasible routinely and axenically
- Rabbit Infectivity Test: Gold Standard but costly, lengthy, technically-demanding
- Direct detection can be performed by dark-field microscopy (DF) or Direct fluorescence assay (DFA)
  - Both are rather obsolete with poor performance characteristics
  - Technically demanding
  - Hard to obtain reagents

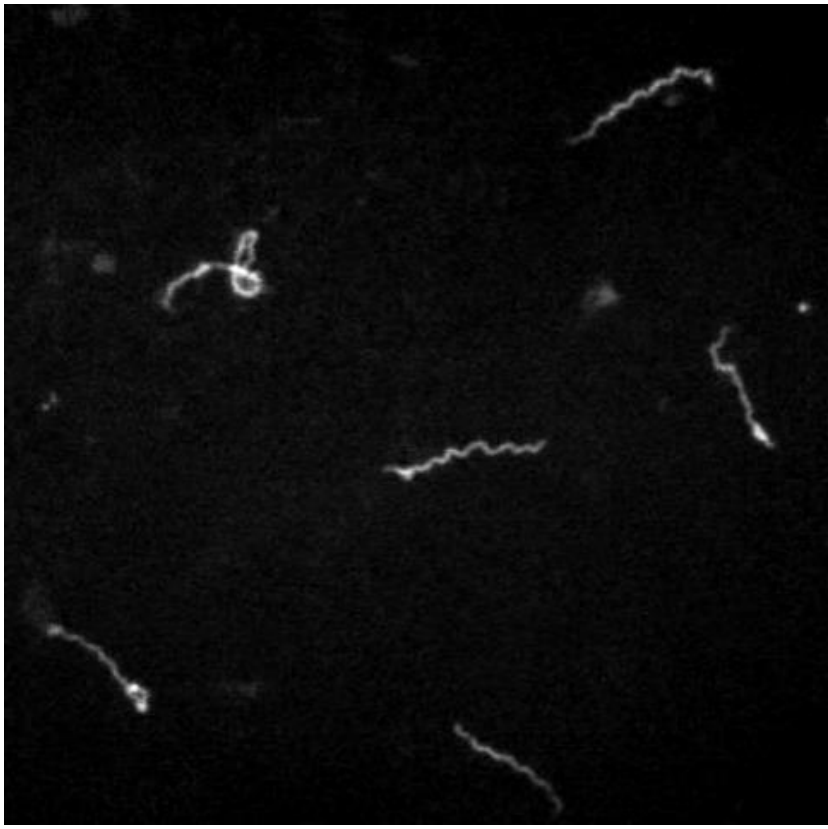


  - Discontinued @ CPL in 2011

Dark-field microscopy (DF)



Bright Field **Dark Field**



Specimen should be examined within 20 minutes as motility has to be seen

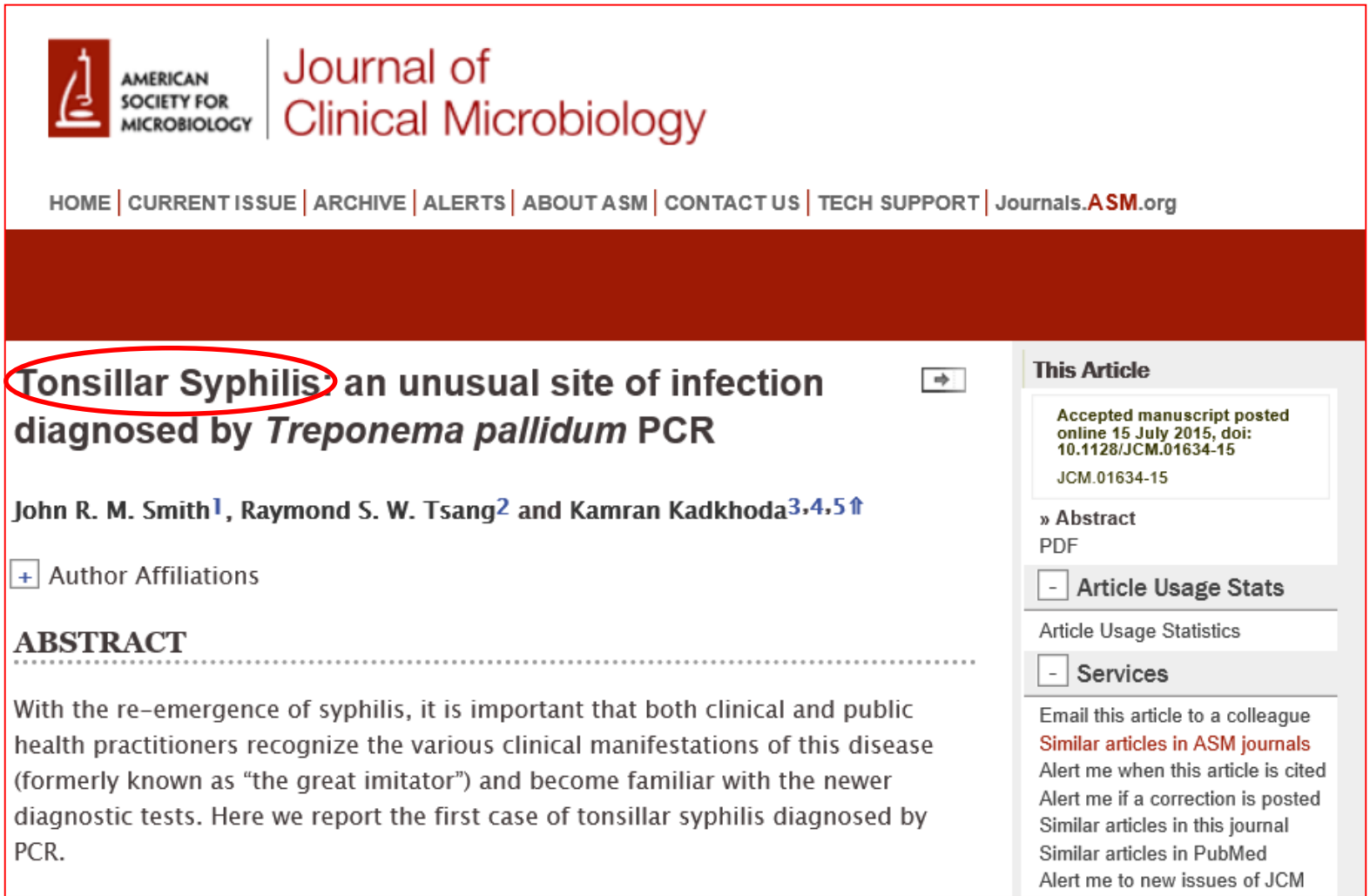
Almost completely obsolete

LOD: 10^4-10^5 spirochetes/ml

Syphilis PCR is available

PCR using specific primers (*bmp*, *tpp47*, *polA*) is highly sensitive and specific; **available through CPL** (→ NML)

Flocked-, Dacron-, Rayon-tipped swabs in VTM on cold packs → sent to CPL preferably on the same day



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Tonsillar Syphilis: an unusual site of infection diagnosed by *Treponema pallidum* PCR

John R. M. Smith[1], Raymond S. W. Tsang[2] and Kamran Kadkhoda[3,4,5]

Author Affiliations

ABSTRACT

With the re-emergence of syphilis, it is important that both clinical and public health practitioners recognize the various clinical manifestations of this disease (formerly known as "the great imitator") and become familiar with the newer diagnostic tests. Here we report the first case of tonsillar syphilis diagnosed by PCR.

This Article

Accepted manuscript posted online 15 July 2015, doi: 10.1128/JCM.01634-15

JCM.01634-15

» Abstract

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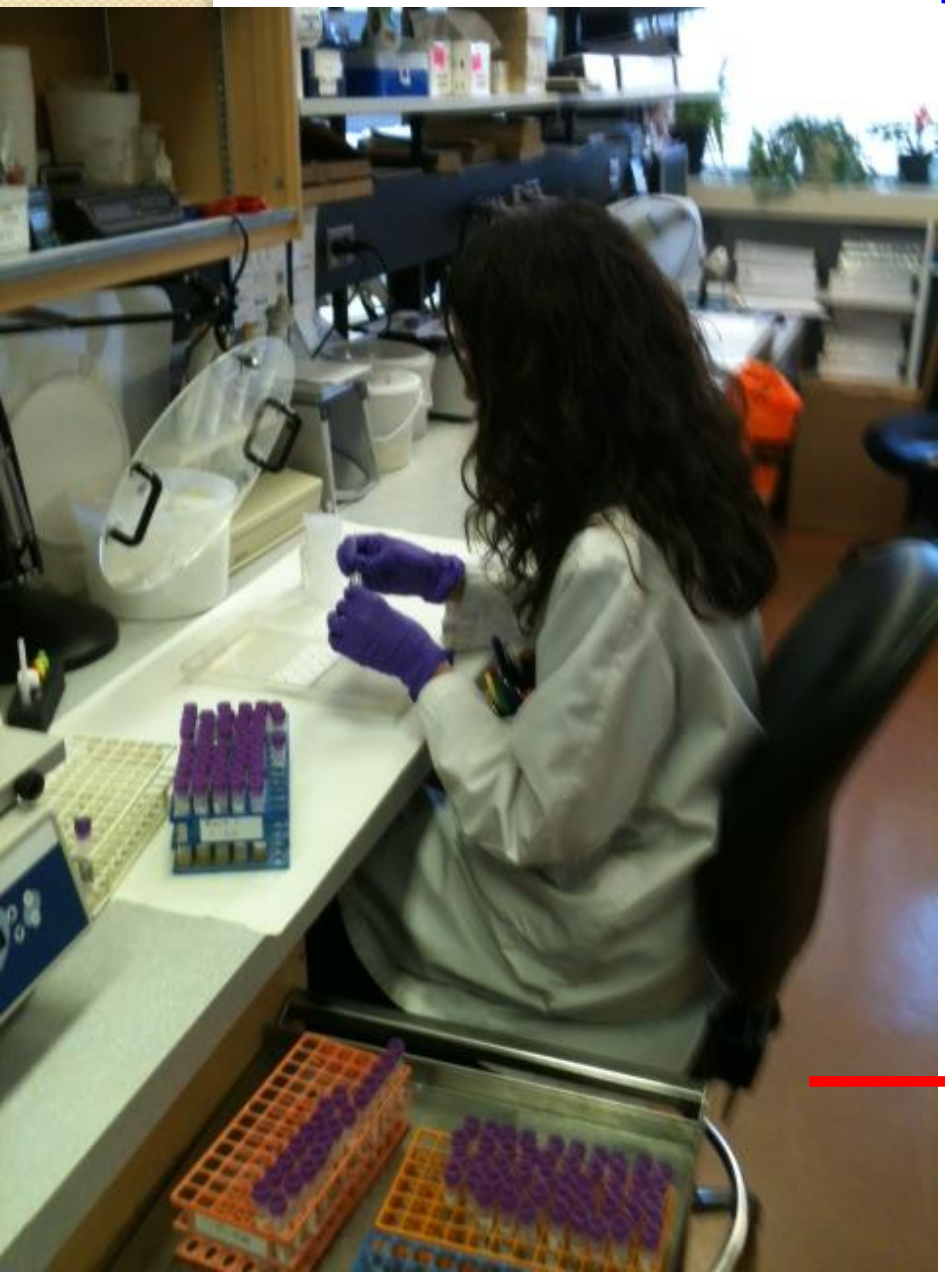
Serology

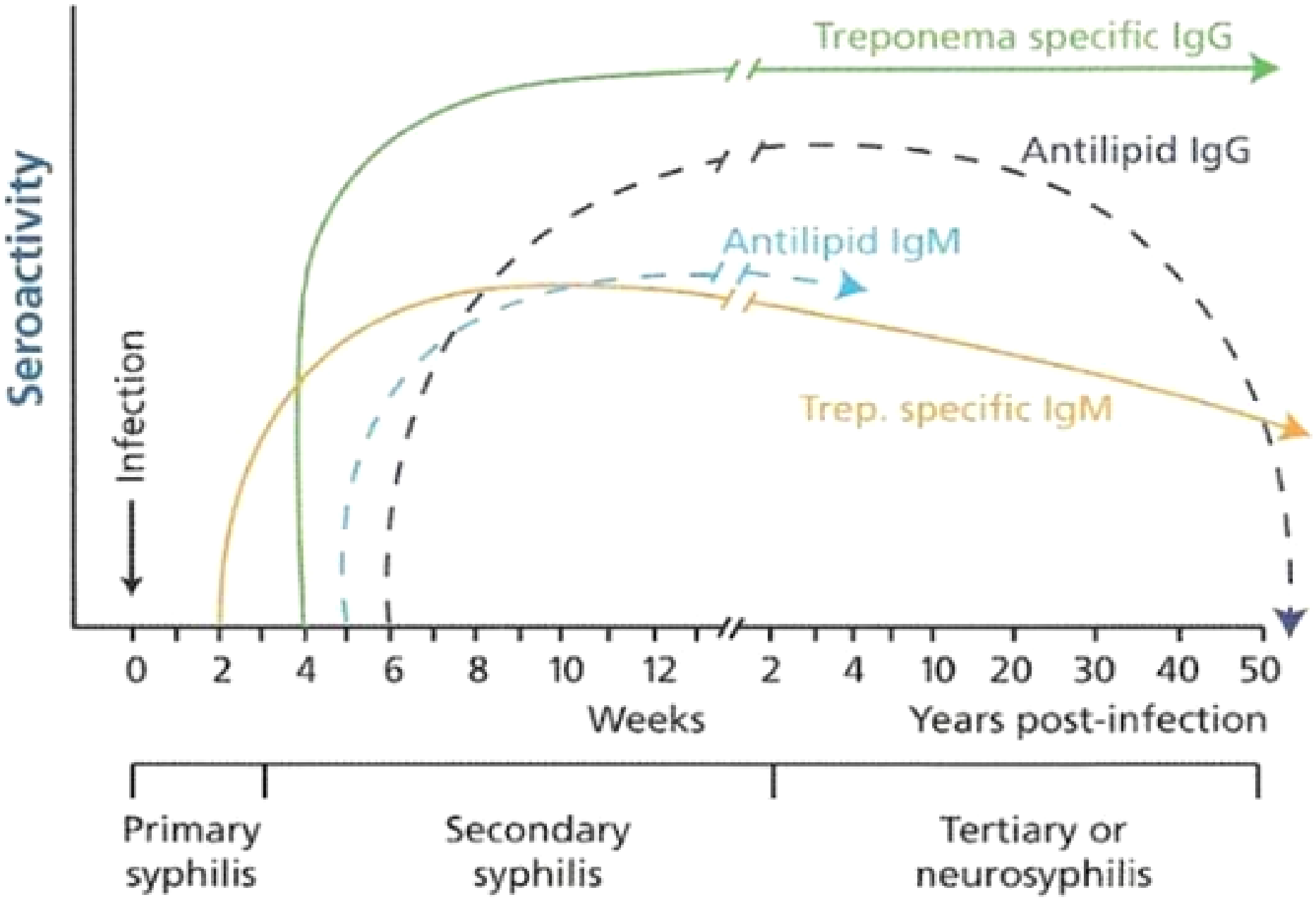
Serology: the mainstay of syphilis diagnosis

- **Treponemal tests** (e.g.,TP-PA, FTA-ABS, EIA, CMIA)
  - Specific antibodies
  - Typically appear within 2-3 weeks after infection
  - Remain positive for life in ca. 85% of healthy subjects
  - Not affected by treatment
  - Not affected by "biological false positivity" phenomenon
- If the screen test starts with Treponemal tests, the algorithm is called: "**reverse-sequence algorithm**"

FROM manual RPR TO walk-away, automated, random-access Syphilis screening

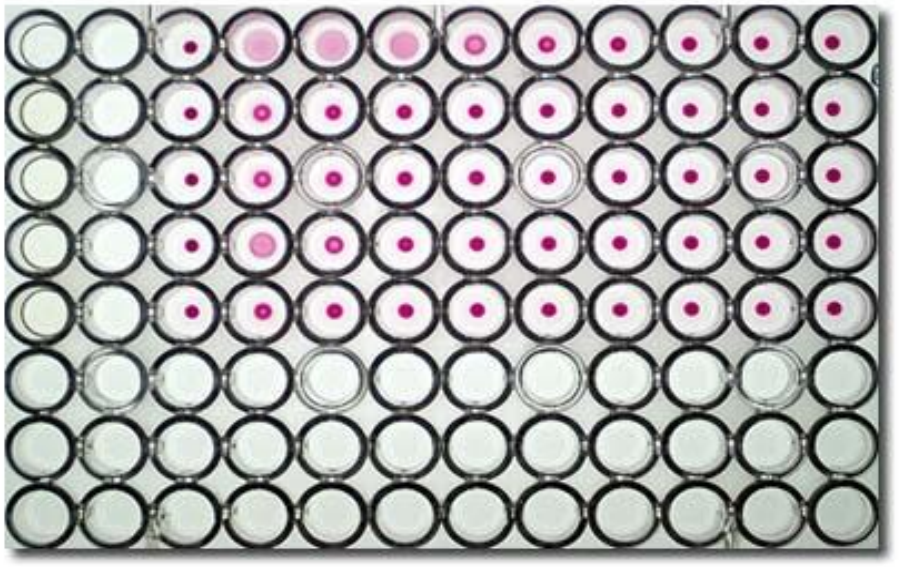


Antibody patterns during treponemal infection[2]

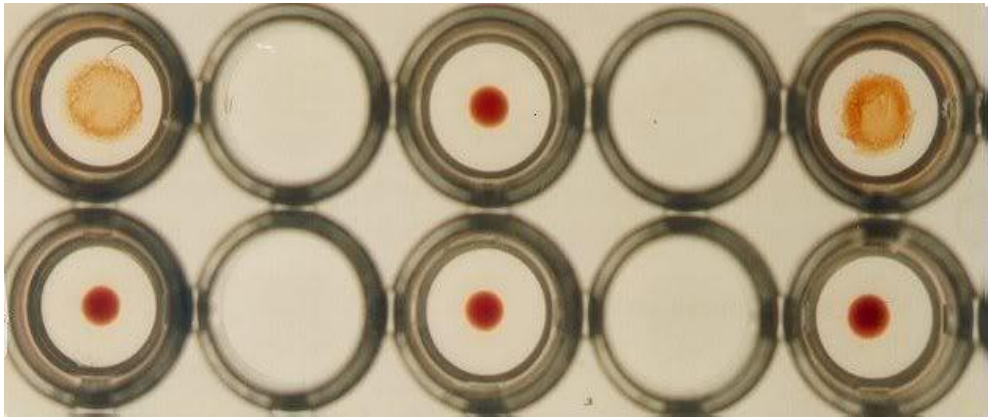


Examples of Treponemal tests

Treponema pallidum – Particle Agglutination



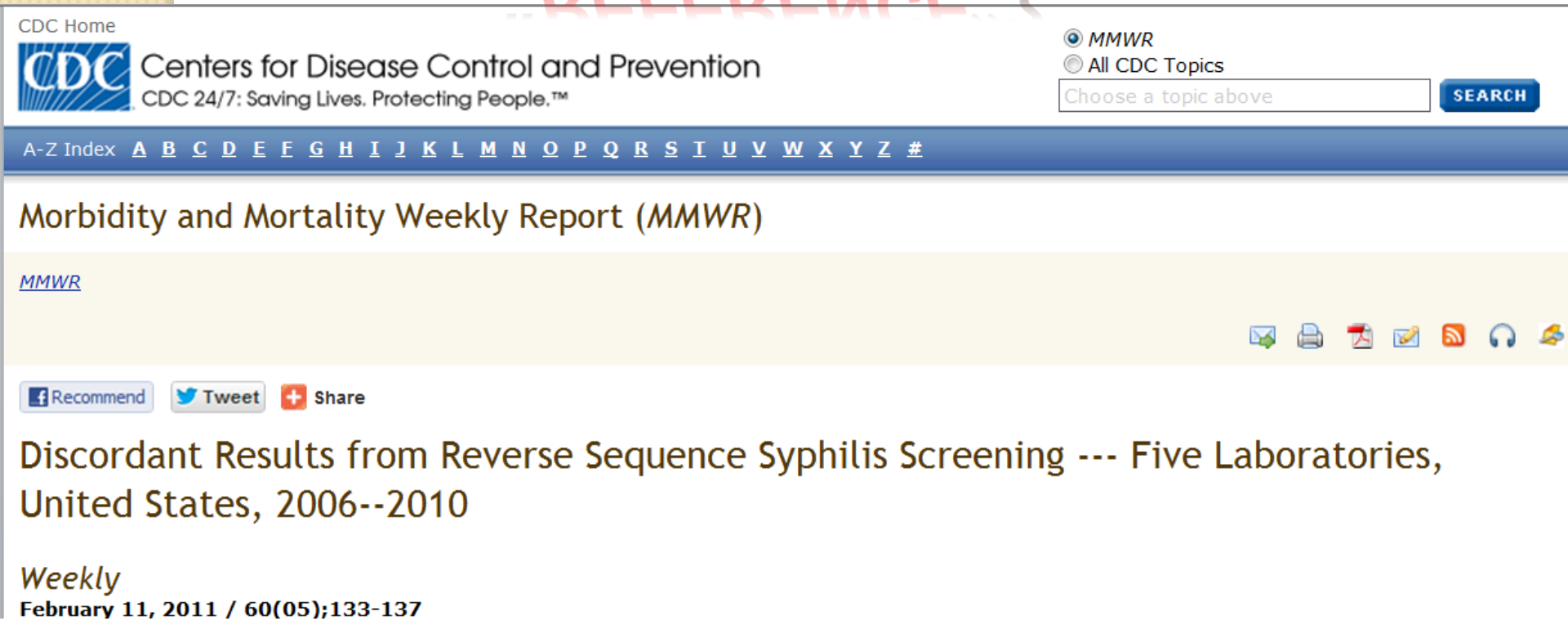
- It is relatively labour-intensive and time-consuming
- Takes up to 3 hours as opposed to 30 minutes on the automated/random-access platforms



The old version using RBCs: MHA-TP

- **Using sheep RBCs**
- **Now obsolete**

FTA-ABS USED TO BE CALLED "*REFERENCE*"?



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Morbidity and Mortality Weekly Report (*MMWR*)

MMWR

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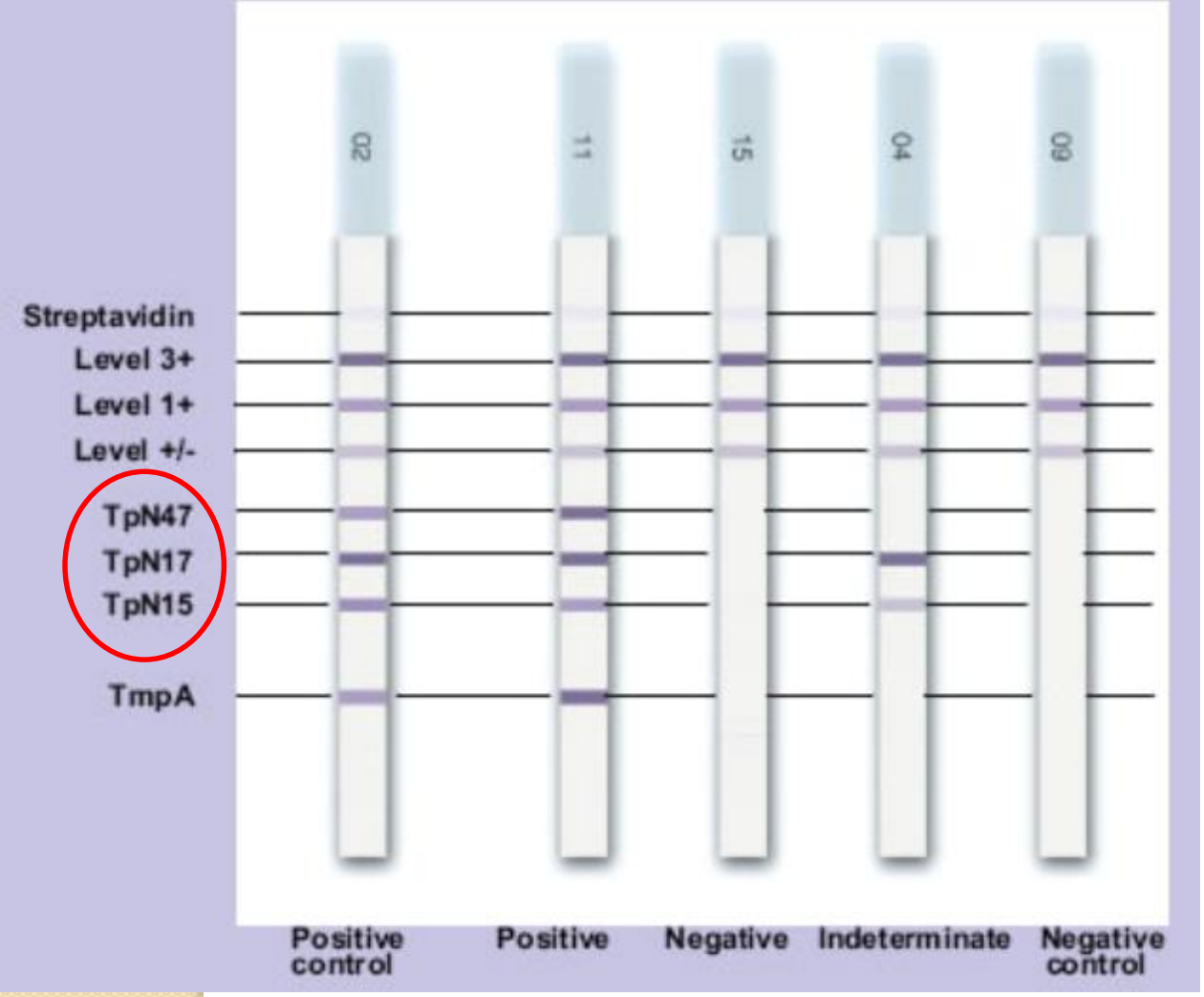
Discordant Results from Reverse Sequence Syphilis Screening --- Five Laboratories, United States, 2006--2010

Weekly
February 11, 2011 / 60(05);133-137

Traditionally, the FTA-ABS test has been considered the gold standard treponemal test and still is used by some laboratories. However, the FTA-ABS test has lower specificity than other treponemal tests and probably lower sensitivity (*9*). In addition to inherent subjectivity, the FTA-ABS test also requires trained personnel and a dedicated fluorescence microscope. For these reasons, CDC recommends that the FTA-ABS test not be used to confirm discordant treponemal screening results. Based on published sensitivity and specificity data, the TP-PA test currently is considered to be the most suitable confirmatory treponemal test (*10*).

Discontinued at CPL but it is still done on CSF samples at NML



Syphilis Immunoblot: as a confirmatory test

- Still labour-intensive
- Not-approved by Health Canada

Modern automated random-access platforms

Current CMIA test used at CPL uses:

- **Takes only 30 min**
- **Walk-away system**

3 recombinant antigens from *Treponema pallidum*:
TpN 15
TpN 17
TpN 47

CMIA specificity: 99.85%

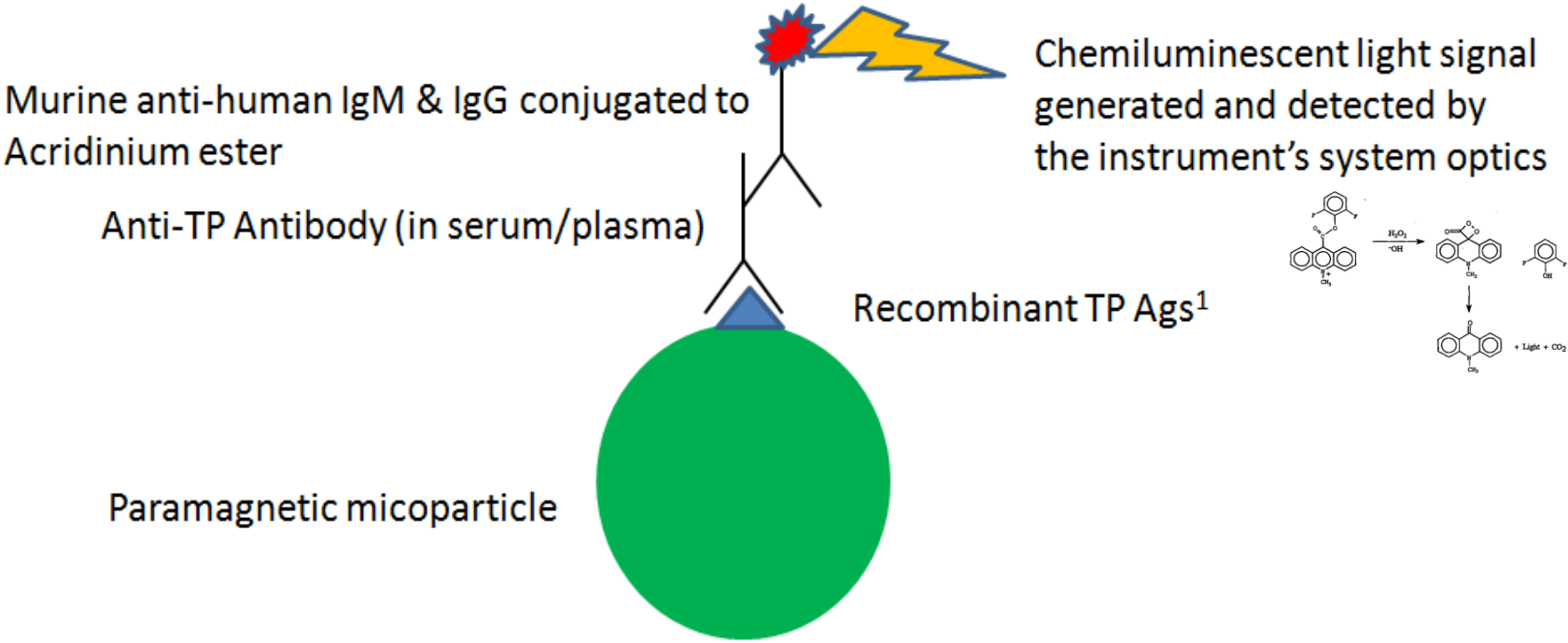
Serology cannot differentiate venereal syphilis from endemic treponematoses

An example of a random-access automated platform (currently used @ CPL)

What is CMIA?

What is CMIA anyways?
Cash Management Improvement Act?
Or? Chemiluminescent Microparticle Immunoassay

Principle of the test



[1], *E. coli*-expressed recombinant antigens: **TpN 15; TpN 17; TpN 47**

Conventional/traditional algorithm

- Screening with a non-treponemal test (RPR) → and if reactive, then followed by a treponemal test (*e.g.*,TP-PA)

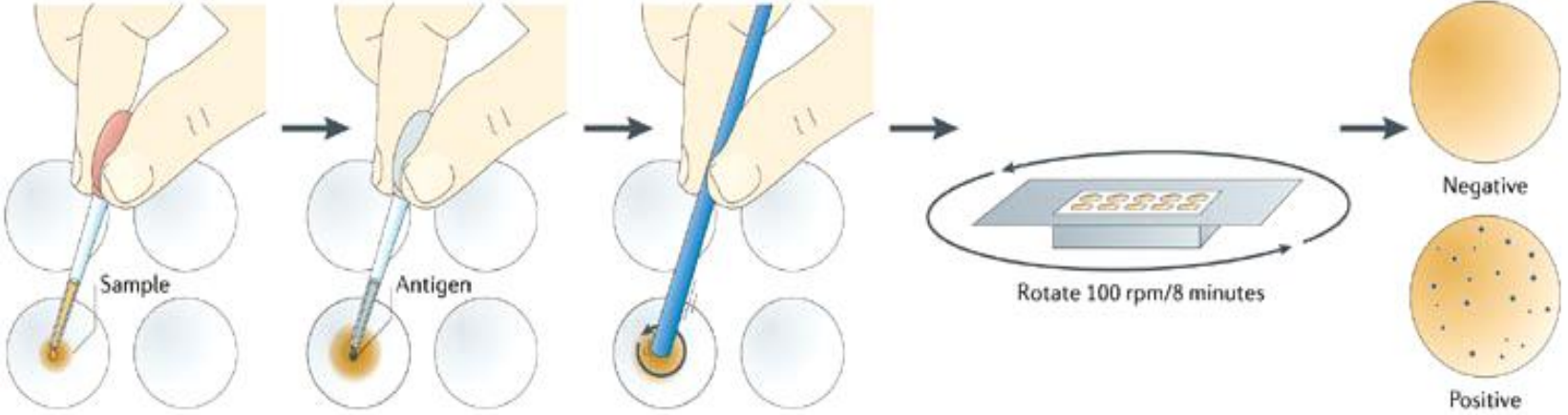
- **RPR**: Rapid Plasma Reagin
- **VDRL**: Venereal Disease Research Laboratory

- Antigens: Cholesterol, Cardiolipin, Lecithin

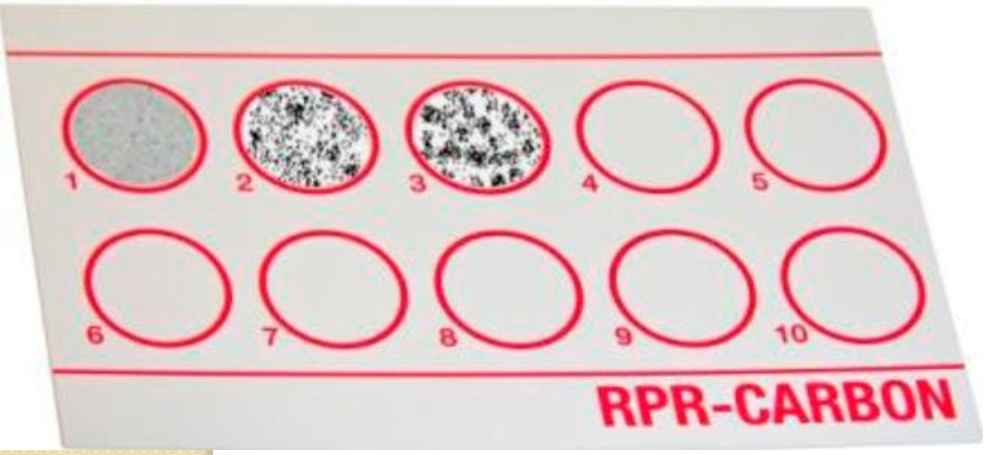
- Based on Flocculation (Wassermann test): as opposed to agglutination

  - Flocculation is based on the presence of lipoidal antigens in liquid phase

- RPR/VDRL look for "Reagin" antibody (misnomer)



RPR: Macroscopic



VDRL: Microscopic

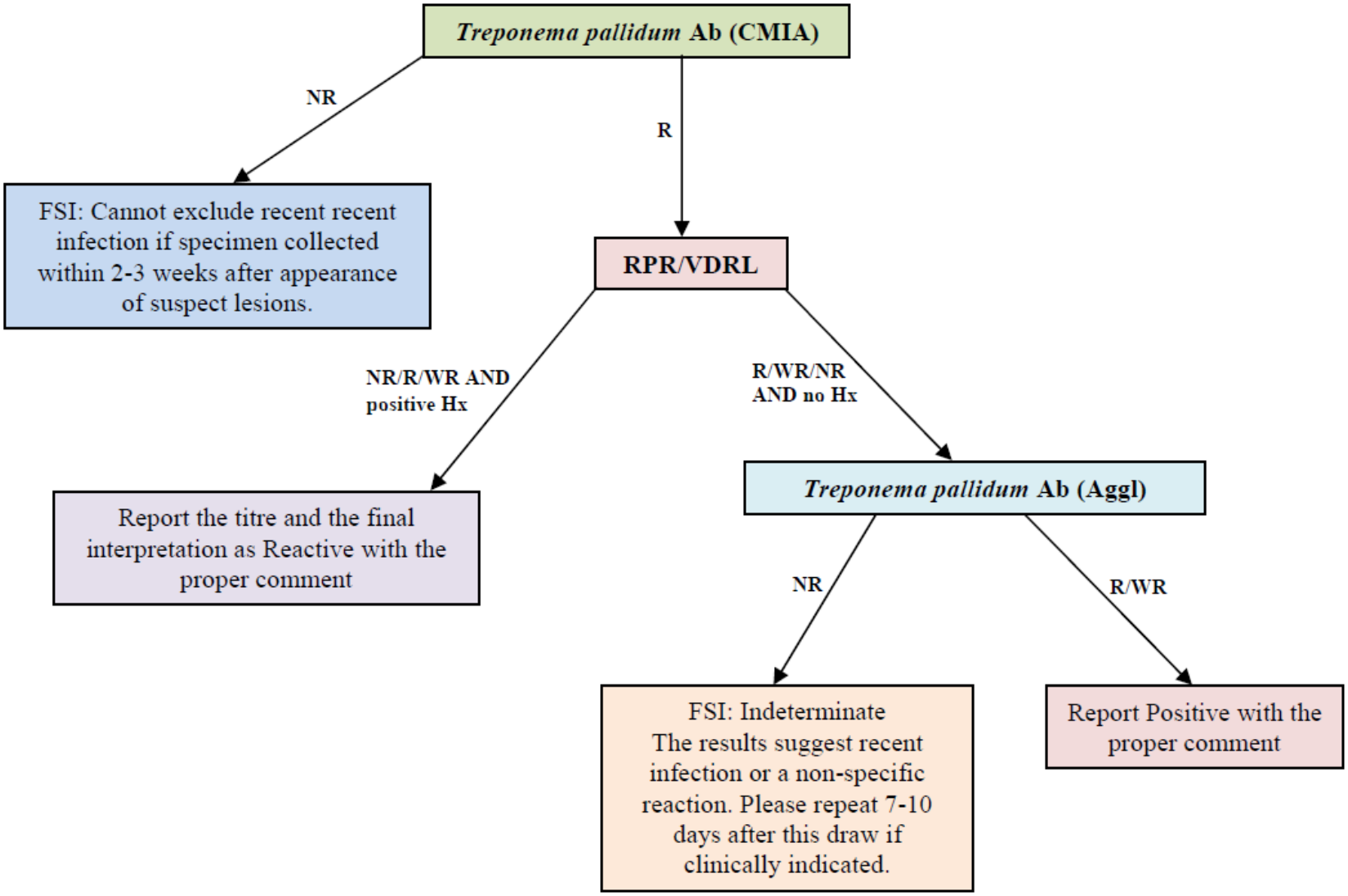
RPR is used to screen CMIA positive samples → if Reactive → VDRL to determine the end titre

Advantages of reverse-sequence testing algorithm

- Very early detection of treponemal infection (treponemal antibodies appear earlier than non-treponemal ones)
- Higher sensitivity during latent and late syphilis
- Less labour and cost for the laboratory especially with random access platforms
- Improved turnaround time
- Less detection of biological false positives →
  - BFPs: Acute vs. Chronic:
    (< or > 6 months duration)

Infectious Diseases
Lyme disease*
Leptospirosis
Relapsing fever
Ratbite fever (*Spirillum minus*)
Leprosy
Tuberculosis
Pneumonococcal pneumonia
Subacute bacterial endocarditis
Chancroid
Scarlet fever
Rickettsial disease
Malaria
Trypanosomiasis
Mycoplasma pneumonia
Chickenpox
Lymphogranuloma venereum
Hepatitis (especially hepatitis C)*
Infectious mononucleosis
Noninfectious Diseases
Drug addiction
Any connective tissue disease disorder
Rheumatoid heart disease
Blood transfusions (multiple)
Pregnancy
"Old age"
any vaccination
Chronic liver disease (noninfectious)

An example of a syphilis reverse sequence algorithm



TP-PA is done for specimens with no previous positive Hx of Syphilis to check for CMIA false positive results

How to request syphilis testing?

Cadham Provincial Laboratory

General Requisition

Manitoba Health

ONLY ONE SPECIMEN TYPE PER REQUISITION

All areas of the requisition must be completed (please **print** clearly)
See back for requisition/specimen instructions

Cadham Provincial Laboratory
P.O. Box 8450
750 William Avenue
Winnipeg, MB R3C 3Y1

Tel: (204) 945-6123
Fax: (204) 786-4770
E-mail: cadham@gov.mb.ca
Website: www.gov.mb.ca/health/publichealth/cpl

SEROLOGY

Serology Test Panels (see #1 over)

- ☐ STI Panel
- ☐ Post Exposure: Source Panel (1, 3)
- ☐ Post Exposure: Exposed Panel (1)
- ☐ Prenatal Panel
- ☐ Prenatal HIV OPT OUT (2)
- ☐ Blood-borne Pathogen

HIV (4) ☐ HIV1/2Ab ☐ Syphilis Screen

Hepatitis

- ☐ HAV IgG (Immunity)
- ☐ HAV IgM (acute HAV)
- ☐ HBcAb (Total)
- ☐ HBsAb (Immunity)
- ☐ HBsAg
- ☐ HCV Ab

Nucleic Acid (Plasma Only) (5)

- ☐ HBV PCR/Quant
- ☐ HCV PCR/Qual
- ☐ HCV PCR/Quant
- ☐ HCV Genotyping
- ☐ WNV PCR

Miscellaneous Serology

	Acute	Immune Status		Acute	Immune Status
CMV	☐ IgM	☐ IgG	Parvo B19	☐ IgM	☐ IgG
EBV	☐ IgM	☐ IgG	Rubella	☐ IgM	☐ IgG
HSV	☐ IgM	☐ IgG	Toxoplasma	☐ IgM	☐ IgG
Measles	☐ IgM	☐ IgG	Varicella	☐ IgM	☐ IgG
Mumps	☐ IgM	☐ IgG	WNV	☐ IgM	

☐ Lyme Ab ☐ H. pylori Ab ☐ Mycoplasma pneumoniae IgM

How to interpret Syphilis results

Examples of True and False positive CMIA results

Final Syphilis Interpretation	1	**The results suggest either recent or previous Treponemal infection.**
Treponema pallidum antibody (CMIA)		**Positive**
Reagin antibody (VDRL)		**Reactive**
		1:64 dilution, 64 dils.
Treponema pallidum antibody (Aggl) TP-PA		**Reactive** ←

Final Syphilis Interpretation	2	Indeterminate Syphilis results. This result suggests very recent infection or a non-specific reaction. Repeat testing should be considered after 7 - 10 days if clinically indicated.
Treponema pallidum antibody (CMIA)		**Positive**
Reagin antibody (RPR)		Non-Reactive
Treponema pallidum antibody (Aggl) TP-PA		Non-reactive ←

Final Syphilis Interpretation	3	Indeterminate. Combined with previous test results, these findings suggest a non-specific screening test result.
Treponema pallidum antibody (CMIA)		**Positive**
Reagin antibody (RPR)		Non-Reactive

CPHLN Guidelines: Further Reading

CPHLN LABORATORY GUIDELINES

Canadian Public Health Laboratory Network laboratory guidelines for the use of serological tests (excluding point-of-care tests) for the diagnosis of syphilis in Canada

Paul N Levett DSc (D)ABMM FCCM FAAM[1], Kevin Fonseca PhD D(ABMM)[2], Raymond SW Tsang PhD[3], Kamran Kadkhoda PhD FCCM[4,5], Bouchra Serhir MSc PhD[6], Sandra M Radons BSc[7], Muhammad Morshed PhD SCCM[8,9*]

CPHLN LABORATORY GUIDELINES

Canadian Public Health Laboratory Network laboratory guidelines for the use of direct tests to detect syphilis in Canada

Raymond SW Tsang PhD[1*], Muhammad Morshed PhD SCCM[2,3], Max A Chernesky PhD FIDSA FAAM FCCM[4], Gayatri C Jayaraman PhD MPH[5], Kamran Kadkhoda PhD FCCM[6,7]

CPHLN LABORATORY GUIDELINES

Canadian Public Health Laboratory Network laboratory guidelines for congenital syphilis and syphilis screening in pregnant women in Canada

Ameeta E Singh BMBS MSc FRCPC[1*], Paul N Levett DSc (D)ABMM FCCM FAAM[2*], Kevin Fonseca PhD D(ABMM)[3], Gayatri C Jayaraman MPH PhD[4], Bonita E Lee MD FRCPC MSc[5]

Rx Monitoring using serology: RPR/VDRL

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Canadian Guidelines on Sexually Transmitted Infections

Table 6. Monitoring of serologic tests and other follow up

Primary, secondary, early latent	(*), 3, 6, 12 months after treatment
Late latent, tertiary (EXCEPT NEUROSYPHILIS)	12 and 24 months after treatment

Table 7. Adequate serologic response

Primary	4-fold* drop at 6 months; 8-fold drop at 12 months; 16-fold drop at 24 months
Secondary	8-fold drop at 6 months and 16 fold drop at 12 months
Early latent	4-fold drop at 12 months

In Case Needed

Thanks for your attention

Office #: (204)945-7545

email:
kamran.kadkhoda@gov.mb.ca