BC PUBLIC HEALTH MICROBIOLOGY & REFERENCE LABORATORY

Vancouver, BC

LABORATORY TRENDS

September 19, 2013

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Specialized Serology Update

Syphilis Reverse Testing Algorithm

Treponema pallidum is a spirochete bacterium that causes diseases such as syphilis. Since *T. pallidum* cannot be cultured, testing is reliant on antibodies produced in response to infection. Serology remains the mainstay for syphilis diagnosis due to the ease of blood collection, compared to the difficulty of obtaining lesion specimens.

Serological tests can be divided into non-treponemal and treponemal tests. Non-treponemal tests measure immunoglobulin G (IgG) and IgM antibodies formed by the host in response to lipoidal material (cardiolipin) released from damaged host cells. Each public health laboratory determines the algorithm based on their population, laboratory infrastructure, staffing needs and costs.

Traditionally, syphilis serology screening in British Columbia (BC) started with a non-treponemal test such as the rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL) test; when positive, results were confirmed using specific treponemal tests such as *T. pallidum* Particle Agglutination (TPPA), or fluorescent antibody-absorption (FTA-Abs) (Figure 1).

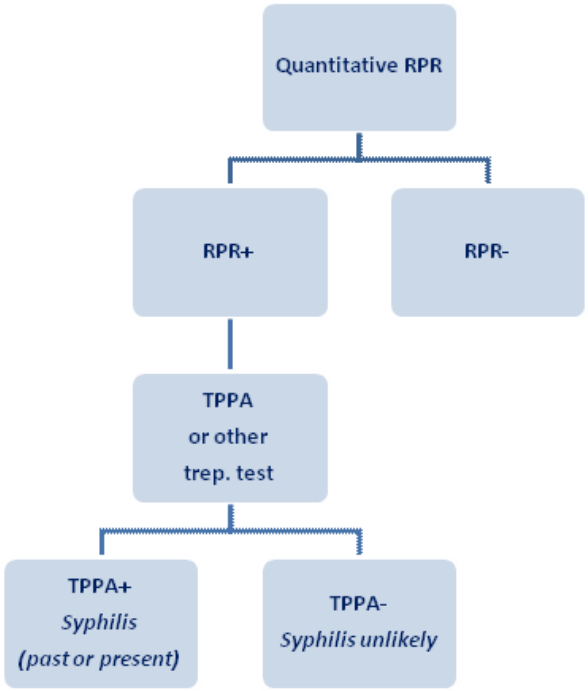

Figure 1
Traditional syphilis serology algorithm.

Since RPR positive titres correlate with disease activity, they can be used to monitor treatment or reinfection. The RPR test is comparatively inexpensive and simple. However, traditional approaches are not without issues. Some older treated or untreated cases may be missed if this algorithm is used. RPR may result in false-positive results where yaws, pinta, non-venereal spirochete disease are endemic. False-positive reactions may also occur with other diseases such as lupus infections, mononucleosis, malaria, leprosy, viral pneumonia, rickettsial infection etc. False negative patterns (*e.g.* RPR negative, TPPA reactive) may also occur with early syphilis and some latent infections.

Recently, due to the need for efficiencies in high-volume screening and the need to address ergonomic stresses (pipetting large numbers of specimens) and with the development of better tests, many laboratories in Europe and North America have changed their diagnostic approach to

Serological Diagnosis of *Treponema pallidum* Infection continued...

screening using automated or semi-automated treponemal antibody assays. The blood specimen is screened using an enzyme-linked immunosorbent assay (EIA). Positive EIA specimens are then tested with a quantitative non-treponemal test (*e.g.* RPR or VDRL). If test results disagree, the specimen is then tested using the TPPA (confirmatory treponemal test) (Figure 2). If TPPA is 1+ or equivocal, laboratories may use another test such as INNO-LIA for confirmatory testing.

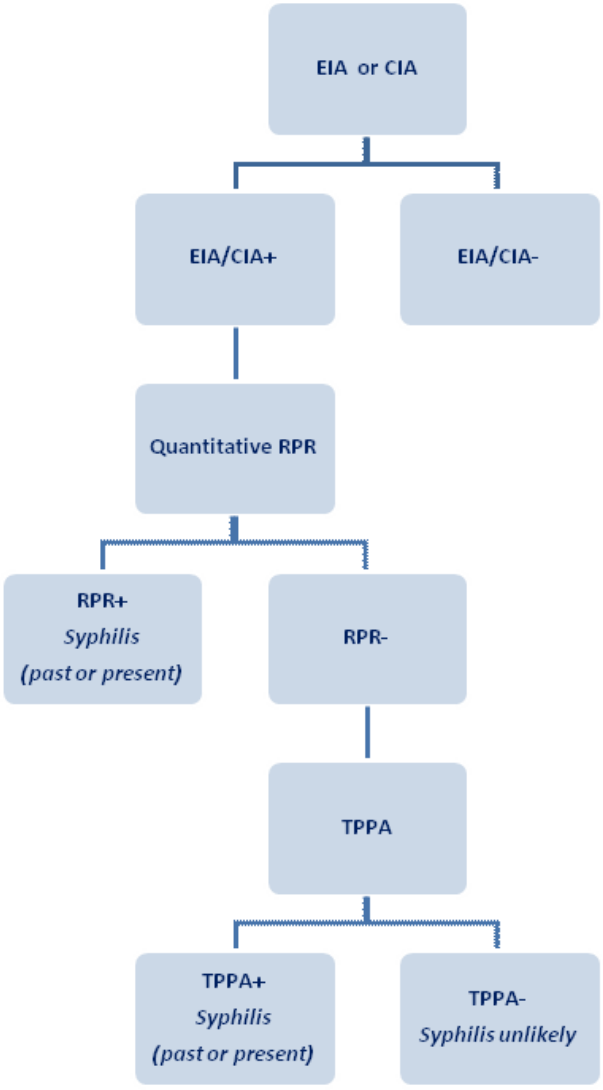

Figure 2
Reverse sequence serology algorithm.

Real-Time Polymerase Chain Reaction (PCR) for Syphilis Diagnosis

A number of recent articles have demonstrated that PCR-based technologies may be helpful in detecting *T. pallidum* in skin or mucous membrane lesion specimens. Using specific gene target primers and probes, Real-Time (RT)-PCR detects *T. pallidum* DNA from ulcerative lesion swabs, lymph node aspirates, CSF, blood, amniotic fluid, and other tissue specimens. However, the specificity varies by specimen type. Theoretically PCR is reported to detect 1-2 gene copies. The test specificity, while intrinsically very high, depends on primer selection, skill of the laboratory technician, sample type, quality, and handling. A few commercial tests are available but need to be verified before implementation. In our experience, laboratory-developed RT-PCR works well but results need to be aligned with serological findings as well as clinical information. While PCR methods are not routinely used for the diagnosis of syphilis these tests offer distinct advantages as an adjunct test to Darkfield (DF) and Direct Fluorescent Antibody-*Treponema pallidum* (DFA-TP) in the following cases: First, for oral lesion specimens (obviating the DFA-TP test). Second, sequence analysis of the DNA of *T. pallidum* can be done and this assists in understanding the molecular epidemiology of spread. Finally, PCR methods identify macrolide resistance and this provides antibiotic surveillance data. Currently, the Zoonotic Diseases & Emerging Pathogens (ZEP) Program is validating a RT-PCR method for *T. pallidum* detection which will provide these advantages, as well as better case information.

Syphilis Point-of-Care Testing

Obtaining specimens from sex trade workers, a population that often has barriers in accessing health services is an ongoing public health challenge. Point-of-Care (POC) testing in this group might help with early syphilis detection, resulting in earlier treatment and decreased transmission particularly in Vancouver's Downtown Eastside. The ZEP Program has evaluated a POC test kit that worked well. However, implementation of this test is challenging since no POC tests for syphilis have been approved by Health Canada. The ZEP Program is working together with the BC Centre for Disease Control (BCCDC) Sexually Transmitted Infections Clinic to address this challenge.

Integrated Data Analysis: A Look at *Helicobacter pylori* Diagnostic Algorithm

Helicobacter pylori is a microaerophilic, slow-growing, spiral-shaped, gram-negative bacterium that colonizes the mucous lining of the human stomach. It causes gastritis, peptic ulcer disease, and is associated with gastric mucosa lymphoid tissue lymphoma. Standard tests for the diagnosis of *H. pylori* in dyspeptic patients include serological screening, the urea breath test (UBT), and the faecal antigen test.

The ZEP Program has assessed trends in the use of these tests in BC (2001-2005) and a cost-effective algorithm building on the diagnostic strengths and weaknesses of all methods was proposed. Serology, proposed as an initial screening test (patients visiting physicians for the first time or after 2 years from the initial visit) was reiterated by the BC Medical Association as a screening test in their dyspepsia guideline (www.bcguidelines.ca/pdf/dyspepsiahpylori.pdf).

Data analysis for an extended period (up to 2011) will review the trend of diagnostic test usage for *H. pylori*. Combining this dataset with the BCPHMRL serology data will allow us to evaluate the *H. pylori* testing algorithm further and communicate the findings to the community to improve test utilization.

H. pylori Drug Susceptibility

H. pylori is notorious for acquiring drug resistance resulting in patient treatment failures. Varying by country and depending on antibiotic regimen used, treatment failure rates of 20-40% have been documented. In BC, treatment failure has been reported. Monitoring the drug susceptibility patterns of commonly used antibiotics will be a step towards addressing this problem. The ZEP Program is currently working on this surveillance.

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Recent Outbreaks and Clusters

Escherichia coli O157:H7 Linked to Cheese

The BCPHMRL Public Health Advanced Bacteriology & Mycology and Environmental Microbiology Programs along with the BCCDC, affected Health Authorities, the Public Health Agency of Canada, the Canadian Food Inspection Agency, and Health Canada are investigating an outbreak of *E. coli* serotype O157:H7. There are currently four confirmed cases of illness in BC linked to consumption of cheese from Gort's Gouda Cheese Farm; other cases are being followed up. For more information please see the BCCDC website.

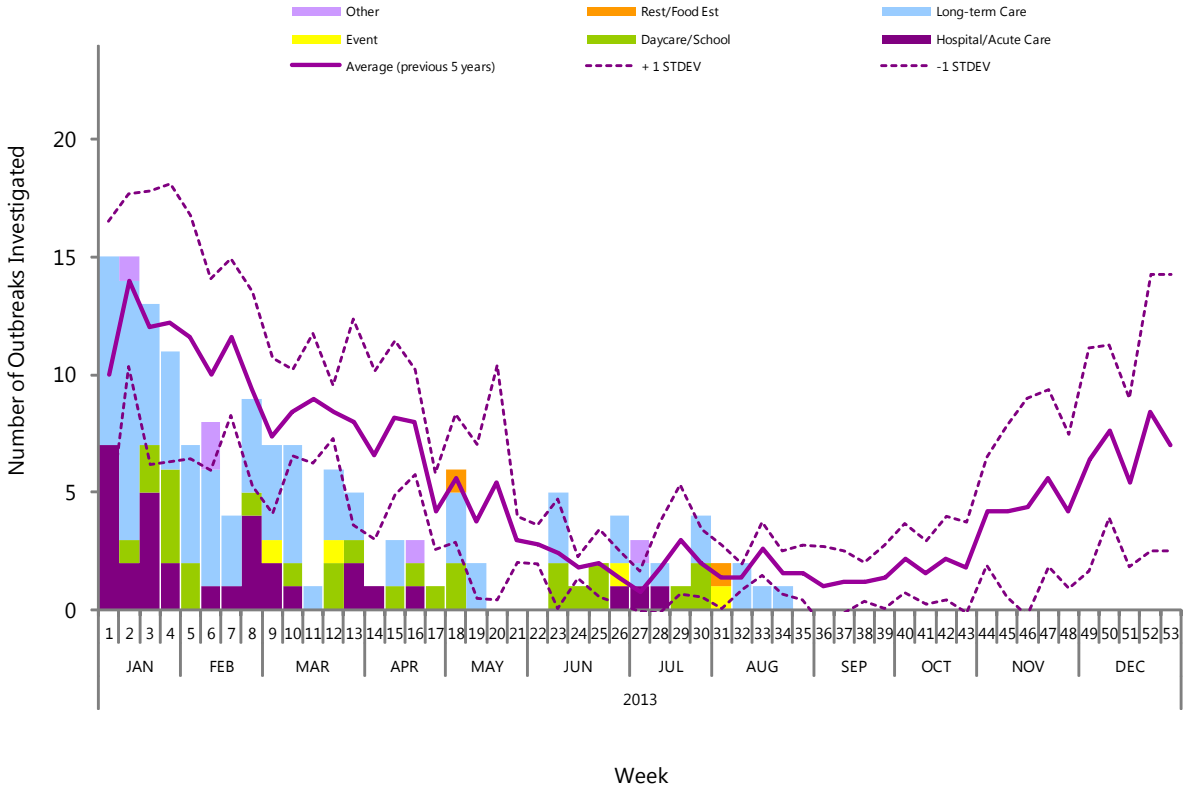
Measles

Our last update in August reported on a measles case linked to an outbreak in the Netherlands (genotype D8). Since then three additional lab confirmed measles cases have been reported in the Fraser Health region with several more suspect cases being followed up. The genotype for two of the three recent cases is B3 and results are pending for the third case. More information on the investigation and guidelines on vaccination can be found on the Fraser Health Authority website.

Other Gastrointestinal Outbreaks

In August, the Environmental Microbiology Program at the BCPHMRL investigated six gastrointestinal (GI) outbreaks. Outbreaks were identified from four long-term care facilities, one restaurant and one event (camp) (Figure 3). Samples for laboratory testing were submitted for three (50%) of these outbreaks. Norovirus was confirmed in two (67%) of these outbreaks at two longterm care facilities.

Figure 3 _____
Gastrointestinal outbreaks investigated* since January, 2013, Environmental Microbiology, Public Health Advanced Bacteriology & Mycology, Parasitology and Virology Programs, BCPHMRL.

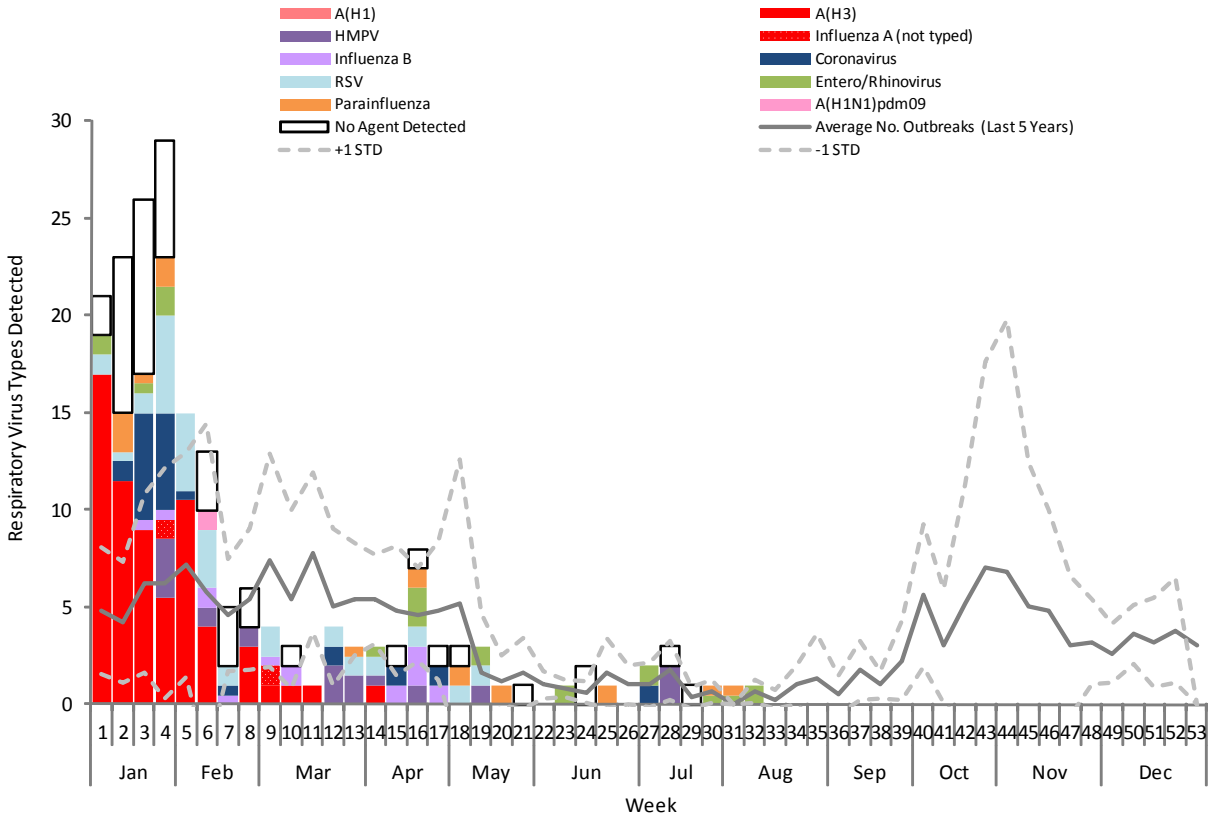


* The data available are from outbreaks in which the BCPHMRL has been notified. Some acute care microbiology laboratories are also testing for norovirus in the province and these data may not include outbreaks from all Health Authorities. Given the nature of GI outbreaks, samples are not always available for testing.

Other Respiratory Outbreaks

Typical of this time of the year (Figure 4), there were few respiratory outbreak investigations. In August samples were submitted to the BCPHMRL for only one respiratory outbreak investigation from a camp setting. Enterovirus/rhinovirus was detected for this outbreak.

Figure 4
Respiratory outbreaks investigated* by respiratory season, Virology Program, BCPHMRL.



* Figure 5 reflects respiratory sample results submitted for investigation to the PHMRL and may not be representative of respiratory outbreaks in the entire BC community.

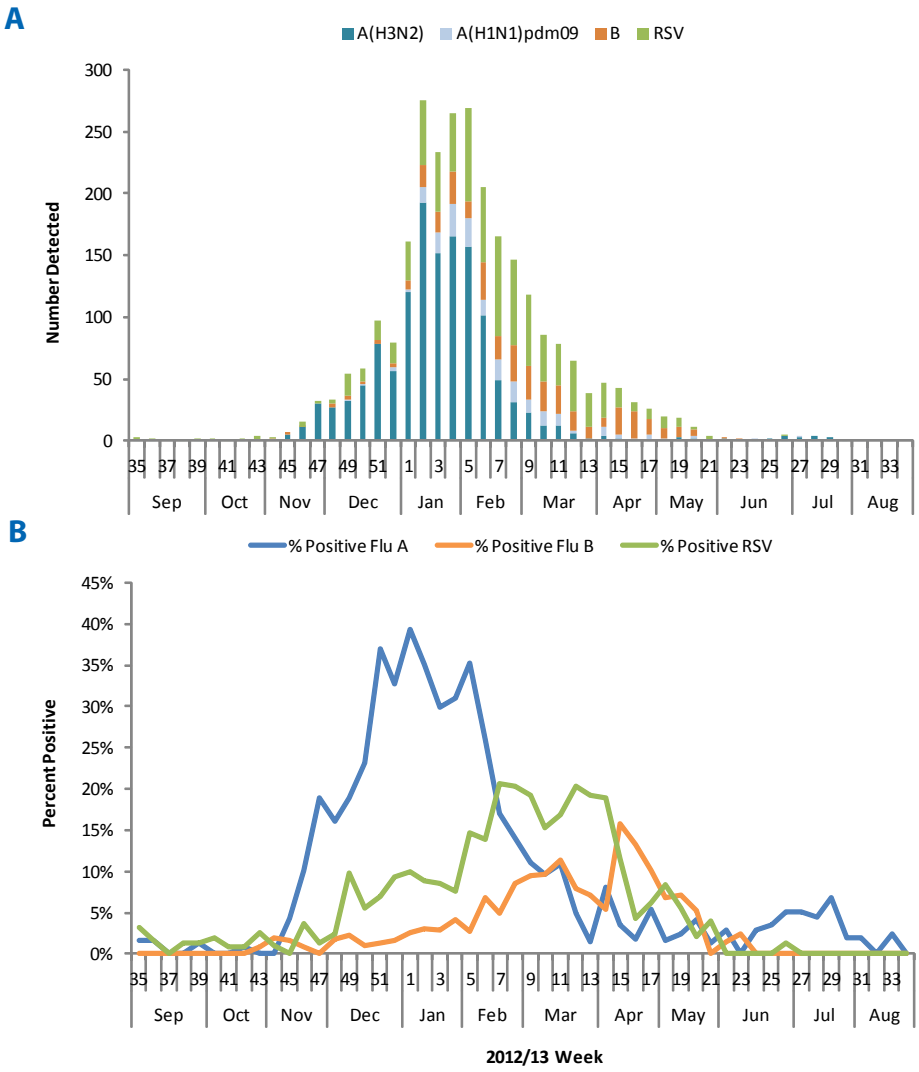
LABORATORY TRENDS

BRITISH COLUMBIA PUBLIC HEALTH MICROBIOLOGY & REFERENCE LABORATORY
Vancouver, BC

Influenza Surveillance

The 2012/2013 influenza season began in November in BC with high percent positivity of influenza A detected (Figure 5). Influenza A detection increased through December, peaking with detection rates of nearly 40% (Figure 5B) in January before falling to lower levels over the course of the season. Influenza A(H3N2) was the predominant strain this season with some A(H1N1)pdm09 also detected to a lesser degree (Figure 5A). As common in other seasons, influenza B appeared at lower rates in the season until it became the dominant influenza virus in March with detection rates peaking at nearly 16%. Respiratory syncytial virus (RSV) was also present throughout the season, peaking February-March with rates up to 21% positivity before disappearing in July.

The BCPHMRL Virology Program in the 2013/14 season will continue to use a four-plex RT-PCR assay to detect influenza A, B and RSV and further subtype any influenza A viruses. Testing for other respiratory pathogens will be performed on limited specimens from sentinel physicians, respiratory outbreaks, children under age 5 and hospitalized patients using the Luminex xTAG-fast Respiratory Viral Panel multipathogen testing system.



Clients are reminded to use the Influenza-Like Illness Outbreak Form when submitting samples for outbreak investigation.

Figure 5
Influenza and RSV surveillance, 2012-2013 season by week with **A**. numbers of sH3N2, pH1N1, influenza B and RSV detected and **B**. percent positivity of influenza A, influenza B and RSV, Virology Program, BCPHMRL.

September 19, 2013

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BRITISH COLUMBIA PUBLIC HEALTH MICROBIOLOGY & REFERENCE LABORATORY
Vancouver, BC

A Report of the BC Public Health Microbiology & Reference Laboratory, Vancouver, BC

The BC Public Health Microbiology Reference Laboratory (BCPHMRL) at the BCCDC site provides consultative, interpretative testing and analyses for clinical and environmental infectious diseases in partnership with other microbiology labs and public health workers across the province and nationally. The PHMRL is the provincial communicable disease detection, fingerprinting and molecular epidemiology centre providing advanced and specialized services along with international defined laboratory core functions province-wide.

This report may be freely distributed to your colleagues. If you would like more specific information or would like to include any figures for other reporting purposes, please contact us.

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