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Changes in Syphilis Testing

Syphilis is a sexually transmitted infection (STI) caused by the bacterium *Treponema pallidum*. Symptoms of infection are often subtle and easily confused with other STI's such as genital herpes infection. Syphilis is passed from person to person through direct contact with infectious exudates from obvious or concealed, moist, early lesions of skin and mucous membranes of infected people during sexual contacts. Transmission of the organism occurs during oral, anal, or vaginal intercourse. A pregnant woman with the disease can pass it to her newborn child.

The diagnosis of syphilis is most commonly made by serologic testing, and is typically performed in two settings: screening of patients at increased risk and evaluation of patients with suspected disease. The United States Preventive Services Task Force (USPSTF) recommends screening all pregnant women and people at higher risk of acquiring syphilis (MSM who engage in high risk behaviors, commercial sex workers, persons who exchange sex for drugs, and those in adult correctional facilities).

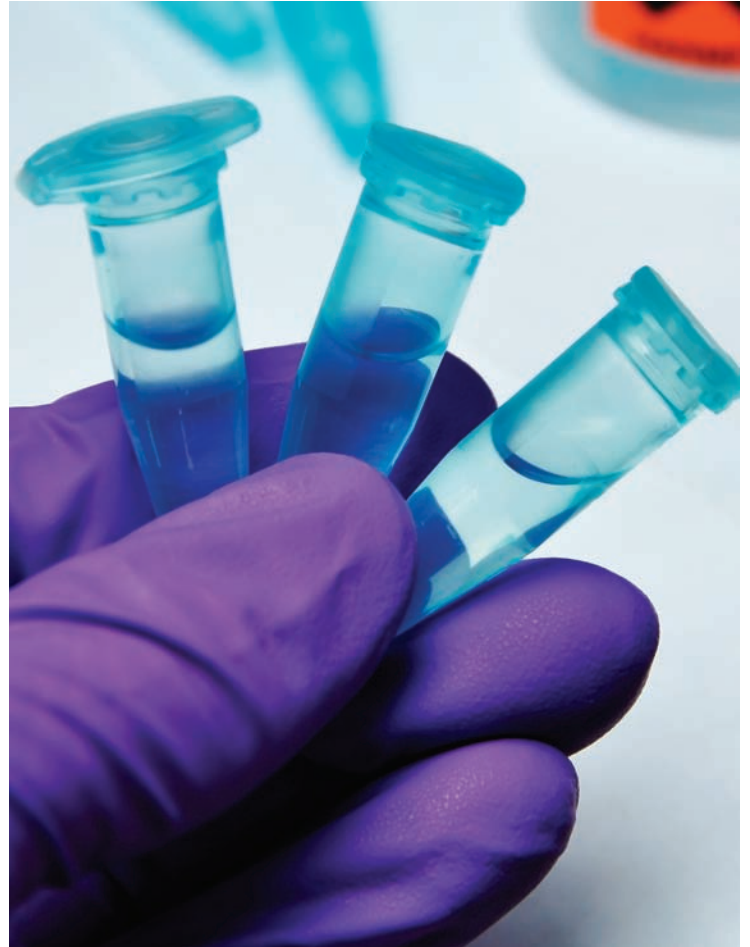


Photo: Kevin Vance

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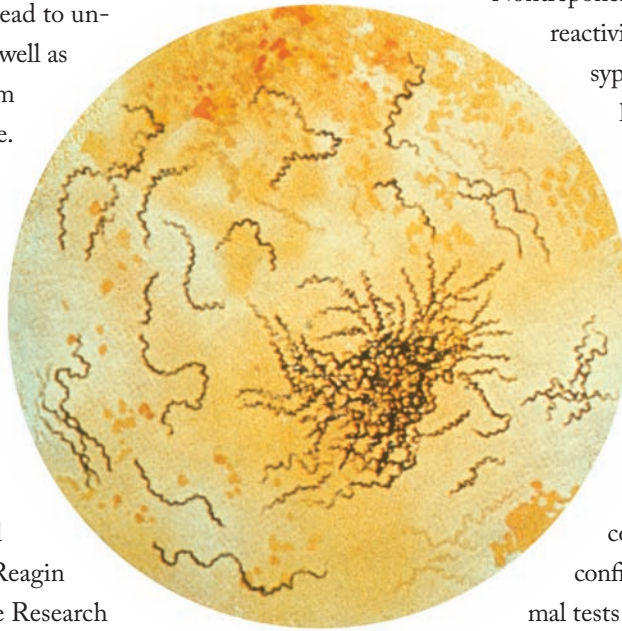
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The task force recommended against routine screening of asymptomatic persons who are not at increased risk of syphilis, since most positive tests in this setting represent false positive and can lead to unnecessary anxiety for patients as well as increased costs and potential harm from inappropriate antibiotic use.

The serologic detection of specific antibodies to *T. Pallidum* is of particular importance in the diagnosis of syphilis, as the natural course of infection is characterized by periods without clinical manifestations.

There are two types of serologic tests for syphilis: nontreponemal tests such as the Rapid Plasma Reagin (RPR) test and Venereal Disease Research Laboratory (VDRL) test, and specific treponemal tests such as the *Treponema pallidum* particle agglutination assay (TPPA), fluorescent treponemal antibody absorption (FTA-ABS) test, and the micro-

hemagglutination test for antibodies to *Treponema pallidum* (MHA-TP).

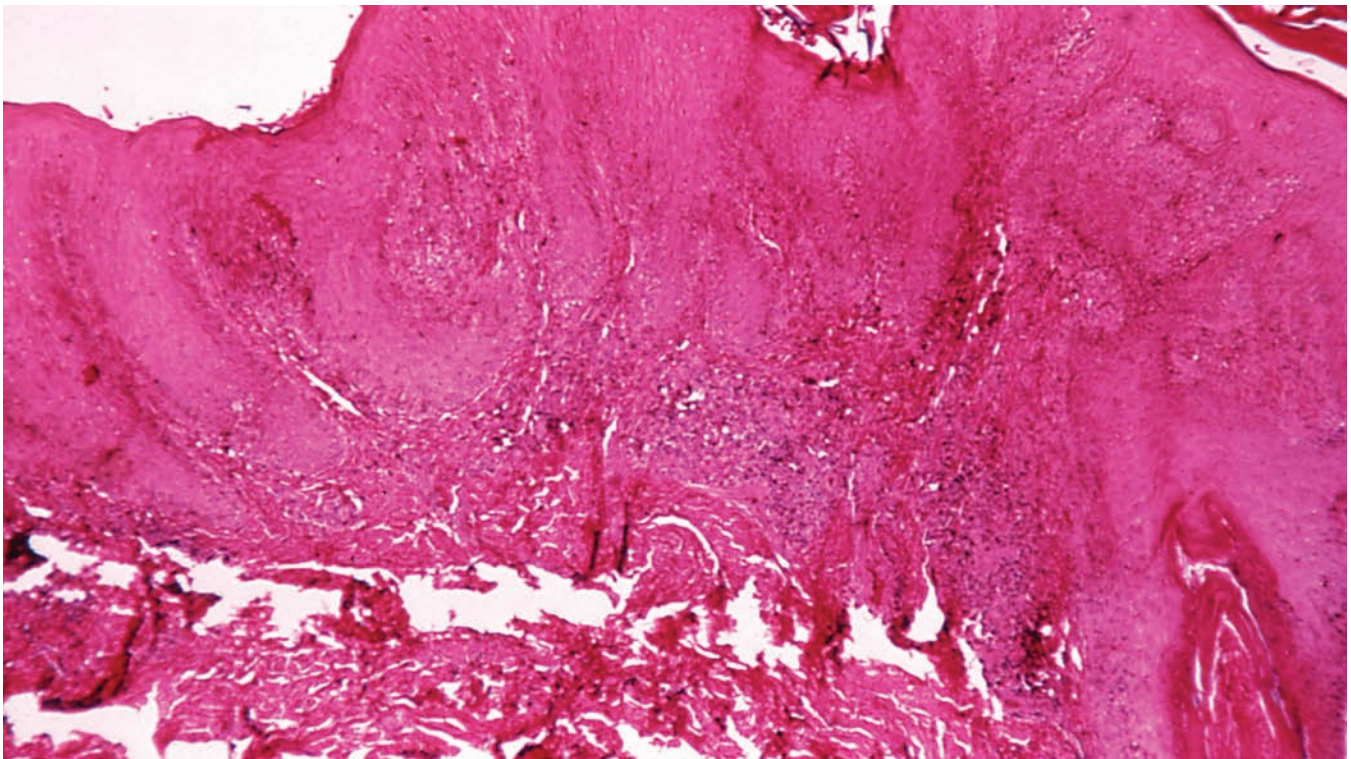


Nontreponemal tests are based upon the reactivity of serum from patients with syphilis to a cardiolipin-cholesterol-lecithin antigen. These tests measure IgG and IgM antibodies and are used as the screening test for syphilis in most settings. Positive tests are usually reported as a titer of antibody, and they can be used to follow the response to treatment in many patients.

Treponemal tests are more complex and are usually used as confirmatory tests when the nontreponemal tests are reactive. These tests all use *T. pallidum* antigens and are based upon the detection of antibodies directed against treponemal cellular components. These tests are qualitative and are reported as reactive or nonreactive.

Above image is courtesy of Department of Health and Human Services Public Health Image Library (PHIL)





Courtesy of Department of Health and Human Services Public Health Image Library (PHIL)

Algorithms for Screening and Testing

Standard algorithms — The use of a single serologic test to diagnose syphilis is generally inadequate because of the potential for false-positive results. However, it is highly unlikely that any one patient will have false positive tests using both reagin and treponemal techniques. Thus, the usual testing algorithm is to screen with a nontreponemal test such as the RPR; a reactive specimen is then confirmed as a true positive with a treponemal test such as the TP-PA. When results are reactive to both treponemal and RPR tests, persons should be considered to have untreated syphilis unless this is ruled out by treatment history. Persons who were treated in the past are considered to have a new syphilis infection if quantitative testing on an RPR test (or another nontreponemal test) reveals a four-fold or greater increase in titer.

In 2008, the CDC reported that four New York City laboratories had reversed the traditional order of screening and confirmatory tests for syphilis (i.e., specific treponemal testing (EIA) prior to non-specific treponemal testing).

However, this change in diagnostic procedures resulted in test results that would NOT have been identified by the traditional testing algorithm. For example, three percent of test results have a reactive treponemal test result and non-

reactive nontreponemal test result. The importance of these paired testing results is unclear because no specific prognostic information exists to guide patient evaluation. Such testing algorithms can lead to confusion regarding patient management. **The CDC has published some suggestions for diagnostic management for clinicians who receive these discordant results:**

When results are reactive to the treponemal test (EIA) but nonreactive to an RPR test, patients with a history of previous treatment require no further evaluation.

For persons without a history of treatment, a second, different treponemal test should be performed. If the second treponemal test is nonreactive, the clinician may decide that no further evaluation or treatment is indicated, or may choose to perform a third treponemal test to help resolve the discrepancy.

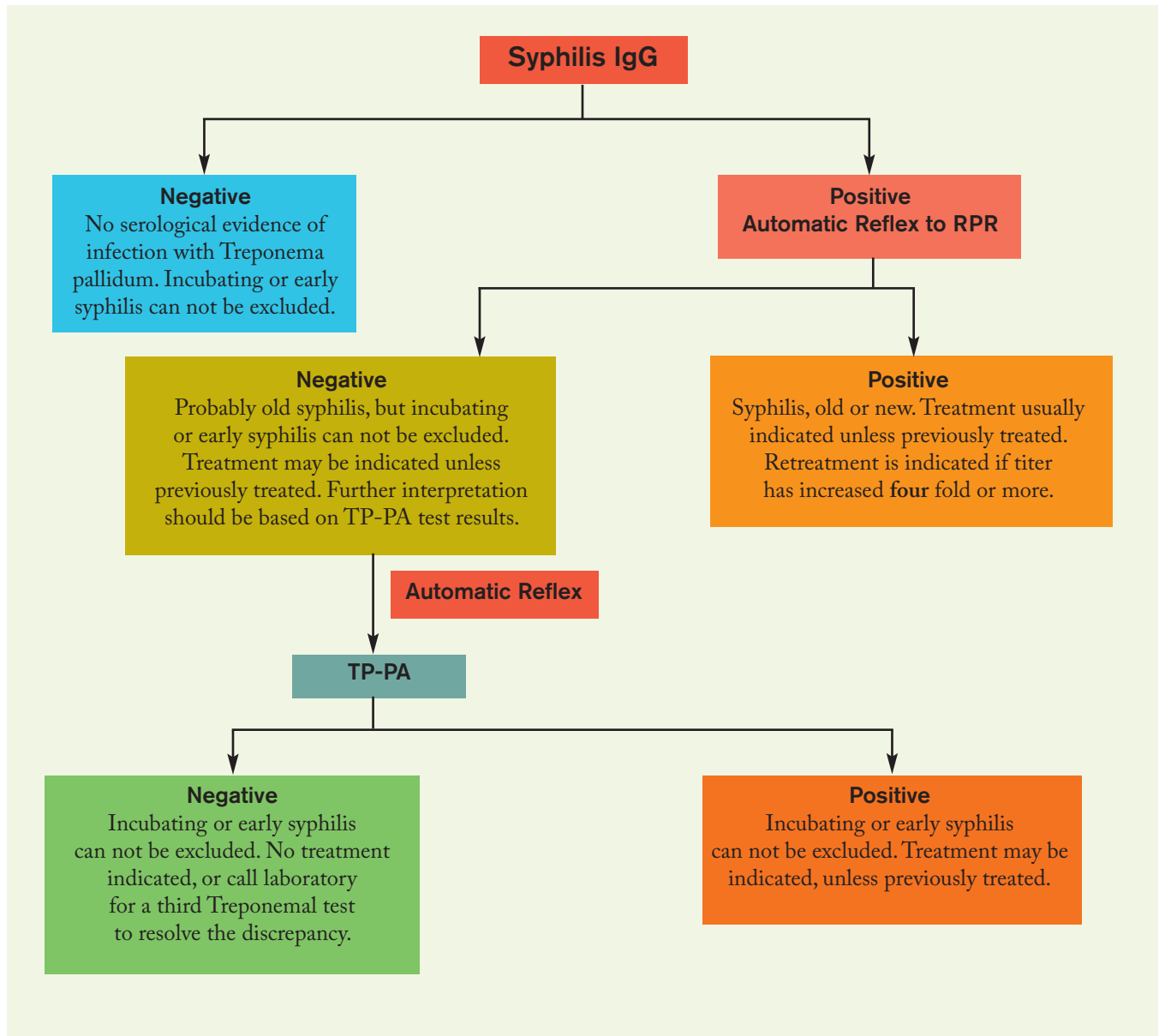
If the second treponemal test is reactive, clinicians should discuss the possibility of infection and offer treatment to patients who have not been previously treated. Such patients are unlikely to be infectious (unless history or physical examination suggests otherwise) and should be treated for late latent infection, even though they do not meet standard surveillance criteria.

Once syphilis has been diagnosed, the response to treatment can be assessed by changes in the titer of reagin antibodies. It is important that the same testing method be used for all follow-up examinations since titers may vary by 1 to 2 dilutions if different tests are used. The response to treatment is not uniform among all patients, and it may be difficult to decide when retreatment or further work-up (such as cerebrospinal fluid examination) is needed.

The current labor-intensive and subjective tests for syphilis at UMass Memorial Laboratories will be replaced by sensitive, fully automated assays. There are no changes in the specimen collection requirements (one SST/Serum tube). This change will greatly improve quality and turn around time of the test reports.

Effective August 24, 2009, UMass Memorial Laboratories will be offering a new algorithm for Syphilis testing. All current RPR test requests will be initially screened for Syphilis IgG (Mnemonic: SYPG) by a very sensitive and automated multiplex immunoassay (BioPlex 2200; BioRad Labs, CA). Only positive Immunoassay test results will be reflexed to traditional RPR test with titers. If RPR is negative, then only second Treponemal test (TP-PA) will be performed.

If you have questions, comments or suggestions, please contact:
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Antinuclear Antibody (ANA) Testing: Update

Screening for ANA has been performed for many years to identify patients with autoimmune diseases. ANAs are directed against a variety of nuclear antigens and have been detected in the serum of patients with many rheumatic and non rheumatic diseases, as well as in patients with no definable clinical syndrome. The strong association of ANA with systemic lupus erythematoses (SLE) is well established, and this finding satisfies the 1 of 11 criteria available for diagnosis.

The traditional tool used to detect ANAs is indirect immunofluorescence (IFA) which is a labor intensive microscopic technique and the test interpretation is operator dependent. This assay is considered the **gold standard** for ANA testing with high sensitivity. The IFA testing currently performed using Hep-2 cells, which contain approximately 100-150 possible antigens and most of them are not well defined and or characterized. When performed with a history and physical,

it identifies almost all patients with SLE (95% sensitivity), although the specificity of this assay is only 57%. In addition, ANA by IFA has the sensitivity of 85% for systemic sclerosis, 61% for polymyositis/dermatomyositis (PM-DM), 48% for Sjogren's syndrome, 57% for Juvenile idiopathic arthritis, 100% for drug induced lupus, 100% for mixed connective tissue disease (MCTD), and autoimmune hepatitis as well as being important in monitoring and assessing prognosis in individuals with Raynaud's phenomenon.

Multiplex Immunoassay (MIA) tests have been recently developed for use in clinical laboratory. It utilizes individually identifiable, fluorescence micro spheres (beads), each coupled with a different antigen or antigen mixture to test for multiple antibodies simultaneously in the same tube. This Multiplex ANA screen is intended for qualitative screening of specific ANAs, the quantitative detection of DSDNA Ab,

| Test Name | Mnemonic | Comment |
|---------------------------|----------|--|
| ANA Multiplex | ANAMP | The test result, negative or positive is dependent upon the presence or absence of 11 specific clinically relevant antibodies as mentioned above. The positives will not be automatically reflexed. You can order individual tests up to 30 days of the initial test result. |
| ANA Multiplex with Reflex | ANAREF | If ANA test result is positive, it will be automatically reflexed to all 11 antibodies (DsDNA, Chromatin, Sm, Ribo- P, SS-A, SS-B, SmRNP, RNP, Scl-70, Centromere B, and Jo-1) as mentioned above. |
| Lupus Panel | LUPUS | ANA Reflex to dsDNA, Chromatin, Sm, Ribosomal P, SS-A, and RNP. |
| MCTD Panel | MCTD | ANA Reflex to RNP, Sm, SmRNP, and Chromatin. |
| Sjögren's Panel | SJOGREN | ANA Reflex to SS-A and SS-B. |
| Scleroderma Panel | SCLEROD | ANA Reflex to Scl-70 and Centromere B |
| DS DNA Abs | DSDNA | Can be ordered individually |
| SSA Abs | SSA | Can be ordered individually |
| SSB Abs | SSB | Can be ordered individually |
| Sm RNP Abs | SMRNP | Can be ordered individually |
| RNP Abs | RNP | Can be ordered individually |
| Sm Abs | SM | Can be ordered individually |
| Scl-70 Abs | SCL70 | Can be ordered individually |
| Chromatin Abs | CHROMAT | Can be ordered individually |
| Ribosomal P Abs | RIBOP | Can be ordered individually |
| Jo-1 Abs | JO1 | Can be ordered individually |
| Centromere Abs | CENTRO | Can be ordered individually |



and semi quantitative detection of 10 separate antibody assays (Chromatin, Ribosomal-P, SSA, SSB, Sm, SmRNP, RNP, Scl-70, Jo-1 and Centromere-B). This ANA by MIA Screen detects the presence of clinically relevant circulating auto antibodies in serum. These auto antibodies may be useful as an aid in the diagnosis of systemic rheumatic diseases such as SLE, MCTD,UCTD, Sjogren's Syndrome, Scleroderma (Systemic Sclerosis), PM-DM and others. These assays are specific compared to IFA, and they are not as sensitive as IFA, because it is not looking at 100-150 possible antigens in the Hep-2 cells, rather specifically looking at 11 specific targeted antibodies. These assays have typical sensitivities of 66-94% for SLE, 94% Sjogren's, 68% for systemic sclerosis, 48% for PM-DM. They are specific compared to IFA for detecting specific targeted connective tissue disorders. In persons with no connective disease the specificity of MIA ranged from 77-91% and in apparently healthy individuals it is at 93%.

UMass Memorial Laboratories offers both IFA and MIA testing for ANAs. All ANA- IFA testing requests are screened by an ELISA assay initially by the reference laboratory. This ELISA test use a purified Hep-2 Cell extract similar to IFA, and by this ELISA the detection of ANA positivity is 99% SLE, 85% in Sjogren's, 88% in scleroderma, 55% in RA, and

40% in JRA. All specimens that screen positive or equivocal are confirmed by IFA using HEp-2 cells, and if positive, the titer (1:40 on wards) and pattern type will be reported.

ANA by MIA testing also have several choices: (see page 6).

Once ANA by MIA is positive, the positive ness of individual antibodies can be associated with specific disease and/or ANA by IFA pattern as below.

The physicians have a choice for ordering ANA testing. They should specifically request whether testing be done by IFA or MIA. The internal validation studies performed on simultaneous measurement of ANA by IFA and MIA showed an agreement of 62% (1:40 IFA+ve titer) to 70% (1:160 IFA +ve titer) depending upon what titer considered positive by IFA. The ANA screen is not, in itself, diagnostic for autoimmune disease and should be considered in conjunction with other laboratory test results and clinical presentation of the patient.

If you have questions, comments or suggestions, please contact:

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| Association of Disease and Antibody Patterns | | | | | | |
|--|--------------|-------------------------------------|------|--------------------|-------------|--------------|
| IFA Pattern | Antibody | Targeted Connective Tissue Diseases | | | | |
| | | SLE | MCTD | Sjogren's Syndrome | Scleroderma | Polymyositis |
| Speckled | SSA | 52% | 13% | >80% | 23% | 42% |
| | SSB | 27% | <2% | >80% | 5% | <2% |
| | Sm | 42% | 31% | <2% | 5% | 8% |
| | SmRNP | 45% | >80% | <2% | 7% | <2% |
| | RNP | 48% | >80% | <2% | 9% | 8% |
| Homogenous | Chromatin | 73% | >80% | 12% | 14% | 8% |
| | dsDNA | 45% | 12% | 6% | 9% | <2% |
| Mixed | ScI-70 | 3% | 7% | <2% | 16% | <2% |
| Centromere | Centromere B | 12% | 7% | <2% | 27% | <2% |
| Cytoplasmic | Ribosomal P | 30% | 7% | <2% | <2% | <2% |
| | J0-1 | <2% | 7% | <2% | <2% | 17% |

 Diagnostic Criteria

 Frequently Elevated

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We are one of the largest laboratory providers in New England

UMass Memorial Laboratories is opening a Patient Service Center (phlebotomy draw station) at 109 Beechwood Ave., Rhode Island.

The vision of UMass Memorial Laboratories is:

- To be a leading provider of laboratory services throughout New England, meeting the needs of patients and providers in the region, and
- To be one of the top ten academic medical center-based laboratories in the United States



Pawtucket PSC 109 Beechwood Ave., Pawtucket, Rhode Island

Pawtucket PSC is located at 109 Beechwood Ave., Pawtucket, Rhode Island. The hours and phone number at Pawtucket PSC are to be determined.