Clinical White Paper

Automated Detection of Acid-Fast Bacilli Stained with Auramine O under a Fluorescence Microscope

Summary

An automated real time detection system, called Signature Mapping TBDx™, has been developed to detect AFB (acid-fast bacilli) from digital images of auramine O stained slide captured under a fluorescence microscope system. Current results demonstrate that the detection system accurately detects the positivity and classifies the severity load. Future system configurations will incorporate bright field microscopy for Ziehl-Neesen stained slides, LED light sources, fully automated slide and FOV acquisition, and integration with laboratory information systems to provide fast and accurate results. Successful deployment of Signature Mapping TBDx™ is expected to provide a consistent, more accurate result, while improving productivity significantly. Additionally, Signature Mapping core technology is extensible to other disease applications. The current results have been published for presentation at the 47th Annual Meeting of the Infectious Diseases Society of America (IDSA), October 29 - November 1, 2009, Philadelphia, Pennsylvania.

Purpose of the Project

To develop a computer automated detection system to detect acid-fast bacilli (AFB) on multiple auramine O stained slides using the Olympus BX41 fluorescence microscope with mercury vapor light source.

Background

Founded in 2003 to solve complex detection and quantification problems in digital imaging, Guardian has successfully developed an automated screening system based on its breakthrough iterative transformational divergence process for the detection of various explosives in baggage. Currently, the technology is protected by 12 awarded and pending patents.

In 2008, Guardian applied its proven technology platform from the detection of explosives to the detection of TB. To accomplish this objective, Guardian collaborated with TB experts and key institutions in South Africa including: The National Health Laboratory Systems (NHLS) and the Aurum Institute for Health Research. The partnerships created an advisory board consisting of members from The National Institute of Communicable Diseases (of the National Health Laboratory Services, NHLS), the Aurum Institute and other leading experts worldwide, to assist in developing the highest performance and most cost-effective TB detection solution. During the R&D process Guardian visited TB hospitals, research centers, and rural/central clinics throughout South Africa to enable a full understanding of the current challenges and detection problems, acquire clinical and operational workflow knowledge, to collect TB cases, to demonstrate preliminary results, and to obtain feedback from researchers and clinicians for further improvement. Scientific experts from the US Center for Disease Control and Prevention (CDC), National Institute of Infectious and Allergy Diseases (NIAID) of US NIH, Global HIV/TB Initiative of Clinton Foundation, and StopTB Partnerships of World Health Organization (WHO) continue to provide Guardian with valuable encouragement, clinical, epidemic, technical, and operational expertise. The system is developed and productized as Signature Mapping TBDx™. The system has been evaluated in a series of pilot clinical evaluations by Guardian and clinical partners in South Africa in April and October 2009. A very encouraging result was obtained and reported in the section below.

Technology Development

Working with the NHLS TB Re-Check program which periodically provided a wide variety of cases, Guardian has collected and digitized 554 cases from both rural and central laboratories. Ranging from five to one-hundred FOVs were captured for each case dictated by the slide quality, positivity and severity classification, current and desired clinical workflow. Totally, Guardian has collected over 1,800 positive FOV and over 8,000 negative FOV for the development of the detection algorithm. The positivity and severity classification of these FOV have been confirmed by either consensus experts or culture. These FOVs were captured at 40x magnification power at normal objective lens and 10x at the eye piece of standard fluorescence microscope system using 1.2 mega pixel digital camera with Peltier cooling filter appropriate for high intensity fluorescence. Each FOV covers 166 μm x 221 μm squared area at 160 nm pixel resolution sufficient for the detection of AFB with typical width (0.2~1.0 μm) and length (1~10 μm). Over 40,000 individual AFB were extracted from positive digitized FOVs by experts and close to 80,000 non-AFB objects were extracted from negative FOVs as training objects to develop and test the robustness of the algorithms. These digitized FOVs cover a comprehensive variety of macroscopic slide quality and microscopic field quality based on different staining processes and chemicals. These training AFB objects cover well-recognized varieties of morphology, contrast, and size of AFB. These FOV and AFB were separated into training, validation, and evaluation groups to form the development set. By analyzing the contrast levels for different microscopic quality from both positive and negative FOVs and various backgrounds (e.g., debris, artifacts, etc.), a staining-based image processing method has been developed to normalize all the fields into a set of standard images representing different contrast ranges. These AFB objects were used to train the machine-learning engine (specifically, the support vector machine - SVM); which is capable of learning about specific information within images. The machine-learning engine first learns from a predetermined training sample, which is a majority of the total development set. During the learning process of the sample, a small validation set is then used to enhance each individual learning process. After successful sessions of several learning processes, the evaluation set (another small independent set of total development cases previously not seen by the developers and machine-learning engine). is then used to further test and confirm the accuracy and robustness of learning. After the successful training of the machine-learning engine, the algorithm is encapsulated into a complete system which is capable of determining AFB and non-AFB based on either previously seen or not-seen image/features.

Detection Technologies for TB AFB

Guardian-developed machine-learning algorithms are based on Signature Mapping technology. Signature Mapping is a dynamic and iterative transformation process in which specifically designed transformation algorithms impact image pixel data causing the group of pixels representing each AFB to respond in a unique collective way. This response-based reaction generates new groups of "self-classifying" pixel data. These self-classifying groups provide a unique set of signatures for each object. The resulting "signature mapped" data, enables the implementation of a rich set of imaging tools that provide new clinical approaches for computer aided diagnosis efforts. This technology is able to enhance the image properties that human eyes can not see. The core Signature Mapping technology "the detection algorithm" consists of two main modules: (1) individual bacilli detection and (2) FOV/Case detection modules. The bacilli detection module first applies image segmentation to select as many AFB candidates as possible in a single FOV. A segregation process applies to all of the candidate regions of interest (ROI) to enhance the appearance as well as characteristics of the AFB candidates. Feature extraction process extracts the representative feature parameters associated with each candidate and sends these parameters to compare against the trained machine-learning engine parameters. The system accurately identifies individual AFB along with very little false positive object in each FOV. The FOV/Case detection module then rolls up results from bacilli detection module to determine the positivity of each FOV and to each Case. When a single AFB is found in a FOV, this FOV will be identified as TBDx -positive FOV. The system will track all detected AFB's. When any one of the FOVs in a particular case is determined as a TBDx-positive FOV, this case is then identified as a TBDx-positive case. In other words, after each individual AFB is detected by the system, the detection

results are then aggregated from AFB level to the field of view level then to the case level. This provides the information necessary to determine the positivity (positive vs. negative) and severity level (scanty, P+, P++, etc.) for each case.

Independent Detection Results and General Performance

The current results demonstrate that TBDx is able to identify 92.86% positive cases (39/42 positive cases) with 3.75% false positive field of view (45/1,200 FOVs on negative cases) on independent case sets consisting of 42 positive and 40 negative cases, confirmed by NHLS experts in Johannesburg, South Africa, Oct 10 – Oct 15, 2009. These 82 independent cases were randomly selected by NHLS personnel from total of 1,856 cases (1,659 negative and 197 positive cases) following the inclusion criteria: (a) numbers of negative and positive cases should be equal and (b) numbers of scanty and non-scanty positive cases also should be equal. These independent cases were collected at NHLS Mycobacteriology Referral Laboratory Braamfontein, Johannesburg South Africa during March 2009. These independent cases were unseen by TBDx and have never been used for development or validation testing of TBDx. About 30 FOVs were captured and digitized from each case. Among 42 positive cases, 22 are scanty and 20 are P+/P++/P+++ cases. The sensitivity is 86.36% and 100% for scanty and for P+/P++/P+++ case, respectively. The existence of a single FP in a single FOV can turn one negative case, which consists of multiple FOVs, into a false positive case. It was noted that some of FP AFB's detected have rod shapes and some of them do not possess any morphology and contrast of AFB. For the positive cases having a few AFB, e.g., scanty and P+ cases, TBDx can easily detect isolated individual AFB's, whereas for the positive cases having large amount of AFB, e.g., P++ and P+++, TBDx is designed to detect sufficient numbers of AFB merely to assure these TBDx-positive cases are true positive cases and the load has been correctly identified. Guardian continues to improve the detection accuracy focusing on the increase of detection specificity.

Potential Benefits

In addition to detecting AFB, the TBDx system serves as a work station providing decision support tools and integrates imaging and information management software and provides real-time detection to ensure faster results and significant improvement in results turnaround time. It also increases the volume of slides screened, leverages existing laboratory microscopy equipment and utilizes stablished smear microscopy processes. The displaying and viewing images on a monitor also eliminates eye fatigue and makes the slide review process much easier.

Retrofit System

A TBDx retrofitted system has been developed to leverage existing fluorescence microscopes by adding a digital camera, a standard PC along with the Signature Mapping automatic detection software. After the operator uses the system to acquire digital FOV, from the slide specimen the system automatically performs the TB analysis and detection, aggregates the detection results from each FOV into a case and then generates the diagnostic results for patient. This retrofit system can be deployed in laboratories, hospitals, clinics that have less skilled technicians. The retrofit system will also be used for the clinical study evaluation of the detection performance. The retrofitted TBDx system can be migrated to a fully automatic detection system (i.e. automatic and systematic slide loading, microscope stage navigation and FOV acquisition) to provide a high volume scaled-up screening system.

Clinical Deployment Strategies

The Signature Mapping application affords many opportunities to provide dependable, consistent results, with improved detection accuracy reducing the human work load and provide faster access to patient information. Here are some examples.

1. Signature Mapping TBDx can be deployed as a mass screening tool for the purpose of eliminating absolutely negative cases from technologists reviewing. Since, in general, only 12 to 15 percent of cases are positive this should have a major impact on case load work.

2. Signature Mapping TBDx can be deployed as a re-check tool which would enable the validation of all visually analyzed negative cases. Such a program would enable an effort to find additional scanty cases or cases which might fall into the sputum negative/culture positive diagnostic category.
3. Signature Mapping TBDx can be utilized as a scanty sputum slide analysis support tool, utilized in a re-check configuration where the digitized FOV can be image processed and visually enhanced to eliminate the mistakenly identified scanty positive sample, i.e., reduction in false positive.
4. Remote support: in many rural laboratories, technologist may have little or no access to experts. Using the digital FOV and standard medical viewing image format, TB experts can support rural labs with remote sputum slide viewing and consultation.

Future Development and Impact to Care

Digital virtual laboratory analysis is poised to have a major impact on laboratory medicine. The combination of digital imaging with automatic computer-aided analysis in TB detection have the ability to profoundly reduce costs, improve diagnostic consistency and accuracy while permitting the heavy burden countries of the world to keep pace with increasing demand.

The Signature Mapping detection technology platform may be packaged in a number of hardware configurations enabling both semi and fully automatic batch processing which can be deployed in rural and central laboratories. In addition, the system can be easily migrated from auramine O staining technology to the detection of AFB on Ziehl-Neelsen stained slides using bright field microscopy to address different sputum staining methods used in majority of world. Furthermore, the system can be augmented to accommodate other lighting sources including LED. This system will provide fast and accurate results in real time for the direct observed therapy for the treatment of TB.