ABOUT HAITI

With an estimated population of 9,980,000, Haiti is the poorest country in the Western Hemisphere (WHO, 2008).

The incidence and prevalence of tuberculosis (TB) is the highest in the region with an annual estimated 306,336 and 366 per 100,000 population, respectively. Of all new TB cases, 6.7% are estimated to be HIV-positive patients (WHO, 2008).

A national public health laboratory, the Laboratoire National de Santé Publique (LNSP), was established in 2006 to provide national reference testing center, including direction and organization of Quality Assurance (QA) programs. As of 2008, the network of microscopy centers consisted of 238 public and private, with one private center providing culture and limited drug susceptibility testing.

BACKGROUND

Direct smear microscopy of respiratory specimens has been the main case detection method and used for the diagnosis of TB in Haiti, making quality assessment of sputum smear microscopy an important activity to evaluate technical proficiency.

For the past year, with support from the Massachusetts Supranational TB Reference Laboratory (MSRL), the LNSP has established an External Quality Assessment (EQA) program using panel testing slides set. This is the first measurement of AFB performance in Haiti.

Twenty high volume smear microscopy centers were evaluated. The International Union Against Tuberculosis and Lung Disease (IUATLD) grading scale and scoring system were adapted for evaluation of panel testing results.

METHODS

Preparation of AFB Smears:

Significant changes to the current protocol have been made in order to manufacture large amounts of highly uniform slides with desirable bacterial loads. (See more about the method in the RESULTS section). The initial set contained a total of 400 slides with varying AFB quantities were prepared at the MSRL. This set was comprised of 5 groups with various AFB load ranging from scanty (1-9 AFB/500 fields) to 1+, 2+ 3+ or negative. Half of all slides were stained using Kinyoun or Ziehl Neelsen (ZN) methods, while the other half were unstained.

Panel Composition & Validation:

Standardized panels containing 5 stained and 5 unstained slides with varying AFB quantities were validated by the MSRL and co-validated by the LNSP Haiti as follows: (a) five stained and five unstained slides were randomly selected from each group, the latter were stained, read and quantified; (b) a slide group was considered valid and was included into the panel if the results from 5 independent readers did not exceed one standard deviation from the mean.

LOCATION

Study Design:

Validated panels were distributed to a selected group of 20 high volume microscopy centers. Results were assessed and a site score provided. Points were deducted based on the degree of variance (max value of 10 points per slide) to the correct score (see the Scoring System for additional detail).

After pre-intervention measurement, on-site corrective action activities were formulated with LNSP. The LNSP initiated re-fresher course training activities, reinforcing standardized ZN staining technique.

Post-intervention performance were evaluated using a second panel testing slide set with the same degree of challenge.

Scoring System:

100 points per microscopy center/10 per slide
- Perfect Score for a single slide: 10 Points
- Any Negative reported as Rare: 5 Points
- Any Negative reported as 1+ or above: 0 Points
- Any Rare reported as Negative: 5 Points
- Any 1+/2+/3+ reported as Negative: 0 Points
- Any deviation of 1 level: 8 Points
- Any deviation of 2 or more levels: 5 Points

RESULTS

Pre-intervention measurement revealed a proficiency average of 60%. Most centres had one or more major errors, predominantly false-negative. At set point of 80% sensitivity, the numbers of laboratories with performance below 80% were eighteen (90%) when the least stringent scoring criteria was applied. This early data demonstrated performance issues.

Post-intervention measurement revealed an overall improvement increase of 27%. At set point of 80%, the numbers of laboratories with performance below 80% decreased from eighteen to four (20%).

This study suggests evidence that EQA panel testing is feasible for implementation in Haiti. The use of panel testing and on-site corrective activities allow for an unbiased representative evaluation of the overall quality of smear microscopy.

“Scanty” or Rare Slide Protocol Modification

As it has been recently demonstrated, “scanty” smears play a pivotal role in TB diagnostics as well as treatment control. Therefore it is imperative to include scanty slides into EQA panels in order to adequately challenge labs under assessment. Preparation of scanty smears with equal AFB distribution among the slides in a selected group and their even spread throughout the smear area of each slide is a notoriously difficult task. Due to the clumping effect, the number of AFB distributed between the slides is somewhat unpredictable since one clump can contain more than 10 AFB which puts it into a category different from “scanties”. To avoid clumping, effect the following protocol has been developed, validated and implemented:

a) a clinical MAC isolate was subcultured in a modified 7H9 liquid medium with aeration until it reached the early log phase;

b) bacterial suspension was left motionless for 3-4 hours after which the upper layer was transferred to a fresh tube;

c) serial dilutions where made in order to achieve the desirable density that was adequate to make slides with 1-9 AFB;

da) AFB-negative sputum obtained from a patient with benign pulmonary abscess was spread on a microscope slide and air dried;

e) diluted bacterial suspension was distributed evenly over the sputum covered area and heat fixed.

LESSONS LEARNED

- Mechanisms to deliver panel testing slide sets must take local logistical complexities into account.

- Reference laboratory staff should be involved in the development of program guidelines to ensure proper evaluation, reporting, and implementation of corrective actions.

- Attention should be given to timely reporting of results to the participating labs, departmental directors, and the National TB Program.

NEXT STEPS

- Gradual EQA expansion in 2010 based on annual volume, programmatic importance, and geographic diversity.

- Build capacity for the reference staff to prepare panel testing slide sets to microscopy centers at regular intervals, including results evaluation, reporting, and on-site corrective activities.

- Network training of TB laboratory supervisors, clinicians, and Departmental Directors on role of TB EQA activities.