

Poster 1:

Testing Algorithms for *Mycobacterium tuberculosis* Complex using Real Time-Polymerase Chain Reaction: When to implement GeneXpert assay?

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Objective: To determine the most effective and efficient algorithm to perform PCR analysis by GeneXpert assay on tuberculosis specimens.

Study Design: Traditionally, *Mycobacterium tuberculosis* complex (MTB-complex) is identified by conventional methods. However, molecular methods are now being used more often. The previous algorithm of acid fast bacilli (AFB) smear positive patients who were new to the Bureau of Clinical Laboratories (BCL) was run on the GeneXpert automatically. The Xpert® MTB/RIF assay, performed on the GeneXpert® Instrument Systems, is a qualitative, nested real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for the detection of MTB-complex DNA in raw sputum or concentrated sputum sediment prepared from induced or expectorated sputum. In specimens where MTB-complex is detected, the Xpert MTB/RIF assay also detects the rifampin- resistance associated mutations of the *rpoB* gene. This assay was used to test 551 tuberculosis specimens between January 8, 2015 and October 12, 2016 using the new algorithm that is based on AFB smear results in addition to physician requests. If a patient is AFB smear positive and is not on file at the BCL, PCR by GeneXpert is performed. If a patient is smear negative and a physician requests PCR by GeneXpert based on clinical presentation of the patient, PCR is also performed.

Results: Of the 551 specimens, 108 were positive by GeneXpert yielding a positivity rate of nearly 20%. The 98 positives from the 304 AFB smear positive specimens had a positivity rate of 32% and the 10 positives from the 247 AFB smear negative specimens had a positivity rate of 4%. Of the 108 PCR positives, 105 grew MTB-complex. In addition, there were two cultures that were AFB smear negative and PCR negative that grew only a few colonies of MTB-complex which were below the limit of detection by the GeneXpert assay.

Conclusion: The new algorithm of utilizing the AFB smear result in addition to the physicians' requests yielded an additional 10 positives. Using the old algorithm of AFB smear result only, MTB-complex would not have been identified in the 10 specimens until final culture results. Utilizing the AFB smear result and the physicians' requests yielded a high rate of positive PCR by GeneXpert samples.

Poster 2:

Evaluation of a Multiplexed PCR and Melt Curve Analysis Method for Rapid Identification of *Mycobacterium tuberculosis* Complex (MTBC) from Positive Broth

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Objective: To evaluate the performance characteristics of a relatively inexpensive, simple, PCR/meltcurve method described in Kim et. al. in the rapid identification of MTBC from positive MGIT broths at the Arizona State Public Health Laboratory (ASPHL).

Study Design: The analytical sensitivity, specificity and reproducibility of the PCR/meltcurve method was performed by spiking negative MGIT broths with known concentrations of organisms and tested. In addition, clinical MGIT broths inoculated with patient samples were tested in parallel with the new method and current method in their ability to identify MTBC. From optimization experiments, a PCR/meltcurve reaction was considered positive for MTBC if the Ct value is ≤ 40 and the melting temperature was between 84-87 °C. All positive clinical MGIT broths were sub cultured onto solid media to confirm presence of MTBC. Samples from failed HPLC runs were reflexed to MALDI-TOF for identification.

Results: The limit of detection of the assay was determined to be 8.6×10^3 CFU/ml. The assay was highly specific, reproducible and reliably detected MTBC from different lineages. A total of 269 positive clinical MGIT samples were tested, 105 of which were positive for MTBC based on recovery on solid media and identification by MALDI-TOF. The PCR/meltcurve method detected MTBC in 103 of the 105 samples (98%), whereas HPLC could detect MTBC in 43 of the 105 samples (40%) successfully on the first attempt.

Conclusions: Here, we present a simple, rapid, cost effective PCR/meltcurve method for the detection of MTBC in positive MGIT broths. The HPLC method is technically demanding and labor intensive. A successful run of ten samples takes a full day for extraction and analysis on the instrument, with interpretation and reporting completed the next day. The meltcurve/PCR method, by comparison, takes less than 2 hours for results and is simple to perform, requiring very minimal hands on time. Furthermore, when comparing costs between the two methods, the PCR/meltcurve method is relatively inexpensive by an estimated four fold when based on consumables alone. Our study demonstrates that this method is a reliable replacement for the HPLC when detecting MTBC in positive MGIT broths.

Poster 3:

Identification and Validation of New Mechanisms of Isoniazid Resistance in *Mycobacterium tuberculosis*

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Objective: Globally, ~14% of all tuberculosis (TB) cases are isoniazid (INH)-resistant, and ~10-12% of INH resistance is unexplained. The validation of novel INH resistance mechanisms can enhance the accuracy of rapid molecular tests for INH resistance including those for detection of both INH and rifampin resistance (i.e., multidrug resistance).

Study Design: We identified 13 *M. tuberculosis* clinical isolates from archived samples that were previously classified as INH resistant (INHR) by phenotypic testing but lacked mutations commonly associated with INH resistance. The MIC for INH was determined using a microdilution assay and whole genome sequencing (WGS) was performed to identify mutations associated with INH resistance.

Results: Four strains were susceptible to INH while most exhibited low-level (4/13) or high-level (5/13) resistance. We identified numerous distinct mutations in the INH resistance-associated loci *katG*, *ahpC*, *ahpD*, *fabG1*, and *fabG3* (but none in *furA*, *inhA*, or *Rv1910c*). One high-level INHR strain did not harbor mutations in any aforementioned loci. We performed functional genetics to determine if the *katG* V1A mutation confers resistance to INH. We confirmed the mutation was successfully introduced into the pansusceptible strain H37Rv by Sanger sequencing and found that it does indeed confer INH resistance.

Conclusion: A mutation discovered by WGS (*katG* V1A) was shown by functional genetics to confer INH resistance. Additional analyses will be conducted to determine the contribution of other mutations identified for INH resistance. Examination of isolates with unknown mechanisms of resistance by WGS could aid assay development by identifying novel resistance markers.

Poster 4:

The Molecular 'MDR Screen' is an Important Tool in the Diagnosis and Initiation of Appropriate Therapy in TB Patients in the State of Florida

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Objective: The Florida Department of Health Bureau of Public Health Laboratories (BPHL) algorithm for the diagnosis and anti-mycobacterial susceptibility testing (AST) of tuberculosis (TB) includes a molecular 'MDR (multi drug resistance) screen' for the rapid and accurate detection of drug resistance in TB. This study reviews the performance of the MDR screen and initiation of appropriate TB patient therapy.

Study Design: Analysis of all AST data over a 2-year period was performed, followed by review of patient treatment records for MDR cases. The following data were analyzed: date of MDR screen result by Hain Genotype® MTBDRplus assay, version 2 (Hain LifeScience); date of phenotypic AST result by Sensititre MIC assay (TREK Diagnostic Systems, Thermo Fisher); and date of initiation of appropriate MDR treatment regimen.

Results: From January 1, 2014 to December 31, 2015, MDR screen was performed on n=618 primary clinical specimens. Of 618 primary specimens a result could not be determined for n=84 (indeterminate or positive control band for TB missing). Of the 534 primary specimens for which a result was known, n=55 showed resistance to one or both rifampin (RIF) and isoniazid (INH): 14 were MDR (resistant to both RIF and INH), 5 were RIF mono-resistant, and 36 were INH resistant. In-depth review of the MDR cases showed that 9 were Florida patients, for which treatment data was available. Of these patients, the average number of days from date of MDR screen result to change to appropriate therapy was n=6.9 days (range 1-16 days). The number of days from change in appropriate therapy to when the phenotypic AST was available was n=30.2 days (range 11-77 days).

Conclusions: From 2014-2015, 10.3% of primary clinical specimens tested by the MDR screen showed resistance to one or both RIF and INH, MDR-TB was detected in 2.6%. The MDR screen provided results for change to appropriate therapy for MDR patients that would not have been available for several days, and in most cases weeks, if clinicians had waited for phenotypic AST results. Ultimately placing patients on appropriate therapy in a timely manner has a positive impact on patient and public health outcomes.

Poster 5:

Evaluation of Gene Xpert MTB/RIF in 70 Culture Positive Cases of Tuberculous Meningitis

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Objectives: Rapid and specific diagnosis of tubercular meningitis (TBM) is of paramount importance to decrease associated morbidity and mortality. Therefore, the present study was undertaken to compare Gene Xpert MTB/RIF (GX) assay and Multiplex PCR (MPCR) using three targets specific for *Mycobacterium tuberculosis* including IS6110, MPB64 and Protein b for diagnosing TBM as described previously from our center¹.

Study Design: GX and Multiplex PCR using three targets specific for *Mycobacterium tuberculosis* like IS6110, MPB64 and Protein B has been described previously from our center for diagnosis of TBM, published in Journal of neurology 2011¹, were performed on total 90 cerebrospinal fluid samples. These included CSF samples from 70 patients with culture positive TBM, CSF samples from 10 Non-TB infectious meningitis and CSF samples from 10 Non-infectious neurological disorder samples. These 20 non TB CSF samples were included in control group. Phenotypic testing by 1% proportion method was carried out for rifampicin and isoniazid from the 70 CSF culture isolates and the *rpoB* gene sequencing was also done for diagnosing rifampicin resistance. We have taken culture as gold standard and all results were compared to culture to calculate sensitivity. For specificity we have taken 20 non TB control group CSF samples as described previously¹.

Results: GX and MPCR were positive in 58/70 (82.85%) and 65/70(92.85%) patients respectively. Both tests were negative in all controls (20 control CSF samples). Rif resistance was detected in 8 of 70 (11.42%) by GX, and in 7 MPCR positive with *rpoB* gene sequencing. Out of the 70 isolates subjected to phenotypic drug susceptibility testing, 63 were sensitive to Rif and INH and 7 were found to be resistant to both Rif and INH. Thus, there was one case of false Rif resistance detected by GX, which was RIF sensitive on *rpoB* gene sequencing and phenotypic drug susceptibility testing. False RIF resistance was observed with probe B of Gene Xpert. Resistance mutations detected on *rpoB* gene sequencing were at 531 codon (3 cases), 533(2 cases) and 516 codon (1 case) and 526 codon (1 case) respectively. These mutations are picked up by GX as well. Cost of doing MPCR is less than 1 dollar where as GX is \$10.

Conclusion: MPCR had a higher sensitivity than GX for diagnosing TBM. MPCR is robust and cost effective method for diagnosis of TBM in low resource and high endemic country.

Poster 6:

Evaluation of Multi targeted Loop-Mediated Isothermal Amplification (LAMP) for Rapid Diagnosis of Extra-pulmonary Tuberculosis

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Objectives: Rapid and accurate diagnosis of extra-pulmonary tuberculosis (EPTB) is imperative for early treatment and better patient outcome. LAMP assay can be carried out in simple water bath under isothermal conditions in 60 minutes in any laboratory, even at rural setting in resource poor, endemic countries. We aimed to evaluate the LAMP test using IS6110 and MPB64 targets for M. tuberculosis complex diagnosis. Comparison of results of IS6110 LAMP, MPB64 LAMP with IS6110 PCR, culture and ZN (Ziehl-Neelsen) smear examination.

Study Design: LAMP assay using 6 primers was performed on various EPTB samples (CSF, Synovial fluid, Lymph node and tissue biopsies and various other samples like gastric aspirate, pus samples and urine samples) from patients suspected of EPTB. 200 patients, clinically suspected of EPTB (75 culture positive, 125 culture negative) and 100 non-TB control subjects were included in the study. Suspicion of EPTB was based on the clinical presentation, radiological examination and histopathological examination in all 200 patients. On evaluation 75 were found to be culture positive while the 125 culture negative patients included based on the composite reference standard (CRS) and response to ATT. Culture was used as reference standard in culture positive cases and CRS with response to ATT was used as reference standard in 125 clinically suspected cases of EPTB. For specificity calculations 100 non TB control were used and specificity was calculated with respect to that.

Results: Overall LAMP test (using either IS6110 or MPB64 targets) had sensitivity and specificity of 96% and 100%, respectively, for culture confirmed EPTB cases and 84% and 100%, respectively for culture negative, clinically suspected EPTB cases. Sensitivity of IS6110 LAMP, MPB64 LAMP, any target LAMP is used (either IS6110 or MPB64) and IS6110 PCR for culture negative EPTB (125) cases was 78.4% (98), 82%(103), 84%(105), and 70.4% (88) respectively. There were 7 cases which were missed by IS6110 LAMP and 2 cases by MPB64 LAMP. This LAMP assay is evaluated earlier by IS6110 LAMP primers in various pulmonary conditions and few EPTB cases. LAMP assay using two targets like IS6110 and MPB64 together at the same time for large number of EPTB samples have not been evaluated earlier.

Conclusion: LAMP assay using two targets at the same time is a promising technique for rapid diagnosis of EPTB in 60 minutes especially in a resource poor setting who are still battling with this deadly disease.

Poster 7:

Evaluating the Significance of Equivocal Mycobacterial Smear Results

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Objective: To determine the significance of mycobacterial smear results when very few acid fast bacilli are observed in the concentrated smear.

Study Design: All sputum specimens were digested and decontaminated using standard protocol. Concentrated smears were prepared, fixed using 5% phenol in 70% ethanol, and stained using Auramine O-phenol stain. Using a fluorescent microscope, smears were examined for AFB at 200x magnification for a minimum of 30 fields. When AFB were observed, they were counted at 500x magnification, and the results were reported qualitatively. All results were blindly verified by a second analyst. When reading discrepancies occurred, a third analyst served as a tie-breaker. A smear was reported as equivocal if only one, two, or three AFB were observed on the entire slide, and as positive if greater than three AFB were observed. Smear and culture data was collected for all specimens tested during a twelve month period and the smear-to-culture correlation was calculated separately for smear positives, smear negatives, and equivocal smears. Equivocal results were counted as smear positives for the purpose of calculating the culture correlation rate.

Results: From September, 2015 through August, 2016, a total of 2097 sputum specimens were processed for smear and culture. Of these, 24.6% (n=516) were smear positive, 69.7% (n=1462) were smear negative, and 5.7% were equivocal (n=119). 68.2% of the smear positive sputa resulted in a culture that was positive for one or more mycobacteria. 13.7% of the smear negative sputa resulted in a culture that was positive, and 36.2% of the equivocal sputa resulted in a positive culture. Of the 77 equivocal smears that did not result in a positive culture, 71.6% (n=53) were collected from a previously diagnosed MTBC patient or were accompanied by one or more positive NTM cultures.

Conclusion: In this data set, equivocal smears resulted in positive cultures approximately three times as often as negative smears. Therefore, even smears with very few visible AFB should be considered significant, as they are more likely to indicate an infectious patient status than a negative smear. For equivocal smear results collected from patients with an unknown TB status, reflex PCR testing should be performed to rule out MTBC, increasing the accuracy and speed of a TB diagnosis.

Poster 8:

***Mycobacterium canetti* Case Identified in Southeastern Michigan**

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Objective: The first reported case of *Mycobacterium canetti* was in 1969. There have been rare reports since of *M. canetti*, which is a progenitor species to *M. tuberculosis*, originating from the Horn of Africa. The Michigan Department of Health and Human Services identified a case of *M. canetti* in 2016 from a patient seen at a Michigan hospital.

Study Design: A 14 month old male from Yemen presented to a large southeastern hospital system in Michigan with lymphadenitis. The patient lived in a crowded house in Djibouti prior to arrival in Michigan. The family noticed swelling in the left submandibular region a month after his arrival. His primary care physician prescribed Augmentin, but another area of swelling appeared. His purified protein derivative TB skin test (PPD) and QuantiFERON-TB Gold test were positive.

Results: Growth characteristics, High Performance Liquid Chromatography (HPLC) mycolic acid testing, and Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) testing, indicated *Mycobacterium tuberculosis* complex (MTBC). Antimicrobial susceptibility testing was performed by *Mycobacterium* growth indicator tube (MGIT). Pyrazinamide results indicated resistance. Per Michigan Department of Health and Human Services (MDHHS) protocol, the sample was forwarded to CDC for additional testing. CDC returned results of a silent mutation, Ala46Ala, in the *pncA* region. MIRU genotyping, performed at MDHHS, indicated *M. canetti*.

Conclusions: *Mycobacterium canetti*, which is intrinsically resistant to pyrazinamide and pyrazinoic acid, was identified in a Michigan patient originating from Yemen. Although *M. canetti* is part of the MTBC, there are differences in growth characteristics, intrinsic resistance, and molecular markers that are useful in the identification. The patient in this case recovered following appropriate organism identification and treatment of nine months with Rifampin and Isoniazid.

Poster 9:

Frequency of Mixed Mycobacterium species Infections Detected by the INNO-LiPA MYCOBACTERIA v2 Hybridization Line Probe Assay From 2015 to 2016

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Objective: To evaluate the frequency of mixed mycobacteria infections detected from 2015 to 2016 using the Fujirebio (Ghent, Belgium) manufactured INNO-LiPA MYCOBACTERIA v2 line probe hybridization assay (LPA), which identifies 18 clinically relevant Mycobacterium sp.

Study Design: PCR is used to amplify the 16s-23s internal transcribed spacer region of Mycobacteria spp. This biotinylated amplified material is reverse hybridized with specific oligonucleotide probes immobilized on membrane strips. A final colorimetric step produces characteristic bands that are associated with specific Mycobacterium sp. All mycobacteria in specimens should be represented on the strip, even mixed infections.

Two years of data (2015 to 2016) was assessed for the number of specimens where more than one species of mycobacteria was detected by the LPA, compared to the total number of LPA positive specimens.

Results: It was determined that for years 2015 and 2016, the MDH TB Laboratory LPA detected approximately 5% mixed mycobacterial infections. Most of the mixed populations were *M. avium* complex with *M. intracellulare* infections followed by *M. avium* complex with *M. abscessus* infections. Other clinically relevant mixed infections were also detected in this study such as *M. tuberculosis* complex with *M. avium* complex but in far lower numbers but nonetheless considered significant for patient care.

Conclusions: In Minnesota, mixed mycobacteria infections do occur with low frequency, which can be easily and rapidly detected using the INNO-LiPA MYCOBACTERIA v2 hybridization line probe assay. Accurate mycobacterial identification and detection of mixed infections is essential for not only epidemiological investigations but for correct diagnosis and appropriate patient treatment.

Poster 10:

T-SPOT.TB Screening in High Risk School Children in Clark County, Nevada: Evaluation of Logistics and Cost Effectiveness Relative to an Onsite QuantiFERON-TB Gold Test

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Objective: To assess whether conducting latent tuberculosis infection (LTBI) screening using a contracted service for the T-SPOT.TB test versus in-house utilization of a QuantiFERON-TB Gold (QFT-G) test is a logistic and cost-effective method in testing and identifying high risk school children.

Study Design: From February-August 2016, 604 high risk school children were screened for LTBI using a brief survey assessing TB risk factors. Participants were recruited for the study by outreach efforts conducted by a community agency at designated venues targeting primarily high risk immigrant children. Questionnaires were assessed to determine participant eligibility for T-SPOT.TB testing. Specimen collection was conducted in-house by the SNHD TB clinic. Specimen testing was conducted by a contracting vendor located 1,500 miles away. Following LTBI screening using the T-SPOT.TB test, the cost-effectiveness and feasibility of using this method versus future use of the QFT-G test was evaluated. A quantiferon analyzer was purchased and validated on a total of 44 test samples following the culmination of the T-SPOT.TB screening. The Nevada Bureau of Health Care Quality and Compliance is in the process of inspecting and reviewing QFT-G protocols prior to conducting patient testing.

Results: Of the 604 participants screened, 253 were assessed for eligibility and 88 (35%) received the T-SPOT.TB test. Evaluation of this screening strategy found that the limited testing results using the T-SPOT.TB method were due to logistic barriers involving restrictive specimen collection and processing turn-around times, difficulty in organizing schedules for the target population, and obstacles in training staff to adhere to newly developed protocols. Specimen processing time utilizing the T-SPOT.TB method for this study was 48-72 hours versus 24 hours attainable by implementation of the QFT-G. The costs of screening using the single T-SPOT.TB test was \$107 per client, while the estimated cost of using an in-house QFT-G screening is \$44 per client. The contracted T-SPOT.TB test was a labor intensive screening method which limited the number of tests performed per day, while the QFT-G, performed locally appears to offer increased efficiency and versatility.

Conclusions: Successful detection of LTBI in high risk groups during a limited time span requires implementing a targeted screening strategy utilizing a logistic and cost-effective method to impact overall TB incidence. Based upon these findings, instrumentation has been purchased by the SNHD laboratory for performance of the QFT-G testing, enabling the SNHD TB Clinic to comfortably expand the capability for LTBI screening.

Note: This research was supported by a grant award, a "Proposal for the Expanded Access to Interferon Gamma Release Assays (IGRA)" from the Association of Public Health Laboratories.

Poster 11:

Tennessee Department of Health Laboratory Services Implementation of GeneXpert MTB/RIF Testing in Non-sputum Sources

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Objective: A study was undertaken to compare recovery rates of *Mycobacterium tuberculosis* in non-sputum sources. Cepheid (the manufacturer of the GeneXpert MTB/RIF kit) only has FDA approval for testing sputum sources. TDH Laboratory Services wanted to evaluate non-sputum sources for the following reasons:

1. Mycobacterium can disseminate throughout the body and to various body fluids. It may present at detectable levels prior to the development of respiratory symptoms.
2. Frequently, a non-sputum source may be the only specimen available for testing and therefore may be the only chance for laboratory confirmation of infection.

Study Design: Non-sputum sources received between July 2014- September 2015 were tested on GeneXpert. Sources varied from: bronchial washings, lymph nodes, urine, stool, blood, CSF, bone marrow and endotracheal aspirate. Twenty-five non-sputum specimens were received from various hospitals and health departments throughout the Tennessee. Specimens tested included both positive and negative specimens by fluorochrome smear. GeneXpert results were compared to reported culture results.

Results: Specimens tested for GeneXpert MTB/RIF had smear results ranging from negative to 10+. Seventeen specimens were reported as smear negative, GeneXpert negative and no growth by culture. Eight specimens had smear positive results with 6 positive GeneXpert results (2 negative). Five out of six positive GeneXperts had culture confirmation of *Mycobacterium tuberculosis* complex; 1 was contaminated and unable to identify further. The contaminated culture had a previous sample test positive for MTBC. Two GeneXpert negative results were confirmed as *M. intracellulare*. 100% correlation between the Positive GeneXpert and culture results.

Conclusions: Reliable results can be obtained from non-sputum sources utilizing GeneXpert MTB/RIF. Therefore, GeneXpert testing will be routinely performed on acceptable respiratory and non-respiratory specimens received for Mycobacteriology Smear and Culture testing. Non-sputum sources will also have the following disclaimer added:

This test has been approved by U.S. Food and Drug Administration for analysis of sputum specimens. Performance characteristics from specimen types other than sputum have been determined by TDH Laboratories Services.

Poster 12:

Evaluation of a Two Week 7H10 Agar Proportion Method for Isoniazid and Rifampin Drug Susceptibility Testing of *Mycobacterium tuberculosis* complex

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Objective: The Centers for Disease Control and Prevention recommend that first-line drug susceptibility test (DST) results be reported within 17 days from the identification of *Mycobacterium tuberculosis* complex (MTBC) from culture by the use of a rapid growth-based DST method. 7H10 agar proportion (AP) DST is not considered a rapid DST method since conventionally, results are not reported until 3 weeks. The objective of this study was to determine the accuracy of INH and rifampin (RMP) AP results reported at 2 weeks.

Study Design: From 10/6/2015 through 3/30/2016, MTBC isolates were tested by examining 7H10 AP DST growth at 2 and 3 weeks at high power magnification (up to 100X). The data represented 26 consecutive weeks (6 months) of routine testing. The total number of AP tests for 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP was 553, 550, and 552, respectively.

Results: Of the total tests, the number (%) of tests with conclusive (susceptible or resistant) 3 week results for 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP was 529 (95.7%), 526 (95.6%), and 531 (96.2%), respectively. Of the tests with conclusive results at 3 weeks, the number (%) of tests with conclusive results at 2 weeks for 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP was 521 (98.5%), 525 (99.8%), and 529 (99.6%), respectively. For all three drug concentrations, the sensitivity and specificity of the 2 week results were 100%. The prevalence of resistance to 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP in the study was 10.0%, 5.4%, and 3.8%, respectively.

Conclusions: This study demonstrated that 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP AP results can be determined at 2 weeks with 100% sensitivity and specificity for at least 98.5% of tests reported at 3 weeks. A limitation of this study is that agar proportion is performed at relatively few U.S. Public Health Laboratories; most labs use MGIT 960 or VersaTREK systems for rapid growth-based first-line DST. However, these broth systems are known to miss clinically significant rifampin and ethambutol resistance detected by solid medium proportion methods. Compared to broth-based DST, 2 week AP DST for INH and RMP may allow more accurate detection of drug resistance and susceptibility in a similar timeframe.

Poster 13:

Optimizing the Public-Private Partnership for Rapid and Accurate TB Laboratory Testing

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Background: In California, specimens from patients with suspected tuberculosis (TB) are tested by both public health and private sector laboratories for the presence of mycobacteria and undergo drug susceptibility testing (DST) as appropriate. TB control programs have noted delays with some private sector laboratories. Delays can impact public health because drug-resistant organisms may be ineffectively treated, acquire further resistance, and be transmitted within the community for prolonged periods of time.

Study Design: We analyzed California TB Registry data to describe the proportion of cases with sputum cultures performed outside a public health laboratory. We solicited reports of substantial delays in private sector laboratory results from the six regions served by the California TB Control Program. Reports were reviewed by program and laboratory staff to identify common contributors to delays. Several actions were taken to address these contributors.

Results: Among culture positive cases reported during 2010–2015, an increasing proportion of cultures were performed outside of public health laboratories (52% in 2010 vs 60% in 2015; Cochran-Armitage trend: $p < .0001$). Six of six regions reported instances of substantial delays. Recurring contributors were: transport, subculturing among multiple labs, underutilization of rapid tests, not reporting preliminary results, complex communication procedures, difficulty obtaining an isolate for molecular testing, and lack of reporting to public health. In response, we initiated a conference call with a large commercial laboratory to discuss instances of delay, better understand work distribution, and establish communication. Additionally, written notification and a webinar were presented to laboratories performing mycobacteriology testing in California which included a review of California regulations, an explanation of benefits of rapid TB laboratory results, and the availability of molecular testing in public health laboratories. Over 150 laboratorians attended from over 55 laboratories, and 72 of 72 (100%) attendees who evaluated the webinar indicated the presentation increased their knowledge of the subject matter.

Conclusion: Private sector laboratories play a critical role in the identification and diagnosis of TB in California. Through future review and continued efforts to build communication and partnerships between private and public laboratory networks, we aim to improve timeliness in TB specimen flow, processing, and reporting.

Poster 14:

Public Health and Corrections: A Model of Program Collaboration and Service Integration (PCSI)

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Objective: In 2009, CDC's National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHHSTP) introduced a service mechanism called "Program Collaboration and Service Integration" (PCSI). Public health programs provide categorical services to persons who have multiple related disease risks, but often miss significant opportunities. PCSI's strategic priority strengthens collaboration across disease program areas at the client level. Incarcerated populations have higher risk for HIV/AIDS, STDs and TB due to increased risk of disease transmission. Incarceration provides important opportunities for disease surveillance, diagnosis, treatment and prevention. In October 2015, the TB Elimination Program, HIV/STD & Viral Hepatitis Program, Information Technology Services Division, and Laboratory Services of Tennessee Department of Health (TDH) collaborated with the Tennessee Department of Correction (TDOC) to screen all new inmates for TB infection, HIV, syphilis, gonorrhea and chlamydia. The collaborative efforts and results of TDH's and TDOC's implementation of intake screening for TB, HIV, and STDs for all inmates entering the TDOC system are described.

Study Design: Multidisciplinary planning included feasibility, resource assessment, protocol development, intake flow analysis, information technology installation, staff training and data analysis at two intake prisons in Tennessee – The Tennessee Prison for Women and Bledsoe County Correctional Complex.

Results: Following pilot testing at both facilities, from December 2015 through May 2016 a total of 16,166 tests for five diseases were obtained. The aggregate test positivity rates were: TB infection – 5.0%; HIV infection – 0.8%; syphilis infection – 1.0%; gonorrhea infection – 2.2%; and chlamydia infection – 0.2%; positivity rates differed by gender.

Conclusion: A unique multi-program and agency collaboration successfully implemented integrated prison intake screening for five (5) diseases of public health importance in Tennessee – including *Mycobacterium tuberculosis*. Multi agency collaboration continues and testing for additional diseases is being explored.

Poster 15:

Adaptation of the use of the GeneXpert Assay for the Detection of Rifampin-Resistant *Mycobacterium tuberculosis* Complex in Culture

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Objective: To detect Rifampin-resistant *Mycobacterium tuberculosis* in culture using the Cepheid GeneXpert assay. This approach provides a rapid and cost-effective means for the diagnosis and treatment of patients.

Study Design:

Sample Preparation: A multi-step sample preparation was developed to ensure that no cultures were viable when tested using the GeneXpert assay. Cultures were dispersed in TE buffer and heated for 30 minutes at 100°C and then quickly frozen at -20°C for 30 minutes. Finally, 2ML of the sample reagent provided in the GeneXpert assay kit was added to 20ul from the specimen and incubated at room temperature for 15 minutes. To test if any organisms remained viable following the sample preparation, 200µl of each sample was inoculated on solid media and monitored for growth for 6 weeks. None of the cultures grew (see Table 1)

GeneXpert Assay: A total of 60 cultures were prepared as described above and analyzed using the GeneXpert assay following methods described by the manufacturer. The cultures were previously tested by conventional methods which verified that 26 were rifampin-sensitive *Mycobacterium tuberculosis*, 5 were rifampin-resistant *Mycobacterium tuberculosis*, and 29 were non-mycobacterium (NTM) cultures.

Results: The results of this study are shown in Table 1. A total of 31 *Mycobacterium tuberculosis* identified using conventional means were correctly identified using the GeneXpert assay. Of these cultures, 26 were rifampin-sensitive and 5 were rifampin-resistant. The GeneXpert assay correctly identified all of these cultures. Finally, the GeneXpert assay failed to detect *Mycobacterium tuberculosis* in all (29/29) of the NTM cultures.

Conclusion: The results of this study show that we have successfully adapted the use of the Cepheid GeneXpert assay for the detection of rifampin-sensitive and rifampin-resistant *Mycobacterium tuberculosis* in culture. This test algorithm reduces the time for culture identification from 48hr to 3:00hr and saves money that would otherwise be used for test employing additional assays and the cost to maintain other instrumentation other than the GeneXpert system. Also this approach reduces staff time and it does not require molecular expertise for the detection of rifampin resistance. The benefit from testing solid not liquid is because on the solid culture you can confirm the colony morphology with the result obtained although the procedure could be easily adapted to test liquid cultures.