UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Evaluation of performance of GENEXPERT MTB/RIF assay versus conventional diagnosis tests of Tuberculosis and Rifampicin resistance detection in Ecuador

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniero Biomédico

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DEDICATORIA

Deseo dedicar el presente trabajo al profundo amor incondicional, soporte y al incansable apoyo de mi familia en especial de mi madre Juana Mercedes Espinoza y al de mi padre Raúl Heriberto Rodríguez, infinitas gracias ya que por su guía y conocimiento, sacrificio e inmenso amor que me han demostrado cada día de mi vida y lo siguen haciendo, es que se ha hecho posible este resultado. Dedico el trabajo también a mis hermanas Elizabeth Rodríguez y Mayra Rodríguez quienes me han guiado moralmente y emocionalmente durante toda la carrera y lo continúan haciendo. Dedico el trabajo también a mi primo Santiago Rodríguez quien ha sido un soporte importante y guía en este sueño llamado Yachay.

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Raúl Heriberto Rodríguez Espinoza

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RESUMEN

En todo el mundo, la tuberculosis (TB) es la segunda causa principal de muerte por una sola infección por patógeno, solo superada por el VIH-SIDA. En 2017, la tuberculosis causó aproximadamente 1.3 millones de muertes. 10 millones de personas desarrollaron la enfermedad. 558,000 pacientes tenían TB resistente a Rifampicina (RIF). En Ecuador, 7200 personas tubieron tuberculosis en 2017. En el presente estudio se evalúa el rendimiento de GeneXpert frente a los métodos convencionales para el diagnóstico a TB y el diagnóstico de resistencia a RIF. En el análisis principal se estudiaron un total de 4,929 muestras, de las cuales 4,494 (91.17%) fueron pulmonares y 435 (8.83%) extrapulmonares. Para todas las muestras fue desarrollado cultivo y el ensayo GeneXpert en el caso de la detección de tuberculosis, y para las muestras de cultivos positivos se desarrolló las pruebas de resistencia a Rifampicina mediante la prueba de sensibilidad a fármacos (DST) y GeneXpert. El estudio estadístico se desarrolló principalmente en el software IBM SPSS Statistics 25 y Excel 2013.

Los resultados obtenidos para la detección de TB contrastando los resultados de GeneXpert con Cultivo fueron los siguientes: para sensibilidad y especificidad, se obtuvo 98.17% y 90.34%, (95% of IC, valor p < 0.001), respectivamente. 92.59% de precisión que representa el rendimiento casi perfecto del dispositivo para detectar casos verdaderos positivos y verdaderos negativos de TB. A partir del índice Kappa Cohen se generó un resultado del 83,10% de acuerdo entre las variables.. Los siguientes resultados se obtuvieron para la detección de resistencia a la Rifampicina contrastando con los resultados de GeneXpert contra DST: sensibilidad, especificidad, fueron 94.4% y 95.26% (95% de IC, valor p < 0.001), respectivamente. Del estudio estadístico de precisión y correlación, se obtuvieron los resultados consecuentes: 95.11% y 84.1respectivamente. Los resultados generales evidenciaron que el dispositivo podría ser adecuado para la detección rápida, pero debe ser llevado a cabo a la vez, la prueba de cultivo y la prueba de laboratorio de resistencia a Rifampicina para un diagnóstico efectivo. El uso de la prueba GeneXpert MTB / RIF podría ser ventajoso para detener la progresión de la epidemia, además, acelerar el sistema de tratamiento para los pacientes, sin embargo, no podría reemplazar los métodos convencionales.

Palabras clave: Tuberculosis, Ensayo GeneXpert MTB / RIF, Cultivo, Prueba de Susceptibilidad a Fármacos, Resistencia a Rifampicina, Estadísticas, Ecuador.

ABSTRACT

Worldwide, tuberculosis (TB) is the second leading cause of death from a single pathogen infection, only surpassed by HIV-AIDS. In 2017, tuberculosis caused approximately 1.3 million deaths. 10 million people developed the disease. 558,000 patients had TB that were resistant to Rifampicin. In Ecuador, 7,200 had tuberculosis in 2017. In the present study, the performance of GeneXpert is assessed against conventional methods for diagnosing TB and diagnosing of RIF resistance. In the main analysis of our assessment were studied a total of 4,929 samples, corresponding of data from 2012 to 2019 patients of whom, 4,494 (91.17%) were pulmonary and 435 (8.83%) extra-pulmonary. For all samples were developed culture and GeneXpert assay in the case of tuberculosis detection, and for samples positive-culture tested were processed to detect Rifampicin resistance by Drug Susceptibility Test (DST) and GeneXpert. The statistical study was mainly developed in IBM SPSS Statistics 25 and Excel 2013 software.

The results obtained for TB detection contrasting GeneXpert results against Culture were the following: sensitivity, specificity, were 98.17% and 90.34% (95% CI, p-value < 0.001), respectively. 92.59% of accuracy that represents the performance from the device to detect true positive and true negative cases of TB. From Kappa Cohen Index was generated a magnitude of 83.10% of agreement among variables. Consecuently, the following results were obtained for Rifampicin resistance detection contrasting GeneXpert results against DST results were: sensitivity, specificity, were 94.4% and 95.26% (95% CI, p-value < 0.001), respectively. From accuracy and correlation statistical study, the outcomes were: 95.11% and 84.1%, respectively. The overall results evidenced that the device could be suitable for fast screening, nonetheless require to be followed by Culture test and Rifampicin resistance laboratory test for an effective diagnosis. The usage of GeneXpert MTB/RIF test could be advantageous in stopping the progression of the epidemic, furthermore, accelerate the treatment system to patients, nevertheless could not replace conventional methods.

Keywords: Tuberculosis, GeneXpert MTB/RIF assay, Culture, Drug Susceptibility Test, Rifampicin Resistance, Statistics, Ecuador.

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INTRODUCTION

GLOBALLY OVERVIEW OF TUBERCULOSIS AND DRUG SUSCEPTIBILITY

Worldwide, tuberculosis (TB) is the second leading cause of death from a single pathogen infection, only surpassed by Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV-AIDS) (Tola et al., 2015). Regarding to the World Health Organization (WHO), TB has generated roughly 1.3 million deaths in 2017. Further in that year, 10 million people developed the disease, of whom 5.8 million were men, 3.2 million women, and 1 million children, Moreover ninety percent of register infected people were older than fourteen years old (WHO, 2018). Drug-resistant tuberculosis (DRT) continue being a public health emergency. Estimations from WHO remarked, 558,000 patients had TB that were Rifampicin resistant tuberculosis (RR-TB) which is the most effective first-line drug for its treatment, of whom, eighty-two percent had multi drug-resistant tuberculosis (MDR-TB) (WHO, 2018). Moreover, prevalence MDR-TB is raising with 0.5 million reported new cases reported in 2008. To control this emergency, WHO has created an urgency for developing an accurate diagnostic method for TB and drug susceptibility detection (Chang 2012).

In Ecuador, TB persists as a severe health problem, additionally aggravating by the emergence of multidrug resistance tuberculosis (MDR-TB) (Pardón et al., 2017). In Ecuador in 2002 were established 7.3 % of people with TB had multidrug resistance (MDR). Furthermore in 2012 that magnitude raised until 28% (Saeed et al., 2017). In 2016, Ecuador reported to the WHO 5,284 cases of TB, where 72% of the patients were successfully treated.

In high-income countries, the tuberculosis diagnostic methods include nucleic acid probes, amplification tests, high-performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), and automated systems for radiometric and non-radiometric detection of growth of mycobacteria in liquid culture (Schoclnik-Cabrera, Orduna, de Leon & Lopez-Vidal, n.d). In low-income countries like Ecuador, TB diagnosis is developed by culture method and microscopy (Colebunders & Bastian, 2000); the latter is selected by its low cost, fast time response and reduced technology requirements (Groeschel, 2013). Nonetheless, studies evidenced a sensitivity minor than 50% from microscopy. A count greater than 5,000 bacilli per ml is required of the sputum sample so that the smear test is positive. Culture has greater sensitivity than the previously evaluated diagnosis, since it can detect from 10 up to 100 bacilli per ml. sample; nonetheless, due to the slight growth of the bacillus, it is regarded as a late detection method (Llerena, 2015).

HIGHLIGHT INFORMATION ABOUT GENEXPERT MTB/RIF ASSAY

The analyzed machine is a diagnostic test equipment that executes specimen processing, through real-time Polymerase chain reaction (real-time PCR) procedure, for detection of TB, further the fast determination of Rifampicin susceptibility in clinical samples (Zeka et al., 2011). The expenditure of this device is compensated by the fast response time, which is less than two hours. Besides, the management of the machine affords minimize biohazard danger and minimal training needed to manage (Vadwai et al, 2011). The apparatus employ five nucleic acid hybridization probes, where in response to a match in a determined sequence within the hot-spot rpoB gene of MTB produce a colored fluorophore. The fluorescent colors result during PCR amplification specify the presence of Rifampicin susceptible MTB. Moreover, the mutation in the hot-spot region means that the probe did not attach to that gene (Weyer et al., 2012). Therefore the

lack of color evidence mutations in that specific region associated with RIF resistance (RR) (Zeka et al., 2011). The machine detects MTB and RIF resistance by PCR amplification of the 81 base pairs (bp) hot spot fragment of the *rpoB* gene.

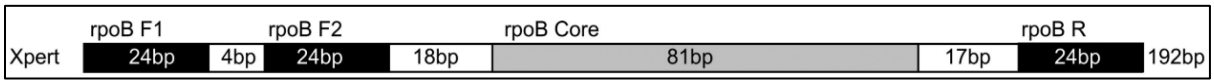

Figure 1. rpoB gen core (81bp), F1 (24 bp), F2 (24 bp) and R (24 bp), amplicon obtained in Xpert (Blakemore et al., 2010).

The performance of the machine is upper 70% detecting positive cases of TB. The equipment consists of a closed molecular biology system that employs single-use disposable cartridges that are independent for test processing with minimum biosafety and infrastructure requirements (Ortiz et al. 2019). Moreover, GeneXpert reduces the infection risk, and averts cross-contamination among samples due to closed working cartridge (Al-Ateah et al., 2012).

The WHO suggests the rapid diagnostic (WRD) test for the diagnosis of tuberculosis and Rifampicin susceptibility currently available in the GeneXpert device. From 48 countries, 32 has adopted national algorithms placing the WRD as a first detection assay for whole people with related symptomatology of pulmonary and extra-pulmonary TB by the end of 2017 (WHO, 2018). In 2017, the amount of MDR/RR-TB cases was around 25% of an estimated of 558,000 cases of MDR (WHO, 2018).

The Ecuadorian Public Health Ministry endorsed the equipment at 2011, and it was enforced in 2012, derived from reinforcement the “Stop Tuberculosis” strategy program, promoted by the WHO, which resulted in the application of the method in different laboratories at a national

level (Ortiz et al. 2019). The expected outcome from GeneXpert assay implementation and validation was to endorse a fast and reliable test method to be inserted in the diagnostic algorithms of TB in respiratory samples (WHO, 2018).

CONVENTIONAL DIAGNOSIS METHODS EMPLOYED IN ECUADOR FOR TUBERCULOSIS AND DRUG RESISTANCE DETECTION

Tuberculosis has been informed worldwide throughout the ages; nevertheless, it has not been well-known as well as HIV or dengue. Tuberculosis spreads quickly, thence a considerable amount of people are infected. Spite of these, they neither seek immediate attention; therefore they contribute, among other factors, to increase the prevalence of this disease (Llerena, 2015). Marked differences of wealth and human development were evident in Ecuador during the decade from 1997 to 2006. This country has one of the highest incidence grades for TB (131/100,000 inhabitants) and TB mortality (27/ 100,000 inhabitants). Furthermore, upgrading disease prevention and prompt diagnosis is mandatory, especially in countries with a high risk of infections like the evaluated country (Armijos et al., 2008).

HIGHLIGHT INFORMATION ABOUT CULTURE (GOLD STANDARD TEST) AND BACILLOSCOPY

The culture method provides the foremost diagnosis technique available. Therefore, M. tuberculosis culture is a gold standard technique for clinical and research diagnosis of active TB (Cheon et al., 2016). Regarding to Ecuadorian information about TB and the methods of diagnosis accessible; although there are low sources concerning to it, some of them were the following: within Ecuador from the central Andean province of Cotopaxi which its indigenous population predominantly, evidenced that until 2006, Tuberculosis Disease Prevention and Control Program only attended patients with respiratory conditions. For instance, ill people with cough with more than fifteen days were attended (Sánchez-Pérez et al., 2013). The previous citation proved the inefficiency to detect people with TB in Ecuador. In a study carried out in China, found over the years in Ecuador, two hundred and two patients had a chronic cough, and thus, 44 (48%) had pulmonary TB positive, that represent 6.7% of the whole population estimated, further the specimens belonged to low-income people of Ecuador (Sánchez-Pérez et al., 2013). In the community of Chine-Ecuador, were accomplished three smear and culture tests during the Tuberculosis treatment at the end of the medication. Consequently, they obtained a cure percentage of 100% (Romero-Sandoval et al., 2009). Once the symptomatic respiratory patients had been identified, the confirmatory diagnosis of pulmonary tuberculosis was done by smear and culture in expectoration samples (Llerena, 2015).

The leading problem of culture is the delay in generating outcomes, obtaining results from two to four weeks in liquid media and from four to eight weeks in solid media, due to the slow growth of the Mycobacterium tuberculosis (Groeschel, 2013). Culture test reveals the specific mycobacteria that could be identified, which is an advantage (Luna, 2016). Nevertheless, Culture

does not was used like standard assay until 2017. Ecuadorian hospitals maintained the employing of sputum method from TB detection, instead of culture as standard, Groeschel (as was cited in Romero and Sandoval et al., 2013). There was a problem with TB control and prevention (Groeschel, 2013). On the other hand, Bacilloscopy is a quick, low cost and simple diagnosis method to detect TB, providing results within 24 hours (Ortiz, Franco-Sotomayor & Ramos-Ramirez, 2019). The Bacilloscopy test is an effective method of diagnosis (Llerena, 2015). Despite this, it is a test with low sensitivity. (Luna, 2016).

HIGHLIGHT INFORMATION REGARDING TO DRUG SUSCEPTIBILITY TEST

The detection of Mycobacterium tuberculosis by culture test takes approximately 3 weeks in standard conditions and the detection of drug sensibility takes around 4 weeks more (Caviedes, 2000). The MDR/RR-TB cases, has raised hurriedly, from 2002, 7.3 % of total people infected with TB has raised to 28% in 2012 (World Health Organization, 2018). Therefore, timely Drug Susceptibility Test (DST) outcomes are mandatory for adequate patient management (Caviedes, 2000). The WHO, Lung Disease Organization and the International Union against Tuberculosis had established a reference laboratory for the reliability of DST efficiency testing. The study was developed in the period from 1994 to 2002. The sensibility obtained for Rifampicin was 97.2%, further the predictive values of the drug susceptibility test (DST) were major of 93% (Kim, 2005). In a study implemented to detect drug resistance, manifested the corresponding results: according to grouped values of resistance to Rifampicin were 99% and 100%, of sensitivity and specificity, respectively (Bwanga, 2009). MDR-TB is an emergency worldwide problem due to current DST requires sixty days to assure the multidrug resistance detection of patients. Furthermore, in

Ecuador, only INSPI is the only one center available the developing that specific test (Saeed et al., 2017).

ABOUT THE DEVELOPED THESIS WORK

There is a similar published project, about the equipment GeneXpert in Ecuador. That work evaluated a total of 1592 sputum respiratory samples collected from patients suspicious of tuberculosis around the country, including data in a period of 4 months from 2012. In the study, the analysis was developed among the analyzed device against traditional methods as Bacilloscopy, Culture, and DST. Furthermore evaluating the specificity and sensitivity (Ortiz-Jiménez, 2019). Indeed, the project could be immersed with clusters of strains and due to a short period of time of the data analyzed, it might be biased and the statistical analysis evidenced not enough to assess the equipment. Despite that study, the present study, established here, developed with 5,043 cases examined with suspicion TB and/or MDR-RR, from an extended period of time, evaluated belong to the period among from 2012 to 2019 with pulmonary and extra-pulmonary samples. Moreover were developed a complete statistical analysis of the performance of GeneXpert and concordance against conventional methods in Ecuador. The statistical analysis was accomplished to assess the performance of the device against conventional methods of TB and Rifampicin resistance detection. Besides, the parameters of accuracy and agreement assessed were: Chi-square, Complete Confusion matrix, Matthews correlation coefficient, Gini coefficient, ROC curve, Kappa Kohen Index.

PROBLEM STATEMENT

PERFORMANCE OF GENEXPERT DIFFER AMONG COUNTRIES CONSEQUENTLY, THE NECESSITY TO DEVELOP A COMPLETE STATISTICAL STUDY OF THE ACCURACY OF THIS DEVICE FOR ECUADOR.

Globally, the assessment of the machine is encouraging. The device has been approved by WHO since 2010 for fast diagnosis of MDR-TB (Cheon et al., 2017). Indeed until the end of March 2012, average of 61 countries had started employing GeneXpert (Pantoja et al., 2012). A study accomplished with 6,648 individuals from Peru, South Africa, India, Philippines, Uganda and Azerbaijan, comparing Xpert MTB/RIF against culture and Drug Susceptibility Test. Xpert MTB/RIF registered a sensitivity of 90.3% from culture confirmed MTB cases, and specificity was 99.0%. Furthermore, the sensitivity and specificity of the machine against DST for Rifampicin resistance determination were: 94.4% (236/250), 98.3% (796/ 810), respectively (Weyer et al., 2012).

The mutations, almost entirely of them, are present in the hot spot rpoB gene that GeneXpert detects to diagnosis TB and RIF resistance. Nevertheless the remaining mutations are immersed in another genes. Furthermore the mutations detected at rpoB gene could vary slightly among countries. Therefore the performance of the artifact could differ in the diagnosis of MTB and Rifampicin susceptibility. For instance, among the 90 rifampicin-resistant specimens isolated in a study carried out in Brazil there were mutations in the 531 codon of rpoB in 42 isolated specimens that represents 46.6% (Senna et al., 2006). In Ecuador the frequency of the mutations associated with rifampicin were 30.3% (10/33) specimens that had mutations in 531 codon from rpoB (Franco-Sotomayor et al., 2018). Comparatively, in the region: Brazil, Argentina and Peru,

have different frequencies of mutations. For instance, from katG gene mutations to INH resistance as a first line drug as RIF, used against of tuberculosis, the frequencies are: Brazil (81.3%), Argentina (71.4%) and Peru (82.4%) (Dalla et al., 2009).

The outcomes of performance of GeneXpert have an established scale from 70% to 100% for sensitivity. Besides, a specificity domain from 91% to 100%, for MTB detection. Furthermore, a sensitivity and specificity of 98% and 99% respectively, for RIF resistance detection. According to several analysis from 12 studies among results from Xpert against culture MTB and DST (Weyer et al., 2012); some of them contrast the previous results. For example in a study developed in Bangladesh with 107 cases suspected of MDR-TB, GeneXpert displayed the following results: 87.64% and 75%, from sensitivity and specificity respectively, evaluated against culture gold standard (Laskar, 2017).

In the Region, the validation of the analyzed device also differ. In a developed study carried out in Medellín Colombia, with 103 samples in the main analysis; 34% of them were positive by culture MTB detection. The overall test about sensitivity, specificity, PPV and NPV were 91%, 92%, 83% and 96%, severally (Atehortúa et al., 2014). And this Information contrast with the results obtained in a study in Cuba in 2018; where 374 analyzed cases from patients during the period of June 2014 to December 2016 evidenced the consequently results: 91.67% sensitivity, 95.68% specificity, 88% PPV and 97.08% NPV for MTB detection (Martínez et al., 2018). In Ecuador from 1592 cases studied showed that 99.8% of sensitivity and 93.2% for specificity. The detection of RIF resistance was contrasted by GeneXpert against DST presenting the following results for sensitivity 91.4% and a specificity of 95.5% (Ortiz-Jimenes, 2019).

OBJECTIVE

General objective

- To assess the performance of GeneXpert MTB/RIF assay for diagnosis of Tuberculosis and Rifampicin resistance in Ecuador.

Specific objectives

- To study GeneXpert effectiveness, against conventional diagnosis methods as Culture and Drug susceptibility test.
- To develop a complete statistical analysis of concordance among the analyzed equipment against conventional methods.
- To determine the efficiency of GeneXpert to detect tuberculosis and RIF resistance in Ecuador.

METHODOLOGY

DATA COLLECTION AND CASE SELECTION

The database containing 5,043 examined patients with suspicion TB and/or MDR-RR that was requested by the National Institute of Public Health and Research (INSPI, in Spanish). Completely cases from database were anonymized at the starting of the statistical study. From this database, 114 cases were excluded from the main study, because 107 cases had contaminated tubes in culture, and 7 had nontuberculous mycobacteria in culture media; remaining with a total of 4,929 cases. From these samples, were developed Culture, and GeneXpert procedures, to each case, for TB diagnosis. For Rifampicin resistance detection was started with 1,419 of confirmed cases by culture, of whom 703 were excluded for the following reasons: no developed culture samples, and dead bacterium; remaining with a total of 716 cases. From these samples, DST and GeneXpert procedures were developed for each patient data.

Culture outcomes were collected from 13 different INH around Ecuador, further from Infectology Hospital of Guayaquil, Provincial laboratory of Guayas, Valenzuela Hospital of Guayaquil and Los Rios Hospital; overall 17 localities. GeneXpert data outcomes were collected by five devices; three located in Guayaquil: two in Valenzuela Hospital and the latter in Infectology hospital. The other two in hospitals from Esmeraldas and Los Rios provinces. The entire samples evaluated to detect DST-RR were developed in INSPI located in Guayaquil. The samples were pulmonary and extra-pulmonary. The presented cases belong to the period among April 4, 2012, to May 18, 2019. The data contained various variables including: date, sample, age, sex, provenance, doctor/area, Bacilloscopy results, Culture results, DST results, RIF resistance results, GeneXpert results of TB detection, and GeneXpert results of RIF resistance. Some of these

variables contained biased information or no useful information for the present study, thus, some of them were eliminated for the analysis.

Concerning to sample variable was restricted to pulmonary and extra-pulmonary. For the study was analyzed a total of 4,929 samples, of whom, 4,494 (91.17%) were pulmonary and 435 (8.83%) extra-pulmonary. In addition, Culture results and DST results were summarized as either positive or negative; representing the presence or absence of MTB infection and the DST Rif resistance, respectively. To be implemented in the final study population, the cases must accomplished two conditions: be reported in the database by INSPI among 2012 to 2019, and present either positive or negative data-information in both variables; Furthermore for the case of DST-RR be culture positive. A total of 1,419 (28.79%) were positive culture (figure 4).

Specifications about the entire database, include the aspect that INSPI is the unique Ecuadorian institution developing drug susceptibility tests (DST). Moreover, the information outcomes regarding to MTB detection by culture and GeneXpert were previously managed by two institutions before to be delivered to INSPI, because in other hospitals and clinics is developed culture and GeneXpert tests as was pre-specified formerly. Therefore, some bias and/or missed data are inherently, due to processed data of DST, Culture and GeneXpert.

VARIABLES EMPLOYED FOR THE STATISTICAL ANALYSIS

Regarding to database information analyzed, following variables were considered important for the insertion in the main statistical analysis: Culture results like positive and negative, DST results for Rifampicin resistance positive and negative, and GeneXpert results positive and negative.

PERFORMANCE OF STATISTICAL ANALYSIS AMONG GENEXPERT AGAINST CONVENTIONAL METHODS OF TB AND DST-RR DETECTION

The statistical comparative analysis was mainly implemented in IBM SPSS Statistics for Windows (version 25.0). Moreover, all confusion matrix parameters were calculated using Excel 2013 software (Tang, Liu, Lu, & Huang, 2017**).** The whole statistical calculations correspond to 95% confidence intervals (95% CI) percent of agreement, and differences among variables were tested for statistical significance ($p<0.05$) using a Pearson chi-square test (Einstein et al., 2014). The comparison analysis was developed among Culture against GeneXpert to detect MTB; and DST against GeneXpert in the case of Rifampicin resistance detection. The assessment of the analyzed machine to diagnosis MTB and RIF resistance was developed employing the following tests: Chi-square test, complete Confusion matrix, Kappa Kohen Index, Matthews correlation coefficient, Area Under the Curve (AUC) from Receiver Operating Characteristic (ROC) curve and Gini coefficient.

Chi-square test was implemented to analyze measures of dependence among variable and provides useful information of significance agreement among variables (McHugh, 2013), 95% of **confidence interval** (CI) was consider. A confusion matrix was executed to analyze parameters of estimations (Townsend, 1971). The following its define the parameters of the confusion matrix to assess the performance of Genexpert: **sensitivity, specificity, positive predictive value** (PPV), **negative predictive value** (NPV). The **sensitivity** is the magnitude of efficacy of the device to detect people with the disease, a percentage major from 90% to 100% is consider almost perfect effectivity, and close to 0% a slight or null efficiency. The **specificity** is the percentage of efficiency of the apparatus to detect people without the disease, a percentage major from 90% to 100% is consider almost perfect effectivity, and close to 0% a slight or null efficiency. **Positive predictive value (PPV)** is the predictive efficacy of the machine to detect people with the disease, a scale of 90% to 100% is consider almost perfect effectivity, and close to 0% a slight or null efficiency to detect people with the disease. **Negative predictive value (NPV)** is the predictive efficacy of the equipment to detect people without the disease, a quantity from 90% to 100% is consider almost perfect effectivity to detect people without the disease and close to 0% a slight or null efficiency.

Moreover, **false negative rate** (FNR) is the amount of positive cases wrongly classified as negative, a measure minor than 10% is required to have an almost perfect positive agreement among GeneXpert against conventional methods to be a significant measure. **False positive rate** (FPR) is the magnitude of negative cases wrongly categorized like positive, a percentage minor than 10% is required of an almost perfect concordance among the equipment against traditional methods to be a significant estimation. **False discovery rate** (FDR), is the prediction measure of positive cases wrongly categorized as negative, a percentage minor than 10% is required of an almost perfect prediction positives values from GeneXpert to be a significant magnitude. **False**

omission rate (FOR) is the prediction percentage of negative cases wrongly categorized as positive, a magnitude minor to 10%, is required of an almost perfect prediction negative outcomes from the device.

Furthermore, **positive likelihood ratio** (LR+), represents the possibility to obtain a positive result, in patients with the disease, and compared that result in patients without the disease (McGee, 2002), and this measure is required be minor than 10 units to be a significant outcome. **Negative likelihood ratio** (LR-), represents the possibility to obtain a negative result, in patients with the disease, and compared that result in patients without the disease (McGee, 2002), and this magnitude must be less than 0.1 to be a significant result. **Diagnostic odd ratio** (DOR) indicates the performance of the device, this measure ranges from zero to infinity, where a magnitude major than 1 is consider an almost perfect performance, otherwise and inefficient performance (Glas et al., 2003). **Prevalence**, is only a parametric data of the proportion of patients with the disease among the total study population. **Critical success index (CSI)** is the measure of people with the disease against people with the disease added besides false positive, and negative results of the device. A percentage of 90% is consider relevant significant test in detect people with the disease. **Markedness** (MK), is a magnitude of marked significance of the device like a good predictor. An amount over 90% is consider almost perfect performance predictor.

Besides, were developed another parameters from the confusion matrix to study the performance and agreement of GeneXpert against culture and DST, like: **accuracy**, is the measure of performance of the device to detect people with and without the disease, uppermost 90% is consider almost perfect performance. **Balanced accuracy** (BA), is the average accuracy of the device to detect people with and without the disease, above 90% is consider significance accuracy. **Bookmaker Informedness** (BM), is other parameter to estimate the amount of how much effective is the device to detect people with the disease and without the disease, an percentage over

90% is consider almost perfect accuracy. **F1 score**, is another magnitude to define device accuracy, upper 90% is consider almost perfect performance. Moreover, overall confusion matrix parameters were calculated using Excel 2013 software.

To assess the concordance of the device among conventional methods, were implemented the following parameters: **kappa Cohen index** (KCI) to study the percentage of concordance between variables, to interpret the Kappa values are suggested as follows: values lower and equal to 0, is equivalent to there is no agreement, from a range of 1% to 20% have slightly agreement, 21% to 39% minimal agreement, 40% to 59% weak agreement, 60% to 79% moderate agreement and 80% to 90% as strong agreement and above 90% near perfect agreement. (McHugh, 2012). This parameter was assessed using SPSS Software**.** Another parameter employed to detect the agreement among variables was **Matthews correlation coefficient** (MCC), it reflects the concordance among variables, where more than 90% represents almost perfect agreement among GeneXpert and traditional methods. Besides, was implemented the graphic of **ROC curves** that is a graphic plot that represents the diagnostic reliability of the device analyzed to detect TB and RIF (Park et al., 2004), further, an upper value of 90% is consider almost perfect performance. Consequently, was calculated the area under the curve (**AUC**) of (ROC) curve where results over the diagonal line estimates discriminating proficiency to diagnose patients with or without the disease (Mandrekar, 2010). Moreover, was implemented **Gini coefficient** to measure inequality among variables (Silber, 1989), a value less than 10% is consider almost perfect agreement among the device and the conventional methods. Additionally, formulas and nomenclature employed to calculate some important parameters for this statistical analysis among GeneXpert against Culture of TB and DST for RIF resistance detection, respectively, as well as values of confusion matrix and Matthews correlation coefficient was developed using (table 1) in Excel 2013 software.

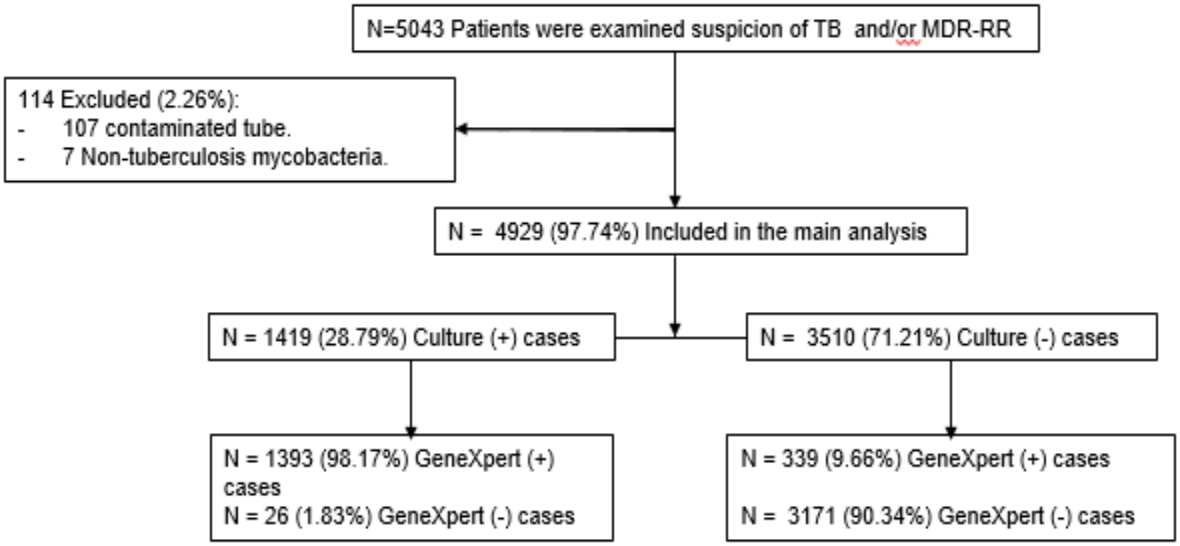

Figure 2. Workflow description the selection procedure, from Culture and GeneXpert for TB detection, from Ecuadorian patients, 2012-2019.

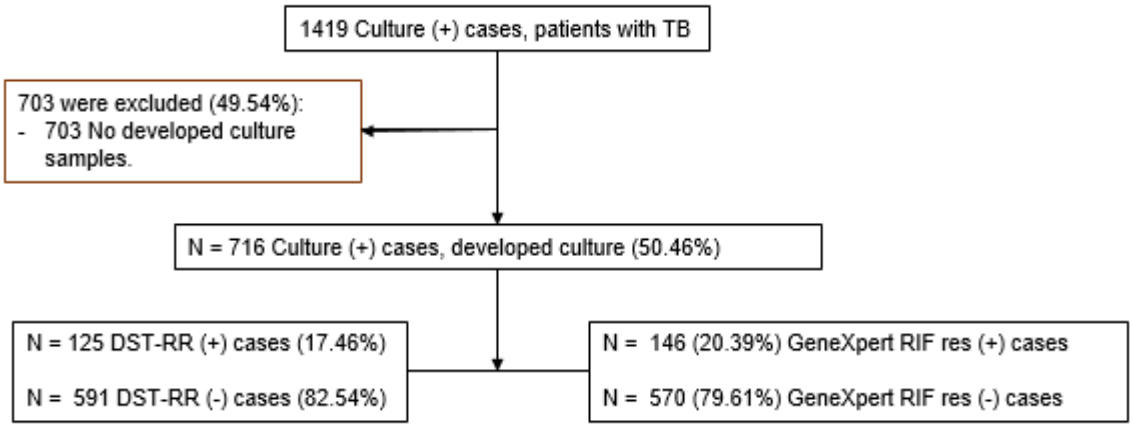

Figure 3. Workflow description the selection procedure, from Drug susceptibility test and GeneXpert for RIF resistance detection, from Ecuadorian patients, 2012-2019.

Nomenclature	**Formula =**
True Positives (TP)	
True Negatives (TN)	
False Positives (FP)	
False Negatives (FN)	
Sensitivity (TPR)	TP / (TP+FN)
Specificity (TNR)	TN / (TN+FP)
Positive predictive value (PPV)	TP / (TP+FP)
Negative predictive value (NPV)	TN / (TN+FN)
False negative rate (FNR)	FN / (FN+TP)
False positive rate (FPR)	FP / (FP+TN)
False discovery rate (FDR)	FP / (FP+TP)
False omission rate (FOR)	FN / (FN+TN)
Positive likelihood ratio(LR+)	TPR / FPR
Negative likelihood ratio(LR-)	FNR / TNR
Diagnostic odds ratio (DOR)	LR+ / LR-
F1 score	(2*PPV*TPR) / (PPV+TPR)
Prevalence	(TP+FN) / (TP+TN+FP+FN)
Critical Success Index (CSI)	TP / (TP+FN+FP)
Accuracy (ACC)	(TP+TN) / (TP+TN+FP+FN)
Balanced Accuracy (BA)	(TPR+TNR)/2
Mattews correlation coefficient (MCC)	((TP*TN)-(FP*FN)) / (((TP+FP)*(TP+FN) *(TN+FP)*(TN+FN))^(1/2))

Bookmaker Informedness (BM)	TPR+TNR-1
Markedness (MK)	PPV+NPV-1

Table 1. Formulas and nomenclature used to calculate some important parameters for statistical analysis (Chicco & Jutman, 2020).

RESULTS

Foremost, one filter was applied to database in both cases, for TB diagnosis and RIF resistance detection, in accordance to the specifications pre-specified in the preceding segment (Fig. 2), and (Fig. 3). The assessment of the analyzed device for TB detection, were developed against culture (Ali, Hassan, & Shehata, 2018), and performance of GeneXpert to RIF susceptibility determination was accomplished among the machine against DST-RR outcomes. According to the statistical analysis, there is significant association with 95% CI, and a p-value < 0.05 (0.0001 in this study), among variables for both cases.

From overall patients (4,929), including in the main analysis of tuberculosis detection. 1,419 cases (28.79%) were culture-positive and 3,510 (71.21%) culture-negative (fig 3.). Mycobacterium tuberculosis registered by the device were 1,732 (35.14%) positives and 3,197 (64.86%) negatives (fig 2.). For the entire cases including in the analysis of RIF resistance detection, 716 cases culture positive were analyzed by DST-RR, of which, 125 (17.46%) were positive and 591 (82.54%) negative by DST (fig 3.). Rifampicin resistance outcomes by GeneXpert were 146 (20.39%) positives and 570 (79.61%) negative cases (fig 3.).

PERFORMANCE ANALYSIS OF GENEXPERT AGAINST CULTURE

The confusion matrix (Table 2), was generated to assess the performance parameters of the device for the case of TB detection, consequently the following results was acquired: a **sensitivity of 98.17%,** this measure present a significant agreement among GeneXpert against Culture to detect positive cases. **Specificity of 90.34%,** nevertheless this percentage is major of 90%, is close

to it, evidencing not perfect strong correlation against variables in detecting negative cases**. Positive predictive value (PPV) was 80.43%**, this amount less than 90% confirm a low strong capacity of prediction of positive cases from GeneXpert. **Negative predictive value (NPV) was 99.19%**, this percentage close to 100% confirm an almost perfect performance prediction from the device to detect true negatives. **False negative rate (FNR)** was 1.83%, such this value is close to zero it refer to almost perfect performance to detect true positives from the device. Regarding to **False positive rate (FPR) of 9.66%,** although the quantity is minor than 10%, is close to it, consequently the detection of true negatives is almost perfect but is not enough efficient like the detection of true positives. **False discovery rate (FDR) was 19.57%,** the performance of the device concerning to positive prediction is not significant, because the magnitude is major of 10%, nevertheless continuing being strong prediction. About to **False omission rate (FOR) was 0.81%,** as this value is near to zero the device performance is significant almost perfect predictor to detect negative results.

Additionally, another useful parameters of Confusion matrix was calculated: **positive likelihood ratio (LR+) was 10.16**, the measure is major of 10 so the performance of the device is significant. The **Negative likelihood ratio (LR-) was 0.02**, the percentage is minor than 0.1 therefore the performance of the device is significant. **Diagnostic odd ratio (DOR) was 501.16**, the resulted value is strongly major from 1, and hence the performance of GeneXpert is significant. **Prevalence of 28.79%,** almost quarter of total population, enrolled in this study have tuberculosis. **Critical success index (CSI) was 79.24%,** evidencing a moderate efficiency from the device, due mainly to the false positives results. **Markedness (MK) was 79.61%,** due to PPV outcome makes this magnitude a moderate performance of GeneXpert.

Moreover, were developed additional parameters to study the performance and agreement of GeneXpert among culture TB diagnosis method (Table 2) like: **accuracy of 92.59%,** this magnitude represents an almost perfect performance of the analyzed device. **Balanced accuracy (BA) was 94.26%,** the percentage result constitute an almost perfect performance of GeneXpert. The percentage **Bookmaker informedness (BM) was 88.51%,** and is strong accuracy of detection true positive and negative cases, nonetheless is not almost perfect performance. **F1 score was 88.42%**, like the previous one is strong accuracy, however is not almost perfect accuracy of the device due to the PPV result.

Table 2.

CONFUSION MATRIX from GeneXpert MTB-RIF assay against Culture test.

		CULTURE				
	Total population N:	Positive	Negative	Prevalence	**Accuracy (ACC)**	Critical Success Index (CSI)
	4929	**(+)**	**(-)**	28.79%	**92.59%**	79.24%
Gene Xpert MTB/ RIF	Positive (+)	1393	339	**Positive predictive value (PPV)**	False discovery rate (FDR)	Balanced accuracy (BA)
				80.43%	19.57%	94.25%

	Negative (-)	26	3171	False omission rate (FOR)	**Negative predictive value (NPV)**		Bookmaker Informedness (BM)
				0.81%	**99.19%**		88.51%
		Sensitivity, True positive rate (TPR)	False positive rate (FPR)	Positive likelihood ratio (LR+)	Diagnostic odds ratio (DOR) = LR+/LR−	F1 score	Markedness (MK)
		98.17%	9.66%	10.16			
		False negative rate (FNR)	**Specificity (SPC), Selectivity, True negative rate (TNR)**	Negative likelihood ratio (LR-)	501.16	88.42%	79.61%
		1.83%	**90.34%**	0.02			

CONCORDANCE ANALYSIS OF GENEXPERT AGAINST CULTURE

To assess the agreement among variables, GeneXpert against Culture. Were employed kappa Cohen index (KCI), developed in SPSS Software, obtaining a quantity of 83.1%, therefore the concordance between variables is strong, nevertheless not is totally strong as was expected. The Mattews correlation coefficient (MCC) measure was evaluated with outcomes from confusion matrix as another magnitude of concordance among variables generating the level agreement of 83.94%, this amount evidence the strong concordance of the device against Culture (Table 3).

Table 3.

Concordance analysis among GeneXpert against Culture

Comparison	Kappa Cohen Index	Matthews Correlation Coeficient (MCC)
GeneXpert - Culture	83.10%	83.94%

ACCURACY AND INEQUALITY STUDY OF GENEXPERT AGAINST CULTURE

To assess the diagnostic accuracy of the analyzed device was implemented the Receiver Operating Characteristic (ROC) curve, that is a graphic plot that represents the diagnostic concordance of the machine against culture to detect TB (Park et al., 2004), (Fig 4). Consequently, was calculated the area under the curve (AUC), that is an estimation of discriminating accuracy of GeneXpert, this measure was 94.26% from (ROC) curve, (Table 4). This quantity represents an

almost perfect accurate of the device to diagnosis true positive and true negative cases. Moreover was developed Gini coefficient that was 9.68%, to measure inequality among variables (Silber, 1989), (table 4), this amount represents an almost perfect equality among GeneXpert and Culture.

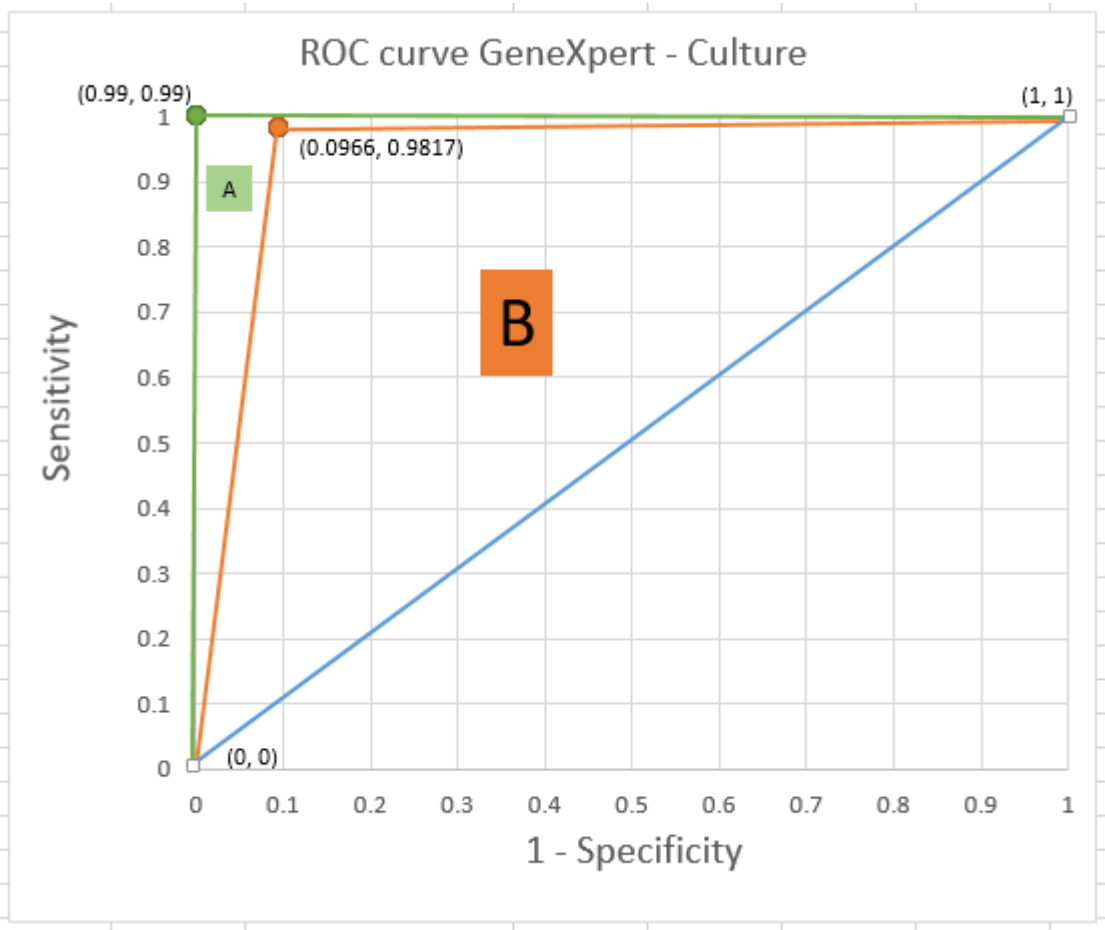

Figure 4. ROC curve graphic by comparison of GeneXpert against Culture (orange curve), in green curve is only the performance of culture.

Table 4. Area under the curve (AUC) of GeneXpert against culture. Further the Gini Coefficient value that measure the percentage of inequality among variables.

Compared Variables	AUC	Gini Coefficient
Genexpert -Culture	94.26%	9.68%

PERFORMANCE ANALYSIS OF GENEXPERT AGAINST DST-RR

The confusion matrix of the performance analysis of GeneXpert against DST-RR (Table 5), was developed to assess the performance of GeneXpert MTB/RIF assay against DST (gold standard), consequently, the following results were generated: **sensitivity was 94.4%,** this measure represents an almost perfect significance concordance among GeneXpert against DST to detect positive cases. **Specificity of 95.26%,** evidences a significant value of almost perfect correlation among variables in detecting negative cases**. Positive predictive value (PPV) was 80.82%,** this amount less than 90% confirms a low significance, nevertheless is a strong prediction from the machine to determine positive cases. **Negative predictive value (NPV) of 98.77%,** the magnitude is close to 100% confirming an almost perfect performance from the device to predict true negative RIF resistance cases. **False negative rate (FNR) was 5.6%,** such this magnitude is minor than 10%, therefore the device have significant almost perfect performance to detect true positives cases of RIF resistance. **False positive rate (FPR) of 4.74%,** like this amount is minor than 10%, hence the artifact have significant almost perfect performance to detect true negative cases. **False discovery rate (FDR) was 19.18%,** the performance of positive prediction is not total significant, because the quantity is major of 10%, nevertheless continuing being strong

prediction but not almost perfect. **False omission rate (FOR) of 1.23%,** this amount is close to zero, thence is significant, and the device reaches an almost perfect prediction capacity to detect true negative results.

Additionally, other useful parameters of Confusion matrix were calculated among them: **Positive likelihood ratio (LR+) was 19.93,** the measure is major of 10, indeed almost twice the value, consequently the performance of the device is significant. **Negative likelihood ratio (LR-) was 0.06,** the magnitude is minor than 0.1 therefore represents an almost perfect performance from the device. **Diagnostic odd ratio (DOR) was 338.95**, the resulting value is strongly, evidencing the efficacy performance of GeneXpert. **Prevalence of 17.46%,** this is the percentage of cases with RIF Resistance, from the total study. **Critical success index (CSI) was 77.12%,** the low strong performance detected in this parameter is due mainly to some many false positive results, nevertheless, is consider moderate efficiency. **Markedness (MK) was 79.59%,** represents a moderate performance of the machine.

Furthermore, were developed another parameters to study the performance and concordance of GeneXpert against DST-RR diagnosis method (Table 5) like: **accuracy that was 95.11%,** this outcome represents an almost perfect performance of the analyzed device. **Balanced accuracy (BA) was 94.83%,** the quantity result constitute an almost perfect performance of the machine. The percentage of **Bookmaker informedness (BM) was 89.66%,** is strong accuracy of detection people with and without RIF resistance, nonetheless is not almost perfect performance. **F1 score was 87.08%,** like the previous one is strong accuracy, however is not almost perfect accuracy of the device, due mainly to some false positives detections.

Table 5.

CONFUSION MATRIX from GeneXpert MTB-RIF assay against Drug susceptibility test *of Rifampicin resistance (DST-RR).*

		DST-RR				
	Total population N:	Positive	Negative	Prevalence	**Accuracy (ACC)**	Critical Success Index (CSI)
	716	(+)	(-)	17.46%	**95.11%**	0.77124183
GeneXpert MTB/RIF	Positive (+)	118	28	**Positive predictive value (PPV)**	False discovery rate (FDR)	Balanced accuracy (BA)
				80.82%	19.18%	94.83%
	Negative (-)	7	563	False omission rate (FOR)	**Negative predictive value (NPV)**	Bookmaker Informedness (BM)
				1.23%	**98.77%**	89.66%

Sensitivity, True positive rate (TPR)	False positive rate (FPR)	Positive likelihood ratio (LR+)	Diagnostic odds ratio (DOR) = LR+/LR−	F1 score l	Markedness (MK)
94.40%	4.74%	19.93			
False negative rate (FNR)	**Specificity (SPC), Selectivity, True negative rate (TNR)**	Negative likelihood ratio (LR-)	338.95	87.08%	79.59%
5.60%	**95.26%**	0.06			

CONCORDANCE ANALYSIS AMONG GENEXPERT AGAINST DST-RR

To study the agreement among the variables. Was employed kappa Cohen index (KCI) in SPSS Software, generating a magnitude of 84.1%, hence the concordance between variables is strong, nonetheless, not almost perfect. The Mattews correlation coefficient (MCC) amount was assessed with results from confusion matrix as another measure to determine the concordance

among variables, obtaining the level agreement of 84.48%, this quantity evidences the strong concordance of the device against DST, nevertheless is not an almost performance (Table 6).

Table 6.

Concordance analysis among GeneXpert against Drug susceptibility test of Rifampicin resistance

Comparison	Kappa Cohen Index	Matthews Correlation Coeficient (MCC)
GeneXpert – DST-RR	84.10%	84.48%

ACCURACY AND INEQUALITY AMONG GENEXPERT AGAINST DST-RR

To evaluate the diagnostic accuracy of the machine was implemented ROC curve, represents a graphic plot of the diagnostic performance of the device to RIF resistance detection (Park et al., 2004), (fig 5). Consequently, was calculated the area under the curve (AUC) from ROC curve, that is an estimation of discriminating proficiency of GeneXpert, this amount was 94.83%, (Table 7). This amount represents an almost perfect performance of the device to diagnosis true positives and true negative cases. Moreover was developed Gini coefficient 8.51%

to measure inequality among variables (Silber, 1989) (table 7), this quantity represents an almost perfect equality among GeneXpert against DST.

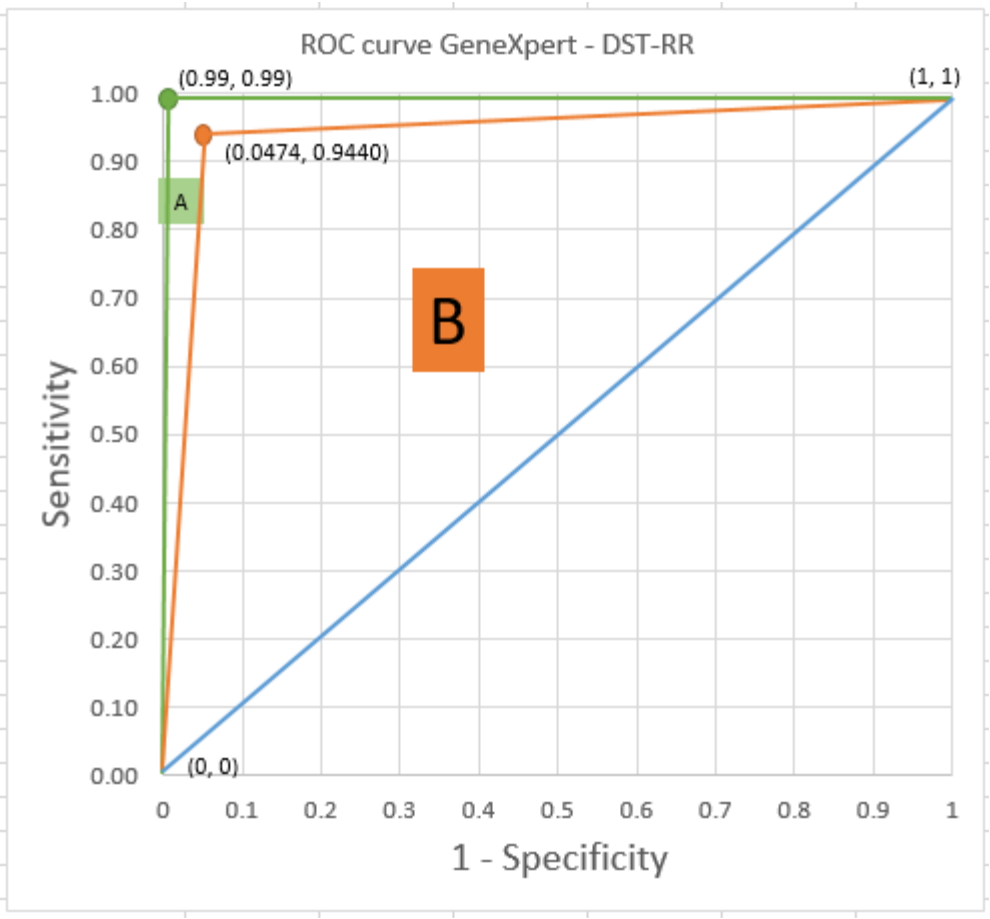

Figure 5. ROC curve by comparison among GeneXpert against DST-RR (orange curve), is the comparison among variables, in green curve is only the performance of DST-RR.

Table 7. Area under the curve (AUC) among GeneXpert and DST-RR, and the Gini Coefficient, magnitude of inequality among the variables.

Compared Variables	AUC	Gini Coefficient

GeneXpert - DST	94.83%	8.51%

DISCUSSION

Foremost, there are to emphasize about the entire statistical results in developing of performance analysis of GeneXpert device. Pearson Chi-square test was developed to assessed significant agreement among GeneXpert against the conventional methods, culture test (gold standard) in the case of TB diagnosis and Drug susceptibility test (gold standard) to RIF resistance detection (Sarfaraz et al., 2018). For whole statistical values outcomes was consider a 95% of Confidence Interval and was obtained a p-value < 0.001 that represents an outstanding dependence among analyzed variables, GeneXpert against conventional methods.

PERFORMANCE STUDY OF GENEXPERT AGAINST CULTURE AND DRUG SUSCEPTIBILITY TEST OF RIFAMPICIN RESISTANCE

- **PERFORMANCE OF GENEXPERT AGAINST CULTURE FOR TB DETECTION**

According to the confusion matrix, about performance of GeneXpert against Culture for TB detection, is remarkable the following results: for sensitivity and specificity, 98.17% and 90.34% respectively. Besides, PPV and NPV were 80.43%, and 99.19%, respectively. These results represent an almost perfect performance by the device to correctly patient's classification, from people that have the disease, but not almost perfect performance to people that is not infected

by Tuberculosis. Moreover, these measures demonstrated the efficacy of GeneXpert prediction from not infected people; nevertheless not strong prediction from infected people. The outcomes of specificity and PPV are due to so many false positives. GeneXpert must improve its specificity specially, to have a better overall performance.

From accuracy and concordance statistical study of GeneXpert against Culture, were revealed the following results: 92.59% of accuracy that represents the performance of GeneXpert to detect true positive and true negative cases of TB. The device, does not display a perfect accuracy, due to some false positives, hence does not have a strong marked accuracy. From Kappa Cohen Index (KCI) was generated a concordance of 83.10%. The concordance value is due to false negatives results by the detection from the device, even though, principally the maximum difference are due to 339 false positives. The Matthews correlation coefficient (MCC) was 83.94% of correlation, this value is similar to (KCI) evidencing that the device does not had an outstanding agreement with culture; due to the outcomes of some false positives.

Moreover, the performance assessed by AUC from Receiver operating characteristic (ROC) curve among variables was 94.26% (95 CI, $p<0.001$), this percentage correspond to correctly classified patients. The previous results evidence a good performance discriminating ability of the apparatus. Is evident that GeneXpert does not have a perfect diagnosis because detects some false positives in the case of TB detection. The Gini Coefficient was developed employing the area under the curve from ROC curve obtaining an amount of inequality among variables of 9.68%. Not is close to zero, again due to some false positives outcomes.

- **PERFORMANCE OF GENEXPERT COMPARED TO DST-RR**

According to confusion matrix, performance of GeneXpert compared to DST-RR detection, the following outcomes were generated: sensitivity and specificity, 94.4% and 95.26%, respectively. These results represent a significant performance from correctly patient's classification. Furthermore, positive predictive value (PPV) and negative predictive value (NPV) were 80.82%, and 98.77%, respectively. These values evidenced the efficacy of prediction classification of cases. Nevertheless, the positive prediction not is almost excellent, as was pre-specified due to some false positives detected by GeneXpert.

From Accuracy and correlation statistical study the consequently results were obtained: 95.11% of accuracy, it display the efficiency of the device to detect true positive and true negative cases of Rifampicin resistance. Due to some false positives outcomes, the equipment does not has an almost perfect accuracy. The Kappa Cohen Index was 84.1%, this is not an almost perfect concordance, due to false negatives and mainly for some false positives results registered by the device. The Matthews correlation coefficient (MCC) was 84.48%, of correlation coefficient among variables, this value like the previous one evidences not close to almost perfect agreement. The device does not has an outstanding concordance with DST-RR detection due to the presence of some false positives.

Furthermore, the accuracy of the machine was assessed by AUC from ROC curve among GeneXpert against DST-RR, the concordance was 94.83% (95 CI, $p<0.001$). Therefore, represents an almost perfect performance of the device to correctly classified patients of RIF resistance. The Gini Coefficient was developed employing the area under the curve from ROC graphic, obtaining an inequality of 8.51%. It proved an almost perfect equality, nevertheless, not totally marked strong, due to some false positives detected.

PERFORMANCE OF GENEXPERT WORLDWIDE AND THE REGION

- **GENEXPERT PERFORMANCE FOR TB DIAGNOSIS**

Globally**,** performance of GeneXpert have stablished scale from 70% to 100% for sensitivity, and a specificity domain from 91% to 100%, for TB detection; according to several analysis from 12 studies among results from the device against culture (Weyer et al., 2012). Another studies reported a good specificity (92.1%) and sensitivity (83.9%) along with PPV (81.3%) and NPV (93.3) of the machine (Reechaipichitkul, 2016). These studies conformed an essential evidences by endorsement of the assay by World Health Organization in 2010 (Lawn et al., 2013). Nevertheless, the performance contrast among regions. In a several study enrolled with 6,648 cases undertaken in Philippines, India, Azerbaijan, South Africa, Uganda and Peru, the device detected 90.3% from culture positive TB samples. The evaluated equipment against culture reveled the following results in other study: a sensitivity and specificity of 76.9% and 99.0%, respectively (Weyer et al., 2012). According to the literature from several analysis that were generated by 15 studies from China was obtained 90.4% and 98.4% for sensitivity and specificity, respectively (Chang et al., 2012). From another study, undertaken at San Francisco United States were recruited 217 samples, were obtained the following results: 89% and 95.3% of sensitivity and specificity, respectively, for Mycobacterium tuberculosis detection from pulmonary samples (Marlowe, 2011). In the present study were obtained a sensitivity and specificity, 98.17% and 90.34% respectively. Reveling an agreement accuracy according to worldwide literature.

Nonetheless, globally some literature not promotes encouraging GeneXpert results. In a study developed in Bangladesh from 107 sputum samples, suspected of MDR-TB were obtained the following results: 87.64% and 75% were the sensitivity and the specificity respectively,

evaluated against culture (gold standard), (Laskar, 2017). Moreover**,** other analysis developed in Montreal, Canada, were assessed 502 cases with suspicious of TB, of whom were generated the following results: sensitivity 46% and specificity of 100% for TB detection (Sohn, 2014). In another analysis were enrolled 6,648 cases, where the results were: 67.1% and 99.5% for sensitivity and specificity, respectively (Boehme, 2011). Furthermore in another study developed in China were undertaken 295 sputum samples in the period 2014-2015, these were the results: 94.64%, 82.97%, 77.37% and 96.18% for sensitivity, specificity, PPV and NPV, respectively (Shao, 2017).

Regarding to likelihood ratios, the results from a study revealed a positive likelihood ratio (LR+) that was 10.6 and the negative likelihood ratio (LR-) 0.18 (Reechaipichitkul, 2016). In our case was 10.16 and 0.02 respectively; the outcomes are similar to pre-specified study results, suggesting a significant performance of GeneXpert. In addition, according to positive likelihood ratio (LR+), likelihood negative ratio (-LR), and Odd Ratio (OR) were obtained the following results: in a study developed in Italia undertaken with 1,474 samples was obtained a (LR+) of 28.8 and (LR-) of 0.2 (Tortoli et al., 2012). Besides, from another study with 682 patients enrolled was got a sensitivity of 100%, specificity 99.4%, PPV of 98.4%, NPV of 100%, positive likelihood ratio (LR+) of 167, and negative ratio (-LR) 0, (Moraa et al., 2017). In a study developed from 36 studies, with a total samples of 9,523 were identified among GeneXpert against Culture the following results: Kappa value 0.80, pooled (LR+) of 29 and (LR-) was 0.22 (Penz et al., 2015). From enrolled 88 samples was developed a study in London, United Kingdom, with extra-pulmonary specimens displaying the consequent outcomes: for (LR+) was 17.8, and (LR-) was 0.35, and Odd Ratio (OR) was 51.3 (Dhasmana et al., 2014). In this thesis the outcomes achieved were the following: (LR+) 10.16, (LR-) 0.02, and Odd Ratio (OR) of 501.16. Nonetheless, (LR+) is major of 10, according to bibliography this value should be upper; (LR-) this quantity in the

present study is superior to the references and OR from literature evidenced an elevated significance; further from OD results of the present analysis display a strong significant amount.

- **GENEXPERT PERFORMANCE FOR RIFAMPICIN RESISTANCE DETECTION**

Worldwide, regarding to Saeed from some assessments the sensitivity of the machine has a scale of 90-98%, further a specificity of 95-100% for Rifampicin resistance diagnosis (Saeed et al., 2017). Moreover, other studies revealed encouraging results for sensitivity, specificity, PPV and NPV of the device for Rifampicin susceptibility diagnosis, these were: 127 (98.3%), 704 (99.1%), 127 (94.7%) and 704 (99.4%), respectively, by comparing the results with drug susceptibility testing (Saeed et al., 2017). Besides, from another analysis, the sensitivity of Rifampicin resistance was 94.4%, and 98.3% for specificity (Weyer et al., 2012). The sensitivity, specificity of detecting RIF-resistance were 94.1%, 97.0% respectively (Chang et al., 2012), in a study developed in China. In other study the specificity was 98% and sensitivity 95% (Steingart et al., 2014). Furthermore, in another analysis developed in United States, the apparatus proved has a sensitivity of 96.8% and specificity of 96.2% for rifampicin susceptible specimens (Boehme, 2011) by a study assessed with data recollected from India, South Africa, and Peru. Moreover in a study developed to register the performance of GeneXpert against DST undertaken from Pakistan with 125 sputum samples from confirmed pulmonary TB patients and they were not responding to first line drug treatment, displayed the following results: a sensitivity of 92.1%, the specificity was 93.5%, the PPV was 98.3%, and NPV was 95.1% **(**Munir et al., 2018). Consequently the findings from the present study have a significant concordance among variables according to the bibliography data.

Furthermore, in a study developed in Seoul-Korea, from 321 samples, was obtained the following outcomes: (LR+) of 74, (LR-) was 0 (Kim et al., 2015). In a study generated in United States displayed the following results: (LR+) of 59, (LR-) was 0, (Rice et al., 2017). Besides, in a study carried out from Lithuania evidenced (LR+) of 49.594, (LR-) of 0.013, (Pimkina et al., 2015). In the present study were generated the following results: (LR+) of 19.93, and (LR-) was 0.06, evidencing according to literature that the value registered in this analysis is low to the standard globally values, due to some false positives generated by GeneXpert.

ACCURACY AND CONCORDANCE ANALYSIS OF GENEXPERT AGAINST CONVENTIONAL METHODS

Assessing to performance of GeneXpert MTB/RIF against culture: In a study generated in China with 851 patients suspicious of TB, developed from 2015 to 2016, was obtained the concordance percentage employing Kappa Cohen Index, which was 84.7% (Liu et al., 2017). Another assessment from China undertaken with sputum specimens of 240 related symptomatology of TB cases, the concordance of outcomes among the device assay against Culture using Kappa value was 73% (Tang et al., 2017). In a study developed in Irak with 182 cases among pulmonary and extra-pulmonary samples; the results were: sensitivity of 94.2%, specificity was 85.7%, PPV of 95.7%, NPV was 81.8% and diagnostic accuracy was 92.3% of GeneXpert compared to culture (Ahmed, 2019). The previous values mainly of concordance and accuracy of GeneXpert evidenced similar results to the present study from a concordance of 83.1%, further an accuracy of 92.59% of the equipment.

Concerning to performance of the machine against DST-RR: In an undertaken study from Pakistan with 125 cases with MDR-RR were revealed the following results: accuracy of 96.7%, a concordance of 92.07% **(**Munir et al., 2018). Besides, from another performance analysis from Kenya with 682 patients enrolled, Kappa value was 98.88% with 95% CI, and a p-value less than 0.0001 (Moraa et al., 2017). In another study of concordance, developed in China, the following result was generated: 90.4% from Kappa Cohen index (Liu et al., 2017). Furthermore, exist localities where were registered perfect concordance among GeneXpert assay against DST-RR, like the case of South Korea, where had reported an outstanding concordance of 100%, with drug resistance test confirmed by culture (Kim et al., 2012). These contrasted the results of concordance principally, with the concordance among GeneXpert and DST present in this study, which was 84.1%, and an accuracy of 95.11%. These results are due to false negatives and mainly for some false positives results detected by the machine. It must be clarify, that there is low literature about statistical studies related to accuracy value among the device against DST-RR.

EFFICIENCY OF GENEXPERT AGAINST CONVENTIONAL METHODS ACCORDING TO AUC FROM ROC CURVE GRAPHIC

Assessing the GeneXpert results against culture, of a study done in South Africa, with 108 cases of culture positive tuberculosis; the device evidenced a strongly association with culture, obtaining an average AUC of 0.91, from ROC curve (Shenai et al., 2016). In a research developed with 25 previous studies was registered an average of AUC of 0.9897 among the apparatus against Culture (Yan, L., Xiao, H., & Zhang, Q., 2016). In a research developed with fifteen data sets were obtained an AUC of 0.85 among the device against Culture. Regarding to another research, generated with 97 studies were involved 26,037 samples for RIF resistance diagnosis and the AUC

result was 0.98 (Zong et al., 2017). In the present statistical analysis the AUC's was 0.94255, and 0.9483 for the case of the machine against Culture and, the case of RIF resistance, respectively. Overall the literature results the AUC from the apparatus presents a better association in this study among the machine and Culture. Nevertheless, does not has the similar agreement among the device and DST-RR. Is evident the low perfect significance, due to some false positives results that the device registered, and limitations from the device, especially in the performance of RIF resistance.

PROCEDURES AND TESTS LIMITATIONS FROM GENEXPERT, CULTURE, AND DST TO DISPLAY NOT PERFECT PERFORMANCE FROM GENEXPERT

From not totally outstanding outcomes of mainly, specificity and PPV and another estimations parameters derived from them, the root from these results are principally for the false positives outcomes that GeneXpert detects; in the case of the device against Culture were 339, in total. These could be explained by failure in the development of Culture and GeneXpert procedures. Two methods of culture were developed Ogawa Kudoh and Lowenstein Jensen that were used as gold standard. These two different types of culture have different efficiency, generating positive culture. In these two methods exists an excess of decontamination that could kill MTB. According to literature, decontamination eliminates microorganisms while enable M. tuberculosis to survive, this procedure must be carefully timed, due to survival advantage MTB from alkaline conditions is relative, nevertheless, not absolute (Burman & Reves, 2000). Consequently, GeneXpert detects killed MTB as a positive that explain some false positives results detected.

GeneXpert presented not totally strong results from sensitivity and PPV mainly and derived parameters from them of Confusion matrix due high false positives and false negative results, 28 and 7 in total, respectively; for the case of RIF resistance detection. The false positives outcomes could be explained by technician error, cross-contamination., and contamination during samples procedures (Bhattacharya, 1998), in the case of DST-RR, and the limitations of DST remarked in methodology like some bias and/or missed data is inherently due to the performance DST and Culture. Another reasons of false positive are: contamination in laboratories, and procedure of thus from the samples during mucolytic, or decontamination, the contamination rates of 16% have been reported (Wurtz et al., 1996). Besides, in some cases had reported some false positives due to inappropriate procedures, further in the compilation of processing of specimens, and defective modules or cartridges (Helb et al., 2009). The GeneXpert algorithm accuracy could develop false positives of detection TB and RIF resistance (Theron, et al., 2016). According to Boehme a modification in the software of GeneXpert increased the specificity of the device from 94.4% to 98.3% (Boehme, 2011), nevertheless the sensitivity decreased. Evidencing that device algorithm have to be improved.

There is low, almost null literature studies developed in Ecuador dedicated to the complete analysis of GeneXpert. Nevertheless, one of them evidenced that mutations were not localized in amplified region of the rpoB gene from that study was the amount of 5.7%, (Franco-Sotomayor, et al., 2018). Is well known that the device detects the mutations from rpoB gene (hot spot) because are associated with RIF resistance. The machine only detects this specific gene because approximately 95% of the mutations in the rpoB gene are within the hyper-short polymorphic region, while 5% of mutations are outside this region (Ortiz -Jimenez et al., 2019). In the present study, false negative rate (FNR) was 5.60% that have close relationship with the cases of RIF resistance that the apparatus cannot detect. The previous literature developed in Ecuador ratify too

about exposed. Regarding to false negative results are generated because GeneXpert only detects mutations from rpoB gene that is responsible from of 95% of RIF resistance. The machine detections suggest that specificity could be lower in patients previously treated (Boyles et al., 2014), **for the development of the present study such information was not available, that could be another bias in the assessment of the performance of GeneXpert of this Thesis.**

Moreover, other analysis had affirmed too, the functional imperfections of the GeneXpert system. Especially in the conditions presented in poor countries, such as the design which is not suitable for employ in peripheral laboratories (Ortiz-Jimenez et al., 2019) because it is a sophisticated device and its handling requires care. It requires an immovable continuous electricity provision to prevent a disturbance in the procedure and consequently loss of outcomes (Boehme et al. 2011). The manufacturer's recommended temperature control below 30 ° C and the cartridges were affirmed stable at 2-28 ° C (Boehme et al. 2011). Further, the machine requires an annual calibration, which needs access to an equipment distributor to substitute the modules concerning to the manufacturer's instructions (Boehme et al. 2011). Besides, the machine assessed must have appropriate deposit place for the equipment and sufficient staff members to perform the diagnosis (Weyer et al., 2012).

SUGGESTIONS TO IMPROVE GENEXPERT PERFORMANCE IN DIAGNOSIS TB AND RIF RESISTANCE

Despite of overall GeneXpert presents an almost perfect performance, there are many features about the device that have to be improved. Analysis accomplished at 2011 display that improvements have been made in the machine analyzed, especially in the reagents used in the process and also in the software to decrease the frequency of false-positive outcomes of Rifampicin resistance detection (Weyer et al., 2012). The insertion of the newest model of GeneXpert cartridge at December 2011 mainly reduced the amount of signal loss mistakes (Weyer et al., 2012). In spite of the improvements developed in that year to the device, have continued the errors of false positives as the evidenced outcomes, in the present thesis project. Therefore is necessary the continued improvement of algorithm of the device.

The competitive development of technologies of related accuracy with GeneXpert is recommended to generate greater demand and competition in the market to improve the efficiency and diminish the expensive of devices to TB and RIF Susceptibility diagnosis. This will require considerable increment in funding to perform and generate technological improvements to develop innovative detection devices. In the next few years, GeneXpert is liable to replace traditional diagnostic methods such as smear microscopy, which is the initial test of all patients suspected of having TB disease (Luna, 2016). The accuracy outcomes by GeneXpert described in the previous sections evidence a mandatory confirmation by culture testing (Evans, 2011). Patients found by the device to have rifampicin-resistant TB still require specialist laboratory facilities for more extensive drug resistance testing (Evans, 2011).

REGARDING TO THE PRESENT STATISTICAL STUDY

The present study is one of the initials complete statistical studies about TB and DST-RR detection assessed in Ecuador and Latin America from national surveillance data collected. The overall tuberculosis detection found by this study achieved 28.79% of the entire study TB Suspicious population and reached 17.46% of the whole DST-RR study population. Exhibiting a more recent screening of TB and RIF resistance burden in Ecuador. This statistical study was performed with last reported data. Although, could be helpful to perceive the current situation of tuberculosis and Rifampicin susceptibility at Ecuador, considered limitations were inherent during the accomplishment of this thesis. Such limitations include the lack of reliable information concerning the current situation of TB and Rifampicin resistance in Ecuador or surrounding countries, the reduced performance of data handling, and the absence of uniformity among government information and independent studies. For instance GeneXpert detections suggest that specificity could be lower in patients previously treated (Boyles et al., 2014), for the development of this study such information was not available. The present study depended in the reliability of the data registered from different health establishments, as the case of Culture outcomes that were collected from an overall of seventeen institutions around Ecuador. Furthermore GeneXpert data outcomes were collected by five different localities. And samples evaluated to detect DST-RR were developed in INSPI.

CONCLUSIONS

- The performance of GeneXpert MTB/RIF assay presents a considerable reliability, but not perfect reliability. Nevertheless is a method with low biohazard risk, and faster compared with traditional diagnostic method Culture and DST-RR.
- GeneXpert evidenced a strong, although not perfect performance to detect TB and RIF resistance.
- The overall concordance of the device displayed an almost perfect agreement among the analyzed equipment against traditional methods.
- Despite the entire outcomes about GeneXpert, not could replace the culture test and Drug susceptibility test, mainly due to false positive results.
- The GeneXpert diagnosis, must be followed by conventional test Culture and Drug susceptibility test, to improve the correct diagnosis of patients.

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ANNEXES

Annex 1. Pearson *Chi-Square test from SPSS statistical software, Independency test among GeneXpert MTB/RIF assay and Culture test*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3473.270[a]	1	.000		
Continuity Correction[b]	3469.388	1	.000		
Likelihood Ratio	3902.579	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases	4929				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 498.62.

b. Computed only for a 2x2 table

Annex 2. *Pearson Chi-Square test, from SPSS statistical software, Independency test among GeneXpert MTB/RIF assay and Drug susceptibility test*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	510.978[a]	1	.000		
Continuity Correction[b]	505.470	1	.000		
Likelihood Ratio	444.887	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases	716				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 25.49.

b. Computed only for a 2x2 table

Annex 3. *Kappa Kohen Index from SPSS statistical software. Concordance test among GeneXpert MTB/RIF assay and Culture (gold standard)*

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.831	.008	58.934	.000
N of Valid Cases		4929			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Annex 4. *Kappa Kohen Index from SPSS statistical software. Concordance test among GeneXpert MTB/RIF assay and Drug susceptibility test.*

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.841	.026	22.605	.000
N of Valid Cases		716			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.