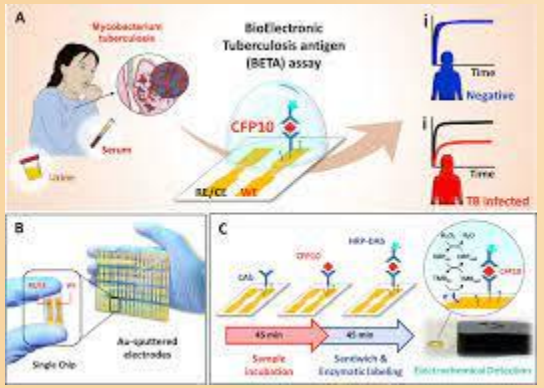
Tuberculosis Diagnostics with Modern Solutions

Z.G. Aytasheva

March 2024

Short pre-history



Tuberculosis (TB) is a life-threatening infectious disease. It is caused by *Mycobacterium tuberculosis (M. tuberculosis)*. Timely diagnosis and effective treatment are critical in the TB controlling.

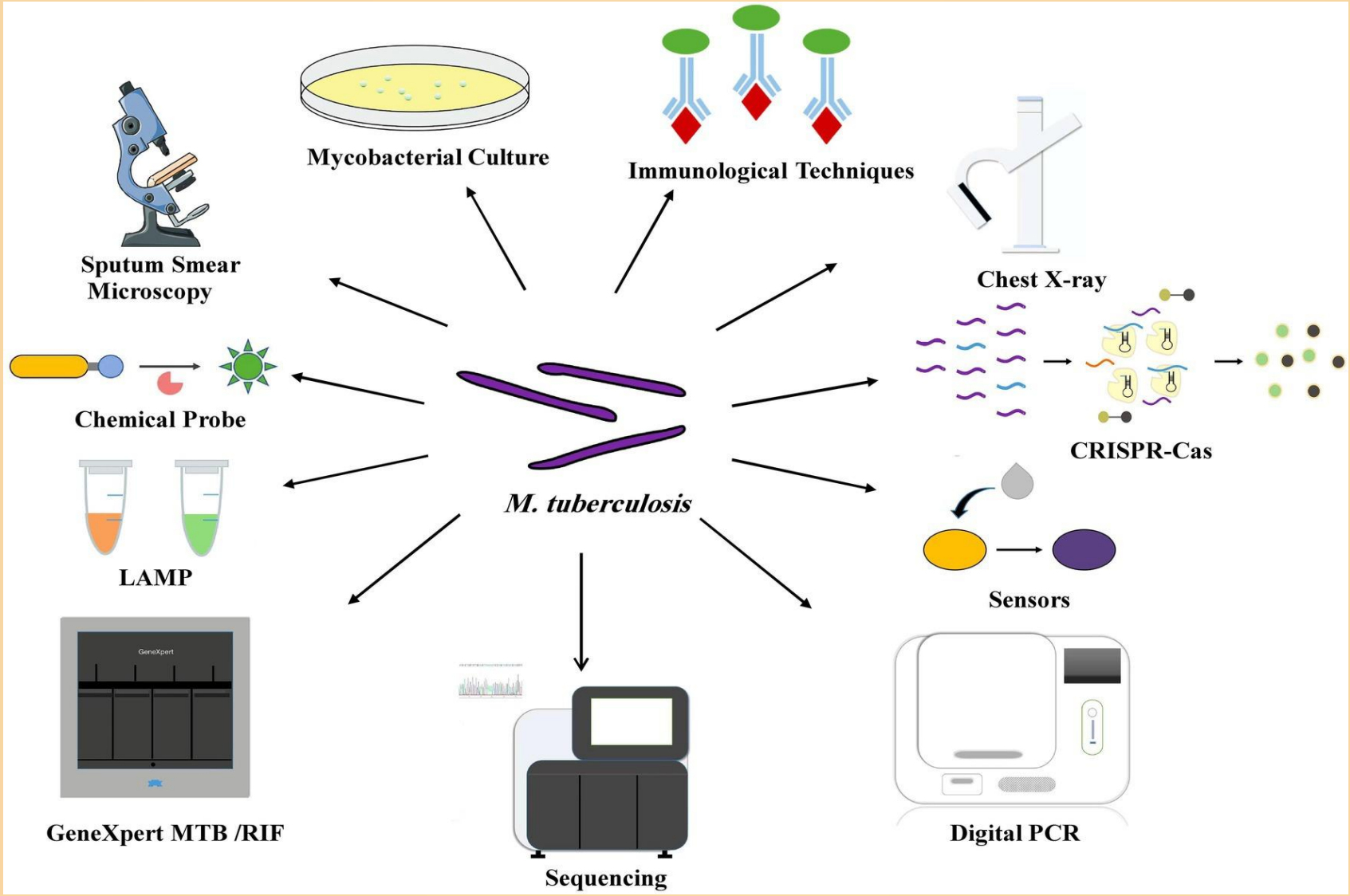
Conventional smear microscopy still has low sensitivity and is unable to display the drug resistance of this bacterium. The traditional culture-based diagnosis is time-consuming, as the results are available in a month. Molecular biology methods can not distinguish living from dead mycobacteria, whereas immunological methods also fail to differentiate active bacteria from those latent. From this point, counting the ongoing emergence of multidrug resistance and extensive cases of drug-resistant TB, the demand for simple, rapid, accurate and economical point-of-care approaches has increased over past years. This ppt highlights the development, evaluation, and implementation of progressive as conventional diagnostic methods for TB and the rapid detection of *M. tuberculosis*.

Early Notification

The express method for diagnosis of contagious tuberculosis is microscopic examination of sputum smears (search for acid-fast bacilli, or AFB) (Truffot-Pernot et al, 2006).

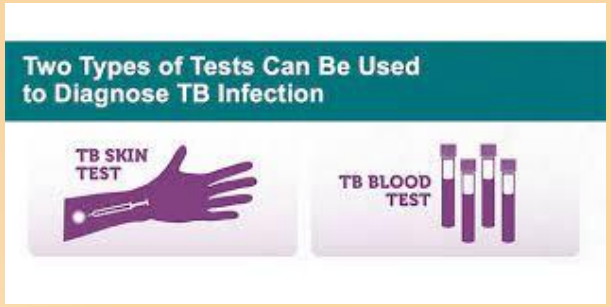
Gene amplification is not yet reliable for direct detection of *M. tuberculosis* in clinical specimens that are AFB smear-negative. 3-8 weeks cultures onLowenstein-Jensen medium or 1-4 weeks cultures in liquid media are used to determine AFB and reveal antibiotic susceptible microorganisms. Thereby AFB is identified in a few hours using specific DNA probes. However, results of susceptibility testing, even in liquid media, are not available up to 2-4 weeks after the recovery of specimens, irrespective of the mutated rpoB and katG 315 genes, which are in charge of rifampin and isoniazid resistance. These mutated genes can be detected within hours by molecular hybridization with specific probes fixed on strips. Immunologic tests indicating the quantity of interferon gamma synthtesized by immune sensitive (sensitized) lymphocytes are ascribed to promising diagnostic tools of latent tuberculosis.

Various diagnostic tools for TB point-of-care testing (https://doi.org/10.3389/fmicb.2022.924410).



Early notification (cond.)

The gap between conventional TB-diagnostic methods and real clinical needs dictates the development of new diagnostic methods which are precise, fast and cheap. This presentation reflects both the improvement and optimization of conventional methods, and the development of new diagnostic approaches (see figure above).



Optimization of Present TB-Diagnostic Techniques

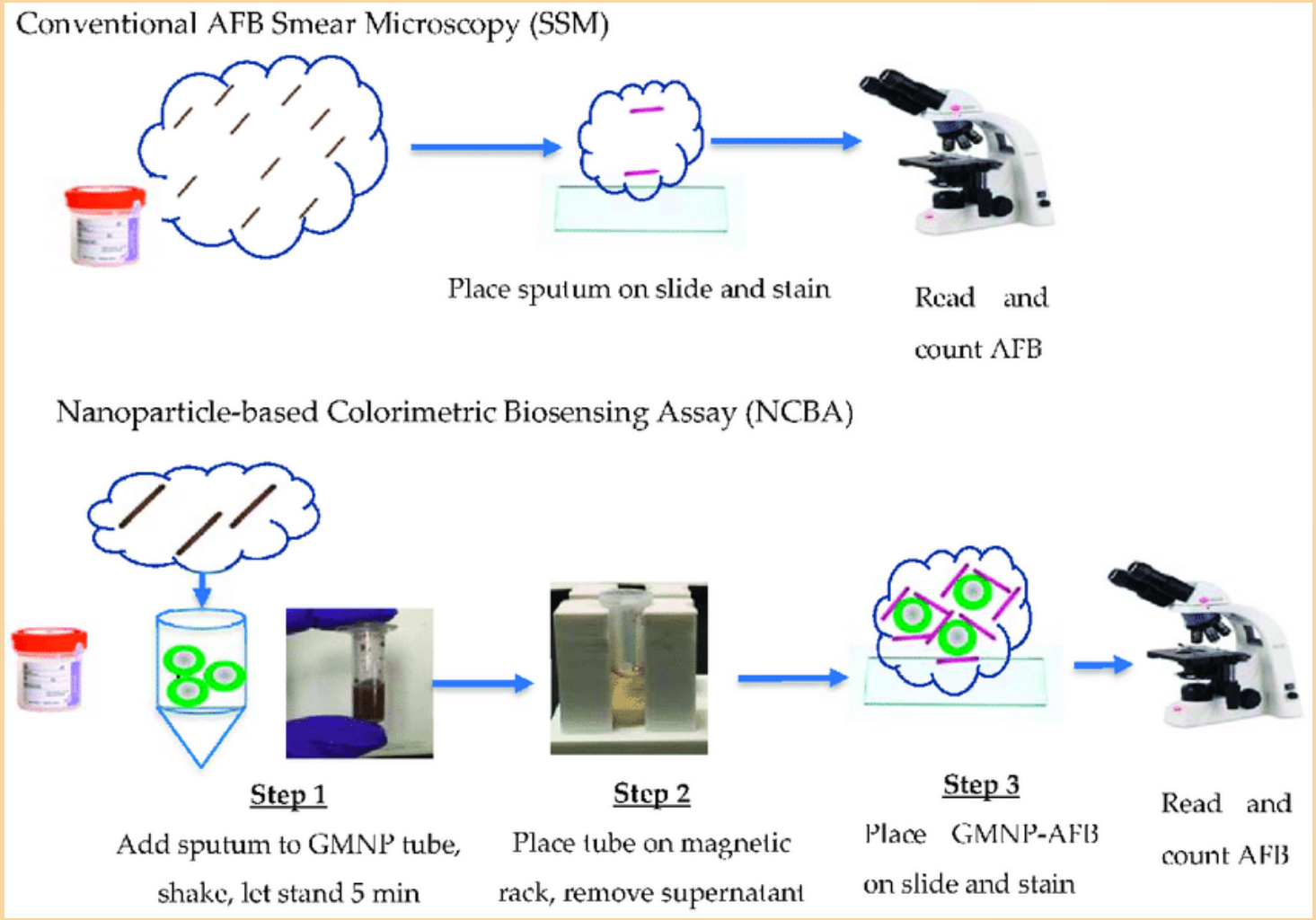
Etiological Diagnostic Techniques

Sputum Smear Microscopy (SSM)

SSM is the favotite method for detecting pulmonary TB. In some remote areas of developing with countries SSM acts as the only approach for TB diagnosis (Ben-Selma et al., 2009). The conventional staining methods are Ziehl-Neelsen (ZN) staining and fluorescent staining (Auramine-O / Auramine-rhodamine). SSM is cheap and comfortable despite its low sensitivity (the operator requires $>10^4$ bacilli•mL-1 of sputum to get positive response and has a high false negative response, which is prone to lead to misdiagnosis. In recent years, the operator-independent SSM based on the ZEISS Axio Scan has been developed to automatically detect and count acid-fast bacilli with a high sensitivity, 97.06% and a high specificity, 86.44% (Zingue et al., 2018). This SSM method rises the detection efficiency and saves time.

The laser confocal microscopy (LCM) with specific fluorescent antibody labeling has been developed for *M. tuberculosis*-affected lung tissue samples. This tool is useful for mycobacteria with weak ZN staining (Erokhina et al., 2019). The Pat-Scan program designed using digital pathology helps in detecting and quantifying the bacteria in the paraffin-embedded ZN-stained tissue. This LCM assists in reducing the diagnostic time (Sua et al., 2021). Noteworthy, SSM is unable both to distinguish dead and live bacteria, and separate *M. tuberculosis* from non-tuberculosis mycobacteria. At the same time, ZN staining provides the data on the acid-fast motility of bacilli from the patient's sample. This information is necessary for the patients that have been pre-treated with anti-TB drugs. In addition, such information can be obtained from only ZN staining, but not from fluorescent SSM.

Sputum Smear Microscopy (SSM) as a scheme



Mycobacterial Culture

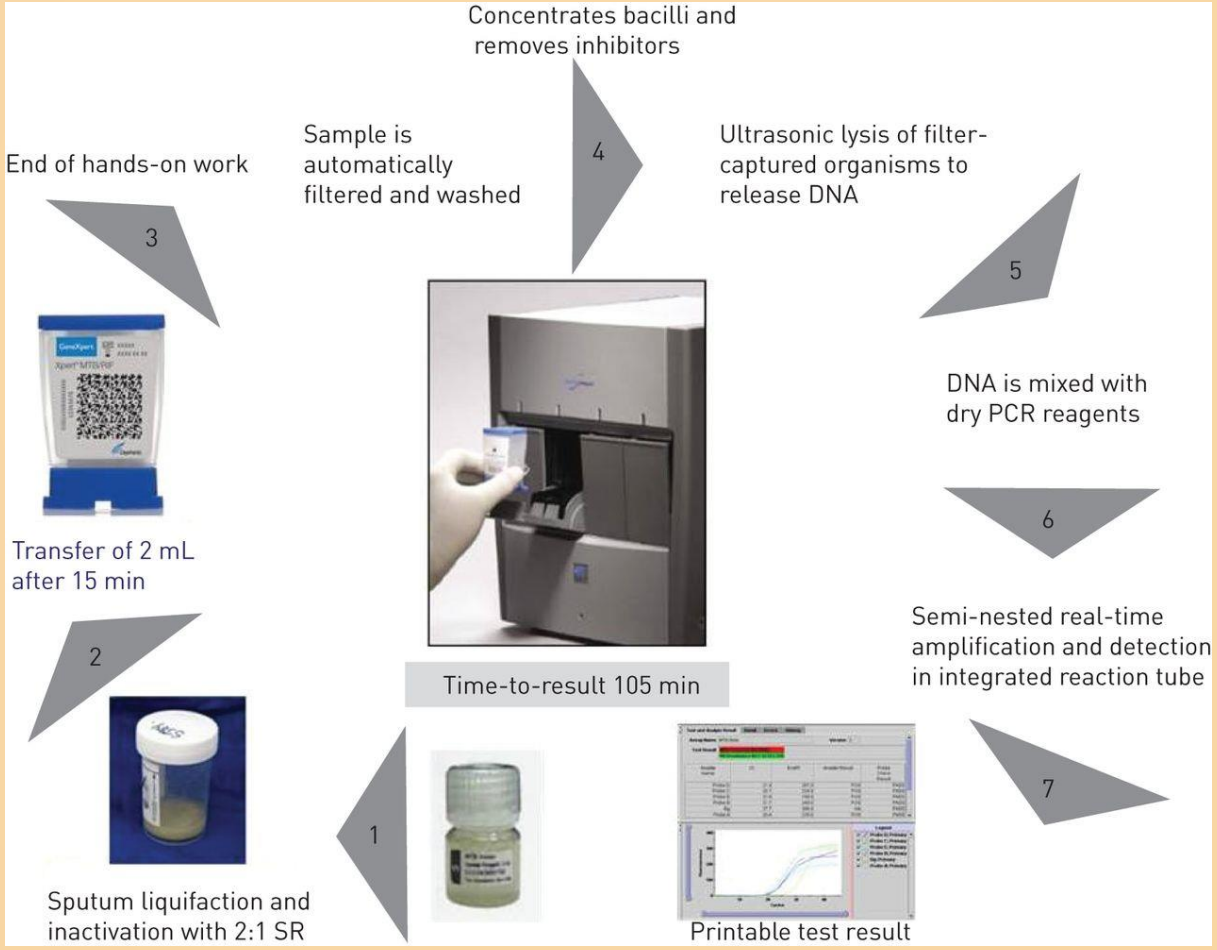
Patients' samples can be gathered for mycobacterial culture (10^2 bacilli•mL^{-1} of sputum). Its cultivation is still the gold standard for TB diagnosis. *M. tuberculosis* is usually cultivated on a solid medium. Then it is detected and tested for drug sensitivity. The information obtained enables the clinicians work out effective antibacterial treatment guidance (Kenaope et al., 2020). The liquid culture of *M. tuberculosis (*for instance, BACTEC MGIT 960, VersaTREK, and MB/BacT Alert 3D) allow to define *M. tuberculosis* in a few days. The BACTEC MGIT 960 automated culture system measures the oxygen quenching fluorescence. The signal is detected upon the mycobacterial growth in the tube. MGIT 960 is more effective for rapid identification of mycobacteria and early TB diagnosis than the Löwenstein-Jensen selective solid agar medium (L-J solid medium, Hasan et al., 2013). The VersaTREK system is sensitive to pressure variation. So, it shows the growth of the inoculated specimen by detecting the pressure change beyond the broth medium (Espasa et al., 2012). The MB / BacT Alert 3D system uses a colorimetric CO_2 sensor to control the growth of *M. tuberculosis* (Piersimoni et al., 2001). Considering the slow growth of the *M. tuberculosis* complex (MTBC), most MT-positive cultures appear in a week, whereas MT-negative cultures occur later, in 2 month (Lee et al., 2003).

MB-diagnostic techniques

Xpert MTB/RIF

is the most popular detection method in molecular diagnostics. It is a semi-nested real-time fluorescent PCR detecting *M. tuberculosis* and rifampin resistance simultaneously. The Xpert MTB/RIF **Ultra** provides two different multi-copy amplifications and a larger DNA reaction chamber (World Health Organization, 2017). The limit for the Xpert Ultra goes down to 15.6 CFU/ml compared to the sensitivity limit of 112.6 CFU/ml for Xpert MTB/RIF (Chakravorty et al., 2017). This method detects MTBC DNA in sputum or concentrated sputum samples and rifampin resistance. The results are deady in 2 hours (Bodmer and Ströhle, 2012). In December 2010, the WHO recommended Xpert MTB/RIF in the diagnosis of TB and drug resistance, especially in HIV patients and suspected patients with multidrug-resistant TB (WHO Guidelines Approved by the Guidelines Review Committee, 2011). The technique is limited due to high demands for professional testing personnel and supporting infrastructure. So medical institutions face difficulties to meet the WHO requirements for Xpert MTB/RIF and ensure the quality of diagnostic outputs (Gidado et al., 2019).

Xpert MTB/RIF steps

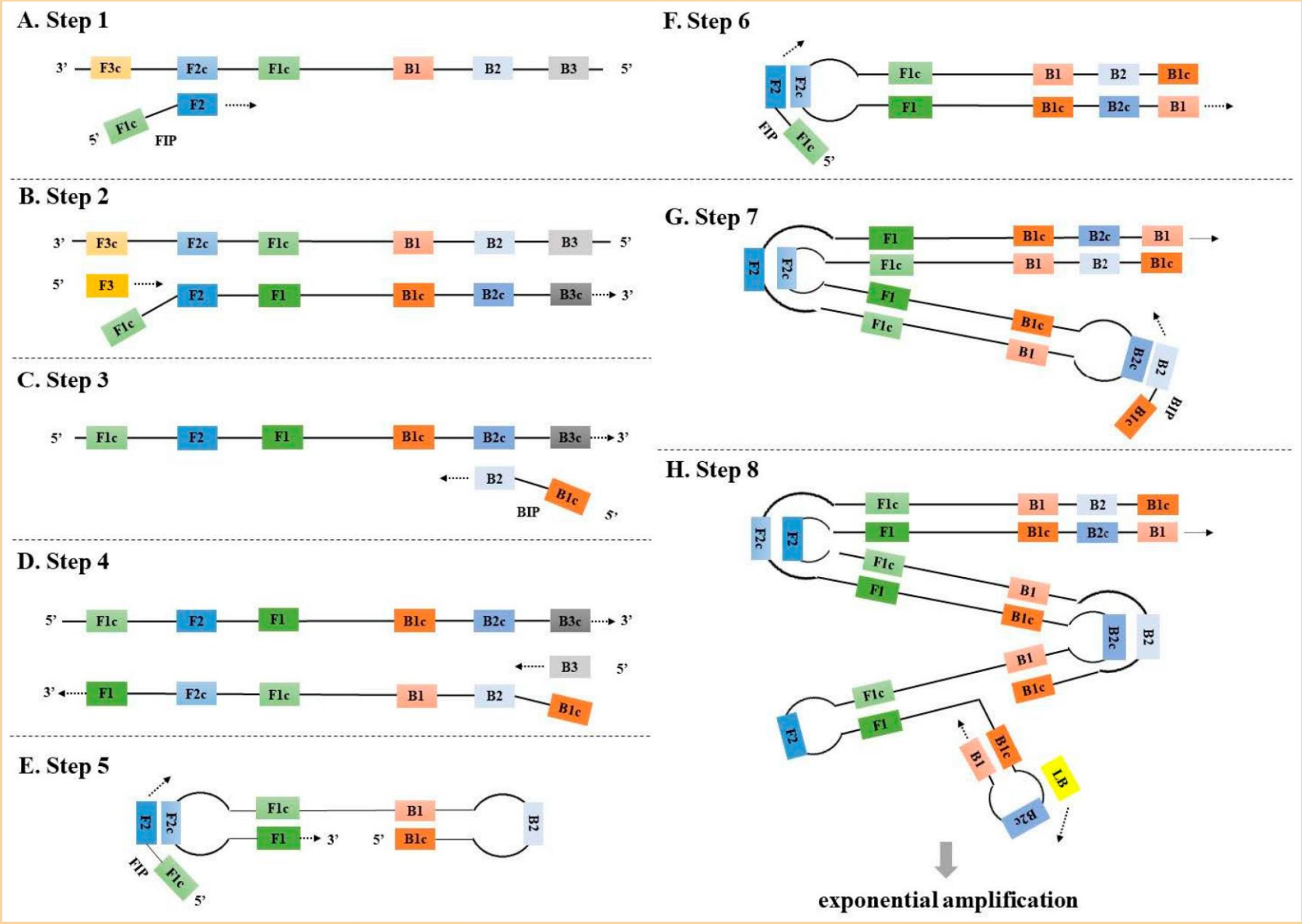


MB-diagnostic techniques (cond.)

Loop-Mediated Isothermal Amplification

LAMP employs the DNA polymerase reaction and a set of specific primers to detect the pathogenic DNA in a patient sample. The SS-LAMP (Single Standed Loop-mediated isothermal amplification) is designed with a set of six specific primers to identify eight different regions on the MTBC-specific repeats 6,110 (IS6110), which is aimed at detecting the MTBC DNA from dissolved sputum samples (Bentaleb et al., 2016). LAMP assessment was performed using 157 liquefied sputum specimens from Moroccan suspected TB patients. SS-LAMP is faster, more specific (99.14%) and sensitive (82.93%) comparing to the conventional L-J solid culture method. LAMP is suitable for areas with scarce medical resources.

The principle of conventional LAMP. F3 and B3: Forward and backward outer primers, respectively; FIP (F1c + F2) and BIP (B1c + B2): Forward and backward inner primers, respectively; LF and LB: Forward and backward loop primers, respectively (Zhang et al., 2023).

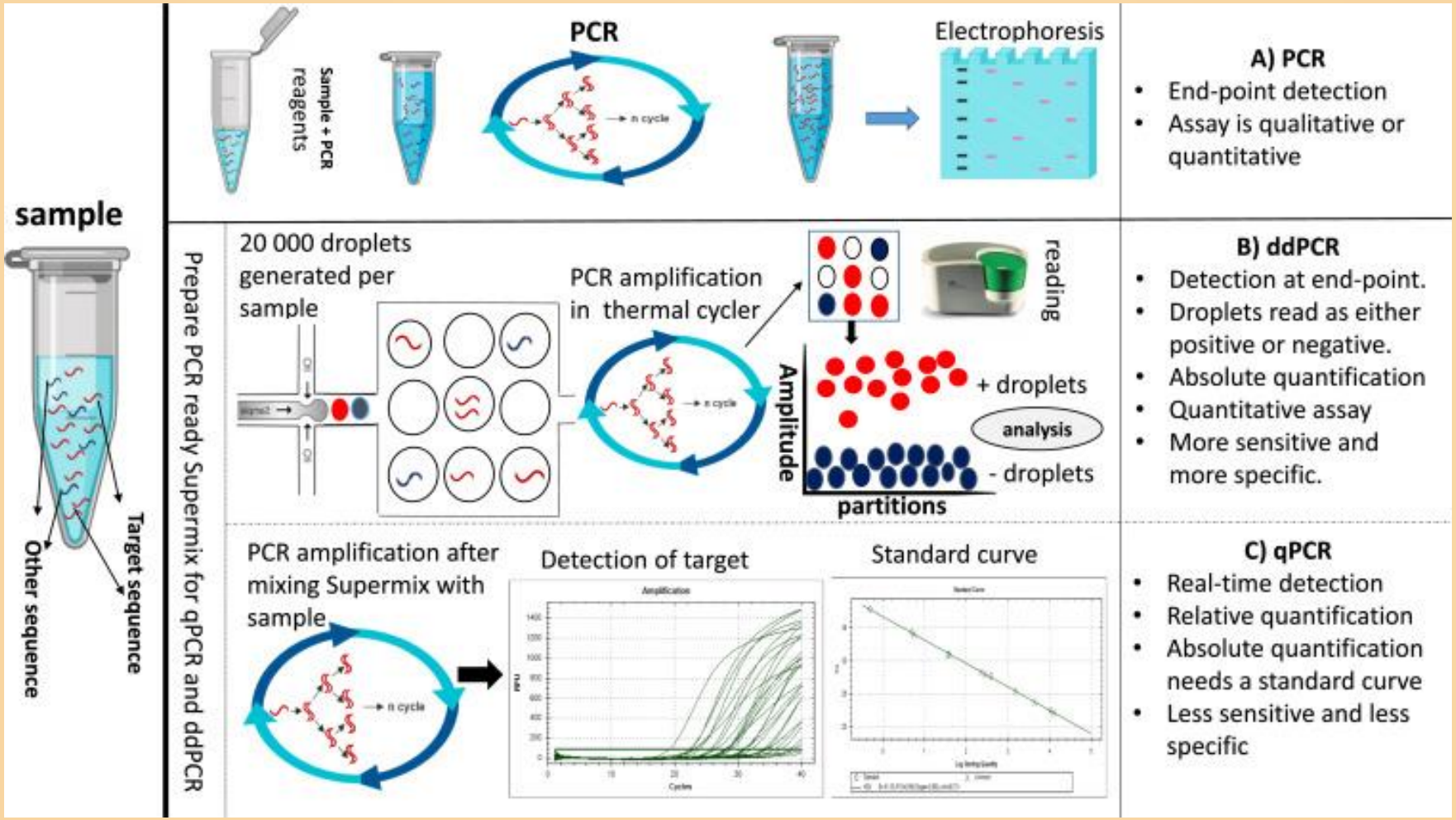


MB-diagnostic techniques (cond.)

Digital PCR

dPCR is a relatively modern nucleic acid quantification technology requiring tiny amounts DNA. It provides full quantification without a pre-built standard curve (Kuypers and Jerome, 2017). Therefore, dPCR is precise and sensitive while detecting **single copies** of DNA (Nyaruaba et al., 2019). Sputum, blood, formalin fixed paraffin embedded tissue, and exhaled breath may be used as the dPCR samples. The drug sensitivity testing can also be conducted by this method (Surat et al., 2014; Ushio et al., 2016; Patterson et al., 2017; Yang et al., 2017a; Luo et al., 2019; Cao et al., 2020; Cho et al., 2020). For dPCR amplification, IS6110 is a common sequence target. But in combination with IS1081 and IS6110 repeats, the dPCR sensitivity is higher than purely IS6110 qPCR. Then this method is applicable for the diagnosis of smear-negative TB (Lyu et al., 2020). Moreover, this technique is useful for examining the lung, extrapulmonary, latent TB infection, and active TB. However, it is also rather prone to error in the inexperienced operator's hands (Nyaruaba et al., 2019).

Digital PCR steps (Nyaruaba et al., 2019)



Immunological Diagnostic Techniques

Tuberculin Skin Test and Interferon-γ Release Assay

The tuberculin skin test and the following tuberculin protein derivative test are the auxiliary diagnostic tools for TB, especially popular in the TB pediatrics. However, these methods are unable to effectively distinguish the positive results due to BCG vaccination or *M. tuberculosis* infection. Moreover, these methods can't provide reliable data for potential immunocompromised TB patients.

CFP-10, ESAT-6, Ag85A, Ag85B, CFP-7, and PPE18 are the most widely studied antigens in *M. tuberculosis* (Fan et al., 2017; Ren et al., 2018).

The interferon-γ release assay detects TB by measuring the IFN-γ secretion of in lymphocytes after stimulating with ESAT-6 and CFP-10 antigens. These two antigens are quite specific for *M. tuberculosis*. The analytical power of the two commercial kits is different. However, they reveal a close sensitivity in diagnosing latent and active TB infection. The methods have certain limitations while applied to HIV patients, immunocompromised adults and children (Ayubi et al., 2016; Benachinmardi et al., 2019). Besides, these methods claim a professional staff and good equipment.

Immunological Diagnostic Techniques(cond.)

Immuno-PCR

I-PCR detects potential mycobacterial antigens and antibodies circulating in the body fluids of TB patients. This technique is based on magnetic beads (MBs)/gold nanoparticles (GNPs) in liquid form. These structures generate a reduced background signal. Automated one-step I-PCR gains also in shorter detection time (Mehta et al., 2017). Singh et al. prioposed an I-PCR (MB-GNP-I-PCR) detection kit using MBs coupled with GNP to detect the early secreted antigen ESAT-6 (Singh et al., 2018). Later Dahiya et al. developed a GNP-RT-I-PCR with GNPs targeted to the CFP-10 protein of *M. tuberculosis* in clinical samples of TB patients (Dahiya et al., 2020a). The sensitivity comprised 83.7 and 76.2%, and the specificity was 93.5 and 93.8% in 49 cases of pulmonary TB and 42 cases of extrapulmonary TB, respectively. The MB coupled AuNP-based I-PCR (MB-AuNP-I-PCR) method has been elaborated for *M. tuberculosis* MPT64 and CFP-10 proteins from the body fluids of TB patients. The sensitivity of MB-AuNP-I-PCR in smear-negative pulmonary TB and extrapulmonary TB patients was much more higher than that of Magneto-ELISA and GeneXpert analysis (Dahiya et al., 2020b).

The PCR-ELISA induced by Zhou et al. reveals mutations of the rpoB, katG, and inhA genes caused by rifampicin and isoniazid resistance. This method predicts also the drug susceptibility in clinical isolates of *M. tuberculosis* (Zhou et al., 2020). Ultrasensitive ELISA with a thioNAD-cycling method is highly sensitivite for detecting TB without *M. tuberculosis* culture (Watabe et al., 2014). MPT64 protein is specifically excreted only from live *M. tuberculosis*, if the bacteria are heated (46°C). Ultrasensitive ELISA detects the MPT64 protein secreted by live *M. tuberculosis* (Wang et al., 2020; Cao et al., 2021). In that case the sensitivity is comparable with that one of SSM (sputum smear microscopy) and Xpert MTB/RIF.

Immunological Diagnostic Techniques(cond.)

Lateral Flow Urine Lipoarabinomannan Assay

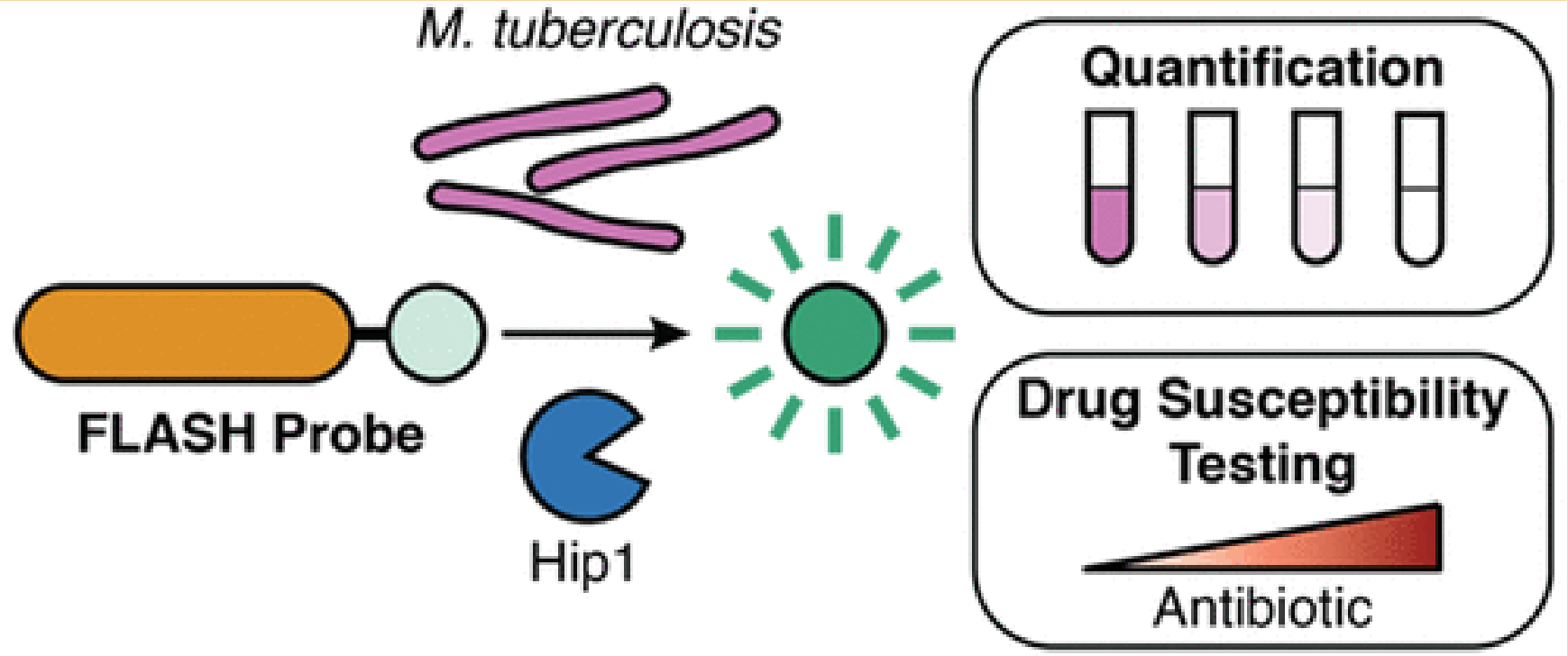
LF-LAM is based on lipoarabinomannan as a pathogenic factor. Lipoarabinomannan is a key pathogenic lipopolysaccharide built in the cell wall of mycobacteria. It has a representative structural epitope of *M. tuberculosis* (Sarkar et al., 2014). LF-LAM is known for its high sensitivity in the TB diagnostics in patients co-infected with HIV, especially those with low CD4 counts. (HIV patients with a CD4 count over 500 cells per cubic mm are usually in pretty good state. People a CD4 cell count lower than 200 are at high risk of developing serious illnesses).

WHO recommends the LF-LAM application to reveal active TB in HIV-infected patients (World Health Organization, 2015), though the sensitivity and specificity ofLF-LAM-tests need further adjustments confirming the method's diagnostic prospect (Sigal et al., 2018).

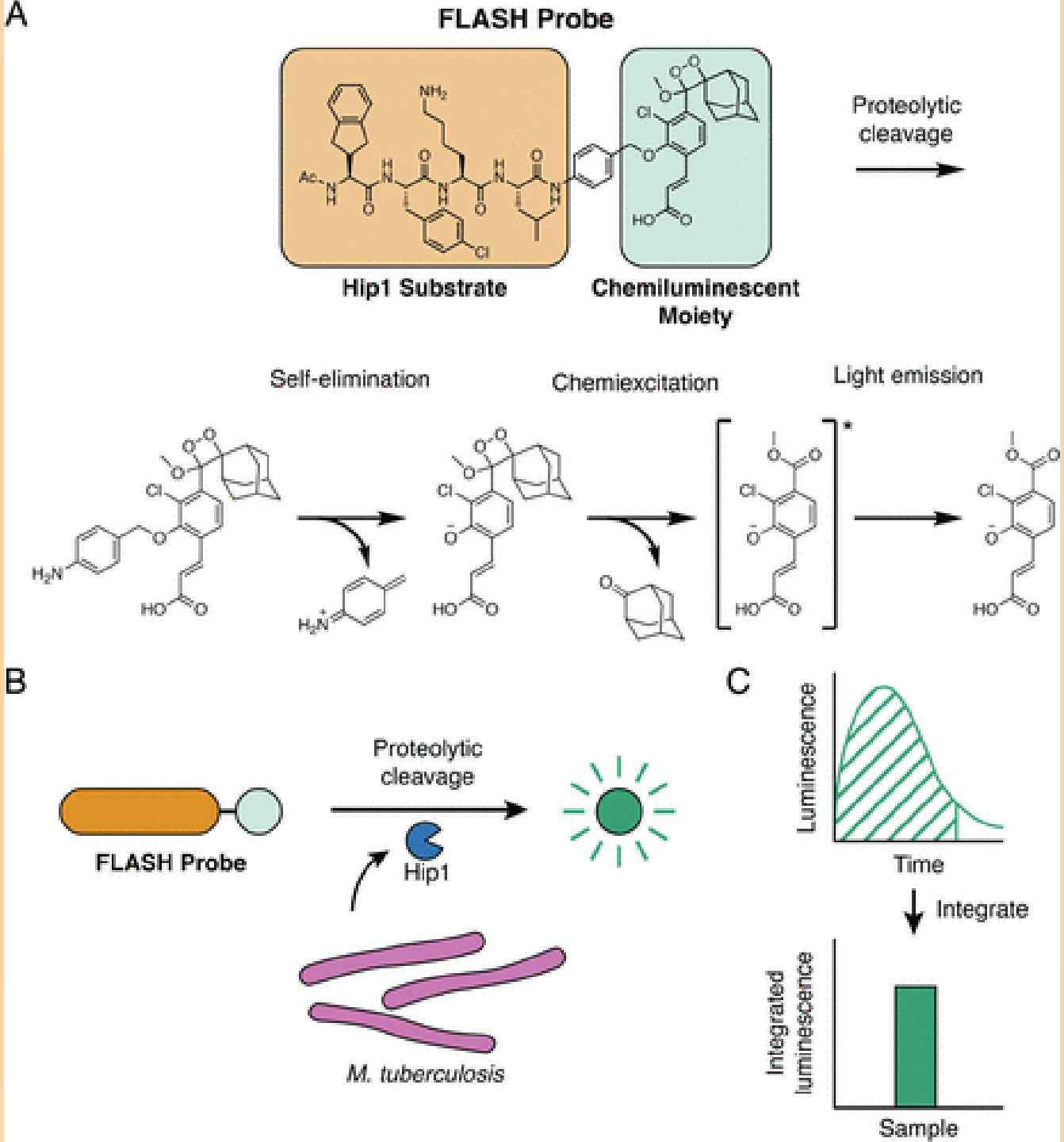
New Diagnostic Methods

Chemical Probe Methods

The chemical probes implied in radiology and optical imaging may help in real-time estimation of bacteria and other pathogenic agents. This is an alternative tool to bacterial cultures (Heuts et al., 2009; Andreu et al., 2012). Chemical probes are applicable to fluorescence, chemiluminescence, light scattering, and radioactivity. Fluorescence is regarded as the most useful tool, as it is able to be followed up using fluorescence scanners/plate readers, time-lapse fluorescence microscopes, ultra-high-resolution microscopes, and flow cytometers (Hira et al., 2020).



1) Proteolytic cleavage of the FLASH probe by *Mtb* Hip1.
2) Chemiexcitation and light emission.
3) Light produced by probe cleavage is quantified in a given time interval as integrated luminescence (Babin et al., 2021).



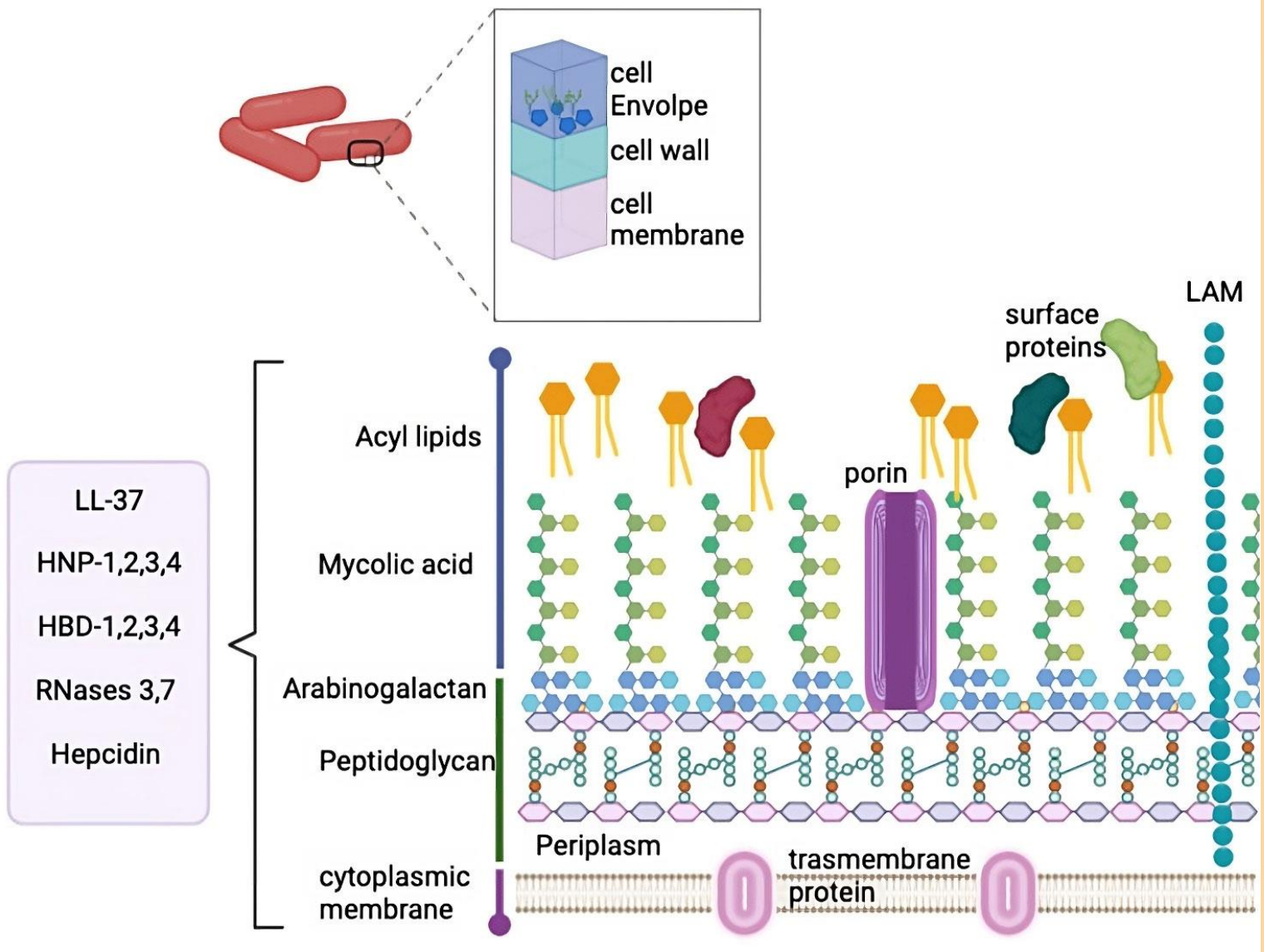
Fast luminescent affordable sensor of Hip1 (FLASH).
(A) Following proteolytic cleavage of the FLASH probe by *Mtb* Hip1, self-elimination and chemiexcitation steps ultimately lead to light emission.
(B) *Mtb* produces Hip1 protease which cleaves the FLASH probe, producing light.
(C) Light produced by probe cleavage is measured over time. Total light output in a given time period (dashed area) is summed to yield integrated luminescence (Babin et al., 2021).

New Diagnostic Methods (cond.)

Cell envelope-dependent probes

Mycobacteria exhibit a distinct extracellular structuring. The arabinogalactan, long-chain mycolic acid, and trehalose-enriched glycolipid are involved in the composition of outer membrane layer. The trehalose mycosyl esterase situated on the cell membrane demonstrates conservative substrate specificity. It allows the external tailing of synthetic probes (FITC-trehalose) thereby to get incorporated specifically into bacteria. Such specific inclusion is the principle of detection of bacterial growth traced with fluorescent labeling. The FITC-trehalose reliably indicated *M. tuberculosis* in a macrophage infection model (Backus et al., 2011). DMN-Tre (the 4-N,N-dimethylamino-1,8-naphthalimide conjugated trehalose probe) sensitively detects *M. tuberculosis* in sputum samples of TB patients. The fluorescence intensity is significantly higher (>700 fold) under the transition from aqueous to hydrophobic solvents. DMN-Tre labeling ensures fast visualization of mycobacteria and corynebacteria (within 1 hr) (Kamariza et al., 2018). DMN-Tre is specific for metabolically active mycobacteria and corynebacteria. This allows to proceed to the drug sensitivity after treatment.

M. tuberculosis cell wall



MTb cell wall is composed by several complex layers, including cell envelope, cell wall and cell membrane. These layers are composed by multiple lipids and peptidoglycan. The peptides that have been described to interact with this complex cell wall are listed in the box

Jacobo-Delgado et al., 2023

Cell envelope-dependent probes (cond.)

DLF-1, another high affinity stoichiometric probe is targeted to the D-Ala-D-Ala motif of bacterial peptidoglycan. The fluorescent probe directly labels the cell wall components of *M. tuberculosis*, representing an alternative approach for a rapid quantitative analysis of active and dormant *M. tuberculosis in vitro* and *in vivo* (Yang et al., 2016). The D-Ala-D-Ala motif is also present in other bacterial strains besides *M. tuberculosis*; thus, DLF-1 cannot be specifically used to label *M. tuberculosis*.

BlaC-Specific Fluorogenic Probes

BlaC is an Ambler Class A β-lactamase, highly conserved among clinical isolates of *M. tuberculosis*, which effectively hydrolyzes β-lactam antibiotics (Kwon et al., 1995). The crystal structure of BlaC indicates the unusual glycines in the BlaC active site, which make *M. tuberculosis* β-lactamase a unique biomarker for *M. tuberculosis* detection (Wang et al., 2006).

BlaC-Specific Fluorogenic Probes (cond.)

Xie et al. developed a fluorescent probe for *M. tuberculosis* by advancing the flexible substrate-specific loop of BlaC enzyme. Then the authors induced a methoxyl substituent at the position 7 of the lactam ring. It has made BlaC 1,000 times more selective than TEM-1 β-lactamase (Xie et al., 2012). The fluorescence rised 100–200 after activation of BlaC. This has reduced false positives.

The green fluorescent probe CDG-OME was developed for growing sensitivity and specificity of extremely small number of live pathogens in the sputum of patients within 10 min, even in unprocessed sputum, making it a quick and cheap diagnostic tool for TB. After that Cheng et al. designed a CDG-3 probe with the substitution of the cyclopropane ring at the position 2 , which completed the initial substitution of the methoxide ring at the position 7. CDG-3 probe's selectivity to BlaC is 120,000 times greater compared with TEM-1 β-lactamase (Cheng et al., 2014). CDG-3 detected *M. tuberculosis* in a trial with 50 clinical samples in 1 h, with a sensitivity and specificity of 90 and 73%, respectively.

Cheng et al. developed later a dual-target fluorescent probe CDG-DNB targeting BlaC and DprE1. BlaC hydrolyzes the lactam ring to activate the fluorophore, while DprE1 covalently bind to an anchor element for the steady fluorescence signal (Cheng et al., 2018). The combination of BlaC and DprE1 enhances *M. tuberculosis* fluorescent labeling. The CDG-DNB probe specifically and precisely labels the single live *M. tuberculosis* in less than 1 h. The specificity of CDG-DNB probe has been demonstrated on selected labels of *M. tuberculosis* among other bacteria (including 43 non-TB mycobacteria).

BlaC-Specific Fluorogenic Probes (cond.)

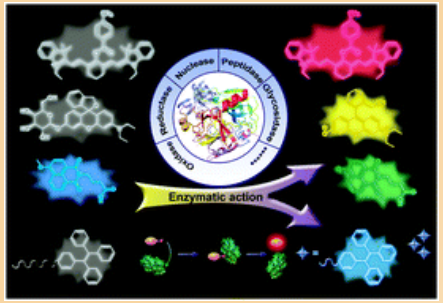
CDG-3 is a fluorescent reporter enzyme substrate specific for BlaC (REF) and a TB diagnostic tool used in sputum specimens (Sule et al., 2016). *M. tuberculosis* is also detected in clinical samples containing other bacteria. The CDG-3 probe is highly selective to BlaC, used to measure the enzyme levels of *M. tuberculosis* in sputum. The sensitivity and specificity for CDG-3 were 88.1 and 86.1% in 160 clinical specimens from potential TB patients, with a negative predictive value of 93%; thus, it could be used to predict potential TB patients (Sule et al., 2019). BlaC is secreted by the Tat secretion system in live *M. tuberculosis* (McDonough et al., 2005); therefore, the CDG-3 test is suitable for estimation and treatment up to analyzing the phenotypic drug susceptibility. REF substrate CNIR800 with the near-infrared (NIR) fluorescent dye IRDye 800CW (Yang et al., 2017b) is based on the quenching agent of IRDye 800CW which is bound to a lactam ring hydrolyzed by BlaC. The CNIR800's emission wavelength is 795 nm, which significantly improves the signal reducing its noise during the *M. tuberculosis* detection. The threshold of CNIR800 detecting is ~100 CFU (colony-forming units) *in vitro* and < 1,000 CFU in the lungs of mice. The CNIR800's fluorescence signal produced by cleavage reaches its maximum level in 4–6 h since its administration in live animals, allowing the accurate assessment of the effectiveness for anti-TB drugs.

Probes Dependent on Sulfatase, Esterase, Protease, and Nitroreductase

A sulfate activation probe (7-hydroxy-9H -(1, 3-dichloro-9, 9-dimethylacridin-2-ketone)-sulfate target mycobacterial sulfotransferases (Beatty et al., 2013) and conserved sulfatases (Mougous et al., 2002). Mycobacteria have unique sulfatase fingerprints to be used for determining mycobacteria and their lineage and TB patients. The highly conserved esterase activity of the MTBC has been discovered (Tallman and Beatty, 2015). These authors synthesized new C4- and C8-masked probes using four-carbon (C4) and eight-carbon (C8) acyl-oxymethyl ether derivatives of long-chain fluorescent substrates to analyze the lysates of macrophages infected with *M. tuberculosis*, and identified the patterns of *M. tuberculosis* esterase and lipase bands (Tallman et al., 2016).

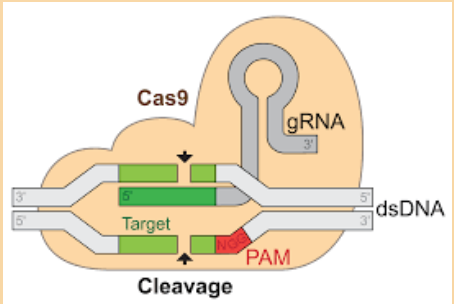
Probes Dependent on Sulfatase, Esterase, Protease, and Nitroreductase (cond.)

A rapid and economical chemiluminescent protease Hip1 probe (FLASH) (Babin et al., 2021) is cleaved by *M. tuberculosis* protease Hip1. After that, the aniline linker is eliminated to release the activated phenoxy-dioxetane luminophore. FLASH allows to estimate active Hip1, detecting and quantifying *M. tuberculosis* in 1 h. It is useful for clinical sputum samples. FLASH distinguishes live and dead cells, and monitor the drug susceptibility of clinical *M. tuberculosis* isolates. A nitrooxidoreductase Rv2466c-dependent fluorescent probe (Mu et al., 2019) is a small molecule mycothiol (MSH) of *M. tuberculosis.* It binds to Rv2466c; its sulfhydryl group forms a disulfide bond with the Cys19 to activate Rv2466c, allowing the entrance of the coumarin-based nitrofuranyl calanolides (NFCs) into Rv2466c and interact with W21, N51, and Y61 of Rv2466c to form the Rv2466c-mycothiol-NFC ternary complex. Rv2466c reduces the nitro group of NFCs to an amino group, agitating a high level of fluorescence, thus it is useful for a rapid diagnosis and drug sensitivity test of clinical sputum samples.



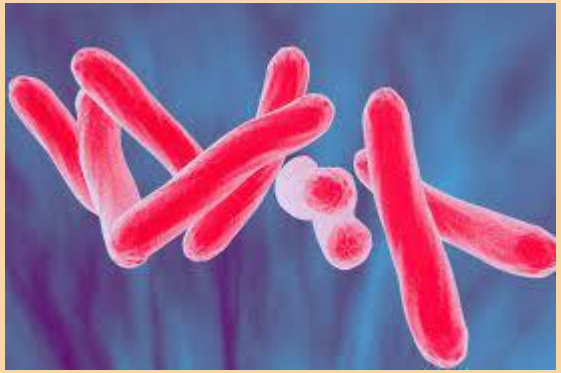
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

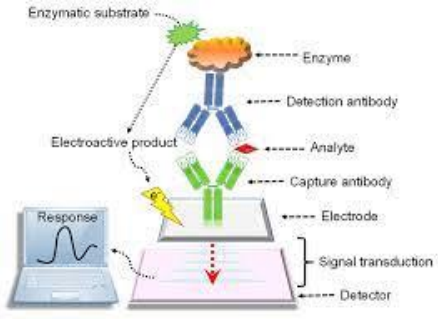
CRISPR-Cas operates in a sequence-specific manner by finding out and cleaving DNA or RNA. This procedure allows to adjust and improve the target. The sensitivity and specificity of CRISPR-Cas are comparable to conventional PCR. However, CRISPR doesn't require expensive equipment as PCR and is very cheap. The CRISPR-MTB detection system applies Cas12a endonuclease to recognize dsDNA and cuts it into ss DNA. This system combines then isothermal amplification technology to achieve nearly single copy level sensitivity. Additional plus is only 500 µl sample (Ai et al., 2019). Yet, amplifications are attributed as indicating high false positives and being unable to distinguish live bacteria from dead.



Mass Spectrometry

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) is used to identify tuberculous complex and most atypical mycobacteria from cultures (Lotz et al., 2010; El Khéchine et al., 2011). The identification of *M. tuberculosis* from a L-J solid culture is better than that from liquid culture due to the interference of some components of the liquid medium. Exhaled breath-collected samples with subsequent high-resolution mass spectrometry is used to identify *M. tuberculosis* (Chen et al., 2020). Currently, the FDA-approved two MALDI-TOF platforms (namely MALDI Biotyper and Vitek MS, BioMérieux) are used to detect mycobacteria and a few other bacteria. The MS approach can be implied in a clinical laboratory in a few minutes (Seng et al., 2010), although it requires expensive laboratory equipment and specialized staff, as well as shorts for an available extensive library for data alignment.





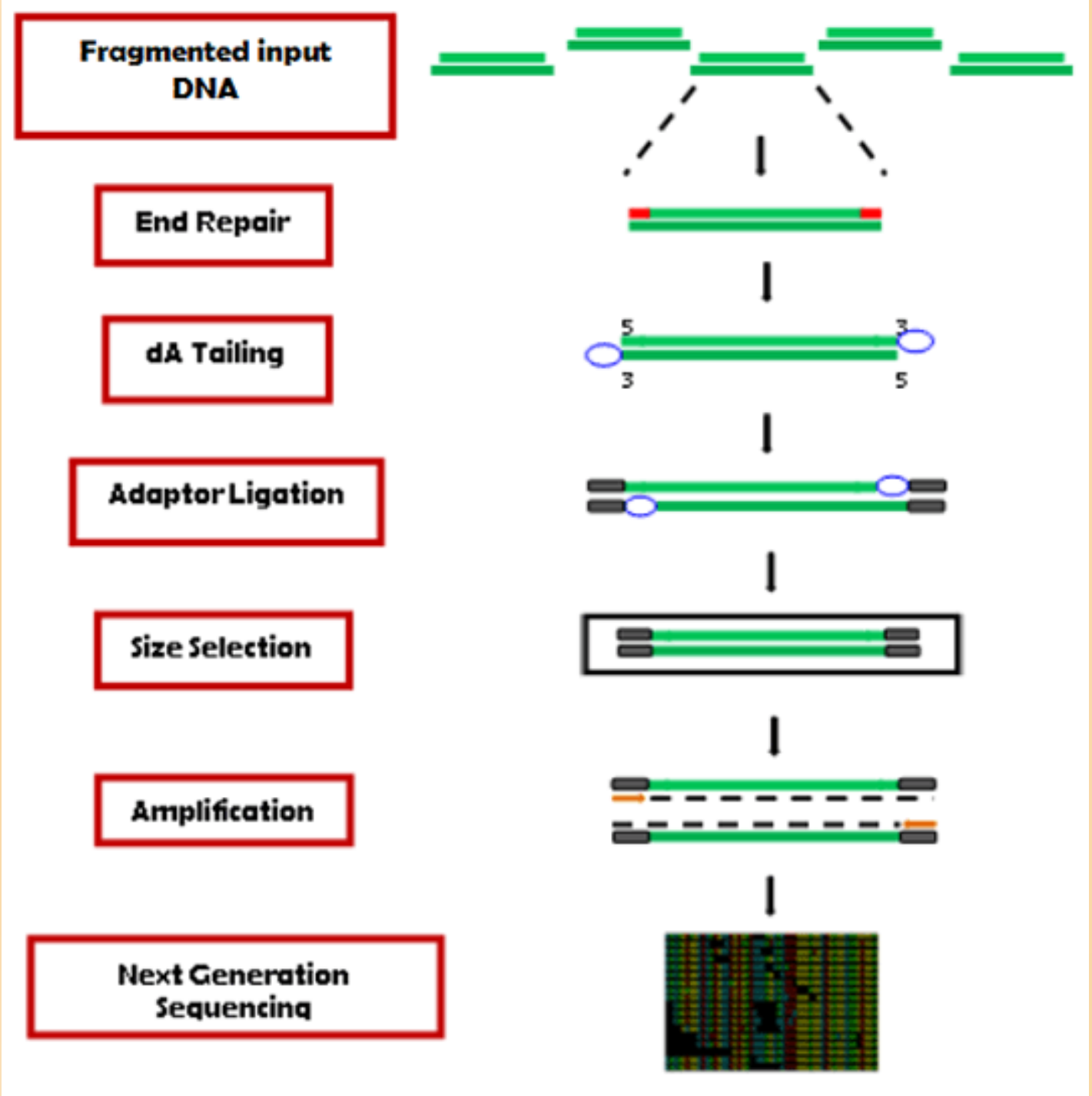
Immunosensors

Immunosensors (Iss) are often used to detect and quantify disease-related agents in clinical diagnosis due to their high affinity to the antigen and antibody complex with great selectivity. Electrochemical immunosensors used in TB diagnosis are based on the use of monoclonal antibodies (MABs) to detect specific proteins secreted by *M. tuberculosis* (Montoya et al., 2017; Mohd Bakhori et al., 2019; Peláez et al., 2020). ISs quantitatively monitor the electrical signals generated by the binding between antibodies and target molecules or antigens of *M. tuberculosis*. Current nanomaterial IS are designed based on the specific chemical, physical and electronic properties of the nanomaterials to improve the sensing device and diagnose TB in real-time (Mohd Bakhori et al., 2019; Hatami et al., 2020; Kahng et al., 2020). Although Iss are promising, portable, easy to operate, with no amplification steps, they did not meet the same success as immunoassays (Shin et al., 2015). It is explained possibly by the detection limit of 10^3 copies to diagnose *M. tuberculosis* (Jaroenram et al., 2020). In addition, identification of latent infections, as well as pediatric and immunocompromised HIV patients may be a problem for Iss (MacGregor-Fairlie et al., 2020). Therefore, further experiments with ISs in diagnosing TB are needed.

Next-Generation Sequencing Technology

Next-generation massively parallel sequencing provides sequencing of millions of fragments simultaneously in each run. NGS has been proposed to set up profiles of drug resistance in a single analysis of drug-resistant TB (Tafess et al., 2020). Drug susceptibility testing is achieved by the targeted or whole-genome sequencing (Papaventsis et al., 2017). Conventional whole genome sequencing of *M. tuberculosis* depends on bacterial cultivation (Iketleng et al., 2018), so, the direct whole genome sequencing of the sputum makes unnecessary bacterial incubation (Doyle et al., 2018). It saves detection time, and significantly shortens the detection of drug resistance comparing to the whole genome sequencing of MGIT (mycobacterial growth indicator tube) or culture-based drug resistance phenotype test in clinical practice.

At the same time, manufacturing of more effective DNA isolation devices, the lower cost of throughput sequencing, suitable databases, and better trained personnel would pave way to the clinical application of NGS technology (Smith et al., 2020).



Each preparatory step of DNA library formation leads to the end repair, dA tailing, the adaptor ligation, cleaning up, size selection and the library amplification.
https://www.enzolifesciences.com/science-center/technotes/2020/august/what-is-next-generation-sequencing-ngs?/

Conclusion

1. Tuberculosis (TB) is a life-threatening infectious disease representing a continuous challenge to the global public health. It is caused by *Mycobacterium tuberculosis (M. tuberculosis)*. Timely diagnosis and effective treatment are critical in the TB controlling.

Several key factors need find their solution in nearest future to hold techniques allowing a rapid TB diagnosis:

2. Diagnosis of extrapulmonary TB, TB in children and compromised adult patients, people combining TB with AIDS, and TB in pregnant women.

Tests for drug response or drug-resistant TB.

3. Effective and affordable test materials and reagents.

4. Sensitive and specific methods for *M. tuberculosis* pdifferent from previously BCG vaccinated.

Therefore, more precise, rapid, tuned, selective, sensitive, cost-effective and versatile diagnostic methods and probes are needed for the recognition of positive cases and reliable detection of drug-resistant TB. Further development, validation, and modification of diagnostic methods should be closely associated clinical application.

Literature

1.Acharya, B., Acharya, A., Gautam, S., Ghimire, S. P., Mishra, G., Parajuli, N., & Sapkota, B. (2020). Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. *Molecular biology reports*, *47*, 4065-4075.

2.Babin, B. M., Fernandez-Cuervo, G., Sheng, J., Green, O., Ordonez, A. A., Turner, M. L., ... & Bogyo, M. (2021). Chemiluminescent protease probe for rapid, sensitive, and inexpensive detection of live mycobacterium tuberculosis. *ACS central science*, *7*(5), 803-814.

3.Brossier, F., Sougakoff, W., Aubry, A., Bernard, C., Cambau, E., Jarlier, V., ... & Veziris, N. (2017). Molecular detection methods of resistance to antituberculosis drugs in Mycobacterium tuberculosis. *Medecine et maladies infectieuses*, *47*(5), 340-348.

4.Campelo, T. A., Cardoso de Sousa, P. R., Nogueira, L. D. L., Frota, C. C., & Zuquim Antas, P. R. (2021). Revisiting the methods for detecting Mycobacterium tuberculosis: what has the new millennium brought thus far?. *Access Microbiology*, *3*(8), 000245.

5.Dong, B., He, Z., Li, Y., Xu, X., Wang, C. and Zeng, J. (2022). Improved Conventional and New Approaches in the Diagnosis of Tuberculosis. *Frontiers in Microbiology*, 13. doi:https://doi.org/10.3389/fmicb.2022.924410.

6.Gill, C. M., Dolan, L., Piggott, L. M., & McLaughlin, A. M. (2022). New developments in tuberculosis diagnosis and treatment. *Breathe*, *18*(1).

7.Huang, Y., Ai, L., Wang, X., Sun, Z., & Wang, F. (2022). Review and Updates on the Diagnosis of Tuberculosis. *Journal of clinical medicine*, *11*(19), 5826.

8.Ismoilovich, A. F. (2022). Tuberculosis Diagnostics with Modern Solutions (Literature Review). *Central Asian Journal of Medical and Natural Science*, *3*(3), 377-383.

9.Jacobo-Delgado, Y. M., Rodríguez-Carlos, A., Serrano, C. J., & Rivas-Santiago, B. (2023). Mycobacterium tuberculosis cell-wall and antimicrobial peptides: a mission impossible?. *Frontiers in immunology*, *14*, 1194923.

10.Kadura, S., King, N., Nakhoul, M., Zhu, H., Theron, G., Köser, C. U., & Farhat, M. (2020). Systematic review of mutations associated with resistance to the new and repurposed Mycobacterium tuberculosis drugs bedaquiline, clofazimine, linezolid, delamanid and pretomanid. *Journal of Antimicrobial Chemotherapy*, *75*(8), 2031-2043.

Literature (cond.)

11. MacGregor-Fairlie, M., Wilkinson, S., Besra, G. S., & Goldberg Oppenheimer, P. (2020). Tuberculosis diagnostics: overcoming ancient challenges with modern solutions. *Emerging Topics in Life Sciences*, *4*(4), 435–448. https://doi.org/10.1042/ETLS20200335
12. MacLean, E., Kohli, M., Weber, S. F., Suresh, A., Schumacher, S. G., Denkinger, C. M., & Pai, M. (2020). Advances in molecular diagnosis of tuberculosis. *Journal of clinical microbiology*, *58*(10), 10-1128.
13. Nyaruaba R., Mwaliko C., Kering K.K., Wei H. (2019). Droplet digital PCR applications in the tuberculosis world. Tuberculosis,117,85-92. https://doi.org/10.1016/j.tube.2019.07.001.
14. Truffot-Pernot, C., Véziris, N., & Sougakoff, W. (2006). Diagnostic moderne de la tuberculose [Modern diagnosis of tuberculosis]. *Presse medicale (Paris, France : 1983)*, *35*(11 Pt 2), 1739–1746. https://doi.org/10.1016/S0755-4982(06)74892-8
15. Xu, G., Liu, H., Jia, X., Wang, X., & Xu, P. (2021). Mechanisms and detection methods of Mycobacterium tuberculosis rifampicin resistance: The phenomenon of drug resistance is complex. *Tuberculosis*, *128*, 102083.
16. Zhang, X.; Zhao, Y.; Zeng, Y.; Zhang, C. Evolution of the Probe-Based Loop-Mediated Isothermal Amplification (LAMP) Assays in Pathogen Detection. *Diagnostics* **2023**, *13*, 1530. https://doi.org/10.3390/diagnostics13091530

Web resources

https://www.who.int/teams/global-tuberculosis-programme/data

https://wonder.cdc.gov/tb.html

https://hub.tbdiah.org/data-explorer

https://mycobacterium.biocyc.org/

https://www.cdc.gov/tb/statistics/default.htm

https://www.stoptb.org/resources/interactive-tb-data

https://www.ucl.ac.uk/global-health/research/tb-ipd-platform

https://www.gov.uk/government/collections/tuberculosis-and-other-mycobacterial-diseases-diagnosis-screening-management-and-data

https://www.enzolifesciences.com/science-center/technotes/2020/august/what-is-next-generation-sequencing-ngs?/

Best AI Facilities for research on tuberculosis

Nijiati, M., Ma, J., Hu, C., Tuersun, A., Abulizi, A., Kelimu, A., Zhang, D., Li, G., & Zou, X. (2022). Artificial Intelligence Assisting the Early Detection of Active Pulmonary Tuberculosis From Chest X-Rays: A Population-Based Study. *Frontiers in molecular biosciences*, *9*, 874475. https://doi.org/10.3389/fmolb.2022.874475

https://stoptb.org/assets/documents/news/Tackling%20TB%20with%20Practical%20Innovation_%20Infervision%E2%80%99s%20AI%20Approach.pdf

https://www.hsph.harvard.edu/india-center/artificial-intelligence-and-tuberculosis-test/

https://appliedradiology.com/articles/ai-based-system-comparable-to-radiologists-in-tuberculosis-detection

https://publichealth.uga.edu/artificial-intelligence-could-reduce-barriers-to-tb-care/

Acharya, V., Dhiman, G., Prakasha, K., Bahadur, P., Choraria, A., M, S., J, S., Prabhu, S., Chadaga, K., Viriyasitavat, W., & Kautish, S. (2022). AI-Assisted Tuberculosis Detection and Classification from Chest X-Rays Using a Deep Learning Normalization-Free Network Model. *Computational intelligence and neuroscience*, *2022*, 2399428. https://doi.org/10.1155/2022/2399428

Mobile applications

Global TB report