

The Next-Generation Bacterial Identification and Antibiotic Sensitivity Test (AST)

B-SMART™ is a fully automatable nucleic acid-encoded system to measure viability and drug resistance of bacteria. By combining the accuracy of functional drug susceptibility assays with the speed and sensitivity of molecular diagnostics, B-SMART™ can accurately and simultaneously report microbial identification and drug susceptibility in a matter of hours or days, depending upon the bacterial species.

How B-SMART™ works

Viruses rely on the protein and nucleic acid synthesis machinery of their host cell to manufacture their virus progeny because they don't carry these processes in their genetic makeup. The extent to which an infecting virus can synthesize and produce new viral components depends entirely on the metabolic activity of the cell it infects.

B-SMART™ uses bacteriophage (viruses that infect bacteria) to assess the metabolic capacity of bacteria in a sample exposed to one or more antimicrobial antibiotics. B-SMART™ phages are designed to create a new and novel nucleic acid, the Surrogate Marker Locus (SML). Because phage infection requires a metabolically active cell, synthesis of the SML does not happen in drug-susceptible bacteria exposed to an effective antibiotic. If the organism is drug-resistant, the antibiotic has no effect on bacterial metabolism and the SML is synthesized efficiently.

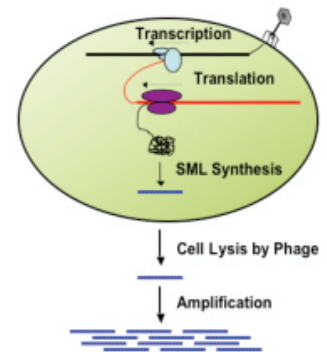
The essential component of B-SMART™ is the synthesis of SML inside bacteria that are alive. SML is the bridge between two general strategies for determining cellular viability or detecting drug resistance: functional assays (to assess the ability of bacteria to grow in the absence or presence of antibiotics and molecular assays) or molecular assays (to amplify and detect pathogen-specific nucleic acid sequences or resistance-conferring mutations).

B-SMART™ can be adapted to determine antibiotic susceptibility of any microorganism infected by a species-specific bacteriophage. B-SMART™ is especially useful for bacterial pathogens that are difficult to culture, or for which the gene(s) that encode drug resistance are not known, or for which drug resistance is controlled by multiple genes. The SML-generation module used in B-SMART™ functions in diverse bacterial species, including *Mycobacteria* and *Escherichia coli*, and even functions in eukaryotic cells infected by recombinant viruses containing the genetic material to produce SML.

Market Opportunity for Infectious Disease Nucleic Acid Testing

Nucleic acid-based diagnostics are well established in laboratory medicine, and many are used to screen blood supplies for a variety of infectious diseases. In the U.S., the market for hospital and laboratory-based infectious disease nucleic acid tests is a \$1.1 billion industry. This market can quickly expand to include:

- Antibiotic susceptibility testing
- Replacement/augmentation of sputum smear or culture for diagnosis of TB
- Potable water screens
- Food pathogen screens (dairy products, meat)



SML-Phage Technology. Upon infection of a viable, metabolically active bacterium, a new DNA sequence is created (the SML), which can be detected by PCR.

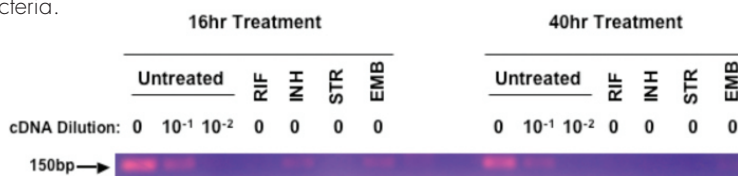
Compared to existing TB diagnostics, B-SMART™ TB is differentiated by the following attributes:

System	Type	Detection	Automation	Drug(s)	Time to Detection (TTD)
FastPlaque	Functional	Plaque Formation	No	Rifampin	2-3 days
Luciferase Reporter Assay	Functional	Light Production	Yes	Any	Culture 1-2 wk + 2 days*
Genotype MTBDRplus	Nucleic Acid	Line Probe Assay	Automatable	Rifampin + Isoniazid	1 day
GeneXpert – TB	Nucleic Acid	Fluorescent Probes(FP)	Yes	Rifampin	1 day
BACTEC	Functional	Fluorescent Metabolites	Yes	Any	Culture 1-2 wk
B-SMART™	Functional + Nucleic Acid	Lateral flow, FP, and others	Yes	Any	Hours to <2 days

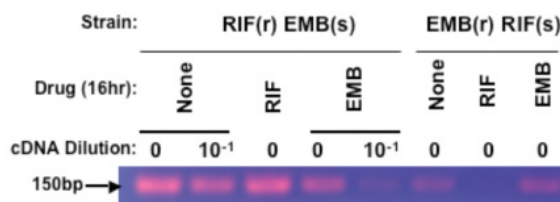
*TTD after initial culture period (1-2 weeks) to obtain enough bacteria to detect light by a (Luciferase Reporter Assay).

Proof of Principle

Determination of Drug susceptibility. Ability of SML-phage to function as an AST to determine susceptibility of drug-sensitive *Mycobacterium tuberculosis* to the front-line antitubercular drugs used in TB therapy is shown below. *Mycobacterium tuberculosis* bacteria were treated for 16 hours or 40 hours with Rifampin (RIF), Isoniazid (INH), Streptomycin (STR), or Ethambutol (EMB). Then they were infected with the SML-phage for 4 hours. At both 16 and 40 hours of drug exposure, there is a clear difference in SML generation between the untreated and drug-treated bacteria.



Determination of Drug Resistance. Ability of SML-phage to detect drug-resistant *Mycobacterium tuberculosis* is demonstrated below. One clinical strain of *Mycobacterium tuberculosis* was resistant to RIF and the other is resistant to EMB. The bacteria were left untreated or were treated with RIF or EMB for 16 hours and then infected with SML-phage for 4 hours. For the RIF-resistant strain, treatment with EMB resulted in ~10 fold reduction in SML generation compared to untreated bacteria, and no reduction in SML occurred after treatment with RIF (RIF-treated bacteria were not killed). For the EMB resistant strain, treatment with RIF resulted in no detectable SML generation, which reflects the activity of RIF against this strain, while bacteria treated with EMB showed the same SML generation as the untreated bacteria. RIF and EMB resistance is easily detected by SML-phage after only 16 hours of drug treatment.



Intellectual Property

B-SMART™ is protected by US and international patent filings and trademarks.

For information on alliance opportunities, contact:

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