

BEFORE THE HON'BLE NATIONAL GREEN TRIBUNAL,
Principal Bench, New Delhi

In

Original Application No. 426/2018

In the Matter of: -

Mohammed Nayeem Pasha & Ors.

Applicant(s)

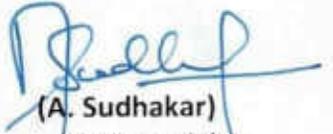
Vs.

The State of Telangana & Ors.

Respondent(s)

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(A. Sudhakar)
Scientist 'E'

Central Pollution Control Board,
Parivesh Bhawan, East Arjun Nagar,
Delhi-110032

Place: Delhi

Date: 18th June, 2020

BEFORE THE NATIONAL GREEN TRIBUNAL
PRINCIPAL BENCH, NEW DELHI

O.A. No.426 OF 2018

IN THE MATTER OF:

MOHAMMED NAYEEM PASHA & ORS.Applicant (S)

VERSUS

THE STATE OF TELANGANA & ORS.....RESPONDENT (S)

Proposal for Validation of Right Biotic System
For Carrying out Quick Hygienic Survey of Rivers



Central Pollution Control Board

(Ministry of Environment, Forest & Climate Change, Govt. of India)

Parivesh Bhawan, East Arjun Nagar,

Delhi – 110032

Proposal for Validation of Right Biotic System in compliance to Hon'ble National Green Tribunal, PB, New Delhi Order dated 29.11.2019 (uploading date 06.12.2019) in Original Application No. 426/2018 in the matter of Mohammed Nayeem Pasha & Anr Versus State of Telangana & Ors.

1. Background

Central Pollution Control Board (CPCB) and Telangana State Pollution Control Board (TSPCB) in association with BITS-Pilani, Hyderabad Campus, Telangana carried out a study entitled "Quick Hygienic Survey of River Musi" utilising indigenously developed automated platform called "Right Biotic method" during May to July 2019 in compliance to Hon'ble NGT Order dated 05.04.2019 passed in O A No 426/2018 in the matter of Mohammed Nayeem Pasha & Anr Versus the State of Telangana & Ors.

Subsequently, CPCB filed findings of the report entitled 'Quick Hygienic Survey of River Musi and Krishna' before Hon'ble NGT. As CPCB do not have expertise for validation of quick hygienic survey of river using right biotic system and thus CPCB has suggested Department of Science and Technology (DST) or concerned Ministry in Central Government may validate right biotic system developed by BITS-Pilani, Hyderabad Campus for carrying out quick hygienic survey of rivers for its adoption in the Country.

Hon'ble National Green Tribunal (NGT) considered the report and passed Order dated 29.11.2019 (uploading date 06.12.2019) in O A No 426/2018 in the matter of Mohammed Nayeem Pasha & Anr Versus the State of Telangana & Ors. Relevant portion of the order pertaining to CPCB is reproduced below:-

"CPCB may itself validate the Right Biotic System for Quick Hygienic Survey of Rivers by seeking opinion of experts on the subject rather than referring it to the Department of Science and Technology, Government of India for its adoption in the country".

A copy of the Hon'ble NGT Order dated 29.11.2019 (uploading date 06.12.2019) passed in O A No 426/2018 in the matter of Mohammed Nayeem Pasha & Anr Versus the State of Telangana & Ors is enclosed as **Annexure-I**.

2. Actions Initiated by CPCB

In pursuance to Hon'ble NGT Order dated 29.11.2019 (uploading date 06.12.2019) actions taken by CPCB in afore-said matter are detailed in subsequent paras.

Letters written to Experts or Expert Institutions seeking Comments/ Suggestions on the Right Biotic System:

In pursuance to Hon'ble NGT Order dated 29.11.2019 (uploading date 06.12.2019), CPCB forwarded a report along with the information brochure of Right Biotic System to the experts or expert institutions or organizations having expertise in the field of microbiology in the Country seeking comments or suggestions for validation of the right biotic system developed by BITS- PILANI, Hyderabad campus for identification and estimation of AMR pattern of pathogenic bacteria in riverine system, vide CPCB letter dated 27.12.2019. A copy of the CPCB letter dated 27.12.2019 written to the experts or expert institutions or organizations is enclosed as **Annexure-II**.

Letters written to selected SPCBs/PCCs for Comments/ Suggestions Right Biotic System:

CPCB also forwarded a copy of the report along with the information brochure of Right Biotic system vide letter dated 08.01.2020 seeking comments or suggestions of selected SPCBs/PCCs (such as Andhra Pradesh, Assam, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Puducherry, Tamil Nadu, Telangana, Tripura, Uttar Pradesh and West-Bengal) for initiating the procedure of validation of the Right Biotic System for carrying out Quick Hygienic Survey developed by BITS- PILANI, Hyderabad Campus. A copy of the letter dated 08.01.2020 is enclosed as **Annexure-III**.

Responses received from Experts or Expert Institutions and SPCBs/PCCs on the Right Biotic System:

In response to CPCB letters dated 27.12.2019 and 08.01.2020, response as received from the Expert Institutions and few SPCBs/PCCs are detailed in the table given below:-

S. No.	Name of the Expert/Expert Institution/ Organisation	Comments
1	The Joint Secretary, Ministry of Jal Shakti, DoWR,RD & GR, Shram Shakti Bhawan, Rafi Marg, New Delhi - 110001	Considering the fact that there has been an increasing trend in the population of Antibiotic Resistant microbes in the water bodies posing a threat to human health, biodiversity and economy of the country, it is felt that there is an urgent need to come up with a pronounced strategy and a right technology that can be used in analyzing the root cause of the problem and to achieve success in addressing this particular issue. On this note, <i>Right Biotic System mentioned above may be considered for using in quick hygienic survey in the country depending on its accuracy, cost effective and user friendly.</i>
2	The Director, National Environmental Engineering Research Institute (NEERI), Nagpur-440020, Maharashtra	As per copy of guidelines /SOP procedure entitled Monitoring of river Hygiene following clarification are sought prior to approval of such report. ➤ Right Biotic System has been developed for Clinical and Urine samples and provided solution for finding AMR in fastest mode and low cost in clinical samples/ body fluids. ➤ Difference in report submitted by BITS and CPCB and one submitted by TSPCB with respect to no presence of bacteria in three sites out of nine. (page 28, Annexure E)

		<ul style="list-style-type: none"> ➤ Right Biotic System claims to work on principle of visible Light scattering by Bacteria. In this regard, how it discriminates other microbes present in sample may be clarified for special media used for detecting pathogens only, scattering of visible light only by pathogens, differentiating bacterial pathogens and other bacteria, assessing diversity with respect to antibiotic resistance profile, considering of COD in rivers water samples during detection, culturable and non-culturable microbial communities which play role in terms of Horizontal gene Transfer (HGT) of antibiotic gene, considering of prevalence of HGT event in environmental samples, developing significant Confidence level for dynamic system like rivers, Environmental bacteria which are VBNCs which develops virulence with time and behave differently than clinical pathogen in terms of their viability and statistical analysis carried out for desired confidence level of monitoring. ➤ In view of the above it still remains uncertain to use Right Biotic platform for Quick Hygienic Survey of ambient water resources
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3	<p>The Joint Secretary, Department of Biotechnology (DBT), 6th-8th Floor, Block 2, CGO Complex, Lodhi Road, New Delhi - 110 003.</p>	<p>In order to follow the order of Hon'ble NGT, CPCB may itself seek the opinion of experts on the subject of Validation of Right Biotic for Quick Hygienic Survey of rivers.</p>
4	<p>Prof. Anand K. Kondapi, Senior Professor, Department of Biotechnology & Bioinformatics University of Hyderabad, CR Rao Road P O, Gachibowli, Hyderabad, Telangana 500046</p>	<p>Following observation, which may need to be addressed by the concerned authorities.-</p> <ul style="list-style-type: none"> ➤ As right biotic system was developed for analysis of urine, pus/ swab cultures for diagnosis of UTI and other body fluids on turbidometric principle. I assume appropriate validation has been carried out by Ministry of Health in consideration on any nonspecific leachates from filters/ sample adhered to filters interfere in optical measurements. I assume that Health Ministry has done calibration of Right Biotic System with other Gold Standard approved for Urinary Track Infection (UTI) evaluation. ➤ Regarding use of Right Biotic System for monitoring river and polluted water, following parameters required to be considered. <ul style="list-style-type: none"> ○ Whether a control from the respective river water

		<p>filtered through 0.22 micron filter is used as a background to rule out any specific species that absorbs light at 595/600 nm where turbid metric measurements are carried out.</p> <ul style="list-style-type: none">○ River water / Waste water have large proportion of suspended matter, inorganic and organic matter with optical activity as absorbers or quenchers, these would absorb to the filters, when filters are exposed to the medium for growth, these may leach out and interfere. So one needs to have controls for each site of collection from 0.22 micron, 0.45 micron filtered water to measure these interferences.○ It would be better to calibrate Right Biotic System for different water systems with another ISO approved culture method and BOD to correlate performance and limitation and corrections if any required.○ The sample should be collected from a given site at least for five to ten different time intervals before confirming the
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		contamination status of the site, double blind random sampling measurement would remove bias.
5	The Member Secretary, SPCBs /PCC (Himachal Pradesh, Karnataka, Odisha and UP)	<p>Himachal Pradesh, Karnataka, Odisha and UP State Pollution Control Boards have responded and provided comments as detailed below:-</p> <p>Himachal Pradesh SPCB:-</p> <ul style="list-style-type: none"> ➤ Process is lacking in validation of Right Biotic Machine. ➤ Procedure of testing of pathogen needs to be as per some standard procedure i.e. BIS/WHO/USEPA or procedure developed & validated by some govt. recognized agencies dealing with pathogens. ➤ The guidelines should include the methods to inactivate all types of pathogens/bacteria in source water generated from pharma/hospital /labs etc including methods to break all types of antibiotics during the treatment of waste water in ETP/STPs. ➤ The instrument mentioned in the guidelines and the procedure once got approved by some validating agency will be a very useful tool for SPCB's for rapid assessment of health of aquatic system w.r.t bacteriological population on 4 hours.

Karnataka SPCB:-

- Right Biotic Assay can be used for finger Print analysis of microbes in the field of water bodies
- Right biotic assay cannot be compared with the Laboratory test method i.e. Multiple Tube Dilution technique method.
- Clarity is required for expression of Right Biotic Assay result since the method reduces the time of analysis as compared to the present 92 hrs as in MPN, this Right biotic Assay can be used as a field kit to assess the microbial load in Lakes/Rivers and Sewage treated waters.
- At present the standard for lakes/Rivers and Sewage treated waters is available, hence it is required to prescribe standards for this right Biotic Assay also.
- Clarification whether this Right Biotic Assay can be used for surface water and STP to assess the pathogenic microbial load.

PCB, Odisha:-

- Method Very less time taking, silent on unit of quantification of bacterial population.

Uttar Pradesh PCB:-

- As proposed, the monitoring of

		<p>river hygiene through the Right Biotic System is an efficient, rapid and cost effective technique to check bacteriological quality (Bacterial load, tier identification and AMR pattern) of water.</p> <ul style="list-style-type: none"> ➤ Although this Guidelines for monitoring of river hygiene based on RBS is highly impressive and informative but needs some clarity in terms of presentation or is highly impressive and informative, but needs some clarity in terms of presentation or demonstration about its functioning (procedure, precaution and its disposal), result validation and applicability in comparison with other traditional methods. ➤ It is suggested that appropriateness of this method with regards to States having large number of pharmaceutical industries to be checked. ➤ It is concluded that this real time and quick technique can prove useful for monitoring of river hygiene through rapid analysis of antibiotic resistance pathogenic bacterial load in water, which can minimise effect of water pollution on human health also.
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3. Constraints of CPCB

As a follow-up measure, experts from different organization were requested to provide comment or feedback and suggestions with regard to validation of Right Biotic System, but none of the organization has clearly dictated the method or procedure to be followed for validation of the Right Biotic System for carrying out quick hygienic survey of the river bodies and for its inclusion in routine / field analysis.

4. Proposed proposal for validation of the quick hygienic survey of river using Right Biotic System

As CPCB do not have expertise for validation of quick hygienic survey of river using Right Biotic System, requisite infrastructure and well equipped molecular labs, trained manpower and statistician to perform all the test and then extrapolate the results to be utilized for validation of new system. The Observations made by NEERI, Hyderabad University and some of the State Boards clearly indicates limitation of right biotic system and the studies conducted till date. Therefore, validation work need to be performed by the facility having all the requisite set up for completing the experiments as well as incorporate views of experts for final assessment. Therefore, for ensuring compliance to Hon'ble NGT Order dated 29.11.2019 (uploading date 06.12.2019), a proposal for validation of Right Biotic System for carrying out Quick Hygienic Survey of Rivers, as detailed in subsequent paras (Detailed proposal is enclosed as **Annexure-IV**) .

- a) For validation of Right Biotic System, both the methods viz., (i) Right Biotic System method and (ii) Most Probable Number (MPN) will be used
- b) Procurement of right biotic system and joint sampling and assessment through CSIR laboratories having requisite infrastructure as well as expertise in addition to CPCB and Bits-Pilani, Hyderabad Campus.
- c) Modalities for procurement of right biotic system as well as parameters to be analysed and sampling protocols will be decided by CPCB in consultation with Bits-Pilani, Hyderabad Campus as well as CSIR Laboratories participating in the assessment.
- d) Declaration of the assessment results and validation of the right biotic system developed by Bits-Pilani, Hyderabad Campus through Expert

Committee members representing DST, MoH & FW, MoJS, MoE & CC, IIT (Delhi), CSIR Laboratory, ICMR and BIS.

e) *Time required for validation of right biotic system is about one year.*

5. Prayer

That as the Government of India (GoI) imposed a nationwide lockdown since 25 March 2020 as a preventive measure against the COVID-19 pandemic and further stipulating lockdown measures in the Country.

That Considering quantum of work involved for having consultation meetings with all the concerned organizations for planning the activity, procurement of right biotic system by all the participating organizations, joint sampling, analysis and interpretation of data as well as apprising proposed Committee for ensuring compliance to Hon'ble NGT directions dated 29.11.2019 (uploading date 06.12.2019), considering the present circumstance, the proposal submitted for validation of right biotic system within one year time from the date of lifting of lockdown restrictions please be granted to CPCB for submitting its comprehensive report before this Hon'ble NGT for consideration and to pass such other and further orders as this Hon'ble NGT may deem fit and proper in the facts and circumstances of the case.

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Item Nos. 03

Court No. 1

**BEFORE THE NATIONAL GREEN TRIBUNAL
PRINCIPAL BENCH, NEW DELHI**

Original Application No. 426/2018
(M.A. No. 986/2018)

(With Report dated 18.11.2019)

Mohammed Nayeem Pasha & Anr.

Applicant(s)

Versus

The State of Telangana & Ors.

Respondent(s)

Date of hearing: 29.11.2019

Date of uploading of order: 06.12.2019

CORAM: HON'BLE MR. JUSTICE ADARSH KUMAR GOEL, CHAIRPERSON
HON'BLE MR. JUSTICE S.P WANGDI, JUDICIAL MEMBER
HON'BLE MR. JUSTICE K. RAMAKRISHNAN, JUDICIAL MEMBER
HON'BLE DR. NAGIN NANDA, EXPERT MEMBER
HON'BLE MR. SAIBAL DASGUPTA, EXPERT MEMBER

ORDER

1. The issue raised in this application relates to remedial action against pollution of river *Musi* at Hyderabad (Telangana) which is reported to be contaminated due to industrial and domestic sewage discharged into the river or into the drains connected thereto. The industries in the catchment areas are bulk drug and pharmaceutical units. Further allegation is that the flood plain zones are encroached and there is also dumping of Construction and Demolition (C&D) waste and solid waste. Water quality is not even fit for irrigation as per water quality criteria of CPCB as per reports of samples of water.
2. The proceedings were initiated by way of *O.A. No. 426/2018* filed on 05.07.2018. Notice was issued on 06.07.2018. The Tribunal noted that as per water quality assessment report of the SPCB during 2014-

18, water was not fit even for irrigation (Class-E). The matter was then considered on 05.04.2019 in the light of pleadings on record. The Tribunal noted the stand of the Municipal Administration/Urban Development Department of the State that steps taken included construction of Sewage Treatment Plants (STPs) and laying of sewer lines but the existing capacity of the STPs was inadequate. There was plan to set up more STPs. Detailed Project Reports (DPRs) were being prepared for the purpose which will also cover control of pollution of 23 lakes in the area. The Telangana High Court has also dealt with the matter. The observations of this Tribunal are:

"With regard to sewage treatment and disposal which is the main cause of pollution of river Musi it is to be noted that total sewerage generated is about 1400 mld which is being discharged in the catchment area of Musi river from either of the banks through Nalas. Already 592 mld capacity sewerage treatment plant have been built along the river Musi. DPRs are also prepared at a cost of Rs. 1200 crores for creation of additional STPs at 10 locations, to treat total sewage so as to prevent pollution into river Musi. Since there is no regular sewer network system, the board is said to have undertaken a survey with a technical expertise and prepared the DPRs for sewerage system of the peripheral areas.

According to MAUD, HMWSSB has appointed Shah Technical consultant to prepare a detailed Project Report(DPR) for the master sewage plan, duly reviewing the available DPRs for integration covering the entire GHMC area up to ORR plus 4 IT hubs. The consultants are preparing DPRs for 27 STPs at 23 lakes in the GHMC area for the combined capacity of 450 mld under Phase-I, along the Musi river and further upgradation of the existing STPs will be taken up in Phase-III. The Phase-I STPs are planned to be taken up in 4 month's time, depending upon the funds availability and Phase-II in one year time. The upgradation of STPs from the secondary level to the tertiary level 3 in one year time including the policy on recycling of water for the usage for the purpose of non-drinking, construction activity, gardening, industrial etc. Due to financial constraint, the possibility of private investments through the transaction advisor under PPP mode is being explored. The HMWSSB is planning to implement the comprehensive sewage master plan within one and half year time.

In view of the aforesaid facts and circumstances, we order that as has been made clear in the order dated 19.12.2018 passed in OA no. 673/2018 BOD will not be the sole criteria to determine

whether a particular river stretch is a polluted one. Other parameters including Faecal Coliform (FC) bacteria will also be the criteria for classifying a stretch as polluted or otherwise. CPCB may devise within two weeks a mechanism for classification wherein two criteria pollutants that is BOD and FC shall henceforth be the basis of classification in priority classes.

Therefore, we direct CPCB and Telangana Pollution Control Board to carry out a quick hygienic survey of the River by engaging Professor Suman Kapur, Dean, International Programmes and Collaborations and Senior Professor, Department of Biological Sciences, BITS- Pilani, Hyderabad Campus, Jawahar Nagar, Shameer Pet, Hyderabad-500078, skapura@hyderabad.bits-pilani.ac.in and submit a report for river Musi and any other clean river in the state falling in the category of 'A' and or 'B.' For this survey we request Professor Suman Kapoor to execute the survey at a cost 9.5 lakhs which will be paid by CPCB out of its environment compensation fund. The scientist of CPCB and TSPCB will be associated during the survey and field testing of pathogenic bacteria."

3. Accordingly, CPCB filed status report dated 28.06.2019 observing as follows:

"3.0 Observations:

- i. There are 24 STPs in and around Musi River. Out of 24 STPs, two STPs namely Lingamaiah Kunta and Goppanpally STPs having total treatment capacity of 8 MLD are under construction stage.
- ii. Out of 22 STPs monitored, two STPs namely M/s Krishnakanth Park and M/s Pragathi nagar STPs having total treatment capacity of 3 MLD were not in operation. No officials and operators were present in the site during inspection.
- iii. Out of 20 Operational STPs, seventeen STPs are operational without consent. However, TSPCB has issued notice to all seventeen STPs for obtaining CFO.
- iv. Three STPs namely M/s Amberpet, M/s Nalla Cheruvu and M/s Nagole are operating with valid consent.
- v. Except Vikarabad STP, KIMS STP & Khairatabad STP, all nineteen STPs are operated and maintained by Hyderabad Metropolitan Water Supply and Sewerage Board (HMWSSB).
- vi. KIMS and Khairatabad STPs are operated and maintained by Hyderabad Metropolitan Development Authority (HMDA).
- vii. Vikarabad STP is operated and maintained by Vikarabad Municipality.
- viii. Only 49% of the sewage generated from the city is being treated with the existing STPs. 51% of untreated sewage is discharged to River Musi directly or through drains/nallah.
- ix. Out of 22 STPs, 13 STPs are not operational to full designed capacities.

- x. Out of 20 Operational STPs, following five STPs are not complying with the discharge norms.
- ✓ M/s Rangadhamuni STP
 - ✓ M/s Nalla Cheruvu STP
 - ✓ M/s Amberpet STP
 - ✓ M/s Langer house STP
 - ✓ M/s Patel Cheruvu STP
- xi. Most of the STPs are not having flow measuring devices and no proper records are maintained, so actual flow for treatment could be assessed.
- xii. The treated sewage from all STPs are discharged into River Musi directly or through nallah that finally joins river Musi
- xiii. The Sludge generated from the STPs are sold to farmer utilize as manure in free of cost except M/s Amberpet and M/s Nallacheruvu STP.
- xiv. M/s Amberpet and M/s Nallacheruvu STPs generates revenue of Rs. 11.40 Lakhs & 3 Lakhs respectively.
- xv. No STPs has installed OCEMS in compliance to NGT directions in the matter of O.A 593/2017 Paryavaran Suraksha Samiti Vs UOI
- xvi. There is a proposal from four STPs to enhance the existing treatment capacity. The details are as follows:

S. No.	Name of STP	Existing	treatment	Additional capacity*	proposed
1	M/s Amberpet STP	339 MLD		142 MLD	
2	M/s Nagole STP	172 MLD		140 MLD	
3	M/s Nalla Cheruvu	30 MLD		88 MLD	
4	M/s Durgam Cheruvu	9 MLD		12 MLD	

as Informed the proposal is still under consideration.

4. The suggestions of the CPCB in the said report are as follows:

"4.0 Suggestions:

- 1) HMWSSB & HMDA shall enhance the existing treatment capacity to treat the 100 % of the sewage generated in the city.
- 2) HMWSSB & HMDA shall prepare an time bound action plan for collection and treatment of 100% sewage generated in the city
- 3) Seventeen STPs shall obtain consent for operation from Telangana State pollution control hoard (TNPCCB) immediately without any delay.
- 4) All STPs should ensure 100% treatment of designed capacity and operated scientifically to ensure compliance to discharge norms.
- 5) All STPs should install flow measuring devices at inlet and outlet and maintain the log records.

- 6) All STPs should install OCEMS in compliance to NGT order in the matter O.A. No. 593 of 2017 *Paryavaran Suraksha Samiti Vs. Uol*.
- 7) HMWSSB shall prepare action plan for utilization of treated sewage and utilize the treated sewage to the maximum extent instead of discharging into River Musi.
- 8) Most of the STPs are operated by untrained officials. HMWSSB shall ensure capacity building programmes to all STP operators on scientific operation and maintenance of STPs.
- 9) *Telangana State pollution control board shall ensure the compliance of all STPs to operate with valid consents, installation of OCEMS and utilisation of treated sewage.**

5. Apart from the above, CPCB has also filed a consolidated report dated 18.11.2019 in O.A. No. 673/2018 wherein reference has also been made to status of Musi river subject matter of O.A. No. 426/2018 and O.A. No. 829/2019. On the subject of quick hygienic survey, it is stated as follows:

****C3. Assessment of Quick Hygienic Survey of River Musi and River Krishna using Right Biotic Method***

(i) Major findings of the study carried out by BITS —Pilani, Hyderabad campus

*Final report entitled "Quick Hygienic Survey of River Musi" carried out by BITS Pilani, Hyderabad Campus has been received by CPCB vide letter dated 04.09.2019 from Prof. Suman Kapoor, BITS Pilani. Comments on the report were communicated to Prof S.Kumar vide CPCB letter dated 17.10.2019 for incorporation. A copy of the quick hygienic survey of river Musi as received by CPCB from Prof. Suman Kapur, BITS Pilani, Hyderabad Campus vide letter dated 24.10.2019 is enclosed at **Annexure-XIX**. Salient features of the quick hygienic survey of river Musi and river Krishna are detailed below:*

Five out of the nine sites namely, Nagole, Pratapsingaram, Pillaipalli, Rudravelly, and Kasaniguda had higher diversity of pathogenic bacteria and overall load all through the study period (page 21 of the report under serial no. 6).

Five pathogenic strains (such as E. coli, Klebsiella spp., Pseudomonas spp., Staphylococcus spp., and Enterococcus spp.) were isolated in six sampling locations, and remaining 3 did not grow any bacteria which includes sampling location on river Musi at Wadapally (before confluence),

sampling location on river Krishna at Wadapally (before confluence), Musi after joining Krishna at Wadapally (after confluence). (Details given at Page No. 103 and Table 40 of the assessment report).

Profile of isolated pathogenic strains predominantly include gram negative organisms such as E. Coll, Klebsiella spp. and Pseudomonas spp. as well as gram positive organisms such as Enterococcus spp. and Staphylococcus spp.

A panel of antibiotics (total 14) including major class of antibiotics relevant to water bodies and important representative from each class was tested for determining Anti-Microbial Resistance (AMR) pattern. Out of the 14 antibiotics tested, Multi Drug Resistance (resistance to 2 or more antibiotics) was observed at the rate of 29% at Nagole, in Klebsiella spp. (4/14 equivalent to 28.57%) to 93% at Pillaipalli in Pseudomonas spp. (13/14 equivalent to 93%) against frontline antibiotics. (Details given at Annexure 13, Page No. 64 to 65 of the assessment report).

Several strains of Staphylococcus spp. showed very high resistance ranging from 79% to 86% indicating an alarming pan-drug resistance like situation. (Details given at Annexure -13, Page No.67 of the assessment report).

Right Biotic assay can be used for rapid and quick hygienic survey of river and other surface and ground water surveys for assessing presence of pathogens resistance to any given panel of antibiotics for assessing the health hazard posed by ABM with accuracy of >70% (Details given at Page No. 26 of the assessment report).

Out of 90 bacterial strains isolated from 54 water samples all fall in multi drug resistance (MDR) and some even fall in Extensively Drug Resistance (XDR) category (as mentioned in Figure 19 of the assessment report).

(ii) Major Findings of General Parameters carried out by TSPCB during the study period

A Joint Team comprising RD, CPCB, Bengaluru, TSPCB have participated along with BITS-Pilani, Hyderabad Campus team and carried out sampling at 9 locations namely Gandipet, Nagole, Pratapsingaram, Pillaipalli, Rudravelly, Kasaniguda, Musi at Wadapally (before Confluence), Krishna at Wadapally (before Confluence), Musi+Krishna at Wadapally (after confluence) on 6 different visit dates during May to July 2019. Collected samples were stored at 40 C and sent to TSPCB Central Laboratory for investigation of general parameters. TSPCB submitted the report vide letter dated 28.08.2019 on the water quality data of river Musi at all 9 selected locations. A copy of the detailed report on the general parameters

carried out by TSPCB is annexed as Annexure-XX. Main observations of TSPCB on the water quality of river Musi and River Krishna during the study period are as follows:-

The compiled average BOD value throughout the stretch from Gandipet (Osmansagar) i.e., upstream of river Musi to river Krishna after confluence with river Musi at Wadapally during the period 9th May 2019 to 23rd July 2019 is ranging between 3 to 44 mg/L.

The BOD of 3mg/L which falls under Class -B i.e., outdoor bathing quality was recorded at upstream of river Musi at Gandipet (Osmansagar), river Krishna before confluence and after confluence with river Musi at Wadapally.

River Krishna water quality before confluence with river Musi and after confluence with river Musi at Wadapally falls in Class -C i.e., drinking water source after conventional treatment and disinfection.

River Musi at Rudravalli at Kasaniguda and before confluence with river Krishna at Wadapally falls under Class -D (i.e., Propagation of Wild life and Fisheries) with BOD at 22, 34 and 8 mg/L respectively.

River Musi at Nagole, Pratapasingaram, Pillaipally falls under Class - E, the BOD observed are 44, 27, 25 mg/L respectively.

Musi river at Pratapasingaram has highest Total Coliform of 1250 MPN/100ml followed by Pillaipalli with 1133 MPN/100ml.

It is observed that the Total Coliform count in river Musi stretch from Nagole to Kasaniguda (Monitoring locations at Sl. No. 2,3,4 &5) is more when compared to other part of river Musi stretch which could be due to discharge of treated and untreated sewage into river Musi.

(iii) One-day hands-on training on Quick Hygienic Survey of River Body using Right Biotic method

A one-day hands-on training on Quick Hygienic Survey of River Body using Right Biotic System for the officials of SPCBs/PCCs in association with BITS-Pilani, Hyderabad campus was organized on 26.08.2019 at BITS-Pilani Hyderabad Campus. 8 officials from different SPCBs attended the training programme at BITS-Pilani, Hyderabad campus.

(iv) Analysis of extracted water samples for pharmaceuticals

The extracted and vacuum dried water samples of river Musi and river Krishna (54 nos) which were collected

during quick hygienic survey of river Musi and river Krishna by BITS -Pilani were also analyzed for 21 different pharmaceuticals in NRTOL laboratory in CPCB. The analysis results reveal that the concentration of 21 pharmaceuticals in all the 54 samples is observed as below detection limit (BDL). The analysis results of 21 pharmaceuticals in all the 54 samples is enclosed at Annexure-XXI.

(v) Views of CPCB on Quick Hygienic Survey of River using Right Biotic System

Broadly, the most prevalent methods used to detect E.-Coli or Fecal Coliforms are based on Membrane Filter technique or Multiple Tube Fermentation techniques (or Most Probable Number method). However, both the methods are having its own advantages and disadvantages. Other than available methods for detection of pathogenic bacteria, there are rapid methods available for identification of pathogenic bacteria including fecal coli and fecal streptococci which include (a) Radiometric methods, (b) Glutamate decarboxylase, (c) Electrochemical, (d) Impedance, (e) Gas Chromatographic assay, (f) Colorimetric, and (g) Potentiometric. These methods generally take time ranging from 3 hrs to 15 hours with varying sensitivity (0.1 to $> 10^7$ cells /mL), but are very costly, require lab setup & expertise for analysis of the assay and are lab based only.

Quick Hygienic Survey of river Musi using Right Biotic method involves Optical Detection System which converts the optical signal into electrical signal. Optical density is used as a measure of the concentration of bacteria in a suspension. As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacteria or other material is present. Right Biotic Platform neither work on the principle of Multiple Tube Fermentation (MPN method) nor Membrane Filtration Technique (MF). Right Biotic method utilises disposable membrane filters of 0.45µm for filtering the water sample in initial steps of sample preparation before incubation at the ambient temperature and thus associated with quick measurement utilizing Optical Density of the pathogenic bacteria in short duration (4-5 hours).

Quick Hygienic study of river Musi carried out during 9th May, 2019 - 23rd July, 2019 (over 10-week period) reveals presence of pathogenic (diseases causing) bacteria apart from pathogenic indicator bacteria i.e. fecal coli & fecal Streptococci. Majority of the selected sampling locations i.e. 5 out of 9 selected sampling locations namely Nagole, Pratapsingaram, Pillaipalli, Rudravelly and Kasaniguda showed higher diversity of pathogenic bacteria which were multi drug resistant (resistance to atleast one agent in three or more antimicrobial class of antibiotics) showing 4 out of 14 antibiotics tested

Right Biotic Method developed by BITS-Pilani, Hyderabad extended for carrying out quick hygienic survey of river require further validation jointly in association with at least 2 independent agencies having similar facilities for comparison with the analysis results of conventional methods being followed for assessment of Fecal Coll and Fecal Streptococci with different water samples with species both sensitive (non-acclimatizing) and non-sensitive (acclimatizing) for estimating Fecal Coli and Fecal Streptococci. Applicability of Right Biotic method in turbid waters also needs to be assessed as suspended solids matter present in water sample may clog the pores and thereby inhibits free passage of specific volume of water sample.

Right Biotic method also be compared in terms of sensitivity and specificity by utilizing commonly available conventional methods such as Multiple Tube Fermentation (MPN- most trusted method) and other approved rapid and automated methods.

Quick Hygienic Survey of River using Right Biotic System developed by BITS-Pilani, Hyderabad campus enable to analyse microbial profile of a water body just in 4 to 6 hours. However, for wide use and applicability of Right Biotic method for quick assessment of hygienic survey of rivers, it would be advisable if it is validated and or approved by Department of Science and Technology, Government of India, for its adoption in the Country."

6. The report of CPCB dated 28.6.2019 clearly indicates that only 49% of the sewage generated in the city is treated and remaining 51% of untreated sewage is discharged directly into the River Musi. The STPs are not granted with the Consents under the Water Act and the 5 STPs are found to be not complying with the discharged norms out of 20 operational STPs. The STPs need up-gradation as applicable to meet with the standards with reference to faecal coliform and the treated water will have to be utilized for the non-portable use.

The report of CPCB dated 18.11.2019 describes about the Quick Hygienic Survey carried out by engaging Birla Institute of Technology and Science (BITS). The purpose of the survey was to

design quick method of detection of pathogenic bacteria as the existing methods are time consuming and also involves, long transportation distances in many cases. The method developed by BITS which has been witnessed by Scientists of CPCB and Telangana SPCB, may now further workout appropriate mechanism to adopt the Method and widely practice to carry out such survey of Indian Rivers following the standardized protocol and its procedures which may remain inconsonance and matchable with the Internationally adopted Standard Methods.

7. We have heard the matter along with O.A. No. 673/2018. While general directions to the extent relevant for the above case will govern the present matter as river *Musi* is one of the 351 polluted river streches and for this purpose this matter need not be heard along with O.A. No. 673/2018 henceforth, the individual issue relating to *Musi* River raised in this application is being dealt with by this separate order without prejudice to the general order in O.A. No. 673/2018. Relevant part of the directions in the said case is as follows:

**47. We now sum up our directions as follows:*

- i. 100% treatment of sewage may be ensured as directed by this Tribunal vide order dated 28.08.2019 in O.A. No. 593/2017 by 31.03.2020 atleast to the extent of in-situ remediation and before the said date, commencement of setting up of STPs and the work of connecting all the drains and other sources of generation of sewage to the STPs must be ensured. If this is not done, the local bodies and the concerned departments of the States/UTs will be liable to pay compensation as already directed vide order dated 22.08.2019 in the case of river Ganga i.e. Rs. 5 lakhs per month per drain, for default in in-situ remediation and Rs. 5 lakhs per STP for default in commencement of setting up of the STP.*
- ii. Timeline for completing all steps of action plans including completion of setting up STPs and their commissioning till 31.03.2021 in terms of order dated 08.04.2019 in the present*

case will remain as already directed. In default, compensation will be liable to be paid at the scale laid down in the order of this Tribunal dated 22.08.2019 in the case of river Ganga i.e. Rs. 10 lakhs per month per STP."

8. In view of status report of CPCB dated 28.06.2019, let necessary remedial steps be taken by the Urban Development Department, Telangana, Municipal Corporation, Hyderabad and the TSPCB. The nodal agency will be TSPCB for the purpose. The CPCB may itself validate the Right Biotic system for Quick Hygienic Survey of rivers by seeking opinion of experts on the subject rather than referring it to the Department of Science and Technology, Government of India for its adoption in the country.
9. Further reports may be filed by CPCB and TSPCB in above terms before the next date by e-mail at judicial-ngt@gov.in.

List again on 22.04.2020.

Adarsh Kumar Goel, CP

S.P Wangdi, JM

K. Ramakrishnan, JM

Dr. Nagin Nanda, EM

Saibal Dasgupta, EM

December 06, 2019
Original Application No. 727/2018
With O.A. No. 426/2018
DV

HON'BLE NGT MATTER
TIME BOUND
27.12.2019

F.No.14011/ WQM - I/ 2019 | 10325-10336

To

The Experts,
 (As per the Institutes list enclosed)

Sub: Compliance to Hon'ble NGT order dated 06.12.2019 in OA No.426/2018 in the matter of Mohammed Nayeem Pasha & Anr. Vs State of Telangana & Ors

Sir,

Please refer to Hon'ble NGT order dated 06.12.2019 passed in OA 426/2018. The relevant portion of Para 8 of the Hon'ble NGT order is reproduced below:

Para 8: *The CPCB may itself validate the Right Biotic system for Quick Hygienic Survey of rivers by seeking opinion of experts on the subject rather than referring it to the Department of Science and Technology, Government of India for its adoption in the country.*

Professor Suman Kapur, BITS-Pilani, Hyderabad has developed Right Biotic System for quick microbial examination in the field of medical sciences. The system is used for quick hygienic survey of rivers-Musi and Krishna in Telangana. A copy of Guidelines/ Standard Operating Procedures entitled "Monitoring of River Hygiene" for carrying out quick Hygienic Survey of rivers are enclosed for ready reference.

It is kindly requested to provide your valuable comments/feedback and suggestions if any within fifteen days for initiating further action to adopt Right Biotic platform for quick hygienic survey of ambient water resources.

Yours faithfully,

(Signature)

(A. Sudhakar)
 DH, WQM-I Division
 etc

Encl as above

Copy to:

1. PS to 'MS' : For information of 'MS' please.
2. DH-Law Division, CPCB : For information, please

pl. follow-up

(in B. Agemil)

(Signature)
 26/12/2019

केन्द्रीय प्रदूषण नियंत्रण बोर्ड
 निर्गत...
 दिनांक...

31/12/19

(A. Sudhakar)
 etc

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M: 9246212654

F.No.14011/ OA-426/2018/WQM - I/ 2020 (0882-10898)

08.01.2020

To

The Member Secretary,
SPCBs/PCC

(Andhra Pradesh, Assam, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Puducherry, Tamil Nadu, Telangana, Tripura, West-Bengal and Uttar Pradesh)

Sub: Compliance to Hon'ble NGT order dated 06.12.2019 in OA No.426/2018 in the matter of Mohammed Nayeem Pasha & Anr. Vs State of Telangana & Ors

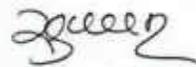
Sir,

Please refer to Hon'ble NGT order dated 06.12.2019 passed in OA 426/2018. The relevant portion of Para 8 of the Hon'ble NGT order is reproduced below:

Para 8: *The CPCB may itself validate the Right Biotic system for Quick Hygienic Survey of rivers by seeking opinion of experts on the subject rather than referring it to the Department of Science and Technology, Government of India for its adoption in the country.*Professor Suman Kapur, BITS-Pilani, Hyderabad has developed Right Biotic System for quick microbial examination in the field of medical sciences. Above system is already used for conducting quick hygienic survey of rivers-Musi and Krishna in Telangana. Also Draft guidelines entitled "**Monitoring of River Hygiene**" prepared by BIT's Pilani, Hyderabad based on the Quick Hygienic Survey of River Musi is uploaded on CPCB website at the following link. [https://cpcb.nic.in/wqm/Draft Guidelines Monitoring of River Hygiene.pdf](https://cpcb.nic.in/wqm/Draft%20Guidelines%20Monitoring%20of%20River%20Hygiene.pdf), for reference.

It is requested to provide your valuable comments/feedback and suggestions if any, within fifteen days on the afore-said mentioned-guidelines for initiating further action for its validation and to adopt Right Biotic platform for Monitoring of hygiene of ambient water resources.

Yours faithfully,



(A. Sudhakar)

DH, WQM-I Division

o/c

Copy to:

1. PS to 'MS'

: For information of 'MS' please.

2. DH-Law Division, CPCB

: For information, please

(A. Sudhakar)

o/c

09/01/2020

h

Pl. follow up

DA (Dr. D. Aganwal) / J. S. S.

Dr

IC- Bady

AD, WQM-I

1. The Member Secretary, Andhra Pradesh State Pollution Control Board, D.No.33-26-14 D/2, Near Sunrise Hospital, Pushpa Hotel Centre, Chalamvari Street Kasturibaipet, Vijayawada- 520 010	2. The Member Secretary, Pollution Control Board Assam, Bamunimaidan, Guwahati – 21
3 The Member Secretary, Gujarat State Pollution Control Board, Paryavan Bhavan, Sector 10-A, Gandhi Nagar-382 010	4 The Member Secretary, Haryana State Pollution Control Board, C-11, Sector 6, Panchkula, Haryana 134109
5. The Member Secretary, Himachal Pradesh Pollution Control Board Him Parivesh, Phase-III, New Shimla Himachal Pradesh – 171009	6. The Member Secretary, Karnataka State Pollution Control Board Parisara Bhavan, 49, 4th & 5th Floor, Church Street, Bengaluru-560001
7. The Member Secretary, Kerala State Pollution Control Board, Pattom P.O, Thiruvananthapuram-695 004.	8. The Member Secretary, Maharashtra Pollution Control Board, Kalpataru Point, 3rd& 4th floors, Opp.Cine Planet, Sion Circle, Mumbai- 400 022
9. The Member Secretary, Madhya Pradesh Pollution Control Board, Paryavaran Parisar, E-5 Arera Colony, Bhopal – 462016	10. The Member Secretary, Odisha Pollution Control Board, Plot No. B-59/2 & 59/3, Chandaka Industrial Estate, Patia, Bhubaneswar- 75
11.The Member Secretary, Punjab Pollution Control Board, Vatavaran Bhawan, Nabha Road, Patiala – 147 001 Punjab	12. The Member Secretary, Tripura Pollution Control Board, Parivesh Bhawan, Pandit Nehru Complex, P.O., Kunjaban, Gorkhabasti Agartala (Tripura) – 799 006
13. The Member Secretary, Tamil Nadu Pollution Control Board, No.76, Mount Salai, Guindy, Chennai-600 032.	14. The Member Secretary, Telangana State Pollution Control Board, Payavarana Bhavan, A-3, Industrial Estate, Sanathnagar, Hyderabad – 500 018
15. The Member Secretary, Uttar Pradesh Pollution Control Board, Building.No. TC-12V Vibhuti Khand, Gomti Nagar, Lucknow – 226010, U.P.	16.The Member Secretary, West Bengal Pollution Control Board, Paribesh Bhavan Building, No.10-A, Block –LA, Sector 3, Salt Lake City, Kolkata – 700 106
17. The Member Secretary, Department of Science, Technology and Environment, Housing Board Complex, 3 rd Floor, Anna Nagar, Puducherry-600 005.	



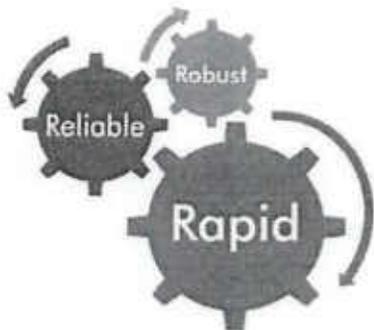
69

RightBiotic

The Fastest Antibiotic Finder

Ready to Use Results ~ 4 Hours

User Friendly Portable Device



No Need To Wait 72 Hours
For Culture And Sensitivity Report



Department of
Biotechnology
Department of Science and
Technology
Technology Development
Board



64

The Problem

3 Days to Finish

Pathogen growth

Selection of media

Crowding of antibiotic discs

Zone measurement

Identification

Susceptibility

Manual reporting

The Solution

In ~4 Hours

Special lyophilized media

For accelerated growth

Functionalized strips

Optical reader

Special analytical algorithm

Mechanism to harvest bacteria

Identification and susceptibility

Ready to use report

Samples Require Only 4 Hours Incubation Time

It combines both colorimetry and nephelometry to detect bacteria and report AST



- It provides information about microbial load type and antibiotic sensitivity of bacteria causing UTI. It can test for upto 42 antibiotics.
- Kit for Urine/Pus/Swab culture are available.
- Machine is future ready as Kits for other body fluids are under trial.

Pidtraent
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Proteus
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Amoxicillin
Gentamicin
Amikacin
Cefepime
Ofloxacin
Ciprofloxacin
Ceftriaxone
Pip-Tazobac
Cefotaxime
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Tobramycin
Levofloxacin
Cefazolin
Imipenem

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Xcellence in Bio Innovations and Technologies

Promoter Founder and Scientific Advisor : Dr. Suman Kapur

Consultants : Mr. Suresh Kabra and Dr. Shashwat Sharad

Contact address : Room No. A101, BITS Pilani, Hyderabad Campus, Jawahar Nagar

Shameerpet Mandal, Dist. Ranga Reddy Telangana - 500078, India

Email : suman.kapur@xcelbits.com | mobile : +91 9010202863

For Further Query & Demo Contact Tek Medicare@8303260372

www.xcelbits.com

www.facebook.com/rightbiotic

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The Only Solution

RightBiotic: Fastest Antibiotic Finder

Ultra Rapid

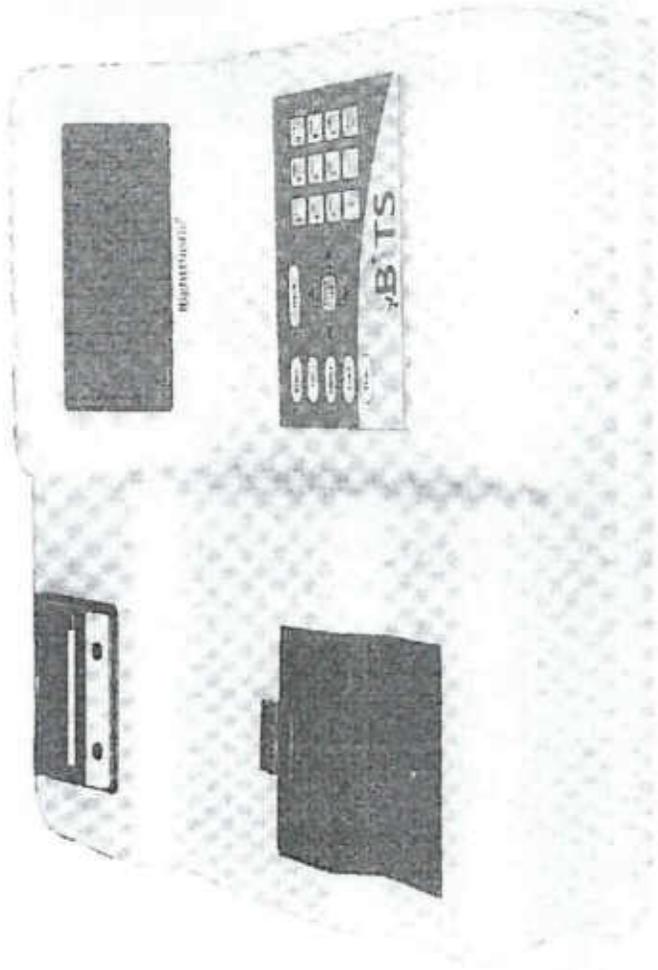
Reliable

Low Cost

User friendly

Point of Care

Ready to use Results in 4hrs



Confidential, Restricted circulation only

1

[www://xcelbits.com/](http://www.xcelbits.com/)

63

62

Healthcare @ Doorstep

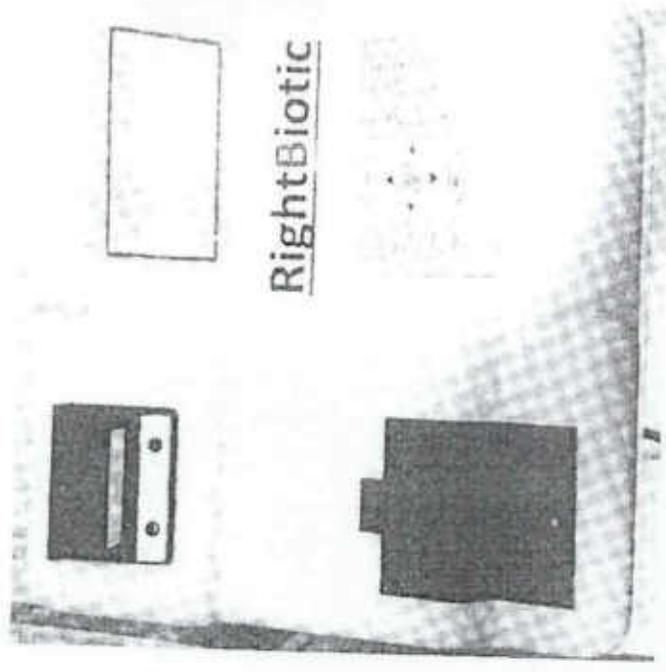
ATS

RightBiotic:

Identify Urinary Tract Infection (UTI) in 2 to 4 hours

- ✓ Get patients on right therapy faster
- ✓ Improve patient outcomes*
- ✓ Enhance Antimicrobial Stewardship
- ✓ Reduce the cost of UTI management

*Based on data derived from several hospitals including AIIMS Jodhpur and Gandhi Hospital Hyderabad, India



RightBiotic



RightBiotic: Instrument

Breakthrough technology enabling direct detection of UTI from Urine in 4 hours

The RightBiotic Instrument is a fully automated, clinical multiplex benchtop diagnostic system capable of running tests directly from urine.

The RightBiotic Instrument utilizes **Optical Detection Method.**

This proprietary technology enables a broad menu of 42 antibiotic assays that can be tested on a single platform, which greatly reduces the complexity of the consumables and the operator interface.

61

Faster Targeted Therapy Earlier detection of UTI with **RightBiotic** Diagnostics

Speed is critical in UTI management: the faster patients receive effective treatment, no more Anti Microbial Resistance (AMR).

Early detection and identification of clinically important organisms frequently missed by initial empiric therapy, provides an opportunity for earlier targeted therapy.

Early Targeted Therapy Starts in the Lab

- ❖ Clinicians typically wait 1 to 5 or more days for species identification results based on urine culture. Now with **RightBiotic** Diagnostics, labs can provide rapid species identification to inform therapy decisions within hours of urine collection, **without waiting for a urine culture result.**
- ❖ Patients diagnosed with **RightBiotic** Diagnostics can be treated in a median of 5 hours – more than **8x faster** compared to urine culture. Waiting for urine culture delayed appropriate therapy by a median of 44 hours.

Improve Patient Outcomes Faster targeted therapy can save lives

Improving patient outcomes is at the heart of xBITS / RightBiotic mission.

- Today, empiric therapy misses 40% of UTI.
- RightBiotic Diagnostic provides faster UTI identification, allowing earlier targeted therapy.
- In addition, the ability to quickly rule-out clinically important pathogens can positively impact patient management.

RightBiotic Proven to Deliver Improved Patient Outcomes

58

B.T.S

Reduce the Cost of UTI Management
Faster patient data for quicker
treatment decisions and cost savings

The value of the RightBiotic is clear: multiple hospitals have shown that making targeted treatment decisions faster leads to improved outcomes, better stewardship, and reduced cost of UTI management.

RightBiotic has proven to:

- Significantly reduce hospital and ICU lengths of stay
- Reduce the use of antimicrobial drugs

A rapid UTI negative result reduces the costs associated with anti-microbial drugs.

RightBiotic Bacteria Panel

Healthcare @ Doorstep

Sensitivity: 95% and Specificity: 98%

E. faecium

S. aureus

K. pneumoniae

P. aeruginosa

E. coli

The fastest UTI identification of major bacteria directly from urine without the wait for urine culture

The RightBiotic Panel is the first and only, urine bacteria pathogen identification test that does not require urine culture.

is now available commercially in India and identifies many of the most deadly and prevalent UTI bacteria species.

By screening suspected patients at the first sign of infection, physicians can start targeted therapy faster, potentially preventing the AMR.

Excellent performance of the RightBiotic Panel and noted several shortcomings of urine culture:

- ✓ Urine culture was found to be limited as a gold standard for detecting UTI, with "suboptimal sensitivity" and "slow turnaround time."
- ✓ Urine culture ID results took an average of 3 days while **RightBiotic** took only **4 hours** in the clinical trial.
- ✓ **RightBiotic** detected infected patients on antibiotics who were missed by urine culture.
- ✓ **RightBiotic** results may expedite life-saving interventions such as the targeting of therapy within hours of urine collection.

Timely, accurate RightBiotic Diagnosis results can improve hospital stewardship

The CDC has declared antimicrobial resistance a serious health threat and the World Health Organization states that "antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.

Many initiatives have been launched to combat this threat, most notably a 2014 U.S. Executive Order, which emphasizes the importance of addressing the threat of antimicrobial resistance.³

In 2015, the U.S. released a National Action Plan for Combating Antibiotic Resistant Bacteria with a goal for every U.S. hospital to have an Antimicrobial Stewardship Program by the year 2020.

Antimicrobial resistance, however, continues to grow and complicate patient management.

The **RightBiotic** is a major advance toward helping improve stewardship by expediting access to accurate patient data to aid in the decision-making process.

Rapid negative UTI test results lead to a reduction in the use of unnecessary antimicrobials.
Eliminating unnecessary drugs and the overtreatment of patients reduces hospital-wide resistance.

Advantage over other Technologies

VITEK 2

Growth based method
Colonies obtained after 18-24hrs

Reagent cards 64 wells are used

4 reagent cards used for antibiotic sensitivity, GN, GP, BCL and YST, AST

Turbidity needs to be adjusted-human intervention

Results after 18+2+6 to 9= 26 to 30 hours after the start of the assay

Requires human intervention

Accessory Instruments required: Centrifuge and laminar flow

Infrastructure required: Air-conditioned labs

Not Portable

Cost of equipment: Rs 15-29L

Cost to Kit: Rs 600-800

Total time required:

18+2+6 to 9 hours ~ 26-29hrs

MicroPro BCS

Turbidity based method

Require 100µl urine specimen each in cuvette A and cuvette B

Uses 2 3ml barcoded cuvettes

Uses Kirby Bauer disc diffusion method for antibiotic sensitivity

Turbidity needs to be adjusted by McFarland's standard--human intervention

Growth checked in 4 hours in cuvette A & B; Pellet of bacteria in cuvette A mixed in saline water, spread on

Mueller Hinton Agar, antibiotic discs dispensed,

incubated at 37°C overnight

Accessory Instruments required:

Centrifuge and laminar flow

Infrastructure required: Air-conditioned labs

Not portable

Cost of equipment: Rs 06L

Cost to Kit: Rs 500-600

Total time required:

5+18hours + 23hrs

RightBiotic

Growth & turbidity based method

Requires 10ml urine sample

Uses a combination of lyophilized media and functionalized strips

3 pre-functionalized strips are used

No human intervention required

Results: identification with antibiotic sensitivity in 4 hour

Accessory Instruments required: None

None

Portable

Cost of equipment: 03L

Cost to Kit: Rs 400-450

Total time required:

4hrs only

[www://xcelbits.com/](http://www.xcelbits.com/)

Advantage over other Technologies

VITEK 2

MicroPro BCS

RightBiotic

<p>Growth based method</p> <p>Colonies obtained after 18-24hrs</p> <p>Reagent cards 64 wells are used</p> <p>4 reagent cards used for antibiotic sensitivity, GN, GP, BCL and YST, AST</p> <p>Turbidity needs to be adjusted-human intervention</p> <p>Results after 18-2+6 to 9= 26 to 30 hours after the start of the assay</p> <p>Requires human intervention</p> <p>Accessory Instruments required: Centrifuge and laminar flow</p> <p>Infrastructure required: Air-conditioned labs</p> <p>Not Portable</p> <p>Cost of equipment: Rs 15-29L</p> <p>Cost to Kit: Rs 600-800</p> <p>Total time required: 18-2+6 to 9 hours ~ 26-29hrs</p>	<p>Turbidity based method</p> <p>Require 100µl urine specimen each in cuvette A and cuvette B</p> <p>Uses 2-3ml barcoded cuvettes</p> <p>Uses Kirby Bauer disc diffusion method for antibiotic sensitivity</p> <p>Turbidity needs to be adjusted by McFarland's standard--human intervention</p> <p>Growth checked in 4 hours in cuvette A & B; Pellet of bacteria in cuvette A mixed in saline water, spread on Mueller Hinton Agar, antibiotic discs dispensed, incubated at 37°C overnight</p> <p>Accessory Instruments required: Centrifuge and laminar flow</p> <p>Infrastructure required: Air-conditioned labs</p> <p>Not portable</p> <p>Cost of equipment: Rs 06L</p> <p>Cost to Kit: Rs 500-600</p> <p>Total time required: 5+18hours + 23hrs</p>	<p>Growth & turbidity based method</p> <p>Requires 10ml urine sample.</p> <p>Uses a combination of lyophilized media and functionalized strips</p> <p>3 pre-functionalized strips are used</p> <p>No -human intervention required</p> <p>Results: identification with antibiotic sensitivity in 4 hour</p> <p>Accessory Instruments required: None</p> <p>None</p> <p>Portable</p> <p>Cost of equipment: 03L</p> <p>Cost to Kit: Rs 400-450</p> <p>Total time required: 4hrs only</p>
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59

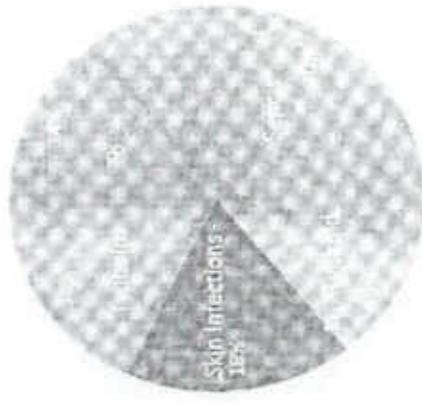
54

8:15

Rationale

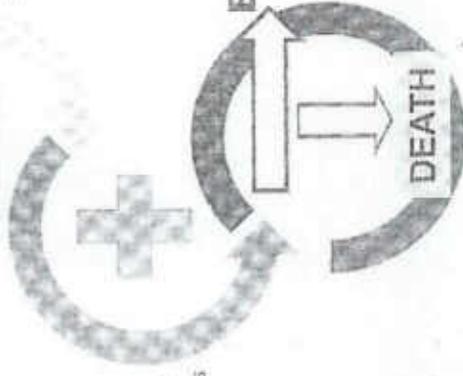
An understanding of the incidence of diseases is a key for decision making in prescribing the right antibiotic in timely manner for clinical care & avoid Anti Microbial Resistance (AMR).

BURDEN OF INFECTIOUS DISEASES



Widespread use of antibiotics

Suspected Infection



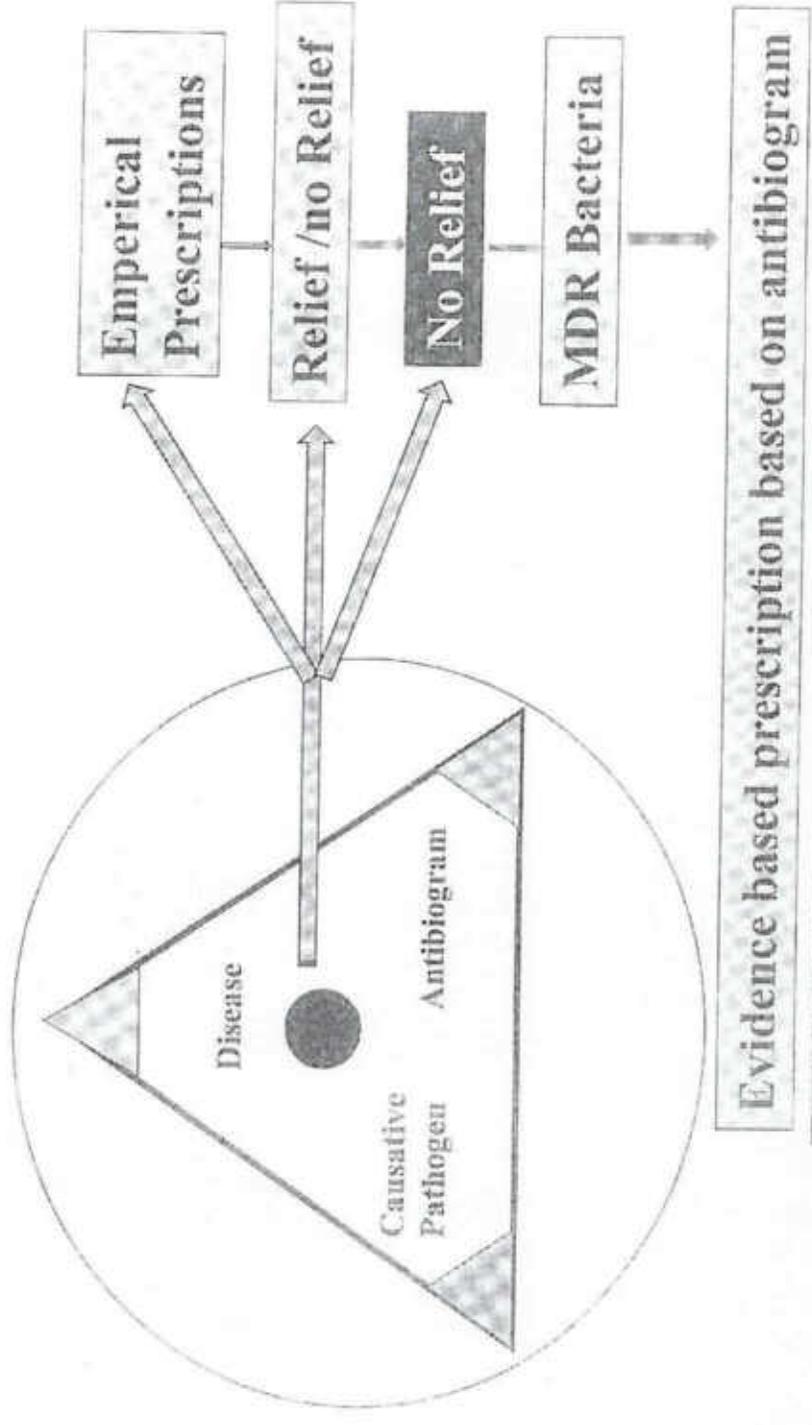
No new Antibiotics
No new antibiotics in last 1 decade

Emergence of Superbugs

AMR MAKES TREATABLE INFECTION UN-TREATABLE

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Emerging Drug Resistance



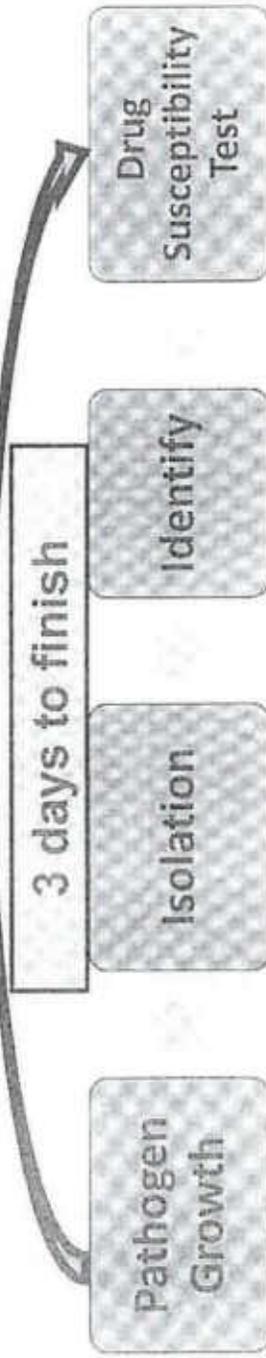
Several countries do not allow sale of antibiotics without AST report!

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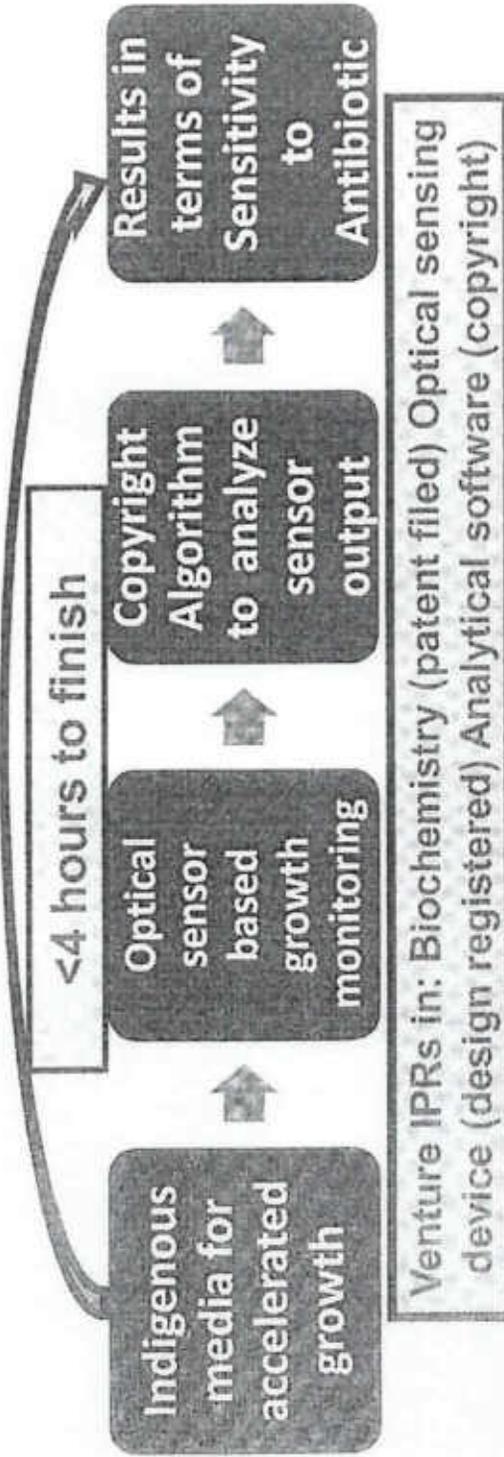
[www://xcelbits.com/](http://www.xcelbits.com/)

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Problem with today's method



RightBiotic: The Solution

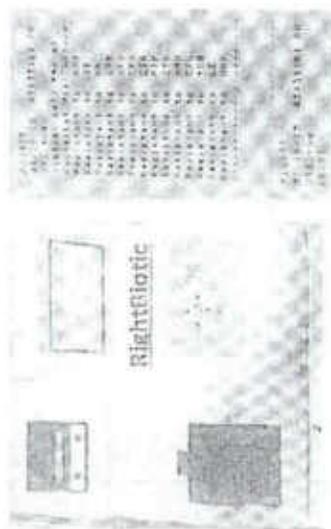


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RightBiotic Comprises of:

RightBiotic Machine

- Optical Reader



RightBiotic Kit



- Pre loaded antibiotic strips for UTI
- P-1 to P-7, Panel of 42 antibiotics
- Dehydrated BITGEN vial (1Pc)
- Syringe (1Pc)
- Sterile Water ampule (1Pc)
- Filter (1Pc)

Sample preparation	20 min
Incubation	03 hrs
Optical readout	10 min
Analysis of results	10 min

Identification of Right Antibiotic for treatment



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Background Work

❖ Technology tested in 2277 clinical cases of Urinary Track Infection (UTI)

N=2277 urine samples (at several centers*)

❖ Sensitivity: 78.33% (95% CI- 74.88% to 81.5%)

❖ Specificity: 51.15%

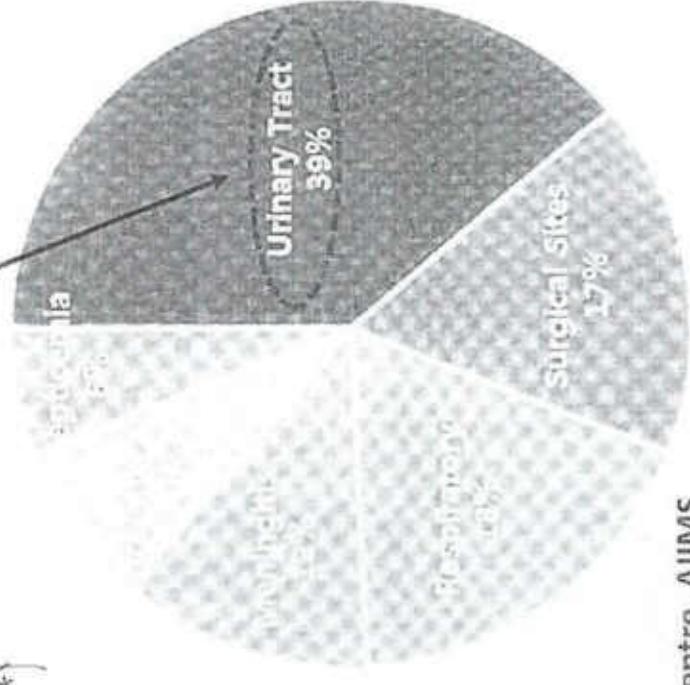
❖ Positive predictive value: 37.65%

❑ Disease prevalence: 27.36%

✓ RightBiotic picked up: 808 additional UTI cases

✓ Antibiotics match rate: 72.2%

✓ Power of the study: 94.07



* Gandhi Medical College, Yashoda Hospital, Medicis Labs, Tapadia Diagnostic centre, AIIMS Jodhpur, AIIMS Delhi, Lifeline labs, New Delhi

Comparison with gold standard (Kirby Bauer Method)

Sensitivity of current urine culture is 51% at a cut-off of 10^5 CFU/ml

Reference:

1. Book Supplement on Laboratory and Other Studies for Acute Cystitis or Asymptomatic Bacteriuria in Women. Internal Medicine Essentials for Clerkship Students. American College of Physicians. 2006
2. University of Michigan Health Sciences. Urinary Tract Infection Guideline, Sept. 2016

With this low sensitivity, it is bound to miss many cases.

Our multi-centric diagnostic accuracy study suggests that the Rapid Strip Test is a feasible alternative to conventional urine culture and antibiotic sensitivity for screening asymptomatic bacteriuria and symptomatic UTI.

xBITS

Background Intellectual Property (IP) details:

No. 2783/DEL/2013;

No. 267834, dt 01-12-2014;

No. 2195/DEL/2015;

PCT No. PCT/IN2016/000153;

US Patent No. 15746424, dated 21.01.2018

IP owned by: BITS (one)

xBITS (four)

Licensing details, if any: 2783/DEL/2013 licensed to xBITS, all others already owned by xBITS, promoted by Dr. Suman Kapur

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[www://xcclbits.com/](http://xcclbits.com/)

bits

Healthcare @ Doorstep

Laurels earned by this technology



Receiving the National Technology Startup Award for 2018 bestowed by TDB
(potential for commercialization) on May 11th '18

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'S In Conclusion.....

AMR has to be prevented at any cost
Economist Ramanan Laxminarayan of CDC, USA says that antibiotics will stop working for everyone, as the bacteria they target are now resistant to all available drugs.

Anti Microbial Resistance is the biggest threat to mankind

RightBiotic averts this threat by **reducing the clinical testing time from 72 hrs to 4 hrs**. The patented technique has been validated by field trials on 2277 clinical cases of UTI.

This venture presents an opportunity to win the war against AMR (Super Bugs) by adoption of evidence based antibiotic prescription in India and several other countries

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W4

Advantages of RightBiotic Assay

Step 1: Identification and Bacterial Load: Within 2 Hours
Identification Kit reports:

Presence and Absence of Bacteria:	UTI Positive and Negative
Type of Bacteria:	Gram Positive or Gram Negative
Bacterial Load/Count:	Number of Bacteria Present

RightBiotic Identifies Bacteria Accounting for 97-98% Cases of UTI

If UTI Positive; Step 2: Antibigram: Another 2-3 Hours

Antibiotic Panels: 42 antibiotics

- 1st Line Antibiotics: Gram Positive + Wide Spectrum 07 antibiotics
- 1st Line Antibiotics: Gram Negative + Wide Spectrum 07 antibiotics
- 2nd Line Antibiotics: Gram Positive + Wide Spectrum 07 antibiotics
- 2nd Line Antibiotics: Gram Negative + Wide Spectrum 07 antibiotics
- End Line Antibiotics: Gram Positive + Wide Spectrum 07 antibiotics
- End Line Antibiotics: Gram Negative + Wide Spectrum 07 antibiotics

PIGMENTED ASSAY FOR URINE

1. Rehydrate the media in the vial with 3ml water.
2. Swirl the vial and keep it for 5 mins.
3. Ensure that the urine sample is about 10ml.
4. The sample should not be older than 1 day and fresh sample is preferred for best use.
5. Now attach the filter provided in the kit to the syringe and then needle to the filter.
6. Aspirate the sample as much as 10ml and then detach the filter and keep it in its sterile casing.
7. Discard the filtrate in the biological discard.
8. Charge the syringe with ready media and attach the same filter and recover the bacteria dropwise.
9. Push only 1.5ml of media with the syringe, then remove the filter and keep it safely in its sterile casing.
10. Attach the dropper cap and dispense equally in Pi strip only.
11. Keep the syringe with 1.5ml in dark.
12. Now switch on the machine and wait for 120 secs for LED stabilization and when machine prompts for "Insert QC strip", slide in the strip and press Enter.
13. The platform comes out, remove the strip and press enter machine displays "QC Strip Validation OK"
14. The machine then reaches home screen.
15. Press Test button to enter ID screen, press Test button again to enter Patient ID.
16. Repeat step 15 to enter to more PIDs.
17. Move to desired patient ID by up and down keys.
18. Press Enter and a message with 3 options will be displayed.
 1. Run Fresh Pi Panel
 2. Run Incubated Panel
 3. Run Final Pi and antibiotic panels
19. Press 1 and machine prompts for blanking, ensure that there is no strip in the platform and press Enter.
20. When machine prompts insert fresh strip in strip holder and press the enter key.
21. The platform comes out, remove the strip and press enter machine displays in status "Fresh strip done"
22. Cover the strip with cello tape and incubate at 37°C along with the remaining bacterial suspension in the media vial.
23. After 2 hours take out the strip and go to the ID and press Enter and Press 2.
24. Repeat step 19 for blanking.
25. The strip platform comes out and machine prompts to insert Incubated Pi panel.
26. Insert the strip and Press Enter.
27. The machine displays either Bacteria Absent or Bacteria Present.
28. If Bacteria Present then: -

29. Choose two antibiotic panels of your choice and attach the filter to the syringe kept in dark and push in the same vial.
30. Using the same dropper cap dispense in equal volumes to all 16 wells.
31. Incubate all 3 strips at 37°C for 2 more hours.
32. After total 4 hours take out the strips from incubator.
33. Select the sample ID on machine and press Enter.
34. This time Press 3 to read the final Pi and antibiotic panels.
35. Repeat Step 19 for blanking.
36. The strip platform will come out and the machine will prompt to insert Final Pi panel.
37. Insert the strip and press Enter.
38. The choices for different antibiotic panels will be displayed.
39. Choose the two panels which was setup 2 hours before.
40. Press Enter.
41. The machine will prompt to insert "Panel-P1" or "Panel P3"
42. Press enter after loading the strip.
43. Then machine will prompt to insert "Panel-P2" or "Panel P4"
44. Remove the strip and press Enter.
45. The result will be displayed and printed by the thermal graphic printer.

Note: If the result is displayed on the screen then Press Print button to get more print copies of the result.



RIGHT IOTIC ASSAY FOR PUS/SWAB CULTURE

1. Collect the swab/pus in the transport media provided in the vial in the kit.
2. Mix the content of the tube by vortex machine (shake vigorously) for 5 min.
3. Remove the swab stick by squeezing it against the walls of the tube so that bacteria/pus is completely dissolved in the transport media.
4. Now rehydrate the media vial by adding 3ml sterile using sterile syringe water provided in the kit.
5. Wait for 5-10 mins so that media is ready for use.
6. Attach the filter provided in the kit with the syringe and needle to the filter.
7. Aspirate the swab/pus suspension (~3 ml).
8. Detach the needle and keep it in its sterile casing and then detach the filter and keep it in its sterile casing.
9. Charge the syringe with the ready media and recover the bacteria drop wise.
10. Attach the dropper cap and dispense the media+pus in 24 wells equally.
11. Then switch on the Right iotic machine from the switch located at the back.
12. Then the machine prompts for LED stabilization for 120 sec.
13. After 120 sec machine prompts "Insert QC strip".
14. Then insert the QC strip provided with machine and press Enter.
15. After reading is complete machine displays "QC strip OD validation OK"
16. Then the machine reaches the home position.
17. Press Test button to enter the ID screen, press Test button again to enter Patient ID.
18. After entering the patient ID select the Pus/Swab as the sample.
19. The status gets updated as PID entered.
20. Repeat Step 17 for entering more PIDs.
21. Go the desired ID by Up and Down Keys.
22. Press Enter and the following message will be displayed: -
 1. Run Fresh Pi Panel
 2. Run Final Pi and Antibiotic panels
23. Press 1 to run fresh Pi Panel
24. The machine will prompt for "Blanking".
25. Ensure that no strip has been loaded in the strip platform before blanking.
26. Press Enter and machine will start blanking.
27. After the blanking is completed the strip platform will come and then place the Pi strip in the strip holder.
28. After reading is complete the machine will display "Incubate Pi panel for 4 hours"
29. Then place the strips with all strips covered with cello tape in an incubator for 4 hours at 37°C.
30. After 4 hours take out the strips and remove the cello tape.
31. In the machine go on the desired ID and press Enter
32. Again, the same message will be displayed and Press 2.
33. Repeat step 25 for blanking.
34. After blanking is complete the strip platform will come out and machine prompts "Insert Final Pi Panel"
35. Insert the Pi panel and press Enter.
36. After reading is complete the screen will show choices for different antibiotic panels.

37. Set the panels which you have chosen to Yes and other to no by entering the no.
38. Press enter and machine will prompt to "Insert Panel-P (_)" as chosen by the user
39. Insert the Panel and Press Enter.
40. Then remove the panel and insert next antibiotic panel and Press Enter.
41. After the reading is complete, remove the strip and press Enter.
42. The result will be displayed on the screen and also printed by the thermal graphic printer.

Proposal for Validation of Right Biotic System for conducting Quick Hygienic Survey of Rivers

1. Introduction:-

Central Pollution Control Board (CPCB) and Telangana State Pollution Control Board (TSPCB) in association with BITS-Pilani, Hyderabad Campus carried out the study entitled "Quick Hygienic Survey of River Musi" utilising indigenously developed method called "Right Biotic method" during May to July 2019 for ensuring compliance to Hon'ble National Green Tribunal (NGT) Order dated 05.04.2019 passed in O A No 426 of 2018 in the matter of Mohammed Nayeem Pasha & Anr Versus the State of Telangana & Ors.

Right Biotic method to conduct Quick Hygienic Survey of rivers involves optical detection system which converts the optical signal into electrical signal and thus facilitate quick measurement utilizing optical density of the pathogenic bacteria in a short duration of 4-5 hours. Subsequently, the findings of the study submitted by BITS-Pilani, Hyderabad campus on Quick Hygienic Survey of River Musi and Krishna was filed before Hon'ble NGT on 18.11.2019 by CPCB and the guidelines entitled "monitoring of river water hygiene" were prepared also uploaded in CPCB website. As CPCB do not have expertise for validation of quick hygienic survey of rivers using right biotic system and thus CPCB has suggested Hon'ble NGT to consider to assign the task of validation of quick hygienic survey developed by BITS-Pilani, Hyderabad campus for its adoption in the country to Department of Science and Technology (DST) or relevant Ministry in Central Government..

Hon'ble NGT vide order dated 06.12.2019 directed CPCB to validate right biotic method for carrying out quick hygienic survey of rivers by itself or by involving experts from subject field so that the said method can be adopted across the country. For ensuring compliance to Hon'ble NGT order, therefore, following activities are proposed for validation of right biotic system for conducting quick hygienic survey of rivers.

2. Selection of Methods –

Presently, there is no unified method to encompass the identification and analysis of all pathogenic microorganisms present in water bodies. The challenges of the detection methods are the physical differences between the major pathogen groups, low concentration of pathogens in a large volume of water which usually requires enrichment and concentration of the samples prior to detection & processing. The presence of inhibitors from the sample (especially if it comes from polluted water), established general protocols for sample collection, culture-independent detection method, as well as detection of the host origin of pathogens. The most important requirements for reliable analysis include: specificity, sensitivity, reproducibility of results, speed, automation and low cost.

Even though culture dependent methods are extensively used for pathogens detection in water, these methods are limited by their low sensitivity and the excessive time needed to obtain reliable results. Furthermore, since there is a broad environmental distribution of human pathogens that exist in a viable but non-cultural (VBNC) state such as *E. coli*, *Helicobacter pylori* and *V. cholerae*, false negative results may arise from culture dependent methods. Alternatively, molecular and enzymatic methods have been developed to overcome the less favourable aspects of conventional ones. In the molecular methods, the objective is to label the large microorganism by binding it to molecular structures on the exterior surface or to structures within the interior of a cell. The first ones can be classified as immunological methods, and the others as nucleic acid methods. The range of these available methods has increased, because they are able to achieve a high degree of sensitivity and specificity. Also, they are adaptable to a wider set of indicators and pathogens at a low cost. However, they may not distinguish live from dead bacteria and they require extensive training and highly specialized equipment.

Since the method of choice for routine analysis of water sample is Multiple Tube Fermentation Technique (or) Most Probable Number (MPN) method, which is also the compendial method and provides number of indicators of pathogenic bacteria. These indicator bacteria belong to coliform and non-coliform (Streptococcus) groups. The indicator bacteria which are generally used for water quality assessment are Total & Fecal coliform, Fecal Streptococci and E. coli. In this method, water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilize the lactose present in the medium to produce acid and gas. The presence of acid is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction and comparing the pattern of positive results with standard statistical tables. Although this method is slower and requires 48 hours for a positive, labour intensive, require more culture medium and low precision but has very high sensitivity around (85-92%) and is applicable to all type of water.

On the other hand Right Biotic system involves the **Optical Detection System** which converts the optical signal into electrical signal. This utilizes disposable membrane filters of 0.45 µm for filtering the water sample in initial steps of sample preparation before incubation at the ambient temperature and thus measures quickly the Optical Density of the pathogenic bacteria in very short duration (within 4-6 hours). Additionally this method may not work with turbid water due to presence of particulate matter in water sample which could block the pores and inhibits passage of the specific volume of water sample required for processing further. **Table 1** below details about the test available for identification and load determination of microorganism the advantages and disadvantages these method offer, are detailed below:

Test category	Type of test	Advantages (what these test offers)
Established Standard methods (Standard most accepted Method: MPN method)	MPN, Membrane Filtration technique Presence absence test (using liquid media), Pour Plate technique, Spread plate method, Liquid Enrichment confirmation or isolation on solid media.	MPN -Low precision but high Sensitivity which further depends upon volume analysed, dilution levels and replicate tubes Other methods- Slow growing , weak level of detection, many cultivation & confirmatory steps required, scoring of colonies difficult
Alternative methods	Molecular-Immunological methods (ELISA & various Immuno-fluorescent methods)	Low selectivity and low specificity, pre-enrichment needed and no differentiation between Viable and Non-Viable Microorganism, Existence of a threshold below which the method cannot be applied
	Molecular Nucleic acid methods (PCR, DNA Microarray based)	<ul style="list-style-type: none"> • Sensitive and Rapid pathogen confirmation • Detection is strictly taxonomic and requires specific and expensive equipment • takes long time in optimisation • negatively affected by many environmental conditions, • No indication of viability of the microorganism • Highly trained personnel, and cost equipment
	Enzyme/Substrate methods (with chromogenic and /or fluorogenic substrate) Commercially available test: Enterolert®, Colisure®, Colilert® manufactured by IDEXX, ColiComplete® manufactured by Bio Control, Chromocult® by Merck, Microsure® manufactured by	<ul style="list-style-type: none"> • simple and rapid (18-24 hrs) has potential for routine microbial water quality assessment as well • eliminate the need for subculture and further biochemical tests to establish the identity of certain MO • Interference can be caused by presence of other bacteria (less than conventional method)

Test category	Type of test	Advantages (what these test offers)
	Gelman, m-ColiBlue® manufactured by Hach	
Emerging methods	Ultrasensitive Detection approaches: Biosensors, Microfluidics, Bioluminescence, Mass spectrophotometry based detection	<ul style="list-style-type: none"> • Highly sensitive • complex instrumentation, • long time optimisation requirements, • highly expensive and require specialised skill in processing and interpretation
Automated method	Growth based methods: Vitek 2 compact from Biomerieux, france	<ul style="list-style-type: none"> • Colonies obtained after 18-24 hrs, • Processing with specialised cards for sensitivity • total time taken is around 30 hours after start of assay
	Turbidity based methods: MicroPro blood culture system	<ul style="list-style-type: none"> • Periodic growth check requirements • Culturing on MHA plate and sensitivity determination. • Takes around 35-40 hrs
Rapid Automated method	& Right Biotic System	<ul style="list-style-type: none"> • Rapid assay (takes 4-6 hours) • Determines bacterial load and type of bacteria • Also determine antibiotic sensitivity • Require validation

From the list mentioned above, we may compare Right Biotic System (RBS) with the traditionally used conventional method for the bacterial load (MPN) and counter confirmed for presence of heterotrophic bacteria by any enzyme substrate method and PCR (Molecular method) for confirmation of identified bacteria. The results thus obtained may be assessed or evaluated statistically for both linearity (regressions among bacterial concentrations) and accuracy (significant correlation between a measured value and a value used as reference) which may confirm the validity of the RBS method.

As it is realised that there is an urgent need for improving current detection methods, particularly in issues related to speed, sensitivity, specificity, accuracy and precision, without the need for sophisticated equipment or skilled analysts and with a reduced cost, therefore validating this rapid method viz., right biotic system helps in determining the pathogenic index of water bodies. Therefore, following methods will be used for carrying out comparative study as a part of validation of quick hygienic survey of rivers using right biotic system developed by BITS-PILANI, Hyderabad campus comprising the results

- Right Biotic System method.
- Most Probable Number

3. Laboratories to be selected for carrying out quick hygienic survey of river samples:

Right Biotic System may be procured at CPCB lab (either at Delhi/Bengaluru) where the processing for RBS method and MPN could be done apart from BITS-Pilani, Hyderabad campus. It may also be counter checked by any of the above Enzyme substrate assay for the presence of bacterial species, and also confirmed by any of the molecular based method like PCR. The results obtained should be statistically validated by the experts of the field. The same may be outsourced to CSIR funded laboratories having requisite infrastructure as well as expertise facility for statistical evaluation.

S.No	Name of the Institute	Address	Website details
1	Central University	The University of Hyderabad Prof. C.R. Rao Road,	http://acad.uohyd.ac.in/contact.html

		P.O. Central University, Gachibowli, Hyderabad 500046, Telangana	
2	Centre for Cellular & Molecular Biology (CCMB)	Uppal Road, Hyderabad -500007 Telangana	www.ccmb.res.in
3	National Centre for Biological Science	Rajiv Gandhi Nagar, Kodigehalli, Bengaluru, Karnataka 560065	www.ncbs.res.in
4	Central Pollution Control Board (CPCB)	Regional Directorate CPCB, Bengaluru	www.cpcb.nic.in
5	Bits-Pilani	Hyderabad Campus	

(iii) Parameters to be analysed during the validation period:

As a part of Quick Hygienic Study, the selected laboratories may work on the following parameters

- Bacterial identification along with species (such as *E.coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Staphylococcus spp.*, *Enterococcus spp* etc.)
- Bacterial load determination/ counts (colony forming units)
- Confirmatory tests like PCR may be processed for pathogenic bacteria such as *E.Coli*, *Staphylococcus spp* and *Enterococcus spp.* using specific primer and known positive and negative controls.

However, a consultation meeting will be held with the representatives of the selected laboratories to decide on the uniform parameters to be analysed during the study period.

(iv) Sample Collection/Preservation/Transportation and analysis of samples:

Sample shall be collected from 3 to 4 selected locations from the selected 2 river bodies representing clean water, moderately polluted, highly polluted and severely polluted water jointly by CPCB, TSPCB, Bits Pilani and the representatives of the selected labs, at-least 4 replicates. As RBS is portable, and it should also be tried in the field and the results obtained should be compared with the testing done at labs. Besides natural samples few synthetic samples with known concentration of bacteria shall also be analysed. Volume of sample collected be around 2 liter (in sterilised bottle from one source and afterwards distributed in smaller containers 500 ml bottles under sterilised conditions (in Laminar flow) after ensuring proper homogenisation). Volume of sample collected be around 100 ml and should be carried always on ice cooler kept in cooled thermocol boxes (from field to the lab) . All the collected samples are either saved in nutrient agar slants (most preferred option) maintained at 4-8 °C or should be lyophilised (vacuume dried) to be kept at controlled room temperature for future use if required.. The sample should be streaked on the media plate as well as on the slants for storage, purification and characterization (if required).

Freezing at -20C may be used to preserve microorganism for longer periods, where viability may be maintained for 1-2 years but damage is caused by ice crystal formation and electrolyte fluctuation resulting in poor long term survival therefore other than agar slants, glycerol stocks could also be prepared)

All the collected samples will be analysed for the related parameters (pre-fixed by CPCB and Bits-Pilani) at the same time and date as fixed by CPCB. For reliability of data each involved laboratory required to be instructed to initiate analysis using identified methods (including RBS) on the specified date and time.

(v) Declaration of Results: The results obtained by individual laboratories may be analysed by comparing it with reference method and then analysed for linearity and accuracy using Statistical tools followed by interpretation of the results with respect to bacterial type and concentration.

(vi) **Total time period required for validation of quick hygienic survey of river samples.**

Item	Tentative time required for the activity
Strategy finalisation with experts	1-2 weeks
Training of staff for handling, sample processing and result interpretation by RBS method	2-3 weeks
Sample collection from different sites (4-5 different sites)	3-4 weeks
Sample preparation and processing (for 3 different methods)	3-4 weeks
Result Analysis & Statistical Correlation	3-4 weeks
Sum-up of results obtained from all test & laboratories	1-2 weeks
Total	19 weeks approx

(vii) Additional Requirement:

Consultation meeting with the CSIR Laboratories for joint assessment and finalisation of SOP for carrying out the validation process.

Procurement of RBS at selected centres (3) and training of the staff for handling and processing for generating data as per requirement of CPCB- **Six months.**

(viii) Constitution of the Committee:-

Expert committee members comprising representative of DST, MoH & FW, MoJS, MoE & CC, IIT (Delhi), CSIR Laboratory, ICMR, BIS to be constituted for deliberating the issues relating to joint assessment findings and for validation of the right biotic system and for submission of the comprehensive report before the Hon'ble NGT.