USP Technology Review: Speedy Breedy

This report is one of an ongoing series of reports evaluating the capabilities of various screening technologies, performed under USP's established Technology Review program (see Introduction for details).

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Executive Summary

A technology review was carried out on Speedy Breedy, a portable respirometer manufactured by Bactest. The objective of this review was to determine whether Speedy Breedy can feasibly be used as a first-line screening technology to detect microbial contamination in sterile liquid samples. The performance evaluation involved analysis of three liquid samples (water for injection, artesunate for injection, and oxytocin injection). To mimic potential contamination of samples, some preparations were spiked with bacteria of varying starting concentrations, measured as colony forming units (CFUs). Using standard protocols developed by Bactest, results showed reliable detection of *E.coli* in sterile water for injection, using the Tryptic Soy Broth (TSB) media vessels, at concentrations as low as 1 CFU and up to 1,000 CFU. *E.coli* was also detected in sterile water for injection using MacConkey Broth (MCC) media vessels and artesunate for injection and oxytocin injection samples in water for injection using the TSB media vessels, while *P. aeruginosa* was detected in water for injection, artesunate for injection, and oxytocin injection using Cetrimide Broth (CB) media vessels. All of these bacteria were spiked into the liquid samples at starting concentrations of 20 CFU. Negative controls were run in parallel and confirmatory analysis of samples using an incubator to culture the bacteria and a spectrophotometer to measure optical density at 600 nm to avoid false positives and negatives and confirm viability of the inoculated bacteria. A blinded test was performed with the analyst successfully able to identify contaminated samples and their relative levels of contamination. The field evaluation indicated that inspectors, chemists, microbiologists, and pharmacists with various levels of technical expertise from the regulatory authorities of two countries, India and Zimbabwe, could become either basic, intermediate, or advanced users of the technology within approximately 2 weeks. Speedy Breedy was able to run samples and generate results consistently in uncontrolled field settings, provided a continuous power source was present. Although the instrument has some limitations related to sample throughput and analysis of low volume samples, overall it was able to effectively detect contamination in spiked liquid samples.

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1. Introduction

Assuring the quality of medicines along all points of the supply chain is vital for promoting positive health outcomes for patients around the world [1]. The importance of medicine quality screening technologies in this endeavor is becoming increasingly recognized [2]. USP has launched the Technology Review program, an initiative guided by a technical expert panel established through the organization's collaborative and volunteer-driven governance and working towards four objectives:

1. Develop standards and guidelines for evaluating medicine quality screening technologies
2. Generate and disseminate tailored information on the capabilities of these technologies through a two-step review process; a lab-based technical performance evaluation and a collaborative field-based utility evaluation.
3. Build the knowledge of key stakeholders to appropriately procure and sustainably utilize screening technologies for the purposes of combating substandard and falsified medicines
4. Foster the development and enhancement of new and emerging screening technologies.

This report contributes directly to objectives two, three and four and is the first in what will become an ongoing series evaluating the capabilities of various promising screening technologies.

Most of the screening technologies currently in use by regulators, manufacturers and other stakeholders focus on identification of active pharmaceutical ingredients, excipients and other raw materials. Little attention has been paid to the issue of microbial contamination or sterility of liquid samples, a particularly germane problem in low and middle income countries (LMICs) where the security and integrity of the medical product supply chain is difficult to maintain. In addition, the currently available methods and instrument require dedicated clean laboratory space and trained staff to perform the required assessment. To date, there has been little emphasis on developing methods and instrumentation to perform tests in the field However, a portable respirometer called Speedy Breedy may present a solution to this concern. Speedy Breedy is a portable respirometer that claims to detect microbial contamination in liquid samples through pressure change measurements over time, which represents microbial respiration [3]. The program, with input from the expert panel and other stakeholders, therefore decided to review Speedy Breedy.

2. Methodology

2.1. General Information

Table 1 provides general information on Speedy Breedy, namely how it functions, its basic specifications, and the upfront and recurring costs of using the instrument. All data in this section were collected through email exchange, telephone conversations, and review of the vendor's website between July 2017 and October 2017.

Table 1: General Information

Technology	Speedy Breedy is a portable respirometer, manufactured by Bactest. Each instrument has two chambers, which detect microbial contamination through the measurement of pressure changes in a sample (solution) of interest.
Specifications	*Dimensions:* 13.3 cm (H), 31 cm (W), 11.2 cm (D) *Weight:*2.75 kg *Power source:* Local mains AC power supply or 12V DC (car adapter is available) *Voltage:* Variable (230V / 50Hz – 120V / 60Hz)
Relative Cost	*Upfront cost* • 1 unit: $4,700 USD *Recurring costs* • 8 pack of media vessels – $80 USD • 30 pack of 50 ml sterile plastic water bottles – $40 USD • 50 mL sterile syringe[1] – $2 USD *Approximate cost per test[2] (not including cost of sample)* • $10–$15 USD

2.2. Performance Evaluation

Acronyms and Definitions

AS1	Artesunate injection medicine 1
CB	Cetrimide broth (*P. aeruginosa*) culture media pellet
CFU	Colony forming units
MCC	MacConkey broth (*E. coli*) culture media pellet
N	Number of runs
OXY1	Oxytocin injection medicine 1
TSB	Tryptic soy broth media pellet
WFI1	Water for injection brand 1
WFI2	Water for injection brand 2

Speedy Breedy Operating Procedure

1. Culture media pellets were allowed to equilibrate to room temperature for 30 minutes.

[1] Not provided by vendor.
[2] Cost per test was calculated by adding the cost of one media vessel to the cost of one sterile syringe, providing some margin for the cost of sterile syringe

2. Stock controls were prepared at required concentrations for each bacterial cell culture.
3. For positive control (spiked) samples, the product was spiked with the required concentration of stock control. If necessary, the sample was diluted to obtain sufficient volume of the solution for accurate inoculation of required CFU for the Speedy Breedy and UV-Visible spectrophotometer analysis.
4. The cap was removed from the selected vessel, and 50 mL of the positive control (spiked sample) was introduced into the first vessel through the vessel port using a sterile 50 mL syringe. The cap was then replaced.
5. The cap was removed from the selected vessel and 50 mL of a negative control (un-spiked sample) was introduced into the second vessel through the vessel port using a fresh pipette or sterile syringe. The cap was then replaced.
6. Both vessels were placed into the Speedy Breedy chambers and firmly closed (a click sound confirmed proper closure).
7. The Speedy Breedy analysis was started, using the appropriate test:
    a. "24h General Contamination Test" for TSB.
    b. "E. coli contamination test" for MCC.
    c. "P. aeruginosa contamination test" for CB.
8. At the end of a run, the data file was saved and the time of event was recorded.

Time of analysis for Speedy Breedy can exceed 24 hours, and each protocol has its own predetermined run time. All samples were allowed to run for their complete protocol run times. For example, the general contamination test, which uses a TSB media vessel, has a 24-hour run time. See Annex 1 for details about the equipment, consumables, samples, and supplies used during the review.

Confirmatory Analysis

All Speedy Breedy results were confirmed using a shaking incubator and UV-Visible spectrophotometer. After sample preparation, a 50 mL aliquot was transferred into a Speedy Breedy vessel, and an additional 50 mL aliquot was transferred into a tissue culture tube or flask. This flask was then incubated in the shaking incubator at 35 degrees Celsius at 200 RPM. After the appropriate time, growth was determined by visual detection and at an OD of 600 nm using a UV-Visible spectrophotometer. A blank preparation was also run on the UV-Vis.

Methodology Limitations

Certain limitations were encountered during this performance review, which were inevitable given the nature of the technology and the objectives of the review. They are identified below:

1. Preparing and diluting low concentration CFU samples inherently meant that there was the possibility that the CFUs within a given preparation were not fully or reproducibly transferred to the spiked sample. This was observed in certain samples, which did not exhibit a pressure event or turbidity after analysis. This absence of contamination was confirmed through confirmatory analysis and, in situations where this occurred, these data were not used.

2. Not all available media vessels were used for this review; as a result, not all related bacteria were "spiked." However, the researchers selected two of the most common bacteria found in water, *E. coli* (ATCC 8739) and *P. aeruginosa* (ATCC 9027), which were used for the purposes of the analytical work. The related media vessels—Cetrimide broth for *P. aeruginosa*, MacConkey broth for *E.coli*, and Tryptic Soy Broth for general microbial contamination—were therefore used.

3. Results

3.1. General Information

Data

Bactest can provide the hardware and software in English, simplified Chinese, German, Romanian, and Spanish. Currently, there are no software permissions or instrument locks on Speedy Breedy, and the instrument does not have Internet capabilities; however, if a computer is connected to the Internet, end-of-test notifications can be received However, data can be transferred between devices using the provided SD card or between a device and a PC or laptop using a connection cable provided. There are four types of data files, listed below:

- .SBX are protocol files
- .SBC are calibration files
- .SBR are test results files
- .SB1 and .SB2 are raw data files

Access, Handling, Maintenance, and Repair

Speedy Breedy is available for procurement and shipment anywhere in the world through Bactest headquarters in the U.K. or the company's distributor network. Repairs currently cannot be performed in the field, so malfunctioning instruments need to be returned for corrective action.

Durability

Speedy Breedy is not waterproof and unshielded, so above normal electromagnetic interference could result in ineffective tests. The instrument is robust but not ruggedized and has not been drop tested. However, provided temperature, humidity, dust, and vibration changes are not too rapid or severe, the instrument can tolerate fluctuations very well.

Use

Speedy Breedy can analyze liquids, macerated materials, powders, filter membranes, swabs, and bodily fluids. The instrument has media vessels for the following general and specific bacteria and yeasts:

- Broad spectrum (covers aerobic bacteria and yeasts)
- General coliforms and *E. coli*
- *Salmonella* spp.
- *Staphylococcus* spp.
- *Enterococci*
- *Clostridium perfringens*
- *Listeria* spp.

- *Pseudomonas aeruginosa*
- *Campylobacter* spp.
- Toxigenic *Vibrio cholera*
- Lactic Acid bacteria
- General and wild yeasts

The performance evaluation did not include evaluation of Speedy Breedy's ability to detect anaerobic growth. However, detection of anaerobic growth can be made through the measurement of a pressure differential, irrespective of whether growth is under aerobic or anaerobic conditions. Broad spectrum vessels are recommended to be used, which can be made to be anaerobic. Further details, as well as informational guides, videos, and the latest software, firmware, protocols, and calibration curves can be found on Bactest's website: http://www.speedybreedy.com.

3.2. Performance Evaluation – Application III: Identification of Contaminants or Impurities

All data below were collected between May 2017 and July 2017. Application III is per the USP Stimuli to the Revision Process: Evaluation of Screening Technologies for Assessing Medicine Quality [4].

Speedy Breedy is a portable, precision respirometer, which detects and monitors microbial activity. Detection is observed through pressure transients relating to gaseous exchanges within a 50 mL closed culture vessel as a result of microbial respiration. The instrument uses a motor and stir bar to mix sample solutions, which creates culture conditions that stimulate growth of microbes. This growth facilitates the conversion of gaseous exchange into pressure variances in the headspace of the culture vessel, which are subsequently measured and recorded and can be visualized on a computer using the instrument software. If the variance exceeds the noise threshold defined by a given protocol linked to a particular media pellet measured in pressure change over time (typically more than 0.1 mbar per minute for at least 7 minutes), it is recognized as a pressure event, which signifies contamination.

Analysis Conditions

Table 2 summarizes the various products that were used as samples and analyzed and highlights the number of runs that were performed under each condition, the media pellet that was used, and whether or not an event was observed for those samples that were spiked.

Negative controls were prepared in parallel with all conditions, and no negative control samples had pressure events.

Table 2: Condition Details and Presence of an Event

Condition	Product	Media pellet	Bacterial Contaminant	Spiked Conc. (CFU)	N	Pressure Event
A	Water for injection	TSB[3]	*E. coli*	1, 10, 20, 50, 100, 1000	29	Yes
B	Water for injection	MCC	*E. coli*	1, 20, 50	16	Yes

[3] See section 2, "Methodology," for acronyms.

C	Water for injection	CB	*P. aeruginosa*	1, 20, 50	3	Yes
D	Oxytocin injection	CB	*P. aeruginosa*	20	5	Yes
E	Oxytocin injection	TSB	*E. coli*	20	1	Yes
F	Artesunate injection	TSB	*E.coli*	20	1	Yes
G	Artesunate injection	CB	*P. aeruginosa*	20	1	Yes

Reproducibility and Reliability

Table 3 provides statistical data of the samples that were run under condition A.

Table 3: Reproducibility, Range, and Reliability of Speedy Breedy under Condition A

			Time to Pressure Event (minutes)							
Spiked Conc. (CFU)	**Log_{10} Conc. (CFU)**	**N**	**Min**	**Max**	**Range**	**Mean**	**Stdev**	**% RSD**	**Days[4]**	**Instruments**
1	0	8	714	842	128	792.4	42.2	5.3	4	3
10	1	2	668	720	52	694.0	36.8	5.3	1	2
20	1.3	8	615	842	227	749.4	72.2	9.6	4	3
50	1.7	6	504	688	184	644.7	69.8	10.8	2	3
100	2	3	624	650	26	635.3	13.3	2.1	3	2
1,000	3	2	474	578	104	526.0	73.5	14.0	2	2
Log transformed Pearson's correlation coefficient (all conc.) – R^2								-0.95		

Runs at all six concentrations gave pressure events on different days and using different instruments. Three of the spiked concentrations—1, 20, and 50 CFUs—had more than three runs (N), and their time to pressure event ranges and percentage relative standard deviations were 128, 227, and 184 minutes and 5.3 percent, 9.6 percent, and 10.8 percent, respectively. Results for the 20 CFU dataset were also collected by two analysts. The Pearson's correlation coefficient of −0.95 was calculated using the log-transformed concentrations and means as comparator variables.

Figure 1 shows a screenshot of one of the results run under condition A at 20 CFU. The first two gray lines denote the start and end times of the run, while the red line denotes a pressure event. The dark purple and green lines display the pressures in the right and left chambers, respectively, while the faint purple and green lines display the temperatures in the right and left chambers, respectively. The left chamber was spiked, while the right chamber was a negative control. The two peaks at the end of the run represent the optional pasteurization cycle, which can be run after a test to kill non-spore-forming bacteria, such as *E.coli*.

[4] Represents the number of different days experiments were run on.

Figure 1: Screenshot of Speedy Breedy Results from an Analysis under Condition A

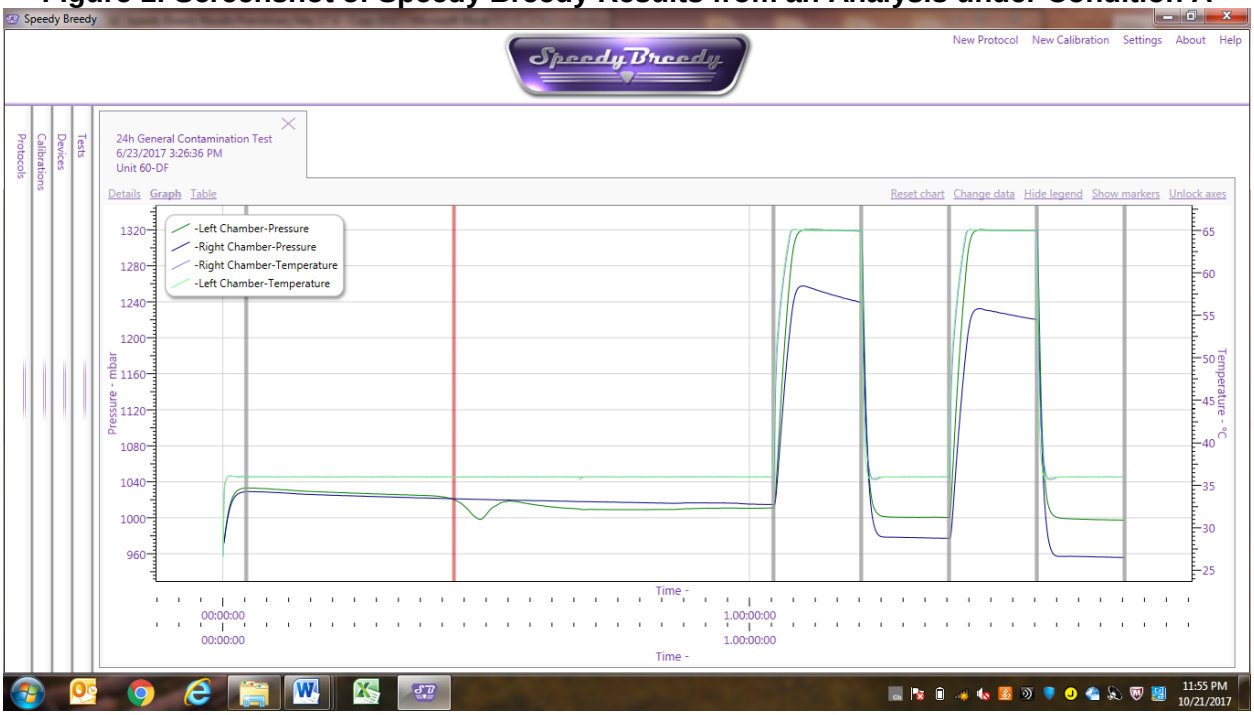

Table 4 provides statistical data of the samples that were run under condition B.

Table 4: Reproducibility, Range, and Reliability of Speedy Breedy under Condition B

	Time to Pressure Event (minutes)								
Spiked Conc. (CFU)	**N**	**Min**	**Max**	**Range**	**Mean**	**Stdev**	**RSD**	**Days**	**Instruments**
1	1	932	932	N/A	N/A	N/A	N/A	1	1
20	14	730	1448	718	914.1	178.8	19.6	3	3
50	1	642	642	N/A	N/A	N/A	N/A	1	1

A total of 14 runs at 20 CFU gave pressure events on 3 different test days and using all 3 instruments. The range for this dataset was 718, and the percentage relative standard deviation was 19.6 percent. However, this included a possible outlier at 1,448 minutes. The second highest time to pressure event in this dataset was 1,048.

Figure 2 shows a screenshot of one of the results run under condition B. The gray lines denote the start and end times of the run, while the red lines denote pressure events. The right chamber contained a sample spiked with *E.coli* at 50 CFU, while the left chamber contained a sample spiked with *E.coli* at 1 CFU.

Figure 2: Screenshot of Speedy Breedy Results from an Analysis under Condition B

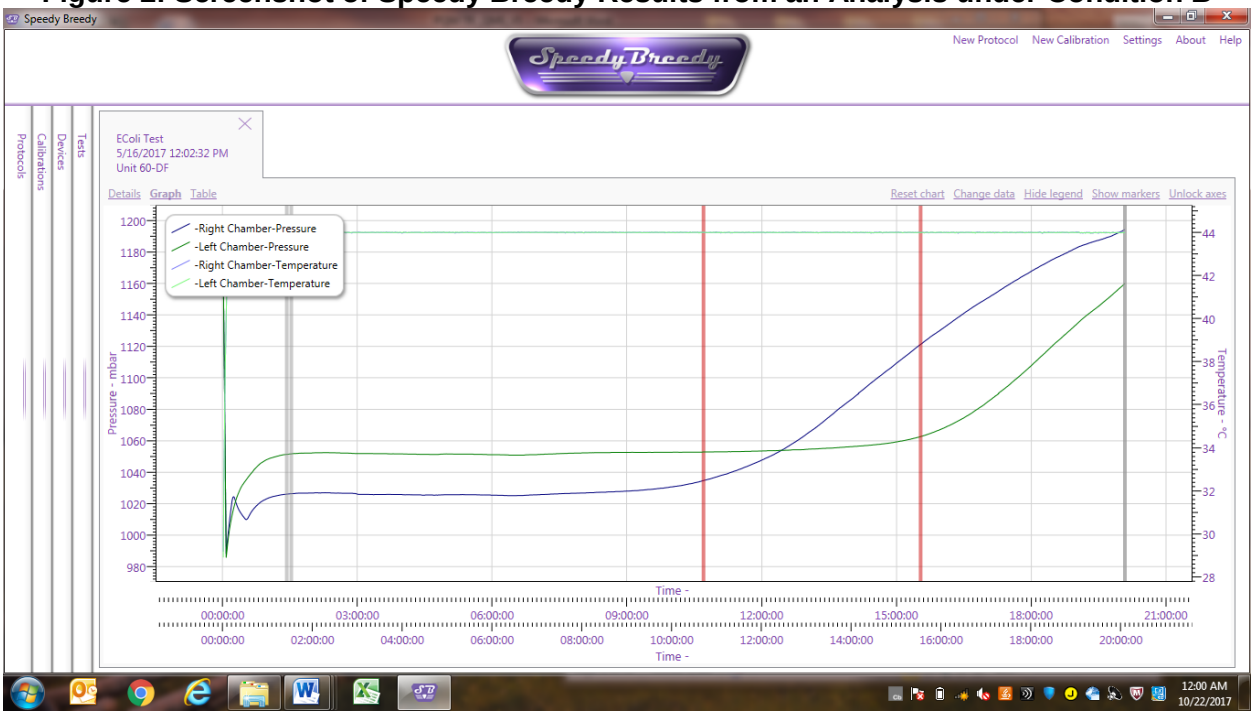

Table 5 provides data of the samples that were run under conditions C, D, E, F, and G.

Table 5: Speedy Breedy Results under Conditions C and D

Condition	Spiked Conc. (CFU)	N	Mean	RSD
C	1	1	1000	N/A
C	20	1	881	N/A
C	50	1	670	N/A
D	1	1	1001	N/A
D	20	3	863	7.0
D	50	1	668	N/A
E	20	1	632	N/A
F	20	1	889	N/A
G	20	1	899	N/A

All spiked samples gave pressure events, while none of the negative controls did. However, the time to event is a function of the media being used and as a consequence of the bacteria being screened for. The three runs at 20 CFU under condition D gave pressure events on 3 different test days and using two different instruments. The range for this dataset was 120 minutes, and the percentage relative standard deviation was 7.0 percent. Results for the 20 CFU dataset under condition D were also collected by two analysts.

Sensitivity and Specificity

Table 6 and Table 7 provide the true positive and negative rates for the two conditions under which more than three spiked samples were run. Rates were not calculated for conditions where three or less spiked samples were run. True positives were spiked samples that gave pressure events within the protocol run time. True negatives were negative control samples that did not give a pressure even within the protocol run time.

Table 6: True Positive and Negative Rates of Three Concentrations under Condition A

	True Positive		True Negative	
Spiked Conc. (CFUs)	**N**	**Rate**	**N**	**Rate**
1	8	1.00	2	1.00
20	8	1.00	4	1.00
50	6	1.00	1	1.00

Table 7: True Positive and Negative Rates of One Concentration under Condition B

	True Positive		True Negative	
Spiked Conc. (CFUs)	**N**	**Rate**	**N**	**Rate**
20	14	0.93	4	1.00

All three concentrations (1, 20, and 50) analyzed under condition A gave true positive rates of 1.00 and true negative rates of 1.00. Under condition B, 1 of the 14 spiked sample runs gave a false negative, while the true negative rate was 1.00.

Limit of Detection

Table 8 provides results for the eight runs that were carried out on spiked samples with starting concentrations of 1 CFU under condition A. Limit of detection was defined as the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated under the state experimental conditions.[5]

Table 8: Limit of Detection under Condition A

Spiked Conc. (CFUs)	Time to Pressure Event (mins)	Mean (Time to Pressure Event)	SD	%RSD
1	752			
1	**799**			
1	**842**			
1	**820**	792.4	42.2	5.3
1	**789**	**812.5**	**23.5**	**2.9**
1	714			
1	793			
1	830			

Reproducible events over 4 days using all three instruments were observed when spiking samples with 1 CFU under condition A. The intermediate precision of the eight runs was 5.3 percent. Furthermore, when calculating the precision for the four runs that took place on the same day on two instruments (data in bold), a precision of 2.9 percent was determined.

[5] Definition per USP <1225>

Table 9: Results of Blinded Test

Time to Pressure Event (mins)	True Concentration	Analyst's Determination
830	1	1
709	20	20
632	100	100
–	Negative control	Negative control

Table 9 provides the data of a blinded test. After preparation of four samples (a negative control and spiked samples with concentrations of 1, 20, and 100 CFUs, respectively), they were blinded by one analyst and then run by a second analyst. The analyst running the samples was informed that there were four different samples. After running the samples, he evaluated the data and determined the blinded samples he thought corresponded to the four concentrations. Table 9 indicates that the analyst correctly identified all four samples based on the results obtained with the two Speedy Breedy instruments used.

3.3. Field Evaluation

This field evaluation reviewed two major parameters: training requirements and field utility. This work was performed in India and Zimbabwe in August 2017. India and Zimbabwe were selected because they represent two countries with vastly different regulatory environments where screening technologies have not been used extensively in the past but have the potential to be deployed effectively to combat substandard and falsified medicines.

Training Requirements

This first component of the field evaluation involved working with and training local staff in India and Zimbabwe to assess the amount of training required to enable staff to reliably and productively utilize Speedy Breedy in the field. The training involved 5 total days of work, which included 3 days of hands-on and theoretical work followed by 2 days in the field collecting and testing samples. Overall across both countries, 10 staff from the Telangana Drug Control Authority and Medicines Control Authority of Zimbabwe were trained; of these, 6 were laboratory staff (either microbiologists or chemists) and 4 were inspectors. To evaluate the perceived training timeframes for three levels of use of the instrument (basic, intermediate, and advanced), two data sources were used to develop a training timeframe requirements matrix: a survey completed by trainees following the training (Annex 2) as well as the observations of the trainer. Two variables were used to develop the matrix:

1. User experience (prior to training):
    a. *Non-technical experience*: A trainee with no prior laboratory experience and no background in one of the physical sciences (e.g., chemistry, biology).
    b. *Technical experience*: A trainee with prior experience working in a laboratory and/or a background in one of the physical sciences.
    c. *Specialized experience*: A trainee with theoretical and practical experience utilizing the technology or the technique underpinning the technology.
2. User type[6] (following training):

[6] The user type abilities build upon the previous level (e.g., an advanced user can perform the functions of an advanced user as well as a basic and intermediate user).

a. *Basic user*: A user with the ability to follow a standard operating procedure or work instruction to set up and run the instrument and collect data.
b. *Intermediate user*: A user with the ability to develop and modify methods and evaluate and interpret results.
c. *Advanced user*: A user with the ability to train other staff and perform basic troubleshooting.

Table 10 provides recommended training timeframes for trainees to reach one of three user levels—basic, intermediate, or advanced—based on the performance evaluation, field evaluation, survey given to trainees and local staff, and trainer observations.

Table 10: Training Timeframe Requirements

User Experience	User Type		
	Basic	***Intermediate***	***Advanced***
Non-technical	Between 1 day and 1 week	1 to 2 weeks	More than 2 weeks
Technical	1 day	Between 1 day and 1 week	1 to 2 weeks
Specialized	1 to 2 hours	2 to 3 days	1 week

Field Utility

The second component of the field evaluation involved running samples using Speedy Breedy in field settings and determining the utility of the instrument in these environments. It also included identifying any challenges associated with traveling with Speedy Breedy.

No problems were encountered during routine international air transportation, which included security checks and hand and checked luggage storage on long-haul flights. Travel by vehicle to various sampling sites also did not involve any challenges, and the instrument withstood temperatures between room temperature and approximately 40 degrees Celsius. One current potential limitation of transporting Speedy Breedy and maintaining the instrument is its travel case, which is currently made of cardboard. Perhaps as a result of this, upon inspection of one of the instruments prior to the commencement of training in Hyderabad, a hinge on one of the chamber lids had broken off, disabling this chamber. A spare lid had been included in the case, which was used to easily replace the broken lid with a small screwdriver. Follow-up communication with Bactest confirmed that the manufacturer is in the process of developing a robust travel case, which will include space for consumables. Throughout the course of the field evaluation work, the vendor was contacted numerous times to address concerns and questions. Communication was through email, and responses were received within 24 hours on all occasions. Furthermore, during the field evaluation a new 16-hour *E.coli* protocol was developed by the manufacturer, shared as an email attachment, and subsequently utilized during one of the training site runs (see Table 10). This protocol was not used as part of the performance evaluation, so data obtained using this protocol were not used to evaluate the analytical performance of the instrument. However, it presented an opportunity to identify any challenges associated with the deployment of a new protocol in the field. There were no challenges

encountered either in uploading the new protocol remotely or subsequently utilizing it for sample analysis.

Because there was no guarantee that contaminated samples would be found during the field work, some spiked samples, negative control samples, and regular samples were run at the training sites to demonstrate the difference between pressure events and non-events to trainees. Table 11 provides the details of these runs. Apart from the first runs in each country, all samples were prepared and run by the trainees.

Table 11: Spiked Samples Run at Training Facility in India and Zimbabwe

Run (Cbr[7])	Training Site	Sample	Spiked with	Protocol	Result
T1 (1)	India	Gentamicin inj.[8]	N/A	24h GC	No event
T1 (2)	India	Sterile WFI	N/A	24h GC	No event
T2 (1)	India	Ringer lactate solution	N/A	24h GC	No event
T2 (2)	India	Sterile WFI	*S. aureus*	24h GC	Event
T3 (1)	India	Milli-Q water	*E. coli*	16h EC	Event
T3 (2)	India	Milli-Q water	N/A	16h EC	No event
T4 (1)	India	Sterile WFI	*E. coli*	24h GC	Event
T4 (2)	India	Sterile WFI	N/A	24h GC	Event
T5 (1)	India	Sterile WFI	N/A	24h GC	Event
T5 (2)	India	Sterile WFI	N/A	24h GC	No event
T6 (1)	Zimbabwe	Sterile NaCl	N/A	24h GC	No event
T6 (2)	Zimbabwe	Sterile NaCl	N/A	24h GC	No event
T7 (1)	Zimbabwe	Sterile NaCl	N/A	24h GC	No event
T7 (2)	Zimbabwe	Sterile NaCl	N/A	24h GC	No event
T8 (1)	Zimbabwe	Tap water	N/A	24h GC	Event
T8 (2)	Zimbabwe	Bottle water	N/A	24h GC	Event
T9 (1)	Zimbabwe	Tap water	N/A	24h GC	Event
T9 (2)	Zimbabwe	Bottle water	N/A	24h GC	Event
T10 (1)	Zimbabwe	Artesunate injection[8]	N/A	24h GC	Event
T10 (2)	Zimbabwe	Artesunate injection[8]	N/A	24h GC	No event

Although several unexpected results were obtained during these runs, notably runs T2(2), T4(2), and T5(1), no results for either the training site runs or field evaluations were used for the performance evaluation data analysis, as conditions for these runs were deliberately uncontrolled. The purpose of these runs was to determine whether trainees could operate the instrument and whether the instrument could operate in true field settings.

In follow-up to the trainings, Speedy Breedy units were taken to pharmacies and rural retail outlets, as well as a parenteral manufacturer where samples were run overnight due to protocol run times. Table 11 provides details of these runs. None of these samples were spiked.

Table 12: Samples Run at Field Sites in India and Zimbabwe

Run (Cbr)	Training Site	Location	Sample	Protocol	Result

[7] Cbr = chamber

[8] Due to small sample unit volumes, these samples were filled to 50 mL with either sterile water for injection (India) or sterile NaCl (Zimbabwe) to enable analysis.

F1 (1)	India	Rural health outpost	Metronidazole inj.[8]	24h GC	No event
F1 (2)	India	Rural health outpost	Ciprofloxacin inj.[8]	24h GC	No event
F2 (1)	India	Parenteral Mfr	Sterile NaCl	24h GC	Event
F2 (2)	India	Parenteral Mfr	Purified water	24h GC	Event
F3 (1)	Zimbabwe	Retail Pharmacy	Metronidazole inj.[8]	24h GC	No event
F3 (2)	Zimbabwe	Retail Pharmacy	Metronidazole inj.[8]	24h GC	No event

Although no malfunctions were encountered as a result of the instruments, several external factors led to some invalid tests, which were subsequently repeated. These factors are listed below:

- At one facility, a power interruption early during a run meant the analysis needed to be repeated the next day.
- At another facility, a ciprofloxacin injection was analyzed. Review of the results the next day showed a foamy solution with what appeared to be some precipitate. A pressure change had taken place early during the run but, as the system had not stabilized, an event was not recorded. The 24-hour general contamination test protocol had been run using TSB.

4. Review and Conclusions

4.1. Performance Evaluation

Conditions A and B had 29 and 16 data points, respectively, which have been used to draw general conclusions about the functionality of Speedy Breedy as a screening technology. As mentioned earlier, these two conditions were chosen because the authors felt water for injection presented an excellent initial case study sample considering its breadth of use globally. Furthermore, *E.coli* is one of the common microorganisms found in contaminated water. However, data were collected for five other conditions, including two additional samples, an additional media vessel, and bacteria (see Table 1). Additional protocols and media vessels exist for other common bacteria, enabling targeted contamination detection based on the previous experience of prospective users.

Condition A results (see Table 2) included samples with six different starting concentrations of bacteria, as low as 1 CFU and as high as 1,000 CFU. Three different instruments were used to collect data over the course of 16 days of work. All 29 spiked samples run under condition A gave pressure events reflected in the 1.00 true positive rates seen in Table 6. Furthermore, none of the negative controls gave pressure events leading to true negative rates of 1.00, as seen in Table 5, implying no false positive results. Although samples with starting concentrations of 10, 100, and 1,000 CFU were excluded from the true positive and true negative calculations because only two, three, and two runs were conducted for these concentrations, respectively, their true positive and negative rates were also 1.00. Table 8 also shows that the instrument could reliably detect contamination down to a starting concentration of 1 CFU. Although the %RSD was quite low for the eight runs (5.3 percent) and even lower for the three runs taking place on the same day (2.9 percent), putting the results into the context of the instrument's possible field use, the important point is that all eight runs resulted in a pressure event.

Condition B results (see Table 4) included samples with three different starting concentrations of bacteria; 1, 20, and 50 CFU. Only one run was conducted for starting concentrations of 1 and 50 CFU. However, three different instruments were used to collect data over the course of 3 days of work at the starting concentration of 20 CFU. Under condition B, 13 of the 14 runs gave pressure events, reflecting a true positive rate of 0.93. None of the negative controls gave pressure events. This analysis indicates that Speedy Breedy seems to reliably and reproducibly detect various level of *E.coli* contamination in water for injection using the MCC and TSB media vessels.

A review of the quantitative capabilities of the instrument was not performed, as any detectable contamination in a sterile product means it is no longer sterile. However, the Pearson's correlation coefficient (R^2) was calculated using the data collected under condition A (see Table 3) and gave an R^2 of −0.95. These data indicate that there is a linear inverse relationship between the log-transformed amount of contamination and the time to detection. These data, coupled with the low %RSDs seen for those samples run at least six times under condition A, provide some basis for the possibility that semi-quantitative protocols could be developed in situations where knowledge of the concentration of contamination is needed.

Of the four remaining conditions, under which data were collected, only the 20 CFU starting concentration for condition D had more than one result. However, each of the spiked samples for conditions C, E, F, and G, including those of condition D, gave a pressure event, and no pressure event was observed for any of the negative controls; this indicates that at least two additional sample matrices do not seem to inhibit the ability of Speedy Breedy to detect contamination.

Lastly, to mimic what a user would encounter in the field, one analyst was given four blinded samples to analyze (see Table 9); three of these were contaminated (at starting concentrations of 1, 20, and 50 CFU), and one was not. Using the results, the analyst was able not only to identify the three contaminated samples but also to correctly identify their relative concentrations based on their times to detection.

4.2. Field Evaluation

Based on feedback from trainees and the ongoing observations of the trainer, the training required to become a basic, intermediate or advanced user of the instrument was manageable. More specifically, a variety of staff with both technical and non-technical backgrounds can become either basic, intermediate or advanced users within approximately 2 weeks of training. The software was easy to download onto a PC and intuitive to use. Additional work by Bactest could assess the feasibility of developing a smartphone application to enhance the field utility of the instrument. Furthermore, as advanced users continue to refine their deployment of Speedy Breedy, and in environments where the supply chain is a particular challenge, users can develop their own media using empty aerobic or anaerobic vessels. Protocols include the option of a pasteurization cycle, which increases the heat of the vessels to 65 degrees Celsius for several hours post-run, ostensibly killing any non-spore-forming bacteria within a sample, aiding in the disposal of the media. Particularly in field settings where biological waste containers may not be available, this is a very useful feature. It is important to note, however, that the pasteurization cycle does not kill spore-forming bacteria or extreme thermophiles. Vessels must still be disposed of according to local regulations.

Although Bactest has 43 distributors globally, currently there are only 2 in low- or low-middle-income countries. This could present problems particularly in countries where shipping and import delays are common. However, media vessels and an instrument were shipped from Bactest headquarters to Zimbabwe in preparation for the field evaluation and arrived within a week of the order being confirmed. Furthermore, the technical support provided by Bactest during the field evaluation was prompt and efficient.

Some challenges were encountered during the field evaluation. The existing carry case is made of cardboard and not particularly sturdy, which could limit the lifespan of the instrument if it is being used in challenging environments. Moving forward, a pelican case would help preserve the integrity of the instrument and may perhaps have prevented one of the chamber lids from breaking. Additionally, at this stage, media vessels do not have a mark identifying where 50 mL of solution is. This would be particularly useful in situations where graduated syringes are not available or samples are transferred directly into vessels. Related to this, vessels are currently only available in 50 mL volumes. For small volume samples, this presents both an opportunity and a challenge. It provides the potential for pooling samples, for example combining 10 5-mL injectable samples to increase throughput in each chamber. If an event is detected, then the samples can be broken into smaller sample sets to ultimately identify the contaminated product. However, decreasing the number of samples being run decreases the total volume in a vessel for a run, which currently needs to be 50 mL. Researching the possibility of developing smaller volume vessels would allow users to customize their systems. For example, hospital pharmacies working predominantly with 1-L sterile saline bags would have no problem using the current 50-mL vessels. However, inspectors working in rural areas, where the samples in a health outlet are limited and generally small, volume injectables could use smaller volume vessels rather than risking a false positive by filling the sample to volume with bottled water.

Although unavoidable, the run time of protocols does limit the effectiveness of the instrument in true field settings where reliable power may not exist even though analysis times are significantly shorter than current confirmatory sterility testing procedures. This was reiterated by several trainees, one of whom suggested “a rechargeable battery or solar power source” as a solution. The current configuration of only two chambers also limits the sample throughput of the instrument. However, sample pooling is one possible solution to mitigate this limitation, and increasing the number of chambers would commensurately increase the footprint of the instrument, which currently is small, light, and easy to transport.

References

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[4] USP Review of Surveillance and Screening Technology for the Quality Assurance of Medicines Expert Panel, "Stimuli to the Revision Process: Evaluation of Screening Technologies for Assessing Medicine Quality (USP PF 43 (5))," September 2017. [Online]. Available: http://www.usppf.com/pf/pub/index.html. [Accessed 29 November 2017].

Annex 1. Equipment, Consumables, Samples and Supplies Used during Performance Evaluation

Item	Manufacturer	Expiry Date	Other details
Speedy Breedy – unit 1	Bactest	N/A	Serial No: 40-D8-55-00-60-E4
Speedy Breedy – unit 2	Bactest	N/A	Serial No: 40-D8-55-00-60-EB
Speedy Breedy – unit 3	Bactest	N/A	Serial No: 40-D8-55-00-60-DF
CB media vessel	Bactest	12 October 2017	Part No: BAC023-1
MCC media vessel	Bactest	11 December 2017	Part No: BAC022-1
TSB media vessel	Bactest	12 October 2017	Part No: BAC021-1
Sterilized water for injection (WFI 1)	Marck Bioscience Ltd.	March 2018	Batch No: 2T545054
Sterilized water for injection (WFI 2)	Nirma Ltd.	November 2017	Batch No: 2501112
Glunate 60 mg Artesunate for injection (AS 1)	Guilin Pharmaceutical Co. Ltd.	23 March 2018	Batch: LA150556
Syntocinon 10 IU/UI (OXY 1)	Novartis	October 2020	Batch No: SO634
Analytical balance	Mettler Toledo	N/A	Model: ML3001E
Autoclave	All American	N/A	Model:75X
Fridge	Thermo Scientific	N/A	Model: Revco
Incubating shaker	Eppendorf	N/A	Model: New Brunswick
Incubator	Lovibond	N/A	Model: TC175S
UV-Vis spectrophotometer	Merck	N/A	Model: Pharo 300
Escherichia coli	ATCC (8739)	30 April 2018	Batch No: 61726100
Pseduomonas aeruginosa	ATCC (9027)	31 July 2020	Batch No: 61461178

Annex 2. TR Field Evaluation Training Survey

Q1 - Which of the following roles best represents your current position?

#	Answer	%	Count
1	Analyst / Chemist / Microbiologist	66.67%	6
2	Inspector	33.33%	3
3	Customs officer	0.00%	0
4	Other. Please specify	0.00%	0
	Total	100%	9

Q2 - Please indicate to what extent you agree with the statements below.

#	Question	Strongly disagree		Somewhat disagree		Neither agree nor disagree		Somewhat agree		Strongly agree		Total
1	I better understand how this technology can be used in my work after this training.	0.00%	0	0.00%	0	0.00%	0	33.33%	3	66.67%	6	9
2	I better understand the basics of operating this screening technology after this training.	0.00%	0	0.00%	0	0.00%	0	11.11%	1	88.89%	8	9
3	I better understand the theory (e.g. analytical technique such as spectroscopy) underpinning this screening technology after this training.	0.00%	0	0.00%	0	11.11%	1	11.11%	1	77.78%	7	9
4	I feel more confident preparing and analyzing samples using this screening technology after this training.	0.00%	0	0.00%	0	0.00%	0	11.11%	1	88.89%	8	9
5	I feel more confident interpreting the results obtained using this screening technology after this training.	0.00%	0	0.00%	0	0.00%	0	33.33%	3	66.67%	6	9

Q3 - Please indicate to what extent you agree with the statements below.

#	Question	Strongly disagree		Somewhat disagree		Neither agree nor disagree		Somewhat agree		Strongly agree		Total
1	The training provided sufficient time to understand the basics of operating this screening technology.	0.00%	0	0.00%	0	0.00%	0	11.11%	1	88.89%	8	9
2	The length and level of detail covered in this training would be sufficient to train colleagues in similar professional positions to myself.	0.00%	0	0.00%	0	0.00%	0	22.22%	2	77.78%	7	9
3	I feel confident teaching someone else how to use this screening technology.	0.00%	0	0.00%	0	0.00%	0	22.22%	2	77.78%	7	9
4	I think this technology would be valuable in helping me carry out aspects of my work related medicine sampling and testing.	0.00%	0	0.00%	0	0.00%	0	44.44%	4	55.56%	5	9

Q4 - In your opinion, how long would you need training on this screening technology to be for you to become a basic user?

#	Answer	%	Count
1	Less than one day	44.44%	4
2	Between one day and one week	55.56%	5
3	More than one week	0.00%	0
	Total	100%	9

Q5 - In your opinion, how long would you need a training on this screening technology to be for you to become an advanced user?

#	Answer	%	Count
1	Less than one day	0.00%	0
2	Between one day and one week	77.78%	7
3	More than one week	22.22%	2
	Total	100%	9

Q6 - In your opinion, how long would you need a training on this screening technology to be for you to be able to train colleagues?

#	Answer	%	Count
1	Less than one day	0.00%	0
2	Between one day and one week	88.89%	8
3	More than one week	11.11%	1
	Total	100%	9

Q7 - Are there any additional comments you have regarding the training and/or screening technology?

Good timing of the training
The training was very effective and it gave us an opportunity to learn how important these technologies are
the device should be equipped with rechargeable batteries such as in mobile devices to make it easier to use especially if one is in remote places where access to shops to procure the AA batteries may be limited