

MIT 1000 Rapid Microbial Identification System User Guide



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Introduction

Congratulations on purchasing the revolutionary MIT 1000 System. This instrument uses the principles of light scattering to identify a variety of bacteria in less than five minutes.

Purpose of This Guide

This Guide describes how to prepare, operate and troubleshoot the MIT 1000 Rapid Microbial Identification System.

Audience

This Guide is for users, technicians, microbiologists, scientists and researchers of all skill levels who will use and maintain the MIT 1000 System.

Assumptions

This Guide assumes the User is familiar with:

- Basic Microsoft[®] Windows[®] operations such as using a mouse, choosing and selecting commands, working with Windows
- Basic computer set-up skills that include connecting power module and USB cable to the appropriate ports on the computer
- Basic laboratory techniques and has a basic microbiological background

Conventions Used In This Guide

This Guide uses the following conventions:

- <u>Notes are underlined</u> and provide useful information or information that requires special emphasis.
- Warnings are in yellow and provide information to help avoid injuries to the User, damaging the equipment, or reducing the System's performance.
- When using the MIT 1000 Application, items that you select or type are shown in **bold**.

User Guide Organization

This Guide is organized as follows:

- Introduction Describes the major features and benefits of the MIT 1000 System.
 - Chapter 1 Getting Started provides a list of components and materials included with the MIT 1000 System and those standard laboratory equipment and materials needed to complete an Identification Test.
 - Chapter 2 Quick Reference Operation Summary provides a quick reference guide to operate the System.
 - Chapter 3 Describes the features of the MIT 1000 Application.
 - Chapter 4 MIT Vial Cleaning and Storage
 - Chapter 5 Water Quality Test
 - Chapter 6 Sample Preparation
 - Chapter 7 Performing an Identification Test
 - Chapter 8 Troubleshooting
- Appendix A MIT Products
- Appendix B Sample Test Report
 - Glossary



Customer Support

If the System appears to be operating incorrectly or you have specific operational questions, please contact Customer Service at <u>service@micro-identification.com</u> or call us at 949-388-4546.

Precautions

The MIT 1000 System is a universal system designed to identify various bacteria, many of which are pathogenic. Take extreme care in handling all specimens, microbial cultures and inoculated MIT Vials. Aseptic technique and usual precautions for handling the bacteria group should be observed throughout the Identification Test process. Ensure all biohazardous waste is disposed of appropriately.

Environment

This System operates on various power services (110/220 VAC, 50/60 Hz, 15/10 amps), requires no reagents or chemicals, has a small footprint of 1.25 cubic feet and weighs less than 25 pounds. The software included in the System operates on a Windows 7 platform in a personal computer.

MIT recommends that the MIT 1000 be placed in an environment that does not exceed the following parameters.

Environmental Factor	Temperature Range	Relative Humidity	Altitude	Vibration
Operating	41°F to 95°F (5°C to 35°C)	20% to 90% (non-condensing)	Sea level to 10,000 ft (3 km)	Vibration sensitive. Do not operate near fans or motors. Normal keyboard and mouse activities are OK.
Non-Operating	-4°F to 140°F (20°C to 60°C)	95% (non-condensing)	Sea level to 10,000 ft (3 km)	

The System uses MIT's proprietary MIT 1000 Application software and Identifiers[™] to identify organisms. Updates of the MIT 1000 Application software and a list of available Identifiers is available on the MIT web site: www.micro-identification.com.

MIT offers all accessories (i.e. additional test vials, culturing media, pipettes and other lab supplies) necessary to operate the System. Should you wish to purchase any of these items, please contact your sales representative or contact us directly via email at <u>service@micro-identfication.com</u> or telephone us at 949-388-4546.



Chapter 1: Getting Started

You will receive the MIT 1000 System in two boxes.

Inside the Large Box

The large box contains the MIT 1000 System and Starter Kit. The following items are included in the large box:

- MIT 1000 System
- Power Supply Module
- USB Cable
- Key
- Starter Kit The starter kit provides the items needed to immediately use the MIT 1000 System. The kit includes the following items:
 - o Twelve 0.6 mL micro-centrifuge tubes
 - o Twenty-five sterile inoculating loops
 - o Six MIT Vials¹
 - o 10 MIT Vial Caps
 - o 12 Eppendorf 0.5 10 μL pipette tips
 - o 15 2" x 2" Parafilm squares
 - o One MIT Vial Holder²
 - o 15 #10 rubber bands
 - o One small vial of 1.59 micron PSL sphere suspension

Inside the Small Box

The small box contains the MIT 1000 Mini Laptop computer. The following items are included in the small box:

- The MIT 1000 Mini Laptop computer
- Computer battery
- Power module

1.59 Micron PSL Sphere Suspension Referigeration

Immediately refrigerate the 1.59 micron PSL sphere suspension.

¹ MIT Part Number 20007

² MIT Part Number 30003 is used for both vial cleaning and ID Test preparation



System Set-up Connect the MIT 1000 System, Personal Computer and External Power Supply as shown in the diagram below:



Warning The MIT 1000 System uses a Class IIIb laser with an output of up to 45 milliwatts. Direct eye contact with the laser beam may cause damage to your eye!



Equipment and Materials Required For an Identification Test

Equipment Required

- MIT Vial holder (MIT Part Number 30003)
- Vortex mixer (See specifications in Appendix B)
- Pipette 0.5µL to 10 µL (See specifications in Appendix B)
- Incubator
- Autoclave
- BioSafety Cabinet

Material Required

- Low Particle Filtered (LPF) water³
- 0.6 mL micro-centrifuge tube See specifications in Appendix B
- Parafilm See specifications in Appendix B
- 1 µL sterile disposable loop See specifications in Appendix B
- #10 rubber band
- Kimwipes
- Pipette tips See specifications in Appendix B
- MIT Vial (MIT Part Number 20007)
- 70% IPA
- 10% solution of Distilled White Vinegar⁴
- Powder free gloves
- Trypticase Soy Agar with 5% sheep blood or BHI culture plate
- Biohazard disposal container

³ Low Particle Filtered Water. Typically, this water is the product of a water filtering system with a final filter of 0.1 micrometers absolute or smaller. The MIT 1000 Water Test function is the final decider on whether or not water can be used for Identification tests with the MIT 1000 System. ⁴ 5% to 8% acetic acid in water with a pH of about 2.4. Available at most grocery stores.



Chapter 2: Quick Reference Identification Test

- 1. Clean and rinse a MIT Vial with LPF water, see Chapter 4.
- 2. Add about 15 mL of LPF Water to the MIT Vial as shown in Figure 20 on page 29. The water level must new the middle of the vial neck.
- 3. Cover with Parafilm and secure with rubber band.
- 4. Clean and rinse a 0.6 mL micro-centrifuge tube with LPF water.
- 5. Fill the micro-centrifuge tube to about 0.2 mL with LPF water.
- 6. Remove a single colony from a culture plate with a 1-µL sterile inoculating loop.
- 7. Dislodge the colony material on the loop into the water in the micro-centrifuge tube.
- 8. Close the micro-centrifuge tube
- 9. Vortex the micro-centrifuge tube
- 10. Remove the rubber band and gently lift the Parafilm covering the MIT Vial and pipette 1- μ L from the micro-centrifuge tube into the MIT Vial.
- 11. Re-cover the MIT Vial with Parafilm and replace the rubber band.
- 12. Lift up the tails of the Parafilm to get the Parafilm off the neck.
- 13. Wipe the round bottom of the MIT Vial with a Kimwipe dampened with 70% IPA.
- 14. Wipe dry the round bottom of the MIT Vial with a second Kimwipe.
- 15. Place the MIT Vial in the MIT 1000 System.
- 16. Start the MIT 1000 Application.
- 17. Click the **Identifiers** button and select the appropriate Identifiers[™] to use for the identification test.
- 18. Select IDENTIFY.
- 19. Fill in the Sample Identification form.
- 20. Click **OK**.



Chapter 3: MIT 1000 Application

There are three ways to start the MIT 1000 Application:

- Double click the **MIT 1000 Application** icon (Figure 1) on the desktop.
- Right click on the desktop MIT 1000 Application icon and select the Open menu item.

Click on MIT 1000 Application icon in the MIT folder in the All Programs



Figure 1 – MIT 1000 Application Icon

MIT 1000 Application Display

menu under the Start button.

•

The MIT 1000 Application Display as shown in Figure 2 and 3 appears after the MIT 1000 Application has started.



Figure 2 – MIT Central Control Display

The six buttons on the left side of the Display provide an easy method to configure the MIT 1000 Application. They provide the User with the capability to troubleshoot, review test log data, configure the MIT 1000 System and connect to the MIT 1000 website for technical updates and other announcements.

- Click the Identifiers button to select the Identifiers[™] to use for Identification Testing.
- Click the **User Guide** button to display this User Guide.
- Click the Troubleshooting button to display the Troubleshooting Guide.
- Click the Log button to display the Sample Test Log.
- Click the **Configure** button to configure the MIT 1000 Application.
- Click the **MIT Web** button to display the Micro Identification Technologies web page: <u>www.micro-identification.com</u>.





Figure 3 – MIT Central Control Display

The System operation (Figure 3) status is shown on the right side of the Display:

- The **Power** indicator is green when the MIT 1000 System is powered on and shows no color when the power is off.
- The MIT Cloud indicator is green when the MIT 1000 Application is connected to the MIT 1000 Cloud⁵ and flashed red when it is not connected.
- The **Ready** indicator is green when the MIT 1000 Application is properly configured and the MIT 1000 System is connected and operating properly. It blinks red when a configuration or connection error occurs.
- The Active Identifiers pane in the middle of the Display shows the Identifier[™] that will be used during an Identification Test.
- Clicking the **Identify** button begins the identification process. <u>Note, once the **Identify** button is</u> <u>clicked, the Sample Information form will appear. The User must enter the required information</u> <u>before the Identification Test can start</u>.
- Clicking the **Water Test** button begins the Water Quality test. Water that does not pass this test MUST NOT be used for Identification Tests.
- Clicking the **Close Program** button will terminate the MIT 1000 Application.

⁵ The MIT 1000 Application MUST be connected to the MIT Cloud to operate.



MIT 1000 Application Display Button Functions

License

Upon clicking the **Identifers**⁶ button (Figure 2), the Identifiers Form shown in Figure 4 will appear.

The upper list, under the "Licensed Identifiers" caption, lists all the IdentifiersTM for which you have a current license to use. The MIT 1000 System cannot be used to identify a specific species or genus unless the license for the IdentifierTM for that species or genus is current. Visit the MIT website for a complete list of available IdentifiersTM and to purchase a license to use them.

The MIT 1000 System will identify only those bacteria that are in the bottom list below the Active Identifiers caption. The User must select the IdentifiersTM that are to be active for Identification tests.

In the example shown in Figure 4, the System has current license to the following Identifiers[™]:

- E. coli
- Listeria spp
- Salmonella spp



Figure 4 – License Display

To use a specific IdentifierTM during an Identification test, simply double-click the desired IdentifierTM in the Licensed Identifiers list or highlight the IdentifierTM and then click the Down (\downarrow) Arrow. This moves the IdentifierTM to the Active Identifiers list. The Active Identifiers list can contain more than one IdentifierTM. The MIT 1000 System will test for the presence of species and genus associated with all selected IdentifiersTM during a single Identification test. In the example in Figure 4, the *E. coli* and *Listeria* spp IdentifiersTM are selected for an Identification Test.

To remove an IdentifierTM from the Active Identifiers list, simply double click it or select it by clicking on it and then click the Up (\uparrow) Arrow.

⁶ For specific installation, operation and precautionary statements please refer to the Insert that accompanied the IdentifierTM.



To purchase additional licenses, contact your local representative or Customer Service.

Logs and Reports

Sample Test Log

The MIT 1000 Application records all Identification Test activities. Click **Log** in the MIT 1000 Application Display to view the Sample Test Log, Figure 5. Maximizing the window enables the User to view more rows and columns of the Log. Figure 6 below shows a partial view of the Sample Test Report that can be viewed and printed for each entry in the Sample Test Log.



Figure 5 – Sample Test Log Display



Recorded Log Information

Log entry	Source of information	Definition		
Test #	MIT 1000 Application	A unique index assigned to an Identification Test by the MIT 1000 Application program.		
Time	MIT 1000 Application	The time and date the Identification Test started.		
Sample	User	Sample name or type.		
Result	MIT 1000 Application	The result of the Identification Test.		
Gram Stain	User	The result of a Gram Stain performed on the sample. The possible values are: Not done GNR : Gram Negative – Rod GPR : Gram Positive – Rod GNC : Gram Negative – Coccus GPC : Gram Positive – Coccus		
Customer Name	User	The organization or company that provided the sample being tested.		
Project Number	User	The Project Number assigned to the purchase order or work order.		
PO Number	User	The Purchase Order number for the work being performed.		
Task Number	User	The Task Identifier in the Project.		
Lot Number	User	The Lot Number of the item(s) being tested for the selected Task.		
Identifiers	MIT 1000 Application	The Identifiers TM that were used during the Identification Test.		
User	User	The User that performed the Identification Test.		
Media	User	The culture media used for bacteria growth.		
Media Lot	User	The lot number of the culture media.		
Media Expiration	User	The expiration date of the culture media.		
Incubation Temp	User	The temperature used for bacteria growth.		
Incubation Start	User	The date and time the incubation period started.		
Incubation End	User	The date and time the incubation process ended.		
Termination	MIT 1000 Application	The reason the Identification Test was terminated, e.g., high concentration, low concentration, etc.		

Table 1 - Log Entry



Finding a Test in the Sample Test Log

The sample Test Log provides two independent methods of finding the tests: By date and by Sample.

By Date

Click the **Year** the test was performed in the Year list in the upper left corner of the form. Then click the date the test was performed in the **Date** list next to the Year list.

After each year and date selection, all tests performed on the selected date will be listed.

By Sample

- First, select the client name in the Customer list. All Project and PO numbers associated with this client will be listed below the Project No. – PO No caption.
- Select the Project and PO Numbers associated with the test. All Task and Lot Numbers associated with this Project and PO Number will be listed below the Task No. – Lot No. caption.
- Select the Task and Lot Numbers associated with the test. All Samples measured associated with the Task and Lot Numbers will be listed below the Sample Name caption.
- Select the Sample Name associated with the test.

After each selection, all tests performed for the selected Sample job information will be listed.

Test Report

The MIT 1000 Application can create a test report for each test in the log as shown in Figure 6. To display the report, find the test in the Sample Test Log as described above.

Then either:

• Click on the test entry and click on **Show Report** at the bottom of the page. The Show Report button is visible only when a test is selected.

OR

• Double-click on the test entry.

The Test Report Form appears, Figure 6.

Test Report			
	MIT Foods		
System #: 000020	Laboratory Report	Printed: 10/23/12 10:42	
Customer:	MIT Foods		The report header is
Project Number:	Burrito		determined by the text shown
PO Number:	12-MITF		in the Header Text hav
Task Number:	002-Burrito		
Lot Number:	2		
Sample Identifier:	Cooking-2	1	To change the header text.
lest #:	000211		
Identification Result:	Listeria spp		enter the new neader in the
			Header Text box then click
Commonte			Apply
<u>comments</u> .			,
Selected Identifiers:			
E, coli	Listeria spp		
Salmonella spp			
		· /	
Test Details:			
Test Time:	2012-10-23 09:40:37		
User:	G. Kessler		
Termination:	Normal		
Sample Details:			
Gram stain result:	Not performed		
1			
Header Text MIT Foods	Ap	bly	Click Print to print a copy
Drint		01	choix i finit to print a copy.
Print		OK	

Figure 6 – Sample Test Report – Partial View



Note: A full view of the Sample Test Report is shown in Appendix C.

Test Report Header

The text in the Header Text box will be displayed at the top of the Test Report. Follow the procedure below to customize the header:

- Enter the header text in the Header Text box and then click Apply.
- To remove the header, delete the text in the Header Text box and then click **Apply**.



Configuring the System

Click the **Configure** button to display the MIT 1000 Configuration Options Form as shown in Figure 7. A description of the options is shown in Table 2.



Figure 7 – Configure Form



General Function Descriptions:

MIT 1000 S/I	N	This is the serial number of the MIT 1000 System. This MUST match the six-digit serial number located on the back of the System. The MIT 1000 Application automatically fills in this value.		
Chime on C	ompletion	Checking this option causes the System to sound a chime when the Identification Test or Water Test is complete.		
User Termination Reason Checking this option will require that the User specify a reaterminating an Identification Test.				
Required Log Entries	Media	Checking this option will require that the User specify the culture media used to grow the bacteria when an Identification Test is performed.		
	Media Lot Number	Checking this option will require that the User specify the lot number of the media used to grow the bacteria when an Identification Test is performed.		
Media Expiration DateChecking this option will require expiration date of the media used to when an Identification Test is perforIncubation TemperatureChecking this option will require temperature the bacteria being test		Checking this option will require that the User specify the expiration date of the media used to grow the bacteria being tested when an Identification Test is performed		
		Checking this option will require that the User specify the temperature the bacteria being tested was incubated.		
	Incubation Time	Checking this option will require the User to specify the incubation start and stop time and date for the bacteria being tested.		

 Table 2 – General Function Descriptions

Note: The Diagnostic Tools are for MIT Technical Representative usage only.



Medium Configuration

To configure the **Medium**, follow the instructions in Table 3.

Medium	List box	The Medium list box shows the available options that the User can select prior to starting an Identification Test and the Required Log Entries – Media check box is selected.		
		The MIT 1000 Application comes preconfigured with two predefined entries:		
		BHI Brain Heart Infusion, and		
		TSA w/5% SB Trypticase Soy Agar with 5% Sheep blood.		
	New	Click New to add a new media selection option.		
		When the Media Name Form (Figure 8) appears, fill in a name for the new Media. Then click OK to accept the new Media or click Cancel to close the form without accepting the new Media.		
	Edit	Click Edit to modify the name of the selected media.		
		When the Media Name Form (Figure 8) appears, the current Media Name is filled in. Modify the name and click OK to accept the change or click Cancel to close the form without accepting the edit.		
		For example, "TSA with 5% SB" can be changed to "Blood agar."		

Table 3 – Media Configuration Options

Warning The MIT 1000 System currently only supports Tryptic Soy Agar with 5% sheep blood and BHI.

Upon clicking the **New** or **Edit** button associated with **Media**, the Form shown in Figure 8 will open:







User Configuration:

To add a User's Name, follow the instructions in Table 4 below.

User	List box	The User list box shows the available names that the User can select prior to starting an Identification Test.	
	New	Click New to add a new User option.	
		When the User Name Form (Figure 9) appears, fill in a name for the new User. Then click OK to accept the new User or click Cancel to close the form without accepting the new User.	
	Edit	Click Edit to modify the User name.	
		When the User Name Form (Figure 9) appears, the current User Name is filled in. Modify the name and click OK to accept the change or click Cancel to close the form without accepting the change.	
		For example, "Susan B. Anthony" can be changed to "SBA."	

Table 4 – User Configuration Options

Upon clicking the **New** or **Edit** button associated with **User**, the Form shown in Figure 9 will open:



Figure 9 – User Name Information Entry

Customer Configuration

To input **Customer** information, follow instructions in Table 5 below:

Customer	List box	Click the Down Arrow to show the list of Customers that have already been entered. Selecting a Customer will allow the entered Projects for that Customer to be viewed or changed, and new Projects added. The selected Customer is also shown in the lower left of the form.	
	New Click New (right of the Customer list box) to add a new Customer to the		
		When the Customer Form (Figure 10) appears, fill in the Customer Name and enter any comments or notes on the Customer. Then click OK to accept the new Customer or click Cancel to close the form without accepting the new Customer.	
Edit Click Edit (right of the New button) to mod		Click Edit (right of the New button) to modify the selected Customer.	
		When the Customer Form (Figure 10) appears, the Customer Name and Notes fields will be fill in with the selected Customer's information. Modify the fields as needed and click OK to accept the changes or click Cancel to close the form without accepting the changes.	

Table 5 – Customer Configuration Options



Upon clicking the **New** or **Edit** button associated with **Customer**, the Form shown in Figure 10 opens.



Figure 10 – Customer Information Entry

Project Configuration

To input a **Project** Name, follow the instructions in Table 6:

Project	List box	Click the Down Arrow to show the list of Projects that have already been entered for the selected Customer. Selecting a Project will allow the entered Tasks for that Project to be viewed or changed, and new Tasks added. The parameters for the selected Customer and Project are shown in the lower left of the form.
	New Click New (right of the Project List box) to add a new Project selected Customer to the list.	
		When the Project Form (Figure 11) appears, fill in the Project Number and/or Purchase Order Number and enter any comments or notes on the Project. Then click OK to accept the new Project or click Cancel to close the form without accepting the new Project.
	Edit	Click Edit (right of the New button) to modify the selected Project.
		When the Project Form (Figure 11) appears, the Project Number, PO Number, and Notes fields will be filled in with the selected Project's information. Modify he fields as needed and click OK to accept the changes for click Cancel to close the form without accepting the changes.

Table 6 – Project Information Entry



Upon clicking the **New** or **Edit** button associated with a Project, the Form shown in Figure 11 will open.

Project 💌	To add a new or edit the Project:
Project Number	 Enter the new Project Number
PO Number	and/or Enter a Purchase Order Number Enter any notes or comments. Click OK to accept the new entry,
Notes	or click Cancel to close the dialog
Cancel OK	without accepting the new entry.

Figure 11 – Project Information Entry

Task Configuration

To input a Task Name, follow the instructions in Table 7:

Task	List box	Click the Down Arrow to show the list of Tasks that have already been entered for the selected Customer and Project. Selecting a Task will allow the entered Samples for that Task to be viewed or changed, and new Samples added. The parameters for the selected Customer, Project, and Task are shown in the lower left of the form.
	New	Click New (right of the Task List box) to add a new Task for the selected Customer's Project to the list.
		When the Task Form (Figure 12) appears, fill in the Task Number and/or Lot Number and enter any comments or notes on the Task. Then click OK to accept the new Task or click Cancel to close the form without accepting the new Lot Number.
Edit Click Edit (right of the New button) to n		Click Edit (right of the New button) to modify the selected Task.
		When the Task Form (Figure 12) appears, the Task Number, Lot Number, and Notes fields will be filled in with the selected Task's information. Modify the fields as needed and click OK to accept the changes or click Cancel to close the form without accepting the changes.

Table 7 – Task Configuration Options



Upon clicking the **New** or **Edit** button associated with **Task**, the Form shown in Figure 12 will open:

Task 🗨	To add a new or edit the Task:
Task Number	 Enter the new Task Number and/or Enter a Lot Number Enter any notes or comments. Click OK to accept the new entry, or click Cancel to close the dialog without accepting the new entry.

Figure 12 – Task Entry Information

Sample Configuration

To input a **Sample** Name and other related information, follow the instructions in Table 8:

Sample	List box	Click the Down Arrow to show the list of Samples that have already been entered for the selected Customer, Project, and Task. The parameters for the selected Customer, Project, Task, and Sample are shown in the lower left of the form.		
	New	Click New (right of the Sample List box) to add a new Sample for the selected Customer's Task to the list.		
		When the Sample Form (Figure 13) appears, fill in the Sample Name and enter any comments or notes on the Sample. Then click OK to accept the new Sample or click Cancel to close the form without accepting the new Sample.		
	Edit	Click Edit (right of the New button) to modify the selected Sample. When the Sample Form (Figure 13) appears, the Sample Name and Notes fields will be filled in with the selected Sample's information. Modify the fields as needed and click OK to accept the changes or click Cancel to close the form without accepting the changes.		

 Table 8 – Sample Configuration Options



Upon clicking the **New** or **Edit** button associated with Sample, the Form shown in Figure 13 will open:



Figure 13 – Sample Entry Information

Show in the Lab Test Report

The Lab Test Report always shows the name of the Sample tested. In addition, the User has the option of displaying the Customer Name, Project Name, PO Number, Task Number and Lot Number on the Lab Test Report simply by checking the appropriate box in the Configure Form as shown in Figure 14.



Figure 14 – Configure Form – Show in Lab Report



Chapter 4: MIT Vial Cleaning and Storage

Sterilizing the MIT Vial

Items Needed

- Autoclave
- Contaminated MIT Vial
- Spray bottle with 5% bleach
- Kimwipe
- Biohazard Bin with Biohazard Bag
- MIT Vial Holder

Procedure

- 1. Place a Kimwipe on the tabletop.
- 2. Soak the Kimwipe with 5% bleach.
- 3. Remove the Parafilm and rubber band.
 - a. Deposit the contaminated Parafilm into the Biohazard Bag.
 - b. Place the rubber band from the contaminated MIT Vial onto the 5% bleached soaked Kimwipe.
- 4. Carefully place the contaminated vial on the MIT Vial Holder.
- 5. Place the MIT Vial Holder(s) (with vials) into the autoclave.
- 6. After all autoclaved vials have been processed, soak the collection of contaminated rubber bands on the 5% bleach soaked Kimwipe by spraying them with more 5% bleach. Note: Allow the rubber bands to sit untouched for at least 20 minutes.
- 7. The rubber bands may be reused after at least a 20 soaking time.
- 8. Autoclave the contaminated MIT Vials at 121°C for 45 minutes.
- 9. Carefully remove the MIT Vial Holder after the sterilization cycle.
- 10. Allow the sterilized vials to cool down to room temperature (~37°C, approximately 2 hours cool time).
- 11. Discard the sterile solution in each vial.
- 12. Rinse the vial with LPF water.
- 13. Proceed to the MIT Vial Cleaning Procedure.

Warning The MIT Vials MUST be cleaned using the procedure below before they can be used for Identification Tests.

MIT Vial Cleaning

Items Needed

- Autoclave
- Sterile MIT Vial
- MIT Vial Holder
- LPF water
- Dish Soap
- Distilled White Vinegar solution of about 5% to 8% acetic acid in water and a pH of about 2.4
- 2-2000 mL beakers or equivalent.



Figure 15 – MIT Vial Holder



Procedure

- 1. In one beaker, make a soapy solution by filling it with 2 liters of lukewarm water and ONE drop of dish soap.
- 2. Stir the soap drop into solution without making excessive bubbles.
- 3. In the second beaker, make at least one liter of a 10% Vinegar solution of LPF water and distilled white vinegar. (Example: 900 mL of LPF water + 100 mL of Vinegar.)
- 4. Stir the LPF Water and Vinegar solution to mix.
- 5. Hold the MIT Vial firmly and fully submerge the MIT Vial in the soapy water so that the vial neck is pointing upward.
- 6. When the MIT Vial is fully filled with soapy water, lift the filled vial out, quickly turn the vial upside down, and **VIGOROUSLY SHAKE** the content out so the content ejects with three shakes. <u>Note: Do not return the vial content back into the beaker</u>.
- 7. Repeat step 6 at least five times.
- 8. Rinse and fill the MIT Vial to the top with LPF Water and discard the content with **VIGOROUS SHAKING** so that the content ejects with three shakes.
- 9. Repeat step 8 at least five times.
- 10. Fully submerge the MIT Vial in the 10% vinegar solution with the neck pointing upward.
- 11. When the MIT Vial is fully filled with the 10% vinegar solution, lift the vial with the vinegar content out, quickly turn the vial upside down, and **VIGOROUSLY SHAKE** the content out so the content ejects with three shakes. <u>Note: Do not return the vial content back into the beaker</u>.
- 12. Repeat step 11 at least five times.
- 13. Rinse and fill the MIT Vial to the top with LPF Water and discard the content with **VIGOROUS SHAKING** so that the content ejects with three shakes.
- 14. Repeat step 13 at least five times.
- 15. Place the MIT Vial upside down in an MIT Vial Holder to air dry.

Storing MIT Vials

When stored in a clean room that has HEPA filters in continuous operation, the MIT Vials can be stored on an MIT Vial Holder.

When the MIT Vials are stored in other, dirtier environments, cover the vial opening with a 2" x 2" square of Parafilm and hold it in place with a #10 rubber band, then store it on an MIT Vial Holder.



Chapter 5: Water Quality Test

The MIT 1000 System requires LPF water for proper operation. MIT recommends that a water quality test be performed before **every** Identification Test. The water filled MIT Vial can be used for Identification Tests only if it passes the 30-second Water Test. If a test failure occurs, dump the water in the vial, then clean, rinse, and fill the vial as described in Step 1 of Chapter 7, on page 29.

To perform a Water Test:

- 1. Clean, rinse, and fill the MIT Vial as described in Step 1 in Chapter 7, on page 29.
- 2. Place the MIT Vial in the MIT 1000 System as described in Step 9 in Chapter 7 on page 34 and click **Water Test** as shown in Figure 16.



Figure 16 – Water Test

Upon clicking the **Water Test** button, the following changes occur in the MIT 1000 Application window:

- Both the **Identify** and **Close** Program buttons are removed from the dialog
- The word "Busy" with a flashing green light appears.
- The Progress Bar appears
- After a few seconds, the Sample Concentration Gauge (SCG) appears. The indicator in the Gauge should remain at the lowest condition (see Figure 17) during the test



Figure 17 – Water Testing In Operation



Warning To conduct a valid test, verify that the System Lid is seated properly and MIT Vial is not glowing red.

<u>Note:</u> If the Check Vial indicator blinks during the Water Test, the MIT 1000 Application will terminate the test. This means that there is a scratch on the MIT Vial in the path of the laser beam. The easiest corrective action is to rotate the flask until the "glare" is eliminated (see Figure 28 & Figure 29) and then restart the Test.

Water Test Results:

• If the water quality is good, the test will require 30 seconds to execute and the MIT 1000 Application will display "Water Test: Pass." (refer to Figure 18)



Figure 18 – Pass Water Test

 If the water quality is not adequate to conduct an Identification Test, the Water Test will self-terminate as soon as that judgment is determined and will display "Water Test: Fail." (refer to Figure 19)



Figure 19 – Fail Water Test



Chapter 6: Sample Preparation

Prior to preparing a sample for MIT 1000 Identification Testing, it is imperative that prior microbiological functions were performed to create a single species incubated in MIT approved solid medium.⁷

Step 1: Fill the MIT Vial with Water

Items Needed

- MIT Vial
- 2" x 2" square of Parafilm
- ~ 15 mL of LPF water
- #10 Rubber band

Procedure

- 1. Using LPF water,
 - a. Start rinsing the MIT Vial by holding the vial upside down in the water stream.
 - b. Slowly rotate the vial in the LPF water stream to the vertical position.
 - c. Fill the vial with LPF Water.
 - d. Quickly turn the vial upside down and **VIGOROUSLUY SHAKE** the water out of the vial. This should be quick enough that it requires three shakes to empty the vial.
 - e. Repeat steps c through d five times.
- 2. Fill the vial with LPF Water it as shown in Figure 20. When properly filled, the vial neck should be about half full.



The System will operate properly as long as the level is above the vial neck bottom and below the top lip.

Figure 20 – Fill the MIT Vial to the indicated "Fill" Level

Warning Under-filling and overfilling the Vial may cause ID inaccuracies and overfilling may result in spillage.

⁷ Current MIT approved medium is BHI or TSA with 5% sheep blood.



3. Immediately center the Parafilm square, with the paper protected side down, over the top of the vial and press it into place forming a seal with the outside of the neck as shown in Figure 21. Secure, by double wrapping the vial neck with a #10 rubber band.



Figure 21 – MIT Vial with Parafilm Cover

Warning Immediately cover the filled MIT Vial with the Parafilm (paper "protected side" down) over the lip to avoid air particulates from entering the Vial and contaminating the water.

4. Perform a Water Quality Test as described in Chapter 5:.

Note: Perform Steps 2 & 3 within 1 hour after completing Step 1.

Warning DO NOT use the LPF Water filled MIT Vial for an Identification Test if it does not pass the Water Quality Test.

Step 2: Clean the Micro-Centrifuge Tube

Items Needed

- 0.6 mL micro-centrifuge tube
- LPF Water

Procedure

Using LPF Water:

- 1. Rinse the outside of the tube first starting from the bottom.
- 2. Rinse the inside of the tube by repeating the following sequence at least five times:
 - a. Completely fill the tube with LPF Water.
 - b. Turn the tube upside-down and VIGOROIUSLY SHAKE out all of the water.

Step 3: Fill the Micro-Centrifuge Tube with Water

Items Needed

- 0.6 µL micro-centrifuge tube
- LPF Water

Procedure

1. Immediately after rinsing the tube, fill the tube with about 0.2 mL (about 1/3 full) with LPF Water, Figure 22.





Figure 22 – Micro-Centrifuge Tube with 0.2 mL of LPF Water

2. Snap the micro-centrifuge tube cover closed.

Warning To prevent random air particulates from entering the micro-centrifuge tube and contaminating the water, close the micro-centrifuge tube immediately after filling.

Step 4: Collect a Test Sample

Items Needed

- Bacteria in isolated colonies
- 1 µL sterile inoculating loop
- Biohazard Bin and Biohazard Bag

Procedure

Examine the culture plate (Note: Cultures should be less than 48 hours after first appearance on culture plate) and select the isolated colony to be identified. Then:

- 1. Place the culture plate upside down (lid down) in the BioSafety Cabinet.
- 2. Using a septic techniques hold the culture plate in your hand and slowly lift the culture plate off the lid.
- 3. Remove a small portion of a single colony by lightly placing the loop at the edge of the colony and dragging the loop through the colony to collect organism cells, as shown in Figure 23. Note: Avoid collecting culture media.
- 4. Put the culture plate back on the lid.
- 5. Immediately proceed to Step 5.



Figure 23 – Collecting a Test Sample



Step 5: Prepare the Micro-Centrifuge Tube Suspension

Items Needed

- Inoculating loop containing organism cells
- Micro-centrifuge tube from Step 3

Procedure

While holding the inoculating loop that contains the organism cells:

- 1. Hold the water-filled micro-centrifuge tube in one hand.
- 2. Open the tube and tilt it no more than 45° making sure not to spill the contents.
- 3. Insert the inoculating loop into the water in the microcentrifuge tube and dislodge the sample into the water by agitating the loop in the water as shown in Figure 24.
- 4. Withdraw the loop and immediately close the tube.
- 5. Properly dispose of the contaminated loop in a Biohazard Bag.

Step 6: Vortex the Sample

Items Needed

- Micro-centrifuge tube with the organism cells in dilution from step 5.
- Vortex mixer

Procedure

- 1. Vortex the micro-centrifuge tube for at least 20 seconds, Figure 25.
- 2. Proceed directly to Step 7.

Note: To ensure optimum performance, do not delay proceeding to Step 7.



Figure 24 – Dislodge the Organism Cells into the Water in the Microcentrifuge Tube



Figure 25 – Vortex the Microcentrifuge Tube



Step 7: Prepare the MIT Vial Suspension

Items Needed

- Micro-centrifuge tube with vortexed organism cells (suspension)
- 0.5 10 µL adjustable pipette with tip
- Covered MIT Vial containing LPF Water from Step 1

Procedure

- 1. Always vortex the micro-centrifuge tube immediately before this step.
- 2. Remove the rubber band from the MIT Vial Parafilm cover.
- 3. Remove or gently lift one side of the Parafilm.
- 4. Pipette 1-μL of the suspension into the MIT Vial as shown in Figure 26.
- 5. Properly dispose of the used tip into a biohazard container.
- 6. Immediately re-cover the MIT Vial in the same manner as done in Step 1 (on page 29) and shown in Figure 21.

Warning

DO NOT retract water back into the micro-centrifuge tip.

Warning DO NOT allow the LPF Water filled MIT Vial to remain uncovered for more than 30 seconds.

Note: To ensure optimum performance, it is recommended that Steps 8 and 9 are completed within 1 hour of completing step 7.

Step 8: Clean the MIT Vial Outside Surface

Items Needed

- MIT Vial
- Kimwipes
- 70% IPA

Procedure

- 1. Dampen a Kimwipe with 70% IPA.
- 2. Wipe the entire exterior of the spherical part of the MIT Vial.
- 3. Immediately dry the MIT Vial with a clean dry Kimwipe.



Figure 26 – Inoculating the Colony Dilution into the MIT Vial



Step 9: Place the MIT Vial into the MIT 1000 System

Items Needed

• MIT Vial

Procedure

- 1. Start the MIT 1000 Application.
- 2. Lift the lid.
- 3. Place the MIT Vial in the MIT 1000 System in an upright position as shown in Figure 27.
- 4. Rotate the MIT Vial and stop at a point where laser glare on the MIT Vial is minimal. See Figure 28 and Figure 29 for unacceptable and acceptable glare.
- 5. Replace the lid.
- 6. Proceed to Chapter 9 to conduct an Identification Test.



Figure 27 – Placing the MIT Vial into the System

Warning The MIT Vial neck must be vertical.



Figure 28 – MIT Vial Incorrectly Oriented with Unacceptable Glare





Figure 29 – MIT Vial Correctly Oriented with Acceptable Glare

Warning The MIT 1000 System uses a Class IIIb laser with an output of up to 45 milliwatts. Direct eye contact with the laser beam may cause damage to your eye!



Chapter 7: Performing an Identification Test

This chapter will describe how to perform Identification Testing using the MIT 1000 Application.

MIT 1000 Application Start-up

Start the MIT 1000 Application by clicking the icon (Figure 1, Page 10) on the computer desktop.

Identification Test Sample Information

Upon clicking the Identify button, the Sample Information Form below will appear.

As a prerequisite to the Identification Test, the User is required to enter the information in Figure 30.

Click **OK** to accept the Sample Information and continue with the Identification Test. Click **Cancel** to abort the test.

	Sample Information		Optional Sample
Required Sample	User: G. Kessler New	Media	Tracking
Information 1. User 2. Sample source information 3. Gram Stain results	Sample Tracking Customer MIT Foods Project 12-MITF-Burrito Task 002-Burrito-2 Sample Cooking-2 Gram Stain Results	Media New Lot Number Expiration Date Expiration Date 10/22/2012 Incubation Temperature *C Start Time 12:00 AM	Information 1. Media parameters 2. Sample incubation parameters 2. Commonto
	© Not done ⊂ GNR ⊂ GNC ⊂ Yeast C GPR ⊂ GPC Comments	Start Time 12/30/99 I2/00 AM End Timer 12/30/99 I2/00 AM	 Comments

Figure 30 – Identification Test Sample Information Form



Identification Test Operation

During the Identification Test, the MIT 1000 Application Display changes to show test status and progress (Figure 31):



Figure 31 – Identification Test Progress Display

- The Busy indicator appears and flashes green.
- The Identification Test progress indicator appears and will change as the test progresses.
- The SCG provides a real-time estimate of the concentration of the sample being tested. If the indicator is in the "blue" or "red" zone, it is recommended that the User take action as described in the "Indicator Explanations" in the next section.
- The **Identify** button changes to a **Stop Identify** button. Click the **Stop Identify** button to stop and cancel the Identification Test at any time during the identification process.
- The Close Program and Water Test buttons are hidden.

When the Identification Test completes, the Display reverts to its original state (Figure 32) and the sample is identified in bold blue letters above the **Close Program** button. If the sample is an Species, Genus, or Subspecies not identified by one of the Active Identifiers, the MIT 1000 Application will display "Test Result: Unknown."



Figure 32 – Identification Completion Display



Identification Test Gauges and Indicators

Identification Test Progress Indicator

This indicator provides the User with a coarse estimate of the test completion progress. Once the progress has reached the halfway mark, completion may occur abruptly. Typically, the test process takes 2 minutes but occasionally may complete as quickly as 30 seconds or as long as 5 minutes. <u>Note: The Identification Test will terminate automatically after 5-minutes and display in blue "Terminated: time out</u>."

Sample Concentration Gauge (SCG)

During the identification process, the MIT 1000 Application estimates the concentration of the sample being tested. The Sample Concentration Gauge (SCG) gives a real-time indication of this estimate. The gauge pointer will point in the middle of the green zone when the sample is properly made.

Warning	
The identification process will terminate automatically if a test does not	
complete in five minutes time indicating that the Test Sample concentration	on
was too low and the test accuracy is compromised. A new Test Sample	;
must be made.	

Concentration Indicator

Low Concentration Indication

When the sample concentration is too low, (Figure 33) the SCG will point in the blue zone and the Concentration indicator will blink blue. If this condition persists, stop the identification process by clicking **Stop Identify** and prepare a new sample.

Note: If the gauge pointer drifts in and out of the blue zone, the test will likely be performed properly but could take several minutes longer than usual to complete.



Figure 33 – Low Concentration Condition



High Concentration Indication

When the sample concentration is too high (Figure 34), the SCG indicator will point in the right side red zone and the Concentration Indicator will begin blinking red. If this condition persists, stop the identification process by clicking **Stop Identify** and prepare a new sample with less concentration.



Note: It is permitted to have the gauge pointer drift into and out of the red zone.

Figure 34 – High Concentration Display

Warning	
---------	--

The identification Progress will terminate automatically if the Low or High Concentration continues for more than 10 consecutive seconds indicating that the Test Sample was not made properly and may compromise the test accuracy. A new Test Sample must be made.



Check Vial indicator

When the Check Vial indicator begins blinking red (Figure 35), MIT Vial was positioned in the System such that a vial surface scratch or defect is in the path of the laser beam. This causes excessive glare and prevents the System from completing an Identification Test. The Check Vial indicator will blink red and the SCG will point to the far left red zone, Figure 35. After five seconds, the MIT 1000 Application will terminate the Identification Test and display the text "Terminated: vial glare" in blue, Figure 36. To correct this, reposition the MIT Vial. If the condition persists, a different MIT Vial must be used.



Figure 35 – Check Vial Display during a Water Quality Test or Identification Test



Figure 36. The MIT 1000 Application terminates the Water Quality Test or Identification Test and displays the Vial Glare warning when it detects vial glare.



Chapter 8: Troubleshooting

Condition	Probable Cause	Recommended Action
MIT Cloud Light blinks red	 The System has no connection to the internet Your network Firewall prohibits the MIT 1000 Application from communicating on port 12000-12010 	 Verify that the MIT 1000 Computer has network access. Configure your firewall to allow the MIT 1000 Application to have access to TCPIP Ports 12000-12010.
Ready Light blinks red	 System is not powered or cables are not properly connected The MIT 1000 System was powered on after the MIT 1000 Application was started MIT 1000 Application is corrupted 	 Verify that power on the MIT 1000 System is ON. Verify cables are connected properly. Turn on the power to the MIT 1000 System then Restart the MIT 1000 Application. If items 1- 3 do not correct the problem, contact MIT Customer Service.
Identifier [™] list does not match with what was ordered	Possible MIT Cloud configuration error	1. Consult MIT Customer Service.
System does not Identify or frequently does not recognize any bacteria examined	 Power is off Water particulate concentration is too high MIT Vial is dirty Lid is not seated properly Laser is not operating 	 Verify the System is powered on. Run a Water Quality Test. Verify the lid is seated properly on the System. Verify the laser is operating – look for red glow with Lid up. If none of the above solves the problem, contact MIT Customer Service.
Check Vial light blinks red or "Terminated: vial glare" is displayed The Identification or Water Test will terminate when this condition persists for more than 10 seconds and displays "Terminated: vial glare."	 The laser beam is illuminating a scratch or other defect on the surface of the MIT Vial causing it to glow red The water in the MIT Vial contains too many particulates 	 Stop the Identification or Water Test. The test will automatically terminate after 5 seconds. Rotate the MIT Vial until the red glare seen in the vial neck is not visible. If 2 does not solve the problem, replace the MIT Vial with a new one. Restart the Identification or Water Test. If none of the above solves the problem, contact MIT Customer Service.



Condition	Probable Cause	Recommended Action	
Concentration light blinks red or "Terminated: high concentration" is displayed	• Excessive concentration bacteria or foreign particles in the MIT Vial	 Discard the Sample and make a new one. Re-test. If this does not solve the problem, contact 	
The Identification Test will terminate when this condition persists for more than 10 seconds and display "Terminated: high concentration".		MIT Customer Service.	
Concentration light blinks blue or "Terminated: low concentration"	 Too low concentration of bacteria in the MIT Vial 	 Discard the Sample and make a new one. Re-test. If this does not solve the problem, contact 	
automatically terminate when this condition persists for more than 10 seconds and display "Terminated: low concentration".		MIT Customer Service.	
Terminated: time out The Identification Test will automatically terminate an Identification Test if there is no result after 5 minutes and display "Terminated: time out."	• The sample was contaminated with particles (agar, more than one species of bacteria, dirt, etc.) that prevented identification	 Discard the Sample and make a new one. Re-test. If this does not solve the problem, contact MIT Customer Service. 	



Appendix A - MIT Products

ltem	Description	Catalog #
MIT 1000 System	MIT 1000 system	
	Power supply module	1000
	MIT Mini Notebook w/MIT 1000 Application	
	Starter Kit	
	Listeria spp Identifier ^{1M}	
Starter Kit	Micro centrifuge tubes	1001
	Sterile loops	
	Sample Vials	
	Pipette tips	
	Parafilm 2" X 2" squares	
	MIT Vial Holder	4000
Yearly Maintenance		1002
Software Upgrades		1003
Calibration	6,9, or 12 month	1004
Identifiers		
	E. COli	10000
	Listeria spp	10001
	Salmonella spp	10002
Supplies		
Supplies		00004
Plated media	Trypticase Soy Agar (TSA) with 5% Sheep Blood, 15 x	20001
	100 mm plates, pack of 10	00000
Micro centrifuge tubes	1.5 µL Micro centrifuge tubes, Locking lid/pack of 500	20002
Disposable loop	1 µL Loop, 96 Packs of 10	20003
Vial Caps		20004
filtere	3 Stage	20005
Dipotto tipo	10 ul Aarosal barriar 10 racks of 06	20006
MIT Viale	15 ml	20000
Pinette Volume Device	MIT 200	20007
Parafilm	2" X 250 ft roll	20000
Power Supply Module	MIT 1000 power supply	20000
		20010
Equipment		
MIT Computer	Mini Notebook with MIT 1000 Application	30000
Pipette	0.5 to 10 µL variable	30001
Vortex Mixer	Fixed Speed	30002
MIT Vial Holder	6 Vail holder TBD	30003
MIT Water filtration	Wall mounted filtration system periodically	30004
System		



Appendix B - Sample Test Report

	MIT Foods	
System #: 000020	Laboratory Repo	Printed: 10/24/12 11:36
Customer:	MIT Foods	
Project Number:	Burrito	
PO Number:	12-MITF	
Task Number:	002-Burrito	
Lot Number:	2	
Sample Identifier:	Cooking-2	
Test #:	000211	
Identification Result:	Listeria spp	
Comments:		
Selected Identifiers : Listeria spp		
Test Details [.]		
Test Time	2012-10-23 09:40:37	
llser	G Kessler	
Termination:	Normal	
Sample Details:		
Gram stain result:	Not performed	
User	Date Appro	val Date
VIT 1000 RMID version 1.0		Page 1 of 1



Glossary

μL	Microliter
Basic Laboratory Procedures	Basic microbiology procedures that include microbial handling, culturing, disposing
Calibration	The method that uses calibrated polystyrene spheres to determine if the MIT 1000 is working properly
Equipment required	The Equipment needed to perform an Identification Test that is NOT supplied by MIT
ID	Identification Test
IPA	Isopropyl Alcohol
Identification Result	The name of the bacteria, sphere or Unknown organism determined by an Identification Test
ldentifier™	An MIT proprietary set of data that is used by the MIT 1000 System to identify the Genus, Species, or Subspecies of a bacterial specimen
Identification Test	The test performed by the MIT 1000 System to determine if the microbial sample being tested contains the bacteria or particle associated with the selected Identifiers TM .
Inside the Box	The MIT 1000 System list of equipment and materials that was included in your purchase order
License	Provides access to Identifiers ^{IM} . Visit the MIT web site for available Identifiers ^{TM} .
Light Scattering	The phenomena that occurs when a particle passes through the laser beam inside the MIT 1000 System
LPF Water	Low Particle Filtered Water. Typically, this is water that is the product of a water filtering system with a final filter of 0.1 micrometers absolute or smaller. The MIT 1000 Application Water Test function is the final decider on whether or not water can be used for Identification tests with the MIT 1000 System.
Material required	The Material needed to perform an Identification Test that is NOT supplied by MIT
MIT website	www.micro-identification.com
mL	Milliliter
Notes	Useful information or information that requires special emphasis
Power Services	The AC electrical power that is typical for your country of usage
PSL	Polystyrene Latex spheres
MIT 1000 Application	The program used to control the MIT 1000 System
MIT 1000 System	MIT 1000 Rapid Microbial Identification System

Micro Identification

MIT Vial	The vessel used to prepare a sample and conduct an Identification Test – MIT Part Number 10007 $$	
MIT Vial Holder	A device to hold the MIT Vials.	
Sample Test Log	The Log used to store pertinent test information	
SCG	Sample Concentration Gauge. The meter used to provide a visual assessment of the quality of the Sample made by the User. The Sample is considered acceptable if the pointer in the gauge is consistently in the Green area.	
Setting up the System	The schematic to connect all of the sub-systems of the MIT 1000 System	
Soapy Water Solution	Approximate ratio of 1 drop dish detergent (Dawn, Joy, etc.) to 2 liters of water	
Starter Kit	Materials needed to for first operation of the MIT 1000 System	
Lid	The flap on top of the MIT 1000 System cover used for placing and removing MIT Vials	
Warning	Information to help avoid injuring the User, damaging the equipment, or reducing the System's performance	
Water Test	The test performed to verify that the water being used in the Sample to be tested has no more than 3 particles after a 5 minute test	



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