VereMTB™ Detection Kit Instructions for Use

For Research Use Only

REF	VMTB-RA50
Σ	50
	Store at -25°C to -15°C (frozen components) Store at 15°C to 25°C (ambient components)
	83 Science Park Drive #04-02 Singapore (118258)

November 2018

IFU-MTB-RA01-1010

Contents

Purpose

VereMTB™ Detection Kit is a multiplex PCR/microarray-based research use only test. This nucleic acid-based test is intended for the simultaneous qualitative detection of *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium tuberculosis* (MTB) resistance or sensitivity to rifampicin and isoniazid from smear-positive sputum samples or cultured (liquid or solid) samples. The test also detects and differentiates 14 nontuberculous mycobacteria (NTM) – (*M. abscessus, M. chelonae), M. avium, (M. intracellulare, M. vulneris, M.chimaera, M.colombiense), (M. simiae, M. kansasii, M. scrofulaceum, M. gastri, M. mantenii), M. xenopi, and M. fortuitum*. This test is for use in conjunction with the VerePLEX™ Biosystem.

Summary and Explanation

Tuberculosis (TB) is a contagious disease that is spread mainly through the air and caused primarily by *Mycobacterium tuberculosis*.

TB Treatment is straightforward but lengthy- by using a combination of anti-TB drugs which has been effective. However, TB still remains a major global health problem. According to WHO Global Tuberculosis Control Report, in 2014, it is estimated that 9.6 million people had developed TB and there were 1.5 million deaths from TB. TB is the leading cause of death worldwide.

A greater challenge is posed by the emergence and spread of multidrug-resistant TB (MDR-TB) which is defined as TB having resistance to both first-line anti-TB drugs- Rifampicin and Isoniazid. In 2014, there were 480,000 estimated cases of MDR-TB. A significant portion of TB patients are still not tested for drug resistance (in 2014, worldwide, only 58% of previously treated patients and 12% of new cases have been tested). Undiagnosed MDR-TB leads to the continuous use of ineffective drugs which may promote the spread of the resistant bacteria and expansion of resistance.

In addition, there is an emergence of NTM lung disease which may bear similar symptoms as TB. The incorrect diagnosis of NTM lung disease as pulmonary TB has led to inappropriate and irrelevant treatment with TB drugs, which requires a different drug regimen.

Facing with the threat of both MDR-TB and rising NTM diseases has propelled the need of a new diagnostic test system that should be able to provide a fast and reliable detection and identification of *Mycobacterium tuberculosis*, with the capability to differentiate it from clinically relevant NTM species, and as well as detecting multidrug resistance.

VereMTB™ Detection Kit uses the lab-on-Chip (LOC) platform for simultaneous and qualitative detection and identification of MTBC and MTB's resistance to Rifampicin and Isoniazid, as well as, NTM based on selected target genes.

Any positive samples should be forwarded to a certified laboratory for confirmation.

Principle of the Procedure

VereMTB™ Detection Kit includes a single-use disposable VereChip™ as well as reagents and consumables necessary for nucleic acid amplification and DNA hybridization. PCR primers and probes are designed to target the different genes of MTB and the 16S rDNA gene of NTM (*Table 1 & Table 2)*

Table 1: Mycobacterial species identified* and differentiated** by VereMTB™ Detection Kit

Mycobacterium species	Target Genes
M. tuberculosis complex	*IS6110*
M. abscessus, M. chelonae	*16S rDNA*
M. avium	
M. intracellulare, M. vulneris, M. chimaera, M. colombiense	
M. simiae, M. kansasii, M. scrofulaceum, M. gastri, M. mantenii	
M. xenopi	
M. fortuitum	

* Test is unable to co-detect (1) *M. avium* with *M. intracellulare, M. vulneris, M. chimaera* and *M. colombiense*; and (2) *M. fortuitum* with *M. abscessus* and *M. chelonae.*
** Test is unable to differentiate (1) *M. abscessus* and *M. chelonae,* (2) *M. intracellulare, M. vulneris, M. chimaera* and *M. colombiense, and* (3) *M. simiae, M. kansasii, M. scrofulaceum, M. gastri* and *M. mantenii.*

Table 2: Target sequences (wild type and mutant) relevant for multi-drug resistance in M. tuberculosis identified by the VereMTB™ Detection Kit

	***M. tuberculosis* target sites**	
Genes	*Wild type**	*Mutations*
rpoB **(hot-spot region)**	Codons 510 to 513	ctg/ccg(L511P)
	Codons 515 to 518	gac/gtc (D516V)
	Codons 523 to 526	cac/gac (H526D)
		cac/tac (H526Y)
	Codons 530 to 533	tcg/ttg (S531L)
katG **(coding region)**	Codons 313 to 317	agc/acc (S315T1)
		agc/aca (S315T2)
inhA **(promoter region)**	Nucleotides -21 to -7	c-15t
		t-8c
		t-8a

* Absence of wild type probe indicates a mutation not identified by the specific mutation probes. Thus, this is interpreted as resistance of the tested strain to the respective antibiotic.

This test is for use in conjunction with the VerePLEX™ Biosystem.

The VerePLEX™ Biosystem consists of the following components:

- Temperature Control System (TCS), which is a system that thermally drives the VereChip™. It consists of five Temperature Control Modules (TCMs) that allow five independent temperature programs to be run independently
- Optical Reader (OR), which detects and analyzes the microarray fluorescence in the range between 670 and 730 nm
- Touch Monitor (TOM), which connects to the TCS for display and input via touch
- Barcode Reader
- VerePLEX™ Biosystem Software

The system requires a VereChip™, on which a miniaturized reactor for PCR amplification and a DNA microarray are integrated.

The DNA microarray consists of microscopic spots of DNA oligonucleotides called probes that are fixed on a silicon substrate. These probes are short sections of target genes which hybridize to a complementary strand of amplified product. The amplified product is labeled with a fluorophore dye and probe-product hybridization is detected as a fluorescent signals and captured by CCD camera in the OR.

The probes for targets are spotted in triplicates in a 6 rows x 21 columns layout. If target mycobacterium is in the sample and captured, respective probes will light up in a particular pattern on the microarray. For a sample to be positive for a particular gene or mycobacterium, certain criteria must be satisfied. The criteria are written in "*Diagnostic Rule files*" provided in the BioApplication. The VerePLEX™ Biosystem Software relies on this set of rules for pattern interpretation.

Kit Content

Kit Catalog no.		**VMTB-RA50**
Tests		**50**
Ambient Components (15°C to 25°C)	**Part Number**	**Quantity**
Box 1 of 3		
VereMTB™ Chip	MTB-43	2 boxes
PCR Clamp	CPR-01	1 pack
IN Clamp	CIN-02	2 packs
HYB Clamp	CHY-01	1 pack
Washing Solution Concentrate	VCP-WSC02/ VCP-WSC03	1 bottle
Frozen Components (-25°C to -15°C)	**Part Number**	**Quantity**
Box 2 of 3		
VereMTB™ Premix A	MTB-PXA02	2 tubes
VereMTB™ Premix B	MTB-PXB02	2 tubes
2X Hyb Buffer	VCP-2XB03	2 tubes
MTB Hyb Probe	MTB-HYP01	2 tubes
VeTaq™ DNA Polymerase	VBI-TDP01	1 tube
VeDirect™ PCR Buffer (2X)	VSH-ADP01	1 tube

Storage Condition

- Store all Frozen Components at -25°C to -15°C upon receipt.
- Keep the Premix A/B and MTB Hyb Probe away from light until ready to use.
- After initial thawing, Premix A/B can be stored at 2°C to 8°C for up to 60 days. For longer storage (>60 days), Premix A/B should be stored at -25°C to -15°C; however, keep the number of freeze-thaw cycles to <6 cycles.
- Store reconstituted Microarray Hybridization Mix at 2°C to 8°C for up to 60 days.
- Store VeDirect™ PCR Buffer (2X) and VeTaq™ DNA Polymerase at -25°C to -15°C. Keep the number of freeze-thaw cycles to <11 cycles.

NOTE: **Repeated thawing and freezing beyond recommendation may reduce the sensitivity of the assay.**

- Store all Ambient Components at room temperature (15°C to 25°C).
- After opening the aluminum packaging, the Chips are stable for 60 days when stored in its original box at room temperature.

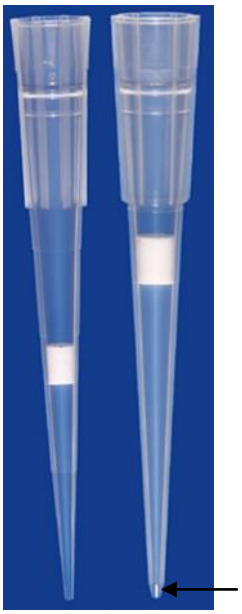
NOTE: **Keep Chips away from light.**

- Precipitation or crystallization may occur in Washing Solution Concentrate if the storage temperature is low. Should this occur, please refer to important note before Chip washing step (page 27).
- After initial opening, Washing Solution Concentrate can be stored at 15°C to 25°C for up to 60 days.
- Store diluted Wash Buffer at room temperature (15°C to 25°C).
- If left unopened, all reagents are stable until the expiration date indicated on the respective labels.

Materials Required but Not Provided

a) Reagents
- PCR Grade Water
- Distilled/Reverse Osmosis (RO)/Ultrapure Water
- 4% NaOH (Sodium hydroxide)
- 2.9% Na citrate (Sodium citrate)
- NALC (N-Acetyl-L-Cysteine)
- Sterile 0.067M Phosphate Buffer Solution (PBS pH 7.0)

b) Consumables
- Personal protective equipment
- Sterile beveled pipette tips[1] for Chip loading
- Sterile filter pipette tips
- Serological pipette
- 1.5 mL low DNA binding safe-lock cap microcentrifuge tubes
- 0.2 mL PCR tubes
- Sterile 50 mL centrifuge tube (non-skirted)
- 1 L Bottle
- 2 mL screw cap centrifuge tubes
- Decontamination products



c) Equipment
- Centrifuge with rotor and adapter for 50 mL centrifuge tube (non-skirted)
- Microcentrifuge for 1.5 mL tube
- Micropipettes (0.5-10 µL, 2-20 µL, 10-100 µL, 100-1000 µL)
- Serological pipette controller
- Freezer/Refrigerator (-20°C/4°C)
- Vortex mixer
- Boiling water bath or Heating block
- Sonicator bath

d) Additional Accessories
- Tube rack/stand
- Tweezers

[1] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) is recommended

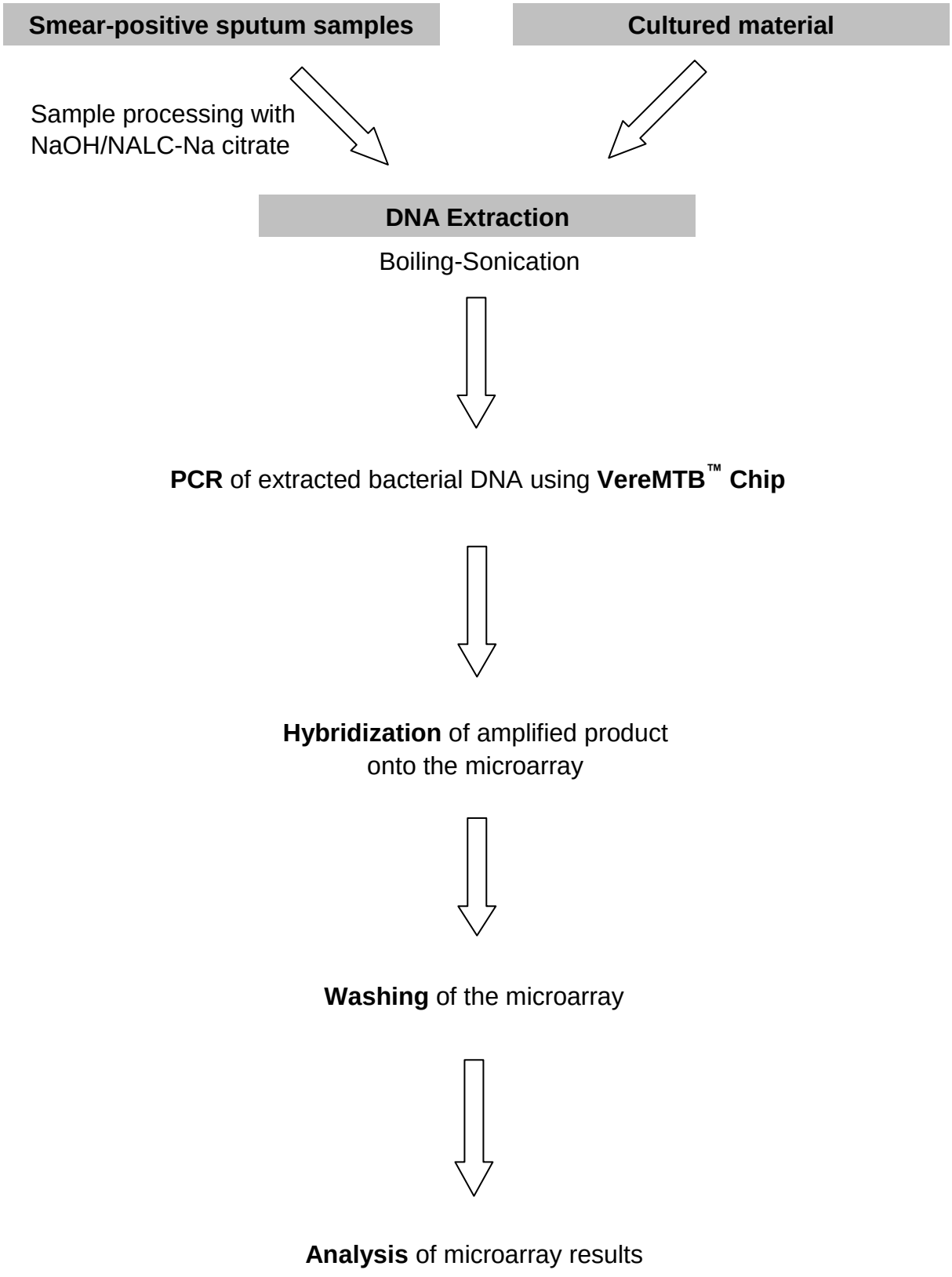
Warnings and Precautions

- All samples should be treated as potentially infectious.
- Wear appropriate personal protective equipment, including (but not limited to) protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents. Wash hands thoroughly after handling specimens and reagents.
- Clean and decontaminate work area and instruments, including pipettes, with commercially available decontamination products.
- 2X Hyb Buffer contains hazardous component, do not breathe vapor.
- A designated working area should be dedicated for processing specimens and to add extracted specimens to PCR Reaction Mix.
- Use sterile pipette tips with filters.
- Handle Chip with care, avoid contact with the microreactor.
- Each Chip is used to process one test. Do not reuse processed Chips.
- Do not use kit, Chips or reagents after the expiration dates shown on the respective labels.
- Follow laboratory safety rules and procedures as defined by approved biohazard safety guidelines or regulations.
- Discard waste according to your local safety regulations.
- Material Safety Data Sheets (MSDS) are available upon request.

Quality Control

Under Veredus' quality assurance program, the performance of our VereMTB™ Detection Kit is monitored routinely to ensure consistent product quality. Sampling is done on each lot and tests carried out via amplification of the respective control nucleic acid fragment.

Workflow


Smear-positive sputum samples
Cultured material
Sample processing with NaOH/NALC-Na citrate
DNA Extraction
Boiling-Sonication
PCR of extracted bacterial DNA using VereMTB™ Chip
Hybridization of amplified product onto the microarray
Washing of the microarray
Analysis of microarray results

Specimen Collection, Handling and Storage

Pulmonary smear-positive sputum specimens and cultured material (solid/liquid medium) specimens can be used as starting material for DNA extraction.

Specimens should be collected, handled and stored following the user institution's standard procedures. Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative results. Training in specimen collection is highly recommended because of the importance of specimen quality.

Recommended sputum specimen collection procedure:

i) Have the patient rinse his or her mouth thrice with plain water.
ii) Unscrew the lid on the sputum collection container.
iii) Instruct patient to take deep breaths, force out deep coughs vigorously, and expectorate the sputum into the container. Avoid spills or soiling the outside of the container.
iv) Collect a minimum of 2 mL of sputum per patient.
v) Secure the lid on the collection container.
vi) Specimens should be held at 2°C to 8°C whenever possible including during transport to the laboratory.
vii) Sputum specimen has to be processed (i.e. decontamination and DNA extraction) within 4 days.

Extracted nucleic acids can be used immediately upon extraction. Store extracted nucleic acids at 2°C to 8°C and use within 24 hours. For longer storage, store extracted nucleic acids at ≤ -70°C. Repeated thawing and freezing may affect the quality of the nucleic acid. They should be tested before use.

Sample Preparation

Extracted DNA is the starting material for the VereMTB™ Detection Kit. To obtain maximum performance, it is very important to establish the extraction process. Specimens can be processed by following the user institution's standard procedures. The following sample processing methods are recommended.

1. Sample processing for smear-positive sputum specimen

1.1 Prepare NaOH/NALC-Na Citrate Solution (Digestant-Decontaminant Solution)

i) Prepare 4% NaOH: dissolve 40 g NaOH in 1 L distilled water.

ii) Prepare 2.9% Na citrate: dissolve 29 g Na citrate dihydrate in 1 L distilled water.

iii) Sterilize the reagents by autoclaving.

NOTE: **Store 4% NaOH and 2.9% Na citrate at 2°C to 8°C. These reagents can be prepared in advance.**

iv) Determine the amount of reagent required (refer to Section 1.2) and mix equal volumes of 4% NaOH and 2.9% Na citrate solutions to desired amount.

v) Add appropriate amount of NALC to NaOH-Na citrate mixture to achieve 0.5% NALC (e.g. add 5 g NALC to 1 L NaOH-Na citrate solution).

vi) Mix gently to dissolve NALC.

NOTE: **Use the Digestant-Decontaminant Solution within one day. Discard any unused solution.**

1.2 Liquefy and Decontaminate Sputum

i) Aliquot at least 2 mL of smear positive sputum specimen into 50 mL centrifuge tube.

ii) Using disposable transfer pipette, add to sputum specimen a volume of Digestant-Decontaminant Solution that is equal (or double, if sputum is very viscous) to the specimen volume (1:1 or 2:1 volume).

iii) Screw cap tightly and vortex suspension for 30 seconds (till specimen is liquefied). Let specimen stand for 15 minutes at room temperature.

Do not incubate specimen in Digestant-Decontaminant Solution beyond 15 minutes. High pH rapidly kills microorganisms in the specimen including mycobacteria and over processing may result in reduced recovery of mycobacteria.

iv) After incubation, fill centrifuge tube with 0.067 M PBS pH 7.0 to 45 mL mark. Mix by inversion.

v) Centrifuge the tube at 3,000 x g at 4^{o}C for 15 minutes.

vi) Upon completing centrifugation, decant the supernatant.

vii) Add 2 mL sterile PBS to sediment pellet and resuspend the sediment.

viii) Transfer 1 mL of resuspension to 1.5 mL microcentrifuge tube for DNA extraction. Remaining sediment can be used for culture or stored in 2 mL screw cap centrifuge at 4°C for later use.

1.3 Extract DNA

i) Centrifuge 1.5 mL microcentrifuge tube at 12,000 rpm for 5 minutes to pellet sediment.

ii) Remove the supernatant by pipette. Do not agitate sediment pellet.

iii) Resuspend pellet with 100 µL sterile PCR grade water.

iv) Boil sediment at 95°C-100°C for 20 minutes.

NOTE: **It is recommended to use parafilm to seal the tubes, to prevent them from opening during 95°C-100°C incubation.**

v) Sonicate sediment in water bath sonicator for 15 minutes.

vi) Centrifuge 1.5 mL microfuge tube at 12,000 rpm for 5 minutes to pellet sediment.

vii) Transfer DNA-containing supernatant to fresh 1.5 mL microcentrifuge tube.

viii) Use extracted DNA for VereMTB™ chip runs.

ix) If not used immediately, store extracted DNA at -25°C to -15°C.

2. Sample processing for cultured material

2.1 Extract DNA

i) When using bacteria from solid medium, take 2-3 loops of bacterial culture and put them into 500 µL of PCR grade water in fresh 1.5 mL microcentrifuge tube. Vortex lightly.

ii) When using bacteria from liquid medium, aliquot 1 mL into fresh 1.5 mL microcentrifuge tube, pellet bacteria at 13,000 rpm at 4^{o}C for 10 minutes. Replace supernatant with 500 µL PCR grade water in tube. Resuspend.

iii) Boil bacterial suspension at 95-100°C for 30 minutes.

NOTE: **It is recommended to use parafilm to seal the tubes, to prevent them from opening during 95°C-100°C incubation.**

iv) Immediately, sonicate samples for 15 minutes.

v) Centrifuge 13,000 rpm for 10 minutes (4^{o}C).

vi) Remove and keep DNA-containing supernatant in fresh 1.5 mL microcentrifuge tube.

vii) Use extracted DNA for VereMTB™ chip runs.

viii) If not used immediately, store extracted DNA at -25°C to -15°C.

Protocol

Important notes before starting

- Thaw all frozen components thoroughly at room temperature before use.
- After thawing, briefly mix and centrifuge the components.
- Vortex briefly or pipette up and down 5-6 times when mixing reagents with enzymes. Avoid making bubbles.
- Precipitation or crystallization may occur in Washing Solution Concentrate if the storage temperature is low. Should this occur, please refer to important note before Chip washing step (page 27).
- User intervention is required:
  - After the PCR protocol is completed, user to load hybridization mix into the Chip and return Chip to the TCM for hybridization step.
  - After hybridization protocol is completed, user to wash the Chip and place the Chip into the OR for detection.
- Use current VereMTB™ Chip version as follows:
  - MTB-4.3
- For software, use current version or higher as follows:
  - VerePLEX™ Biosystem Version 5.3.X[2]
  - BioApplication VereMTB_4.3.1
- Screenshots are for illustration purposes only, and individual installations may vary.

[2] Current VerePLEX™ Biosystem version. Subject to minor changes.

1. Prepare PCR Reaction Mix

i) Depending on the number of samples, prepare the required volume of PCR Reaction Mix in a sterile PCR tube according to the following pipetting scheme:

Number of Reactions		1 reaction	
PCR Reaction Mix Components		**Tube A (µL)**	**Tube B (µL)**
VeDirect™ PCR Buffer (2X)		6.25	6.25
VereMTB™ Premix	A	2	-
	B	-	2
VeTaq™ DNA Polymerase		0.1	0.1
Extracted DNA Sample		x*	x*
PCR Grade Water		y**	y**
Total		**12.5**	**12.5**

* *Add 1-4.15 µL. When using extracted DNA sample from cultured material, it is recommended to use 1 µL*

** *Add water to adjust total volume to 12.5 µL*

ii) Mix thoroughly by pipetting or brief vortexing.

iii) Briefly centrifuge to pull contents down to bottom of tube.

2. Load PCR Reaction Mix into Chip

i) Insert a VereMTB™ Chip into the Chip Holder and ensure a secure hold.

NOTE: **To ensure a secure and firm positioning of the Chip, the holder has a pin fastener, press to release holder (*Figure 1*) when inserting and removing the Chip.**

Figure 1: *Chip Holder*

ii) Draw **11.5 µL** of the PCR Reaction Mix in **Tube A** with a pipette.

NOTE: **Use a 20 µL pipette and recommended pipette tips[3] for Chip loading.**

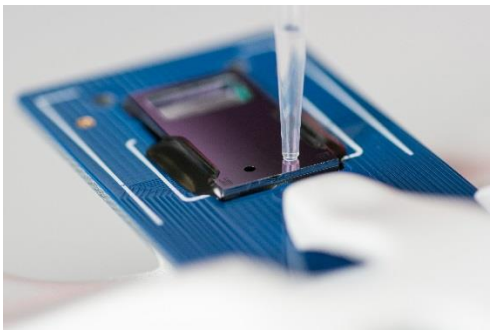
iii) Hold the pipette in a vertical position, in such a way that the tip is perpendicular to the surface.

iv) Fit the tip into one of the inlet holes (see *Figure 2a*).

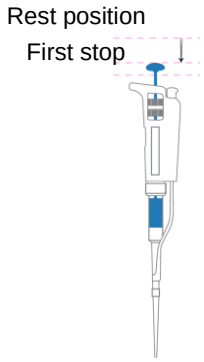
v) Applying slight pressure onto the tip, press the plunger smoothly to the first stop position (see *Figure 2b*), allowing the mix to flow into the PCR chamber.

Do not press the plunger <u>beyond the first stop</u> as this will introduce air into the chamber and mix will flow into the microarray chamber. Keep the plunger at the first stop until you remove the tip from the inlet (this procedure avoids spilling and the injection of air inside the chambers).

vi) Using a new pipette tip, draw **11.5 µL** of the PCR Reaction Mix in **Tube B**, repeat steps (ii) to (v). Load the Reaction Mix into the other inlet.



(a)



(b)

Figure 2: *(a) Tip placement into an inlet hole during sample loading; (b) Pipette sketch indicating the different stop positions*

Press and release the pipette plunger <u>slowly</u> at all the times. Never allow the push button to snap back. Check for foreign particles in the tip. Hold the pipette in an upright position while aspirating liquid.

[3] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) is recommended

3. Seal Chip for PCR

i) The IN and PCR sealing clamps are shown in *Figure 3*. The IN clamp (labeled "**2 IN**") is dedicated to seal the inlet holes, and the PCR clamp (labeled "**1PCR**") to seal the outlet holes in the microarray chamber.

Figure 3: *IN and PCR clamps*

ii) The clamp undersides are different, owing to their specific sealing function:

- The "**2 IN**" clamp (*Figure 4*a) has an elastomer with a rectangular protrusion that seals the inlet holes, and one alignment pin that fits the corresponding hole on the Chip (*Figure 5*);
- The "**1PCR**" clamp (*Figure 4*b) has an elastomer with a rectangular protrusion that seals the outlet holes, and two alignment pins that fit the corresponding holes on the Chip (*Figure 5*).

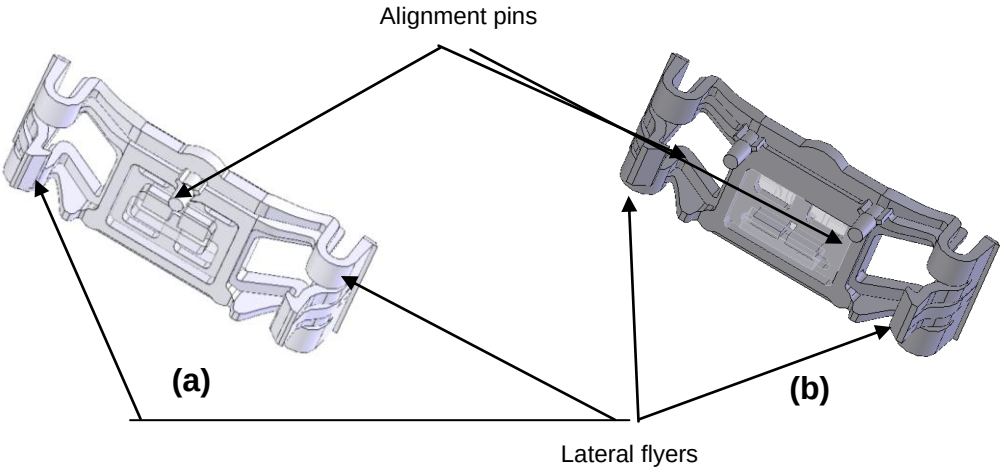


Figure 4: *Bottom view of (a) "**2 IN**" and (b) "**1PCR**" clamps*

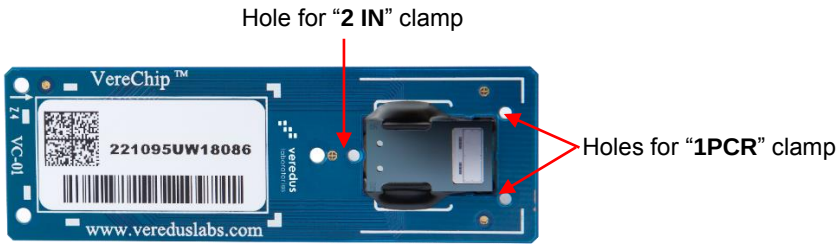


Figure 5: *Alignment holes for "**2 IN**" and "**1PCR**" (or Hybridization) clamps*

iii) Attach the "**1PCR**" first by pressing the lateral flyers and placing the pins into the alignment holes (*Figure 4* and *Figure 5*). After reaching the final position (the solid part of the clamp touches the edge of the Chip) release the flyers and press the upper part of the clamp until a 'click' sound is heard (*Figure 6*).

Figure 6: *"**1PCR**" clamp attached to Chip*

iv) Repeat step (iii) with "**2 IN**" clamp.

v) After the Chip is sealed (*Figure 7*), remove Chip from the Chip Holder.

Figure 7: *Sealed Chip ready for PCR*

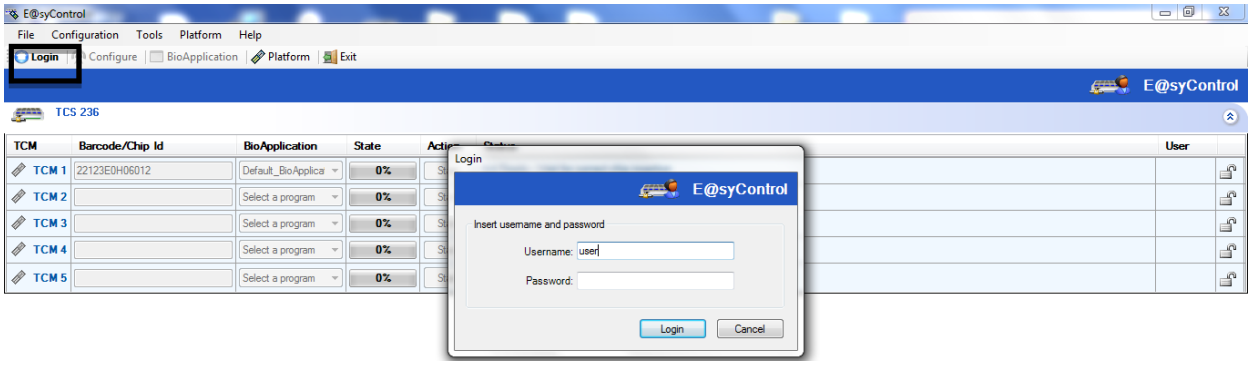
4. Run Chip (PCR)

i) Switch on the TCS.

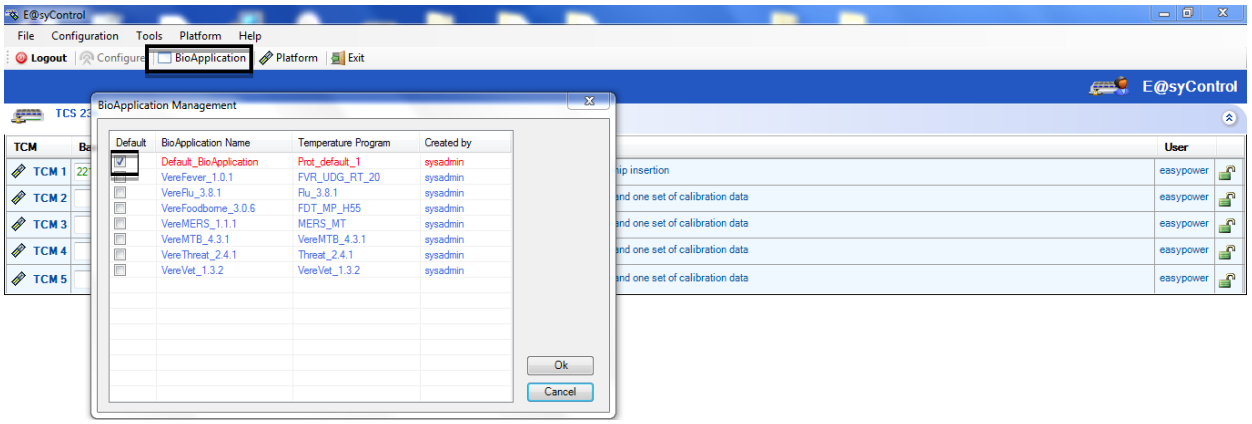
ii) Switch on the computer and launch "*E@syControl*" software by clicking on the icon  on the computer desktop.

The program will start searching for connected TCSs and the green TCS icon  will be displayed in the "*E@syControl*" window when TCS is connected. TCM will display "*READY TO USE*" message on the LCD screen.

iii) Click "*Login*" on the toolbar. The "*Login*" window will be displayed. Log in with the correct username and password.



iv) Select "*BioApplication*" from the toolbar and check the "**VereMTB**" (version 4.3.1 or higher) BioApplication.



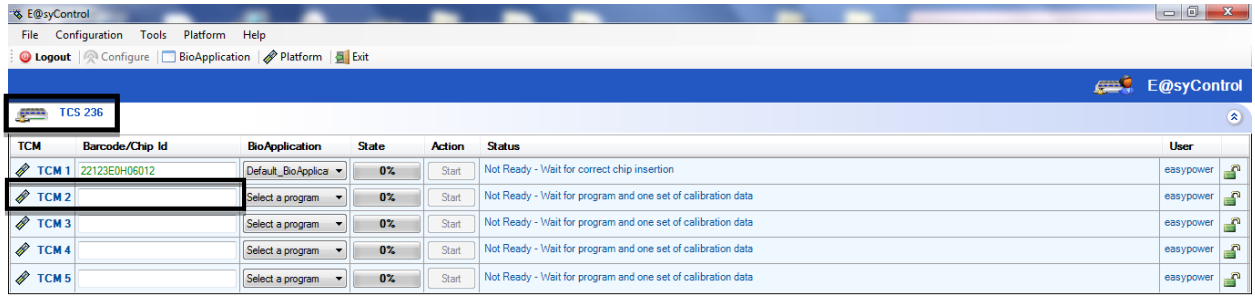
v) Open the lid of the TCM (if not already open).

vi) Place Chip into TCM.

NOTE: **Ensure the alignment pins on the TCM are inserted into the corresponding alignment holes on the Chip (*Figure 8*).**

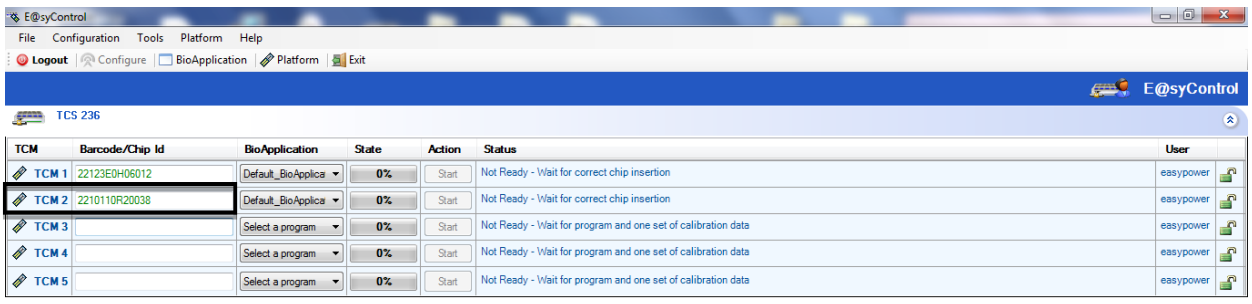
Figure 8: *Chip inserted into the TCM*

vii) In the "*E@syControl*" window, select the appropriate TCS and place the cursor in the "*Barcode/Chip Id*" field of the respective TCM.



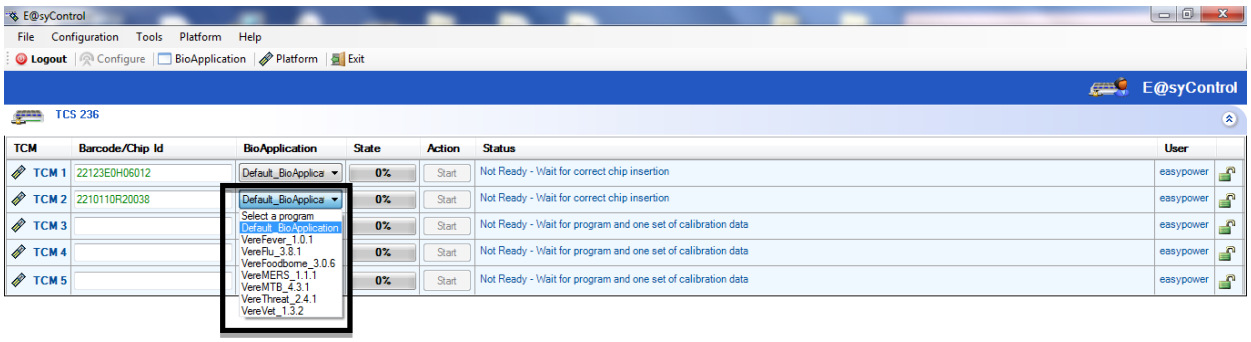
viii) Scan the 2-D barcode on the Chip using the barcode scanner.

NOTE: **Wait for the program to register the Chip calibration data before scanning the next Chip. The Chip ID should become "green" color.**

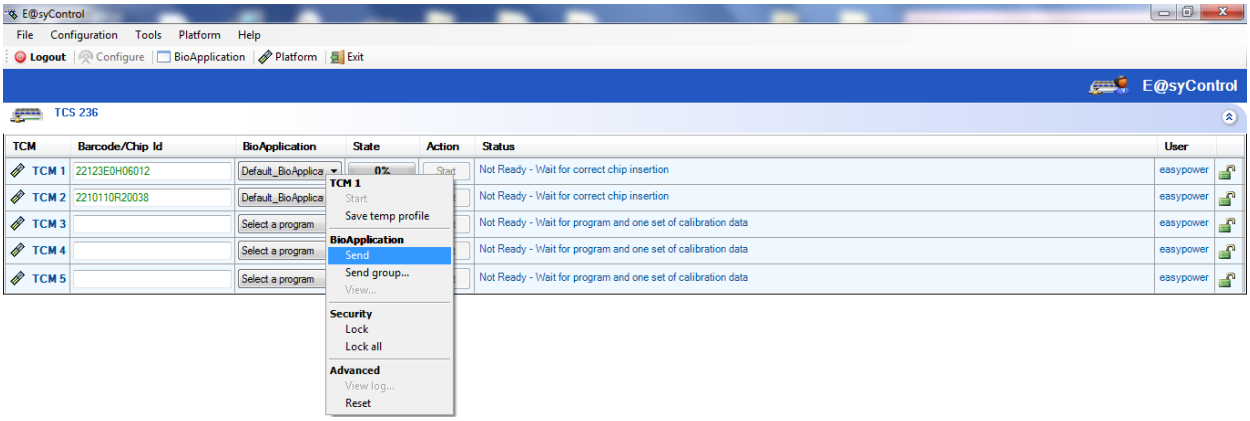


ix) Repeat step (v) to (viii) to register all of the Chips to be run.

x) For random access, select the relevant BioApplication from the dropdown list under "*BioApplication*" for each TCM.



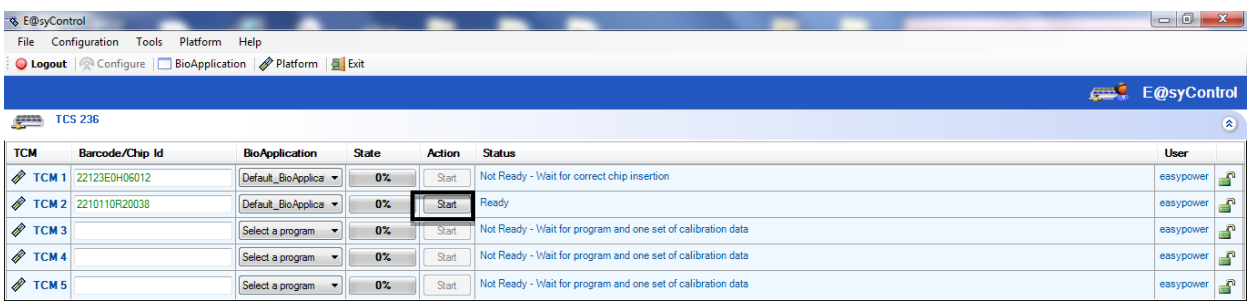
xi) After selection, right-click the selected BioApplication. An option menu will be displayed. Select "*Send*" to load the selected program to the corresponding TCM.



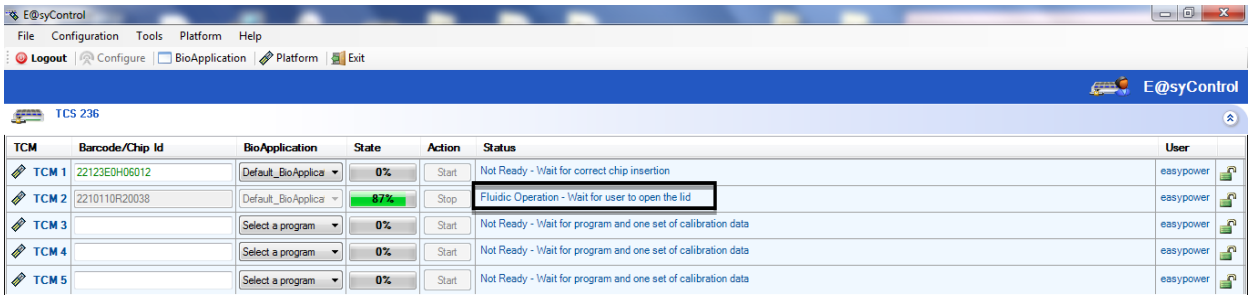
xii) Close the lid of TCM. The TCM will validate the Chip against its calibration data and the TCM will display "*CHIP VALIDATION*" message on LCD screen.

xiii) Once the Chip is validated, the TCM will display "*CHIP INSIDE PRESS START*" on the LCD screen or "*Ready*" in the "*Status*" field in the "*E@syControl*" window.

xiv) Press "*Play*" button (▶) on the TCM front panel or click "*Start*" in the "*E@syControl*" window to begin thermal program.



xv) Once the PCR protocol is completed, "*WAITING FLUIDIC OPERATION*" will be displayed on the LCD screen of the TCM or "*Fluidic Operation – Wait for user to open the lid*" will be displayed under the "*Status*" field in the "*E@syControl*" window.



5. Prepare Hybridization Mix

i) Prepare Microarray Hybridization Mix by transferring **870 µL** of 2X Hyb Buffer to 1 tube of **30 µL** MTB Hyb Probe:

Number of Reactions	25 reactions
Hyb Mix Components	**Volume (µL)**
2X Hyb Buffer	870
MTB Hyb Probe	30
Total	**900**

ii) Leave Microarray Hybridization Mix at room temperature to equilibrate for at least 20 minutes.

Microarray Hybridization Mix must be equilibrated at room temperature for 20 mins before use. Mix well before use.

iii) Mix thoroughly by vortexing the tube briefly (~10 seconds) or inverting it 4-6 times before spinning down.

6. Load Hybridization Mix into Chip

i) Remove Chip from the TCM when prompted.

ii) Insert Chip onto the Chip Holder and ensure a secure fit.

iii) Remove "**2 IN**" and "**1PCR**" clamps and discard them. **DO NOT** reuse the clamps.

iv) Draw **14.5 µL** of the Microarray Hybridization Mix with a pipette.

NOTE: **Use a 20 µL pipette and recommended pipette tips[4] for Chip loading.**

v) Hold the pipette in a vertical position, in such a way that the tip is perpendicular to the surface.

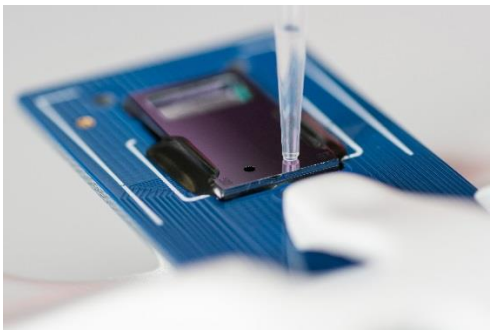
vi) Fit the tip into one of the inlet holes (see *Figure 9a*).

vii) Applying slight pressure onto the tip, press the plunger smoothly to the first stop position (see *Figure 9b*), allowing the mix to flow into the PCR chamber. The PCR mix inside the PCR chamber will be displaced by the Microarray Hybridization Mix and will be observed to fill up the microarray chamber (*Figure 10*).

Do not press the plunger <u>beyond the first stop</u> as this will introduce air into the chamber and mix will flow into the microarray chamber. Keep the plunger at the first stop until you remove the tip from the inlet (this procedure avoids spilling and the injection of air inside the chambers).

viii) Using a new pipette tip, repeat steps (vi) and (vii) for another chamber. Load the mixture into the other inlet.

NOTE: **Use a new tip for every loading to prevent carryover of the PCR product.**



(a)

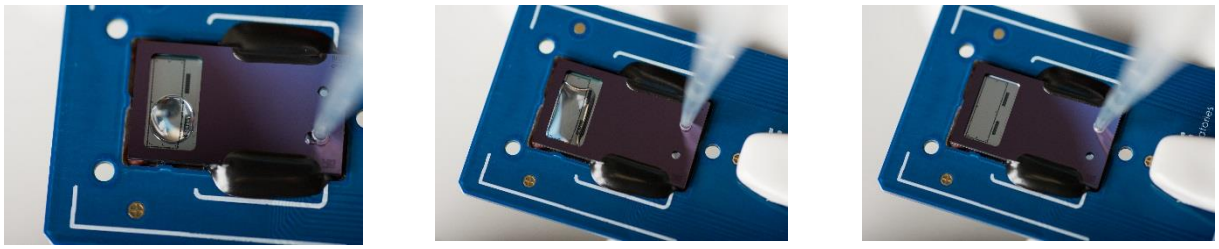
(b)

Figure 9: *(a) Tip placement into an inlet hole during sample loading; (b) Pipette sketch indicating the different stop positions*

Press and release the pipette plunger <u>slowly</u> at all the times. Never allow the push button to snap back. Check for foreign particles in the tip. Hold the pipette in an upright position while aspirating liquid.

[4] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) is recommended

ix) Tap the Chip gently at the side if the solution in the microarray chamber does not fully fill the microarray chamber.



(a) **(b)** **(c)**

Figure 10: *Microarray chamber filling – (a): filling of first inlet; (b) filling of second inlet (c): completely filled*

7. Seal Chip for Hybridization

i) Prepare a new IN clamp (labeled "**2 IN**") and HYB clamp (not labeled) as shown in *Figure 11*.

Figure 11: *IN and HYB clamps*

ii) The HYB clamp has a flat PDMS surface, 100 µm deep elicited in the gasket, and a surrounding trench used to accommodate the air displaced by the solution (*Figure 12*).

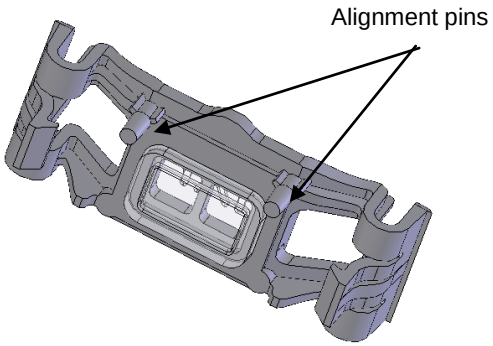


Figure 12: *Bottom view of HYB clamp*

iii) Attach the "**2 IN**" clamp first (*Figure 13*).

Figure 13: *"**2 IN**" clamp attached to Chip*

iv) Seal the microarray chamber carefully using the HYB clamp, making sure that no bubbles are introduced into the chamber (*Figure 14*).

Figure 14: *Sealed Chip ready for the hybridization*

NOTE: **Should any bubbles form during the sealing of the microarray chamber, tap the Chip gently on the workbench, microarray closest to the bench surface. This will force the bubbles to migrate to the outlet edge of the PCR chamber where there are no probes (*Figure 15*).**

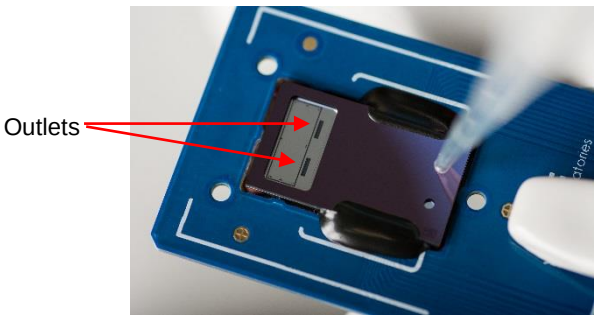
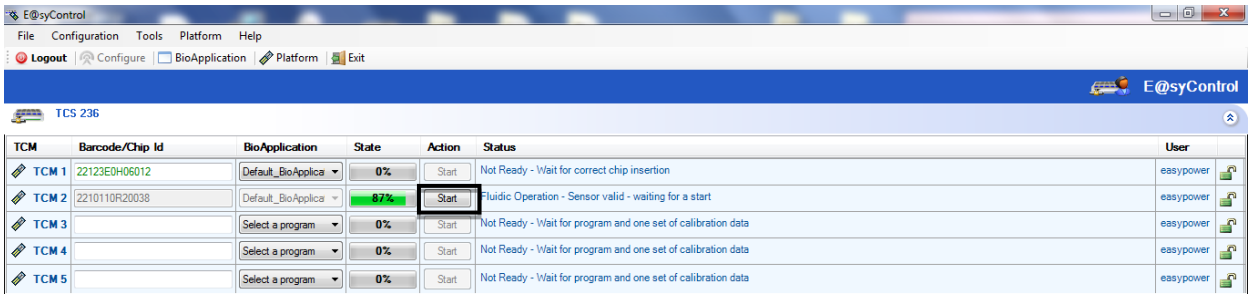


Figure 15: *Outlet edge of the PCR chamber on the Chip*

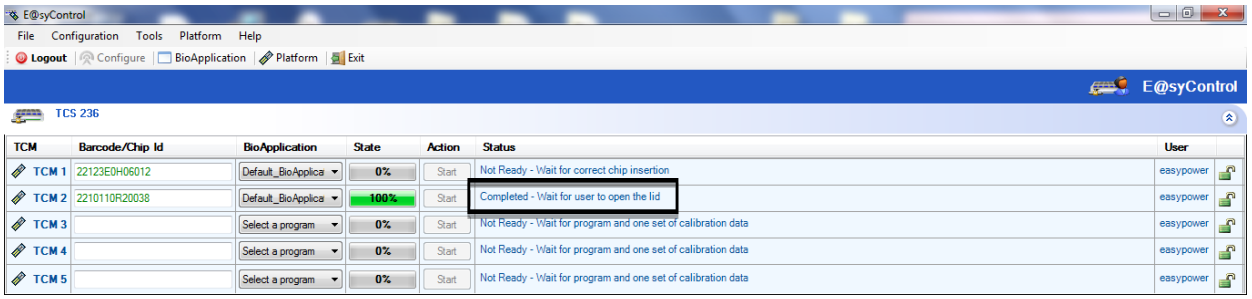
v) Remove Chip from the Chip Holder, making sure the clamps are tightly held in place.

8. Run Chip (Hybridization)

i) Load the sealed Chip into the respective TCM. Press "*Play*" button (▶) on the TCM front panel or press '*Start*' when prompted.



ii) Once the hybridization protocol is completed, "*COMPLETED*" message will appear on the LCD screen of the TCM or "*Completed – Wait for user to open the lid*" will be displayed in the "*E@syControl*" window. Remove the Chip from TCM and proceed to washing step (Section 9) immediately.



Temperature is one of the biggest factors to control hybridization process. TCM will not maintain the temperature after hybridization process is completed. To avoid exposure to the lower temperature, it is highly recommended to start washing step immediately after the hybridization protocol is completed.

9. Wash Chip

In case of precipitation or crystallization in the Wash Buffer Concentrate, warm up the entire bottle of buffer in a water bath set to 40°C for approximately 15 minutes or until the crystals dissolve. Mix thoroughly before use.

i) Measure 50 mL of the supplied Washing Solution Concentrate into a 1 L empty bottle. Top up the 1 L bottle with Distilled/RO/Ultrapure Water to 1 L. Mix well.

Components	Volume (mL)
Washing Solution Concentrate	50
Distilled/RO/Ultrapure Water	950
Total	**1000**

ii) Prepare and fill non-skirted 50 mL centrifuge tubes with 50 mL of the prepared Wash Buffer from step i *(Figure 16*).

NOTE: **Fill Wash Buffer to 50 mL mark on centrifuge tube to completely submerge the Chip. Place only ONE Chip per tube.**

iii) Remove the "**2 IN**" and HYB clamps, paying attention that no liquid spills out. Discard clamps. **DO NOT** reuse the clamps.

iv) Insert Chip with the microarray end at the top into the centrifuge tube *(Figure 17)*. Screw the tube cap on.

Figure 16

Figure 17

Figure 18

v) Place the tube with the microarray side facing towards the rotor axis *(Figure 18)*.

vi) Centrifuge the tube at 3000 rpm for 2 minutes.

The centrifuge spins at high speeds. Ensure that the lid is closed properly and that all the buckets are correctly balanced.

vii) After centrifugation, empty the tube of Wash Buffer and place tube back into the centrifuge with the microarray in the same orientation as step (v).

viii) Centrifuge the tube at 3000 rpm for 2 minutes to spin-dry the microarray.

ix) After centrifugation, remove the Chip using a pair of tweezers.

x) Proceed to detection step immediately.

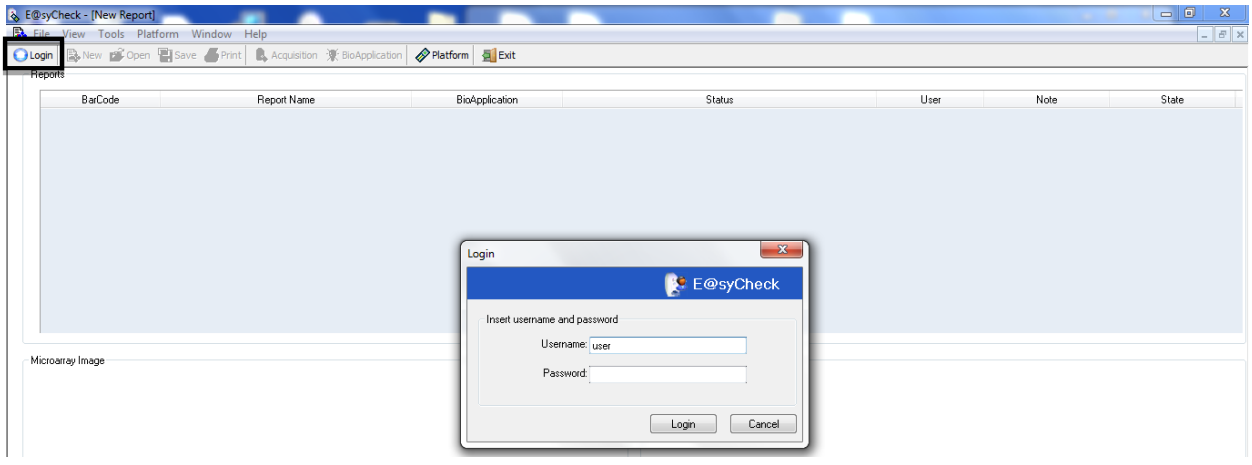
Fluorescent dye is used and is prone to degradation upon ozone exposure. It is highly recommended to proceed to the detection step immediately after washing. Minimize exposure of hybridized arrays to light, high temperatures and high ozone levels after washing. Place the Chip with the microarray face down on a clean paper towel or in a container.

10. Detection

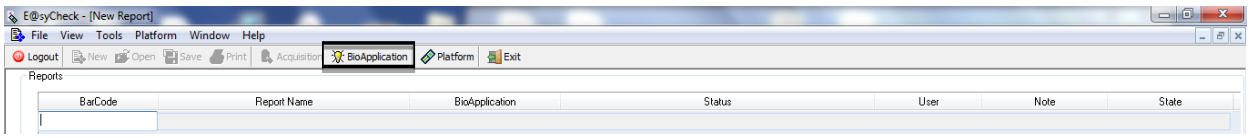
i) Switch on the Optical Reader (OR).

ii) Launch "*E@syCheck*" software by clicking on the icon  on the computer desktop.

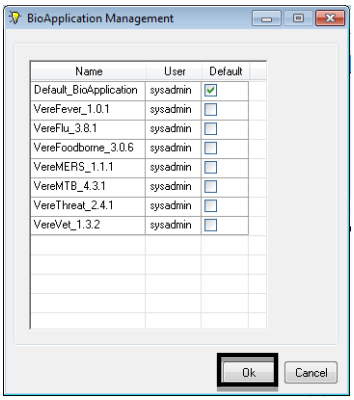
iii) Click "*Login*" on the toolbar. The "*Login*" window will be displayed. Log in with the correct username and password.



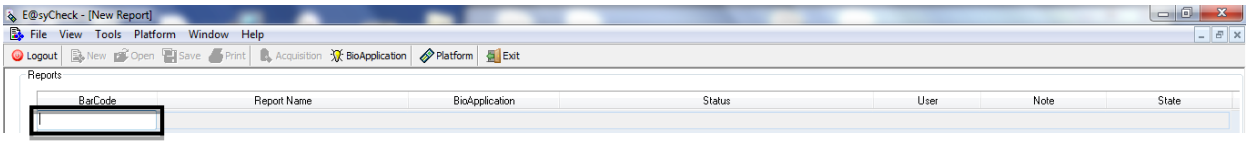
iv) Click "*BioApplications*" on the toolbar.



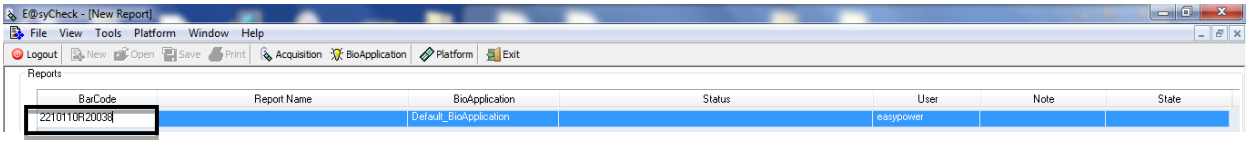
v) The "*BioApplication Management*" window will be displayed. Check the "**VereMTB**" (version 4.3.1 or higher) BioApplication. Click "*Ok*" to proceed.



vi) Click on the "*Barcode*" field to bring the cursor to this location.



vii) Scan the 2-D barcode on the respective Chip.

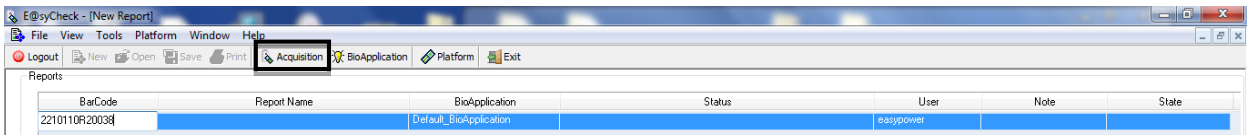


viii) Open the OR lid (if not already open).

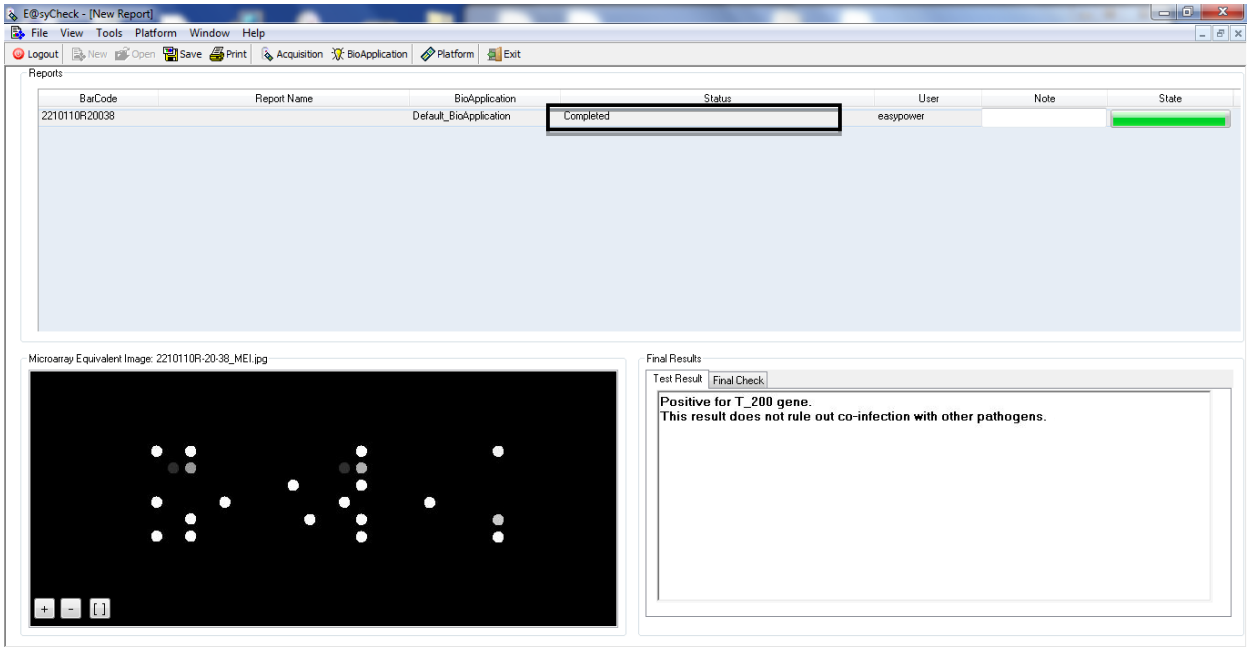
ix) Insert Chip into the OR with the microarray facing up (*Figure 19*).

Figure 19: *Chip inserted into OR*

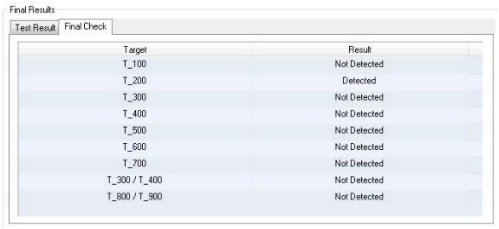
x) Click on "*Acquisition*" button on the toolbar. The OR will begin image acquisition and image analysis immediately.



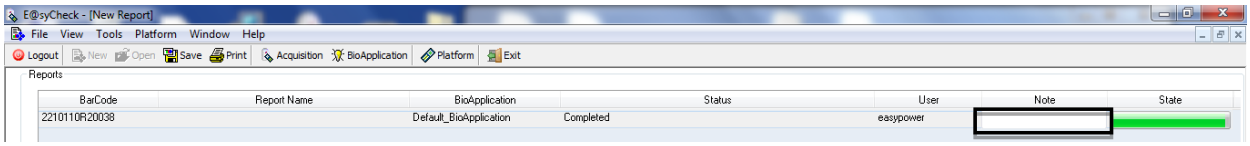
xi) After "*Acquisition*" operation, "*Completed*" will be displayed in the "*Status*" field and the results will be displayed.



Clicking the "*Final Check*" tab shows target/control detection summary.

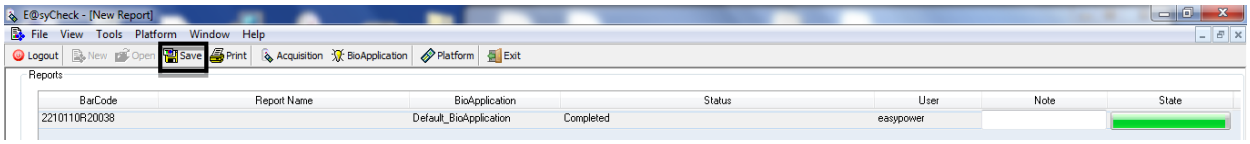


xii) Comments on this particular Chip run can be recorded under the "*Note*" field and this will be printed on the final report.

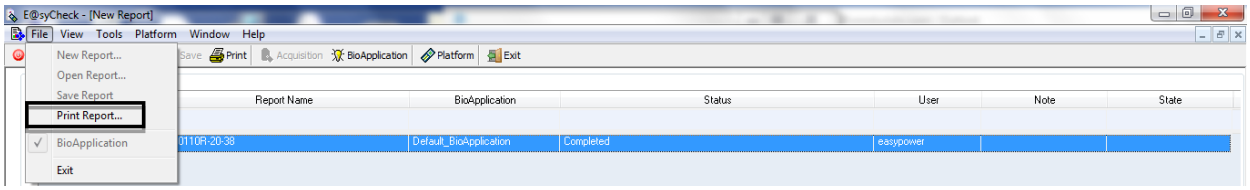


NOTE: **"*Note*" field can be modified only BEFORE saving the analysis.**

xiii) To save the analysis, press "*Save*" button on the toolbar. All the information associated to the analysis will be stored in a local database.

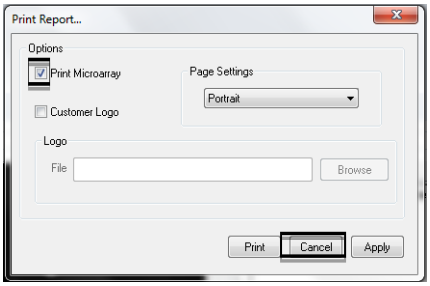


xiv) Click "File" on the top toolbar and select "Print Report" from the context menu.

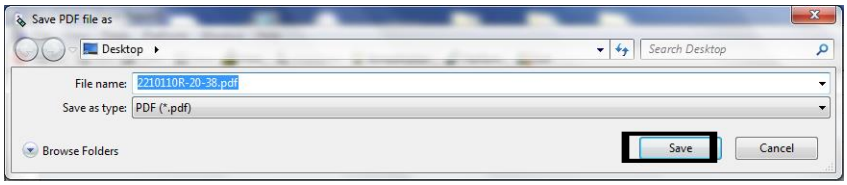


xv) The "*Print Report*" window will be displayed. Click "*Print*" to proceed.

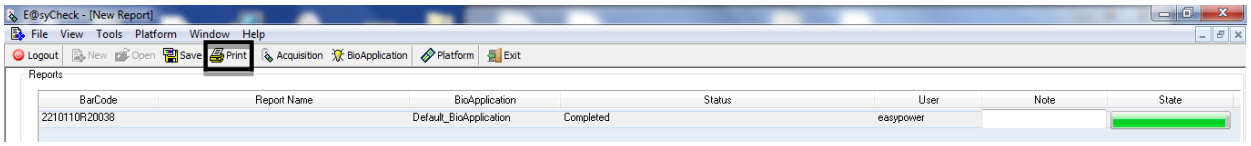
NOTE: **By default, the microarray equivalent image is not included in the PDF report, check "*Print Microarray*" to print report with the microarray equivalent image.**



xvi) A "*Save PDF file as*" window will be displayed. Save file in the desired destination folder. Click "*Save*" to proceed.



xvii) Alternatively, press "*Print*" button on the toolbar to display and print the PDF report.



Assay Controls

The following controls are included in each test:

- Internal PCR controls to check for succesful nucleic acid amplification reaction
- Amplicon controls to detect a gene region specific for *rpoB*, *katG* and *inhA* locus
- Positive and negative hybridization controls to check for hybridization-related issues
- Orientation controls for microarray grid alignment

It is recommended to include a negative control sample for each test run to check for possible contamination.

Interpretation of Results

The VerePLEX™ Biosystem software provides a qualitative result for the presence (Detected) or absence (Not Detected) of the target gene/organism under the "*Test Result*" and "*Final Check*" tab on the software interface and the final printed report. "Inconclusive" will be displayed for presence of bad spot signal(s) and the number of bad spot fails to meet the set criteria.

The control fields will display "Valid", "Not Valid" and "Inconclusive". If the hybridization control and/or and negative control is "Not Valid" or "Inconclusive", the software will not proceed with any further data analysis and the result of the control will only be displayed in the "*Test Result*" section. No result will be displayed in the "*Final Check*" section. The validity of PCR control has no influence of the outcome of the result if the target is "Detected".

For amplicon controls, a positive test result (evaluable wild type and/or mutation probes pattern) may cause the amplicon control signal to be weak or absent (especially katG amplicon control). If wild type and mutation probes are negative, and amplicon control signal is absent, the test cannot be evaluated (Results: "Indeterminate").

For further details regarding the interpretation of the results and recommended actions, please refer to Troubleshooting Guide or contact our Technical Support.

Troubleshooting Guide

The troubleshooting guide may be helpful in solving problems that may arise.

	Comments and Recommended Actions
Instrument and Software Issues	
1. Hardware failure	Make sure that the instruments (TCS and OR) are properly maintained. Refer to the Troubleshooting section of the VerePLEX™ Biosystem SYSTEM & SOFTWARE IFU.
2. Error message displayed on the screen	Refer to the Troubleshooting section of the VerePLEX™ Biosystem SYSTEM & SOFTWARE IFU.
Chip 'NOT Valid'	
1. Poor contact between Chip electrodes and pogo pins on TCS	Re-seat the Chip in the TCM and make sure the Chip has good contact with the pogo pins.
2. Small particles or stains blocking the Chip electrodes	Use 70% ethanol to wipe the Chip electrodes.
3. Mechanical damage due to improper storage or manipulation of Chip	Use another Chip to run test. Keep Chips in chip box until use. Do not put extra pressure on the Chips during Chip loading.
No Results	
1. Negative control "Not Valid" OR "Inconclusive"	1. Make sure the correct BioApplication is used. 2. One or more negative control probes have a fluorescent signal due to a high fluorescent background or fluorescent artifacts in the microarray surface. This problem may occur in the following situations: a. Dust or fiber-like material is found on the microarray area. Inspect the area and repeat detection step. If the high background persists repeat the test. b. The Chip is not washed properly. Repeat the washing step following carefully the Instructions for Use (IFU). If the high background persists repeat the test. c. The Wash Buffer is not diluted according to the instructions. Prepare a new bottle of Wash Buffer following carefully the IFU and repeat the washing step using the newly prepared Wash Buffer. If the high background persists repeat the test. d. The Hybridization clamps are not properly inserted causing partial or total evaporation of the Microarray Hybridization Mix with a consequent drying of the

	Comments and Recommended Actions
	fluorescent mix on the microarray surface. Repeat the test. e. Contamination. Repeat the test. Ensure that the workspace and instruments are decontaminated at regular intervals. Refer to the Cleaning section of the respective IFU.
2. Hybridization control “Not valid” OR “Inconclusive”	1. Make sure the correct BioApplication is used. 2. Some of the hybridization control probes are not lit up. This problem may occur when the labeled hybridization probes are partially degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 3. High fluorescent background or fluorescent artifacts in the microarray surface (see negative controls not valid or inconclusive comments and suggestions).
3. No signal	1. Make sure the correct BioApplication is used. 2. Photo-bleaching of the dye signal in the microarray detection area due to high level of ozone in the lab. Repeat the test and minimize the microarray surface exposure to the light as much as possible. 3. The labeled primers and hybridization probes are degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 4. The Wash Buffer is not diluted according to the instructions. Prepare a new bottle of Wash Buffer following carefully the IFU and repeat the washing step using the newly prepared Wash Buffer. 5. Defective Chip. Repeat test with new Chip. 6. The kit has expired. Check the expiry date of the kit and use a new kit, if necessary.
PCR control “Not Valid”	
1. No fluorescent signals for PCR control probes but the specific target probes give signals	1. Make sure the correct BioApplication is used. 2. This may occur when the target nucleic acid is much more concentrated than the PCR control. This has no influence on the outcome of the test. 3. The PCR control is degraded due to wrong storage conditions. This has no influence on the outcome of the test but use new reagents in the next run.
2. No fluorescent signals for	1. Make sure the correct BioApplication is used.

	Comments and Recommended Actions
both the PCR control probes and the specific target probes	2. The PCR control and the target nucleic acid are degraded due to wrong storage conditions. Repeat the test using new reagents and sample and follow carefully the IFU on Storage Condition. 3. The PCR control and the target nucleic acid are degraded due the presence of RNase. Check quality of nucleic acid sample or use fresh nucleic acid sample. Repeat the test and follow carefully the IFU on Warnings and Precautions. 4. The labeled primers are degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 5. PCR was inhibited. Use recommended extraction methods. Repeat the test. 6. Defective Chip. Repeat test with new Chip. 7. The kit has expired. Check the expiry date of the kit and use a new kit, if necessary.
Inconclusive/Ambiguous results for specific probes	
1. One or more specific probes have bad spots	Some of the spots of one or more specific probes are not recognized as a spot by the E@syCheck software. This problem may occur when the spot morphology is not good mainly due to a high fluorescent background or fluorescent artifacts on the microarray surface (see negative controls not valid or inconclusive comments and suggestions).
2. One or more probes have one replica of a specific spots pair not lit up	This problem may occur when there is not enough amplified dye-labaled target to hybridized the microarray due to the following reasons: 1. The labeled primers are partially degraded due to wrong storage conditions. Repeat the test using new reagents and follow carefully the IFU on Storage Condition. 2. The target nucleic acid is partially degraded due the poor sample preparation and storage. Check quality of nucleic acid sample or use fresh nucleic acid sample. Repeat the test and follow carefully the IFU on Warnings and Precautions. 3. Insufficient starting material. Repeat test with increased amount of nucleic acid sample.

Limitations of the Test

- Use of this kit should be limited only to trained personnel.
- This test is a qualitative test and does not provide a quantitative value for the detected pathogen in the sample.
- Strict compliance with the IFU is required for optimal results. Modifications to these procedures may alter performance of the test.
- Appropriate specimen collection, handling, storage and processing procedures are required for the optimal performance of this test.
- This test is not to be used on specimen directly. Specimen needs to be processed using appropriate nucleic acid extraction methods prior to using this test.
- The dye used is susceptible to degradation upon exposure to ozone. Strict compliance with the processing procedures is required for optimal performance of the test. If possible, procedures should be done in a reduced ozone environment to eliminate degradation of the dye molecule.
- It is advised to scan each microarray only once; subsequent scans may not yield similar results as fluorescence intensity may decrease due to decay of the fluorophore.
- Results from the test should be interpreted with other laboratory data available.
- Although the kit is highly specific and sensitive, a low incidence of false results can occur. A negative result does not preclude the possibility of existence of the target organisms in the sample. Other available tests are required if questionable results are obtained.
- A specimen yielding a negative result may contain other organisms other than the target organisms.
- False negative results may occur due to presence of sequence variants in the gene targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate nucleic acids for amplification.
- False positive results may occur due to cross-contamination by target organisms, their nucleic acids, amplicons, or from non-specific signals in the test.
- Cross-reaction with NTM probes may occur (refer to Analytical Specificity section for more information).
- *M. phlei* may cross-react with *M. abscessus/M. chelonae* probe.
- *M. lentiflavum* may cross-react with *M. intracellulare/M. vulneris/M. chimaera/M. colombiense* probe.
- *M. szulgai*, *M. shimoidei* and *M. lentiflavum* may cross-react with *M. simiae/M. kansasii/M. scrofulaceum/M. gastri/M. mantenii* probe
- *M. mucogenicum*, *M. peregrinum* and *M. porcinum* may cross-react with *M. fortuitum probe.*

- Test is unable to co-detect (1) *M. avium* with *M. intracellulare, M. vulneris, M. chimaera* and *M. colombiense*; and (2) *M. fortuitum* with *M. abscessus* and *M. chelonae.*
- Test is unable to differentiate (1) *M. abscessus* and *M. chelonae,* (2) *M. intracellulare, M. vulneris, M. chimaera* and *M. colombiense, and* (3) *M. simiae, M. kansasii, M. scrofulaceum, M. gastri* and *M. mantenii.*

Disposal

Dispose of hazardous or biologically contaminated materials according to local safety regulations.

Technical Assistance

If you have any questions or technical issues regarding the use of the kit, or any other Veredus products, please contact our technical support department.

Contact

Your opinions, comments, questions or feedback are important to us and all Veredus' customers. Please contact us if you have any suggestions about product performance or new applications and techniques.

For information and technical assistance, please contact us via:

Telephone : +65 6496 8600
Fax : +65 6779 2680
Email : info@vereduslabs.com

Visit our website : www.vereduslabs.com

Understanding the Symbols

Symbol	Meaning
	Catalog number
	Lot number
	Contains sufficient for <n> tests
	Do not re-use
	Date of manufacture
	Manufacturer
	Temperature limitation
	Use-by date (YYYY-MM-DD)
	Consult Instructions for Use
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	This side up
	Caution

Product Use Limitations, Warranty Disclaimer

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