VereBeef™ Detection Kit Instructions for Use

VBEF-AA50

50

Store at -25°C to -15°C (frozen components)
Store at 15°C to 25°C (ambient components)

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Intended Use

VereBeef™ Detection Kit is a multiplex PCR/microarray-based test. This nucleic acid-based test is intended for qualitative detection and differentiation of multiple food-borne pathogens in raw beef trim samples. The following organisms are identified: *E. coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* (STEC) serogroups (O26, O45, O103, O111, O121 and O145) and *Salmonella* species. The virulence genes targeted for *E. coli* analysis are *eae* (intimin), *stx* (Shiga-like toxins). This test is for use in conjunction with the VerePLEX™ Biosystem. This kit is intended for trained laboratory personnel who follow standard microbiology laboratory practice to detect the presence of target organisms in raw beef trim. This kit is not for animal or human therapeutic or diagnostic use.

Summary and Explanation

STEC may also be referred to as Verocytotoxin-producing *E. coli* (VTEC) or Enterohemorrhagic *E. coli* (EHEC), are important causes of diarrheal illness. Approximately 5%–10% of people with STEC infection will develop hemolytic-uremic syndrome (HUS), ~10% of those who develop HUS will die or have permanent renal failure, and up to 50% of those who develop HUS will develop some degree of renal impairment[1].

Although *E. coli* O157:H7 has been most commonly identified as the cause of STEC infection, isolation of non-O157 STEC strains from clinical cases, outbreaks and environmental sources has been increasing. At least 150 STEC serotypes have been associated with outbreaks and sporadic illness[2]. In the United States, six non-O157 serogroups (O26, O45, O103, O111, O121, and O145) account for the majority of reported non-O157 STEC infections.

The main virulence factor of STEC is *stx1* or *stx2*, which has cytotoxic effect on human vascular endothelial cells. Human virulent STEC strains often may also contain other virulence factors such as intimin (*eae*), a protein essential for the intimate attachment and the formation of attaching and effacing lesions on gastrointestinal epithelial cells.

US Food Safety and Inspection Service (FSIS) has been testing beef manufacturing trimmings for six non-O157 STECs (O26, O45, O103, O111, O121, and O145) in addition to *E. coli*

[1] Thorpe CM. Shiga toxin-producing Escherichia coli infection. Clin Infect Dis. 2004;38(9):1298–303. doi: 10.1086/383473.
[2] CDC. Recommendations for Diagnosis of Shiga Toxin--Producing Escherichia coli Infections by Clinical Laboratories. MMWR 2009; 58 (RR12):1-14.

O157:H7 since May 2012. FSIS declared these six non-O157 STECs adulterants in raw, non-intact beef products and product components.

In September 2013, FSIS announced that all ground beef and beef trim that are tested for STEC will also be tested for Salmonella, the most common cause of foodborne illness in the meat and poultry industry.

VereBeef™ Detection Kit uses the lab-on-Chip (LOC) platform for simultaneous and qualitative detection and identification of *E. coli* O157:H7, big six STEC and *Salmonella* based on selected target genes.

Principle of the Procedure

VereBeef™ 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 target organisms listed in Table 1 as well as internal controls.

Table 1: Targets identified and differentiated by VereBeef™ Detection Kit

Targets	Genes/regions
E. coli O157:H7	O antigen gene cluster for O typing, *fliC* for H typing
E. coli O26	O antigen gene cluster
E. coli O45	O antigen gene cluster
E. coli O103	O antigen gene cluster
E. coli O111	O antigen gene cluster
E. coli O121	O antigen gene cluster
E. coli O145	O antigen gene cluster
STEC virulence factors	*stx1A, stx2A, eae*
Salmonella	*invA, ttr*

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 drives thermal control on the VereChip™. It consists of five Temperature Control Modules (TCMs) that allow five independent temperature programs to be run independently
- Optical Reader (OR), which captures fluorescence image of the microarray
- Laptop computer, which connects to the TCS and OR for display and input
- Barcode Reader for Chip ID and calibration data input
- 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 hybridizes to a complementary strand of amplified product. The amplified product is labeled with a fluorophore and probe-product hybridization is detected as fluorescent signals and captured by CCD camera in the OR.

The probes for targets are spotted in duplicates in a 6 rows x 21 columns layout. If target pathogen 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 pathogen, 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. **Tests**		**VBEF-AA50** **50**
Ambient Components (15°C to 25°C)	**Part Number**	**Quantity**
Box 1 of 2		
VereBeef™ Chip	BEF-12	2 boxes
PCR Clamps	CPR-01	1 pack
IN Clamps	CIN-02	2 packs
HYB Clamps	CHY-01	1 pack
Wash Buffer Concentrate	VCP-WBC03	1 bottle
VereBeef™ Lysis Buffer	BEF-LYB02	4 tubes
Frozen Components (-25°C to -15°C)	**Part Number**	**Quantity**
Box 2 of 2		
VereBeef™ Primer Mix A	BEF-PXA03	2 tubes
VereBeef™ Primer Mix B	BEF-PXB03	2 tubes
VCP Hyb Buffer	VCP-15B01	2 tubes
Hyb Probe Mix	VCP-AHP01	2 tubes
VeTaq™ One-Step Mix (2x)	VBI-OSM04	1 tube
CRD	VCP-CRD02	1 tube

Storage Condition

- Store all Frozen Components at -25°C to -15°C upon receipt.
- Keep the Premix and Hyb probe mix away from light until ready to use.
- After initial thawing:
    - Store Premix A and B at -25°C to -15°C for up to 2 months. Keep the number of freeze-thaw cycles to < 6 cycles.
    - Store VeTaq™ One-Step Mix (2x) at -25°C to -15°C for up to 12 months. Keep the number of freeze-thaw cycles to < 12 cycles.
    - Store CRD at -25°C to -15°C for up to 12 months.
    - Store reconstituted Microarray Hybridization Mix at -25°C to -15°C for up to 2 months. Keep the number of freeze-thaw cycles to < 6 cycles.
- Store Mastermix A and B aliquots at -25°C to -15°C for up to 2 months without thawing.

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

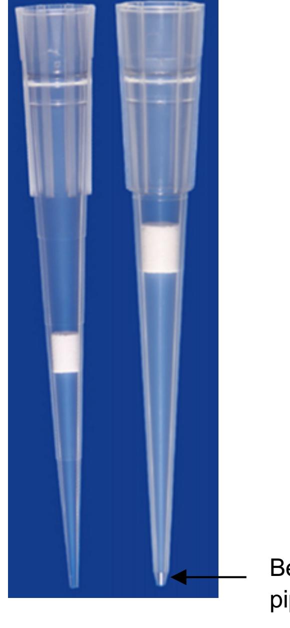
- Store all Ambient Components at room temperature (15°C to 25°C).
- After opening the aluminum packaging, the Chips are stable for 2 months when stored in its original box at room temperature.

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

- Precipitation or crystallization may occur in Wash Buffer Concentrate if the storage temperature is low. Should this occur, please refer to important note before Chip washing step (page 24).
- Once opened, Wash Buffer Concentrate can be kept at room temperature for up to 1 year or until expiration date, whichever comes first.
- Store diluted Wash Buffer at room temperature for up to 2 months. Precipitation or crystallization may occur in diluted Wash Buffer if the storage temperature is low. Should this occur, please refer to important note before Chip washing step (page 24)
- If left unopened, all reagents are stable until the expiration date indicated on the respective labels.

Materials Required but Not Provided

a) Reagents
- Modified Tryptone Soya Broth (mTSB)
- PCR Grade Water
- Distilled/Reverse Osmosis (RO)/Ultrapure water



Bevelled pipette tip

b) Consumables
- Personal protective equipment
- Sterile plain, clear polypropylene bags, or Whirl-Pak™ type bags, or equivalent
- Sterile beveled pipette tips[3] for Chip loading
- Sterile filter pipette tips
- 1.5 mL or 1.7 mL microcentrifuge tubes
- 0.2 mL PCR tubes
- Sterile 50 mL centrifuge tube (non-skirted)
- 1 L Bottle
- Decontamination products

c) Equipment
- Centrifuge with rotor and adapter for 50 mL centrifuge tube (non-skirted)
- Microcentrifuge for 1.5 mL or 1.7 mL tube
- Micropipettes (0.5-10 µL, 2-20 µL, 10-100 µL, 100-1000 µL)
- Freezer/Refrigerator (-25°C to -15°C / 2°C to 8°C)
- Vortex Mixer
- Stomacher, masticator or equivalent for homogenizing test samples
- Incubator or water bath, 42 ±1°C
- Heating block, 95 ± 0.5°C
- Balance, 2000 g capacity, sensitivity of 0.1g

d) Additional Accessories
- Tube rack/stand
- Tweezers

3. GoldenGate Bioscience. (Cat. No. BUN020GL-MRS) or VWR (Cat. No. 10126-388) is recommended.

Warnings and Precautions

a) Safety precautions

- The VereBeef™ Detection Kit should be disposed of following procedures for infectious or potentially infectious products.
- All samples should be treated as potentially infectious.
- Guidelines for manipulating Biosafety level 2 pathogens should be followed whenever live cultures are used.
- 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.
- 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.

b) Additional precautions

- 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.

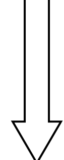
Quality Control

Under Veredus' quality assurance program, the performance of our VereBeef™ 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.

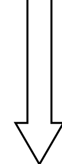
Workflow

Raw beef trim (25 g, 325 g or 375 g)
+ pre-warmed mTSB

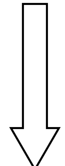
Enrichment at 42 ± 1 ºC for 10 hours - 20 hours[4]



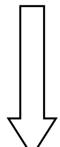
Lysis with VereBeef™ lysis buffer



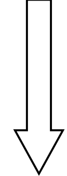
PCR of extracted bacterial DNA using **VereBeef™ Chip**



Hybridization of amplified product
onto the microarray



Washing of the microarray



Analysis of microarray results

[4] Incubation time can be reduced to 8 hours for *E. coli* O157, *E. coli* O157:H7, *stx* and *eae* detection only.

Specimen Collection, Handling and Storage

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.

Store extracted nucleic acids at 2°C to 8°C and use within 24 hours. For longer storage, store extracted nucleic acids at ≤ -20°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 VereBeef™ 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 method is recommended.

1. Sample Processing for Beef Trim Specimen

1.1 Sample preparation and Enrichment

Intact packages must be disinfected at the incision sites immediately prior to incision for sampling using an appropriate disinfectant, e.g., 3% hydrogen peroxide, ca. 70% ethanol or ca. 70% isopropanol. If the package does not appear to be clean, scrub gently using soapy water and rinse thoroughly prior to disinfection. A sterile scalpel may be helpful for cutting the packaging.

Sample preparation and enrichment incubation may vary by portion size, refer to Table 2 for additional details.

Table 2 Sample Preparation and Enrichment Guide

Sample	Portion Size (g)	Pre-warmed mTSB Volume (mL)	Incubation
Beef trim	375 ± 37.5	1125 ± 22.5	42±1 ºC for 10 hours – 20 hours[5]
	325 ± 32.5	975 ± 19.5	
	25 ± 2.5	75 ± 1.5	

i) Weigh the beef trim into a sterile polypropylene bag, add the pre-warmed mTSB. Stomach, blend or hand massage until clumps are dispersed.

[5] Incubation time can be reduced to 8 hours for *E. coli* O157, *E. coli* O157:H7, *stx* and *eae* detection only.

ii) Incubate at 42 ± 1 ºC for 10 hours – 20 hours.[6]

1.2 Lysis

i) Transfer 1 ± 0.1 mL of beef trim culture from into 1.5 mL or 1.7 mL microcentrifuge tube.

ii) Centrifuge at 1,500 x g for 1 minute to pellet large debris.

iii) Transfer 800 µL supernatant to a new sterile 1.5 mL or 1.7 mL microcentrifuge tube.

NOTE: **Make sure that none of the pelleted debris is carried over with the supernatant.**

iv) Centrifuge supernatant at 10,000 x g for 5 minutes.

v) Discard the supernatant by pipetting.

vi) Suspend the pellet in 100 µL of VereBeef™ Lysis Buffer by using the pipette tips.

vii) Heat the tubes in heating block for 10 minutes - 12 minutes at 95 ± 0.5 ºC.

NOTE: **It is recommended to use parafilm to seal the tubes, to prevent them from opening during 95 ± 0.5 °C incubation.**

viii) Vortex the tubes, and centrifuge at 10,000 x g for 2 minutes to pellet sediment.

ix) Transfer 50 µL – 75 µL of DNA-containing supernatant to a fresh 1.5 mL or 1.7 mL microcentrifuge tube.

x) Use extracted DNA for VereBeef™ chip runs.

xi) If not used immediately, store extracted DNA at ≤ -20°C.

[6] Incubation time can be reduced to 8 hours for *E. coli O157*, *E. coli O157:H7*, *stx* and *eae* detection only.

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 Wash Buffer Concentrate if the storage temperature is low. Should this occur, please refer to important note before Chip washing step (page 24).
- 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 VereBeef™ Chip version as follows:
  - BEF-12
- For software, use current version or higher as follows:
  - VerePLEX™ Biosystem Version 5.3.1
  - BioApplication VereBeef_1.2.2
- Screenshots are for illustration purposes only, and individual installations may vary.

1. Prepare PCR Reaction Mix

Users may choose Option 1 or 2 for PCR Reaction Mix preparation.

1.1 Option 1: Mastermix Preparation

i) Prepare Mastermix A and B in sterile PCR tubes according to the following pipetting scheme:

PCR Reaction Mix Components		Mastermix A (µL)	Mastermix B (µL)
VeTaq™ One-Step Mix (2x)		175	175
VereBeef™ Premix	A	70	-
	B	-	70
CRD		5.6	5.6
PCR Grade Water		29.4	29.4
Total		**280**	**280**

ii) Mix thoroughly by pipetting or brief vortexing.

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

iv) Aliquot Mastermix into 10 µL in sterile PCR tubes. If not use immediately, store aliquots at -25°C to -15°C for up to 2 months without thawing.

v) On the day of testing, thaw Mastermix A and B aliquots at room temperature.

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

vii) Add **2.5 µL of extracted DNA** samples into Mastermix A and B aliquots each for testing.

1.2 Option 2: PCR Reaction Mix Preparation

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 (µL)	
PCR Reaction Mix Components		**Tube A**	**Tube B**
VeTaq™ One-Step Mix (2x)		6.25	6.25
VereBeef™ Premix	A	2.5	-
	B	-	2.5
CRD		0.2	0.2
Extracted Sample		2.5	2.5
PCR Grade Water		1.05	1.05
Total		**12.5**	**12.5**

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 VereBeef™ 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 **Tube A** with a pipette.

NOTE: **Use a 20 µL pipette and recommended pipette tips[7] for Chip loading.**

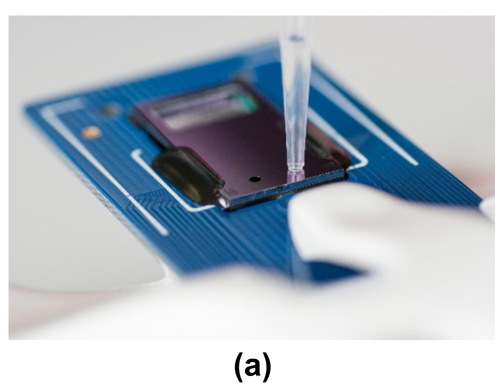
iii) Hold the pipette in a vertical position, in such a way that the tip is perpendicular to the surface.

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

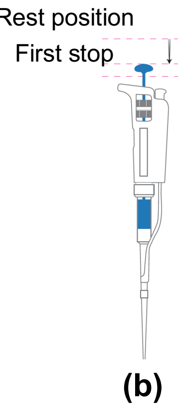
v) Applying slight pressure onto the tip, press the plunger smoothly to the first stop position (see *Figure 2b*), allowing the mix to flow into the PCR chamber.

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, repeat steps (0 to (v) for another chamber. Load the Reaction Mix **Tube B** 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.

[7] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) or VWR (Cat.No. 10126-388) 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.

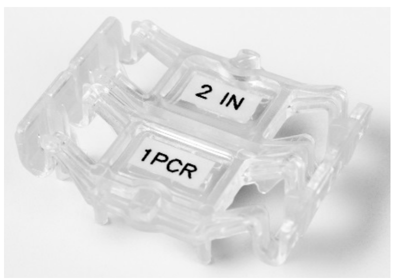


Figure 3: *IN and PCR clamps*

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*).

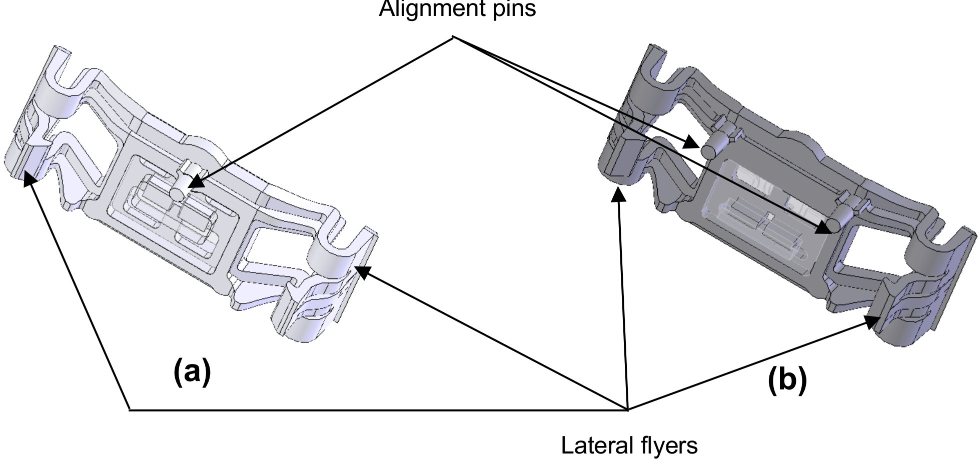


Figure 4: *Bottom view of (a) “**2 IN**” and (b) “**1PCR**” clamps*

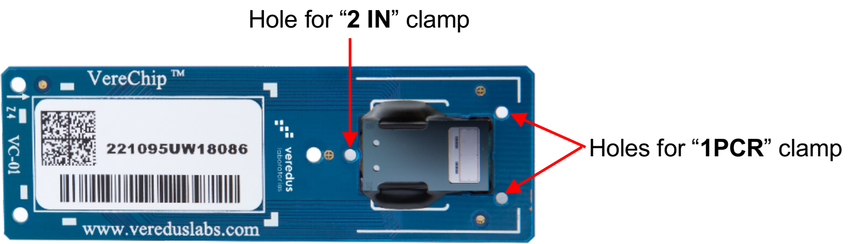


Figure 5: *Alignment holes for "**2 IN**" and "**1PCR**" (or Hybridization) clamps*

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*

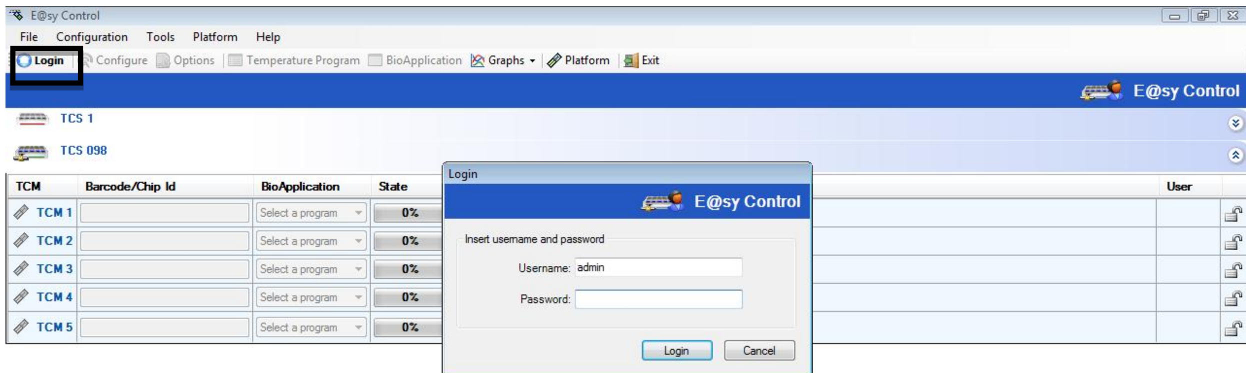
4. Run Chip (PCR)

i) Switch on the TCS.

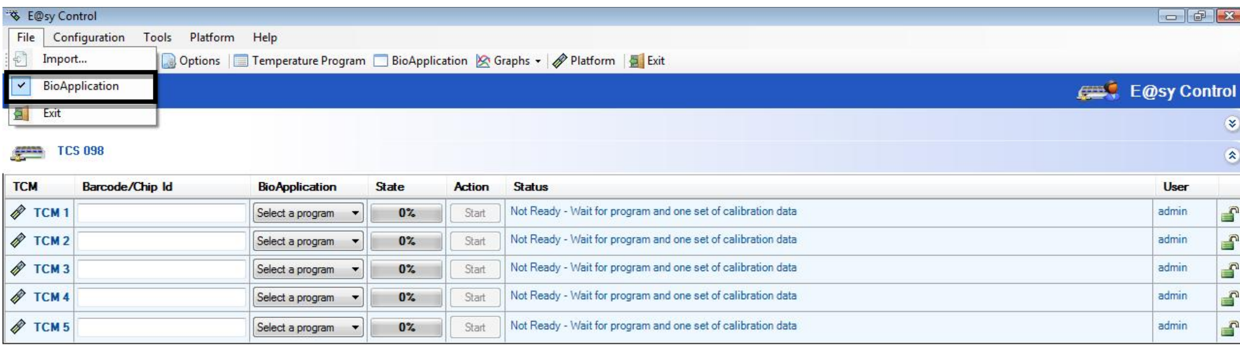
ii) Switch on the computer and launch "*E@syControl*" software by clicking on the icon on the computer desktop.

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.

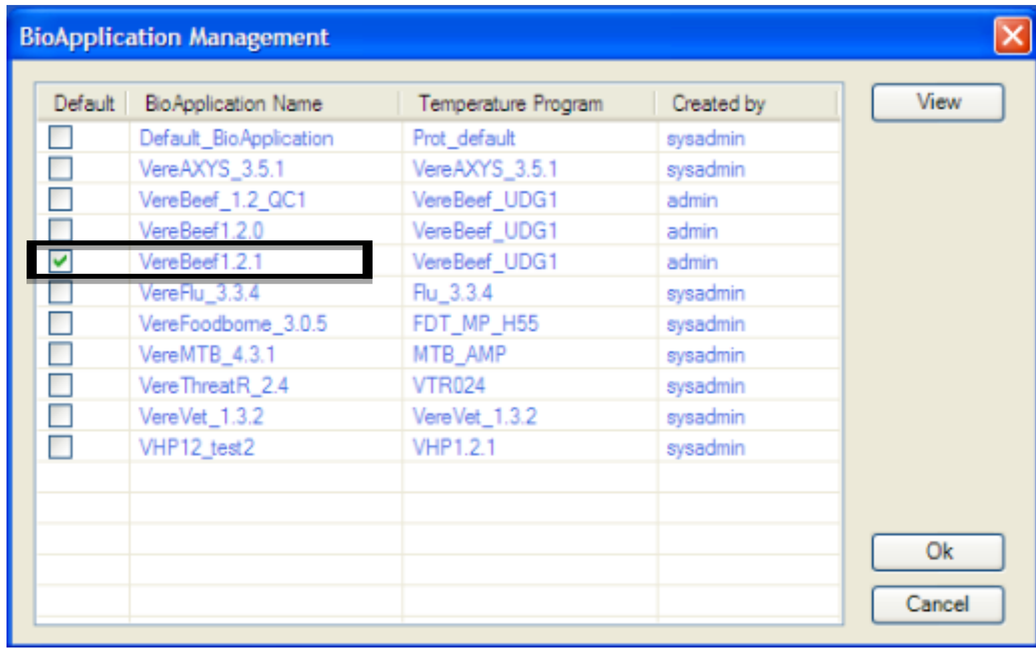
iii) Click "*Login*" on the top menu bar. The "*Login*" window will be displayed. Log in with the correct username and password.



iv) Click "*File*" on the top menu bar and ensure the "*BioApplication*" function is checked.



v) Select "*BioApplication*" from the toolbar and check the "**VereBeef**" (version 1.2.1 or higher) BioApplication.



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

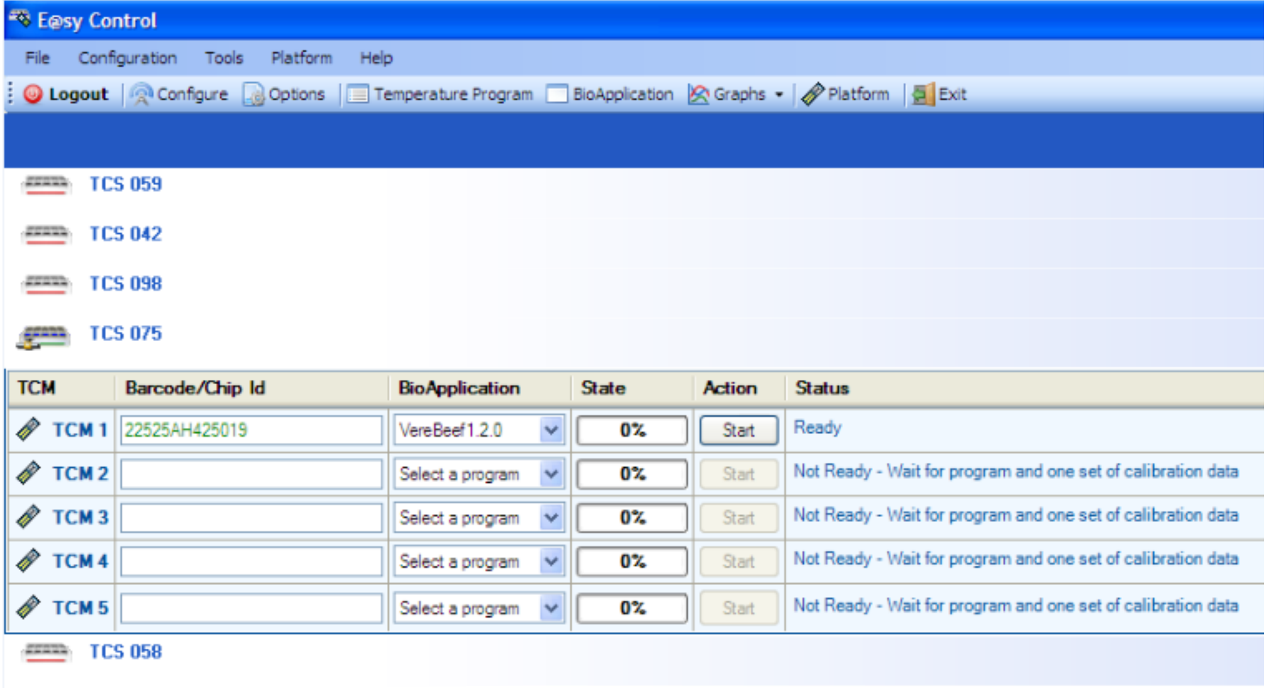
vii) Place Chip into TCM.

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

Figure 8: *Chip inserted into the TCM*

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

ix) 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.**

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

xi) 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.

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

xiii) Proceed to prepare hybridization mix (Section 5).

NOTE: **PCR run time is 47 minutes. Waiting time between the START of PCR reaction and Loading Hybridization Mix (Section 6) should be within 2 hours.**

xiv) 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 VCP Hyb Buffer to 1 tube of **30 µL** Hyb probe mix:

Number of Reactions	25 reactions (µL)
VCP Hyb Buffer	870
Hyb probe mix	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 minutes 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

Waiting time between the START of PCR reaction and Loading Hybridization Mix should be within 2 hours.

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[8] 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*).

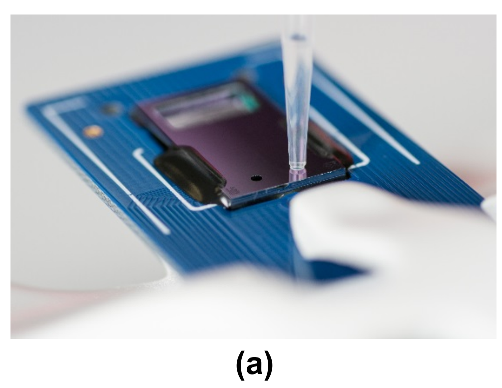
[8] National Scientific Supply Company, Inc. (Cat. No. BUN020GL-MRS) or VWR (Cat.No. 10126-388) is recommended

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*).

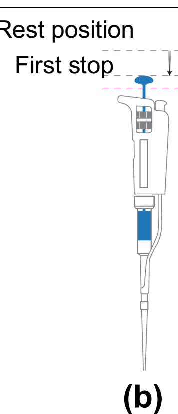
Do not press the plunger beyond the first stop as this will introduce air into the chamber and mix will flow into the microarray chamber. Keep the plunger at the first stop until you remove the tip from the inlet (this procedure avoids spilling and the injection of air inside the chambers).

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 slowly 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.

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

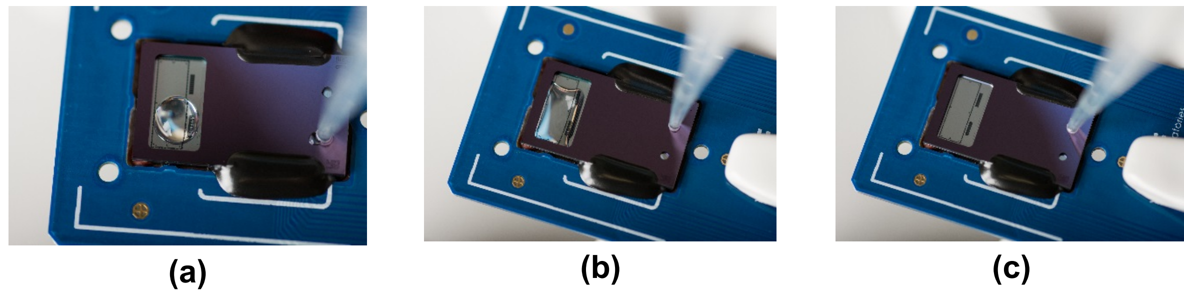


Figure 10: *Microarray chamber filling – (a): filling of first inlet; (b) filling of second inlet (c): completely filled*

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*).

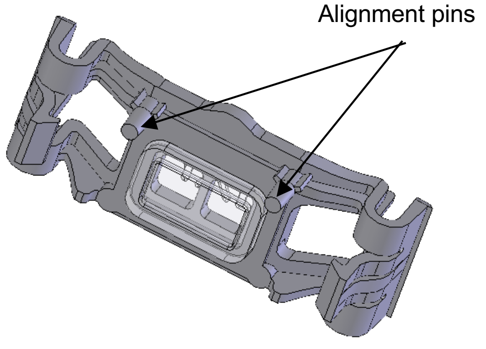


Figure 12: *Bottom view of HYB clamp*

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*).**

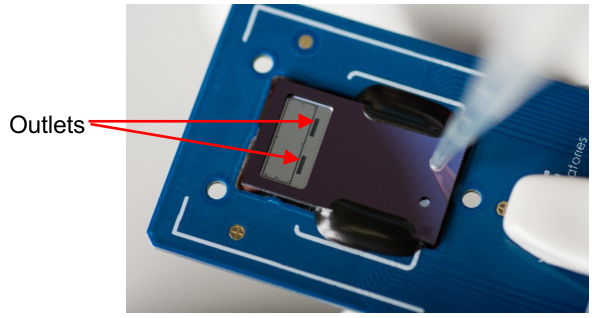


Figure 15: *Outlet edge of the PCR chamber on the Chip*

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 (with triangle) 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 "*PCR-Ended – 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 42°C for at least 1 hour with occasional shaking until all the precipitate is dissolved. Mix thoroughly before use.

i) Measure 50 mL of the supplied Wash Buffer 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)
Wash Buffer 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)*.

In case of precipitation or crystallization in the diluted Wash Buffer, warm up the entire bottle of buffer in a water bath set to 42°C with occasional shaking until all the precipitate is dissolved. Equilibrate at room temperature for at least an hour and mix thoroughly before use.

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. **<u>DO NOT</u>** 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 1157 x g 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 1157 x g 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.

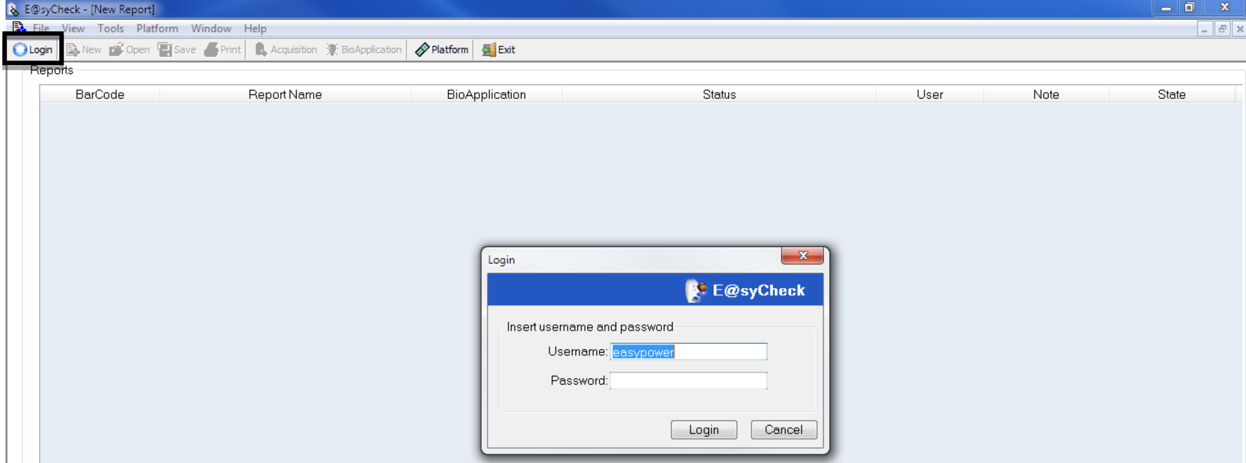
Fluorescent dye is used and is prone to degradation upon ozone exposure. It is highly recommended to proceed to the detection step immediately after washing. Minimize exposure of hybridized arrays to light, high temperatures and high ozone levels after washing. Place the Chip with the microarray face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

10. Detection

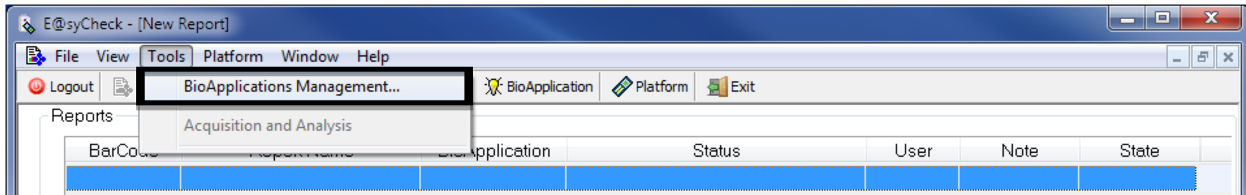
i) Switch on the Optical Reader (OR).

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

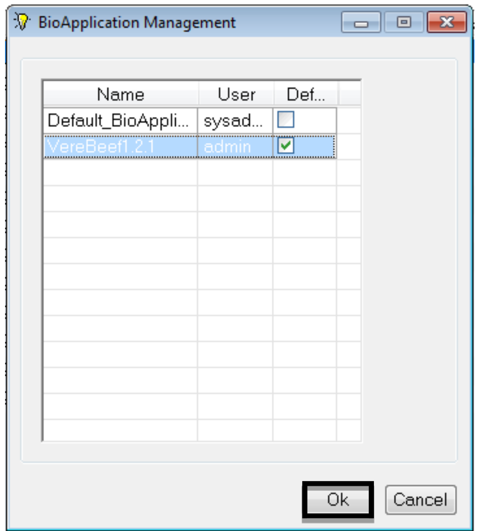
iii) Click "*Login*" on the top menu bar. The "*Login*" window will be displayed. Log in with the correct username and password.



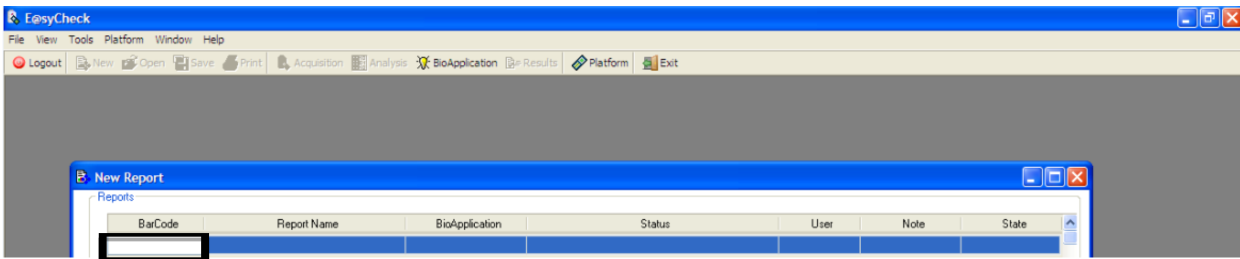
iv) Click "*Tools*" on the top menu bar, and select "*BioApplications Management*" from the context menu.



v) The "*BioApplication Management*" window will be displayed. Check the "**VereBeef**" (version 1.2.1 or higher) BioApplication. Click "*Ok*" to proceed.



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



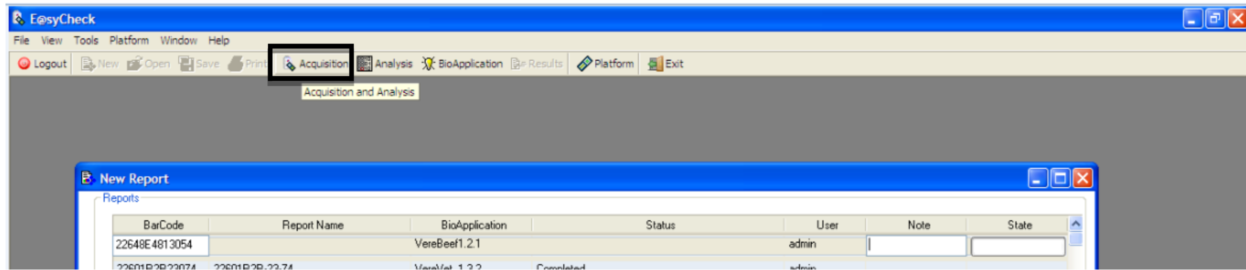
vii) Scan the 2-D barcode on the respective Chip.

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

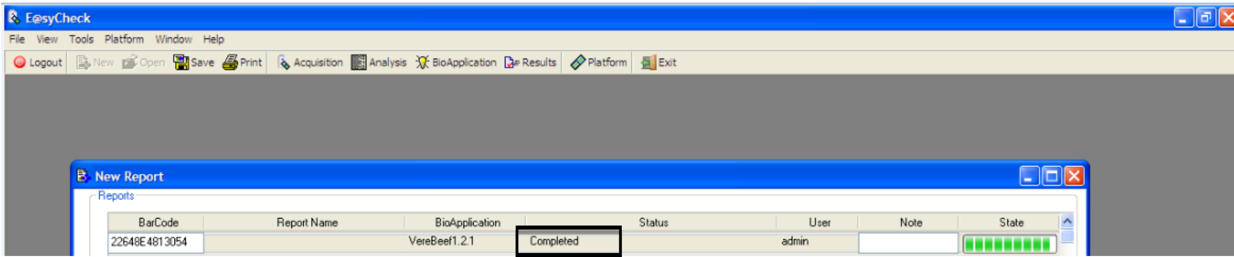
ix) Insert Chip into the OR with the microarray facing up (*Figure 19*).

Figure 19: *Chip inserted into OR*

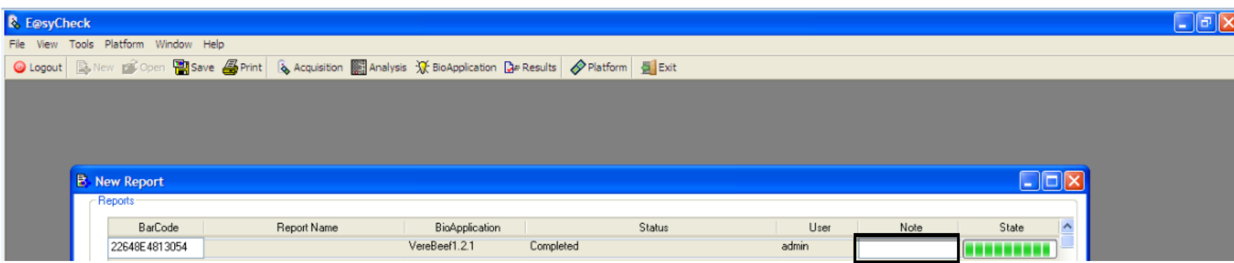
x) Click on "*Acquisition*" button on the toolbar. The OR will begin image acquisition and image analysis immediately.



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

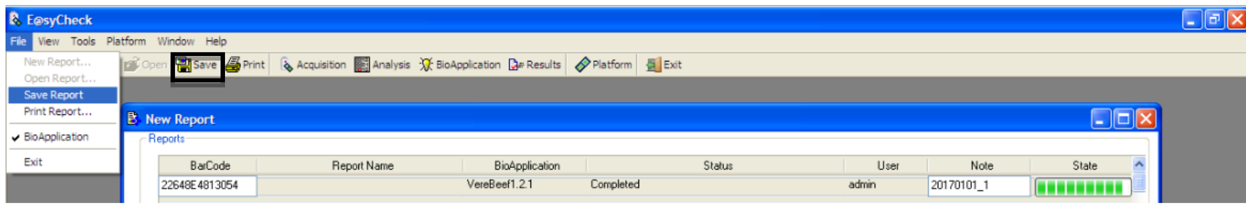


xii) Comments on this particular Chip run can be recorded under the "*Note*" field and this will be printed on the final report.

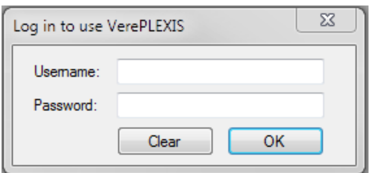


NOTE: **"*Note*" field can be modified only BEFORE saving the analysis.**

xiii) To save the analysis, press "*Save*" button on the toolbar or select "*File*" from the top toolbar and select "*Save Report*" from the context menu. All the information associated to the analysis will be stored in a local database.

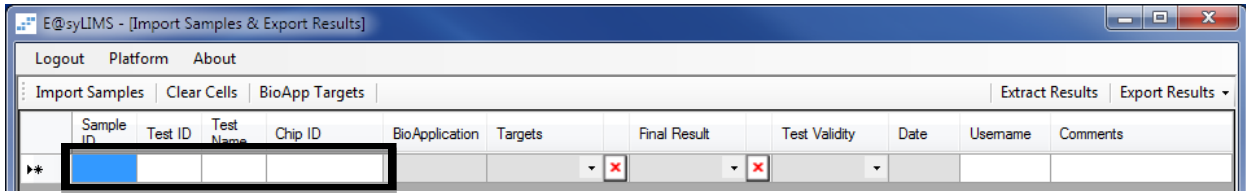


xiv) Launch "*E@syLIMS*" software by clicking on the icon on the computer desktop. Log in with the correct username and password.



xv) Upon logging in, there are 4 main fields for input:

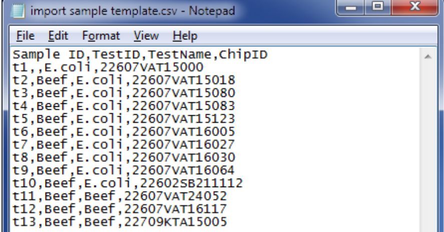
- SampleID (Mandatory)
- TestID (Optional)
- TestName (Optional)
- ChipID (Mandatory)



NOTE: **"*SampleID*", "*TestID*" and "*TestName*" are free text fields, which can take up to 11 characters.**

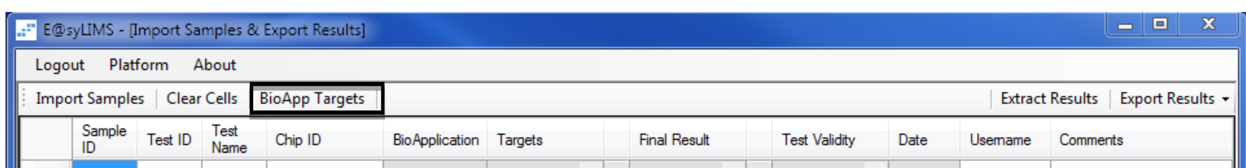
"*ChipID*" field can be entered manually and the recommended method is to use provided 2D-Barcode reader to scan the chips that were previously analysed in "*E@syCheck*".

For importing in CSV file format, the field header names have to be exact. The "*SampleID*", "*TestID*" and "*TestName*" fields are <u>without spacing</u>. Example of CSV below.

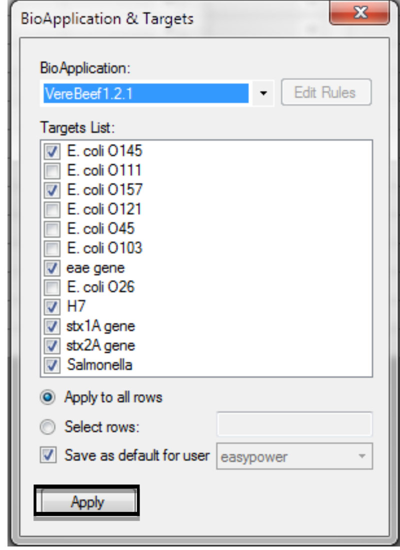


"*E@syLIMS*" will match the "*ChipID*" in "*E@syCheck*" and populate the result fields accordingly.

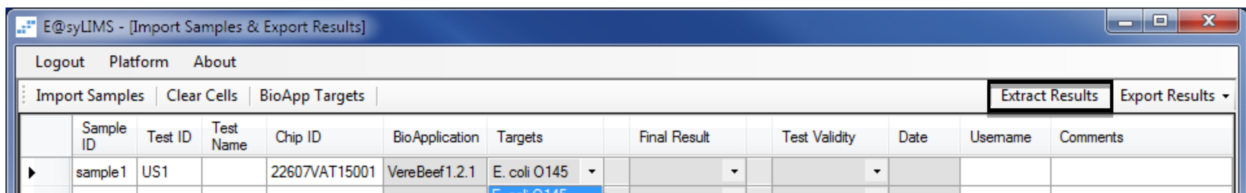
xvi) After importing or entering the required fields, click on the "*BioApp Targets*" to bring up the Targets List.



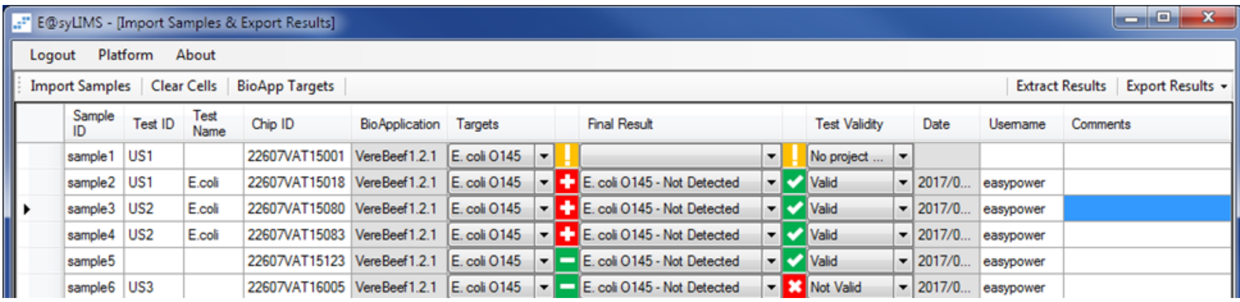
xvii) The Targets List is a list of genes or pathogens defined by the BioApplication and chip type. Select target(s) of interest by checking or unchecking and click on the "*Apply*" button.



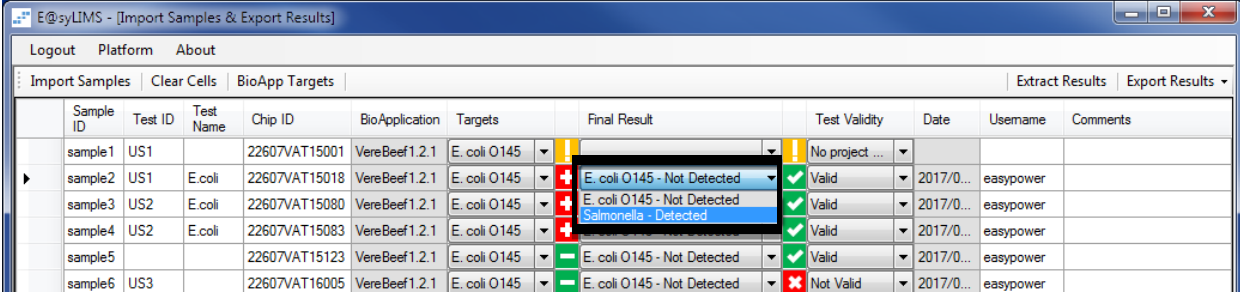
xviii) Click on the "*Extract Results*" button.



xix) Results will be extracted from *E@syCheck* and displayed in the "*Final Result*" and "*Test validity*" columns.

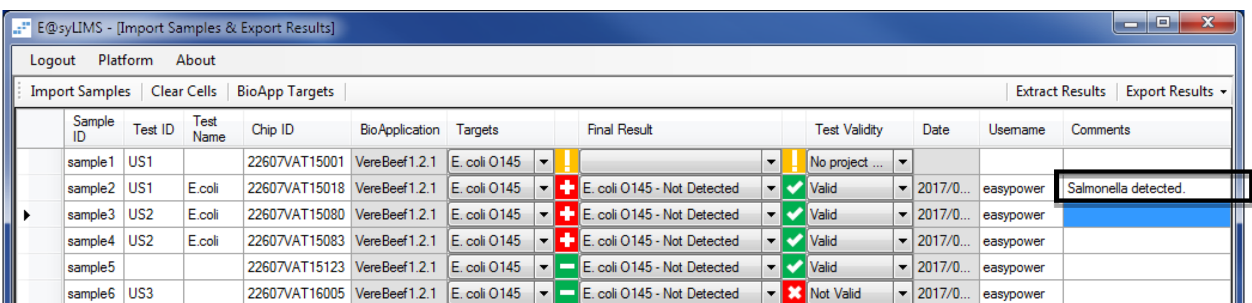


NOTE: Click on the drop-down button to display more results.



xx) Enter comments after the results have been extracted (optional).

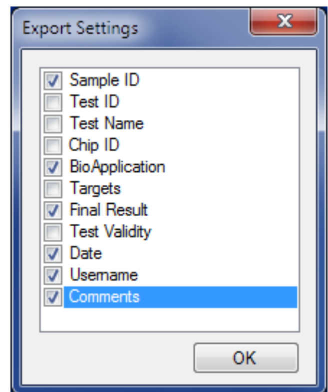
NOTE: The comments sections are a free text field which allows up to 140 characters.



xxi) To save the results, select the samples that are to be exported by clicking on the left most cell. For multi-select, click and drag. Ctrl + click can also be used to select specific rows. The selected samples can be now exported in the preferred format. (i.e. CSV or PDF). To do this, click on the "*Export Results*" button and there would be the option for CSV or PDF.

NOTE: Samples exported will be in chronological order.

xxii) The CSV file generated is a comma separated text file similar to what is displayed in *E@syLIMS*. After selecting "*Save as CSV*", a dialog box will pop-up for user to select what columns or fields to export.



Example of CSV file:

exported results.csv - Notepad

File Edit Format View Help

```
SampleID,BioApp,FinalResult,Date,Username,Comments,
sample2,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Detected,2017/08/28
10:37:02,easypower,Salmonella detected.,
sample3,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Detected,2017/08/28 10:37:18,easypower,,
sample4,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Detected,2017/08/28 10:37:33,easypower,,
sample5,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28 10:39:51,easypower,,
sample6,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28 10:40:07,easypower,,
sample7,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Detected,2017/08/28 10:40:22,easypower,,
sample6,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28 10:41:28,easypower,,
sample8,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28 10:41:45,easypower,,
sample10,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28
10:43:02,easypower,,
sample11,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28
10:43:14,easypower,,
sample10,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28
11:28:54,easypower,,
sample10,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28
13:46:19,easypower,,
sample11,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/28
13:47:16,easypower,,
sample11,VereBeef1.2.1,E. coli O145 - Not Detected \ Salmonella - Not Detected,2017/08/29
17:30:49,easypower,,
```

NOTE: **This format would be most suitable for importing into the results into a Laboratory Information Management System (LIMS).**

xxiii) The PDF option will generate one file containing the results of the selected samples sorted in chorological order.

exported results.pdf - Adobe Reader

File Edit View Document Tools Window Help

3 / 14 100% Find

Bookmarks

Options

Sample: sample2
Sample: sample3
Sample: sample4
Sample: sample5
Sample: sample6
Sample: sample7
Sample: sample6
Sample: sample8
Sample: sample10
Sample: sample11
Sample: sample10
Sample: sample10
Sample: sample11
Sample: sample11

Chip ID: 22607VAT15080 2 of 14

veredus laboratories

Sample Details

Chip ID:	22607VAT15083	Sample ID:	sample4
BioApp:	VereBeef1.2.1	Test ID:	US2
User:	easypower	Test Name:	E.coli
Date:	2017/08/28 10:37:33	Test Validity:	Valid
Comments:			

Final Results

E. coli O145	–	Not Detected		Salmonella	+	Detected

8.27 x 11.69 in

Assay Control

The following controls are included in each test:
1. Internal PCR controls to check for succesful nucleic acid amplification reaction
2. Positive and negative hybridization controls to check for hybridization-related issues
3. 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*” with the symbol) or absence (“*Not Detected*” with the symbol) of the target gene/organism under the “*Final Result*” column on the software interface and the final printed report. “ ” or “ ” will be displayed for inconclusive results. Refer to table below for possible outcomes:

Symbols	Status
	None of the targets selected are detected in the sample.
	One or more of the targets selected are detected in the sample.
	One or more of the targets selected is inconclusive. Inconclusive results are mainly due to bad spots in the microarray and the number of bad spot fails to meet the set criteria. Recommended action is to re-run the test. This symbol is also shown when no project is found.
	This icon indicates one or more targets selected are detected and also inconclusive results due to bad spots in the microarray and the number of bad spot fails to meet the set criteria.

"*Test Validity*" column displays the control fields with three possible outcomes for each test:

Symbols	Status
✔	Test is valid.
✖	Test is not valid.
!	This symbol is also shown when no project is found. No project found can be cause by hybridization and/or negative controls being invalid. Another possible cause is when the BioApplication selected in *E@syLIMS* is different from the BioApplication selected in *E@syCheck*.

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 VerePLEX™ Biosystem SYSTEM & SOFTWARE IFU.
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 HYB clamps are not properly inserted causing partial or total evaporation of the Microarray Hybridization Mix with a consequent drying of the 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	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

	Comments and Recommended Actions
"Inconclusive"	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 fluorescent 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 both the PCR control probes and the specific target probes	1. Make sure the correct BioApplication is used. 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 DNase. Check quality of nucleic acid sample or use fresh nucleic acid sample. Repeat the test and follow carefully the IFU on Warnings and Precautions.

	Comments and Recommended Actions
	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 kit. Refer to the manufacturer's handbook for detailed extraction procedure. 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 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 Fluoresecent-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.
- Fluorescent dye 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 Fluorescent 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.
- Positive results for both virulence factors and any of the *E. coli* targets does not necessarily indicate the presence of STEC. It is possible that the virulence factors are from other non-target *E. coli.*
- 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-reactivity with organisms not tested can lead to erroneous results.
- Bacteria nucleic acids may persist *in vivo* independent of organism viability. Detection of analyte target(s) do not imply that the corresponding organisms are infectious.

References

1. United States Department of Agriculture, Food Safety and Inspection Service. Microbiology Laboratory Guidebook – Chapter 4.09 - Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges (Jan 2, 2017).
2. United States Department of Agriculture, Food Safety and Inspection Service. Microbiology Laboratory Guidebook – Chapter 5.09 - Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges (Jan 15, 2015).
3. United States Department of Agriculture, Food Safety and Inspection Service. Microbiology Laboratory Guidebook – Chapter 5B.05 - Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges (Jun 29, 2014).

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:

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Understanding the Symbols

Symbol	Meaning
REF	Catalog number
LOT	Lot number
Σ	Contains sufficient for <n> tests
2	Do not reuse
	Manufacturer
	Temperature limitation
	Use by (YYYY-MM-DD)
i	Consult Instructions for Use
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	This side up
!	Caution

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