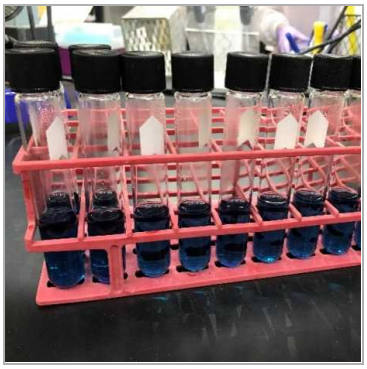

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Salmonella detection with Kingfisher Flex/Apex extraction and 7500-FAST enrichment PCR V.1

Forked from Extraction of bacterial DNA using MagMAX™ CORE Nucleic Acid Purification Kit on KingFisher™ Flex Instrument

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Vet LIRN

Laura Goodman

DISCLAIMER

Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

ABSTRACT

This procedure is used to test enrichment broth from environmental or animal specimens using the MagMAX™ CORE Nucleic Acid Purification Kit and the MicroSEQ Salmonella spp. Detection Kit.

The RVS enrichment broth should be prepared in advance to allow for quality control. The rest of the method is performed over two days:
Day 1 - sample setup and enrichment
Day 2 - DNA purification and real-time PCR

The steps for Kingfisher automated extraction were forked from "Extraction of bacterial DNA using MagMAX™ CORE Nucleic Acid Purification Kit on KingFisher™ Flex Instrument" (https://dx.doi.org/10.17504/protocols.io.81wgb781ovpk/v2)

GUIDELINES

Wipe down sample tubes and gloves with 10% bleach to minimize cross-contamination.

Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 68254

Keywords: KingFisher Flex, MagMAX CORE, Salmonella detection

MATERIALS

MagMAX™ CORE Nucleic Acid Purification Kit (A32702 or A32700)

MicroSEQ™ Salmonella Spp. PCR kit (Thermo/ABI cat# 4403930) contains 96 reactions pre-loaded into tube strips in a lyophilized format. A negative PCR setup control is also provided.

SAFETY WARNINGS

Follow biosafety procedures consistent with your institution.

Preparation of RVS broth and Quality Check

1 This broth is used for the enrichment of specimens for *Salmonella* spp. isolation. The Rappaport Vassiliadis (RVS) media has an expiration date of **6 months** from the preparation date.

Determine batch size:

A	B	C	D	E	F	G
RO water	0.5 L	1 L	2 L	3 L	4 L	5 L
RVS broth powder	13.55 g	27.11 g	54.22 g	81.33 g	108.44 g	135.55 g

RVS powder (Rappaport Vassalidius) can be obtained from HIMEDIA (#M1491)

2 Add half the amount of RO water needed for the batch and a sterile stir bar to a sterile flask of appropriate size

3 Place the flask on a stir plate and start rotating the stir bar to “stir” the water

4 Add the entire amount of RVS powder to the flask while stirring

5 Add the remaining amount of water to the flask

6 Heat the flask until the powder is completely dissolved

7 Dispense **10 ml** of the broth from the flask into sterile 20 x 125 mm screw-capped tubes using sterile tubing and the DoseIt. Loosely cap each tube.

8 Autoclave the tubes of broth for **15 minutes** on liquid cycle @ 121^{o}C

9 Allow to cool to RT and then tighten caps.

10 Label racks of tubes as “RVS Broth”, date made, initials,& expiration date (months)

11 Perform QC using the fields below according to the manufacturer’s directions

12 Store RVS Broth @ 2-8^{o}C protected from light

13 **Quality Control Procedure:RVS Broth**

RVS Broth/BP inoculated:Initials:Date:

BP examined:Initials:Date:
BP :☐growth ☐no growth

BG/XLD inoculated:Initials:Date:

BG/XLD Subculture plates examined: Initials:Date:

Positive Control plates:
BG: ☐typical colonies ☐atypical colonies ☐no growth ☐mixed growth
XLD:☐typical colonies ☐atypical colonies ☐no growth ☐mixed growth

Final Results(check one) Pass ☐ Fail/Batch Discarded ☐

Date:
Initials:

Sample processing and enrichment

14 Thoroughly disinfect a biosafety cabinet (BSC) using a disinfectant spray. Place a piece of absorbent bench paper in the BSC after cleaning. Allow the BSC to run for several minutes before placing items in it.

15 Obtain the following and place into the running BSC:

- Prepared aliquots of RVS (9ml per sample) to accommodate the total number of samples being tested plus one negative enrichment setup control. Positive controls are optional for this exercise. Retrieve additional aliquots of RVS if you will be adding positive controls.
- Racks to hold 15 ml tubes
- Lab towels soaked with 10% bleach contained within a plastic holder.
- Small biohazard bag (to collect waste)
- Labeled samples (organized in appropriate sized rack)
- Optional: one positive control culture each for *Salmonella* Typhimurium and *Salmonella* Dublin grown on blood agar
- Sterile 10 µL loop (optional if using an in-house positive culture control)

16 For the Vet-LIRN Salmonella Interlaboratory Comparison Exercise, samples to be tested will be liquid suspensions of bovine intestinal scrapings. The test sample tubes contain 1ml of sample in a 15 ml tube. **Important!** The entire sample will be used; do not transfer the sample out of this

tube at this time.

17 Place the first sample in a tube rack and loosen the cap. Clean your entire gloved hand with the 10% bleach towel.

18 Chose one 9ml RVS aliquot and pour the entire volume into the first sample tube. Use care to avoid back splashing. Recap the tube. Discard the empty aliquot tube in the biohazard waste.

19 Clean your entire gloved hand with the 10% bleach towel. Place the inoculated tube, which now has both sample and RVS broth, back into the original rack. Clean your gloves again.

20 Repeat steps 16-19 with the remaining samples.

21 Reserve one aliquot of RVS broth and label as negative setup enrichment control: this tube will remain un-inoculated. *Note! After over-night incubation, the negative setup enrichment control will be extracted and tested by PCR along with the samples.*

22 Optional: Inoculate one positive control using a sterile loop.

23 Move the rack(s) to a 40-44^{o}C incubator, with shaking preferred. Incubate overnight, at least 18-24 hours.

DNA Purification

24 **Prepare plates for Robot**

24.1 Prepare Wash Plate 1 by adding 500 µL of MagMAX™ CORE Wash Solution 1 to each well for each sample to be extracted plus controls.

24.2 Prepare Wash Plate 2 by adding 500 µL of MagMAX™ CORE Wash Solution 2 to each well for each sample to be extracted plus controls.

24.3 Prepare Elution Plate by adding 100 µL of MagMAX™ CORE Elution Buffer to each well for each sample to be extracted plus controls.

24.4 Set Tip Comb in a 0.5 ml 96-Well Plate

	A	B	C	D
	Plate setup of Processing Plates: KingFisher™ Flex instrument			
	Plate ID	**Plate Type**	**Reagent**	**Volume Per Well**
	Wash Plate 1	Deep Well	MagMAX™ CORE Wash Solution 1	500 µL
	Wash Plate 2	Deep Well	MagMAX™ CORE Wash Solution 2	500 µL
	Elution Plate	Standard	MagMAX™ CORE Elution Buffer	100 µL
	Tip Comb Plate	Standard	Place a tip comb in the plate	

24.5 Prepare Bead/PK mix by adding 20 µL of MagMAX™ CORE Magnetic Beads and 10 µL of MagMAX™ CORE Proteinase K for the required number of samples plus 10% overage.

24.6 Prepare Lysis/Binding solution by adding 350 µL of MagMAX™ CORE Lysis Solution and 350 µL of MagMAX™ CORE Binding Solution for for the required number of samples plus 10% overage.

Mix by inverting the tube or bottle at least 10 times.

24.7 Invert the tube of Bead/PK Mix several times to resuspend the beads, then add 30 µL of the Bead/PK Mix to the required wells in the 2.4 mL 96- deep well Sample Plate.

24.8 Transfer 200 µL of RVS enrichment broth to a well with 30 µL Bead/PK mix in the Sample Plate. Seal the plate and shake vigorously for 2 minutes on a plate shaker at room temperature, or pipette mix them and incubate for 2 mins at room temperature.

> Note
>
> Use extended-reach filter tips to pull the aliquot of broth out of the tubes in order to avoid contaminating the pipettor. Clean gloved fingertips with a 10% bleach towel in-between samples.

24.9 Add 700 µL of Lysis/Binding Solution to each well containing sample.

25 Operate on KingFisher machine

25.1 Select the appropriate script for your instrument if not already loaded:

Download the **KingFisher Flex** heated script: MagMAX CORE_Flex.bdz at https://www.thermofisher.com/order/catalog/product/A32700#/A32700, and then use BindIt sofware to transfer this script from a computer to the machine with the connection line.

For the **KingFisher Apex**, use the following script instead: https://assets.thermofisher.com/TFS-Assets/BID/Methods-&-Protocols/MagMAX_CORE_Heated_v1.kfx

BindIt software can be requested and downloaded at the website https://www.thermofisher.com/us/en/home/global/forms/life-science/download-bindit-software-kingfisher-instruments.html

25.2 Start the run, then load the prepared plates in the appropriate positions when prompted by the instrument. The instrument will direct the user as to which plate to load in what position.

25.3 Load the Tip Comb Plate

25.4 Load the Elution Plate

25.5 Load the Wash Plate 2

25.6 Load Wash Plate 1

25.7 Lastly, load the Sample Plate and press Start. The program takes about 20 min.

25.8 The elution plate contains the eluted DNA to be run on qPCR.

PCR Setup

26 Program the ABI 7500-FAST machine with the following settings (use fast mode):

	A	B	C	D

	A	B	C	D
		Reps	**Temperature**	**Duration (sec)**
	Stage 1	1	95 C	20
	Stage 2	40	95 C	03
			60 C	30

27 Set up reactions at ROOM TEMPERATURE (do not use cold blocks or ice). Chilled nucleic acid and lyophilized components will make resuspension of the reaction mixture difficult.

Slowly remove the rounded caps on the tube strips, one row at a time, and discard them.

28 Add 30 µl of sample nucleic acid elution to the PCR tube containing the lyophilized master mix. Wait 3-5 seconds and then resuspend the reagent pellet by pipetting up and down 2-3 times.

Important! It is critical to wait 3-5 seconds to avoid aspirating part of the still lyophilized PCR master mix into the pipette tip.

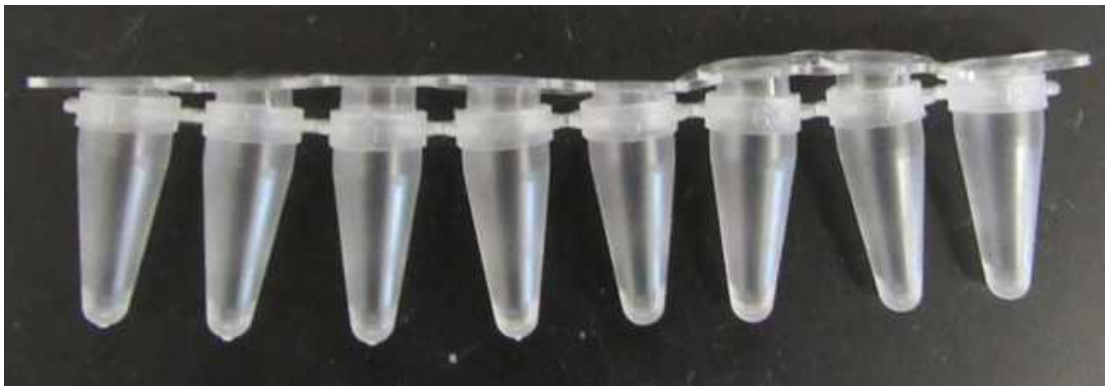
29 Add 30 µl of the negative amplification control (supplied with the kit) to a PCR tube containing the lyophilized master mix. Wait for 3-5 seconds and then resuspend the reagent pellet by pipetting up and down 2-3 times.

30 Add 30 µl of the positive amplification control to a PCR tube containing the lyophilized master mix. Wait for 3-5 seconds and then resuspend the reagent pellet by pipetting up and down 2-3 times.

31 Cap the tubes with the flat caps provided with the kit. The cap strip must be seated level as shown in the picture.

Place a small mark at one end of each of the strip caps in order to keep proper orientation and order of the tubes. Do not mark the sides of the tubes or place any marks over the main surface of the optical cap.

Proper seating of cap strip



Poor seating, strip is not horizontal
Lips of caps not secure inside of tubes

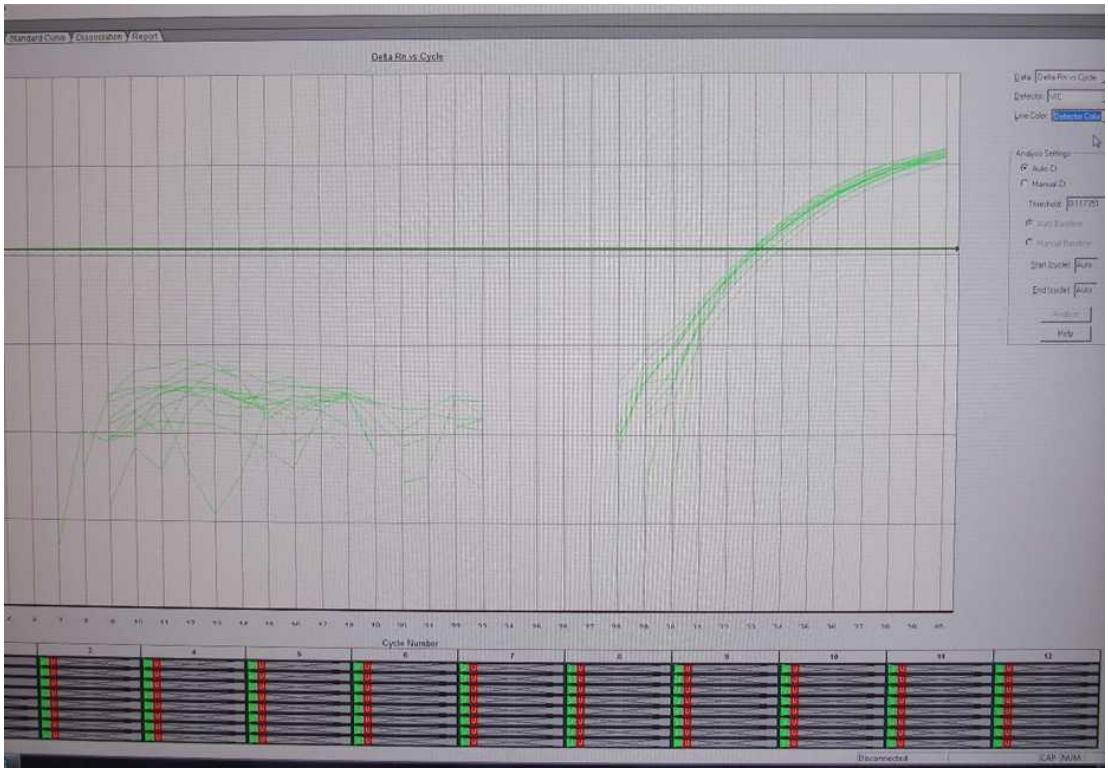
32 Pulse spin the strip tubes in order to collect liquid in the bottom of the tubes and displace any air bubbles.

33 Place Samples in ABI-7500 FAST thermocycler using the tube strip adapter. Balance the tubes on each side according to the manufacturer's instructions (blank tubes may be used for this purpose if necessary). If you do not have this adapter or are using a different machine, you can transfer the reactions into a plate that is compatible with your setup if needed.

Balance the tubes on each side of the 7500-FAST insert tray for tube strips

34 Start the run.

35 Following run, analyze the results using the automatic analysis setting. All samples should have a VIC (internal control) signal. If the VIC is >35 or undetermined, repeat the PCR using a 1:5 dilution of the sample in nuclease-free water and interpret based on the result that gives the lowest Ct for the VIC.



36 Save a copy of the original run file and export the data into an Excel file. Interpret the results according to the following guidelines:

	A	B	C	D
	FAMa	**VICb**	**Interpretation**	**Action**
	undetermined	detected	Not Detected	None – this result is final
	< 35	disregard	Positive	Culture confirmation is optional
	≥ 35	detected	Suspect	Culture confirmation is optional
	undetermined	undetermined	Inconclusive	Culture confirmation is optional

[a]FAM (6-carboxyfluorescein) is the most commonly used fluorescent dye attachment for oligonucleotides and is calibrated as part of the standard set of dyes for the ABI 7500-FAST

machine. Its max excitation wavelength is 495 nm and emission wavelength is 520 nm

[b]VIC is an ABI-proprietary dye that with excitation wavelength 488 nm/Emission Wavelength 552 nm that is also calibrated as part of the standard set of dyes for the ABI 7500-FAST machine.

37 Copy the results into the provided SecureSheet file.

38 Optional: proceed with culture confirmation for samples with a *Salmonella* (FAM) Ct detected