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USDA
FSIS

Isolation, Identification and Enumeration of *Salmonella* from meat, poultry, egg products and catfish products

V. Prencipe

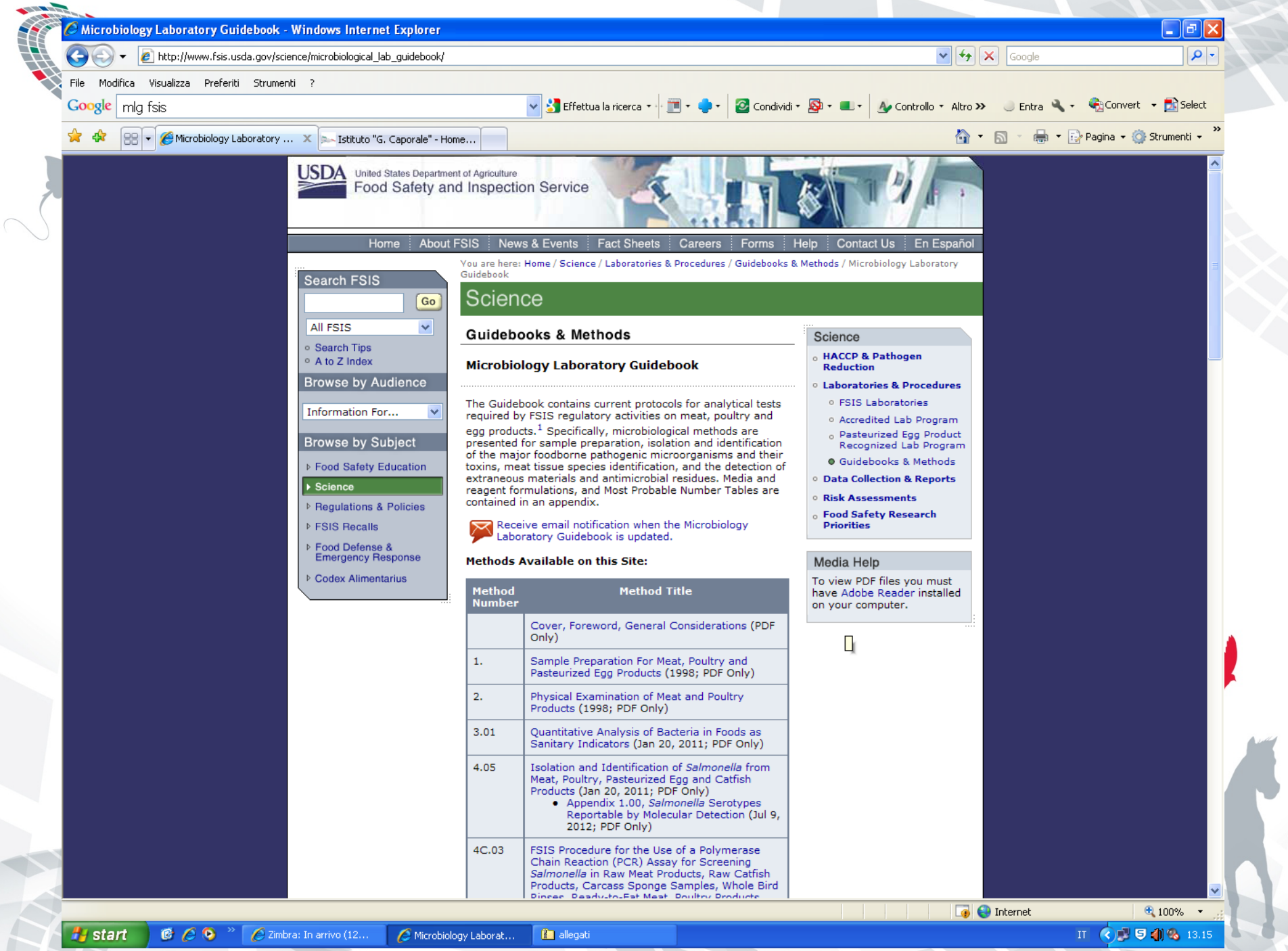
IZS A&M - LNR *L. monocytogenes*

FSIS Official Methods can be found in the FSIS Microbiology Laboratory Guidebook (MLG). The MLG is updated to incorporate improvements in analytical methods. The latest version can be found at: http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp

Resources:

USDA Microbiology Laboratory Guidebook (MLG) 4.05 Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products (Jan 20, 2011)

Appendix 1.00, Salmonella Serotypes Reportable by Molecular Detection (Jul 9, 2012)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Microbiology Laboratory Guidebook
The Guidebook contains current protocols for analytical tests required by FSIS regulatory activities on meat, poultry and egg products.[1] Specifically, microbiological methods are presented for sample preparation, isolation and identification of the major foodborne pathogenic microorganisms and their toxins, meat tissue species identification, and the detection of extraneous materials and antimicrobial residues. Media and reagent formulations, and Most Probable Number Tables are contained in an appendix.
Receive email notification when the Microbiology Laboratory Guidebook is updated.
Methods Available on this Site:
Method Number
Method Title
Cover, Foreword, General Considerations (PDF Only)
1. Sample Preparation For Meat, Poultry and Pasteurized Egg Products (1998; PDF Only)
2. Physical Examination of Meat and Poultry Products (1998; PDF Only)
3.01 Quantitative Analysis of Bacteria in Foods as Sanitary Indicators (Jan 20, 2011; PDF Only)
4.05 Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products (Jan 20, 2011; PDF Only)
Appendix 1.00, Salmonella Serotypes Reportable by Molecular Detection (Jul 9, 2012; PDF Only)
4C.03 FSIS Procedure for the Use of a Polymerase Chain Reaction (PCR) Assay for Screening Salmonella in Raw Meat Products, Raw Catfish Products, Carcass Sponge Samples, Whole Bird Rinses, Ready-to-Eat Meat, Poultry Products
Science
HACCP & Pathogen Reduction
Laboratories & Procedures
FSIS Laboratories
Accredited Lab Program
Pasteurized Egg Product Recognized Lab Program
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Data Collection & Reports
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Safety precautions ☑

- Salmonella are generally categorized as Biosafety Level 2 pathogens.
- CDC guidelines for manipulating Biosafety Level 2 pathogens
- A Class II laminar flow biosafety cabinet
- The Material Safety Data Sheet (MSDS) must be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis.
- The personnel who will handle the material should read the MSDS prior to startup.

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Quality Control

- One H2S+ positive control strain (S. minnesota)
- One H2S- positive control strain (S. paratyphi A)
- Blank

- Each set of concurrently analyzed samples

- Enviromental samples of working areas

Quality Control

- The biochemical and serological tests used for confirmation of the sample isolates require the use of appropriate controls to verify that the results are valid. Salmonella ‘O’ antisera should be tested with QC control cultures or sera before initial use, and with a saline control for each test.

- Biochemical kit and rapid test manufacturers may specify control cultures for use with their products. If not specified, quality control procedures for biochemical tests and test media should include cultures that will demonstrate pertinent characteristics of the product.

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Media

- Buffered peptone water (BPW)
- TT broth (Hajna)
- Modified Rappaport Vassiliadis (mRV) broth, Rappaport-Vassiliadis R10 broth, or Rappaport-Vassiliadis Soya Peptone Broth (RVS)
- Brilliant green sulfa agar (BGS; contains 0.1% sodium sulfapyridine)
- Xylose lysine TergitolTM 4 agar (XLT4) or Double modified lysine iron agar
- (DMLIA)
- Triple sugar iron agar (TSI)
- Lysine iron agar (LIA)
- Trypticase soy broth (TSB) or Tryptose broth
- Trypticase soy agar (TSA)
- Nutrient agar slants
- Nutrient broth, semi-solid
- Tryptic soy agar with 5% sheep blood agar

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Sample preparation

Product	Sample Preparation		Incubation Time
	Portion Size	BPW Amount	Cultural or PCR rapid screen
Breading Mixes, Dehydrated Sauces and Dried Milk	325 ± 6.5 g	2925 ± 58.5 ml	18-24 h
Ready-to-Eat Foods	325 ± 6.5 g	2925 ± 58.5 ml	18-24 h
Fermented Products	325 ± 6.5 g + 10 g of sterilized calcium carbonate	2925 ± 58.5 ml of BPW that contains 1 ml of a 1% aqueous solution of crystal violet per liter	18-24 h
Raw Meat Products	25 ± 0.5 g HACCP: 25 ± 2.5 g	225 ± 22.5 ml	20-24 h
Carcass Sponge and Environmental Swabs	1 sponge	50 ± 1 ml (brings total volume to 60 ml)	20-24 h
Whole Bird Rinses	30 ± 0.6 ml sample rinse fluid	30 ± 0.6 ml	20-24 h
Pasteurized Liquid, Frozen or Dried Egg Products	100 ± 2 g	900 ± 18 ml	18-24 h
Raw Catfish Products	25 ± 2.5 g	225 ± 22.5 ml	22-26 h

Sample preparation

Ready-to-Eat Foods

•Follow additional program requirements for preparing sample and sub-sample composites. Outbreak samples may require a different sample preparation. Follow customer specifications.

- Weigh 325 ± 6.5 g of the composite sample into a Stomacher bag (or sterile blender jar if required by the customer or sample type).
- Instructions for multi-component RTE products:
  - If the meat or poultry component is separate and distinct from other non- meat ingredient, analyze only the representative meat/poultry portion of the RTE product.
  - When meat/poultry is combined with other ingredients to form the product, analyze representative meat/poultry portions in combination with other ingredients.
- Add approximately one third to one-half of 2925 ± 58.5 ml of ambient temperature sterile BPW. Blend or stomach approximately 2 minutes then add the remainder of the 2925 ml of BPW.
- Incubate at 35 ± 2
- Proceed to Section 4.6 to continue the cultural analysis or refer to MLG 4C for use of the BAX® PCR Assay.

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Enrichment step

- Transfer 0.5 ± 0.05 ml of sample into 10 ml TT broth and 0.1 ± 0.02 ml into 10 ml mRV broth.
- b. Incubate at 42 ± 0.5 C for 22-24 h or in a water bath at 42 ± 0.5 C for 18-24 h.
- c. Carefully mix contents of tube by vortexing or equivalent means. Streak to BGS and either DMLIA or XLT4 agar plates using a 10 ul loopful of inoculum for each plate. Streak the entire agar plate with a single sample enrichment.
- Examination of and Picking Colonies from Plating Media 4.7.1 Picking Colonies
- After the recommended incubation interval, examine the selective-differential agar plates and controls for the presence of colonies meeting the description for suspect Salmonella colonies. Pick well-isolated colonies.
- BGS. Select colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that give a tan appearance against a green background.
- XLT4. Select black colonies (H2S-positive) or red colonies with (H2S- positive) or without (H2S-negative) black centers. The rim of the colony may still be yellow in 24 h; later it should turn red.
  DMLIA. Select purple colonies with (H2S-positive) or without (H2S- negative) black centers. Since Salmonella typically decarboxylate lysine and ferment neither lactose nor sucrose, the color of the medium reverts to purple.

Examination of and Picking Colonies from Plating Media

- Pick up to three colonies from each plate, if available.

NOTE: Before any sample is reported as Salmonella-negative, a total of three typical colonies, if available, from each selective agar plate must be examined

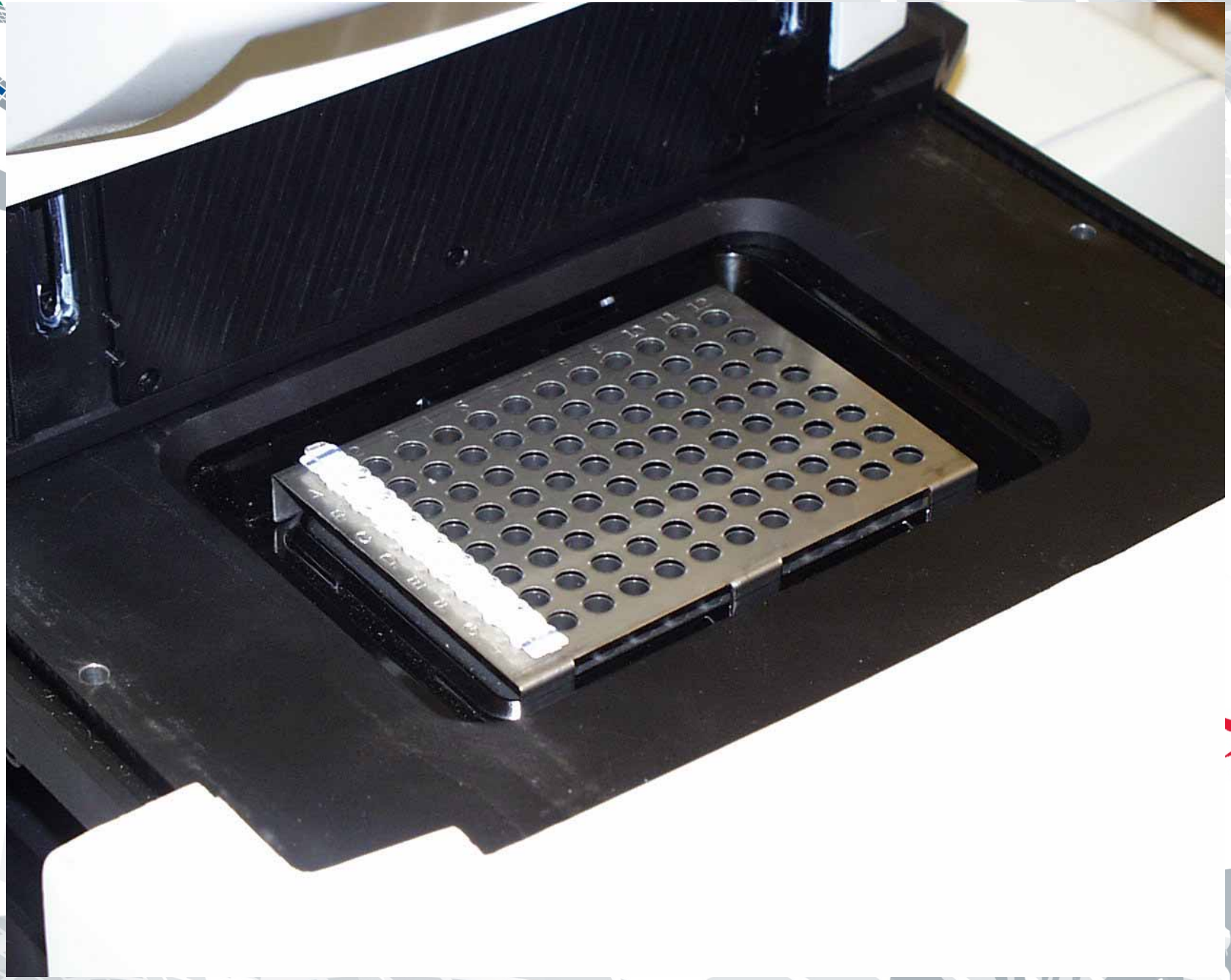
- Pick only from the surface and center of the colony. Avoid touching the agar because these highly selective media suppress growth of many organisms that may be viable.
- If there are typical colonies on a plate that are not well isolated, pick from the typical colonies and re-streak directly to selective agar plates.

Alternatively, Incubate at 35 ± 2 C for 18-24 h. e. Select typical colonies.

Automated PCR Detection System

Salmonella PCR screening

- 5 microl of enriched sample + 200 microl lysis buffer
- Extraction
  - 37° C x 15-25 min
  - 95° C x 10-20 min



Screening Media

- Inoculate TSI and LIA slants in tandem with a single pick from a colony by stabbing the butts and streaking the slants in one operation.. Incubate at 35 ± 2

- Examine TSI and LIA slants as sets. Note the colors of butts and slants, blackening of the media and presence of gas as indicated by gas pockets or cracking of the agar. Note also the appearance of the growth on the slants along the line of streak. A typical control on LIA should produce a purple butt with (H2S-positive) or without (H2S-negative) blackening of the media. A typical control on TSI should produce a yellow butt and red slant, with (H2S-positive) or without (H2S-negative) blackening of the media.

Screening Media

Screening Media

- Discard, or re-streak for isolation, any sets that show "swarming" from the original site of inoculation. Discard sets that show a reddish slant in lysine iron agar. Isolates giving typical Salmonella spp. reactions an isolates that are suggestive but not typical of Salmonella spp. should be confirmed by a combination of biochemical and serological procedures.

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CONFIRMATION & IDENTIFICATION

- SEROLOGICAL TEST
- BIOCHEMICAL TEST

Table 2. Potential *Salmonella* Biochemical Reactions

Triple Sugar Iron Agar			Lysine Iron Agar		Polyvalent Sera		Further Testing/ Disposal
Butt	Slant	H_2S	Butt	H_2S	O	H	
Y	R	+	P	+	+	+	B. & M. T.
Y	R	+	P	+	+	-	B. & M. T.
Y	R	-	P	-			B. & M. T.
Y	R	-	Y	-	+	+	* B. & M. T.
Y	R	-	Y	-	-	-	** B. & M. T.
Y	R	+	Y	+/-			B. & M. T.
Y	Y	-	Y or P	-			Discard
Y	Y	+	P	+			*** B. & M. T.
NC	NC						Discard

Y = Yellow; R = Red; P = Purple; B. & M. T. = Biochemical and (optional) motility tests;
NC = No change in color from uninoculated medium.

* *Salmonella* Typhisuis (found seldom in swine in U.S.)
** *Salmonella Para-typhi A* (example)
*** *Salmonella enterica* subsp. *arizonae* or *S. enterica* subsp. *diarizonae* (occasional reaction)

4.10 Storage of Cultures

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Storage of Cultures

Do not store cultures on TSI agar because this tends to cause roughness of O antigens.

For short-term (2-3 months) storage, inoculate a nutrient agar slant, incubate at 35 ± 2C overnight and then store at 2° -8° C.

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Storage of Cultures

- Store "working" *Salmonella* stock cultures on nutrient agar slants or equivalent.
- Transfer stocks monthly onto duplicate nutrient agar slants, incubate overnight at 35 ± 2C, and then store them at 2-8C.
- Use one of the slants as the working culture. Use the other slant for sub-culturing to reduce the opportunity for contamination. Cultures may be subcultured up to 5 times. After this period, the culture must be re-confirmed biochemically or a new culture initiated.
- For long-term storage, lyophilize cultures or freeze using cryo-beads, i.e. Cryostor™ or equivalent.