



Salmonella Isolation and Detection Methods

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NPIP Laboratory Survey

48 labs responded (95 labs)

- 13 University
- 24 State
- 11 Private

NPIP Laboratory Survey

No. Samples per month	No. Laboratories			No. Samples (NPIP)	No. Samples (FDA)
	NPIP	FDA	Total		
0	17	36	9		
<50	10	4	8		
50 - 100	5	3	6		
100 – 500	8	3	10		
>500	8 *	2**	15	*2340, 4600, 2475, 1300, 1044, 1500, 4000, 2500	**9000 52,000
				7/8 Private	2/2 State

NPIP Laboratory Survey

No. Suspect colonies screened

No.	Labs	No. samples per month
1	2	
2	0	
3	18	
4	4	
5	7	300, 71, 120, 50, 3200, 1500, 1370
>5	5	300, 6, 21, 3, 6
3-5	3	

Screening Method

Method	Labs
TSI	5
TSI + serogroup	19
TSI + chromogenic	3
TSI + Chromogenic + Serogroup	4
Chromogenic + Serogroup	3
Chromogenic only	1
Serogroup only	5
Biochem ID	33

NPIP Laboratory Survey

Serogroup

- No 4
- Yes 40
- In house 33
- Send out 5

Serotype

- No 11
- Yes 33
- In house 16
- Send out 20

NPIP Laboratory Survey

Types of Samples	No. Labs	No. Labs using Rapid Method							
		BAX	“PCR”	SDIX	SDIX-SE	Neogen	LT SE-RTPCR	SE-PCR	VIDAS
NPIP	16	2	4	4	8	1	2	3	0
FDA	10	0	0	0	7	1	1	2	0
Other	15	5	4	6	2	1	1	1	2
Total	27	7	5	7	13	3	3	3	2

NPIP Laboratory Survey Concerns and Comments

- Develop more sensitive rapid methods
- Need validation criteria for molecular tests
- Increase molecular training at workshops
- Screen with PCR and confirm with culture
- Need confirmatory PCR

- Better quality typing antisera
- Faster, cheaper, more definitive serotyping
- Increase serotyping training at workshops

NPIP Laboratory Survey Concerns and Comments

- Confusion over what is approved
- Need faster culture methodology
- Need harmonized culture method
- NPIP and FDA need to communicate and work together

- Cost is important

NPIP Laboratory Survey Concerns and Comments

- What would you like to see in the future?

NPIP Laboratory Survey Concerns and Comments

- What would you like to see in the future?
 - “Common sense, cook your eggs and chicken ...
Can't fix stupid!”

NPIP Approved Salmonella Culture Methods

Bird Samples

Pullorum Reactors
SE Positive environments

NPIP “white book”

Subpart B

Sections 147.10 and 147.11;
Illustration 1

NPIP Program Standards

Subpart B

(1) and (2); Illustration 1

Method:

Direct plating (PT)

Direct enrichment followed by DSE (PT,SE)

NPIP Approved Salmonella Culture Methods

House Environment and Hatchery

NPIP “white book” Subpart B

- Section 147.12 – Methods for the collection, isolation and identification of Salmonella from environmental samples, cloacal swabs, chick box papers and meconium samples
 - (a) For egg-and meat-type chickens, waterfowl, exhibition poultry, and game birds
 - (b) Isolation and identification of Salmonella
 1. Tetrathionate enrichment with delayed secondary enrichment (DSE)
 2. Pre-enrichment followed by selective enrichment
 3. Approved rapid detection methodIllustration 2
 - (c) For turkeys

NPIP Approved Salmonella Culture Methods

House Environment and Hatchery

Section 147.12 revised 2010 Conference

- Procedures for collection, isolation, and identification of Salmonella from house environmental samples, cloacal swabs, and hatchery samples
 - (a) For egg- and meat-type chickens, turkeys, waterfowl, exhibition poultry, and game birds
 - (b) Isolation and identification of Salmonella
 1. Direct tetrathionate enrichment followed by MSRV enrichment
 2. Pre-enrichment followed by selective enrichment
 3. Approved rapid methods for the detection of Salmonella
- Illustration 2 (revised)

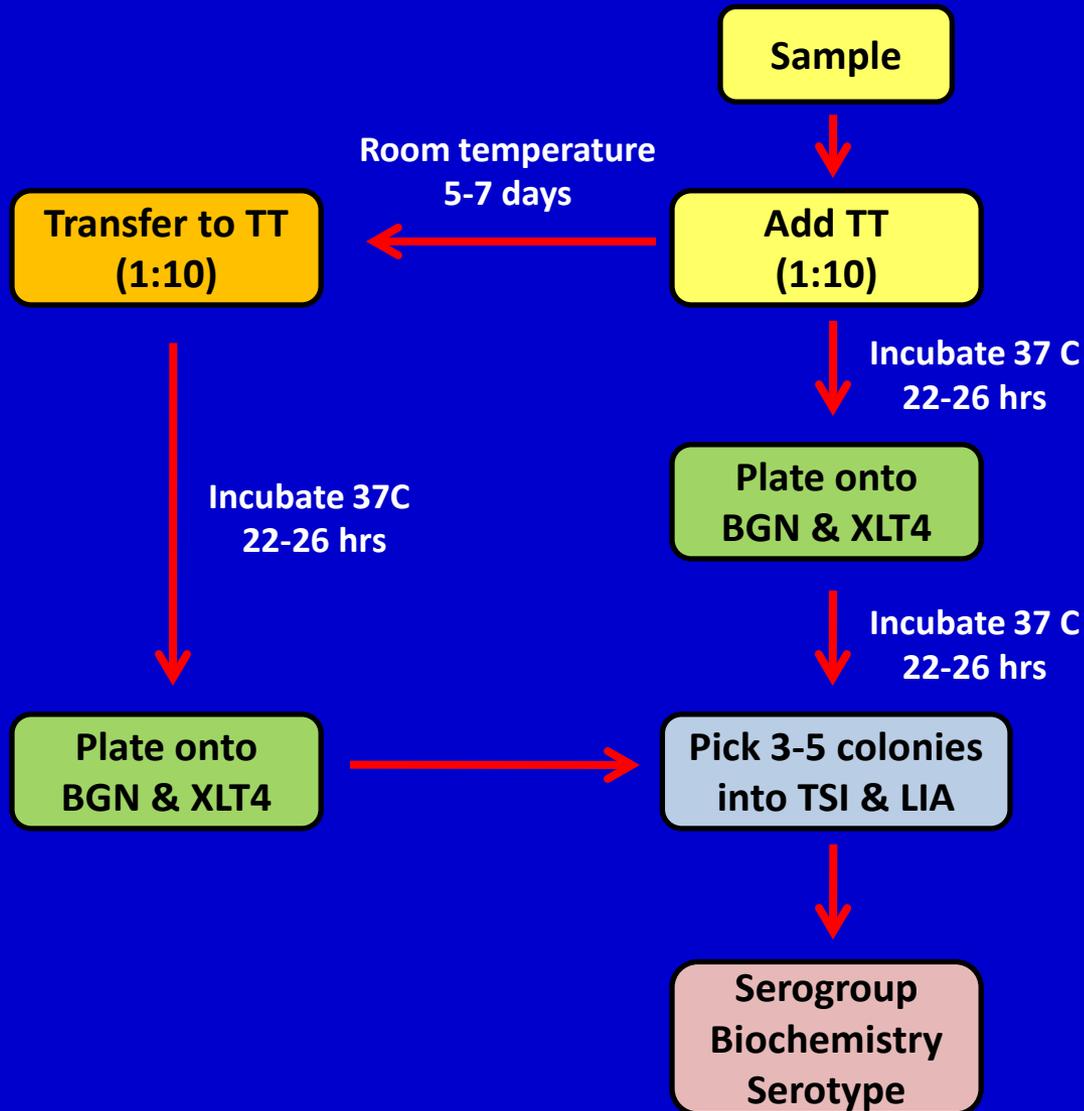
NPIP Approved Salmonella Culture Methods

House Environment and Hatchery

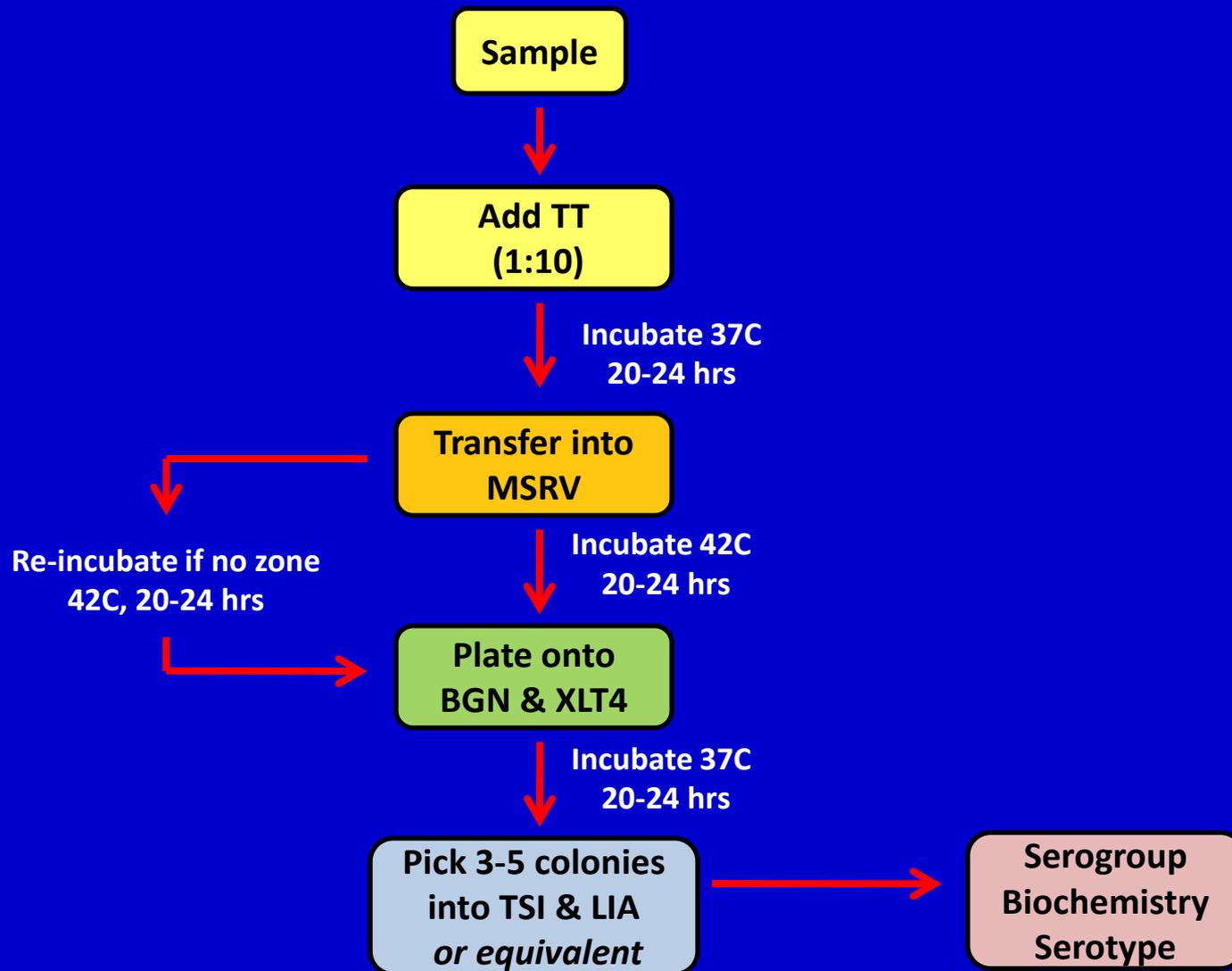
NPIP Program Standards

- Procedures for collection, isolation, and identification of Salmonella from environmental samples, cloacal swabs, chick box papers, and meconium samples
 - (a) For egg- and meat-type chickens, turkeys, waterfowl, exhibition poultry, and game birds
 - (b) Isolation and identification of Salmonella
 1. Direct tetrathionate enrichment followed by MSR/V enrichment
 2. Pre-enrichment followed by selective enrichment
 3. Approved rapid methods for the detection of Salmonella
- Illustration 2 (needs revising)

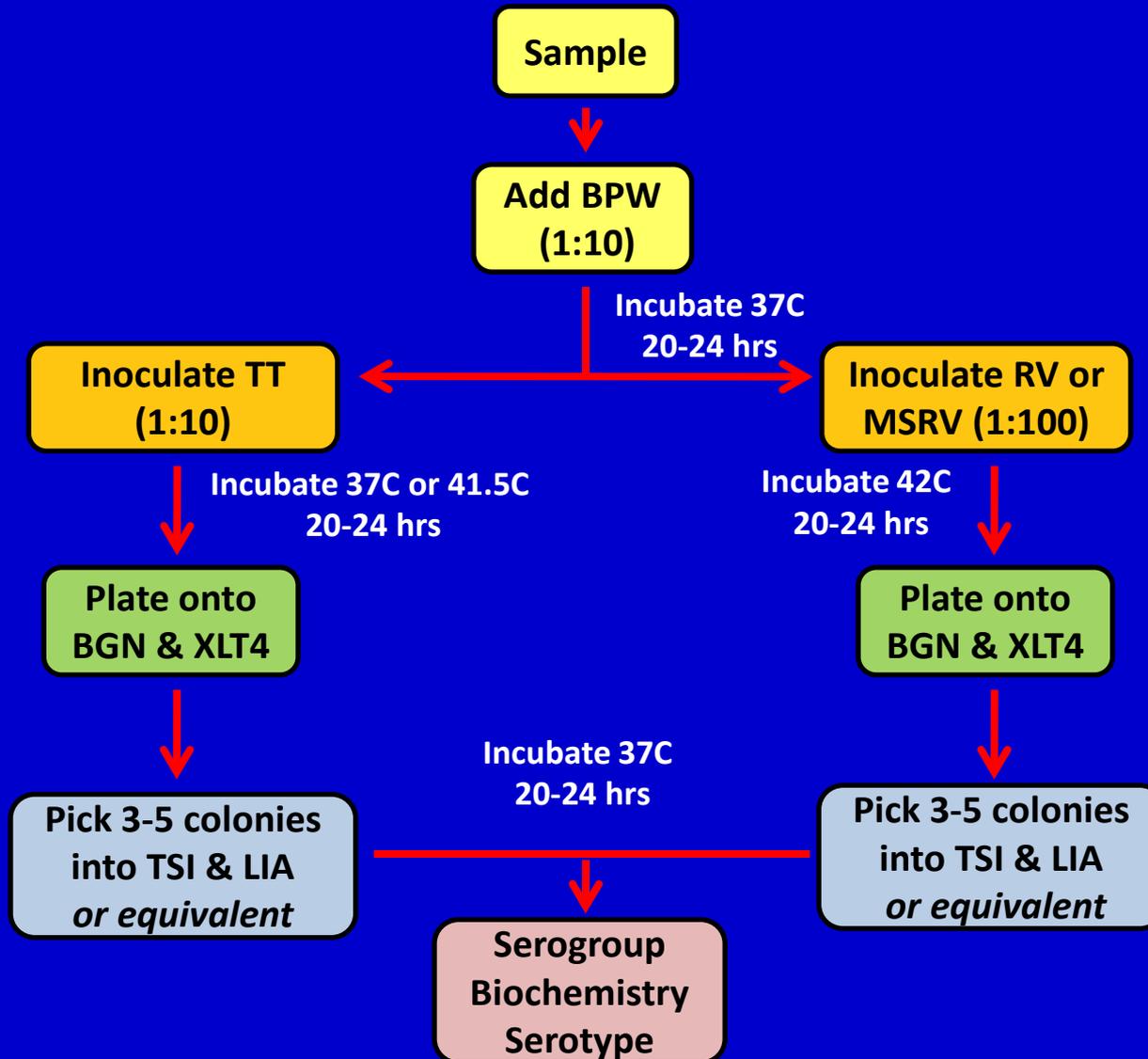
NPIP Method for the isolation of Salmonella from Bird samples: DSE



NPIP Method for the isolation of Salmonella from Environmental samples: MSRV



NPIP Method for the isolation of Salmonella from environmental samples: Pre-enrichment



NPIP Approved Rapid Assays for Salmonella

- RapidChek Select Salmonella Test Kit - Strategic Diagnostics, Inc.,
- ADIAFOOD Rapid Pathogen Detection System for Salmonella spp. - AES Chemunex
- DuPont Qualicon BAX Polymerase Chain Reaction (PCR) - based assay for Salmonella - DuPont Qualicon,

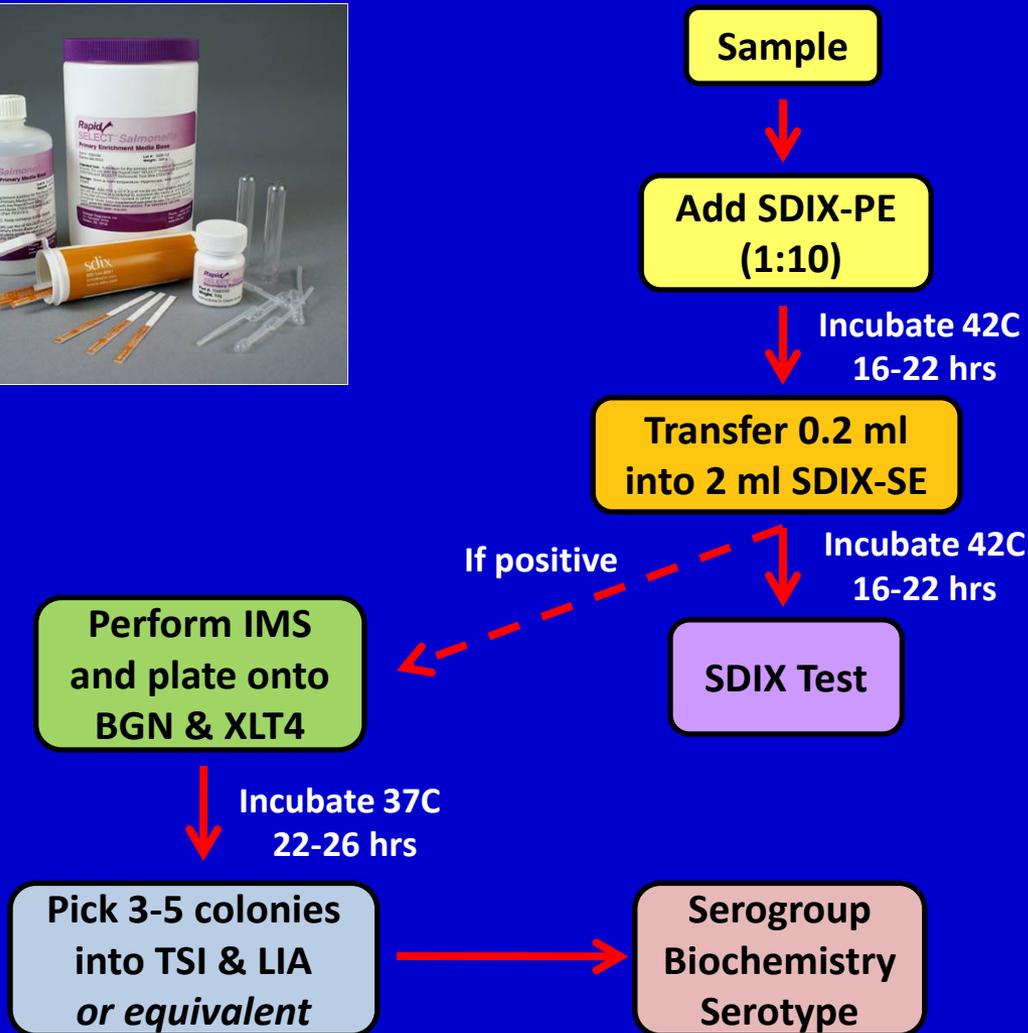
NPIP Approved Rapid Assays for Salmonella

- Salmonella enteritidis specific PCR – Dr. Bruce Charlton
- Group D specific RT-PCR – Seo et al. (2004)

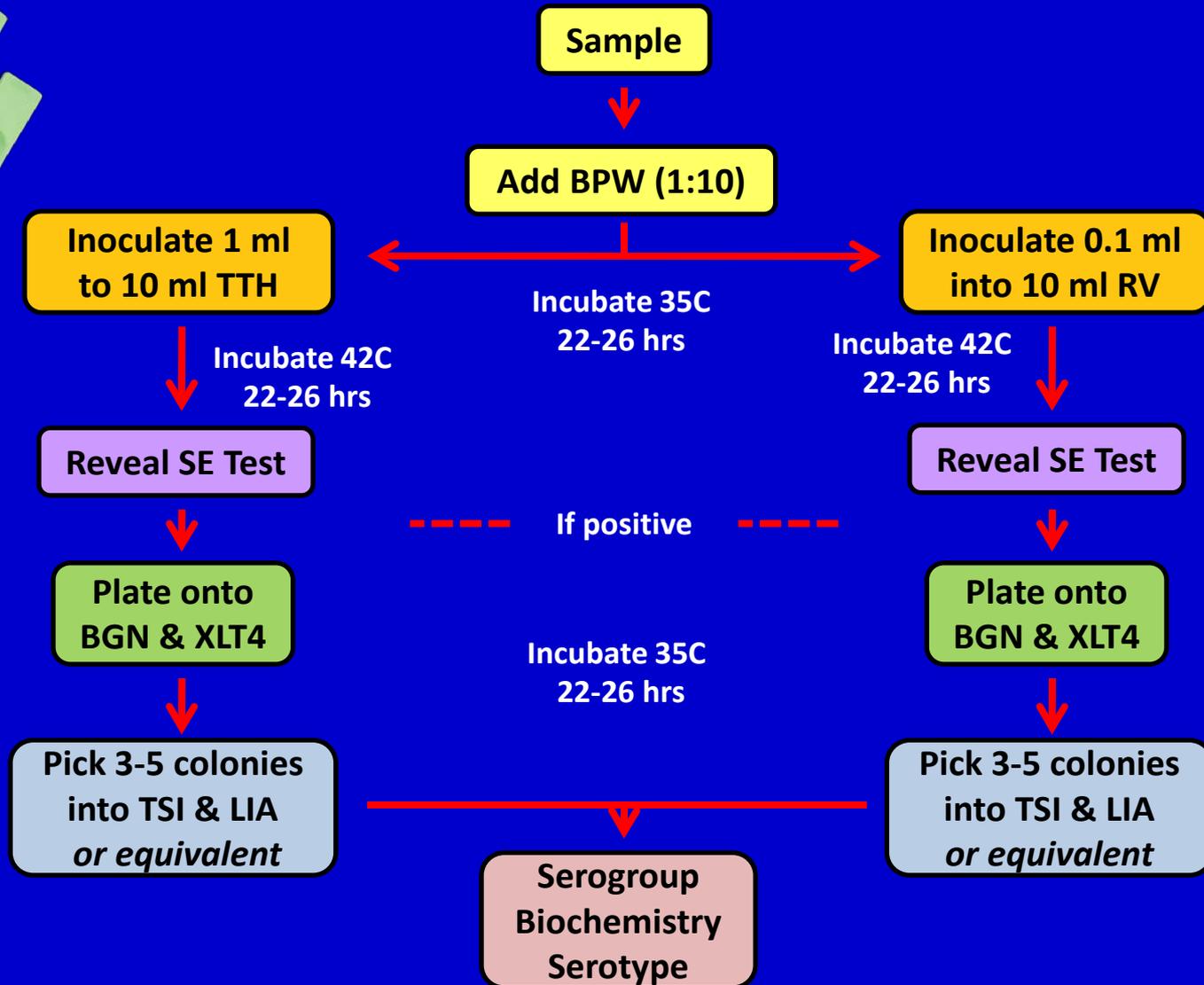
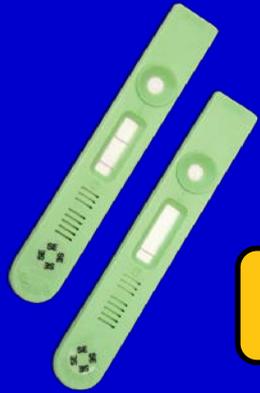
NPIP Rapid Assays for Salmonella: Interim Approval

- RapidChek Select Salmonella Enteritidis Test Kit
- Strategic Diagnostics, Inc.
- Neogen's Reveal Salmonella Enteritidis kit
- Applied Biosystems TaqMan SE-Specific RT-PCR assay – Life Technologies

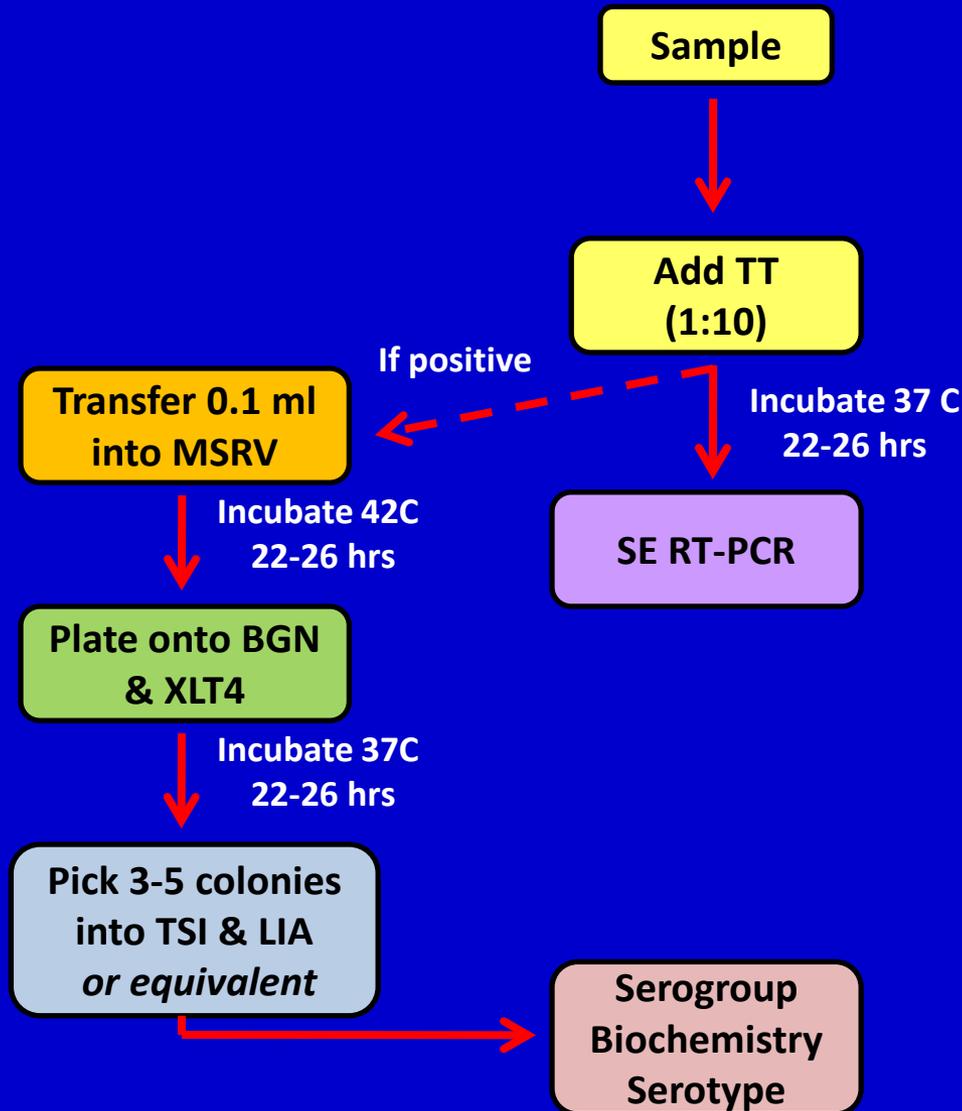
SDIX RapidChek SE Method for the detection of *Salmonella* Enteritidis from environmental samples



Neogen Reveal SE Method for the detection of *Salmonella* Enteritidis from environmental samples



Life Technologies SE RT-PCR for the detection of Salmonella Enteritidis from environmental samples



FDA Approved Salmonella Culture Methods

- Environmental samples
 - FDA Method
 - NPIP culture methods
 - “Equivalent” Rapid Methods
- Egg samples
 - BAM Method
 - “Equivalent” Rapid Methods

FDA Testing methodology for *Salmonella* Enteritidis (SE) from Environmental samples

FDA has determined that the following methods are equivalent to "Environmental Sampling and Detection of *Salmonella* in Poultry Houses" (April 2008) in accuracy, precision, and sensitivity in detecting *Salmonella* Enteritidis:

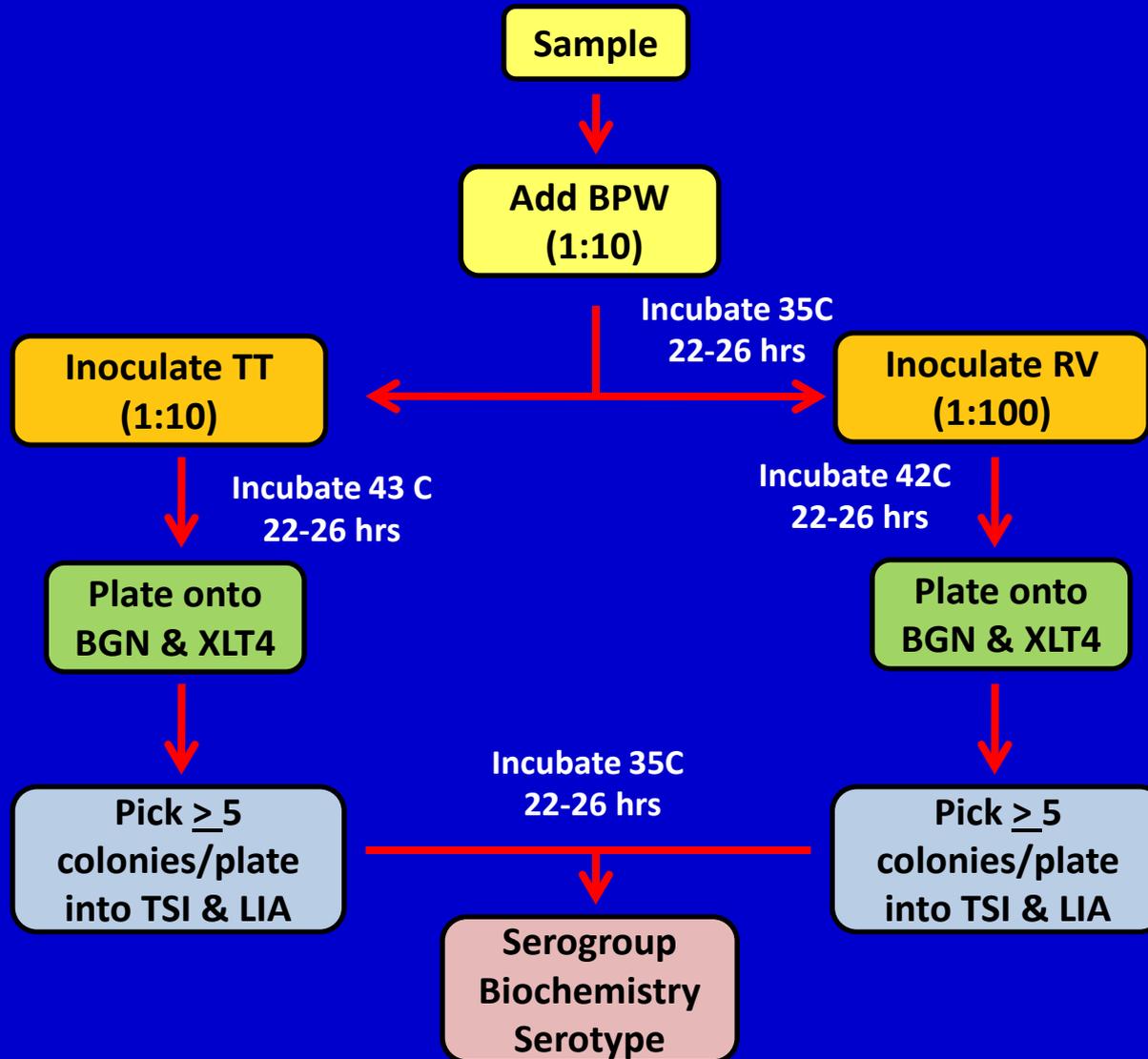
- "Procedures for collection, isolation and identification of *Salmonella* from environmental samples, cloacal swabs, chick box papers, and meconium samples," 9 CFR 147.12.(September 2010)
- SDIX RapidChek SELECT™ *Salmonella* Enteritidis Test System
- Neogen Reveal *Salmonella* Enteritidis (SE) Test System
- Applied Biosystems TaqMan® *Salmonella* Enteritidis Detection Kit from Life Technologies

FDA Testing methodology for *Salmonella* Enteritidis (SE) from Egg samples

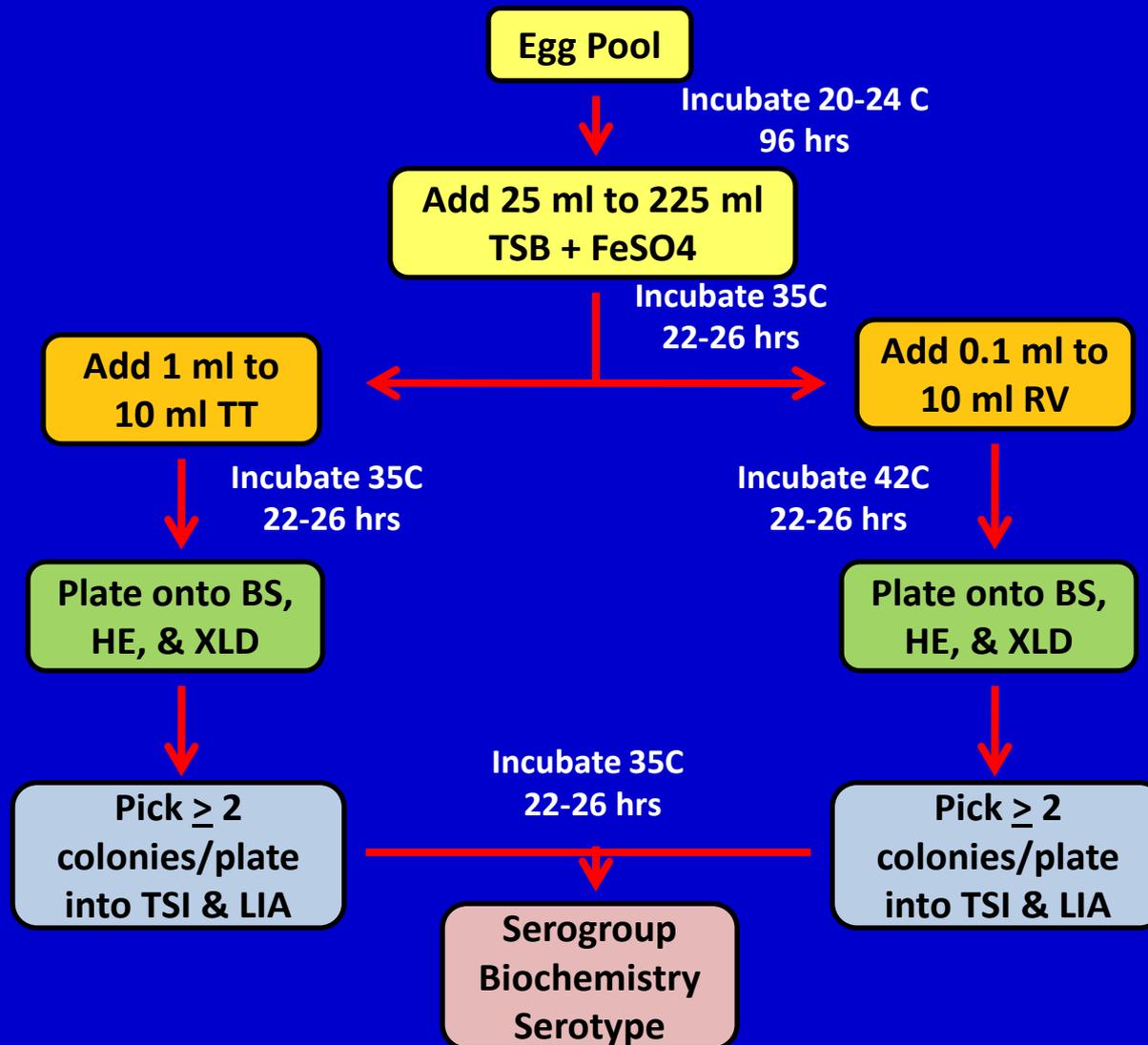
FDA has determined that the following methods are equivalent to Chapter 5 (*Salmonella*) of FDA's Bacteriological Analytical Manual (BAM, December 2007 Edition) in accuracy, precision, and sensitivity in detecting *Salmonella* Enteritidis:

- ABI Life Sciences Real-time PCR *Salmonella* Enteritidis Detection Kit, both with and without the 96-hour hold time recommended by the BAM.
- SDIX RapidChek SELECT™ *Salmonella* Enteritidis Test System, without the 96-hour hold time recommended by the BAM.
- Neogen Reveal *Salmonella* Enteritidis (SE) Test System, but only with the 96-hour hold time recommended by the BAM. It is not considered equivalent without the 96-hour hold time.
- The BAX® System PCR Assay for *Salmonella* and the BAX® System PCR Assay for *Salmonella* 2, without the 96-hour hold time recommended by the BAM

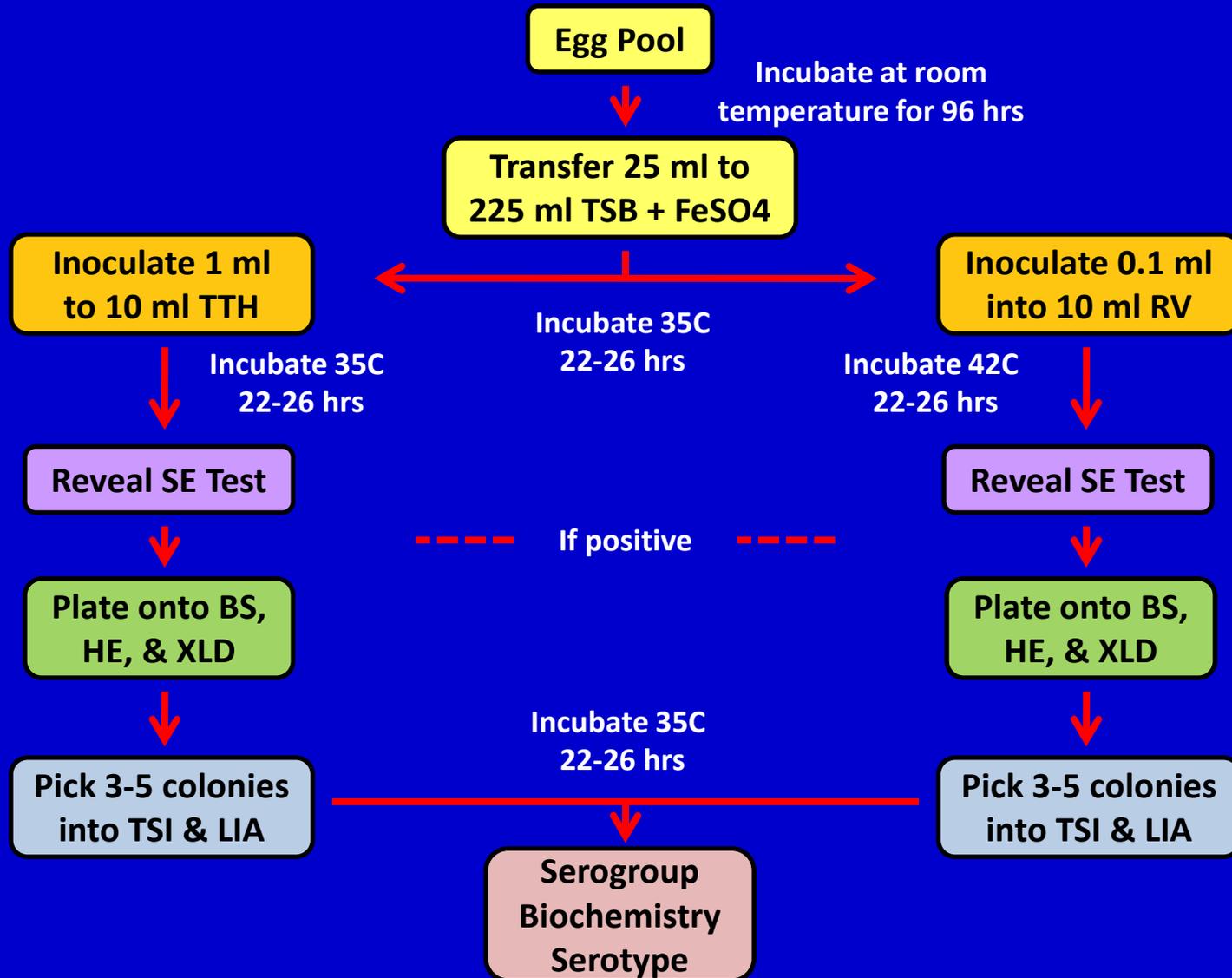
FDA Method for the isolation of Salmonella from environmental samples



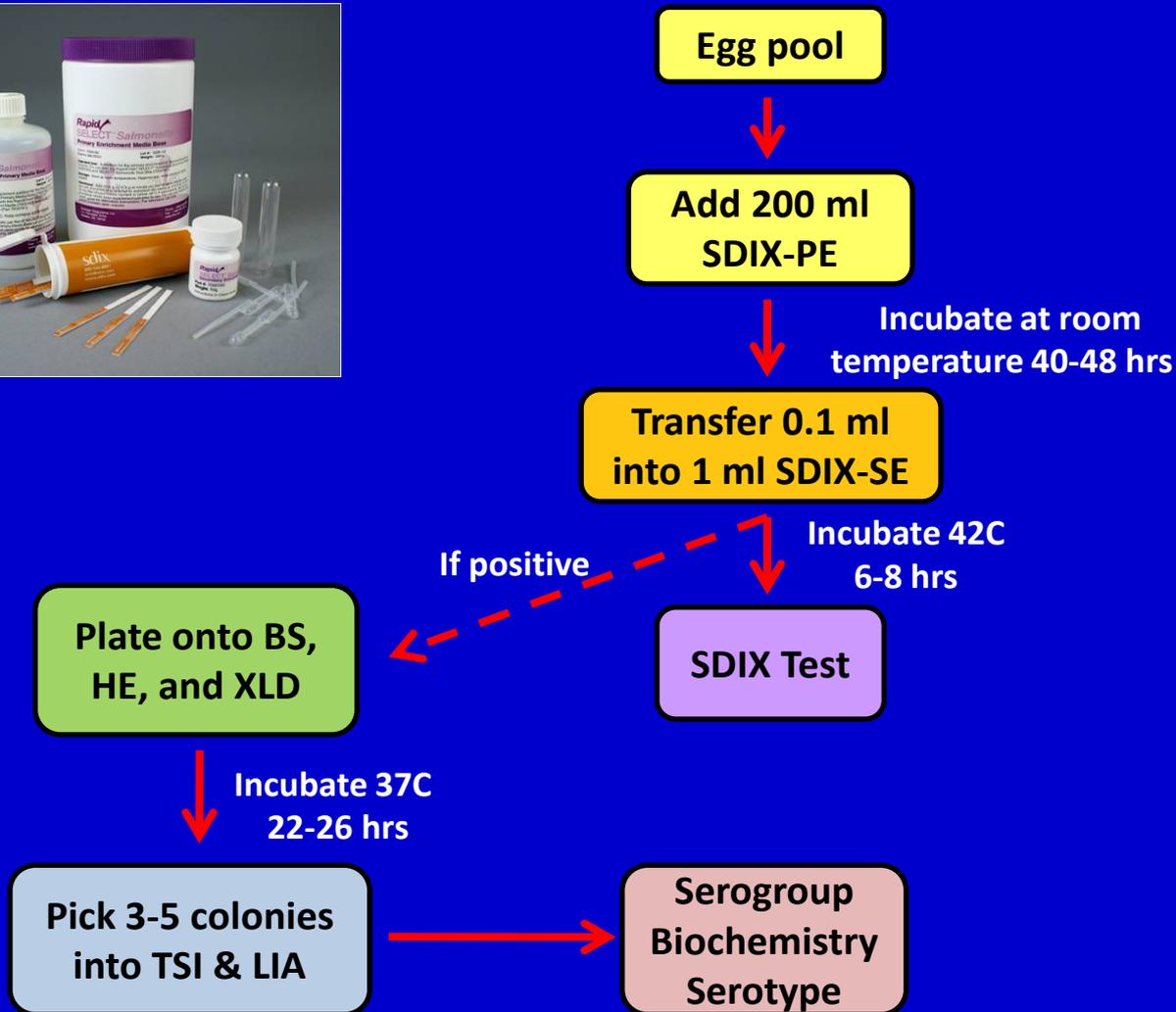
FDA Method for the isolation of Salmonella from Egg pools: BAM



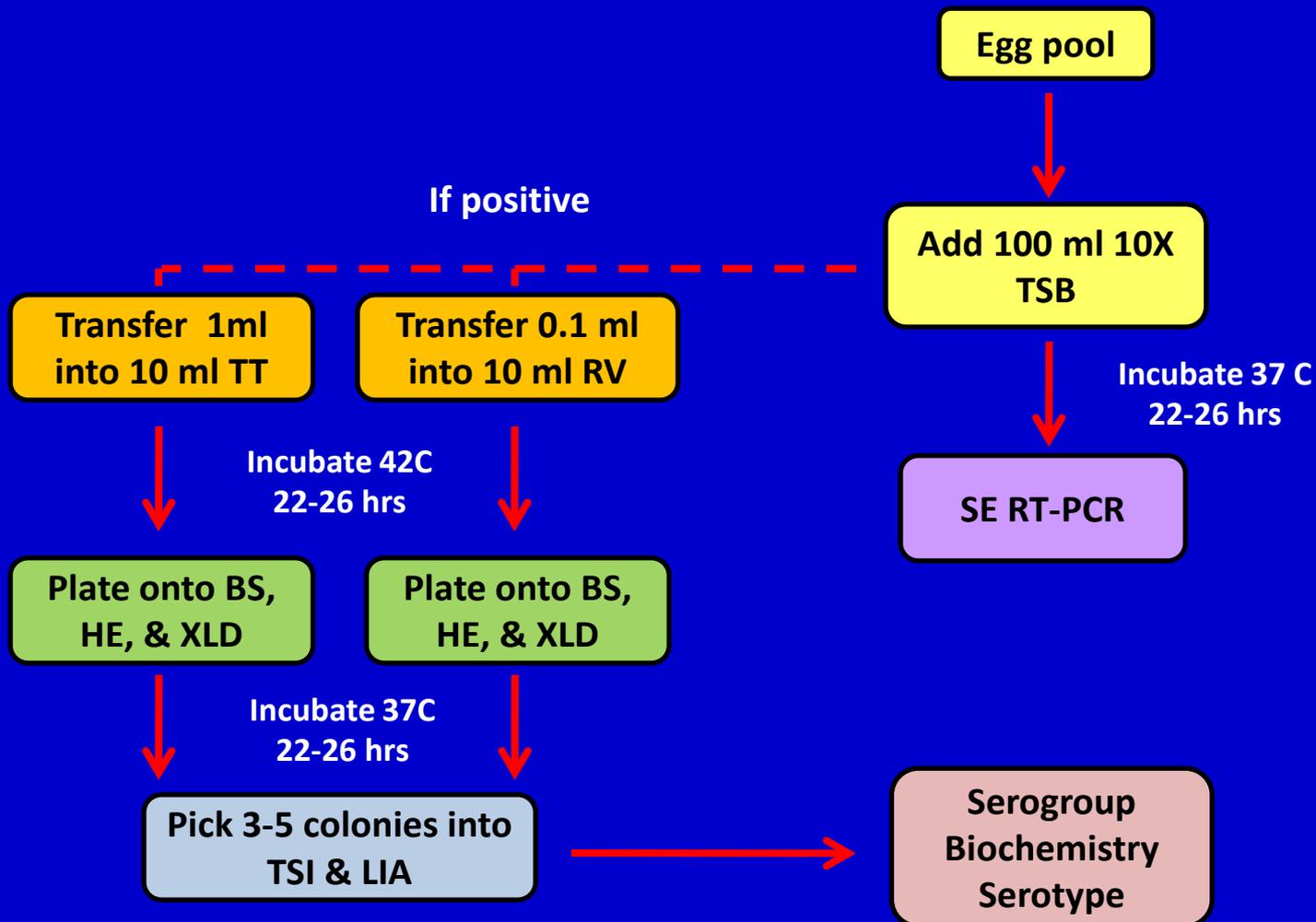
Neogen Reveal SE Method for the detection of Salmonella Enteritidis from Egg Pools



SDIX RapidChek SE Method for the detection of Salmonella from Egg Pools



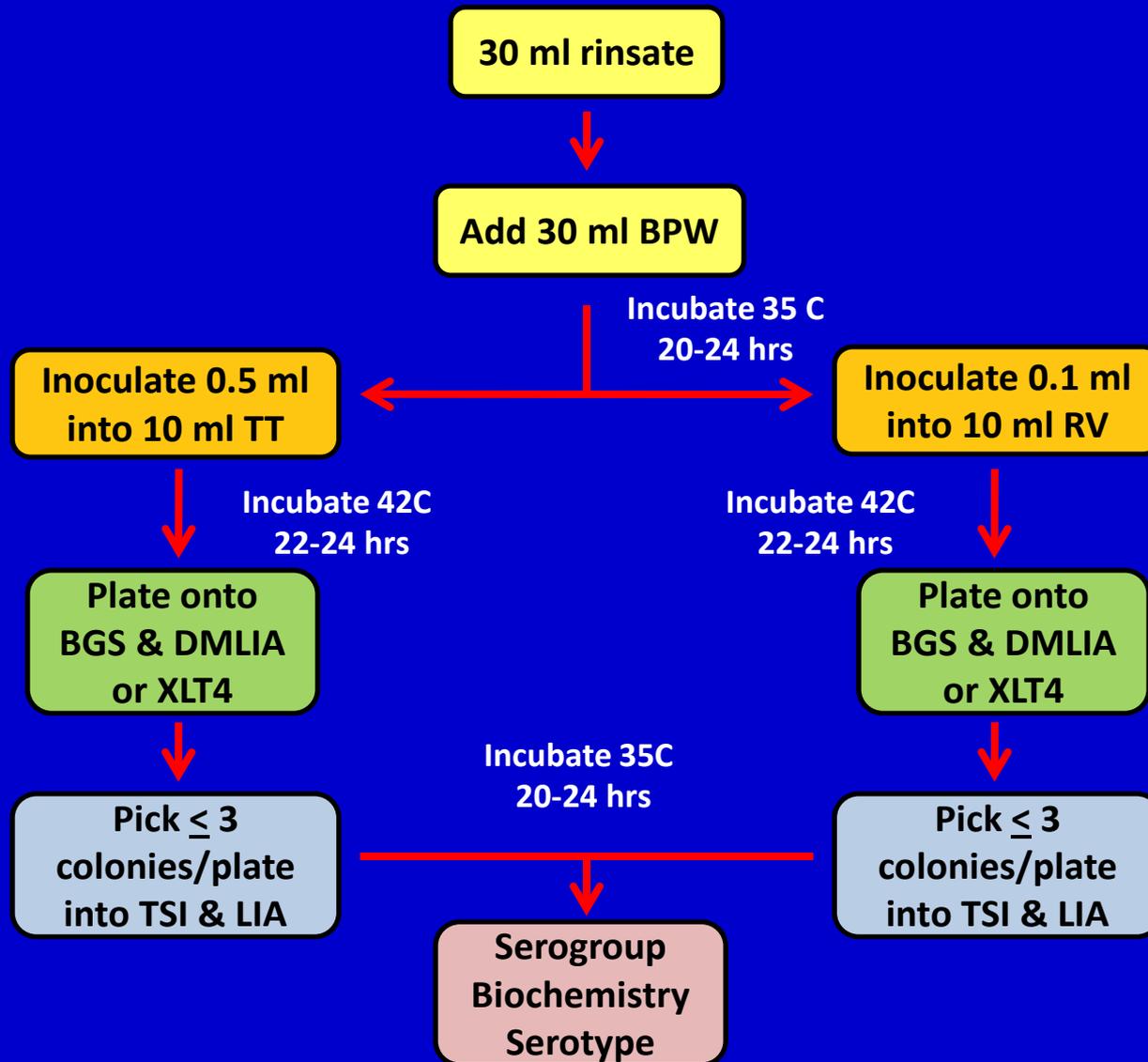
Life Technologies SE RT-PCR for the detection of Salmonella Enteritidis from egg pools



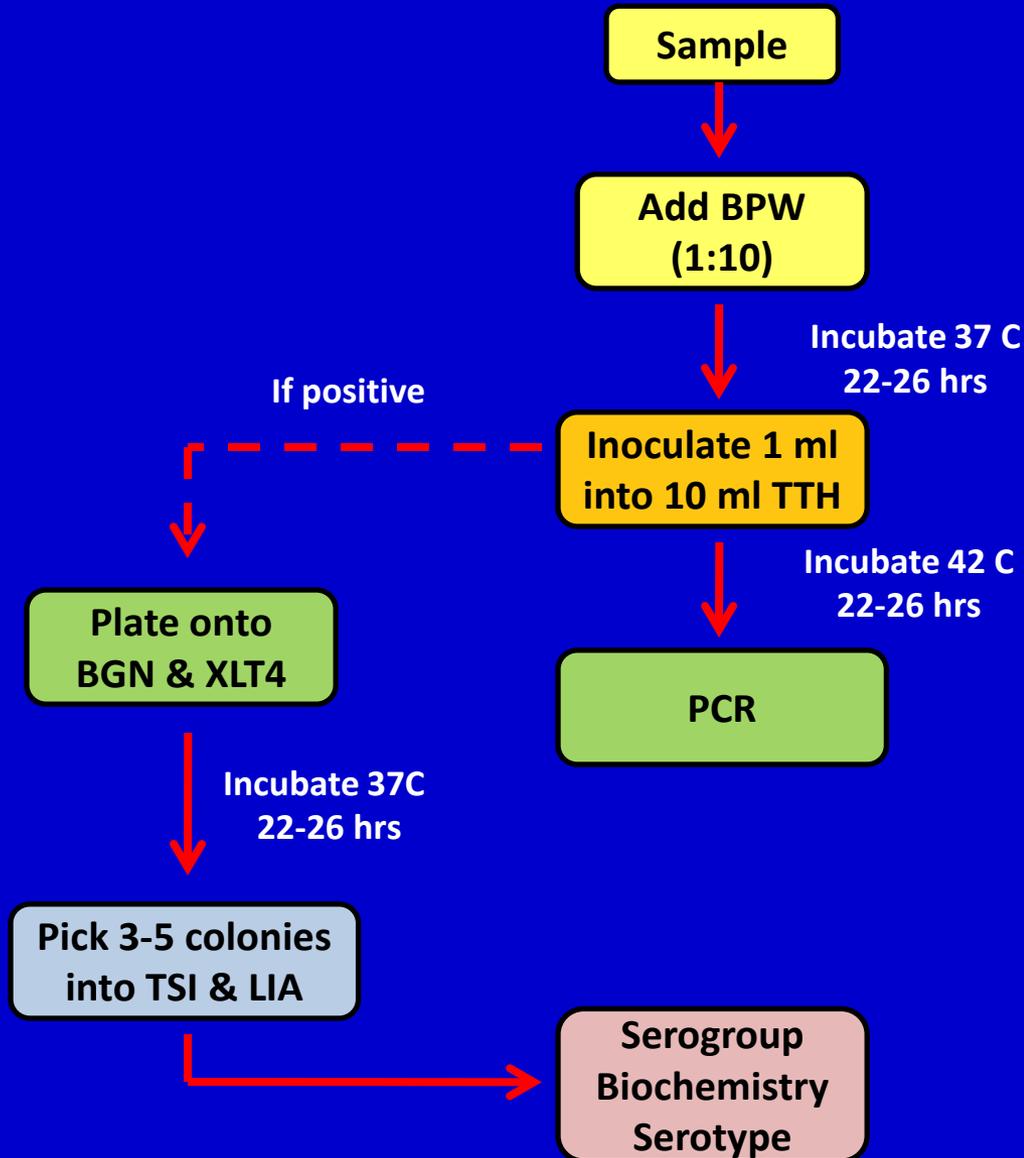
FSIS Approved Salmonella Culture Methods

- Carcass Rinses
 - Pre-enrichment followed by selective enrichment
 - BAX PCR

FSIS Method for the isolation of Salmonella from carcass rinse samples



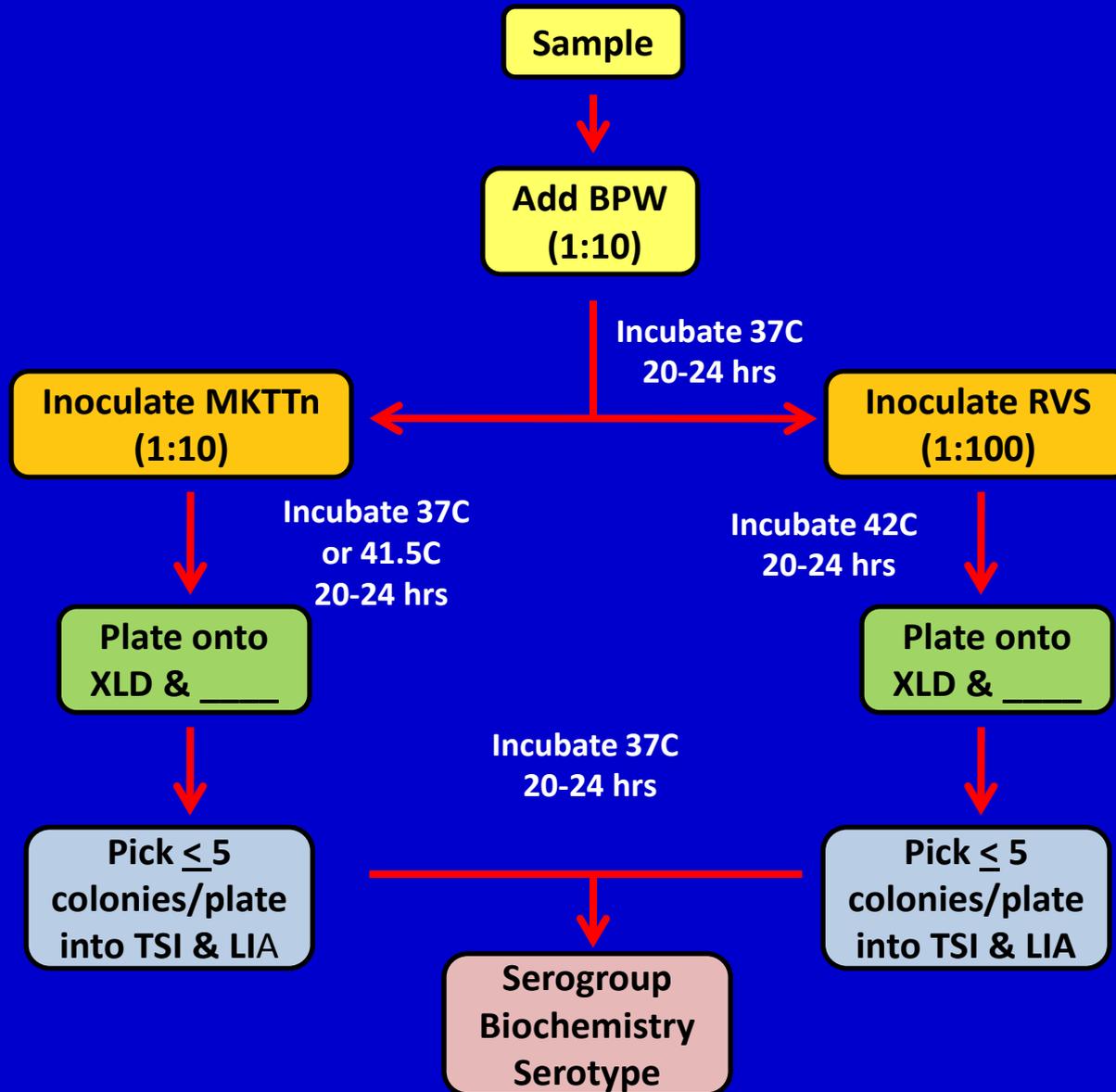
BAX PCR for the detection of Salmonella from environmental samples



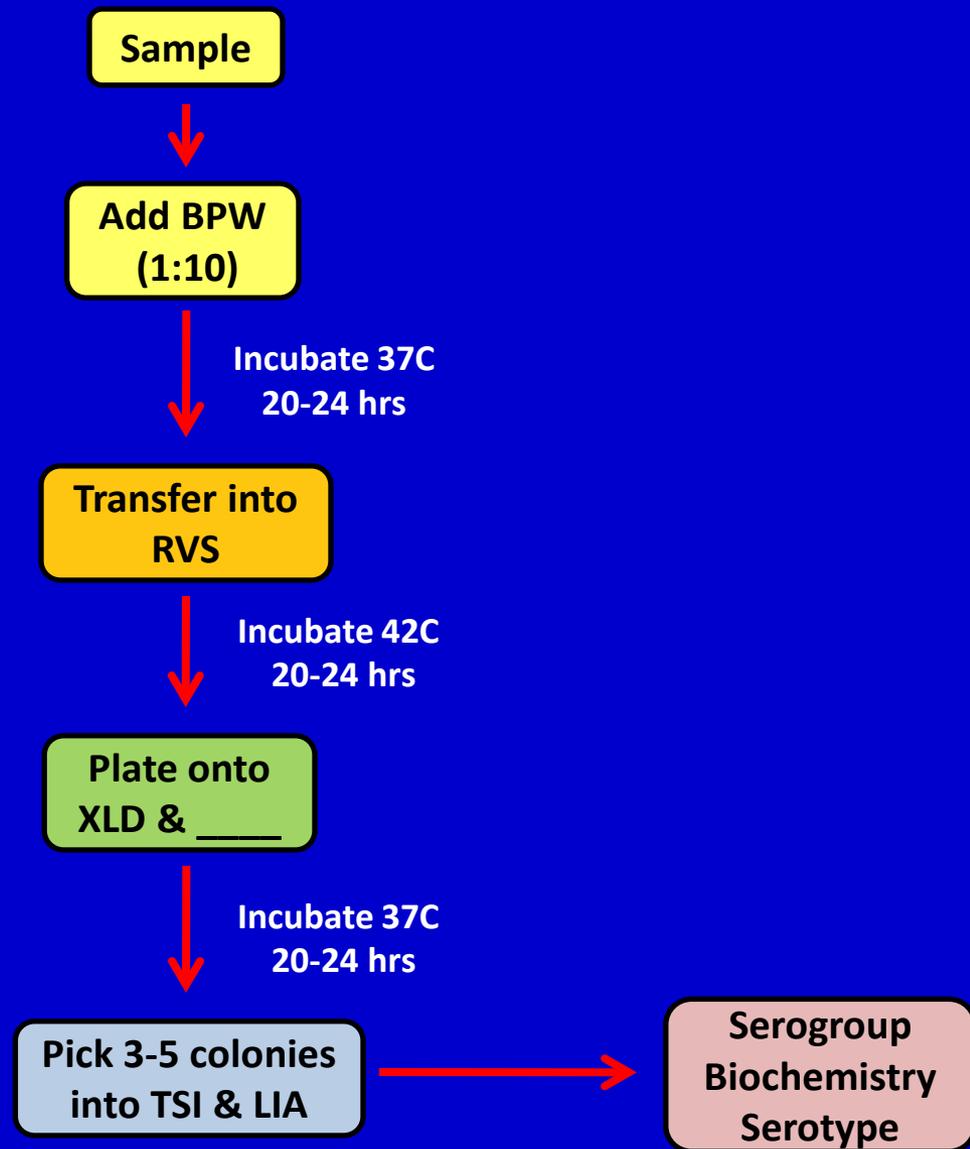
Internationally Approved Salmonella Culture Methods

- ISO 6579:2002 Method
 - Pre-enrichment followed by selective enrichment
 - Pre-enrichment followed by MSRV
- NMKL-71
 - Pre-enrichment followed by RVS

ISO 6579:2002 Method for the isolation of Salmonella from environmental samples



NMKL 71 Method for the isolation of Salmonella from environmental samples



NPIP

FDA

Birds

Environment

Environment

Eggs

Feed

**Direct
DSE**

**TT/MSRV
Pre/Selective
SDIX
BAX PCR
Chemunex PCR
SE-PCR
Group D PCR
SDIX-SE
Neogen-SE
LT SE-RTPCR**

**FDA method
NPIP methods
SDIX-SE
Neogen-SE
LT SE-RTPCR**

**FDA BAM
SDIX-SE
Neogen-SE
LT SE-
RTPCR
BAX**

FDA BAM

FSIS



Carcass Rinse

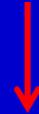


**USDA/MLG
BAX PCR**

NCC



Environment



**TT/MSRV
Pre/Selective
SDIX
BAX PCR
Chemunex PCR
SE-PCR
Group D PCR
SDIX-SE
Neogen-SE
LT SE-RTPCR**

Thank you

