Finding Salmonella in feed
What's the problem?

Simon Carlton

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EMEA & Russia

Our Focus

- Anitox Focus is on FEED Safety

- Includes control of Salmonella, Clostridia (Necrotic Enteritis), E.coli, Campylobacter, Mold etc…….
- Viruses including AIV, ASF, PEDv, PRRS

Sources of Pathogen Challenge

Feed

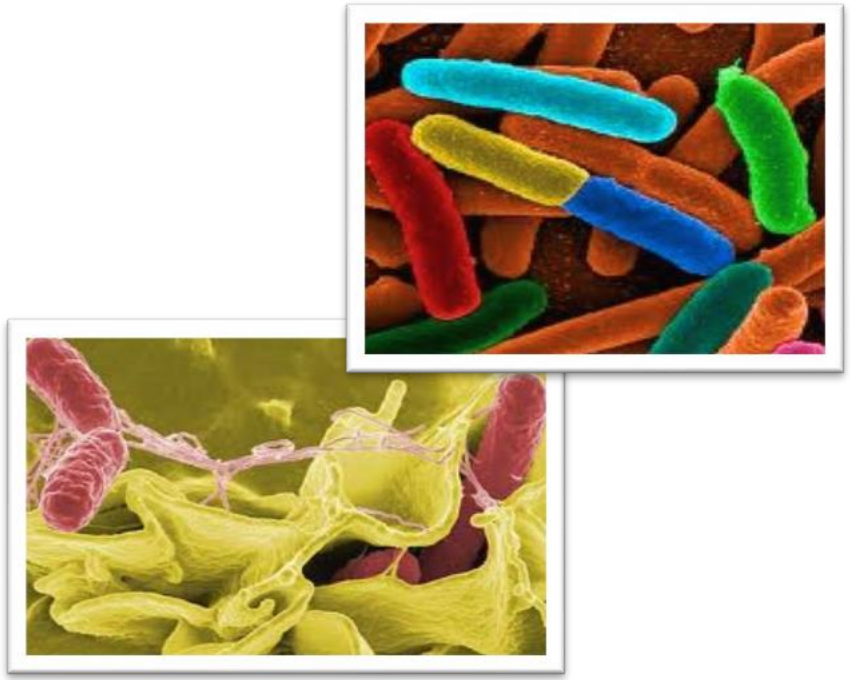
Water

Environment

Hatchery

Feed is a Fomite

- *Escherichia coli*
- *Clostridium*
- *Staphylococcus*
- *Streptococcus*
- *Campylobacter*
- *Salmonella*
- *Molds & Mycotoxins*
- *Listeria*
- *Pseudomonas*
- *Pasteurella*
- *Yeasts*
- ***Viruses!***



When & How do Feed Ingredients Become Contaminated?

- When?
  - Growing
  - Harvesting
  - Processing
  - Storage/transport
  - Feed mill intake and storage
- How?
  - Environmental contact
    - Direct - soil, rodents, birds, dust, humans, water
    - Indirect – water, sewage, animal manure
  - Cross contamination
    - Storage, processing, transport, mill intake

Does feed get contaminated by birds/vermin?

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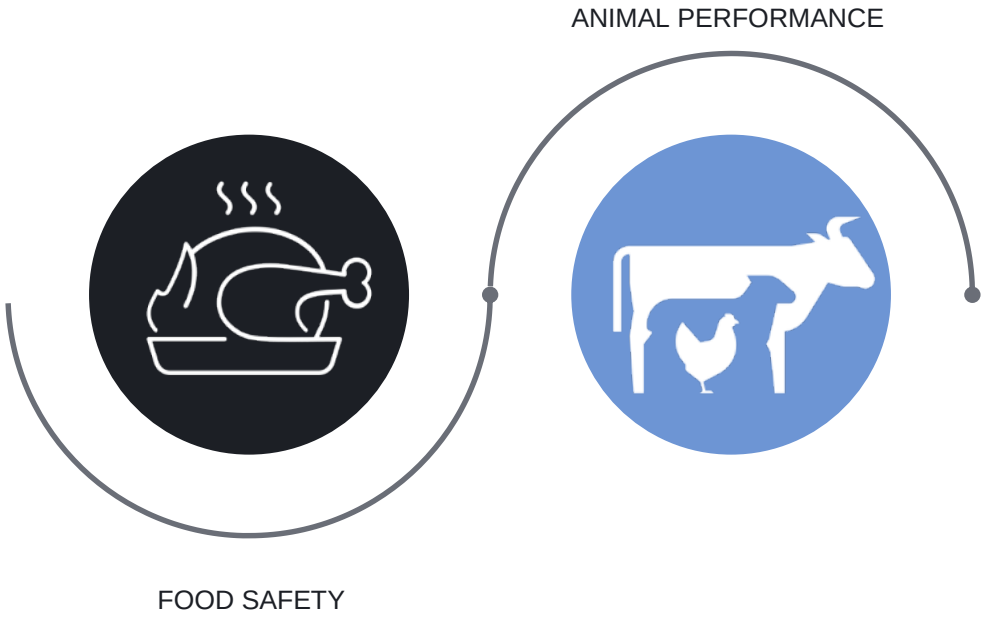
Impact of microorganisms in feed



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Salmonella in Feed and Food Safety

1948	*Salmonella* isolated from animal feed (Univ. Kentucky Exp. Res. Sta.)
1969	*S. agona* in Peruvian fishmeal incorporated into US broiler feed associated with US human disease outbreak

However, the significance of *Salmonella* in feed on carcass/egg contamination and human food safety is often debated

Do In Feed Pathogens affect Poultry?

Can Salmonella Contamination of Poultry Carcasses come from Feed?

Yes

Reference	% of Isolates in Feed Found on Carcasses
Morris *et al*, 1969	50
McKenzie and Bains, 1976	100
Lahellic and Collins, 1985	50
Corry *et al*, 2002	55

Do In Feed Pathogens affect Turkeys?

Yes

Reference	% of Isolates in Feed Found in Turkey Poults
Boyer et al 1958	56
Boyer et al 1962	50
Sanad et al 2016	60

Do in Feed pathogens affect Breeders?

Yes

Breeders – **80%** of the Serotypes of *Salmonella* from Feed were Found Weeks Later in the Breeding Flock or Their Offspring (Jenson and Rosales, 2002)

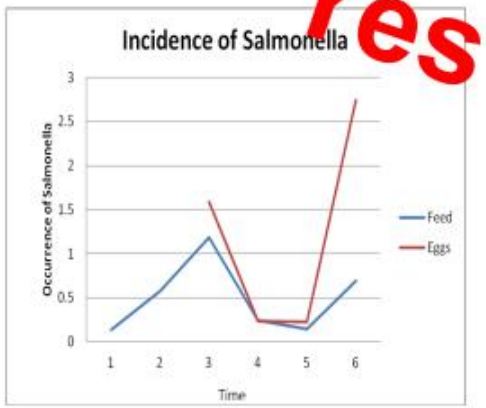
Do in Feed Pathogens Contaminate Eggs?

Yes

Shirota *et al* (2001) observed that:

1) 58.5% of the serotypes in the egg were the same as the feed

2) The incidence of Salmonella in feed and the incidence of Salmonella in eggs were directly correlated



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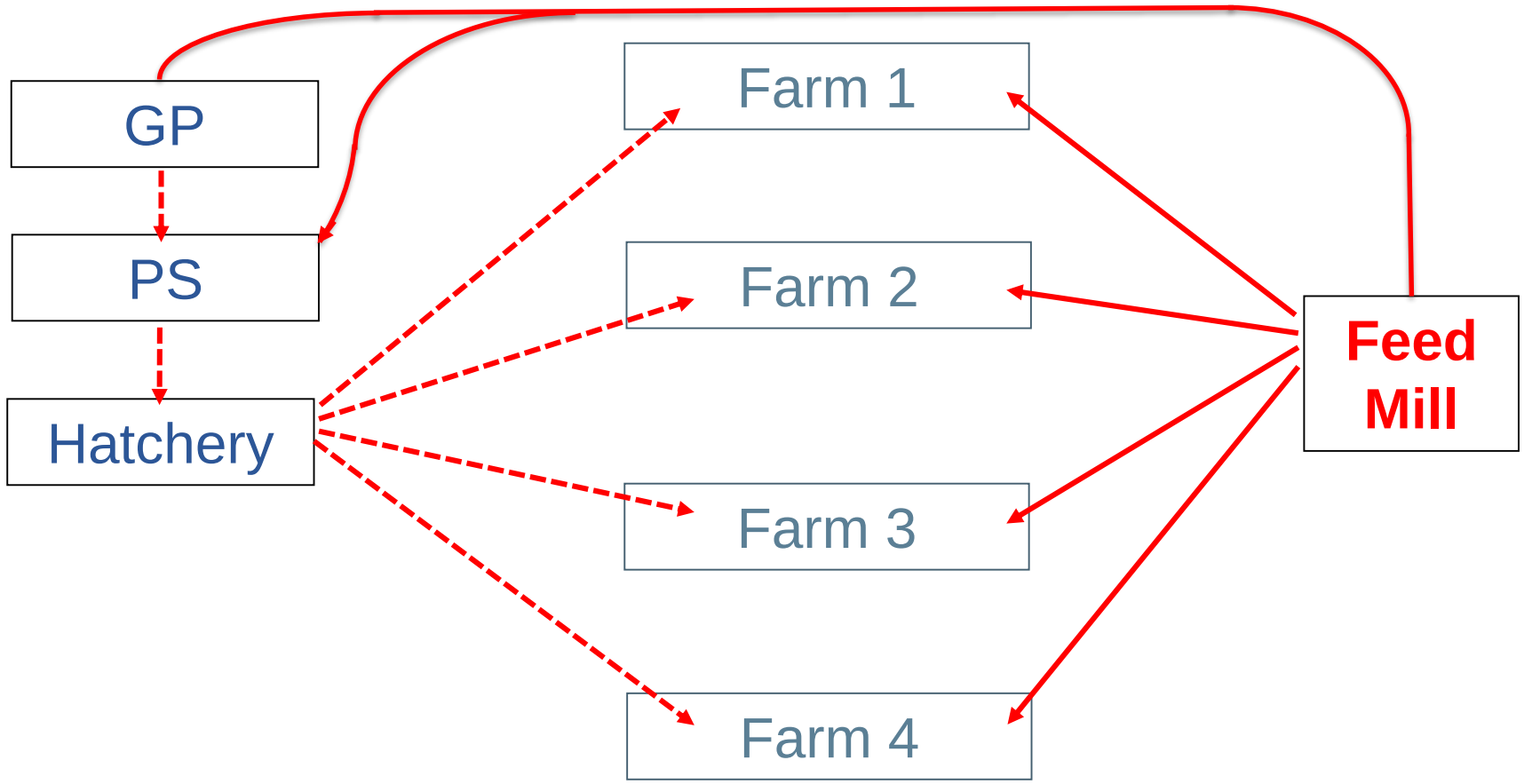
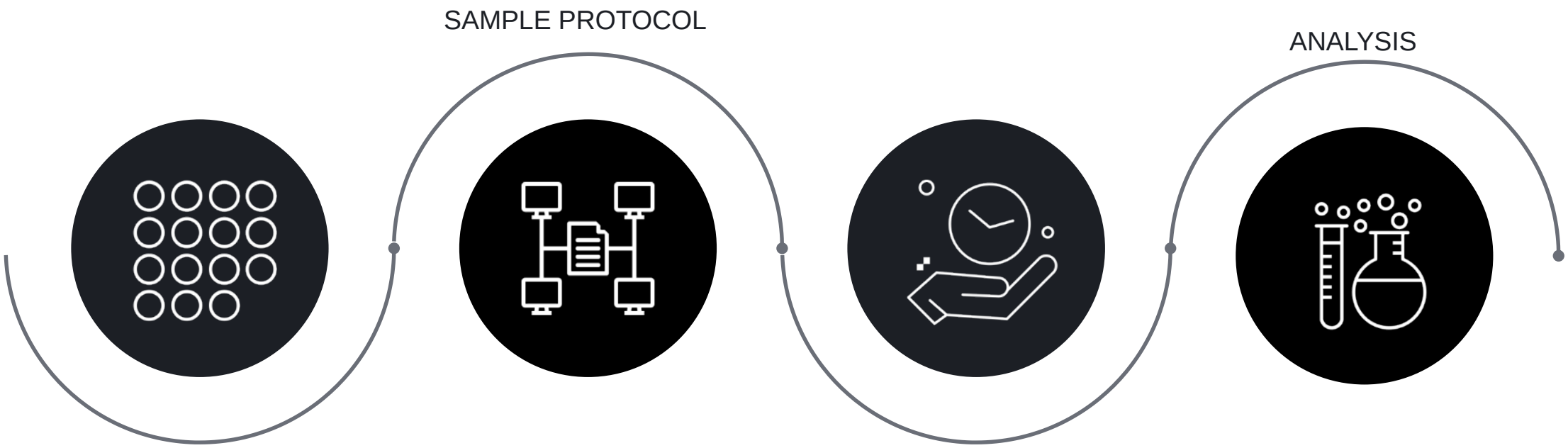
Why do we need to control pathogens in Feed?



Four Keys to Salmonella Detection



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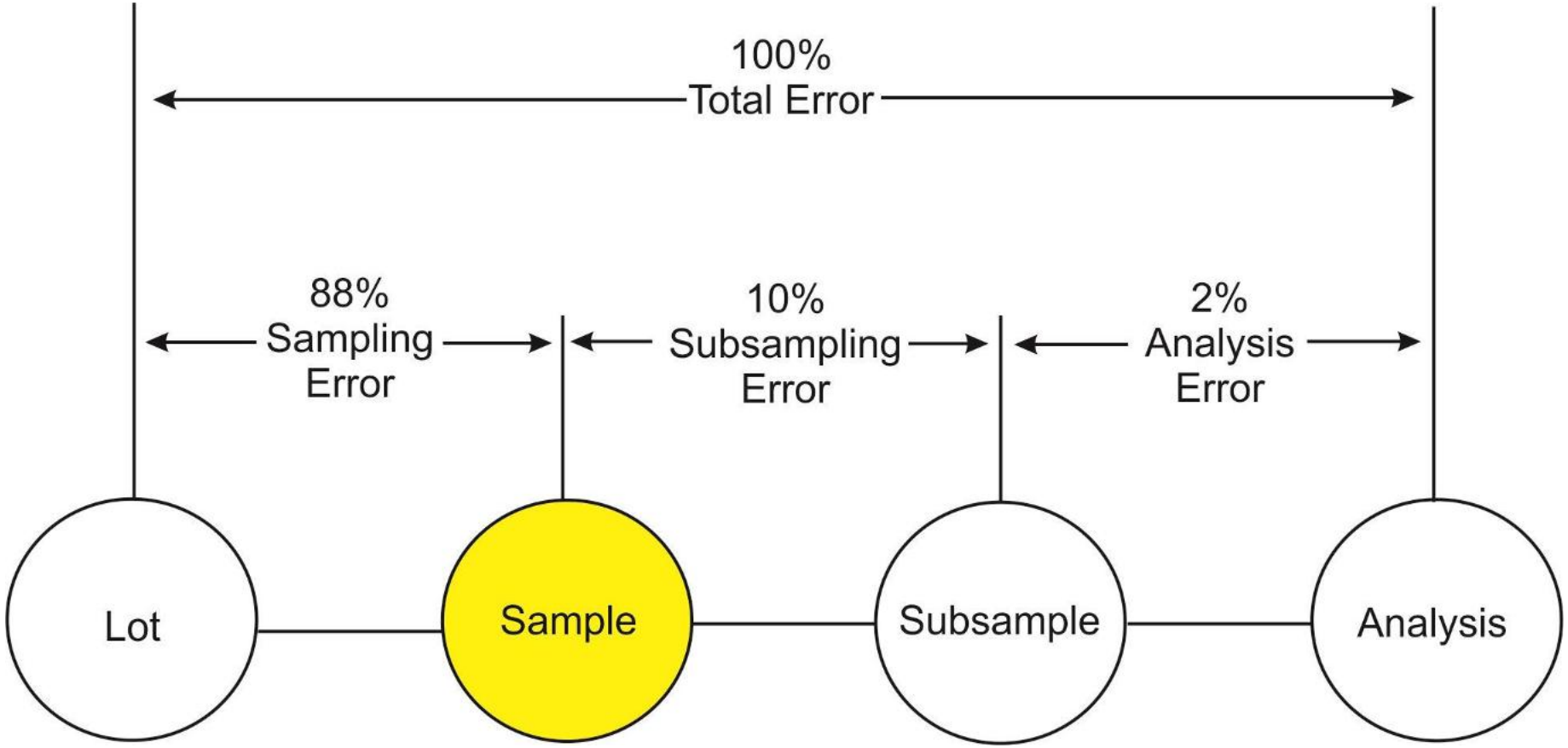
Challenges in Recovery



Whitaker and Dickens, 1974

Sampling Difficulties

- The level of Salmonella in feed/ingredients is extremely low (2-4 cfu/100g; Izat and Waldroup, 1990)
- Salmonella in feed/ingredients is not uniformly distributed
- If 100 Salmonella are added to 1 ton of feed and evenly distributed. The odds of finding Salmonella in a single 25g sample are 1/400

Uneven distribution = Uncertainty in detection

Sampling vs Probability of Detection

Probability of Accepting a Contaminated Lot Depending on the Number of Samples tested and Distribution of Contamination

Percent of samples Contaminated	Number of samples tested					
	1	5	10	15	30	60
1	0.99	0.95	0.90	0.86	0.74	0.55
2	0.98	0.90	0.82	0.74	0.55	0.30
5	0.95	0.77	0.60	0.46	0.21	0.05
10	0.90	0.59	0.35	0.21	0.04	<0.005
20	0.80	0.33	0.11	0.04	<0.005	<0.005

NGFA 2013 (adapted form the ICMSF)

Sampling – Increasing Sensitivity

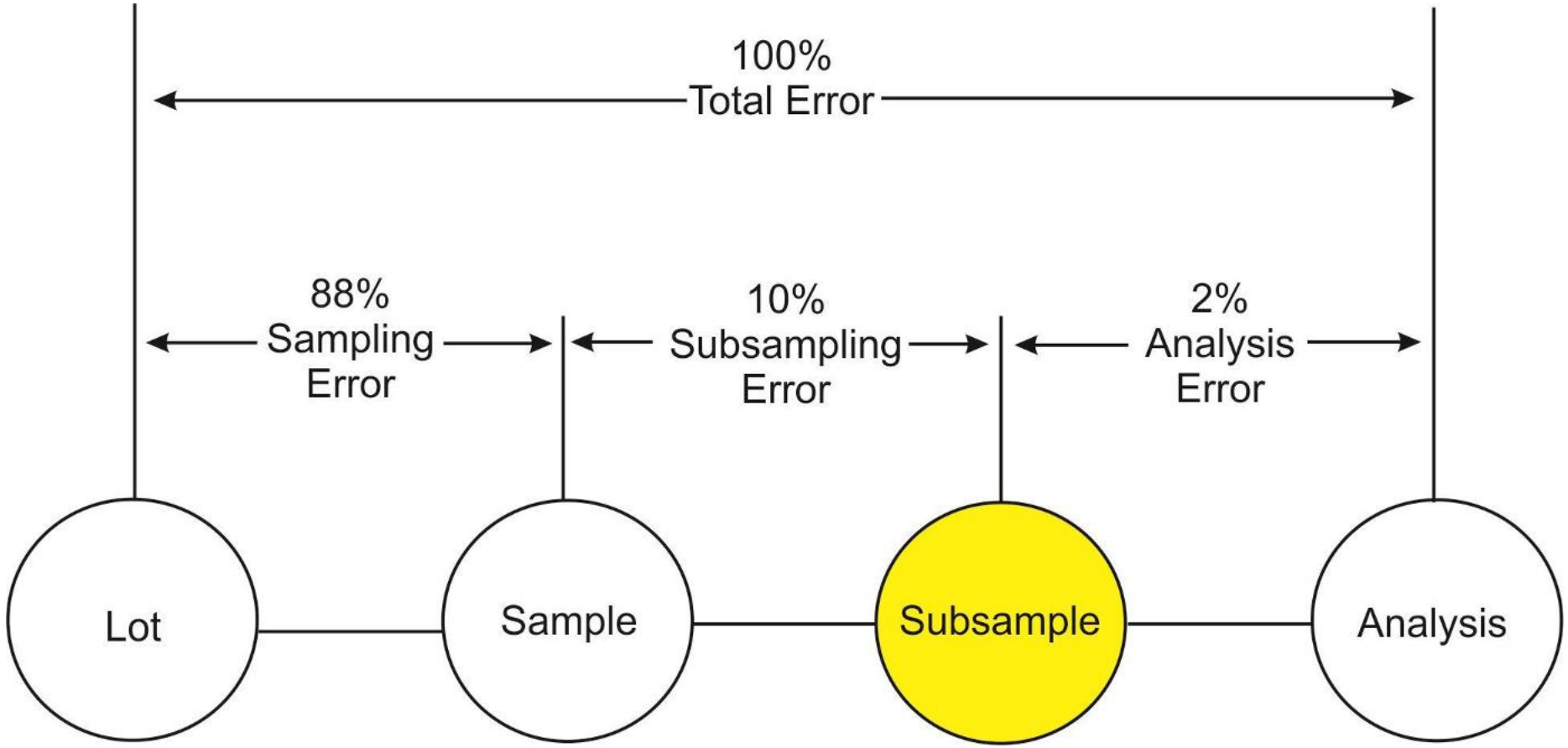
- Environmental sampling is often used when the incidence of Salmonella in feed is low (<2-5%)
  - Residues in coolers, tops of auger covers, and feed bins
  - Dust samples inc from vacuum cleaner bags
  - Large moist swabs

Overcoming Challenges in Recovery (Scale)

Sample site Mill 3	No. samples	Salmonella isolated
Intake area	120	25 (Dur,Typ,New,Aga,Wag)
Post intake/pre Loadout	160	13 (Aga,Kot,Bin,Giv)
Loadout area	128	5 (Aga)
Total	408	43

Davies and Wales 2010

Subsample traps



Sample Preparation

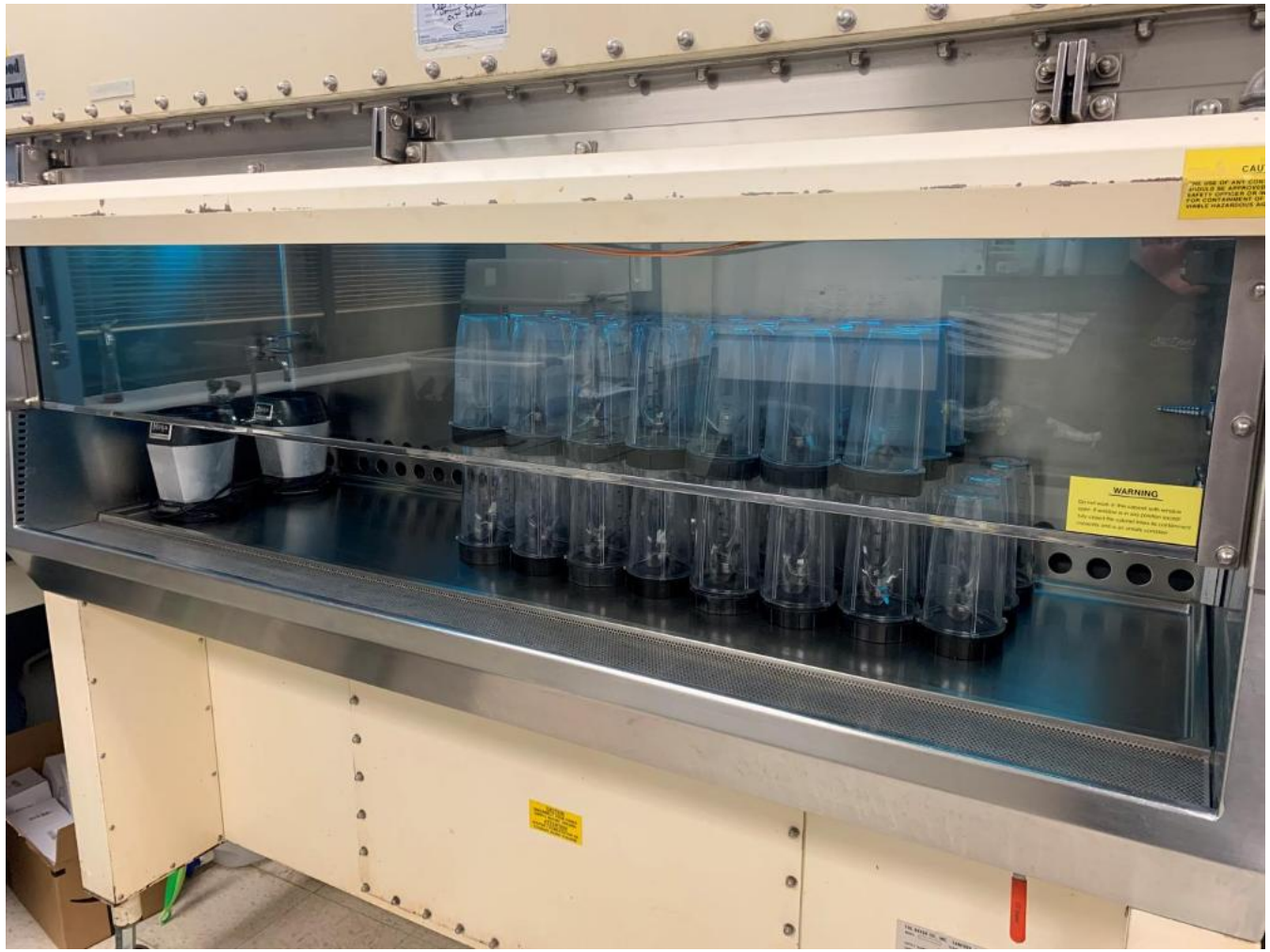
- Reduction of particle size
- Reduction of sample size (subsamples) for analysis

Particle Size Reduction and Salmonella

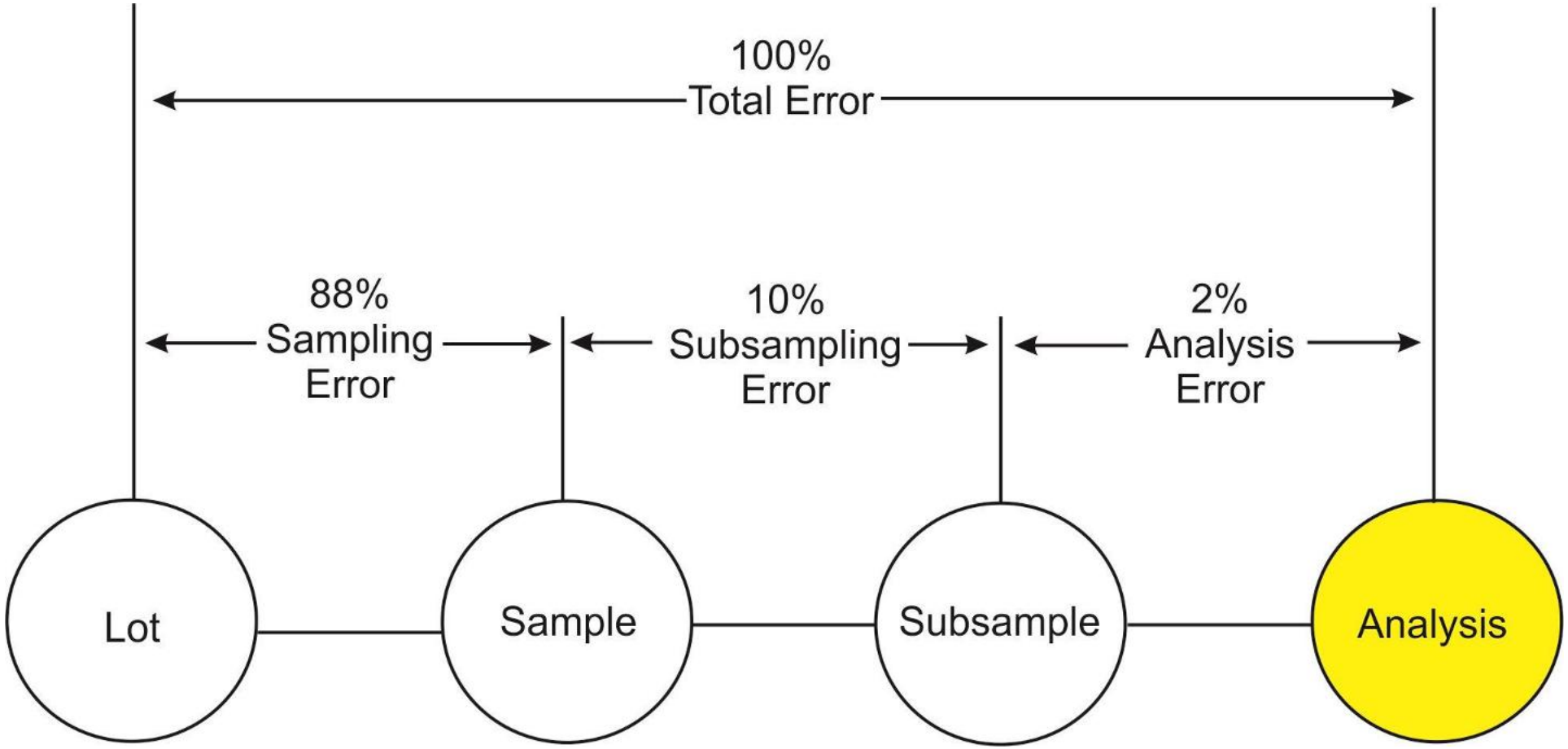
Relationship of Particle Size to the Level of Salmonella	
Particle Size (mm)	Salmonella (MPN/100g)
>4.0	4
2.8 – 4.0	92
2.0 – 2.8	92
1.0 – 2.0	171
<1.0	1,850

Israelson *et al*, 1997

Grinder - Flasks



Analysis Process



Microbiological Challenges

- Salmonella in feed/ingredients is usually in an injured or stressed state requiring resuscitation (pre-enrichment media)
- Most pre-enrichment media were designed for the recovery of Salmonella from human foods
- However, feed and ingredients poses unique challenges

Complications During Pre-enrichment

- The level of Salmonella in feed/ingredients is extremely low (2-4 cfu/100g)
- The level of other bacteria in feed/ingredients can range from <100 to >1,000,000 cfu/g
- **pH changes of pre-enrichment media can occur due to other competing bacteria and the composition of the feed/ingredient**

Pre-enrichment pH of Feed

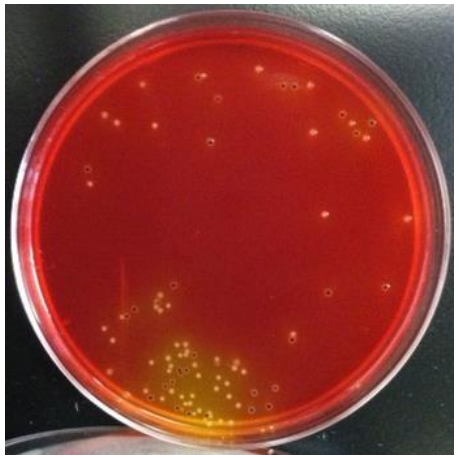
pH of Pre-enrichment Media after 24 hrs @ 35ºC				
Feed Type	Pre-enrichment Media			
	LB	BPW	M-9	UPB
Broiler Breeder	4.1	4.8	5.1	5.5
Broiler Grower	4.1	4.3	5.0	5.1
Layer	4.7	4.8	4.8	5.2
Turkey Breeder	5.5	5.9	6.7	6.0
Turkey Grower	5.9	6.6	5.6	5.8

Initial pH ranged from 6.2 – 7.1

Cox et al 2013

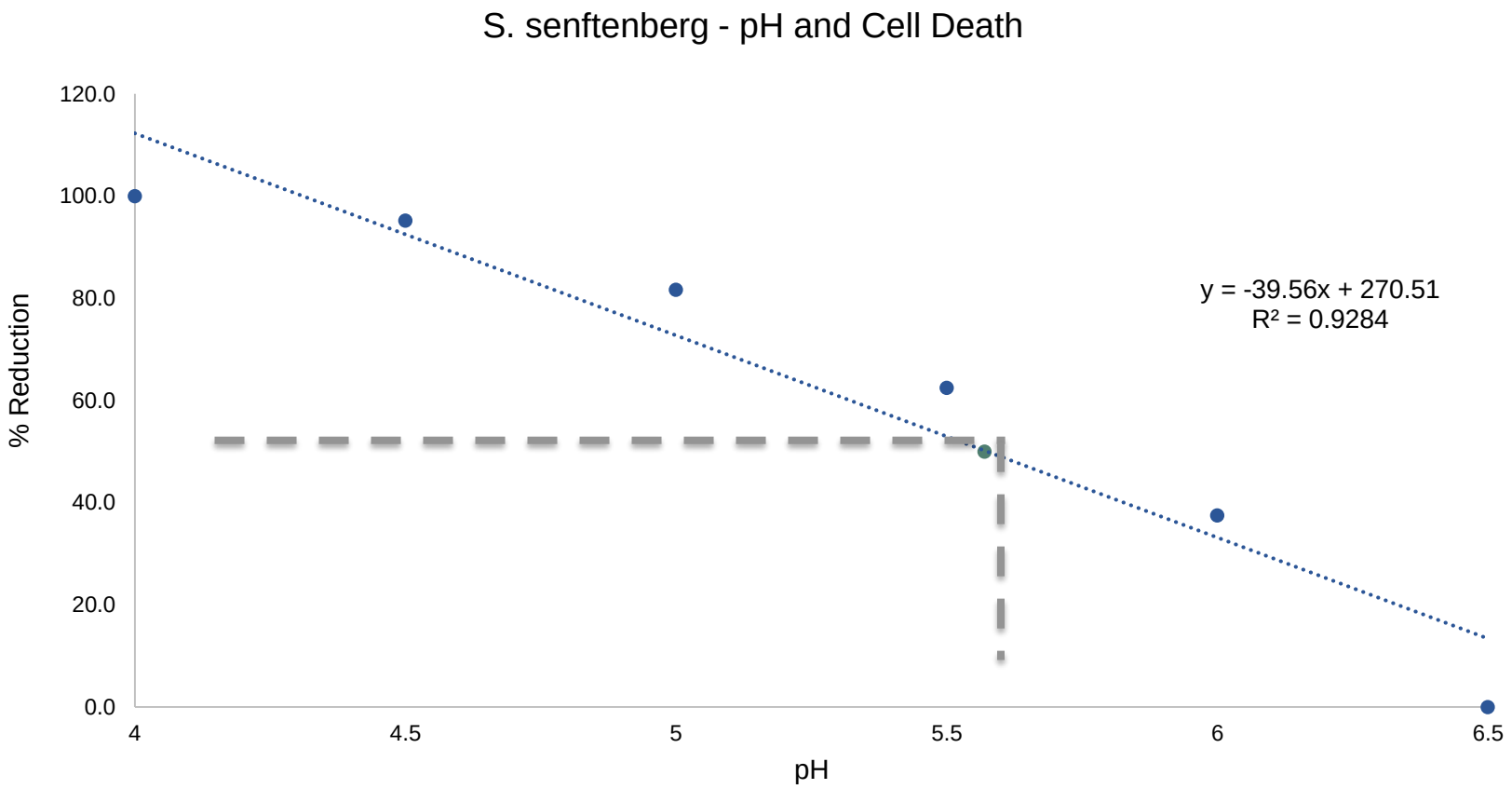
Pre-enrichment Media and Acid Injury

- Exposure of S. bareilly to low pH (pH 3.0 – 3.5) can result in:
  - Cell death
  - Cell injury
  - Change in biochemical reactions (i.e. H_2S production, ornithine decarboxylase, lysine decarboxylase, arabinose fermentation, inositol fermentation, sorbitol fermentation)



Blankenship, 1981

Impact of pH on Cell Death – pH_{50}



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Acid Exposure of Salmonella

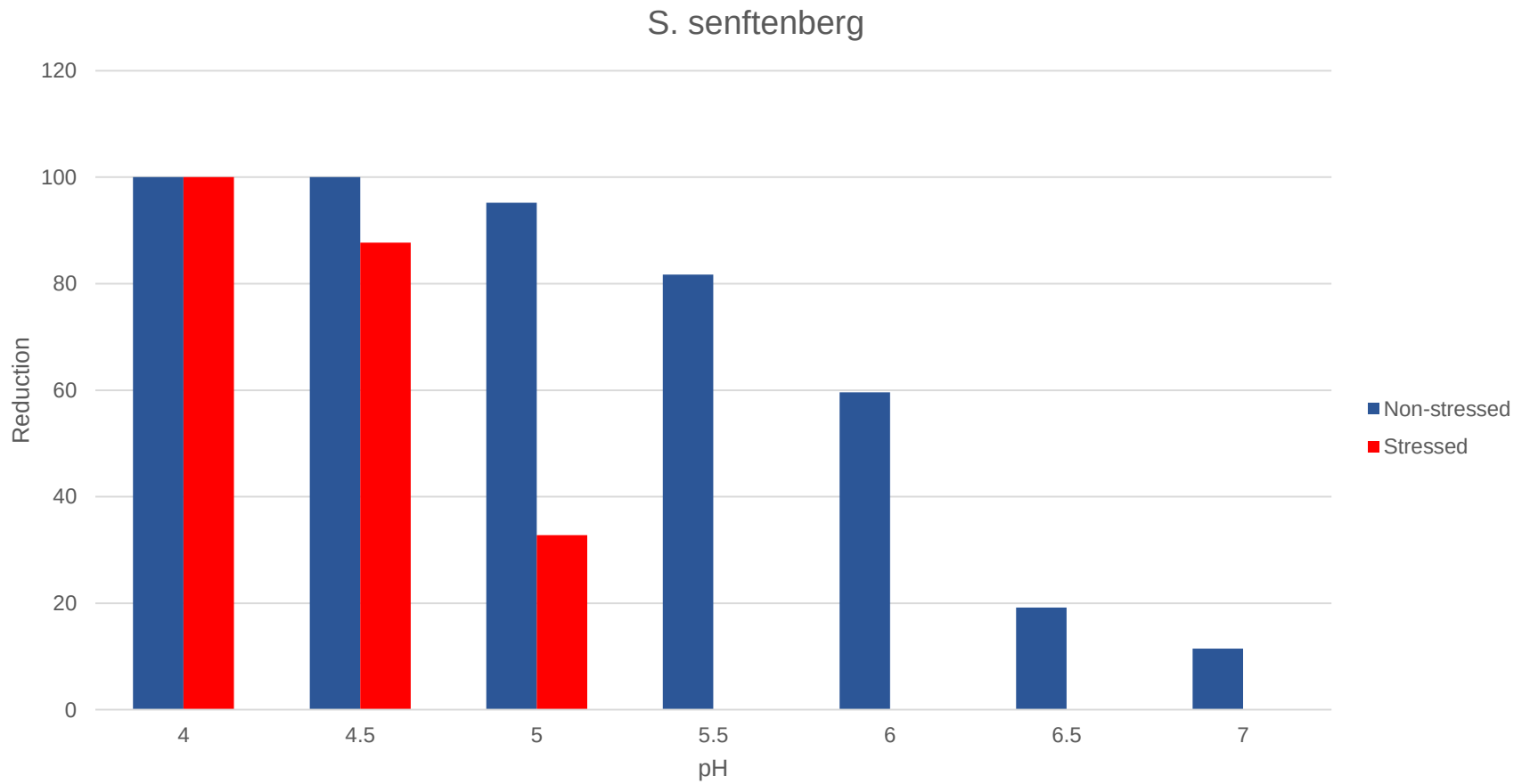
pH Required to Cause a 50% Reduction in the Recovery of Salmonella		
Isolate	Cell Death	Cell Injury
S. typhimurium	4.85	5.21
S. tennessee	4.86	5.48
S. senftenberg	5.57	6.01
S. schwarzengrund	5.69	6.23
S. montevideo	5.75	6.22
S. enteritidis	5.75	4.98
S. infantis	5.78	6.18
S. heidelberg	5.93	6.29

Richardson et al 2018

Challenges in Recovering Salmonella from Feed/Ingredients

- The level of Salmonella in feed/ingredients is low (2-4 cfu/100g)
- **Salmonella in feed/ingredients is usually in an injured or stressed state**
- The level of other bacteria in feed/ingredients can range from <100 to >1,000,000 cfu/g
- pH changes due to feed composition or growth of other competing bacteria

Stressing Affects Acid Tolerance



Richardson et al 2018

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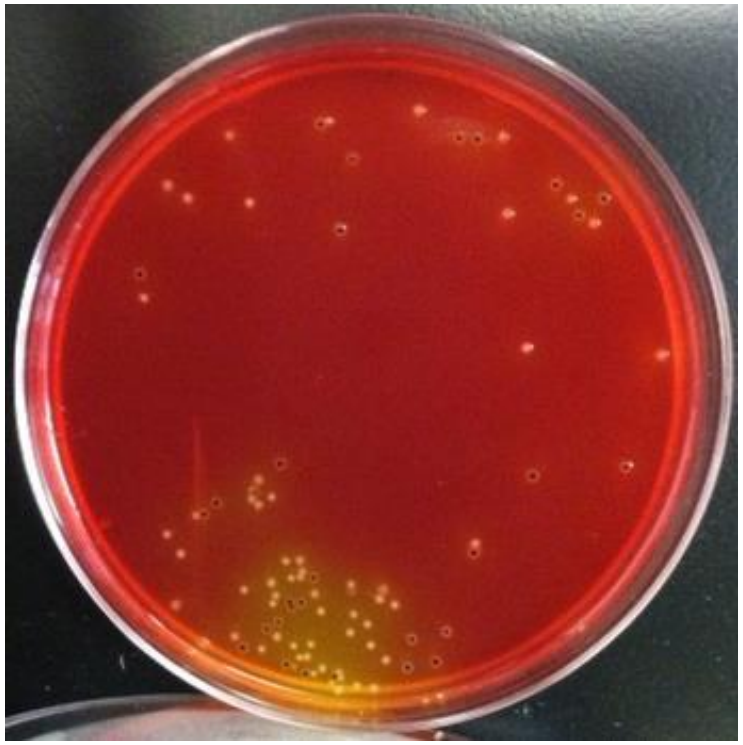
Acid Exposure and Biochemical Reactions



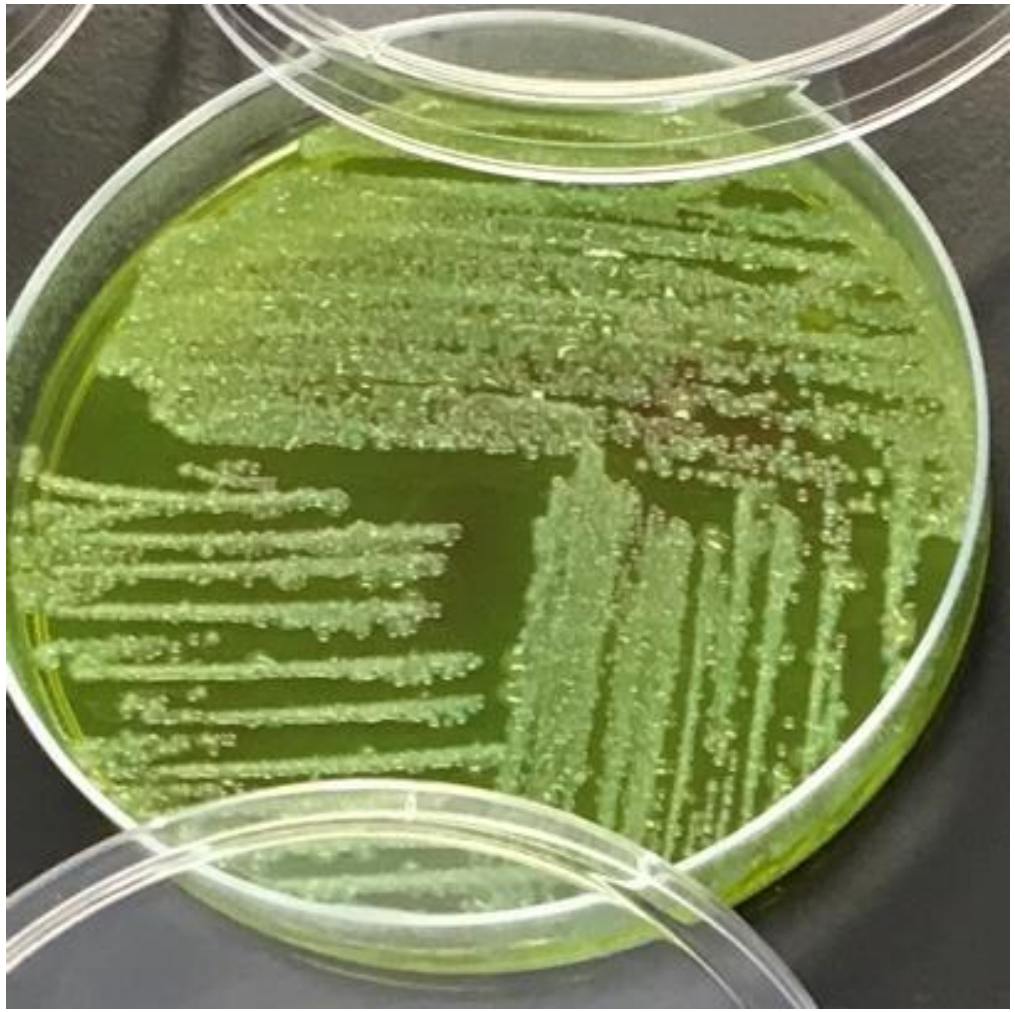
Acid Exposure Impact on H_2S Production		
Isolate	Non-stressed	Stressed
S. typhimurium	-	-
S. tennessee	-	-
S. enteritidis	-	+
S. senftenburg	-	-
S. montevideo	-	+
S. schwarzengrund	+	+
S. heidelberg	-	-
S. infantis	+	+

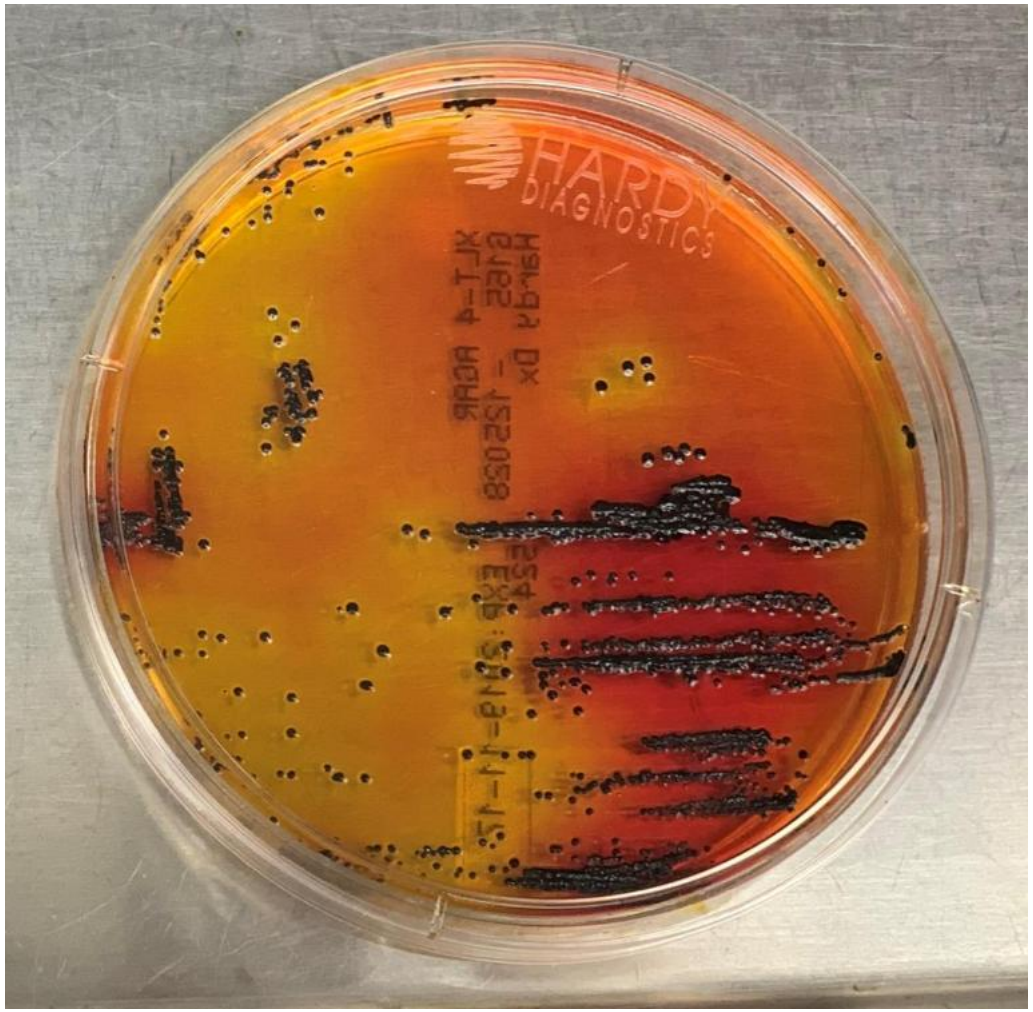
Putting it All Together

PRE-ENRICHMENT	ENRICHMENT	SEROGROUP (%)				
		A	B	C	D	E
M-9 media	Tetrathionate 35° C	5.3	8.0	74.7	8.0	4.0
M-9 media	Tetrathionate 42°C	6.7	6.7	65.3	6.7	14.7
M-9 media	Selenite cystine	1.3	36.0	49.3	5.3	8.0
M-9 media	Rappaport Vassiliadis	8.0	8.0	49.3	8.0	24.0
Buffered peptone	Tetrathionate 35° C	0	5.3	84.0	5.8	5.3
Buffered peptone	Tetrathionate 42°C	1.3	14.7	74.7	4.0	5.3
Buffered peptone	Selenite cystine	0	12.0	85.3	2.7	0
Buffered peptone	Rappaport Vassiliadis	0	20.0	78.7	0	1.3
Lactose broth	Tetrathionate 35° C	6.7	28.0	61.3	1.3	2.7
Lactose broth	Tetrathionate 42°C	1.3	18.7	72.0	1.3	6.7
Lactose broth	Selenite cystine	0	48.0	48.0	4.0	0
Lactose broth	Rappaport Vassiliadis	0	33.3	61.3	4.0	1.3

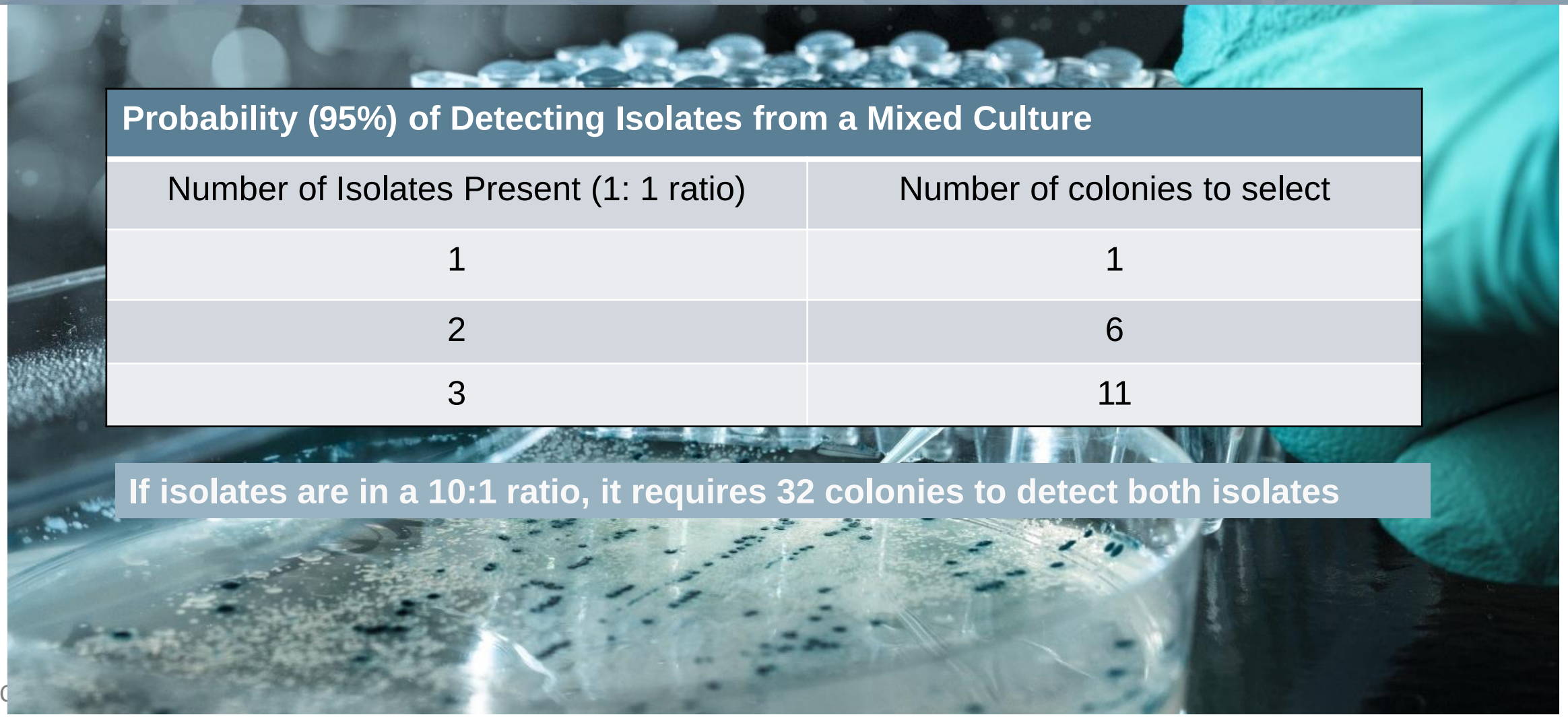
MLIA and XLT4 agar – 3 reps/treatment and 25 colonies/rep

BGS and XLD agar plates





Salmonella Serology



Probability (95%) of Detecting Isolates from a Mixed Culture	
Number of Isolates Present (1: 1 ratio)	Number of colonies to select
1	1
2	6
3	11

If isolates are in a 10:1 ratio, it requires 32 colonies to detect both isolates

Variation in labs using same procedure

Table 6. Reported technical deviations from the prescribed procedures

Lab code	BPW		RVS	MKTTn		MSRV	
	Incubation time (h:min)	pH	pH	pH	Novo-biocin	pH	Novo-biocin
ISO 6579-1	16–20 h	6.8–7.2	5.0–5.4	Complete 7.0-8.2 *	40 mg/l	5.1–5.4	10 mg/l
1	18:00	7.0	5.4	7.8	5	5.2	1
2	19:15	7.2	NO	NO	NO	5.2	10
3	18:30	7	5.2	6.6	40	NO	NO
4	19:30	7.1	5.4	8.0	1	NO	NO
5	18:30	7.2	NO	8	10	5.2	10
9	18:00	7	5.2	8	0.04	5.2	0.05
14	22:00	7.0	5.4	8.0	20	8.0	10
15	18:30	7.2	5.3	6.7	4	NO	NO
20	18:00	-	NO	-	40	-	10
21	17:10	7.1	5.2	8.2	4	5.2	10
26	20:00	7.0	NO	7.9	40	5.3	20
27	17:30	7	NO	-	-	5.2	10
31	21:00	7.3	5.4	7.8	0	NO	NO
35	22:00	7.1	7	7	5	NO	NO

Grey cells: Deviating from EN ISO 6579-1:2017
-: No information
NO: Did not use this selective enrichment medium (MKTTn or MSRV or RVS)

EURL Salmonella ring test

Table 11. Mean number of positive samples found by all participants with the artificially contaminated chicken feed samples

	Percentage positive samples	**Mean no. of positive samples/total no. of samples**
S. Mbandaka high level (SMb high)	50%	3.1/6
S. Mbandaka low level (SMb low)	5%	0.3/6

The Methodology/Serology Dilemma

We have to be careful and methodical when sampling, subsampling and use appropriate methods in order to find all Salmonella serotypes

People get sick; the processor says we didn't find that serotype, the farmer says we didn't see it, the feed miller says we didn't find that one!

CRISPR technology can identify multiple serotypes in a sample by looking at the genetic information present

Anitox and PDRC Ga. working together on Crispr project to identify all Salmonella serotypes in feed samples

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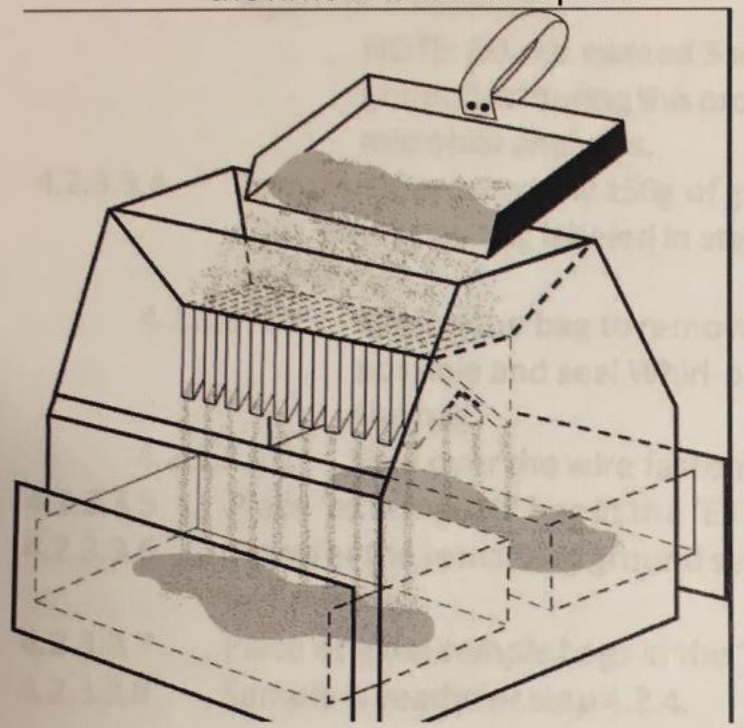
Thank you

Questions?

4.2.3.2.1 Using the Humboldt sample splitter, mix and divide the entire sample by passing the sample through the full width of the riffle into the feed pans.



4.2.3.2.2 Return the contents of both pans into the splitter three times.

- 4.2.3.2.2.1 Do not shovel the feed sample into the riffle
- 4.2.3.2.2.2 Do not dribble the feed into the riffle from a small mouth container
- 4.2.3.2.2.3 Do not allow the feed to build up in or above the riffle slots
- 4.2.3.2.2.4 If the feed does not flow freely through the slots, shake or vibrate the riffle to facilitate an even flow

4.2.3.2.3 Thoroughly mix the final sample obtained from the sample splitter.

Mill 3 results Davies & Wales 2010

M3	Intake pits & augers	44	Aga (2), Dur (3), Typ (1), New (2)	Weighers, blender	56	Aga (6), Kot (1), Bin (1)	Outloading gantries	52	Aga (4)
				Grinders	16	Aga (1)			
	Augers	56	Aga (2)	Augers/ elevators	8	Aga (1)	Augers	36	
	Bins/silos	12	Aga (10), Dur (2), Typ (2), Wag (1)	Pellet presses	8		Bins	40	Aga (1)
				Coolers	52	Aga (2)			
	Dust/waste	8		Fat coater, shaker	20	Giv (1)			