

Detección MÁS rápida y Confirmación y tipado de Salmonella

Montserrat Enjuto PhD,

22 de Noviembre de 2023, MRAMA, UAB

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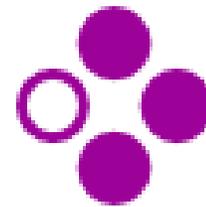
Agenda

Detección más rápida de Salmonella y Listeria:
DNA Diagnostic

Confirmación y tipado molecular de Salmonella más rápido:
Check&Trace

Otras marcas líderes: QIAGEN, NEB, INDICAL

DNA
DIAGNOSTIC



Check & Trace

DNA Diagnostic product line:



Mastit 4 Range

Detection of Mastitis Bacteria at farm level in milk

Lacti Range

Detection of bacterias at dairy level, with focus on thermoturics

Infant 3

Detection of Cronobacter spp., Enterobacteriaceae, and Salmonella spp., in infant formula-related products

Salmonella 4 cows – Milk

Detection of Salmonella in milk



Salmonella Velox

Detection of Salmonella in meat, pork, poultry, fish, seafood, swabs, environment/ primary production

Salmonella Velox SE+ST

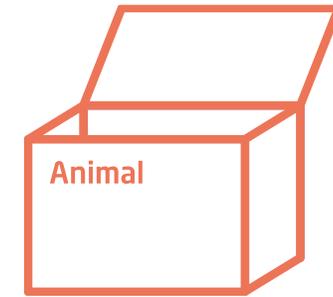
Detection of Salmonella Enteritidis and Typhimurium

Salmonella Velox Cocoa

Detection of Salmonella in cocoa and chocolate

Listeria Velox

Detection of Listeria spp./Monocytogenes in meat, pork, poultry, fish, seafood and swabs



Pneumo 4

Detection of viral and bacterial pathogens in calf pneumonia

ParaTB

Detection of paratuberculosis

Enterit 4 calves

Detection of pathogens causing diarrhea in calves

Enterit 4 swine

Detection of pathogens causing diarrhea in swine

Salmonella 4 cows

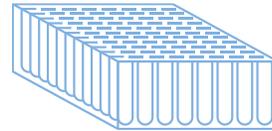
Detection of Salmonella Dublin and Salmonella spp., in cows

Todo incluido en el kit "All in one"



All reagents included

- ✓ DNA extraction
- ✓ Media
- ✓ qPCR mastermix
- ✓ Internal PCR Control



All consumables are included

excepts pipette tips

- ✓ 96 deepwell plates
- ✓ qPCR plates
- ✓ Lids
- ✓ Adhesive seals



qPCR mix are pre-aliquoted into wells

- ✓ Less risk of cross-contamination
- ✓ Time reducing

All the above must be considered when comparing price

Notice: Most competitors don't provide "all-in-one" packages

Kits para la industria alimentaria

	Salmonella Velox	Salmonella Velox SE+ST	Salmonella Velox Cocoa	Listeria Velox
Targets	<i>Salmonella spp.</i>	<i>Salmonella Enteritidis and Typhimurium</i>	<i>Salmonella spp.</i>	<i>Listeria spp./ Monocytogenes</i>
Usage	Meat, poultry, fishery, swabs, environment/ primary production (PPS)	Meat, poultry, fishery, swabs, environment/ primary production (PPS)	Cocoa containing products	Meat, poultry, fishery & swabs
Sample to result (Incl enrichment)	5.5 hrs. (9 hrs. PPS)	5.5 hrs. (9 hrs. PPS)	7h 15 min	20 hrs.
NordVal certification	X	X		X
!	Fastest test on market	Import into EU	Fastest test on market	

Salmonella Velox

Salmonella Velox

Fastest certified qPCR method on the market

Result in 5.5 hrs (9 hrs for primary production)

Salmonella Velox SE+ST

Serotyping of Salmonella Enteritidis and Typhimurium

High sensitivity

Detects 1 CFU in 25 g sample → No confirmation needed according to NordVal certificate

Salmonella 4 powder (Environment) & Salmonella 4 feces

NordVal certified

For testing raw or ready-to-eat meat, pork, poultry and fish/seafood, primary production samples and swabs



qPCR in Food Industry

Farm level

(Poultry, pork, meat, fish, seafood)



- Environment
(Swabs / Dust / Bedding)
- Feces
- Boot swabs



Processing plant



- Equipment
- Environment/hygiene
- Products (5.5 h)



Retail



- Finished products
- Ready-to-eat
- Ready-to-reheat



Why to use qPCR in Food Industry?

Farm level



- *Surveillance*
- *Prevention*
- *Hygiene control*
- *Animal welfare*

Processing plant



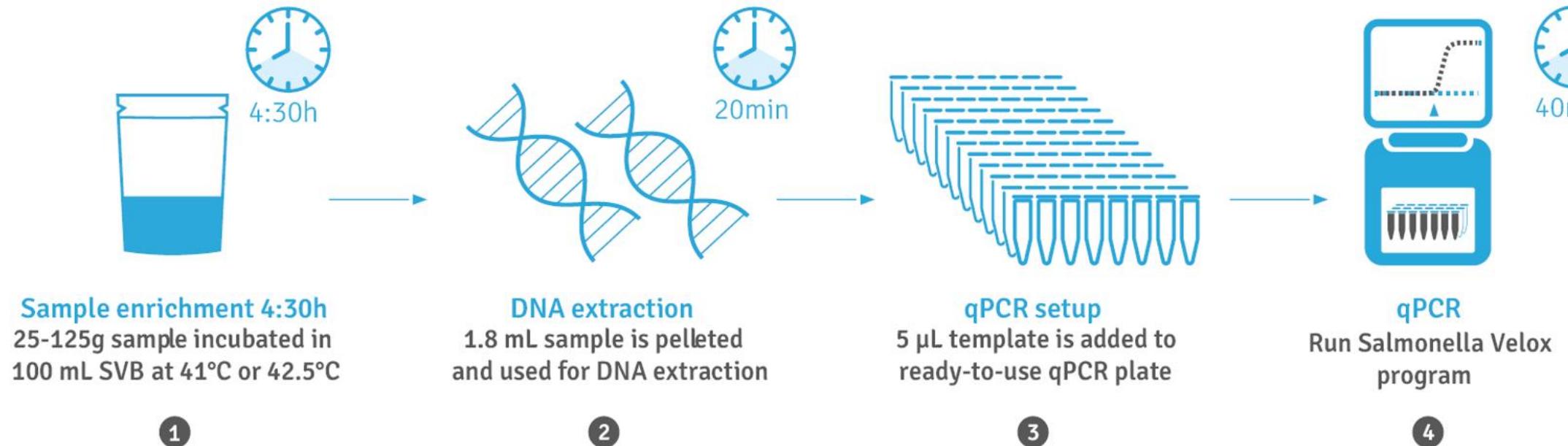
- *Surveillance*
- *Rapid decision making*
- *Environment control*
- *Reduce waste*

Retail



- *Certification*
- *Fast release*
 - ↓
Longer shelf-life
 - ↓
Minimize storage capacity
 - ↓
Reduce CO₂ emission

Easy and Fast Workflow



External Validation of Salmonella Velox – Internat Certification

	NMKL - NordVal International c/o Institute of Marine Research P.O. box 1870 Nordnes, N-5817 Bergen, Norway www.nmkl.org											
<h2>NordVal International Certificate</h2>												
<table border="1"><tr><td>Issued for:</td><td>Salmonella Velox</td></tr><tr><td>NordVal No:</td><td>046</td></tr><tr><td>First approval date:</td><td>7 June 2016</td></tr><tr><td>Renewal date:</td><td>11 August 2021</td></tr><tr><td>Valid until:</td><td>11 August 2023</td></tr></table>			Issued for:	Salmonella Velox	NordVal No:	046	First approval date:	7 June 2016	Renewal date:	11 August 2021	Valid until:	11 August 2023
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Renewal date:	11 August 2021											
Valid until:	11 August 2023											
Salmonella Velox spp. and Salmonella Velox SE + ST												
<u>Manufactured and supplied by:</u> DNA Diagnostic A/S Voldbjergvej 14 8240 Risskov Denmark												
<p>fulfils the requirements for NordVal Certification. The reference method was EN ISO 6579:2002: Microbiology of food and animal feeding stuffs – Horizontal method for the detection of <i>Salmonella</i> spp. For the matrices included in the study, the comparisons are also valid for the current reference method; EN ISO 6579:2017. AnalyTech Miljølaboratorium A/S, Denmark and ISI Food Protection ApS, Denmark have conducted validation studies according to ISO 16140-2. The interlaboratory study was carried out according to NordVal Protocol of 2009.</p>												
<p>Salmonella Velox spp. and S. Velox SE + ST are rapid qPCR tests that can be carried out within 5.5 hours, validated for raw meat, poultry and fishery products as well as ready-to-cook, ready-to-reheat and ready-to-eat meat, on swabs performed on raw food samples and in environmental and primary production samples.</p>												
<p>According to ISO 20838:2006, a method using a fluorescent DNA probe specific for a target gene sequence can be used without confirmation of the positive result itself. The production of the kits is fulfilling the requirements given in ISO 13485.</p>												

Salmonella Velox Cocoa

Samonella Velox cocoa

Sample to result in 7h 15 min, including enrichment

Faster decision-making, less hold-time in the production and storages



No need for secondary enrichment step

Test matrix - Not certified

All products containing cocoa and cocoa related products

Intermediate products and hygiene control

High sensitivity

Detects 1 CFU in 25 g sample

Listeria Velox

Listeria Velox

Result within a day

Results in only 20 hours (incl. enrichment)

Multiplex detection

Detects *Listeria* spp., and *Listeria Monocytogenes*

High sensitivity

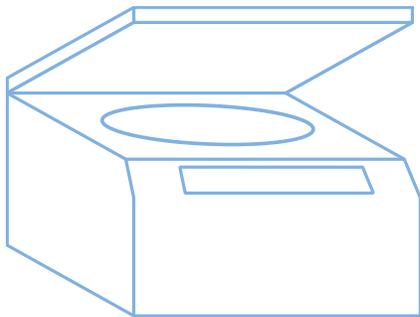
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NordVal certified

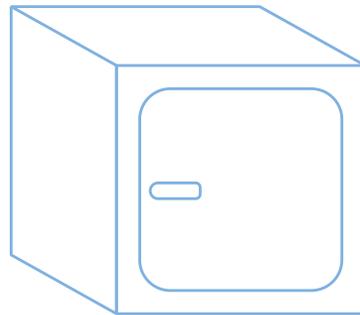
For testing raw or ready-to-eat meat, poultry, pork and fish/seafood and swabs



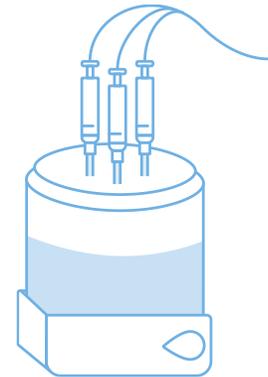
Laboratory equipment* (applicable for all DNA-D kits)



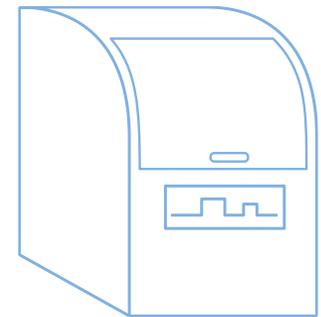
Centrifuge for deepwell plates, 5000 x g



Incubator



Vacuum system for deepwell plates

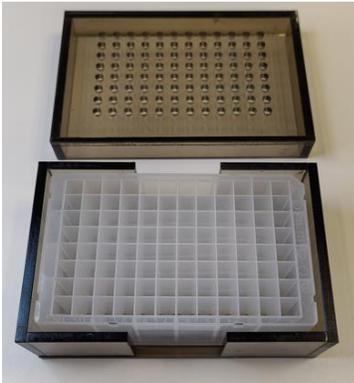
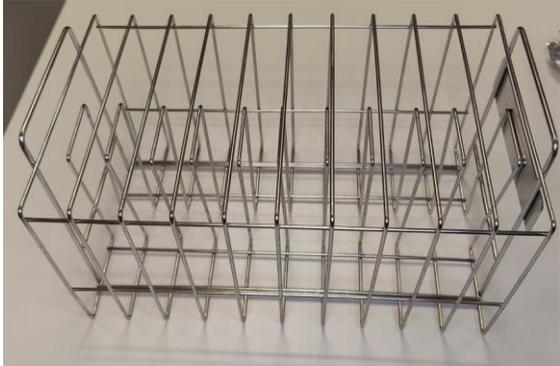
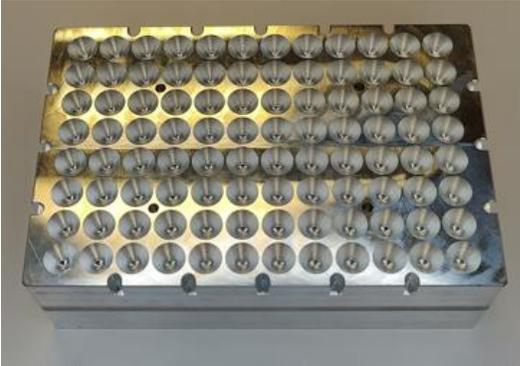


qPCR instrument
(2-3 filters depending on kits)

*Only shows major equipment, for all required equipment and materials see IFU's

Food range accessories

Only used for specific kits – See IFU or contact DNA Diagnostic



Cooling block for deepwell plates/qPCR*
Cat no: SV Block

Aspiration Assisting Box for deepwell plates
Cat no: AAB96

Rack for 5 media bags
Cat no: SV Rack

Why choose DNA Diagnostic

- ✓ **Short lead time**
Often within 2 weeks
- ✓ **Fast and reliable qPCR test kits**
Optimized for high-throughput
- ✓ **Ready-to-use kits**
All reagents and consumable included
- ✓ **Diverse range of kits**
Food, Animal, Leukemia, COVID19
- ✓ **Multiplex kits**
Detection of multiple pathogens in one reaction
- ✓ **Open platform**
No locked equipment needed
Any Real Time PCR
- ✓ **Rapid tests**
Sample to result within hours

The logo consists of four purple circles arranged in a cross pattern. The top, right, and bottom circles are solid purple, while the left circle is a white outline.

Check & Trace 2.0



Agenda

Detección más rápida de Salmonella y Listeria:
DNA Diagnostic

Confirmación y tipado molecular de Salmonella más rápido:
Check&Trace

DNA
DIAGNOSTIC



Check & Trace

Check & Trace Salmonella vs2.0

Ensayo cualitativo de RealTime PCR, q permite la confirmación y el tipado de presuntos aislados de Salmonella que provienen de medios de enriquecimiento. Es una alternativa rápida a los métodos convencionales de serotipado

Antecedentes

La información que nos da el serotipado de Salmonella, para la seguridad alimentaria, es poder detectar serotipos recurrentes de Salmonella, pudiendo trazar la contaminación, y detectar la fuente de contaminación. Esto nos permite el mapeo de las instalaciones, y nos permite tomar decisiones de impacto, de manera más rápida y más informada.

Es importante para la seguridad alimentaria y para el manejo operacional.



Previo Check & Trace Salmonella vs1

Plataforma de microarray de DNA que, por primera vez, permite serotipar el DNA de Salmonella diferenciando distintas secuencias. Está presente desde 2010 en grandes empresas de procesamiento de alimentos y productores de proteína de origen animal.

DNA MARKERS

The innovative Check&Trace Salmonella method detects and confirms Salmonella and discriminates over 300 serotypes. The serotype identification relies on molecular markers targeting different regions of the Salmonella genome. Even strains with incomplete antigenic formulation can be assigned to a serotype.

SAVE VALUABLE ANALYSIS TIME

No need for biochemical tests, Check&Trace Salmonella identifies and serotypes suspected Salmonella colonies from MSRV and selective agar samples.

RESULTS / SOFTWARE

The presence or absence of genetic markers creates a unique profile that is automatically interpreted by the software and assigned to a serotype.

EASY TO PERFORM

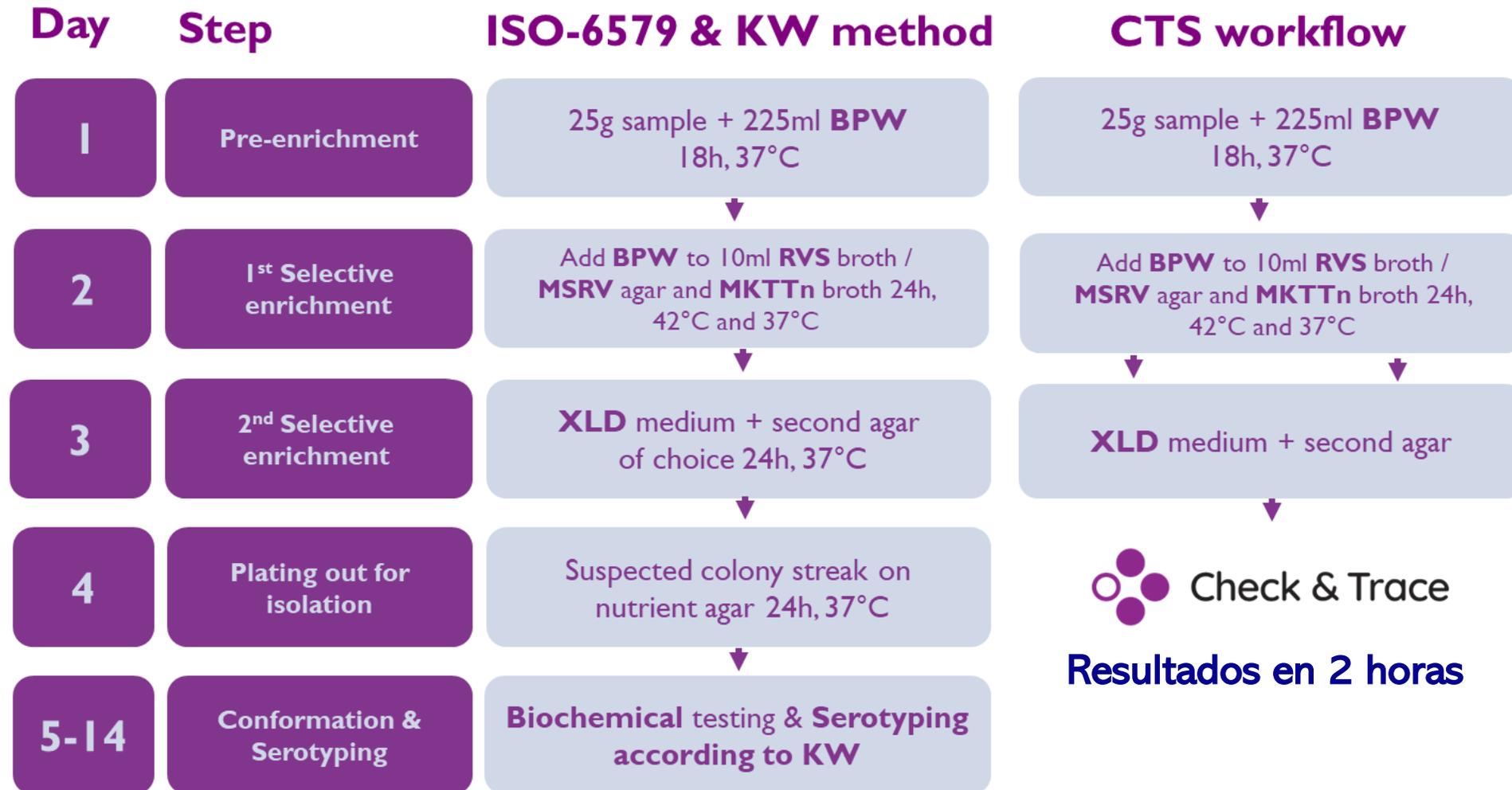
No previous expertise with molecular biology needed, easy to learn within one day.

Step	01	02	03	04	05
	Sample prep	Ligation	Endonuclease	Detection	Results
Equipment required	<ul style="list-style-type: none"> Heating block Vortex mixer 	<ul style="list-style-type: none"> Thermocycler Mini-centrifuge Flow cabinet 	<ul style="list-style-type: none"> Thermocycler 	<ul style="list-style-type: none"> Thermocycler with active cooling Vortex mixer Mini-centrifuge 	<ul style="list-style-type: none"> Check-Points Tube Reader + Software Computer with USB drive & Internet connection (Barcode reader)
Total time per step*	30 min	330 min (5.5 h)		1.5 h	5 min
Hands-on time per step*	15 min	2 x 15 min	15 min	3 x 15 min	5 min

* Subject to variation depending on the number of samples analyzed. Approx. time for 12 samples. Please contact info@checkandtrace.com for more information.



Check & Trace Salmonella vs2.0



Check & Trace Salmonella 2.0 - Serovares certificados

Abaetetuba	Choleraesuis	Havana	Molade	Paratyphi B. (Java)	Tennessee
Agona	Corvallis	Heidelberg	Montevideo	Poona	Thompson
Alachua	Cubana	Idikan	Muenchen	Pullorum	Typhimurium
Albany	Derby	Infantis	Muenster	Reading	Typhimurium monophasic (1,4,[5],12:i:-)
Anatum	Dublin	Javiana	Newport	Rissen	Uganda
Bovismorbificans	Enteritidis	Kentucky	Ohio	Saintpaul	Virchow
Braenderup	Gallinarum	Livingstone	Oranienburg	Sandiego	Worthington
Brandenburg	Give	London	Orion	Schwarzengrund	Yoruba
Bredeney	Goldcoast	Mbandaka	Ouakam	Senftenberg	4,[5],12:d:-
Cerro	Hadar	Minnesota	Panama	Stanley	



Se trata de una plataforma abierta, que ofrece la oportunidad de añadir más serotipos de Salmonella, según se requiera

Check & Trace Salmonella vs2.0

Statement:

Quick turnaround

Strict regulations, and rising market pressure, demand a fast testing solution

Allows rapid release of tested food products

State of the Art

CTS 2.0 is qPCR based detecting 27 target genes in parallel. Presence/absence of these markers generates a genetic fingerprint linked to the various *Salmonella* types, ensuring reliability

Plug & Play

The One-Two step workflow means a reduction in media prep time, minimizing hands-on time, and reducing human errors

Características Check & Trace vs2.0

SIMPLE y OBJETIVO

- Uses an uncomplicated sample preparation process minimizing the technician intervention
- Easy to learn
- Automated and objective result calling

ANALISIS CLOUD-BASED

- A suited cloud-based analysis software provides reliable results and securely store data

RAPIDO Y EXTENSO

- Up to 59 serovars are covered with one test
- Those with the most regulatory significance
- Fast turnaround time: less than 2 hours

CONFIRMACIÓN Y TIPADO

- De Salmonella a partir de colonias de bacterias en medio agar: NA o XLD

Check & Trace Salmonella vs2.0

Kauffman White

- Depends on phenotypical expressions of antigens: $\pm 10\%$ untypeable isolates
- Results in from 2 up to 5+ days
- Subjective results
- Expertise required (difficult to learn)
- Different tests for different serovars
- False positive reactions may occur as a result of a weak, nonspecific agglutination
- It requires the use of over 150 specific antisera with variable quality between providers

Check&Trace Salmonella

- Based on DNA markers, providing higher sensitivity/specificity: no untypeable isolates
- Results in hours from a pure culture
- Objective software analysis
- Automated and objective results calling
- One test for 59 serovars
- Easy to learn
- Low Hands-on time



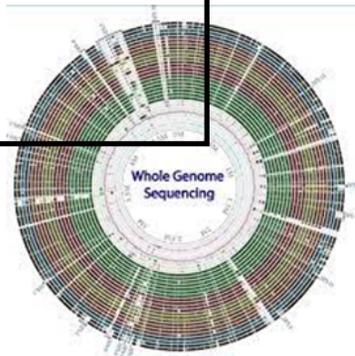
Molecular profiling to identify and differentiate Salm serotypes is more consistent, reliable and traceable than the classical method K&W

30

Check & Trace Salmonella vs2.0

WGS

- WGS (sequencing) for typing is Time Consuming
- 1-2 days depending of technology
- Accreditation may be difficult to achieve
- Labour Intensive
- Expensive: high capital investment and reagents costs

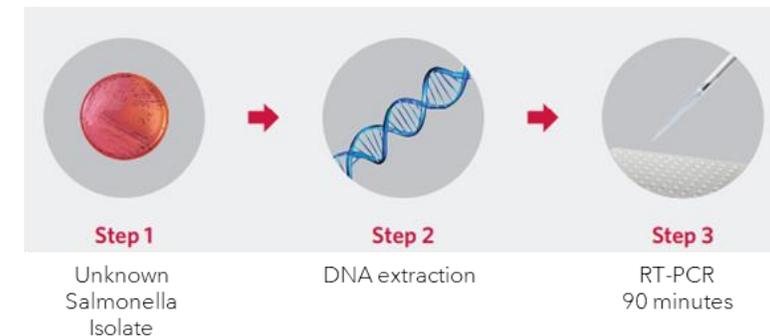


Check&Trace Salmonella

- Results in hours from a pure culture
- Automated and objective results calling
- Simple workflow
- Easy to learn
- Low Hands-on time
- No investment necessary if RT-PCR equipment available

Flujo de trabajo del Check & Trace vs2.0

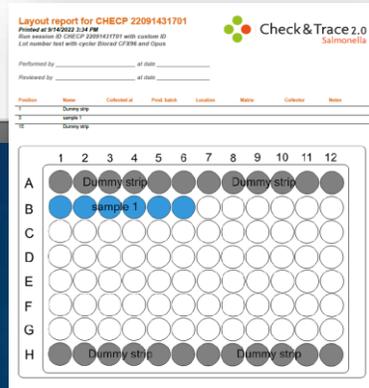
- Prepare work list in "CTS 2.0 Analysis Portal"
- Simple DNA extraction
- Mix DNA with Mastermix
- Aliquot DNA/Mastermix in provided strips and run experiment
- Upload CFX results file in Check-Points' Portal
- Automated results in few minutes



Flujo de trabajo paso a paso del C&T vs2.0

Log in to the Check & Trace Analysis Portal online: analysis.check-points.com

- Generate a worklist and run session
- Print out worklist for sample preparation and PCR run in the lab

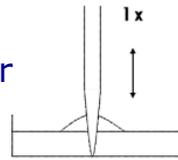


1 Sample prep/extraction

A) Aliquot 200 μ L Sample Buffer into a microcentrifuge tube



B) Pick a bacterial colony using the colony sampler

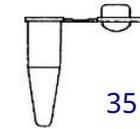


C) Resuspend into the 200 μ L of Sample Buffer and boil for 10 minutes



2 Amplification (Prep and run PCR)

A) Aliquot 35 μ L of the PCR master mix into the PCR tube



35 μ L PCR master mix

B) Add 140 μ L of the bacterial lysate into the PCR tube and mix with the pipette



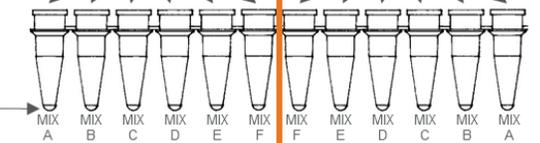
Add 140 μ L bacterial lysate

C) Take 25 μ L of master mix/sample and pipetted into the PCR strip, well A-F



D) Run the PCR

Specific primers and probes
MIX A, B, C, D, E, and F



Check & Trace Salmonella 2.0 workflow

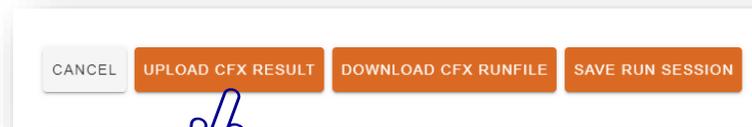
Log in to the Check & Trace Salmonella Analysis Portal online:
analysis.check-points.com

- Enter the verification code received by email when the message "Login verification required" appears.

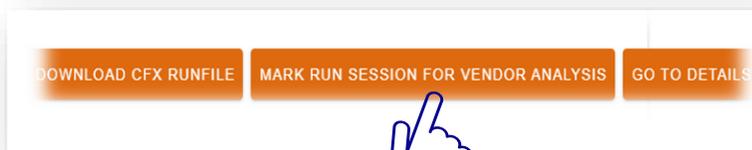


3 Detection (Data processing and analysis)

A) Select the run session of the experiment and click the "Upload CFX Result file" tab to upload the .pcrd file containing the corresponding PCR run data.



B) Click the "Mark run session for vendor analysis" tab to start the analysis.



C) Results pop up after 3 minutes and can easily be downloaded (MS Word/PDF) or exported (CSF/Excel) via the Reporting tab.



Check & Trace Salmonella 2.0 workflow

Results reporting



DETAILS ANALYSIS REPORTING MESSAGES

	1	2	3	4	5	6	7	8	9	10	11	12
A	Grey	Dummy strip	Grey	Grey	Grey	Grey	Grey	Dummy strip	Grey	Grey	Grey	Grey
B	Green	Sample 1	Green	Green	Green	Green	Green	Sample 2	Green	Green	Green	Green
C	Red	Sample 3	Red	Red	Red	Red	Red	Sample 4	Red	Red	Red	Red
D	Red	Sample 5	Red	Red	Red	Red	Red	Sample 6	Red	Red	Red	Red
E	Red	Sample 7	Red	Red	Red	Red	Red	Sample 8	Red	Red	Red	Red
F	White	White	White	White	White	White	White	White	White	White	White	White
G	White	White	White	White	White	White	White	White	White	White	White	White
H	Grey	Dummy strip	Grey	Grey	Grey	Grey	Grey	Dummy strip	Grey	Grey	Grey	Grey

- #3 Sample 1 - No salmonella
- #4 Sample 2 - No salmonella
- #5 Sample 3 - Salmonella Enteritidis
- #6 Sample 4 - Salmonella Senftenberg
- #7 Sample 5 - Salmonella Typhimurium
- #8 Sample 6 - Salmonella Typhimurium monopha
- #9 Sample 7 - Salmonella Schwarzengrund
- #10 Sample 8 - Salmonella Heidelberg

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Aprobaciones/certificados de C&T Salmonella vs2.0



- ✓ **Certified by Microval** for confirmation and typing of *Salmonella*.
- ✓ Check&Trace Salmonella 2.0 has been successfully validated according to ISO 16140/6 protocol for 59 unique serotypes and can be used by any laboratory as alternative for ISO 6579-1 and ISO 6579-3, for Salmonella confirmation and typing.



- ✓ **AOAC certified:** as determined by the inclusivity study, the Check&Trace Salmonella 2.0 was proven to be effective at detecting Salmonella species and typing 59 serovars of Salmonella
- ✓ **License number:** 072301

Aprobaciones/certificados de C&T Salmonella vs2.0

Check & Trace® 2.0 Salmonella

RT-PCR para la confirmación y tipificación de cepas aisladas de Salmonella procedentes de muestras de producción primaria y calidad ambiental. Uso veterinario.

HCMR-0216
Nº de Reg. Pendiente de asignación

werfen

Werfen España, S.A.U.
Plaza de Europa, 21-23
08908 L'Hospitalet de Llobregat (Barcelona)

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HCMR-0216



✓ **Ministerio de Agricultura Pesca y Alimentación**

✓ autorización para aves de corral y muestras ambientales de producción primaria

✓ Registro de Prod Zoosanitarios. Nº Trámite: 23-0536. Autorización Producto RD. Check & Trace Salmonella 2.0



Rápido y accionable



Equipos Bio-Rad CFX96 / Opus96 (*)



(*) for now, more RT-PCR platforms in development



Check & Trace Salmonella 2.0 - Serovares certificados

Abaetetuba	Choleraesuis	Havana	Molade	Paratyphi B. (Java)	Tennessee
Agona	Corvallis	Heidelberg	Montevideo	Poona	Thompson
Alachua	Cubana	Idikan	Muenchen	Pullorum	Typhimurium
Albany	Derby	Infantis	Muenster	Reading	Typhimurium monophasic (1,4,[5],12:i:-)
Anatum	Dublin	Javiana	Newport	Rissen	Uganda
Bovismorbificans	Enteritidis	Kentucky	Ohio	Saintpaul	Virchow
Braenderup	Gallinarum	Livingstone	Oranienburg	Sandiego	Worthington
Brandenburg	Give	London	Orion	Schwarzengrund	Yoruba
Bredeney	Goldcoast	Mbandaka	Ouakam	Senftenberg	4,[5],12:d:-
Cerro	Hadar	Minnesota	Panama	Stanley	



Se trata de una plataforma abierta, que ofrece la oportunidad de añadir más serotipos de Salmonella, según se requiera

Thanks
Gracias

werfen

Powering Patient Care