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Manual

Salm-SeroGenoTyping AS-1 Kit

Array Hybridisation Assay for DNA-based serogenotyping and detection of resistance genes of *Salmonella* and assignment of unknown *Salmonella* isolates to known strains

Kit order number: 245700096

96 reactions (ArrayStrip format)

For Research Use Only. Not Intended for Use in Clinical Diagnostics.

CONTENT

BACKGROUND

The Alere SeroGenoTyping AS-1 Kit for *Salmonella* allows DNA-based serogenotyping according to the Kauffmann-White scheme and an assignment of unknown *Salmonella* isolates to known strains as well as, simultaneously, the detection of resistance genes of *Salmonella*.

RNA-free, unfragmented genomic DNA from pure and monoclonal *Salmonella* culture material is amplified approximately 50-fold and internally labelled with biotin-11-dUTP using a linear amplification protocol. In contrast to standard PCR, only one antisense primer per target is used resulting in single stranded (ss) DNA reaction products. This allows a simultaneous sequence specific labelling and amplification of an essentially unlimited number of targets. However, sensitivity is lower than in a standard PCR (whereas contamination with undesired amplicons is nearly impossible) and for that reason the method is restricted to clonal culture material and cannot be performed on samples such as swabs or other patient samples (e.g. faeces). Resulting biotin labelled ssDNA is transferred and hybridised to DNA oligonucleotide microarrays with 329 covalently immobilised probes for different genetic markers and a biotin staining control. All of them are printed in two duplicate spots.

The target set consists of a variety of species and serotyping markers including genes encoding 28 O-antigens and 86 H-antigens (H1 and H2). Additionally, 77 targets analysing antimicrobial resistance (AMR) genes were included (Braun *et al.* 2012).

Spot recognition is performed automatically based on a digital image of the arrays. The overall pattern is analysed automatically for the presence or absence of specific genes and it is compared to a database of strain profiles. This allows the assignment of the serovar, the antigenic formula to Kauffmann-White and the AMR profile.

GENERAL INSTRUCTIONS FOR USE

Intended Use

For Research Use Only. Not Intended for Use in Clinical Diagnostics.

This kit allows genotypic characterisation of bacterial cultures from *Salmonella* isolates for research and epidemiological applications. It must not be used as a substitute for phenotypic susceptibility tests and for the guidance of antibiotic therapy. It cannot be used for bacteria other than *Salmonella.*

Specifications

Upon receipt, the kit components need to be stored at different temperatures as specified on the package insert. The kit is to be performed at an ambient temperature of 18-28 °C.

Technical Support

If you require any further information on this product please contact:

email: cct.home@clondiag.com

phone: +49 (0) 36 41 3111-155

Fax: + 49 (0) 36 41 3111-120

For up-to-date information regarding the kit, please visit our website at

http://www.alere-technologies.com

Safety Precautions

The kit is intended for use by personnel that are trained in microbiological and molecular methods. Preparation of DNA from pure *Salmonella* colonies (clones) requires expertise in

microbiology and the local regulations for handling of pathogenic microorganisms (biosafety level 2) are to be obeyed.

Isolated, cell-free *Salmonella* DNA may be processed without further biosafety precautions, although contamination with *Salmonella* or other bacteria needs to be ruled out.

Always wear protective clothing as required for laboratory work according to your local regulations.

Material Safety Data Sheets (MSDS)

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)] and the latest amendments to the European Union Directives 67/548/EC and 1999/45/EC, the enclosed reagents do not require a Material Safety Data Sheet (MSDS). They do not contain more than 1% of a component classified as hazardous and do not contain more than 0.1 % of a component classified as carcinogenic. MSDS therefore are not provided. Nevertheless, the buffers may cause irritation if they come into contact with eyes or skin, and may cause harm if swallowed. The regular precautions associated with laboratory work should be obeyed (e.g. wear protective goggles, gloves and lab coat and avoid contact with the reagents). In case, any liquids are spilled, clean with disinfectant and/or laboratory detergent and water.

Alere assumes no liability for damage resulting from handling or contact with these products. If you have any questions please contact our Technical Support (see above).

Shipping Precautions

RID/ADR: *Kein Gefahrgut* / No dangerous goods
IMDG: No dangerous goods
IATA: No dangerous goods

REAGENTS AND DEVICES

Assay Components, Storage and Stability

All reagents are provided in surplus (see below). If necessary, all components may also be ordered separately; please refer to the order numbers at the end of this manual. For pricing please contact your local representative or our customer service, respectively.

The expiry date can be found on each bottle and on the outer packaging. All components were tested for stability for short term shipment (<1 week) at ambient temperature (< 37 °C). The kit components with limited stability are D1 and C3. The other components proved to be stable even six months after passing the kit expiry date.

Cell Lysis (optional order)

- A1: Lysis Buffer (cat# 245101000)
  Store at 18-28 °C (ambient temperature). Surplus: 50 %.
- A2: Lysis Enhancer (lyophilised, cat# 245102000)
  Store at 18-28 °C (ambient temperature). Centrifuge A2 tubes shortly prior to opening. Add 200 µl Buffer A1 to Lysis Enhancer before use. Mix well and store for less than 1 week at 2-8 °C. Sufficient for 96 isolations.

DNA Labelling and Amplification

- $B1^{+}$: Labelling Buffer, Store at 2-8 °C. Surplus: 40 %.
- B2: Labelling Enzyme, Store at 2-8 °C. Surplus: 100 %.
- $B3^{Salm}$: lyophilised Labelling Primermix, three tubes,
  dilute each in 70 µl molecular grade water. Store at -20 °C. Surplus: 100%.

Hybridisation and Detection

- ArrayStrips (12 x 8 samples),

  Protected against light and sealed under inert gas. Store at 18-28 °C. After opening to be used within two weeks. Close the unused wells with caps to protect against humidity and dust, and store in the dark. *Avoid any touching or scratching of the microarray surface at the bottom of the well. Do not store or handle unused wells at more than 60 % relative humidity since this may irreversibly corrode the spots.*

- StripCaps (24 units)

- C1: Hybridisation Buffer

  Store at 18-28 °C, protect against sunlight. Surplus: 150 %.

- C2: Washing Buffer 1

  Store at 18-28 °C, protect against direct sunlight. Surplus: 200 %.

- C3: HRP Conjugate 100 x

  Store at 2-8 °C, protect against direct sunlight. Surplus: 100 %.

- C4: Conjugate Buffer

  Store at 18-28 °C, protect against direct sunlight. Surplus: 200 %.

- C5: Washing Buffer 2

  Store at 18-28 °C, protect against direct sunlight. Surplus: 200 %.

- D1: Horseradish Peroxidase Substrate

  Store at 2-8 °C, protect against direct sunlight. Surplus: 50 %.

- **optiona**l: CM^{LT2}: Reference DNA from *S.e.e.* Typhimurium LT2 (GenBank accession number NC_003197.1), c_{DNA} = 0.1-0.4 µg/µl. Store at 2-8 °C. Sufficient for 5-6 tests.

Instrumentation and Software

- ArrayMate Reader (to be ordered separately, for details see below)

  The Salm-SeroGenoTyping AS-1 Kit may be used on the ArrayMate reader only. The alternative devices ATR01/03 are not suitable for reading ArrayStrip based assays. In case of any questions please contact us.

- Iconoclust software (provided with the reader)

- Test specific software plug-in (can be downloaded from Alere website, check periodically for updates, for details see below). Information (such as spot names, marker names, location of the spots on the array, size of the image taken by the reader's specific camera) is delivered with the reader or can be downloaded from our website. These test specific plug-ins will occasionally be updated. Please check the NEWS section of our website http://www.clondiag.com. Support is available under cct.home@clondiag.com.

Components required but not provided

- Growth media for the cultivation of *Salmonella.* The test should be performed with colonies harvested from 2 x TY Agar. Other rich media (e.g. Standard 1 or LB) may also suffice, but have not systematically been tested. Liquid media should also not been used because contaminations or mixed cultures cannot easily be ruled out.

- Equipment and consumables needed for the cultivation of *Salmonella* (incubator, inoculation loops, Petri dishes)

- DNA preparation kits:
  The kit was tested with the DNeasy Blood & Tissue Kit from Qiagen (cat# 69504) and High Pure DNA Isolation Kit from Roche (cat# 11796828001).

  Please note: *The DNA specimen needs to be free of RNA. Recommendation: a pre-treatment with the cell lysis components A1 / A2 (see below) or a standard RNase A treatment while DNA preparation.*

- Equipment needed for DNA isolation, e.g. pipettes, centrifuge, thermoshaker or automated device (see above)
- Photometer ($OD_{260\ nm}$) for measuring the concentration of DNA
- Equipment for non denaturing agarose DNA gel electrophoresis for quality control of DNA
- Thermocycler for PCR
- Thermoshaker

  We strongly recommend the BioShake iQ by Quantifoil Instruments (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayStrips. Alternatively, you may use Eppendorf's Thermomixer Comfort, equipped a heating block for microtitre plates.
- Pipettes: suitable for 1-5 µl volumes, 90 µl, 100 µl, 200 µl, 1000 µl
- Multichannel pipettes for 100-200 µl
- Reaction vials suitable for PCR
- Ultrapure (PCR grade) water
- RNAse A (we recommend Qiagen's RNase A solution, 100 mg / mL, Qiagen, cat# 19101).
- Pasteur pipettes (VWR, cat# 612-2856).

PROTOCOL

Culturing and Harvesting Bacterial Cells

Serovars of the genus *Salmonella* are potential pathogens. All procedures for cultivation of the bacterium and DNA preparation need to be performed by properly trained staff in a biosafety level 2 facility.

Grow *Salmonella* on 2 x TY agar (overnight at 37 °C or 48 hrs at room temperature). Obtain confirmation of the identification as *Salmonella* (EN ISO 6579:2002/Amd 1:2007, Anonymous 2007) and make sure that you have a pure, monoclonal culture of *Salmonella*. Contamination with other bacteria, especially with other *Enterobacteriaceae*, needs to be strictly avoided as they might carry the same resistance genes as certain *Salmonella* strains and thus can introduce false positive signals and patterns.

Extraction of DNA

The required sample quantity is 0.5-2 µg (c_{DNA} = 0.1-0.4 µg / µl) of intact genomic DNA from a **single clone**.

The DNA specimen needs to be free of RNA and it should not be fragmented.

This can be determined by agarose gel electrophoresis. DNA should not be prepared by disrupting *Salmonella* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. Most performance problems with the *Salmonella* Serogenotyping kit are due to insufficient amounts or quality of DNA preparation. We therefore strongly recommend to obey the protocols outlined below.

DNA Extraction by spin columns (e.g. Qiagen)

- Add an inoculating loop full of monoclonal colony material of the *Salmonella* isolate to 0.2 ml 1 x PBS and vortex thoroughly.

loop empty loop full

It is important to harvest enough bacteria; this is prerequisite for extraction of a sufficient amount of DNA.

Take an inoculating loop 1 mm in diameter filled with bacteria as shown in the righthand picture.

Optional cell lysis with A1 / A2 reagent (instead of 1 x PBS):

- *Centrifuge A2 tube shortly, open it, add 0.2 ml of Lysis Buffer A1 to Lysis Enhancer A2 and dissolve.*
- *Add an inoculating loop full of monoclonal colony material of the Salmonella isolate to A1 / A2 reagent and vortex thoroughly.*
- *Incubate the colony material of the Salmonella isolate in A1 / A2 for 30-60 min at 37 °C and 550 rpm in the thermoshaker.*

- Proceed with DNA preparation protocol of the DNA preparation kit. For the Qiagen DNeasy Blood & Tissue Kit it is as follows:
- Add 20 µl proteinase K (Qiagen Kit, or equivalent) and add 200 µl buffer AL (Qiagen Kit).
- Vortex briefly or shake vigorously.
- Incubate for 30-60 min at 56 °C and 550 rpm in the thermoshaker.
- **Important**: If A1 / A2 reagent not used, add 4 µl RNase A (100 mg / ml), mix by vortexing, and incubate for 2 min at room temperature before continuing.
- Add 200 µl ethanol (96-100 %).
- Vortex the sample and centrifuge (quick spin).
- Transfer the complete content of the tube (including any precipitate) into a spin column

that is placed in a 2 ml collection tube.

- Centrifuge at room temperature, time and speed need to be determined depending on viscosity of the sample and type of centrifuge used. All liquid should be collected in the collection tube afterwards.
- Discard collection tubes with liquid.
- Place the spin column in a new 2 ml collection tube (provided with the kit).
- Add 500 µl Buffer AW1.
- Centrifuge (8,000 rpm, 1 min) at room temperature.
- Discard collection tubes with liquid.
- Place the spin column in a new 2 ml collection tube (provided with the kit).
- Add 500 µl Buffer AW2.
- Centrifuge (14,000 rpm, 3 min) at room temperature, the membrane of the spin column should be dry, and all liquid should be in the collection tube.
- Discard collection tube with liquids.
- Place the spin column in a clean 1.5 ml tube (provided with the kit).
- Add 50 µl Buffer AE (or PCR grade distilled water) directly onto the membrane of the spin column.
- Incubate at room temperature for 1 min to elute DNA.
- Centrifuge (8,000 rpm, 1 min) at room temperature.
- Optional: add another 50 µl Buffer AE (or PCR grade distilled water) directly onto the membrane, incubate at room temperature for 1 min and centrifuge again.
- Discard the spin column.

***Please note**: Ethanol from Washing Buffers strongly inhibits the enzymes used in the assay.*

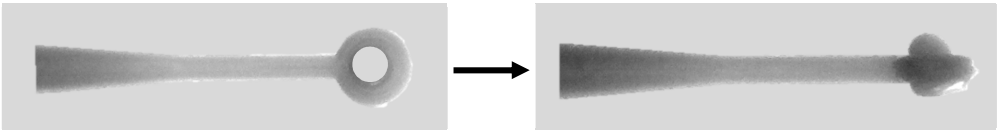
Contamination with Washing Buffer might occur during elution of prepared DNA by droplets adhering to the funnel of the spin columns. Thus these funnels should be gently touched and dried with sterile filter paper or wipes prior to the elution step. Alternatively, prepared DNA can be heated to evaporate ethanol (e.g. 10 min at 70 °C).

- Check for DNA integrity and absence of RNA (e.g. agarose gel). If necessary, you might perform another digestion step with additional RNase A (not provided). Measure DNA concentration (A_{260} method), it should not be less than 0.1 µg / µl. The concentration might be increased by heating and evaporation of water, or by using a speed vac centrifuge (not recommended when the same preparation shall be used in PCR experiments).

DNA Extraction by Heat Lysis

*Please Note: Only a **fresh** overnight culture can be used. After DNA extraction by heat lysis the linear amplification must be done **immediately**. Storage of extracted DNA is not recommended.*

- Add a 1 µl inoculating loop (*Please Note: do not use too much culture material, see figure below*) of a monoclonal *Salmonella* isolate to 50 µl PCR-grade distilled water and vortex thoroughly.



- Incubate at 99 °C, 15 min at 550 rpm in a thermoshaker.
- Centrifuge for 5 min at 13,600 rpm at room temperature.
- Carefully pipette 25 µl supernatant into a new 1.5 ml tube and discard the old tube with the pellet.
- Add 0.25 µl RNase A (not provided / see above) with a stock concentration of 1 mg / ml.
- Incubate at 37 °C, 5 min at 550 rpm in a thermoshaker.

- Use 5 µl of this DNA suspension for the linear amplification and internal biotin labelling process.

Linear Amplification and Internal Biotin Labelling

Please keep in mind the limited surplus of reagents whilst pipetting. The surplus of $B1^+$ labelling reagent is 40 %.

- Prepare a Master Mix by combining 3.9 µL of $B1^+$ labelling reagent, 1 µl $B3^{Salm}$ Labelling Primermix and 0.1 µl of B2 (DNA polymerase) per sample.
- Add 5 µl of *Salmonella* DNA (c_{DNA} = 0.1-0.4 µg/µl) prepared as described above to 5 µl of the Master Mix ($B1^+$/B2/$B3^{Salm}$). Do **not forget** to label the vial!
- Perform amplification in a pre-programmed thermocycler (e.g. Eppendorf Mastercycler gradient with heated lid, VWR, cat# 460-0108) according to the following protocol:

Pre-heat cover / lid to 105 °C	
300 sec at 96 °C	
50 cycles with	20 sec at 50 °C
	40 sec at 72 °C
	60 sec at 96 °C
Cool down to 4 °C, hold	

- The samples can be stored frozen until usage.

Please note: When using another device, some adaptations might be necessary. Before starting routine use, please test the protocol with a few known reference strains.

Hybridisation

General Remarks - Handling of Arrays

Never touch the array surface!

Avoid complete drying of the array surface during processing!

Do not allow it to stay without liquid for more than two minutes!

Never rinse the wells with distilled water after the hybridisation step, use only C2 Washing Buffer!

Unused wells should be capped during the whole procedure. The strips may be processed up to three times without a loss of quality of properly capped unused arrays. Close all wells that will not be used with a cap and leave it there until you use these wells (for storage conditions after use: see section “Kit Components, Storage and Stability / Hybridisation and Detection”).

Always label your ArrayStrips with a laboratory marker at the recommended position. **Never** label them on the bottom or across the data matrix barcode! This may cause errors.

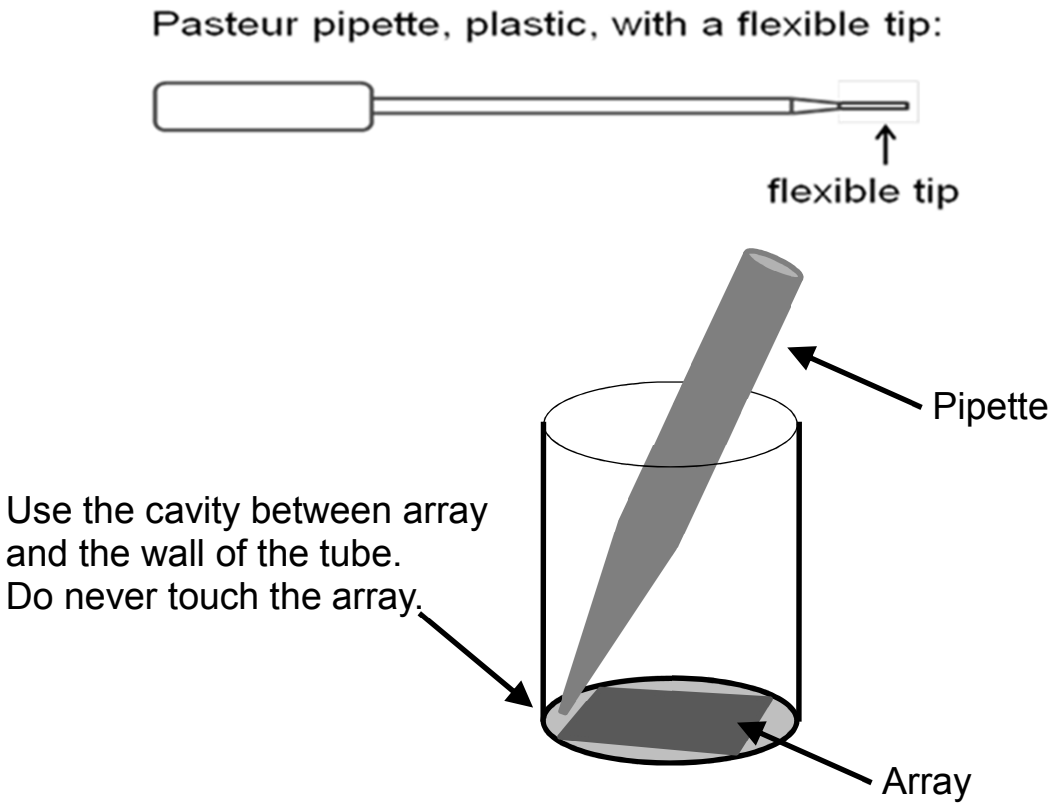
Avoid contact of data matrix code with organic solvents! The ArrayMate needs the information encoded in the data matrix to perform the assay and the analysis afterwards.

Avoid touching the bottom of the microarray strip and keep it clean.

General Remarks - Handling of Liquids

We recommend the use of a multichannel pipette and reagent reservoirs.

We strongly recommend that the liquid is removed by pipetting rather than by inverting the strips and flicking the liquids out. Fine tipped soft, disposable Pasteur pipettes are best suited (such as VWR, cat# 612-2856). Always place the pipette tip at the cavity between the array and the wall of the reagent well. If you touch the array surface, probes may be scratched off and this may cause an error.



General Remarks - The Substrate (Precipitating Dye) D1

It is recommended to fill an appropriate amount of substrate (precipitating dye D1) into a reaction tube and taken out of the refrigerator when starting the procedure allowing it to acclimatise it to room temperature / 25 °C. Cold D1 may yield weak signals. D1 should be centrifuged (quick spin) prior to use to remove bubbles as well as possible precipitates.

Triggered by peroxidase, the dye precipitates in the case of positive reactions, but it is not covalently bound. The precipitate can be dissolved by vigorous shaking. Thus, the arrays must **not be shaken**, dropped or moved abruptly during the staining procedure or thereafter.

After completion of staining, remove and discard reagent D1 as completely as possible and scan immediately (ArrayMate). The dye precipitate fades slowly in presence of liquids.

General Remarks - Thermoshakers

The correct temperature within the vessels is essential; therefore always use appropriate equipment for heating. Because of the possibility of inhomogeneous distribution of temperature within the heating block, as well as possible differences between displayed and actual temperatures, the use of different brands of thermoshakers might affect test performance. We tested the kit with BioShake iQ by Quantifoil Instruments, see figure below (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayStrips (recommended) and Eppendorf's Thermomixer Comfort, equipped with a heating block for microtitre plates. When using other devices, some modifications to the protocol might be necessary. Before starting routine use, please test the protocol with a few known reference strains or the control DNA CM^{LT2} (*S.e.e.* serovar Typhimurium LT2).

BioShake iQ by Quantifoil Instruments equipped with a customised heating block designed to fit ArrayStrips (http://www.qinstruments.com/).

Protocol for Quantifoil's BioShake iQ and Eppendorf's Thermomixer Comfort with microtitre plate adapter

- Switch on the thermoshaker and pre-heat it to 55 °C.
- Remove the amount of ArrayStrip(s) needed from the pouch.
- Insert the ArrayStrip(s) into the white frame. Ensure the correct orientation (data matrix code close to row (A) and proper fit.
- Pre-wash the array in two steps:
    - First, PCR-grade distilled water, 200 µl per well at 55 °C, 5 min and 550 rpm. Remove the water from the well.
    - Second, C1 Hybridisation Buffer, 150 µl per well at 55 °C, 5 min and 550 rpm
- Add 90 µl of buffer C1 to each tube with 10 µl labelled amplification product, mix gently.
- Remove the buffer from the well with the array and add the mixture of C1 and labelled amplification product.
- Incubate at 55 °C, 60 min and 550 rpm.

- **Meanwhile, log on to the ArrayMate device and prepare your worklist (see section "Data Analysis" p. 17)**
- Remove liquid and add 200 µl C2 Washing Buffer. Incubate at 45 °C, 5 min and 550 rpm, remove and discard.
- Add another 200 µl C2 Washing Buffer. Incubate at 45 °C, 5 min and 550 rpm, remove and discard.
- Meanwhile, prepare the conjugate: For each experiment, add 1 µl C3 HRP conjugate to 99 µl of C4 Conjugation Buffer. This mixture is stable for around one working day at room temperature; C3 is delivered with a surplus of 100 %, C4 with a surplus of 200 %.

  Suggested pipetting scheme:

	1 well	2-3 wells	4-6 wells	7-10 wells	11-15 wells	16-20 wells	21-30 wells	31-40 wells
C3	1.5 µl	3.5 µl	7 µl	11 µl	16 µl	21 µl	32 µl	42 µl
C4	150 µl	350 µl	700 µl	1100 µl	1600 µl	2100 µl	3200 µl	4200 µl

- Remove and discard the Washing Buffer, and add 100 µl diluted conjugate C3 / C4 to each well, incubate at 30 °C, 10 min and 550 rpm.
- Remove liquid and wash with 200 µl C5 Washing Buffer, just pipette up and down once, remove and discard.
- Add another 200 µl C5 Washing Buffer. Incubate at 30 °C, 2 min and 550rpm.
- Remove and discard Washing Buffer, add 100 µl of D1 substrate (precipitating dye, at 25 °C, see above) per well.
- Incubate at 25 °C, 10 min *but* ***do not shake!***
- Remove liquid completely.
- The outside of the bottom of the ArrayStrips may be carefully cleaned with wipes.
- Scan and process (ArrayMate, see below).

Data Analysis

Starting the ArrayMate Reader

We recommend to start the ArrayMate Reader after starting the hybridisation; this allows the convenience of starting the device and importing the worklist file.

Please note: This is a short instruction only. For more detailed information please refer to the ArrayMate User Manual.

- Switch on the ArrayMate (1st: main switch on the rear below the electric cable plug, 2nd: operating switch on the bottom left corner of the front side).
- Switch on the screen (switch righthand side below the screen).
- Log on as **R&D User** (Research and Development User) for full access to test specific software (a default password will be provided together with the ArrayMate device). If you log on as **User**, you will obtain raw values only, but neither interpretation of positives / negatives nor strain assignment. The **Administrator** log on will allow the installation of a new assay specific plug-in, which can be downloaded at http://alere-technologies.com (see p. 28).
- The user interface will be loaded, the ArrayMate performs internal testing. It requires slightly less than a minute.
- Click on the icon **New Run** (left upper edge of the screen). A suggestion for a run name / folder name for the new run appears in the top line of the screen. You may modify or change the experiment name at your convenience.
- Type in your operator ID (**optional**).
- You may enter a comment into the **memo** field **(optional)**.

Worklist

A **Worklist** file allows to link an identifier such as a laboratory or sample number to a position of an array within the ArrayStrip. Please respect the rules of confidentiality and data protection.

Worklists can be generated using spreadsheet software such as EXCEL (see below) but must be saved in the *.txt file format that can be imported into the test specific ArrayMate software. **Do not use special characters** (such as: ; ()[] / \ ä ü etc.).

- Create a list with at least three columns with obligatory headers in the following order: position / sample ID / assay ID (table 1).
- Positions are consecutively numbered from 1 to a maximum of 96. Position 1 would correspond to A1, 8 to H1, 9 to A2 and 96 to H12 (table 2). Do not leave empty lines in the worklist. If you use EXCEL, position numbers should be entered into column A.
- Sample ID is strain / sample / laboratory number such as exported from your LIMS (or assigned in any different way). Patient name should not be used as sample ID.
- The Assay ID enables the system to identify the current test and to correctly use information on layout, spot number, and identity etc. The Salm-SeroGenotyping AS-1 Kit has the Assay ID: 10624. ***Please note:*** *Assay ID numbers must not be confused as this could lead to errors or loss of data.*
- You may add further columns and headers with notes and comments at your convenience. Information from these columns will not appear on the result screen or in the Test Report.
- We recommend using a printout of the worklist as a template for pipetting.
- Save the worklist as **tab separated *.txt** file on the memory stick provided together with the ArrayMate.
- To avoid confusion, make sure that worklists are named unambiguously or that worklists from earlier experiments are deleted.

Table 1: Example worklist. *Please note: Table header must be written exactly as shown.*

Position	Sample ID	Assay ID	Comment
1	2013-12345	10624	
2	2013-12346	10624	
3	2013-12347	10624	
4	2013-12348	10624	
5	2013-12349	10624	

6	2013-12350	10624	
7	987654	10624	*Isolate referred from Dr. J. Doe.*
8	CM^{LT2}	10624	*control strain*

Table 2: Positions in the 96 well format

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
H	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Data Acquisition in the ArrayMate Reader

- Insert your memory stick containing the worklist into any of the USB ports down to the right hand side of the ArrayMate.
- Press the button  ; a folder selection dialogue will open.
- Select your worklist (path: “My Computer / Removable Disk”).
- Open your selected worklist by pressing **Enter** or **Open**.
- Press the button  (your imported worklist opens in a separate window). Proofread. If the new window is empty, or if it was the wrong worklist, repeat the import.
- Press the button **OK**; the worklist window will close.
- Leave the memory stick in the ArrayMate if you intend to export Salm-SeroGenotyping AS-1 Test Reports afterwards (check the memory stick for computer viruses and malware using an appropriate program on a regular basis).
- Press the button **Next** (at the bottom right on the screen; reader opens).
- Carefully insert the appropriate metallic adapter / frame into the ArrayMate. Do not apply strong force. Assure proper fit, otherwise the images may be out of focus.

- Carefully insert the white frame with the ArrayStrips into the metallic adapter. Ensure the correct orientation (position A1 in the frame next to the data matrix code on the adapter) and proper fit; otherwise the images may be out of focus.

ArrayStrip frame with strips inserted in accordance with the Worklist.

Please note: ArrayStrips must be clean. They should not contain any liquids during analysis. Data matrix codes must be clean. There must be no StripCaps on the wells to be analysed (however, unused wells should remain capped).

- Press the button **Next** (at the bottom right on the screen; reader closes, analysis program starts, it takes about 2-10 min, depending on the number of strips; the reader takes images and automatically analyses the data). The progress of the reading is indicated by the following symbols:

photographed:  in analysis:  ready: 

- The reader indicates the end of the entire process with an acoustic signal (beep).
- Press **Next** (at the bottom right on the screen; reader opens).
- Remove the white frame with the ArrayStrip(s).
- Press **Next** (at the bottom right on the screen; reader closes).

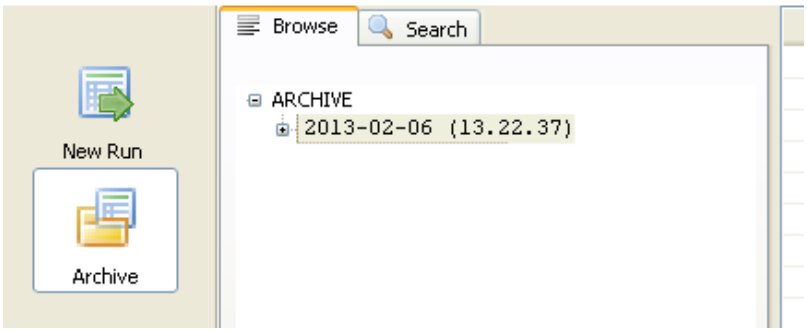
Results

On the lefthand side of the screen, you will see a list showing all runs stored on the ArrayMate´s hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not displayed:

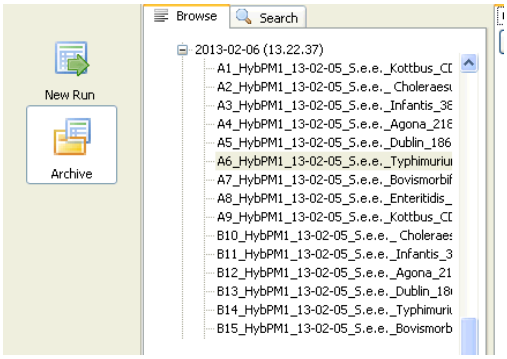
- Press the button **Archive** (lefthand) and activate the flag **Browse** (top left).

The runs are organised like folders in **Windows Explorer,** and named **by default** according to the date of acquisition.

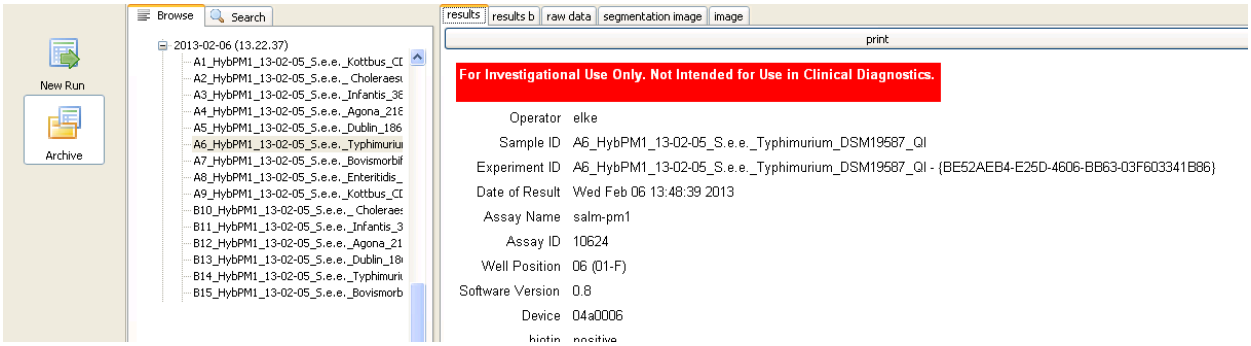
Example: There is one experiment run in this archive:



If you click on the plus symbol left of the run name, the folder opens and you will see a list of the individual arrays ordered by the sample ID.



Click on a Sample ID, and the Salm-SeroGenotyping AS-1 Test Report for this array is shown in the window on the right:



Export of Salm-SeroGenotyping Test Reports

Two result files in HTML format will be generated. The shorter report includes a summary on typing information. A longer HTML (*.html) result sheet ("result_B.res.html") show information on all probes.

Possible error messages that might occur in these reports will be explained below (see section "Troubleshooting").

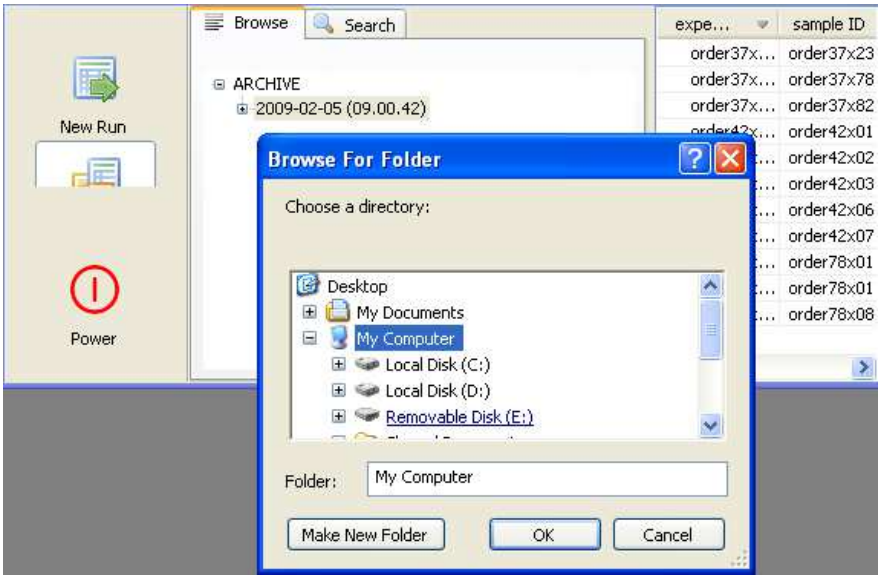
Other files that are generated and that can be exported include:

- A text file (*.txt) with the raw measurements,
- An image file (*.bmp) showing the actual photo of the array,
- A second image file (*.png) in which the coordinate grid is superimposed and the recognised spots are circled, and
- A XML file (*.xml) that contains the same information like the HTML result sheets for future export into databases etc.,
- An *.out file containing output log data which helps our service to trace imgae evaluation errors.

Please note: Only complete runs can be exported. The export of individual Salm-SeroGenotyping AS-1 Test Reports is not possible.

- Right-click on the selected run (a menu appears with the option **Export Run Reports**).

- Right-click on **Export Run Reports** (a file browser opens).



- Click **My Computer**, subsequently on **Removable Disk** and choose the folder where to save or click **Make New Folder** (on the bottom, a new folder icon appears).
- Rename the new folder (e.g. with the experiment name or date).
- Click on the **OK** button (data are exported into the new folder on your memory stick).
- Do **NOT** remove the memory stick as long as the hourglass symbol is visible.
- Switch off the device by pressing **Power** (at the bottom left on the screen): 

- Switch off the screen. There is no need to physically switch off the ArrayMate Reader.

TROUBLESHOOTING

In case of trouble always make sure that the reagents are within the recommended shelf-life and stored under appropriate conditions.

Should you encounter a problem, we will always be happy to support you. Please contact cct.home@clondiag.com and include a description of the problem as well as the array images (*.bmp files) in your question.

Staining Control

A staining control is included to check whether possible problems originate from the hybridisation or the staining procedure. If the staining control has “Failed” proceed as follows:

Horseradish peroxidase conjugate may have degraded during storage. Add 1 µl buffer C3 / C4 to 9 µL D1 (substrate). If the solution turns green within 3-5 seconds, the horseradish peroxidase still has sufficient enzymatic activity.

Enzymatic reaction is inhibited by carryover of buffer C1. Ensure proper washing of the wells with Buffer C2 to remove all of Buffer C1 prior to adding horseradish peroxidase conjugate.

If the staining control has “Passed”, refer to the following hints.

Image Quality

In case of poor image quality we recommend to re-check DNA quantity and quality first by loading leftover DNA on an agarose gel.

In order to determine whether any problems originated from the DNA preparation, perform an experiment with the CM^{LT2}. This contains DNA from the reference strain S.e.e. Typhimurium LT2 (GenBank accession number NC_003197.1) and should be identified by the assay as “*Salmonella enterica* spp. *enterica* serovar Typhimurium”. If the control experiment yields a valid result and a correct identification, there was probably an issue with DNA preparation. If the control experiment fails as well, an error affecting later steps or a degradation of reagents applied in later steps is likely.

DNA Quality

The amount of DNA is crucial because of the linear kinetics of amplification (see introduction). DNA should be free of RNA, as RNA reduces the efficiency of amplification and labelling by effectively removing primer from the reaction mix due to competitive hybridisation. A_{260}

readings will cover RNA and other contaminants as well. Therefore pure DNA preparations without RNA contamination are prerequisite for proper DNA concentration measurement. RNase treatment prior to A_{260} reading therefore is necessary (component A2 contains RNAse).

DNA must be unfragmented, as fragmentation reduces the efficiency of amplification and labelling due to the distance between primer and probe binding sites. For this reason DNA should not be prepared by disrupting *Salmonella* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. We evaluated the manual QIAGEN DNeasy kit and the Roche High Pure Kit.

DNA must be free of any trace of ethanol, as ethanol strongly influences the amplification. It is possible to heat the sample prior to adding it to the labelling mix (5-10 minutes at 70 °C) to evaporate the ethanol.

Physical Damage to the Array

Scratching of the array surface with a pipette tip may damage array spots which may lead to the impairment or absence of a valid signal. In this case the respective marker is not assigned as "Negative", but instead the message "none" appears next to the marker name.

Report Unavailable

If the ArrayMate indicates that no report is available for an array (or multiple arrays on one strip), please check that the strip positioned properly into the frame. Scratches or drops of condensed water might render the data matrix code identifier unreadable, please wipe it carefully or try to manually identify the test. If no obvious reason for the fault can be discovered, please contact the technical service.

ADDITIONAL INFORMATION

Warranty

Alere Technologies GmbH guarantees the performance as described in this manual. Usage of the Kit was successfully tested at ambient temperatures up to 37 °C, a guarantee is limited to ambient temperatures in the laboratory between 18 to 28 °C. Kit components comprise the arrays and their caps, the Lysis Enhancer, the reagents for DNA labelling and for detection of labelled DNA products on the array, the ArrayMate Reader and its software. In case one of these components fails within the expiry date due to other reasons other than misuse, contact Alere Technologies GmbH for replacement or refund. Terms and conditions apply.

If you have any problem or question, please contact the technical service.

Disclaimer

This system is for research use only.

We do not accept any liability for damages caused by misuse. Misuse comprises, especially but not exclusively, of a use of the system for the detection of resistance genes in order to predict phenotypic antibiotic resistances or susceptibilities for the guidance of an antibiotic chemotherapy.

Since resistances might be caused by genes or mutations not covered by this array or by hitherto unknown genes or mutations, any antibiotic chemotherapy MUST be guided by phenotypic susceptibility tests.

Furthermore, we do not accept any liability for damages caused by inappropriate use of the device as a personal computer, for instance related to the use of additional software, to network connections, or to a breach of privacy related to the storage of confidential information (such as names of patients from whom *Salmonella* was isolated) on its hard disk and / or to the use of external storage devices that might be contaminated with spyware.

Quality Control

Each batch is stringently tested with the use of standard *Salmonella* DNA preparations for good performance and correctness of results.

List of Components for Separate Order

If required, these reagents for the Salm-SeroGenotyping Kit may be ordered separately:

Component	Name	Category	Amount	Cat#	Storage
A1	Lysis Buffer	buffer	30 ml	245101000	18-28 °C
A2	Lysis Enhancer	lyophylised enzymes	96 units	245102000	18-28 °C
$B1^{+}$	Labelling Buffer	buffered reagents	550 µl	245103000	2-8 °C
B2	Labelling Enzyme	buffered enzyme	20 µl	245104000	2-8 °C
$B3^{Salm}$	*Salmonella* Primermix	labelling primers	70 µl	245703500	-20 °C
C1	Hybridisation Buffer	buffered reagents	30 ml	245105000	18-28 °C
C2	Washing Buffer 1	buffer	120 ml	245106000	18-28 °C
C3	HRP Conjugate 100 x	buffered enzyme	200 µl	245107000	2-8 °C
C4	Conjugate Buffer	buffered reagents	30 ml	245108000	18-28 °C
C5	Washing Buffer 2	buffer	120 ml	245109000	18-28 °C
D1	HRP Substrate	buffered reagents	15 ml	245110000	2-8 °C
CM^{LT2}	Control Material	S.e.e. Typhimurium LT2 DNA (c_{DNA} = 0.1-0.4 µg/µl)	30 µl	245711000	2-8 °C
ArrayStrip	Salm AS-1	plugged microarrays	1 Strip	240009642	15-28 °C
StripCaps	StripCaps	plasticware	24 units	245112000	18-28 °C

For pricing please contact your local representative or our customer service, respectively.

Legal Manufacturer

Alere Technologies GmbH

Loebstedter Str. 103-105

07749 Jena, Germany

Contact

If you require any further information on this product please contact: cct.home@clondiag.com

LITERATURE

Literature quoted in this manual:

Anonymous (2007) Microbiology of food and animal feeding stuffs – horizontal method for the detection of *Salmonella* (EN ISO 6579:2002/Amd 1:2007). Geneva: International Organization for Standardization. 40 p.

Braun SD, Ziegler A, Methner U, Slickers P, Keiling S, et al. (2012) Fast DNA serotyping and antimicrobial resistance gene determination of *Salmonella enterica* with an oligonucleotide microarray-based assay. PLoS One 7: e46489.

UPDATES AND SOFTWARE

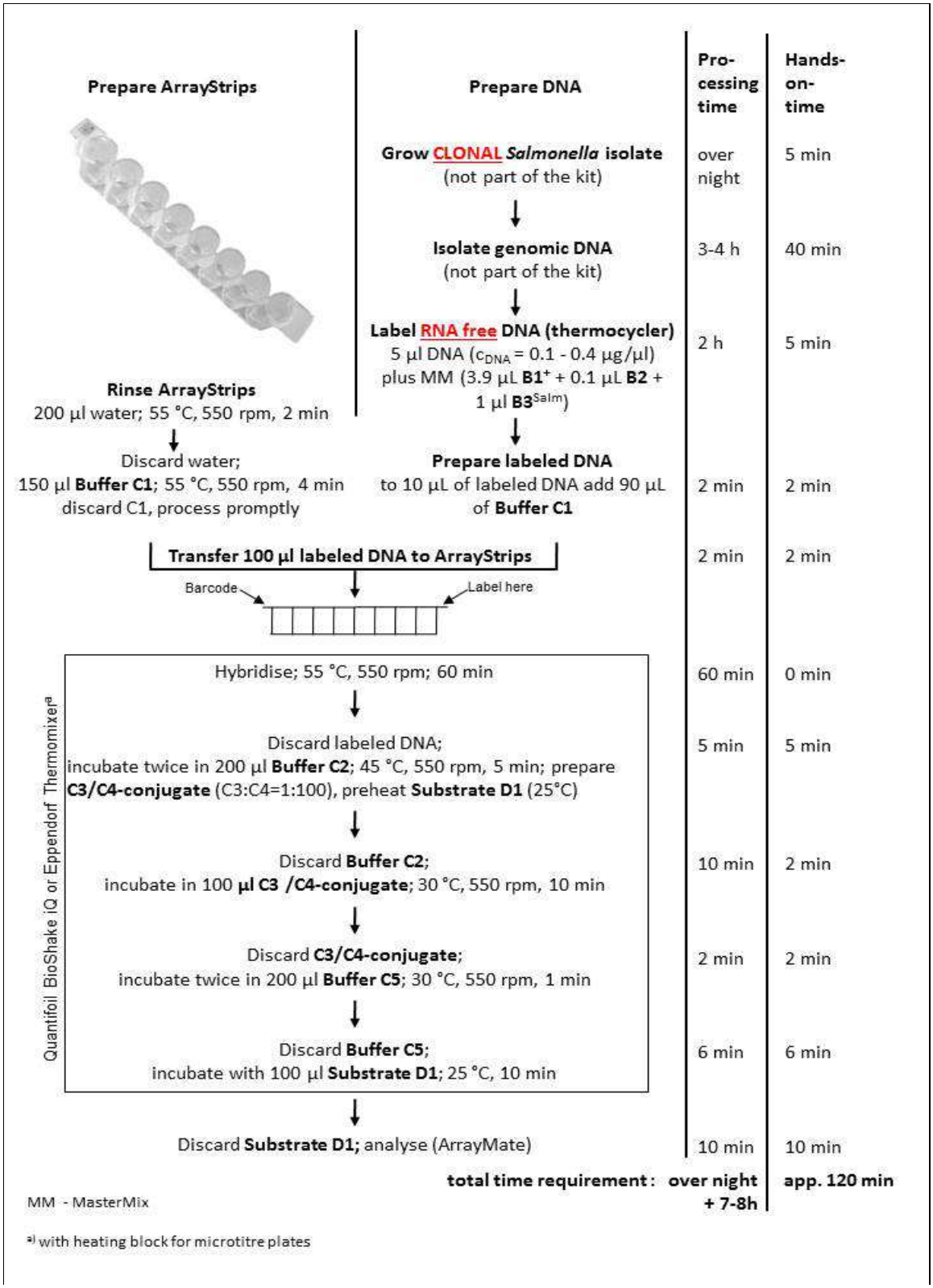
Notifications on database / software updates and freeware tools can be found at:

http://alere-technologies.com/en/products/lab-solutions/salmonella.html and / or

http://alere-technologies.com/en/news.html.

APPENDIX 1 – Flow chart

The figure on this page summarises the test procedure. However, please refer to the text section of this user guide at any step of the test protocol for further important details.


Prepare ArrayStrips
Prepare DNA
Pro-cessing time
Hands-on-time
Grow CLONAL Salmonella isolate
(not part of the kit)
over night
5 min
Isolate genomic DNA
(not part of the kit)
3-4 h
40 min
Label RNA free DNA (thermocycler)
5 µl DNA (c_{DNA} = 0.1 - 0.4 µg/µl)
plus MM (3.9 µL B1+ + 0.1 µL B2 + 1 µl B3Salm)
2 h
5 min
Rinse ArrayStrips
200 µl water; 55 °C, 550 rpm, 2 min
Discard water;
150 µl Buffer C1; 55 °C, 550 rpm, 4 min
discard C1, process promptly
Prepare labeled DNA
to 10 µL of labeled DNA add 90 µL of Buffer C1
2 min
2 min
Transfer 100 µl labeled DNA to ArrayStrips
2 min
2 min
Barcode
Label here
Quantifoil BioShake iQ or Eppendorf Thermomixer[a]
Hybridise; 55 °C, 550 rpm; 60 min
60 min
0 min
Discard labeled DNA;
incubate twice in 200 µl Buffer C2; 45 °C, 550 rpm, 5 min; prepare C3/C4-conjugate (C3:C4=1:100), preheat Substrate D1 (25°C)
5 min
5 min
Discard Buffer C2;
incubate in 100 µl C3 /C4-conjugate; 30 °C, 550 rpm, 10 min
10 min
2 min
Discard C3/C4-conjugate;
incubate twice in 200 µl Buffer C5; 30 °C, 550 rpm, 1 min
2 min
2 min
Discard Buffer C5;
incubate with 100 µl Substrate D1; 25 °C, 10 min
6 min
6 min
Discard Substrate D1; analyse (ArrayMate)
10 min
10 min
total time requirement:
over night + 7-8h
app. 120 min
MM - MasterMix
[a] with heating block for microtitre plates

APPENDIX 2 – PROBE TO TARGET TABLE

Genoserotyping		
No.	**Probes**	**Targets**
1	hp-3001-FL-1+e,n,x	e,n,x; z6; e,n,x,z15
2	hp-3003-FL-1+e,n,x	e,n,x; z6; e,n,x,z15; 1,5; 1,6
3	hp-3004-FL-1+e,n,x	1,2; 1,5; 1,2,7; 1,5,7; 1,6; 1,7; z; 1,11,16; 1,12
4	hp-3005-FL-1+e,n,x	1,11,16; 1,2; 1,5; 1,12; 1,2,7; 1,5,7; 1,6; 1,7; z
5	hp-3006-FL-1+e,n,x	z; 1,5; 1,2; 1,2,7; 1,5,7; 1,6; 1,7; 1,11,16; 1,12
6	hp-3007-FL-1+e,n,x	1,5; 1,6
7	hp-3008-FL-1+e,n,x	1,5; 1,6; e,n,x; z6; e,n,x,z15; 1,2
8	hp-3009-FL-1+e,n,x	1,5; 1,6; e,n,x; z6; e,n,x,z15; 1,2
9	hp-3012-FL-1+e,n,x	1,2; e,n,x; z6; e,n,x,z15; z; 1,11,16; 1,12; 1,5; 1,2,7; 1,7; 1,5,7; 1,6
10	hp-3013-FL-1+e,n,x	1,2; e,n,x,z15; e,n,x; z6; z; 1,11,16; 1,12; 1,2; 1,2,7; 1,5,7; 1,5; 1,6; 1,7
11	hp-3014-FL-1+e,n,x	e,n,x,z15; 1,2; e,n,x; z6; z; 1,11,16; 1,12; 1,5; 1,2,7; 1,7; 1,5,7 1,6
12	hp-3015-FL-1+e,n,x	1,5; 1,6; 1,2; e,n,x; z6; e,n,x,z15; z; 1,11,16; 1,12; 1,2,7; 1,7; e,n,x,z15
13	hp-3016-FL-c	c
14	hp-3017-FL-c	c
15	hp-3018-FL-d+j	d
16	hp-3019-FL-d+j	d
17	hp-3020-FL-d+j	d
18	hp-3021-FL-d+j	d; j
19	hp-3022-FL-d+j	d
20	hp-3023-FL-d+j	d; j
21	hp-3024-FL-e,h	e,h
22	hp-3025-FL-e,h	e,h
23	hp-3026-FL-e,n,x	e,n,x,z15
24	hp-3027-FL-e,n,x	e,n,x; e,n,x,z15; e,n,x,z15,
25	hp-3029-FL-g,z51	g,z51
26	hp-3032-FL-i+r	i
27	hp-3033-FL-i+r	i
28	hp-3034-FL-i+r	i
29	hp-3035-FL-i+r	r
30	hp-3036-FL-i+r	r
31	hp-3038-FL-k+z	k; z44; z58
32	hp-3039-FL-k+z	z35; l,v; z39; z65; z10
33	hp-3040-FL-k+z	z35
34	hp-3041-FL-k+z	k
35	hp-3042-FL-k+z	k; z41
36	hp-3043-FL-k+z	(k)
37	hp-3044-FL-z	z41
38	hp-3045-FL-k+z	z10
39	hp-3046-FL-k+z	z10
40	hp-3047-FL-k+z	z81
41	hp-3048-FL-k+z	a, z10

42	hp-3049-FL-k+z	z35
43	hp-3050-FL-k+z	k; z58; z44; z41
44	hp-3051-FL-k+z	a; z10
45	hp-3052-FL-z	z41
46	hp-3053-FL-k+z	z81
47	hp-3054-FL-k+z	z35
48	hp-3055-FL-k+z	z35
49	hp-3056-FL-k+z	z35
50	hp-3057-FL-k+z	(k)
51	hp-3058-FL-k+z	z10
52	hp-3060-FL-k+z	k; z41
53	hp-3061-FL-k+z	(k)
54	hp-3062-FL-l+z39+z52	z39
55	hp-3063-FL-l+z39+z52	l,v; l,z13; l,z28; l,z13,z28; l,w
56	hp-3065-FL-l+z39+z52	z52
57	hp-3066-FL-l+z39+z52	l,v; l,z13; l,z28; l,w; l,z13,z28
58	hp-3067-FL-y	y
59	hp-3068-FL-y	y
60	hp-3069-FL-z29	z29
61	hp-3070-FL-z29	z29
62	hp-3071-FL-z36+z38	z38
63	hp-3072-FL-z36+z38	z36; z36,z38
64	hp-3073-FL-z36+z38	z36; z36,z38
65	hp-3074-FL-z36+z38	z36; z36,z38
66	hp-3075-FL-z36+z38	z36,z38; z38
67	hp-3076-FL-z4	z4,z23; z4,z23,z32; z4,z24; z4,z32
68	hp-3077-FL-z4	z4,z24
69	hp-3078-FL-z4	z4,z23,z32
70	hp-3080-FL-z65	z65
71	hp-3085-FL-g	f,g; g,m,p,q; f,g; f,g,s,t; f,g,t; g,z62; g,m,t
72	hp-3086-FL-g	f,g,s,t; g,m,s; g,m,s,t; f,g; f,g,t; g,m,t; g,m,p,q; g,t; g,z62
73	hp-3087-FL-g	g,m,p,q; f,g; f,g,s,t; f,g,t; g,t; g,z62; g,m,t
74	hp-3089-FL-g	f,g,t; m,t; g,m,t
75	hp-3090-FL-g	g,m,t; f,g,t; m,t
76	hp-3091-FL-g	g,m,t; f,g,t; f,g; f,g,s,t; g,m,p,q; g,t; g,z62; g,m,s; g,m,s,t
77	hp-3092-FL-g	f,g; f,g,s,t; g,m,p,q; g,t; g,z62; f,g; g,m,t; g,m,s; g,m,s,t
78	hp-3103-FL-g	f,g; f,g,s,t; f,g,t; g,m,s; g,m,p,q; g,t; f,g,t; g,m,t; g,m,s,t; g,z62
79	hp-3104-FL-g	f,g,t; g,m,p,q; f,g; f,g,s,t; g,m,s; g,m,t; g,t g,z51
80	hp-3105-FL-g	g,z51; g,m,s,t; g,m,t; g,z62; f,g; f,g,s,t; f,g,t; g,m,s; g,m,p,q; g,t
81	hp-3106-FL-g	g,m,s,t; g,m,t; g,z62; f,g; f,g,s,t; f,g,t; g,m,s; g,m,p,q; g,t; g,z51
82	hp-3107-FL-g	m,t
83	hp-3108-FL-g	m,t
84	hp-3109-FL-g	m,t
85	hp-3113-FL-l+z39+z52	z39
86	hp-3117-FL-l+z39+z52	z39

87	hp-3118-FL-l+z39+z52	z52
88	hp-3120-FL-g,z51	g,z51
89	hp-3121-FL-g,z51	g,z51
90	hp-3124-FL-e,n,x	e,n,x; e,n,z15
91	hp-3125-FL-b+z91	b; z91
92	hp-3126-FL-b+z91	b; z91
93	hp-3128-FL-b+z91	b; z91
94	hp-3129-FL-b+z91	b; z91
95	hp-3130-FL-b+z91	b; z91
96	hp-3134-FL-z	z6
97	hp-3135-FL-z	z6
98	hp-3136-FL-z	z69
99	hp-3138-FL-z	z
100	hp-3139-FL-z	z
101	hp-3140-FL-z	z
102	hp-3141-FL-z	z50
103	hp-3142-FL-z	z; z35
104	hp-3144-FL-z	z50
105	hp-3145-FL-z	z
106	hp-3146-FL-z	z
107	hp-3149-FL-l+z39+z52	z39
108	hp-3150-FL-z	z
109	hp-3152-FL-i+r	i
110	hp-3153-FL-l+z39+z52	l,v; l,w; l,z13; l,z28; l,z13,z28; l,z28
111	hp-3154-FL-k+z	z10
112	hp-3155-FL-z4	z4,z23; z4,z24; z4,z32; z4,z23
113	hp-3157-FL-1+e,n,x	e,n,x,z15; 1,2; e,n,x; z6; z; 1,11,16; 1,12; 1,2; 1,2,7; 1,5; 1,7; 1,5,7; 1,6
114	hp-3158-FL-1+e,n,x	1,5,7; 1,2; e,n,x; z6; e,n,x,z15; z; 1,11,16; 1,12; 1,2,7; 1,5; 1,7; 1,6
115	hp-3161-FL-1+e,n,x	1,5; 1,6
116	hp-3163-FL-1+e,n,x	1,2; 1,12; 1,2,7; 1,5,7; 1,5; 1,6; 1,7; z; 1,11,16
117	hp-3165-manC	manC species marker
118	hp-3166-wbyJ	O41
119	hp-3167-wbyJ	O41
120	hp-3168-manC-O16+39	O16; O39
121	hp-3169-manC-O16+39	O16; O39
122	hp-3170-manC-O7	O7
123	hp-3171-manC-O7	O7
124	hp-3172-manC-O11	O11
125	hp-3173-manC-O11	O11
126	hp-3174-manC-O18	O18
127	hp-3175-manC-O18	O18
128	hp-3176-manC-O41	O41
129	hp-3177-manC-O41	O41
130	hp-3178-manC-O41	O41
131	hp-3179-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50

132	hp-3180-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
133	hp-3181-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
134	hp-3182-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
135	hp-3183-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
136	hp-3184-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
137	hp-3185-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
138	hp-3186-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
139	hp-3187-manC-O13+O30+O43+O45+O50	O13; O30; O43; O45; O50
140	hp-3188-manC-O2+4+9+3,10	O2; O4; O9; O3,10
141	hp-3189-manC-O2+4+9+3,10	O2; O4; O9; O3,10
142	hp-3190-manC-O40	O40
143	hp-3191-manC-O40	O40
144	hp-3192-rfbV-O2+9+9,46	O2; O9; O9,46
145	hp-3193-rfbV-O2+9+9,46	O2; O9; O9,46
146	hp-3194-rfbV-O4	O4
147	hp-3195-rfbV-O4	O4
148	hp-3196-wbuH-O41+62	O41; O62
149	hp-3197-wbuH-O41+62	O41; O62
150	hp-3198-weiB_O66	O66
151	hp-3199-weiB_O66	O66
152	hp-3200-wzx_O13	O13
153	hp-3201-wzx_O13	O13
154	hp-3202-wzx_O16	O16
155	hp-3203-wzx_O16	O16
156	hp-3204-wzx_O17	O17
157	hp-3205-wzx_O17	O17
158	hp-3206-wzx_O18	O18
159	hp-3207-wzx_O18	O18
160	hp-3208-wzx_O2+9	O2; O9
161	hp-3209-wzx_O2+9	O2; O9
162	hp-3210-wzx_O28_Dakar	O28 serovar Dakar
163	hp-3211-wzx_O28_Dakar	O28 serovar Dakar
164	hp-3212-wzx_O28_Pomona	O28 serovar Pomona
165	hp-3213-wzx_O28_Pomona	O28 serovar Pomona
166	hp-3214-wzx_O3,10	O3,10
167	hp-3215-wzx_O3,10	O3,10
168	hp-3216-wzx_O30	O30
169	hp-3217-wzx_O30	O30
170	hp-3218-wzx_O35	O35
171	hp-3219-wzx_O35	O35
172	hp-3220-wzx_O4	O4

173	hp-3221-wzx_O4	O4
174	hp-3222-wzx_O4	O4
175	hp-3223-wzx_O41+62	O41; O62
176	hp-3224-wzx_O41+62	O41; O62
177	hp-3225-wzx_O50	O50
178	hp-3226-wzx_O50	O50
179	hp-3227-wzx_O55	O55
180	hp-3228-wzx_O55	O55
181	hp-3229-wzx_O56	O56
182	hp-3230-wzx_O56	O56
183	hp-3231-wzx_O58	O58
184	hp-3232-wzx_O58	O58
185	hp-3233-wzx_O6,14	O6,14
186	hp-3234-wzx_O6,14	O6,14
187	hp-3235-wzx_O66	O66
188	hp-3236-wzx_O66	O66
189	hp-3237-wzx_O7	O7
190	hp-3238-wzx_O7	O7
191	hp-3239-wzx_O7	O7
192	hp-3240-wzx_O8	O8
193	hp-3241-wzx_O8	O8
194	hp-3242-wzy_O13	O13
195	hp-3243-wzy_O13	O13
196	hp-3244-wzy_O16	O16
197	hp-3245-wzy_O16	O16
198	hp-3246-wzy_O17	O17
199	hp-3247-wzy_O17	O17
200	hp-3248-wzy_O18	O18
201	hp-3250-wzy_O28_Dakar	O28 serovar Dakar
202	hp-3251-wzy_O28_Dakar	O28 serovar Dakar
203	hp-3252-wzy_O28_Pomona	O28 serovar Pomona
204	hp-3253-wzy_O28_Pomona	O28 serovar Pomona
205	hp-3254-wzy_O3,10+9,46	O3,10; O9,46
206	hp-3255-wzy_O3,10+9,46	O3,10; O9,46
207	hp-3256-wzy_O3,10+9,46	O3,10; O9,46
208	hp-3257-wzy_O30	O30
209	hp-3258-wzy_O30	O30
210	hp-3259-wzy_O35	O35
211	hp-3260-wzy_O35	O35
212	hp-3261-wzy_O38	O38
213	hp-3262-wzy_O38	O38
214	hp-3263-wzy_O41+62	O41; O62
215	hp-3264-wzy_O41+62	O41; O62
216	hp-3265-wzy_O50	O50
217	hp-3266-wzy_O50	O50

218	hp-3267-wzy_O55	O55
219	hp-3268-wzy_O55	O55
220	hp-3269-wzy_O56	O56
221	hp-3270-wzy_O56	O56
222	hp-3271-wzy_O58	O58
223	hp-3272-wzy_O58	O58
224	hp-3273-wzy_O6,14	O6,14
225	hp-3274-wzy_O6,14	O6,14
226	hp-3275-wzy_O7	O7
227	hp-3276-wzy_O7	O7
228	hp-3277-wzy_O8	O8
229	hp-3278-wzy_O8	O8
230	hp-3279-wzy_O18	O18
231	hp-3280-SSPAI	Paratyphi A
232	hp-3281-SSPAI	Paratyphi A
233	hp-3282-Q8ZK10	Typhimurum
234	hp-3287-lygA	Enteritidis
235	hp-3288-lygD	Enteritidis
236	hp-3289-Q8ZK15	Typhimurium
237	hp-3290-tviA	plasmid Vi
238	hp-3292-tviA	plasmid Vi
239	hp-3293-stgA	Typhi
240	hp-3294-stgA	Typhi
241	hp-3297-sefB	Enteritidis
242	hp-3298-sefA	Enteritidis
243	hp-3299-sefC	Enteritidis
244	hp-3300-galF	species marker for *Salmonella*
245	hp-3301-B5FQV7	Dublin
246	hp-3302-B5R5L5	unknown
247	hp-3306-B5R7B6	Gallinarum, Weltevreden
248	hp-3307-B5R7C1	unknown
249	hp-3308-ISR1	Infantis
250	hp-3310-ISR1	Infantis
251	hp-3311-Q57QY4	Choleraesuis
252	hp-3312-Q57QY4	Choleraesuis
253	hp-3314-invA	species marker for *Salmonella*
254	hp-3315-invA	species marker for *Salmonella*
255	hp-3316-invA	species marker for *Salmonella*
Genes associated with antibiotic resistance		
No.	**Probes**	**Targets**
1	hp_aac3_611	3-N-aminoglycoside acetyltransferase; associated with resistance to gentamycin (U90945.1)
2	hp_aac3_614	3-N-aminoglycoside acetyltransferase; associated with resistance to gentamycin (U90945.1)
3	prob_aac3Ia_1	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin;
4	hp_aac6_612	aminoglycoside 6'-N-acetyltransferase, associated with resistance to amikacin; dibekacin;
5	hp_aac6_615	aminoglycoside 6'-N-acetyltransferase, associated with resistance to amikacin; dibekacin;

6	hp_aac6_618	aminoglycoside 6'-N-acetyltransferase, associated with resistance to amikacin; dibekacin;
7	prob_aac6Ib_1	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin
8	hp_aadB_611	2''-aminoglycoside nucleotidyltransferase (L06418.4)
9	hp_aadB-2_611	2''-aminoglycoside nucleotidyltransferase (L06418.4)
10	hp_armA_611	16S rRNA methylase, associated with aminoglycoside resistance (AB117519.1)
11	prob_aadA1_1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin
12	prob_aadA2_1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin
13	prob_aadA4_1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin
14	prob_ant2Ia_1	aminoglycoside (2'') adenylyltransferase; associated with resistance to dibekacin; gentamicin;
15	hp_aphA_611	aminoglycoside 3'-phosphotransferase; kanamycin resistance protein (AY260546.3)
16	hp_ble_611	associated with bleomycin resistance (X01702.1)
17	hp_sph_611	streptomycin 3''-phosphotransferase; associated with resistance to streptomycin (U00004.1)
18	prob_strA_611	aminoglycoside-3''-phosphotransferase (locus A) associated with resistance to streptomycin
19	prob_strB_611	aminoglycoside-6''-phosphotransferase associated with resistance to streptomycin (EF090911.1)
20	prob_tetA_11	tetracycline resistance protein A, tetracycline efflux protein (CP000971.1)
21	prob_tetB_11	tetracycline resistance protein A, class B (V00611.1)
22	prob_tetC_11	tetracycline resistance protein A, class C (EU751612.1)
23	prob_tetD_1	tetracycline resistance protein A, class D (X65876.1)
24	prob_tetG_11	tetracycline resistance protein A, class G (AF261825.2)
25	hp_kpc4_611	carbepenem-hydrolyzing beta-lactamase
26	hp_blaCMY_611	consensus sequence for blaCMY-13, blaCMY-2, blaCMY-24, blaCMY-35
27	prob_cmy_11	consensus sequence for blaCMY-13, blaCMY-2, blaCMY-24, blaCMY-35
28	prob_acc1_11	class C beta-lactamase blaACC-1 (EF554600.1)
29	prob_acc2_11	class C beta-lactamase blaACC-2 (EF554600.1)
30	hp_per2_611	class A beta-lactamase PER-1; extended-spectrum beta-lactamase (Z21957.1)
31	prob_ctxM1_11	class A extended-spectrum-beta-lactamase (X92506.1), including blaCTX-M15 (HQ202266.1)
32	prob_ctxM2_11	class A extended-spectrum-beta-lactamase (AY517475)
33	prob_ctxM9_11	class A beta-lactamase (AF174129.3)
34	prob_ctxM26_11	class A extended-spectrum-beta-lactamase (AF518567)
35	prob_dha1_1	class C beta-lactamase (EF406115.1)
36	prob_oxa1_21	class D beta-lactamase blaOXA-1 (AY458016.1)
37	prob_oxa2_11	class D beta-lactamases blaOXA-2/blaOXA-15 (U63835.1)
38	prob_oxa7_11	class D beta-lactamase blaOXA-7 (AY866525.1)
39	prob_per2_1	class A beta-lactamase PER-2; extended-spectrum beta-lactamase (X93314.1)
40	prob_pse1_1pm	class A beta lactamase (carbenicillinase) (Z18955.1)
41	prob_shv1_11	class A beta-lactamase (consensus)
42	prob_tem1_1	class A beta-lactamase (consensus)
43	prob_catA1_11	chloramphenicol acetyltransferase (group A) (V00622.1)
44	prob_catB3_11	chloramphenicol acetyltransferase (group B) (AJ009818.1)
45	prob_catB8_12	chloramphenicol acetyltransferase (AF227506.1)
46	prob_cmlA1_11	chloramphenicol transporter (EF113389.1)
47	prob_floR_11	chloramphenicol and florfenicol efflux protein
48	hp_mphA_611	macrolide 2'-phosphotransferase (EF102240.1)
49	hp_ereA_611	type I erythromycin resistance (AY183453.1)
50	prob_qnr_12	quinolone and fluoroquinolone resistance protein (AY931018.1)

51	prob_qnrB_12	quinolone and fluoroquinolone resistance protein (AB281054.1)
52	hp_qnrD_611	quinolone and fluoroquinolone resistance protein (FJ228229.1)
53	prob_qnrS_11	quinolone and fluoroquinolone resistance protein (AM234722.1)
54	prob_sul1_11	dihydropteroate synthetase type 1 (AJ698325.1)
55	prob_sul2_11	dihydropteroate synthetase type 2 (DQ464881.1)
56	prob_sul3_11	dihydropteroate synthetase type 3 (AJ459418.2)
57	prob_dfrA1_21	dihydrofolate reductase type 1 (AJ884723.1)
58	prob_dfrA1_22	dihydrofolate reductase type 1 (AJ884723.1)
59	prob_dfrV_21	dihydrofolate reductase type 5 (AB188269.1)
60	prob_dfrA7_11	dihydrofolate reductase type 7 (AB161450.1, AM237806.1)
61	prob_dfrA7_12	dihydrofolate reductase type 7 (AB161450.1, AM237806.1)
62	prob_dfr12_11	dihydrofolate reductase type 12 (AB154407.1)
63	prob_dfr13_11	dihydrofolate reductase type 13 (synonym A21) (Z50802.3)
64	prob_dfrA14_21	dihydrofolate reductase type 14 (AJ313522.1)
65	prob_dfrA15_1	dihydrofolate reductase type 15 (Z83311.1)
66	prob_dfrA17_11	dihydrofolate reductase type 17 (AF169041.1)
67	prob_dfrA19_1	dihydrofolate reductase type 19 (AJ310778.1)
Genes encoding **virulence factors**		
No.	**Probes**	**Targets**
1	astA_consens_10	heat stable enterotoxin (consensus sequence)
2	prob_intI1_1	class 1 integron integrase (AY260546.3)
3	prob_intI2_11	class 2 integron integrase (AY183453.1)

APPENDIX 3 – TYPING INFORMATION

Definitions and Explanations

The displayed result will yield following typing information:

- Discrimination of the 46 described O-serotypes is mainly determined by the genes *wzy* (polymerase) and *wzx* (flippase). The 114 known H-antigens are encoded by two genes; *fliC* (phase 1 flagellin) and *fljB* (phase 2 flagellin).
- The probes immobilized on the current array version can discriminate 28 O-antigens: A (O:2), B (O:4), C1 (O:7), C2-C3 (O:8), D1 (O:9), D2 (O:9,46), E1/E4 (O:3,10/O:1,3,19), F (O:11), G (O:13), H (O:6,14), I (O:16), J (O:17), K (O:18), M (O:28), N (O:30), O (O:35), P (O:38), Q (O:39), R (O:40), S (O:41), U (O:43), W (O:45), Z (O:50), O55, O56, O58, O62 and O66.
- The following flagellar antigens can be identified on the array: a; b; c; d; e,h; e,n,x; e,n,x,z15; f,g; f,g,m,t; f,g,s; [f],g,[t]; f,g,t; g,[s],t; g,m; g,m,[p],s; g,m,[t]; g,m,q; g,m,s; g,m,s,t; g,m,t; g,p; g,p,s; g,p,u; g,q; g,s,t; g,t; g,z51; g,z62; i; j; k; (k); l,v; l,w; l,z13; l,z13,z28; l,z28; m,p,t,[u]; m,t; r; r,[i]; y; z; z10; z29; z35; z36; z36,z38; z38; z39; z4,z23; z4,z23,z32; z4,z24; z4,z32; z41; z44; z47; z52; z58; z6; z65; z69; z81; z91; 1,11 (AY353292); 1,16 (AY353263); 1,2; 1,[2],7; 1,2,7; 1,5; 1,5,(7); 1,5,7; 1,6; 1,7; e,n,x; e,n,x,z15; e,n,z15; k; l,w; l,z13,z28z; z10; z35; z39; z41; z50 and z6.
- Probes specifying *invA*, *galF* and *manC* that were introduced to confirm the identity of *Salmonella* and to serve as genus controls.
- Different probes were used to detect the following antimicrobial resistance genes: *aac3Ia*, *aac3Ie*, *aac6Ib*, *aac6II*, *aadA1*, *aadA2*, *aadA23b*, *aadA3*, *aadA5*, *aadB*, *ant2Ia*, *aphA*, *armA*, *sph*, *strA*, *strB* (resistance to various aminoglycosides); *catA1*, *catB3*, *catB8*, *cmlA*, *floR* (chloramphenicol); *tetA*, *tetB*, *tetC*, *tetD*, *tetG* (tetracyclines); *sul1*, *sul2*, *sul3*, *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA13*, *dfrA14*, *dfrA15*, *dfrA17*, *dfrA19* (sulfonamide/trimethoprim); *ble* (glycopeptides: bleomycin); *qnrA*, *qnrB*, *qnrD*, *qnrS* (quinolones); *acc1*, *carB2*, *cmy2*, *ctxM1*, *ctxM2*, *ctxM26*, *ctxM9*, *dha1*, *oxa1*, *oxa2*, *oxa10*, *oxa53*, *per2*, *shv*, *tem1* (beta-lactam

compounds); *kpc4* (carbapenems) and *ereA*, *mphA* (macrolides). Additionally, two probes were designed to determine the presence of genes *intI1* and *intI2* possibly mediating an integrase function

- Using a PatternMatch module, a software package was developed to analyze *Salmonella* serovars directly at the ArrayMate device after scanning and calculating signals of the stained arrays.
- The detection software used a database comprising 168 reference *Salmonella* strains (representing 132 *Salmonella* serovars) which were classically serotyped. Patterns of unknown *Salmonella* were compared to the whole database and the two best hits were given in an overview result sheets (result_A) and in a detailed result sheet with all probes listed in a table (result_B).
- Assignment score. This is a score for the similarity to the average hybridisation result for a given strain. Scores above 6.5 exclude reliable strain identification, and could be attributed either to technical reasons or to the presence of a yet unknown strain.

List of Currently Recognised Strains

If you have array images of a strain not yet covered or if you have additional information on strain you wish to be included please contact: cct.home@clondiag.com

Species	Serovar	Strain	Results of classical Serotyping		Results of microarray based Serotyping			
			Serogroup	Antigenic Formula	Serogroup	*invA/galF/manC*	Unique Pattern	Pattern similar to Serovars
S.e. enterica	Paratyphi A	CDC1	A (O:2)	1,2,12:a:[1,5]	A (O:2)	+/+/+	Yes	
S.e. enterica	Nitra	CDC1280	A (O:2)	2,12:g,m:-	A (O:2)	+/+/+	No	Enteritidis, Blegdam
S.e. enterica	Kiel	CDC09-1879; CDC674	A (O:2)	1,2,12:g,p:-	A (O:2)	+/+/+	No	Dublin, Naestved, Moscow
S.e. enterica	Koessen	CDC2417	A (O:2)	2,12:l,v:1,5	A (O:2)	+/+/+	No	Panama
S.e. enterica	Abony	CDC103; DSM4224	B (O:4)	1,4,[5],12,[27]:b:e,n,x	B (O:4)	+/+/+	Yes	
S.e. enterica	Paratyphi B	CDC3	B (O:4)	1,4,[5],12:b:1,2	B (O:4)	+/+/+	Yes	

S.e. enterica	Wien	SGSC2528	B (O:4)	1,4,12,[27]:b:l,w	B (O:4)	+/+/+	Yes	
S.e. enterica	Jericho	CDC621	B (O:4)	1,4,12,27:c:e,n,z15	B (O:4)	+/+/+	Yes	
S.e. enterica	Duisburg	SGSC2472	B (O:4)	1,4,12,[27]:d:e,n,z15	B (O:4)	+/+/+	Yes	
S.e. enterica	Schwarzengrund	CDC1629; SGSC2514	B (O:4)	1,4,12,27:d:1,7	B (O:4)	+/+/+	Yes	
S.e. enterica	Stanley	CDC000477; SGSC2517	B (O:4)	1,4,[5],12,[27]:d:1,2	B (O:4)	+/+/+	Yes	
S.e. enterica	Chester	CDC17	B (O:4)	1,4,[5],12:e,h:e,n,x	B (O:4)	+/+/+	Yes	
S.e. enterica	Reading	CDC19; SGSC2510	B (O:4)	1,4,[5],12:e,h:1,5	B (O:4)	+/+/+	Yes	
S.e. enterica	Saintpaul	CDC108	B (O:4)	1,4,[5],12:e,h:1,2	B (O:4)	+/+/+	Yes	
S.e. enterica	Sandiego	CDC18	B (O:4)	1,4,[5],12:e,h:e,n,z15	B (O:4)	+/+/+	Yes	
S.e. enterica	Derby	CDC20	B (O:4)	1,4,[5],12:f,g:[1,2]	B (O:4)	+/+/+	Yes	
S.e. enterica	Agona	CDC1636	B (O:4)	1,4,[5],12:f,g,s:[1,2]	B (O:4)	+/+/+	Yes	
S.e. enterica	California	CDC1109	B (O:4)	4,12:g,m,t:[z67]	B (O:4)	+/+/+	Yes	
S.e. enterica	Budapest	CDC23	B (O:4)	1,4,12,[27]:g,t:-	B (O:4)	+/+/+	Yes	
S.e. enterica	Travis	CDC990318	B (O:4)	4,[5],12:g,z51:1,7	B (O:4)	+/+/+	Yes	
S.e. enterica	1,4,[5],12:i:-	CDCQA126; NRL688; NRL813	B (O:4)	1,4,[5],12:i:-	B (O:4)	+/+/+	Yes	
S.e. enterica	Agama	CDC513	B (O:4)	4,12:i:1,6	B (O:4)	+/+/+	Yes	
S.e. enterica	Gloucester	CDC443	B (O:4)	1,4,12,27:i:l,w	B (O:4)	+/+/+	Yes	
S.e. enterica	Typhimurium	CDC14; DSM10506; DSM17058; DSM17058; DSM19587; DSM554; LT2	B (O:4)	1,4,[5],12:i:1,2	B (O:4)	+/+/+	Yes	
S.e. enterica	Brandenburg	CDC2519; SGSC2460	B (O:4)	4,[5],12:l,v:e,n,z15	B (O:4)	+/+/+	Yes	
S.e. enterica	Bredeney	CDC112	B (O:4)	1,4,12,27:l,v:1,7	B (O:4)	+/+/+	Yes	
S.e. enterica	Heidelberg	CDC16; DSM9379	B (O:4)	1,4,[5],12:r:1,2	B (O:4)	+/+/+	Yes	
S.e. enterica	Indiana	CDC377; SGSC2482	B (O:4)	1,4,12:z:1,7	B (O:4)	+/+/+	No	Kiambu
S.e. enterica	Kiambu	CDC399	B (O:4)	1,4,12:z:1,5	B (O:4)	+/+/+	No	Indiana
S.e. enterica	Haifa	SGSC2479	B (O:4)	1,4,[5],12:z10:1,2	B (O:4)	+/+/+	Yes	
S.e. enterica	Stanleyville	CDC223; SGSC2518	B (O:4)	1,4,[5],12,[27]:z4,z23:[1,2]	B (O:4)	+/+/+	Yes	
S.e. enterica	Maska	CDC2349	B (O:4)	1,4,12,27:z41:e,n,z15	B (O:4)	+/+/+	Yes	
S.e. enterica	Ohio	CDC710	C1 (O:7)	6,7,14:b:l,w	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Choleraesuis	CDC34; DSM14846	C1 (O:7)	6,7:c:1,5	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Paratyphi C	CDC33; SGSC3592	C1 (O:7)	6,7,[Vi]:c:1,5	C1 (O:7)	+/+/+	Yes	

S.e. enterica	Typhisuis	SGSC2527	C1 (O:7)	6,7:c:1,5	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Kambole	CDC1863	C1 (O:7)	6,7:d:1,[2],7	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Livingstone	NRL720	C1 (O:7)	6,7,14:d:l,w	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Braenderup	CDC49	C1 (O:7)	6,7,14:e,h:e,n,z15	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Nola	CDC2206	C1 (O:7)	6,7:e,h:1,7	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Rissen	CDC955	C1 (O:7)	6,7,14:f,g:-	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Montevideo	CDC1904	C1 (O:7)	6,7,14:g,m,[p],s:[1,2,7]	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Singapore	CDC010011	C1 (O:7)	6,7:k:e,n,x	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Thompson	CDC000342	C1 (O:7)	6,7,14:k:1,5	C1 (O:7)	+/+/+	Yes	
S.e.diarizonae	6,7:l,v:z53	DSM14847	C1 (O:7)	6,7:l,v:z53	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Bonn	CDC344	C1 (O:7)	6,7:l,v:e,n,x	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Potsdam	CDC876	C1 (O:7)	6,7,14:l,v:e,n,z15	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Kenya	CDC497	C1 (O:7)	6,7:l,z13:e,n,x	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Haelsingborg	CDC586	C1 (O:7)	6,7:m,p,t,[u]:-	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Oranienburg	CDC1271	C1 (O:7)	6,7,14:m,t:[z57]	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Infantis	CDC1428	C1 (O:7)	6,7,14:r:1,5	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Virchow	CDC2688	C1 (O:7)	6,7,14:r:1,2	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Bareilly	NRL608	C1 (O:7)	6,7,14:y:1,5	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Mbandaka	CDC1906	C1 (O:7)	6,7,14:z10:e,n,z15	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Tennessee	CDC155	C1 (O:7)	6,7,14:z29:[1,2,7]	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Tienba	CDC2425	C1 (O:7)	6,7:z35:1,6	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Lille	CDC354	C1 (O:7)	6,7,14:z38:-	C1 (O:7)	+/+/+	Yes	
S.e. enterica	Manhattan	CDC122	C2-C3 (O:8)	6,8:d:1,5	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Muenchen	CDC54; SGSC2243	C2-C3 (O:8)	6,8:d:1,2	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Virginia	CDC189	C2-C3 (O:8)	8:d:1,2	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Kottbus	CDC52	C2-C3 (O:8)	6,8:e,h:1,5	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Newport	CDC2434	C2-C3 (O:8)	6,8,20:e,h:1,2	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Emek	SGSC2477	C2-C3 (O:8)	8,20:g,m,s:-	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Kentucky	CDC2590; Eng196[b]	C2-C3 (O:8)	8,20:i:z6	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Lindenburg	CDC334	C2-C3 (O:8)	6,8:i:1,2	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Blockley	CDC448; Eng23[b]; Eng24[b]	C2-C3 (O:8)	6,8:k:1,5	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Litchfield	CDC000462	C2-C3 (O:8)	6,8:l,v:1,2	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Manchester	Eng205[b]	C2-C3 (O:8)	6,8:l,v:1,7	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Breukelen	CDC1699	C2-C3 (O:8)	6,8:l,z13,[z28]:e,n,z15	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Goldcoast	NRL852	C2-C3 (O:8)	6,8:r:l,w	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Bovismorbificans	CDC2201	C2-C3 (O:8)	6,8,20:r,[i]:1,5	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Hidalgo	CDC2359	C2-C3 (O:8)	6,8:r,[i]:e,n,z15	C2-C3 (O:8)	+/+/+	Yes	

S.e. enterica	Hadar	CDC347;	C2-C3 (O:8)	6,8:z10:e,n,x	C2-C3 (O:8)	+/+/+	No	Istanbul
S.e. enterica	Istanbul	CDC1466	C2-C3 (O:8)	8:z10:e,n,x	C2-C3 (O:8)	+/+/+	No	Hadar
S.e. enterica	Uno	CDC1697	C2-C3 (O:8)	6,8:z29:[e,n,z15]	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Corvallis	CDC1770	C2-C3 (O:8)	8,20:z4,z23:[z6]	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Duesseldorf	CDC130	C2-C3 (O:8)	6,8:z4,z24:-	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Tallahassee	CDC196	C2-C3 (O:8)	6,8:z4,z32:-	C2-C3 (O:8)	+/+/+	Yes	
S.e. enterica	Gallinarum	CDC74; DSM13674	D1 (O:9)	1,9,12:-:-	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Berta	CDC69	D1 (O:9)	1,9,12:[f],g,[t]:-	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Miami	CDC198; SGSC2485	D1 (O:9)	1,9,12:a:1,5	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Goeteborg	CDC696	D1 (O:9)	9,12:c:1,5	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Typhi	No. 1[c]	D1 (O:9)	9,12[Vi]:d:-	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Enteritidis	CDC64; DSM14221; DSM17420	D1 (O:9)	1,9,12:g,m:-	D1 (O:9)	+/+/+	No	Nitra, Blegdam
S.e. enterica	Blegdam	CDC090361; CDC68	D1 (O:9)	9,12:g,m,q:-	D1 (O:9)	+/+/+	No	Nitra, Enteritidis
S.e. enterica	Dublin	CDC10-0635; CDC65	D1 (O:9)	1,9,12[Vi]:g,p:-	D1 (O:9)	+/+/+	No	Kiel, Naestved, Moscow
S.e. enterica	Naestved	CDC559; SGSC3612	D1 (O:9)	1,9,12:g,p,s:-	D1 (O:9)	+/+/+	No	Kiel, Dublin, Moscow
S.e. enterica	Moscow	CDC67	D1 (O:9)	1,9,12:g,q:-	D1 (O:9)	+/+/+	No	Kiel, Dublin, Naestved
S.e. enterica	Panama	CDC73; SGSC2496	D1 (O:9)	1,9,12:l,v:1,5	D1 (O:9)	+/+/+	No	Koessen
S.e. salamae	9:l,w:e,n,x	DSM9220	D1 (O:9)	9:l,w:e,n,x	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Javiana	CDC146	D1 (O:9)	1,9,12:l,z28:1,5	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Ottawa	CDC1934	D1 (O:9)	1,9,12:z41:1,5	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Franken	CDC2570	D1 (O:9)	9,12:z6:z67	D1 (O:9)	+/+/+	Yes	
S.e. enterica	Fresno	CDC1412	D2 (O:9,46)	9,46:z38:-	D2 (O:9,46)	+/+/+	Yes	
S.e. enterica	Anatum	CDC78	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:1,6	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Meleagridis	NRL737	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:l,w	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Muenster	CDC79	E1 (O:3,10)	3,{10}{15}{15,34}:e,h:1,5	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Amsterdam	CDC070756	E1 (O:3,10)	3,{10}{15}{15,34}:g,m,s:-	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Westhampton	CDC326	E1 (O:3,10)	3,{10}{15}{15,34}:g,s,t:-	E1 (O:3,10)	+/+/+	No	Senftenberg
S.e. enterica	Bessi	CDC1999	E1 (O:3,10)	3,10:i:e,n,x	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Give	CDC495; CDC77	E1 (O:3,10)	3,{10}{15}{15,34}:l,v:1,7	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	London	NRL700	E1 (O:3,10)	3,{10}{15}:l,v:1,6	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Weltevreden	CDC147	E1 (O:3,10)	3,{10}{15}:r:z6	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Orion	CDC321	E1 (O:3,10)	3,{10}{15}{15,34}:y:1,5	E1 (O:3,10)	+/+/+	Yes	
S.e. enterica	Pietersburg	CDC2258	E1 (O:3,10)	3,{10}{15,34}:z69:1,7	E1 (O:3,10)	+/+/+	Yes	

S.e. enterica	Senftenberg	CDC87; DSM10062	E4 (O:1,3,19)	1,3,19:g,[s],t:-	E4 (O:1,3,19)	+/+/+	No	Westhampton
S.e. enterica	Westerstede	CDC607	E4 (O:1,3,19)	1,3,19:l,z13:1,2	E4 (O:1,3,19)	+/+/+	Yes	
S.e. enterica	Missouri	CDC2309	F (O:11)	11:g,s,t:-	F (O:11)	+/+/+	Yes	
S.e. enterica	Connecticut	CDC2392	F (O:11)	11:l,z13,z28:1,5	F (O:11)	+/+/+	Yes	
S.e. enterica	Rubislaw	CDC102; SGSC2511	F (O:11)	11:r:e,n,x	F (O:11)	+/+/+	Yes	
S.e. enterica	Mississippi	CDC154	G (O:13)	1,13,23:b:1,5	G (O:13)	+/+/+	Yes	
S.e. enterica	Havana	NRL607	G (O:13)	1,13,23:f,g,[s]:-	G (O:13)	+/+/+	Yes	
S.e. enterica	Idikan	CDC1690	G (O:13)	1,13,23:i:1,5	G (O:13)	+/+/+	Yes	
S.e. enterica	Kedougou	CDC1523	G (O:13)	1,13,23:i:l,w	G (O:13)	+/+/+	Yes	
S.e. enterica	Poona	CDC1243	G (O:13)	1,13,22:z:1,6	G (O:13)	+/+/+	Yes	
S.e. enterica	Cubana	CDC207	G (O:13)	1,13,23:z29:-	G (O:13)	+/+/+	Yes	
S.e. enterica	Ajiobo	CDC527	G (O:13)	13,23:z4,z23:-	G (O:13)	+/+/+	Yes	
S.e. indica	6,14:a:e,n,x	DSM14848	G (O:13)	6,14:a:e,n,x	G (O:13)	+/+/+	Yes	
S.e. enterica	Blijdorp	CDC765	H (O:6,14)	1,6,14,25:c:1,5	H (O:6,14)	+/+/+	Yes	
S.e. enterica	Carrau	CDC93	H (O:6,14)	6,14,[24]:y:1,7	H (O:6,14)	+/+/+	Yes	
S.e. enterica	Grancanaria	CDC2506	I (O:16)	16:z39:[1,6]	I (O:16)	+/+/+	Yes	
S.e. enterica	Cerro	CDC990087	K (O:18)	6,14,18:z4,z23:[1,5]	K (O:18)	+/+/+	Yes	
S.e. enterica	Pomona	CDC2473A	M (O:28)	28:y:1,7	M (O:28)	+/+/+	Yes	
S.e. enterica	Morocco	CDC694	N (O:30)	30:l,z13,z28:e,n,z15	N (O:30)	+/+/+	Yes	
S.e. enterica	Ealing	CDC745	O (O:35)	35:g,m,s:-	O (O:35)	+/+/+	Yes	
S.e. enterica	Alachua	CDC325	O (O:35)	35:z4,z23:-	O (O:35)	+/+/+	Yes	
S.e. enterica	Kasenyi	NRL878	P (O:38)	38:e,h:1,5	P (O:38)	+/+/+	Yes	
S.e. enterica	Lansing	CDC634	P (O:38)	38:i:1,5	P (O:38)	+/+/+	Yes	
S.e. enterica	Inverness	CDC171	P (O:38)	38:k:1,6	P (O:38)	+/+/+	Yes	
S.e. enterica	Gera	CDC1316	T (O:42)	1,42:z4,z23:1,6	T (O:42)	+/+/+	Yes	
S.e. enterica	Niederoderwitz	CDC2579	U (O:43)	43:b:-	U (O:43)	+/+/+	Yes	
S. bongori	66:z41:-	DSM13774	O:66	66:z41:-	O:66	+/+/+	Yes	