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Manual

E. coli SeroGenoTyping AS-1 Kit

Array Hybridisation Assay for DNA-based serogenotyping of *Escherichia coli*

Kit order number: 246300096

96 reactions (**ArrayStrip** format)

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

CONTENT

BACKGROUND

The Alere **E. coli SeroGenoTyping AS-1 Kit** allows DNA-based serogenotyping of most known O- and H-antigens.

RNA-free, unfragmented genomic DNA from pure and monoclonal *E. coli* 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 producing single stranded (ss) DNA reaction products. This allows 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 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 265 probes for different genetic markers and a biotin staining (positive) and buffer (negative) control. All probes for serogenotyping are printed in two duplicate spots.

Targets include a variety of species and serotyping markers including genes encoding 93 different O-antigens and 47 H-antigens (see also PROBE TO TARGET TABLE, page 40). Based on a digital image of the arrays, spot recognition is performed automatically and results are given as an HTML-file with a description of each analysed target.

GENERAL INSTRUCTIONS FOR USE

Intended Use

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

This kit allows genotypic characterisation of *E. coli* 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.

Specifications

Upon receipt, the kit components need to be stored at different temperatures as specified on the package insert. The assay is to be performed at an ambient temperature of 18 °C to 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 assay is intended for use by personnel trained in microbiological and molecular methods. Preparation of DNA from pure *E. coli* 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 *E. coli* DNA may be processed without further biosafety precautions, although contamination with *E. coli* or other bacteria needs to be ruled out.**

- **Always wear protective clothing as required for laboratory work according to your specific regulations on laboratory safety.**

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

Kit Components, Storage and Stability

All reagents are provided in surplus (see below). If necessary, all components may be ordered separately. Please refer to the catalogue reference numbers (Cat#) 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 short term shipment (< 1 week) at ambient temperature (< 37 °C). The assay components with limited stability are D1 and C3. The other kit components proved to be stable six months after post expiry.

Cell Lysis (optional order)

- A1: Lysis Buffer (Cat# 245101000)
  Store at 18 to 28 °C (ambient temperature). Surplus: 50 %.
- A2: Lysis Enhancer (lyophilised, Cat# 245102000)
  Store at 18 to 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 %.
- $B3^{Ec01}$: lyophilised Labelling Primermix, two tubes,
  dilute each in 70 µl molecular grade water. Store at -20 °C. Surplus: 50%

Hybridisation and Detection

- ArrayStrips (12 x 8 samples),

  Protected against light and sealed under inert gas. Store at 18 to 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 an air humidity of more than 60 % since this may irreversibly corrode the spots.*

- StripCaps (24 units)

- C1: Hybridisation Buffer

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

- C2: Washing Buffer 1

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

- C3: HRP Conjugate 100 x

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

- C4: Conjugate Buffer

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

- C5: Washing Buffer 2

  Store at 18 to 28 °C, protect against direct sunlight. Surplus: 500 %.

- D1: Horseradish Peroxidase Substrate

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

- **Optional: CM^{EDL}**: Reference DNA from *E. coli* EDL933 (GenBank accession number NC_002655.2), c_{DNA} = 0.1-0.4 µg/µl. Store at 2 to 8 °C. Sufficient for 5-6 tests.

Instrumentation and Software

- ArrayMate Reader (to be ordered separately, for details see below)
  The ArrayStrip based **E. coli SeroGenoTyping AS-1 Kit** can 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 Technologies GmbH 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://alere-technologies.com/. Support is available via cct.home@clondiag.com.

Components Required but not Provided

- Growth media for the cultivation of *E. coli.* The test should be performed with colonies harvested from 2 x TY or Columbia Blood Agar. Other rich media (e.g. Standard 1 or LB) may also suffice, but have not been tested systematically. Liquid media should not be used because contaminations or mixed cultures cannot be ruled out easily.
- Equipment and consumables needed for the cultivation of *E. coli* (incubator, inoculation loops, Petri dishes)
- DNA preparation kits:
  The assay has been tested using the DNeasy Blood & Tissue Kit by Qiagen (Cat# 69504) and High Pure DNA Isolations Kit from Roche (Cat# 11796828001).

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

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

  Please note: *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 with a heating block for microtiter plates.*
- Pipettes: Suitable for 1 µl-5 µl volumes, 90 µl, 100 µl, 200 µl, 1000 µl
- Multichannel pipettes for 100-200 µl
- Sterile 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).

PROTOCOLS

Culturing and Harvesting Bacterial Cells

Members of the genus *Escherichia* 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 *E. coli* on 2 x TY or Columbia Blood agar (overnight at 37 °C or 48 h at room temperature). Obtain confirmation of the identification as *E. coli* (API, VITEK, MALDI) and make sure that you have a pure, monoclonal *E. coli* culture. Contamination by other bacteria, especially other *Enterobacteriaceae,* must be strictly avoided.

DNA Extraction

The required sample type for the **E. coli SeroGenoTyping AS-1 Kit** 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 RNA-free and it should not be fragmented. This can be determined by agarose gel electrophoresis.

Additionally, the microarray includes probes to internally check for RNA contamination. The automatic software analysis will give an “invalid” if high RNA contamination occurred during DNA isolation.

DNA **should not be** prepared by disrupting *E. coli* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. Most performance problems with the **E. coli SeroGenoTyping AS-1 Kit** are due to insufficient amounts or quality of DNA preparation. We therefore strongly recommend to obey the protocols outlined below.

Please note: To yield more genomic DNA, we recommend the optional cell lysis step with A1 / A2 reagent.

DNA extraction using spin columns produce higher amounts of pure DNA, which can be stored (4-8 °C) whereas DNA extraction with the fast and economic boiling procedure results in DNA for direct subsequent use without storage.

DNA Extraction by Spin Columns (e.g. Qiagen)

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

Loop empty Loop full

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

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

Optional cell lysis with A1/A2 reagent (instead of 1x 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 culture material of the E. coli isolate to this A1 / A2 reagent and vortex thoroughly.*
- *Incubate the culture material of the E. coli isolate in A1 / A2 for 30-60 min at 37 °C and 550 rpm in the thermoshaker.*

- Proceed with the DNA preparation protocol of the DNA preparation kit. For the Qiagen DNeasy Blood&Tissue Kit it is as follows:
- Add 20 µl proteinase K (from Qiagen Kit, or equivalent) and add 200 µl buffer AL (Qiagen Kit).
- Vortex shortly or shake vigorously.
- Incubate for 30-60 min at 56 °C and 550 rpm in the thermoshaker.

- **Important**: If A1/A2 reagent is not used, add now 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 tube content (including any precipitate) into a spin column that is placed in a 2 ml collection tube.
- Centrifuge (8,000 rpm, 1 min) at room temperature. Time and speed need to be determined depending on the sample viscosity and the type of centrifuge used. All liquid should be collected in the collection tube afterwards.
- Discard collection tube 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 tube 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 (not provided with the kit).
- Add 100 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 (8000 rpm, 1 min) at room temperature.

- Optional: Add another 100 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.

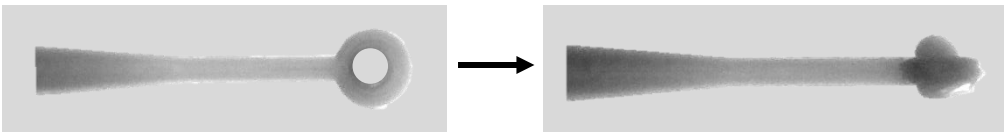
Please note: Ethanol from Washing Buffers strongly inhibits the enzymes used in the assay. Contamination with Washing Buffer might occur during the elution of prepared DNA by drops adhering to the spin columns funnels. Therefore these funnels should be gently touched and dried with sterile filter paper or wipes prior to the elution step. Alternatively, prepared DNA can be heated shortly 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 lower than 0.1 µg / µl. The concentration might be increased by heating and evaporating 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 boiling 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 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 directly 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 $B3^{Ec01}$ Labelling-Primermix and 0.1 µl of B2 (DNA polymerase) per sample.
- Add 5 µl of *E. coli* DNA (c_{DNA}=0.1-0.4 µg/µl) prepared as described above to 5 µl of the Master Mix ($B1^{+}$ / B2 / $B3^{Ec01}$). Do **not forget** to label the vial!
- Perform amplification in a pre-programmed thermocycler (e.g., Eppendorf Mastercycler gradient with heated lid) 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 they will be used.

Please note: *When using a different device, some adaptations, such as an increase of the number of cycles, might be necessary. Before establishing routine use, please test the protocol with a few known reference strains and the control DNA (CM) supplied with the kit.*

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, only use 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 und leave them 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.

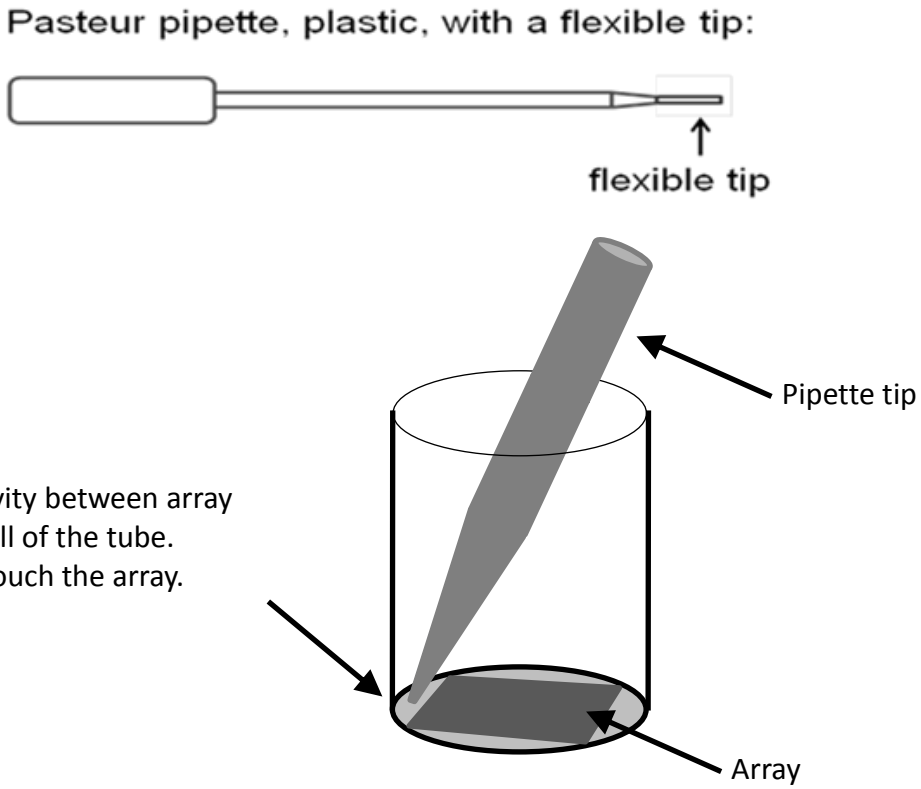
Avoid contact of data matrix barcode with organic solvents! The ArrayMate needs the information encoded in the data matrix to perform the assay and the analysis afterwards.

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 suited best (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 errors.



General Remarks - The Substrate (Precipitating Dye) D1

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

Triggered by peroxidase, the dye precipitates in 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 the appropriate equipment for heating. Because of a possibly inhomogeneous distribution of the temperature within the heating block and because of possible differences between displayed and actual temperatures, the use of different brands of thermoshakers might affect test performance. We tested the assay with BioShake iQ by QInstruments (see picture below) (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayStrips and Eppendorf's Thermomixer Comfort, equipped with a heating block for microtiter 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^{EDL} (*E. coli* EDL933 ATCC700927). The difference between the protocols for QInstrument´s BioShake iQ and Eppendorf's Thermomixer Comfort with microtiter plate adapter is only the washing temperature after the hybridisation step.

Please note: The Quantifoil's BioShake iQ has no active cooling function. Please use the second pre-temperatured passive cooling-block to reduce the incubation temperature quickly.

Protocol for Quantifoil's BioShake iQ

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

- Switch on the thermoshaker and let it pre-heat to 50 °C.
- Remove the amount of ArrayStrip(s) needed from the pouch.
- Insert the ArrayStrip(s) into the white frame. Assure the correct orientation (data matrix barcode close to row A) and proper fit.
- Pre-wash the array(s) in two steps:
  - First, PCR-grade distilled water, 200 µl per well at 50 °C, 5 min at 550 rpm. Remove the water from the well.
  - Second, C1 Hybridisation Buffer, 150 µl per well at 50 °C, 5 min at 550 rpm.
- Add 90 µl of C1 buffer to each tube with 10 µl labelled amplification product, mix gently.
- Remove the buffer from the well and add the mixture of C1 and labelled amplification product.
- Incubate at 50 °C, 60 min at 550 rpm.
- **Meanwhile, login to the ArrayMate device and prepare your worklist (see section "Data Analysis" p. 22)**
- Remove the liquid and add 200 µl C2 Washing Buffer. Incubate at 45 °C, 10 min at 550 rpm, remove and discard.

- Add another 200 µl C2 Washing Buffer. Incubate at 45 °C, 10 min at 550 rpm.
- Meanwhile, prepare conjugate: For each experiment add 1 µl C3 conjugate 100 x HRP to 100 µl C4 Conjugation Buffer. This mixture is stable for one working day at room temperature; C3 is delivered with a surplus of 100 %, C4 with a surplus of 200 %.

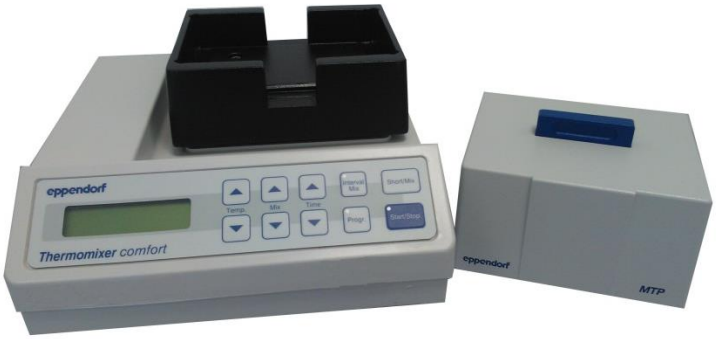
  Suggested pipetting scheme:

	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 the Washing Buffer, and add 100 µl diluted conjugate to each well, incubate at 30 °C, 10 min at 550 rpm.
- Remove the conjugate (C3 / C4), add 200 µl C5 Washing Buffer. Incubate at 30 °C, 5 min at 550 rpm.
- Remove the Washing Buffer, add 100 µl of D1 (HRP substrate, precipitating dye, at 25 °C, see above) per well.
- Incubate at 25 °C for 10 min *but* ***do not shake!***
- Remove liquid completely.
- The bottom of the ArrayStrips (outside surface) may be cleaned cautiously with wipes. Bubbles may be removed by removing and adding D1.
- Scan and process (ArrayMate, see below).

Please note: *Check immediately all images for cleanliness (i.e., absence of dust particles, residual liquids) and for good focus. Dust particles and residual fluids inside the vial can be removed by cautiously washing twice with 200 µl PCR-grade distilled water. If necessary, scan and process again (For Troubleshooting see p. 29 and 38).*

Protocol for Eppendorf's Thermomixer Comfort with Microtiter Plate Adapter



Eppendorf Thermomixer with thermoblock for MTPs and deepwell plates.

- Switch on the thermoshaker and let it pre-heat to 50 °C.
- Remove the amount of ArrayStrip(s) needed from the pouch.
- Insert the ArrayStrip(s) into the white frame. Assure the correct orientation (data matrix barcode close to row (A) and proper fit.
  - First, add PCR-grade distilled water, 200 µl per well at 50 °C, 5 min at 550 rpm. Remove the water from the well.
  - Second, add C1 Hybridisation Buffer, 150 µl per well at 50 °C, 5 min at 550 rpm.
- Add 90 µl of C1 buffer to each PCR tube with 10 µl labelled amplification product, mix gently.
- Remove the buffer from the well and add the mixture of C1 and labelled amplification product.
- Incubate at 50 °C, 60 min at 550 rpm.
- **Meanwhile, login to the ArrayMate device and prepare your worklist (see section "Data Analysis" p. 22)**
- Remove the liquid and add 200 µl C2 Washing Buffer. Incubate at 50 °C, 10 min at 550 rpm, remove and discard.
- Add another 200 µl C2 Washing Buffer. Incubate at 50 °C, 10 min at 550 rpm.

- Meanwhile, prepare conjugate: For each experiment add 1 µl C3 conjugate 100 x HRP to 100 µl C4 Conjugation Buffer. This mixture is stable for 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 the Washing Buffer, and add 100 µl diluted conjugate to each well, incubate at 30 °C, 10 min at 550 rpm.
- Remove the conjugate (C3/C4), add 200 µl C5 Washing Buffer. Incubate at 30 °C, 5 min at 550 rpm.
- Remove the Washing Buffer, add 100 µl of D1 (HRP substrate, precipitating dye, at 25 °C, see above) per well.
- Incubate at 25 °C for 10 min *but* ***do not shake!***
- Remove liquid completely.
- The bottom of the ArrayStrips (outside surface) may be cleaned cautiously with wipes. Bubbles may be removed by removing and adding D1.
- Scan and process (ArrayMate, see below).

Please note: *Check immediately all images for cleanliness (i.e., absence of dust particles, residual liquids) and for good focus. Dust particles and residual fluids inside the vial can be removed by cautiously washing twice with 200 µl PCR-grade distilled water. If necessary, scan and process again (For Troubleshooging see p. 29 and 38).*

Data Analysis

Starting the ArrayMate Reader

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

Please note that 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 is on the right hand side below the screen).
- Log-in 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-in as **User**, you will obtain only raw values, but neither positives/negatives interpretation nor strain assignment. The **Administrator** log-in will allow the installation of a new assay specific plug-in, which can be downloaded at http://alere-technologies.com (see p. 31).
- The user interface will be loaded, the ArrayMate performs internal testing. It requires slightly less than a minute.
- Click **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**).

Worklist

A **Worklist** file allows linking an identifier such as a laboratory or sample number to a position of an array within the ArrayStrip. For privacy reasons, arrays should not be identified by patient names. 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 that have headers written in the first line. The following headers are obligatory (in this order): position / sampleID / assayID (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 IDs are strain/sample/laboratory numbers such as exported from your LIMS (or assigned in any different way). Patients' names should not be used as sample IDs.
- The Assay ID allows the system to identify the current test and to correctly use information on layout, spot number, and identity etc. The **E. coli SeroGenoTyping AS-1 Kit** has the Assay ID: **10630**. ***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.
- You may use the software tool **Worklist Generator** to create a worklist easily.

  http://alere-technologies.com/en/products/lab-solutions/software-tools/worklist-generator.html

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

position	sampleID	assayID
1	2015-12345	10630
2	2015-12346	10630
3	2015-12347	10630
4	2015-12348	10630
5	2015-12349	10630
6	2015-12350	10630
7	987654	10630
8	*E. coli* EDL933	10630

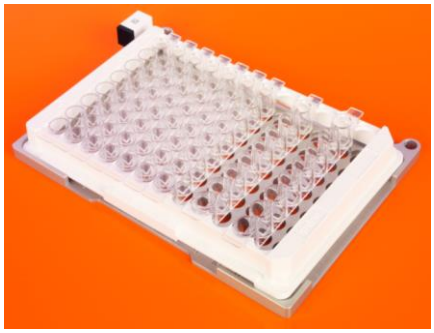
Table 2: Positions in the 96 well format

	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 flash drive containing the worklist into any of the USB ports on the lower right-hand side of the ArrayMate.
- Press  ; a folder selection dialogue will open.
- Select your worklist (path: ‘My Computer/Removable Disk’).
- Open your selected worklist by pressing **Enter** or **Open**.
- Press  (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 **OK**; the worklist window will close.

- Leave the flash drive in the ArrayMate if you intend to export **E. coli SeroGenoTyping AS-1 Kit** reports afterwards (Check the flash drive regularly for computer viruses and malware using an appropriate program.).
- Press **Next** (at the bottom right on the screen; reader is opening).
- Carefully insert the appropriate metallic adapter/frame into the ArrayMate. Do not apply strong force. Ensure proper fit, otherwise the images may be out of focus.
- Carefully insert the white frame with the array strips into the metallic adapter. Ensure the correct orientation (Position A1 in the frame next to the data matrix barcode on the adapter) and proper fit; otherwise the images may be out of focus.



ArrayStrip frame with inserted strips. Strips are 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 Array StripCaps on the wells to be analysed (however, unused wells should remain capped).

- Press **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 is opening).
- Remove the white frame with the ArrayStrip(s).

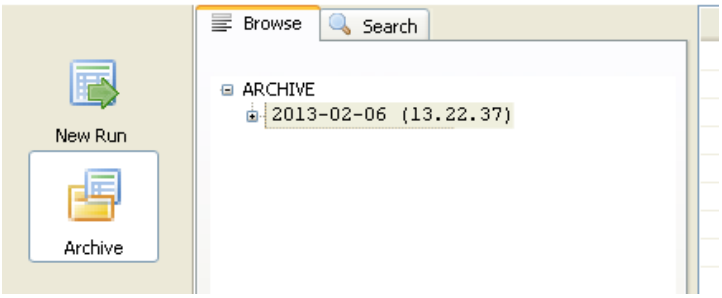
- Press **Next** (at the bottom right on the screen; reader is closing).

Results

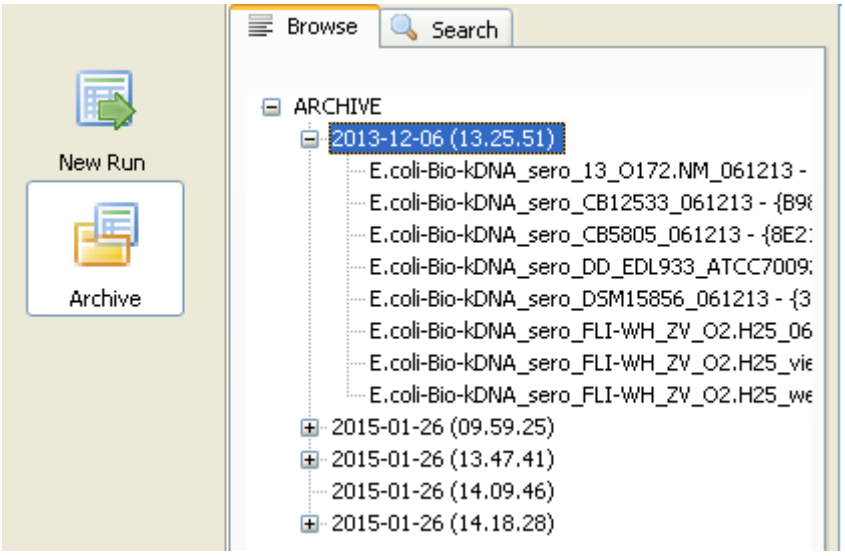
On the left-hand side of the screen, there you will see a list showing all runs stored on the ArrayMate´s hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not displayed:

- Press **Archive** (left hand side) and activate the flag **Browse** (at the top left).
- The runs are organised like folders in **Windows Explorer,** and named **by default** according to the date of acquisition.

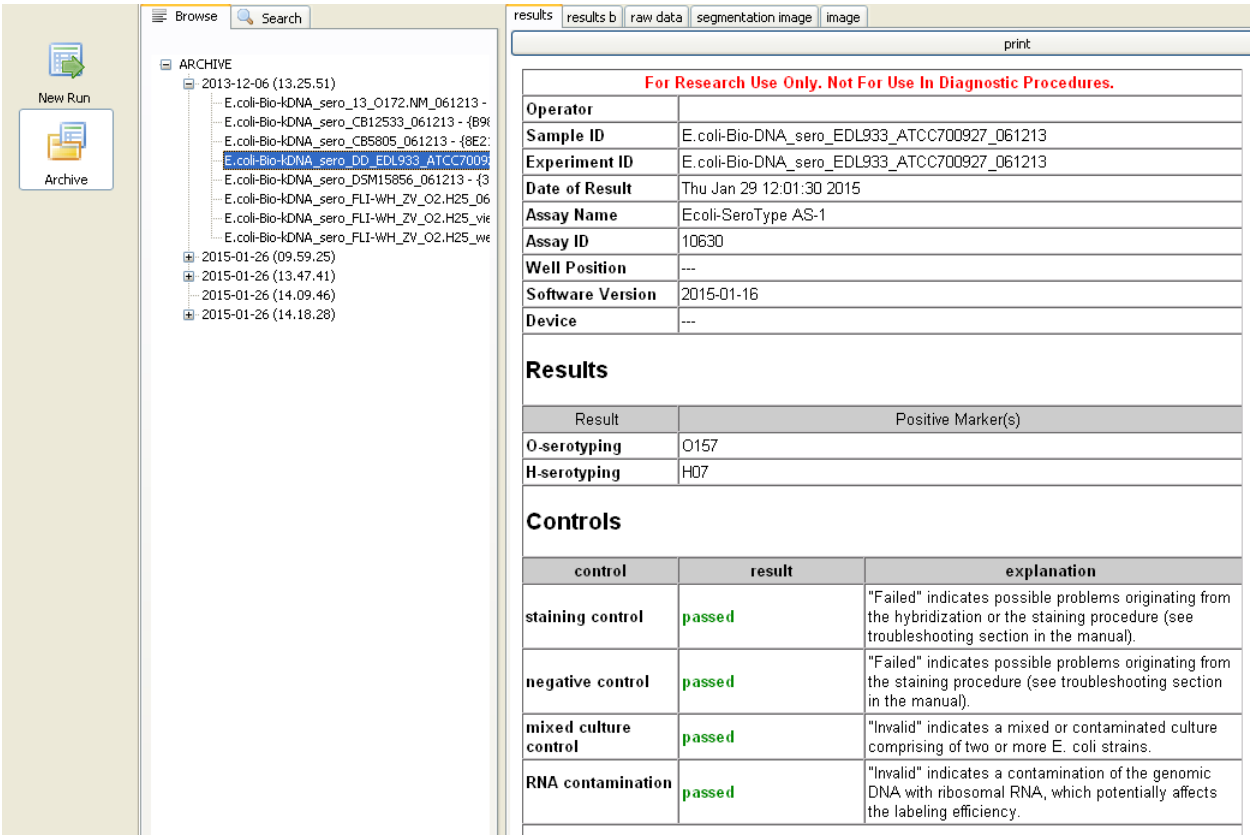
Example: There is one experiment run in this archive:



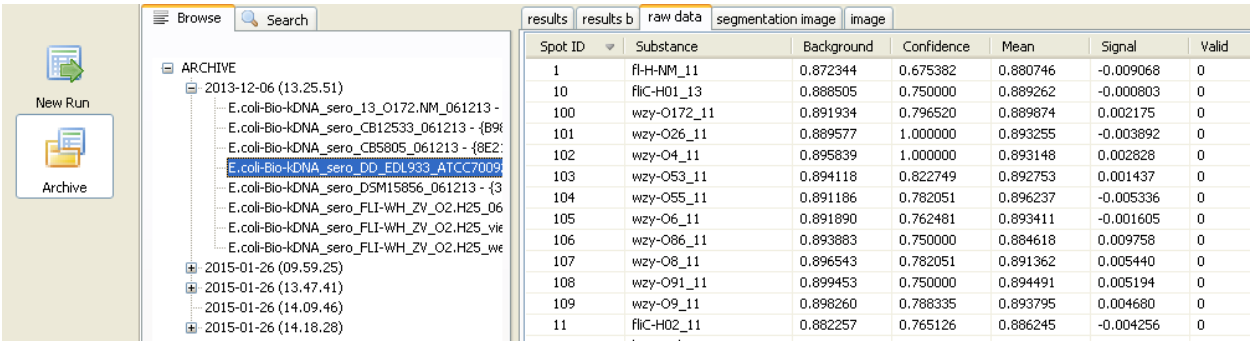
If you click on the plus symbol left to the run name, the folder opens and you will see a list of the individual arrays alphabetically ordered by Sample ID.



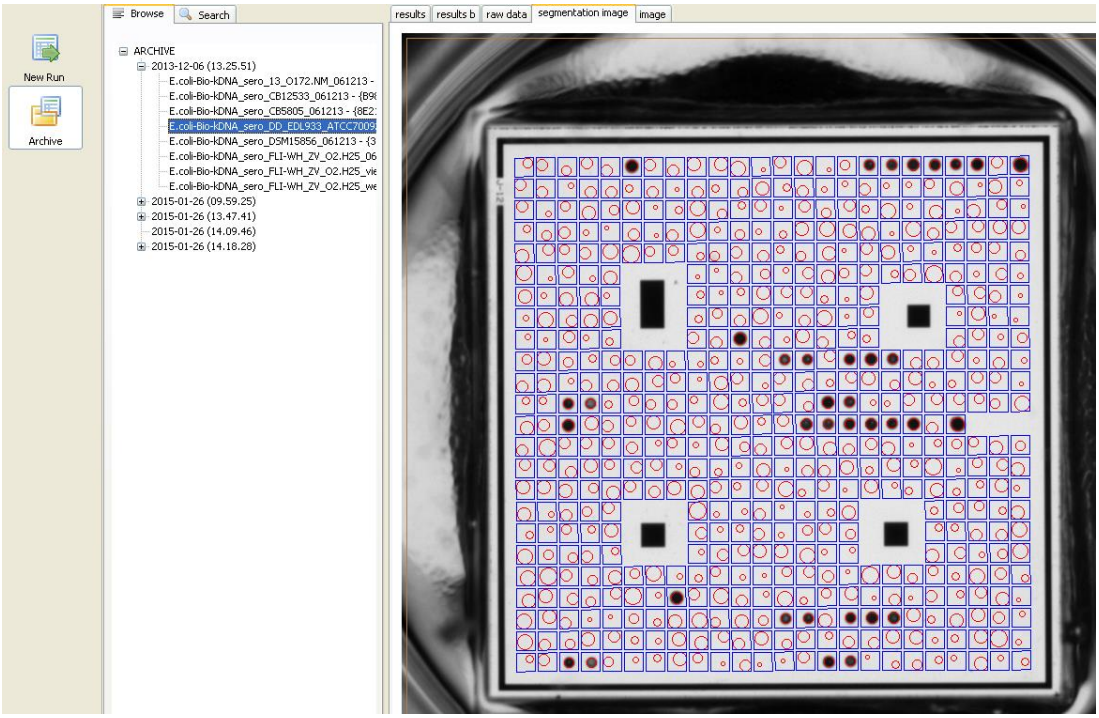
Click on a Sample ID and the **E. coli SeroGenoTyping AS-1 Kit** Test Report for this array is shown in the window on the right:



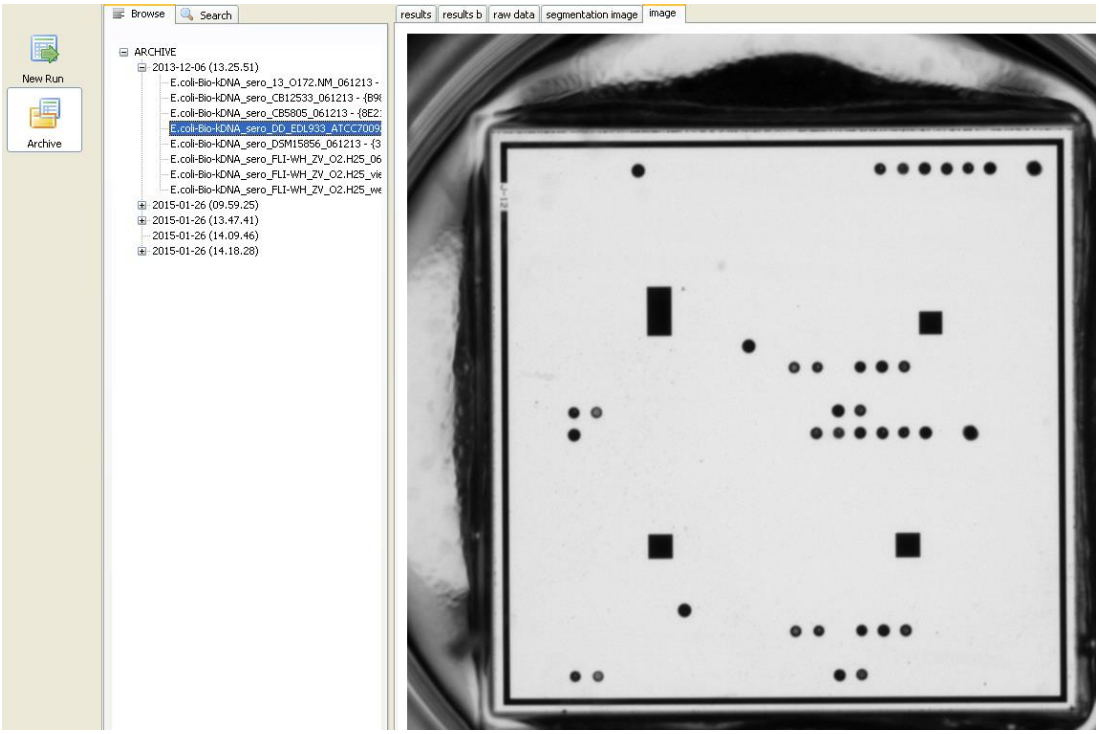
Click on flag raw data and the raw data is shown in the window on the right:



Click on flag segmentation image and the grid alignment accuracy is shown in the window on the right:



Click on flag image and the image file (*.bmp) is shown in the window on the right:



This image of the reference isolate *E. coli* EDL 933 shows an example for a valid test without any dust particles or non-specific background.

The image file is automatically analysed be the ArrayMate software and a HTML report is provided that lists all markers that have been analysed.

Export of E. coli SeroGenoTyping AS-1 Kit Test Reports

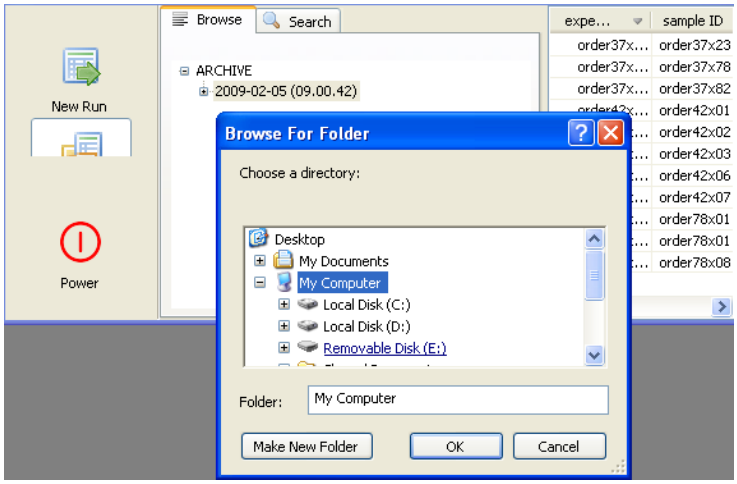
The generated result files in an html format will show information of all target genes. Possible invalid controls that might display in this report will be explained below (see Troubleshooting).

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

- A *.txt file with the raw measurements,
- An image file (*.bmp) with 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 (*.xml) files that contains the same information as the html result sheets for future export into databases etc.

*Please note: Only complete runs can be exported. The export of individual **E. coli 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**, then **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 **Ok** (data are exported into the new folder on your flash drive).
- Do **NOT** remove the flash drive as long as the hourglass symbol is visible.
- Switch off the device by clicking **Power** (at the bottom left on the screen): 
- Switch off the screen. There is no need to physically switch off the ArrayMate Reader.

TROUBLESHOOTING

When troubleshooting, always make sure that reagents are within the recommended shelf-life and stored appropriately.

Should you encounter a problem, we will always be happy to support you. Please e-mail to cct.home@clondiag.com and include a description of the problem as well as the array images (*.bmp files) in 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 C2 buffer to remove all C1 buffer 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 Control material (CM^{EDL}). This is DNA from the *E. coli* EDL933 reference strain (GenBank accession number NC_002655.2), which should be identified by the assay as *E. coli* with O157 and H7. If the control experiment yields a valid result and a correct identification, there was probably an issue with DNA preparation. If the control experiment also fails, an error affecting later steps or a degradation of reagents from later steps is likely.

See also Appendix 2 – Images for troubleshooting (p. 38 and 39).

DNA Quality and RNA Contamination Control

The amount of DNA is crucial because of the linear kinetics of amplification (see Introduction). DNA should be RNA-free as free RNA reduces the amplification and labelling efficiency 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 contaminations are a prerequisite for proper DNA concentration measurement. Therefore, RNase treatment is necessary prior to A_{260} reading (component A2 contains RNAse).
DNA must be unfragmented as fragmentation reduces the amplification and labelling efficiency due to the distance between primer and probe binding sites. For this reason DNA should not be prepared by disrupting *E. coli* cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. We made good experiences with positive experiences when using the manual QIAGEN DNeasy Kit and the Roche High Pure Kit.
DNA must be free of any trace of ethanol as ethanol has a strong impact on the amplification. It is possible to heat the sample prior to adding it to the labelling mix (5-10 minutes at 70 °C) in order to evaporate any traces of ethanol.

Physical Damage to the Array

Scratching 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 will not be 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 was 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.

Ambiguous Results

Apart from a “positive” or “negative” result for the individual markers on **E. coli SeroGenoTyping AS-1** Test Report, the result can also be “ambiguous”.

This can be caused by poor sample quality, poor signal quality or less then optimal stringency of the hybridisation process (temperatures in hybridisation process are too low or too high).

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 °C - 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 reason 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. The assay must not be used as a substitute for phenotypic susceptibility tests.

We shall not be held liable for damages caused by an 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 *E. coli* 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 for good performance and correctness of results using standard *E. coli* DNA preparations.

List of Components for Separate Order

If required, these reagents for the **E. coli SeroGenoTyping AS-1 Kit** may be ordered separately:

component	name	amount	cat#	storage
A1	Lysis Buffer	30 ml	245101000	18-28 °C
A2	Lysis Enhancer	96 units	245102000	18-28 °C
$B1^{+}$	Labelling Buffer	550 µl	245103000	2-8 °C
B2	Labelling Enzyme	20 µl	245104000	2-8 °C
$B3^{Ec01}$	E. coli_sero1_AS Primermix	70 µl / tube	246303500	2-8 °C
C1	Hybridisation Buffer	30 ml	245105000	18-28 °C
C2	Washing Buffer 1	120 ml	245106000	18-28 °C
C3	HRP Conjugate 100x	200 µl	245107000	2-8 °C
C4	Conjugate Buffer	30 ml	245108000	18-28 °C
C5	Washing Buffer 2	120 ml	245109000	18-28 °C
D1	HRP Substrate	15 ml	245110000	2-8 °C
CM^{EDL}	E. coli EDL933 DNA (c_{DNA} = 0.1-0.4 µg/µl)	30 µl	245911000	2-8 °C
ArrayStrip	E. coli SeroGenoTyping AS-1	1 strip	240009681	15-28 °C
StripCap	StripCap	24 units	245112000	15-28 °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 e-mail to cct.home@clondiag.com

LITERATURE

Anjum MF, Mafura M, Slickers P, Ballmer K, Kuhnert P, et al. (2007) Pathotyping *Escherichia coli* by using miniaturized DNA microarrays. Appl Environ Microbiol 73: 5692-5697.

Anjum MF, Tucker JD, Sprigings KA, Woodward MJ, Ehricht R (2006) Use of miniaturized protein arrays for *Escherichia coli* O serotyping. Clin Vaccine Immunol 13: 561-567.

Ballmer K, Korczak BM, Kuhnert P, Slickers P, Ehricht R, et al. (2007) Fast DNA serotyping of *Escherichia coli* by use of an oligonucleotide microarray. J Clin Microbiol 45: 370-379.

Geue L, Monecke S, Engelmann I, Braun S, Slickers P, et al. (2013) Rapid Microarray-Based DNA Genoserotyping of *Escherichia coli*. Microbiol Immunol. 2013 Dec 3. doi: 10.1111/1348-0421.12120.

Geue L, Schares S, Mintel B, Conraths FJ, Muller E, et al. (2010) Rapid microarray-based genotyping of enterohemorrhagic *Escherichia coli* serotype O156:H25/H-/Hnt isolates from cattle and clonal relationship analysis. Appl Environ Microbiol 76: 5510-5519.

Korczak B, Frey J, Schrenzel J, Pluschke G, Pfister R, et al. (2005) Use of diagnostic microarrays for determination of virulence gene patterns of *Escherichia coli* K1, a major cause of neonatal meningitis. J Clin Microbiol 43: 1024-1031.

Monecke S, Mariani-Kurkdjian P, Bingen E, Weill FX, Baliere C, et al. (2011) Presence of enterohemorrhagic *Escherichia coli* ST678/O104:H4 in France prior to 2011. Appl Environ Microbiol 77: 8784-8786.

Schilling AK, Hotzel H, Methner U, Sprague LD, Schmoock G, et al. (2012) Zoonotic agents in small ruminants kept on city farms in southern Germany. Appl Environ Microbiol 78: 3785-3793.

Wu G, Ehricht R, Mafura M, Stokes M, Smith N, et al. (2012) *Escherichia coli* isolates from extraintestinal organs of livestock animals harbour diverse virulence genes and belong to multiple genetic lineages. Vet Microbiol 160: 197-206.

For further literature please refer to:

http://alere-technologies.com/en/science-technologies/publications/downloads.html.

UPDATES AND SOFTWARE

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

http://alere-technologies.com/en/products/lab-solutions/e-coli/e-coli-serotyping.html

http://alere-technologies.com/en/products/lab-solutions/software-tools.html

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

Currently available freeware programs are:

- Alere **Result Collector** for the conversion of multiple *result.xml files from the ArrayMate into spreadsheet tables. This should make it easier to compare isolates or to determine relative abundances of genes or strains etc.
- Alere **Worklist Generator** is a tool which helps you to create a well formatted worklist for the Arraymate.
- Alere **Report Generator** is a software tool to create reports using the assay software normally used and installed on the ArrayMate. It uses an image taken by the ArrayMate or a txt file (raw signal data file) and generates a report from the raw signal data.

APPENDIX 1 – FLOW CHARTS

The figure summarise the test procedure for the thermoshaker **BioShake iQ by Quantifoil**. Please always refer to the text section of this manual for further important details.

Protocol : Quantifoil BioShake iQ

prepare ArraysStrips	prepare DNA	pro-cessing time	hands-on-time
	grow CLONAL *E. coli* isolate (not part of the kit)	over night	5 min
	↓		
	isolate genomic DNA (not part of the kit)	3 h	40 min
	↓		
	label RNA free DNA in thermocycler 5 µl DNA (c_{DNA} = 0.1 - 0.4 µg/µl) plus Mastermix (3.9 µl **B1^{+}** + 0.1 µl **B2** + 1 µl **B3^{Ec01}**)	3 h	5 min
	↓		
rinse ArrayStrips 200 µl water; 50 °C, 550 rpm, 5 min	**prepare labeled DNA** to 10 µl of labeled DNA add 90 µl of **Buffer C1**	15 min	2 min
↓			
discard water; 150 µl **Buffer C1**; 50 °C, 550 rpm, 5 min discard C1, process promptly			
transfer 100 µl labeled DNA to ArrayStrips		2 min	2 min
Barcode / Label here			
hybridise; 50 °C, 550 rpm, 60 min **Meanwhile**: *Login to the ArrayMate device and prepare your worklist.*		60 min	10 min
↓			
discard labeled DNA; incubate twice in 200 µl **Buffer C2**; 45 °C, 550 rpm, 10 min; prepare **C3/C4-Conjugate** (C3:C4=1:100), preheat **Substrate D1** (25°C)		20 min	5 min
↓			
discard **Buffer C2**; incubate in 100 µl **C3/C4-Conjugate**; 30 °C, 550 rpm, 10 min		15 min	2 min
↓			
discard **C3/C4-conjugate**; incubate once in 200 µl **Buffer C5**; 30 °C, 550 rpm, 5 min		5 min	2 min
↓			
discard **Buffer C5**; incubate with 100 µl **Substrate D1**; 25 °C, 10 min		15 min	2 min
↓			
discard **Substrate D1;** analyse (ArrayMate)		10 min	5 min
	total time requirement :	**over night + app. 8 h**	**app. 120 min**

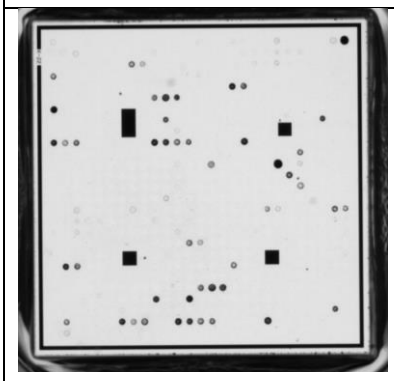
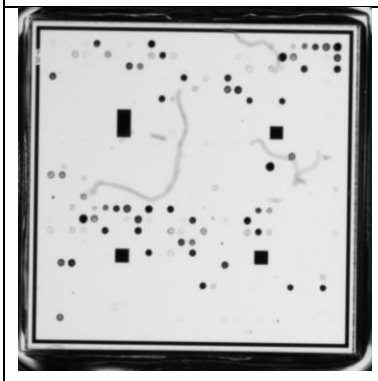
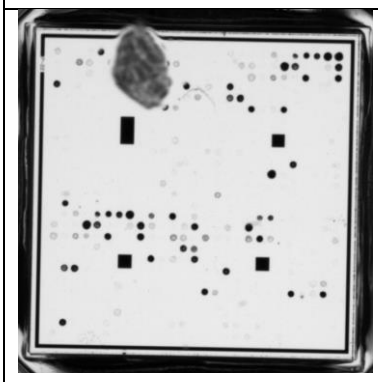
The figure summarise the test procedure for the thermoshaker **Thermomixer Comfort by Eppendorf** (with heating block for microtiter plates). Please always refer to the text section of this manual for further important details.

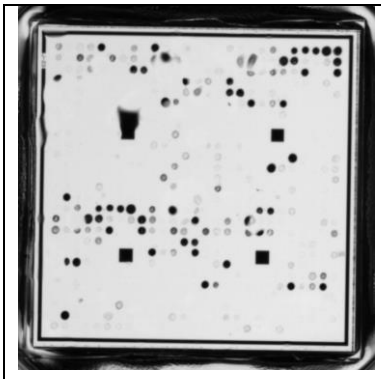
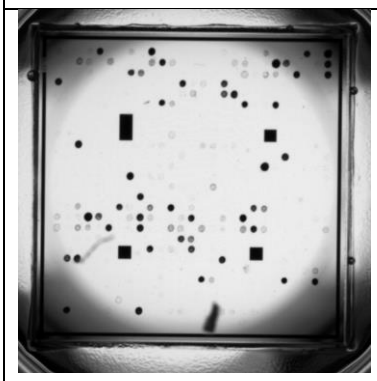
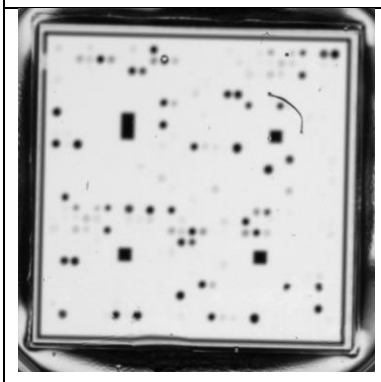
Protocol : Eppendorf Themoshaker comfort[a)]

prepare ArraysStrips	prepare DNA	pro-cessing time	hands-on-time
	grow CLONAL *E. coli* isolate (not part of the kit)	over night	5 min
	↓		
	isolate genomic DNA (not part of the kit)	3 h	40 min
	↓		
	label RNA free DNA in thermocycler 5 µl DNA (c_{DNA} = 0.1 - 0.4 µg/µl) plus Mastermix (3.9 µl **B1**$^{+}$ + 0.1 µl **B2** + 1 µl **B3**^{Ec01})	3 h	5 min
rinse ArrayStrips 200 µl water; 50 °C, 550 rpm, 5 min	↓		
discard water; 150 µl **Buffer C1**; 50 °C, 550 rpm, 5 min discard C1, process promptly	**prepare labeled DNA** to 10 µl of labeled DNA add 90 µl of **Buffer C1**	15 min	2 min
transfer 100 µl labeled DNA to ArrayStrips		2 min	2 min
hybridise; 50 °C, 550 rpm, 60 min **Meanwhile**: *Login to the ArrayMate device and prepare your worklist.*		60 min	10 min
↓			
discard labeled DNA; incubate twice in 200 µl **Buffer C2**; 50 °C, 550 rpm, 10 min; prepare **C3/C4-Conjugate** (C3:C4=1:100), preheat **Substrate D1** (25°C)		20 min	5 min
↓			
discard **Buffer C2**; incubate in 100 µl **C3/C4-Conjugate**; 30 °C, 550 rpm, 10 min		15 min	2 min
↓			
discard **C3/C4-conjugate**; incubate once in 200 µl **Buffer C5**; 30 °C, 550 rpm, 5 min		5 min	2 min
↓			
discard **Buffer C5**; incubate with 100 µl **Substrate D1**; 25 °C, 10 min		15 min	2 min
↓			
discard **Substrate D1;** analyse (ArrayMate)		10 min	5 min
	total time requirement :	**over night + app. 8 h**	**app. 120 min**

[a)] with heating block for microtiter plates

APPENDIX 2 – IMAGES FOR TROUBLESHOOTING

Image	Comment	Handling
	Valid experiment.	Valid results, no error messages.
	The bottom of the well is contaminated with dust particles.	Please clean the bottom of the well, scan and process again.
	The microarray surface is contaminated with dust particles.	If the microarray surface is contaminated with particles, wash the microarray with double distilled water (pipetting water carefully up and down, remove), scan and process again.

	The bottom of the well is contaminated with a liquid (e.g. buffer).	Please clean the bottom surface with a cleanroom wipe, scan and process again.
	Substrate D1 residue in the array edges.	Remove substrate D1 completely with a Pasteur pipette.
	Signal intensity is too low.	This could be due to low DNA concentration, fragmented DNA, ethanol trace contaminations in DNA sample or expired reagents. The experiment should be repeated with a new DNA preparation. If this also fails, try an experiment with EDL933 control DNA (CM, available on request).
	Chip was not in focus during image acquisition.	Repeat image acquisition after fitting the ArrayStrip in the frame.

APPENDIX 3 – PROBE TO TARGET TABLE

Target genes	Probes	Function
Family and Species Marker		
gad	gad_10	glutamate decarboxylase (AE014075.1, locus tag c4328) - genus-specific marker for Escherichia/Shigella
dnaE	hp_dnaE_612, hp_dnaE_613	DNA polymerase III subunit alpha - genus-specific marker for Escherichia/Shigella (U00096.3)
rrs	hp_rrs_611, hp_rrs_612	16S rRNA - species-specific marker for Escherichia coli (U00096.3)
gapA	prob_gapA_611	glyceraldehyde 3-phosphate dehydrogenase A (CP000468.1, locus tag APECO1_847)- family-specific marker for Enterobacteriaceae
O-serotyping		
O1	hp1021|wzx_O001, hp1087|wzy_O001	O-antigen - O1 (GU299791.1)
O2	hp1022|wzx_O002, hp1088|wzy_O002	O-antigen - O2 (GU299792.1)
O3	hp1023|wzx_O003, hp1089|wzy_O003	O-antigen - O3 (EU694097.1)
O4	hp1024|wzx_O004, wzy-O4_11	O-antigen - O4 (U39042.1; AY568960.1)
O6	wzx-O6_11, wzy-O6_11	O-antigen - O6 (AJ426045.2; CP000247.1)
O7	wzx-O7_11, hp1090|wzy_O007	O-antigen - O7 (AB490074.1)
O8	hp1012|wzm_O008, hp1015|wzt_O008, wzx-O8_11, wzy-O8_11	O-antigen - O8 (AF013583.1)
O9	hp1013|wzm_O009, hp1016|wzt_O009, wzx-O9_11, wzy-O9_11	O-antigen - O9 (wzm, wzt - D43637.1; wzx - AF104912.2; wzy - AB031867.1)
O11	hp1025|wzx_O011, hp1091|wzy_O011	O-antigen - O11 (HQ388393.1)
O13; O129; O135	hp1026|wzx_O013+O129+O135, hp1092|wzy_O013+O129+O135	O-antigen - O13; O129; O135 (EU296422.1; EU296423.1; EU296424.1)
O15	wzx-O15_11, wzy-O15_11	O-antigen - O15 (CP002291.1) (CP002291.1)
O17; O44; O73; O77; O106	hp1027|wzx_O017+O044+O073+O077 +O106, hp1093|wzy_O017+O044+O073+O077 +O106	O-antigen - O17; O44; O73; O77 or O106 (CU928163.2; FN554766.1; DQ000313.1; DQ000314.1; DQ000315.1)
O18	hp1028|wzx_O018, hp1094|wzy_O018	O-antigen - O18 (GU299793.1)
O21	hp1167|wzx_O021, hp1095|wzy_O021	O-antigen - O21 (EU694098.1)
O22	hp1030|wzx_O022, hp1096|wzy_O022	O-antigen - O22 (DQ851855.1)
O24	hp1164|wzx_O024, hp1097|wzy_O024	O-antigen - O24 (DQ220292.1)
O25	hp1032|wzx_O025, hp1098|wzy_O025	O-antigen - O25 (GU014554.1)
O26	wzx-O26_11, wzy-O26_11	O-antigen - O26 (AF529080.1)

O27	hp1033|wzx_O027, hp1099|wzy_O027	O-antigen - O27 (GU014555.1)
O28; O42	hp1034|wzx_O028+O042, hp1100|wzy_O028+O042	O-antigen - O28; O42 (DQ462205.1; FJ539194.1)
O29	hp1035|wzx_O029, hp1101|wzy_O029	O-antigen - O29 (EU294173.1)
O32	hp1036|wzx_O032, hp1102|wzy_O032	O-antigen - O32 (EU296410.1)
O35	hp1037|wzx_O035, hp1103|wzy_O035	O-antigen - O35 (FJ940774.1)
O40	hp1038|wzx_O040, hp1104|wzy_O040	O-antigen - O40 (EU296417.1)
O45	hp1039|wzx_O045, hp1105|wzy_O045	O-antigen - O45 (CU463050.1)
O52	wzm-O52_11, hp1017|wzt_O052	O-antigen - O52 (AY528413.1)
O53	hp1040|wzx_O053, wzy-O53_11	O-antigen - O53 (AF402312.1)
O55	wzx-O55_11, wzy-O55_11	O-antigen - O55 (AF461121.1)
O56	hp1041|wzx_O056, hp1106|wzy_O056	O-antigen - O56 (DQ220293.1)
O58	hp1042|wzx_O058, hp1107|wzy_O058	O-antigen - O58 (EU294175.1)
O63	hp1043|wzx_O063, hp1108|wzy_O063	O-antigen - O63 (FJ539195.1)
O66	hp1044|wzx_O066, hp1109|wzy_O066	O-antigen - O66 (DQ069297.1)
O70	hp1045|wzx_O070, hp1110|wzy_O070	O-antigen - O70 (FN995094.1)
O71	hp1159|wzx_O071, hp1111|wzy_O071	O-antigen - O71 (GU445927.1)
O75	hp1047|wzx_O075, hp1112|wzy_O075	O-antigen - O75 (GU299795.1)
O78	hp1048|wzx_O078, hp1113|wzy_O078	O-antigen - O78 (FJ940775.1)
O79	wbdU-O79_11, wzx-O79_11, hp1114|wzy_O079	O-antigen - O79 (EU294162.1)
O81	hp1049|wzx_O081	O-antigen - O81 (CU928162.2)
O83	hp1050|wzx_O083, hp1050|wzx_O083	O-antigen - O83 (CP001855.1)
O85	hp1051|wzx_O085, hp1116|wzy_O085	O-antigen - O85 (GU299798.1)
O86	wzx-O86_11, wzy-O86_11	O-antigen - O86 (AY670704.1)
O87	hp1052|wzx_O087, hp1117|wzy_O087	O-antigen - O87 (EU294177.1)
O91	wzx-O91_11, wzy-O91_11	O-antigen - O91 (AY035396.1)
O98	hp1054|wzx_O098, hp1118|wzy_O098	O-antigen - O98 (DQ180602.1)
O99	hp1014|wzm_O099, hp1018|wzt_O099	O-antigen - O99 (FJ940773.1)
O101	wz-O101_11, wbdA-O9_11	O-antigen - O101 (wzt - AFAH02000001.1; wbdA - AB031867.1)
O103	wzx-O103_11, wzy-O103_11	O-antigen - O103 (AY532664.1)
O104	wzx-O104_11, wzy-O104_11	O-antigen - O104 (AF361371.1)
O105	hp1055|wzx_O105, hp1119|wzy_O105	O-antigen - O105 (EU294171.1)
O107; O117	hp1056|wzx_O107+O117, hp1120|wzy_O107+O117	O-antigen - O107; O117 (EU694095.1; EU694096.1)
O109	hp1057|wzx_O109, hp1121|wzy_O109	O-antigen - O109 (HM485572.1)
O111	wbdH-O111_11, wbdM-O111_11, wzx-O111_11, wzy-O111_11	O-antigen - O111 (AF078736.1)
O112	hp1059|wzx_O112, hp1123|wzy_O112, hp1058|wzx_O112ac, hp1122|wzy_O112ac	O-antigen - O112 (EU296413.1)
O113	hp1060|wzx_O113, wzy-O113_11	O-antigen - O113 (AF172324.1)
O114	wzx-O114_11, wzy-O114_11	O-antigen - O114 (AY573377.1)

O115	hp1061|wzx_O115, hp1124|wzy_O115	O-antigen - O115 (GQ499339.1)
O118; O151	hp1062|wzx_O118+O151, hp1125|wzy_O118+O151	O-antigen - O118; O151 (DQ990684.1; DQ990685.1)
O119	hp1063|wzx_O119, hp1126|wzy_O119	O-antigen - O119 (GQ499368.1)
O121	wzx-O121_11,wzy-O121_11	O-antigen - O121 (AY208937.1)
O123	hp1064|wzx_O123, hp1127|wzy_O123	O-antigen - O123 (DQ676933.1)
O124; O164	hp1065|wzx_O124+O164, hp1128|wzy_O124+O164	O-antigen - O124; O164 (EU296419.1; EU296420.1)
O126	hp1066|wzx_O126, hp1129|wzy_O126	O-antigen - O126 (DQ465248.1)
O127	hp1067|wzx_O127, hp1130|wzy_O127	O-antigen - O127 (AY493508.1)
O128	wzx-O128_11, wzy-O128_11	O-antigen - O128 (AY217096.1)
O130	hp1068|wzx_O130, hp1131|wzy_O130	O-antigen - O130 (EU296421.1)
O138	hp1069|wzx_O138, hp1132|wzy_O138	O-antigen - O138 (DQ109551.1)
O139	hp1070|wzx_O139, hp1133|wzy_O139	O-antigen - O139 (DQ109552.1)
O141	hp1071|wzx_O141, hp1134|wzy_O141	O-antigen - O141 (DQ868765.1)
O143	hp1072|wzx_O143, hp1135|wzy_O143	O-antigen - O143 (EU294164.1)
O145	hp1073|wzx_O145, hp1136|wzy_O145	O-antigen - O145 (AY863412.1)
O146	hp1074|wzx_O146, hp1166|wzy_O146	O-antigen - O146 (DQ465249.1)
O147	hp1075|wzx_O147, hp1138|wzy_O147	O-antigen - O147 (DQ868766.1)
O148	hp1076|wzx_O148, hp1139|wzy_O148	O-antigen - O148 (DQ167407.1)
O149	hp1077|wzx_O149, hp1140|wzy_O149	O-antigen - O149 (DQ091854.2)
O150	hp1155|wzx_O150, hp1142|wzy_O150	O-antigen - O150 (EU294168.1)
O152	hp1081|wzx_O152, hp1144|wzy_O152	O-antigen - O152 (EU294170.1)
O157	rfbE-O157_11, wzx-O157_11, hp1145|wzy_O157	O-antigen - O157 (AB008676.1)
O159	hp1082|wzx_O159, hp1146|wzy_O159	O-antigen - O159 (EU294176.1)
O167	hp1083|wzx_O167, hp1147|wzy_O167	O-antigen - O167 (EU296408.1)
O168	hp1084|wzx_O168, hp1148|wzy_O168	O-antigen - O168 (EU296403.1)
O172	wzx-O172_11, wzy-O172_11	O-antigen - O172 (AY545992.1)
O174	hp1085|wzx_O174, hp1149|wzy_O174	O-antigen - O174 (DQ008592.1)
O177	hp1086|wzx_O177, hp1150|wzy_O177	O-antigen - O177 (DQ008593.1)
H-serotyping		
H01	fliC-H01_11, fliC-H01_12	flagellin C H-antigen H01 (AE014075.1)
H02	fliC-H02_11	flagellin C H-antigen H02 (AF543692.1)
H03	flkA-H03_11	flagellin A H-antigen H03 (AB128916.1) (Note: If repressor FljA "positive", the gene fliC H16 is repressed; H-phenotype is H03)
H04	fliC-H04_11	flagellin C H-antigen H04 (AY249989.1)
H05	fliC-H05_11	flagellin C H-antigen H05 (AY249990.1)
H06	fliC-H06_11	flagellin C H-antigen H06 (AY249991.1)

H07	fliC-H07_11, fliC-H07_12	flagellin C H-antigen H07 (AB028474.1)
H08	fliC-H08_11	flagellin C H-antigen H08 (AJ884571.1)
H09	fliC-H09_11	flagellin C H-antigen H09 (AY249994.1)
H10	fliC-H10_11	flagellin C H-antigen H10 (AY249995.1)
H11	fliC-H11_11	flagellin C H-antigen H11 (AY337465.1)
H12	fliC-H12_11	flagellin C H-antigen H12 (AY249997.1)
H14	fliC-H14_11	flagellin C H-antigen H14 (AY249998.1)
H15	fliC-H15_11	flagellin C H-antigen H15 (AY249999.1)
H16	fliC-H16_11	flagellin C H-antigen H16 (AB128919.1), Note: If repressor fljA "positive", fliC H16 might be repressed, H-phenotype is than determined by flkA H03.
H18	fliC-H18_11	flagellin C H-antigen H18 (AY250001.1)
H19	fliC-H19_11	flagellin C H-antigen H19 (AY250002.1)
H20	fliC-H20_11	flagellin C H-antigen H20 (AY250003.1)
H21	fliC-H21_11	flagellin C H-antigen H21 (DQ862122.1), Note: If repressor fljA "positive", fliC H21 might be repressed, H-phenotype is than determined by flmA H54 or flkA H53.
H23	fliC-H23_11	flagellin C H-antigen H23 (AY250005.1)
H24	fliC-H24_11	flagellin C H-antigen H24 (AY250006.1)
H25	fliC-H25_11	flagellin C H-antigen H25 (ADUP01000024.1)
H26	fliC-H26_11	flagellin C H-antigen H26 (AY250008.1)
H27	fliC-H27_11	flagellin C H-antigen H27 (CU928162.2)
H28	fliC-H28_11	flagellin C H-antigen H28 (AY250010.1)
H29	fliC-H29_11	flagellin C H-antigen H29 (AY250012.1)
H30	fliC-H30_11	flagellin C H-antigen H30 (AY250011.1)

H31	fliC-H31_11	flagellin C H-antigen H31 (AY250013.1)
H32	fliC-H32_11	flagellin C H-antigen H32 (AY250014.1)
H33	fliC-H33_11	flagellin C H-antigen H33 (AY250015.1)
H34	fliC-H34_11	flagellin C H-antigen H34 (AY250016.1)
H37	fliC-H37_11	flagellin C H-antigen H37 (AY250017.1)
H38	fliC-H38_11	flagellin C H-antigen H38 (AY250018.1)
H39	fliC-H39_11	flagellin C H-antigen H39 (AY250019.1)
H40	fl-H40_11	flagellin C H-antigen H40 (AJ865464.1), Note: If repressor fljA "positive", fliC H40 might be repressed, H-phenotype is than determined by flkA H53.
H41	fliC-H41_11	flagellin C H-antigen H41 (AY250020.1)
H42	fliC-H42_11	flagellin C H-antigen H42 (AY250021.1)
H43	fliC-H43_11	flagellin C H-antigen H43 (AY250022.1)
H45	fliC-H45_11	flagellin C H-antigen H45 (AY250023.1)
H46	fliC-H46_11, fliC-H46_12	flagellin C H-antigen H46 (AY250024.1)
H48	fliC-H48_11	flagellin C H-antigen H48 (AY250025.1)
H49	fliC-H49_11	flagellin C H-antigen H49 (AY250026.1)
H51	fliC-H51_11	flagellin C H-antigen H51 (AY250027.1)
H52	fliC-H52_11	flagellin C H-antigen H52 (AY250028.1)
H53	flkA-H53_11	flagellin A H-antigen H53 (AB128917.1) (Note: If repressor FljA "positive", the genes fliC H40 or fliC H21 are repressed; H-phenotype is H53))
H54	flmA-H54_11	flagellin A H-antigen H54 (AB128918.1) (Note: If repressor FljA "positive", the gene fliC H21 is repressed; H-phenotype is H54)
H56	fliC-H56_11	flagellin C H-antigen H56 (AY250029.1)

H-serotyping (additional marker)		
fliC Marker	fliC-5p_11, fliC-5p_12, fliC-5p_13, fliC-5p_14, fliC-5p_15	fliC marker - consensus sequence for all fliC genes
fljA Repressor	fljA_11	FljA represses the expression of fliC genes, if "positive" the genes flkA (H03 or H53) or flmA (H54) represent the H-serotype (AB128916.1)
fliC non-motile	fl-H-NM_11	flagellin C from non-motile isolates (AY337480.1)

APPENDIX 4 – TYPING INFORMATION

Definitions and Explanations

The displayed result will yield the following typing information:

- Discrimination of the 93 described O-serotypes is mainly determined by the genes *wzy* (polymerase) and *wzx* (flippase). The 47 known H-antigens are encoded by the gene *fliC*.
- The probes immobilized on the current array version can discriminate 93 O-antigens:

specific probes O-typing:

O:1, O:2, O:3, O:4, O:6, O:7, O:8, O:9, O:11, O:15, O:18, O:21, O:22, O:24, O:25, O:26, O:27, O:29, O:32, O:35, O:40, O:45, O:52, O:53, O:55, O:56, O:58, O:63, O:66, O:70, O:71, O:75, O:78, O:79, O:81, O:83, O:85, O:86, O:87, O:91, O:98, O:99, O:101, O:103, O:104, O:105, O:109, O:111, O:112, O:113, O:114, O:115, O:119, O:121, O:123, O:126, O:127, O:128, O:130, O:138, O:139, O:141, O:143, O:145, O:146, O:147, O:148, O:149, O:150, O:152, O:157, O:159, O:167, O:168, O:172, O:174, O:177

grouped (consensus) probes O-typing:

{O:13, O:129, O:135}
{O:17, O:44, O:73, O:77, O:106}
{O:28, O:42}
{O:107, O:117}
{O:118, O:151}
{O:124, O:164}

- The following flagellar antigens can be identified on the array:

H:01, H:02, H:03, H:04, H:05, H:06, H:07, H:08, H:09, H:10, H:11, H:12, H:14, H:15, H:16, H:18, H:19, H:20, H:21, H:23, H:24, H:25, H:26, H:27, H:28, H:29, H:30, H:31, H:32, H:33, H:34, H:37, H:38, H:39, H:40, H:41, H:42, H:43, H:45, H:46, H:48, H:49, H:51, H:52, H:53, H:54, H:56

- The mix culture control is based on 5 probes located in consensus sequences on the 5' end of the *fliC* genes. These probes were divided into two groups which are correlated with two groups of *fliC* genes (fliC-5p_11, fliC-5p_12 and fliC-5p_13, fliC-5p_14, fliC-5p_15). Only one group should be positive, if not an *E. coli* mix culture was probed.
- The gene *fljA* encodes for a repressor protein which repressed some of *fliC* genes. If the *fljA* probe positive may two H- serotype probes are positive. In this case the genes *flkA* and *flmA* encodes for the H-serotype. Such strains are described as biphasic (Wang *et al.* 2003, Tominaga 2004).
- Probes specifying *gapA, gad* and *dnaE* that were introduced to confirm the identity of *E. coli* and to serve as genus-specific controls.