Instructions for use

eazyplex® EHEC

Molecular biological rapid test for the detection of enterohaemorrhagic *Escherichia coli*

for use with Genie® II Mk2 devices

For in vitro diagnostic use

CE

eazyplex® EHEC complete	REF: 7655
eazyplex® EHEC basic	REF: 7656
eazyplex® EHEC basic plus	REF: 7657
eazyplex® EHEC classic	REF: 7658
eazyplex® EHEC classic plus	REF: 7659
eazyplex® EHEC expert	REF: 7660
eazyplex® EHEC expert plus	REF: 7661
language: english	valid from: April 2019

Explanation of symbols

IVD	in vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by
	Temperature limitation
TESTSTRIP	Teststrip
	Contains sufficient for <n> tests
	Consult instructions for use
	No re-use
	Manufacturer

Document Revision Information:
centrifuge added (4.2), software version actualised (4.2), „select the test manually“ deleted (8.1), Documentation under 9. amended by PDF report and CSV file, Warning messages inserted under 9.

1. Intended Use

eazyplex® EHEC is a qualitative in vitro diagnostic medical device for the detection of different kind of pathovars of *Escherichia coli* and their pathogenic factors in stool samples or bacterial colonies.

The following variants are available:

	EHEC complete	**EHEC basic**	**EHEC basic plus**	**EHEC classic**	**EHEC classic plus**	**EHEC expert**	**EHEC expert plus**
REF	**7655**	**7656**	**7657**	**7658**	**7659**	**7660**	**7661**
Verotoxin 1	x	x	x	x	x	x	x
Verotoxin 2	x	x	x	x	x	x	x
Intimin	x			x	x	x	x
Haemolysin	x					x	x
EIEC/ Shigella	x						
EAggEC	x						
Verotoxin 2f	x		x		x		x

The test can be performed at any time by qualified professional staff in a medical laboratory. The intended use includes:

- screening of symptomatic patients (acute diarrhea) via stool samples.
- diagnosis (confirmatory assay to verify results of previous testing) and aid to diagnosis (providing additional information to assist in the determination or verification of a patient´s clinical status, test is not the sole determinant) of all kind of patients via testing bacterial colonies as well as stool samples.

2. Pathovars of *Escherichia coli*

Escherichia coli (*E. coli*) are gram negative, facultatively anaerobic rod bacteria, which move by peritrichal flagellation and belong to the Enterobacteriaceae family. *E. coli* are part of the normal intestinal flora of humans and many farm animals and are generally nonpathogenic. Some *E. coli* strains are pathogenic to humans through the acquisition of certain pathogenic factors (e.g. genes for toxins).
The six known intestinal pathogenic *E. coli*: enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) can be differentiated by the specific pathogenic factors.

a) enterohaemorrhagic *E. coli* (EHEC)

Enterohaemorrhagic *E. coli* (EHEC) are currently the most important intestinal pathogenic *E. coli*. Every year about 1000 cases of illness due to an infection with enterohaemorrhagic *E. coli* (EHEC) are reported in Germany.
EHEC are a subgroup of the Shiga toxin or Verotoxin producing *E. coli* (STEC or VTEC) and are capable to produce two cytotoxins, Verotoxin 1 and 2. Due to the similarity of the Verotoxins to the Shiga toxin of *Shigella dysenteriae*, the VTEC are also called STEC.
The clinical symptoms which are caused by EHEC range from mild diarrhoeas and severe gastroenteritis to haemorrhagic colitis which occurs in approx. 10 to 20 % of cases of infection. With 5 -10 % of infections, in babies and small children in particular as well as old patients or patients with weakened immune systems, this may also lead to a hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) as a life-threatening post-infectious complication. With HUS and TTP, mortality is particularly high among infants (approx. 10 - 15%). Acute kidney failure with a temporary need for dialysis or an irreversible loss of the kidney function resulting in a constant need for dialysis may occur. The incubation period is approximately 2 to 10 days.
Because of the high environmental resistance and the infective dose for EHEC is only at about 100 organisms. Sources of infection are contaminated foods from cattle, sheep or goats, particularly raw meat or meat products which have not been heated sufficiently, non-pasteurised raw or certified milk and contaminated fruits and vegetables. Infective chains from human to human, particularly in communal facilities such as Kindergartens, homes for the elderly or hospitals, as well as direct contacts to animals are also important.

A further characteristic of EHEC is the production of haemolysin, which causes a specific haemolysis on blood agar plates and which is termed the enterohaemolytic phenotype. In this connexion, after an 18- to 24-hour incubation, small cloudy haemolytic halos are formed around the colonies. In wild type bacteria, the EHEC haemolysin responsible for this is a pore-forming cytotoxin that occurs both cell-associated and, in lower amounts, extracellularly. The genes, including *hly*A, that are responsible for the synthesis, activation and transport of EHEC haemolysin were identified by Karch et al. (for example, 6) and could be detected in 94.3 % of the EHEC isolates from HUS patients and in 72.7 % of the enteritis cases. These genes are located on the approximately 90 kb-large virulence plasmid (pO157), which is found in almost all EHEC and was implicated through its pathogenetic properties.

A further variant of Verotoxin 2 (so-called stx2f) was isolated first in 2000 out of pigeon faeces. Later on, it was observed sporadically within human infections, especially in the Netherlands.

b) Enteropathogenic E. coli (EPEC)

Enteropathogenic *E. coli* (EPEC) cause diarrhea, particularly in infants. Intimin is a virulence factor of EPEC and EHEC, coded by the eae gene. It is a protein which serves for the attachment on intestinal epithelial cells and –together with other virulence factors- is responsible for the development of haemorrhagic *Escherichia coli* enteritis. Thus, it is a bacterial adhesin, which is expressed on the bacterial cell surface. Intimin binds via the Tir (Translocated intimin receptor) to the host cell. Tir is secreted from the bacterium and inserted into the plasma membrane oft he host cell. Intimin-Tir interaction mediates tight binding of the bacteria to intestinal epithelial cells via formation of so-called "pedestals".

c) Enteroinvasive *E. coli* (EIEC)

Enteroinvasive E. coli (EIEC) are responsible for Shigellose-like disease in developing countries and among travellers to these less developed regions. EIEC strains are biochemically and genetically related to Shigella spp. The pathogenic features of EIEC and Shigella spp. are based on plasmid-mediated capability to invade the colonic epithelium for destruction. By the detection of the ipaH gene (invasion plasmid antigen H gene) EIEC/Shigella spp. can be differentiated from ETEC. Shigellose caused by EIEC is characterized by abdominal pain and watery diarrhea, sometimes containing blood. Sources of infection are contaminated water, food and infective chains from human to human.

d) Enteroaggretative *E. coli* (EAEC or EAggEC)

Infections with EAEC are poorly investigated. EAEC causes persistent diarrhea in children and is the effective agent for traveller´s diarrhea in North Africa and Central America. Pathogenesis of EAEC is not completely clarified at present, but the molecular biological detection of a distinct pathogenic plasmid (pCVD 432) serves as reliable diagnostic marker for EAEC. Normally, the genes aatA or aggR serve as target genes.

3. Principle of the test

A single **eazyplex®** test strip contains six oligonucleotide primers in each filled cap and these provide the means for simultaneous, specific amplification of different genes in a single isothermal amplification reaction. In the presence of relevant DNA sequences, specific amplification products are generated and visualised by real-time fluorescence measurement of a fluorescence dye bound to double-stranded DNA. Thus, positive signals indicate the presence of the different specific *E. coli* associated pathogenic factors in the sample to be investigated.
Data interpretation is based on an algorithm in the eazyReport™ software.

The following protective mechanisms prevent the use of false results:
Performance of “inhibition control” with each sample prevents the use of false negative test results due to inhibition of the amplification reaction and simultaneously serves as reagent control.
As required, a test strip can be processed as negative / contamination control by testing **RALF** without addition of sample material. In this case, only the inhibition control is allowed to create a positive signal.

eazyplex® EHEC basic:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Verotoxin 1	stx1 (all variants)	**red**
2	Verotoxin 2	stx2 (-a;-b;-c;-d;-e)	**orange**
3	Inhibition control	Inhibition control	**yellow**
4-8	-	-	

eazyplex® EHEC basic plus:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Verotoxin 1	stx1 (all variants)	**red**
2	Verotoxin 2	stx2 (-a;-b;-c;-d;-e)	**orange**
3	Inhibition control	Inhibition control	**yellow**
4	Verotoxin 2f	stx2f	**light green**
5-8	-	-	

eazyplex® EHEC classic:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Verotoxin 1	stx1 (all variants)	**red**
2	Verotoxin 2	stx2 (-a;-b;-c;-d;-e)	**orange**
3	Intimin	eae	**yellow**
4	Inhibition control	Inhibition control	**light green**
5-8	-	-	

eazyplex® EHEC classic plus:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Verotoxin 1	stx1 (all variants)	**red**
2	Verotoxin 2	stx2 (-a;-b;-c;-d;-e)	**orange**
3	Intimin	eae	**yellow**
4	Inhibition control	Inhibition control	**light green**
5	Verotoxin 2f	stx2f	**dark green**
6-8	-	-	

eazyplex® EHEC expert:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Verotoxin 1	stx1 (all variants)	**red**
2	Verotoxin 2	stx2 (-a;-b;-c;-d;-e)	**orange**
3	Intimin	eae	**yellow**
4	Haemolysin	hlyA	**light green**
5	Inhibition control	Inhibition control	**dark green**
6-8	-	-	

eazyplex® EHEC expert plus:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Verotoxin 1	stx1 (all variants)	**red**
2	Verotoxin 2	stx2 (-a;-b;-c;-d;-e)	**orange**
3	Intimin	eae	**yellow**
4	Haemolysin	hlyA	**light green**
5	Inhibition control	Inhibition control	**dark green**
6	Verotoxin 2f	stx2f	**turquois**
7-8	-	-	

eazyplex® EHEC complete:

tube-n°	Assay parameter (abbreviation in result display)	Specificity	Colour of curve
1	Verotoxin 1	stx1 (all variants)	**red**
2	Verotoxin 2	stx2 (-a;-b;-c;-d;-e)	**orange**
3	Intimin	eae	**yellow**
4	Haemolysin	hlyA	**light green**
5	Inhibition control	Inhibition control	**dark green**
6	EIEC / Shigella	ipaH	**turquois**
7	EAggEC	aatA	**purple**
8	Verotoxin 2f	stx2f	**pink**

4. Reagents

4.1 Content

The reagents contained in one kit are sufficient for 24 determinations.
Each kit contains:

TESTSTRIP	Test strips with 8* filled tubes, each containing lyophilized, ready-to-use mix for isothermal amplification. The mix contains DNA-polymerase, buffer components, Mg_2SO_4, dNTPs, oligonucleotide primers and a fluorescence dye.	24 strips
RALF	2ml –tubes with 500 µl „RALF - **R**esuspension **a**nd **L**ysis **F**luid“ each (ready-to-use)	24 x 500 µl

* eazyplex® EHEC complete; in the variants: 3 (eazyplex® EHEC basic), 4 (eazyplex® EHEC basic plus / classic), 5 (eazyplex® EHEC classic plus / expert) or 6 (eazyplex® EHEC expert plus).

4.2 Additional accessories required

- GENIE® II Mk 2 with eazyReport™ - software version 2.34 or higher (including Instructions for Use in PDF format)
- Heating block for 2 ml tubes
- If necessary: Centrifuge for 2 ml tubes
- Inoculation needle
- Swab with Liquid Amies Medium
- Pipettes with sterile disposable filter tips
- optional: USB bar code scanner
- optional: printer DYMO® Labelwriter 450 (Dymo) with labels 54x101mm

5. Warnings and precautions

- All reagents and materials which come into contact with potentially infectious samples must be treated with suitable disinfectant or autoclaved.

- Suitable disposable gloves must be worn during the entire test.

- **Never** open a test strip after use! Autoclave used test strips!

6. Handling notes – preparation of assay realization

The components of **eazyplex® EHEC** have to be stored from 15°C to 30°C.
The kits have an expiry date. Quality cannot be guaranteed after this date.
Before beginning the test, remove a test strip from the bag. The test strip may only be used if the white pellets in the filled tubes are visible.

As with any test procedure, good laboratory practice is essential to the proper performance of this assay.

7. Sample material

The sample material has to be:

- stool samples in Liquid Amies Medium:
Coat a swab (with Liquid Amies Medium) lightly, by dipping it carefully into three different positions of a stool sample. Put the swab into the swab tube according to the Instructions for Use of the manufacturer. Vortex briefly or shake to transfer the sample material into the liquid medium.
Optimally, the sample is not older than 12 hours.

☝ **Caution! Too much cell material on the swab may considerably reduce the effectiveness of the reaction and may lead to invalid test runs.**

- bacterial colonies from agar plates.

Any swabs, liquid culture or blood samples **CANNOT** be used.

8. Test procedure

8.1 Preparation of the system

- Turn on GENIE® II Mk2
- Touch the Screen
- Enter user name and password
- Select „Run“
- Scan test barcode via barcode scanner or enter test barcode manually
- Check display if the right test profile is selected
- Enter patient´s / sample ID (via barcode scanner or keyboard)
- Confirm the selection with “Enter”

8.2 Preparing the amplification reaction

Stool samples:

Briefly mix/vortex the Liquid Amies Medium, which contains the sample (swab, see 7.). Transfer 25 µl of the Liquid Amies Medium into 500 µl **RALF**.

☝ **Caution! Too much cell material on the swab may considerably reduce the effectiveness of the reaction and may lead to invalid test runs.**

Incubate this **RALF**-suspension at 99°C for 3 minutes for cell lysis and centrifuge the suspension at 1000x g for 1 minute afterwards.

Carefully remove the protective foil from the test strip.

Pipette 25 µl of the **RALF**-suspension onto the ready-to-use mix in each filled tube of the test strip, taking care not to allow the pipette tip to make contact with the pellet. Do not vortex, shake heavily or pipette up and down. Remove any air bubbles by tapping the test strip gently.

Once the pellets have dissolved, place the test strip immediately into the GENIE® II Mk2 device and start the run (8.3).

Bacterial colony:
Suspend a small part of a single bacterial colony in 500 µl **RALF** by an inoculation needle. As soon as a little amount of cell material is visible on the inoculation needle, it is sufficient sample material for the test.

☝ **Caution! Too much cell material may considerably reduce the effectiveness of the reaction and may lead to invalid test runs.**

Incubate this **RALF**-suspension at 99°C for 2 minutes for cell lysis.
If you wish further microbiological testing, transfer 50 µl of the cell suspension in a sterile tube **before** cell lysis.

Carefully remove the protective foil from the test strip.

Pipette 25 µl of the **RALF**-suspension onto the ready-to-use mix in each filled tube of the test strip, taking care not to allow the pipette tip to make contact with the pellet. Do not vortex, shake heavily or pipette up and down. Remove any air bubbles by tapping the test strip gently.

Once the pellets have dissolved, place the test strip immediately into the GENIE® II Mk2 device and start the run (8.3).

8.3 Realization of the amplification reaction

- Select „Start“
- Select block A or B
- Place the test strip into the selected block
- Close the lid
- Start test run by selecting „Yes“.
- If the second block is not in use, a second test run can be initiated by the button “start test run” (see 8.1)
- Note: the directory path and number of test run data file can be seen on the display
- Once the run is complete, open the lid and remove the test strip, taking care as the block could still be hot!
- **Never** open a test strip after use! Danger of contamination!

9. Evaluation

The test run can be monitored in real time mode (choose „Amplification“). Positive results are indicated by a strong rise in fluorescence signal (in the form of a typical amplification curve). Unambiguous assignment of the curves to the test parameters takes place by coloring.

After completion of the test run select „Results“:

Valid test run:
Positive results are colored red.

Invalid test run:
The result of „Inhibition Control“ is colored red (invalid). In this case, data interpretation of the eazyReport™ software is displayed above the result table as follows:
„Invalid control” (colored red)

Warning messages:
„WARNING! Kit expired!“:
A kit has been used which is out of date (see 6.).

„WARNING! Too much sample material!“:
Too much sample material was used; this could have been the reason for an invalid test run (see 11.)

Documentation:

- Result printout via Dymo® Labelwriter 450 printer: select „print“
  or
- create a PDF result report: select „PDF“; the generated PDF file is stored in the folder „Report“ on the device and can be exported via USB stick (according to Instructions for Use Genie® II Mk2).
  or
- create a result file in CSV format: select „CSV“; the generated CSV file is stored in the folder „Report“ on the device.

The stored run file can be reviewed at any time:

- select symbol „Folder“
- select „LOG“ and confirm with „✓ “
- the data files are numbered consecutively and archived according to creation date

10. Interpretation of the test results

The **eazyplex® EHEC** system is a rapid screening and confirmatory test for the specific detection of different pathovars of *Escherichia coli* and their corresponding pathogenic factors.
Positive results in the **eazyplex® EHEC** system demonstrate the presence of the respective pathogenic factor(s) of *Escherichia coli* in a sample.

The **eazyplex® EHEC** system is neither intended to diagnose an infection with *E. coli* pathovars (it cannot be distinguished between colonisation and infection) nor to guide or monitor medical treatment.
The test solely generates a test result. The attending doctor is responsible for achieving a decision about diagnosis or treatment of a patient or taking hygienic measures.

The **eazyplex® EHEC** system is able to detect some few genome copies within 25 minutes under optimal conditions. But it is not possible to detect one single copy of the respective pathovar in a stool sample. Therefore, a negative test result can be generated despite a weak colonization of the patient´s intestinal tract.

	Verotoxin 1 (stx1)	Verotoxin 2 (stx2a-f)	Intimin	Haemolysin	EIEC/Shigella (ipaH)	EaggEC (aatA)
EHEC/STEC	x*	x*	(x)	(x)		**
EPEC			x			
EIEC					x	
EAEC						x

*if at least one of the verotoxins is detected, a STEC is present.
() Intimin and/or Haemolysin can be additionally present in the case of STEC.
** probably, crossing of different pathogenic *E. coli* types may occur, as well, e.g. see statement No. 019/2011 from BfR of 2011/June/7.

11. Troubleshooting

All signals negative (incl. inhibition control):

- Reaction is inhibited due to inhibitory substances in the sample and must not be interpreted (invalid test).

- In case of testing bacterial colonies: Too much cell material was used for amplification ➔ suspend just a small part of one colony in 500 µl RALF or dilute already suspended cell material with RALF
- If assay with diluted sample is invalid again ➔ perform assay with RALF without sample
- If inhibition control is negative again ➔ device may be damaged, please contact our support team.

- In case of testing stool samples: Too much sample material was used for amplification ➔ dilute 50 µl of remaining **RALF**-suspension in 500 µl RALF
- If assay with diluted sample is invalid again ➔ perform assay with RALF without sample; if this assay with diluted sample is valid, further dilution of the sample is necessary.
- If inhibition control is negative again ➔ device may be damaged, please contact our support team.

All signals positive, incl. inhibition control (in the case of testing bacterial colonies with eazyplex® EHEC complete):

- Very occasionally cell lysis in a sample will not complete within the first 3 minutes of the test run (signals are not displayed at the beginning of the run). If cell lysis occurs later on during the amplification reaction, newly appearing dsDNA will lead to unspecific amplification signals. This phenomenon can be identified easily because it will happen simultaneously in all wells (but the IC curve or correct positive amplification curves could appear earlier). It is recommended to repeat the test run with fresh sample material with an extended incubation time of 10 minutes for complete cell lysis. If the phenomenon reoccurs, the sample should be interpreted as "invalid“ or "not valid".

12. Performance data

A) As part of a prospective evaluation study at the „Institut für medizinische Mikrobiologie des Universitätsklinikum Jena" (study coordinator: Prof. Dr. Rödel) 52 *E. coli* strains, characterized relating to their pathogenic factors, were tested in comparison to other commercial molecular biological methods. The following Analytical Performance data result:

Verotoxin 1	Reference method positive	Reference method negative	Σ	
eazyplex® EHEC positive	19	0	19	**Sensitivity: 100 %**
eazyplex® EHEC negative	0	33	33	**Specificity: 100%**
Σ	19	33	**52**	

Verotoxin 2 (ohne 2f)	Reference method positive	Reference method negative	Σ	
eazyplex® EHEC positive	15	0	15	**Sensitivity: 100 %**
eazyplex® EHEC negative	0	37	37	**Specificity: 100%**
Σ	15	37	**52**	

Intimin (eae)	Reference method positive	Reference method negative	Σ	
eazyplex® EHEC positive	25	0	25	**Sensitivity: 100 %**
eazyplex® EHEC negative	0	27	27	**Specificity: 100%**
Σ	25	27	**52**	

Haemolysin (hlyA)	Reference method positive	Reference method negative	Σ	
eazyplex® EHEC positive	3	0	3	**Sensitivity: 100 %**
eazyplex® EHEC negative	0	6	6	**Specificity: 100%**
Σ	3	6	**9**	

EIEC / Shigella (ipaH)	Reference method positive	Reference method negative	Σ	
eazyplex® EHEC positive	4	0	4	**Sensitivity: 100 %**
eazyplex® EHEC negative	0	48	48	**Specificity: 100%**
Σ	4	48	**52**	

EAggEC (aatA)	Reference method positive	Reference method negative	Σ	
eazyplex® EHEC positive	4	0	4	**Sensitivity: 100 %**
eazyplex® EHEC negative	0	48	48	**Specificity: 100%**
Σ	4	48	**52**	

Verotoxin 2f	Reference method positive	Reference method negative	Σ	
eazyplex® EHEC positive	1	0	1	**Sensitivity: 100 %**
eazyplex® EHEC negative	0	51	51	**Specificity: 100%**
Σ	1	51	**52**	

B) As part of a prospective evaluation study at the „Institut für medizinische Mikrobiologie des Universitätsklinikum Jena" (study coordinator: Prof. Dr. Rödel) a total of 216 stool samples was tested with regard to *E.coli* pathovars. The following data in comparison to other commercial molecular biological methods result:

- 1 sample showed two times an invalid test result. This corresponds to a inhibition rate of 0,46%.
- 168 samples were concordantly completely **negative**.
- **Verotoxins (1 / 2 / 2f)** have been detected in 17 samples by commercial RT-PCR. 12 of these also showed a positive result with eazyplex® EHEC (ten times VT1, two times VT2). In two out of the five discrepant cases a germ could be isolated, which was confirmed by a molecular biological reference method, as well as with eazyplex® EHEC, to be a STEC. In three cases no Verotoxin-positive isolate was found.
- **Intimin** (no screening reference method in the study) was detected 23 times with eazyplex® EHEC. In three cases, culture was not applied. In 15 out of the other 20 cases a germ could be isolated, which was diagnosed Intimin positive by commercial RT-PCR.
- **Haemolysin** (no screening reference method in the study) was detected 14 times with eazyplex® EHEC. In 12 cases, other pathogenic factors (eight times Verotoxin 1 or 2, three times Intimin and EAEC once) have been detected in parallel. Due to the lack of a commercial assay, confirmation was not possible.
- **EIEC/Shigella** (screening with commercial RT-PCR): 1 sample was concordantly positive.
- **EAEC** (no screening reference method in the study): 8 samples were positive using eazyplex® EHEC. In all cases, a germ could be isolated, which was confirmed to be EAEC by a molecular biological reference method.

13. References

1. Schmidt H, Scheef J, Morabito S, Caprioli A, Wieler L, Karch H (2000) A New Shiga Toxin 2 Variant (Stx2f) from *Escherichia coli* Isolated from Pigeons. Applied and Environmental Microbiology Mar. 2000, Vol. 66, No. 3, 1205–1208.

2. Farfan MJ, Torres AG (2012) Molecular mechanisms that mediate colonization of Shiga toxin-producing *Escherichia coli* strains. Infect Immun. 2012 Mar; 80(3):903-13.

3. van den Beld MJ, Reubsaet FA (2012) Differentiation between *Shigella*, enteroinvasive *Escherichia coli* (EIEC) and noninvasive *Escherichia coli.* Eur J Clin Microbiol Infect Dis. 2012 Jun;31(6):899-904.

4. Friesema I, van der Zwaluw K, Schuurman T, Kooistra-Smid M, Franz E, van Duynhoven Y, van Pelt W (2014) Emergence of *Escherichia coli* encoding Shiga toxin 2f in human Shiga toxin-producing E. coli (STEC) infections in the Netherlands, January 2008 to December 2011. Euro Surveill. 2014 May 1;19(17):26-32.

5. Friesema I, Keijzer-Veen MG, Koppejan M, Schipper HS, van Griethuysen AJ, Heck M and van Pelt W (2015) Hemolytic Uremic Syndrome Associated with *Escherichia coli* O8:H19 and Shiga Toxin 2f Gene. Emerg Infect Dis. 2015, Jan; 21(1):168-9.

6. www.brf.bund.de (2011) Enterohämorrhagische Escherichia coli (EHEC) O104:H4: ein erstes bakteriologisches Kurzporträt. Statement No. 019/2011 from BfR of 2011/June/7.

7. Bryan A, Youngster I, McAdam AJ (2015) Shiga Toxin Producing *Escherichia coli*. Clin Lab Med. 2015 Jun;35(2):247-72.

8. Freedman SB, Xie J, Neufeld MS, Hamilton WL, Hartling L, Tarr PI; Alberta Provincial Pediatric Enteric Infection Team (APPETITE), Nettel-Aguirre A, Chuck A, Lee B, Johnson D, Currie G, Talbot J, Jiang J, Dickinson J, Kellner J, MacDonald J, Svenson L, Chui L, Louie M, Lavoie M, Eltorki M, Vanderkooi O, Tellier R, Ali S, Drews S, Graham T, Pang XL (2016) Shiga Toxin-Producing *Escherichia coli* Infection, Antibiotics, and Risk of Developing Hemolytic Uremic Syndrome: A Meta-analysis. Clin Infect Dis. 2016 May 15; 62(10):1251-1258.

9. Köckerling E, Karrasch L, Schweitzer A, Razum O, Krause (2017) Public Health Research Resulting from One of the World's Largest Outbreaks Caused by Entero-Hemorrhagic *Escherichia coli* in Germany 2011: A Review. Front Public Health. 2017 Dec 11; 5:332

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