



**Kliniken der Stadt Köln gGmbH**



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Rapid diagnostics from patients  
suspected to suffer from  
Norovirus infection reveals  
novel EHEC variant

LIVE AUS  
JETZT

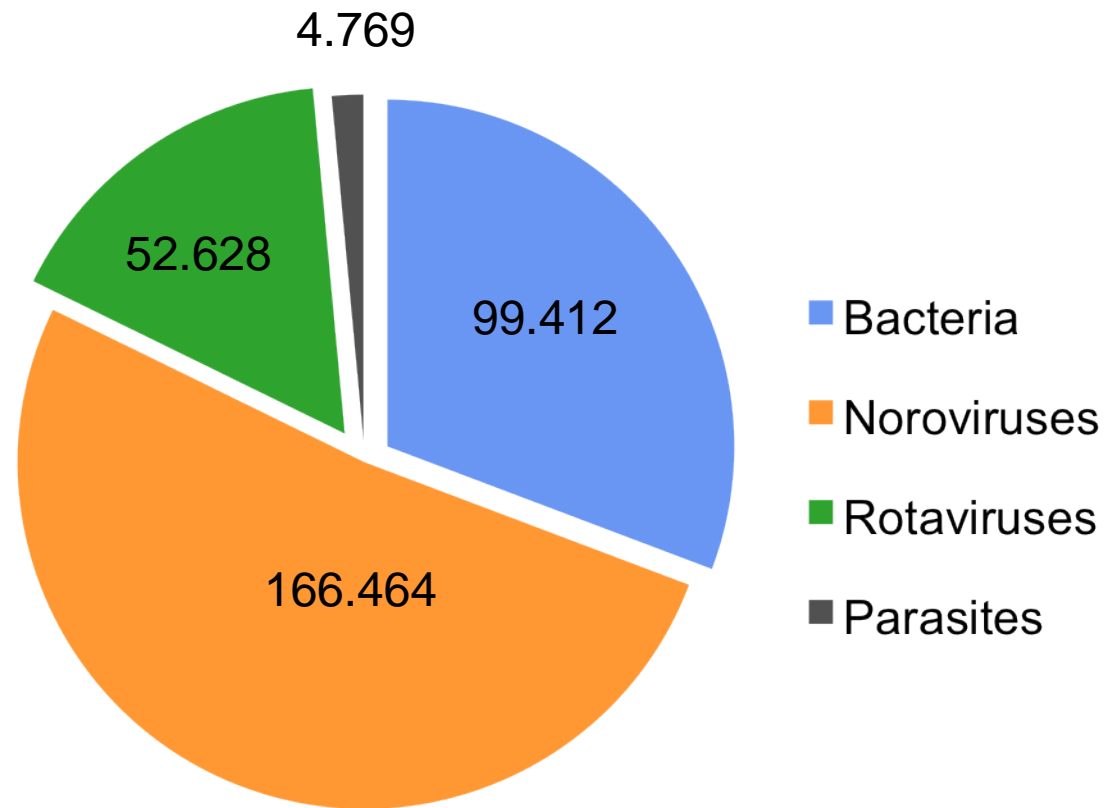
Live from the Robert  
Koch Institute: The  
next drawing of the  
EHEC Source...



# Increased gastroenteritis within the German population

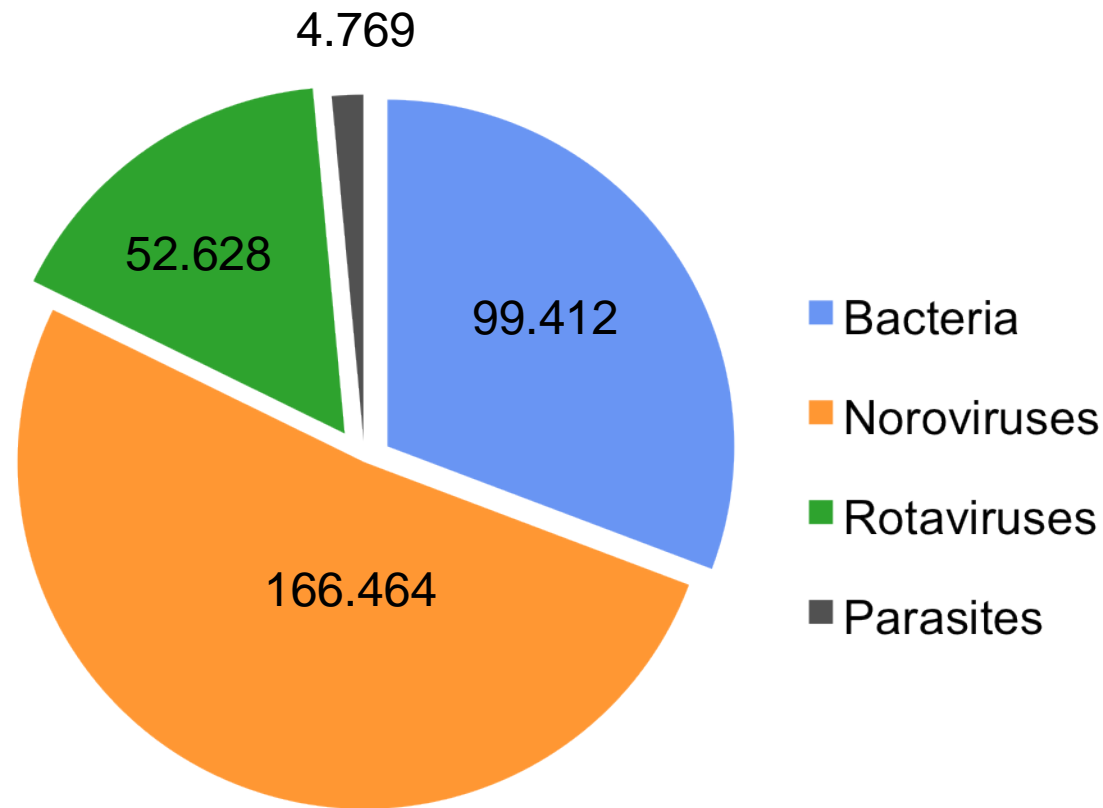
- Onset of acute diarrhoea combined with vomiting
- Often watery and bloody diarrhoea

# Increased gastroenteritis within the German population



Pathogens of enteral infections in Germany, 2010.

# Increased gastroenteritis within the German population



Are noroviruses the causative agent for the outbreak?

Increased gastroenteritis within  
the German population

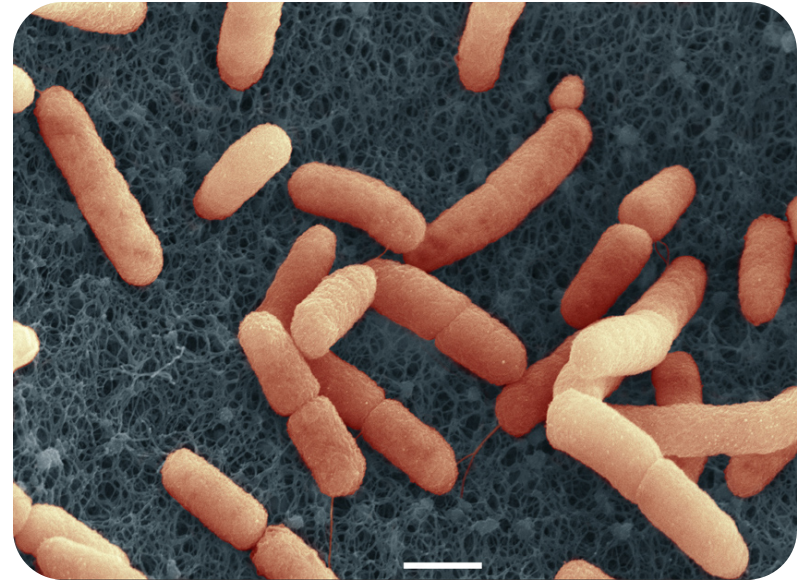
Exploding numbers of patients with Hemolytic-  
uremic syndrome (HUS)

Increased gastroenteritis within  
the German population

Exploding numbers of patients with Hemolytic-  
uremic syndrome (HUS)



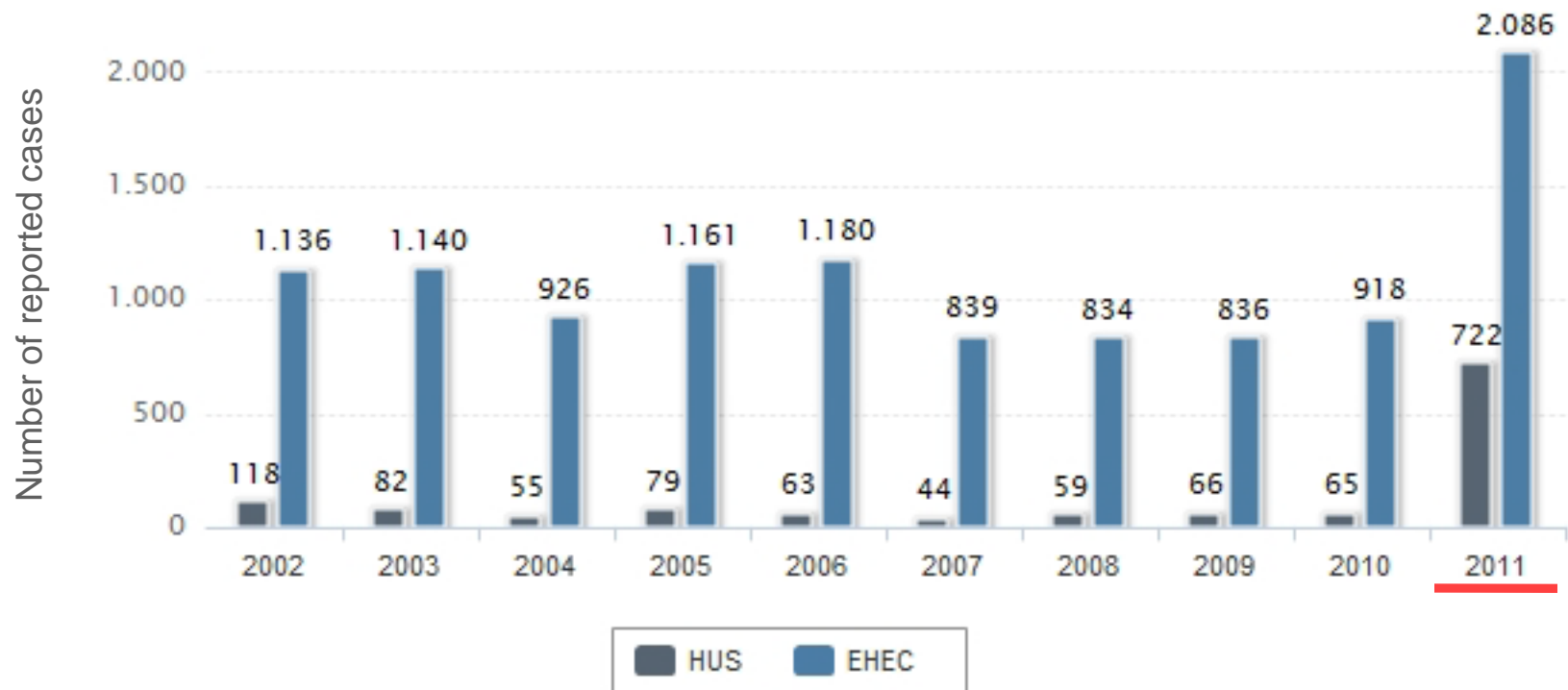
Outbreak caused by  
an enterohemorrhagic  
*E. coli* (EHEC)



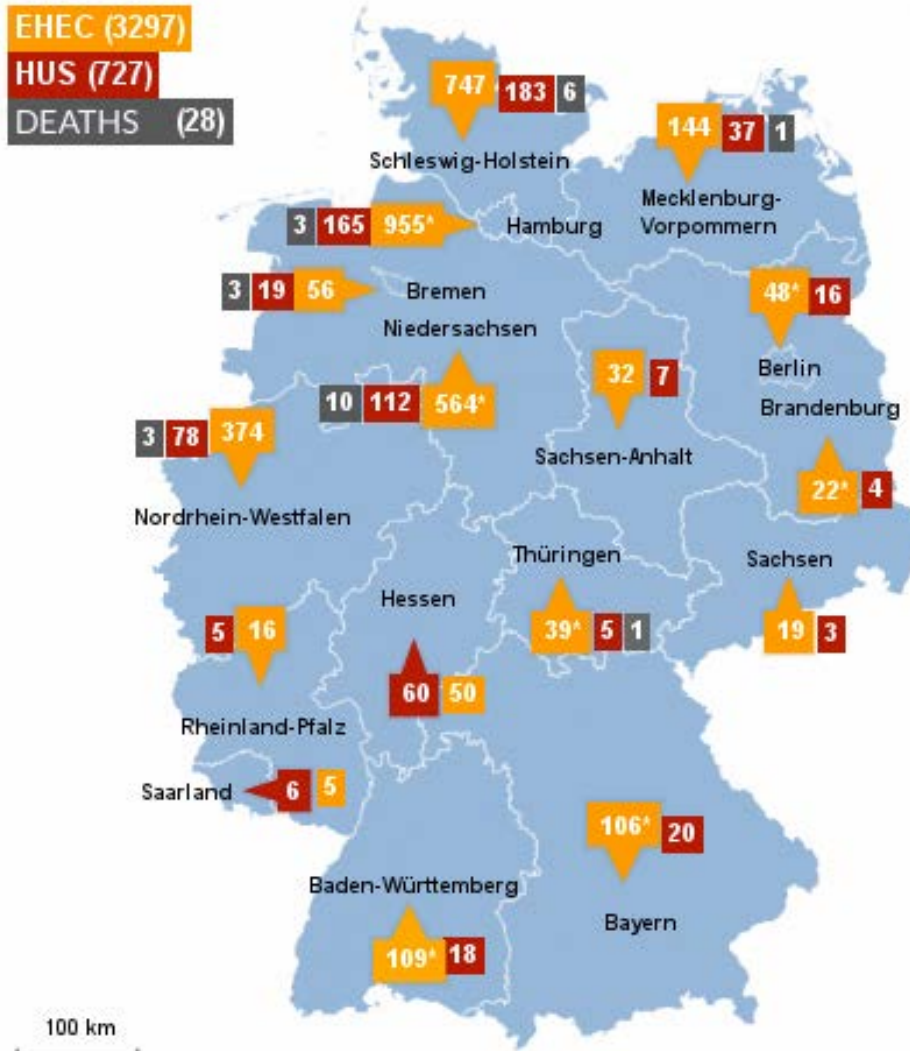
# May 2011: EHEC Outbreak in Germany



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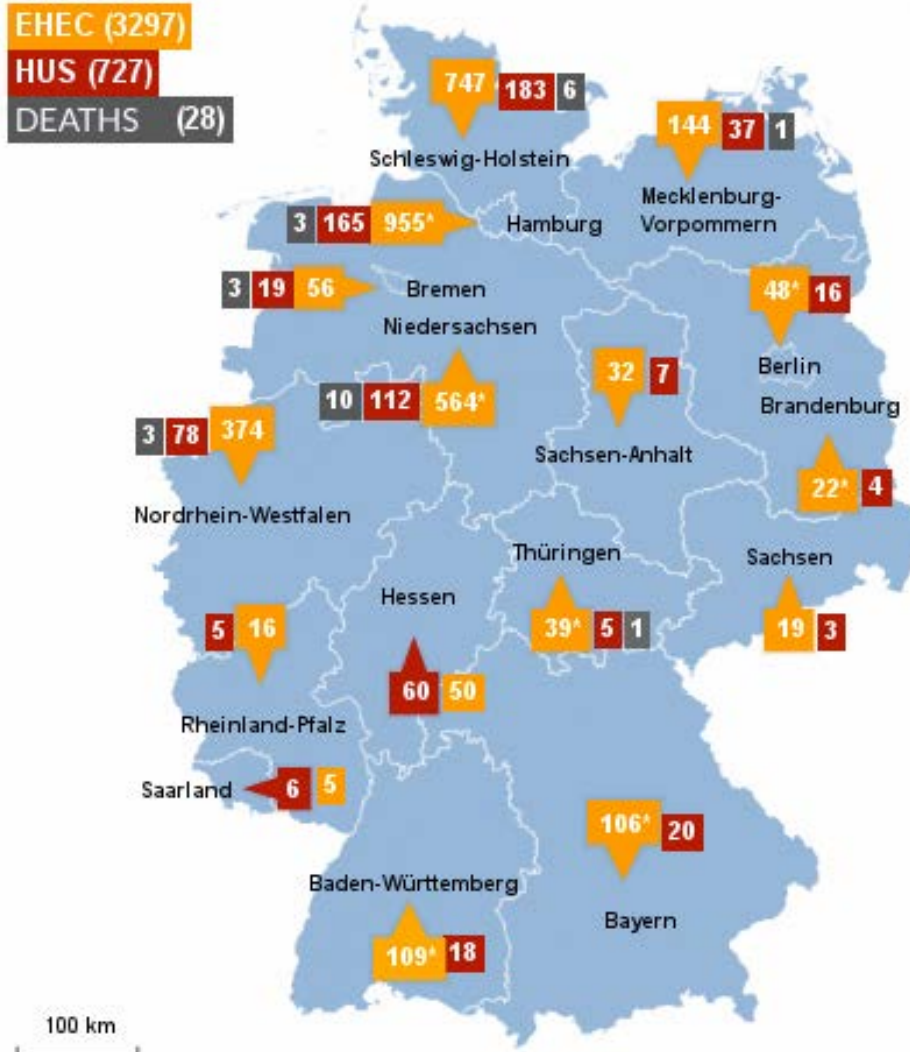


# Novel EHEC strain O104:H4



Source: RKI, 09.06.2011, incl. suspicious cases

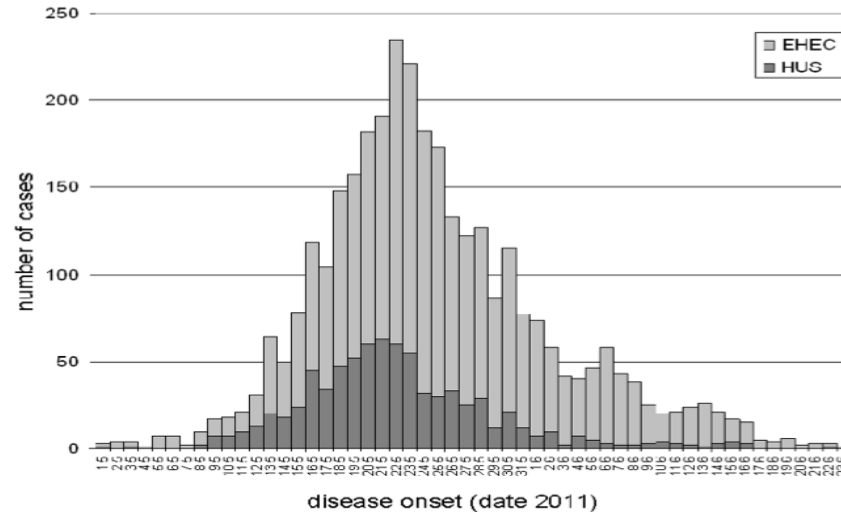
# Novel EHEC strain O104:H4



Source: RKI, 09.06.2011, incl. suspicious cases

- Stx-2 locus (EHEC)
- Stacked brick aggregative adherence (EaggEC)
- Extended spectrum  $\beta$ -lactamase (ESBL) antibiotic resistance profile
- Intimin-negative (eae) and enterohaemolysin (hyl)-negative

# May 2011: EHEC Outbreak in Germany



- National Reference Center overloaded
- No sufficient diagnostic capabilities at resident laboratories
- Increasing crisis within the population: Every diarrhoea = EHEC?

# May 2011: EHEC Outbreak in Germany

## Diagnostic challenge:

- Rapid detection of the new EHEC strain O104:H4
- Simultaneous exclusion from a differential diagnostic standpoint

Luminex xTAG GPP

# Gastroenteric pathogens detected by xTAG GPP

## **BACTERIA**

*Campylobacter*

*Clostridium difficile* toxin A/B

E. Coli O157

Enterotoxigenic *E. coli* (ETEC)  
LT/ST

*Salmonella*

Shiga-like Toxin producing *E. coli*  
(STEC) stx 1/stx 2

*Shigella*

*Vibrio cholerae*

*Yersenia enterocolitica*

## **VIRUSES**

Adenovirus 40/41

Norovirus GI/GII

Rotavirus A

## **PROTOZOA/PARASITES**

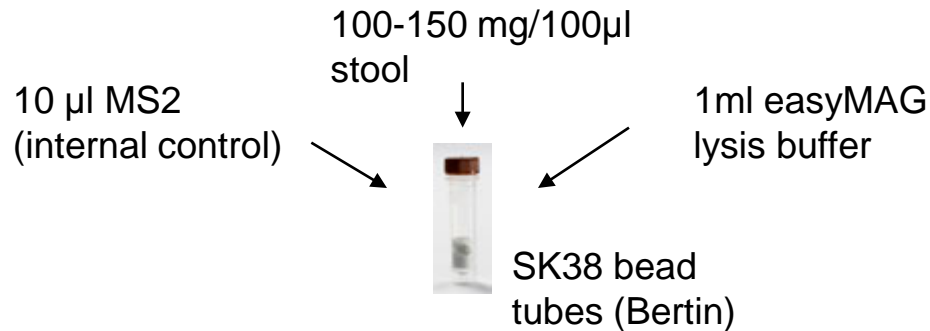
*Cryptosporidium*

*Entamoeba histolyca*

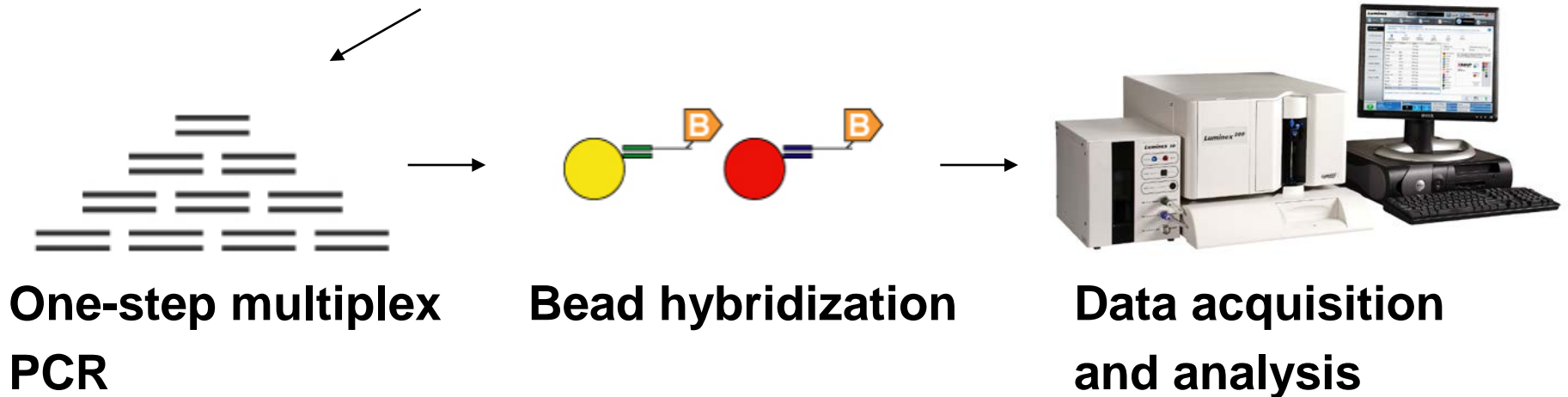
*Giardia*

# Workflow of the GPP xTAG assay

## Pretreatment



## Extraction of nucleic acid



Approx. 5 h handling time!

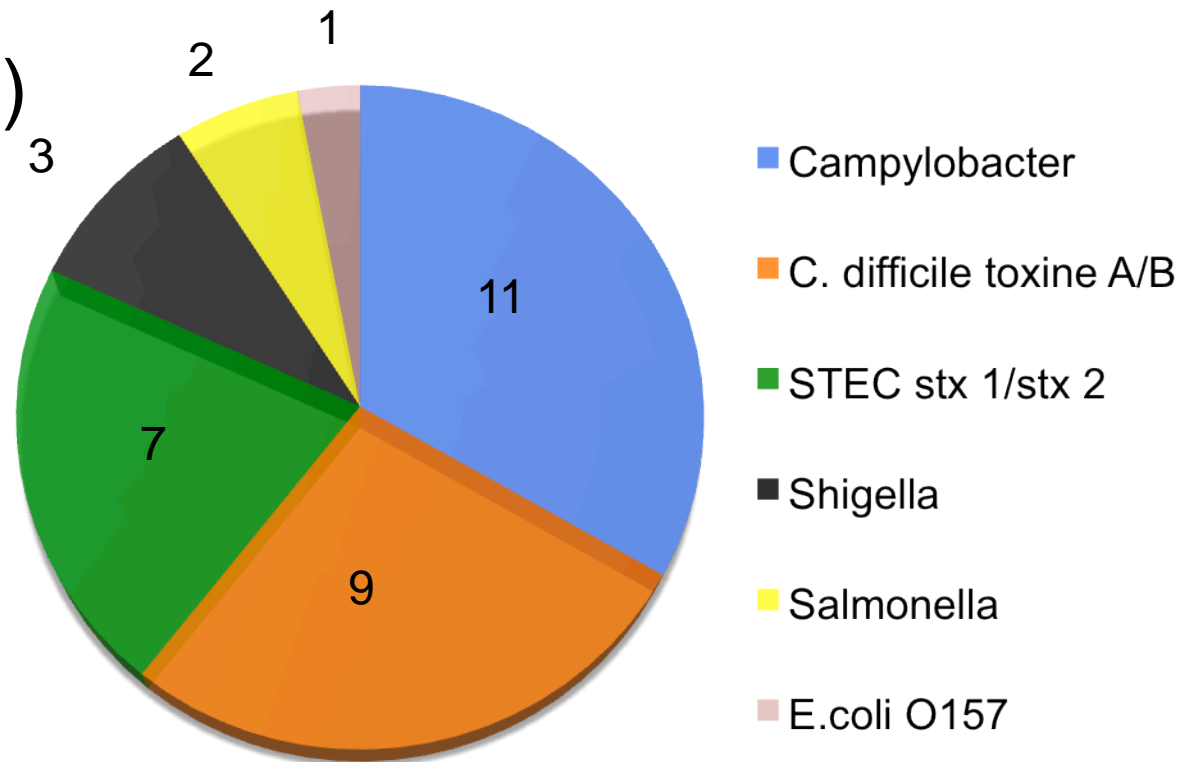
# Results

# Incidence of diarrhoea in June – August in Merheim, Cologne

92 stool samples:

32 samples (29%)  
were positive

34 pathogens  
were detected



STEC = Shiga-like toxin-producing *E. coli*

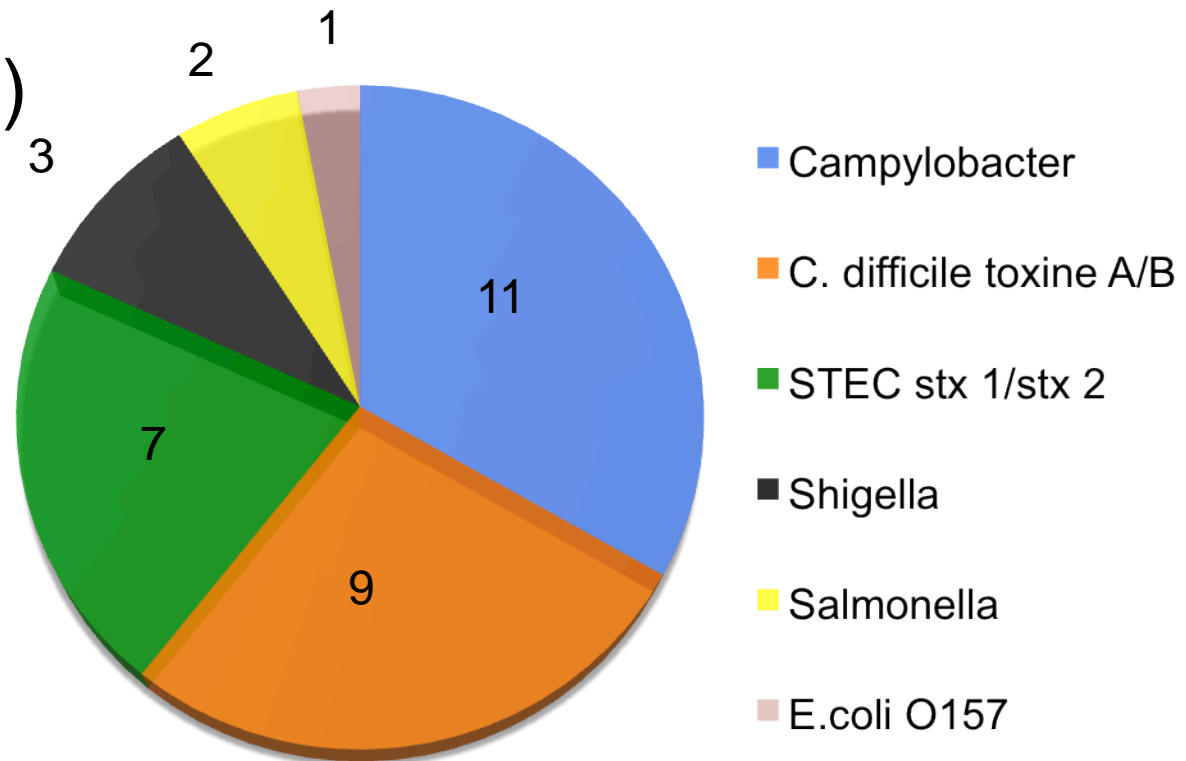
# Incidence of diarrhoea in June – August in Merheim, Cologne

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34 pathogens  
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Stx 2 positive:  
4 samples



STEC = Shiga toxin like-producing *E. coli*

# Limit of detection of O104:H4 detected by xTAG GPP determined with LX100/200

LoD by Luminex:

STEC stx 1/stx 2 LoD:  $8.59 \times 10^5$  CFU/ml

# Limit of detection of O104:H4 detected by xTAG GPP determined with LX100/200

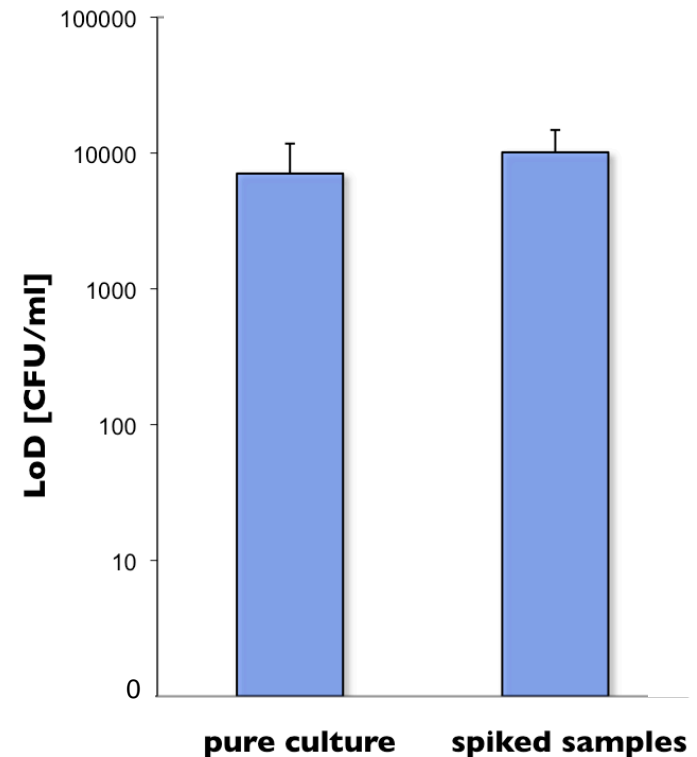
LoD by Luminex:

STEC stx 1/stx 2 LoD:  $8.59 \times 10^5$  CFU/ml

## O104:H4

pure culture LoD:  $7.07 \times 10^3$  CFU/ml

spiked preparation LoD:  $1.01 \times 10^4$  CFU/ml



# Stool samples adopted for the respiratory viruses panel (RVP)

|                                   |
|-----------------------------------|
| Respiratory Syncytial Virus (RSV) |
| RSV A                             |
| RSV B                             |
| Influenza A                       |
| Non-specific influenza A          |
| H1 subtype                        |
| H3 subtype                        |
| H5 subtype                        |
| Influenza B                       |
| Parainfluenza 1                   |
| Parainfluenza 2                   |
| Parainfluenza 3                   |
| Parainfluenza 4                   |

|                        |
|------------------------|
| Metapneumovirus (hMPV) |
| Adenovirus             |
| Enterorhinovirus*      |
| Corona NL63            |
| Corona HKU1            |
| Corona 229E            |
| Corona OC43            |
| Bocavirus              |

# Stool samples adopted for the respiratory viruses panel (RVP)

62 stool samples



2 samples were positive for Enterovirus/Rhinovirus

From Norovirus to the new EHEC  
variant O104:H4

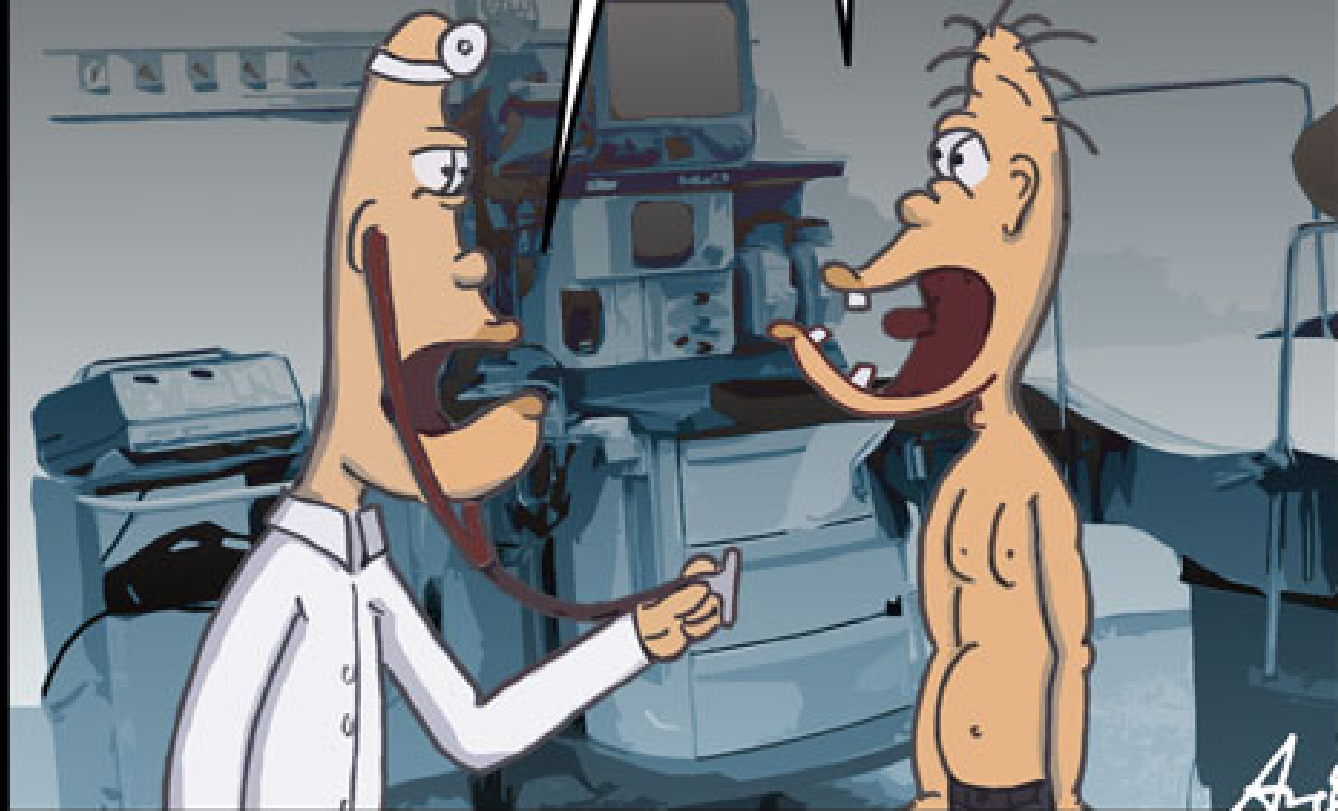
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Luminex xTAG GPP can identify both

When did you eat  
vegetables for the  
last time?

Do I have EHEC?

No, scurvy.



Arjo