German Experience with non-O157:H7 STEC

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The USDA/FSIS meeting „The Public Health Significance of non-O157 Shiga Toxin-Producing *E. coli* (STEC)“
Washington, D.C., October 17, 2007

Notifiable microorganisms according to § 7 of the German Protection against Infection Act

1. Adenoviruses
2. Bacillus anthracis
3. Borrelia recurrentis
4. Brucella sp.
5. Campylobacter sp.
6. Chlamydia psittaci
7. Clostridium botulinum or toxin detection
8. Corynebacterium diphtheriae, toxigenic
9. Coxiella burnetii
10. Cryptosporidium parvum
11. Ebolavirus
12. a) STEC/EHEC
    b) E. coli, other enteropathogenic
13. Francisella tularensis
14. FSME-Virus
15. Yellow fever virus
16. Giardia Lamblia
17. Haemophilus influenzae (liquor, blood)
18. Hantaviruses
19. Hepatitis A virus
20. Hepatitis B virus
21. Hepatitis C virus (not chronic infection)
22. Hepatitis D virus
23. Hepatitis E virus
24. Influenza viruses (only direct evidence)
25. Lassavirus
26. Legionella sp.
27. Leptospira interrogans
28. Listeria monocytogenes (blood, liquor, newborns)
29. Marburgvirus
30. Measles virus
31. Mycobacterium leprae
32. Mycobacterium tuberculosis/africanum, Mycobacterium bovis
33. Neisseria meningitidis (liquor, blood)
34. Norwalk-like virus (stool)
35. Poliovirus
36. Rabiesvirus
37. Rickettsia prowazekii
38. Rotavirus
39. Salmonella Paratyphi (direct evidence)
40. Salmonella Typhi (direct evidence)
41. Salmonella, others
42. Shigella sp.
43. Trichinella spiralis
44. Vibrio cholerae O 1 und O 139
45. Yersinia enterocolitica
46. Yersinia pestis
47. Other causes of haemorrhagic fever

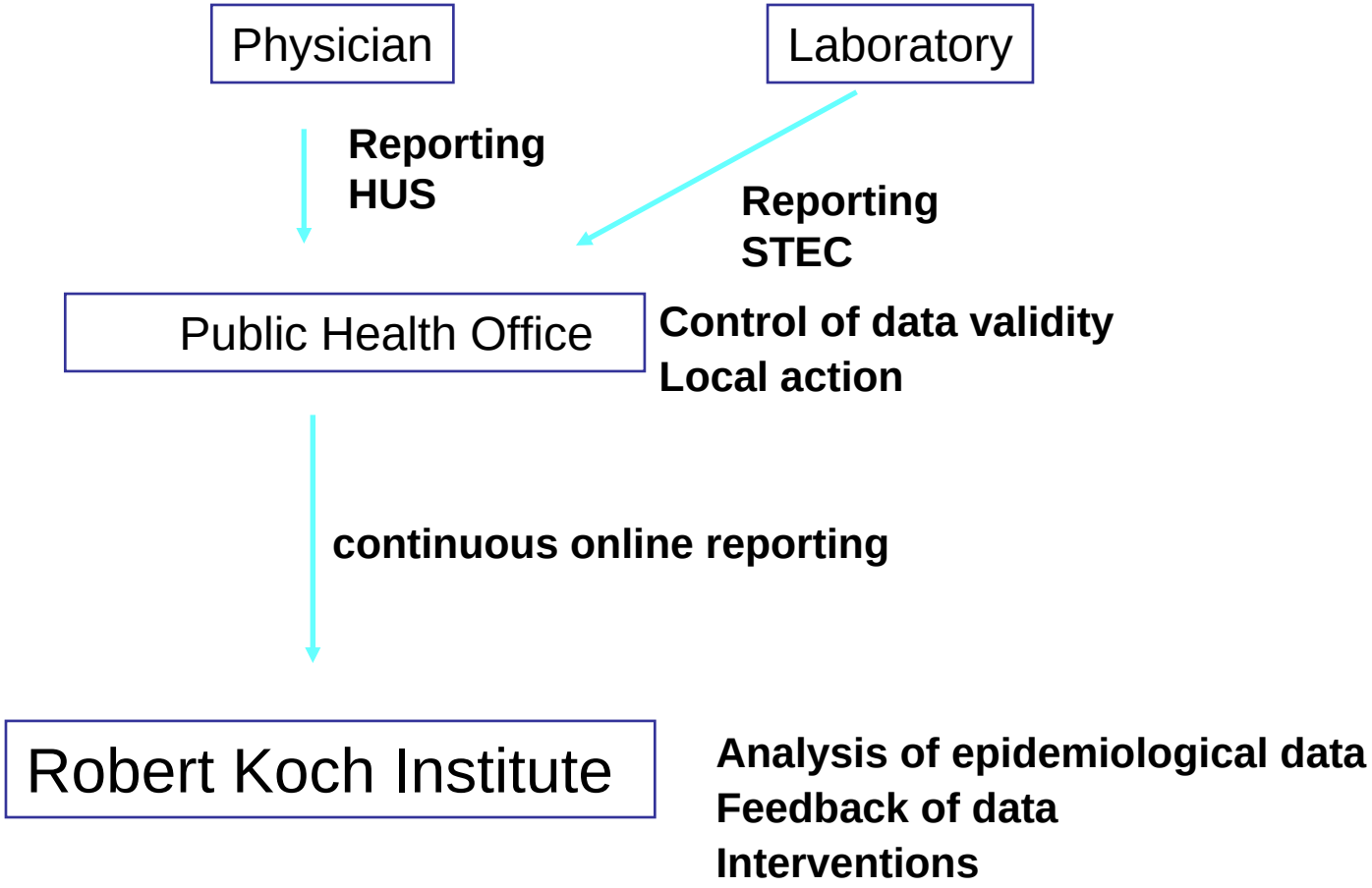
Notifiable Diseases according to § 6 of the German Protection against Infection Act

Suspicion, disease and death § 6 Abs.1 Nr.1

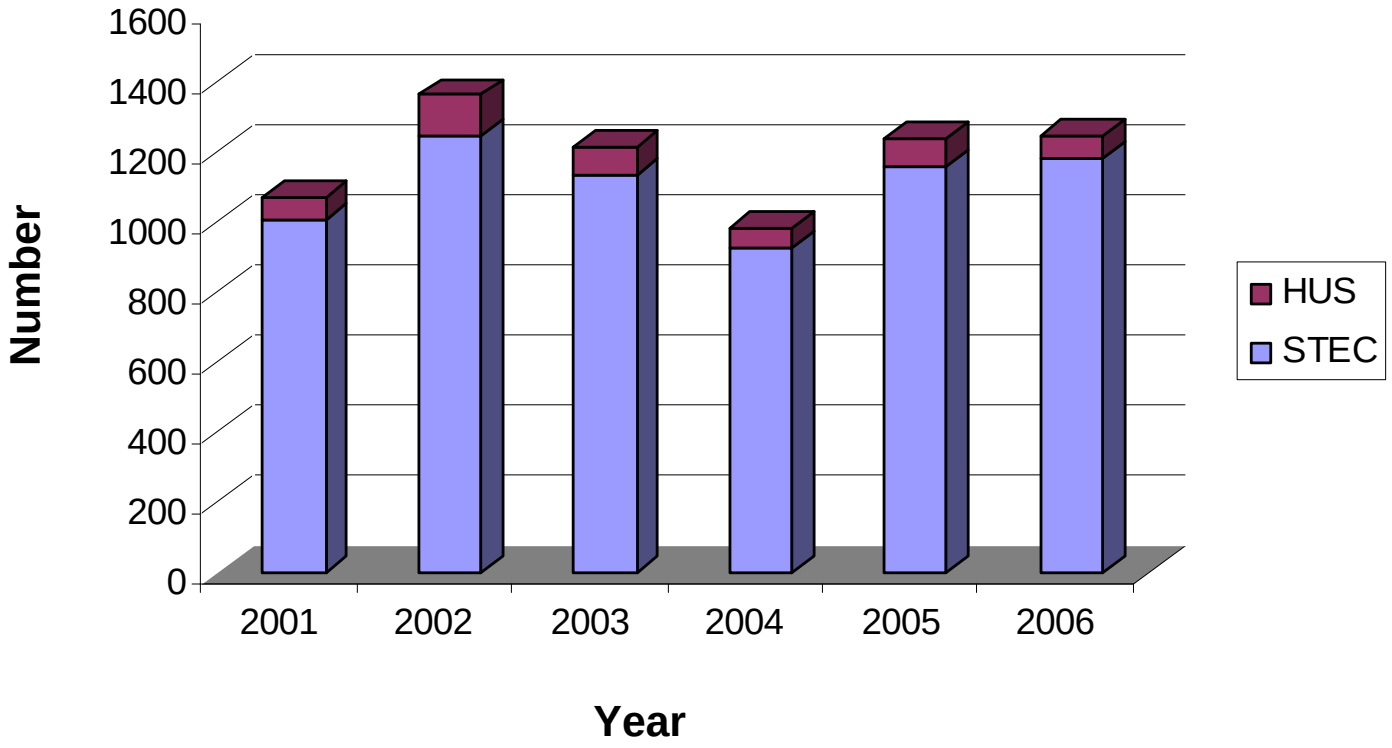
a) **Enteropathic haemolytic-uraemic syndrome (HUS)**
b) Botulisms
c) Cholera
d) Diphtheria
e) Humane spongiform enzephalopathy
f) Acute viral hepatitis
g) Viral haemorrhagic fevers
h) Measles
i) Meningococcal meningitis or -septicaemia
j) Anthrax
k) Poliomyelitis
l) Plague
m) Rabies
n) Typhus abdominalis/ Paratyphus

- Disease and death from tuberculosis

Dual communication flow according to the German Protection against Infection Act



Reported STEC infections and HUS cases in Germany (2001-2006)



- 80% of reported STEC are non-O157

Epidemiol. Bulletin Robert Koch Institute

Risk factors for STEC infections in Germany

Laboratory-based sentinel surveillance study in 14 of 16 German federal states (April 2001-March 2003)

- 202 cases (3 mo-89 yrs, median 2.5 yrs) (86% non-O157 infection, 5 HUS)
- 202 age- and region-matched controls

Age-specific risk factors

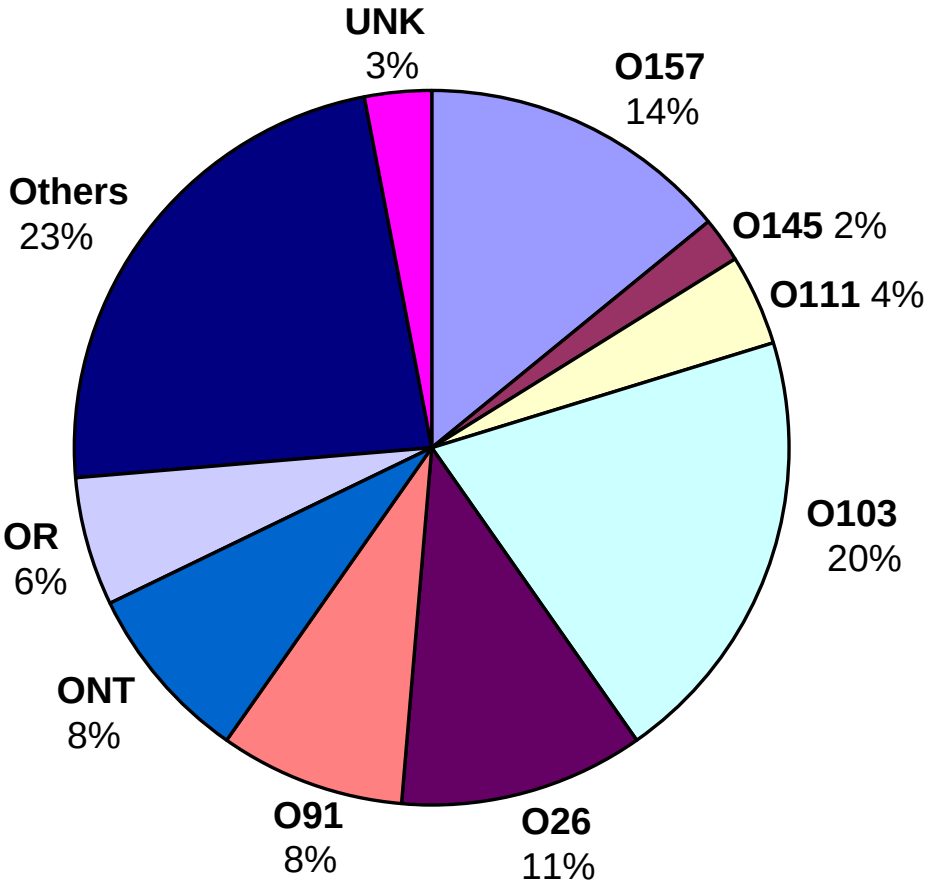
<3 yrs: direct contact with ruminants
playing in sandbox
drinking raw milk

3-9 yrs: playing in sandbox

> 10 yrs: eating lamb meat
eating raw spreadable sausages

Werber et al., Am. J. Epidemiol. 2007

STEC serogroups

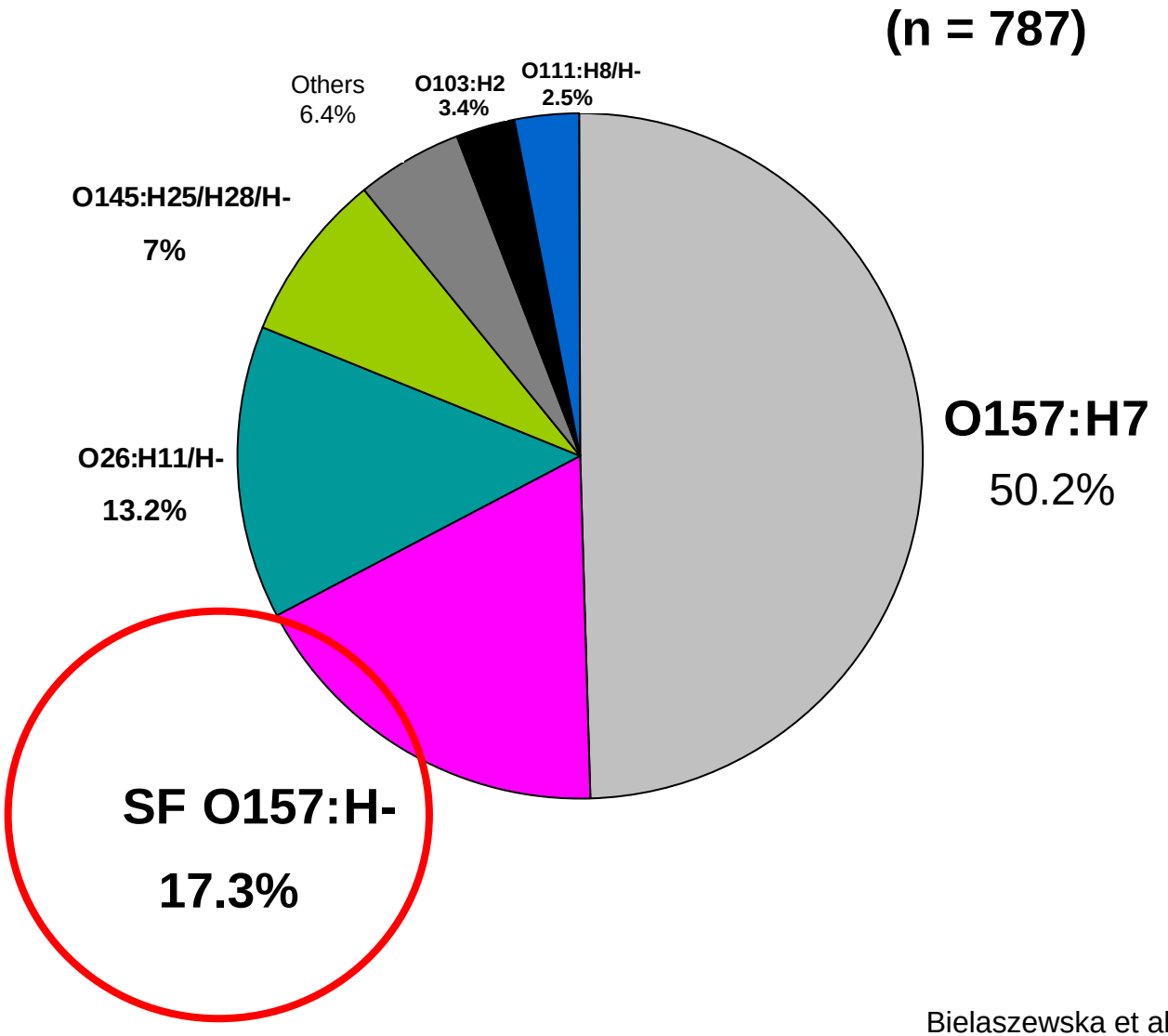


Sero-group	Disease (%)			
	D	BD	HUS	Others*
O157	48	34	10	7
Non-O157	**76**	**10**	**1**	**12**

* Abdominal pain, nausea, vomiting, fever, blood in stool, or incomplete clinic

Werber et al., Am. J. Epidemiol. 2007

Prevalence of STEC serotypes in sporadic cases of HUS in Germany (1996-2006)



Bielaszewska et al., PLoS ONE, 2007

Outbreaks caused by SF STEC O157:H$^-$ in Germany

Year (mo)	Location	HUS cases (n)	Deaths (n)	Case-control study
1995/96 (12-03)	Bavaria	28	3	Teewurst*
2002 (03)	Münsterland	6	0	Unknown
2002 (10-12)	Bavaria, B-W	38	4	Apple juice* Curdled cheese*
2006 (04-08)	North Germany	18	0	Unknown

* SF STEC O157 never isolated from any food or environmental samples

Ammon et al., J. Infect. Dis. 1999; Epidemiol. Bull. of Robert Koch Institute, 2002, 2003; Fruth et al., 3rd International Workshop on Thrombotic Microangiopathies, Jena, Germany, October 2007

Differences between SF STEC O157:H$^-$ and STEC O157:H7

	SF STEC O157:H$^-$	STEC O157:H7
Sorbitol fermentation	+	-
β-Glucuronidase	+	-
Motility	-	+
Seasonality	Winter	Summer
Age of patients	< 3 yrs	> 3 yrs
Reservoirs	Unknown	Ruminants
Transmission routes	Unknown	Food, water, direct contact
Risk for HUS	?	~ 10%

Karch & Bielaszewska, J. Clin. Microbiol., 2001; Karch et al., Int. J. Med. Microbiol., 2005; Tarr et al., Lancet, 2005; Werber et al., Am. J. Epidemiol. 2007

SF STEC O157:H$^-$ are spreading!

Countries with reported isolation of SF EHEC O157:H$^-$ (2007)

Outbreaks caused by non-O157 STEC

Serotype	Year	Settlement	Cases/HUS	Source
O22:H8	1989	family	3/1	cattle (milk)
O26:H11	1999	community	3/3	unknown
O26:H11	2000	day care centres, hospitals (3 states)	11/0	Seemerrolle* (beef)
O111:H8	1986	family	4/1	unknown
O145:H28	1999	family	2/0	unknown
	2001	kindergarten	6/1	unknown
	2002	family	2/0	unknown

*** Identified as a possible source based on comparison of invoices of purchased food, but the strain not isolated from the food**

Bockemühl et al. Bundesgesundhbl. 1990; Misselwitz et al., Pediatr. Infect. Dis., 2003; Sonntag et al., J. Clin. Microbiol., 2004; Werber et al., J. Infect. Dis. 2002

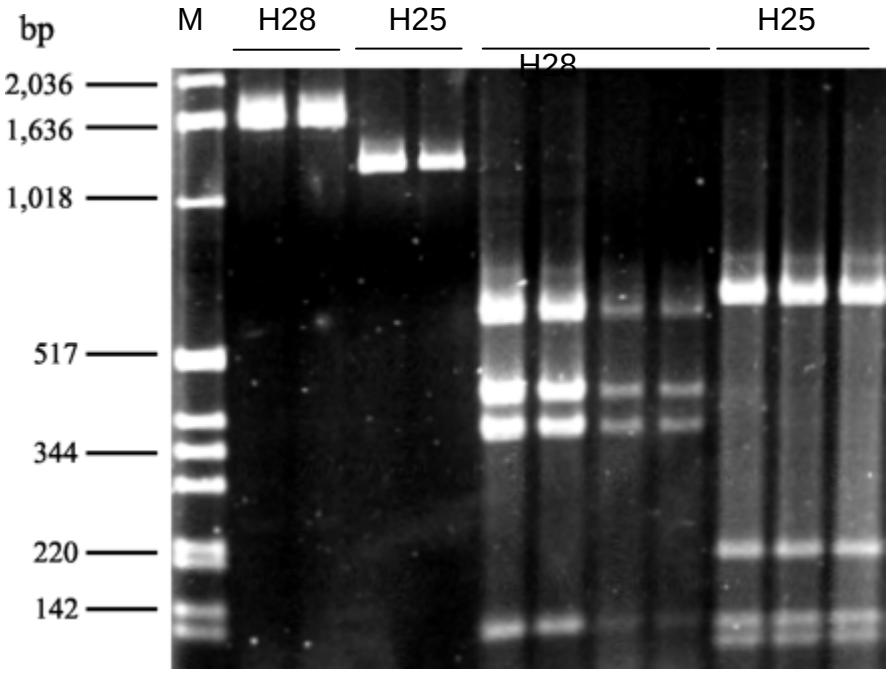
STEC O26

- **The most frequent non-O157 STEC isolated from HUS patients (> 1/3 of non-O157 STEC, 13.2% of all STEC)**
- **Serotypes O26:H11 and O26:H$^-$, both possess *fliC*$_{H11}$**
- **Stx production: Stx1, Stx2, Stx1+Stx2**
- **Temporal shift from *stx*$_1$ to *stx*$_2$ since 1990th**
- **The *stx*$_2$ genotype is associated with HUS ($P < 0.001$)**

Zhang et al., J. Clin. Microbiol. 2000
Bielaszewska et al., J. Clin. Microbiol. 2005

STEC O145

- **Second most frequent HUS-associated non-O157 STEC (7%)**



fliC PCR *fliC* RFLP (*Hha*I)

- Two different serotypes (*fliC* types)
- Two different *eae* types

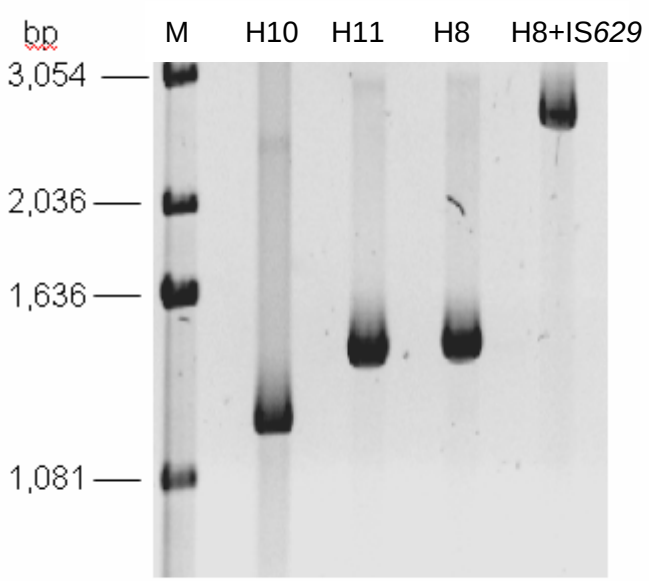
O145:H28 (98%), *eae* γ
O145:H25 (2%), *eae* β

- 5 *stx* genotypes

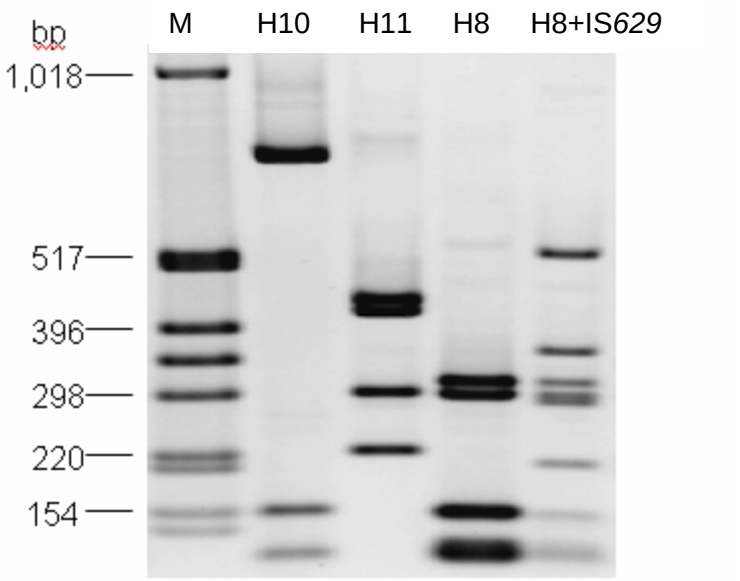
stx_2	(70.8%)
stx_1	(21.7%)
stx_1+ stx_2	(2.5%)
stx_{2c}	(4.2%)
stx_1+ stx_{2c}	(0.8%)

Sonntag et al., J. Clin. Microbiol. 2004

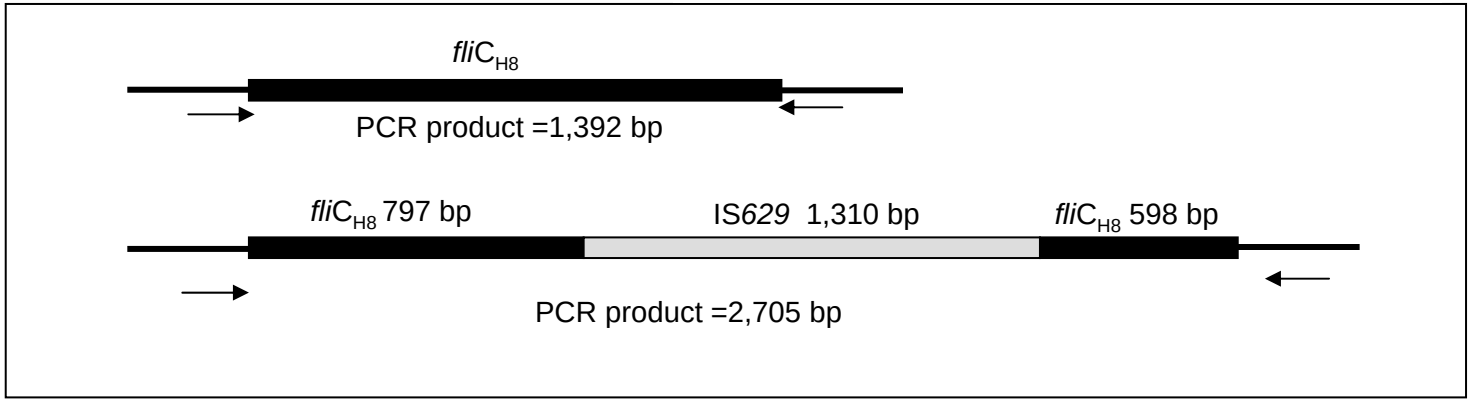
STEC O111



fliC PCR



fliC RFLP (*Hha*I)



Zhang et al., Int. J. Med. Microbiol., 2007

Subtyping scheme for STEC O111

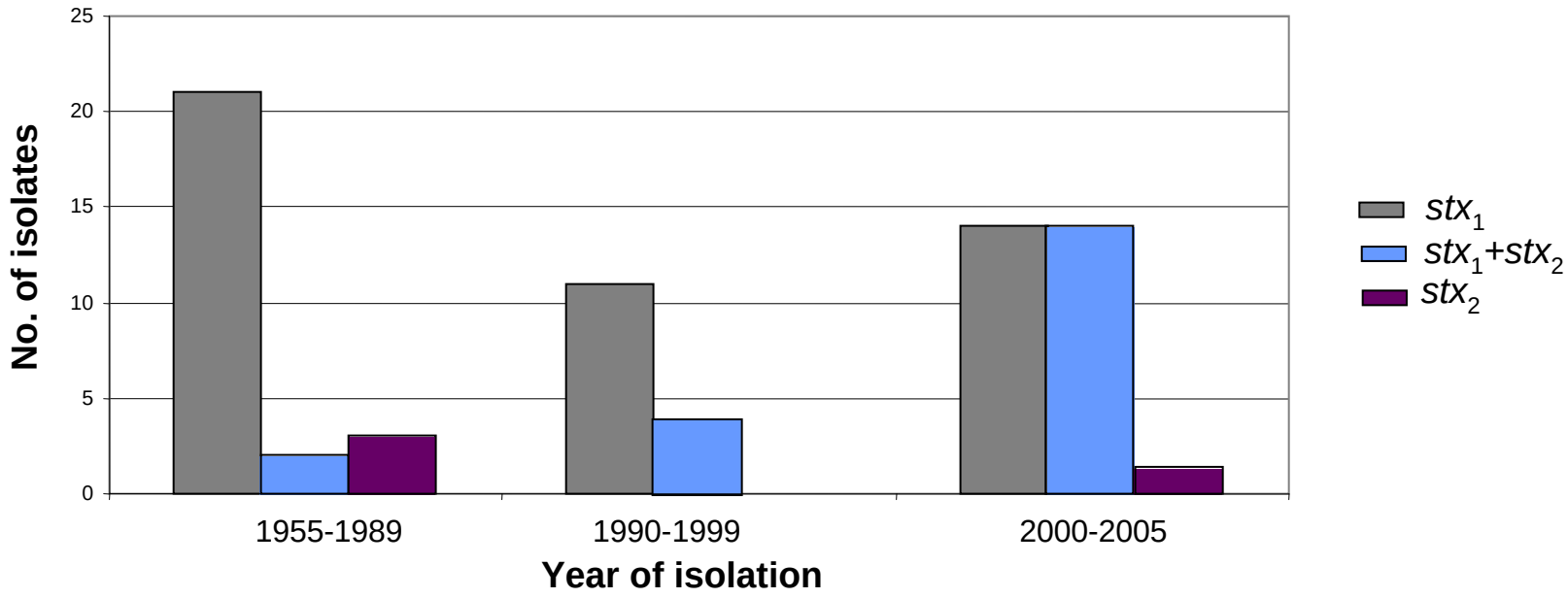
Serotype	*fliC*	*stx*	*eae*	OI-122 (*efa-1/pagC/sen*)	*cad* genes (A/B/C)	LDC*
O111:H8/H$^-$ (n = 64)	H8	1,2,1+2	+	+/+/+	-/+/-	-
O111:H10/H$^-$ (n = 5)	H10	2	-	-/-/-	+/+/+	+
O111:H11/H$^-$ (n = 3)	H11	1	+	+/+/+	+/+/+	+

*** Lysin decarboxylation**

Zhang et al., Int. J. Med. Microbiol., 2007

stx genes in STEC O111:H8/H$^-$

- Stx production: Stx1, Stx2, Stx1+Stx2
- Temporal shift from *stx*$_1$ to *stx*$_1$+*stx*$_2$ since 2000



- The introduction of *stx*$_2$ is associated with HUS (*P* < 0.001)

Zhang et al., Int. J. Med. Microbiol., 2007

Production of $Stx2d_{activatable}$ is a predictor for a severe outcome of infections caused by *eae*- STEC

Clinical outcome	Total isolates	No. (%) of *eae*-	No. (%) of *eae*-negative STEC producing		Clinical associations of $Stx2d_{activatable}$-producing STEC with outcome	
			$Stx2d_{activatable}$*	non-activatable Stxs **	Outcome	*P*
HUS	337	11 (3)	7 (64)	4 (36)	HUS vs. others	< 0.00001
BD	50	6 (12)	6 (100)	0 (0)	Severe (HUS+ BD) vs. milder (D) disease	0.0001
D	397	113 (29)	17 (15)	96 (85)	BD vs. D	0.0009
A	108	79 (73)	0 (0)	79 (100)	Disease vs. A	< 0.0001

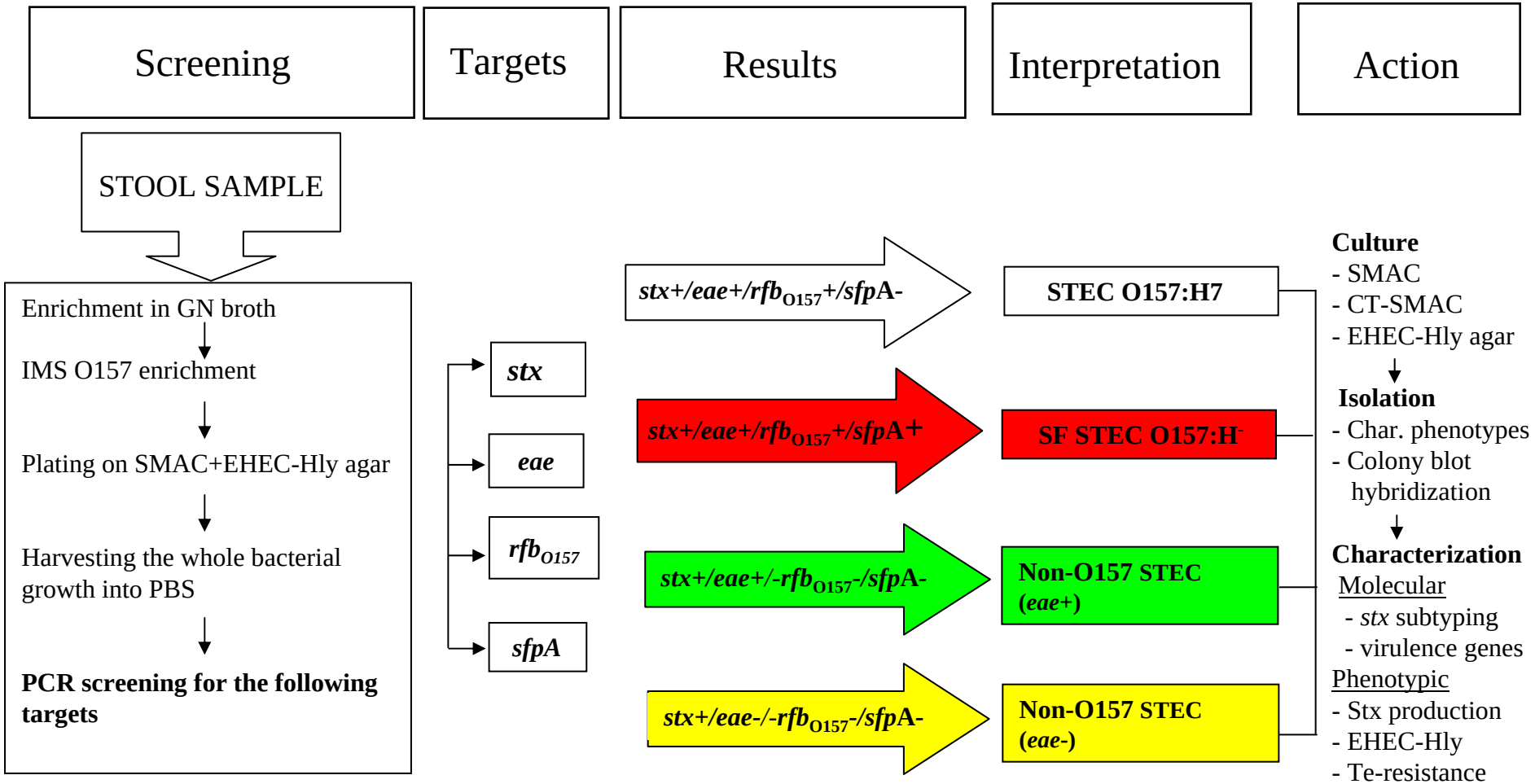
*** Most common serotypes O91:H21, O113:H21**

**** Stx1, Stx1c, Stx1d, Stx2, Stx2c, $Stx2d_{EH250}$, Stx2e, Stx2f**

- **Rapid and comprehensive subtyping of *stx* genes in STEC isolates is necessary to alert a clinician about the risk of HUS development**

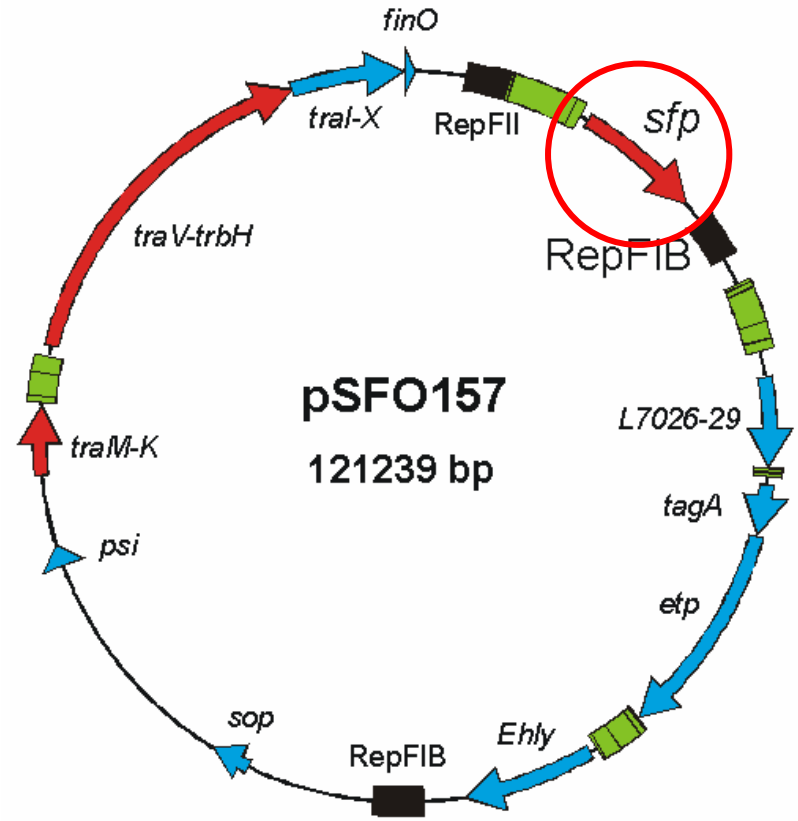
Bielaszewska et al., Clin. Infect. Dis. 2006

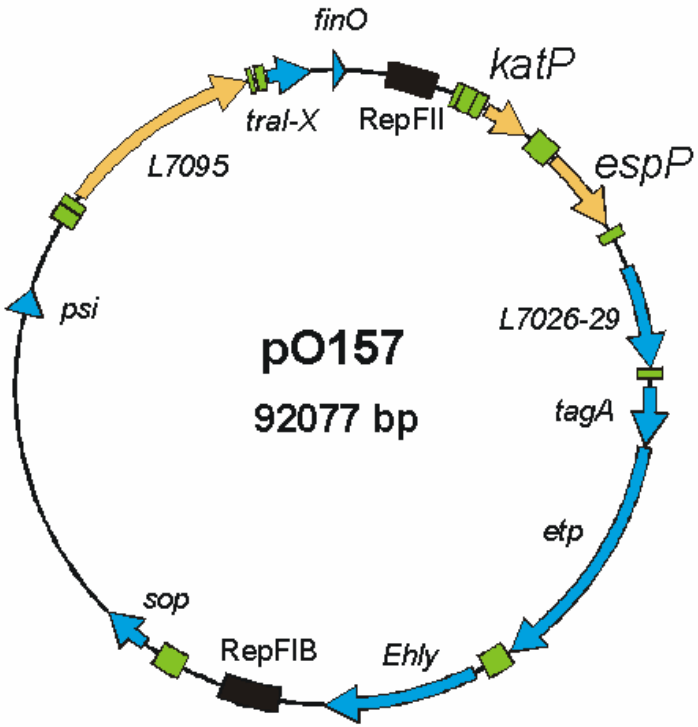
Diagnostic scheme for the detection of STEC in stools

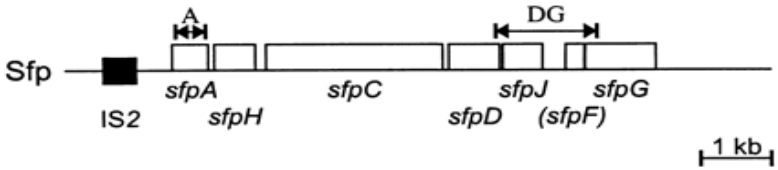


Adapted from Friedrich et al., J. Clin. Microbiol. 2004

Nucleotide sequence analysis of pSFO157





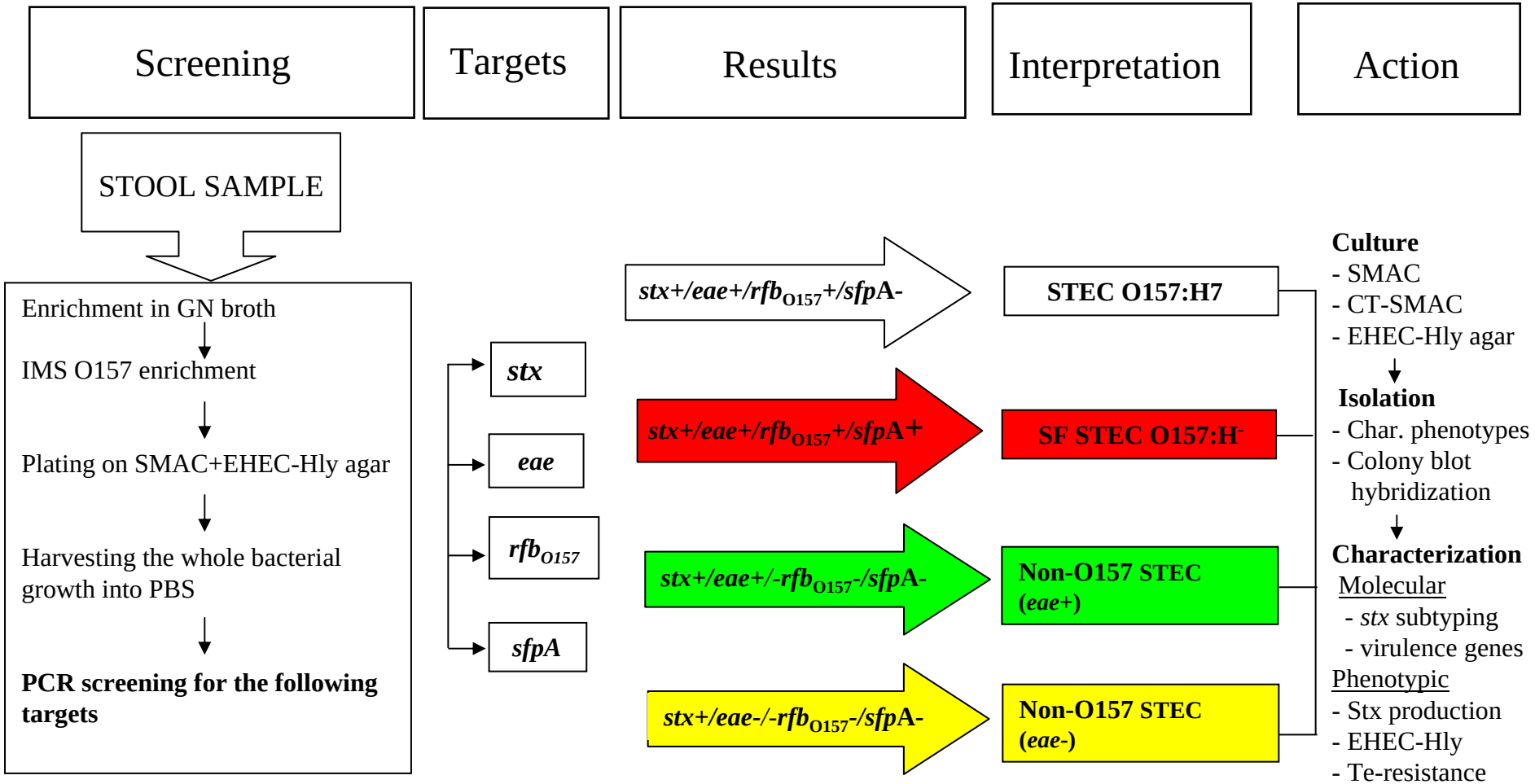


Brunder et al., Infect. Immun. 2001
Brunder et al., Int. J. Med. Microbiol. 2006

Schmidt et al., Infect. Immun. 1995
Brunder et al., Microbiology 1996
Brunder et al., Mol. Microbiol. 1997

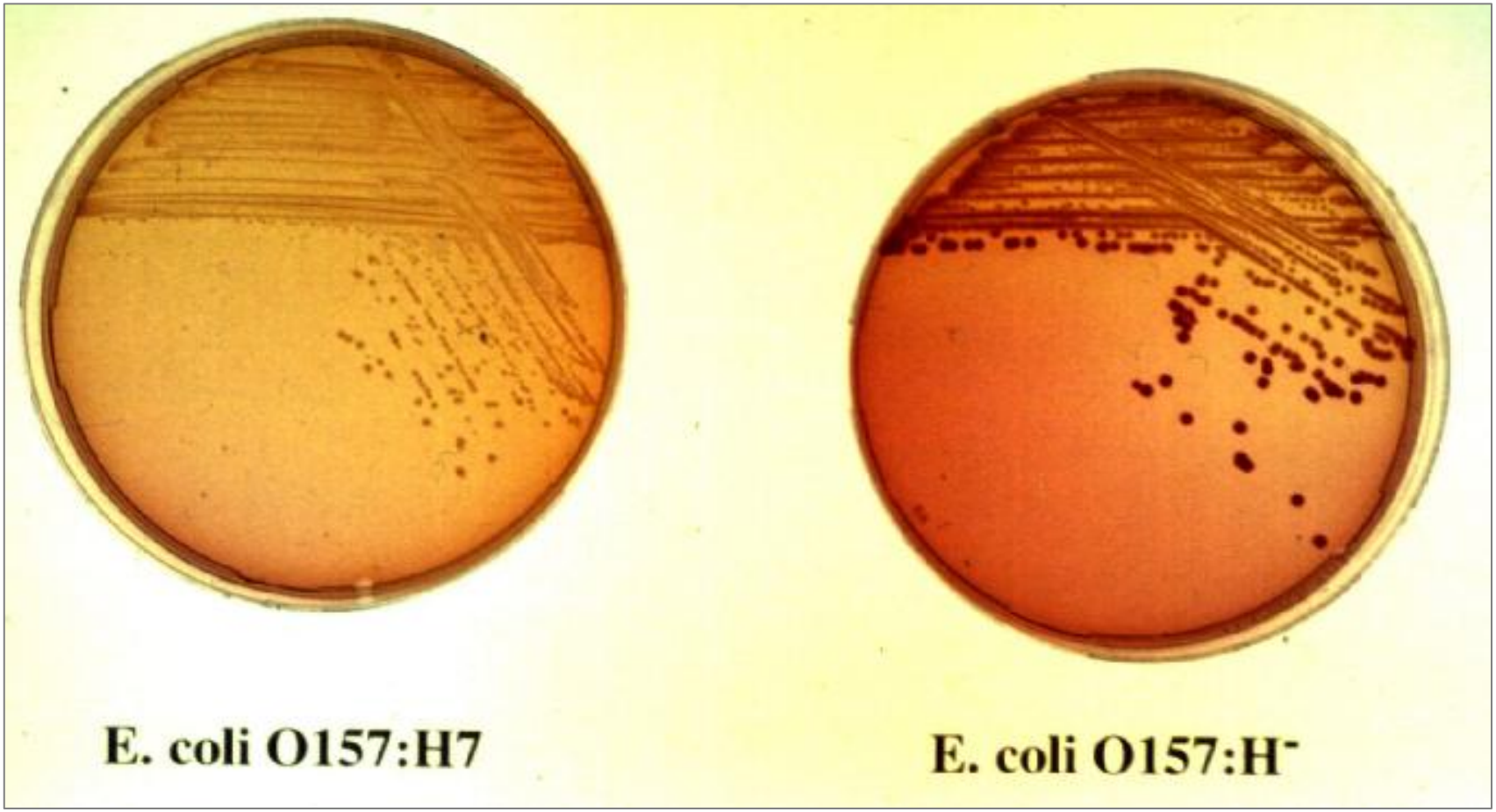
Burland et al., Nucleic Acids Res. 1998

Diagnostic scheme for the detection of STEC in stools



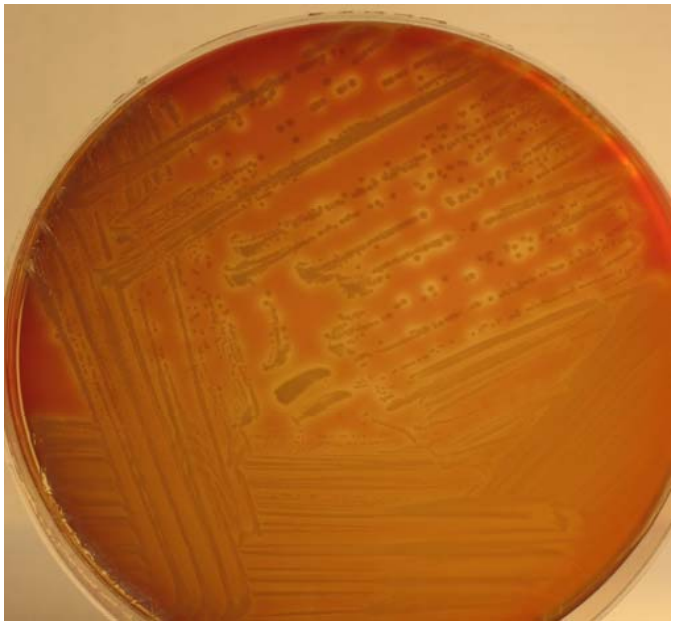
Adapted from Friedrich et al., J. Clin. Microbiol. 2004

Isolation of STEC O157- SMAC



Non-O157 STEC

Isolation of non-O157 STEC



- **EHEC hemolysin is expressed**

  O26, O103, O111, O145 = *eae*+
  O91:H21, O113:H21 = *eae*-

→ **Isolation on enterohemolysin agar**

- **EHEC hemolysin not expressed**

  SF EHEC O157:NM

→ **Isolation on SMAC (not CT-SMAC ← tellurite-sensitive!)**

- Slide agglutination
- Colony hybridization (*stx*, *eae* probes)

Selective/diagnostic medium needed

Bielaszewska et al., J. Clin. Microbiol. 2005
Mellmann et al., Clin. Infect. Dis. 2005
Sonntag et al., J. Clin. Microbiol. 2004
Zhang et al., Int. J. Med. Microbiol. 2007
Bielaszewska et al., PLoS ONE 2007

Isolation of STEC strains is a requisite, not a luxury!

Isolation of STEC from stools is necessary for:

- **Epidemiological studies**
- **Public health actions**
- **Monitoring of virulence**
- **Emergence of highly pathogenic clones**

Centers for Disease Control and Prevention (CDC).

Importance of culture confirmation of Shiga toxin-producing *Escherichia coli* infection as illustrated by outbreaks of gastroenteritis-New York and North Carolina, 2005.

MMWR Morb Mortal Wkly Rep. 2006;55(38):1042-5.

Acknowledgment

IFH Münster
Helge Karch
Alexander Friedrich
Alexander Mellmann

Stockholm
Andrea Ammon

Innsbruck
Bernd Zimmerhackl

RKI Berlin
Dirk Werber
Katharina Alpers

RKI Wernigerode
Angelika Fruth
Rita Prager
Helmut Tschäpe