

# **Studies on a rapid immuno-chromatographic detection system for Shiga toxins and the *E. coli* O157 antigen**

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# STEC: more than 400 serotypes from human patients (2005)

from: Scheut + Strockbine. Genus I. Escherichia, Bergey's Manual of Systematic Bacteriol pp 607ff (2005)

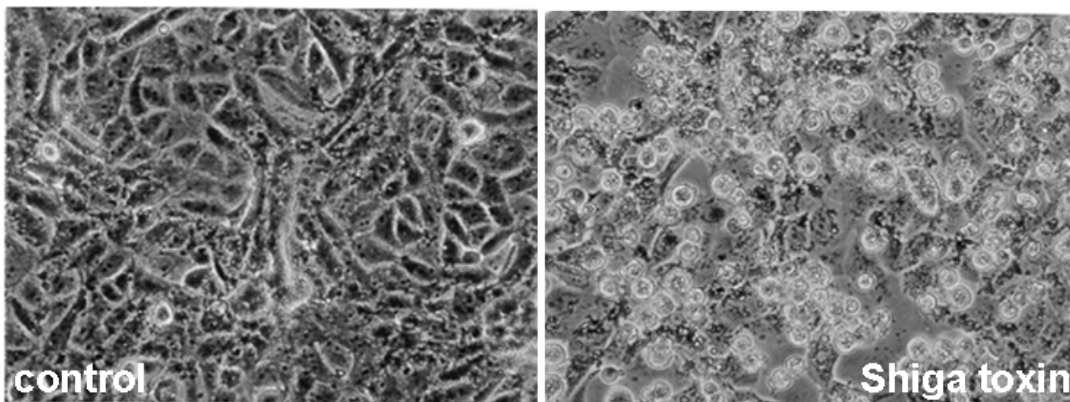
TABLE BXII.γ.205. Serotypes of non-O157 STEC/VTEC isolated from humans<sup>a,b,c</sup>

Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype	Serotype
O1:H <sup>-</sup>	O8:H21	O25:K2:H2	O52:H23	O83:H1	O103:H18	O114:H4	O126:H20	O146:H11	O169:H <sup>-</sup>
O1:H1	O8:H25	O25:H14	O52:H25	O84:H <sup>-</sup>	O103:H21	O114:H48	O126:H21	O146:H14	O171:H <sup>-</sup>
O1:H2	O9ab:H <sup>-</sup>	O26:H <sup>-</sup>	O54:H21	O84:H2	O103:H25	O114:H7	O126:H27	O146:H21	O171:H2
O1:H7	O9:H7	O26:H2	O55:H <sup>-</sup>	O84:H20	O103:HNT	O115:H10	O127	O146:H28	O172:H <sup>-</sup>
O1:H20	O9:H21	O26:H8	O55:H6	O85:H <sup>-</sup>	O104:H <sup>-</sup>	O115:H18	O128:H <sup>-</sup>	O148:H28	O172:H7
O1:HNT	O11:H <sup>-</sup>	O26:H11	O55:H7	O85:H10	O104:H2	O116:H <sup>-</sup>	O128ab:H2	O150:H <sup>-</sup>	O173:H2
O2:H <sup>-</sup>	O11:H2	O26:H12	O55:H9	O85:H23	O104:H7	O116:H4	O128:H7	O150:H8	O174:H <sup>-</sup> d
O2:H1	O11:H8	O26:H32	O55:H10	O86:H <sup>-</sup>	O104:H16	O116:H10	O128:H8	O150:H10	O174:H2 d
O2:K1:H2	O11:H49	O26:H46	O55:H19	O86:H10	O104:H21	O116:H19	O128:H10	O152:H4	O174:H8 d
O2:H5	O12:H <sup>-</sup>	O27:H <sup>-</sup>	O55:H7	O86:H40	O105ac:H18	O117:H <sup>-</sup>	O128:H12	O153:H2	O174:H21 d
O2:H6	O14:H <sup>-</sup>	O27:H30	O60:H <sup>-</sup>	O87:H16	O105:H19	O117:H4	O128:H25	O153:H11	O175:H16 e
O2:H7	O15:H <sup>-</sup>	O28ab:H <sup>-</sup>	O64:H25	O88:H <sup>-</sup>	O105:H20	O117:H7	O128:H31	O153:H12	OX176:H <sup>-</sup> f
O2:H11	O15:H2	O28:H25	O65:H16	O88:H25	O106	O117:K1:H7	O128:H45	O153:H21	OX177:H <sup>-</sup> f
O2:H27	O15:H8	O28:H35	O68:H <sup>-</sup>	O89:H <sup>-</sup>	O107:H27	O117:H8	O129:H <sup>-</sup>	O153:H25	OX177:H11 f
O2:H29	O15:H27	O30:H2	O69:H <sup>-</sup>	O90:H <sup>-</sup>	O109:H2	O117:H19	O130:H11	O153:H30	OX178:H7 f
O2:H44	O16:H <sup>-</sup>	O30:H21	O69:H11	O91:H <sup>-</sup>	O109:H16	O117:H28	O131:H4	O153:H33	OX179:H8 f
O3:H10	O16:H6	O30:H23	O70:H11	O91:H4	O110:H <sup>-</sup>	O118:H <sup>-</sup>	O132:H <sup>-</sup>	O154:H <sup>-</sup>	OX181:H15 f
O4:H <sup>-</sup>	O16:H21	O37:H41	O71:H <sup>-</sup>	O91:H10	O110:H19	O118:H2	O133:H <sup>-</sup>	O154:H4	OX181:H49 e
O4:H5	O17:H18	O38:H21	O73:H34	O91:H14	O110:H28	O118:H12	O133:H53	O154:H19/20	ONT:H <sup>-</sup>
O4:H10	O17:H41	O38:H26	O74	O91:H15	O111:H <sup>-</sup>	O118:H16	O134:H25	O156:H <sup>-</sup>	ONT:H2
O4:H40	O18:H <sup>-</sup>	O39:H4	O75:H <sup>-</sup>	O91:H21	O111:H2	O118:H30	O137:H6	O156:H4	ONT:H8
O5:H <sup>-</sup>	O18:H7	O39:H8	O75:H1	O91:H40	O111:H7	O119:H <sup>-</sup>	O137:H41	O156:H7	ONT:H18
O5:H16	O18:H12	O39:H28	O75:H5	O91:HNT	O111:H8	O119:H5	O138:H2	O156:H25	ONT:H19
O6:H <sup>-</sup>	O18:H15	O40:H2	O75:H8	O92:H3	O111:H11	O119:H6	O141:H <sup>-</sup>	O156:H27	ONT:H21
O6:H1	O18:H7	O40:H8	O76:H7	O92:H11	O111:H21	O119:H25	O141:H2	O156:HNT	ONT:H25
O6:H2	O20:H <sup>-</sup>	O41:H2	O76:H19	O95:H <sup>-</sup>	O111:H30	O120:H19	O141:H8	O160:HP	ONT:H41
O6:H4	O20:H7	O41:H26	O77:H <sup>-</sup>	O96:H10	O111:H34	O121:H <sup>-</sup>	O142	O161:H <sup>-</sup>	ONT:H47
O6:H12	O20:H19	O44	O77:H4	O98:H <sup>-</sup>	O111:H40	O121:H8	O143:H <sup>-</sup>	O162:H4	ONT:K39:H48
O6:H28	O21:H5	O45:H <sup>-</sup>	O77:H7	O98:H8	O111:H49	O121:H11	O144:H <sup>-</sup>	O163:H <sup>-</sup>	Orough:H <sup>-</sup>
O6:H29	O21:H8	O45:H2	O77:H18	O100:H25	O111:H7	O121:H19	O145:H <sup>-</sup>	O163:H19	Orough:H2
O6:H31	O21:H7	O45:H7	O77:H41	O100:H32	O112ab:H2	O123:H19	O145:H4	O163:H25	Orough:H5
O6:H34	O22:H <sup>-</sup>	O46:H2	O78:H <sup>-</sup>	O101:H <sup>-</sup>	O112:H19	O123:H49	O145:H8	O165:H <sup>-</sup>	Orough:K1:H6
O6:H49	O22:H1	O46:H31	O79:H7	O101:H9	O112:H21	O124:H <sup>-</sup>	O145:H16	O165:H10	Orough:K1:H7
O7:H4	O22:H5	O46:H38	O79:H14	O102:H6	O113:H2	O125:H <sup>-</sup>	O145:H25	O165:H19	Orough:H11
O7:H8	O22:H8	O48:H21	O79:H23	O103:H <sup>-</sup>	O113:H4	O125:H8	O145:H26	O165:H21	Orough:H16
O8:H <sup>-</sup>	O22:H16	O49:H <sup>-</sup>	O80:H <sup>-</sup>	O103:H2	O113:H5	O125:HP	O145:H28	O165:H25	Orough:H18
O8:H2	O22:H40	O49:H10	O81:H7	O103:H4	O113:H7	O126:H <sup>-</sup>	O145:H46	O166:H12	Orough:H20
O8:H9	O23:H7	O50:H <sup>-</sup>	O82:H <sup>-</sup>	O103:H6	O113:H21	O126:H2	O145:HNT	O166:H15	Orough:H21
O8:H11	O23:H16	O50:H7	O82:H5	O103:H7	O113:H32	O126:H8	O146:H <sup>-</sup>	O166:H28	Orough:H28
O8:H14	O23:H21	O51:H49	O82:H8	O103:H11	O113:H53	O126:H11	L	O168:H <sup>-</sup>	Orough:H46
O8:H19	O25:H <sup>-</sup>	O52:H19	O83:H <sup>-</sup>						

## Official food control for STEC / EHEC according to the German legislation (§ 64 LFBG)

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**Detection of Shiga (Vero) Toxins or their genes is the only method to detect all variants of STEC /EHEC**



**Gold standard: Verocelltest**

## STEC screening in German food inspection laboratories

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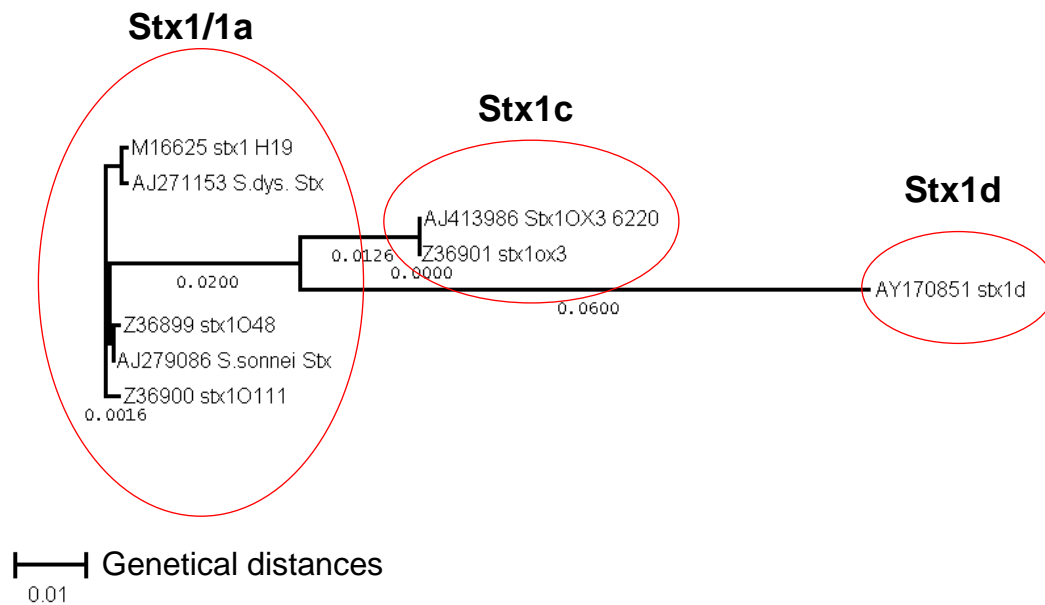
**More than 60% of the government laboratories use commercially obtainable test kits for detection of STEC from food samples.**

STEC detection method	RV2008	RV2009
Stx-ELISA (4 commercial tests)	11 (47.8%)	13 (50,0%)
stx-PCR	8 (34.8%)	8 (30.8%)
Stx-ELISA+stx-PCR	4 (17.4)	5 (19.2%)

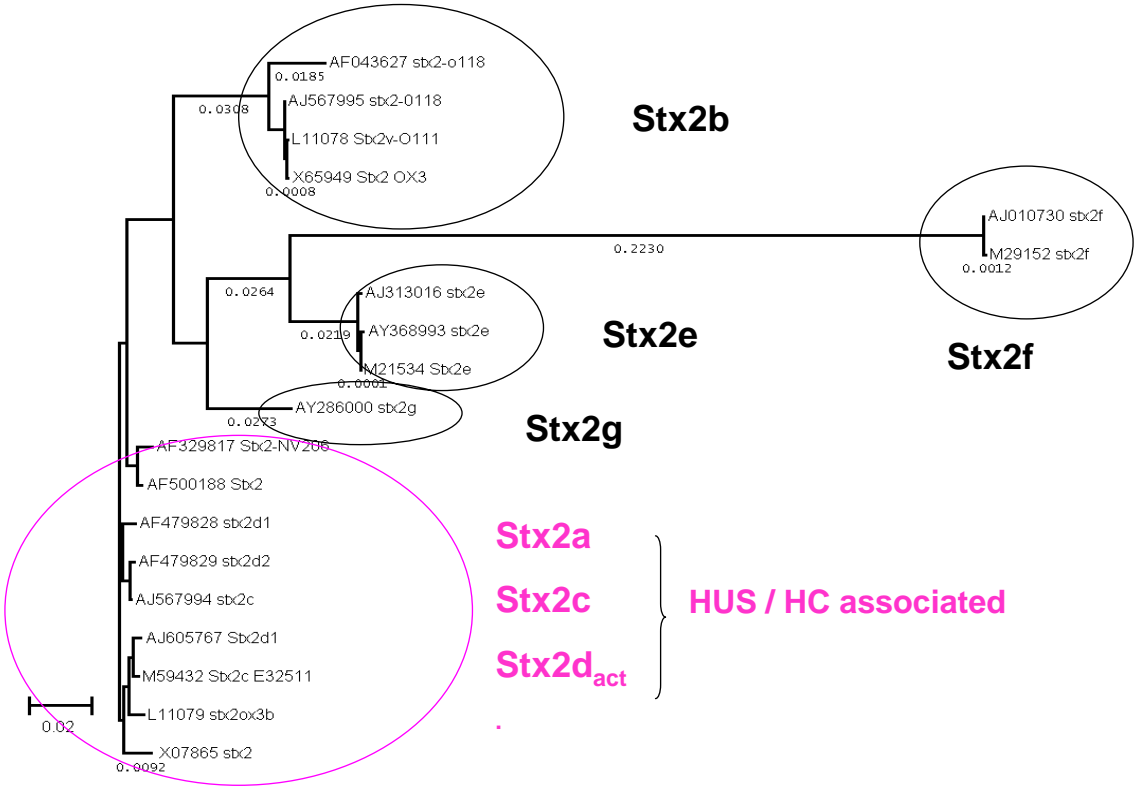
Beutin et al., J. Verbrauchersch. + Lebensmittelsich. 5: 21-34, 2010

**A task of the NRL-E.coli is to evaluate existing and new Stx typing systems for their specificity and sensitivity**

## Stx1 family: three major groups

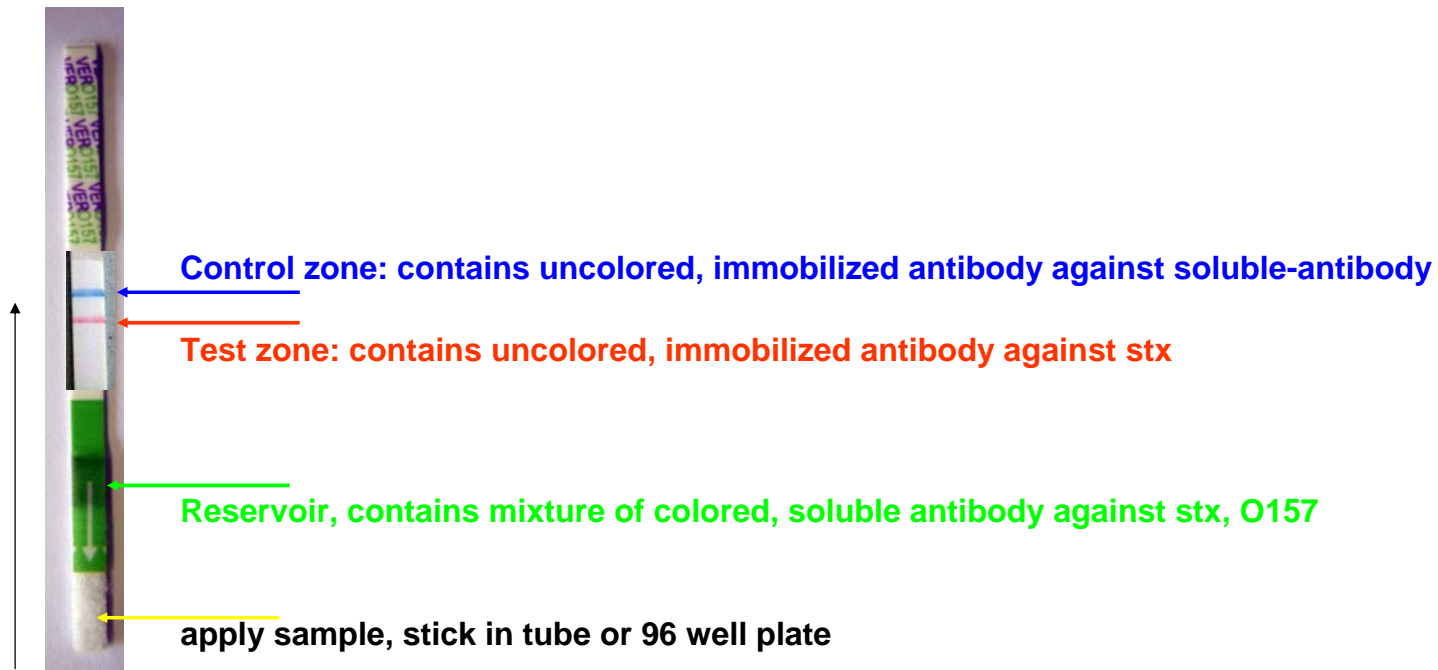


# Stx2 family: seven major groups



# Principle of Immunochromatography

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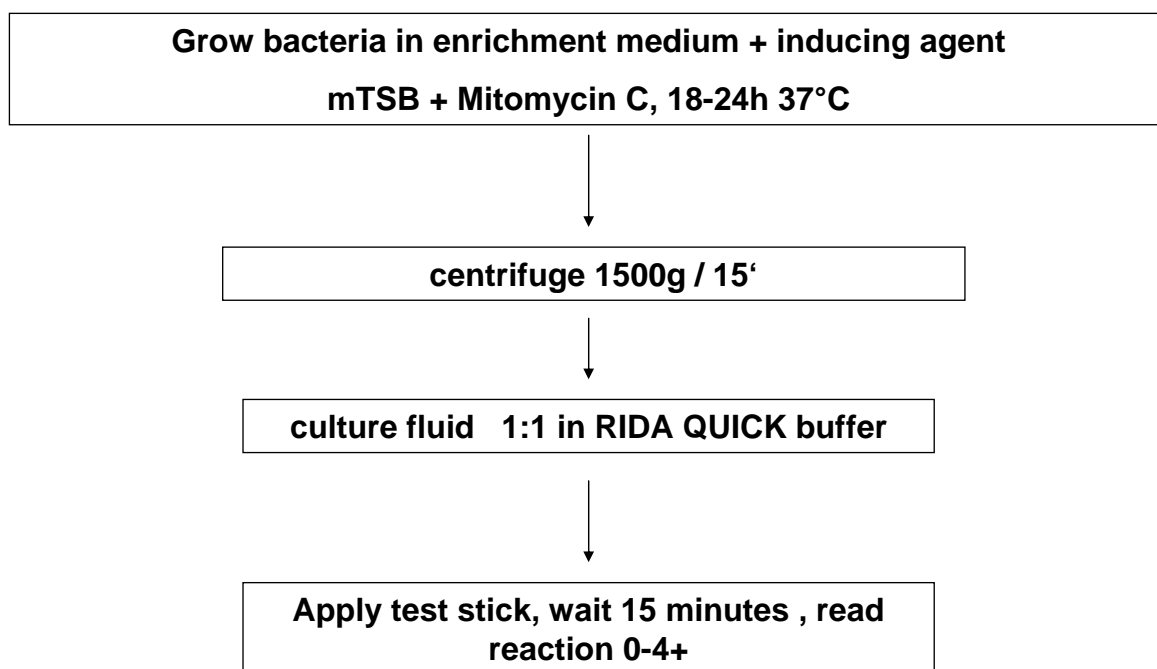


**Migration by  
diffusion**

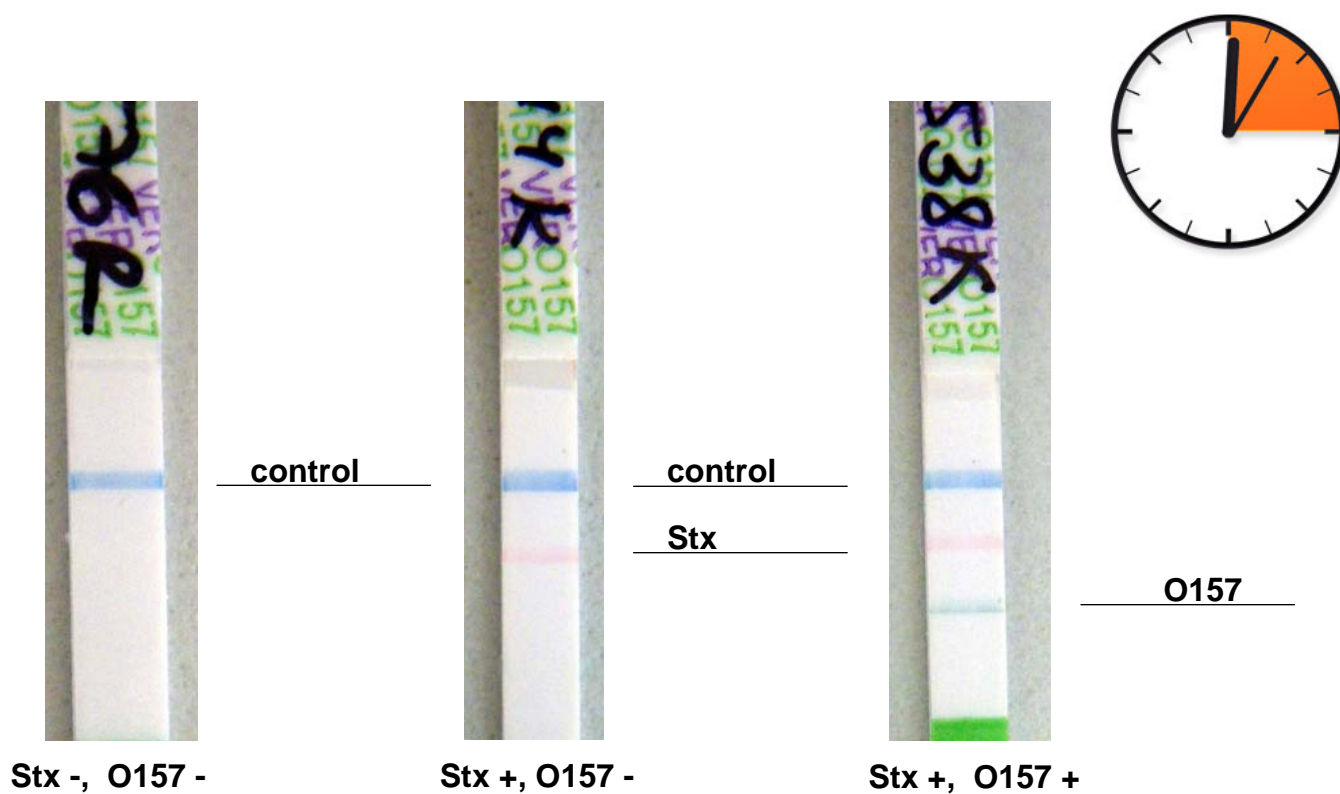
## RIDA-QUICK Verotoxin/O157 Combi, test procedure

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**Rapid Immunochromatographic test for detection of Stx1 und Stx2 family toxins  
and for the O157 antigen**



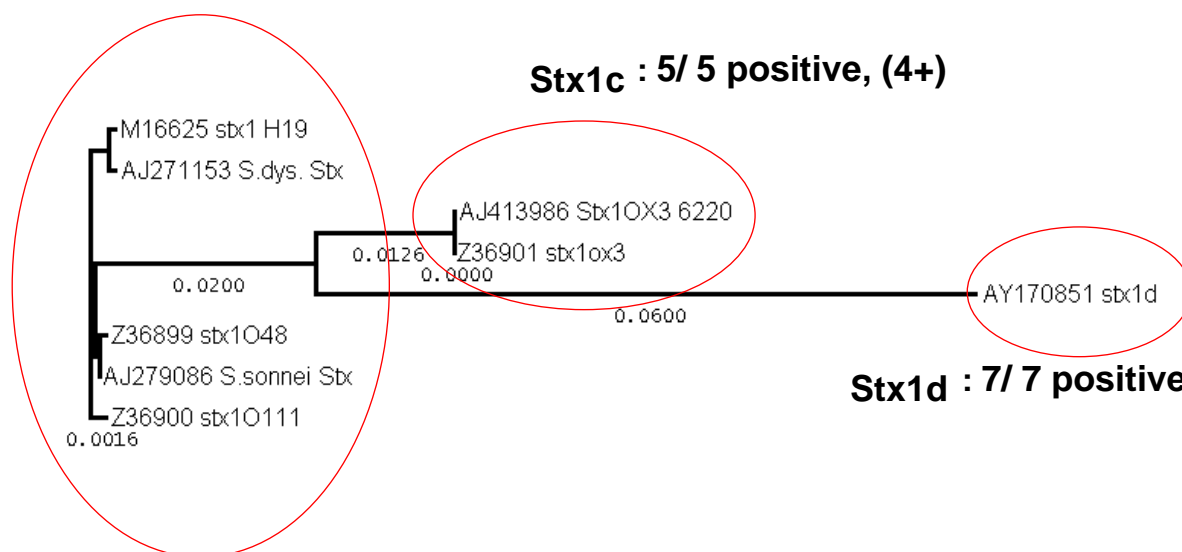
## RIDA-QUICK Verotoxin/O157 Combi, reading the results



## RIDA QUICK: specificity Stx1-family (n=23)

**Stx1/1a : 11/ 11 positive, (4+)**

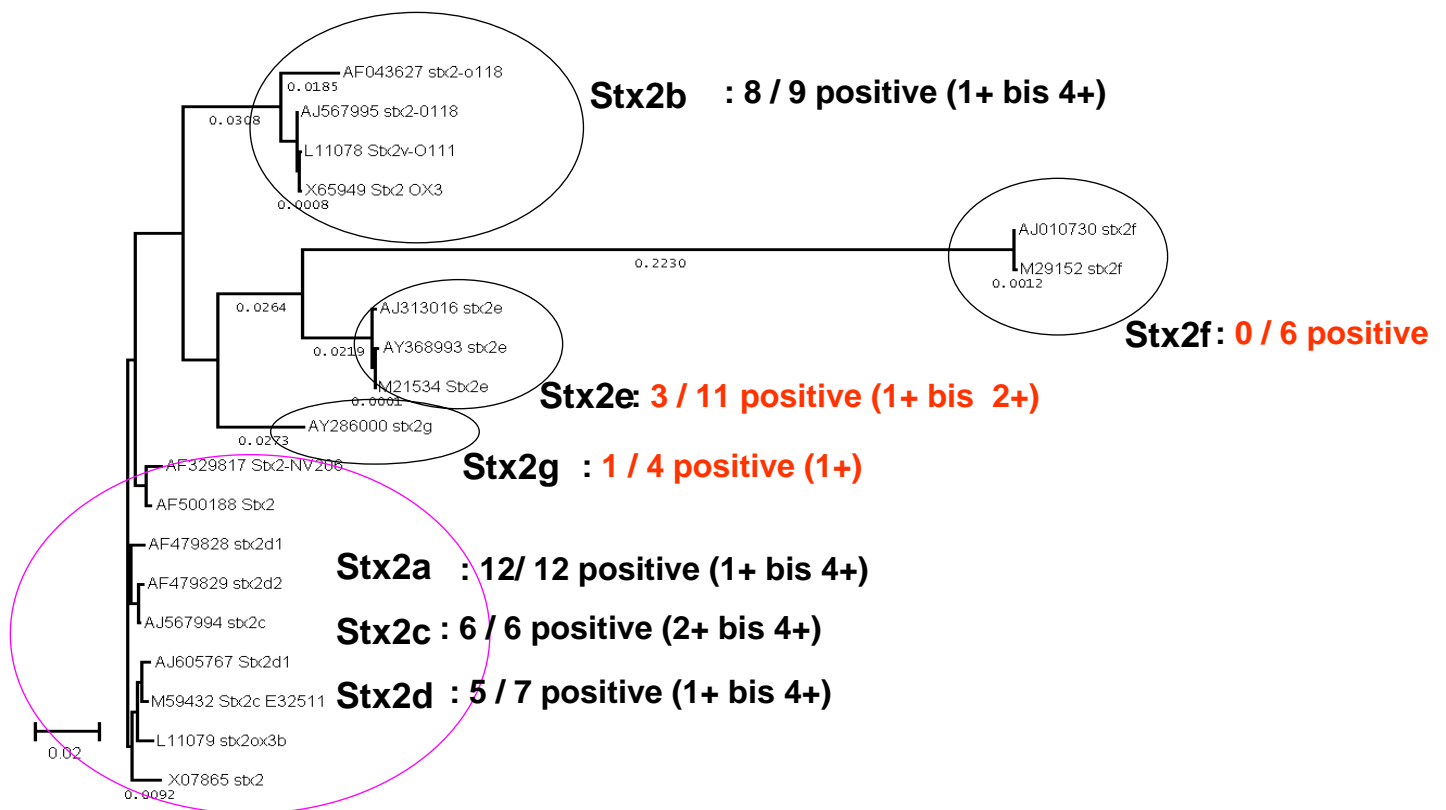
**Stx1c : 5/ 5 positive, (4+)**



**Stx1d : 7/ 7 positive, (1+ bis 3+)**

0.01

## RIDA QUICK: specificity Stx2-family (n=55)



## **RIDA QUICK: controls + strains with multiple Stx**

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**Stx-negative controls:**

**4/4 negativ**

**STEC with more than one stx gene: 33/33 positive**

## RIDA QUICK: specificity for *E. coli* O157 antigen (n=134)

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**tested: 117 strains from 45 O-groups**

**Including 17 *E. coli* O157**

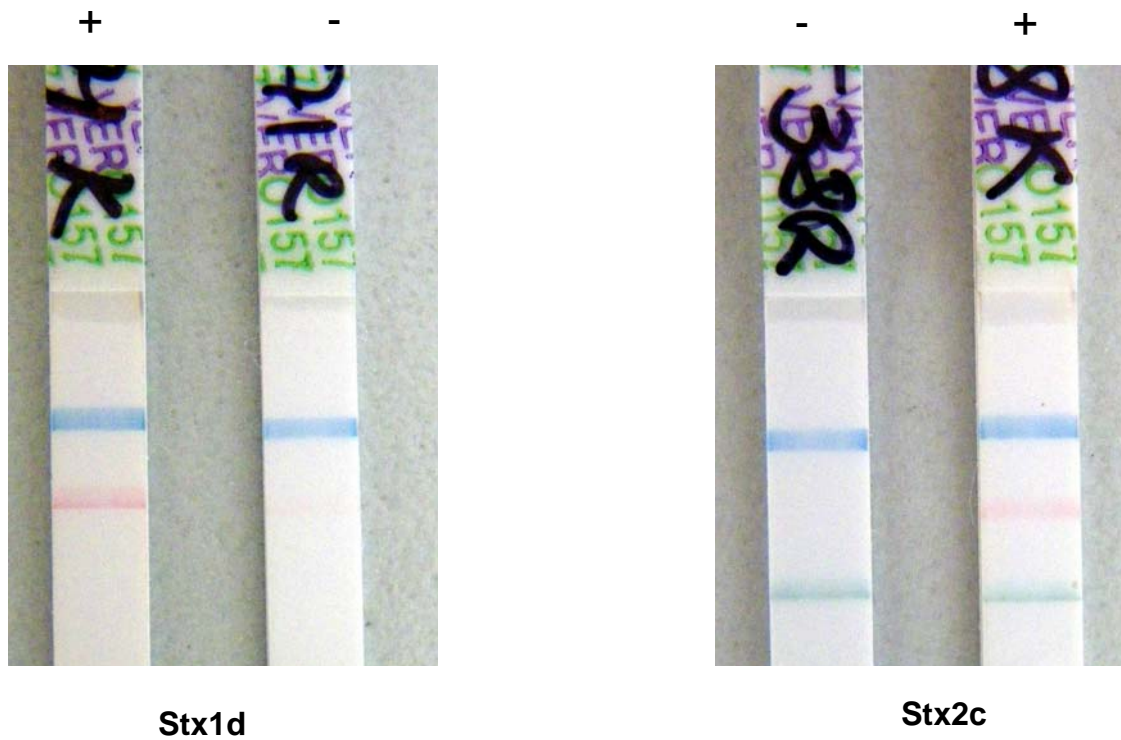
**alle 17 O157 were identified**

**There were no false positive reactions**

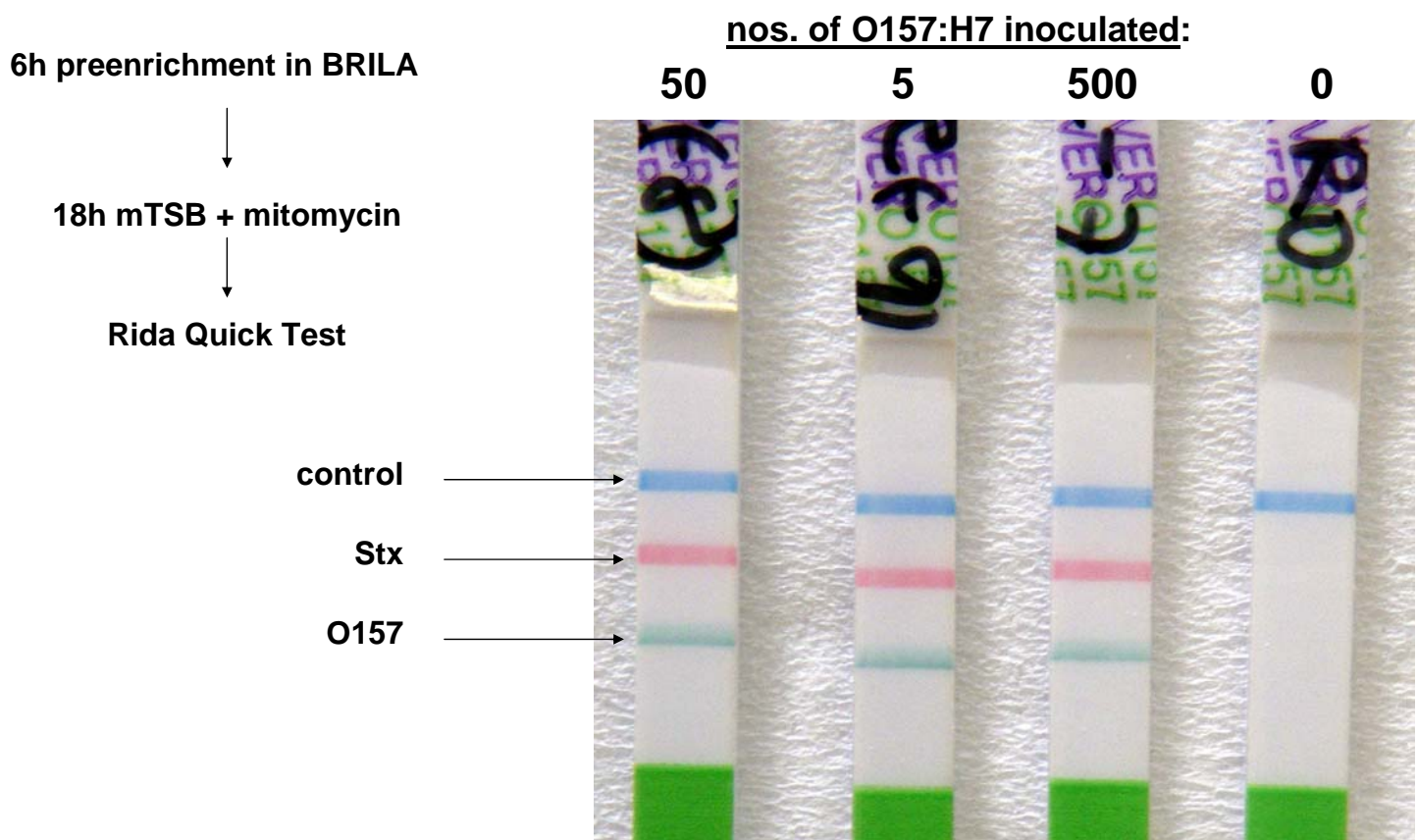
← **O157-Antigen**

## Importance of effective Stx induction, influence of enrichment media

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## Detection of EHEC O157 from spiked food samples after enrichment



## Summary of the major finding

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**Rapid and lab-apparatus independent, qualitative test system for Stx and O157 antigen**

**Sensitivity comparable to RIDA Screen Verotoxin, Stx-EIA of the same producer, dependent on amount of Stx produced by the bacteria**

**Application area: screening test for STEC from pure and mixed cultures of bacteria**

**Induction of Stx production (mitomycin C) is needed for best results**

**Testkit includes Stick, buffer, induction medium can be purchased from the same producer**

Thank you for your attention



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