

# Russian approaches to VTEC control

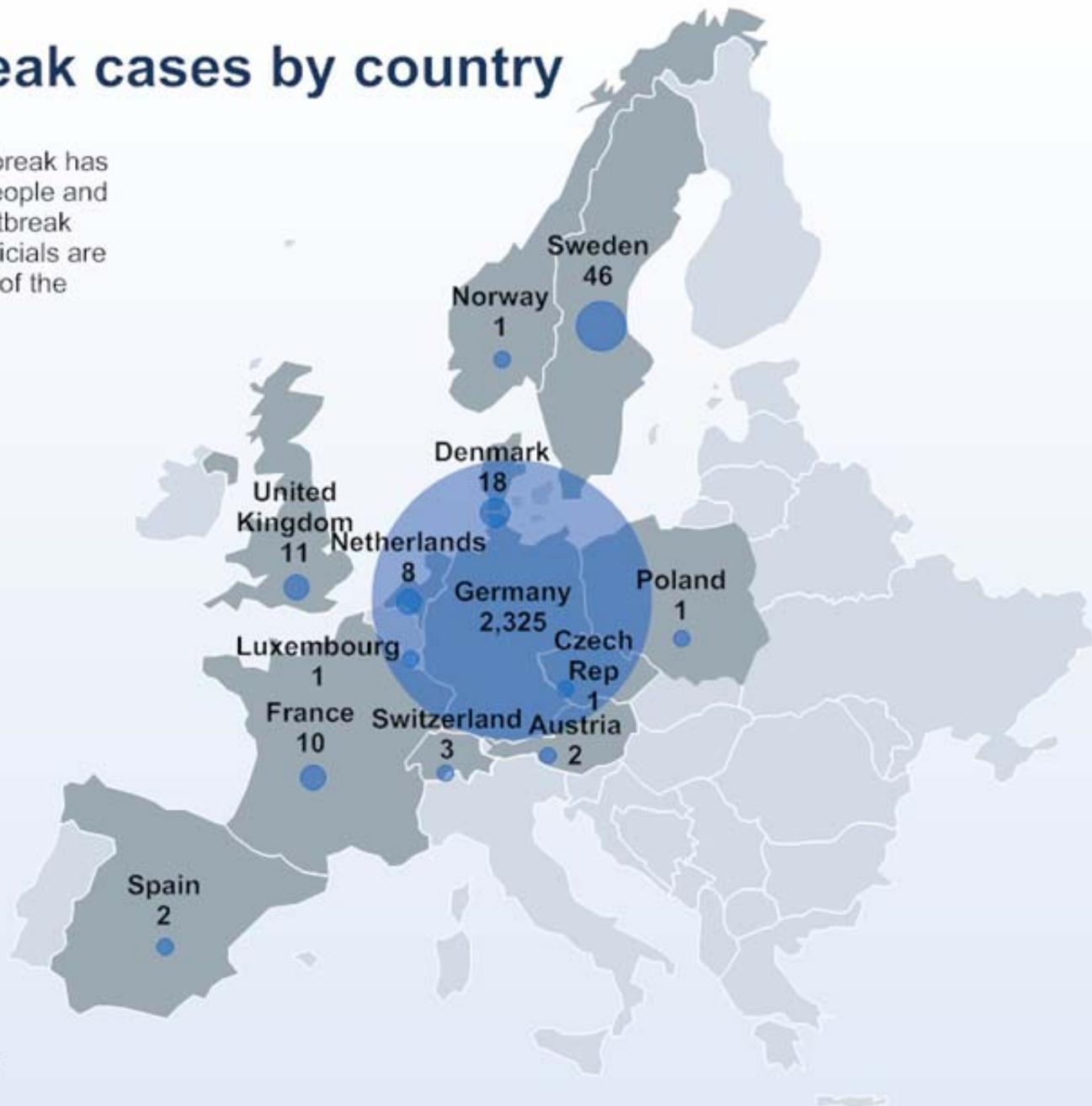
Ivan Dyatlov, Andrey Anisimov, Edward Svetoch  
State Research Center for Applied Microbiology &  
*Biotechnology (Obolensk, Russia)*



6<sup>th</sup> Annual Workshop of the National Reference Laboratories  
for *E. coli* in the EU (4<sup>th</sup> November, 2011)

# E. coli outbreak cases by country

Europe's food poisoning outbreak has sickened more than 2,400 people and killed 24. Weeks after the outbreak began on May 2, German officials are still searching for the source of the contamination



Source: World Health Organization; The Robert Koch Institute  
Visual made with SmartDraw VP. [www.smartdraw.com](http://www.smartdraw.com)

# June 02, 2011

# Order # 583

## Import of vegetables from the EU was prohibited in Russia

Russia says "nyet" to European vegeta...

**Minyanville**

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### Ernst's Blog

Russia says "nyet" to European vegetables, as a consequence of the EHEC bacterium outbreak. Scientists in the meantime, are nowhere near finding a smokin' gun.

Posted on June 3, 2011 by Ernst Labruyere

Share Tweet share share

Last week I wrote a number of articles on the EHEC bacterium that has Germany and the Western European countries in its grip:

Dutch market gardeners want social support  
Forget the data economy and look at pharmacy



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## About federal service

Federal service on customers' rights protection and human well-being surveillance had been founded based on the decree of the President of Russian Federation, dated by March, 9, 2004, №314 <About executive power federal departments system and structure>

The Federal service had been partially transferred the functions of the Ministry of Healthcare of Russian Federation, the Ministry of economical development and trade of Russian Federation, the Ministry of antimonopoly policy of Russian Federation.

June 07, 2011

Order # 583

# “On holding of short-term extension courses on laboratory diagnosis of acute enteric infections caused by enterohemorrhagic and enterotoxigenic *Escherichia coli*”

<http://rospotrebnadzor.ru/>



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## MAIN DIRECTIONS OF ACTIVITY

**115 bacteriologists from 87 Russian local and regional labs underwent  
72-hour extension courses on laboratory diagnosis of acute enteric  
infections caused by *Escherichia coli* O104:H4 (Obolensk, 2011)**



June 07, 2011

Letter # 01/6970-1-32

# “On organization of laboratory diagnosis of acute enteric infections caused by *Escherichia coli* O104:H4”

<http://rospotrebnadzor.ru/>



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# Three National Reference Laboratories

- (i) State Research Center for Applied Microbiology & Biotechnology, Obolensk
- (ii) Central Research Institute of Epidemiology, Moscow
- (iii) Antiplague Research Institute of Siberia and Far East, Irkutsk



# Main laboratory building of the State Research Center for Applied Microbiology & Biotechnology



# Biosafety level (BSL-2, BSL-3)



Actual BSL-3 space  
is c. 1500 m<sup>2</sup>



# June 17-June 24, 2011



**EU Reference Laboratory for *E.coli***  
Department of Veterinary Public Health and Food Safety  
Unit of Foodborne Zoonoses  
**Istituto Superiore di Sanità**



To the recipient laboratory:

**State Research Centre for Applied Microbiology & Biotechnology**  
**Moscow Region, Russia**  
**Dr Anisimov**

Please find enclosed a stabbed culture of the German outbreak strain RKI No. 11 2027. The original strain has been provided by the National Reference Centre for Salmonella and other Enterics at the Robert Koch Institute and has the following characteristics:

Strain	Serogroup	Genotype
11 2027	O104:H4	eae- vtx2+, H4



June 29, 2011

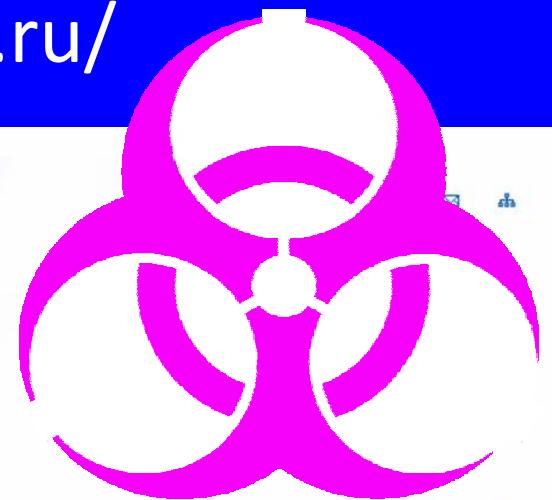
Decree # 86

VTEC strains were classified as belonging to the Russian second pathogenicity group = international risk group level 3

<http://rosпотребнадзор.ru/>



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PROTECTION AND HUMAN WELL-BEING SURVEILLANCE



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## Methodological instructions on laboratory diagnosis of infections caused by *Escherichia coli* producing Shiga toxins (STEC-cultures) and indication of causative agents of STEC-infections in foodstuffs



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Методические указания МУК 4.2.2963-11 по лабораторной диагностике заболеваний, вызываемых энтерогеморрагическими *E. coli*

# Diagnostic kit for the identification of Escherichia coli O104:H4 in the reaction of latex agglutination

## The kit reagents

Vial №1 – 0,5% suspension of latex particles sensitized with rabbit immunoglobulin G against *E. coli* O104:H4 antigens (**latex test**);

Vial №2 – 0,5% suspension of latex particles sensitized with normal rabbit immunoglobulin (**control test**);

Vial №3 – inactivated microbial suspension of *E. coli* O104:H4 at a concentration of 10<sup>9</sup> CFU/ml (**positive control**).

Date of manufacture 08.2011

Best before 08.2012

Storage temperature 4 °C



CERTIFICATE OF COMPLIANCE  
(ISO 9001:2000)



**FBIS SRCAMB**

Obolensk, Serpukhov district, Moscow region, 142279, Russia

Phone: +7 (4967) 36-00-03, Fax: +7 (4967) 36-00-10

E-mail: [info@obolensk.org](mailto:info@obolensk.org), [www.obolensk.org](http://www.obolensk.org)

Diagnostic kit to identify DNA of Escherichia  
coli O104:H4 in bacterial cultures and clinical  
specimens by multiplex PCR  
“TЭК-O104”

Manuf. date: 07.2011

Best before: 01.2012

Storage: at +4 °C

**FBIS SRCAMB ROSPOTREBNADZOR**

Manufactured & packed: FBIS SRCAMB

142279, Obolensk, Serpukhov district, Moscow region, Russia.  
Phone: +7(4967) 36-00-03. Fax : +7(4967) 36-00-10  
E-mail:[info@obolensk.org](mailto:info@obolensk.org), [www.obolensk.org](http://www.obolensk.org)

Diagnostic kit to identify DNA of Escherichia  
coli O157 in bacterial cultures and clinical  
specimens by multiplex PCR  
“TЭК-O157”

Manuf. date: 07.2011

Best before: 01.2012

Storage: at +4 °C

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**Diagnostic kit to identify DNA of Escherichia coli O157 in bacterial cultures and clinical specimens by multiplex PCR  
“TЭК-O157“**

Manuf. date: 07.2011

Best before: 01.2012

Storage: at + 4 °C

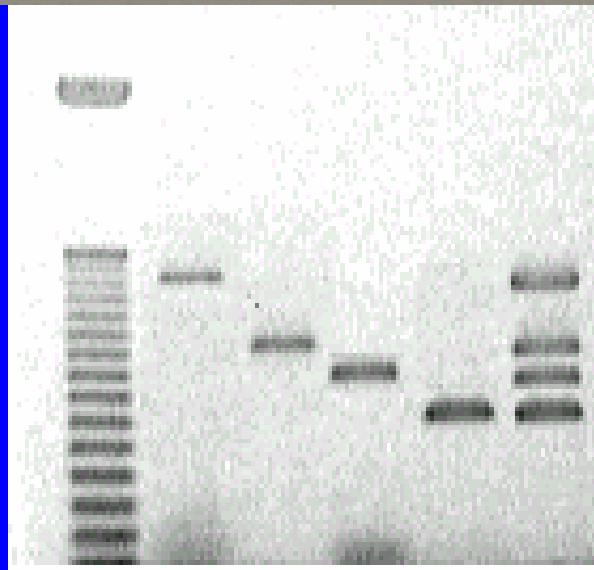
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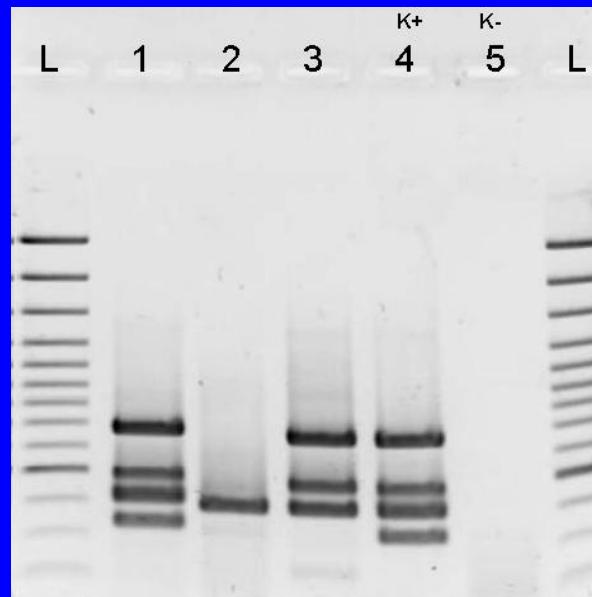
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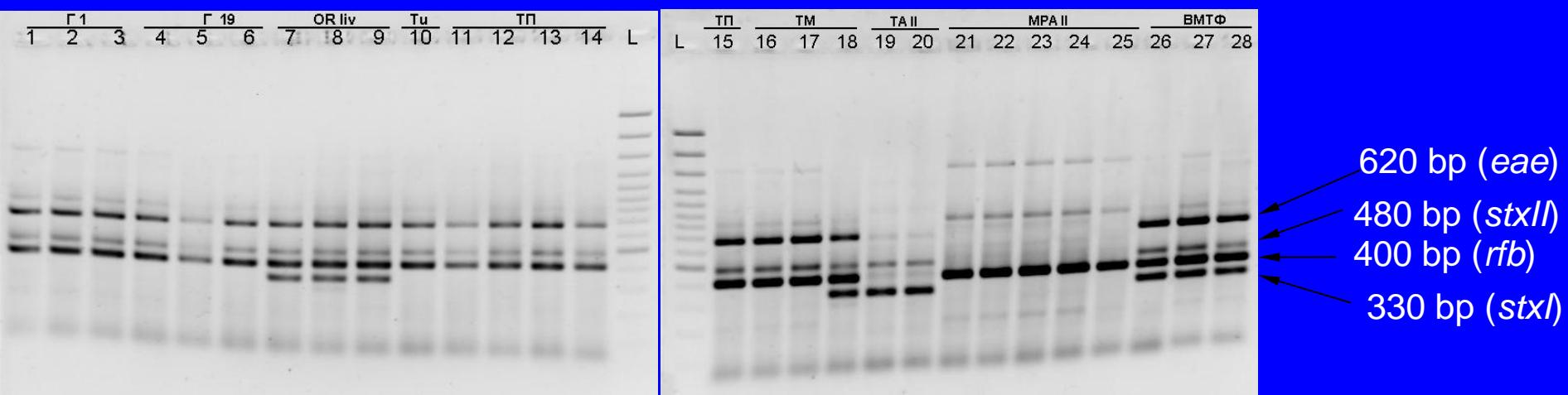
620 bp (*eae*)  
480 bp (*stxII*)  
400 bp (*rfb*)  
330 bp (*stxI*)

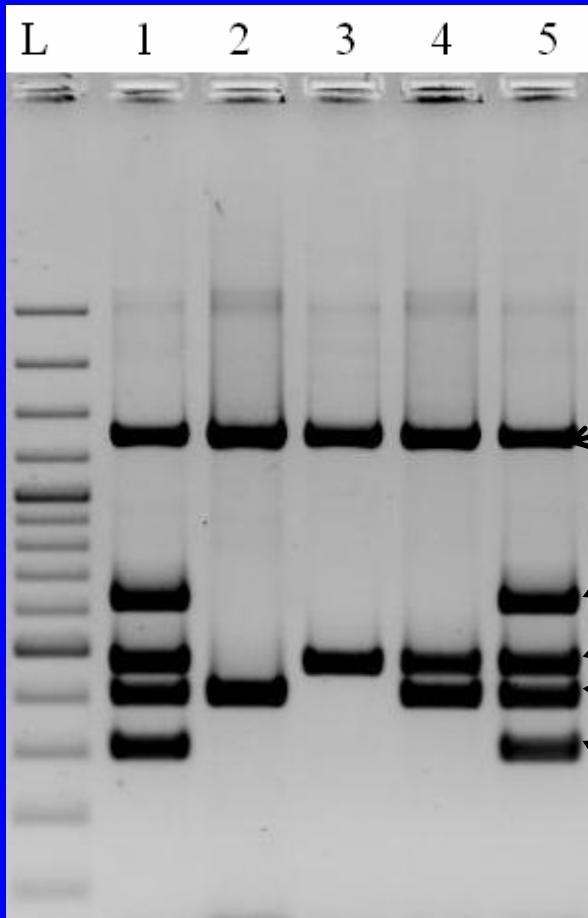


- 1 - *E.coli* O157:H7 (StxI+ StxII+)
- 2 – *E.coli* O157
- 3 – *E.coli* O157:H7 (StxII+)
- 4 – positive control
- 5 – negative control
- L – molecular markers

**Identification *E. coli*  
O157:H7 in multiplex PCR**

## Genotyping of Russian *E.coli* O157:H7 isolates in multiplex PCR





- 1, 5 – *E.coli* O104:H4
- 2 – *E.coli* O104:H12
- 3 – *E.coli* O157:H7
- 4 – mixture of *E.coli* O104:H12 & *E.coli* O157:H7
- L – molecular markers

Diagnostic kit to identify DNA of *Escherichia coli* O104:H4 in bacterial cultures and clinical specimens by multiplex PCR  
“TЭK-O104”

Manuf. date: 07.2011

Best before: 01.2012

Storage: at +4 °C

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## Konsiliarlabor für Hämolytisch-Urämisches Syndrom (HUS)

am Institut für Hygiene · Universitätsklinikum Münster

Direktor: Prof. Dr. Dr. h.c. H. Karch

Robert-Koch-Str. 41 · 48149 Münster

Tel. 0251 83 55367 (Labor) Fax: 0251 83 55688

[www.ehec.org](http://www.ehec.org)

### PCR zum spezifischen Nachweis von HUSEC041 (O104:H4)

Primerbezeichnung	Primersequenz (5'—3')	Target	Länge des PCR-Produkts (bp)	Referenz	Primerkonzentration
LP43	ATC CTA TTC CCG GGA GTT TAC G				
LP44	GCG TCA TCG TAT ACA CAG GAG C	<i>stx2</i>	584	Cebula et al. 1995	150 nM
TerD1	AGT AAA GCA GCT CCG TCA AT				
TerD2	CCG AAC AGC ATG GCA GTC T	<i>terD</i>	434	Diane E et al. 2002	240 nM
104rfbO-f	TGA ACT GAT TTT TAG GAT GG				
104rfbO-r	AGA ACC TCA CTC AAA TTA TG	<i>rfbO104</i>	351	Hier	360 nM
fliCH4-a	GGC GAA ACT GAC GGC TGC TG				
fliCH4-b	GCA CCA ACA GTT ACC GCC GC	<i>fliC H4</i>	201	Hier	240 nM

### Reaktionsbedingungen

25 µl PCR Ansatz mit REDTaq ReadyMix (Sigma-Aldrich)  
mit 2 µl DNA Template

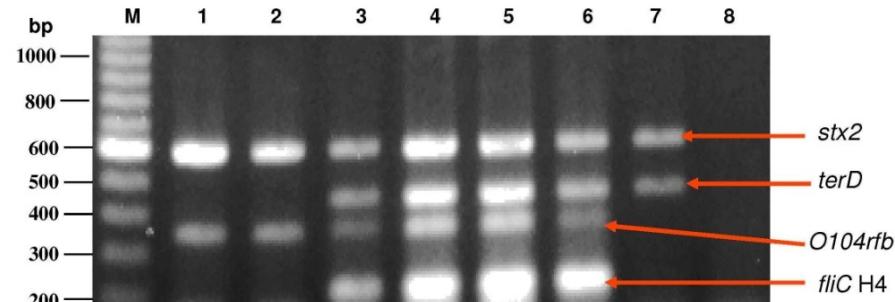
94 °C 5 min Denaturierung

30 Zyklen mit

- |                  |       |      |
|------------------|-------|------|
| 1. Denaturierung | 94 °C | 30 s |
| 2. Annealing     | 55 °C | 60 s |
| 3. Elongation    | 72 °C | 60 s |

72 °C 5 min. finale Elongation

### Nachweis im 2%igen Agarosegel (Exemplarisch in der Abbildung rechts)



1. HUSEC037, ST: 672, (O104:H21) *stx2+*, *terD-*,
2. HUSEC038, ST: 672 (Ont:H21) *stx2+,terD-*
3. HUSEC041, ST: 678 (O104:H4) *stx2+, terD+*
4. 226692 (O104:H4) *stx2+,terD+*
5. 226541(O104:H4) *stx2+, terD+*
6. 226806 (O104:H4) *stx2+,terD+*
7. HUSEC003 (O157:H7) *stx2+, ter+*
8. C600

gene	Länge des PCR Produkte
<i>stx2</i>	584 bp
<i>terD</i>	434 bp
<i>O104rfb</i>	351 bp
<i>fliC H4</i>	201 bp