Legionella analysis

Standardization?

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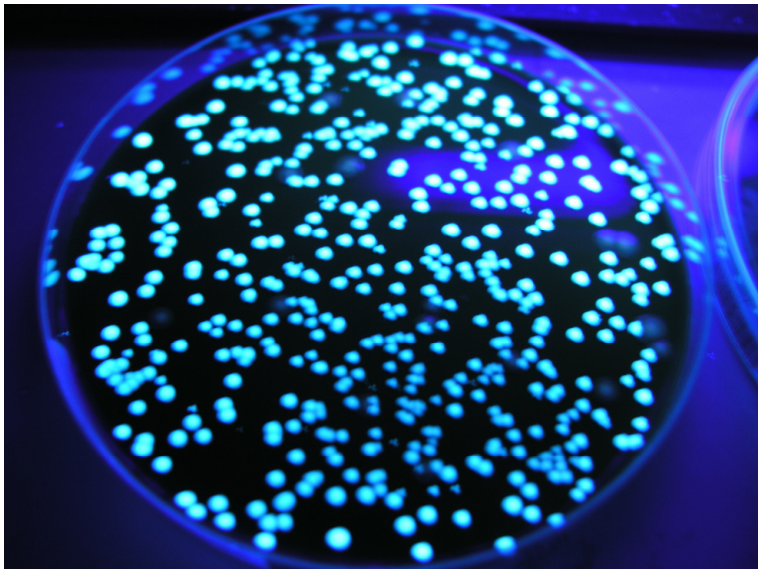
Vitens Laboratory

Presentation

- Introduction Vitens
- Introduction Legionella
- Overview (inter)national standards
- Comparison tests Germany-the Netherlands
- Critical steps Legionella analysis
- Recent developments



Vitens Drinking Water Company

Legionella (1)

- 1976 Philidelphia
- 1977 Isolation Legionnaires Disease Bacterium
- 1978 First solid medium
- 1979 Establishment Legionella genus

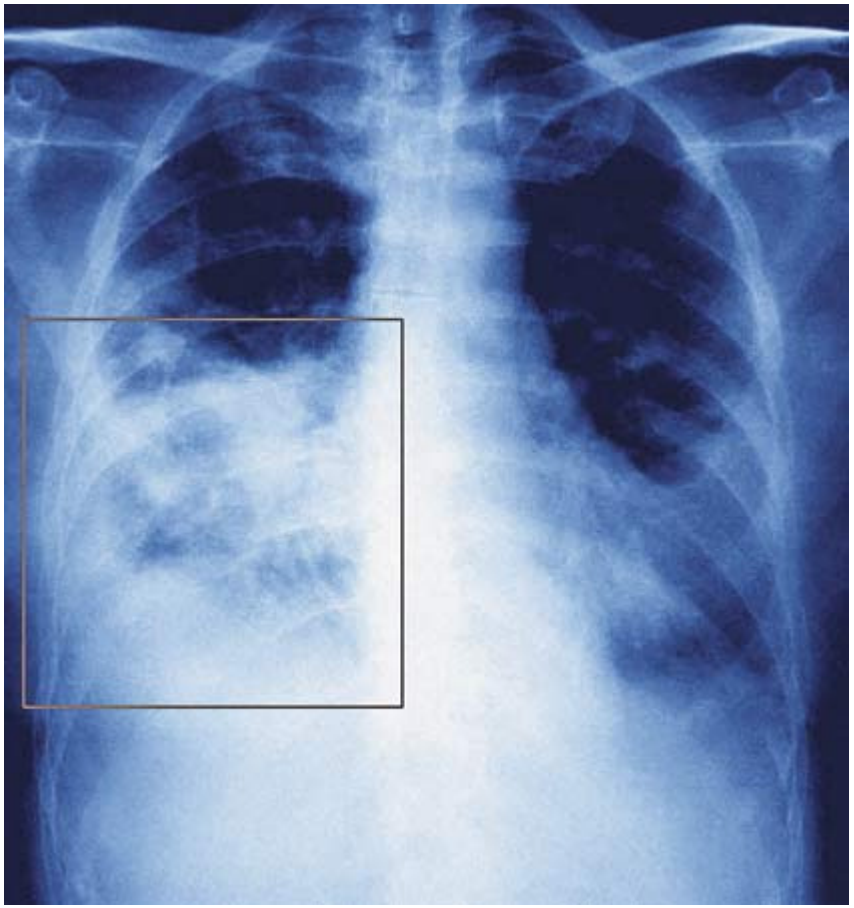
At present:

- At least 50 Legionella species
- 21 species known pathogenic
- 70% Legionella pneumophila serogroup 1
- 20%-30 % Legionella pneumophila serogroup 2-16
- 5%-10% Legionella non-pneumophila

Legionella (2)

- Legionnaires disease
  - pneumonia
  - fever
  - headache
  - malaise
  - nervous system symptoms
  - antibiotic treatment

- Pontiac Fever
  - flu-like illness
  - no antibiotics necessary

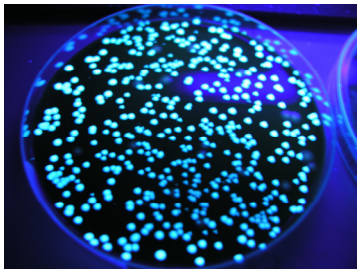
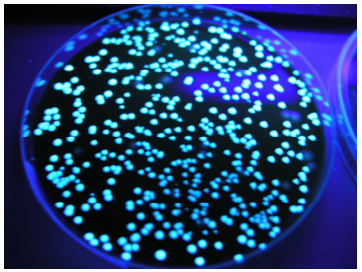
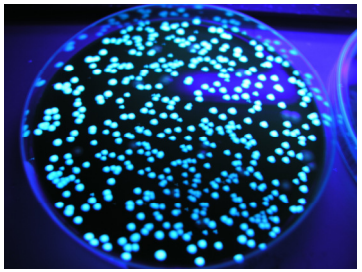


Overview standards

- ISO 11731
- ISO 11731-2
- NEN 6265
- AFNOR NF T 90-431
- Standard Methods (USA)
- Blue Book (UK)
- Standards Australia
- …….

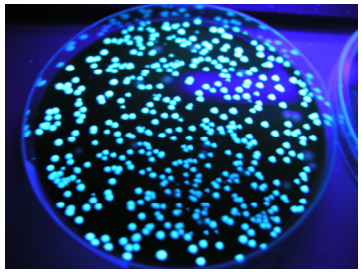
ISO 11731; 1998
Detection and enumeration of Legionella

- Direct plating (10^5 cfu/l), concentration or dilution
- Untreated, heat and acid treatment
- One plate GVPC

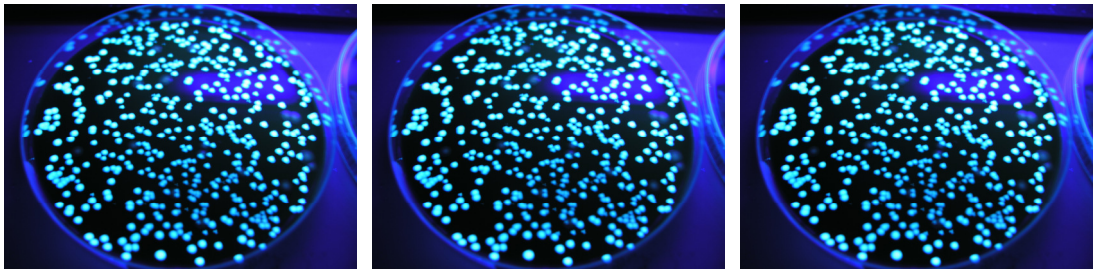
ISO 11731-2;2008
Detection and enumeration of Legionella; Direct membrane filtration method for waters with low bacterial counts

- Filtration (cellulose nitrate filter, 0.45 µm)
- Acid treatment
- One plate GVPC or BCYE



Standard Australia/New Zealand

- Direct plating
  - **Untreated and Heat treated**
    - 0.1 ml on MWY or GVPC
    - 0.01 ml on BMPα
  - **Acid treated (1:9 sample/acid)**
    - 0.1 ml onto BMPα



Standard the Netherlands (NEN 6265)

- Direct plating, filtration or dilution "clean water"

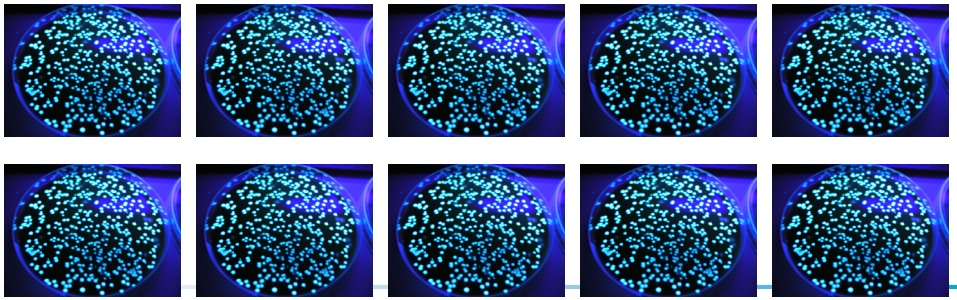
  Untreated and Heat treated

  – 0.1 ml on BCYE –cys, 2*BCYE and 2*BCYE+ab

- Sample high background flora; filtration 50 ml

  Heat treatment

  – 0.2 ml on BCYE –cys, 5*BCYE+ab and 5*MWY



Legionella isolations
Germany vs the Netherlands

	Vitens
• Jan2006-July2009	•Jan2006 – Dec 2008
• 28 % Legionella positive	• 15 % Legionella positive
• 89 % Legionella pneumophila	• 20% Legionella pneumophila
• 17 % Legionella species	• 80% Legionella species

Overview

STOP
Amsterdam
Almere
Haarlem
Haarlemmermeer
Amstelveen
Hilversum
Amersfoort
Utrecht
Leiden
Zoetermeer
Delft
Rotterdam
Dordrecht
's-Hertogenbosch
Breda
Tilburg
Eindhoven
Nijmegen
Alkmaar
Hoorn
Purmerend
Lelystad
Leeuwarden
Zwolle
Enschede
Arnhem
Apeldoorn
Amersfoort
Gelsenkirchen
Dortmund
Essen
Duisburg
Oberhausen
Recklinghausen
Mülheim an
Witten
Bergkamen
Soest
Werl
Arnsberg
Warstein
Menden (Sauerland)
Emden
Delfzijl
Moormerland
Uplengen
Leeuwarden
Franeker
Boarnsterhim
Sneek
Heerenveen
Waddenzee
Texel
Den Helder
Anna Paulowna
Schagen
Harenkarspel
Medemblik
IJsselmeer
Bergen
Heerhugowaard
Castricum
Velsen
Noordoostpolder
Dronten
Huizen
Alphen aan Den Rijn
Katwijk
Teylingen
Leiden
Woerden
Zeist
Barneveld
Ede
Gouda
Nieuwegein
Veenendaal
Schiedam
Barendrecht
Spijkenisse
Moerdijk
Heusden
Oss
Tiel
Overbetuwe
Halderberge
Roosendaal
Etten-Leur
Essen
Zundert
Woensdrecht
Veldhoven
Geldrop
Bakel
Venray
Deurne
Uden
Dinslaken
Lünen
Wad
Ferwerderadiel
De Marne
Winsum
Loppersum
Krummhörn
Großefehn
Wiesmoor
Friedeburg
Varel
Raste
Old
Wardenburg
Osnabrück
Bad Essen
Bielefeld
Harsewinkel
Gütersloh
Oelde
Beckum
Lippstadt
Ahlen
Hamm
Bochum
Wuppertal
Düsseldorf
Krefeld
Mönchengladbach
Aachen
Maastricht
Venlo
Roermond
Zwolle
Deventer
Apeldoorn
Arnhem
Nijmegen
Tilburg
Breda
Eindhoven
Helmond
Venlo
Roermond
Maastricht
Aachen
Heerlen
Sittard
Geleen
Kerkrade
Vlaardingen
Schiedam
Spijkenisse
Dordrecht
Gouda
Zoetermeer
Leiden
Haarlem
Amsterdam
Almere
Amstelveen
Hilversum
Utrecht
Amersfoort
Zwolle
Enschede

Comparison tests (1)

	1372		1373		1374		1375	
Direktansatz	9.000		158.000		<1000		5.000	

1372 1373 1374 1375

Direktansatz	9.000		158.000		<1000		5.000	
20 ml Filtration	<50		<50		<50		4.350	
100 ml Filtration	<10		10		<10		2.000	

NL PW	BCYE (NL)	7.200	13.500	100.000	162.200	2.300	9.200	2.700	16.900
	MWY (NL)	6.400	16.600	100.000	219.200	1.700	10.300	2.200	22.200
	GVPC (D)	1.750		72.500		300		1.750	
	BCYE (D)	4.500		50.000		300		1.500	

Comparison tests (2)

	P376	P377	P380	P384
Average direct plating (ml)	**166**	**8**	**146**	**12**
100 ml MF with acid-treatment	**>200**	**179**	**6**	**0**
Result [cfu/100 ml)	**16583**	**750**	**14583**	**1167**

Results Vitens Laboratory [cfu/100 ml]	**10300**	**670**	**25360**	**470**
Process water method				

Additional tests influence acid treatment

untreated	heat treatment	acid treatment
MWY	MWY	MWY
88	100	85
89	78	99
91	101	104
103	121	94
87	123	97
95	109	104
93	88	90

Filter untreated	Filter acid treatment
MWY	MWY
1	1
2	1
2	5
8	3
7	8
4	0
11	2

Influence membrane filters

- Study 1993 (Smith)

Membrane filter	Recovery (%) 0,2 µm	Recovery (%) 0,4 µm
Nucleopore PC	66	49
Gelman Supor	56	40
Millipore cellulose ester	49	43
Schleicher & Schuell cellulose acetate	41	21

Influence culture media

Research Vitens/KWR

- Comparison culture media *Legionella pneumophila*

	BCYE non-selective	BCYE +selective (=NEN)	BMPα (Oxoid SR111)	GVPC (Oxoid SR152	MWY (Oxoid SR118)
	cfu	%	%	%	%
L. pneumophila	100 ± 6	107 ± 6	***75 ± 3***	***79 ± 11***	86 ± 5
serogroup 1	100 ± 17	105 ± 19	***69 ± 8***	95 ± 4	***80 ± 12***
L. pneumophila	100 ± 25	90 ± 18	73 ± 10	75 ± 8	86 ± 18
serogroup 3	100 ± 20	103 ± 14	88 ± 13	81 ± 28	71 ± 18
L. pneumophila	100 ± 20	110 ± 4	92 ± 20	66 ± 21	76 ± 3
serogroup 6	100 ± 17	99 ± 12	***64 ± 18***	74 ± 15	***64 ± 23***
average of	100 ± 18	102 ± 7	*77 ± 12*	***78 ± 10***	***77 ± 9***
L. pneumophila					

Research KWR/Vitens

- Comparison culture media *Legionella non-pneumophila*

	BCYE non-selective	BCYE +selective (=NEN)	BMPα (Oxoid SR111)	GVPC (Oxoid SR152	MWY (Oxoid SR118)
	cfu	%	%	%	%
L. dumoffii	100 ± 9	90 ± 6	111 ± 7	52 ± 10	60 ± 9
	100 ± 9	92 ± 15	98 ± 9	47 ± 8	45 ± 8
L. longbeachae	100 ± 8	88 ± 6	71 ± 6	52 ± 6	70 ± 5
serogroup 1	100 ± 25	111 ± 5	88 ± 10	77 ± 2	91 ± 11
L. longbeachae	100 ± 16	99 ± 15	54 ± 9	94 ± 7	95 ± 11
serogroup 2	100 ± 20	130 ± 6	122 ± 10	79 ± 17	114 ± 20
L. anisa	100 ± 5	91 ± 10	88 ± 9	78 ± 9	79 ± 4
	100 ± 12	91 ± 5	113 ± 6	71 ± 6	90 ± 2
L. gormanii	100 ± 9	117 ± 15	125 ± 7	35 ± 9	54 ± 8
	100 ± 20	114 ± 12	96 ± 3	40 ± 8	49 ± 3
L. jordanis	100 ± 16	7 ± 3	0	77 ± 7	94 ± 12
	100 ± 3	66 ± 7	0	101 ± 8	123 ± 1
L. micdadei		0	0	87 ± 13	81 ± 4
	100 ± 3	0	2 ± 2	95 ± 14	98 ± 11
L. bozemanii	100 ± 8	99 ± 18	0	87 ± 7	88 ± 14
serogroup 1	100 ± 19	101 ± 15	0	51 ± 16	106 ± 5
L. bozemanii	100 ± 8	122 ± 7	0	119 ± 14	107 ± 10
serogroup 2	100 ± 14	112 ± 28	0	72 ± 26	119 ± 9

Influence pH on growth Legionella species

legionellasoort	pH van de voedingsbodem								
	6,09	6,29	6,50	6,79	6,91	7,03	7,21	7,27	7,34
L. pneumophila serogroep 1	-	±	+	+	+	+	+	+	+
L. pneumophila serogroep 3	±	+	+	+	+	+	+	+	+
L. pneumophila serogroep 6	±	+	+	+	+	+	+	+	+
L. bozemanii serogroep 1	±	+	+	+	±	±	±	±	-
L. bozemanii serogroep 2	±	+	+	+	±	±	±	±	-
L. longbeachae serogroep 2	-	-	±	+	+	+	±	±	-
L. gormanii	-	±	±	+	+	+	±	-	-
L. dumoffii	±	+	+	+	+	±	-	-	-
L. micdadei	-	±	+	+	+	+	+	±	-
L. jordanis	-	±	+	+	+	+	+	+	+
L. anisa	-	±	+	±	±	±	-	-	-

Source:KWR

Further experiments

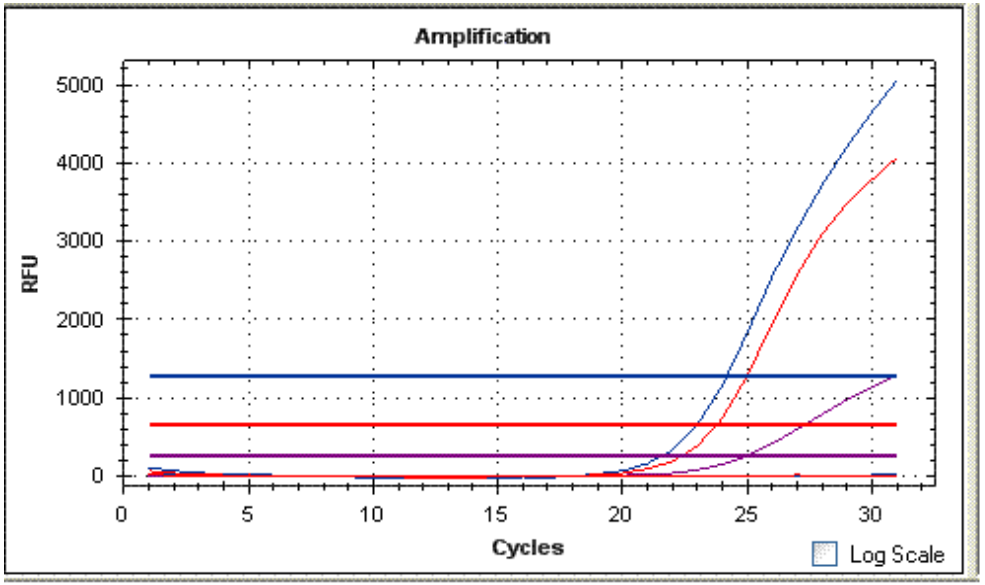
- Influence acid/cellulose nitrate filter
- Recovery centrifugation step
- Recovery different culture media
- Recovery media commercial suppliers
- ……..

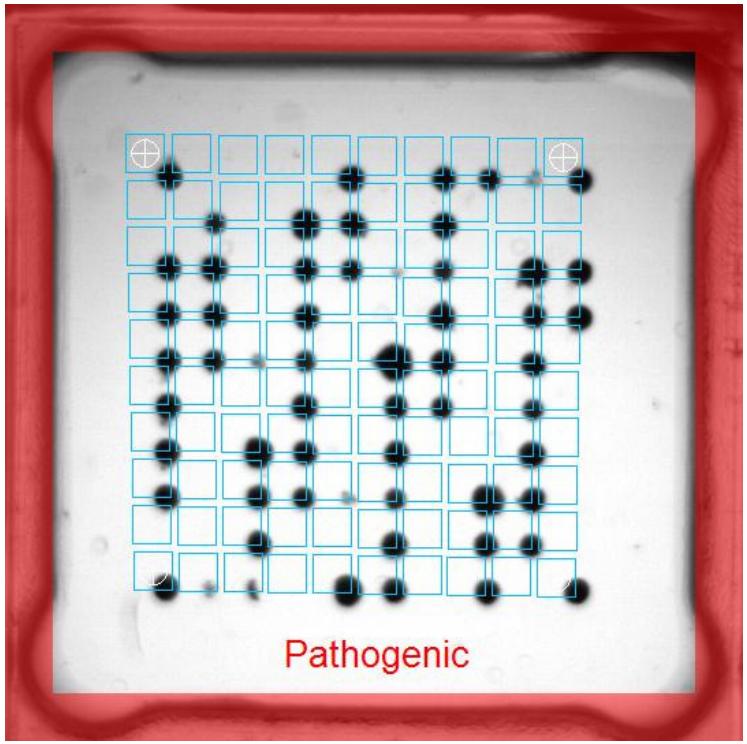
Draft NWIP 11731

- ✓ Merge two ISO 11731 standards
- ✓ Low Background
  - ✓**Direct Plating without treatment**
  - ✓**Concentration with heat treatment**
- ✓ High Background
  - ✓**Direct plating or concentration**
    - ✓Heat treatment
    - ✓Acid treatment
- ✓ Extreme High Background
  - ✓**Combined heat and acid treatment**

Recent developments

- Confirmation colonies with Real Time PCR
- Serotyping L. pneumophila with Real Time PCR
- Introduction Q-PCR method L. pneumophila
- Introduction Legionella chip





Standardization of Legionella analysis: a way to go

Vitens