

Der *aqua-checker*.

**Die einfache Art
Wasser zu testen**



Der *aqua-checker* – ein neuartiges Gerät zum Nachweis von mikrobiologischen Verunreinigungen im Trinkwasser

F. Stauffer

Enzymatische Bestimmung von Indikator-Bakterien am Beispiel des Anreicherungs-Mediums ReadyCult im *aqua-checker*.

M. Manafi



Die Idee

Ein handliches und tragbares Analysegerät ,daß

- nach einer anerkannten Methode arbeitet
- zuverlässige und aussagekräftige Ergebnisse liefert
- sehr einfach zu Bedienen ist
- unabhängig von Laborinfrastrukturen eingesetzt werden kann

Das Resultat – der *aqua-checker* .

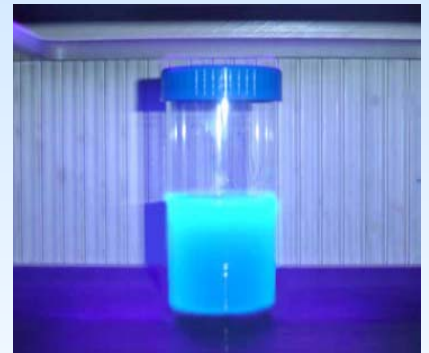


Der *aqua-checker* .

- arbeitet mit einem anerkannten Nährboden => Readycult von Merck KGaA
- ist auch von ungeschultem Personal problemlos zu bedienen
- ist unabhängig von Labors und technischer Infrastruktur: er kann mit z.B. einer Autobatterie betrieben werden.
- ist geeignet für Online Monitoring: die Messergebnisse können z.B. auf ein Handy überspielt werden.

Der *aqua-checker*.

sein Anreicherungsmedium: READYCULT



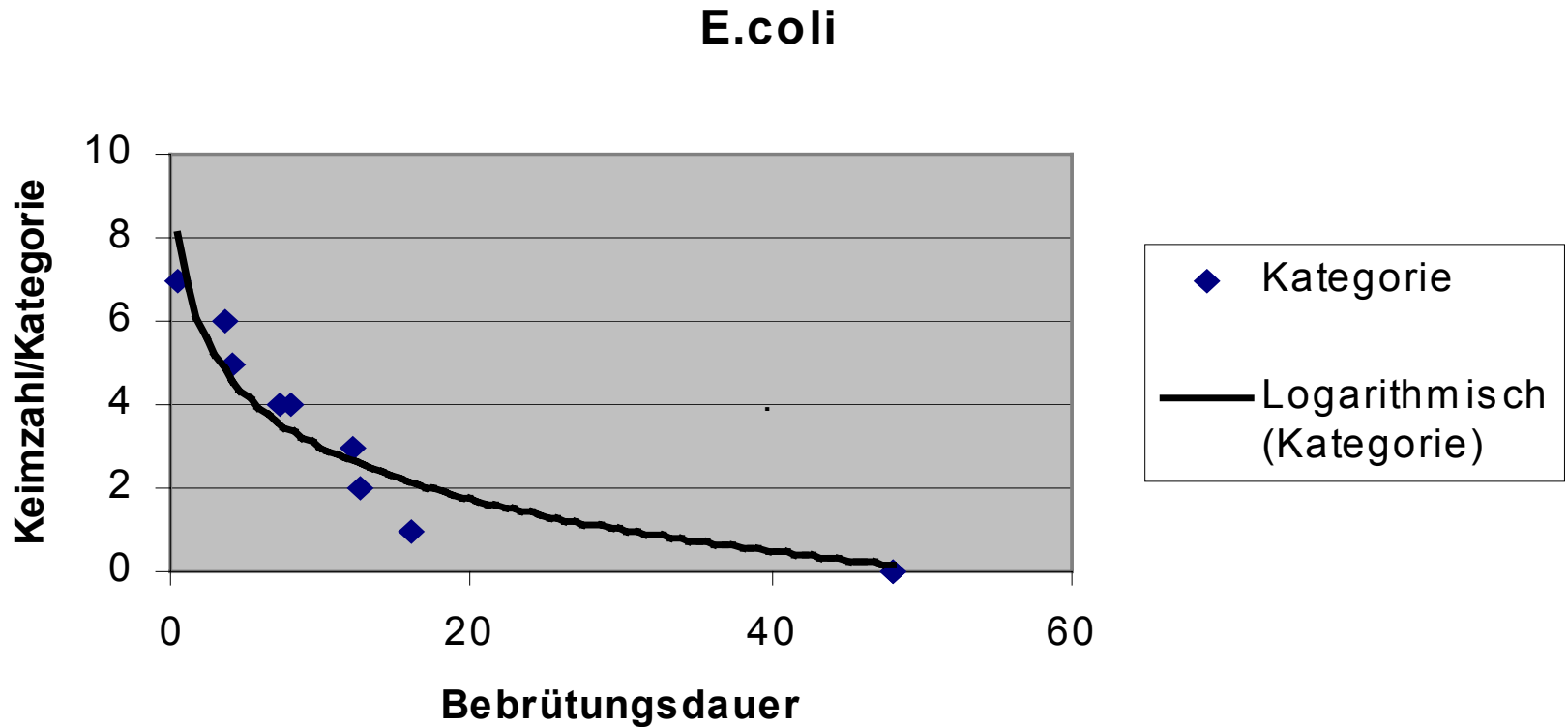
Der *aqua-checker* .

Seine Messungen sind automatisiert



Der *aqua-checker* .

Sein Meßverfahren - semiquantitativ

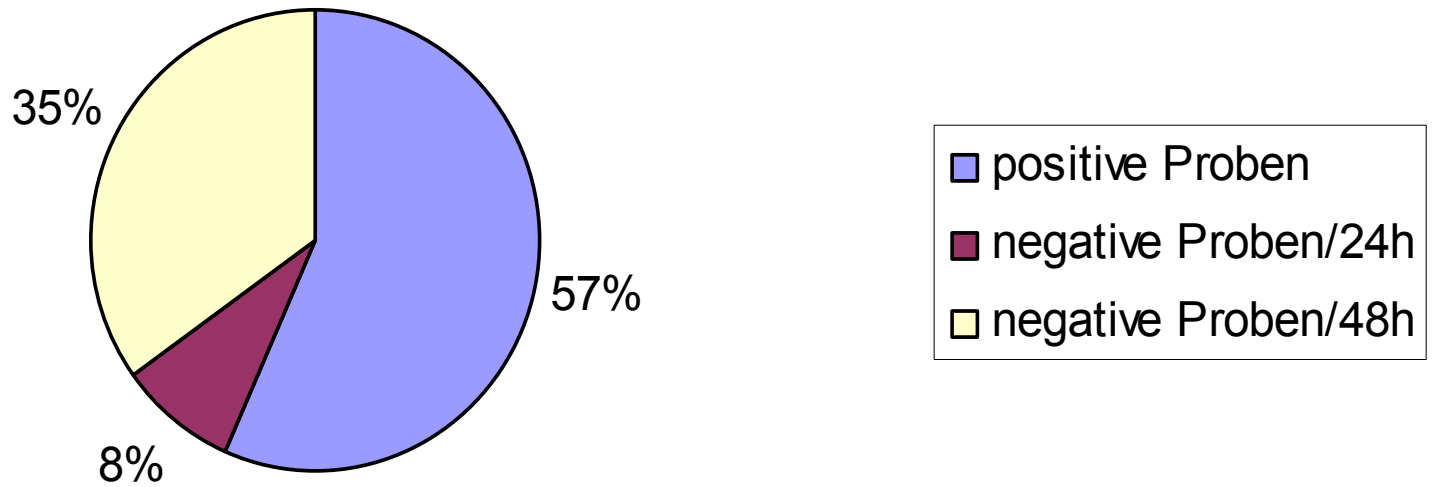


Der *aqua-checker*. –

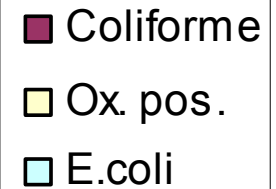
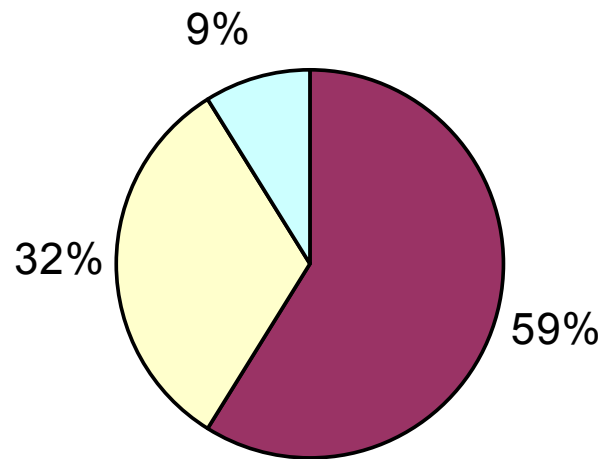
Seine Untersuchungsergebnisse sind aussagekräftig und zuverlässig

1. Automatisierter Nachweis einer Kontamination mit *E. coli*, Coliformen-Bakterien, Pseudomonaden, Aeromonaden, Vibrionen
2. Automatisierte Differenzierung von *E. coli* und Coliformen-Bakterien
3. Bestätigung von *E. coli*
4. Bestätigung von z.B. Pseudomonaden

60 Trinkwasserproben



Verteilung der Bakterienstämme bei den positiven Proben



Der *aqua-checker*. ist

- Ein österreichisches Produkt der Firma Kroneis
- Ein universell einsetzbares Messgerät für Eigenüberwachung, Online Monitoring und Katastropheneinsätze
- Eigenüberwachung:
 - von „sensiblen“ Bereichen im Versorgungsnetz
 - nach Rohrbrüchen oder Rohrarbeiten
 - bei sich plötzlich ändernder Wasserqualität
 - Hotels, Spitälern und Freizeiteinrichtungen
 - Überprüfung von Bade- und Rekreationswässern

Enzymatische Bestimmung von
Indikatorbakterien am Beispiel
des Anreicherungsmediums
Readycult im *aqua-checker*.



"Markerorganismus"

Index und Indikator (Mossel 1982)

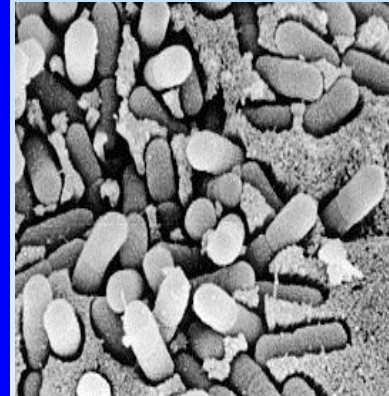
- **"Indexorganismen" stehen direkt oder indirekt entweder mit der Gefährdung der Gesundheit oder mit der Anwesenheit der Krankheitserreger in Verbindung.**
- **"Indikatororganismen" wiederum weisen auf die mögliche Verunreinigung, ineffiziente Behandlung des Lebensmittel oder ungenügende Desinfektion des Wassers hin.**

- **β -D-glucuronidase**

- Hartman 1989, Manafi et al. 1991, Frampton & Restaino 1993.

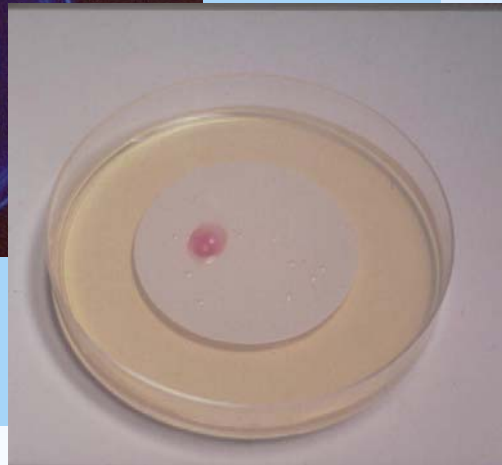
Summary of β -glucuronidase positive Gram-negative bacteria

Organism	% Phenotypically Positive
<i>Escherichia coli</i>	94-97%
<i>Escherichia non-E. coli</i>	0%
<i>Shigella</i> spp.	40-67%
<i>Salmonella</i> spp.	17-29%
<i>Yersinia, Citrobacter, Flavobacter, Bacteroides</i>	Occasional species positive



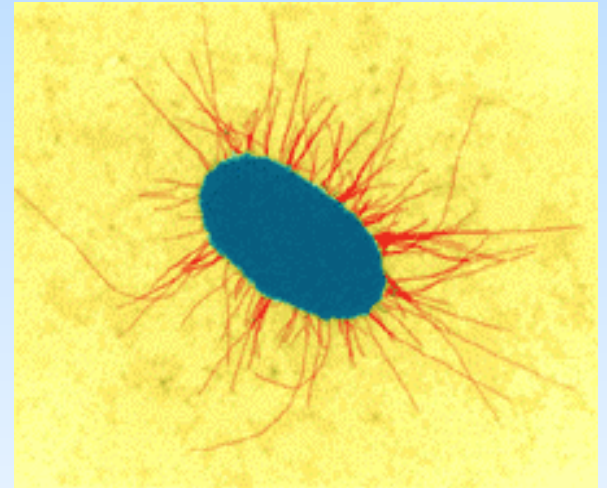
E. coli media

- **Fluorocult media (Merck)**



E.coli/Coliforme Bakterien

Durch den Nachweis von β -D-Glucuronidase und β -D-Galactosidase ist es möglich, *E.coli* und coliforme Bakterien simultan aus einer Mischflora zu bestimmen.



**Coliforme Bakterien, ein
zeitgemäßes Konzept?**

Definition und Klassifikation der coliformen Bakterien

Abbau von Laktose in Gas und Säure innerhalb von 24-48 Stunden bei $36\pm 2^{\circ}\text{C}$, thermotolerante oder fäkal coliforme Bakterien ($44.5\pm 0.2^{\circ}\text{C}$).

- ***Escherichia coli***
- ***Klebsiella***
- ***Enterobacter***
- ***Citrobacter***

Definition und Klassifikation der coliformen Bakterien

6. Ausgabe vom UK, *Bacteriological Examination of Drinking Water Supplies*.

Definition von Coliformen wurde zu Säurebildung aus Laktose geändert.

- ***Escherichia coli***
- ***Klebsiella***
- ***Enterobacter***
- ***Citrobacter***
- ***Yersinia***
- ***Serratia***
- ***Hafnia***
- ***Pantoea***
- ***Kluyvera***

Definition und Klassifikation der coliformen Bakterien

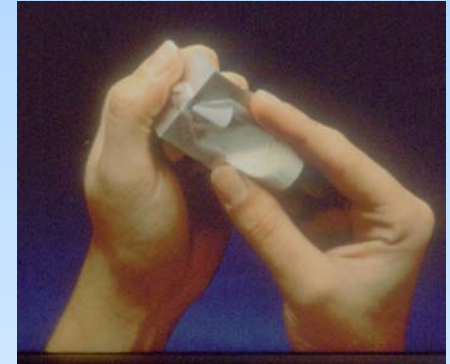
Neuerdings werden Bakterien, die das β -Galactosidase Gen (lac Z gene) enthalten, zur Gruppe der coliformen Bakterien gezählt.

Escherichia coli

- *Klebsiella*
- *Enterobacter*
- *Citrobacter*
- *Yersinia*
- *Serratia*
- *Hafnia*
- *Pantoea*
- *Kluyvera*
- *Cedecea*
- *Ewingella*
- *Moellerella*
- *Leclercia*
- *Canella*
- *Yokenella*

Readycult Coliforms Procedure „EPA approved method“

- **Selektivbouillon in Blisterform zum gleichzeitigen Nachweis von gesamtcoliformen Bakterien (X-GAL Hydrolyse) und E.coli (MUG Hydrolyse)**
- **Inhalt eines Blister zu 100 ml Wasser geben**
- **Bebrüten (18-24 h bei 35-37°C)**
- **Interpretation der Ergebnisse**



Interpretation der Ergebnisse

- COLIFORME BAKTERIEN: JEDGLICHE VERFÄRBUNG DER BOUILLON NACH **BLAU - GRÜN**
- *E.coli*: **BLAU – GRÜN**
- **FLUORESZENZ (365nm)**
- **INDOLPOSITIV**



Identification of bacteria isolated from water and the results of enzymatic reactions using LMX broth (n=2441)

Genus/Species ^a	No. of strains	No. (%) strains positive for ^b		
		GAL	GUD	IND
<i>Acinetobacter</i> spp	18	0 (0%)	0 (0%)	0 (0%)
<i>Aeromonas hydrophila</i>	105	82 (78%)	0 (0%)	100 (95%)
<i>Aeromonas sobria</i>	7	7 (100%)	0 (0%)	7 (100%)
<i>Budvicia aquatica</i>	2	0 (0%)	0 (0%)	0 (0%)
<i>Buttiauxella agrestis</i>	10	8 (80%)	0 (0%)	0 (0%)
<i>CDC gr. IV C-2</i>	1	0 (0%)	0 (0%)	0 (0%)
<i>Cedecea davisae</i>	1	0 (0%)	0 (0%)	0 (0%)
<i>Cedecea lapagei</i>	1	0 (0%)	0 (0%)	0 (0%)
<i>Cedecea</i> sp	1	1 (100%)	0 (0%)	1 (100%)
<i>Chromobacterium violaceum</i>	2	0 (0%)	0 (0%)	0 (0%)
<i>Chryseomonas luteola</i>	3	0 (0%)	0 (0%)	0 (0%)
<i>Citrobacter diversus</i>	10	9 (90%)	0 (0%)	9 (90%)
<i>Citrobacter amalonaticus</i>	3	3 (100%)	0 (0%)	3 (100%)
<i>Citrobacter freundii</i>	138	138 (100%)	1 (1%)	0 (0%)
<i>Comamonas acidovorans</i>	3	0 (0%)	0 (0%)	0 (0%)
<i>Edwardsiella</i> spp	8	2 (25%)	0 (0%)	2 (25%)
<i>Enterobacter aerogenes</i>	9	8 (88%)	0 (0%)	0 (0%)
<i>Enterobacter agglomerans</i>	48	44 (92%)	0 (0%)	2 (4%)
<i>Enterobacter amnigenus</i>	57	57 (100%)	0 (0%)	0 (0%)
<i>Enterobacter cloacae</i>	94	92 (98%)	0 (0%)	0 (0%)
<i>Enterobacter intermedius</i>	18	12 (67%)	0 (0%)	0 (0%)
<i>Enterobacter sakazakii</i>	26	26 (100%)	0 (0%)	1 (4%)
<i>Enterobacter</i> sp.	32	32 (100%)	0 (0%)	0 (0%)
<i>Enterobacter taylorae</i>	2	2 (100%)	0 (0%)	0 (0%)
<i>Erwinia nigrifluens</i>	3	0 (0%)	0 (0%)	0 (0%)
<i>Erwinia</i> spp.	1	0 (0%)	0 (0%)	0 (0%)
<i>Escherichia adecarboxylata</i>	1	1 (100%)	0 (0%)	1 (100%)
<i>Escherichia coli</i>	1246	1230 (99%)	1240 (99%)	1236 (99%)
<i>Escherichia hermannii</i>	2	2 (100%)	0 (0%)	0 (0%)
<i>Escherichia vulneris</i>	14	11 (79%)	0 (0%)	0 (0%)
<i>Flavimonas oryzae</i>	8	0 (0%)	0 (0%)	0 (0%)
<i>Hafnia alvei</i>	102	20 (20%)	0 (0%)	0 (0%)
<i>Klebsiella oxytoca</i>	16	14 (88%)	0 (0%)	16 (100%)

***E. coli*: GUD 99%, GAL 98%, IND 99%**

<i>Morganella morganii</i>	12	0 (0%)	0 (0%)	12 (100%)
<i>Ochrobacterium anthropi</i>	10	0 (0%)	0 (0%)	1 (10%)
<i>Pasteurella aerogenes</i>	1	0 (0%)	0 (0%)	0 (0%)
<i>Proteus mirabilis</i>	14	0 (0%)	0 (0%)	0 (0%)
<i>Proteus vulgaris</i>	10	0 (0%)	0 (0%)	10 (100%)
<i>Providencia alcalifaciens</i>	2	0 (0%)	0 (0%)	2 (100%)
<i>Providencia rettgeri</i>	11	0 (0%)	0 (0%)	11 (100%)
<i>Providencia stuartii</i>	1	0 (0%)	0 (0%)	1 (100%)
<i>Pseudomonas aeruginosa</i>	66	0 (0%)	0 (0%)	0 (0%)
<i>Pseudomonas alcaligenes</i>	4	0 (0%)	0 (0%)	1 (25%)
<i>Pseudomonas aureofaciens</i>	1	0 (0%)	0 (0%)	0 (0%)
<i>Pseudomonas cepacia</i>	5	0 (0%)	0 (0%)	0 (0%)
<i>Pseudomonas fluorescens</i>	15	1 (7%)	0 (0%)	1 (7%)
<i>Pseudomonas paucimobilis</i>	1	0 (0%)	0 (0%)	0 (0%)
<i>Pseudomonas pseudomallei</i>	1	1 (100%)	0 (0%)	0 (0%)
<i>Pseudomonas putida</i>	4	0 (0%)	0 (0%)	0 (0%)
<i>Pseudomonas stutzeri</i>	13	1 (7%)	0 (0%)	0 (0%)
<i>Pseudomonas testosteroni</i>	1	0 (0%)	0 (0%)	0 (0%)
<i>Salmonella arizonae</i>	3	3 (100%)	0 (0%)	0 (0%)
<i>Salmonella</i> spp	3	0 (0%)	0 (0%)	0 (0%)
<i>Serratia ficaria</i>	1	1 (100%)	0 (0%)	0 (0%)
<i>Serratia fonticola</i>	25	18 (72%)	0 (0%)	0 (0%)
<i>Serratia liquefaciens</i>	58	15 (26%)	0 (0%)	0 (0%)
<i>Serratia marcescens</i>	14	11 (78%)	0 (0%)	0 (0%)
<i>Serratia odorifera</i>	1	1 (100%)	0 (0%)	1 (100%)
<i>Serratia</i> sp.	25	10 (40%)	0 (0%)	0 (0%)
<i>Shigella sonnei</i>	1	1 (100%)	0 (0%)	0 (0%)
<i>Shigella</i> spp.	1	0 (0%)	0 (0%)	0 (0%)
<i>Sphingomonas paucimobilis</i>	10	0 (0%)	0 (0%)	0 (0%)
<i>Tatumella ptyseos</i>	4	0 (0%)	0 (0%)	0 (0%)
<i>Vibrio metschnikovii</i>	4	4 (100%)	0 (0%)	0 (0%)
<i>Vibrio parahaemolyticus</i>	1	1 (100%)	0 (0%)	1 (100%)
<i>Vibrio vulnificus</i>	1	1 (100%)	0 (0%)	1 (100%)
<i>Xanthomonas maltophilia</i>	5	0 (0%)	0 (0%)	0 (0%)
<i>Yersinia enterocolitica</i>	64	17 (26%)	3 (4%)	45 (70%)
<i>Yersinia intermedia</i>	1	0 (0%)	0 (0%)	1 (100%)
<i>Yersinia kristensenii</i>	1	0 (0%)	0 (0%)	1 (100%)

^a All genus/species names given refer to the API 20E Manual.
^b GAL, β-galactosidase; GUD, β-glucuronidase; IND, tryptophanase



Mikrobiologische Untersuchung von 106 Trinkwässern

COLIFORMS Readycult coliforms	E.COLI Readycult coliforms	ENTEROKOKKEN Readycult enterococci	ENTEROKOKKEN Enterococci agar
+	+	+	16
+	+	+	92
+	+	+	3250
+	+	+	95
+	+	+	75
+	+	+	61
+	+	+	2504
+	+	+	47
+	+	+	14
+	+	+	47
+	+	+	95
+	+	+	1520
+	+	+	100
+	+	+	5
+	+	+	225
+	+	+	46
+	+	+	88
+	+	+	86
+	+	+	RASEN
+	+	+	200
+	+	+	79
+	+	+	52
+	+	+	19
+	+	+	RASEN
+	+	+	RASEN
+	+	+	310
+	+	+	RASEN
+	+	+	RASEN
+	+	+	295
+	+	+	55
+	+	+	RASEN
+	+	+	14
+	+	+	265
+	+	+	188
+	+	+	200
+	+	+	170
+	+	+	7
+	+	+	21

Eine Studie in Zusammenarbeit mit den Studenten
d. Inst f. Ernährungswissenschaft, Diätakademie
und MTA-akademie der Stadt Wien



Mikrobiologische Untersuchung von 106 Trinkwässern

Eine Studie in Zusammenarbeit mit den Studenten
d. Inst f. Ernährungswissenschaft, Diätakademie
und MTA-akademie der Stadt Wien

COLIFORMS		E.COLI		ENTEROKOKKEN		ENTEROKOKKEN	
Readycult coliforms		Readycult coliforms		Readycult enterococci		Enterococci agar	
54 +	52-	45+	61-	43+	63-	51+	55-
	48-		48-		48-		48-
-		-		-		5	
-		-		-		2	
-		-		-		5	
+		+		-		2	
+		-		-		2	
+		+		-		1	
+		+		-		4	
+		+		-		5	
+		+		-		-	
+		-		-		-	
+		-		-		-	
+		-		-		-	
+		+		-		-	
+		+		-		-	
-		-		+		96	
+		-		+		48	
+		-		+		36	
+		-		+		1	
+		-		+		2	

Fecal Coliform MF-Method acc. to US-EPA 9222 D



Filtration of 100 ml Water 0,45µm Filter

MF onto M-FC Agar
24 h at 44,5 +/- 0,2°C

COUNTING
24 h

Non fecal Coliforms
gray to cream-colored colonies

Fecal Coliforms
blue colonies

Subculture into LSTB
48 h at 35 +/- 0,5° C

Verification
72 h

GAS formation

Subculture in BG
Broth
at 35 +/-

Subculture in EC-MUG
Broth
44,5 +/- 0,2° C

Confirmation
96 h

Coliform → **GAS**

E.coli → **MUG pos.**

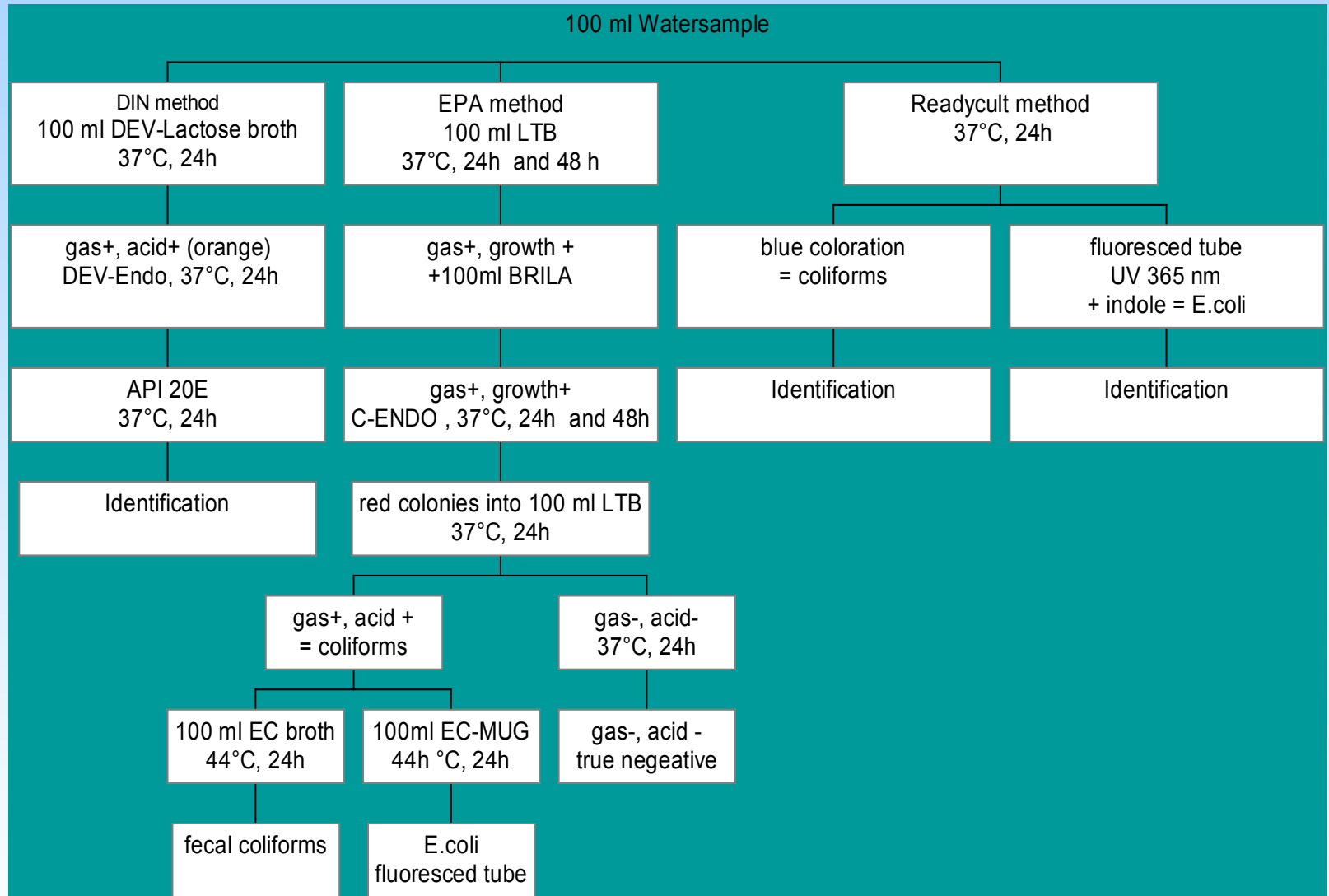
Approvals / References

- **USEPA: 40 CFR Part 141 (Sec.141.21) Federal Register / Vol. 67, No. 209, Tuesday, October 29, 2002 / Rules and Regulations**
- **Microbiology Manual for the Meat Industry, 3rd Edition April 2000, New Zealand, Chapter 11 Testing Potable Waters for Indicator Bacteria, A1.1 Chromogenic Substrate Methods for Total Coliforms and E.coli**
- **Ministry of Health, New Zealand, Drinking Water Standard for New Zealand 2000 (DWSNZ:2000), Enzyme Substrate Coliform Test**
- **Ministry of Health, Colombia, Potable Water Quality Regulations, National Standard 475, 2001, Defined Substrate Technology for Total Coliforms and *E.coli***
- **Manafi, M., Rosman; Evaluation of ReadyCult® Presence/Absence Test for the Detection of Total Coliforms and E.coli in Water. 98th American Society of Microbiology, Atlanta, 17-21st May 1998**
- **Lee, J.V., Lightfoot, N.F., Tillet, H.E.; An evaluation of presence/absence tests for coliforms and Escherichia coli. International Conference on Coliforms and E.coli: Problem or solution?**

TOTAL COLIFORMS/E.COLI TEST (USA-EPA) LS & BRILLANTGREEN BROTH resp. LTB & ECMUG (E. coli) IN COMPARISON TO READYCULT COLIFORMS 100

	No.of positive samples RC Coliforms	No.of positive samples LTB+BG broth
Total Coliforms	98	96
<i>E. Coli</i>	72	67
Results of EPA Approval performance Study,		August 2000, USA

DIN, EPA and READYCULT



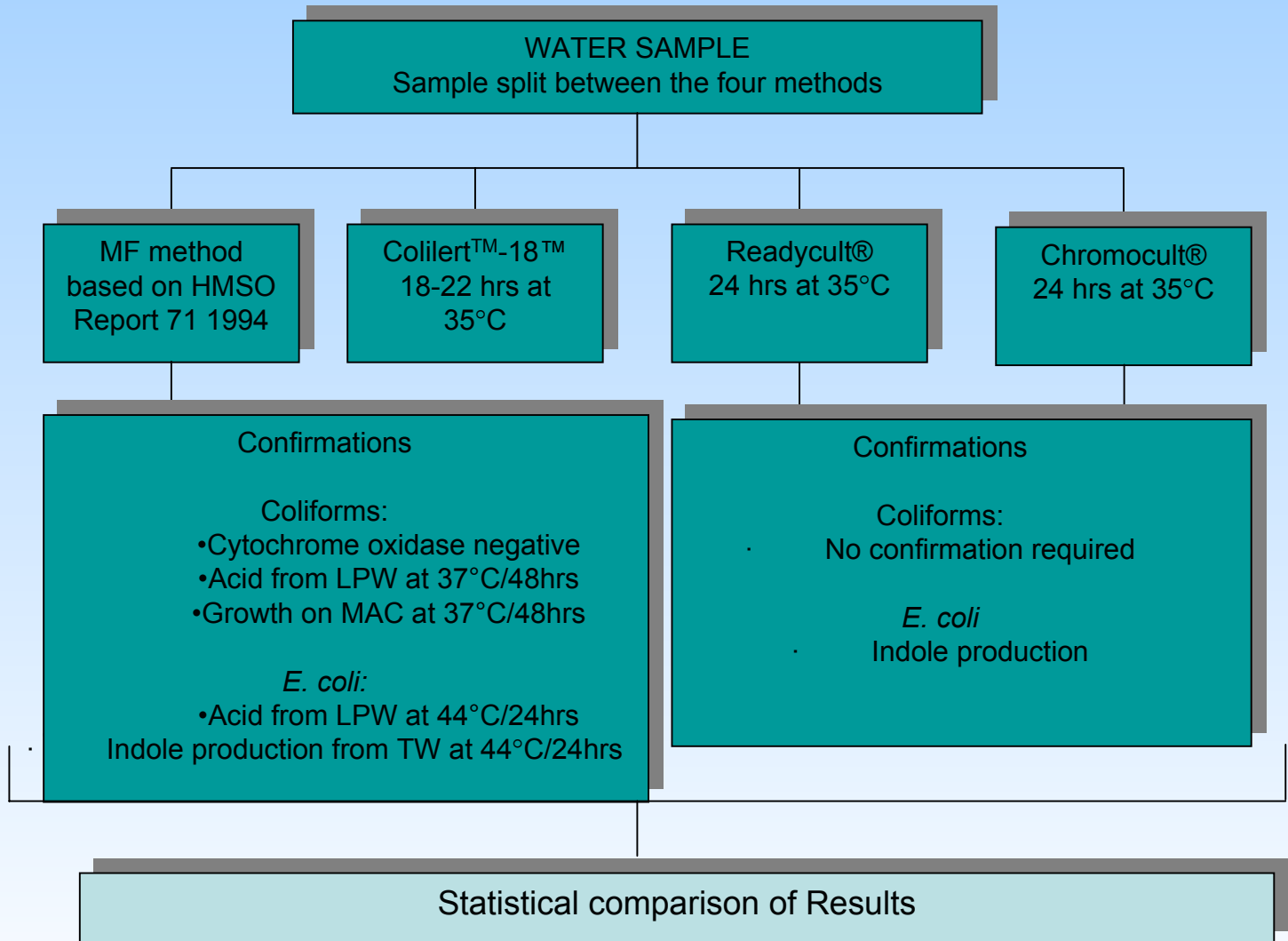
THE COMPARISON OF TESTED METHODS FOR DETECTING *E. COLI* AND COLIFORMS IN WATER. SAMPLES, NUMBER OF CONFIRMED POSITIVE AND FALSE POSITIVE SAMPLES^a.

Methods	presumptive positive		confirmed positive		false- positive	
EPA	91	63	90	62	1 b	1
DIN	95	60	94	60	1 b	2
RC	103	63	100	63	3 c	0

- **a** strains grown on EMB were identified by Gram stain, oxidase test and API 20E System
- **b** Api test was not detectable
- **c** false positive results with strains of *A. hydrophila*

An evaluation of a new chromogenic/fluorogenic microbiology media system for detection of total coliforms and *E. coli* in water. Asian Environmental Technology, 3:7-8 (1999).

The Comparative Trials of Enzyme Substrate Technologies in Drinking Water Supplies, Dean Comrie, WSL Consultants,



LPW = Lactose Peptone Water
TW = Tryptone Water
MAC = MacConkey Agar

Table 1: Summary of the results of the Coliform analysis for the reference (Colilert™) and alternate (Readycult®) methods.

Colilert™ (Reference Method)	Readycult® (Alternate Method)	
	Positive	Negative
Positive	146	12
Negative	15	496

Table 2: Summary of the results of the *E. coli* analysis for the reference (Colilert™) and alternate methods (Readycult®).

Colilert™ (Reference Method)	Readycult® (Alternate Method)	
	Positive	Negative
Positive	67	0
Negative	9	593

Table 3: Summary of the results of the McNemar's Change Test.

	Coliforms	<i>E. coli</i>
Difference between proportions	-0.004	-0.013
97.5% CI	-0.020 to 0.012	-0.013 to -0.004
p-value	0.7011	0.0039

The Comparative Trials of Enzyme Substrate Technologies in Drinking Water Supplies

- The trial was conducted over a four-month period with a total of 669 water samples tested. The statistical analysis of the results of the qualitative methodologies demonstrate no significant difference between the Colilert™ and Readycult® methods for coliforms analysis and that the Readycult® appears to be more equivalent or better than Colilert™ with respect to the analysis of *E. coli*.

The Comparative Trials of Enzyme Substrate media and Testkits



LBTB, NISSUI, COLILERT, READYCULT



LBTB, NISSUI, COLILERT, READYCULT



LMX, LMX*, RC, NISSUI

Comparison IDEXX Colilert-18 vs Readycult Coliforms 100, 120ml IDEXX bottles



Results after 18 h at 35-37°C

5 x E. coli



5 x Klebsiella



5x citrobacter

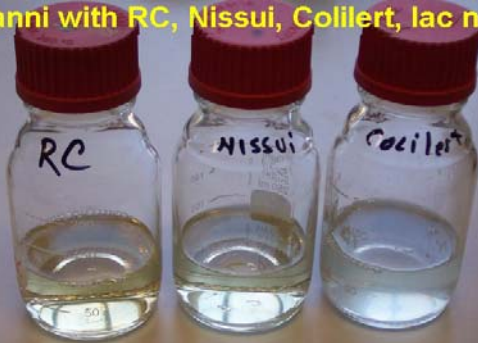


5 x Enterobacter



Ac.baumannii with RC, Nissui, Colilert

Ac. baumannii with RC, Nissui, Colilert, lac negative



Ac. baumannii, false positive with Colilert



Ac. baumannii, RC, Nissui, colilert



Ac baumannii, lac+, ind+, -glu, -lbn, false positive with colilert



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Fluorogenic and chromogenic substrates used in bacterial diagnostic. **Microbiol. Rev. 55:335-348, (1991).**

Fluorogenic and chromogenic substrates in culture media and identification tests. **Int. J. Food Microbiol. 31:45-58, (1996).**

Use of enzyme tests in characterization and identification of aerobic and facultative anaerobic gram positive cocci. **Clin. Microbiol. Rev. 11:318-340 (1998).**

New developments in chromogenic and fluorogenic culture media. **Int. J. Food Microbiol. 60:205-218 (2000).**

[Http://www.univie.ac.at/chromogenic](http://www.univie.ac.at/chromogenic)