



DESDE 1995

RICOQUÍMICA

RICHTER INDÚSTRIA & COMÉRCIO / DISTRIBUIDOR



ENVELOPE Nº 1 (PROPOSTA DE PREÇOS)

MUNICÍPIO DE MERCEDES

ESTADO DO PARANÁ

RICOQUÍMICA LTDA.

Endereço: RUA PARAÍBA, Nº 390

B. CENTRO - CEP-85960-000

MARECHAL CÂNDIDO RONDON-PR

CNPJ: 02.775.279/0001-00

PREGÃO PRESENCIAL Nº 69/2017

DATA DE ABERTURA: 10/07/2017

HORÁRIO: 09:00 h



RICOQUÍMICA

Soluções para
Água Pura.

“O Elemento da Vida.”

PROPOSTA DE PREÇOS

Ao Pregoeiro do Município de Mercedes - PR
Pregão Presencial n.º 69/2017
Processo Licitatório n.º 104/2017

RICOQUÍMICA LTDA, inscrita no CNPJ/MF n.º **02.775.279/0001-00**, Inscrição Estadual n.º **90169627-64**, neste ato representada por seu representante legal, o Sr. **EDERSON ARNO RICHTER**, portador da Carteira de Identidade n.º 5.638.366-2, expedida pela SSP/PR, e do CPF n.º 797.381.929-00, em atendimento ao disposto no Edital em epígrafe, apresenta a seguinte Proposta de Preços para a venda de MATERIAIS E PRODUTOS QUÍMICOS PARA TRATAMENTO DA ÁGUA, conforme descrição a seguir:

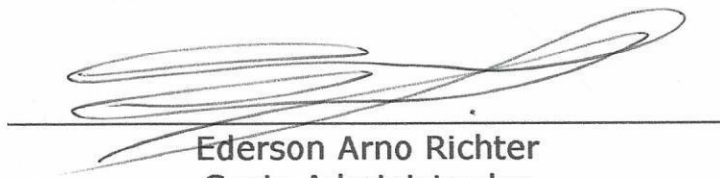
Item	Qtd	Unid	Descrição	R\$ Unit	R\$ Total
1	30	unid	Ácido Dicloro Isocyanurato de Sódio Anidro, granulado, com 99% de pureza e teor de cloro ativo 60%; em bombonas de 50Kg (a granel).Marca.B&L	1.445,00	43.350,00
2	10	unid	Ácido Tricloro – S – Triazina Triona, com 99% de pureza e teor de cloro ativo 90%. Em bombonas de 50Kg (a granel).Marca.Lonza	1.816,83	18.168,30
3	20	unid	Fluossilicato de sódio a granel, com pureza superior a 98%, com mais de 59% de íon fluoreto; acondicionado em sacos de 25 kg.Marca.Prayon.	591,93	11.838,60

Valor Total: R\$ 73.356,90 (Setenta e três mil, trescentos e cinquenta e seis reais e noventa centavos).

No preço estão incluídos, além do lucro, todas as despesas e custos como frete, seguro, tributos de qualquer natureza e todas as demais despesas, diretas ou indiretas, relacionadas com o fornecimento do objeto da presente licitação.

Prazo de validade da Proposta: 60 dias, estendendo-se à etapa de lances verbais.

Marechal Cândido Rondon-PR, 07 de julho de 2017.


Ederson Arno Richter
Socio Administrador



Página 1 de 1

Ricoquímica Ltda.

Rua Paraíba, 390 - Centro - Fone/Fax (45) 3254-0500
CEP 85960-000 Mal. Cândido Rondon - Paraná

www.ricoquimica.com.br

000121

ENVELOPE N.º 1 (PROPOSTA DE PREÇOS)

MUNICÍPIO DE MERCEDES

ESTADO DO PARANÁ

SLO CONSTRUTORA DE OBRAS LTDA EPP

ENDEREÇO: AV. BRASIL, 1701, 1º ANDAR, SALA 01, CENTRO, S/

CNPJ: 21.586.333/0001-14

PREGÃO PRESENCIAL N.º. 69/2017

DATA DE ABERTURA: 10/07/2017.

HORARIO: 09h00min


600132

SLO CONSTRUTORA DE OBRAS LTDA EPP

AV. BRASIL, 1701, 1º ANDAR, SALA 01, CENTRO, SANTA HELENA/PR

FONE: 45-3268-2593

CNPJ: 21.586.333/0001-14 - IE: 90682813-82

ANEXO III

PROPOSTA DE PREÇOS

Ao Pregoeiro do Município de Mercedes - PR
Pregão Presencial n.º 69/2017

SLO CONSTRUTORA DE OBRAS LTDA EPP, inscrita no CNPJ/MF n.º 21.586.333/0001-14, Inscrição Estadual n.º 906.82813-82, neste ato representada por seu representante legal, o Sr. Sérgio Luiz de Oliveira, portador da Carteira de Identidade n.º 5.989.186-3, expedida pela SSP/PR, e do CPF n.º 968.499.919-49, em atendimento ao disposto no Edital em epígrafe, apresenta a seguinte Proposta de Preços para a formalização de Ata de Registro de Preços para eventual contratação de empresa para fornecimento de produtos químicos e materiais para laboratório, para manutenção da qualidade da água potável disponibilizada pelo SEMAE – Serviço Municipal de Água e Esgoto, do Município de Mercedes, durante o exercício de 2017, conforme especificações técnicas constantes deste Edital e Anexos, conforme descrição a seguir:

Item	Qtde	Unid	Descrição do Produto	R\$ Unit	R\$ Total
1	30	Unid	Ácido Dicloro Isocyanurato de Sódio Anidro, granulado, com 99% de pureza e teor de cloro ativo 60%; em bambonas de 50Kg (a granel). Adequado para tratamento de água para consumo humano. Marca: Hidroall	1.445,00	43.350,00
2	10	Unid	Ácido Tricloro – S – Triazina Triona, com 99% de pureza e teor de cloro ativo 90%. Em bambonas de 50Kg (a granel). Adequado para tratamento de água para consumo humano. Marca: Hidroall	1.816,83	18.168,30

No preço estão incluídos, além do lucro, todas as despesas e custos como frete, seguro, tributos de qualquer natureza e todas as demais despesas, diretas ou indiretas, relacionadas com o fornecimento do objeto da presente licitação.

Prazo de validade da Proposta: 30 (trinta) dias, estendendo-se à etapa de lances verbais.
A remessa de eventuais ordens de compra e demais comunicações poderão ser encaminhadas para o e-mail: Giovani.agualuz@hotmail.com

Mercedes, 10 de julho de 2017.

21.586.333/0001-14

SLO CONSTRUTORA DE OBRAS LTDA EPP
SERGIO LUIZ DE OLIVEIRA
RG 5.989.186-3/SESP-PR
SOCIO ADMINISTRADOR

SLO CONSTRUTORA DE
OBRAS LTDA - ME

Av. Brasil, 1701 1º Andar - Sl 1 - Centro
85.892-000 Santa Helena, PR

000129

ENVELOPE N.º 1 - PROPOSTA DE PREÇOS
MUNICÍPIO DE MERCEDES
ESTADO DO PARANÁ
PR LABOR COMÉRCIO DE PRODUTOS E
EQUIPAMENTOS PARA LABORATÓRIOS LTDA - EPP
ENDEREÇO: R. PRUDENTE DE MORAIS, N.º 323 -
PARQ. SÃO PAULO - CASCAVEL - PR
CNPJ: 15.188.525/0001-70
PREGÃO PRESENCIAL N.º 104/2017
DATA DE ABERTURA: 10/07/2017
HORÁRIO: 09:00h

000124





PR LABOR COM DE PROD E EQUIP PARA LABOR LTDA ME

Endereço: RUA PRUDENTE DE MORAIS, 323
Bairro: PARQUE SAO PAULO
Cidade: CASCAVEL
Telefone: (45)3096-8907
Site: www.prlabor.com.br
E-mail: prlabor@prlabor.com.br
CNPJ: 15.188.525/0001-70

CEP: 85.803-680
UF: PR
IE: 9058908913

Orçamento N°: 6718

Data de emissão: 10/07/2017

Comissionado: HERMANN CRISTOPHER BRUN

Data de validade: 09/08/2017

E-mail: hermann@prlabor.com.br

Cliente: PREFEITURA MUNICIPAL DE MERCEDES
Telefone: (45)3256-8033
E-mail: cristianekerkhoven@gamil.com
Endereço: RUA DOUTOR OSWALDO CRUZ, 555
Cidade: MERCEDES
CNPJ: 95.719.373/0001-23

Fax: A/C:
Bairro: CENTRO
Estado: PR
Insc. Estadual: ISENT0

CEP: 85998-000

Tipo frete: CIF - Por conta do emitente

Item	Código	Descrição	Marca	Und.	Quant.	Preço unit.	Total
4	4	MEIO DE CULTURA ESPECÍFICO , BASEADO NO SISTEMA DE SUBSTRATO DEFINIDO ONPG/MUG, CONSTITUID O POR DOIS OU MAIS SUBSTRATOS ESPECÍFICO S PARA DETERMINAÇÃO, VIA ENZIMÁTICA , SIMULTÂNEA DE COLIFORMES TOTAIS E ESCHERICHE A COLI, COMPOSTO DE INIBIÇÃO SOLANUM. Não necessita confirmação com o reagente Kovac. Utilização: para análise de presença ou ausência em amostras de água em meio normal, com resultados em amarelo para coliformes totais e azul fluorescente para Escherichea coli. Tempo de incubação: 24 horas. Embalagem: em blister individual, meio pulverizado para uma amostra de 100mL de água. Estabilidade: por 10 meses. Entrega: 15 dias úteis.	CPI	UN	2.600,00	8,50	22.100,00
5	5	BOLSA PLÁSTICA LACRADA E ESTÉRIL. COM TIOSSULFAT O DE SÓDIO E TARJA DE IDENTIFICAÇÃO, CAPACIDADE 100ML, PARA COLETA DE AMOSTRAS DE ÁGUA PARA EXAME BACTERIOLÓGICO, CONFORME EXIGÊNCIAS EPA E APHA. Entrega: 15 dias úteis.	NASCO	UN	2.800,00	2,50	7.000,00

Condição de pagamento: 30 DIAS

Observações: Pregão Presencial nº 104/2017.

15.188.525/0001-70

PRLABOR COM. DE PRODUTOS E EQUIPAMENTOS PARA LABORATÓRIOS LTDA EPP.

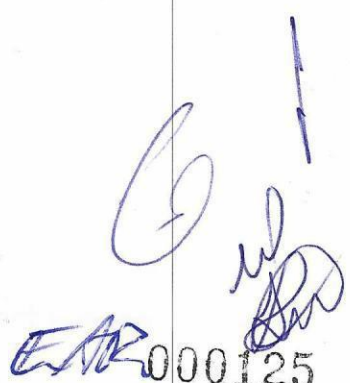
Rua Prudente de Moraes, 323
Parque São Paulo - CEP 85.803-680
CASCAVEL - PARANA

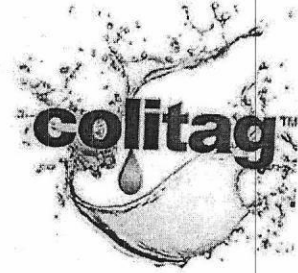
Valor bruto:	R\$ 29.100,00
Desconto:	R\$ 0,00
Valor total:	R\$ 29.100,00

(VINTE E NOVE MIL E CEM REAIS)


HERMANN CRISTOPHER BRUN

PR LABOR COM DE PROD E EQUIP PARA LAB. LTDA


000125



CERTIFICATE OF ANALYSIS

Product: Colitag™
 Lot Number: 060116
 Expiration Date: 06/2018

PHYSICAL CHARACTERISTICS

SPECIFICATION

Appearance
 Hydrated color
 Weight of media

EXPECTED RESULTS

Granular to coarse powder, free flowing
 Colorless to pale straw
 1.2 – 1.4 g

ACTUAL RESULTS

Granular to coarse powder, free flowing
 Colorless to pale straw
 Conforms

CULTURAL RESPONSE

<u>MICROORGANISM</u>	<u>ATCC</u>	<u>APPROX. INOCULUM (CFU)</u>	<u>EXPECTED RESULTS</u>			<u>ACTUAL RESULTS</u>		
			<u>Growth</u>	<u>ONPG</u>	<u>MUG</u>	<u>Growth</u>	<u>ONPG</u>	<u>MUG</u>
<i>Escherichia coli</i>	25922	10-300	Good	+	+	Good	+	+
<i>Citrobacter freundii</i>	8090	10-300	Good	+	-	Good	+	-
<i>Pseudomonas aeruginosa</i>	10145	10-300	None to Poor	-	-	None	-	-

Physical characteristic testing was performed in compliance with ISO/TS 11133-1 and BS EN 12322:1999 + A1:2001 standards. For culture response, the medium was weighed out for 100-mL samples and rehydrated with sterile, processed water and inoculated with the organisms listed above. Cultures were incubated aerobically at 35 ± 0.5°C and examined for growth and reactions after 24 hours.

Product Storage: Store at -20°C to 30°C, (preferably 4°C to 7°C), away from light and moisture.

The product has been gamma irradiated in accordance with FDA, QSR, ISO 9001, and EN 46001 guidelines. CPI certifies that the material listed above received the specified targeted dose according to a dosimetry monitoring system.

For questions or comments on CPI or Colitag™, please call 1-800-878-7654 in the USA, +31 20 638 05 97 in Europe or visit our web-site at www.cpiinternational.com.

000126



colitagTM

O revolucionário
16 - 48 horas teste em água

Ed. 1000



O Revolucionário 16-48 Horas Teste de Água

O Colitag™ é um teste de água de 16-48 horas (P/A e NMP) que detecta quantidades de 1 NMP de E. coli e outros coliformes em 100 mL de água. O pacote contém blisters com validade de 22 meses da data de fabricação. O Colitag™ é aprovado pelo US EPA para o uso nos testes de presença/ausência e Número Mais Provável, como especificado no Standard Method 9221, para o monitoramento de conformidade de coliformes totais e E. coli em água potável.

Instruções

Para leitura de amostras entre 16 e 22 horas:

1. Assepticamente adicione o Colitag™ a 100 mL da amostra de água em um frasco estéril.
2. Tampe o frasco e agite para começar a dissolução.
3. Se a amostra não estiver dissolvida de 33-38 °C, coloque o frasco em um banho de água a 44,5 °C dura
4. Incube a amostra a 35 °C ± 0,5 °C, durante o restante do tempo de incubação desejado; 16 a 22 horas.
5. Leia os resultados de acordo com o método de interpretação da amostra abaixo.

Para leitura de amostras entre 22 e 48 horas:

1. Assepticamente adicione o Colitag™ a 100 mL da amostra de água em um frasco estéril.
2. Tampe o frasco e agite para começar a dissolução.
3. Incube a amostra a 35 °C ± 0,5 °C por 22 a 48 horas.
4. Leia os resultados de acordo com o método de interpretação da amostra abaixo.

Interpretação da amostra

Aparência	Resultado
Menos amarelo que o comparador do Colitag™ (P/A)	Negativo para coliformes totais e E. coli
Amarelo igual ou mais intenso que o comparador do Colitag™ (P/A)	Positivo para coliformes totais
Usando uma lâmpada de UV (365nm), fluorescente igual ao mais intenso que o comparador do Colitag™ (P/A)	Positivo para E. coli

Armazenamento do produto

Armazene de 4 °C a 30 °C, ao abrigo de luz. A validade do Colitag™ é de 22 meses da data de fabricação. A data de expiração do produto está impressa na caixa e nos blisters individuais.

Para leitura de amostra entre 16-48 horas *

Adicionar Colitag™
Para 100 mL de amostra de água

Incubar
durante
16 -48
horas
a 35 ±
0,50°C

Verifique visualmente a
amostra quanto à cor
amarela

Se a amostra
for amarela
do que a
Colitag
Comparador
(P/A),
a amostra
é negativa para
coliformes totais

Se Amostra
for igual ou
superior a
amarelo
que o colitag
Comparador
(P/A) a amostra
é positiva para
coliformes totais,
verifique a
fluorescência
usando uma
lâmpada UV
de onda longa
(365nm)

Se a
amostra
não
fluoresce,
a amostra
negativo
para *E. coli*
bactérias

Se a
amostra
fluoresce,
a amostra é
positiva
para *E. coli*
bactérias



colitag™

O revolucionário
16 - 48 horas teste em água

APROVADO PELA US EPA

6
EPA
000159



**The Revolutionary
16 - 48 Hour Water Test**

P/N	Description	Thiosulfate	Tamper Seal
4600-0009	Colitag™ Test Kit, P/A, 100mL format, 5/pk	—	—
4600-0011	Colitag™ Test Kit, P/A, 100mL format, 20/pk	—	—
4600-0013	Colitag™ Test Kit, P/A, 100mL format, 100/pk	—	—
4600-0012	Colitag™ Test Kit, P/A, 100mL format, 200/pk	—	—
4600-0040	Colitag™ Comparator, P/A, ea.	—	—
4600-0121	120mL Sterile Colitag Sample Vessel, w/ Thiosulfate, Screw Cap, Tamper Seal, Polystyrene, 100/pk	YES	YES
4600-0121FT	120mL Sterile Colitag Sample Vessel, Flip Top in a High Clarity Polypropylene, 200/pk	YES	YES
4600-0111	120mL Sterile Colitag Sample Vessel, w/o Thiosulfate, Screw Cap, Tamper Seal, Polystyrene, 100/pk	NO	YES
4600-0026	Thio-bag, with Thiosulfate, 100mL, 100/pk	YES	—
4600-95000602	365nm Handheld UV Lamp, 115V	—	—
4600-95000603	365nm Handheld UV Lamp, 230V	—	—

The Material Safety Data Sheet for Colitag™ is available upon request.
www.cpiinternational.com

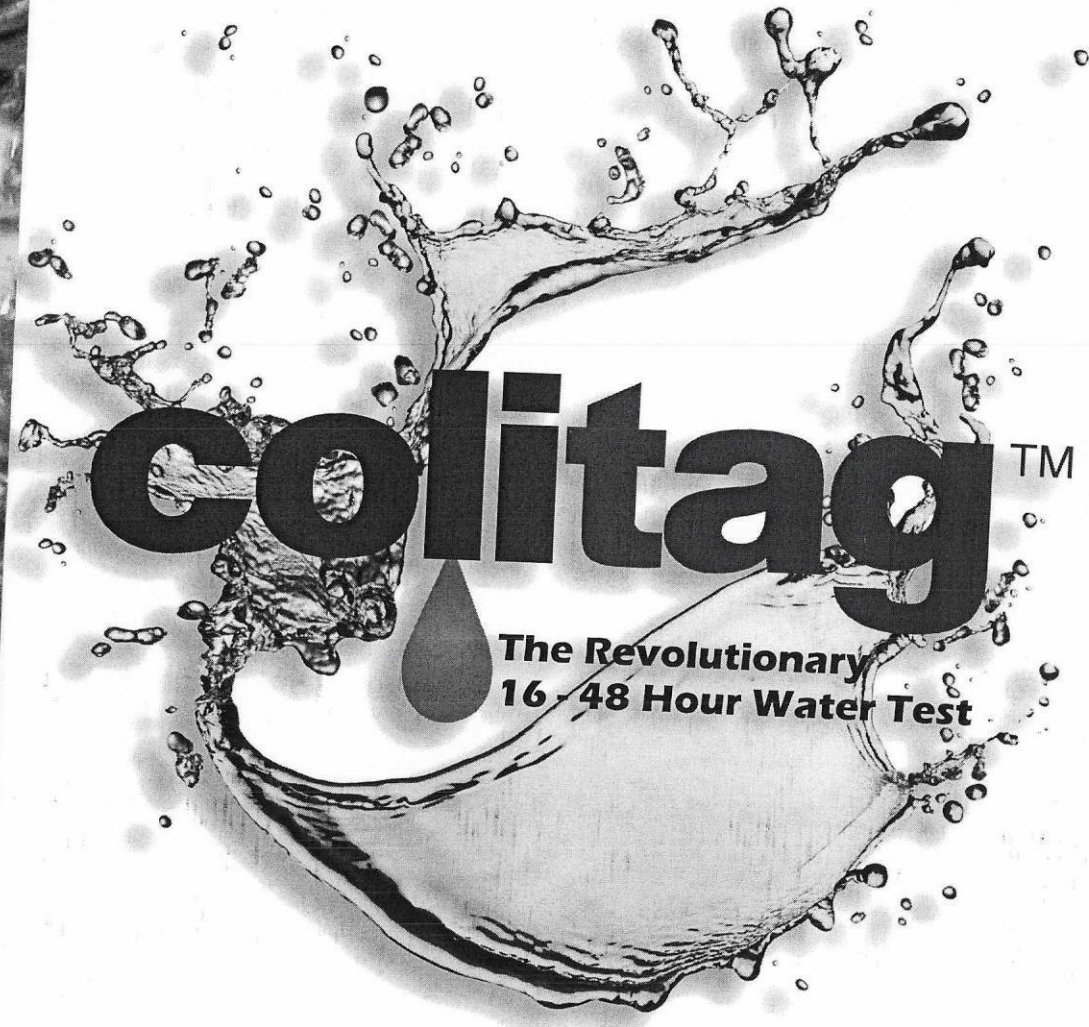
USA

5580 Skylane Blvd.
Santa Rosa, CA 95403
P: 800.878.7654
F: 707.545.7901




Europe

P.O. Box 2704
1000 CS Amsterdam
The Netherlands
P: +31 20 638 05 97
F: +31 20 420 28 36



Handwritten notes in blue ink, including a signature and the number '000130'.



The Revolutionary 16 - 48 Hour Water Test

Colitag™ is a 16 - 48 hour water test (P/A and MPN) that detects as few as 1 MPN of *E. coli* and other coliform bacteria in 100mL of water. This package contains snap-top blisters with a shelf life of 22 months from the manufacture date. Colitag™ is US EPA approved for use as a presence absence test and in the Most Probable Number (MPN) format as specified in Standard Method 9221 for compliance monitoring of total coliforms and *E. coli* in drinking water.

Instructions

For sample reading between 16 and 22 hours:

1. Aseptically add the Colitag™ to a 100mL water sample in a sterile vessel.
2. Secure vessel top and agitate to begin dissolution.
3. If sample is not already at 33-38°C, then place vessel in a 44.5°C water bath for 7 to 10 minutes.
4. Incubate sample at 35°C ± 0.5°C for the remainder of the desired incubation time; 16 to 22 hours.
5. Read results according to the sample interpretation method listed below.

For sample reading between 22 and 48 hours:

1. Aseptically add the Colitag™ to a 100mL water sample in a sterile vessel.
2. Secure vessel top and agitate to begin dissolution.
3. Incubate sample at 35°C ± 0.5°C for 22 to 48 hours.
4. Read results according to the sample interpretation method listed below.

Sample Interpretation

Appearance	Result
Less yellow than the Colitag™ Comparator (P/A)	Negative for total coliforms and <i>E. coli</i>
Yellow equal to or greater than the Colitag™ Comparator (P/A)	Positive for total coliforms
Using a longwave (365nm) UV lamp, fluorescence equal to or greater than the Colitag™ Comparator (P/A)	Positive for <i>E. coli</i>

Product Storage

Store at 4.0°C to 30.0°C, away from light. The shelf life of Colitag™ is 22 months from the manufacture date. The product's expiration date is printed on the box and individual blisters.

The Material Safety Data Sheet for Colitag™ is available upon request.

www.cpiinternational.com

USA

5580 Skylane Blvd. P: 800.878.7654
Santa Rosa, CA 95403 F: 707.545.7901



Europe

P.O. Box 2704 P: +31 20 638 05 97
1000 CS Amsterdam F: +31 20 420 28 36
The Netherlands

For Sample Reading Between 16-48 Hours*

Add Colitag™
media to 100mL water sample

Incubate
for 16 -48
hours at
35 ± 0.5°C

Visually Check
the sample for
yellow color

If sample is
less yellow
than the
Colitag™
Comparator
(P/A), the
sample is
negative for
total coliforms

If sample
is equal to or
greater
yellow
than the Colitag™
Comparator
(P/A),
the sample is
positive for total
coliforms, check
for fluorescence
using a longwave
(365nm)
UV lamp

If sample
does not
fluoresce,
the sample is
negative
for *E. coli*
bacteria

If sample
fluoresces,
the sample is
positive
for *E. coli*
bacteria



The Revolutionary
16 - 48 Hour Water Test

US EPA Approved

* For reading between 16 and 22 hours, place the vessel into a 44.5°C water bath for 7-10 minutes prior to incubation.

000131

**Modified Colitag™ Test Method for the Simultaneous Detection of
E. coli and other Total Coliforms in Water
(ATP D05-0035)**

August 28, 2009

Modified Colitag™ Test Detection of *E. coli* and other Total Coliforms in Water as required in National Primary Drinking Water Regulations

1.0 Scope and Application

- 1.1 Modified Colitag™ is a selective and differential medium for the simultaneous determination of the presence or absence or enumeration of *E. coli* and other total coliforms in water.^{1,2,3} Modified Colitag™ is for use in the Environmental Protection Agency's survey and monitoring programs under the Safe Drinking Water Act. This method is for use in accordance with the National Primary Drinking Water Regulations at 40 CFR Part 141.⁴
- 1.2. This method allows for the detection of coliform bacteria and/or *E. coli* from 16 - 48 hours and does not require further confirmation or verification steps.
- 1.3 Modified Colitag™ is US EPA approved for compliance monitoring of public water systems as required by the Total Coliform Rule. This method can be used as a Presence/Absence test or for quantification of bacteria in a most probable number format (MPN) format.
- 1.4 Modified Colitag™ is capable of detecting 1 colony forming unit (CFU) of chlorine-injured *E. coli* or other coliform bacteria per 100-mL of water sample. There is no upper limit of detection in the MPN format as dilutions are used to detect and enumerate bacteria.

2.0 Summary of Method

- 2.1 Modified Colitag™ is a one-step, ready-to-use, dehydrated medium for analysis of water samples. For presence/absence testing, one packet of medium is mixed with a 100-mL sample of water and incubated at $35 \pm 0.5^\circ\text{C}$ for 16 to 48 hours. If results are intended to be read before 22 hours incubation, samples must be pre-warmed for 7-10 minutes in a $44.5 \pm 0.2^\circ\text{C}$ water bath. If yellow color is observed, coliforms are present. If blue fluorescence is observed under 365 nm ultraviolet (UV) light, *E. coli* are present. Complete directions for use are available from CPI International, 5580 Skylane Blvd., Santa Rosa, CA, 95403, (800) 878-7654, <http://www.colitag.com/colitag-instructions.pdf>.

EAP. [Signature]
000133

- 2.2 For enumeration of *E. coli* and other coliforms, Modified Colitag™ is suitable in a variety of MPN formats. In one format, a 100-mL of water sample would be mixed with one packet of Modified Colitag™ and divided into ten 10-mL volumes before incubation. Based on the number of positive tubes, an MPN value for this number of tubes can be looked up in Table 9221:III of Section 9221C Estimation of Bacterial Density in the Standard Methods for the Examination of Water and Wastewater.⁵ Other most probable number formats suitable with Modified Colitag™ can be found in the same reference and further information is available from CPI International, 5580 Skylane Blvd., Santa Rosa, CA, 95403, (800) 878-7654, www.colitag.com.
- 2.3 Modified Colitag™ is based on the detection of two enzymes, β -glucuronidase and β -galactosidase, which are characteristic of *E. coli* and the coliform group respectively.^{5,6} For detection of β -galactosidase, which is an enzyme indicative of the coliform group, Modified Colitag™ utilizes the chromogenic substrate, ortho-nitrophenyl- β -D-galactopyranoside (ONPG). Upon hydrolysis of ONPG by β -galactosidase, a distinctly yellow-colored compound, ortho-nitrophenol, is released indicating the presence of coliforms. For detection of β -glucuronidase, which is the enzyme specific to *E. coli*, Modified Colitag™ utilizes the fluorogenic enzyme substrate, 4-methylumbelliferyl- β -D-glucuronide (MUG). Upon hydrolysis of MUG by β -glucuronidase, 4-methylumbelliferone is released, a compound which fluoresces when exposed to ultraviolet light. The β -glucuronidase enzyme is specific to *E. coli* and observation of this fluorescence differentiates this organism from other members of the coliform group.
- 2.4 Quality of the detection process is assured through resuscitative components in the medium that promote recovery of chlorine-injured *E. coli* and other coliforms.

3.0 Definitions

- 3.1 The definition and purposes below are specific to this method but conform to common usage as much as possible.

Hydrolyzable Substrate: A chemical substrate (i.e. ONPG, MUG) that is capable of being hydrolyzed by bacteria such as coliforms and *E. coli*.

Chromogenic Enzyme Substrate: A substrate which can be hydrolyzed by an enzyme releasing a colored (or chromogenic) compound.

Fluorogenic Enzyme Substrate: A substrate which can be hydrolyzed by an enzyme releasing a fluorescent compound.

Ortho-nitrophenyl-β-D-galactopyranoside (ONPG): A chemical substrate which is hydrolyzed by β-D-galactosidase activity in coliforms. When a drinking water sample containing coliform bacteria is added to Modified Colitag™ and subjected to incubation, enzymatic hydrolysis of ONPG causes production of ortho-nitrophenol, a yellow colored compound indicating the presence of coliforms.

4-Methylumbelliferyl-β-D-glucuronide (MUG): A chemical substrate which is hydrolyzed by β-glucuronidase activity in *E. coli*. When a drinking water sample containing *E. coli* bacteria is added to Modified Colitag™ and subjected to incubation, enzymatic hydrolysis causes production of 4-methylumbelliferon, which is a compound that fluoresces when exposed to long wavelength ultra violet light (365 nm). Fluorescence by 4-methylumbelliferone indicates the presence of *E. coli*.

Proton Gradient Resuscitation: Modified Colitag™ utilizes a patented method of resuscitating chlorine-injured bacteria, whereby a slightly acidic medium allows injured coliforms to more easily maintain a proton gradient across the cell membrane³.

Coliform bacteria: Bacteria, which hydrolyze ONPG at 35±0.5 degrees C after 24 ± 2 h of incubation.

***E. coli*:** Bacteria, which hydrolyze MUG at 35±0.5 degrees C after 24 ± 2 h of incubation.

4.0 Interferences

- 4.1 **Chemical/Physical:** There are no known chemical interferences that would be found in drinking water or source water that would inhibit or interfere with the development of color or the production of fluorescence when one uses Modified Colitag™ medium. If a water sample has background color prior to analyses, a control blank (same water sample not inoculated with Modified Colitag™ medium) should be run and used as the control blank.
- 4.2 **Biological:** Heterotrophic bacteria greater than 10⁴/ml can yield positive coliform reactions⁷.

5.0 Safety

- 5.1 None of the components used in Modified Colitag™ are listed as a carcinogen or suspected carcinogen. Reference should be made to the

Material Safety Data Sheet provided with the product for specific information.

- 5.2 The analyst should follow the recommended safety guidelines described in *Standard Methods for the Examination of Water and Waste Water* (American Public Health Association 2005), 21st edition, section 1090).⁸
- 5.3 When working with Modified Colitag™ it is important to know and practice normal safety procedures for working in a microbiology laboratory. Routine biosafety procedures should be followed when handling this medium and related samples.

6.0 Instrumentation, Equipment and Supplies

- 6.1 Incubator or water bath set to $35 \pm 0.5^\circ\text{C}$
- 6.2 Circulating water bath set to $44.5 \pm 0.2^\circ\text{C}$
- 6.3 Long wavelength 6-watt UV light (365 nanometers)
- 6.4 Non-fluorescing sterile glass or plastic sample collection vessels (120-mL or larger bottles or bags) with thiosulfate. All glassware and plastic vessels used for determining the presence of coliform bacteria or *E. coli* should be handled according to recommended guidelines.⁵ All glassware and containers should be washed with a suitable laboratory detergent, rinsed thoroughly with tap water followed by rinsing with reagent water⁹ and sterilized by autoclaving at 121°C for 15 minutes before use. Certified sterile plastic disposable coliform sample vessels with 100-mL fill line, thiosulfate tablet, and tamper seals are available through a variety of commercial suppliers and CPI International. Sterile Coli-Test bags are available through Nasco or CPI International.

7.0 Reagents and Standards

- 7.1 Modified Colitag™ product for P/A or MPN testing is provided as ready-to-use, pre-measured dehydrated media. One packet dissolves directly into a 100-mL sample for presence/absence testing or is dissolved with 100-mL sterile reagent water to make 100-mL of single strength medium. The medium is stable when stored at 4 to 30 degrees Centigrade away from light. The expiration date and lot number are indicated on each unit. Modified Colitag™ dehydrated medium has a shelf life of at least 18 months from the date of manufacture under proper storage conditions.
- 7.2 Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) is used to dechlorinate drinking water samples.

- 7.3 A reagent blank (one Modified Colitag™ test packet mixed with 100-mL of sterile distilled water) may be incubated along with the test samples as a negative control standard. A Modified Colitag™ color comparator may be used as a positive control standard. If the sample has yellow color equal or greater than the comparator, coliforms are present. If the fluorescence of the sample is equal or greater than the fluorescence of the comparator then *E. coli* are present in the sample.

8.0 Sample Collection, Dechlorination, Preservation, Shipment and Storage

- 8.1 Water Sample Collection: Sampling procedures are described in the USEPA Manual for the Certification of Laboratories Analyzing Drinking Water and the Standard Methods for the Examination of Water and Wastewater, part 9000⁵.
- 8.1.1 Water samples should be collected in sterile, plastic or glass leakproof containers and sufficient sodium thiosulfate to neutralize chlorine at the time of collection. For drinking water, 0.1-mL of a 3% solution of Na₂S₂O₃ in a 120-mL bottle will neutralize up to 5 mg/L of residual chlorine.
- 8.1.2 Storage temperature and handling conditions: Samples should be tested as soon as possible after collection and should be placed on ice or refrigerator at a temperature of 1-10°C during transit to the laboratory. Use insulated containers to transport sample vessels to maintain proper storage temperatures. Sample vessels should be packed so they do not become immersed should the ice melt during transit or storage.
- 8.1.3 Drinking water samples should be analyzed within 30 h of collection.

9.0 Quality Control (QC):

Each lot of Modified Colitag™ should be tested for quality. Four presence/absence packs or single strength MPN tubes will be needed; the first as a blank (uninoculated), and the others to be inoculated with a representative positive and negative cultures. For additional guidance or clarity regarding QC, refer to *Standard Methods*, Sections 9223 B and 9020 A. The QC results for a particular lot are valid until the expiration date is realized.

- 9.1 Incubate the individual tubes or bottles containing Modified Colitag™ medium with a loopful of growth from an 18-24 hour broth culture of the following: *E. coli* (ATCC 25922 or a characterized MUG positive strain), *Klebsiella pneumoniae* (ATCC 13883 or another characterized non-

fluorescing coliform species), *Salmonella* subsp. *enterica* serovar *Typhimurium* (ATCC 14028 or another characterized non-coliform). Include an uninoculated tube or bottle of Modified Colitag™ as an additional control.

- 9.2 Incubate QC samples at 35±0.5°C for 16-48 hours. If results are intended to be read before 22 hours incubation, samples must be pre-warmed for 7-10 minutes in a 44.5 ±0.2°C water bath. Observe for the following reactions:

Modified Colitag™ Control Organism Reactions — 24 hours

Organism	Color	Fluorescence
<i>E. coli</i>	Yellow	Positive
<i>Klebsiella</i> species	Yellow	Negative
<i>Salmonella</i> subsp. <i>enterica</i> serovar <i>Typhimurium</i>	Colorless	Negative
None	Clear	Negative

- 9.3 For additional quality control testing, positive Modified Colitag™ may be inoculated into Brilliant Green Lactose Bile, EC-MUG or other media used for confirmation.

10.0 Calibration and Standardization

- 10.1 Check incubation temperatures twice daily (morning and afternoon) to ensure proper temperature regulation.
- 10.2 Temperature measurement devices should be calibrated annually against a NIST-certified thermometer or one traceable to NIST.

11.0 Procedure

- 11.1 Test Procedure for the Presence/Absence method
- 11.1.1 Aseptically add Modified Colitag™ medium from one packet to a 100-mL water sample in a sterile nonfluorescing, leakproof, container.
- 11.1.2 Shake to begin dissolution.
- 11.1.3 Incubate bottles at 35±0.5°C for 16-48 hours. If results are intended to be read before 22 hours incubation, samples must be pre-warmed for 7-10 minutes in a 44.5 ±0.2°C water bath.

- 11.1.4 Read results (refer to section 11.3, below).
- 11.2 Test procedure for the MPN method: Refer to Section 9221C of the *Standard Methods for the Examination of Water and Wastewater*:
- 11.2.1 Method A:
- 11.2.1.1 Select the appropriate number of tubes per sample for the MPN test (10 tubes x 10-mL or 5 tubes x 20-mL).
- 11.2.1.2 Mix 100-mL of a water sample with one packet of Modified Colitag™ medium in a sterile vessel. Shake to dissolve medium.
- 11.2.1.3 Aseptically dispense the sample into the tubes.
- 11.2.1.4 Incubate tubes at 35 ± 0.5 °C for 16-48 hours.
- 11.2.1.5 Read results (refer to section 11.3, below) and report as the most probable number (MPN)/100-mL using values from Table 9221:III, Section 9221 C in the *Standard Methods for the Examination of Water and Wastewater*.
- 11.2.2 Method B:
- 11.2.2.1 Dissolve Modified Colitag™ in sterile distilled water to make up desired quantity of medium. One packet contains sufficient contents to make 100-mL of single strength medium, or 50-mL of double strength medium.
- 11.2.2.2 Perform serial dilutions and inoculations according to the MPN method described in The Standard Methods for the Examination of Water and Wastewater, 21st Ed. Section 9221C, Estimation of Bacterial Density.
- 11.3 Sample Interpretation
- 11.3.1 Visually check each bottle, bag or tube for a yellow color. If the sample (P/A bottles or bags or MPN tubes) are yellow, coliform bacteria are present.
- 11.3.2 The absence of yellow color in the sample after 16-48 hours incubation indicates the sample is negative for coliform bacteria.
- 11.3.3 If samples are yellow, examine for fluorescence using a long wavelength 365 nm UV lamp in a darkened environment. If a bright blue fluorescence is present, the sample is confirmed for the

presence of *E. coli*. As with the ONPG test, known positive and negative cultures may be run parallel to the unknown sample, or the sample may be compared to a Colitag™ comparator (description included in this section).

11.3.4 The absence of fluorescence in the sample under longwave UV light after 16-48 hours incubation indicates the sample is negative for *E. coli* bacteria.

11.3.5 For quality control, the test sample may be compared to any of the following:

- Negative control – either sterile water with CPI Modified Colitag™ and or a negative control culture with Modified Colitag™, incubated at $35\pm 0.5^{\circ}\text{C}$ for 16-48 hours (see Table 9.2 in the quality control section).
- Positive control – Coliform-positive, MUG-positive organism with Modified Colitag™, incubated at $35\pm 0.5^{\circ}\text{C}$ for 16-48 hours (see Table 9.2 in the quality control section).
- Color Comparator -- Colitag™ comparator transferred to a similar test vessel- if the sample is equal or darker yellow than the Colitag™ comparator, the sample is confirmed for the presence of total coliforms. If the sample fluoresces with equal or greater intensity than the comparator, the sample is confirmed for the presence of *E. coli*. The color comparator should be stored protected from light when not in use.

11.3.6 Use Modified Colitag™ medium on or before the printed expire date. For optimal performance, it is recommended that the powdered medium be stored in cool (preferably 4-7 degrees C), dry conditions, protected from light and moisture.

12.0 Data Analysis, Calculation, Interpretation and Reporting Results

12.1 Follow the same interpretation directions from 11.3 above.

12.1.1 Presence/Absence: Report results as presence/absence of total coliforms/*E. coli* per 100-mL. No further data analysis or calculation is required.

12.1.2 MPN Format: Report results as the most probable number of total coliforms/*E. coli* (MPN) per 100-mL. MPN values and further

information are available in Section 9221C of the Standard Methods for Examination of Water and Wastewater, refer to tables 9221: II, III, and IV.

13.0 Method Performance Characteristics of Modified Colitag™

- 13.1 **Specificity:** 93.8% overall (16-48 hours incubation) when compared to SM9221B (LTB, BGLB) and 96.1% overall compared to SM9221F (EC+MUG).
- 13.2 **Comparability:** Modified Colitag was found to have an overall agreement of 94.5% with LTB, BGLB for total coliforms and 95.9% with EC-MUG for *E.coli* detection.
- 13.3 **Sensitivity:** 95.2% overall (16-48 hours incubation) when compared to SM9221B (LTB, BGLB) and 92.8% overall compared to SM9221F (EC+MUG).

14.0 Pollution Prevention

- 14.1 Wherever possible, it is recommended that laboratory personnel use pollution control techniques to minimize waste generation. When waste cannot be reduced at the source, recycling is recommended.

15.0 Waste Management

- 15.1 It is the responsibility of each laboratory to comply with all federal, state and local regulations governing waste management, particularly to hazardous waste identification rules and land disposal restrictions. In addition, it is the responsibility of each laboratory to protect the air, water and land by minimizing and controlling all release from fume hoods and bench operations. Compliance is also required with any National Pollutant Discharge Elimination System (NPDES) Permits and regulations.¹⁰ For further information, Federal, State or local agencies should be contacted.

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16.0 References

- 16.1 Colitag™ product insert, prepared by CPI International.
- 16.2 "Colitag: An Indole- and MUG-based Medium for Detecting and Recognizing Fecal Coliforms and *Escherichia coli*." Abstract with poster presentation, 1991 ASM General Meeting, Dallas TX.
- 16.3 "A Comparison of the Colitag Method and the Standard Methods for the Detection of Fecal Coliforms and *Escherichia coli* in Urban Creeks "WEF/CWEA Collection Systems 2002 Conference. San Francisco, CA.
- 16.4 Chang, G. W. and Lum, R. A., 1992. *Improved Recovery of Chlorine-Injured E. coli on Acidified Media. Colitag™ and mCT3*. Abstract with poster presentation. American Society for Microbiology General Meeting, New Orleans, LA.
- 16.5 United States Environmental Protection Agency. *National Primary Drinking Water Regulations, Total Coliforms (Including Fecal Coliforms and E. coli); Final Rule. Federal Register 54 (124): 27547-27568*. Washington, D. C., Office of Federal Register. June 29, 1989.
- 16.6 American Public Health Association, American Water Works Association, Water Environment Federation. Microbiological Examination, Part 9000. IN: *Standard Methods for the Examination of Water and Wastewater, 21st edition*.
- 16.7 Krieg, N. R. and J. G. Holt., eds. *Bergey's Manual of Systematic Bacteriology*, vol. 1. Baltimore, Williams and Wilkins, 1989.
- 16.8 Unpublished data, CPI International Inc., 5580 Skylane Blvd, Santa Rosa, Ca, 95403.
- 16.9 American Public Health Association, American Water Works Association, Water Environment Federation. Microbiological Examination, *Standard Methods for the Examination of Water and Wastewater, 21st edition*, Sections 1090 H and 1090 J.
- 16.10 American Society of Testing Materials. Specifications for Reagent Water, (Type III Grade), D1193-91. In: *Annual Book of ASTM Standards*, 1991, vol. 11.01. p. 45. Philadelphia, PA.
- 16.11 Code of Federal Regulations Title 40 Protection of the Environment. Chapter I Environmental Protection Agency. Part 122 EPA Administered Permit Programs: The National Pollutant Discharge Elimination System.
<http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=344382d789a386c880645c300a514061&rgn=div5&view=text&node=40:21.0.1.1.12&idno=40>.

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ground water sources, were fortified with known chlorine concentrations and then analyzed by each method. The results are summarized in the validation study report (Palintest Ltd 2009b).

EPA has determined that the ChloroSense Method is equally effective as approved Standard Methods 4500-C1 D and 4500-C1 G. The basis for this determination is discussed in the validation study report (Palintest Ltd 2009b). Therefore, EPA is approving the ChloroSense Method for determining free and total chlorine residuals in drinking water. A copy of the method can be downloaded from NEMI at <http://www.nemi.gov> or obtained by contacting Palintest Ltd, 21 Kenton Lands Road, P.O. Box 18395, Erlanger, KY 41018.

3. Modified Colitag™ (CPI International 2009). Colitag™ (CPI International 2001) is a presence/absence method approved for use under the Total Coliform Rule. It uses enzymatic cleavage of a chromogenic substance to detect total coliforms and enzymatic cleavage of a fluorogenic substance to detect *E. coli* in a 100 mL sample of drinking water. Detection of total coliforms and *E. coli* are performed simultaneously by this method. Colitag™ may also be used in a most-probable-number format provided that the sum of all individual portions of the sample total 100 mL. Modified Colitag™ has a different formulation from the originally approved Colitag™. The purpose of the formula change is to achieve greater selectivity for total coliforms and *E. coli*. Additionally, the Modified Colitag™ provides flexibility in the incubation period (16 to 48 hours), while the approved Colitag™ requires a 24 hour incubation time.

Approved methods for total coliforms are listed at 40 CFR 141.21(f)(3) and approved methods for *E. coli* are listed at 40 CFR 141.21(f)(6). The performance characteristics of Modified Colitag™ were compared to Standard Methods 9221 B (LTB/BGLB) for total coliforms and 9222 G (LTB/EC-MUG) for *E. coli* (APHA 1998). The comparison study involved analyses of twenty replicate drinking water samples that were inoculated with very low densities of chlorine stressed total coliforms or *E. coli* obtained from ten geographically dispersed waste waters. Method specificity was evaluated using 100 positive and 100 negative cultures as determined from analyses by the reference methods.

EPA has determined that the Modified Colitag™ Method is equally effective as approved Standard Methods 9221 B for total coliforms and 9222 G for *E. coli* which are already promulgated in the

regulations at 40 CFR 141.21(f)(3) and 40 CFR 141.21(f)(6), respectively. The basis for this determination is discussed in the study report (USEPA 2009g). Therefore, EPA is approving the Modified Colitag™ Method for determining total coliforms and *E. coli* in drinking water. A copy of the method can be downloaded from NEMI at <http://www.nemi.gov> or obtained by contacting CPI International, 580 Skylane Boulevard, Santa Rosa, CA 95403.

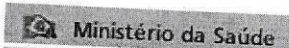
IV. Statutory and Executive Order Reviews

As noted in Section II, under the terms of SDWA Section 1401(1), this streamlined method approval action is not a rule. Accordingly, the Congressional Review Act, 5 U.S.C. 801 *et seq.*, as added by the Small Business Regulatory Enforcement Fairness Act of 1996, does not apply because this action is not a rule for purposes of 5 U.S.C. 804(3). Similarly, this action is not subject to the Regulatory Flexibility Act because it is not subject to notice and comment requirements under the Administrative Procedure Act or any other statute. In addition, because this approval action is not a rule but simply makes alternative (optional) testing methods available for monitoring under SDWA, EPA has concluded that other statutes and executive orders generally applicable to rulemaking do not apply to this approval action.

V. References

- American Public Health Association (APHA). 1997. Standard Method 9223-97. Enzyme Substrate Coliform Test. Approved by Standard Methods Committee 1997. Standard Methods Online. (Available at <http://www.standardmethods.org>.)
- American Public Health Association (APHA). 1998. *20th Edition of Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 800 I Street, NW., Washington, DC 20001-3710.
- American Public Health Association (APHA). 2005. *21st Edition of Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 800 I Street, NW., Washington, DC 20001-3710.
- ASTM International. 2009a. ASTM D 511-09. Standard Test Methods for Calcium and Magnesium in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009b. ASTM D 1688-07. Standard Test Methods for Copper in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009c. ASTM D 2972-08. Standard Test Methods for Arsenic in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009d. ASTM D 3559-08. Standard Test Methods for Lead in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009e. ASTM D 3645-08. Standard Test Methods for Beryllium in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009f. ASTM D 3697-07. Standard Test Methods for Antimony in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009g. ASTM D 3859-08. Standard Test Methods for Selenium in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009h. ASTM D 1253-08. Standard Test Method for Residual Chlorine in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009i. ASTM D 516-07. Standard Test Method for Sulfate Ion in Water. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- ASTM International. 2009j. ASTM D 6581-08. Standard Test Methods for Bromate, Bromide, Chlorate, and Chlorite in Drinking Water by Suppressed Ion Chromatography. ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428-2959. (Available at <http://www.astm.org>.)
- Best, J. 2009. Memo to the record describing basis for expedited approval of Standard Methods 9223 B (20th Edition), 9223 B (21st Edition) and 9223 B-97 for determining *E. coli* as specified at 40 CFR 141.21(f)(6). August 31, 2009.
- CPI International. 2001. Colitag™ Test. Colitag™ Product as a Test for Detection and Identification of Coliforms and *Escherichia coli* Bacteria in Drinking Water and Source Water as Required in National Primary Drinking Water Regulations. August 2001. 580 Skylane Boulevard, Santa Rosa, CA 95403.
- CPI International. 2009. Modified Colitag™ Method. Modified Colitag™ Test Method for the Simultaneous Detection of *E. coli* and other Total Coliforms in Water (ATP D05-0035). August 28, 2009. 5580 Skylane Boulevard, Santa Rosa, CA 95403.
- Edberg, S.C. *et al.* 1989. "National Field Evaluation of a Defined Substrate Method for the Simultaneous Detection of Total Coliforms and *Escherichia coli* from Drinking Water: Comparison with

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VISA LEGIS

Legislação em
Vigilância Sanitária



RESOLUÇÃO DA DIRETORIA COLEGIADA - RDC Nº 206, DE 17 DE NOVEMBRO DE 2006.

Estabelece Regulamento Técnico de Produtos para Diagnóstico de uso in vitro e seu Registro, Cadastramento, e suas alterações, revalidações e cancelamento.

A Diretoria Colegiada da Agência Nacional de Vigilância Sanitária, no uso da atribuição que lhe confere o inciso IV do art. 11 do Regulamento aprovado pelo Decreto nº 3.029, de 16 de abril de 1999, e tendo em vista o disposto no inciso II e nos §§ 1º e 3º do art. 54 do Regimento Interno aprovado nos termos do Anexo I da Portaria nº 354 da ANVISA, de 11 de agosto de 2006, republicada no DOU de 21 de agosto de 2006, em reunião realizada em 6 de novembro de 2006, e considerando as disposições constitucionais e a Lei Federal nº 8080, de 19 de setembro de 1990 que tratam das condições para a promoção, proteção e recuperação da saúde, como direito fundamental do ser humano; considerando a necessidade de atualizar os procedimentos para registro, cadastramento, alteração, revalidação e cancelamento de Produtos para Diagnóstico de uso in vitro de que tratam a Lei nº 6360, de 23 de setembro de 1976, o Decreto nº 79.094, de 5 de janeiro de 1977; adota a seguinte Resolução de Diretoria Colegiada e eu, Diretor-Presidente Substituto, determino a sua publicação:

Art.1º Estabelecer o Regulamento Técnico que disciplina os requisitos necessários para o Registro de Produtos para Diagnóstico de uso in vitro, seu cadastramento, seu cancelamento, sua alteração ou revalidação, na forma do Anexo desta Resolução da Diretoria Colegiada (RDC).

Art.2º Os Produtos para Diagnóstico de uso in vitro estão sujeitos a cadastro ou registro, de acordo com seu enquadramento de classe de risco constante no Anexo, item 2, deste Regulamento Técnico.

§1º Os produtos podem ter a sua classificação alterada, quanto ao risco, a critério da autoridade sanitária.

§2º O Regulamento Técnico estabelecido por esta RDC não se aplica:

I - aos reagentes e materiais de referência, destinados especificamente à avaliação de qualidade em testes de proficiência ou de comparação inter-laboratorial;

II - aos reagentes isolados, comercializados como insumos para fabricação de produtos para diagnóstico de uso in vitro;

III - aos reagentes montados (kit) nos laboratórios de análises clínicas para serem utilizados exclusivamente na mesma instituição, proibida sua comercialização ou doação;

IV - aos reagentes laboratoriais que não sejam destinados ao diagnóstico em amostras humanas;

V - aos reagentes destinados exclusivamente à medicina legal;

VI - aos produtos que não sejam destinados ao diagnóstico em amostras humanas e que sejam utilizados exclusivamente na pesquisa científica;

VII - aos meios de cultura destinados exclusivamente a análises de controle ambiental, industrial, de alimentos e de água;

Art.3º Todos os dizeres e informações que acompanham o produto devem estar em concordância com as declaradas no processo.

Art.4º Toda comunicação ao público ou publicidade referente a Produtos para Diagnóstico de uso in vitro deverá guardar estrita concordância com as informações prestadas à ANVISA e conter o respectivo número do registro ou cadastro, de acordo com Decreto 79.094/77.

Art.5º Os registros concedidos anteriormente à vigência desta Resolução deverão se adequar à mesma no momento

9223 ENZYME SUBSTRATE COLIFORM TEST*

9223 A. Introduction

The enzyme substrate test utilizes hydrolyzable substrates for the simultaneous detection of total coliform bacteria and *Escherichia coli* enzymes. When the enzyme technique is used, the total coliform group is defined as all bacteria possessing the enzyme β -D-galactosidase, which cleaves the chromogenic substrate, resulting in release of the chromogen. *Escherichia coli* are defined as bacteria giving a positive total coliform response and possessing the enzyme β -glucuronidase, which cleaves a fluorogenic substrate, resulting in the release of the fluorogen. The test can be used in either a multiple-tube, multi-well, or a presence-absence (single 100-mL sample) format.

1. Principle

a. Total coliform bacteria: Chromogenic substrates, such as ortho-nitrophenyl- β -D-galactopyranoside (ONPG) or chlorophenol red- β -D-galactopyranoside (CPRG), are used to detect the enzyme β -D-galactosidase, which is produced by total coliform bacteria. The β -D-galactosidase enzyme hydrolyzes the substrate and produces a color change, which indicates a positive test for total coliforms at 24 h (ONPG) or 28 h (CPRG) without additional procedures. Noncoliform bacteria, such as *Aeromonas* and *Pseudomonas* species, may produce small amounts of the enzyme β -D-galactosidase, but are suppressed and generally will not produce a positive response within the incubation time unless more than 10^4 colony-forming units (CFU)/mL (10^6 CFU/100 mL) are present.

* Approved by Standard Methods Committee, 1997.
Joint Task Group: 20th Edition—Carol Palmer (chair), Terry C. Covert, Robert E. Grant, Nancy H. Hall, Bruce M. Roll, Jon Standridge.

b. Escherichia coli: A fluorogenic substrate, such as 4-methylumbelliferyl- β -D-glucuronide (MUG), is used to detect the enzyme β -glucuronidase, which is produced by *E. coli*. The β -glucuronidase enzyme hydrolyzes the substrate and produces a fluorescent product when viewed under long-wavelength (366-nm) ultraviolet (UV) light. The presence of fluorescence indicates a positive test for *E. coli*. Some strains of *Shigella* spp. also may produce a positive fluorescence response. Because *Shigella* spp. are overt human pathogens, this is not considered a detriment for testing the sanitary quality of water.

2. Applications

The enzyme substrate coliform test is recommended for the analysis of drinking and source water samples. Formulations also are available for the analysis of marine waters. Initially, laboratories planning to use this procedure should conduct parallel quantitative testing (including seasonal variations) with one of the standard coliform tests to assess the effectiveness of the test for the specific water type being analyzed and to determine the comparability of the two techniques. This is particularly important when testing source waters.

Water samples containing humic or other material may be colored. If there is background color, compare inoculated tubes to a control tube containing only water sample. In certain waters, high calcium salt content can cause precipitation but this should not affect the reaction.

Do not use the enzyme substrate test to verify presumptive coliform cultures or membrane filter colonies, because the substrate may be overloaded by the heavy inoculum of weak β -D-galactosidase-producing noncoliforms, causing false-positive results.

9223 B. Enzyme Substrate Test

1. Substrate Media

Formulations are available commercially* in disposable tubes for the multiple-tube procedure, in disposable multi-wells† for the multi-well procedure, or in containers that will hold 100-mL samples for the presence-absence approach.* Appropriate pre-weighed portions of the reagent for mixing and dispensing into multiple tubes for 10-mL test portions or other containers for 100-mL samples also are available. The need for good quality

* Colilert® and Colilert 18® for multi-tube, P/A, and tray formats, Colilert MW® for multi-tube format, and Colisure™ for multi-tube and P/A formats, available from IDEXX Laboratories, Inc., Westbrook, ME.

† Quanti-Tray® or Quanti-Tray®/2000, available from IDEXX Laboratories, Inc., Westbrook, ME.

assurance and uniformity requires the use of a commercial substrate medium. Avoid prolonged exposure of the substrate to direct sunlight. Store media according to directions and use before expiration date. Discard discolored media.

2. Procedure

a. Multiple-tube procedure: Select the appropriate number of tubes per sample with predispensed media for the multiple-tube test and label. Follow manufacturer's instructions for preparing serial dilutions for various formulations. Aseptically add 10 mL sample to each tube, cap tightly, and mix vigorously to dissolve. The mixture remains colorless with ONPG-based tests and turns yellow with the CPRG format. Some particles may remain

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- COVERT, T.C., E.W. RICE, S.A. JOHNSON, D. BERMAN, C.H. JOHNSON & P.M. MASON. 1992. Comparing defined-substrate coliform tests for the detection of *Escherichia coli* in water. *J. Amer. Water Works Assoc.* 84(5):98.
- MCCARTY, S.C., J.H. STANDRIDGE & M.C. STASIAK. 1992. Evaluating a commercially available defined-substrate test for recovery of chlorine-treated *Escherichia coli*. *J. Amer. Water Works Assoc.* 84(5):91.
- PALMER, C.J., Y. TSAI, A.L. LANG & L.R. SANGERMANO. 1993. Evaluation of Colilert-marine water for detection of total coliforms and *Escherichia coli* in the marine environment. *Appl. Environ. Microbiol.* 59:786.
- CLARK, J.A. & A.H. SHAARAWI. 1993. Evaluation of commercial presence-absence test kits for detection of total coliforms, *Escherichia coli*, and other indicator bacteria. *Appl. Environ. Microbiol.* 59:380.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1994. National Primary and Secondary Drinking Water Regulation: Analytical methods for regulated drinking water contaminants; Final Rule. 40 CFR Parts 141 & 143; *Federal Register* 59:62456.
- McFETERS, G.A., S.C. BROADWAY, B.H. PYLE, M. PICKETT & Y. EGOZY. 1995. Comparative performance of Colisure[™] and accepted methods in the detection of chlorine-injured total coliforms and *E. coli*. *Water Sci. Technol.* 31:259.

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TABLE 9223:1. COLOR CHANGES FOR VARIOUS MEDIA

Substrate	Total Coliform Positive	<i>E. coli</i> Positive	Negative Result
ONPG-MUG	Yellow	Blue fluorescence	Colorless/no fluorescence
CPRG-MUG	Red or magenta	Blue fluorescence	Yellow/no fluorescence

undissolved throughout the test; this will not affect test performance. Incubate at $35 \pm 0.5^\circ\text{C}$ for period specified by substrate manufacturer.

The procedure also can be performed by adding appropriate amounts of the substrate media to the sample, mixing thoroughly, and dispensing into five or ten sterile tubes. Incubate as stated for multiple-tube procedure.

b. Multi-well procedure: The multi-well procedure is performed with sterilized disposable packets. Add sample to 100-mL container with substrate, shake vigorously, and pour into tray. The tray sealer dispenses the sample into the wells and seals the package. Incubate at $35 \pm 0.5^\circ\text{C}$ for period specified by substrate manufacturer. The MPN value is obtained from the table provided by the manufacturer.

c. Presence-absence procedure (P/A): Aseptically add pre-weighed enzymatic medium to 100-mL sample in a sterile, transparent, nonfluorescent borosilicate glass or equivalent bottle or container. Optionally, add 100-mL sample to the enzymatic substrate in a sterile container provided by the manufacturer. Aseptically cap and mix thoroughly to dissolve. Incubate as specified in manufacturer's instructions.

3. Interpretation

a. Total coliform bacteria: After the minimum proper incubation period, examine tubes or containers for the appropriate color change (Table 9223:1). ONPG is hydrolyzed by the bacterial enzyme to yield a yellow color. CPRG is hydrolyzed by the bacterial enzyme to yield a red or magenta color. If the color response is not uniform throughout the tube, mix by inversion before reading. Read manufacturer's instructions for interpretation guidelines. Some manufacturers suggest comparing sample tubes against a color comparator available through the manufacturer. Samples are negative for total coliforms if no color is observed in ONPG tests or if the tube is yellow when CPRG is used. If a chromogenic response is questionable after 18 or 24 h for ONPG, incubate up to an additional 4 h. If response is negative after 28 h for CPRG, incubate up to an additional 20 h. If the chromogen intensifies, the sample is total-coliform positive; if it does not, the sample is negative.

b. Escherichia coli: Examine positive total coliform tubes or containers for fluorescence using a long-wavelength (366-nm) ultraviolet lamp (preferably 6-W bulb). Compare each tube against the reference comparator available from a commercial source of the substrate. The presence of fluorescence is a positive test for *E. coli*. If fluorescence is questionable, incubate for an additional 4 h for ONPG tests and up to an additional 20 h for CPRG tests; intensified fluorescence is a positive test result.

4. Reporting

If performing an MPN procedure, calculate the MPN value for total coliforms and *E. coli* from the number of positive tubes as described in Section 9221C. If using the presence-absence procedure, report results as total coliform and *E. coli* present or absent in 100-mL sample.

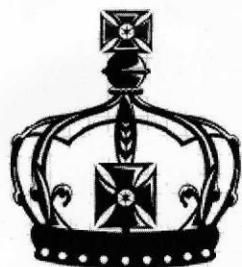
5. Quality Control

Test each lot of media purchased for performance by inoculation with three control bacteria: *Escherichia coli*, a total coliform other than *E. coli* (e.g., *Enterobacter cloacae*), and a noncoliform. Also add a sterile water control. If the sterile water control exhibits faint fluorescence or faint positive coliform result, discard and use a new batch of substrate. Avoid using a heavy inoculum. If *Pseudomonas* is used as the representative noncoliform, select a nonfluorescent species. Incubate these controls at $35 \pm 0.5^\circ\text{C}$ as indicated above. Read and record results. Other quality-control guidelines are included in Section 9020.

6. Bibliography

- EDBERG, S.C., M.J. ALLEN, D.B. SMITH & THE NATIONAL COLLABORATIVE STUDY. 1988. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: Comparison with the standard multiple tube fermentation method. *Appl. Environ. Microbiol.* 54:1595.
- EDBERG, S.C. & M.M. EDBERG. 1988. A defined substrate technology for the enumeration of microbial indicators of environmental pollution. *Yale J. Biol. Med.* 61:389.
- COVERT, T.C., L.C. SHADIX, E.W. RICE, J.R. HAINES & R.W. FREYBERG. 1989. Evaluation of the Autoanalysis Colilert test for detection and enumeration of total coliforms. *Appl. Environ. Microbiol.* 55:2443.
- EDBERG, S.C. & D.B. SMITH. 1989. Absence of association between total heterotrophic and total coliform bacteria from a public water supply. *Appl. Environ. Microbiol.* 55:380.
- EDBERG, S.C., M.J. ALLEN, D.B. SMITH & THE NATIONAL COLLABORATIVE STUDY. 1989. National field evaluation of a defined substrate method for the simultaneous detection of total coliforms and *Escherichia coli* from drinking water: Comparison with presence-absence techniques. *Appl. Environ. Microbiol.* 55:1003.
- EDBERG, S.C., M.J. ALLEN, D.B. SMITH & N.J. KRIZ. 1990. Enumeration of total coliforms and *Escherichia coli* from source water by the defined substrate technology. *Appl. Environ. Microbiol.* 56:366.
- RICE, E.W., M.J. ALLEN & S.C. EDBERG. 1990. Efficacy of β -glucuronidase assay for identification of *Escherichia coli* by the defined-substrate technology. *Appl. Environ. Microbiol.* 56:1203.
- RICE, E.W., M.J. ALLEN, D.J. BRENNER & S.C. EDBERG. 1991. Assay for β -glucuronidase in species of the genus *Escherichia* and its application for drinking water analysis. *Appl. Environ. Microbiol.* 57:592.
- SHADIX, L.C. & E.W. RICE. 1991. Evaluation of β -glucuronidase assay for the detection of *Escherichia coli* from environmental waters. *Can. J. Microbiol.* 37:908.
- EDBERG, S.C., M.J. ALLEN & D.B. SMITH. 1991. Defined substrate technology method for rapid and simultaneous enumeration of total coliforms and *Escherichia coli* from water: Collaborative study. *J. Assoc. Offic. Anal. Chem.* 74:526.
- EDBERG, S.C., F. LUDWIG & D.B. SMITH. 1991. The Colilert® System for Total Coliforms and *Escherichia coli*. American Water Works Association Research Foundation, Denver, Colo.

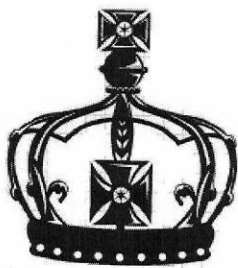
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EDITAL Nº 69/2017 – PREGA
REALIZAÇÃO: Dia 10/07/2017
ENVELOPE 01 - PROPOSTA
PROPONENTE: PRIDE ATACADISTA
ENDEREÇO: RUA SANTA MARIA, 100
FONE/FAX: (045) 3054-5779
CNPJ/MF: 20.732.659/0001-40

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ANEXO III
PROPOSTA DE PREÇOS

PROPONENTE: PRIDE ATACADO LTDA

ENDEREÇO: RUA SANTA MARIA 668 - JD LA SALLE

CNPJ/MF: 20.732.659/0001-40 FONE/FAX: (045) 3054-5779

EMAIL: prideatacado@hotmail.com

Ao Pregoeiro do Município de Mercedes - PR Pregão Presencial n.º 32/2017 PRIDE ATACADO LTDA, inscrita no CNPJ/MF n.º.20732659000140, Inscrição Estadual n.º.9067157599, neste ato representada por seu representante legal, o(a) Sr.(a) CRISTIAN RAFAEL DONASSOLO, portador(a) da Carteira de Identidade n.º88052803, expedida pela SSP/PR, e do CPF n.º. 06689088901, em atendimento ao disposto no Edital em epígrafe, apresenta:

Proposta de Preços conforme descrição a seguir

ITEM	QTD	UNID	DESCRIPTIVO	R\$ UNIT	MARCA	R\$ TOTAL
4	2600	Und	Meio de cultura específico, baseado no sistema de substrato definido ONPG/MUG, constituído por dois ou mais substratos específicos para determinação, via enzimática, simultânea de coliformes totais e Escherichea coli, composto de inibição Solanium, sem a necessidade de confirmação com o reagente Kovac. Utilização: para análise de presença ou ausência em amostras de água em meio normal, com resultados em amarelo para coliformes totais e azul fluorescente para Escherichea coli. Tempo de incubação: 24 horas. Embalagem: em blister individual, meio pulverizado para uma amostra de 100mL de água. Estabilidade: por 10 meses. (O produto deverá ser certificado no Standard Methods ou dispor de laudo de validação da SVS/MS,	8,55	COLITAG	22230,00

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			cumprindo o art. 22 da Portaria 2914/2011 MS)			
5	2800	UND	Frasco ou Bolsa plástica lacrada e estéril. Com tiosulfato de sódio e tarja de identificação; capacidade 100 ml; para coleta de amostras de água para exame bacteriológico, conforme exigências EPA e APHA	2,65	NASCO	7420,00
6	300	UND	Kit para análise e contagem total de bactérias heterotróficas em água; contendo substrato definido enzimático – dose única. O kit deve vir acompanhado de placas estéreis com 84 cavidades. Embalagem: 01 frasco + 01 placa = 01 análise. (O produto deverá ser certificado no Standard Methods ou dispor de laudo de validação da SVS/MS, cumprindo o art. 22 da Portaria 2914/2011 MS)	31,00	SIMPLATE	9300,00

VALOR GLOBAL: R\$ 38950,00 (TRINTA E OITO MIL NOVECENTOS E CINQUENTA REAIS)

No preço estão incluídos, além do lucro, todas as despesas e custos como frete, seguro, tributos de qualquer natureza e todas as demais despesas, diretas ou indiretas, relacionadas com o fornecimento do objeto da presente licitação.

Prazo de validade da Proposta: 60 dias, estendendo-se à etapa de lances verbais.

20.732.659/0001-40

TOLEDO 10 DE JULHO DE 2017
PRIDE ATACADO LTDA.

RUA SANTA MARIA, 668, SL. 10
JARDIM LINDO - CEP: 85902-570
P.R.

Nome: CRISTIAN RAFAEL DONASSOLO
RG/CPF 066890889-01
Cargo: SOCIO-ADM.

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