



# **RBML Food Laboratory: reference Materials challenges and experience**

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May AlKourgie

# Summary

- Introduction about RBML food Laboratory
- Internal quality Control :Reference Materials
  - List
  - type
  - preparation
  - challenges



# About us...

- RBML lab is one of the Lebanese leading providers of **food** and **water testing** services. With vast experience of offering a range of high quality analytical testing services across Lebanon and some middle east countries.

## Testing services include:

- *Microbiological*
- *Chemical and Nutritional*
- *Pesticides residues analysis*
- *Contaminants*
- *Nutrition facts*
- *Air sampling*
- *GMO*
- *Heavy metals*
- *Food Colors*



# RBML is an accredited laboratory

## Food and Feed:

- - Enumeration of Total Coliforms at 37 °C and E. Coli: Chromogenic Rapid E.coli'2 BIO-RAD, AOAC Certification No.050601 AFNOR Validated
- - Detection of Salmonella spp: Elisa-Immunoassay Transia Plate Salmonella Gold kit Nord Val 001
- - Enumeration of Coagulase positive Staphylococci :Rapid Staph BIO-RAD AOAC Certification No.080602 AFNOR Validated P and C
- - Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique :EN ISO 7937(02/2005) Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of Clostridium perfringens – Colony – count technique at 37°C 24 hrs P and C.

## -Fruits and Vegetables:

- - Pesticides residues determination of the analytes of the categories:
- organophosphates, organochlorines, triazines, triazoles, amides, benzimidazoles, carbamates, benzoyl urea, neonicotinoids: Documented in-house multi-residue methods based on the ESYD-G Pesticides Guidance Document ,01/20-10-2016, using the following analytical techniques: LC-MS/MS GC-MS/MS.

## Legumes:

- - Determination of pesticide residues of the categories Organophosphates, organochlorines, carbamates, triazines, triazoles, benzoylureas, neonicotinoids, amides, benzimidazoles, pyrethroids, various: In house multiresidue LC-MS/MS and GC-MS/MS method based on the QuEChERS method (European Union Reference Laboratory

]CYPRUS ORGANIZATION FOR THE PROMOTION OF QUALITY  
CYPRUS ACCREDITATION BODY



**ACCREDITATION CERTIFICATE no. L042-2**

The Board of Governors  
of the Cyprus Organization for the Promotion of Quality  
acting as the authorized Cyprus Accreditation Body  
according to the Article 7 of the Law 156(I)/2002

**grants accreditation to the**

**RBML Labs**

in Beirut, Lebanon

which has been assessed according to the Accreditation Criteria for Testing  
Laboratories as defined in the standard

**CYS EN ISO/IEC 17025:2005**

as **competent to perform the Methods** defined in the Scope of Accreditation  
referred to in the **Annex** of this certificate; the said Annex represents  
inextricable part of the certificate. The **Accreditation Scope** can only be  
modified after a decision of the Cyprus Accreditation Body.

The current Accreditation Certificate, no. **L042-2**, is **replacing the one issued  
on the 1<sup>st</sup> June 2017 and it is valid from the 18<sup>th</sup> September 2018 until the 12<sup>th</sup>  
September 2020.**

Accreditation was granted for the first time on the 13<sup>th</sup> September 2012.



Antonis Ioannou  
Director

Date: 18<sup>th</sup> September 2018

*This laboratory is accredited in accordance with the recognised International Standard  
ISO/IEC 17025:2005. This accreditation demonstrates technical competence for a defined  
scope and the operation of a laboratory quality management System (ISO-ILAC-IAF  
Communiqué, 18/01/2009).*



**Annex**  
**of the Accreditation Certificate number L042-2**

**Scope of Accreditation**  
**of**  
**Laboratory RBML Labs**

Valid from 13<sup>th</sup> September 2016 until 12<sup>th</sup> September 2020

\* Valid from 1<sup>st</sup> June 2017 until 12<sup>th</sup> September 2020

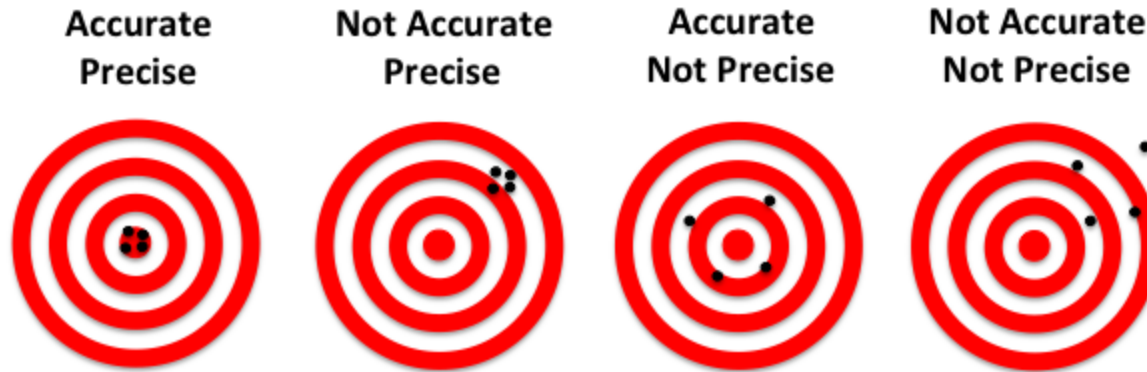
\*\* Valid from 18<sup>th</sup> September 2018 until 12<sup>th</sup> September 2020

Materials / Products	Type of testing / Countable properties	Methods / Techniques
Food	Enumeration of <i>Coliforms</i> and <i>E. Coli</i> at 37 °C	<b>Chromogenic Rapid E.coli'2 BIO-RAD</b> AOAC Certification No.050601, AFNOR Validated BRD 07/07-12/04, NMKL Validated NordVal 020
	Detection of <i>Salmonella</i> spp	<b>Elisa-Immunoassay Transia Plate Salmonella Gold</b> AOAC Certification No.010602 AFNOR Validated TRA 02/08-03/01, NMKL Validated NordVal 001
	Enumeration of <i>coagulase positive Staphylococci</i>	<b>Rapid Staph BIO-RAD</b> AOAC Certification No.080602, AFNOR Validated BRD 07/9-02/05, NMKL Validated NordVal 049
	Horizontal method for the enumeration of <i>Clostridium perfringens</i> — Colony-count technique	<b>EN ISO 7937:2004</b> Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of <i>Clostridium perfringens</i> — Colony – count technique
Fruit and vegetables with high water content *	Pesticides residues determination of the analytes of the categories: organophosphates, organochlorines, triazines, triazoles, amides, benzimidazoles, carbamates, benzoyl urea, neonocotinoids, Pyrethroid**	In-house multi-residue methods based on based on the QuEChERS EN 15662 method using the following analytical techniques: LC-MS/MS and GC-MS/MS
Legumes**	Determination of pesticide residues of the categories: Organophosphates, organochlorines, carbamates, triazines, triazoles, benzoylureas, neonicotinoids, amides, benzimidazoles, pyrethroids, various	Documented in-house multi-residue methods based on the based on the QuEChERS EN 15662 method using the following analytical techniques LC-MS/MS and GC-MS/MS

# Internal Quality Control

QCs are used to measure

- Accuracy
- precision
- contamination effects.



# Reference Materials:

The Use of known materials can be used to assess the accuracy of the method, as well as obtaining information on interferences.

- **Control (-)**
- **Control (+)**
- **Microbial Growth – Productivity**
- **Preparation of Fortified (spiked) samples**





# List of Reference Materials:

Organism	ATCC number	Reference	Lot number	Expiry Date	Concentration	Location	company
Clostridium Perfringens	13124	0318-CRM Swabs	318-226-1	31-8-2020		Micro lab/ microbiology section	Microbiologic s
L. monocytogenes (4b)	19115	0687 Z	687-228-3	31-8-2020	$7.2 \times 10^2$	Micro lab/ microbiology section	Microbiologics
E.coli	8739	0483 E3	483-858-1	31-8-2020	$5.7 \times 10^3$	Micro lab/ microbiology section	Microbiologics
E.coli	8739	0483 Z	483-820-1	31-5-2020	$6.7 \times 10^2$	Micro lab/ microbiology section	Microbiologics
S.aureus	25923	0360E3-CRM	360-421-1	30-9-2020	$5.1 \times 10^3$	Micro lab/ microbiology section	Microbiologics
K.aerogenes	13048	0306 E3-CRM	306-230-1	31-7-2020	$7.8 \times 10^3$	Micro lab/ microbiology section	Microbiologics
K.aerogenes	13048	0306 Z	306-231-2	31-7-2020	$8.9 \times 10^2$	Micro lab/ microbiology section	Microbiologics
S.Typhimurium	14028	0363 E3-CRM	363-369-1	30-9-2020	$5.7 \times 10^3$	Micro lab/ microbiology section	Microbiologics
S.Typhimurium	14028	0363 Z	363-347-2	30-6-2020	$8.0 \times 10^2$	Micro lab/ microbiology section	Microbiologics



## Accredited Reference Material Producer

A2LA has accredited

**MICROBIOLOGICS, INC.**

*St. Cloud, MN*

This accreditation covers the specific materials listed on the agreed upon Scope of Accreditation. This producer meets the requirements of ISO 17034:2016 *General Requirements for the Competence of Reference Material Producers*. This accreditation demonstrates technical competence for a defined scope and the operation of a quality management system.

Presented this 17<sup>th</sup> day of July 2018

President & CEO  
For the Accreditation Council  
Certificate Number 2655.02  
Valid to February 29, 2020



*For reference materials to which this accreditation applies, please refer to the reference material producer's Scope of Accreditation.*

Certificate of Analysis: Lyophilized Microorganism Specification and Performance Upon Release

<b>Specifications</b> <b>Microorganism Name:</b> Escherichia coli <b>Catalog Number:</b> 0483 <b>Lot Number:</b> 483-858** <b>Reference Number:</b> ATCC® 8739™* <b>Purity:</b> Pure <b>Passage from Reference:</b> 3 <b>(7) Mean Assay Value (MAV):</b> 5.7E+03 CFU per pellet	<b>Expiration Date:</b> 2020/8/31 <b>Release Information:</b> <b>Quality Control Technologist:</b> Alexandra D Stensvad <b>Release Date:</b> 2018/11/6
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Performance	
<b>Macroscopic Features:</b> Medium to large, gray, mucoid, convex. <b>Microscopic Features:</b> Gram negative straight rod.	<b>Medium:</b> SBAP <b>Method:</b> Gram Stain (1)

<b>ID System:</b> MALDI-TOF (1) See attached ID System results document.	<b>Other Features/ Challenges: Results</b> (1) Oxidase (Kovacs): negative Beta-glucuronidase (E. coli Broth w/MUG): positive
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Amanda Kuperus  
 Quality Control Manager  
 AUTHORIZED SIGNATURE

\*\*Disclaimer: The last digit(s) of the lot number appearing on the product label and packing slip are merely a packaging event number. The lot number displayed on this certificate is the actual base lot number.

Note for Vitek®: Although the Vitek® panel uses many conventional tests, the unique environment of the card, combined with the short incubation period, may produce results that differ from published results obtained by other methods.

Refer to the enclosed product insert for instructions, intended use and hazard/safety information.

Individual products are traceable to a recognized culture collection.



(\*) The ATCC Licensed Derivative Emblem, the ATCC Licensed Derivative word mark and the ATCC catalog marks are trademarks of ATCC. Microbiologics, Inc. is licensed to use these trademarks and to sell products derived from ATCC® cultures.

(1) These tests are accredited to ISO/IEC 17025:2005.



(7) The Mean Assay Value (MAV) stated above may deviate from the end-user's MAV based on variables inherent to each laboratory environment, such as methods, media type, equipment, pipettes, and individual technician technique.

Bruker Daltonik MALDI Biotyper Classification Results



Meaning of Score Values

Range	Interpretation	Symbols	Color
2.00 - 3.00	High-confidence identification	(+++)	green
1.70 - 1.99	Low-confidence identification	(+)	yellow
0.00 - 1.69	No Organism Identification Possible	(-)	red

Meaning of Consistency Categories (A - C)

Category	Interpretation
(A)	<b>High consistency:</b> The best match is a high-confidence identification. The second-best match is (1) a high-confidence identification in which the species or genus is identical to the best match, (2) a low-confidence identification in which the species or genus is identical to the best match, or (3) a non-identification.
(B)	<b>Low consistency:</b> The requirements for high consistency are not met. The best match is a high- or low-confidence identification. The second-best match is (1) a high- or low-confidence identification in which the genus is identical to the best match or (2) a non-identification.
(C)	<b>No consistency:</b> The requirements for high or low consistency are not met.

Sample Name: Escherichia coli  
 Sample Description: 0483  
 Sample ID: 483-858  
 Sample Creation Date/Time: 2018-10-30T12:46:48.068 ADS  
 Applied MSP Library(ies): BDAL, Mycobacteria Library (bead method), Filamentous Fungi Library 1.0, Listeria

Sample Name	Sample ID	Organism (best match)	Score Value
A7 (+++)(A)	483-858	Escherichia coli	2.44

Comments:

closely related to Shigella / Escherichia fergusonii and not definitely distinguishable at the moment

 **Microbiologics®**  
Statistical Analysis Certificate

Microorganism Name: Escherichia coli  
Reference #: ATCC® 8739™\*  
Catalog #: 0483  
Lot #: 483-858\*\*  
Expiration Date: 2020/8/31  
(7) Mean Assay Value (MAV): 5.7E+03 CFU per pellet  
Standard Deviation: 1.4E+03  
Coefficient of Variation: 24%  
99% Confidence Interval of 5.3E+03 to 6.2E+03 CFU  
95% Confidence Interval of 5.4E+03 to 6.1E+03 CFU

Method used to determine Mean Assay Value: Spiral Biotech Test Method  
Medium Employed: TSA  
Incubation Time and Temp: 24 hrs at 34-38 degrees C



Amanda Kuperus  
Quality Control Manager  
AUTHORIZED SIGNATURE

(7) The Mean Assay Value (MAV) stated above may deviate from the end-user's MAV based on variables inherent to each laboratory environment, such as methods, media type, equipment, pipettes, and individual technician technique.

The last digit(s) of the lot number appearing on the product label and packing slip are merely a packaging event number. The lot number displayed on this certificate is the actual base lot number. The information included in this Statistical Analysis Certificate is strictly based on the product's lot number. A product lot number may be assigned to multiple packaging configurations. As a result, this certificate only lists the lot number and does not include a product description.

© 2012 Microbiologics, Inc. All Rights Reserved. 200 Cooper Avenue North Saint Cloud, MN 56303

# Type Of Reference Materials:




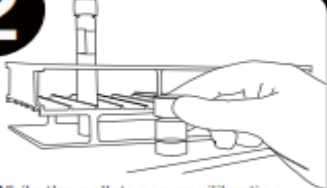
- With hydrating Fluid
- Concentration between 10-100 CFU/0.1ml

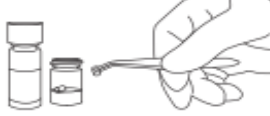


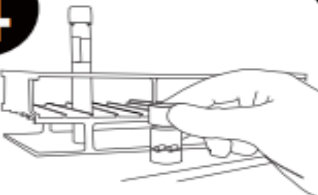
- PBS buffer for hydration
- range of CFU Concentration (proportional to the volume of PBS buffer)


# Reference Materials: Hydration and Dilution

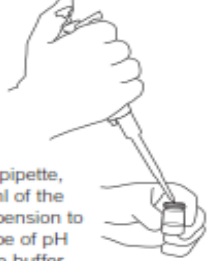
- 


Remove the vial of lyophilized pellets from refrigerated storage. Allow unopened vial of lyophilized pellets to equilibrate to room temperature (about 30 minutes).
- 


While the pellets are equilibrating, prewarm the hydrating fluid vial and a tube of 9.0 ml of phosphate buffer pH 7.2 to 34°C–38°C (at least 30 minutes).
- 

With a sterile forceps, transfer 2 pellets to the 2 ml vial of hydrating fluid. Do not remove the desiccant from the vial. Two pellets must be used to obtain the challenge concentration of 10–100 CFU per 0.1 ml on non-selective media. Immediately recap the pellet vial and return the remaining lyophilized material to refrigerated storage 2°C–8°C.
- 

Immediately recap the vial with the hydrated material and place into a 34°C–38°C incubator for 30 minutes to ensure complete hydration.
- 

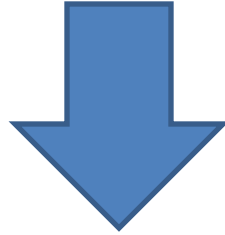
Immediately following incubation, vortex the hydrated material until pellets have completely dissolved and suspension is homogeneous.
- 

With a sterile pipette, transfer 1.0 ml of the hydrated suspension to the 9.0 ml tube of pH 7.2 phosphate buffer.
- 

Vortex working solution well.
- 

With a sterile pipette, transfer 0.1 ml from the working solution to the material being challenged (0.1 ml contains 10–100 CFU).
- Proceed with the challenge procedure according to laboratory protocol. The challenge must be completed within 30 minutes of hydration. Discard any remaining hydrated material in accordance with the laboratory protocol for disposal of biohazard materials.

# Reference materials challenges



**Import and  
clearance**

**Supplier  
Problems**

# Reference materials challenge: Import and Clearance

HS-Code

License is only provided after proving that these dangerous substances are imported for medical purposes only and not for research

UN number to prove that this item is bought for non-military research

Transportation and storage conditions (dry ice)



# Reference materials challenge: Import and Clearance

Import our orders to  
Amman Representative

Issuing invoice from  
Amman without HS code

Shipping reference  
materials by regular  
courier or shipping  
forwarder



# Reference materials challenge: supplier problems

Unsatisfactory concentration

Pending orders

Item discontinued



# Reference materials challenge: unsatisfactory concentration

Each microorganism pellet has a pre-determined range of CFUs as per the certificate of Analysis



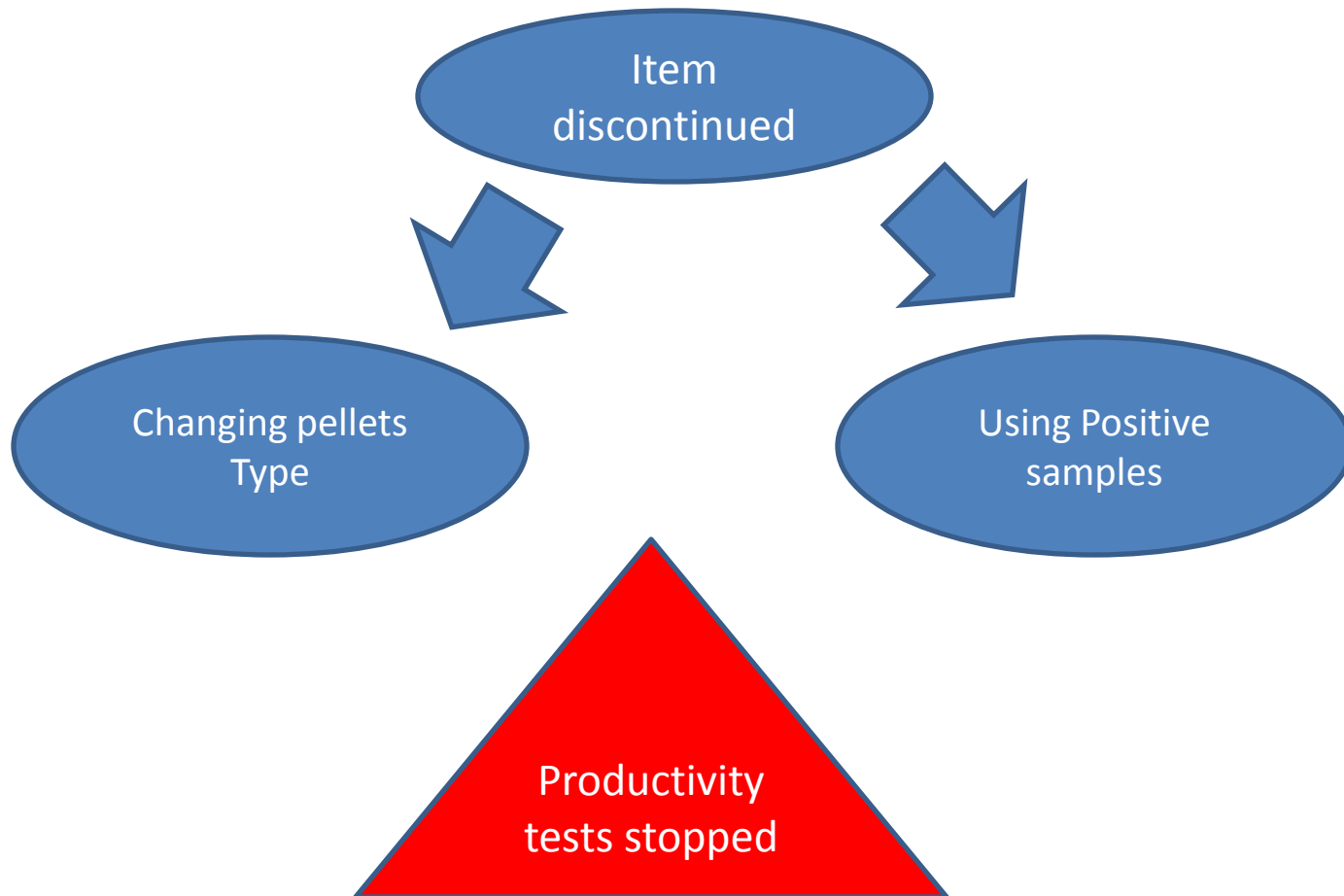
In some received pellets the number of colonies is lower than the assigned value.

Ex: For a pellets with a range of 10-100 CFU/0.1ml the number of colonies observed after culture was 8-15 CFU/0.1 ml



After sending several complaints to the supplier some reference materials was considered as discontinued

# Reference materials challenge: items discontinued



# Example 1: E.Coli

E.Coli ATCC 25922  
Discontinued



ATCC 8739 EZ-  
CFU

ATCC 8739  
E-Power

**for E.coli  
Different ATCC  
Enumerated Range**



200 Cooper Avenue North  
Saint Cloud, Minnesota 56303 USA  
[www.microbiologics.com](http://www.microbiologics.com)

November 5, 2018

Dear Microbiologics Customer,

*Escherichia coli* derived from ATCC® 25922™\*, Microbiologics catalog numbers 0335E3 and 0335C, have continued to encounter unsatisfactory results in recovery. As a result, we have decided to discontinue 0335E3 and 0335C.

At Microbiologics, we strive to provide the highest quality biomaterial for a safer, healthier world. 0335E3 and 0335C were on backorder for a prolonged period while we attempted to produce lots that met our stringent standards. Due to the continued unsatisfactory recovery, all backorders will be cancelled.

We offer *Escherichia coli* derived from ATCC® 25922™\* in our KWIK-STIK™ and LYFO-DISK™ formats. If you are looking for an enumerated format, please consider ordering a different strain of *Escherichia coli*, such as *Escherichia coli* derived from ATCC® 8739™\* in the EZ-CFU™ or Epower™ E3 format (0483C or 0483E3).

Thank you for your patience and understanding. Please reach out to our Customer Service team at 1.320.253.1640 or [info@microbiologics.com](mailto:info@microbiologics.com) with questions or to order an alternative product.

Sincerely,

Megan Murn  
Industrial Market Manager  
Microbiologics

# Example 2: Clostridium Perfringens

Clostridium  
ATCC13124  
enumerated range  
Discontinued



Clostridium  
ATCC13124 swab

Dear Dr. Rami,  
|  
Hope my email finds you well.

Please find my answers below regarding your requests;

1st: Clostridiumperfringens ATCC 13124/ new code 0318E3 OR 0318 E3 / CRM

Microbiologics nowadays have the non enumerated range only;  
<http://www.microbiologics.com/item-type/Product?keywords=13124>

According the enumerated one; they are currently working on a format called EZ-ACCUSHOT Food, which will be dedicated to the food industry with the appropriate strains for this market and concentrations. It is supposed to be ready on 2020

**For Clostridium Perfringens:  
Same ATCC  
Non Enumerated range**



