Microbiology Focus

Volume 6.2, 2014

Fluka® Analytical

Microbiology Proficiency Testing

How correct are your results? ISO 17025 accredited laboratories must regularly undergo proficiency testing (PT) to demonstrate their competency. Correct qualitative and quantitative results in a PT assure the labs that they are doing things the right way.

Contents

Microbiology proficiency testing

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

We are living in a world with an increasing amount of regulation. However, in the field of microbiology, there is still some uncertainty with regard to quality control parameters.

Microbial safety is a major issue for water, plants, and the food and beverage industry, but there is also a great deal of pressure to reduce costs while increasing productivity. Currently, microbiology provides a broad range of recommendations to satisfy the regulations. Even though more methods are known and available on the market, the potential and demand for more rapid methods with greater accuracy is very high. On the one hand, the general trend is toward more standardization, but on the other hand, we have non-regulated new steps, new situations, new samples and new problems. Organizations such as ISO, AFNOR, UKAS, ASTM and ILAC propose guidelines and offer support regarding standardization in microbiology quality control.

The problems in a microbiology QC lab are broad, and compared to chemical analysis, there are more unanswered questions with regard to unknown and uncontrolled variables. It is sometimes difficult to decide if results obtained are correct or if they are the result of errors or a natural phenomenon.

Here are some examples of problems:

- Large deviations in analysis
- Discrepancies between labs
- Difficult to compare results because of many parameters, different methods and different reporting of the results
- Confusion in determining the most suitable methods
- Validation takes a lot of time
- Human error, because of handling, calculation and reporting
- Failures tracing back to equipment, culture media, test, etc.

With the current trend toward standardization, microbiology is moving to the next level, especially due to the fact that knowledge about microbiology quality control has increased over the last 20 years. The methods employed are more accurate and labs do a better job of self-regulation.

The following, however, needs to occur to ensure even more reliable results in microbiology quality control:

- Standardization of methods by following ISO, UKAS, EU Regulation, FDA or other National Accreditation bodies
- Good, defined and stable performance of tests in reference to qualitative and quantitative determinations
- Using standards like certified reference materials (reference strains)
- Participating in regular proficiency testing

Did you know…

More than 5% of false negatives pertain to the most important pathogens?

According to the American Proficiency Institute, a study with the four most common food pathogens - *E.coli* O157:H7, *Salmonella spp.*, *Listeria monocytogenes*, and *Campylobacter spp.*, showed that the average percentage of false negative results was consistently above 5.0% for all four pathogens throughout the study period (data collection over 11 years). Mainly, the issues are seen with *Campylobacter spp.*

Figure 1: Culture plates.

One basic but vital tool needed to improve the accuracy of testing is the use of **standards**. In the UKAS LAB 31 2nd edition, it is stated that control strains should be ISO 17025 certified if available. They should be comprised of material from a recognized national culture collection or a reference materials producer like Sigma-Aldrich, which produces under ISO guide 34 with strains from ATCC and NCTC.

In the case of a microbiology lab, standard means the need for both microorganism standards or a control strain, as well as standards for calibration of equipment, such as balances or incubators. Reference cultures are not only required for testing the performance of traditional culture methods and new techniques, but also for validation of new methods and to confirm the competency of the lab. It is also possible to use reference strains, which are derivatives of national or international reference cultures, as long as it can be proven that the relevant properties for the application still exist. According to ISO 11133-1, it is possible to culture reference strains for a single passage to produce reference stock cultures which are then controlled for purity and for biochemical tests. They should be stored in a freezer or in a freeze-dried form in small aliquots; however, defrosted cultures should not be refrozen. It is preferable for working strain cultures to be made out of stock cultures, and they should not be subcultured again.

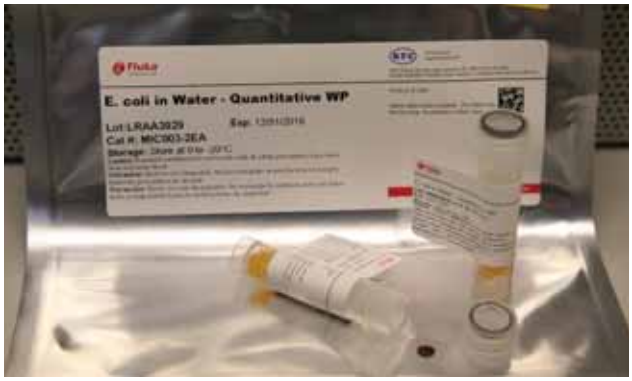

Figure 2: PT samples.

Proficiency Testing (PT) and ISO 17025

PT is an effective tool to help laboratories assure themselves and the accreditation bodies that the results that they report are correct, and to verify the effectiveness of the accreditation process. It is a comparison of results between several labs and demonstrates the technical competence of laboratories. ISO/IEC 17025 describes the general requirements for the competence of testing and calibration laboratories. It is specific as to the requirements for competence, and it applies directly to those organizations that produce testing and calibration results.

Principle: The labs getting samples of known, but undisclosed content, go through the routine procedures. As a result, the testing laboratory gets an assurance of their performance by an independent, external assessment.

The philosophy of ISO/IEC 17025: The same sample at different times, from different analysis, and from many different laboratories should reflect an agreeable result. Participating in a robust proficiency testing scheme not only gives laboratory managers confidence in their laboratory equipment, methodologies and laboratory staff, but also provides assurance that the laboratory is delivering the quality of results demanded by its customers. The accreditation according to ISO 17025 is a certification that guarantees calibrations and PT schemes are regularly performed – a true seal of approval!

How does a PT process work?

In the flow chart of **Figure 4**, the process of a PT cycle from Sigma-Aldrich is shown. It starts as a program and the PT organization assumes the coordination for the participating laboratories. First, the registration form has to be filled in as shown in **Figure 3**. For registration to a PT program go to our web site: **sigma-aldrich.com/proficiencytesting.** Then the kits are sent out to the labs. They contain different microorganisms in different concentrations (or blanks) with a sample matrix. The labs complete their testing and submit the results to the PT organization. The PT organization collects all results and performs a statistical analysis. A report is then generated and sent back to the participating laboratories.

The basis of these tests builds the Vitroids™ technology, which allow us to make a PT material with stable and exact colony forming units (CFU) and a narrow standard deviation (down to ± 4% on the level of 100 CFU). These microorganism standards are produced and sold as certified reference materials and are

Figure 3: Registration form.

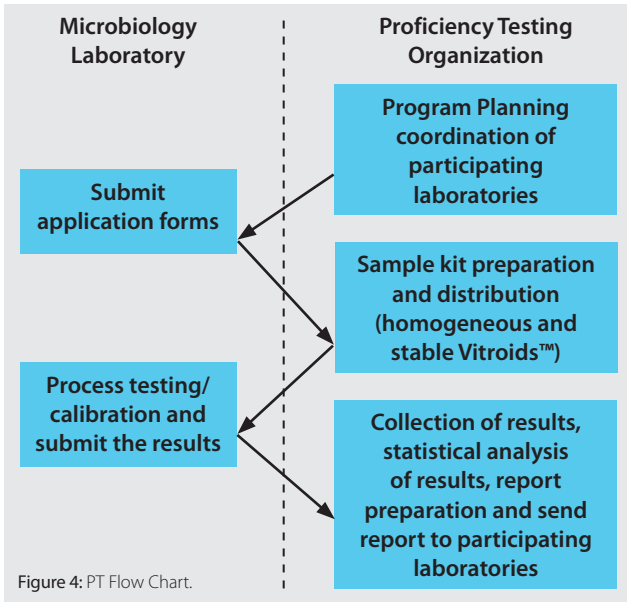

Figure 4: PT Flow Chart.

Figure 5: Vitroids™ Discs.

used for PT schemes. They are made out of reliable reference strains from ATCC and NCTC, are produced under ISO guide 34, and the CFU value is certified under ISO 17025. The organisms are immobilized in a disc in a possible range of 30 to 10^9 CFU per disc.

The discs are easy to use since they can be placed directly in water, diluent, broth, or even on agar plates. The Vitroids™ contain highly viable bacteria and when placed in contact with media, they dissolve rapidly and start to grow without a lag-phase. The viability of the CFU in a disc is stable for at least one year (for most organisms, more than two years) when kept under refrigeration (-20 °C). It is also acceptable for the product to be briefly transported at ambient temperature. Each disc is packed individually with some desiccant and then sealed in mylar foil. Each package comes with a comprehensive certificate of analysis reporting the CFU and standard deviation.

Vitroids help microbiologist have reliable results, save a lot of time (laboratory, documentation), and lower their costs. For additional info visit: **sigma-aldrich.com/vitroids**

PT Cat. No.	QC Cat. No.	Description
MIC020		*Clostridium perfringens* in Water
MIC014	QCMIC014	*E. coli* - Sludge
MIC107	QCMIC107	*E. coli* in Drinking & Surface Water - Quantitative - WS 3 Levels
MIC207	QCMIC207	*E. coli* in Drinking & Surface Water - Quantitative- WS 4 Levels
MIC007	QCMIC007	*E. coli* in Drinking and Surface Water - Quantitative - WS
MIC022		*E. coli* in Seawater - Quantitative WP
MIC122		*E. coli* in Seawater - Quantitative WP 3 Levels
MIC003	QCMIC003	*E. coli* in Water - Quantitative WP
MIC103	QCMIC103	*E. coli* in Water - Quantitative WP 3 Levels
MIC009	QCMIC009	*E. coli* Quantitative - Soil
MIC109	QCMIC109	*E. coli* Quantitative - Soil 3 Levels
MIC209	QCMIC209	*E. coli* Quantitative - Soil 4 Levels
MIC015	QCMIC015	Fungi and Yeast - WS
MIC004	QCMIC004	*Legionella* in Water - WP
MIC019	QCMIC019	*Listeria* - WP
MIC008	QCMIC008	*Pseudomonas aeruginosa* - WS
MIC021		*Salmonella* - P/A 5 samples
MIC013	QCMIC013	*Salmonella* - Sludge
MIC113	QCMIC113	*Salmonella* - Sludge 3 Levels
MIC213	QCMIC213	*Salmonella* - Sludge 4 Levels
MIC106	QCMIC106	*Salmonella* - WS 3 Levels
MIC206	QCMIC206	*Salmonella* - WS 4 Levels
MIC006	QCMIC006	*Salmonella* for Drinking/Surface Water - WS
MIC012	QCMIC012	Standard Plate Count - WP
MIC112	QCMIC112	Standard Plate Count - WP 3 Levels
MIC212	QCMIC212	Standard Plate Count - WP 4 Levels
MIC002	QCMIC002	Standard Plate Count - WS
MIC102	QCMIC102	Standard Plate Count - WS 3 Levels
MIC202	QCMIC202	Standard Plate Count - WS 4 Levels
MIC011	QCMIC011	*Streptococcus/Enterococcus* - Drinking & Surface Water
MIC111	QCMIC111	*Streptococcus/Enterococcus* - Drinking & Surface Water 3 Levels
MIC211	QCMIC211	*Streptococcus/Enterococcus* - Drinking & Surface Water 4 Levels
MIC123		*Streptococcus/Enterococcus* - Seawater WP 3 Levels
MIC105	QCMIC105	*Streptococcus/Enterococcus* - WP 3 Levels
MIC023		*Streptococcus/Enterococcus* in Seawater - WP
MIC205	QCMIC205	Total & Fecal *Streptococcus/Enterococcus* - WP 4 Levels
MIC005	QCMIC005	Total and Fecal *Streptococcus/Enterococcus* - WP
MIC016	QCMIC016	WS-Enterococci -Sample (1-10)
MIC001	QCMIC001	WS-Microbiological - Sample (1-10)

Table 1: Proficiency testing schemes (PT) and Quality Check Sets (QC) for internal validation. The PTs and QCs are used to test the performance of microbial quality control in water supply (WS), water pollution (WP) and other matrices. The levels show how many concentrations (always in duplicate) are present in the set. More detailed information on each PT or QC set can be found on the web (sigma-aldrich.com). Be aware that some products need a buffer for hydrolyzing; we recommend using Z699489 Phosphate Buffer, Magnesium Chloride, volume 72 x 99 mL.

Vitroids™	Origin	Strain No.	CFU	Cat. No.
Aspergillus brasiliensis	ATCC	16404™	80	RQC15003
Bacillus subtilis	ATCC	6633™	10'000	RQC02258
Bacillus subtilis	ATCC	6633™	80	RQC16003
Candida albicans	ATCC	10231™	80	RQC14003
Candida albicans	ATCC	10231™	1'000	RQC14007
Candida albicans	ATCC	10231™	10'000	RQC14008
Citrobacter freundii	ATCC	8090	200	RQC02105
Clostridium perfringens	NCTC	10240	30	RQC02351
Clostridium perfringens	NCTC	10240	200	RQC02355
Clostridium perfringens	NCTC	10240	500	RQC20106
Clostridium sporogenes	ATCC	19404™	80	RQC19003
Enterobacter aerogenes	ATCC	13048™	10'000	RQC01658
Enterobacter aerogenes	ATCC	13048™	100	RQC01654
Enterobacter aerogenes	ATCC	13048™	50	RQC01652
Enterobacter aerogenes	ATCC	13048™	200	RQC01655
Enterobacter aerogenes	ATCC	13048™	1'000	RQC01657
Enterococcus cloacae	ATCC	35030™	50	RQC21102
Enterococcus faecalis	ATCC	19433™	50	RQC01772
Enterococcus faecalis	ATCC	19433™	100	RQC01774
Enterococcus faecalis	ATCC	19433™	200	RQC01775
Enterococcus faecalis	ATCC	19433™	1'000	RQC01777
Escherichia coli	ATCC	11775™	1'000	RQC01707
Escherichia coli	ATCC	11775™	200	RQC01705
Escherichia coli	ATCC	11775™	50	RQC01702
Escherichia coli	ATCC	8739™	80	RQC11003
Escherichia coli	ATCC	11775™	10'000	RQC01708
Heterotrophic Organisms	—	—	100	RQC02504
Fluoribacter bozemanae	NCTC	11368	50'000	RQC02908
Legionella pneumophila (serogroup 1)	NCTC	12821	100'000	RQC02008
Listeria monocytogenes	ATCC	19115™	30	RQC01901
Pseudomonas aeruginosa	ATCC	9027™	30	RQC02202
Pseudomonas aeruginosa	ATCC	9027™	100	RQC02204
Pseudomonas aeruginosa	ATCC	9027™	50	RQC12002
Pseudomonas aeruginosa	ATCC	9027™	200	RQC12005
Pseudomonas aeruginosa	ATCC	9027™	1'000	RQC12007
Salmonella enterica subsp. Enterica serovar Typhimurium	ATCC	14028	50	RQC17002
Salmonella enterica subsp. Enterica serovar Abony	NCTC	6017	80	RQC18003
Salmonella goldcoast	NCTC	13175	30	RQC02301
Staphylococcus aureus susp. Aureus	ATCC	6538	1'000	RQC13007
Staphylococcus aureus susp. Aureus	ATCC	6538	200	RQC13005
Staphylococcus aureus susp. Aureus	ATCC	6538	50	RQC13002
Vitroids™ Blank	—	—	0	RQC0001

Table 2: Range of Vitroids

Confirmation of bacteria by specific enzymes

Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

A quick and easy confirmation of bacteria is always recommended at the end of a bacteria detection process.

Confirmation steps are really important in microbiology, and there is a wide range of methods that can be employed. Often another differential medium or method is used, such as a biochemical or immunological test. In most cases, it is important that the test be performed within a short time in order to release the results as soon as possible, and it should not be too costly. Therefore, Sigma-Aldrich focused their effort on bacteria specific enzymes and a simple detection system with specific chromogenic and fluorogenic substrates. Test cards were developed, where the reaction is visible to the naked eye, and the result is obtained within 10 minutes. A nice product range has been provided to confirm *E. coli, Salmonella, Neisseria gonorrhoea, Staphylococcus aureus, Enterococci*, Group A *Streptococci*, *Total coliform, Fecal coliform* and *Gram negatives*.

These tests utilize a specific substrate which, when hydrolyzed by a specific enzyme of the target organism (during peptide hydrolysis), produces a blue/white fluorescence, or purple/blue color upon the addition of Reagent B (color developer) when applicable.

Content of Kit

The test set contains 12 test cards with 4 test spots on each card (sufficient for 48 tests) and a reagent A (buffer), and in some tests, a reagent B with color developer is provided. Each kit contains a product insert. All materials can be stored at room temperature (away from direct sunlight). Refrigeration storage does not harm the test. The shelf life of the test is more than 2 years. Cards which are no longer white should not be used anymore and could make the results invalid.

Validation

Over 500 isolates were tested with these test cards, with a correlation of greater than 99% when compared to traditional biochemical testing.

Quality Control/Incubation

When using the test, it should be checked each time with positive and negative controls, using known stock strains of the target organism and another strain of bacteria that is not the target organism. Ideally, cultured bacteria should be no more than 18 hours old and grown on an agar plate specified in **Table 1**, otherwise the target enzyme may no longer be present (it is only produced during the exponential growth phase). Using other media

5th Microbiology photo competition

Win an android™ tablet in the fluka microbiology competition.

This photography competition is sponsored by Sigma-Aldrich with the aim of encouraging microbiologists to promote something about their work and their science. The best photographic entries will win prizes such as an Android™ tablet PC, a Giant Microbe, a Swiss army knife and a USB stick. The winning images will be published in Microbiology Focus, and the best one will be featured on the cover.

Rules of the Competition and Conditions of Entry

1. The competition is open to all residents worldwide.
2. Entries should illustrate any microorganisms (living or dead) or a microbiologist in action at work
3. Picture size should be at least 400 dpi and 90 x 120 mm (max 5 MB). The file format must be in jpg, tiff or pdf!
4. The entries will be judged on:
   - Clarity of presentation
   - Composition
   - Illumination and contrast
   - Congruency of subject matter and title of photograph
   - Scientific interest and relevance
   - Originality

5. Winning entries will be retained by Sigma-Aldrich, who will have sole rights of publication, reproduction and display.
6. Closing date will be 31st August 2014
7. Entries after the closing date will not be considered. Entries that are incomplete, illegible, mutilated, altered or not complying exactly with the instructions and theme may be disqualified.
8. Decisions of the judges in all matters affecting the competition will be final and legally binding.

The competition will be judged by:

Dr. Lars Fieseler, Zurich University of Applied Sciences - ZHAW, Supervisor, Department Microbiology

Prof. Mohammad Manafi, Medical University of Vienna, Head of the Department for Food Hygiene

Jvo Siegrist, Sigma-Aldrich, Product Manager, Microbiology

Method of Entry

There is no entry fee, but for each entry, an entry form must be completed (three entries at the most).

Entry form available from

sigma-aldrich/mibi-competition

could also influence the metabolism and cause false results, so it is recommended that only the specified media in **Table 1** be used.

Procedure

Use a culture from an agar plate of the types specified in Table 1. Other types of growth media may not allow the target organism to produce the target enzyme to be detected by the test card. Ideally, the growth should be between 14 to 18 hours old before inoculating the test card. After 24 hours of growth, most bacteria go into a stationary phase where reduced levels of enzymes are present, which may affect the results. If a test of a negative result is done after 24 hours of growth and a concern remains, repeat the culture and test within 14 to 18 hours. (Note: The incubation temperature should be 44 °C for fecal coliform, and 38.5 °C for all other types of bacteria.)

Figure 1: Blue/white fluorescence on an Enzyme Confirmation Card.

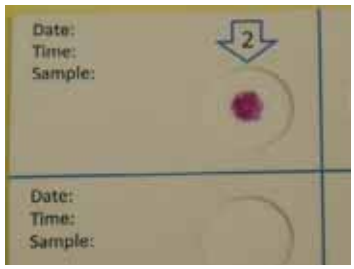

Figure 2: Color reaction on an Enzyme Confirmation Card.

1. Select one test spot as your Control Spot, and other test spots as your Sample Spot or positive control. Add 1 drop of Reagent A to the filter membrane area of the Control Spot and to the filter membrane area of each Sample Spot.
2. Select colonies that morphologically resemble the target organism from the first growth plate and touch the tops of 1-2 colonies with a loop, inoculating needle, swab, or wooden applicator. Smear the colonies onto the filter membrane area of the Sample Spot ONLY.

Set aside the inoculated card at room temperature for 5-10 minutes.

Did you know…

Bacteria do not waste time and energy?

Generally, bacteria do not synthesize enzymes unless the substrates are present and they are needed. The regulation of the enzyme production is an interplay of substrates, inducers and inhibitors.

Figure 3: ATP synthase is an enzyme that provides energy for cells by synthesizing adenosine triphosphate.

Figure 3

A) UV detection method (see **Table 1**)

3. Look at the test card under a long wave (~360nm) UV light. A positive test will be indicated by a fluorescence light (see **Table 1**) around the smeared colonies in the Sample Spot over a blue background. The test is negative if no fluorescence colonies appear in the Sample Spot (which will look like the Control Spot). A positive test is indicative of the target organism. The florescence of a positive result should remain on the Sample Spot for more than 24 hours.

B) Color detection method (see **Table 1**)

4. Add 1 drop of Reagent B to the filter membrane area of the Sample Spot and to the filter membrane area of each Sample Spot.
5. A purple or blue (as stated in **Table 1**) color will immediately form on and around the deposited colonies on the Sample Spot in the presence of the hydrolyzed substrate, indicating a Positive test of the target organism. The test is negative if no purple or blue color appears in the Sample Spot. The test result must be read within 1 minute after the addition of Reagent B, otherwise the test result may be invalid. If purple or blue color shows on the Control Spot, then re-test.

Limitations

Occasionally, some species of other microorganisms may produce small amounts of enzyme, which can produce a positive test from culture. This is extremely rare. The test detects bacterial enzymes, which may dissolve in liquids. Serial dilutions of bacteria may not give a positive reaction.

Part Number	Target Organism	Reagent B	Inspection Method	Shown Color if Positive	Suitable Growth Agar Media									
					B	M	E	Sal	S.Sal	GN	EC	110	LT	CH
*75444	*E. coli*	No	UV Light	Blue/White Fluorescence	X	X				X	X		X	
*77643	Total Coliform	No	UV Light	Blue/White Fluorescence	X	X				X			X	
*40926	*Fecal* Coliform	No	UV Light	Blue/White Fluorescence	X	X				X			X	
*55283	*Salmonella*	No	UV Light	Yellow Fluorescence	X			X	X					
*56305	*Enterococcus*	Yes	Visual	Purple	X		X							
*74203	Gram+/Gram–Differentiation	Yes	Visual	Purple (indicate Gram–)	X					X				
*92598	*Neisseria gonorrhea*	Yes	Visual	Blue										X
*77701	Group A *Streptococcus*	Yes	Visual	Purple	X									
*80031	*Staphylococcus aureus*	Yes	Visual	Purple	X							X		

*Currently not available in the USA.

Table 1: Range of Enzyme Confirmation Test Cards/Soon also available in USA

Index List of Media:

B = Blood Agar (e.g., Fluka 70133)
M = MacConkey Agar (e.g., Fluka 70143)
E = *Enterococcus* Selective Agar (e.g., Fluka 45183)
Sal = *Salmonella* Agar (W/brilliant green) (e.g., Brilliant Green Agar, modified Fluka 70134?)
S.Sal = Selective *Salmonella* Agar (e.g. Fluka 05538 or 738419?)
GN = Gram Negative agar (?)
EC = *E. coli* Agar (e.g., Fluka 44655?)
110 = Staphylococcus Agar No. 110 (e.g., Fluka 70193)
LT = Lauryl Tryptose Agar (e.g., Fluka 17349 + Agar)
CH = Chocolate Agar (e.g., Fluka 70133)

Sigma-Aldrich® Worldwide Offices

Argentina
Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia
Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil
Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

Canada
Free Tel: 1800 565 1400
Free Fax: 1800 265 3858
Tel: (+1) 905 829 9500
Fax: (+1) 905 829 9292

Chile
Tel: (+56) 2 495 7395
Fax: (+56) 2 495 7396

People's Republic of China
Free Tel: 800 819 3336
Tel: (+86) 21 6141 5566
Fax: (+86) 21 6141 5567

Czech Republic
Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark
Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland
Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France
Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany
Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1169

Hungary
Tel: (+36) 1 235 9055
Fax: (+36) 1 235 9068

India
Telephone
Bangalore: (+91) 80 6621 9400
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 4087 2364
Pune: (+91) 20 4146 4700
Hyderabad: (+91) 40 3067 7450
Kolkata: (+91) 33 4013 8000

Fax
Bangalore: (+91) 80 6621 9550
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 2579 7589
Pune: (+91) 20 4146 4777
Hyderabad: (+91) 40 3067 7451
Kolkata: (+91) 33 4013 8016

Ireland
Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: +353 (0) 402 20370
Fax: + 353 (0) 402 20375

Israel
Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4222
Fax: (+972) 8 948 4200

Italy
Free Tel: 800 827 018
Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

Japan
Tel: (+81) 3 5796 7300
Fax: (+81) 3 5796 7315

Korea
Free Tel: (+82) 80 023 7111
Free Fax: (+82) 80 023 8111
Tel: (+82) 31 329 9000
Fax: (+82) 31 329 9090

Luxembourg
Tel: (+32) 3 899 1301
Fax: (+32) 3 899 1311

Malaysia
Tel: (+60) 3 5635 3321
Fax: (+60) 3 5635 4116

Mexico
Free Tel: 01 800 007 5300
Free Fax: 01 800 712 9920
Tel: (+52) 722 276 1600
Fax: (+52) 722 276 1601

The Netherlands
Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

New Zealand
Free Tel: 0800 936 666
Free Fax: 0800 937 777
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Norway
Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland
Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal
Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia
Tel: (+7) 495 621 5828
Fax: (+7) 495 621 6037

Singapore
Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

Slovakia
Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa
Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain
Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden
Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland
Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2511
Fax: (+41) 81 756 5449

Thailand
Tel: (+66) 2 126 8141
Fax: (+66) 2 126 8080

United Kingdom
Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 01747 833 000
Fax: (+44) 01747 833 574

United States
Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

Vietnam
Tel: (+84) 8 3516 2810
Fax: (+84) 8 6258 4238

Internet
sigma-aldrich.com

Enabling Science to
Improve the Quality of Life

Order/Customer Service: sigma-aldrich.com/order
Technical Service: sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries SAFC® safcglobal@sial.com
Safety-related Information: sigma-aldrich.com/safetycenter

World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

QHU
81521/T414019
1104